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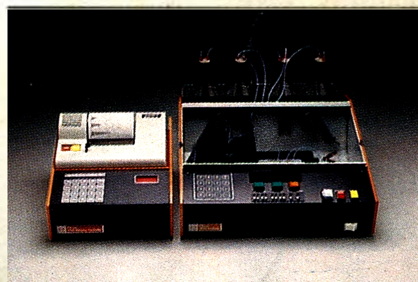

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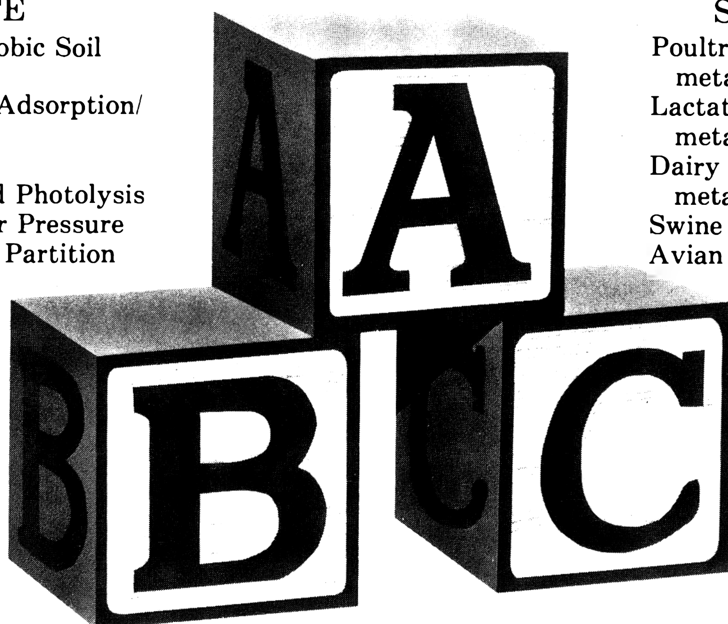

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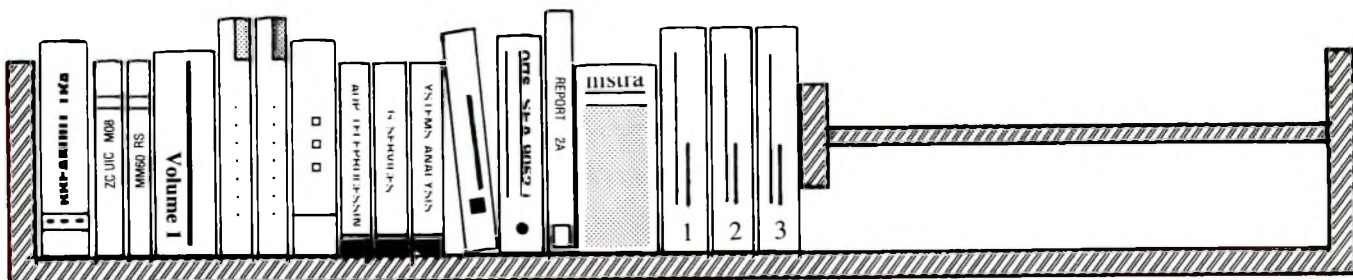

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Circle No. 470

Chromatography Workstation

The Maxima Integrated Chromatography Workstation for the IBM PC, XT, and AT is an advanced hardware/software system that acquires data, performs calculations, and provides control of pump gradients, valves, and other devices for up to 4 chromatographs. This system can be learned in less than 1 hour. Features included are zoom-windowing, pull-down menus, dialog boxes, simulated instrument controls, and simple mouse-driven point-and-select actions are incorporated with the analysis and control software required for most demanding LC, GC, IC, and TLC applications. The maxima also supplies several powerful chromatogram manipulations, such as addition, subtraction, ratioing, derivatives, linear offset, retention time adjustment, and smoothing for noise reduction. Contact: Loring Kutchins, Dynamic Solutions Corp., 2355 Portola Rd, Suite B, Ventura, CA 93003; 805/658-6612.
Circle No. 471

LITERATURE

Color-Detector Tubes Catalog

Bulletin 852 describes color-detector tubes for sampling and analysis of chemicals in air and gases. A direct-reading system enables short-term analysis to determine quickly and precisely the concentration of more than 120 specific gas and chemical vapors at a work site. The catalog contains a selection chart which specifies each tube according to the chemical hazard to be tested and the measuring range. Contact: SKC Inc., RD 1, 395 Valley View Rd, Eighty Four, PA 15330; 412/941-9701.
Circle No. 472

Cryogenic Catalog

The Cryogenic Master Catalog is a 32-page publication which gives in-depth descriptions on a wide variety of cryogenic equipment including containers and instrumentation for liquid nitrogen. Most item descriptions are accompanied by a photograph of the product in question. To order, contact Andonian Cryogenics, 26 Farwell St, Newtonville, MA 02160; 617/969-8010.
Circle No. 473

Short-Form Analysis Catalog

This Products Brief describes the latest in Dohrmann's line of carbon, organic halide, nitrogen, sulfur, and chlorine analyzers. Publication includes photographs and specifications. Instrument applications include: analysis of high-purity, process, wastewater; pollution monitoring; detection of corrosive and polluting elements in crude and refined petroleum; and analysis of coal for sulfur. Contact: Dohrmann Division, Xertex Corp., Marketing Dept, 3240 Scott Blvd, PO Box 58007, Santa Clara, CA 95052-8007; 800/538-7708.
Circle No. 474

BOOKS IN BRIEF

Analytical Solution Calorimetry. Edited by J. K. Grime. Published by John Wiley and Sons, Inc., One Wiley Dr, Somerset, NJ 08873, 1985. 401 pp. Price: $60.00. ISBN 0-471-86942-2.

This volume provides a comprehensive guide for the application of calorimetry to analytical chemistry and the determination of thermodynamic parameters. This publication includes information necessary for readers to understand the principles of the technique, to decide on the instrumental variation best suited to a particular application, and finally to interpret and treat the data. Accordingly, the essential features of 3 major instrumental approaches—isoperibol, heat conduction, and isothermal (heat compensation) calorimetry—are discussed. Also included is a section on flow enthalpimetry and a comprehensive review of applications of many fields of chemistry. The final chapter focuses on the largest single growth area for calorimetry in the last decade—biochemical and clinical analyses.

Molecular Luminescence Spectroscopy: Methods and Applications: Part 1. Edited by S. G. Schulman. Published by John Wiley and Sons, Inc., One Wiley Dr, Somerset, NJ 08873, 1985. 826 pp. Price: $85.00. ISBN 0-471-86848-5.

This volume presents several applications of luminescence spectroscopy. Some of these methods would not have been possible a decade ago and some are recent developments in long-existing methods. The chapters of this work have been written by authors who are active investigators in their area of contribution. These chapters are (1) Luminescence Spectroscopy: An Overview, (2) Fluorescence and Phosphorescence of Pharmaceuticals, (3) Fluorescence of Organic Natural Products, (4) Determinations of Inorganic Substances by Luminescence Methods, (5) Bioinorganic Luminescence Spectroscopy, (6) Excited-State Optical Activity, (7) Fluorescence Detection in Chromatography, and (8) Luminescence Immunoassay.

Bioluminescence and Chemiluminescence: Instruments and Applications. Edited by K. Van Dyke. Published by CRC Press, Inc., 2000 Corporate Blvd, NW, Boca Raton, FL 33431, 1985. Vol I: 256 pp. Price: $83.00 (U.S.)/$95.00 (elsewhere). ISBN 0-8493-5863-9. Vol. II: 288 pp. Price: $93.00 (U.S.)/$107.00 (elsewhere). ISBN 0-8493-5864-7.

These volumes present state-of-the-art applications of chemiluminescence and bioluminescence in measurements of solid phase immunoassay for antigens, hormones, and other biochemicals. Volume I concentrates on the fundamentals of chemistry and measurement of light reactions, including an explanation of available instrumentation and immunoassay, while volume II is dedicated to the applications.

Microbiology of Frozen Foods. Edited by R. K. Robinson. Published by Elsevier Science Publishing Co., Inc., 52 Vanderbilt Ave, New York, NY 10017, 1985. 304 pp. Price: $60.00. ISBN 0-85334-335-7

This book begins with a review of modern food freezing systems, followed by an analysis of the impact of low temperatures on both foodstuffs and contaminating microorganisms. Additional chapters deal with the specific characteristics of meat, fish, and dairy products in relation to cold storage, along with methods for the microbiological examination of frozen foods.

CRC Handbook of Chromatography: Polymers. Edited by G. C. Smith, N. E. Skelly, R. A. Solomon, and C. D. Chow. Published by CRC Press, Inc., 2000 Corporate Blvd, NW, Boca Raton, FL 33431, 1985. 200 pp. Price: $58.00 (U.S.)/$67.00 (elsewhere). Catalog No. 3073AM.

This reference provides important data, such as column packing, temperature, flow rates, column length, retention time, and detection, on 4 chromatographic methods (gas, pyrolysis-gas, liquid column, and thin layer) in accessible, tabular format. Each table concentrates on a polymer or class of compound and is introduced by "cookbook" instructions for the procedure.

Modern Practice of Gas Chromatography, 2nd Ed. Edited by R. L. Grob. Published by John Wiley & Sons, Inc., One Wiley Dr, Somerset, NJ 08873, 1985. 897 pp. Price: $65.00. ISBN 0-471-87157-5.

The chapters in this book have been prepared by member-experts from the Chromatography Forum of the Delaware Valley. This book is an outgrowth of a popular gas chromatography (GC) course sponsored by the Forum. The book is divided into 3 parts: theory and basics; techniques and instrumentation; and applications. In this 2nd edition, the discussion on columns has been expanded to 3 chapters: packed columns, capillary column technology; and optimization of separations in GC. These latter 2 have become very important since the publication of the first edition. The application chapters have been modified, and several new chapters have been added—petroleum and petrochemicals, polymers, and environmental applications.

Organosilicon and Bioorganosilicon Chemistry. Edited by H. Sakurai. Published by Ellis Horwood Ltd, Market Cross House, Cooper St, Chirchester, West Sussex, P019 1EB, England. Distributed by John Wiley and Sons, Inc., One Wiley Dr, Somerset, NJ 08873, 1985. 298 pp. Price: $46.95. ISBN 0-470-20188-6.

This book is a complete compilation of 27 plenary and invited section lectures from the 7th International Symposium on Organosilicon Chemistry held in Kyoto, Japan, September, 1984. Authors were drawn from both academia and industry in order to cover a wide variety of topics. These papers fall into 5 basic categories: stable silicon double bonds, theoretical approach and reactive intermediates, reaction mechanisms and new compounds, silicon in organic synthesis, and bioorganosilicon chemistry and further applications.

Analysis of Drugs in Biological Fluids. By J. Chamberlain. Published by CRC Press, Inc., 2000 Corporate Blvd, NW, Boca Raton, FL 33431, 1985. 320 pp. Price: (prepub.) $92.00 (U.S.)/$106.00 (elsewhere). ISBN 0-8493-5144-8.

This book covers available methodology and equipment for drug analysis. The author guides readers in choosing appropriate methods by reviewing reasons for measuring drugs in biological

fluids, and the applicability limitations, advantages, and disadvantages of various techniques. In addition, this book provides principles for quality control and laboratory management, including guidelines from the FDA Good Laboratory Practice Regulations and Biopharmaceutical Analytical Testing Compliance Program.

Spectral Atlas of Polycyclic Aromatic Compounds. Edited by W. Karcher et al. Published by D. Reidel Publishing Co., member of Kluwer Academic Publishers Group, PO Box 322, 3300 AH Dordrecht, Holland, 1985. 824 pp. Price: Dfl. 280,–/£77.75. ISBN 90-277-1652-8.

This reference source facilitates identification and analysis of polycyclic aromatic compounds. Qualitative and quantitative analyses of polycyclic aromatic hydrocarbons (PAH) and heterocyclic derivatives are complicated by the great numbers of species—often isomeric in composition—which have to be identified and determined; therefore, the systematic determination and collection of the molecular spectra under standard conditions of a selected set of PAH and heterocyclic compounds, as presented in this volume, should be of great help to scientists. In addition to UV-, IR-, mass-, NMR-, and fluorescence spectra, this atlas also contains information on the occurrence and mutagenic/carcinogenic activities of the compounds considered. All spectra have been taken of material of the same origin at known purity as a result of a round robin exercise. Purity in all cases is better than 99% and, in most cases, better than 99.5%.

STP 863/Advances in Luminescence Spectroscopy. Edited by L. J. C. Love and D. Eastwood. Published by ASTM, 1916 Race St, Philadelphia, PA 19103, 1985. 129 pp. Price: $20.80 (member)/$26.00 (nonmember). ISBN 0-8031-0412-X.

In this publication leading experts discuss new, diverse applications and uses of luminescence spectroscopy, new instrumentation, and the latest advances in analytical, physical and biochemistry relating to fluorescence/phosphorescence. The 8 papers in this work cover three basic topics: microenvironmental effects on luminescence properties; indirect probes of luminescence using photoacoustic spectroscopy and immunobilized fluorescent ligands; and transformation of hazardous chemical luminescence spectral data by synchronous excitation fluorescence and pattern recognition.

Microbiology of Fermented Foods, Vols 1 and 2. Edited by B. J. B. Wood. Published by Elsevier Science Publishing Co., Inc., 52 Vanderbilt Ave, New York, NY 10017, 1985. Vol. 1: 368 pp. Price: $81.00. ISBN 0-85334-332-2; Vol. 2: 304 pp. Price: $63.00. ISBN 0-85334-333-0.

These 2 volumes provide a comprehensive overview of microbiology of fermented foods, discussing the major groups of foodstuffs prepared by means of fermentation, including cheese and other milk products, vegetables, vinegar, bread, sausage, and beverages. Several chapters deal with aspects particularly relevant to developing countries. Two chapters on animal production by ensiling and related processes illustrate the microbiological and bio-related chemical similarities between the 2 seemingly different areas of human food fermentation and animal feed production. Finally the potential contribution of genetic engineering to the future development of the fermented food industries is discussed, with special attention to stain maintenance and improvement.

An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. Report of the Subcommittee on Microbiological Criteria, Committee on Food Protection, National Research Council. Published by National Academy Press, 2101 Constitution Ave, NW, Washington, DC 20418, 1985. 456 pp. Price: $32.50. ISBN 0-309-03497-3.

This report examines the role and the value of microbiological criteria in the control of food quality and safety; formulates general principles for the consideration and application of microbiological criteria for foods and food ingredients; provides recommendations for a unified, coordinated approach for implementing the criteria; and puts into perspective the uses of microbiological criteria in a program for microbiological control of food in the United States. The report recommends general adoption in the food industry of the Hazard Analysis Critical Control Point (HACCP) system, a structured approach to the microbiological quality control that emphasizes identification of hazards associated with producing, processing, and retailing foods; determination of critical points at which hazards may be controlled; and establishment of procedures to monitor these control points. These procedures may selectively emphasize inspection, physical and chemical measurements, or microbiological testing, depending on which is most appropriate to the food and the microbiological organism concerned.

STP 867/Quality Assurance for Environmental Measurements. Sponsored by ASTM Committees D-19 on Water and D-22 on Sampling and Analysis of Atmospheres. Published by ASTM, 1916 Race St, Philadelphia, PA 19103, 1985. 448 pp. Price: $40.00 (member)/ $50.00 (nonmember). Pub. Code No. 04-867000-16. ISBN 0-8031-0224-0.

This publication focuses on the importance of reliable measurement data in setting environmental quality standards and establishing regulations to monitor compliance with these standards. This book also evaluates the current environmental measurement systems in terms of: quality of environmental data, data sampling procedures associated with the measurement task, and management and data assessment reports documenting and evaluating the measurement method.

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BAM contains analytical methods for the detection of microorganisms and certain of their metabolic products, primarily in foods. The methods were developed by the U.S. Food and Drug Administration for Federal and State regulatory and industry quality control laboratories. The manual will be updated by supplements issued to users at no additional charge.

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- Aerobic Plate Count
- Coliform Bacteria
- Enteropathogenic *Escherichia coli*
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- *Staphylococcus aureus*
- Staphylococcal Enterotoxins
- *Bacillus cereus*
- *Clostridium perfringens:* Enumeration and Identification
- *Clostridium botulinum*
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To obtain this book, send order and remittance with your name and address to AOAC, 1111 N. 19th Street, Suite 210-J, Arlington, VA 22209 USA (US funds only).

AOAC to hold 11th Annual Spring Training Workshop and Exposition, April 27–30, 1986

The Association of Official Analytical Chemists will sponsor the 11th Annual Spring Training Workshop and Exposition, April 27–30, 1986, at the Stouffer Madison Hotel in Seattle, WA.

In addition to a list of outstanding speakers, this year's workshop will feature the "Leading Edge" Series—a presentation of the newest in LC, GC, GC/MS, AA, ICP, computers, microbiological methods, and more. Also featured is the "Working Smart" Symposium Series—problem solving symposia in the following key analytical areas: LC, AA/ICP, GC/MS, automated analysis, molecular spectroscopy, ion chromatography, and microbiology. The discussion workshop and technical sessions will provide analytical updates in critical areas such as pesticides, foods, drugs, microbiology, feeds, fertilizers, and the environment.

The scientific exposition will be open Sunday evening from 4:00 to 7:00 p.m. and Monday and Tuesday from 10:00 a.m. to 4:00 p.m. A variety of entertainment is scheduled throughout the workshop including boat to Tillicum Island Village for salmon bar-be-que and Indian folklore, theatre, symphony, sports, and tours.

Also, plan now to attend the 1986 World Exposition, which follows the AOAC Workshop, on May 2 in Vancouver, British Columbia, Canada.

For further information, contact H. Michael Wehr, Oregon Department of Agriculture, Laboratory Services Division, 635 Capitol St, NE, Salem, OR 97310; 503/378-3793.

Daniel P. Schwartz Named 1985 Wiley Award Winner

Daniel P. Schwartz, a senior research chemist with the Food Safety Laboratory, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture (USDA), Philadelphia, PA, is the 1985 winner of the AOAC Wiley Award for his development of unique micro methodology. Richard J. Ronk, 1985 AOAC president, will present the award to Dr. Schwartz on Tuesday evening, October 29, at the 99th AOAC Annual International Meeting.

The $2500 award is given annually to a scientist who has made outstanding contributions to the development and validation of methods of analysis for foods, drugs, cosmetics, pesticides, feeds, fertilizers, environmental contaminants, or other related areas. The award was established in 1956 in honor of Harvey W. Wiley, "Father of the 1906 Pure Food and Drug Act" and a founder of AOAC. The award's primary purpose is to emphasize the role of the scientist in protecting the consumer and the quality of the environment.

Dr. Schwartz was responsible for developing the analytical procedures for the quantitative isolation of microgram quantities of carbonyl and hydroxyl compounds from natural products which represented a major breakthrough in methodology for clarifying the role of these compounds in the flavor deterioration of milk and its products. Furthermore, application of the methodology resulted in Dr. Schwartz and his colleagues identifying numerous hydroxyl and oxo-acids at the parts per million levels in milk fat. Many of these compounds have been shown to be precursors to the characteristic flavors of manufactured dairy products. The contributions of the analytical methodology in elucidating the flavor chemistry of dairy products has been recognized by many researchers and led to Dr. Schwartz being awarded the prestigious Hillebrand prize of the Chemical Society in Washington, DC, in 1973.

Dr. Schwartz has also devoted his talents in analytical methodology to developing procedures for performing classical chemical reactions at the submicrogram to micromole levels. The availability of methodology for quantitatively altering the chemical nature of compounds at such minute levels has been indispensible to elucidating the structure of unknown compounds isolated from biological systems.

For the last three years Dr. Schwartz has been involved in the development of new methods for detecting and quantitating drug residues in natural products. His approach has been to devise practical, inexpensive, colorimetric methods that can be applied to a relatively large number of samples per day and which can be adapted readily to field use and possibly to automation in the future.

Dr. Schwartz received his B.S. degree from New Mexico State University in 1950, his M.S. degree from West Virginia University in 1951, and his Ph.D. degree from Ohio State University in 1954. Dr. Schwartz has spent his entire professional career with USDA. In 1966 he worked with the Nobel Laureate, A. I. Virtanen, at the Biochemical Institute, Helsinki, Finland.

Dr. Schwartz is a member of the Americal Chemical Society, American Oil Chemists Society, and the American Dairy Science Association. He is author of approximately 85 publications (about two thirds of which he is senior author) and has one patent (U.S. Pat. No. 3 450 541, 1969).

1985 Fellows of the AOAC

Eleven scientists have been chosen to receive the 1985 Fellow of the AOAC Award, which will be given at the 99th AOAC Annual International Meeting this fall. The following are award recipients.

Charlotte A. Brunner, Chairman of the Archive Committee, has served as Associate Referee (AR) for Digitoxin and Related Glycosides, Mestranol and Sulfonamides. As a member of the Centennial Committee, she coordinated the Hall of Fame display at the Centennial Meeting.

Edwin M. Glocker, former Statistical Consultant for the Committee on Disinfectants and Pesticides (A), has served as Statistician for the Magruder Committee, and was a member of the Committee on Statistics and the Interlaboratory Study Committee.

Prince G. Harrill, Chairman of the Finance Committee and Secretary/Treasurer of the Association, has also served as General Referee (GR) on Cacao Products, as Secretary of the Committee on Foods II (D), and as a member of the Wiley Award Committee and the Ashing Method Committee.

Paul D. Jung, GR for Carbamate and Substituted Urea Insecticides, Pesticide Formulations: General Methods, and Pesticide Formulations: Inorganic Methods, has also served as AR for Atomic Absorption Spectroscopy, and as a member of the Long Range Planning Committee, the Subcommittee on GLC Column Specification, and the Committee on Gas-Liquid Chromatography.

Bernhard Larsen served as Chairman of the Finance Committee and was Treasurer of the Association from 1975 to 1983. He has also been a member of the Committees on the Wiley Award, Fellows, Membership, and International Cooperation, as well as the Long Range Planning and Interagency Committees.

John M. Newton, GR for Nonalcoholic Beverages, is also AR for: Caf-

feine in Coffee and Tea; Nikethamide; Epinephrine and Related Compounds by HPLC-Electrochemical Detectors; Methyl Xanthine Compounds in Nonalcoholic Beverages; and Theophyllin in Tea.

Rodney J. Noel, Chairman of the Committee on Feeds, Fertilizers, and Related Topics (G) and a member of the Official Methods Board, is also a member of the Committee on Safety. He has served as GR on Drugs in Feeds (4 methods adopted in GR topic area) and as AR on Crude Protein in Feeds (3 methods adopted).

Richard J. Ronk is 1985 President of the AOAC.

Leon D. Sawyer is AR for: PCBs; Low Moisture-High Fat Samples for Chlorinated Pesticides; Ethylene Dibromide, and is Co-AR on Comprehensive Multiresidue Methodology (3 methods adopted).

Arnold E. Schulze, AR on Filth in Botanicals, has also served as AR on: Stabilizing Solutions for Mold and Rot Counts; Relation of Mold Count to Dilution of Tomato Catsup; Effects of Comminution of Mold Counts of Tomato Products; Asbestos Fibers; and as GR on Analytical Mycology of Foods and Drugs.

Donald J. Smith is AR for: Chlorpromazine; Epinephrine-Lidocaine Combinations; Thyroid and Thyroxine Related Compounds; and Insulin—Human, Porcine, Bovine HPLC Assay. He has also served as AR for Ion Exchange Resins for Nonalkaloid Drugs and Phenylpropanolamine.

The Fellow of the AOAC award was established in 1961 to recognize those persons giving meritorious service to the Association. Winners of the award have performed notably for 10 years or more, usually as officers, referees, or committee members. Nominations are made by AOAC members, reviewed by the Committee on Fellows, and finally approved by the Board of Directors.

1985 Scholarship Awarded to Maryanne Martling of Longwood, Florida

Maryanne Martling, an outstanding chemistry student at the University of Central Florida, Orlando, FL, is the winner of the 2-year, $1000 scholarship sponsored by AOAC. After completing 60 credit hours at Seminole Community College in Sanford, FL, Maryanne's grade point average was 3.95; and at the end of her freshman year, Maryanne received one of the two "Outstanding Chemistry Student" awards. In addition to her full academic course load, Maryanne has worked as a chemistry laboratory assistant and has devoted much of her time to helping her peers with their studies.

Each year AOAC awards a 2-year scholarship to a college sophomore who is studying a subject important to public health and agriculture. To qualify, the student must be in need of financial aid, maintain at least a B average during the first two years of undergraduate study, and plan to do research, regulatory work, quality control, or teach in an area of interest to the AOAC.

Nominations for the 1986 award must be received before May 1, 1986. Send 6 copies of a nomination letter, and 2 supporting reference letters, to AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209.

Short Courses

The American Association of Cereal Chemists (AACC) announces 2 short courses: (1) "Starch: Structure, Properties, and Food Uses," Dec. 5–6, 1985, at the Holiday Inn O'Hare Kennedy, Chicago, IL. This course is designed for those who wish to have an understanding of the chemical and physical properties of starches, their structure, modification, and food uses. (2) "Milling for Cereal Chemists," January 6–8, 1986, Kansas State University, Manhattan, KS. This course emphasizes experimental milling. All major aspects of the milling process will be discussed and demonstrated with the student observing and then performing the procedure.

For more information, contact Dotty Ginsburg, AACC short course coordinator, 3340 Pilot Knob Rd, St. Paul, MN 55121; 612/454-7250.

IBM Instruments in cooperation with Kent State University's (KSU) Chemistry Dept and the University Conference Bureau will present "Fundamentals of Chromatographic Analysis," December 9–13, 1985. The course will include gas, liquid, and thin layer chromatographic methods, stressing the 3 techniques as complementary rather than competing processes. For more information, contact Carl J. Knauss, Chromatographic Course Coordinator, Chemistry Dept, KSU, Kent, OH 44242; 216/672-2327.

Meetings

December 5, 1985: AOAC New York Regional Section Meeting, World Trade Center, New York, NY. Contact: George Boone, Food and Drug Administration (FDA), HFR-2160, 850 Third Ave, Brooklyn, NY 11232, USA, 718/965-5033.

March 20–21, 1986: National Committee for Clinical Laboratory Standards (NCCLS) Annual Meeting, Omni International Hotel, Baltimore, MD. Contact: NCCLS, 771 E Lancaster Ave, Villanova, PA 19085, USA, 215/525-2435.

April 27–30, 1986: AOAC 11th Annual Spring Training Workshop, Madison Hotel, Seattle, WA. Contact: H. Michael Wehr, Oregon Department of Agriculture, Laboratory Services Division, 635 Capitol St, NE, Salem, OR 97310, USA, 503/378-3793.

April 27–30, 1986: AOAC Northwest Regional Section Meeting, in conjunction with the AOAC 11th Annual Spring Training Workshop, Madison Hotel, Seattle, WA. Contact: H. Michael Wehr, Oregon Department of Agriculture, Laboratory Services Division, 635 Capitol St, NE, Salem, OR 97310, USA, 503/378-3793.

June 24–25, 1986: AOAC Northeast Regional Section Meeting, Canisius College, Buffalo, NY. Contact: Gerald L. Roach, FDA, 599 Delaware Ave, Buffalo, NY 14202, USA, 716/846-4494.

June 1986: AOAC Midwest Regional Section Meeting, Lincoln, NE. Contact: Thomas Jensen, Nebraska Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502, USA, 402/471-2176.

September 15–18, 1986: AOAC 100th Annual International Meeting, The Registry, Scottsdale, AZ. Contact: Margaret Ridgell, AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209, USA, 703/522-3032.

May 18–23, 1986: 10th International Symposium on Column Liquid Chromatography, Sheraton Palace Hotel, San Francisco, CA. Contact: Shirley E. Schlessinger, 400 E Randolph St, Chicago, IL 60601, USA, 312/527-2011.

November 1986: Papers sought for a Symposium on Functional Testing for Hazard Evaluation, Bal Harbour, FL. Authors are requested to submit a title, 3 copies of a 300 to 500 word abstract, and an ASTM Paper Submittal Form by 1 January 1986 to Kathy Greene, ASTM Acquisition and Review, 1916 Race St, Philadelphia, PA 19103, USA; 215/299-5581. For more information, contact John Cairns or James R. Pratt, Virginia Polytechnic Institute and State University, Center for Environmental Studies,

Biology Department, 1020 Derring Hall, Blacksburg, VA 24061, USA, 703/961-5539.

ISO Standard Published

The following standard has been published by the International Organization for Standardization (ISO), Technical Committee 34—Agricultural Food Products. This standard is available for $10.00 from American National Standards Institute, Inc., 1430 Broadway, New York, NY 10018; 212/354-3300.
ISO 5534-1985 Cheese and processed cheese—Determination of total solids content (Reference method).

Standard Reference Materials

The National Bureau of Standards (NBS) Office of Standard Reference Materials announces the availability of the following Standard Reference Materials (SRM) and Reference Materials (RM):

SRM 1960, a 10.0 μm supension of polystyrene spheres in water at a weight concentration of about 0.4%, is intended for use with particle size measuring instruments including electron microscopes. Price: $384.00 per 5 mL vial.

SRM 909, human serum, contains nominal concentrations of copper (1.1 μm/mL), iron (2.0 μg/mL), cadmium (1.2 ng/mL), chromium (91.3 ng/mL), lead (20 ng/mL), and vanadium (2.7 ng/mL) in reconstituted serum. The revised certificate also reports estimated catalytic concentrations of 6 enzymes at 37.0°C. Price: $187.00 per unit of 6 vials of freeze-dried serum and 6 vials of high-purity diluent water.

SRM 8419, inorganic constituents in bovine serum, is intended primarily for use in the determination of major, minor, and trace inorganic constituents in blood serum, plasma, and similar biological fluids. Price: $52 per unit of 3 polyethylene tubes, each containing at least 4 mL serum.

SRM 1583, chlorinated pesticides in 2,2,4-trimethylpentane, was developed for calibrating instrumentation used in the determination of the certified chlorinated pesticides. It is also useful for adding known amounts of these compounds to samples and for determining instrumental response factors. Price: $130 per unit of 6 ampules, each containing about 1 mL solution.

SRM 955, lead in blood, is intended primarily for use in evaluating the accuracy of lead determinations on blood and in calibrating the instrumentation used in these determinations. Four individual concentrations of lead in porcine blood are provided in polyethylene bottles. The nominal certified concentrations, in μg/dL and (μmol/L), are: 5.7(0.27), 30.5(1.47), 49.4(2.38), and 73.2(3.53). SRM 955 consists of four 2 mL samples of blood, each at different concentration. Price: $104.00 per unit.

These SRMs may be purchased from: Office of Standard Reference Materials, Room B311, Chemistry Bldg, National Bureau of Standards, Gaithersburg, MD 20899; 301/921-2045.

EPA Project Summary

In Method 601, the halocarbons are purged by an inert gas which is bubbled through the aqueous sample. The vapors are trapped in a short column containing a suitable sorbent. The trapped components are then thermally desorbed onto the head of a chromatographic column and measured by means of a halide-specific detector. In this study the 28 halocarbon compounds were divided into 3 separate mixes to minimize interferences from co-eluting peaks. The spiking solutions used in the study contained the 29 halocarbons at 6 concentrations. Six water matrices were used in the study: a distilled, drinking, and surface water supplied by the cooperating laboratories; and 3 industrial wastewaters supplied by Monsanto Co.

The object of this study was to characterize the performance of Method 601 in terms of accuracy, overall precision, single-analyst precision, and the effect of water types on accuracy and precision. Through statistical analyses of 26,160 analytical values, estimates of accuracy and precision were made and expressed as regression equations. The accuracy of the method is obtained by comparing the mean recovery to the true values of concentration. For all but 2 of the 27 analytes, the average accuracy over all 6 waters ranged from 83 to 108%. The 2 exceptions were *trans*-1,3,-dichloropropene at 76% and *cis*-1,3 dichloropropene at 56% which were found to be unstable.

The overall standard deviation indicates the precision associated with measurements generated by a group of laboratories. The percent relative standard deviation (%RSD) over 6 waters ranges from 16 to 29% for all but 4 of the 27 analytes. Exceptions include *cis*-1,3 dichloropropene at 40%, bromomethane gas at 42%, *trans*-1,3,-dichloropropene at 45%, and chloromethane gas at 50%.

The gas determinations are expected to be less precise because of handling problems associated with both the sample and standard preparations. The average precision of all analytes in a given water was relatively independent of water type, ranging from 24 to 29% for the 6 water matrices. In all cases, as expected, the highest %RSD (poorest precision) occurred at the lowest Youden pair concentration. The precision is acceptable at all levels when the background interferences are minimal.

The single-analyst standard deviation indicates the precision associated with a single laboratory. The percent relative standard deviation for a single analyst (%RSD-SA), averaged over all the waters, ranged from 10 to 22% for the 27 analytes, while the average value of %RSD-SA for all analytes in a given water ranged from 12 to 15%. In all cases, the highest % RSD-SA (poorest precision) was associated with the lowest Youden pair concentration. The precision is acceptable at all levels where background interference is minimal.

Statistical comparisons of the effect of water type were performed on all analytes. These indicated a practical effect of water matrix on the accuracy or precision of Method 601 in the following cases:

Carbon tetrachloride in wastewater 1
Chlorobenzene in wastewater 1
1,4-Dichlorobenzene in wastewater 3, and
1,2-Dichloroethane in wastewater 3.

Method 601 is recommended for the analysis of purgeable halocarbons in municipal and industrial wastewaters. The accuracy and precision are acceptable, while the matrix effects are significant only at low concentration levels.

The complete report (Order No. PB 84212448) can be obtained for $22.00 (subject to change) from the National Technical Information Service, 5285 Port Royal Rd, Springfield, VA 22161; 703/487-4650. For more information, contact R. Wesselman, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268.

New Private Sustaining Members

AOAC welcomes the following 6 new private sustaining members to the growing list of organizations aware of the need to support an independent methods validation association: Burroughs

Wellcome Co., Research Triangle Park, NC; Mobay Chemical Corp., Kansas City, MO; Waters Chromatography Division of Millipore Corp., Milford, MA; Biochem Laboratorium b.v., 's-Hertogenbosch, The Netherlands; Galbraith Laboratories, Inc., Knoxville, TN; and Silliker Laboratories, Inc., Chicago Heights, IL.

Correction

J. Assoc. Off. Anal. Chem. (1985) **68,** 911–916,

"Analysis of *d*-Phenothrin and Its Optical Isomers in Technical Preparations," by Doi et al., p. 913, left column, line 5,

Change ". . . *d*-phenothrin and total *d*-phenothrin . . . ," to read, ". . . *d*-phenothrin, i.e., total *d*-phenothrin. . . ."

General Referee of the Year Award

Bernadette McMahon, Food and Drug Administration, Washington, DC, was presented with the first annual General Referee of the Year Award at the 99th AOAC Annual International Meeting, October 27–31, 1985. McMahon is General Referee (GR) on Oraganohalogen Pesticides, Committee on Residues.

The General Referee of the Year Award was established by the Board of Directors in response to a recommendation made by the Official Methods Board to recognize the importance of General Referees' roles to the success of the AOAC collaborative/method approval effort and to honor an individual who has significantly contributed to the advancement of methods and who has done an outstanding job as a GR.

The award, which consists of a plaque and financial support for the awardee to attend the annual meeting, is given to the GR who most closely meets the following criteria: Has current GR status, submits oral and written reports in timely and complete manner, meets annually with appropriate Methods Committee, provides assistance in revising pertinent chapters in the *Official Methods of Analysis,* and provides leadership in the investigation of the appropriate analytical fields and new topic identification. In addition, accomplishments of the Associate Referee guided by the GR and AOAC accomplishments related to the GR's topic are considered.

U.S. EPA Brochure Explains New Requirements for Small Quantity Generators

A brochure has been developed by the U.S. Environmental Protection Agency (EPA) to explain the new requirements placed on small quantity generators of hazardous wastes by the Hazardous and Solid Waste Amendments of 1984.

These new amendments to the Resource Conservation and Recovery Act (RCRA) reduce the level at which generators of hazardous wastes become subject to waste management regulations from 1000 to 100 kilograms per month. As a result, many businesses that were previously unregulated will now be required to comply with certain "small quantity generator" provisions.

Some of these provisions became effective on August 5, 1985. To help ensure that all potentially affected businesses are aware of the new regulations, EPA is organizing a massive information dissemination effort and is asking organizations to help distribute the new brochure, which summarizes the new regulations, plus a series of industry-specific inserts explaining how the new provisions will apply to different industry groups.

For a free copy of this brochure, send a stamped, 9 × 12, self-addressed envelope to AOAC, 1111 North 19th St, Suite 210, Arlington, VA 22209.

Please note: A number of states have requirements that differ from those federal requirements for small quantity generators explained in the new EPA brochure. The following states have asked EPA not to distribute the brochure in their states because the federal information it contains might be confusing to residents who must meet the different state requirements: California, Connecticut, District of Columbia, Indiana, Kansas, Kentucky, Louisiana, Maine, Massachusetts, Minnesota, Missouri, Nebraska, New Hampshire, New Mexico, Rhode Island, South Carolina, Vermont, Virginia, and West Virginia.

INFORMATION FOR SUBSCRIBERS, CONTRIBUTORS, AND ADVERTISERS

The Association The primary objective of the Association of Official Analytical Chemists (AOAC) is to obtain, improve, develop, test, and adopt uniform, precise, and accurate methods for the analysis of foods, vitamins, food additives, pesticides, drugs, cosmetics, plants, feeds, fertilizers, hazardous substances, air, water, and any other products, substances, or phenomena affecting the public health and safety, the economic protection of the consumer, or the protection of the quality of the environment; to promote uniformity and reliability in the statement of analytical results; to promote, conduct, and encourage research in the analytical sciences related to agriculture, public health, and regulatory control of commodities in these fields; and to afford opportunity for the discussion of matters of interest to scientists engaged in relevant pursuits.

Membership Membership in AOAC is open to all interested persons worldwide. Sustaining memberships are available to any government agency or private company interested in supporting an independent methods validation program.

The Journal The Journal of the Association of Official Analytical Chemists is published by AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209. The Journal is issued six times a year in January, March, May, July, September, and November. Each volume will contain approximately 1200 pages. The scope of the Journal broadly encompasses the development and validation of analytical procedures pertaining to both the physical and biological sciences related to agriculture, public health and safety, consumer protection, and the quality of the environment. Emphasis is focused on research and development to test and adopt precise, accurate, and sensitive methods for the analysis of foods, food additives and supplements, contaminants, cosmetics, drugs, toxins, hazardous substances, pesticides, feeds, fertilizers, and the environment.

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PLEASE

* NOTE *

The Dates and Location of the
1986 AOAC Annual International Meeting
Have Been Changed to:

September 15-18, 1986
at
THE REGISTRY
Scottsdale, Arizona

Be sure to include
attendance at this meeting
in your 1986 budget!

FOCUS: CHEMICAL CONTAMINANTS IN FOODS

Control of Chemical Contaminants in Foods: Past, Present, and Future

CHARLES F. JELINEK
Food and Drug Administration, Office of Physical Sciences, Washington, DC 20204

In the late 1930s and early 1940s, almost the only analyses carried out for chemical contaminants in foods were for lead arsenate and other arsenical pesticides in fruits. Since then, a tremendous expansion has occurred in the types of chemical contaminants found in foods and in the activities of the U.S. Food and Drug Administration and other organizations responsible for monitoring and controlling the presence of these contaminants in the food supply. This paper describes the findings and control of additional chemical contaminants in foods, including synthetic pesticides, PCBs (polychlorinated biphenyls), other industrial chemicals, fungal metabolites such as aflatoxins, toxic metals, and radionuclides. The common characteristics of problems connected with these defferent types of contaminants include uncontrolled entry into the food supply, incidents causing extreme public worry, and near impossibility in removing these contaminants from the food supply. Problems may also arise from new technologies and environmental developments. New approaches beyond ordinary regulatory activities are being used to meet these problems. Broader analytical methods requiring less time and faster and more sophisticated toxicological methods are needed to assess the hazard of these environmental food contaminants.

It is appropriate, when discussing environmental toxicology and chemical analysis, to review the growth in the responsibilities of the U.S. Food and Drug Administration (FDA) for controlling the chemical contaminants in the nation's food supply in the last 50 years, the way in which FDA has addressed new and emerging problems, and the implications for the future of chemical contaminants analysis. This discussion will cover the broader aspects of the control of chemical contaminants in foods, rather than trace the development of new analytical methods.

In the late 1930s and early 1940s, virtually the only chemical residues in foods which the FDA monitored were the pesticides containing copper, mercury, lead, and/or arsenic, with lead arsenate being by far the most important, because they represented the major pesticides in use at that time. Since those comparatively straightforward days, a tremendous expansion in activities has been necessary for the control of chemical contaminants in the food supply. Both the types of contaminants and the scope of responsibilities have necessitated this expansion.

It is appropriate first to review the different types of residues which FDA now monitors in foods: pesticides, industrial chemicals, toxic metals, natural toxins, and radionuclides. This review will cover our developing awareness of the extent to which foods and feeds are contaminated by these different types, FDA's approach for controlling their levels in the food supply, the common characteristics of the problems connected with these different types of contaminants, and the common strategy that has evolved in dealing with all these contaminants. Because of the overlap in chronology of FDA's involvement with the above types of contaminants, they will not be discussed in chronological order.

Pesticides

With the advent of DDT during World War II for the control of malaria-carrying mosquitoes and typhus-bearing lice, the stage was set for the mass introduction of synthetic organic pesticides as soon as that war ended. By that time, the chemical industry had already instituted a very active program for the development of these synthetic organic products. In 1947, the U.S. Congress passed the Federal Insecticide, Fungicide and Rodenticide Act, under which no pesticide may be shipped in interstate commerce for general use until it has been shown to be safe as used and effective for the purpose claimed. In 1954, Congress amended the Food, Drug and Cosmetic Act to require a company promoting the use of a pesticide to prove that the residues remaining on food are safe for the consumer, to require government clearance before the pesticide is used, and to authorize FDA to establish tolerances for the pesticide on food or feedstuffs. In 1970, the U.S. Environmental Protection Agency (EPA) was given the responsibility for ensuring proper pesticide labeling and use, and for setting food or feed residue tolerances. FDA still enforces pesticide tolerances on all foods except meats, for which the U.S. Department of Agriculture is responsible.

It early became evident to FDA that monitoring for every pesticide on every food for which there was a tolerance would not be possible. Consequently, one of the main objectives guiding FDA's pesticides analytical research has been the development and expansion of multiresidue methods of analysis to enable the simultaneous analysis of a food sample for as many pesticides as possible, rather than a separate analysis for each pesticide by an individual method. At the same time, the agency studied such information as pesticide usage, environmental stability, toxicity, and past monitoring results to determine which pesticide/crop combinations should be monitored for regulatory purposes.

Later, in 1979, it became apparent to FDA that the pesticides to be included in its monitoring programs should be selected on a more systematic basis. As a result, we instituted the development of Surveillance Index documents, which summarize for a given pesticide the pertinent chemical, biological, stability, production/usage, and toxicity data that determine the potential food hazard posed by its residues (1). Our agency has found these Surveillance Index documents to be invaluable in optimizing the development of our pesti-

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cide analytical methodology and our monitoring activities in the field to control pesticide residues in food or feed products.

Currently, FDA analyzes about 12 000 samples of foods and feeds per year for pesticides and PCBs (polychlorinated biphenyls), and takes action on those lots that do not comply with regulations. The agency also furnishes EPA with the data obtained for their use in evaluating current pesticide regulations.

The discovery of the widespread contamination of foods by PCBs was convincing evidence that industrial chemicals can enter the food supply, even though they are not intentionally used in or on agricultural products or processed foods.

Polychlorinated Biphenyls

Following reports of findings of PCBs in fish and wildlife by Swedish investigators in 1966 (2), FDA initiated investigations of PCBs in foods and animal feeds. In 1967, we started the development of a method to separate PCBs from the chlorinated pesticides that were recovered by the multiresidue method for these pesticides (3). As a result of findings of PCBs in subsequent monitoring, FDA established action levels for these products in milk and fish in 1969 and 1970. Based on further results obtained in monitoring programs and on our toxicological evaluation of PCBs, FDA set formal tolerances in 1973 for PCBs in various foods and feeds, prohibited the use of PCBs in "unsealed" equipment in plants processing food, feeds, and food packaging materials, and established an action level for PCBs in paper food-packaging materials (4). Shortly before this, the sole U.S. manufacturer of PCBs ceased marketing these products for all uses except as a fluid for transformers and other enclosed pieces of electrical equipment.

The effects of these actions are shown in Table 1, which depicts the results obtained in FDA's Total Diet or "Market Basket" Studies from 1972 through 1979. In these Studies, we analyzed composites of groups of Market Basket foods which were prepared in the kitchen. The findings of PCBs declined drastically after 1973 in all foods except those in the meat-fish-poultry composite. In most cases, fish are the source of PCBs in this food composite.

The findings in the Total Diet Studies are reflected in FDA's analyses of individual foods; for some years, PCBs have generally been found only in fish and to a lesser degree in animal feeds. Minor increases in findings in milk, eggs, cheese, or meat in certain years usually reflect occurrences such as the contamination of an animal feed component by a leaking transformer.

When new information indicated that PCBs are more toxic (5) and much more persistent in the environment than had previously been thought (6), FDA lowered the tolerances in milk, dairy products, poultry, and eggs in 1979 (7) and fish in 1984 (8). In addition, the possibilities of PCB contamination of foods or feeds from the use, storage, or disposal of transformers and capacitors have been lessened appreciably by regulations established by EPA.

Even so, it will be necessary for FDA to continue monitoring the food supply for PCBs for many years to come.

Unrecognized Contaminants

The discovery of the widespread contamination of foods by PCBs was convincing evidence that industrial chemicals can enter the food supply, even though they are not intentionally used in or on agricultural products or processed foods. Consequently, FDA launched a formal, continuing program in 1971 to search for as yet unrecognized industrial chemical contaminants (9). Its basic aims were to develop background information in a noncrisis atmosphere, to provide FDA and other agencies with an early warning system, and to enable the agency to react promptly and effectively in emergency situations.

In the first step in this program, we were faced with a very challenging situation. About 50 000 industrial chemical products were manufactured in the United States at that time. Since we obviously could not look for all these products in foods, it was necessary to choose those chemicals or chemical families that were the most likely food contaminants. The following criteria were selected in 1972 to make these choices:

- Production volume—the more a product is made and used, the more likely it is to occur in foods
- Toxic by-products
- End-use pattern—the less confined the uses of a product, the more likely it is to cause environmental contamination problems
- Toxicity
- Solubility or partition coefficient—a fat-soluble, water-insoluble material is more likely to concentrate in the fatty portion of fish, meat, milk, and eggs
- Stability
- Means of disposal

These criteria are not noted in any order of priority and are similar to the factors now used by other workers in the environmental field, including FDA's Surveillance Index program for pesticides mentioned earlier.

Table 1. Percent of Total Diet composites containing PCBs

Fiscal year	Food class composite								
	Dairy prods	Meat, fish, poultry	Grains, cereals	Potatoes	Legume vegs	Root vegs	Garden fruits	Oils, fats, shortening	Sugar & adjuncts
1972	6	46	6	—	6	3	3	17	6
1973	10	33	17	3	—	—	—	3	—
1974	—	43	—	—	—	—	—	—	3
1975	—	40	—	—	—	—	—	—	—
1976	5	5	—	—	—	—	—	—	—
1977	—	35	5	—	—	—	—	20	—
1978	—	30	—	—	10	—	—	5	—
1979	5	10	—	—	—	—	—	5	—

The search for a potential industrial chemical contaminant involves several steps (10). First, a satisfactory analytical method to identify and measure trace levels of the compound in the presence of many other chemicals in food such as fish has to be developed, if one is not available. Simultaneously, information about major locations and quantities involved in manufacture and industrial use is developed to identify locations from which to obtain food samples, often with the active assistance of individual states. The freshwater fish has been a prime indicator food because most contaminants eventually enter the waterways. If the suspected contaminants are found, the appropriate state or federal agencies are notified. FDA's monitoring activities may be expanded, up to and including nationwide surveys of various foods. On the basis of these results, FDA toxicologists may then judge whether the incidence and levels of contaminants in foods are of concern, so that FDA can initiate appropriate follow-up.

The entrance of chemical contaminants into foods is difficult to control, they can enter the environment and the food supply in a variety of ways, including misuse, unusual growing conditions, accidents, and legal and illegal disposal. We cannot simply remove a chemical contaminant from foods; we must actually remove the food from the food supply.

Many unrecognized industrial chemical contaminants have been detected in these investigations. Some of the new chemical families found in foods include chlorinated benzenes, chlorinated benzylchlorides and benzotrifluorides, chlorinated cycloaliphatics, chlorinated butadienes, volatile halocarbons, brominated aromatics, orthophosphate esters, and aromatic amines (11). These findings usually do not reflect general public exposure, but may indicate that some chemicals are present in the effluents from nearby plants or are leaching from treatment operations or disposal sites.

Toxic Elements

The discovery in 1969 that mercury in the form of methyl mercury caused hazardous contamination of fish (12), and the subsequent steps that had to be taken by FDA and EPA to protect the public health, emphasized the fact that FDA must pay more attention to the possibility of environmental contamination of foods by toxic elements. The study of toxic element contamination of foods is complicated by the fact that humans experience a finite level of intake of all the elements present in the earth's crust, as demonstrated by the presence of these elements in human tissues (13). Based on consideration of usage, toxicity, and environmental occurrence, FDA accorded first priority in its program on toxic metals to mercury, lead, cadmium, arsenic, selenium, and zinc (14). Of these metals, the agency has devoted by far the most attention to lead and cadmium (11).

Table 2. Decrease in lead content (ppm) in foods for infants

	Mean lead level		
Product	Early 1970s	1976–77	1982–83
Infant formula concentrate[a]	0.10	0.055	0.01
Infant juices	0.30[a]	0.045[a]	0.015[b]
Infant foods, pureed[b]	0.15	0.05	0.02
Evaporated milk[a]	0.52	0.10	0.08

[a]Packed in cans.
[b]Packed in glass.

The single most controllable source of lead in the food supply has been the lead-soldered cans used for canned foods. Data developed by FDA and the industry indicated that canned foods contributed about one-third of all the dietary lead and that the lead solder contributed about two-thirds of this lead (15). Because of lead's toxicity, FDA first expressed its concern to industry about the lead content of canned foods in 1971–1972. More particularly, FDA placed its greatest emphasis on the reduction of lead in foods for infants because of their greater susceptibility to the toxic effects of this metal (16).

Ever since we first contacted industry, our agency has closely followed their programs to decrease lead in foods, especially foods for infants. From time to time, we have monitored the results obtained in these continuing programs by carrying out our own surveys for lead in the various products. As a result of the genuine interest by all parties concerned, lead levels in foods for infants have been substantially reduced (Table 2). In all cases, the levels today are only about one-fifth to one-tenth of what they were when we first contacted the manufacturers of these products. This is one of the real success stories of what can be achieved by a strong government-industry commitment to protect the health of a sensitive group of the consuming public.

Most of the reduction in lead in foods for infants has been achieved by better quality control and by shifting almost all the production from lead-soldered to non-soldered containers. Significantly, this development is now taking place throughout the canned food industry. It is estimated that over 50% of all canned foods are now packed in non-soldered cans—either in welded or drawn two-piece steel cans—contrasted with only 10% five years ago.

FDA will continue to monitor the levels of dietary exposure to lead and closely follow industry's progress in this area.

Mycotoxins

Mycotoxins are toxic substances produced by fungi or molds. Fortunately, relatively few of the many known mycotoxins have been identified as food contaminants. The following discussion will be confined to aflatoxins, because most of the activity on mycotoxins has been concentrated on this group. The aflatoxins are chemically related toxic substances produced by molds such as *Aspergillus flavus* on various foods under favorable conditions of temperature and humidity.

Worldwide interest in mycotoxins was greatly accelerated in 1960 when it was discovered that aflatoxins were responsible for the death of thousands of turkey poults in Great Britain that had been fed contaminated peanut meal. A mixture of the most prevalent aflatoxins, aflatoxins B_1 and G_1, was shown to be carcinogenic to animals in 1963 (17).

FDA became actively involved in research on aflatoxins in the early 1960s. Cooperative research efforts initiated at that time between FDA, USDA, and the peanut industry resulted in the establishment and implementation of programs aimed at keeping the human exposure to aflatoxins through peanut

consumption as low as possible. Later, joint plans for control of aflatoxins by FDA and USDA were extended to other nuts.

In 1965, FDA established an action level of 30 ppb total aflatoxins in affected commodities. In 1969, improvements in analytical techniques enabled the agency to reduce the action level to 20 ppb total aflatoxins.

In 1977, when surveys showed that aflatoxin-contaminated milk in several southeastern states was a potentially serious health problem, FDA established an action level of 0.5 ppb for the metabolite aflatoxin M_1 in whole milk, skim milk, and low fat milk (18).

In 1982, the action level for aflatoxins in cottonseed meal used as a feed ingredient for nondairy animals was increased from 20 to 300 ppb (19). Cottonseed meal normally comprises no more than 11–15% of the total ration of beef cattle, and less for swine and poultry. The higher level of 300 ppb does not result in harmful levels of aflatoxin residues in meat and eggs, and is not considered harmful to the animal.

Emerging problems include the existence of many more chemical dumps than once suspected, resulting in wider and deeper environmental contamination of groundwater, rivers, and lakes; loss of fish and shellfish to the food supply because of naturally occurring seafood toxins; and the possible increase of toxic components through genetic engineering.

Severe drought and other stress conditions in many southeastern states during 1980 resulted in high incidence and levels of aflatoxin contamination in the corn crop from that area. In 1983, a similar phenomenon occurred in the southeastern and also several of the midwestern states. In view of the success of FDA-approved state control procedures which have been used, various options are being considered for the control of corn intended for use as feed for mature beef cattle, swine, and poultry.

Radionuclides

Because of the atmospheric testing of nuclear weapons, FDA initiated a program to monitor for radioactivity in the teenage total diet in 1961 (20). In addition to FDA, 6 federal, state, and private organizations conducted monitoring programs for radionuclides in foods in the 1960s. Because of the downward trend in radionuclide levels as a result of the Nuclear Test Ban Treaty, and to prevent duplication of effort, FDA decided in 1969 to discontinue monitoring for radionuclides in foods. However, with the projected growth of nuclear power, FDA resumed monitoring in 1973 for radionuclides in the food supply, and continues to maintain a radiochemical analytical capability to perform necessary food analyses in the event of a radionuclear accident (21).

The agency's regular monitoring program for radionuclides comprises 3 main segments: radionuclides in imported foods, the Total Diet, and foods collected near nuclear plants (22), as shown in Table 3. FDA monitors imports of various foods and also the Total Diet samples for strontium-90, iodine-131, ruthenium-106, cesium-137, and potassium-40. The first 4 are representative of fall-out products, while potassium-40 is a naturally occurring radionuclide. Tritium is measured in foods grown near nuclear plants as an indicator of reactor leakage. Scanning for beta- and gamma-emitters in these foods is also conducted to detect radioactive corrosion products and fall-out products which would result from an incident or from faulty operations. In these 3 monitoring programs, the radionuclides either have not been detected, or at most have been found at very low levels. No particular trends have been noted in the levels observed.

Table 3. FDA monitoring for radionuclides in foods

Monitoring activity	Radionuclides
Imported foods	^{90}Sr, ^{131}I, ^{106}Ru, ^{137}Cs, ^{40}K
Total Diet	^{90}Sr, ^{131}I, ^{106}Ru, ^{137}Cs, ^{40}K
Food grown near nuclear plants	^{3}H, β-emitters, γ-emitters

In addition to these ongoing radionuclide monitoring programs, FDA has carried out investigations in local areas of interest. Examples include analysis of citrus fruits grown in areas of phosphate mine tailings in Florida for radium-226, determination of plutonium-239 and beta- and gamma-emitters in fish caught in the vicinity of nuclear waste disposal areas in the Atlantic and Pacific Oceans, and analysis of sediments, crops, and fish collected near the Savannah River nuclear facility for plutonium-239. We have not found any levels of radionuclides that would represent a potential hazard for the consumer.

As a result of these radionuclide monitoring activities, FDA was in a position to respond immediately and effectively to such incidents as the iodine-131 contamination of milk in northeastern states several years ago following a Chinese H-bomb test, and even more important, the potential contamination of food in Pennsylvania during the Three Mile Island nuclear power plant crisis (11).

Discussion

As can be seen, the scope of chemical contaminants in foods has grown to include synthetic chemicals varying from radioactive isotopes to pesticides. It also includes chemicals furnished by nature, varying from mold toxins to organometallics formed in the ocean sediments. Despite this tremendous diversity of products, certain characteristics are common to all the major categories of contaminants.

First, FDA must deal with many substances in each of these categories. About 300 pesticides are registered by EPA for use on foods. Moreover, these pesticides are converted to metabolites and other alteration products, many of which are toxic. With regard to industrial chemicals other than pesticides, the Office of Toxic Substances in EPA has about 60 000 commercial chemical products in its registry. We must also consider impurities and by-products, such as chlorinated dioxins and dibenzofurans. Although the number of toxic metals and other elements is smaller, they literally are ubiquitous, and their toxicity is very dependent on their chemical form. We estimate that about 280 natural and 1700 synthetic radionuclides exist, a considerable number. No one has a good estimate of the number of toxic mold metabolites, marine toxins, and plant toxins.

Second, every major contaminant category contains some very toxic substances—for example, aldicarb, dioxins, methyl mercury, aflatoxins, and plutonium, to name a few.

Third, the entrance of these materials into foods is difficult to control, even with the registered pesticides, when it comes to their actual usage. As a result, toxic chemical contaminants can enter the environment and the food supply in a variety of ways, including misuse, unusual growing conditions, accidents, near-miss catastrophes, legal and illegal disposal, and governmental nuclear testing. When such incidents, uncontrolled as they are, occur with these contaminants, as toxic as they are, the media feature them prominently, and the public understandably becomes very edgy. Near hysteria can occur if citizens feel that government agencies do not show proper concern for their safety.

Finally, we cannot prevent these toxic products from being in the food supply after they have entered the environment, in the way that we can ban the use of food additives or animal drugs. If we need to exert control, we cannot simply remove a chemical contaminant from foods; we must actually *remove food from the food supply.*

Because of these common characteristics, the general strategy FDA has used in developing its programs for control of these major categories of contaminants is as follows:

- We develop information to select those contaminants to be investigated.
- By means of toxicological studies, analytical methods development, and monitoring programs, we decide which contaminants pose a potential for hazardous contamination of foods. Where possible, we gear the limits of determination of analytical methods to the contaminant levels of concern proposed by our toxicologists.
- We ensure that these contaminants do not pose a hazard to the consumer.

Even though the general strategy is the same, the tactics used by FDA in regard to the latter step will obviously vary greatly among the different categories of contaminants. With pesticides, for example, regulations prescribe the maximum levels allowed for each pesticide in each food on which it is permitted to be used. In this case, FDA's field programs are carried out to determine whether pesticides are present on a particular food in excessive levels, and to take regulatory action where necessary. At the other end of the spectrum, FDA's search for potential contaminants is exploratory in nature; the follow-up, if any, depends on the nature of the findings, and in any event is usually not regulatory in nature.

The very diversity of the environmental chemical contaminants area has led FDA to be flexible in its control procedures, besides the basic one of carrying out field monitoring programs and taking regulatory action where required. Among these other approaches are the following:

Anticipatory investigations.—The first two parts of FDA's general strategy—developing information and deciding whether the food levels of a contaminant pose a hazard to humans—can provide an early warning in the cases in which the agency has not set an action level or tolerance for the substance. Because of our development of information about a contaminant's use, occurrence, and toxicity, and the possession of a suitable method to measure it in the food supply, FDA is at the very least able to respond promptly when incidents do occur.

Cooperation of industry.—FDA can also use this information more actively by enlisting industry's cooperation in preventing a serious problem from arising. Some cases in point include the reduction of lead in canned foods, the policing of the peanut crop by the peanut industry for excessive aflatoxin contamination, and the reduction of nitrosamines in baby bottle nipples.

Joint activities with States and Federal agencies.—FDA's experience in the previous 10–15 years has shown the benefits to be gained in working with states and other federal agencies in anticipating and preventing problems whenever possible. Some examples of fruitful cooperation are the joint USDA-FDA monitoring for aflatoxins in peanuts; the guidance jointly developed by EPA, USDA, and FDA for use of municipal sludge on cropland; and the exchange of data between FDA and some states on pesticide residues in foods. This approach must be developed and used much more in the future. With the limited resources at the disposal of federal and state agencies, we must jointly continue to make the best use of the information developed and the monitoring we carry out to either prevent or anticipate problems that may arise.

Potential Problem Developments

The United States has been aware of the hazards from toxic dump sites for a long time, and this has been a subject of intense public worry since the Love Canal chemical dump incident. Furthermore, as a result of EPA investigations carried out under the Resource Conservation and Recovery Act and of the cleanup programs being initiated for hazardous sites as part of the Superfund program, it is obvious that there are many more chemical dumps (in operation and abandoned) than were once suspected. In addition, the areas of contamination from these sites are known to be wider and deeper than once thought. In some cases, the groundwater has become contaminated, with every prospect that the toxic plumes from more sites will advance into the aquifers. In other cases, the contaminants have leached into rivers and lakes.

FDA has kept in close touch with EPA concerning the information that has been developed about toxic sites. As a follow-up, our agency plans to investigate the process water and selected products of food processors located near Superfund-designated dump sites which have caused groundwater contamination and also the products of processors in municipalities where drinking water has been contaminated.

Even where these sites are reclaimed, many toxic substances are very stable and will remain in the groundwater and surface water a long time. It will be necessary for FDA to continue to work closely with EPA for many years to determine where food contamination is occurring and to take care of the situation where problems of food contamination do exist.

Even though we do not now hear as many dire predictions of worldwide food shortages and under-nutrition as a decade ago, regional crises such as the one in Ethiopia and also in the Sahara do occur periodically. More and more pressure will be applied to use every available food source as the world population increases.

At present, sizable amounts of fish and shellfish are lost to the food supply annually because of naturally occurring seafood toxins. It is estimated that over 10 000 individuals worldwide are afflicted annually by ciguatera and paralytic shellfish poisoning, leading to more than 100 deaths (23). In the Atlantic Ocean, shellfishing areas from the Maritime Provinces to Long Island are closed temporarily every year to harvesting because of the presence of paralytic shellfish poisons from toxic "red-tide" organisms. In the Pacific Ocean, the Alaskan Coasts, except for 3 small areas, have been closed permanently to shellfishing for the same reason. Likewise, many cases of illness and some deaths have been reported from the ingestion of ciguatera toxins from finfish such as red snapper, grouper, and barracuda in the Caribbean Sea and from amberjack and Spanish mackerel in the Pacific Ocean.

Considerable progress has been made by FDA, the U.S. National Marine Fisheries Service, and other organizations in learning more about the structures of these fish toxins and developing chemical analytical methods to replace the tedious mouse bioassay. We can anticipate improved analytical methods to better protect the public health in the future. However, it is important that many additional studies be carried out to learn more of the propagation of the dinoflagellate organisms and how they develop these toxins. At the least, we will be able to protect the public health more effectively. But even more, we might learn how to prevent or control the propagation of the seafood toxins to make many more tons of seafood available for human consumption.

Biotechnology, or genetic engineering, is the area where the most radically new research and development in foods is being carried out. By the use of techniques such as recombinant DNA, new varieties of food crops are being developed to impart substantial improvements in many properties such as insect resistance, ability to fix nitrogen, drought resistance, ability to grow in saline water, or any other characteristic which may increase the yield or marketability of crops. We can expect that these technical improvements will occur much faster than they would by classical genetic approaches.

In developing these significant changes in a plant, the levels of its toxic components may also increase significantly. Even where ordinary genetic techniques were used, it was necessary some years ago to withdraw a new variety of potato from the market because of the levels of solanine it contained. To be in a position to assure the safety of these new crop varieties, much more information must be developed by academia, the companies engaged in these developments, and agencies such as FDA and USDA to determine the naturally occurring plant toxins in the important crops, and to identify those that may be toxic enough to merit special surveillance. Although many studies have been conducted in this subject area, including some by FDA, many more well thought-out investigations requiring structure determination, analytical methods development, and toxicological screening and testing must be carried out.

All three of the above problems require more attention and increased study now. Toxic dump sites and naturally occurring fish toxins are existing problems which merit the most immediate attention. In the long run, however, new food products resulting from genetic engineering will pose more problems and will require more activity.

Acid rain is another environmental problem that has caused a great deal of concern in recent years. Acid rain results when the acid-causing products emitted in the atmosphere in one area cause acidic precipitation in other locations. In mountainous areas where the topsoil is thin, the run-off has lowered the pH sufficiently in some lakes to kill the fish. If run-off is not a factor, the effects of acid rain are not so dramatic. However, as the soil becomes more acidic, some toxic metals such as cadmium and lead are mobilized and are more readily translocated into food crops or flow into a waterway to a greater extent. This is not likely to pose a problem of food safety. Nevertheless, FDA plans to keep abreast of the information developed by other agencies on acid rain to determine whether any investigations from the standpoint of food safety are warranted.

We have seen how the control of environmental contaminants in foods has grown tremendously in breadth, complexity, and public awareness. Also, we have considered a few developments that will require special attention by all of us in the future. With these challenges facing us, it is obvious that we will need better methods, both chemical and toxicological, to develop needed information faster. The analytical chemists must develop better analytical methods—not so much methods with lower quantitation limits, but ones that are broader in scope, less time-consuming, and greater in specificity. Likewise, toxicologists must develop more fundamental information on the biochemical mechanisms of such chronic manifestations as carcinogenicity, mutagenicity, teratogenicity, effects on the central nervous system, and immune deficiency. More important, they need to develop in vivo and in vitro tests to assess more quickly the toxicity of the different chemicals found in the environment to help guide analytical developments and monitoring activities.

Even more important than amassing information on toxicity and exposure, however, is its use: How do we assess the risks connected with a contaminant or family of contaminants; how do we decide to control this risk, whether by setting regulatory levels for the contaminant in different foods, or by other approaches; and how do we use the activities of the federal agencies, the states, the producers, the processors, and even the consumers themselves in assuring the safety of foods. Public health protection will require an unusual degree of wisdom to properly limit human exposure to the various environmental toxicants.

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Chemical Contaminants in Foods: Some Analytical Considerations

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Data on the incidence and levels of chemical contaminants in foods are needed for continuous assessment of the safety of the food supply and to inform the public about the safety of food. A larger share of the total analytical resource—federal and state government, private sector, and academia—could profitably be directed to collection and publication of data on the occurrence of chemical contaminants in foods. The quest for more data must be accompanied by measures to ensure data reliability and comparability and to estimate the uncertainty of measurements. Research to improve the efficiency of analysis may be the top priority for future methods improvement studies. Analytical chemistry will continue to be an essential factor in assuring a safe food supply and in communicating to the public accurate information and conclusions about food safety.

Analytical chemistry is at the heart of programs to protect the public from toxic chemical contaminants which may enter food. Regulatory limit enforcement, trend monitoring, surveys to determine the extent of contamination, and exploratory investigations all depend on laboratory analysis. Reliable data on the incidence and levels of contaminants are essential for recognition and control of potential food safety problems and for maintaining public confidence in food safety. Consumers, farmers, food manufacturers, chemical manufacturers, toxicologists, and analytical chemists all have an interest in this issue, but each from a different point of view. Yet all must rely on data generated in laboratory analyses.

In this context, the term chemical contaminants includes industrial chemicals with no purpose for being in food, toxic elements naturally present in the earth's crust and in some food, and pesticides and their residues that are expected to be present on food after permitted uses, but which may also enter the food chain by virtue of their presence in the environment.

Publication of Data on Contaminant Occurrence

An immediate challenge to contaminant analytical chemists is to collect and publish data on the incidence and levels of chemical contaminants in foods to help answer contemporary questions. The generation of data must be supported by appropriate quality assurance measures throughout the entire process, from sampling to data reporting. The universe of food is enormous; similarly large and diverse is the array of potential contaminating chemicals. Because of the information-seeking nature of analytical chemistry, chemists should take the lead in conducting studies to fill gaps in our knowledge of contaminant occurrence in food. Analytical chemists will need to interact closely with toxicologists, statisticians, epidemiologists, and administrators in selecting samples, evaluating findings, and recommending actions to protect and promote the public health.

Analytical chemists have made remarkable advances over the past 25 years in developing and applying their technology to the resolution of chemical contaminant issues. But we have not shown the same zeal for publishing the results of surveys for contaminants in foods as for publishing research on methods development. Possibly determination of the occurrence of chemical contaminants has not seemed to offer the opportunity for appropriate recognition, or limited outlets have existed in the scientific literature for data collections. Continuing acquisition of occurrence data for chemical contaminants and publication in peer-reviewed journals are necessary for recognition of and response to emerging problems. Moreover, these data will be essential 10–20 years from now for evaluation relative to expanding toxicological information or changes in human food consumption and other exposures to contaminants.

Consumer interest in knowing which contaminants are present in food and which are not is another reason for timely collection and publication of occurrence data. A Food Marketing Institute poll released in March 1984 concluded that public concern persists about chemical residues in food: 77% of those surveyed said that chemical residues such as, "pesticides and herbicides" are a serious hazard, topping the list of consumer concerns (1).

Importance of Analytical Results

In decisions by regulatory agencies, the quality of analytical data, availability of methodology, and capabilities of laboratories are important issues. Information about contaminant occurrence is the starting point for decision making, and subsequent action to determine the extent of contamination or to establish and enforce a regulatory limit depends on the availability of reliable and practical analytical methodology. The impact of food safety decisions on consumers and industry justifies this emphasis. Often decision makers would like to have much more data with which to operate. If action is taken against a food commodity because of the presence of a potentially toxic amount of contaminant, in effect the food is banned. This is costly to both food producers and consumers, whether the food is a shipment of fresh produce or the fish from an entire lake. Yet if a harmful food is left on the market, consumers are exposed to an unexpected and unnecessary hazard.

Analytical chemists often may not appreciate the significance of the data they generate, for example, that each finding of PCBs in fish, even when well below the former tolerance of 5 ppm, would eventually support the decision to reduce the tolerance for PCBs to 2 ppm, balancing safety against economic impact to the fishing industry, or that their analysis for lead in a canned food would play a vital part in correctly assessing the dietary intake of lead.

Occasionally, the analytical chemist must step back and assess the purpose and significance of his or her work. When a sample is directly related to specific regulatory action, such as a pesticide residue above the tolerance level, the purpose of the analysis and the significance of the result are clear. When no regulation appears to be violated, the significance of the analysis is less obvious. However, *each* analytical finding may be an important building block of a future decision affecting human safety (or the consumer's perception of

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it) and the economy. Thus nondetection of potential contaminants can have just as significant an effect as finding that the contaminant was present. The ultimate purpose of analyzing food for chemical contaminants is to protect the public health, no matter who performs the analysis.

An interested and concerned citizenry expects information about contaminant occurrence in food from well planned and executed studies that have been carefully evaluated and clearly reported. A greater share of the total analytical resource should now be devoted to acquiring more, better, and faster data on the incidence and levels of chemical contaminants in food.

Acceptably Accurate Analytical Data

To ensure the safety of the nation's food, the U.S. Food and Drug Administration (FDA), sometimes in conjunction with the U.S. Environmental Protection Agency (EPA), must obtain acceptably accurate analytical data, i.e., analytical data prepared with care and free of mistakes and error. Data may be obtained from FDA laboratories or other government, academic, industrial, or private laboratories. The issues addressed may range from relatively narrow, such as inquiry into an apparently increased level of a toxic element in a food crop from a specific geographical area, to very broad, such as establishing tolerances for PCBs in several foods and animal feeds.

Data comparability.—Comparability of data is an essential consideration when more than one laboratory is involved. This basic concern led to the establishment of the Association of Official Analytical Chemists 100 years ago. Questions about data comparability fall into 2 categories: sampling and analysis.

The food sample.—In addition to the necessary considerations about representative sampling, it is necessary to take precautions to protect the integrity of sample and analyte from the time of collection until analysis and to be careful in selecting the actual portion of the food commodity analyzed. The use of proven analytical methods, carefully monitored by quality assurance procedures, does not protect against contamination of the sample during collection and transit or loss of the analyte during inappropriate sample storage. An analytical chemist experienced with the analytes sought should always assist in developing the protocol for all phases of sample handling.

Selection of the portion of the food item for analysis should be guided by the U.S. Code of Federal Regulations tolerances for pesticide residues and the FDA *Pesticide Analytical Manual*, Volume I (2, 3). In general these guides also apply to toxic elements or industrial chemicals. The importance of carefully defining the portion of a food analyzed is illustrated by the example of fish. The FDA *Pesticide Analytical Manual* defines the edible portion of fish for analysis as with guts, head, tail, fins, and scales removed; skin remains on the sample (3). Analytical data for fish prepared otherwise, e.g., as the whole fish, would not be comparable and could not be used as part of a data-set evaluated for food safety purposes.

The analytical method.—Because of the physio-chemical variation in foodstuffs, the large number of chemicals of potential interest, and the various purposes of analysis, a large variety of analytical methodology is necessary. Moreover, analytical chemists tend to interject variations into a prescribed analytical process. Sometimes procedural modifications are essential to solve a problem associated with a particular type of sample or analyte, but changes in a method are not always well supported or clearly communicated. It is best to avoid changing an analytical method just because we think the analysis can be done differently. Data often are not usable for food safety decisions because of questions about data comparability or analytical methodology.

Use of AOAC official analytical methods will reduce questions about data quality and interlaboratory comparability. FDA has taken the official position that the agency will use AOAC methods, when available, in its enforcement programs (4).

Official Methods of Analysis, 14th edition, contains methodology for 47 pesticides and alteration products, 12 elements, and one industrial chemical in specified foods (5). Because AOAC official methods are available for relatively few of the potential chemical contaminants and some of these methods are not considered modern or efficient, future data on many chemicals and foods will be generated by methods that have not been subjected to formal interlaboratory collaborative study. Conclusions and significant decisions must be made in spite of this. Analytical chemists share a common duty to ensure the generation of reliable data.

Reliable data on the incidence and levels of contaminants are essential for recognition and control of potential food safety problems. Consumers, farmers, food manufacturers, chemical manufacturers, toxicologists, and analytical chemists all have an interest in this issue, but each from a different point of view.

Measures necessary to obtain reliable data have been addressed in "Principles of Environmental Analysis," a 1983 publication of the American Chemical Society Subcommittee on Environmental Monitoring and Analyses (6), to which many respected scientists from government, industry, and academia have contributed. This comprehensive set of guidelines for planning and executing analytical work contains sections on Planning, Quality Assurance and Quality Control, Verification and Validation, Precision and Accuracy, Sampling, Measurements, and Documentation and Reporting that are applicable to analysis of food as well as environmental samples.

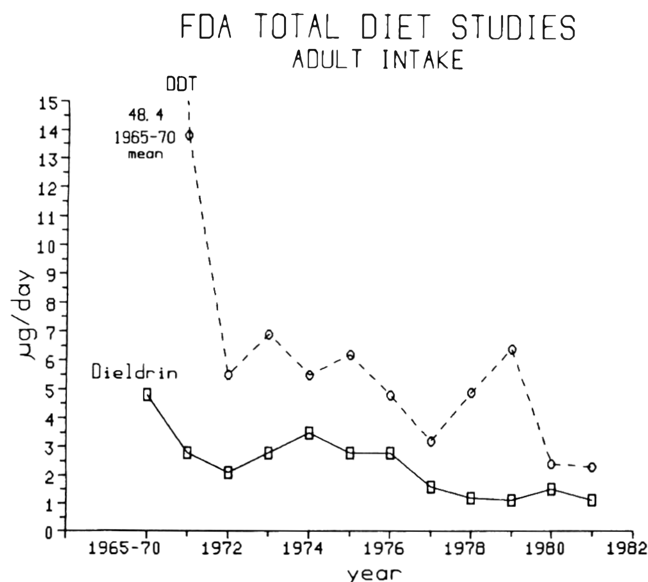

Figure 1. Decline in adult dietary intake of DDT and dieldrin, 1965–1981.

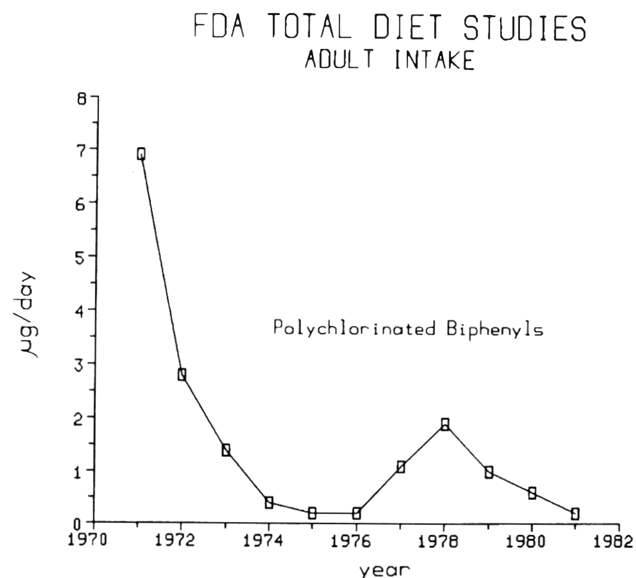

Figure 2. Decline in adult dietary intake of polychlorinated biphenyls, 1971–1981.

Looking Back

During the past 20 years, analytical chemistry has played a major role in governmental and technological processes that have succeeded in substantially reducing the levels of food contaminants, as exemplified by the pesticides DDT and dieldrin, the industrial chemical PCBs, and the toxic element lead in food.

The dietary intakes shown in Figure 1 for DDT and dieldrin and in Figure 2 for PCBs are from FDA Total Diet Studies (periodic analyses of about 120 kitchen-prepared food items grouped into 12 major food categories analyzed as composites).

DDT and dieldrin.—Figure 1 shows the declines in adult dietary intakes of DDT and dieldrin monitored for over 15 years in FDA's Total Diet Studies (7, 8). The need to examine food rapidly for many residues of organochlorine pesticides brought about the development of multiresidue methods that are still being extended and updated, and which prepared the way for chemical contaminant analysis as we know it today. Regulatory issues led to collaborative studies and to AOAC official methods. Data gathered in widespread monitoring of food were used by EPA and regulatory bodies in other countries in decisions to ban or drastically limit the use of these pesticides. However, the need for analysis continues. Although their levels have declined substantially, DDE (the DDT metabolite) and dieldrin are 2 of the 3 most frequently encountered organic residues in the Total Diet Studies, and residues are also found in other foods, especially fish from inland waters. The development of multiresidue methods and conduct of collaborative studies many years ago have proven to be excellent investments.

PCBs.—Figure 2 shows the decline in the adult dietary intake of PCBs over a 10 year period of government and industry action, including technological changes and establishment of tolerances for residues in foods (7, 8). Multiresidue analysis for organochlorine pesticides opened the way for detection of these previously unrecognized contaminants. Jensen's pursuit of an explanation for unidentified peaks in gas chromatograms leading to the identification of PCBs as contaminants of fish (9) is a reminder to analytical chemists that contamination of foods can occur from chemical uses unrelated to food. The analyst must be alert for unusual analytical findings and follow them up to identify previously unrecognized contaminants and to prevent erroneous reporting of the presence of known contaminants. Quantitation of PCB levels when the chlorobiphenyl congener composition has been altered by metabolic or environmental processes is extremely difficult and its accuracy is open to question. In spite of this, however, practical methods have been successfully collaborated and used with sufficient uniformity to generate data necessary for far-reaching decisions, leading to substantially reduced and diminishing PCB levels.

Lead.—Figure 3 shows the decrease in the level of lead in several foods eaten by infants, which resulted from a joint effort by FDA and the infant food and canned food industry that led to substantial changes in canning technology and food packaging. Most of the data shown here were generated by the food industry. Government and industry chemists worked together to achieve and implement the capability to measure lead with acceptable precision at ever-decreasing levels. The major obstacle to be overcome was the relatively high blank that limited the validity of measurements at low levels. Analytical chemists learned to perform analyses at these low levels, introducing special precautions to control contamination of laboratory and reagents, and using sensitive anodic stripping voltammetry or graphite furnace atomic absorption spectrophotometry for determination. Contamination control is a continuing requirement to ensure a sufficiently low limit of quantitation for accurate lead measurements and interlaboratory comparability of data.

Future Choices

Today's managers of analytical laboratories are faced with the question of how best to allocate resources. Technology is available for detection of nanograms and picograms and determination of part-per-million and part-per-billion levels of contaminants in food is commonplace. An interested and concerned citizenry expects information about contaminant occurrence in food from well planned and executed studies that have been carefully evaluated and clearly reported. A greater share of the total analytical resource should now be devoted to acquiring more, better, and faster data on the incidence and levels of chemical contaminants in food.

More data.—There are no strict criteria for selecting analytes and foods for a contaminant occurrence study. For

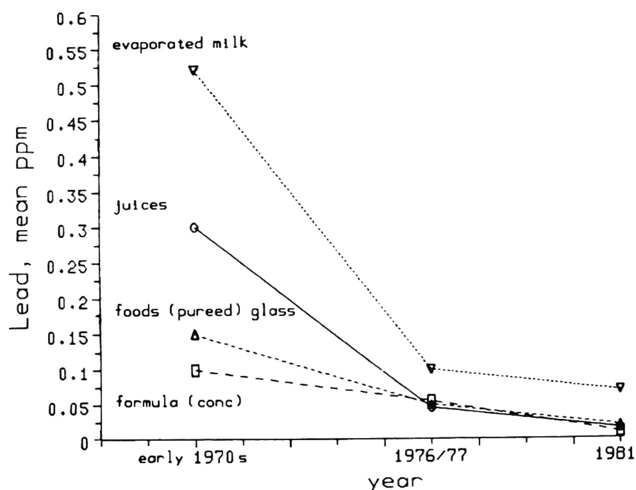

Figure 3. Decrease in levels of lead in some infant foods over an approximately 10-year period.

industrial chemicals, FDA has considered volume of production, associated impurities or by-products, predicted environmental stability, use pattern, oil-water partition coefficients, bioaccumulation potential, known toxicity, and means of disposal (10). Pesticides that are expected to be used on foods present a different situation. FDA is evaluating each pesticide to assure that those with the highest relative hazard potential are included in monitoring programs. A 5-tiered relative hazard classification considers such factors as production and market volume, crop usage, environmental stability, alteration products, toxicity, and potential for dietary exposure. A monograph or "Surveillance Index" prepared for each pesticide evaluated is available from the National Technical Information Service (11). Use of the Surveillance Index to guide monitoring for pesticide residues in food has been discussed by Reed (12). In 1982 the list of foods sampled for the Total Diet Studies was revised to reflect information on food consumption acquired between 1976 and 1980; 234 kitchen-prepared foods were chosen to approximate 90% or more of the weight of food usually consumed by 8 age-sex groups. These foods are now analyzed individually instead of as composites (13).

Although it is important to be aware of the activities of organizations such as FDA, the individual analytical chemist can be a significant driving force in collecting relevant data by identifying some combination of food, geographical location, circumstance of food production, potential chemical contaminants, and unique chemistry for which data are limited or nonexistent.

Better data.—Thoughtful application of the guidelines given in "Principles of Environmental Analysis" (6) will help assure that reliable data are produced by a given study. Careful planning and reporting are absolutely essential for generating purposeful data and avoiding misinformation (6).

Analytical laboratories should have an agenda that includes participation in interlaboratory studies leading to unified use, and recognition by AOAC, of specified analytical methodology. Deliberate selection of the analytical methods and important and representative analytes for study, and working through the AOAC referee process, are requirements for success. Conduct of collaborative studies should be a continuing process, involving a cross section of laboratories, and aimed at attaining a repertoire of AOAC methods.

Beyond the use of standard methods by skilled and experienced analysts, properly equipped and performing in an atmosphere of management concern and support, accuracy and credibility of data require an on-going quality assurance program. Even with official analytical methods, skilled analytical chemists, good management, and sound quality assurance programs, analytical measurements will have a range of uncertainty. For several years, Horwitz has written eloquently about the inevitability of variability. A selection of his papers is included in the bibliography (14–31).

Analytical chemists need better ways to communicate the uncertainty associated with their measurements. Toxic element analysts, with the availability of NBS Standard Reference Materials, are better prepared than are the organic contaminant analysts to express measurement uncertainty and to resolve questions about data comparability. Preparation of standard reference materials for carefully selected chemical-matrix combinations, with emphasis on stable compounds expected to be potential contaminants for many years, could be a goal, perhaps of a joint effort spearheaded by AOAC and the National Bureau of Standards, with sponsors from government and industry. In the absence of standard reference materials, consensus standards based on rigorous analysis by 2 or more laboratories of an appropriate matrix-analyte combination can be used to monitor analytical performance. This approach is now being used by FDA laboratories in the agency's analysis program for TCDD (2,3,7,8-tetrachlorodibenzodioxin).

Because AOAC official methods are available for relatively few of the potential chemical contaminants . . . future data on many chemicals and foods will be generated by methods that have not been subjected to formal interlaboratory collaborative study. Conclusions and significant decisions must be made in spite of this. Analytical chemists share a common duty to ensure the generation of reliable data.

In the quest for better data, we analytical chemists need to improve our use of quantitative analytical results, i.e., significant figures. This was exemplified during the recent interest in ethylene dibromide. Technical reports and stories in the media contained values such as 14,212, and 2.163 ppb. Reporting of excessive numerals complicates our already difficult job of communicating about and effectively dealing with exogenous chemicals in foods; these numbers should have been reported as 14.2 ppm and 2.2 ppb.

Faster data.—Faster data signifies not only increased analytical efficiency but also more timely acquisition of data. Studies to investigate the occurrence of a contaminant are best carried out before a public issue has developed, while

there is time to gather enough information to assess a situation and decide on a course of remedial action. The sooner an emerging contaminant problem is recognized the easier it is to achieve control.

Research is needed to increase the information gained for a given amount of analysis time. The possibilities for increasing analytical efficiency are numerous: computer applications to data reduction, instrument control or report preparation, robotics, chemometrics, automated chromatography systems for unattended operation, increased analysis specificity, streamlining of a particular method, identity confirmation procedures not requiring scarce instruments, an analysis scheme for systematic examination of food for a large number of potential contaminants, and continued expansion of the number of chemicals tested for recovery through established multiresidue methods. However, successes in achieving more efficient analysis will not lessen the requirement for measures to ensure quality and interlaboratory comparability of data.

Implicit in our responsibility as scientists concerned with contaminants in food is the responsibility to protect and promote public health. We can promote public health and help avoid public misperception about food safety by publishing data on those contaminants that are found in food, and equally important, those contaminants that have been sought but which have not been found. Analytical chemists from government, industrial, private, and academic laboratories should be able to find a way to participate in this important research.

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FOOD ADDITIVES

Detection of Tricresyl Phosphates and Determination of Tri-*o*-Cresyl Phosphate in Edible Oils

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Tricresyl phosphate (TCP) in contaminated edible oils was extracted using acetonitrile and detected by thin layer chromatography as well as gas chromatography (GC). The chromatoplate was developed with isooctane–ethyl acetate (90 + 10) and visualized by spraying with 2,6-dichloroquinone chloroimide. TCP gives a characteristic blue-violet spot when heated at 100°C for 15 min. The method is direct and sensitive and can be used to detect as low as 2.5 μg TCP or TOCP (tri-o-cresyl phosphate). GC was carried out using 10% OV-101 as the stationary phase and flame ionization detection for confirmation and quantitation of TOCP in oils.

Tricresyl phosphate (TCP) is widely used in lacquers and varnishes, as a plasticizer in vinyl plastics, and as an additive in lubricating oils (1). TCP usually exists along with its isomeric forms, viz., ortho, meta, and para, of which tri-*o*-cresyl phosphate (TOCP) is a highly toxic and cumulative poison (1, 2). The minimum paralytic dose for humans is about 10–30 mg/kg body weight (3). Cases of TCP contamination in edible oils have been reported from Morocco (4), Sri Lanka (5), and the Maharashtra and West Bengal States in India (6). The reported contamination was attributed to the use of second-hand drums originally used for lubricating oils. TCP in edible oils, whether or not it contains TOCP, has been shown to be injurious to health (4, 5), although the TCP isomers appear to have significantly different toxicological properties.

Methods available (4, 6) for detection of TCP in edible oils involve saponification of the oil, removal of soap, followed by analysis by thin layer chromatography (TLC) (6) or by distillation (4) and reaction with diazotized *p*-nitroaniline. These methods are tedious and do not differentiate some of the natural phenolic components of edible oils and TCP, thus leading to doubtful results. The colorimetric method recently reported (7) involves extraction of TCP by rectified spirit from the oil for 4 h, and saponification of the alcoholic layer, followed by development of color with 2,6-dichloroquinone chloroimide reagent. However, this method suffers from the interference of natural and added phenolic antioxidants; in addition, alcoholic extracts of individual pure oils react with the reagent to give green, pink, and brown colors that mask the blue color given by TCP.

The present study describes TLC and gas chromatographic (GC) methods for the detection of TCP or TOCP and quantitation of TOCP in edible oils.

METHOD

Apparatus and Reagents

(**a**) *Separatory funnels.*—250 mL capacity.

(**b**) *TLC plates.*—Prepare slurry of silica gel G (BDH) with water (1 + 2 w/v) and spread over glass plates (0.3 mm layer on 20 × 20 cm plates) with applicator. Let plates set at room temperature and then dry 1 h at 120°C.

(**c**) *Developing solvent system.*—Isooctane–ethyl acetate (90 + 10). Line developing chamber with filter paper.

(**d**) *Spray reagent.*—0.5% solution of 2,6-dichloroquinone chloroimide (E. Merck, GFR) in absolute ethyl alcohol (Gibbs reagent). Store reagent at < 10°C and use within 5 days.

(**e**) *Tricresyl phosphate and tri-o-cresyl phosphate.*—Ashland Chemicals, Columbus, OH 43216.

(**f**) *Gas chromatograph.*—Chromatography Instruments Co., Baroda, India. Fitted with hydrogen flame ionization detector; stainless steel column (10 ft × 1/8 in.) packed with 10% OV-101 on 60–80 mesh Chromosorb-AW-DMCS; nitrogen carrier gas 30 mL/min; column temperature 250°C; detector and injector temperatures 300°C; chart speed 1 cm/min.

Procedure

Extraction of TCP or TOCP from oil.—Take 10 mL oil sample (containing ca 50 μg TCP or TOCP) into separatory funnel; add 50 mL petroleum ether (40–60°C) to dissolve the oil, followed by 10 mL acetonitrile previously saturated with petroleum ether. Shake contents vigorously and let stand 10 min. Collect lower acetonitrile layer in beaker and evaporate solvent on hot water bath. Dissolve residue in ca 1 mL ethyl or methyl alcohol.

Thin layer chromatography.—Spot ca 0.1–0.2 mL (ca 5 μg TOCP) of solution on TLC plate. Develop plate in glass chamber containing isooctane–ethyl acetate (90 + 10) ca 45 min to height of 10 cm. Remove plate and dry in air. Spray plate with Gibbs reagent and heat in 100°C oven ca 15 min.

Table 1. Recovery of TOCP from fortified groundnut oil

TOCP, μg/g	Recovery, %[a]		
	1 Extn	2 Extns	3 Extns
1.0	60	70	80
2.0	73	76	90
2.5	90	95	100
5.0	90	95	102

[a]Mean of 3 determinations for each series of 10 mL acetonitrile extractions.

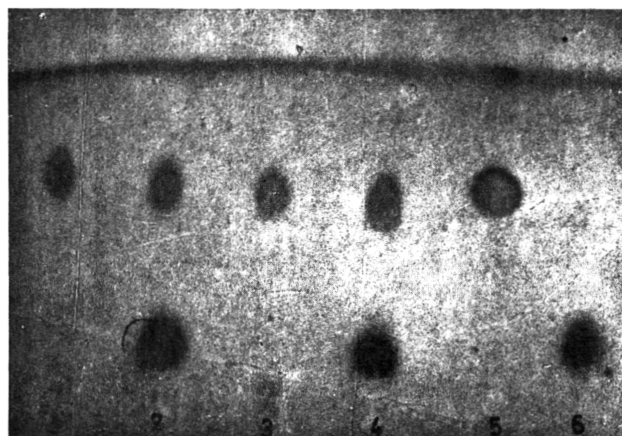

Figure 1. Thin layer chromatography of acetonitrile extract of 1, groundnut oil; 2, groundnut oil spiked with tri-o-cresyl phosphate (TOCP); 3, groundnut oil spiked with butylated hydroxytoluene (BHT); 4, groundnut oil spiked with TOCP and BHT; 5, groundnut oil spiked with DL-tocopheryl acetate; and 6, tricresyl phosphate.

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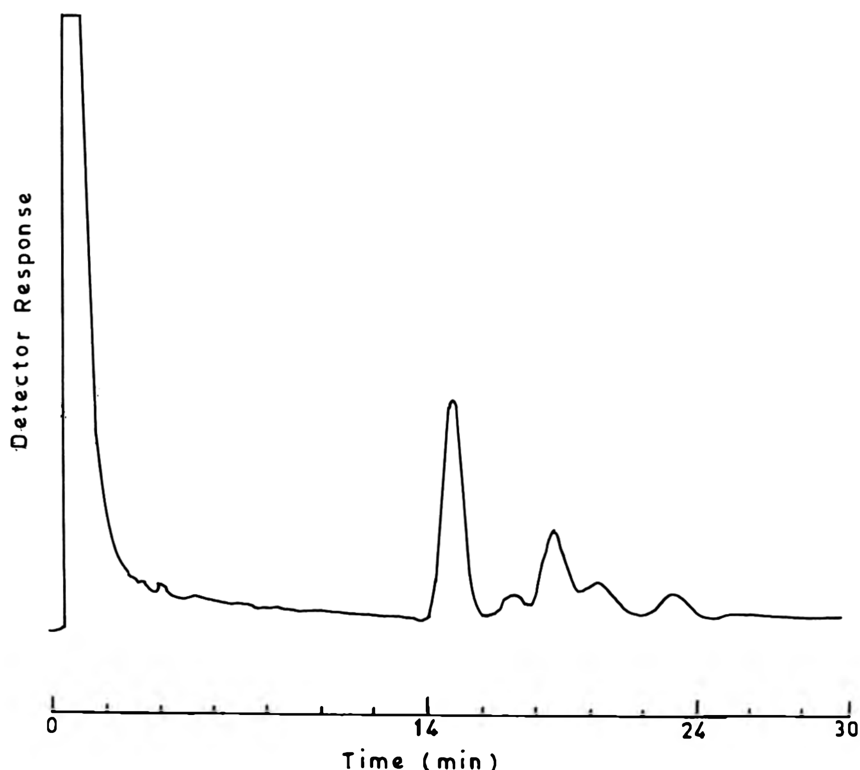

Figure 2. Gas chromatogram of tri-o-cresyl phosphate spiked at 2.5 μg/g groundnut oil.

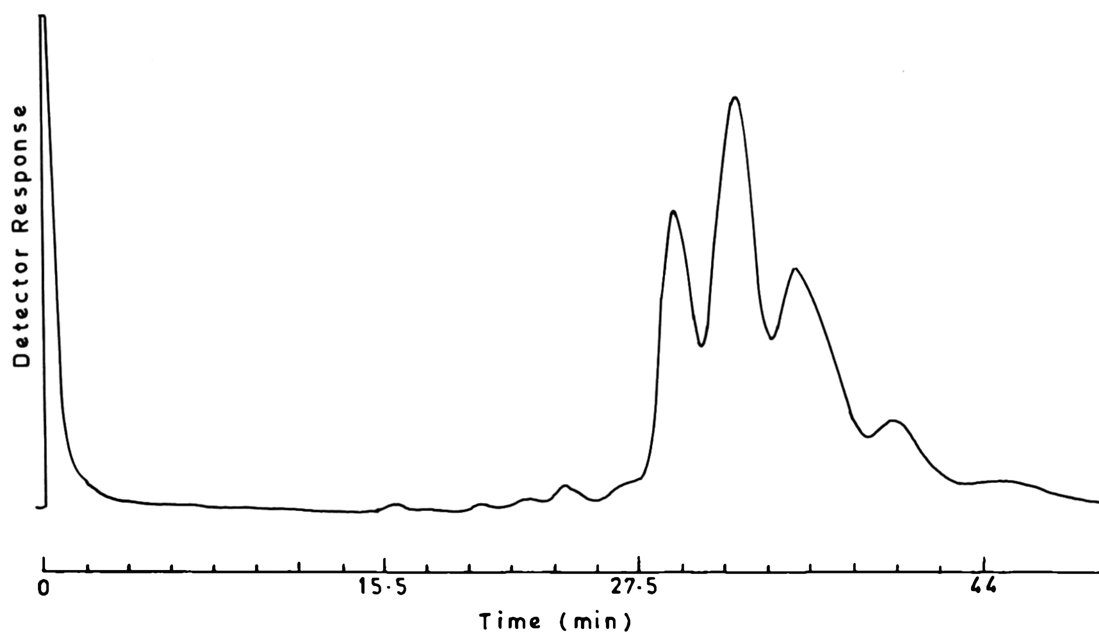

Figure 3. Gas chromatogram of commercial tricresyl phosphates.

Observe for characteristic blue-violet spot at R_f 0.27, corresponding to TCP or TOCP.

Gas chromatography.—Inject ca 1 μL (containing ca 2.5 μg TOCP) of acetonitrile extract into GC apparatus; compare retention time and peak area of sample with that of standard for quantitation.

Results and Discussion

The presence of TCP or TOCP in edible oils can be detected by the characteristic blue-violet spot at R_f 0.27 on the chromatoplate (Figure 1). Both TCP and TOCP gave a single spot at the same R_f. The minimum detectable quantity of TCP or TOCP by thin layer chromatography was 2.5 μg. The phenolic substances naturally present in edible oils, such as tocopherols and sterols, and added antioxidants like butylated hydroxytoluene, propyl gallate, nordihydroguaiaretic acid, do not interfere in the detection of TCP or TOCP. However, butylated hydroxyanisole and also some phenolic substances present in coconut oil obtained from smoked copra (cresols) gave a violet spot at R_f 0.3 immediately after being sprayed with Gibbs reagent; in contrast, TCP or TOCP gave the characteristic blue-violet spot only after the chromatoplate was heated. In such cases, presence of TCP or TOCP could be further confirmed by gas chromatography.

Recovery of TCP or TOCP from groundnut oil spiked at 2.5 μg/g by a single extraction with 10 mL acetonitrile was

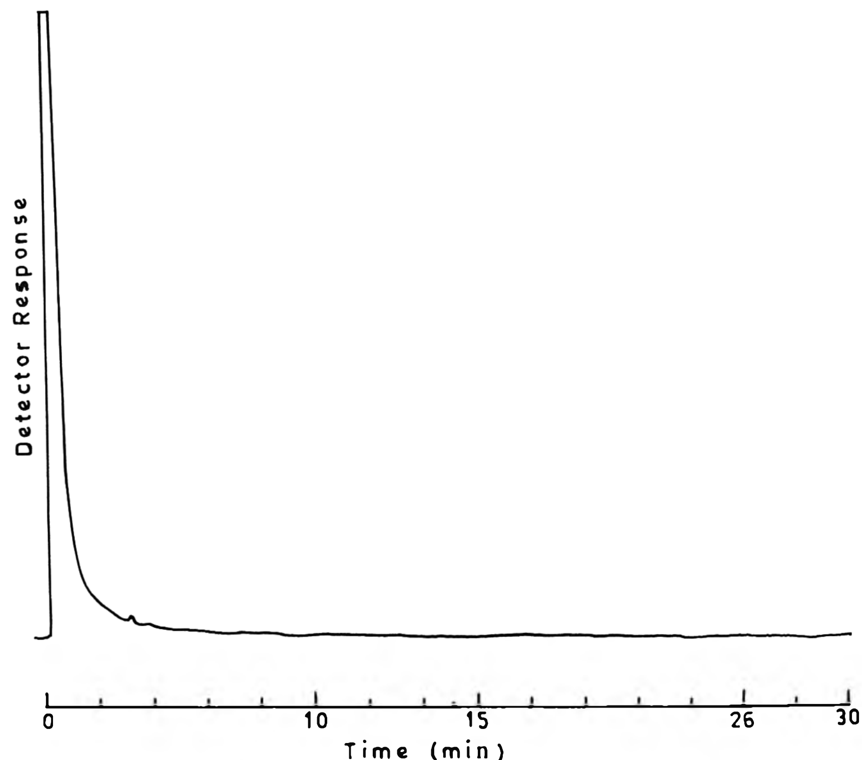

Figure 4. Gas chromatogram of acetonitrile extract of groundnut oil (blank).

90%. As can be seen from Table 1, subsequent extractions with two 10 mL portions of acetonitrile were necessary for quantitative recovery of TCP or TOCP. Low recoveries, 80–90%, were obtained from groundnut oil spiked at 1–2 μg/g of TCP or TOCP even after 3 successive extractions with acetonitrile. This may be caused by improper resolution of complex isomers (8) of TCP or TOCP by gas chromatography. GC patterns of acetonitrile extract of TOCP spiked at 2.5 μg/g groundnut oil, commercial TCP, and a blank are shown in Figures 2, 3, and 4.

The purity of standard TOCP as calculated from the resolved peaks was 68%. However, the total area of all the peaks was taken for quantitation purposes and expressed as TOCP. The other peaks obtained when standard TOCP was injected (Figure 2) can be attributed to the more complex isomers present in TOCP other than the meta and para isomers observed by Deo and Howard (8).

It was observed that about 0.4% of the oil was extracted with acetonitrile. After evaporation of acetonitrile, the residue was dissolved in ethyl or methyl alcohol before GC analysis to eliminate traces of extracted oil.

This is the first reported direct analysis of TCP by TLC. Determination of tricresyl phosphates in water, fish, etc., by GC, using a nitrogen-phosphorus specific detector, has been reported by earlier workers (9, 10). The method described here has been tried on various edible oils like groundnut, sesame, sunflower seed, safflower seed, mustard, coconut, and palmolein. In all, about 120 sample oils received from different parts of the country were screened for TCP or TOCP. Although 22 samples of these edible oils showed the presence of TCP or TOCP when tested by earlier reported methods (4, 6), only one actually contained 35 ppm TOCP.

Acknowledgments

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Effect of *N*-Nitrosothiazolidine-4-Carboxylic Acid on Formation of *N*-Nitrosothiazolidine in Uncooked Bacon

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N-Nitrosothiazolidine-4-carboxylic acid (NTHZC) was recently identified in a variety of smoked fish and cured meat products. While the toxicological properties of NTHZC are currently unknown, it has been speculated that N-nitrosothiazolidine (NTHZ) in fried bacon may be caused in part by decarboxylation of NTHZC. A limited survey of uncooked cured meat products failed to demonstrate any correlation between levels of NTHZC and NTHZ. However, NTHZC levels were significantly higher, ranging from 8 to 1400 ppb, than the corresponding NTHZ levels, which ranged from <2 to 18 ppb. Decarboxylation of NTHZC to NTHZ in a model system occurs at 110°C, which is significantly higher than the average bacon processing temperature of 54°C, but lower than the normal frying temperature of 177°C recommended for bacon. Results from both model system and frying experiments with NTHZC and its precursors suggest that NTHZC decarboxylation to NTHZ is not the principal pathway to NTHZ formation in uncooked bacon.

N-Nitrosothiazolidine-4-carboxylic acid (NTHZC) was recently identified in some smoked fish and cured meat products (1, 2). It has been hypothesized that this nitrosamine can form by the reaction of cysteine in foods with formaldehyde in smoke to yield thiazolidine-4-carboxylic acid (THZC), which can then be nitrosated either by nitrite in the product or by nitrogen oxides generated during the smoking process. The presence of NTHZC in foods is possible because THZC has been shown to nitrosate 250–500 times more rapidly than proline (3), which is thought to be one of the precursors for *N*-nitrosopyrrolidine found in fried bacon. In addition, NTHZC detected in the urine of normal people is thought to have a better potential as an indicator of in vivo nitrosamine formation than nitrosoproline (3). Part of the total amount of NTHZC found in the urine, however, may be contributed by foods containing preformed nitrosamines.

Although the toxicological properties of NTHZC are unknown, it may undergo decarboxylation to form *N*-nitrosothiazolidine (NTHZ), which has been reported to form a mutagenic compound when prepared from cysteamine and formaldehyde (4). In addition, Helgason et al. (1) hypothesized a relationship between NTHZ ingestion and congenital diabetes found in Iceland.

Recently, Sen et al. (2) reported a good correlation between NTHZC levels in raw bacon and NTHZ levels in fried bacon. However, they found no correlation between NTHZC and NTHZ in raw bacon. Previously, we reported (5) that NTHZ levels were higher in the raw bacon than in the fried product, which indirectly suggests that NTHZC was not responsible for NTHZ in fried bacon, contrary to the report by Sen et al. (2). To resolve this discrepancy in results, we developed a method for determining NTHZC that was applicable to a wide variety of smoked cured meat products, and then conducted a limited survey to determine levels of NTHZC in uncooked food products in addition to bacon. We also conducted experiments to determine whether NTHZC had the potential to be a precursor of NTHZ in raw or fried bacon. The results are reported herein.

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Reference to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

METHOD

Note: N-Nitrosoamines are potential carcinogens. Exercise care in handling these materials.

Reagents

(a) *Methanol, ethyl acetate, and dichloromethane.*—Burdick and Jackson Distilled-in-Glass solvents.

(b) *Sulfamic acid.*—1% in 1N sulfuric acid.

(c) *Diazomethane.*—Prepared from Aldrich *N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide as directed.

(d) *N-Nitrosopipecolic acid (NPIC) internal standard solution.*—0.20 μg NPIC/mL in methanol.

(e) *Methyl esters of N-nitrosothiazolidine-4-carboxylic acid (NTHZC) and NPIC.*—GC working standard, each 0.20 μg/mL in dichloromethane.

(f) *Cured meat products.*—Random samples purchased from local retail outlets, ground, and thoroughly mixed before analysis.

(g) *Other reagents.*—Purchased from local suppliers and used without further purification. NTHZ, NTHZC, and NPIC were synthesized from their corresponding amines and sodium nitrite, and purified by either fractional vacuum distillation (NTHZ) or by recrystallization (NTHZC, NPIC), according to general procedure published previously (6).

Apparatus

Usual laboratory equipment and the following items:

(a) *Homogenizer.*—Virtis Co., Inc., Model 45 with 250 mL flask.

(b) *Rotary evaporator and Evapo-Mix.*—Buchler Instrument Co.

(c) *Refrigerated centrifuge.*—Sorvall Model RC-5B.

(d) *Gas chromatograph-thermal energy analyzer (GC-TEA).*—Varian Aerograph gas chromatograph Model 1700, or equivalent, interfaced with thermal energy analyzer Model 502. Operating conditions: 1.8 m × 2 mm glass column packed with 5% Silar 10CP on 100–120 mesh Supelcoport; helium carrier gas, 35 mL/min; column temperature programmed from 150 to 250°C at 4°/min; injector port, 200°C; TEA furnace, 450°C; TEA vacuum, 0.5 mm; liquid nitrogen–ethanol cold trap.

(e) *Gas chromatograph-mass spectrometer (GC-MS).*—Hewlett-Packard Model 5992B low-resolution quadrupole mass spectrometer. Operating conditions: 10 m × 0.20 mm glass capillary column coated with methyl silicone (fused silica); helium flow rate through column, 0.6 mL/min; column temperature maintained at 20°C for 2 min, then programmed at 10°/min to 250°C; injector port, 150°C.

If ions with m/z 30, 45, 59, 87, 146, and 176 were present before and absent after UV photolysis (365 nm), using same procedure described previously (7), the presence of NTHZC as its methyl ester was considered confirmed.

Procedure

(a) *Sample analysis, NTHZC.*—A flow diagram of this method is shown in Figure 1. Accurately weigh 20.0 ± 0.1 g of ground meat sample into a 250 mL Virtis flask. Add 1.0 mL NPIC internal standard solution (equivalent to 10 ppb) to

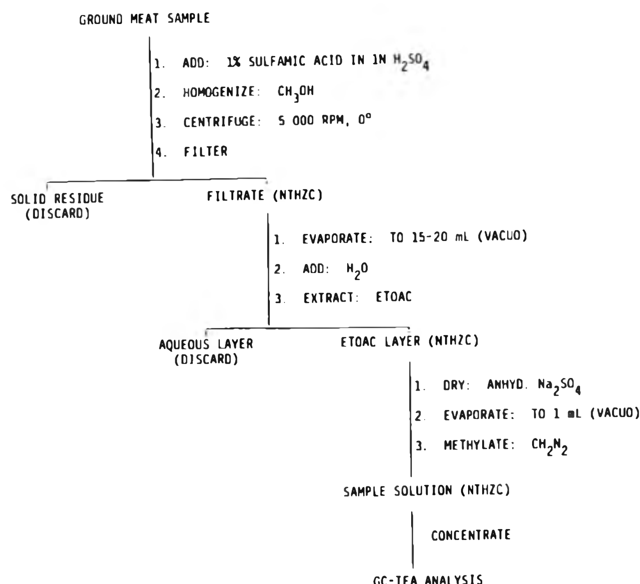

Figure 1. Schematic of procedure for determination of *N*-nitrosothiazolidine-4-carboxylic acid in cured meat products.

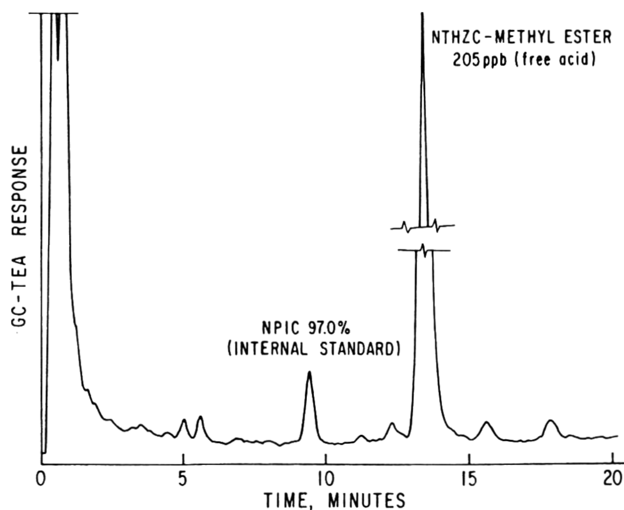

Figure 2. GC-TEA chromatogram of *N*-nitrosothiazolidine-4-carboxylic acid in uncooked bacon.

sample with 1.0 mL transfer pipet, then add 100 mL methanol and 10 mL sulfamic acid solution to flask to destroy residual nitrite. Homogenize sample 3 min at medium setting of 40. Quantitatively transfer sample, using methanol, to 150 mL glass bottle and centrifuge 25 min at 5000 rpm at 0°C. Filter samples through glass wool into 250 mL round-bottom flask, add 2 alundum boiling stones, and then reduce solvent to 15–20 mL on vacuum rotary evaporator (water bath at 40°C). Quantitatively transfer sample with 50 mL water to 125 mL separatory funnel. Extract sample 2 times with 50 mL ethyl acetate. Combine extracts and slowly pass them through 35 g anhydrous sodium sulfate, held in 60 mL coarse fritted-glass funnel. Collect effluent in 250 mL round-bottom flask, and reduce ethyl acetate on vacuum rotary evaporator to 1 mL. Quantitatively transfer sample, using 1–2 mL methanol, to 16 × 145 mm test tube. Reduce volume to ca 0.5 mL on Evapo-mix, and then add 3 mL ether solution containing diazomethane while test tube is heated and shaken at 38°C for 20 min. Quantitatively transfer solution to 4 mL concentrator tube by using dichloromethane, and concentrate solution to 1 mL in 70°C water bath.

(b) *NTHZ analysis and determination.*—Carry out procedure and necessary calculations for determining NTHZ in cured meat products as described previously (8).

(c) *Model system reaction.*—Heat for 6 min in closed glass pressure flask a mixture consisting of 4×10^{-4} mole cysteine, formaldehyde, and sodium nitrite in 10 mL of pH 5.6 phthalate buffer. Extract contents of cooled flask twice with 25 mL DCM. Wash combined DCM extracts once with 10 mL 5N NaOH, then dry and concentrate to 1.0 mL as before.

(d) *Bacon system.*—Add precursor (NTHZC, THZC, THZ) to ground raw bacon containing 36 ppm residual $NaNO_2$ and 870 ppb NTHZC before frying at 177°C for 6 min. Analyze fried product for NTHZ as described previously (8).

(e) *NTHZC determination.*—Inject 8.0 µL GC-TEA working standard at lowest attenuation that yields TEA response at least one-third full scale on recorder; measure peak heights. Repeat standard injection to assure reproducibility of retention time and response. Inject 8.0 µL sample solution and measure peak heights. Calculate NTHZC, from its methyl ester, in ppb. Typical chromatogram from cured meat extract is shown in Figure 2. Minimum level of reliable measurement, using this method, was 5 ppb NTHZC.

(f) *Mass spectral confirmation.*—Mass spectral confirmation was carried out on those samples containing 100 pbb or more NTHZC. All other samples containing lower values for NTHZC were considered "apparent" NTHZC.

(g) *Sodium nitrite analysis.*—Residual sodium nitrite content was determined on 10 g raw, comminuted sample by modified Griess-Saltzman procedure (9).

(h) *Statistical analyses.*—Statistical analyses were carried out according to methods of Snedecor and Cochran (10).

Results and Discussion

To determine the within-laboratory repeatability of the NTHZC method, 8 samples containing NTHZC at levels ranging from 5 to 500 ppb were analyzed in duplicate. An analysis of variance showed that the repeatability coefficient of variation was 5.22% (4.29% in samples corrected for recovery of the internal standard). The repeatability coefficient of variation of the NPIC internal standard recovery was 5.95%.

Table 1. *N*-Nitrosothiazolidine-4-carboxylic acid and *N*-nitrosothiazolidine in uncooked cured meat products

	NTHZC, ppb			NTHZ, ppb			$NaNO_2$, ppm	
Sample type	No. pos./total	Range	Av.	No. pos./total	Range	Av.	Range	Av.
Cure-pumped bacon	10/10	14–1400	370	10/10	2.0–13.1	5.4	5–75	36
Dry-cured bacon	2/2	45, 281	163	2/2	3.6, 6.2	4.9	51, 58	55
Dry-cured ham	4/4	8–103	61	3/4	3.2–17.6	8.9	2–101	50
Lebanon bologna	5/5	8–276	127	4/5	3.0–8.3	5.5	1–2	1
Poultry franks	2/2	69, 173	121	2/2	3.1, 8.3	5.7	34, 45	40
Pepperoni	2/2	39, 79	59	2/2	4.3, 5.8	5.1	2, 2	2
Hot dogs	3/3	12–83	58	2/3	7.2, 9.8	8.5	2–15	8
Beef, pork strips	2/2	19, 28	24	2/2	7.5, 11.8	9.7	4, 6	5
Other cured products	4/4	8–18	12	4/4	1.7–2.2	1.9	2–10	6

Table 2. Effect of added precursor on nitrosothiazolidine formation in fried bacon

Precursor	Added, ppm	NTHZ, ppb
Control	none	8.5
Nitrosothiazolidine-4-carboxylic acid	0.1	11.1
	1	12.2
	10	133.0
	100	1031.0
Thiazolidine-4-carboxylic acid	100	19.0
	1000	46.5
Thiazolidine	100	143.0
	1000	1548.0

Table 3. Effect of frying on nitrosothiazolidine formation in bacon

	Raw bacon			Fried bacon	
Sample	$NaNO_2$, ppm	NTHZC, ppb	NTHZ, ppb	NTHZC, ppb	NTHZ, ppb
1	51	1022	6.9	645	8.0
2	35	798	5.7	403	4.2
3	35	418	ND	271	2.3
4	32	120	4.9	138	2.3
5	47	173	3.8	163	2.8
6	2	223	2.7	169	1.5

The results of our limited survey of smoked cured meat products for both NTHZC and NTHZ are shown in Table 1. All of the samples analyzed contained NTHZC ranging from 8 to 1400 ppb. These values were significantly higher than the NTHZ concentrations in the same samples, which ranged from <2 to 18 ppb in 32 of 34 samples analyzed. Analysis of the data indicated no correlation between residual sodium nitrite in any of the products surveyed, and either NTHZC or NTHZ. It is also interesting to note that the one bacon sample that had the NTHZC value of 1400 ppb had a residual nitrite level of 75 ppm, but other bacon samples that had comparable levels of residual nitrite contained significantly lower levels of NTHZC, which again suggested that no nitrite-NTHZC correlation was evident. Lebanon bologna, which is subjected to the longest smoking period of any of the products surveyed, showed a NTHZC average equivalent to the lightly smoked poultry franks. This is consistent with our previous finding, where we hypothesized that the low NTHZ values observed may be due to the acidic nature of this type of fermented product. Despite the higher levels of NTHZC in the surveyed samples, no correlation between NTHZC and NTHZ was found, which is in agreement with Sen et al. (2), who also found no correlation between NTHZC and NTHZ in the uncooked bacon only. Our results suggest that the NTHZC-amino precursor was either present in higher concentrations or was more readily nitrosatable than the NTHZ precursor in the uncooked meat products.

Although NTHZC does not appear to be the primary precursor for NTHZ in uncooked bacon, its parent compound, thioproline (THZC), could decarboxylate to form THZ, which could then nitrosate to form NTHZ. However, we found by thermogravimetric analysis that THZC does not decarboxylate appreciably until 200°C, which is significantly higher than the average internal temperature (54°C) used in processing bacon. These results support the previous findings and suggest that THZC does not contribute to NTHZ formation unless the product contains some unknown factor(s) that might facilitate decarboxylation.

Mandagere et al. (11) reported that NTHZC decarboxylates to NTHZ at 110°C, which is lower than the bacon frying temperature of 177°C recommended by many bacon producers. However, since our previous work did not show an increase in NTHZ levels when uncooked bacon was fried (5), we investigated, in a model system, whether adding high levels of NTHZC to the uncooked bacon could significantly increase the levels of NTHZ found in the product after it was fried at 177°C. Table 2 shows the NTHZ content after frying when NTHZC, THZC, and THZ were added to ground bacon containing 36 ppm residual nitrite, 870 ppb NTHZC, and 8.5 ppb NTHZ before frying. When 1 ppm NTHZC was added to the bacon, the NTHZ value increased only 3.7 ppb (8.5 to 12.2 ppb); whereas, at the 10 ppm fortification level, NTHZ increased markedly (8.5 to 133.0 ppb). These bacon model system results indicate that the product may need a very high level of NTHZC to make a significant contribution to NTHZ.

As predicted from the decarboxylation temperature (200°C), THZC does not appear to be a major contributor to NTHZ formation, since 100 ppm added to the uncooked bacon only increased the NTHZ level by 10.5 ppb (8.5 to 19.0 ppb). When 100 ppm THZ was added to the bacon, the NTHZ level increased by 134.5 ppb (8.5 to 143.0 ppb), which shows that the bacon used in these experiments was capable of nitrosating the exogenous amines or amine precursors to form NTHZ.

The previous model system results suggest that NTHZC may not be the primary precursor of NTHZ in fried bacon unless present in large amounts or if higher cooking temperatures were employed. To confirm this hypothesis, selected samples of uncooked bacon containing NTHZC levels >100 ppb were fried; NTHZ and NTHZC values were determined both before and after frying. The results in Table 3 show that NTHZC levels decreased in 5 of 6 samples after frying, but NTHZ increased slightly in only 2 of 6 samples; even here, the increase was not considered significant. The difference between our results and those reported by Sen et al. (2) may be due to the higher concentrations of NTHZC or $NaNO_2$ in the bacon that they analyzed. In our case, the samples contained less than 50 ppm residual nitrite, the concentration normally found in bacon at the retail level.

Up to now, we have shown that NTHZC and THZC are not likely precursors for NTHZ in either raw or typically fried bacon. However, we considered it necessary to investigate the remaining pathway to NTHZ formation, that is, via cysteine. In a pH 5.6 aqueous model system, we reacted, at various temperatures, cysteine, formaldehyde, and sodium nitrite to determine if and how much NTHZ would form. The system was closed during heating to prevent loss of any volatile components generated. The results presented in Figure 3 show that the optimum temperature for conversion of the reactants to NTHZ occurred at 215°C, which is higher than either the decarboxylation temperature for NTHZC to NTHZ (110°C) or for THZC to THZ (200°C), but below the reported temperature threshold of 230°C for cysteine decarboxylation (12). Even though formation of NTHZ from cys-

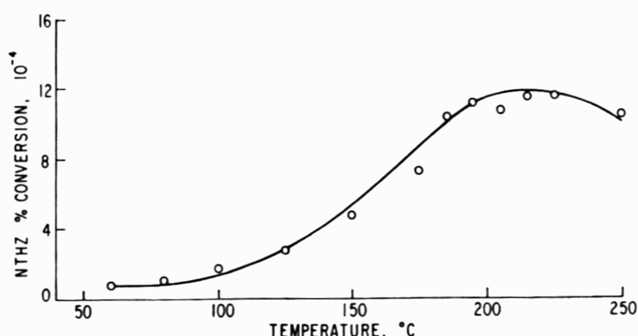

Figure 3. *N*-Nitrosothiazolidine formation from cysteine–formaldehyde–nitrite in a model system.

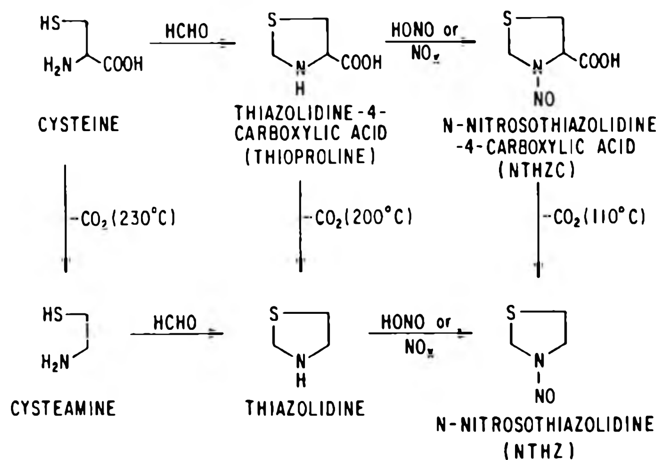

Figure 4. Possible pathways of *N*-nitrosothiazolidine formation.

teine in a simple model system is minor at 215°C (12×10^{-4}%), at normal frying temperatures (177°C), the rate of decarboxylation might be sufficient to form NTHZ, but the rate of decarboxylation at typical bacon processing temperatures (54°C) is far too low to account for formation by this pathway.

The possible pathways of NTHZ formation are presented in Figure 4, with the decarboxylation temperatures indicated in parentheses. We have shown that there is a high probability that NTHZ in raw bacon does not occur by decarboxylation of cysteine, THZC, or NTHZC. As we hypothesized previously (13), NTHZ appears to form via the cysteamine-thiazolidine pathway. However, at present there has been no published data on either cysteamine or thiazolidine content in meat products. Therefore, we are currently evaluating precooked cured bacon for these compounds to confirm that this indeed is the pathway to NTHZ. We have also shown in a bacon model system that NTHZC must be present in large amounts to contribute to NTHZ formation when bacon is fried. However, our results indicated that while NTHZC was present in amounts considerably higher than normally obtained for NTHZ, the concentrations were sufficiently low as not to significantly contribute to NTHZ formation during normal frying.

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PROCESSED VEGETABLE PRODUCTS

Microwave Oven Drying Determination of Total Solids in Processed Tomato Products: Collaborative Study

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Fourteen laboratories analyzed 7 samples of tomato products with total solids content ranging from 6.5 to 40.2%. Samples were analyzed directly with the exception of samples with solids contents greater than 15%, which were diluted with water (1 + 1 for up to 30% solids and 1 + 3 for greater than 30% solids). The 2–4 g samples were dried at 100% power for 4 min. The results of the collaborative study showed good repeatability and reproducibility: S_o = 0.16 (CV = 0.46%) and S_x = 0.36 (CV = 1.06%) for the higher solids samples and S_o = 0.37 (CV = 3.76%) and S_x = 0.14 (CV = 1.41%) for samples with total solids up to 15%. Results were compared with those obtained using vacuum oven drying. No difference was seen in the results by the 2 methods at the 95% level of confidence. The microwave oven drying method has been adopted official first action as an alternative to the vacuum oven drying method for total solids.

The official AOAC method for determination of total solids in tomato products is a vacuum oven procedure (1). This method requires 2 h drying in a vacuum oven with repeated weighing of drying dishes and samples. Besides the long drying time and chances for errors in repeated sample handling, few commercial ovens give the correct shelf temperature (70°C).

Alternative means of determining total solids until now have proven to be unreliable because of the propensity of some food products, particularly those with high contents of carbohydrates, to char.

In recent years, microwave ovens with built-in microprocessors have been developed for determining solids in a variety of food (2) and nonfood items. The advantages of microwave oven drying appear to be short drying times and a minimum of sample handling. In particular, studies comparing results obtained on tomato products by using a vacuum oven and a microwave moisture analyzer have shown remarkable agreement (G. L. Marsh, S. J. Leonard, T. K. Wolcott, J. R. Heil, and J. E. Buhlert, private communications; H. B. Chin and J. R. Kimball, unpublished results). However, in contrast with the vacuum oven method, procedures using the microwave moisture analyzer have not been standardized, thus it is difficult to compare results from different laboratories.

The purpose of this study was to standardize and validate a microwave moisture analyzer method for tomato products and then to study the method collaboratively.

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The recommendation of the Associate Referee was approved by the General Referee and Committee C and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1985) 68, March issue.
This report of the Associate Referee, H. B. Chin, was presented at the 98th Annual International Meeting of the AOAC, Oct. 28–Nov. 2, 1984, at Washington, DC.

Solids (Total) in Processed Tomato Products
Microwave Oven Drying Method
First Action

32.A01 ***Principle***

Tared sample on fiberglass pad is placed in microwave oven 4 min. Instrument automatically weighs sample before and after drying, and calcs % total solids.

32.A02 ***Apparatus***

(a) *Microwave oven.*—CEM Microwave Drying Moisture Solids Analyzer Model AVC-MP (CEM Corp., PO Box 9, Indian Trail, NC 28079), or equiv.
(b) *Fiberglass pads.*—CEM £20-20015, or equiv. Dry in microwave oven before use.
(c) *Jumbo bulb pipet.*—6 in., Beral No. 028-795 (Curtin Matheson Scientific Inc.), or equiv.
(d) *Spatula.*—Plastic or Teflon-coated.
(e) *Top loading balance.*—Accurate to 0.01 g.

32.A03 ***Preparation of Sample***

(a) *Tomato juice.*—Use 4 g, as is.
(b) *Puree.*—10–15% solids. Use 2 g, as is.
(c) *Paste.*—Up to 30% solids. Prep. 1 + 1 diln (w/w) with H_2O by one of following technics: (*1*) blending in mini-cup blender; (*2*) shaking in closed jar; (*3*) mixing with rubber spatula. Use 2 g of diln.
(d) *Paste.*—Over 30% solids. Prep. thoroly mixed diln (1 + 3, w/w) as in (c) above. Use 2 g of diln.

Use calibrated Beral pipet to deposit sample on fiberglass pad. To calibrate pipet, weigh 4 g sample (2 g puree or paste), draw completely into pipet, and mark level. Draw subsequent samples to this line.

32.A04 ***Determination***

Before starting each day's run, place 2 pads on oven balance ring, and run thru complete cycle with oven set at 100% power. Then, place 2 pre-dried pads on balance ring. Press "weigh" button, then press "auto-tare" button until balance displays 0.0000 (±0.0002).

Remove both pads and deposit proper amt of sample on *rough* side of one pad. Use spatula to spread sample evenly over entire pad. Place second pre-dried pad on top of sample—rough side

Table 1. Comparison of results between microwave and vacuum oven methods for determination of total solids (%) in tomato products[a]

Sample	Microwave oven	Vacuum oven
84 TJ-1	6.49	6.47
84 TJ-2	6.23	6.23
84 TPU 1045	12.38	12.33
84 TPU 1060	14.58	14.43
84 TP 2	26.94	26.59
84 TP 3	34.27	33.91
84 TP 4	40.22	40.29

[a]Average of determinations in triplicate.

Table 2. Collaborative results for determination of total solids (%) in tomato products by microwave oven drying method

	Sample							
Lab.	1 84TJ-1		2 84TJ-2		3 84TPU1045		4 84TPU1060	
A	6.44	6.45	6.19	6.22	12.47	12.50	14.67	14.69
B	6.67	6.67	6.42	6.39	12.56	12.52	14.71	14.69
C	6.54	6.54	6.26	6.27	12.46	12.47	14.60	14.65
D	6.48	6.51	6.34	6.27	12.45	12.45	14.33	14.31
E	6.50	6.50	6.31	6.31	12.55	12.54	14.64	14.67
F	6.56	6.53	6.21	6.18	12.41	12.45	14.52	14.55
J	6.48	6.50	6.25	6.21	12.41	12.50	14.54	14.56
K	6.46	6.49	6.18	6.22	12.46	12.53	14.73	14.62
L	6.55	6.58	6.27	6.28	12.15	12.19	14.66	14.82
N	6.49	6.49	6.21	6.21	12.24	12.28	14.47	14.47
O[a]	6.34	6.39	6.19	16.17	12.12	12.20	14.20	14.35
P	6.54	6.50	6.26	6.27	12.37	12.50	14.67	14.72
Q	6.44	6.41	6.13	6.10	12.32	12.31	14.71	14.73
R	6.38	6.31	6.16[b]	6.00[b]	12.34[b]	12.12[b]	14.31	14.14
Mean	6.49		6.23		12.40		14.56	
Repeatability								
Std dev.	0.02		0.02		0.04		0.06	
CV, %	0.34		0.35		0.33		0.41	
Reproducibility								
Std dev.	0.08		0.07		0.13		0.18	
CV, %	1.29		1.13		1.08		1.23	

Lab.	5 84TP2		6 84TP3		7 84TP4	
A	26.94	27.06	34.44	34.08	40.04	40.04
B	27.04	27.20	33.91	33.55	40.45	40.33
C	26.70	26.62	34.08	34.08	40.48	40.44
D	27.04	27.10	33.84	33.60	40.56	40.32
E	27.72[b]	27.06[b]	34.84	34.52	40.16	40.44
F[c]	26.64	26.66	36.68	33.84	40.52	40.20
	26.56	26.50	33.64	34.04	40.20	40.40
J	26.54	26.72	33.72	33.64	40.08	40.32
K	26.66	26.68	31.80[d]	32.21[d]	39.14	39.87
L	27.99	27.95	35.00	34.78	39.99	40.40
N	26.60	26.66	34.48	34.52	40.56	40.12
O[a]	27.80	26.28	33.48	33.55	39.26	39.48
P	27.12	27.12	34.52	34.44	40.84	40.84
Q	26.32	26.38	33.60	33.92	39.84	39.96
R	27.30	27.28	34.98	35.12	36.51[d]	36.27[d]
Mean	26.94		34.27		40.22	
Repeatability						
Std dev.	0.07		0.16		0.22	
CV, %	0.25		0.48		0.54	
Reproducibility						
Std dev.	0.30		0.51		0.37	
CV, %	1.12		1.50		0.91	

[a] Did not follow procedure for samples 5–7; results not included in statistics.
[b] Cochran outlier.
[c] Reported in quadruplicate; extra values eliminated by table of random numbers (underscored values were retained).
[d] Excluded on basis of Dixon test.

against sample. Work *rapidly* to minimize evapn. Invert pads and place on balance ring. Drop cover over samples. Close door securely.

Closing door activates microprocessor. Display will indicate *increasing* wt for 4–5 s, then wt will begin to decrease. At exact point that wt starts to decrease, press "auto-time" button to begin drying cycle. At end of 4 min, record % solids. Det. % total solids on duplicate samples, and correct for sample diln.

Results

The microwave moisture analyzer method and the AOAC vacuum oven method **(32.010)** (1) were used to analyze samples in the laboratory of the Associate Referee. The results (Table 1) are in excellent agreement; there is no difference in results at the 95% confidence level.

The collaborative results are given in Table 2. Thirteen collaborators participated in this study. Laboratory O did not analyze samples 5, 6, and 7 according to the described procedure and those results were excluded from statistical analysis.

To detect laboratories which show consistently high or low results, the ranking test described by Youden (3) was applied. No outlying laboratories were found at the 5% level of significance.

The results were next examined for outliers by the Dixon and Cochran tests. At the 5% level of significance, results for sample 6 from Laboratory K and sample 7 from Laboratory R were Dixon outliers and were excluded from further statistical analysis. Results for sample 5 from Laboratory E and samples 2 and 3 from Laboratory R were Cochran outliers and were likewise excluded from further analysis.

Analysis of variance of the data (Table 3) showed that the between-laboratory variation and the laboratory-sample interaction were significant at the 95% confidence level. Estimates of the repeatability and reproducibility standard deviations were 1.38 and 1.78, respectively.

The determination of total solids in processed vegetables by using the vacuum oven drying method has been an accepted procedure for many years. In a collaborative study of the

Table 3. Collaborative results for determination of total solids by microwave oven: 2-way analysis of variance

Source of Variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Between laboratories	967.594	13	74.430 (MS_L)	503.810 (MS_L/MS_{LS})
Between samples	27092.107	6	4515.351	
Laboratory-sample interaction	10.194	69	0.148 (MS_{LS})	12.848 (MS_{LS}/MS_O)
Between replicates	1.023	89	0.016 (MS_O)	
Total	28070.919	177	158.593	
Repeatability: 1.38				
Reproducibility: 1.78				

method on samples of tomato paste, coefficients of variation in the range 0.47–1.02% were found for samples with solids from 25.41 to 34.31% (4). For samples in a similar range of solids content, the coefficients of variation found in the current study of the microwave moisture analyzer were 0.91–1.50%. When this good reproducibility is considered along with the good agreement obtained between the vacuum oven method and the microwave oven method, when carried out as described, use of the latter method appears to be advantageous.

Recommendation

The results obtained by the microwave oven drying procedure are equivalent to those obtained by the vacuum oven procedure. Because of its inherent speed and ease of use, we recommend that the microwave oven drying method be considered as an alternative to the official vacuum oven method **(32.010)** (1).

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Janet Dudek, National Food Processors Association, Washington, DC

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Rick Falkenburg, Ragu Foods, Merced, CA

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Samuel K. Reeder, Beatrice/Hunt-Wesson, Fullerton, CA

Greg Winters and Adah Tessaro, Gerber Products Co., Fremont, MI

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VITAMINS AND OTHER NUTRIENTS

National Bureau of Standards Reference Materials as Organic Nutrient Standards: A Preliminary Study

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A preliminary study was conducted to determine if the available National Bureau of Standards (NBS) Standard Reference Materials (SRM) Non-Fat Powdered Milk, Oyster Tissue, Wheat Flour, Rice Flour, Spinach, and Albacore Tuna would be suitable for use as organic nutrient standards. These materials were assayed for folates, total pantothenates, vitamin B_6, thiamine, riboflavin, niacin, and protein. Vitamins A, E, D, K, and C were also assayed but, for the most part, were not detected. Based on results from this study, it appears that at least some of the NBS SRMs would be useful as organic nutrient standards.

In October 1980, a workshop was held at the National Bureau of Standards (NBS) to discuss reference materials for organic nutrients. The need for readily available standard reference materials (SRM) certified for these nutrients was recognized. Numerous suggestions were made as to which nutrients and matrices should be used. The NBS SRM Non-Fat Powdered Milk (NFPM), recently certified for elemental composition, was a reasonable starting point.

Subsequently, the Food and Drug Administration (FDA) established an informal working agreement with NBS to investigate the organic nutrient content of NFPM and other SRMs derived from food materials—spinach, tuna, oysters, and rice and wheat flours. The goals are to quantitate the constituents by a variety of techniques and ultimately to provide informational values for as many organic nutrients as possible.

FDA will establish a repository of information on the organic nutrient content of SRMs. To achieve this, input is needed from other laboratories. Interested persons are asked to send to this laboratory any data obtained, stating the individual results for each assay, time period, and a reference for the method of assay. At regular intervals a compilation of all submitted information will be made available. In this way, the SRMs will take on a greater role as laboratory reference materials for both organic and mineral content.

The statistical attributes of Association of Official Analytical Chemists (AOAC) methods have been developed by collaborative studies. The matrix effect on analytical results is often studied by addition of a reference standard of the analyte. As a consequence, the effect of the matrix is diluted. With regard to vitamins, the added reference standard is usually a pure compound. Vitamin activity indigenous to foods may be in a multiplicity of bound and free forms that vary in activity.

In the assay of foods to which vitamins have been added, problems relative to matrix and form are diminished. For assay of naturally occurring vitamin congeners it is especially important that a reference material be available to assess analytical techniques, such as efficiency of enzymolysis; to serve as a basis for in-house quality control; and to use to compare performance between laboratories and methods.

Among the properties of materials considered appropriate for this purpose are homogeneity, representativeness of food substances, microbial stability, and availability in sufficient amounts for wide distribution. The NBS Research Materials (RM) and SRMs have these characteristics. This preliminary investigation was conducted to assess vitamin and protein content of selected materials and to elucidate the stability of nutrients in these substances under ambient and refrigerated conditions.

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Samples

Non-Fat Powdered Milk, SRM 1549; Oyster Tissue, SRM 1566; Wheat Flour, SRM 1567; Rice Flour, SRM 1568; Spinach, SRM 1570; and Albacore Tuna, RM 50, were obtained from the National Bureau of Standards, Gaithersburg, MD 20760.

Sample Preparation and Storage

Sufficient bottles of one lot of each material to provide adequate amounts of sample for all analyses were selected from shelf stock. The bottles were opened and contents were combined and mixed by stirring. The combined sample was divided into 2 portions and transferred to screw-cap clear glass bottles. One portion was held under ambient laboratory conditions and the other was held at 4°C in a refrigerator. No precautions were taken to exclude light.

The initial assay was performed on the day the bottles were opened, and the storage intervals were measured from that day.

Moisture Analysis

Moisture was determined by method **14.003.**

Experimental

Vitamin Analyses

Vitamin C activity was assayed by the official first action automated microfluorometric assay (1). All other vitamins were determined by turbidimetric microbiological assays. Vitamin B_{12} activity, niacin, and riboflavin were determined by AOAC methods (2) **43.139–43.141**, **43.155–43.158**, and **43.173–43.176**, respectively.

The AOAC vitamin B_6 assay, **43.188–43.193**, was followed except that the ion-exchange separation of isomers, **43.192**, was not used, and the following modifications were made in the medium: Acid-hydrolyzed casein solution, **43.188(d)**, was detected; the volume of vitamin solution II was increased from 25 to 50 mL per liter; and 2.0 g L-asparagine, 20 μg $(NH_4)_6Mo_7O_{24}$, 100 μg KI, and 40 μg $CuSO_4 \cdot 5H_20$ were added per liter. Rather than adding sterile basal medium aseptically, the basal medium was added to the standard and test solution and the array was autoclaved before inoculation. The preponderant isomeric form of each material as identified in the literature was used as the standard, namely, pyridoxal for spinach, tuna, and NFPM; pyridoxamine for oyster tissue; and pyridoxine for rice and wheat flours (3–6).

Folacin was extracted with pH 7.0 buffer solution containing 1% ascorbic acid (7). Commercial dried chick pancreas

Table 1. Non-Fat Powdered Milk (NFPM) SRM 1549: average assay results of 2 determinations for vitamin content (μg/g), protein (%), and moisture (%) in NFPM at initial time and after storage

		Storage conditions[a]			
		4 weeks		6 weeks	
Constituent	Initial	Rm temp.[b]	4°C	Rm temp.	4°C
Vitamin C	43.4	50.3[c]	55.1[c]	49.1[c]	50.0[c]
Total folates	(0.64)	[0.68]	(0.70)	(0.73)	(0.76)
Total pantothenates	45.2	45.9	45.7	50.3	48.7
Niacin	9.8	9.8	10.4	9.7	9.6
Thiamine	4.5	4.4	4.5	4.5	4.6
Riboflavin	15.0	13.9	14.2	13.8	13.4
Vitamin B_6	4.8	5.2	5.1	5.4	4.7
Protein	35.8	35.8	36.1	35.7[c]	35.8[c]
Moisture	3.6	3.8	3.6	4.3	3.9

[a]Parentheses signify that assay results did not comply with the requirements of method 43.133. Brackets signify average of one result that did and one result that did not comply with requirements of method 43.133.
[b]Ambient laboratory room temperature.
[c]Single determination.

Table 2. Oyster Tissue SRM 1566: average assay results of 2 determinations for vitamin content (μg/g), protein (%), and moisture (%) in Oyster Tissue at initial time and after storage

		Storage conditions[a]			
		4 weeks		6 weeks	
Constituent	Initial	Rm temp.[b]	4°C	Rm temp.	4°C
Total folates	(1.2)	1.1	[0.86]	[1.0]	
Total pantothenates	10.2	[11.1]	[12.2]	11.0	15
Niacin	101.6	97.9	102.8	98.2	102.8
Thiamine	5.1	[5.6]	[5.6]	4.7	5.2
Riboflavin	9.8	7.8	7.8	[8.4]	8.6
Vitamin B_6	1.4	1.8	1.8	1.9	2.3
Protein	41.4	41.0	41.6	40.8[c]	41.0[c]
Moisture	7.3	6.0	6.4	7.1	6.1
Vitamin B_{12} activity				0.92	1.16

[a–c]See footnotes [a–c], Table 1.

conjugase was used to convert polyglutamates to diglutamates followed by assay using *L. casei* ATCC 7469 as the test organism (8). The folic acid standard solution was also subjected to the conjugase activity to obviate the need to correct for the response of the test organism to growth stimulants in the enzyme preparation. Commercially prepared basal medium was used.

Pantothenic acid was released from coenzyme A by using simultaneous alkaline intestinal phosphatase and pigeon liver (peptidase) enzymolysis (9). The released acid was assayed by AOAC method **43.164–43.167.** The pantothenic acid standard solution was subjected to the enzymolysis step to eliminate the need for blank correction.

Thiamine was assayed by the method of Deibel et al. (10, 11) which used *L. viridescens* ATCC 12706 as the test organism and commercially prepared basal medium.

Protein Analysis

Nitrogen was determined by the AOAC modified Kjeldahl method, **2.057,** using a semi-automated apparatus. Digestion was performed with a Tecator Digestion System 20 1015 Digester (Tecator Inc., Herndon, VA 22070) at 420°C with concentrated H_2SO_4 and a mercury catalyst. Ammonia was distilled into standardized acid in a Tecator Kjeltec System 1003 distilling unit with automatic addition of 50% NaOH. Titration was performed with a Brinkmann Automated Titrator (Brinkmann Instruments, Inc., Westbury, NY 11590). Factors used for the conversion of percent nitrogen to percent protein were 6.25 for spinach, oyster tissue, and tuna; 6.38 for NFPM; and 5.7 for rice and wheat flours.

Results and Discussion

The results of the preliminary analyses of the subject SRMs and the RM are reported in Tables 1–6. All values are reported on an "as is" basis and are not corrected for the indicated moisture content.

The criterion that the minimum fluorescent quantifiable amount of an analyte is that which gives a fluorescent yield equal to 2 times that of the blank was used in evaluating the vitamin C assay results. On the basis of this criterion, only the NFPM had levels of vitamin C high enough for assay by the microfluorometric method (Table 1). NBS liquid chromatographic analyses for vitamin C in this material corroborated the fluorometric assay results. The value of 52.7 ± 6.3 μg/g is included as an informational value on the Certificate of NFPM SRM 1549 (12).

The oyster SRM was the only material assayed in this study that contained enough vitamin B_{12} activity to give a measurable response by the microbiological assay used (Table 2). All of the SRMs and the RM contained assayable amounts of folates, total pantothenates, vitamin B_6, thiamine, riboflavin, niacin, and protein (Tables 1–6). The initial assays for total folates did not meet AOAC criteria for validity (**43.133**). This failure to meet criteria was also encountered in a few of the storage assays. Those values are included in the tables as estimates. Their agreement with AOAC valid assay results attests to the reasonableness of their inclusion.

Because of the time constraints inherent in this type of study, all assays for each nutrient were conducted on one day. The day-to-day analytical variability, therefore, is included in the variability of the storage time results. It is anticipated that when sufficient data have been generated, this variability can be identified and the significance of storage time on analytical results can be evaluated. In general, however, the 2 storage conditions appear to cause little difference in the vitamin and protein content of the test materials.

The SRMs and the RM studied are freeze-dried materials that have been subjected to defatting and gamma irradiation sterilization. These processes avoid the development of rancidity and preserve functional stability of the materials. Determinations of oil-soluble vitamins A, E, D, and K were negative in the RM and all SRMs. Our conjecture is that the stabilization process either extracts or destroys these oil-soluble vitamins.

Conclusion

From this study we found that the NBS SRMs can indeed be used for some organic nutrients. Further study is needed

Table 3. Wheat Flour SRM 1567: average assay results of 2 determinations for vitamin content (µg/g), protein (%), and moisture (%) in Wheat Flour at initial time and after storage

		Storage conditions[a]			
		4 weeks		6 weeks	
Constituent	Initial	Rm temp.[b]	4°C	Rm temp.	4°C
Total folates	(0.22)	0.26	0.26	0.23	0.22
Total pantothenates	3.1	2.0	2.0	3.1	3.0
Niacin	14.7	15.4	14.4	14.0	14.1
Thiamine	2.5	1.8	1.9	1.8	2.0
Riboflavin	0.56[c]	0.58[c]	0.57[c]	0.59[c]	0.54[c]
Vitamin B_6	0.72	0.66	0.66	0.68	0.69
Protein	12.4	12.4	12.4	12.4[c]	12.3[c]
Moisture	9.8	9.8	9.6	9.8	8.0

[a–c]See footnotes [a–c], Table 1.

Table 4. Rice Flour SRM 1568: average assay results of 2 determinations for vitamin content (µg/g), protein (%), and moisture (%) in Rice Flour at initial time and after storage

		Storage conditions[a]			
		4 weeks		6 weeks	
Constituent	Initial	Rm temp.[b]	4°C	Rm temp.	4°C
Total folates	(0.21)	0.21	0.21	0.21	0.20
Total pantothenates	3.8	3.2	3.0	3.9	3.8
Niacin	15.7	16.6	17.0	15.0	15.3
Thiamine	1.4	1.3	1.2	1.2	1.2
Riboflavin	0.33[c]	0.30[c]	0.30[c]	0.31[c]	0.29[c]
Vitamin B_6	1.4	1.4	1.4	1.4	1.4
Protein	8.4	8.5	8.5	8.6[c]	8.4[c]
Moisture	9.9	9.3	9.4	9.4	7.5

[a–c]See footnotes [a–c], Table 1.

Table 5. Spinach SRM 1570: average assay results of 2 determinations for vitamin content (µg/g), protein (%), and moisture (%) in Spinach at initial time and after storage

		Storage conditions[a]			
		4 weeks		6 weeks	
Constituent	Initial	Rm temp.[b]	4°C	Rm temp.	4°C
Total folates	(5.3)	6.2	5.6	6.4	6.0
Total pantothenates	14.3	[6.3]	6.0	7.8	8.0
Niacin	42.4	42.3	43.5	42.9	41.8
Thiamine	5.6	5.5	5.6	5.2	6.3
Riboflavin	17.6	16.2	15.7	15.6	15.5
Vitamin B_6	12.1	13.4	12.8	12.9	12.9
Protein	33.2	32.8	33.0	33.3[c]	32.8[c]
Moisture	6.0	5.7	6.6	6.7	6.9

[a–c]See footnotes [a–c], Table 1.

Table 6. Albacore Tuna RM 50: average assay results of 2 determinations for vitamin content (µg/g), protein (%), and moisture (%) in Albacore Tuna at initial time and after storage

		Storage conditions[a]			
		4 weeks		6 weeks	
Constituent	Initial	Rm temp.[b]	4°C	Rm temp.	4°C
Total folates	(0.42)	0.44	0.47	(0.43)	(0.44)
Total pantothenates	14.8	15.0	15.3	13.0	15.2
Niacin	505.6	533.1	528.2	511.2	519
Thiamine	3.3	3.2	3.1	2.8	2.9
Riboflavin	3.8	3.4	3.3	3.4	3.5
Vitamin B_6	27.5	41.8	42.4	36.9	36.4
Protein	81.2	80.5	82.2	80.4[c]	81.6[c]
Moisture	1.6	1.8	1.6	2.3	1.8

[a–c]See footnotes [a–c], Table 1.

to assess the long-term stability. Researchers are encouraged to inform us of results obtained in their laboratories so that a central clearing house of information can be established and results can be reported at regular intervals. If enough data are obtained, NBS will consider the inclusion of the organic nutrient content on the SRM certification as an informational value. The existing SRMs will thereby be enhanced in value and will serve until more suitable SRMs are developed.

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Liquid Chromatographic Determination of Thiamine in Infant Formula Products by Using Ultraviolet Detection

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A liquid chromatographic (LC) method has been developed for determination of thiamine in infant formula products. The method involves the following steps: (a) dissolution of the formula with water, (b) pH adjustment to induce protein precipitation, (c) filtration, (d) concentration of thiamine by using a cation exchange column and extraction system, (e) cleanup of adsorbed thiamine and other contaminants on the ion exchange column by washing with water and then methanol, (f) elution of thiamine with a mixture of methanol–2M potassium chloride buffer, (g) analysis for thiamine by liquid chromatography. Thiamine is separated from its phosphate esters, the mono-, di-, and triphosphates, as well as its antagonists oxythiamine and pyrithiamine on a 6 μm particle size column and a mobile phase of 40mM triethylammonium phosphate buffer–methanol (pH 7.7) (90 + 10). The method is reproducible, with relative standard deviations ranging from ± 0.76 to ± 1.2%, depending on the infant formula product tested. Recovery of thiamine from various infant formula products is greater than 99%. Analysis for thiamine of several commercially available infant formulas at different levels of fortification gave results that ranged from 122 to 216% of the declared levels. These results agree well with those obtained using the AOAC fluorometric method.

Vitamin B_1 exists in 4 different forms in animal tissues (1): thiamine (T), thiamine monophosphate (TMP), thiamine pyrophosphate (cocarboxylase) or diphosphate (TDP), and thiamine triphosphate (TTP). It is an essential nutrient for most mammalian species and functions in its coenzyme form, thiamine pyrophosphate (2). The classical pathological syndrome of thiamine avitaminosis in humans is called beriberi. Thiamine pyrophosphate is a requirement for carbohydrate metabolism and is recognized as one of the essential cofactors for the enzymatic transfer of acyl groups (3–5). Thiamine, in addition to this function, also plays an important role in the nerve excitation process, independent of its coenzyme role (6).

In the light of its physiological role in mammalian metabolism as well as its antineuritic activity, several methods have been developed to separate and resolve thiamine and its phosphate esters in various matrices. Some of these methods are qualitative and involve the use of paper or thin layer chromatography (1, 7, 8). However, other methods exist for the quantitative determination of thiamine and its analogs in enriched or fortified food (9–15), tissues (16, 17), and urine (18). These methods in general involve (a) acid hydrolysis prior to extraction of thiamine and its phosphate esters, (b) dephosphorylation of the extracted thiamine phosphate esters with the enzyme phosphatase or diastase, (c) oxidation of the thiamine to thiochrome under alkaline conditions, (d) extraction of the thiochrome into isobutanol and measurement of the fluorescence. However, the acid extract from food preparations can contain substances that interfere with the fluorometric determination of thiamine. Therefore, various chromatographic procedures (ion exchange, paper, thin layer, and liquid chromatography (LC)) have been used for purification of the extractions. Ion exchange (cation exchanger) sorbents combined with conventional column chromatography have been used with great success (19) and, in more recent years, LC has been added for the resolution and quantitation of thiamine and its esters (20–24).

Very few methods use direct UV detection of thiamine in food (10, 24, 25) rather than fluorescence detection of thiochrome. Even though UV detection is inherently less sensitive, sample preparation is simpler and it avoids probable interfering compounds in the oxidation reaction leading to the thiochrome formation.

This paper describes a rapid, sensitive, and reproducible LC method for the determination of thiamine in infant formula products, which uses solid phase extraction as a procedure of sample preparation. This technique offers a way of concentrating the thiamine and its phosphate esters from a complex matrix, cleanup of foreign contaminants, and quantitation using UV detection. The LC results obtained with this method are compared with those from the official AOAC method (26).

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METHOD

Reagents

(a) *Solvent.*—Distilled-in-glass methanol (Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442).

(b) *2M KCl solution.*—Dissolve ca 75 g potassium chloride (reagent grade) in 500 mL water; adjust pH to 1.5–2.0 with concentrated HCl.

(c) *Elution solvent.*—Prepare mixture containing 60% 2M KCl solution and 40% methanol (LC grade). Prepare fresh daily.

(d) *Mobile phase.*—Prepare 1 L buffer solution containing 5.5 mL triethylamine and adjust to pH 7.7 with phosphoric acid (87%). Mix buffer and methanol (LC grade) (90 + 10). Adjust pH to 7.7 with phosphoric acid. Filter through 0.45 μm Nylon-66 filter or equivalent and deaerate under vacuum for 15 min.

(e) *Stock standard solution.*—Accurately weigh ca 38 mg thiamine hydrochloride (USP Reference Standard) into 250 mL low actinic volumetric flask. Dilute to volume with acidified (1–2 drops of concentrated HCl) water and shake thoroughly.

(f) *Standard solution.*—Pipet 10.0 mL stock standard solution into 250 mL low-actinic volumetric flask and dilute to volume with elution solvent.

Apparatus

Equipment specified is not restrictive; other suitable equipment may be used.

(a) *Liquid chromatograph.*—LDC Constametric III (Laboratory Data Control, Riviera Beach, FL 33404).

(b) *LC column.*—4.6 × 250 mm Zorbax CN, 6 μm (Dupont No. 880952-705).

(c) *Baker-10 Extraction System™.*—J. T. Baker Co., Phillipsburg, NJ 08865.

(d) *Filter paper.*—Type A/E glass fiber filter paper (Gelman No. 61635).

(e) *Strong cation exchange (SCX) extraction columns.*—2.8 mL disposable solid phase extraction columns (Analytichem International, Harbor City, CA 90710).

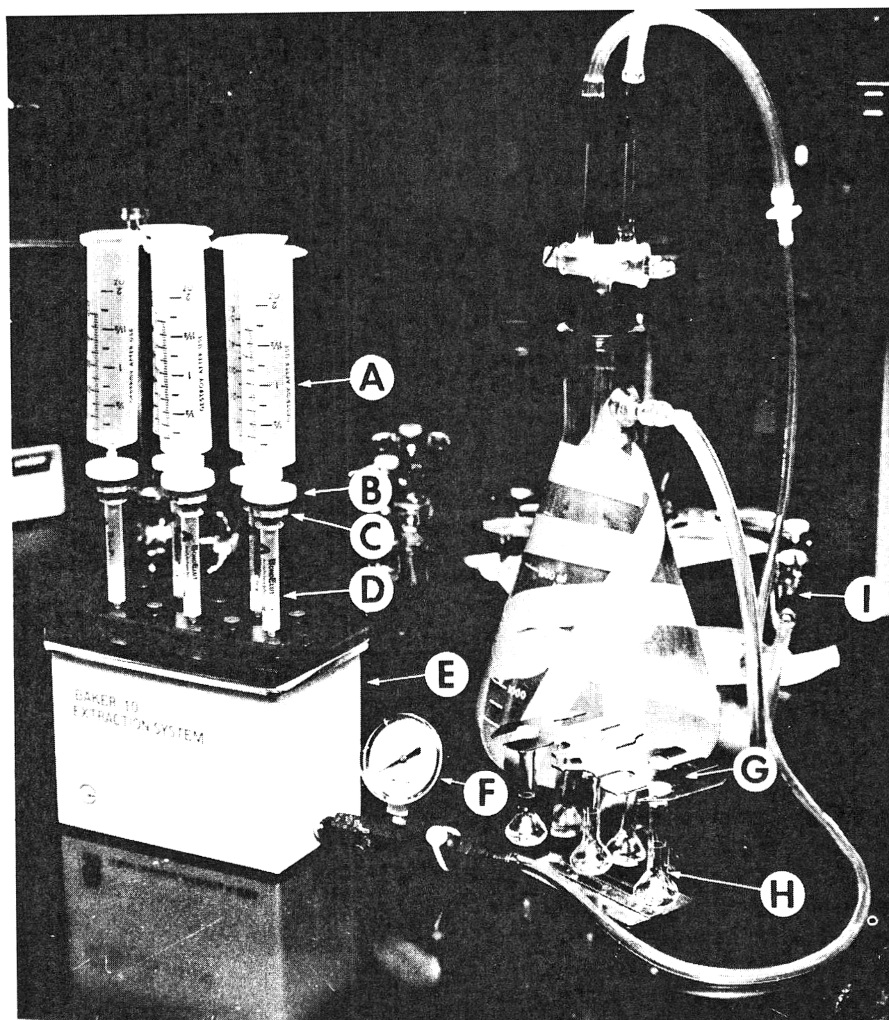

Figure 1. Solid phase extraction system: (A) empty 50 mL syringe barrel; (B) nylon-66 filter unit; (C) adaptor; (D) SCX extraction column; (E) extraction system; (F) vacuum controller/release; (G) collector rack; (H) 5 mL volumetric flask; (I) house vacuum.

(f) *Vacuum controller-release.*—(No. A16077); adaptors (No. 636001); collector racks for 5 mL volumetric flasks (No. A16175) (Analytichem International).

(g) *Syringe.*—Pharmaseal styler disposable 50 mL syringe (Pharmaseal Labs, Glendale, CA 91201).

(h) *Filter units.*—Nylon-66, 0.45 μm (No. 38-150, Rainin Instruments Co., Inc., Mack Rd, Woburn, MA 01801).

Sample Preparation

Perform all sample preparation under subdued light.

Sample size.—For liquid or powder samples, take equivalent of 60–80 μg thiamine HCl (higher concentrations of vitamin lead to overloading of SCX column). For powder samples, reconstitute as per label directions before sampling.

Procedure

Pipet sample into suitable container (e.g., 100 mL beaker) and add enough water to bring total volume to ca 70 mL. Place beaker on magnetic stirrer and, while stirring, adjust pH of solution to 1.7 and then to 4.6, using 1N HCl and 1N NaOH, respectively. Quantitatively transfer sample to 100 mL volumetric flask, washing beaker 3 times with 5 mL portions of water and adding washings to volumetric flask. Dilute to volume with water and shake thoroughly 2 min. Filter ca 60 mL sample solution through type A-E glass fiber filter, collecting filtrate in suitable container.

Equilibrate 2.8 mL SCX extraction column by passing 2 column volumes (ca 6 mL) of methanol followed by 2 column volumes of water through column. This can be done by inserting column tip into one of the holes in top of Baker-10 extraction system and pulling vacuum on it (Figure 1). Do not let column run dry. After column has been equilibrated, remove vacuum, attach disposable 0.45 μm Nylon-66 filter unit directly on top of it using adaptor, and connect filter to 50 mL disposable syringe barrel (Figure 1).

Pipet 50 mL clear filtrate into syringe barrel and apply vacuum. Let all sample pass through column. Release vacuum and remove 50 mL disposable syringe barrel, Nylon-66 filter unit, and adapter. Wash extraction volume with 2 column volumes of water followed by 2 column volumes of methanol and again with 2 column volumes of water. Do not let column run dry until final water wash; at that point, apply vacuum until most of remaining water is removed from column (ca 30 s). Discard column eluates and washings.

Heat portion of elution solvent in suitable glass container on hot plate to 65–75°C, keeping container covered with watch glass to prevent evaporation. Maintain temperature in specified range during the following steps.

Insert 5 mL volumetric flask into Baker-10 extraction system. Elute thiamine from extraction column by passing ca 4.5 mL warm elution solvent through column, collecting eluate in the 5 mL volumetric flask. Vacuum should be adjusted so

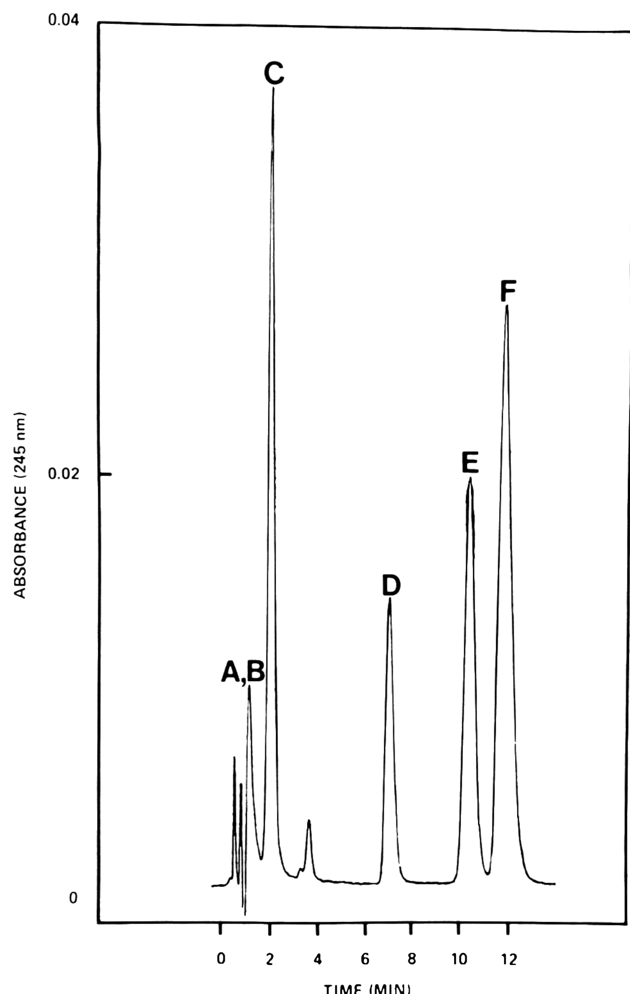

Figure 2. Representative chromatograms of thiamine and related compounds on Zorbax CN column. Mobile phase: triethylammonium phosphate buffer–methanol (pH 7.7) (90 + 10) at flow rate of 1.5 mL/min. Response was monitored at 245 nm. Peak A, thiamine diphosphate; peak B, thiamine triphosphate; peak C, thiamine monophosphate; peak D, oxythiamine; peak E, thiamine; peak F, pyrithiamine.

that this procedure takes at least 30 s, i.e., flow rate should be at most 2 mL/min (vacuum controller reading ca 5–10 in. Hg). Let extracted sample solution cool to room temperature and dilute to volume with cooled elution solvent. Shake flask vigorously to effect mixing. Filter portion of this solution through 0.45 μm Nylon-66 filter unit. Discard first 2–3 mL and collect filtrate in 5 mL vial. Inject 100 μL of standard and sample preparation into liquid chromatograph, using the following chromatographic conditions: solvent flow rate 1.5 mL/min; UV detector 245 nm; sensitivity 0.04 AUFS.

System Suitability Test

(a) Prepare a solution containing 5 μg/mL of oxythiamine chloride (Sigma Chemical Co., St. Louis, MO 63178), 6 μg/mL of thiamine HCl, and 18 μg/mL of pyrithiamine hydrobromide in water. Inject 100 μL of test solution into liquid chromatograph, using chromatographic conditions stated above. Typical retention times for oxythiamine, thiamine, and pyrithiamine are 7.0, 11.0, and 12.5 min, respectively. If resolution factors (as defined by USP) between thiamine and oxythiamine and thiamine and pyrithiamine are greater than 3.0 and 1.0, respectively, then proceed with repeatability of standard injections. If they are less, then decrease amount of methanol and concomitantly increase amount of buffer by ca 3–5%. Prepare system suitability test solution fresh each day.

(b) Inject 100 μL standard preparation into liquid chromatograph 5 times and measure thiamine peak height or area. Calculate relative standard deviation of the 5 replicate injections. If it is 2% or less, then proceed with sample analysis.

(c) *Strong cation exchange (SCX) extraction columns.*—To check suitability of each batch of columns for sample preparation step, carry thiamine standard through procedure. Recovery of thiamine from column should be greater than 95%.

Results and Discussion

The infant formulas used for this study included ready-to-feed liquids, concentrated liquids, and powders prepared in a nonfat milk base or soy base. The declared fortification level for each was 0.67 mg vitamin B_1 per reconstituted quart.

The separation of thiamine from its mono-, di-, and triphosphate esters as well as from its antagonists oxythiamine and pyrithiamine by using this method is shown in Figure 2. Typical sample and standard chromatograms are shown in Figure 3. The method has been shown to be reproducible with relative standard deviations of ± 1.2% for soy-base concentrate, ± 0.78% for milk-base powder, and ± 0.76% for milk-base concentrate (Table 1). The linearity of response for the thiamine standard from 36 to 300% of the stated method sample size had a coefficient of correlation of 0.9999. The linearity of the method was further demonstrated by taking different sample concentrations (volumes) through the procedure. The results (summarized in Table 2) agreed well with each other and the values were not dependent on sample size for volumes ranging from 25–35 mL for milk-base and soy-base concentrates and 50–70 mL for milk-base powders. Since it is not possible to make a true placebo for the various formulations to check for possible interferences from the sample matrix, the method of standard addition was used to analyze one of the products (milk-base powder). The assay results obtained by the method as given and by standard addition differed by less than 1%. Recovery studies were performed by spiking an amount of the milk-base and soy-base liquid concentrates with a known amount of thiamine. The recovery of vitamin B_1 from these formulations was greater than 99% (Table 1).

The official method of analysis (AOAC) for thiamine (26) in infant formula products involves acid hydrolysis followed by dephosphorylation of the phosphate esters of thiamine with an enzyme phosphatase or diastase and then oxidation of the thiamine to thiochrome, which is determined by fluorometry. To demonstrate that any free or bound thiamine was completely extracted and not lost or occluded in the sample matrix, using this LC method, samples were hydrolyzed by the AOAC method of sample preparation (acid hydrolysis) and then taken through this LC method. The results of the thiamine analysis in the hydrolyzed samples as shown in Table 3 agreed well with those analyzed using the LC method of sample preparation. These results also showed that both methods of extraction were comparable.

Thiamine occurs in milk not only as free thiamine, but also in the phosphorylated form and complexed with protein. In whole cow's milk, the total thiamine consists of 50–70% free thiamine, 18–45% phosphorylated thiamine, and 5–17% protein-bound thiamine (27). DeJong (28) found no cocarboxylase present and concluded that the phosphorylated form was thiamine monophosphate. Gregory and Kon (29) found only thiamine monophosphate present in cow's milk during the second week of lactation, and only free thiamine in mid- or late-lactation milk. It had also been reported that cow's milk contains predominantly free thiamine and the thiamine-protein complex (30). It should be noted that this LC method was designed to quantitate the extracted free or bound thia-

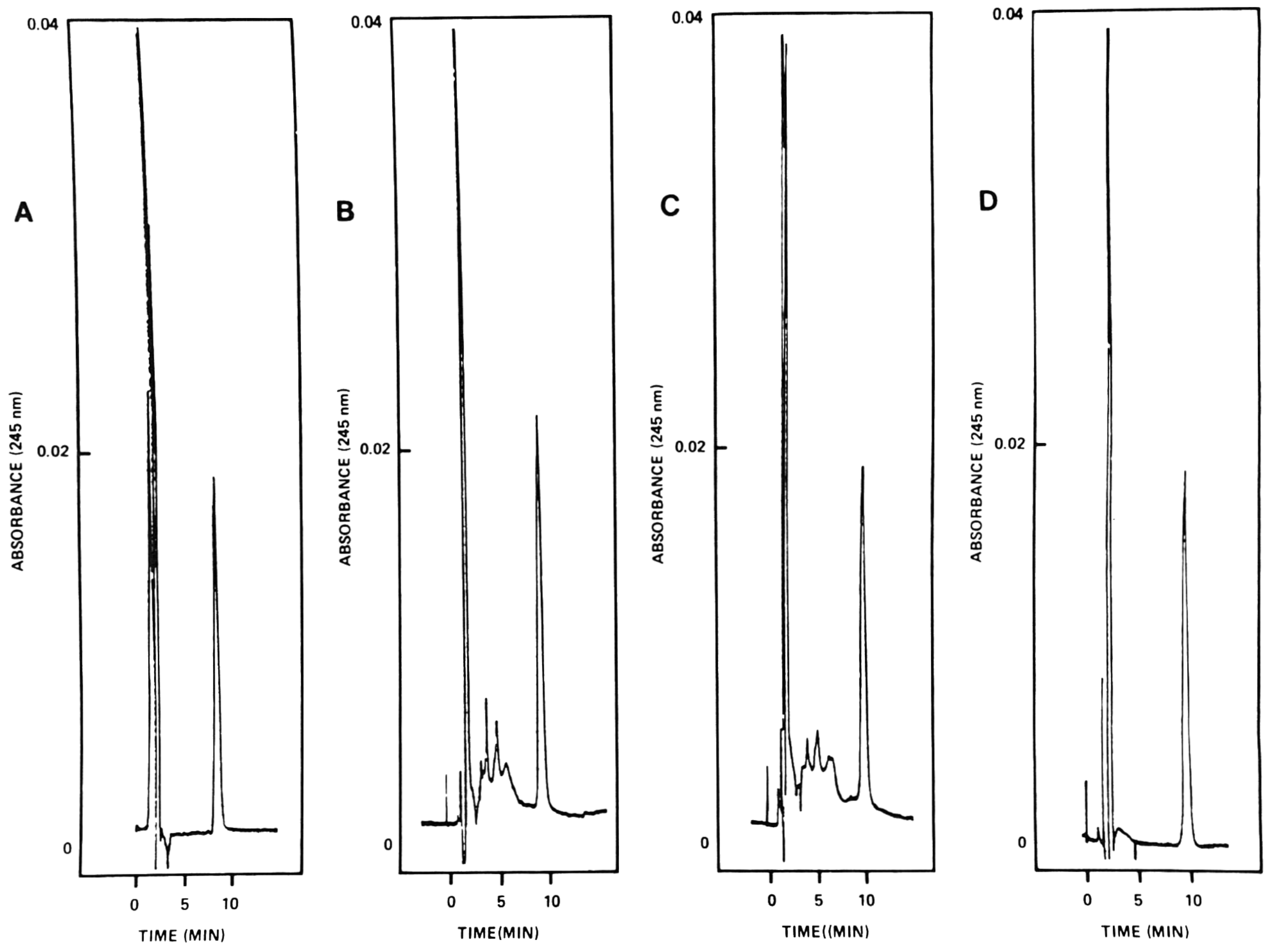

Figure 3. Representative chromatograms of thiamine (A) standard solution; (B) milk-base concentrate; (C) soy-base concentrate; and (D) milk-base powder, using LC method. Chromatographic conditions were identical to those noted in Figure 2.

Table 1. Recovery of thiamine from infant formula products, and reproducibility of LC method

Product	Reproducibility, mg/qt[a]	Recovery				
		Sample size, mL	Found, µg	Added, µg	Sample & spike, µg[b]	Rec., %
Soy-base concentrate	0.99 ± 1.2	30	0.00[c]	62.21	62.41 ± 0.71	100
Milk-base concentrate	1.18 ± 0.76	60	8.10[d]	62.21	71.00 ± 0.74	102
Milk-base powder	1.14 ± 0.78	60	72.0	62.21	135.49 ± 1.05	102

[a]Mean ± % RSD, $n = 5$.
[b]Mean + SD, $n = 6$.
[c]No significant amounts of thiamine found in this product which was unfortified with thiamine.
[d]Product unfortified with thiamine.

Table 2. Results of analysis of infant formula products by LC, at different sample concentrations (volumes) of thiamine

Product	Sample taken, mL[a]	Thiamine HCl, mg (per reconstituted quart)
Milk-base concentrate	25.0	1.19
	30.0	1.19
	35.0	1.20
Soy-base concentrate	25.0	1.03
	30.0	1.00
	35.0	1.04
Milk-base powder	50.0	1.16
	60.0	1.14
	70.0	1.15

[a]Claimed mg thiamine/qt of formula on ready-to-feed basis is 0.67 mg.

Table 3. Comparison of methods of extracting vitamin B_1 from infant formula products—protein precipitation vs acid hydrolysis—both followed by LC analysis

Product	Sample	mg Thiamine/qt	
		Protein precipitation[a]	Acid hydrolysis[b]
Milk-base concentrate	1	1.18	1.17
	2	1.18	1.14
Soy-base concentrate	1	0.99	0.98
	2	0.76	0.78
Milk-base powder	1	1.13	1.13
	2	1.13	1.13

[a]LC method of sample preparation.
[b]AOAC method of sample preparation, no dephosphorylation step.

Table 4. Comparison of results for vitamin B_1 in infant formula products, using LC and AOAC methods

Product	Sample	mg Thiamine HCl/qt	
		LC	AOAC
Milk-base concentrate	1	1.30	1.27
	2	1.32	1.34
	3	1.35	1.40
Soy-base concentrate	1	0.99	1.05
	2	1.06	1.05
	3	1.04	0.95
Milk-base powder	1	1.14	1.19
	2	1.13	1.15
	3	1.14	1.15
Soy-base powder	1	1.14	1.14
	2	1.16	1.10
	3	1.24	1.24
Milk-base ready-to-feed	1	1.40	1.46
	2	1.33	1.36
	3	1.35	1.36
Milk-base powder	1	1.40	1.40
	2	1.32	1.35
	3	1.29	1.25

mine and not its phosphorylated derivatives. If their analyses were desired, one would need to change the chromatographic conditions (mobile phase).

The LC method was used in the analysis of various lots of infant formula products from the same manufacturer. The results of the analysis and their comparison to these lots assayed by the AOAC method are summarized in Table 4. The results from both methods compared favorably. A significant fact that emerged from these results was the very little contribution from the phosphate esters of thiamine to the total amount of vitamin B_1 present in these milk-base infant formula products. If an appreciable level of phosphate esters were present, the results by the AOAC method (which measured total thiamine, i.e., contributions from both free and bound thiamine as well as the phosphate esters) would be significantly higher than the results from the LC method. This LC method was used successfully in the analysis of vitamin B_1 in commercially available products of infant formula. The results as summarized in Table 5 agreed well with the AOAC method.

Having demonstrated that the contributions from the phosphate esters of thiamine to the total amount of vitamin B_1 present in these milk-base infant formula products were not significant, a series of experiments was set up to see what modifications, if any, could be made in the AOAC method in terms of the analysis of vitamin B_1 in infant formula products. The first experiment involved the use of the LC method of sample preparation. The clear filtrate resulting from the protein precipitation was subjected to open column chromatography according to AOAC, with the enzyme dephosphorylation eliminated. The resulting solution containing thiamine

Table 5. Results of analysis of vitamin B_1 in commercially available infant formula products, using AOAC method, LC method, and combination of both methods

Formula type	Declared, mg/qt	mg Vitamin B_1/qt		
		AOAC	LC	LC & AOAC[a]
Manufacturer A:				
Soy-base ready-to-feed	0.38	0.90	0.99	1.09
Milk-base ready-to-feed	0.71	1.51	1.53	1.55
Milk-base concentrate	0.62	1.29	1.33	1.30
Milk-base concentrate	0.62		1.34	
Milk-base powder	0.62	0.73	0.76	
Soy-base powder	0.38		1.01	
Milk-base powder	0.62		1.56	
Manufacturer B:				
Milk-base ready-to-feed	0.50	0.69	0.68	0.73
Soy-base ready-to-feed	0.50	0.74	0.78	0.80
Milk-base concentrate	0.50	0.65	0.65	0.67
Milk-base concentrate	0.50		0.64	
Milk-base powder	0.50	0.59	0.66	

[a] LC method of sample preparation was used. Clear filtrate resulting from protein precipitation was subjected to column chromatography according to AOAC; enzyme dephosphorylation step was eliminated. Resulting solution containing vitamin B_1 was oxidized to thiochrome which is quantitated using fluorometry.

was oxidized to thiochrome which was quantitated using fluorometry. The results obtained (Table 5) agreed well with the LC and the AOAC methods. The next experiment involved the elimination of the column cleanup step in the AOAC method. The resulting solution from the protein precipitation step in the LC method was further diluted with potassium chloride solution and filtered through a 0.45 μm Nylon-66 filter. The thiamine was then oxidized to thiochrome and quantitated according to AOAC. The results as summarized in Table 6 clearly demonstrated the agreement between this shortened version of the AOAC, the LC, and the standard AOAC methods.

In conclusion, a simple, fast, sensitive, and reproducible LC method has been described for the determination of vitamin B_1 in infant formula products. The method separates thiamine from its antagonists and particularly the respective phosphate esters. There is also evidence that the contributions from the phosphate esters of thiamine to the total inherent amount of vitamin B_1 in milk-base infant formula products is not significant. This suggests that it may be feasible to eliminate the cumbersome acid hydrolysis as well as the dephosphorylation steps in the AOAC method, replacing them with a protein precipitation step followed by oxidation of the thiamine to thiochrome. This modification of the AOAC method, however, would have to be shown to be applicable to the particular product in question.

Table 6. Results of analysis for thiamine in commercially available infant formula products, using modified AOAC, official AOAC, and LC methods

Product	Sample	mg Thiamine/reconstituted qt		
		Modified AOAC[a]	Official AOAC	LC method
Milk-base powder	1	1.41	1.48	1.40
	2	1.27	1.30	1.37
	3	1.39	1.40	1.40
Milk-base powder	1	1.17	1.29	1.13
	2	1.14	1.12	1.17
	3	1.16	1.19	1.14
Milk-base concentrate	1	1.08	1.03	1.10
	2	1.04	1.00	1.08
Soy-base concentrate	1	0.99	1.05	0.99

[a] Protein precipitation, followed by oxidation of extracted thiamine to thiochrome and quantitation by fluorometry.

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EXTRANEOUS MATERIALS

Brine Saturation Technique for Extracting Light Filth from Ground Cinnamon: Intralaboratory Study

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An intralaboratory study was performed using the new brine saturation technique for isolating light filth from ground cinnamon. Recoveries of light filth averaged ≥96.5%. The excellent recovery plus improvements in safety and simplicity give this new technique considerable advantage over the present official method.

Extraction of light filth from ground cinnamon is a problem because of the high mucilaginous content of some cassias, which form a slimy gel-mass when mixed with water. The oil content of the spice causes poor separation and makes filter papers heavy and difficult to examine. To circumvent these problems, pancreatin digestions and sodium oxalate additions as well as petroleum ether and chloroform/carbon tetrachloride mixtures have been used to disperse the gel and remove the oils (1, 2). Sodium-EDTA has also been used, unsuccessfully, in efforts to solve these problems (2).

Gel formation ceased to be a problem when hydrochloric acid was included in the method in 1968 (3), and that work resulted in the present AOAC official first action method (4). Average recoveries for the study were 85.7% for insect fragments and 49.0% (3) for rodent hairs.

In comparison, average recoveries for the new brine saturation technique (5–7) are ≥96.5% for both elements. Both oil and mucilage are removed by boiling the sample in acidified isopropanol and then by washing with hot tap water on a 230 mesh sieve. A single brine saturation step before percolator extraction of the filth elements with olive oil results in clean filter papers and excellent recoveries.

METHOD

Reagents

(a) *Brine.*—Dissolve ca 360 g NaCl, AR grade or equivalent, in 1 L water.

(b) *Olive oil.*—NF or equivalent.

Extraction

Weigh 50 g sample into 600 mL beaker; add 300 mL isopropanol plus 30 mL HCl and magnetic stir bar. Cover and boil 5 min with constant stirring on magnetic stirrer hot plate. (Caution: To prevent scorching, mix thoroughly before applying heat.) Transfer to 230 mesh sieve (Note: Avoid transferring stir bar by attracting it to a second stir bar on outside bottom of beaker.) and rinse beaker with hot tap water. Wash sample on sieve with stream of hot tap water until foam disappears and effluent is clear. Remove excess tap water by rinsing with small amount of brine. Quantitatively transfer sample back into original beaker with brine. Dilute to ca 400 mL with brine and return to stirrer hot plate. Boil 3 min with constant stirring; then cool with constant stirring in cold water bath (plastic or glass bowl filled with cold water on magnetic stirrer) until just warm to the touch. Add 30 mL olive oil and continue stirring 5 min (oil will slow the stirring; compensate so that oil is mixed into sample, but not so vigorously as to incorporate air or cause sample to overflow beaker). Transfer sample with tap water, retaining stirring bar in beaker as before, into 2 L percolator containing glass stirring rod [sec. **44.002(h)***(2)*] (4) and 500 mL tap water. Fill percolator to within 5 cm from top with slightly warm (ca 32°C) tap water and stir with glass rod to prevent entrapment of oil in product at bottom of vessel. Let separation continue for 15 min; then drain and discard water until oil layer is within 5 cm of bottom. Refill percolator and repeat draining procedure twice; then drain remaining contents into 400 mL beaker and wash percolator and rod thoroughly with isopropanol and tap water into same beaker. Filter contents of beaker and isopropanol washings from beaker onto lined filter paper and examine as in sec. **44.005**(g)(4).

Results

Two analysts, using only the brine saturation method described here, each examined 12 spiked 50 g subsamples of the product. All subsamples were spiked with 12 insect elytral squares and 12 rodent hair fragments, using the gelatin matrix technique (8). Table 1 shows the excellent recovery of 280 of 288 spiked insect fragments and 272 of 276 spiked rodent hairs by this method.

Recommendations

Because recovery of insect fragments is improved and recovery of rodent hairs is double that reported for the present AOAC official method, we recommend that a collaborative study be conducted on this method. We also recommend that the brine saturation technique for light filth extractions be continued and expanded to alleviate problems with other products and/or methods.

Table 1. Intralaboratory recovery results for insect fragments and rodent hairs (12 each added) from 50 g cinnamon, using brine saturation method

	Number of insect fragments		Number of rodent hairs	
	Analyst		Analyst	
Subsample	1	2	1	2
1	12	12	12	11
2	12	12	12	12
3	12	12	12	12
4	12	12	10	12
5	12	12	12	12
6	11	12	12	12
7	12	12	12	12
8	12	11	12	12
9	12	11	12	12
10	12	12	11	3[b]
11	12	11	12	11
12	11	10	12	12
Total[a]	280		272	
Av., %	97.9	96.5	98.6	98.5
SD	0.6	0.7	0.4	0.4
CV, %	5.3	5.8	3.3	3.4

[a] A total of 288 insect fragments and 276 rodent hairs were spiked.
[b] Deleted from calculation by Dixon's test.

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PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Multiresidue Method for Quantitative Determination of Organophosphorus Pesticides in Foods

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A multiresidue method for the quantitative determination of organophosphorus pesticides in foods from the Food and Drug Administration's Total Diet Study is described. The organophosphorus pesticides are separated on the basis of polarity and determined in both fatty and nonfatty foods with a minimum of interferences. The foods analyzed included raw and cooked individual foods as well as combination dishes, water, and whiskey. Recoveries of 17 organophosphorus pesticides in 41 foods ranged from about 80 to 118%.

The Total Diet Study of the Food and Drug Administration is a surveillance program that monitors the levels of chemical residues in foods. The foods analyzed are representative of the range of raw and cooked foods commonly eaten by the American consumer. These include fruits, vegetables, meats, fatty foods such as peanut butter and grains, recipe items such as lasagna, dairy products, baby foods, and alcoholic and nonalcoholic beverages. Details of the program are presented elsewhere (1–5). The Total Diet Study is a means to monitor, on a yearly basis, the contaminant and selected nutrient content of the United States food supply and to observe trends in the consumption of those substances over time. Information from the study helps to identify potential public health issues that may warrant changes in agricultural or manufacturing practices or in the regulatory policies concerning food additives or nutrient fortification.

The quantitative determination of organophosphorus pesticide residues in foods in the form in which they are eaten is a major part of the Total Diet Study. Routinely, the methods of Storherr et al. (6), Carson (7), and Mills et al. (8) are used to determine organophosphorus pesticide residues in foods. To a lesser degree, the method of Luke et al. (9–11) is also used. These methods were developed primarily for the analysis of raw agricultural crops, i.e., fruits and vegetables, which comprise only a fraction of the total number of food items in the Total Diet Study. Problems have been encountered in the application of these analytical methods to fatty foods and the more complex foods prepared according to recipes. The use of these methods does not assure the quantitative determination of highly polar organophosphorus pesticides from all foods that comprise the Total Diet Study. This paper reports the development of a method to completely recover all organophosphorus pesticides from all foods of the Total Diet Study and to minimize or eliminate the difficulties previously encountered.

METHOD

Apparatus and Reagents

(a) *Gel permeation chromatograph.*—Auto-Prep Model 1001 (Analytical Biochemistry Laboratories, Inc., Columbia, MO), equipped with 30 × 2.5 cm id column (Kontes, Vineland, NJ), slurry packed with 33 g 200–400 mesh Bio-Beads SX-3 (Bio-Rad Laboratories, Richmond, CA), compressed to bed length of ca 20 cm. Eluting solution was methylene chloride–hexane (1 + 1) at flow rate of 5.0 mL/min with operating pressure of ca 10 psi. System was operated with 12 min dump cycle, 20 min collect cycle, and 0 min wash cycle.

(b) *Gas chromatographs.*—*(1)* Tracor Model 560 equipped with ^{63}Ni electron capture (EC) detector and Model 702 N/P alkali thermionic detector. Two m × 4 mm id glass column was packed with 2% stabilized diethylene glycol succinate (DEGS) on 80–100 mesh Chromosorb W-AW and effluent was linked to detectors through 1:1 glass-lined splitter (Scientific Glass Engineering, Austin, TX) with helium flow rate of 60 mL/min. EC detector was operated with purge gas of 10% methane in argon with flow rate of 70 mL/min. Temperatures (°C)—inlet 215, oven 200, EC detector 350, N/P detector 250. *(2)* Varian Vista Model 6000 equipped with flame photometric detector (FPD) and operated with phosphorus-specific filter (526 nm). Two m × 2 mm id glass column was packed with Ultrabond 20SE on 100–120 mesh Chromosorb W and was linked to FPD. Nitrogen carrier gas was used with flow rate of 65 mL/min. Temperatures (°C)—inlet 200, oven 175, FPD detector 300.

(c) *Methylene chloride, hexane, and acetone.*—Pesticide grade (Burdick & Jackson Laboratories, Muskegon, MI).

(d) *Eluting solution.*—Methylene chloride–hexane (1 + 1).

(e) *Extracting solutions.*—Methylene chloride–acetone (3 + 1) and methylene chloride–hexane (1 + 9).

Extraction

Accurately weigh 100 g food into blender jar. If food has low moisture content (<25% moisture), add 100 mL water to jar. Add 200 mL acetone and blend mixture 2 min. Filter solution through sharkskin paper in Buchner funnel and collect filtrate. Wash filter cake with 50 mL acetone, collect washing, and add to filtrate.

Extract 80 mL aliquot of combined washing/filtrate with three 30 mL portions of methylene chloride–hexane (1 + 9). Collect organic layers (eluate 1). Extract aqueous solution remaining after first set of extractions with three 30 mL portions of methylene chloride–acetone (3 + 1). Collect organic layers (eluate 2). Saturate aqueous solution remaining after second set of extractions with sodium chloride. Extract solution with three 30 mL portions of methylene chloride–acetone (3 + 1). Collect organic layers (eluate 3). Discard aqueous layer.

Dry eluates by passing them through anhydrous sodium sulfate in 15 cm × 20 mm id column. Collect dried eluates in Kuderna-Danish concentrator and attach Snyder condensation column. Concentrate eluates on steam bath until top of Snyder column is no longer wetted with solvent. For nonfatty foods, ca 1 mL solution will remain. For fatty foods, <1 mL will remain. Add hexane–methylene chloride (1 + 1) to yield final volume of 10 mL for nonfatty samples or to final volume containing ≤0.2 g fat/mL solution. Mix solution thoroughly.

Gel Permeation Chromatographic (GPC) Cleanup

Maximum of 1 g fat may be loaded on GPC column, necessitating use of multiple GPC loops for cleanup of fatty foods

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Reference to any commercial material, equipment, or process does not constitute endorsement or recommendation by the Food and Drug Administration.

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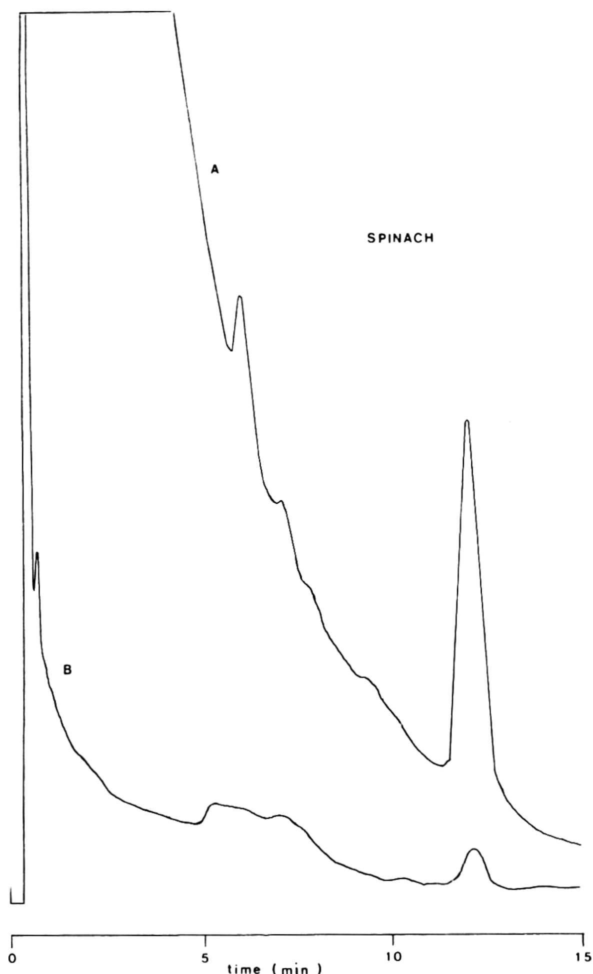

Figure 1. Chromatograms of unspiked spinach sample. A, eluate before GPC cleanup; B, eluate after GPC cleanup. Chromatographic conditions: 2% stabilized DEGS column with alkali thermionic detector, attenuation 8; 5 μL injected.

to obtain final eluate for analysis which contains ≥5 g sample equivalent/mL solution.

Fill 10 mL syringe and load 5 mL holding loops of chromatograph with eluates. Filter each eluate as it is loaded by passing it through 5.0 μm Millipore filter in Swinny filter adapter attached to syringe. Elute each sample with methylene chloride–hexane (1 + 1), using parameters in *Apparatus and Reagents*. Transfer collected solutions, combining multiple fractions if necessary, to Kuderna-Danish concentrator. Add Snyder column and concentrate each eluate to ca 1 mL on steam bath. Add 20 mL acetone and concentrate to 2 mL to remove all traces of methylene chloride.

Gas Chromatographic (GC) Determination

Determine residues by GC, using parameters in *Apparatus and Reagents*. Inject ca 5 μL solution.

Results and Discussion

The extraction methods described by Mills et al. (8) and Storherr et al. (6) are used to remove the organic chemical residues from foods. The extraction also removes a large number of indigenous compounds. These coextractives complicate the quantitative determination of the organic chemical residues. One of the biggest obstacles to the routine determination of organophosphorus pesticide residues in Total Diet foods is the difficulty in rapidly and quantitatively removing all residues from the foods and then retrieving only those residues of interest in the presence of coextractive interferences such as plant pigments or lipids. This can easily be done for organohalogen pesticides, as these compounds are of relatively low polarity and may be quantitatively isolated from the more polar coextractives by Florisil column cleanup. This method is ineffective for the more polar organophosphorus pesticides. Significant losses have also been observed with the use of magnesia-charcoal columns. The sample eluates must be cleaned up, as the "dirty" sample eluates will inhibit quantitative determination by chromatography.

The acetone extraction described by Bates (12) and Luke et al. (9) was used because it offers the greatest potential for simple, quantitative removal of residues from foodstuffs. The pesticides are removed in order of polarity by successively increasing the polarity of the extraction conditions. Food samples representative of the Total Diet Study were fortified with a series of organophosphorus pesticides so that the efficiency of recovery for a range of polarities could be determined. Our emphasis was on the determination of highly polar organophosphorus pesticides from a wide range of food matrices because these pesticides have proven to be the most difficult to determine.

The first partitioning step, extraction with methylene chloride–hexane (1 + 9), removes the less polar pesticides and most of the coextractable materials from the water layer. The coextractable materials can be further separated, if desired, from the less polar pesticides by Florisil column chromatography. These coextractable materials inhibited our recovery of polar pesticides (e.g., methamidophos, acephate, methidathion, monocrotophos) when analysis was attempted by

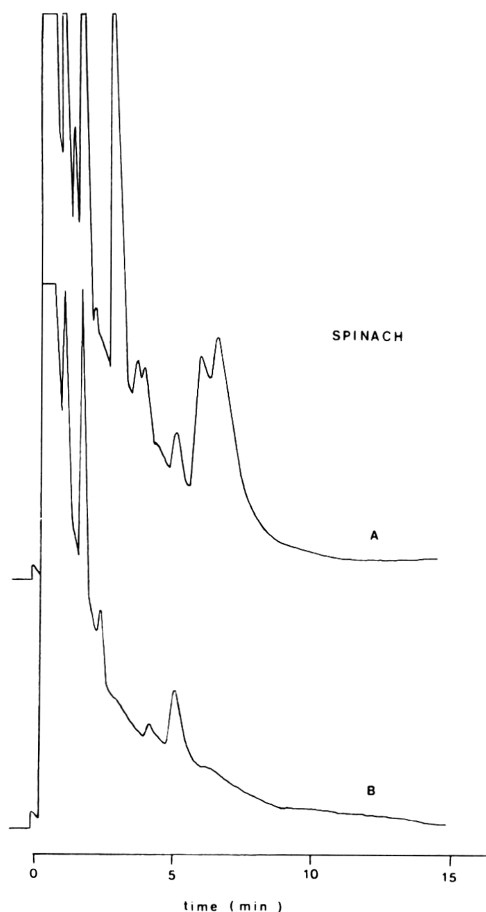

Figure 2. Chromatograms of 1:10 dilution of unspiked spinach sample. A, eluate before GPC cleanup; B, eluate after GPC cleanup. Chromatographic conditions: 2% stabilized DEGS column with EC detector, attenuation 5; 5 μL injected.

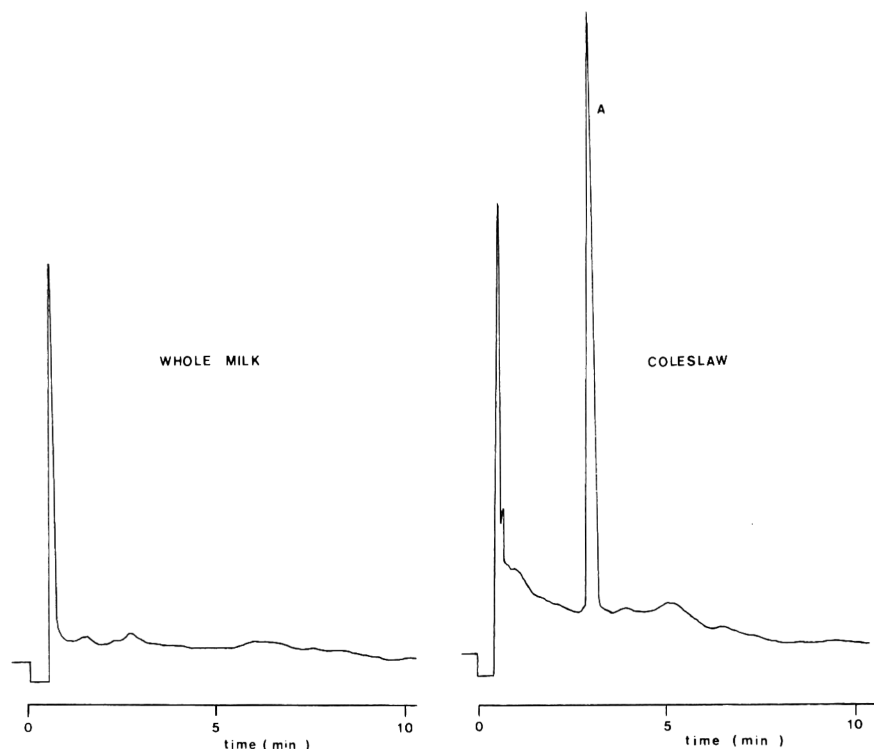

Figure 3. Chromatograms of unspiked whole milk and coleslaw samples. See Figure 1 for chromatographic conditions.

other procedures. After the initial extraction step, the polar pesticides remain in the water layer.

The second step increases the polarity of the extraction solvent and removes the moderately polar pesticides and most of the remaining coextractable materials from the water layer. The use of acetone prevents the formation of the emulsion that may be formed when such naturally occurring materials as pectin from fruits and vegetables, collagen from meats, or starches from vegetables and grains are forced out of the water layer and form a semisolid gel. These substances are normally soluble in water but are insoluble in methylene chloride. This gel formation is exacerbated by the presence of a high sugar concentration and natural fruit acids. The gel has been a formidable obstacle to the efficient separation of pesticides from various foods of the Total Diet Study.

The third step displaces the strongly polar pesticides from the aqueous layer by saturation of the water with sodium chloride. Acetone is again used to prevent the formation of gels. The very polar pesticides (e.g., methamidophos, acephate, monocrotophos) could only be completely displaced from water by using acetone and sodium chloride. All other attempts resulted in diminished recoveries or incomplete extraction of the pesticides from the water layer.

The proposed method directs the analysis of the individual eluates, consisting of the 3 extracts of a given extraction step. All eluates may be combined, if desired, but the advantage of separation of compounds by polarity is then lost. The eluates were dried by passing them through a column of anhydrous sodium sulfate. A 50 mL methylene chloride rinse was used to ensure that all organophosphorus residues were removed from the column. This was done rather than drying each individual eluate as it was obtained because lower recoveries were obtained for the more polar organophosphorus pesticides, i.e., methamidophos and acephate, when the latter procedure was used. In some cases, losses as great as 60% were observed. The lower recoveries were not observed when a single drying step was used. We believe that the pesticides are trapped on the salt column during the multiple drying of the individual eluates and cannot be eluted from the filter cake by the organic solvents used in the wash. To confirm this, we dissolved the filter cakes in water and extracted the salt-saturated water with methylene chloride–acetone (3 + 1). Concentration of the eluates in acetone and subsequent GC analysis showed that the missing pesticides were then recovered.

The GPC cleanup procedure developed by Hopper (13) was originally intended to remove fats from food extracts before further cleanup for GC analysis. Fats were removed in the first 60 mL eluate with methylene chloride–hexane (1 + 1) and all pesticides were collected in the next 100 mL eluate. This procedure was used to analyze fatty foods for organophosphorus pesticides. This procedure also worked well in removing a large portion of the coextractive materials from nonfatty foods. An example is shown in Figures 1 and 2, the chromatograms of an unspiked spinach sample before and after GPC cleanup. The solution analyzed before GPC cleanup was black, while that obtained after GPC cleanup was green. Figure 1 shows the response obtained with a thermionic detector. The eluate obtained after GPC cleanup shows a marked reduction in background. Figure 2 shows the response obtained with an EC detector. These eluates were diluted 10-fold to show the chromatographic response. Again, the eluate obtained after GPC cleanup shows a reduced background, although it is not as clean as that obtained with the element-specific thermionic detector.

Eluates cleaned up via this procedure could be analyzed directly by GC and the components quantitatively determined. The coextractives had not been quantitatively removed from the final eluates after extraction and GPC cleanup but the concentration of these materials had been sufficiently reduced so that there was not a significant interference in the chromatography using element-specific detectors.

Figure 3 shows the results of analysis of fatty foods. The eluates obtained after GPC cleanup of whole milk and cole-

Table 1. Recovery (%) of pesticides from fortified foods

	Pesticide[a]																
Food	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Bananas	95	100	94			80	100			83	95			100			
Beef, sirloin				83		83	86	86		94							
Beef stew			87						90				102		95		
Butter			101			87			98	94					91		
Cheese, American				87		90	85	85	89	96		86				88	
Cherries	87	86	110	83	86	102	89	87	84	82	100		86	107			98
Chicken casserole			100						92				102		104		
Chicken, fried									89			85				87	
Coleslaw			87	85		104	100	90	94	100		100	100		95	102	
Corn bread			90						87				107		102		
Eggs, boiled			101			93			95	98		92			98	87	
Eggs, fried			92	86		90	85	90		63					92		
Eggs, raw				94		90	93	95		89							
Eggs, scrambled			102			93				103					94		
Grapes	93	96	97	105	96	95	92	93	92	99	106		105	100			102
Honey			100			99			91	118		86			96	87	
Lasagna			92						92				92		102		
Lettuce	94	100	94	96		80	94	107	89	84	95	87		94		90	
Meat loaf			96						96				86		96		
Milk				94		84	93	93		97							
Oats, rolled									89			83				88	
Oil, vegetable									98								
Onion rings			93						92				106		102		
Onions	92	97	99	104	97	92	89	92	101	113	110		101	100	87		95
Oranges			89	107		96	97	103		112	100			83			
Peas	82	96	100	113	96	95	90	82	101	100	112		110	100			96
Peppers, green	89	97	94	97		90	94	108		90	89		101	94	97		
Pizza			110	83		83	89	83	98	100			114		104		
Pork chops									85			87				80	
Pork chow mein			84						98				88		91		
Potatoes, baked	81	93	95	100	93	88	114	81	96	96	97		94	94			98
Potatoes, boiled			90			92			94	93		93			85	85	
Potatoes, scalloped			107						97				107		113		
Shrimp			91						106				96		108		
Spaghetti with meat			88						110				95		115		
Spinach	94	94	93			80	93			89	94			97			
Sweet potatoes, candied			85						107				97		108		
Tomatoes	88	94	100			104	90			86	97			94			
Water	101	94	95	88	94	90	88	101	100	95	94		91	114	107		100
Watermelon	95	89	100			80	95			81	97			100			
Whiskey	88	98	89	101	98	81	96	98	85	91	108		104	100			101

[a] 1, malaoxon; 2, phenthoate; 3, diazinon; 4, dimethoate; 5, fenitrothion; 6, methamidophos; 7, monocrotophos; 8, omethoate; 9, chlorpyrifos; 10, acephate; 11, malathion; 12, fonophos; 13, ethion; 14, methidathion; 15, parathion; 16, ronnel; 17, phosalone. Pesticides were added at the following levels (μg): 1 and 2, 0.4; 3–5, 0.6; 6–8, 0.8; 9–12, 1.0; 13 and 14, 1.2; 15, 1.6; 16, 2.0; and 17, 4.0.

Table 2. Comparison of results of pesticide residue determinations, using proposed method and Storherr method

		Found, ppm	
Food	Pesticide	Proposed method	Storherr method
Apples, red	Imidan	0.034	0.029
	Guthion	0.042	0.040
Bread, whole wheat	diazinon	0.010	0.005
	malathion	0.039	0.026
Green beans, boiled	methamidophos	0.055	0.032
	acephate	0.125	—
Oats, rolled	malathion	0.007	0.009
Oranges	acephate	0.010	—
	ethion	0.006	0.007
	carbophenothion	0.005	0.005
Peppers, green	acephate	0.005	—
	dimethoate	0.018	0.008
	omethoate	—	0.014

slaw had relatively clean backgrounds. A large peak (A) in the coleslaw chromatogram was found in all cabbage samples and is believed to be a natural component. We also did not find enhancement due to the presence of coextractive materials in the eluates as has been reported (9, 10). If desired, eluate 1, which contains the less polar pesticides and the majority of the coextracted materials, may be further cleaned up by elution through a Florisil column with either the mixed ether eluant series or the hexane–methylene chloride–acetonitrile eluant series described in the *Pesticide Analytical Manual* (14) or *Official Methods of Analysis* (15). Efficient cleanup could also be obtained if methylene chloride alone was used as the eluting solvent.

Forty-one foods, representing the full range of foods analyzed in the Total Diet Study, were examined using this method. Food samples were spiked with the levels of pesticides indicated in Table 1. Recoveries from fortified samples ranged from about 80 to 118% for most samples.

The determination of the pesticides by fractions offers the advantage of subdividing the number of organophosphorus pesticides so that overlapping of closely eluting compounds can be minimized and quantitation is thus improved. However, analysis by fractions is much more time consuming than analysis of combined eluates, as each fraction must be cleaned up, concentrated, and analyzed individually. The user must decide which approach is best.

There were some difficulties in recovering acephate, a very polar pesticide, from fried eggs. Recoveries were only 63%. However, recoveries from raw, boiled, and scrambled eggs were ≥89%. The reason for this is as yet unknown.

Concurrent tests were run to compare results obtained using the proposed method with those obtained with the methods currently used in our laboratory (Table 2). The results agreed.

Chromatograms from the eluates obtained from the Storherr method and this method are presented in Figure 4. Baked

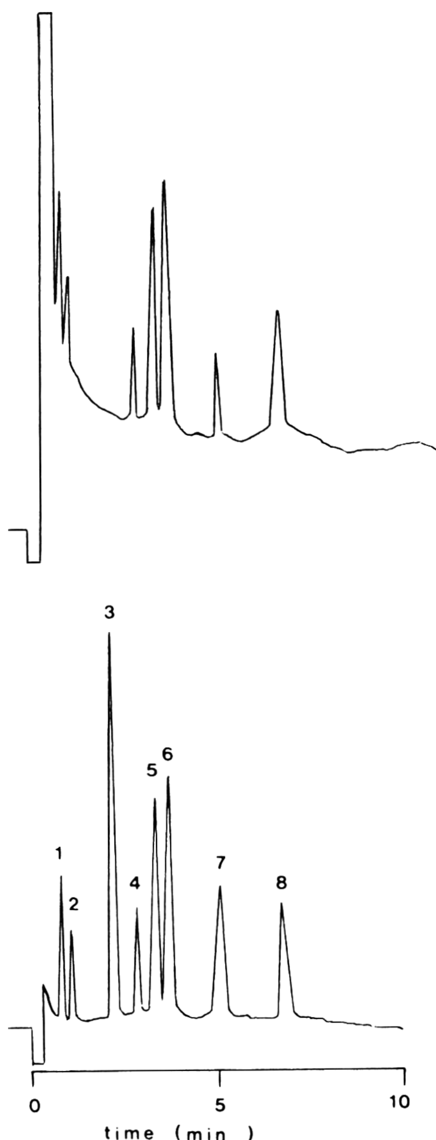

Figure 4. Chromatograms of spiked baked potato sample. Top, eluate obtained by Storherr procedure; bottom, eluate obtained by proposed procedure. See Figure 1 for chromatographic conditions. Peaks and spike level (μg): 1, diazinon, 0.6; 2, methamidophos, 0.8; 3, acephate, 1.0; 4, malathion, 1.0; 5, malaoxon, 0.4; 6, phenthoate, 0.4; 7, monocrotophos, 0.8; and 8, methidathion, 1.2.

potato with no detectable organophosphorus residues was spiked with an 8-component organophosphorus pesticide mixture. In this instance all eluates were combined. A higher relative background is seen in the chromatogram obtained using the Storherr method. Acephate was not detected and the response for methamidophos and monocrotophos was reduced. When the proposed method was used, all compounds were easily detected and quantitation was straightforward.

A level of 0.018 ppm dimethoate was found in green peppers analyzed by the method described. When the peppers were analyzed with the Storherr method, 0.008 ppm dimethoate and 0.014 ppm omethoate were found. Dimethoate metabolizes to omethoate under various conditions (16–18), and this probably happened during the handling of the pepper sample, which was one of a number of samples in a batch process. Most likely, the sample was handled over several days and metabolism occurred during this period. The analysis of green peppers by the method described here was completed within 3 h. No metabolism had occurred.

In addition, several very polar pesticides were detected via this method that had not been found before, for example, acephate in green beans, oranges, and green peppers. Repeated analysis of these samples by the Storherr method showed no acephate. Acephate is apparently lost during sample cleanup by the Storherr method; this has been attributed to the presence of magnesia in the magnesia-charcoal cleanup column (11). Reanalysis of these samples by the method described here showed the presence of acephate.

The extraction procedure, based on separation by polarity, assures the quantitative removal of all organophosphorus pesticides from the foods of the Total Diet Study. These foods include fatty and recipe-composited foods that have been difficult or impossible to analyze by currently used methods. The interferences that have hindered the quantitative determination of pesticide residues in these foods have been minimized and enhancement of the organophosphorus pesticide response to the presence of coextractive materials has been reduced. The method increases the specificity of the analysis and allows rapid, simple quantitation of organophosphorus pesticides in a wide range of foods.

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Gas Chromatographic Relative Retention Data for Pesticides on Nine Packed Columns: I. Organophosphorus Pesticides, Using Flame Photometric Detection

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The retention time relative to parathion, absolute retention time, concentration range, peak asymmetry factor, and peak shape class are given for each of 50 organophosphorus pesticides analyzed by gas chromatography (GC) on 9 different packed columns. The packing materials used were 3% SP-2100, 1% Dexsil-300, 3% OV-17, 1.5% OV-17 + 1.95% QF-1, 4% SE-30 + 6% QF-1, 3% OV-17 + 3% OV-210, 5% DC-200 + 7.5% QF-1, 3% Carbowax 20M, and 4% Reoplex-400. Retention data were determined at 200°C with a carrier gas flow at $\bar{u}_{opt}$, using a flame photometric detector. The results should be useful for choosing the most suitable column for single or multiple pesticide residue analysis.

Several publications contain relative retention data for pesticides on packed columns (1–3), but many of the organophosphorus compounds analyzed have since been withdrawn from the market while others have appeared. According to Bot and Hollings (4), approximately 50 organophosphorus pesticides are currently registered in South Africa. This paper deals with the determination of the relative retention times of 50 such pesticides on 9 commonly used packed columns.

Although the trend in residue analysis is to use capillary columns, many laboratories throughout the world continue to use packed columns. During 1981, 4 interlaboratory calibration exercises were done in South Africa, with a total of 33 analysts from different laboratories participating (5). None of the analysts used capillary columns. During 1982 and 1983, further pesticide residue interlaboratory calibration exercises indicated that out of 37 participants, only 3 used capillary columns (6). Thus, packed columns are still very much in use, and as Fehringer and Walters (7) pointed out, the majority of retention data obtained on packed columns can be used for the tentative identification of pesticides with capillary columns at isothermal conditions, at least for the coatings tested in their report.

The lack of any description of peak shape can be regarded as a deficiency in all previously published relative retention indices (1, 2). We frequently found in our laboratory that a column, which was apparently suitable for the analysis of a specific pesticide, was in practice totally unsuitable for quantitative work because of severe tailing. In this paper, the peak shape of each compound has, therefore, been classified into 1 of 4 categories on the basis of the peak asymmetry factor defined by Kirkland et al. (8).

In multiresidue analysis, maximum resolution between peaks is desirable. Because optimum carrier gas flow influences column efficiency, which is one of the important factors that affect resolution, we decided to determine retention indices at the mean optimum carrier gas velocity ($\bar{u}_{opt}$), based on parathion. Column lengths were standardized at 200 cm, because most authors seem to prefer using fairly long columns, e.g., Bowman and Beroza (1) 240 cm, Tompson et al. (2) 182.9 cm, and Winterlin and Moseman (9) 180 cm. An oven temperature of 200°C was chosen because most analyses in this laboratory are done at this temperature and because Tompson et al. (2) considered it to be optimum for general use. The detectable quantities of pesticides were determined under these conditions of gas flow and temperature, and these levels are reported here. The relative response factors were not determined for reasons that are discussed later. In addition to relative retention times, the absolute retention times were determined, from which it is possible to decide whether a column will be suitable for a specific pesticide analysis without any further calculations.

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Experimental

Apparatus

Varian Model 6000 gas chromatograph, equipped with flame photometric detector (dual flame) operated in the phosphorus mode (530 nm), was coupled to Varian 401 data system (Varian Instrument Group, Palo Alto, CA 94303). The gas chromatograph was operated at the following flow rates (mL/min): hydrogen, 140; air No. 1, 80; air No. 2, 170; and nitrogen (carrier gas) was set at $\bar{u}_{opt}$ for each column, which was determined with parathion. The following temperatures (°C) were used: injection port 225, column oven 200, and detector 250.

Reagents

Pesticide standards were all analytical grade. Parathion was used as reference because it is well known and had been used before as a reference in relative retention tables (1, 2). Analytical grade *n*-hexane and acetone were used for dilution of pesticide standards which were purity tested on the gas chromatograph before analysis. Butane was used for the determination of the gas hold-up time for the calculation of average linear gas velocities. Dimethyldichlorosilane was used to deactivate columns (Glasstreet, Alltech Associates, Deerfield, IL 60015). Hydrochloric acid (30%) was used to clean empty columns.

Column Preparation

Glass columns of 207 cm × 3 mm id × 6.4 mm od were cleaned, deactivated, and dried before they were packed. The length of an empty column was measured by passing a very thin steel wire through it, measuring the length that went into the column, and cutting off that part of the column over 207 cm. Each column was then filled with 30% HCl solution, left overnight, rinsed with deionized water to remove the acid, and rinsed with acetone to remove the water before the column was deactivated with dimethyldichlorosilane. This reagent was left in each column for ca 1 h. The excess was then removed with methanol, after which each column was dried with nitrogen before it was carefully packed by means of a vacuum applied to one end with a minimum of vibration.

Column packings (Chromtek, Cape Town, South Africa) prepared according to Spark's method (10), and the phases were all coated on 80-100 mesh Chromosorb W-HP (Supelco Inc., Bellefonte, PA 16823). A survey of pesticide residue laboratories in South Africa showed that the packing materials listed in Table 1 were those most frequently used.

After it was packed, the ends of each column were plugged with silanized glasswool, 5 cm on the inlet side and 2 cm on the outlet side. Each packed column was heat-conditioned for 24 h at a maximum of 230°C. Where the temperature limit of the stationary phase was lower than 230°C, the column was conditioned 10°C below the limit. A nitrogen carrier gas

Table 1. Number of theoretical plates/2 m column, determined with parathion-ethyl as indicator

Column	As[a]	Theoretical plates (n)[b]
3% SP-2100	1.50	2908
1% Dexsil-300	1.10[c]	3087
3% OV-17	1.30	4645
1.5% OV-17 + 1.95% QF-1	1.50	5633
4% SE-30 + 6% QF-1	2.00	3407
3% OV-17 + 3% OV-210	1.30	3352
5% DC-200 + 7.5% QF-1	1.10[c]	2700
3% Carbowax-20M	1.30	4000
4% Reoplex-400	1.10[c]	3724

[a]Asymmetry factor.
[b]$n = 5.54(t_R/w_h)^2$ where t_R is retention time and w_h is width at ½ peak height.
[c]Essentially symmetrical peaks.

flow of 60 mL/min was maintained throughout the conditioning period.

Column Evaluation

Each column was evaluated at 200°C under optimal carrier gas flow conditions, which were determined by using parathion as reference. The height equivalent to one theoretical plate (HETP) for each column was determined by the method of Ettre (11), using the equations $n = 5.54(t_R/w_h)^2$ and $h = L/n$ at various average linear gas velocities ($\bar{u}$). From these results, Van Deemter curves (12) were constructed to determine the optimum average gas velocity ($\bar{u}_{opt}$) for each column. The average linear gas velocities were determined by injecting ca 15 μL butane, for which the flame photometric detector gave a very fast, narrow peak. The number of theoretical plates (n) at $\bar{u}_{opt}$ for each column is shown in Table 1.

GC Procedure

Pesticides were injected into the gas chromatograph in *n*-hexane solution, except for a few that were incompletely soluble, in which case, acetone was added to obtain complete solubility. To ensure accuracy of the data, the retention time of parathion (reference) was checked between every 4 or 5 pesticide injections. If any parameter had changed, the retention time of parathion also would have changed. Thus, errors in determining relative retention time, were prevented. Each pesticide was injected twice. Retention times were measured from the injection point.

At the first injection of each pesticide, 60 min was allowed for the peak and/or possible impurities to elute. If a pesticide peak did not appear in the first 60 min, an additional 60 min was allowed for possible late-eluting peaks. Peaks that eluted after 120 ± 10 min were not reported.

Peak Shape

To show the kind of peak shapes that can be expected with the various columns and pesticides, a peak classification system was used. For each peak, an asymmetry factor (8) was calculated, which is a measure of the amount of peak tailing that occurs, and the following 4 classes of peak shapes were distinguished on this basis: 1, asymmetry factor < 1.2; 2, > 1.2 but ≤ 2.4; 3, > 2.4 but ≤ 3.6; and 4, > 3.6. Class 1 is essentially a symmetrical peak. Asymmetry factors for all pesticides are given in Table 2.

Detectable Quantity Levels of Pesticides

As a result of the relatively low average linear gas velocities used, the flame photometric detector did not operate at maximum sensitivity, and the response of the detector differed widely according to the different pesticides. It was therefore decided to report the detectable quantity levels of the different pesticides under the described parameters by using a suitable attenuation which gave 2% baseline noise. Under these conditions, we reported the quantity of each pesticide needed to obtain a peak of at least 75% FSD (full-scale deflection). The 75% FSD was chosen because measurements on smaller peaks would not be sufficiently accurate. Four quantity levels were selected and given the following symbols: A = 1–10 ng; B = 10–100 ng; C = 100–1000 ng; D = 1000–10000 ng.

Results and Discussion

The results presented in Figures 1–9 and in Table 2 are in 2 different formats that are complementary to each other; each format presents data not available in the other.

Kirkland et al. (8) have shown that serious errors in plate counts can result if the equation $n = 5.54(t_R/w_h)^2$ is used on even moderately tailing peaks and that the errors are always positive. They therefore suggested that column performance should be determined on only essential symmetrical peaks with asymmetry values less than 1.2. Because parathion was used to determine the theoretical plate values on all columns, some of the values presented in Table 1 are too optimistic. Symmetrical peaks (less than 1.2) were obtained on the 1% Dexsil-300, 4% Reoplex-400, and 5% DC-200 + 7.5% QF-1 columns, which means that the plate counts on these columns are correct. It was decided, however, to continue using parathion because it would have been impossible to predict beforehand which of the 50 pesticides would be suitable in terms of asymmetry values for the determination of n and $\bar{u}_{opt}$ for all columns. Another consideration in using parathion was that possibly more than one pesticide would be needed to determine n. We wanted to avoid that because it would diminish the possibility of comparing all the columns directly, which was one of the primary objectives of this work. When all the results had been obtained, it became clear that one pesticide alone would not have been suitable for determining n on all columns (see Table 2). Vivilecchia et al. (13) proposed another plate-height equation to account for unrealistically high plate counts with asymmetrical peaks, but if manual calculation is used, the accuracy and precision of this method are highly variable, and more significantly, the correlation of $N_5\sigma$ values for tailing peaks with actual column performance has not been established (14). It was therefore decided not to use this equation, but to report the results as obtained.

The effect that a moderately tailing peak, as compared with a symmetrical peak, would have on the determination of $\bar{u}_{opt}$ is not readily accessible from the literature. It was found, however, that the valley of the Van Deemter curves, as determined with parathion, allowed a certain variation in $\bar{u}_{opt}$ without significantly decreasing the plate count and, thus, the column efficiency. Because the Van Deemter curves tend to allow some variation in $\bar{u}_{opt}$, it is questionable whether a symmetrical peak would give $\bar{u}_{opt}$ values which would differ significantly from those of moderately tailing peaks.

Figures 1–9 show the classification into 4 shapes of the pesticide peaks obtained from different columns. These figures give a convenient visual impression of the separation of the different pesticides on a specific column, the approximate absolute retention time, the suitability of the column for a specific application, and the amount of tailing that can be expected of specific pesticides. Various systems of peak classification were considered before the one presented here was adopted. Winterlin et al. (9) classified peak shape from 1 to 6 according to loosely defined peak descriptions. We found this method rather arbitrary. Dal Nogare and Jen Chui (15) devised

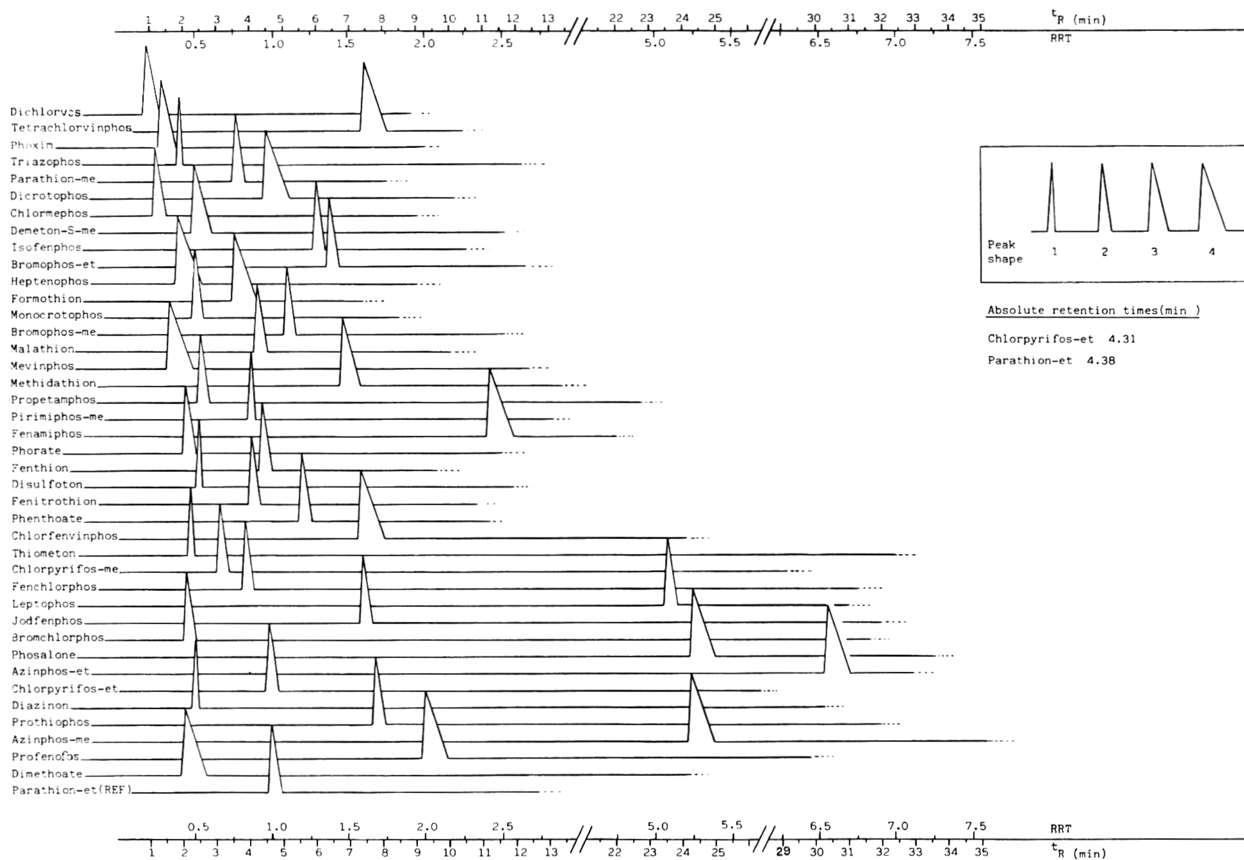

Figure 1. Retention data and peak shape classifications for pesticides analyzed by GC on 3% SP-2100 column at $\bar{u}_{opt}$ of 6.2 cm/s.

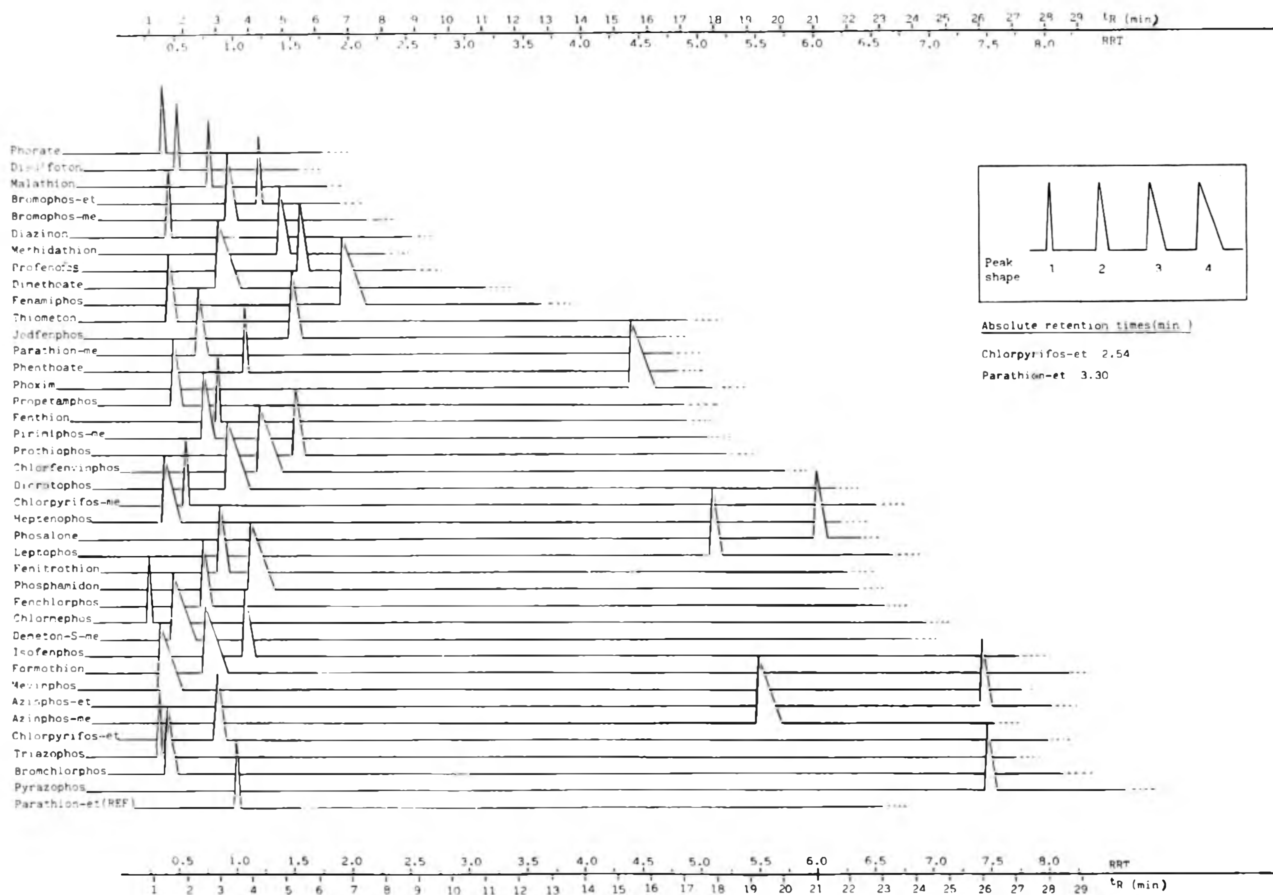

Figure 2. Retention data and peak shape classifications for pesticides analyzed by GC on 1% Dexsil-300 column at $\bar{u}_{opt}$ of 6.30 cm/s.

t_R (min)
RRT

Dichlorvos
Chlormephos
Oxydemeton-me
Monocrotophos
Fenchlorphos
Thiometon
Mevinphos
Heptenophos
Disulfoton
Phosphamidon
Isofenphos
Demeton-S-me
Malathion
Chlorfenvinphos
Parathion-me
Dicrotophos
Triazophos
Fenitrothion
Bromophos-et
Formothion
Diazinon
Phorate
Prothiophos
Tetrachlorvinphos
Pirimiphos-me
Propetamphos
Bromchlorphos
Jodfenphos
Fenthion
Fenamiphos
Chlorpyrifos-me
Dimethoate
Profenofos
Leptophos
Phenthoate
Phosalone
Azinphos-me
Chlorpyrifos-et
Phoxim
Bromophos-me
Azinphos-et
Methidathion
Parathion-et(REF)

Peak shape 1 2 3 4

Absolute retention times (min.)
Chlorpyrifos-et 8.06
Parathion-et 8.43

Figure 3. Retention data and peak shape classifications for pesticides analyzed by GC on 3% OV-17 column and $\bar{u}_{opt}$ of 7.10 cm/s.

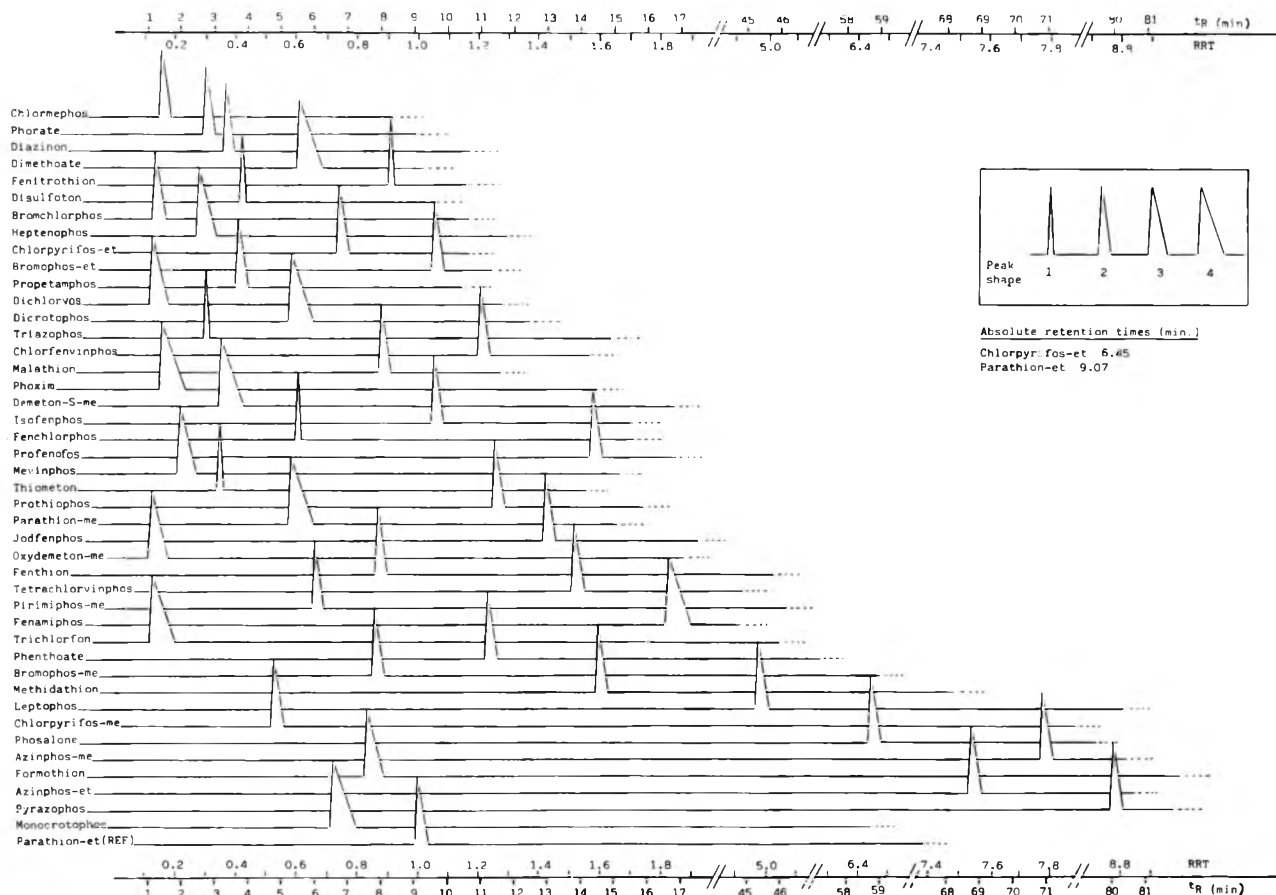

Figure 4. Retention data and peak shape classifications for pesticides analyzed by GC on 1.5% OV-17 + 1.95% QF-1 column at $\bar{u}_{opt}$ of 5.71 cm/s.

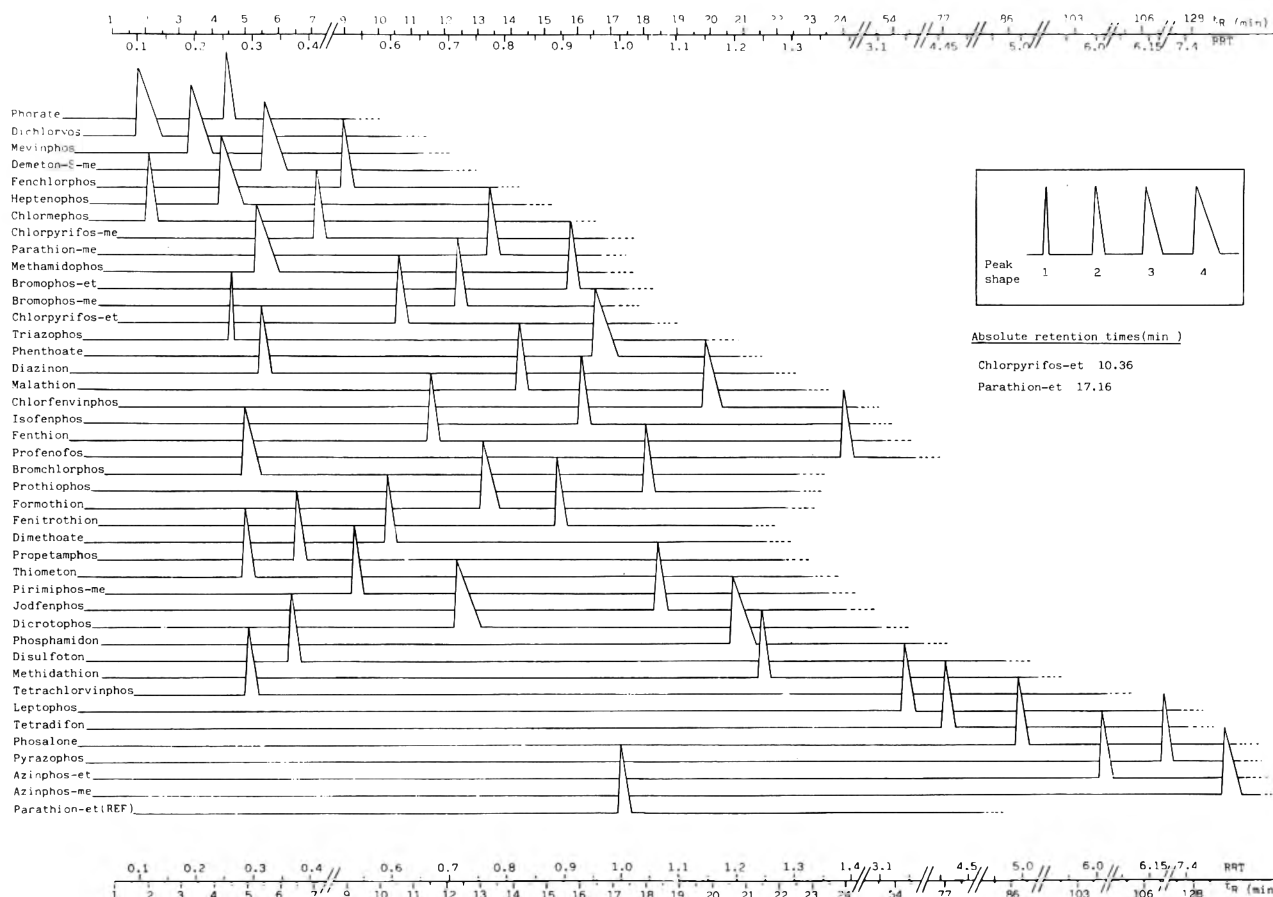

Figure 5. Retention data and peak shape classifications for pesticides analyzed by GC on 4% SE-30 + 6% QF-1 column at $\bar{u}_{opt}$ of 7.71 cm/s.

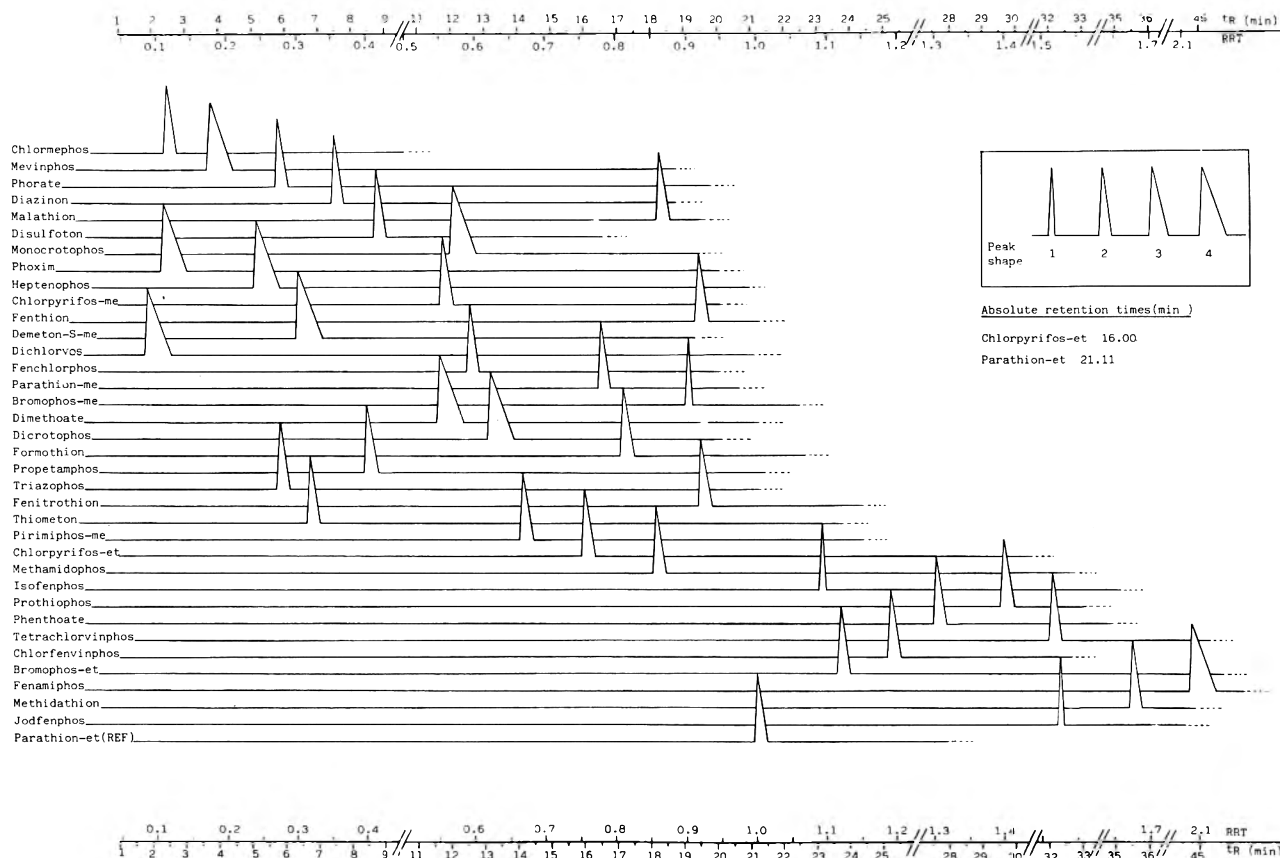

Figure 6. Retention data and peak shape classifications for pesticides analyzed by GC on 3% OV-17 + 3% OV-210 column at $\bar{u}_{opt}$ of 5.67 cm/s.

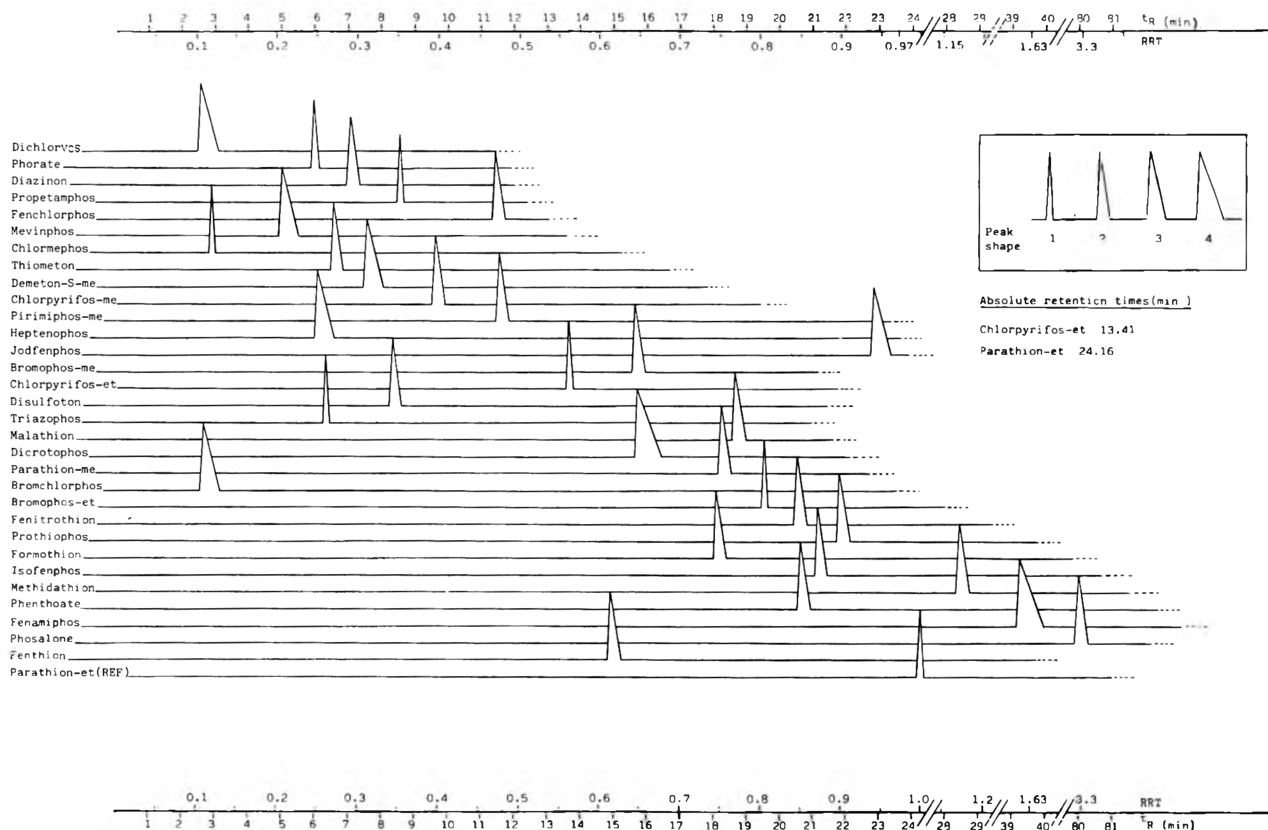

Figure 7. Retention data and peak shape classifications for pesticides analyzed by GC on 5% DC-200 + 7.5% QF-1 column at $\bar{u}_{opt}$ of 4.47 cm/s.

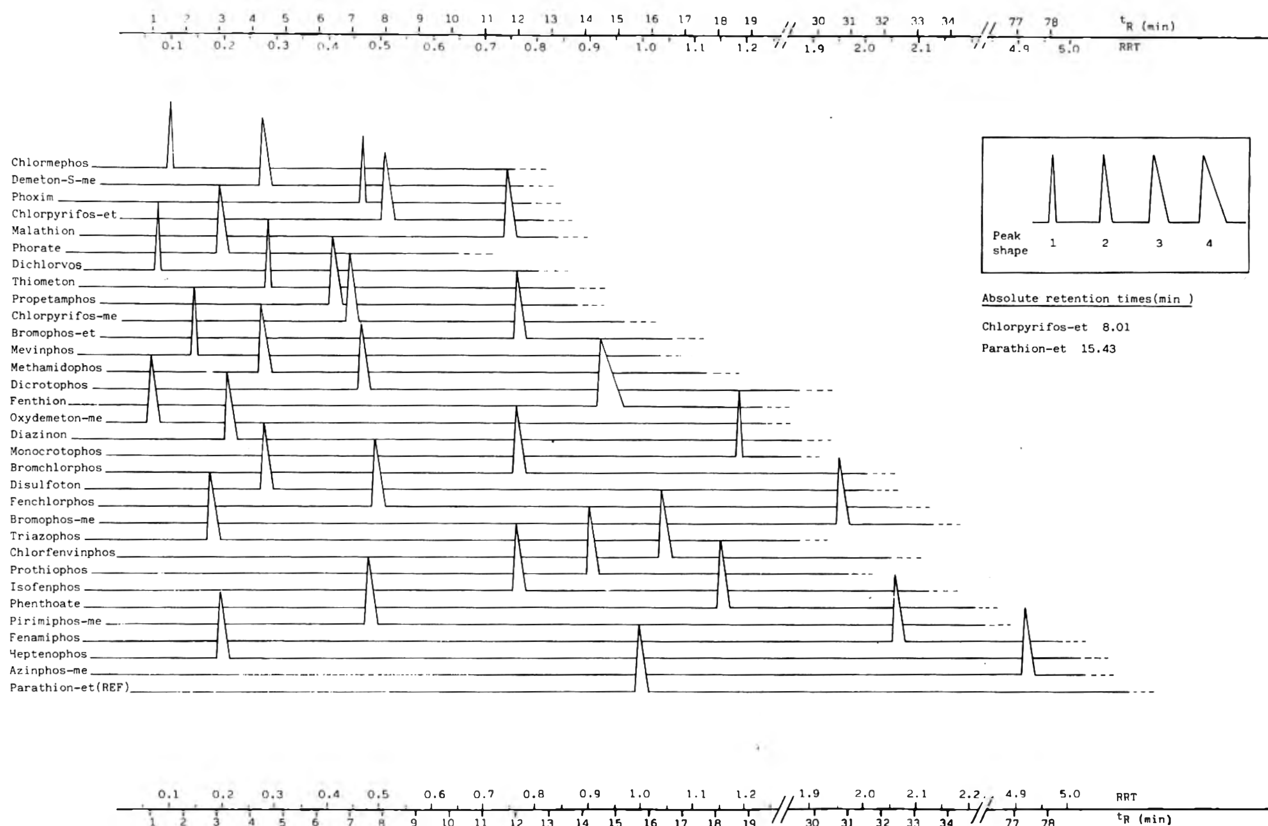

Figure 8. Retention data and peak shape classifications for pesticides analyzed by GC on 3% Carbowax-20M column at $\bar{u}_{opt}$ of 5.80 cm/s.

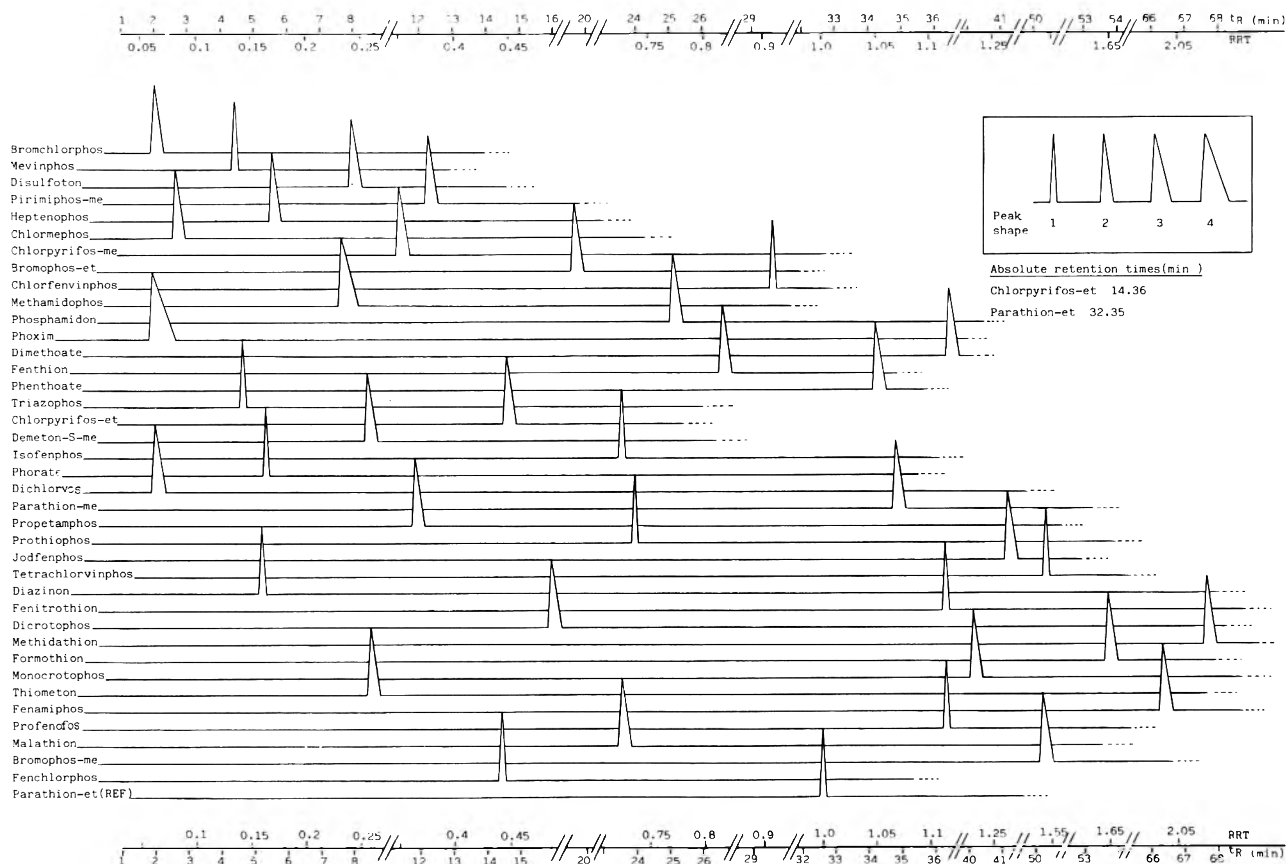

Figure 9. Retention data and peak shape classifications for pesticides analyzed by GC on 4% Reoplex-400 column at $\bar{u}_{opt}$ of 5.40 cm/s.

a mathematical formula for representing peak skew. Their method does not adequately describe the amount of peak tailing, because tangents are drawn to the inflection points, and all calculations are based on measurements made at the baseline of the peak. It was found in a number of examples that different peaks gave the same asymmetry value in spite of the fact that the amounts of tailing differed. It seems that the point where the actual tailing starts is responsible for this anomaly. Kirkland et al. (8) described a much better way of measuring peak asymmetry—horizontally at 10% of total peak height from the baseline. Grushka (16) proposed a method in which peak tailing can be quantitated by calculating a peak skew value. Kirland et al. (8) compared peak asymmetry values and peak skew values and found that the latter can be determined with greater precision and accuracy. Obtaining peak skew values, however, is laborious unless a computer is available. We therefore decided to use peak asymmetry values, because they can easily be calculated and are directly related to peak skew (8).

The $\bar{u}_{opt}$ values, which were used for each column and based on parathion, ensured optimum efficiency, but unfortunately raised the minimum detectability of the flame photometric detector. In every case the carrier gas flow was so low that the detector did not operate at optimum sensitivity. This is reflected in the relatively high quantities of some pesticides needed to obtain measurable peaks (see Table 2). This drawback could be overcome by adding makeup gas between the outlet of the column and the inlet of the detector. The detectable quantity levels presented in Table 2 have a very broad range and are included to illustrate what can be expected by using such low average linear gas velocities. Calculation of relative response levels for the various pesticides was considered, but was rejected because an optimized detector might respond differently to the various pesticides than one that was not optimized.

Recent literature (7) indicates that chlorpyrifos is preferred as a reference compound for relative rentention data; it is detectable by all detectors used in pesticide residue analysis because it contains phosphorus, sulphur, nitrogen, and chlorine atoms. The absolute retention times of both chlorpyrifos and parathion are shown in Figures 1–9 so that the data can easily be converted to those for chlorpyrifos as reference compound.

As Table 2 shows, temephos and omethoate did not elute from any of the columns within 2 h ± 10 min, while other pesticides did not appear to elute from certain columns (shown by dashes); this behavior may have resulted from some pesticides either having very short retention times and eluting with the solvent front, or not eluting within the 2 h limit. Some compounds exhibited multiple peaks, which probably indicate decomposition. In each case the major peak was used to determine relative retention time.

Table 2 and Figures 1 and 2 show that the more apolar column packings, e.g., SP-2100 and Dexsil-300, did not produce a good separation between different pesticides. These columns may be more suited for single-component quantitative analysis. Column packings that were mixtures of stationary phases gave better separations, but in some cases the peak shapes showed much tailing.

The 3% OV-17 column (Figure 3) produced peaks that generally were class 2 and were well-distributed over the time range. Problems with the separation of many pesticides at certain time intervals were evident. All except 7 of the pesticides produced peaks.

The 1.5% OV-17 + 1.95% QF-1 column (Figure 4) produced the most numerous peaks, and only 6 pesticides failed

Table 2. Alphabetical list of all the organophosphorus pesticides tested on nine packed columns

Pesticide	3% SP 2100			1% Dexsil 300			3% OV 17			1.5% OV 17 + 1.95% QF 1			4% SE 30 + 6% QF 1			3% OV 17 + 3% OV 210			5% DC 200 + 7.5% QF 1			3% Carbowax 20M			4% Reoplex 400		
	RRT[a]	As[b]	Dql[c]	RRT	As	Dql	RRT	As	Dql	RRT	As	Dql	RRT	As	Dql	RRT	As	Dql	RRT	As	Dql	RRT	As	Dql	RRT	As	Dql
Acephate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Azinphos-et	6.56	7.7	C	7.43	1.4	C	12.73	2.2	D	9.52	1.3	C	6.01	1.9	D	–	–	–	–	–	–	–	–	–	–	–	–
Azinphos-me	5.23	8.0	C	5.50	6.9	C	10.33	2.2	D	7.78	1.3	C	7.47	2.6	D	–	–	–	–	–	–	4.92	1.5	C	–	–	–
Bromchlorphos	0.45	2.2	B	0.40	1.6	B	0.35	3.2	A	0.13	2.0	B	0.28	2.6	C	–	–	–	0.11	2.5	C	0.76	1.3	B	0.06	1.4	B
Bromophos-et	1.38	1.7	B	1.21	1.2	B	1.34	1.5	A	1.05	1.3	A	0.91	1.4	C	1.12	1.3	C	0.81	1.1	C	0.77	1.3	B	0.55	1.3	A
Bromophos-me	1.10	1.3	B	0.95	0.3	A	1.08	1.3	A	0.86	1.7	A	0.72	1.7	B	0.90	1.2	C	0.64	1.3	C	1.96	2.1	A	1.54	1.4	A
Chlorfenvinphos	1.58	10.5	C	1.20	4.4	B	1.37	1.8	A	1.21	2.0	A	1.15	2.8	B	1.19	2.5	B	–	–	–	1.04	2.0	B	0.91	1.2	B
Chlormephos	0.25	2.2	A	0.25	1.1	A	0.16	2.1	A	0.15	1.4	A	0.12	1.4	B	0.12	1.5	B	0.12	1.1	B	0.10	1.2	A	0.08	1.5	A
Chlorpyrifos-et	0.97	1.7	B	0.83	1.4	B	0.93	1.3	A	0.74	1.5	A	0.61	1.4	A	0.76	1.5	C	0.56	1.1	B	0.51	1.6	A	0.45	1.3	A
Chlorpyrifos-me	0.67	1.8	B	0.57	1.0	A	0.67	1.2	A	0.53	1.5	A	0.41	1.5	A	0.56	1.3	B	0.40	1.5	B	0.44	1.3	A	0.35	1.3	A
Demeton-S-me	0.51	3.2	B	0.46	5.5	B	0.32	1.8	A	0.35	4.2	A	0.32	4.3	C	0.30	4.0	B	0.31	3.1	B	0.28	1.8	B	0.25	1.3	A
Diazinon	0.51	1.1	A	0.43	1.1	B	0.44	1.9	A	0.37	1.5	A	0.31	1.4	B	0.35	1.5	B	0.29	1.3	B	0.21	1.3	A	0.15	1.1	A
Dichlorvos	0.21	3.1	A	–	–	–	0.10	3.8	A	0.12	3.5	A	0.10	4.0	B	0.39	3.9	B	0.11	3.0	B	0.08	1.0	A	0.06	1.5	A
Dicrotophos	0.97	16.3	B	0.92	14.3	B	0.45	7.5	A	0.58	6.3	B	0.71	6.0	D	0.62	5.4	D	0.65	9.0	C	0.46	1.6	B	0.49	1.3	A
Dimethoate	0.44	6.0	C	0.86	15.0	C	0.56	3.0	A	0.61	6.4	A	0.59	2.3	C	0.55	7.4	B	–	–	–	–	–	–	1.12	1.4	B
Disulfoton	0.53	1.1	A	0.52	1.0	A	0.50	1.3	A	0.42	1.2	A	0.37	1.8	B	0.41	1.4	B	0.34	1.5	B	0.28	1.4	A	0.22	1.3	A
Fenamiphos	2.42	33.2	B	1.93	17.1	C	2.04	2.0	B	1.83	5.1	B	–	–	–	2.12	4.9	C	1.62	9.5	D	2.06	1.5	C	1.98	1.6	B
Fenchlorphos	0.83	1.3	A	0.70	1.5	A	0.73	1.4	A	0.60	1.0	A	0.52	1.4	B	0.59	1.3	B	0.47	1.5	B	0.49	1.3	A	0.44	1.2	A
Fenitrothion	0.87	1.4	B	0.86	1.5	B	0.94	1.4	A	0.91	1.1	A	0.89	1.5	C	0.92	1.7	C	0.85	1.4	B	–	–	–	1.12	1.2	B
Fenthion	0.93	1.7	B	0.84	1.2	A	1.10	1.3	A	0.87	1.4	A	0.67	1.7	C	0.92	1.4	C	0.62	1.3	C	0.92	3.8	B	0.81	1.3	B
Formothion	0.76	6.5	C	0.73	4.6	B	0.80	2.2	A	0.83	2.5	B	0.76	2.8	C	0.81	2.3	C	0.75	1.5	D	–	–	–	1.64	1.4	B
Heptenophos	0.40	7.0	B	0.38	2.9	C	0.26	2.2	A	0.28	3.3	A	0.25	4.0	B	0.24	4.6	B	2.51	2.6	C	0.20	1.5	B	0.16	1.3	A
Isofenphos	1.30	1.5	B	1.05	1.5	B	1.25	1.3	A	1.05	1.3	A	0.93	1.5	B	1.09	1.2	C	0.87	1.7	C	0.77	1.3	B	0.72	1.2	A
Jodfenphos	1.58	1.7	B	1.46	1.3	A	1.88	1.4	A	1.42	1.3	B	1.06	1.4	C	1.53	1.0	C	0.92	3.4	C	–	–	–	1.26	1.3	B
Leptophos	5.08	1.9	C	5.03	1.5	C	7.11	1.1	B	4.98	1.5	B	3.15	1.3	D	5.65	1.6	D	–	–	–	–	–	–	–	–	–
Malathion	0.91	2.3	B	0.79	1.2	B	1.00	1.1	A	0.88	1.4	B	0.82	1.8	C	0.86	1.5	A	0.77	1.3	C	0.74	1.3	B	0.72	1.3	B
Methamidophos	–	–	–	–	–	–	–	–	–	–	–	–	0.31	8.1	C	0.86	1.6	C	–	–	–	0.27	2.2	B	0.23	2.7	B
Methidathion	1.46	3.2	B	1.39	2.0	B	1.89	1.5	A	1.60	1.7	A	1.25	2.3	D	1.68	1.8	D	1.17	2.0	D	–	–	–	2.02	1.3	B
Mevinphos	0.34	14.0	A	0.33	9.5	A	0.17	5.6	A	0.22	3.0	A	0.19	5.7	B	0.18	6.1	B	0.21	3.2	B	0.15	1.2	A	0.14	1.1	A
Monocrotophos	0.51	1.7	B	–	–	–	0.51	6.3	A	0.72	15.0	C	–	–	–	0.57	11.6	B	–	–	–	1.19	1.2	C	1.23	1.5	B
Omethoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Oxydemeton-me	–	–	–	–	–	–	0.33	3.3	B	0.12	2.5	B	–	–	–	–	–	–	–	–	–	0.06	1.4	B	–	–	–
PARATHION-ET (ref)	1.00	1.5	B	1.00	1.1	B	1.00	1.3	A	1.00	1.5	A	1.00	2.0	C	1.00	1.3	C	1.00	1.1	C	1.00	1.3	B	1.00	1.1	B
Parathion-me	0.77	1.9	A	0.69	1.4	A	0.79	1.4	A	0.58	7.0	B	0.77	1.9	C	0.78	1.7	B	0.76	1.3	B	–	–	–	1.07	1.5	B
Phenthoate	1.20	2.0	B	1.09	1.0	B	1.55	1.3	A	1.23	1.4	B	0.96	8.0	B	1.30	1.4	C	0.85	1.8	C	1.16	1.3	C	1.05	1.3	B
Phorate	0.44	2.0	A	0.39	1.1	B	0.34	1.5	A	0.30	1.5	A	0.25	1.8	B	0.27	1.3	B	0.25	1.2	B	0.19	1.3	A	0.15	1.1	A
Phosalone	5.24	4.4	C	6.01	1.4	B	7.77	1.1	A	6.45	1.5	B	5.00	1.3	C	–	–	–	3.30	2.0	D	–	–	–	–	–	–
Phosphamidon	–	–	–	1.11	19.5	C	0.79	4.8	B	–	–	–	1.19	4.3	C	–	–	–	–	–	–	–	–	–	0.77	1.3	B
Phoxim	0.28	3.0	C	4.41	12.5	C	1.95	1.6	B	0.16	5.6	C	–	–	–	0.11	6.7	C	–	–	–	0.47	1.1	B	0.06	7.7	B
Pirimiphos-me	0.87	1.2	B	0.72	1.9	A	0.83	1.5	A	0.66	1.5	A	0.53	1.4	B	0.67	1.2	B	0.48	1.4	B	0.48	1.6	B	0.38	1.3	A
Profenofos	2.00	6.5	C	1.57	1.9	C	1.90	1.1	B	1.58	1.8	B	1.39	2.1	C	–	–	–	–	–	–	–	–	–	1.09	1.1	B
Propetamphos	0.54	1.4	A	0.42	2.2	A	0.44	1.4	A	0.41	1.6	A	0.38	1.9	B	0.40	1.6	B	0.35	1.2	A	0.41	1.6	A	0.36	1.3	A
Prothiophos	1.68	1.4	B	1.51	1.3	B	1.63	1.1	B	1.26	1.3	A	1.04	1.3	B	1.40	1.3	B	0.90	1.3	B	0.90	1.5	B	0.74	1.2	A
Pyrazophos	–	–	–	7.47	1.5	C	–	–	–	8.78	1.5	B	6.18	2.0	C	–	–	–	–	–	–	–	–	–	–	–	–
Temephos	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tetrachlorvinphos	1.60	3.9	C	–	–	–	1.74	1.4	A	1.52	2.0	C	0.29	1.4	C	1.52	1.8	C	–	–	–	–	–	–	1.55	1.1	A
Tetradifon	–	–	–	–	–	–	–	–	–	–	–	–	4.46	1.7	D	–	–	–	–	–	–	–	–	–	–	–	–
Thiometon	0.48	1.2	A	0.43	1.4	A	0.40	1.4	A	0.35	1.2	A	0.29	1.6	B	0.32	1.6	B	0.27	1.3	B	0.29	1.2	A	0.26	1.3	A
Triazophos	0.41	1.2	C	0.36	1.0	C	0.33	1.9	B	0.30	1.2	B	0.26	1.1	D	0.27	1.5	D	0.26	1.0	C	0.18	1.6	B	0.14	1.0	B
Trichlorfon	–	–	–	–	–	–	–	–	–	0.13	13.3	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Relative retention time [b] Asymmetry factor [c] Detectable quantity level (see text)

to elute in the specified time. In comparison with the 3% OV-17 column, the separation was not much improved, but the peaks eluted much faster.

Eight pesticides failed to produce peaks from the 4% SE-30 + 6% QF-1 column (Figure 5). Most of the peaks were class 2, and few were class 1. Most of the peaks were well distributed over the time range, but separation problems may be encountered with the relatively early-eluting peaks.

The 3% OV-17 + 3% OV-210 column (Figure 6) produced 37 peaks, which were mostly well-distributed in the relative short time range of about 45 min, but separation problems were encountered at certain time intervals.

The smallest number of pesticides eluted on the 5%DC-200 + 7.5% QF-1 column (Figure 7). The peaks were not very well-distributed, and there were many separation problems.

Eighteen pesticides failed to produce peaks from the 3% Carbowax-20M column (Figure 8), although the pesticides that did produce peaks were well-distributed over the time range. Separation problems were evident at certain time intervals.

The 4% Reoplex-400 column (Figure 9) performed extremely well, and the peaks were well-distributed over the whole time range. Eleven pesticides failed to produce any peaks, but 36 peaks were classes 1 and 2, which is the highest number for any of the columns tested. The 4% SE-30 + 6% QF-1 has always been recognized in this laboratory as an ideal column for multiresidue analysis of organophosphorus pesticides. The results, however, indicate that the Reoplex column is also well-suited for this application, because it produces peaks of better quality in about half the time of those produced by the SE-30 + QF-1 column (see Figures 5 and 9).

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Purge and Trap Method for Determination of Ethylene Dibromide in Whole Grains, Milled Grain Products, Intermediate Grain-Based Foods, and Animal Feeds

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An improved method has been developed for the determination of ethylene dibromide (EDB; 1,2-dibromoethane) in whole grains, milled grain products, intermediate grain-based foods, and animal feeds. Samples are mixed with water and sparged with nitrogen for 1 h with stirring in a water bath at 100°C. The EDB collected on the adsorbent Tenax TA is eluted with hexane and determined by gas chromatography (GC) with electron capture detection (ECD) and confirmed with Hall electrolytic conductivity detection (HECD) using a second GC column. The highest levels of EDB were also confirmed by full scan GC/mass spectrometry (GC/MS). A total of 24 whole grains, milled grain products, intermediate grain-based foods, and animal feeds analyzed by using this method contained EDB levels up to 840 ppb (wheat). Recoveries from fortified samples ranged from 90 to 105%. Values from this method were compared with those obtained from the acetone soak method; for all 24 samples, this purge and trap method gave equivalent or superior recoveries and detected levels of EDB. Chromatograms for this purge and trap method were clean, enabling a quantitation level of 0.5 ppb to be achieved.

Ethylene dibromide (EDB; 1,2-dibromoethane) has been used as a fumigant in both soil and stored food commodities (1). However, recent studies have determined that EDB causes cancer, birth defects, and genetic and reproductive disorders in test animals.

In response to, and in conjunction with, the suspension of registration of fumigation products containing EDB (2) and the establishment of new tolerances for residues of EDB in grain products and citrus fruits (3) by the U.S. Environmental Protection Agency (EPA), the U.S. Food and Drug Administration (FDA) began a limited survey of EDB in grains and grain-based products.

Although several methods for the determination of EDB are available (4–12), the acetone soak procedure developed by Heuser and Scudamore (7) and Clower (10) was considered appropriate for grains and grain-based products. The acetone soak procedure, however, has several shortcomings: (a) it is time consuming, in that 48 h are required for the extraction step alone; (b) the chromatograms often contain interfering coextractive peaks; and (c) the limit of quantitation is not sufficient for ultra-low levels (<1 ppb) of EDB.

In this study, a purge and trap procedure developed for the analysis of table-ready foods (13) has been applied to 24 samples of whole grains, milled grain products, intermediate grain-based foods, and animal feeds. Results are compared with those obtained from the acetone soak procedure (10).

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Table 1. Comparative levels (ppb) of EDB in whole grains and milled grain products determined by acetone soak and purge and trap methods

Item	Acetone soak	Purge and trap[a]
Wheat	36	40, 43
Wheat	84	120, 130
Wheat	840	870, 870
Wheat mids	—[b]	7.0, 7.9
Corn, yellow	90	93, 95
Corn, yellow	120	120, 120
Oats	—[b]	1.3, 1.7
Oats	17	17, 19
Soybeans	—[b]	—[c]
Distiller's dry grain	—[d]	1.8, 2.7

[a]Duplicate determinations.
[b]Less than quantitation limit of 15 ppb.
[c]Less than quantitation limit of 0.5 ppb.
[d]Interfering peaks in chromatograms, calculation not possible.

Table 2. Comparative levels (ppb) of EDB in intermediate grain-based foods determined by acetone soak and purge and trap methods

Item	Acetone soak	Purge and trap[a]
Corn muffin mix	—[b]	—[c]
Corn meal	—[b]	0.62, 0.71
Pancake mix	—[b]	0.76, 0.81
Flour	—[b]	—[c]
Flour	—[b]	0.86, 0.93
Brownie mix, fudge	—[b]	3.6, 4.1
Egg noodles (raw)	—[b]	0.93, 1.2
Spaghetti (raw)	—[b]	1.4, 1.6
Cake mix, yellow	33	40, 41

[a]Duplicate determinations.
[b]Less than quantitation limit of 15 ppb.
[c]Less than quantitation limit of 0.5 ppb.

METHOD

Principle

Samples in water are extracted (sparged) with a stream of nitrogen while being stirred vigorously in a water bath at 100°C. EDB collected on the adsorbent Tenax TA and eluted with hexane is determined by gas chromatography/electron capture detector (GC/ECD) with GC/Hall electrolytic conductivity detector (HECD) and/or GC/mass spectrometry (MS) confirmation.

Apparatus and Reagents

Gas-liquid chromatograph.—Equipped with 180 × 0.2 cm id glass column packed with 1% SP-1000 on Carbopak B (60–80 mesh) (Supelco, Inc.) and constant current ^{63}Ni ECD (Hewlett-Packard 5880, or equivalent). Conditions: injector, 190°C; column, 170°C; detector, 325°C; carrier gas, 5% methane in argon; flow rate, 40 mL/min. Attenuate to obtain ca ½ full-scale deflection for 100 pg EDB with 1 mV recorder. Second instrument equipped with 180 × 0.2 cm id glass column packed with 10% SP-1000 on Supelcoport (80–100 mesh) (Supelco, Inc.) and HECD (Tracor 560, or equivalent). Conditions: injector, 225°C; column, 100°C; detector, 250°C; carrier gas, hydrogen; flow rate, 40 mL/min. Attenuate to obtain ca ½ full-scale deflection for 100 pg EDB with 1 mV recorder.

Additional apparatus and reagents and preparation of Tenax TA and EDB standards are described in ref. 13.

Determination

Accurately weigh ca 10 g homogeneous, frozen or partially thawed sample into 500 mL double-neck, round-bottom flask containing 250 mL deionized water, 1 mL antifoam B, and stirring bar. Using universal adapter, place gas dispersion tube into angled side neck of flask to depth of ca 5 mm above stirring bar. Complete assembly of purge and trap apparatus by addition of Liebig condenser with second universal adapter and trap tube at top, as illustrated in Figure 2, ref. 13.

Place assembled apparatus in a boiling water bath positioned above hot plate with magnetic stirrer. Circulate tap water through Liebig condenser. Adjust nitrogen flow to 100

Table 3. Comparative levels (ppb) of EDB in animal feeds determined by acetone soak and purge and trap methods

Item	Acetone soak	Purge and trap[a]
Poultry ration	97	95, 100
Poultry ration	120	140, 140
Swine ration	—[b]	1.3, 1.6
Rabbit ration	210	230, 230
Cattle ration	—[c]	63, 68

[a]Duplicate determinations.
[b]Less than quantitation level of 15 ppb.
[c]Interfering peaks in chromatogram, calculation not possible.

Table 4. Recoveries from whole grains, milled grain products, intermediate grain-based foods, and animal feeds fortified with EDB determined by purge and trap method

Item	Fortification level, ppb[a]	Rec., %
Wheat	46	98
Wheat	92	97
Corn, yellow	92	100
Distiller's dry grain	4.6	94
Corn muffin mix	2.3	103
Flour	2.3	90
Spaghetti, raw	4.6	96
Swine ration	4.6	105
Cattle ration	92	96

[a]Spiking standard in deionized water.

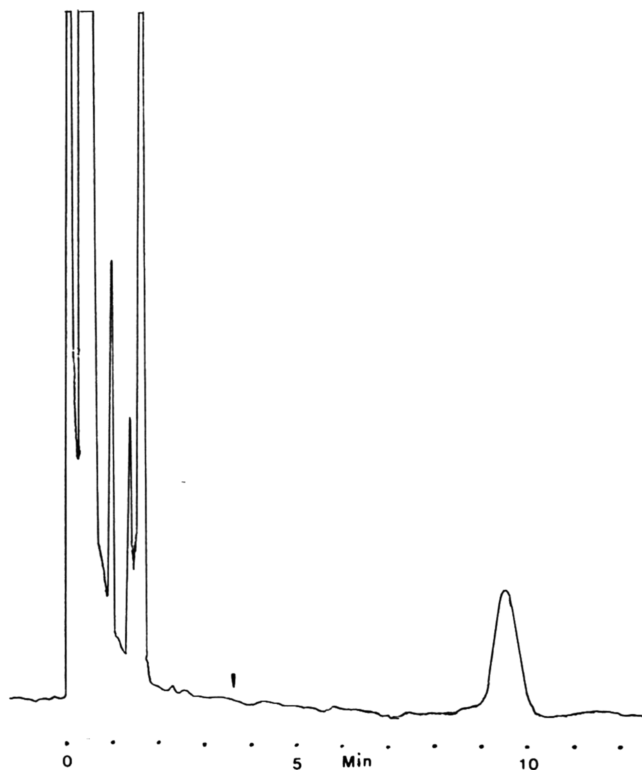
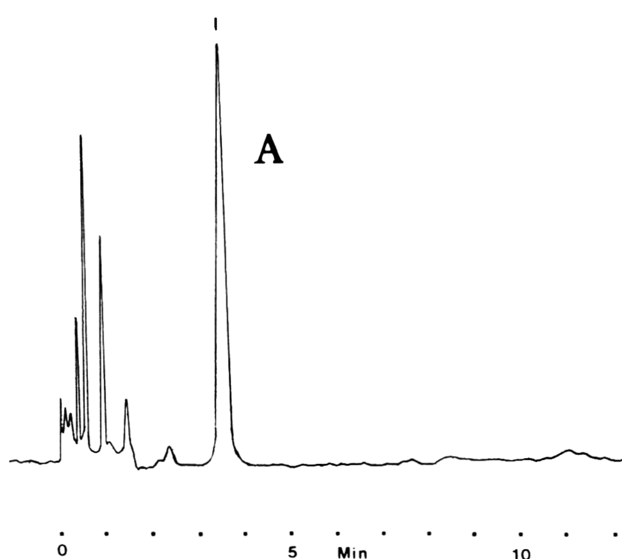


Figure 1. Chromatogram of procedural blank from acetone soak method with electron capture detection (GC/ECD); 1% SP-1000 on Carbopak B (60–80 mesh), 180 × 0.2 cm id, 170°C. Tick indicates position of EDB peak.



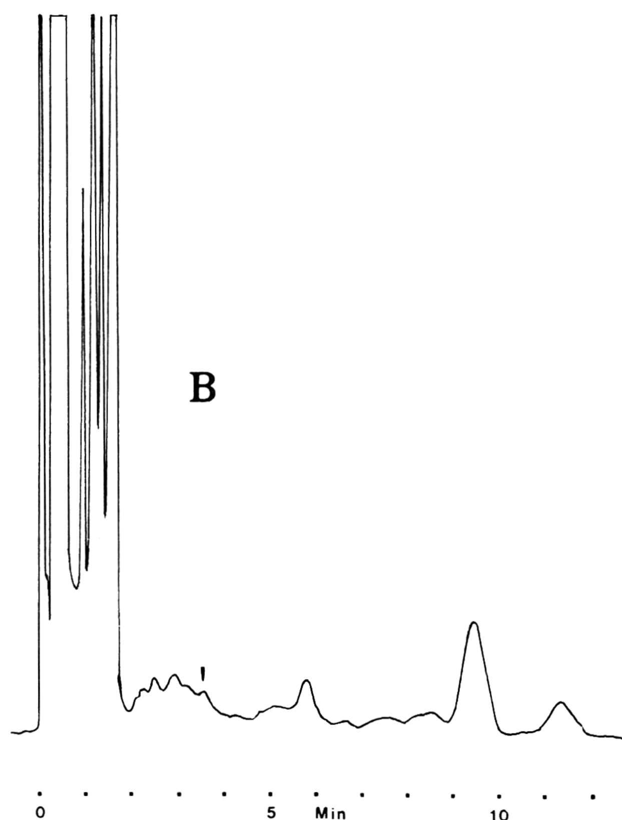


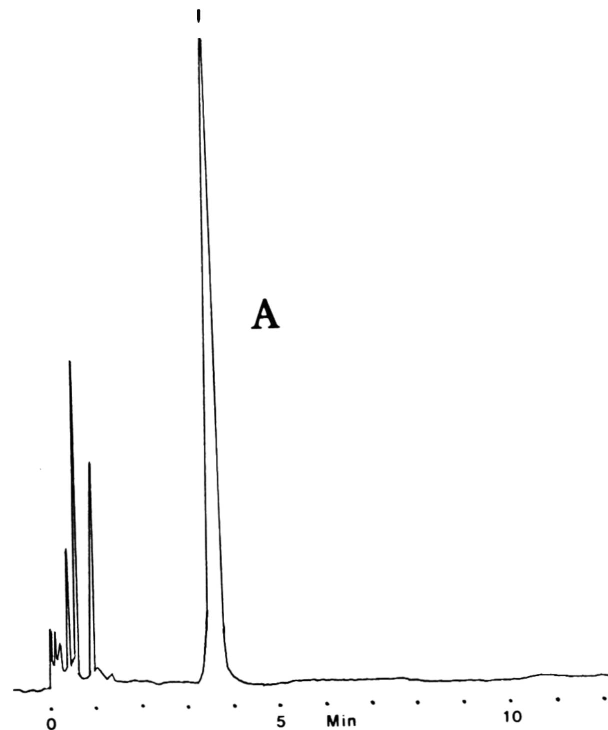
Figure 2. Chromatogram of fudge brownie mix (3.6 ppb EDB) with electron capture detection (GC/ECD); 1% SP-1000 on Carbopak B (60–80 mesh), 180 × 0.2 cm id, 170°C: A, from purge and trap method (equivalent to 28 mg); B, from acetone soak method (equivalent to 1.0 mg). Ticks indicate position of EDB peak.

mL/min through gas dispersion tube and stir vigorously (maximum speed, ca 1200 rpm) to obtain strong vortex.

After 1 h, remove trap tube from universal adapter and elute with at least 1 mL hexane into suitable volumetric flask. Dilute to volume with hexane and quantitate EDB, using GC/ECD. Confirm presence of EDB by using second GC column with HECD and/or GC/MS.

Results and Discussion

The development of specific procedural parameters has been discussed elsewhere (13).



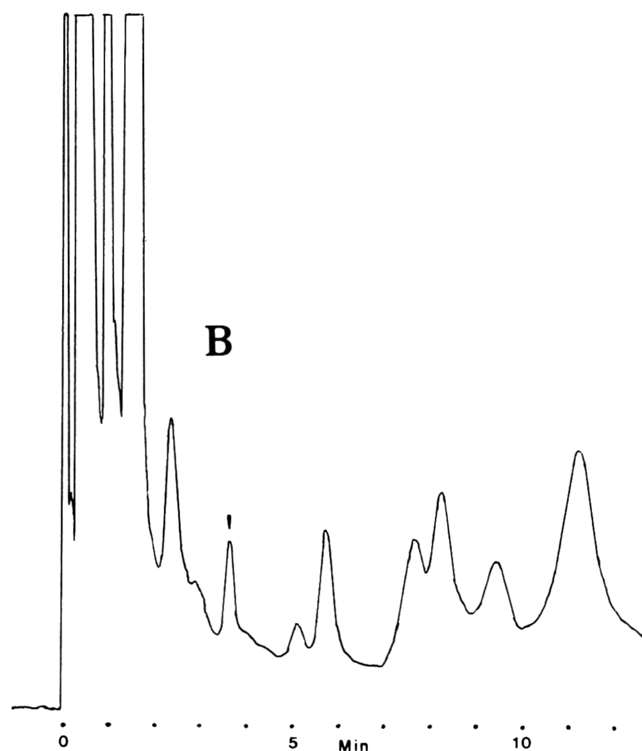


Figure 3. Chromatogram of oats (17 ppb EDB) with electron capture detection (GC/ECD); 1% SP-1000 on Carbopak B (60–80 mesh), 180 × 0.2 cm id, 170°C: A, from purge and trap method (equivalent to 10 mg); B, from acetone soak method (equivalent to 1.5 mg). Ticks indicate position of EDB peak.

A total of 24 samples of grains and grain-based products were analyzed by the acetone soak procedure and the purge and trap method described above. With the chromatographic (GC/ECD) conditions described under *Apparatus and Reagents*, the acetone soak procedure has a quantitation limit of ca 15 ppb. Under the same conditions, the quantitation limit in the purge and trap method was 0.5 ppb.

Table 1 lists comparative levels of EDB found in several grains and milled grain products by using these 2 methods. Comparative results of analysis of intermediate grain-based foods and animal feeds are given in Tables 2 and 3, respectively.

Nine of these samples were also examined by the purge and trap method after fortification with an aqueous EDB spiking solution. After 250 mL water, antifoam, and 10 g sample were added, the aqueous spiking solution was pipetted into the mixture. The apparatus was immediately assembled and placed in the boiling water bath. Table 4 lists spiking levels and recoveries, which ranged from 90 to 105%.

Aqueous spiking solutions should be analyzed daily. When equal volumes of spiking standard and hexane are shaken vigorously 1 min, 98% of EDB is extracted into the hexane layer. The hexane extract is compared by GC to an EDB standard solution prepared in hexane.

Some difficulty was encountered in stirring yellow corn samples; on several occasions, it became necessary to restart the stirring bar. Later, these samples were stirred successfully at a somewhat slower rate (ca 900 rpm), and reproducibility and recovery were satisfactory with this modification.

Acetone produced a very large interfering solvent peak with the GC column of *n*-octane-Porasil C used in the original purge and trap study (13). A 1% SP-1000 column was successfully substituted. A procedural blank from the acetone soak method and the SP-1000 column is shown in Figure 1.

The chromatograms generated by the acetone soak procedure were much "dirtier" than those obtained by the purge and trap method; in fact, 2 samples (distiller's dry grain and cattle ration) contained interfering peaks that prevented quantitation. Figures 2 and 3 are representative chromatograms of fudge brownie mix and oats, respectively, from both methods using GC/ECD, with a 1% SP-1000 on Carbopak B column.

An exhaustive sparging test was performed on all 24 samples. After the samples were sparged for 1 h (as in the method), they were stirred at 100°C overnight without nitrogen flow but with a fresh Tenax TA trap in place. A second hour of sparging the following day produced less than 2% of the level of EDB extracted by the first hour of sparging.

In conclusion, this purge and trap method is suitable for the rapid analysis of EDB in a variety of grains and grain-based products, giving both good reproducibility and good recovery from fortified samples. Results can be obtained in less than 2 h. The procedure is not labor-intensive, in that extractions proceed unattended. Elution of the adsorbent Tenax TA results in a relatively clean, concentrated extract suitable for both low level GC quantitation (0.5 ppb) or GC/MS confirmational analysis. By contrast, the acetone soak procedure requires 3 days to produce a less concentrated extract that often contains interfering coextracted GC peaks.

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Headspace Gas Chromatographic Method for Determination of Methyl Bromide in Food Ingredients

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A headspace gas chromatographic (GC) method, which can be automated, has been developed for determination of methyl bromide. This method has been applied to wheat, flour, cocoa, and peanuts. Samples to be analyzed are placed in headspace sample vials, water is added, and the vials are sealed with Teflon-lined septa. After an appropriate equilibration time at 32°C, the samples are analyzed within 10 h. A sample of the headspace is withdrawn and analyzed on a gas chromatograph equipped with an electron capture detector (ECD). Methyl bromide levels were quantitated by comparison of peak area with a standard. The standard was generated by adding a known amount of methyl bromide to a portion of the matrix being analyzed and which was known to be methyl bromide free. The detection limit of the method was 0.4 ppb. The coefficient of variation (CV) was 6.5% for wheat, 8.3% for flour, 3.3% for cocoa, and 11.6% for peanuts.

As a result of regulatory actions that have removed, or are in the process of removing, liquid fumigants from the ranks of approved pesticides, the use of alternative compounds is expected to increase. Methyl bromide is one such alternative. To assure a food supply that is free of residues of methyl bromide above any established tolerances, methodology is needed which possesses the requisite sensitivity, selectivity, speed, and cost effectiveness for routine application to a variety of matrices.

Currently published methods for the analysis of fumigant residues in commodities typically involve a 24–48 h acetone–water (5 + 1) extraction of the commodity followed by direct injection of the dried acetone extract onto a gas chromatograph (GC) for quantitation (1, 2). Other methods involve extracting methyl bromide by refluxing with acid, followed by cold trapping and direct GC injection (3); extracting methyl bromide with acetone–water followed by conversion of the extracted methyl bromide to methyl iodide with sodium iodide (4); or by trapping methyl bromide from air using Tenax GC resin followed by thermal desorption into a gas chromatograph (5). Each of these methods involved extensive sample preparation time, and resulted in methods of limited sensitivity. King et al. (6) reported analyzing for methyl bromide in fruits by headspace gas chromatography. This method avoided the necessarily long soak period and the need for drying the extracts obtained by the acetone/water soak method. It also resulted in increased sensitivity in the detection of methyl bromide. In anticipation of using the method for large numbers of samples with results desired in minimal elapsed time, we pursued the headspace method of analysis. This approach would handle a large batch of samples, would be capable of automation, would ensure rapid turnaround, and would require minimum analyst time per sample.

METHOD

Apparatus

(a) *Gas chromatograph.*—Model 3700 set up for use with capillary columns, equipped with electron capture detector (ECD) (Varian Inc., Palo Alto, CA 94303). Operating conditions: injector 200°C; detector 270°C; column oven 40°C; carrier gas nitrogen at 1 mL/min (13 psi); injector split ratio 20:1; detector makeup flow 15 mL/min.

(b) *Column.*—Durabond DB1701; 30 m thick film fused silica capillary (Part No. 08573, J and W Scientific Inc., Rancho Cordova, CA 95670).

(c) *Recorder/integrator.*—Compatible with GC system.

(d) *Automated headspace sampler (optional).*—With adjustable temperature bath (Model No. HS 250, Erba Instruments, Peabody, MA 01960). Operating conditions: syringe 55°C; bath 32°C; analysis time 8 min; syringe clean strokes 7; sample flush strokes 4; sample injection size 250 μL.

(e) *Headspace sample vials.*—7 mL (Part No. 66041, Alltech, Deerfield, IL 60015) or 5 mL (No. 24006200, Erba) equipped with Teflon-coated silicone septa (No. 95313, Alltech) and crimp caps (No. 6638, Alltech).

Reagents

(a) *Methyl bromide-free blank samples.*—*(1) Wheat.*—Stir 100 g wheat with 500 mL distilled water. Drain. Rinse with two 100 mL portions of distilled water. Dry in 50°C oven for 3 h or until dry. *(2) Flour.*—Spread sample to depth of no greater than ¼ in. on bottom of large pan or cookie sheet. Heat in 50°C oven overnight. *(3) Cocoa.*—Use same procedure as for flour. *(4) Peanuts.*—Use same procedure as for wheat.

(b) *Distilled water.*—Pretest to assure no peak interferences with methyl bromide peak by placing 2 mL water into a headspace vial; cap and seal. Proceed to step 4 of procedure section.

(c) *Standard.*—Methyl bromide standard solution (0.2 mg/mL in methanol) (Cat. No. 4-8624, Supelco Inc., Bellefonte, PA 16823).

(d) *Standard stock solutions.*—(Prepare fresh daily and keep tightly sealed to prevent rapid dissipation of methyl bromide from solution at room temperature.) *(1)* 400 ppb equivalent MeBr stock: Dilute 100 μL standard (c) to 100 mL with water. *(2)* 200 ppb equivalent MeBr stock: Dilute 25 mL of 400 ppb solution to 50 mL with water. *(3)* 100 ppb equivalent MeBr stock: Dilute 25 mL of 400 ppb solution to 100 mL with water. *(4)* 50 ppb equivalent MeBr stock: Dilute 25 mL of 200 ppb solution to 100 mL with water.

(e) *Working standard solutions.*—Standard A (equivalent to 400 ppb MeBr for 1 g sample): Weigh 1.0 g MeBr-free sample of type to be analyzed (i.e., wheat when wheat is to be analyzed) into headspace sample vial. Pipet 2 mL of 400 ppb stock solution into vial. Immediately seal vial.

Standard B (200 ppb equivalent): Repeat procedure for standard A using 200 ppb stock solution.

Similarly prepare standard C (100 ppb equivalent) and standard D (50 ppb equivalent) using 100 ppb and 50 ppb stock solutions, respectively.

Proceed to step 4 of the procedure with each standard.

Sample Preparation

Store samples in tightly sealed containers until ready for analysis. Store sealed sample in freezer if analysis will not be initiated within 24 h.

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Table 1. Detector area response for aqueous methyl bromide solutions containing various matrices

MeBr, ppb	Water	Wheat & water	Flour & water	Cocoa & water	Peanuts & water
400	1846960	1896673	1579292	1746574	1497916
200	1256121	923594	1064185	957327	745551
100	524709	441457	447056	496336	308774
50	343878	324632	278904	312424	220553

Preparation of Fumigated Samples

If laboratory generation of fumigated sample is necessary, the following procedure may be followed:

Place 100 g clean matrix in an 8 oz glass jar. Cover jar mouth with 1 layer of parafilm. Inject 2 mL MeBr gas through parafilm into jar, using a gas-tight syringe. Cover mouth of jar with second layer of parafilm. Attach screw-cap lid to jar and wrap neck of jar with sealing tape. Let fumigated sample equilibrate at room temperature overnight. After overnight exposure, sample may be exposed to well ventilated air or treated in some other desired fashion.

Procedure

Pipet 2 mL of pretested distilled water into a headspace sample vial. Add 1 g sample to be analyzed to vial. Immediately cap with Teflon-lined septum (Teflon side toward sample) and crimp aluminum cap on tightly with crimping tool. Place vials containing standards and samples into water bath or autosampler bath and let equilibrate for appropriate time listed in Table 2 (i.e., 1 h for wheat, 2 h for cocoa and flour, and 6 h for peanuts).

Inject headspace from standards and samples onto GC column. MeBr will elute in approximately 2.4 min. Be sure all samples and standards are injected before the maximum equilibration time has elapsed (i.e., 10 h for wheat and peanuts, and 12 h for cocoa and flour). Assure against carryover between standards and samples by flushing the syringe with air an appropriate number of times.

Calculation

Plot peak area vs concentration of equilibrated working standard for matrix of interest. Plotting peak area for each matrix is significant because generally the area responses for MeBr are somewhat lower with matrix present than for methyl bromide in water only, as shown in Table 1.

Using MeBr peak area obtained for sample, read MeBr concentration directly from calibration curve plotted in step 1. Note: This calculation procedure assumes constant weights for both standards and samples. It is advisable that identical weights are always used to assure minimal matrix effects on headspace between standards and samples.

Results and Discussion

As anticipated, the high volatility of methyl bromide results in a headspace method which is straightforward, rapid, and efficient to carry out for the matrices studied. The method is capable of detecting methyl bromide at the 0.4 ppb level based on 2× signal to noise on the chromatogram. Figures 1 and 2 show that the chromatograms are uncluttered and easily quantitated. Figure 1 is the chromatogram of the headspace obtained from a sample of wheat containing 0.4 ppb MeBr

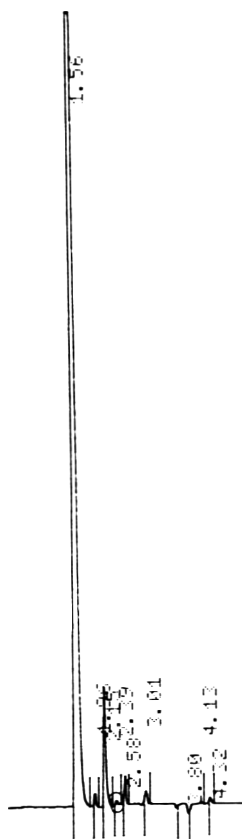

Figure 1. Chromatogram of headspace from wheat sample: 0.4 ppb methyl bromide; methyl bromide retention time 2.39 min. Conditions as listed in text.

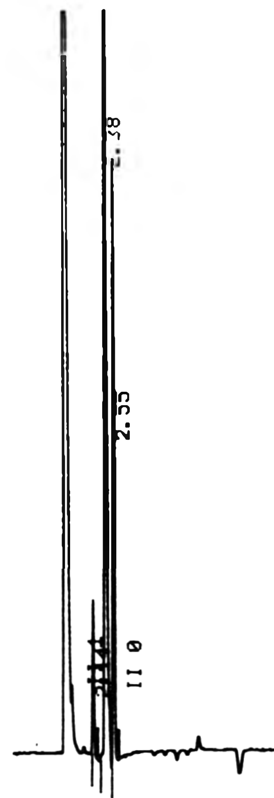

Figure 2. Chromatogram of headspace from peanut sample: 400 ppb methyl bromide; methyl bromide retention time 2.38 min. Conditions as listed in text.

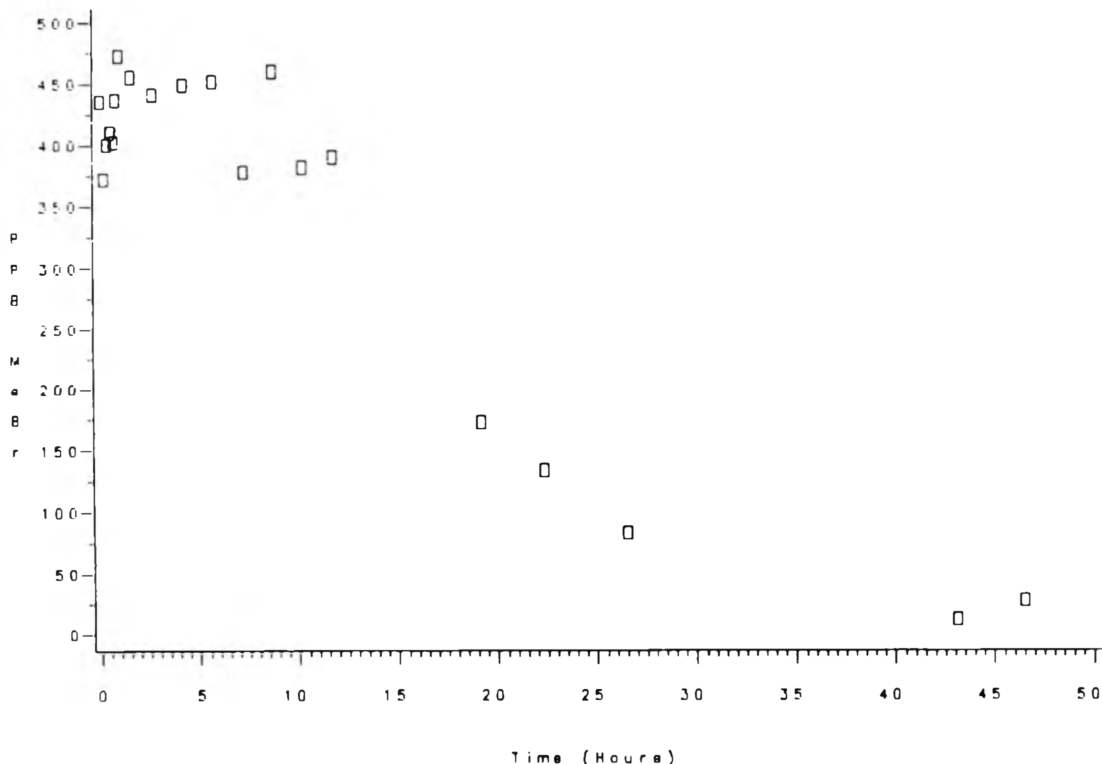

Figure 3. Equilibration study of headspace over wheat. Aqueous methyl bromide added to blank wheat.

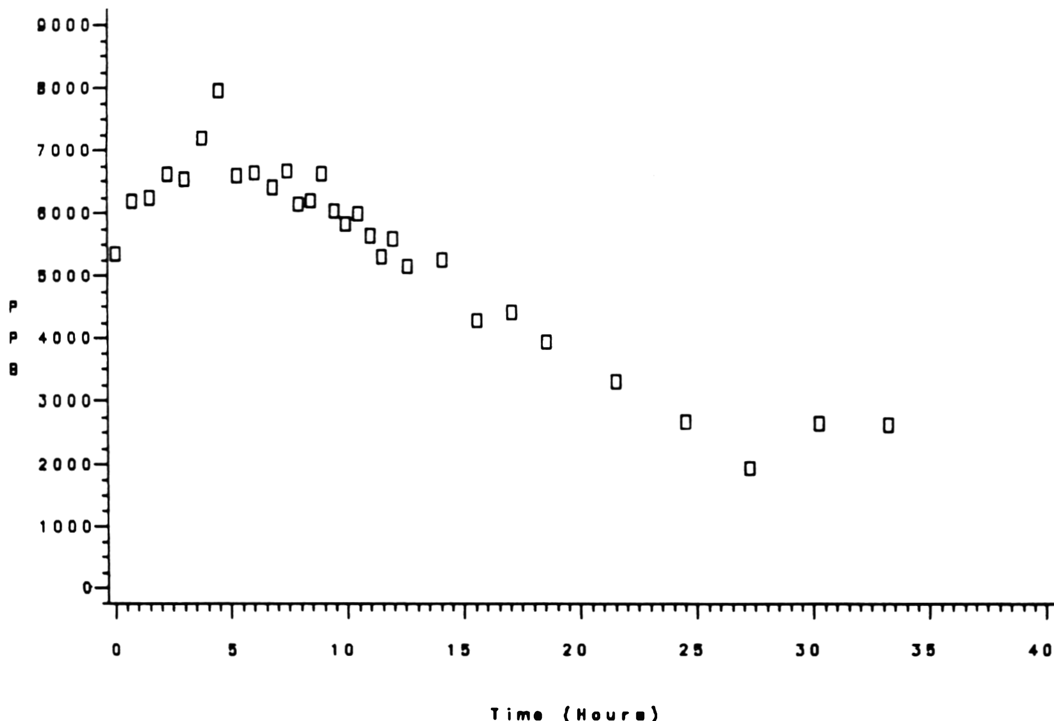

Figure 4. Equilibration study of headspace over wheat. Distilled water added to fumigated wheat.

and Figure 2 is that of the headspace from a sample of peanuts containing 400 ppb MeBr.

Two definite concerns with this headspace method are that the headspace of the standards be equivalent to the headspace of the samples, and that the vial headspace be at equilibrium with the matrix at the time of injection. To address the first concern, samples of methyl bromide-free matrix were treated with aqueous solutions of methyl bromide, placed in the constant temperature bath, and sampled at various time intervals. The rate of equilibration of methyl bromide from the solution to the matrix was monitored by plotting the concentration of MeBr in the vial headspace as a function of time. The second concern was addressed by determining the time required for the methyl bromide from fumigated matrix to equilibrate with the fumigant-free water. The fumigated samples were weighed into sample vials and distilled water was

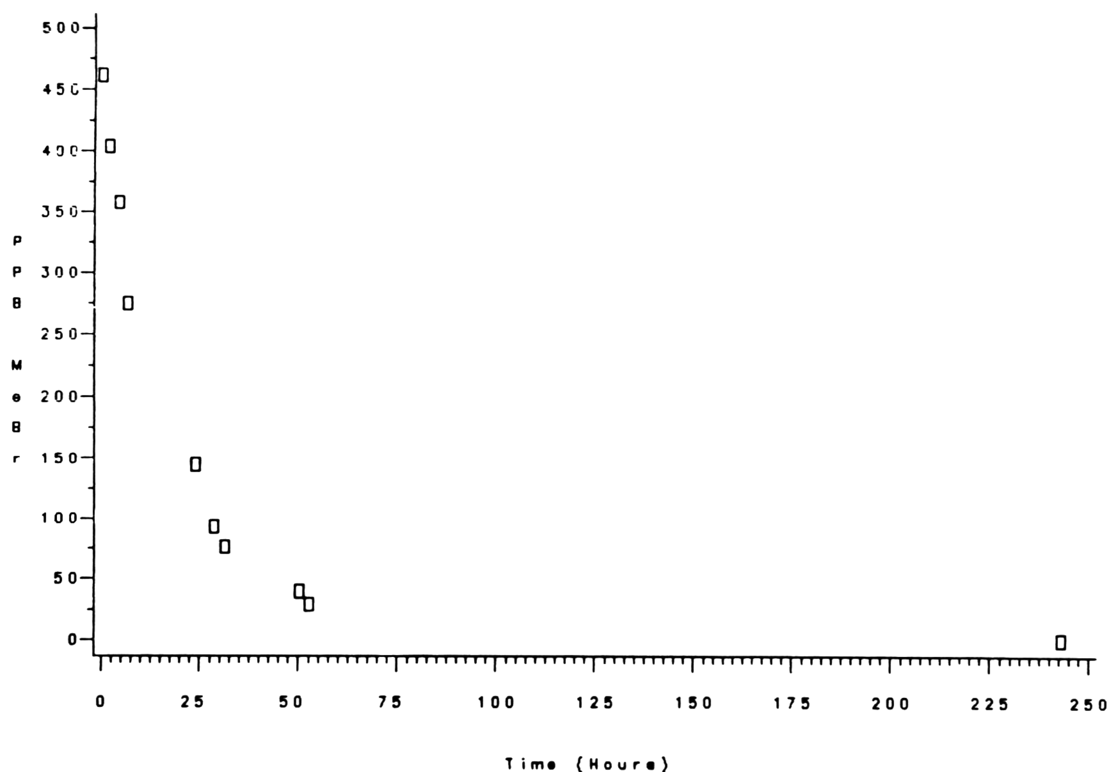

Figure 5. Methyl bromide loss from fumigated wheat over time.

Table 2. Results of methyl bromide equilibration studies

Sample matrix	Aqueous MeBr-to-sample equilibration window (h)	Fumigated sample-to-water equilibration window
Wheat	1–10	1–10
Flour	1–12	2–12
Cocoa	2–12	1–12
Peanuts	1–10	6–17

Table 3. Reproducibility of methyl bromide headspace method

	Spiked samples		Fumigated samples	
Sample	CV, %	Number	CV, %	Number
Wheat	4.4	10	6.5	6
Flour	3.4	10	8.3	4
Cocoa	4.5	10	3.3	10
Peanuts	7.8	10	11.6	10
	Mean CV = 6.0%, n = 70			

Table 4. Disappearance rate of methyl bromide in food commodities

		Air exposure time, h				
Sample	Initial MeBr, ppb	6	12	24	48	240
Wheat	460	400	275	145	40	0
Flour	55	10	6	5	2	0
Cocoa	1600	1500	700	100	25	0
Peanuts	660	540	240	120	60	0

added. The samples were placed in the constant temperature bath and samples were withdrawn and injected at various time intervals. Figures 3 and 4 show the results of these equilibration experiments for wheat. Equilibrium is achieved after 1 h, and no significant loss of methyl bromide occurs until 10 h. Thus, the headspace over wheat samples can be injected anytime from 1 to 10 h. Each of the other matrices were treated in the same manner, with similar results.

Table 2 summarizes the results of the equilibration experiments for wheat, wheat flour, cocoa, and peanuts. These results show that the sample headspace for wheat can be injected between 1 and 10 h, and flour and cocoa can be injected between 2 and 12 h. The fumigated peanuts require a longer equilibration time because of their large particle size and high fat content, both of which tend to slow the equilibration process. Therefore, peanut samples should be injected between 6 and 10 h.

Because of the difficulty of finding samples with incurred methyl bromide, reproducibility studies were carried out on standards (i.e., methyl bromide-free samples with standard added to the sample vial) and on samples fumigated in the laboratory with methyl bromide gas. The results are shown in Table 3. The overall method CV of all samples combined was 6.0%.

To measure the rate of decrease of methyl bromide in wheat, flour, cocoa, and peanuts after fumigation, samples of each were fumigated with methyl bromide gas. Samples were analyzed immediately by this method and then after various time intervals of exposure of the sample to air. Figure 5 shows the results of this experiment for wheat. Results for the other matrices were similar. Table 4 summarizes the results of the fumigant disappearance rate tests. The methyl bromide concentration decreases very rapidly in all cases, and no residual methyl bromide could be found at the 0.4 ppb level in any of the samples after 2 weeks. The rapid disappearance of MeBr indicates that it is highly unlikely that any residual methyl bromide will remain in food products if the starting ingredients are held and aerated for an appropriate time span.

Overall, the headspace approach to the analysis of methyl bromide in the 4 commodities allowed large batches of samples to be handled with a minimal amount of analyst time per sample, was automatable, and gave an elapsed sample turnaround time as short as 1 h.

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FEEDS

Hydrolysate Preparation for Analysis of Amino Acids in Sorghum Grains: Effect of Oxidative Pretreatment

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Sorghum samples were either untreated or oxidized with performic acid (PA) before hydrolysis, and their amino acid contents were determined by cation exchange chromatography using an amino acid analyzer. HCl was used to destroy excess PA. Oxidative pretreatment of the samples resulted in increased yields of Cys (as cysteic acid), Met (as Met dioxide), and His, destroyed Tyr and Phe, and resulted in the appearance of an extraneous peak which most likely consisted of halogenation by-products (HBP) of Tyr and Phe. The destruction of Tyr and Phe occurred despite the presence of phenol, a halogen scavenger, in both the PA and hydrolysis reagents. The higher His values observed in all oxidized samples most likely resulted from the co-elution of His with Tyr and Phe HBP. It was concluded that the complete (except Trp) amino acid content of a feedstuff cannot be accurately determined from only one oxidized hydrolysate preparation by using this particular procedure.

The accurate determination of Met and Cys is probably the most difficult aspect of feedstuff amino acid analysis. Because these sulfur amino acids are destroyed to different degrees by acid hydrolysis, they must be converted to stable compounds such as cysteic acid (Cya) and Met dioxide (Met O_2) before hydrolysis. This is most commonly achieved by oxidizing samples with performic acid (PA) (1–8). Labile amino acids such as Tyr, Phe, His, and Arg may be destroyed to varying extents during this procedure (9). However, inclusion of phenol in the oxidizing and hydrolysis reagents has been reported to completely protect Phe, His, and Arg and to markedly reduce the losses of Tyr (10, 11).

Researchers at the Danish National Institute of Animal Science have conducted an exhaustive series of investigations aimed at developing a "rapid" method of hydrolysate preparation for amino acid determinations in feedstuffs (for summary, see ref. 9). A general overview of their procedure (9) is as follows:

Samples containing 10 mg nitrogen are oxidized with PA (containing phenol) at 0°C for 16 h before acid hydrolysis. Following oxidation, excess PA is destroyed by the addition of sodium disulfite and the sample is acid-refluxed for 23 h. The hydrolysate is then adjusted to pH 2.2, diluted to 200 mL, and filtered or centrifuged before analysis.

This streamlined procedure, which permitted the accurate determination of all amino acids except Tyr and Trp, had one major drawback: Hydrolysates contained high concentrations of formic acid and sodium, which are detrimental to several commercial amino acid analyzers (9, 11). Furthermore, regarding the recent popularity of packed, stainless steel, narrow-bore cation exchange columns for amino acid analyzers and modular liquid chromatographic systems, the application of the streamlined procedure may be severely limited because these expensive columns can be easily damaged by high salt concentrations.

The main objectives of the present study were *(1)* to modify the streamlined procedure such that the hydrolysates obtained would be potentially less destructive to cation exchange columns, *(2)* to determine if accurate and reproducible data could be obtained with small (less than 1 g) samples, and *(3)* to examine the effect on Phe and Tyr stability of including phenol in both the oxidation and hydrolysis reagents. In addition, L-aminohexanoic acid (Ahx) was used as an internal standard to account for small variations in sample preparation, injection volume, and/or chromatography (12). Trp, which is destroyed by acid hydrolysis regardless of oxidative pretreatment and is most commonly determined after basic hydrolysis (13–16), was not analyzed.

Experimental

Reagents and Apparatus

(**a**) *Amino acid analyzer.*—Beckman (Palo Alto, CA) 119 CL amino acid analyzer, previously described (17) except that a Hewlett-Packard (Palo Alto, CA) 3390A integrator replaced the Model 126 data system.

(**b**) *Buffers and chemicals.*—Sodium citrate buffers and STD hydrolysate amino acid calibration solution, Cat. No. 338088 (Beckman Instruments, Inc., Palo Alto, CA). L-Cya, L-Met O_2, and L-Ahx (Sigma Chemical Co., St. Louis, MO). Ninhydrin (Pierce Chemical Co., Rockford, IL). Ethylene glycol monomethyl ether, 30% hydrogen peroxide, 88% formic acid, 1-octanol, and hydrochloric acid (Fisher Scientific Co., Fair Lawn, NJ). Phenol (Mallinckrodt Chemical Works, St. Louis, MO).

Standard Preparation

One mL of STD hydrolysate amino acid calibration solution, 1 mL of pH 2.2, 0.2N sodium citrate buffer (NaC) containing 2.5 μmol each of L-Cya and L-Met O_2, and 1 mL of NaC containing 2.5 μmol of L-Ahx were diluted to a total volume of 5 mL with NaC before injection. Thus, the standard contained 250 nmol/mL of L-Cys and 500 nmol/mL of each of the following: L-Ahx, L-Ala, L-Arg, L-Asp, L-Cya, L-Glu, Gly, L-His, L-Ile, L-Leu, L-Lys, L-Met, L-Met O_2, NH_3, L-Phe, L-Pro, L-Ser, L-Thr, L-Tyr, and L-Val.

Table 1. Dry matter, crude protein, and ether extract contents of the 5 sorghums

Sorghum[a] Variety	Harvest year	Dry matter, %	Crude protein, %	Ether extract, %
RS610	1982	91.11	9.84	2.41
RS610	1983	89.97	10.77	2.86
Savanna	1982	88.99	9.23	2.27
Savanna	1983	87.80	9.39	2.97
BR64	1983	87.40	8.71	2.80

[a]Based on dry matter and ether extract contents, the amount of sorghum analyzed for amino acids per each 50 μL hydrolysate injection was as follows: RS610 (1982), 563 μg; RS610 (1983), 572 μg; Savanna (1982), 575 μg; Savanna (1983), 587 μg; BR64 (1983), 589 μg.

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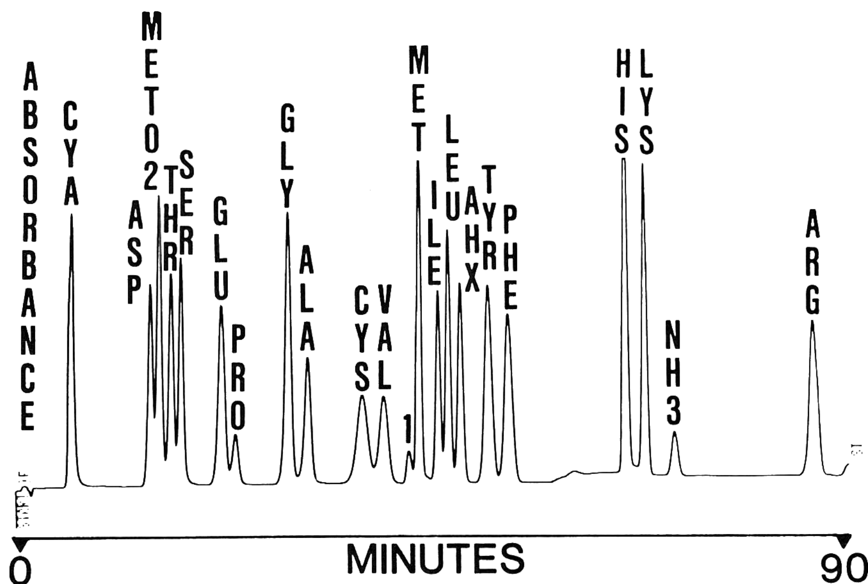

Figure 1. Calibration standard chromatogram. Each peak represents 25 nmol (Cys, 12.5 nmol). Peak 1 is buffer change peak. Cya = cysteic acid; Met O_2 = Met dioxide; Ahx = aminohexanoic acid.

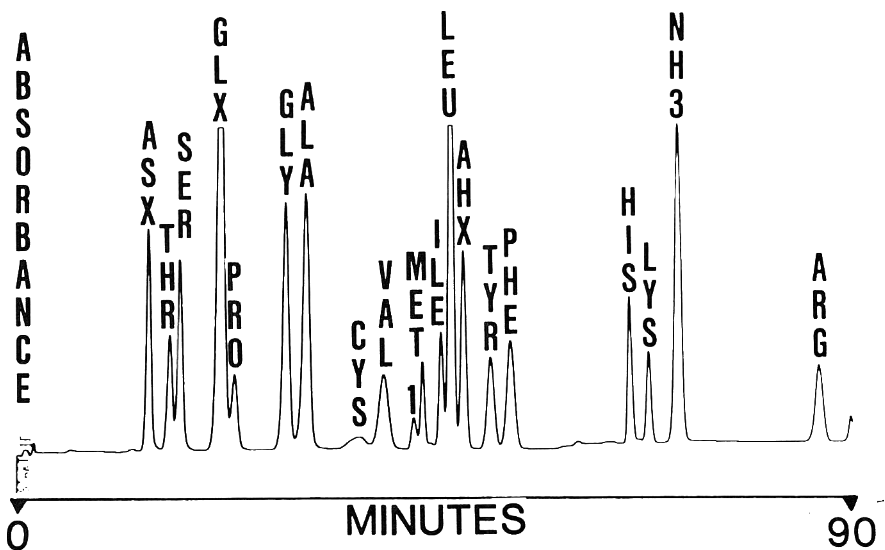

Figure 2. Chromatogram of sorghum (BR64) hydrolysate not subjected to oxidative pretreatment. Peak 1 is buffer change peak. Ahx = aminohexanoic acid.

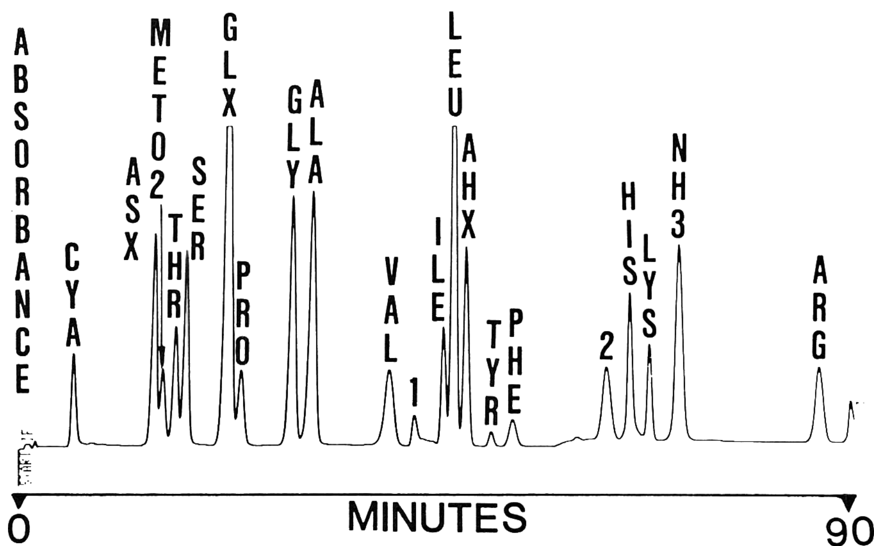

Figure 3. Chromatogram of sorghum (BR64) hydrolysate subjected to oxidative pretreatment. Peaks 1 and 2 are buffer change and (presumably) halogenation by-product peaks, respectively. Cya = cysteic acid; Met O_2 = Met dioxide; Ahx = aminohexanoic acid.

Table 2. Amino acid composition of sorghum variety RS610, 1982 harvest

Amino acid	Oxidized[a,b]	Unoxidized[a,c]
Asx[d]	0.702 ± 0.005	0.683 ± 0.003
Thr	0.319 ± 0.002	0.317 ± 0.006
Ser	0.436 ± 0.002	0.435 ± 0.007
Glx	2.056 ± 0.009	2.091 ± 0.041
Pro	0.803 ± 0.005	0.821 ± 0.021
Gly	0.310 ± 0.003	0.312 ± 0.002
Ala	0.916 ± 0.006	0.934 ± 0.016
Cys[d]	0.164 ± 0.004	0.093 ± 0.001
Val[d]	0.506 ± 0.005	0.516 ± 0.005
Met[d]	0.161 ± 0.004	0.139 ± 0.004
Ile	0.396 ± 0.002	0.400 ± 0.006
Leu	1.371 ± 0.007	1.391 ± 0.029
Tyr[d]	0.024 ± 0.010	0.420 ± 0.011
Phe[d]	0.176 ± 0.015	0.516 ± 0.013
His[d]	0.247 ± 0.009	0.215 ± 0.003
Lys	0.183 ± 0.001	0.183 ± 0.001
Arg[d]	0.372 ± 0.001	0.382 ± 0.002

[a]Values are means ± SD in % ("as is" basis) for duplicate analyses of duplicate samples (thus $n = 4$).
[b]After oxidative pretreatment and hydrolysis.
[c]After direct hydrolysis without oxidative pretreatment.
[d]$P \leq 0.05$ for oxidized vs unoxidized.

Table 3. Amino acid composition of sorghum variety RS610, 1983 harvest

Amino acid	Oxidized[a,b]	Unoxidized[a,c]
Asx	0.723 ± 0.017	0.711 ± 0.007
Thr	0.314 ± 0.005	0.306 ± 0.005
Ser	0.463 ± 0.017	0.480 ± 0.004
Glx[d]	2.140 ± 0.023	2.208 ± 0.018
Pro	0.870 ± 0.013	0.884 ± 0.008
Gly[d]	0.325 ± 0.005	0.333 ± 0.003
Ala[d]	0.960 ± 0.013	0.994 ± 0.007
Cys[d]	0.164 ± 0.004	0.099 ± 0.003
Val[d]	0.539 ± 0.009	0.553 ± 0.003
Met[d]	0.173 ± 0.003	0.129 ± 0.006
Ile[d]	0.391 ± 0.006	0.401 ± 0.005
Leu[d]	1.461 ± 0.005	1.496 ± 0.014
Tyr[d]	0.020 ± 0.004	0.446 ± 0.008
Phe[d]	0.247 ± 0.009	0.554 ± 0.006
His[d]	0.301 ± 0.004	0.241 ± 0.003
Lys	0.207 ± 0.003	0.205 ± 0.004
Arg[d]	0.401 ± 0.008	0.413 ± 0.001

[a–d]See Table 2.

Sample Preparation

(a) *Preliminary*—Five different sorghum samples, representing 3 varieties and 2 harvests, were finely ground in a Burr mill (Laboratory Construction Co., Kansas City, MO) and assayed for dry matter (18) and crude protein (19). Five g portions of each ground, dried sorghum were wrapped in 2 Whatman No. 1 filter paper circles, 15 cm (Whatman Ltd, Kent, UK), and extracted with petroleum ether in a Soxhlet apparatus for 16 h to remove lipid before oxidation and/or hydrolysis.

(b) *Unoxidized samples.*—Two 800 mg moisture-free, defatted samples of each sorghum, corresponding to approximately 10 mg nitrogen, were placed in individual 250 mL round-bottom boiling flasks. Three glass beads and 125 mL 6N HCl containing phenol (1 mg/mL) and L-Ahx (41.98 μg/mL)[2] were added and flasks were placed in a reflux apparatus. Following a 23 h reflux, measured from the time of boiling (9), hydrolysates were filtered, cooled, and diluted to 200 mL with water. Twenty-five mL of each hydrolysate was evaporated to near dryness in a rotary evaporator (50°C). Several mL of water was added, and the sample was evaporated to dryness. Following a second water addition and evaporation, the residue was dissolved in 10 mL of NaC and refrigerated. Each sample was filtered (Millex-HV, 0.45 μm, Millipore Corp., Bedford, MA) before analysis.

(c) *Oxidized samples.*—Two 800 mg moisture-free, defatted samples of each sorghum, corresponding to approximately 10 mg nitrogen, were placed in individual 250 mL round-bottom boiling flasks. PA was prepared by mixing 30% hydrogen peroxide (H_2O_2) and 88% formic acid (FA) containing phenol (5.56 mg/mL) in the ratio of 1 part H_2O_2 to 9 parts FA (1). The mixture was kept at room temperature for 60 min and, then the oxidizing reagent and sorghum samples were cooled in an ice water bath (0°C) for 15 min. Five mL of PA was carefully mixed with each sorghum sample using a bent-tip glass spatula (9, 11). The flasks, still containing the spatulas, were sealed with parafilm "M" (American Can Co., Greenwich, CT) and placed in an ice water bath in a refrigerator at 0°C for 16 h. On completion of the oxidation, excess PA was destroyed by adding 1 mL concentrated HCl to each flask, swirling, and then adding 4 drops of 1-octanol to prevent foaming. Because chlorine gas is released during this step, the HCl addition was conducted in a hood. Each flask was allowed to stand for 15 min at which time the glass spatulas were rinsed with 5 mL water and removed. Samples were then rotary evaporated (50°C) to dryness; extreme care was taken to avoid any sample losses caused by foaming. Beginning with the addition of 3 glass beads and 125 mL 6N HCl

Table 4. Amino acid composition of sorghum variety Savanna, 1982 harvest

Amino acid	Oxidized[a,b]	Unoxidized[a,c]
Asx[d]	0.656 ± 0.009	0.627 ± 0.007
Thr[d]	0.300 ± 0.002	0.291 ± 0.003
Ser	0.411 ± 0.002	0.409 ± 0.004
Glx	1.949 ± 0.011	1.952 ± 0.014
Pro	0.749 ± 0.006	0.751 ± 0.007
Gly	0.294 ± 0.001	0.293 ± 0.002
Ala	0.869 ± 0.007	0.873 ± 0.005
Cys[d]	0.152 ± 0.001	0.084 ± 0.001
Val	0.485 ± 0.003	0.482 ± 0.002
Met[d]	0.159 ± 0.002	0.130 ± 0.004
Ile	0.371 ± 0.003	0.368 ± 0.001
Leu	1.301 ± 0.010	1.298 ± 0.008
Tyr[d]	0.017 ± 0.002	0.393 ± 0.001
Phe[d]	0.291 ± 0.012	0.480 ± 0.002
His[d]	0.288 ± 0.002	0.204 ± 0.003
Lys	0.162 ± 0.001	0.165 ± 0.008
Arg[d]	0.345 ± 0.002	0.354 ± 0.003

[a–d]See Table 2.

Table 5. Amino acid composition of sorghum variety Savanna, 1983 harvest

Amino acid	Oxidized[a,b]	Unoxidized[a,c]
Asx[d]	0.655 ± 0.002	0.628 ± 0.004
Thr[d]	0.299 ± 0.001	0.295 ± 0.002
Ser	0.397 ± 0.003	0.394 ± 0.004
Glx	1.785 ± 0.019	1.779 ± 0.019
Pro	0.675 ± 0.011	0.669 ± 0.008
Gly	0.315 ± 0.001	0.316 ± 0.001
Ala	0.793 ± 0.007	0.792 ± 0.007
Cys[d]	0.160 ± 0.001	0.091 ± 0.002
Val	0.473 ± 0.006	0.469 ± 0.003
Met[d]	0.165 ± 0.002	0.143 ± 0.001
Ile	0.350 ± 0.002	0.347 ± 0.003
Leu	1.165 ± 0.012	1.155 ± 0.014
Tyr[d]	0.017 ± 0.002	0.365 ± 0.003
Phe[d]	0.239 ± 0.018	0.442 ± 0.003
His[d]	0.272 ± 0.010	0.206 ± 0.003
Lys	0.202 ± 0.003	0.205 ± 0.004
Arg[d]	0.396 ± 0.002	0.404 ± 0.001

[a–d]See Table 2.

[2]Prepared by adding equal volumes of concentrated HCl and a solution containing phenol (2 mg/mL) and L-Ahx (83.96 μg/mL) in water.

Table 6. Amino acid composition of sorghum variety BR64, 1983 harvest

Amino acid	Oxidized[a,b]	Unoxidized[a,c]
Asx[d]	0.593 ± 0.009	0.564 ± 0.004
Thr[d]	0.285 ± 0.003	0.278 ± 0.001
Ser	0.394 ± 0.001	0.395 ± 0.002
Glx[d]	1.693 ± 0.005	1.711 ± 0.003
Pro[d]	0.689 ± 0.006	0.700 ± 0.006
Gly	0.298 ± 0.002	0.295 ± 0.003
Ala[d]	0.753 ± 0.004	0.765 ± 0.004
Cys[d]	0.163 ± 0.001	0.099 ± 0.005
Val	0.435 ± 0.004	0.434 ± 0.004
Met[d]	0.164 ± 0.001	0.142 ± 0.001
Ile	0.339 ± 0.001	0.339 ± 0.001
Leu[d]	1.133 ± 0.006	1.143 ± 0.002
Tyr[d]	0.041 ± 0.001	0.357 ± 0.002
Phe[d]	0.097 ± 0.012	0.447 ± 0.003
His[d]	0.249 ± 0.006	0.236 ± 0.003
Lys	0.192 ± 0.001	0.193 ± 0.006
Arg	0.380 ± 0.002	0.381 ± 0.005

[a–d]See Table 2.

Table 7. Average amino acid composition of the 5 sorghum samples

Amino acid	Oxidized[a,b]	Unoxidized[a,c]
Asx	0.666 ± 0.047	0.643 ± 0.052
Thr	0.303 ± 0.013	0.297 ± 0.014
Ser	0.420 ± 0.028	0.422 ± 0.033
Glx	1.924 ± 0.171	1.948 ± 0.192
Pro	0.757 ± 0.075	0.765 ± 0.081
Gly	0.308 ± 0.012	0.310 ± 0.015
Ala	0.858 ± 0.079	0.871 ± 0.088
Cys[d]	0.160 ± 0.005	0.093 ± 0.006
Val	0.488 ± 0.036	0.491 ± 0.042
Met[d]	0.165 ± 0.005	0.137 ± 0.007
Ile	0.369 ± 0.023	0.371 ± 0.027
Leu	1.286 ± 0.127	1.297 ± 0.140
Tyr[d]	0.024 ± 0.010	0.396 ± 0.035
Phe[d]	0.210 ± 0.070	0.488 ± 0.044
His[d]	0.271 ± 0.023	0.221 ± 0.016
Lys	0.189 ± 0.016	0.190 ± 0.016
Arg	0.379 ± 0.021	0.387 ± 0.021

[a]Values are means ± SD in % ("as is" basis) (n = 20).
[b–d]See Table 2.

containing phenol and L-Ahx to each flask, all subsequent steps were identical to those described for the unoxidized samples.

Chromatographic Procedures

Chromatographic procedures for sodium cation exchange chromatography have been previously described (17) except that the initial column temperature was 49°C and the elution time for the first buffer was increased from 22 to 25 min. Calibration runs with 50 μL of the standard solution, corresponding to 25 nmol of each amino acid except Cys (12.5 nmol) per injection were performed daily. Fifty μL aliquots of each sorghum hydrolysate were run in duplicate. The actual amount of sorghum analyzed per injection ranged from 563 to 589 μg, depending on the dry matter and ether extract contents of the sample (Table 1).

Statistics

Student's t-tests (20) were performed to compare oxidized and unoxidized data within and across sorghums.

Results

Representative calibration standard and unoxidized and oxidized sorghum (BR64) hydrolysate chromatograms appear in Figures 1–3, respectively. Sample oxidation before hydrolysis increased yields of Cys (as Cya), Met (as Met O_2), and His, destroyed Tyr and Phe, and resulted in the appearance of a well resolved extraneous peak (peak 2, Figure 3) which most likely consisted of halogenation by-products of Tyr and Phe (5, 9–11, 21). The above results were consistent within (Tables 2–6) and across (Table 7) sorghums.

With regard to the amino acids other than Cys, Met, His, Tyr, and Phe, significant differences resulting from oxidative pretreatment were observed in 22 cases across the 5 sorghum samples (Tables 2–6), although in 16 of these instances the numerical differences between oxidized and unoxidized values were less than 0.020%. This was most likely attributed to the excellent precision attained in this study, as 15 of the 17 amino acid coefficients of variation (CV) averaged across sorghums were between 0.60 and 2.00%. Because oxidation markedly destroyed Tyr and Phe, these amino acids exhibited higher average coefficients of variation.

Discussion

HCl, rather than HBr (1, 8) was used as the reducing agent in the present study to avoid the tendency of the latter to condense in the stem of the rotary evaporator before reaching the condenser (1). Although some sample foaming occurred during the removal of residual chlorine and FA by rotary evaporation, it did not pose a major problem if the flasks were frequently monitored. Sodium disulfite can also be used to destroy excess PA, but the hydrolysate must be neutralized, rather than concentrated by evaporation, which results in high sodium concentrations (9, 11, 22).

The use of HCl to destroy excess PA increased the sample preparation time, but it was felt that this was justified considering the alternatives of potential evaporation problems with HBr or shorter column life, and thus higher costs per analysis, with sodium disulfite. Furthermore, despite the increased amount of sample manipulation, accurate and reproducible data were obtained using small (less than 1 g) samples.

Contrary to previous reports (9, 11), the inclusion of phenol, a halogen scavenger, in both the oxidation and hydrolysis reagents did not prevent the marked destruction of Tyr and Phe. In addition, the higher His values observed for all oxidized sorghums as compared to unoxidized samples can probably be attributed to co-elution with halogenation by-products of Tyr and Phe which have been reported to interfere with the chromatography of His (5, 9, 11). Although Tyr can be mono- or dichlorinated by the action of chlorine liberated when H_2O_2 reacts with chloride (21), Tyr losses caused by oxidation can occur at temperatures above 0°C in the absence of chloride (5, 23). Therefore, the complete (except Trp) amino acid content of a feedstuff cannot be accurately determined from only one oxidized hydrolysate preparation, using this particular procedure.

Considering the recent controversy over guaranteed amino acid levels on commercial feed labels (24–26), the variability of amino acid data between laboratories (6, 22, 25, 27), and the economic importance of analyzing feedstuffs to accurately formulate poultry and livestock feeds (8), a standard method of hydrolysate preparation would be desirable. However, because of the many different chemistries and various instrument configurations presently being used for amino acid analyses, the element of systematic error (28) would remain. This is substantiated by a recent collaborative study on feedstuff amino acid determination, involving a total of 30 laboratories from 8 European Economic Community countries (22), where over 25% of all data obtained were rejected for various reasons.

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Disposable Cartridge Extraction of Retinol and α-Tocopherol from Feedstuffs

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Automated determination of fat-soluble vitamins by modern methods such as liquid chromatography is hampered by the initial extraction step. A simple technique is proposed that allows an appreciable increase in the actual rates of determination. Feedstuff samples are first hydrolyzed in an aqueous alcohol (mainly methanol)–potassium hydroxide solution. Instead of extracting retinol and α-tocopherol from the hydrolysis solution by an organic solvent, an aliquot of the solution is mixed with a small volume of a strong antioxidant solution (ascorbic acid) and pipetted onto a kieselguhr disposable cartridge where it is adsorbed. Retinol and α-tocopherol are eluted with isooctane at normal pressure. The proposed method has been compared with conventional techniques on many feed samples.

Methods for extraction of retinol from feeds and foods have not progressed a great extent in previous decades. Methods used today (1) are quite similar to older ones (2–4). These methods are based on alkaline hydrolysis followed by liquid/liquid partitions between aqueous alcoholic and organic solvent phases. The AOAC method specifies centrifugation after partition, and then pipetting of the lower phase (1). Our own former method necessitated 3 extractions with petroleum ether–diethyl ether and 3 water washings.

In addition, the problem of how much feed sample to weigh and hydrolyze has not been satisfactorily addressed. For a retinol concentration of 5000 IU/lb, the AOAC method simply specifies that sample weight must be ≥40 g. Bourgeois recently demonstrated that the minimum theoretical coefficient of variation for a 40 g sample is equal to 16% for usual commercial forms of vitamin A containing 500 000 IU/g (and even 19.5% for a 750 000 IU/g form) (5).

Tocopherol extraction techniques have also not evolved much. Here the situation is complicated by 2 opposite approaches: One consists of first extracting α-tocopherol with an organic solvent (such as acetone, ethanol) and then hydrolyzing the extracted lipids with a base. The alternative approach is direct hydrolysis of the sample with KOH in aqueous alcohol and extraction of the unsaponifiable matter with an organic solvent.

The first approach has been employed by researchers concerned with vegetable tissues, cereals, grains, and oils (6–9), and animal tissues (10). The AOAC method (11) for foods and feeds consists of an overnight Soxhlet extraction with ethanol followed by hydrolysis of the extracted lipids. The alternative technique has been employed by many authors (12–14). We have been using this approach for about 10 years in foods and feeds with satisfaction.

Recently, McMurrey et al. compared both alternatives and found better results with the direct hydrolysis method (15). Yet the question remains open.

Whatever the approach, the treatment of the unsaponifiable fraction (containing retinol or α-tocopherol) is cumbersome.

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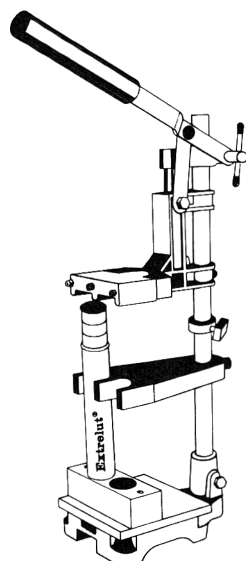

Figure 1. Laboratory-designed loading press for preparing columns.

Table 1. Parallel extractions of retinol with cartridges and conventional liquid/liquid partition[a]

Sample	Conventional technique, IU/kg	Cartridge technique, IU/kg	Cart./Conv. × 100, %
Pig feed	5 220	5 330	102
Chick feed	9 482	9 821	103.6
Pig feed	4 487	4 840	108
Chick feed	13 330	13 500	101.3
Cattle feed	12 260	11 840	96.6
Chicken feed	5 795	6 311	109
Chicken feed	6 467	6 774	104.7
Premix	397 330	405 640	102
Premix	2000 000	2080 000	104
Premix	1700 000	1750 000	103
Growing chick feed	8 271	8 253	99.8
Chick feed	10 131	10 519	103.8
Chicken feed	9 544	9 868	103.4
Growing chick feed	8 922	9 040	101.3
Premix	2238 000	2339 000	104.5
Premix	4920 000	4935 000	100.3
Premix	1850 000	1930 000	104
Average, %			103.0
CV, %			2.8

[a]Results are averages of duplicate determinations.

Table 2. Parallel extractions of α-tocopherol with cartridges and conventional liquid/liquid partition

Sample	Detns	Conventional technique, ppm	Cartridge technique, ppm	Cart./Conv. × 100, %
Broiler premix	2	331.9	364.4	109.8
Laying chick	2	33.3	38.1	114.3
Broiler feed	2	32.55	35.1	107.9
Pig feed	2	22.5	24.35	108.2
Broiler feed	2	66.0	71.9	108.2
Laying chick	2	86.5	91.45	105.7
Broiler premix	2	186.2	199.4	107.1
Laying chick	2	56.3	60.7	107.9
Minerals and vitamins premix (MVP)	2	454.8	492.3	108.2
Milk	3	11.5	12.1	105.4
MVP	4	3 723.1	3 765.4	101.1
MVP	4	6 782.8	6 849.4	101.0
MVP	4	8 891.9	9 075.9	102.0
MVP	3	1 267	1 275.3	100.6
MVP (at 4%)	2	789.8	848.5	107.4
MVP (at 4%)	2	798.5	817.6	102.4
MVP (at 4%)	2	823.15	834.5	101.4
Liquid feed	2	3.6	3.7	102.9
Average, %				105.3
CV, %				3.6

The numerous liquid/liquid extractions and washings are time consuming, and prevent automation of fat-soluble vitamin determinations. Moreover, the solvents used are dangerous and expensive, and the frequent emulsions reduce the analysis rate and affect accuracy of the results.

METHODS

I. Proposed Procedure

Reagents

(**a**) *Pyrogallol solution.*—Dissolve 0.1 g pyrogallol in 4 mL water. Add 20 mL ethanol and adjust to 100 mL with methanol. Prepare daily.

(**b**) *Potassium hydroxide aqueous solution.*—Dissolve 700 g KOH (86% w/w) in 600 mL water and adjust to 1 L.

(**c**) *Ascorbic acid aqueous solution.*—20% (w/v) in water. Prepare daily.

(**d**) *Disposable cartridges.*—Extrelut (E. Merck cartridges 11737 and fillings 11738). Small plastic columns containing kieselguhr (16, 17) packing 8 cm depth, 3 cm diameter, and weighing about 10 g. Operate columns under normal pressure. Columns may be reloaded 20 times or more.

Hydrolysis

Weigh 50 or 100 g feed sample (for α-tocopherol or for retinol and retinol plus α-tocopherol) or 10–20 g premix in 1 L low actinic long-neck boiling flask. Add 400 mL pyrogallol solution and 80 mL KOH solution. Weigh flask, attach reflux condenser, and heat 20 min under nitrogen. Shake flask from time to time or, preferably, use magnetic stirrer. Rinse condenser with 20 mL water and weigh flask again. Bring weight to its initial value plus 20 g with 2–3 mL ethanol if necessary. Heating should last 20 min from beginning of reflux.

Preparation of Cartridges

One can employ ready-to-use columns (already filled), but the kieselguhr weight varies. We usually fill empty columns with kieselguhr powder sold in small bags. It is necessary to weigh 10 g. After filling, the powder is homogenized with a vibrating agitator.

Note: We designed a small loading-press (see Figure 1) to make the loading easier. This press is ca 40 cm high (plus 20 cm for the handle) and 12 cm wide × 18 cm long at the base. It can load 2 columns simultaneously (it is possible to manufacture presses that can load more than 2 columns simultaneously). After column is filled with kieselguhr powder and settled with a vibrating agitator, it is placed into the press and the hand-lever is lowered so as to slightly compress the powder.

Extraction

Let hydrolysis solution cool to room temperature; pipet 40.0 mL aliquot into 50 mL low actinic volumetric flask. Adjust to mark with ascorbic acid solution. Shake and transfer 15.0 or 20.0 mL (see discussion) of this solution onto cartridge. Wait 15 min, set 50 mL volumetric flask under column, and elute with 60 mL isooctane. Elution lasts ca 25 min, and ca 40 mL isooctane is collected. Keep flask in freezer (− 18°C) and adjust volume with isooctane just before determination.

Just after use, discard adsorbent, and dip column in aqueous solution of Mucapur® (Merz Co., GFR) for 1 h. Then column may be washed with rest of glassware. Column can be reloaded several times (at least 20).

Table 3. Reproducibility of proposed method

	Retinol		α-Tocopherol	
Kieselguhr, g	CV, %	IU	CV, %	IU
8	4.4	1045	2.3	2.36
10	2.4	1074	2.0	2.38
12	3.3	1081	1.9	2.37

Table 4. Recovery of retinol

Sample	Concentration, IU /kg	Recovery, %
Chick 1 feed	17 000	113.9
Chick 2 feed	10 000	90.2
Chick 3 feed	18 000	100.2
Starting chick feed	6 400	98.5
Dairy cow feed	3 500	94.1
Premix A	3 000 000	107.7
Premix B	1 900 000	111.0
Premix C	2 100 000	93.4
Premix D	950 000	101.7
Average, %		101.2

II. Conventional Procedure for α-Tocopherol Samples

Reagent

Diethyl ether.—Less than 1 ppm peroxides, analytical grade.

Hydrolysis

Same as in I.

Extraction

Transfer 100 mL aliquot of hydrolysis solution (I) to separatory funnel. Add 50 mL water and 120 mL diethyl ether, and extract unsaponifiable matter. Extract twice again with 120 mL diethyl ether. Collect ether phases and wash 3 times with 100 mL water. Final water phase must be neutral. Take aliquot of ether solution (volume depends on sample concentration and sensitivity of method), add 2–3 mL ethanol, and evaporate at 50°C under vacuum. Dissolve residue in solvent used for determination of α-tocopherol.

III. Conventional Procedure for Retinol Samples

Reagent

Petroleum ether (60–80°C) and anhydrous sodium sulfate.—Both analytical grade.

Hydrolysis

Same as in I.

Extraction

Pipet 100 mL aliquot of hydrolysis solution into separatory funnel. Add 60 mL water. Extract 3 times with 100 mL petroleum ether (60–80°C). Add 20 mL diethyl ether each time, to break emulsion. Wash collected ether phases 3 times with 100 mL water. Final water phase must be neutral. Filter through anhydrous sodium sulfate, evaporate eluate with rotatory evaporator, and dissolve residue with solvent used for determination of retinol.

Results and Discussion

We assayed retinol and α-tocopherol, but our method does not determine the other tocopherol homologs (18).

Since retinol and α-tocopherol are easily oxidizable, it is necessary to stabilize the aliquot taken from the hydrolysis solution with a strong antioxidant such as ascorbic acid, previous to the extraction. Ascorbic acid is not very soluble in ethanol, which is commonly used as hydrolyzing solvent, so it is necessary to use methanol instead. Feedstuff samples must not be too fatty. We have compared the proposed method with our former liquid/liquid partition methods (called here "conventional methods") for either retinol or α-tocopherol. Four aliquots of the same hydrolysis solution were taken: Two were treated according to the proposed technique and the other 2 were extracted according to the conventional technique. Finally, retinol was determined by liquid chromatography and α-tocopherol was determined with our new continuous flow method (18). As can be seen on Tables 1 and 2, the results obtained with the proposed and the conventional techniques are in good agreement.

Statistical Evaluation

(a) *Retinol (see Table 1).*—The mean value obtained with the proposed method is slightly higher (103%) than that obtained with the liquid/liquid partition method. The coefficient of variation (CV) of the ratio of results obtained with both methods is equal to 2.8%. The paired t-test applied to results in Table 1 shows that the compared extraction techniques give significantly different results: The calculated value is equal to 2.36 and the t-value for 16 degrees of freedom and a 5% significance level is 2.12.

(b) *α-Tocopherol (see Table 2).*—The mean value obtained with the proposed method is slightly higher (105.3%) than that obtained with the conventional method. The CV of the ratio of the results obtained with both methods is equal to 3.6%. The paired t-test applied to results in Table 2 shows that the compared extraction techniques give significantly different results: The calculated value of t is equal to 2.67 and the t-value for 17 degrees of freedom and a 5% significance level is 2.11.

The significantly higher results for the proposed method mean either that these vitamins are not completely extracted by liquid/liquid partition, or that a small amount is destroyed during partition.

Reproducibility.—The coefficients of variation for the extractions from hydrolyzed standard solutions containing 1100 IU retinol acetate and 2.50 IU α-tocopherol acetate were determined with cartridges filled with either 8, 10, or 12 g

Table 5. Recovery of α-tocopherol

Sample	Concentration, mg/kg	Recovery, %
Chick feed 1	30	85.0
Chick feed 2	23	103.7
Chick feed 3	34	97.3
Starting chick feed	13	106.5
Layer feed	30	106.6
Dairy cow feed	22	106.1
Premix 1	176	111.1
Premix 2	1800	102.3
Premix 3	350	95.1
Premix 4	1700	102.5
Premix 5	335	94.4
Average, %		101

Table 6. Percentages of retinol in 10 mL isooctane fractions eluted from column

Fraction	Retinol, %	Fraction	Retinol, %
1	67.7	1	64.1
2	26.1	2	27.5
3	6.2	3	7.3
4	0	4	1.1

Table 7. Percentages of α-tocopherol in 10 mL isooctane fractions eluted from column

Fraction	α-Tocopherol, %	Fraction	α-Tocopherol, %
1	79.5	1	77.8
2	12.3	2	13.3
3	4.8	3	5.4
4	3.4	4	3.5

Table 8. Comparison of assays with 15 and 20 mL aliquots layered onto cartridges (standard solutions)

Vitamin	15 mL (A)	20 mL (B)	A – B/B × 100
Retinol	95.74	97.0	– 1.3
	102.8	101.8	+ 0.8
$(\alpha\text{-T})_1$	98.0	97.4	+ 1.0
	96.6	95.7	+ 0.9
	96.0	99.0	– 3.0
	101.0	104.0	– 2.9
$(\alpha\text{-T})_2$	102.0	100.0	+ 2.0
	101.0	103.0	– 1.9
	103.0	102.0	+ 1.0

Retinol = hydrolyzed standard retinol acetate.
$(\alpha\text{-T})_1$ = hydrolyzed standard *d,l*-α-tocopherol.
$(\alpha\text{-T})_2$ = hydrolyzed standard *d,l*-α-tocopherol acetate.
Results expressed in international units.

kieselguhr. Ten assays were run with the 10 g columns and 5 with the 8 and 12 g columns. The obtained CV values and averages are shown on Table 3.

Recovery.—Samples were first assayed to determine actual levels of retinol and α-tocopherol then aliquots of standard solutions were added, after the alkaline hydrolysis step. The known amounts of added vitamins were approximately equal to the amounts originally present in the samples. The recovery factor was calculated by subtracting the result obtained for the unsupplemented sample from the one obtained for the supplemented sample and dividing by the known added amount.

Tables 4 and 5 show the results obtained from double determinations. The average recovery factors were 101.2% for retinol and 101% for α-tocopherol. When 60 mL isooctane is poured onto the cartridge to elute the vitamins only 40 mL is recovered.

To locate the point of elution, we separated eluates in four 10 mL fractions and determined retinol or α-tocopherol in each fraction. This operation was carried out twice, starting from hydrolyzed standard solutions; 60 IU retinol and 70 μg α-tocopherol were layered onto the columns. As can be seen in Table 6, about 65% retinol elutes with the first 10 mL fraction, whereas only 1% remains in the last 10 mL. Table 7 shows that about 79% α-tocopherol elutes with the first fraction and only 3% with the last one. Since the latter is less polar, it elutes faster.

Maximum Capacity of Cartridges

The amounts of retinol and α-tocopherol pipetted onto 10 g columns are usually about 30 IU and 0.1 mg, respectively, for feedstuffs. We have tried to layer amounts of 300 and 3000 IU retinol (5 assays each) as well as 5.0 and 10.0 mg α-tocopherol (4 assays each), from reference standard solutions. The recoveries were 99.2 and 100%, respectively, for retinol and 97 and 99.3%, respectively, for α-tocopherol.

From the results obtained in Tables 1–7, it can be concluded that extractions with disposable cartridges are satisfactory. Yet we had problems with several samples. In these cases, the aliquot layered on top of the cartridge was not completely adsorbed and part of it eluted prematurely. This happened with fatty feedstuffs and large amounts (100 g). In these cases, one can use aliquots of 15 mL instead of 20 mL, or carry out a conventional liquid/liquid extraction.

Tables 8 and 9 show results obtained with 15 and 20 mL aliquots. The assays were run in parallel.

Pure retinol acetate was hydrolyzed and extracted according to the proposed method and aliquots of either 15 or 20 mL were layered onto the columns. Four determinations of retinol were then carried out (by LC); the average results are given in Table 8. The average difference is only – 0.25%. The same procedure was applied to pure *d,l*-α-tocopherol $(\alpha\text{-T})_1$ and pure *d,l*-α-tocopherol acetate $(\alpha\text{-T})_2$. Two α-tocopherol determinations were carried out after extraction. Results given in Table 8 are averages of these 2 determinations. The average difference for $(\alpha\text{-T})_1$ and $(\alpha\text{-T})_2$ together is only 0.4%.

Double determinations of retinol and α-tocopherol were made following extractions. The average difference was only + 1.4% for retinol and + 1.7% for α-tocopherol (Table 9).

To determine how much fat the column can tolerate, we hydrolyzed increasing amounts of lard (a saturated fat) or soya-bean oil (an unsaturated fat) under the conditions given in the proposed procedure, and layered 20 mL aliquots onto the columns. The maximum amounts (in the whole hydrolysis solution) were 2.4 g lard or 3.6 g soya-bean oil. As can be seen in Tables 10 and 11, recoveries of standard retinol and α-tocopherol solutions were satisfactory up to these limits. Duplicate determinations were carried out on the extracts. The natural retinol content of lard and soya-bean oil was

Table 9. Comparison of assays with 15 and 20 mL aliquots layered onto cartridges (feedstuff solutions)

Feedstuff	15 mL (A), IU /kg	20 mL (B), IU /kg	A – B/B × 100
	Retinol		
Sheep	5 322	5 211	+ 2.1
Cattle	18 091	17 292	+ 4.6
Sow	21 316	20 760	+ 2.7
Broiler	8 342	8 458	– 1.3
Broiler	8 622	8 570	+ 0.6
Chick	9 941	9 954	– 0.1
Av. diff.			+ 1.4
	α-Tocopherol		
Laying chick	13.7	13.8	– 0.7
Pig	59.6	57.3	+ 4.0
Type unknown	61.4	62.0	– 1.0
Laying chick	12.6	12.9	– 2.3
Cattle	28.7	28.0	+ 2.5
Pig	28.2	26.3	+ 7.2
Pig	46.2	45.9	+ 0.6
Pig	34.3	34	+ 0.9
Laying chick	11.4	11.6	– 1.7
Pig	50.0	47.2	+ 5.9
Sheep	55.2	52.8	+ 4.5
Pig	23.8	23.4	+ 1.7
Av. diff.			+ 1.7

Table 10. Recoveries of retinol and α-tocopherol from hydrolysis solutions containing soya-bean oil, after extraction on cartridges

Amt oil, g	Retinol, %	α-Tocopherol, %
0	100	100
2.0	100.8	98.7
3.4	100.9	98.1
3.6	104.0	97.6
3.8	—[a]	—[a]

[a]The 20 mL aliquot of hydrolysis solution has not been completely retained by the cartridge.

Table 11. Recoveries of retinol and α-tocopherol from hydrolysis solutions containing lard, after extraction on cartridges

Amt lard, g	Retinol, %	α-Tocopherol, %
0	100	100
1.0	101.7	102.0
2.0	96.0	98.6
2.2	103.5	97.4
2.4	95.0	95.6
2.6	—[a]	—[a]

[a]The 20 mL aliquot of hydrolysis solution has not been completely retained by the cartridge.

zero. The α-tocopherol content of soya-bean oil was determined with a specific method (18). It was first estimated, then the value was subtracted from the results found for the standard α-tocopherol solutions mixed with soya-bean oil. Thus it can be concluded that the column is satisfactory until the aliquot is no longer completely retained.

Notes: (*1*) Results given in Tables 1–7, 10, and 11 were obtained with 20 mL aliquots layered onto the columns. (*2*) When retinol and α-tocopherol concentrations in the hydrolysis solution are sufficiently high, it is preferable to layer 15 mL aliquots onto the column, because of the risk of unsatisfactory column performance. (*3*) Use of disposable columns has another advantage: retinol and α-tocopherol can be hydrolyzed and extracted from a common feed sample together on the same column. We have been routinely following that in our laboratory for a year, with satisfaction.

Conclusions

The proposed extraction technique for retinol and α-tocopherol has been shown to be at least as quantitative as conventional liquid/liquid partition techniques. Extraction cartridges can be operated in series of 10 or more, with a minimum of manipulations; this allows appreciably increased rates of automated determinations (by about a factor of 2). Retinol and α-tocopherol can be hydrolyzed and extracted together from a common feedstuff sample by using the proposed technique.

Premixes as well as many feedstuffs pose no problems, but fatty samples do pose problems, particularly for large amounts (100 g). These problems seem to arise from soap formation and obstruction of kieselguhr pores by micelles. Use of hydrolyzing solutions containing more ethanol and less methanol is recommended since ethanol is known to break up soap micelles. We recently obtained good results with fatty samples when employing as much ethanol as methanol, but a general evaluation remains to be done.

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MYCOTOXINS

Rapid Screening Method for Detection of Deoxynivalenol

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A rapid method for detecting deoxynivalenol (DON), also known as vomitoxin, was developed. DON was extracted from grains and other samples with acetonitrile–4% potassium chloride solution (9 + 1). Impurities that would interfere with detection were removed on a C_{18} silica gel reverse phase column. Water was removed from eluates on a hydrophilic matrix column. DON was detected by thin layer chromatography using an aluminum chloride solution to develop the blue response characteristic of the mycotoxin. Total time involved is approximately 30 min. The method was applicable to corn, wheat, and barley at detection levels of 1 ppm, and oats at 1.5 ppm. It is applicable to environmental samples (soil, green plants, and water) at detection levels of 0.75 ppm.

Deoxynivalenol (DON), also known as vomitoxin, is a trichothecene mycotoxin associated with feed refusal and emesis in swine. The need for a rapid screening method became obvious when DON was reported in corn grown in the United States, Austria, and Canada in 1979; in wheat harvested in 1980 from southeastern Ontario, Canada; and in wheat grown in 1982 in eastern Kansas and Nebraska (1). A number of methods have been proposed for the detection of DON and other trichothecenes by color reagents. Three of these reagents, nicotinamide and 2-acetylpyridine (2), 4-(*p*-nitrobenzyl)pyridine (3), and chromotropic acid (4) are specific to trichothecenes, but reported analyses have been applied to standards, not to mycotoxins extracted from cereal grains and feeds. The 3 color reagents require a number of complicated steps to develop the color on thin layer chromatographic (TLC) plates or are suitable for use only by well trained analysts. Several other color methods have been used to detect trichothecenes in cereal grains and feeds (5–7). In one, *p*-anisaldehyde was used (7) to develop a yellow response with DON on TLC plates, but the color is not always easy to differentiate from yellow formed by other substances in the extract. These methods (5–7) also require setting up a vacuum system to hasten the development of columns.

We now report a screening method applicable to corn, wheat, barley, and oats as well as soil, green plants, and water, which uses relatively nontoxic, inexpensive chemicals and disposable equipment. It is suitable for laboratories with a minimum of equipment and does not require excessive training of personnel.

This research includes 7 types of samples spiked with standard DON solution, 2 types naturally contaminated and 1 type naturally contaminated at a low level (0.73 ppm) and then spiked with DON to reach a level of 1 ppm. A limit of detection of ≥ 1 ppm was achieved for all types, using thin layer chromatography for detection.

METHOD

Reagents

(a) *Solvents.*—Acetonitrile, ethyl acetate, chloroform, acetone, isopropyl alcohol.

(b) *Potassium chloride solution.*—4%. Dissolve 40 g ACS grade KCl in 1 L water.

(c) *Aluminum chloride solution.*—20%. Dissolve 200 g ACS grade $Al_2Cl_3.6H_2O$ in 1 L ethanol.

(d) *C_{18} silica gel.*—Waters Associates, reverse phase, 55–105 μm.

(e) *Hydrophilic matrix.*—Analtichem International, Harbor City, CA.

(f) *Deoxynivalenol standard.*—Myco Lab Co., PO Box 321, Chesterfield, MO 63017. Prepare in chloroform–acetonitrile (4 + 1) to contain 20–25 μg/mL.

(g) *Thin layer plates.*—Whatman, MK 6-F, prepoured TLC plates.

Apparatus

(a) *Hot plate.*—Any hot plate that will reach and hold 250°F.

(b) *Filter paper.*—24 cm S&S 588, or equivalent, and S&S 30 glass microfiber filters, or equivalent, cut to 20 mm diameter circles.

(c) *Dryer.*—Any air dryer that delivers ambient temperature air.

(d) *Disposable micropipets.*—Drummond, Microcaps, 10 μL.

(e) *Extraction bottle or jar.*—Dynalab Corp., PO Box 112, Rochester, NY 14692-0112, 6 oz polyethylene dispensing bottle with spout and extra screw-on cap, and pint Mason jar with screw-on lid.

(f) *Syringes.*—Becton-Dickinson, 10 mL, disposable, and Aldrich, polypropylene, 20 mL, disposable.

(g) *Pipet tips.*—Disposable, cut to fit end of 20 mL syringe and extend 5 mm through No. 1 rubber stopper.

(h) *TLC developing tank.*—Glass, slide staining jar.

Samples

All samples were analyzed for DON by a modified Scott method (8) using gas chromatography with electron capture detection. Seven types of DON-free material were used in the spiking experiments: wheat, barley, oats, corn, soil, lake water, and wild green plants. All except wheat were spiked at 3 levels: 0.75, 1.0, and 1.5 ppm. Using this method, DON cannot be detected in most wheat at a level below 1.0 ppm. Two types of material were naturally contaminated: wheat and corn. Access was limited to samples of these materials contaminated with low levels of DON. There were only 2 corn samples available, one at 0.73 ppm and the other at 1.4 ppm.

The corn sample that was naturally contaminated at 0.73 ppm was spiked with DON to equal 1.0 ppm. This was analyzed 10 times to demonstrate repeatability.

The wild green plant samples were extracted in a jar due to the excessive bulk of this material.

Extraction

Add 50 g material to be analyzed to extraction bottle, or jar. Add 50 mL acetonitrile–4% KCl solution (9 + 1). Seal

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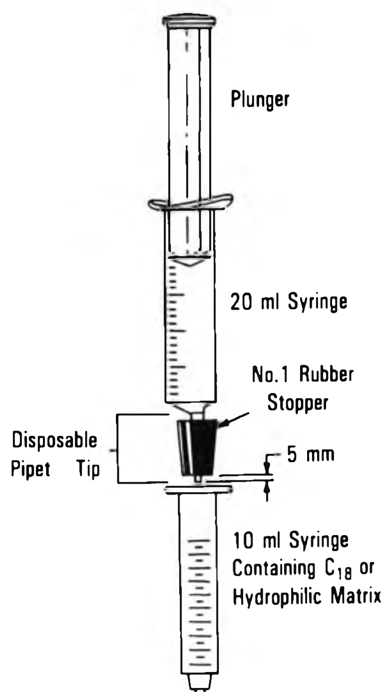

Figure 1. Cleanup apparatus.

with screw-on cap and hand-shake 5 min. Remove cap and replace with spout containing small amount glass wool in tip and 20 mm glass fiber filter. Invert bottle and squeeze gently, collecting 2+ mL filtrate. If using jar, pour through filter paper and collect 2+ mL filtrate. Save for column cleanup. For water analysis, take 2 mL water sample and go to column cleanup step. All soil samples should be poured through filter paper, collecting 2+ mL filtrate for column cleanup.

Column Preparation and Cleanup

A C_{18} silica gel column and a hydrophilic matrix (H. M.) column are needed. Prepare C_{18} column by placing very small glass wool plug in bottom of 10 mL disposable syringe. Add C_{18} silica gel to 4 mL gradation mark (2 g), tapping lightly to settle while adding, then top with another glass wool plug. Prepare H. M. column in same manner except add material up to 10 mL gradation mark.

Add 2 mL water to 2 mL filtrate. Swirl to mix. Transfer mixture to C_{18} silica gel column. Fit C_{18} column on bottom of 20 mL syringe fitted with pipet tip through rubber stopper (see Figure 1). Carefully depress plunger in 20 mL syringe to force slow elution or filtrate mixture to top of C_{18} silica gel layer. Collect total eluante. Rinse filtrate mixture container with 2 mL water and add rinse to C_{18} silica gel column. Again, use pressure to elute. Flush completely. Combine with original eluate.

Transfer 3 mL combined eluates to 20 mL syringe, minus plunger, fitted with H. M. column on bottom. Let stand 5 min. Add 3 consecutive 4 mL ethyl acetate rinses to 20 mL syringe. Collect and combine rinses in small vial. Evaporate to dryness under air dryer, or nitrogen. Do not use heat.

Thin Layer Chromatography

Add 100 μL chloroform–acetonitrile (4 + 1) to sample residue in vial and mix well. Score TLC plate with 2 vertical lines 8 mm apart, resulting in 3 channels. Apply 10 μL sample 5 mm from bottom of plate to each of 2 channels with micropipet. Superimpose 10 μL DON standard on one channel to serve as an internal standard. Also apply 10 μL DON standard in the remaining blank channel.

Develop plate to within 5 mm of top with ethyl acetate in small tank. Do not let solvent depth reach area of sample origin. Remove and air-dry. When the solvent odor is no longer detected, redevelop plate the same distance in chloroform–acetone–isopropyl alcohol (8 + 1 + 1). Remove, air-dry, then dip plate quickly in aluminum chloride solution about equal distance. Wipe back of plate dry and put on preheated (250°F) hot plate for 5–7 min. Remove, let air-cool, and observe under longwave UV light (365 nm) for evidence of DON blue response in sample as compared with the DON standard.

Results and Discussion

The 10 analyses of a single corn sample resulted in DON being easily detected in each extract. It was decided not to spike wheat at a level lower than 1 ppm due to the results from the naturally contaminated wheat samples (Table 1). DON could only be detected in one wheat sample contaminated at a level below 1 ppm, which was 0.8 ppm. Apparently, this particular sample had less interfering background material than the others.

In reference to the spiking experiments, barley and oats also have interfering background material (Table 2), but not enough to complicate analysis at levels of 1 ppm or higher. Detection of 1 ppm DON in all samples was possible.

Acetonitrile–water, Freon 113, and acetonitrile–citric acid solution were some of the experimental extraction solvents. Extraction of DON with acetonitrile–4% KCl solution (9 + 1) resulted in extracts that could be cleaned up for TLC. Development with ethyl acetate, drying plates, and redevelopment with chloroform–acetone–isopropyl alcohol (8 + 1 + 1) separated DON from interfering substances, making detection possible. Pretreatment of the TLC plates used with the aluminum chloride solution caused a streaking problem

Table 1. DON detection in naturally contaminated products

Product	Actual DON, ppm	Detected[a]
Wheat	2.97	+
	2.55	+
	2.39	+
	1.64	+
	1.35	+
	0.5	(−)
	1.24	+
	0.87	(−)
	0.56	(−)
	1.77	+
	0.80	+
	0.53	(−)
Corn	1.4	+
	0.73	+

[a] + = DON presence; (−) = not detected.

Table 2. DON detection in spiked products

Product	DON level, ppm[a] 0.75	1.0	1.5
Wheat		+	+
Barley	TR	+	+
Oats	(−)	TR	+
Water	+	+	+
Soil	+	+	+
Weeds	+	+	+
Corn	+	+	+
Corn	+	+	+
Corn	+	+	+
Corn	+	+	+

[a] + = DON presence; TR = trace amount of DON; (−) = not detected.

on the small plates, which made DON detection impossible. The double development of the TLC plates removes much of the interfering materials from the area of the DON. The use of a syringe as a pressure device, another as a cleanup column, and a third as a dehydrator as well as the other disposable items gives this method additional mobility in the field.

Work is continuing on modification of this method for quantitative analysis incorporating high pressure liquid chromatography and high performance TLC as detection methods.

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Screening and Quantitation of Ochratoxin A in Corn, Peanuts, Beans, Rice, and Cassava

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To answer the need for simple, economical, rapid methods for mycotoxins, a procedure for screening and quantitation of ochratoxin A was developed. A methanol–aqueous KCl extraction is used, followed by cleanup with clarifying agents and partition into chloroform. Part of the chloroform extract is used for screening and the other part for quantitation by thin layer chromatography (TLC). The screening procedure takes 40 min, using a silica gel/aluminum oxide minicolumn developed for this purpose. The limits of detection are 80 and 10 μg/kg, respectively, for minicolumn screening and TLC quantitation. Ammonium sulfate is efficient in cleaning samples of corn and cassava; cupric sulfate is better with peanuts, beans, and rice. Tests were conducted on triplicate spiked samples of yellow corn meal, raw peanuts, dried black beans, polished rice, and cassava flour at different levels (400, 200, 80, 40, and 10 μg/kg). Recoveries ranged from 86 to 160% and the coefficients of variation ranged from 0 to 26%.

It is generally assumed that the mycotoxin problem is more serious in developing countries where the climatic conditions and the agricultural and storage practices are considered conducive to fungal growth and toxin production. Yet, in these countries, data on mycotoxin incidence is either inadequate or unavailable. This is due, at least in part, to the lack of analytical methods suitable to existing laboratory resources and conditions.

Several methods are described in the literature for determining ochratoxins (1–5), but they are expensive, lengthy, and labor-intensive. The need for simple, rapid, economical methods is deeply felt in developing countries where the cost of the analysis is often the first factor considered before a method is adopted. For this reason, we set out to develop a method for determining ochratoxin A, which is uninvolved and inexpensive, but would not compromise precision and accuracy.

The proposed method includes extraction with methanol–aqueous 4% KCl (9 + 1) and cleanup with either $(NH_4)_2SO_4$ or $CuSO_4$. Partition into chloroform follows and the resulting extract is used for both screening and quantitation. The procedure is simple enough to be used by unskilled personnel after a short training.

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We found no report of the use of methanol–aqueous KCl to extract ochratoxins and, to our knowledge, this mixture was used in only one case for sterigmatocystin (6). On the other hand, methanol–4% aqueous NaCl (6 + 4) has been used to extract ochratoxin A from olive oil and olives (7, 8); methanol–water (55 + 45) for ochratoxin A in wheat, corn, barley, and rice (9); and methanol–alkaline water (1 + 1) for ochratoxin A in pig kidney (10). Ammonium sulfate (30% aqueous solution) has been used to clean wheat, barley, corn (11), and mixed feed (12) extracts in trichothecene determinations but not for ochratoxins. Only one minicolumn for ochratoxin A has been reported (13); it consists entirely of Florisil, and is developed with methanol followed by dilute H_2SO_4. The ochratoxin band, however, diffuses throughout the first half of the column with consequent loss of sensitivity.

METHOD

Apparatus and Reagents

(a) *Blender.*—Waring, with 1 qt jar. Explosion-proof not necessary.

(b) *TLC apparatus.*—Stahl-type (Desaga, Heidelberg, GFR).

(c) *TLC plates.*—20 × 20 cm glass, coated with 0.25 mm silica gel G 60 (E. Merck). With scriber, divide plates into twenty 1 cm strips. Activate plates before use in 110°C oven for 1 h.

(d) *Ultraviolet light.*—Longwave tube, 366 nm (Sylvania F8T5 BLB/USA) in suitable stand.

(e) *Minicolumns.*—8 mm (od) × 20 cm, 6 cm neck length after tapering to 4 mm (od).

(f) *Minicolumn packing materials.*—*(1)* Alumina, neutral, 100–200 mesh (E. Merck). *(2)* Silica gel 60, 70–230 mesh (E. Merck). *(3)* Calcium sulfate, anhydrous, 20–40 mesh, prepared from powdered anhydrous calcium sulfate (E. Merck). Dry 1–2 h at 110°C and keep in desiccator.

(g) *Hyflo Super-Cel.*—Or equivalent product.

(h) *Extraction solvents.*—*(1)* Methanol (E. Merck). *(2)* Potassium chloride solution, 4%, 40.00 g KCl/L. *(3)* Chloroform (E. Merck).

Table 1. Evaluation of clarifying agent effectiveness

Product[a]	Clarifying agent		TLC	Minicolumn
Yellow corn meal	$(NH_4)_2SO_4$	30%	no streaking, single spot[b]	no interference, tight band
	$CuSO_4$	10%	no streaking, additional spot	no interference, tight band
Cassava flour	$(NH_4)_2SO_4$	30%	no streaking, additional spot	no interference, tight band
	$CuSO_4$	10%	light streaking, additional spot	no interference, tight band
Raw peanuts	$(NH_4)_2SO_4$	30%	no streaking	interference at silica/alumina interface
	$CuSO_4$	10%	no streaking, single spot	no interference, tight band
Dried black beans	$(NH_4)_2SO_4$	30%	—[c]	—
	$CuSO_4$	10%	no streaking, additional spot	no interference, tight band
Polished rice	$(NH_4)_2SO_4$	30%	no streaking, single spot	no interference, tight band
	$CuSO_4$	10%	no streaking, single spot	no interference, tight band

[a]Each sample was spiked with ochratoxin A to a level of 200 μg/kg.
[b]Single spot corresponds to ochratoxin A.
[c]Partition into chloroform was not possible because of strong emulsion.

(i) *Clarifying agents.—(1)* Cupric sulfate solution, 10%. 100.00 g $CuSO_4$/L. *(2) Ammonium sulfate*, 30%, 300.00 g $(NH_4)_2SO_4$/L (E. Merck).

(j) *TLC developing solvent.*—Toluene–ethyl acetate–formic acid (5 + 4 + 1).

(k) *Minicolumn developing solvent.*—Toluene–ethyl acetate–acetic acid (50 + 49 + 1).

(l) *Ochratoxin A standard solutions.*—10 and 1 μg/mL benzene (Makor Chemicals Ltd, Israel). Prepare from stock solution. Determine exact concentration as described in *Official Methods of Analysis* (5).

Extraction

Prepare sample according to **26.003** (5). Weigh 50 g sample into blender jar. Add 270 mL methanol and 30 mL aqueous 4% KCl solution. Blend 5 min at low speed. Filter mixture through fluted qualitative filter paper. Transfer 150 mL aliquot of filtrate to 600 mL beaker.

Cleanup

Add to this aliquot 150 mL clarifying solution ($(NH_4)_2SO_4$ or $CuSO_4$, depending on sample) and 50 mL Hyflo Super-Cel, measured in beaker. Stir with glass rod and filter through fluted qualitative paper. Transfer 150 mL aliquot to separatory funnel and add 150 mL water. Extract twice with 10 mL portions of chloroform.

Screening Test

Place small plug of glass wool at end of minicolumn. Add calcium sulfate layer ca 5 mm in height, neutral alumina 10 mm, silica gel 20 mm, and calcium sulfate 10 mm.

To prepared minicolumn, add 2 mL chloroform extract from first extraction and let drain until it reaches top of calcium sulfate layer, then add 4 mL toluene–ethyl acetate–acetic acid (50 + 49 + 1). If ochratoxin A is present, a blue, tight fluorescent band will be seen at silica gel/alumina interface. Reference minicolumn may be prepared by letting ochratoxin A standard solution, dissolved in 2 mL chloroform, seep through column, followed by 4 mL toluene–ethyl acetate–acetic acid mixture. Detection limit for pure standard and spiked commodities tested is 80 μg/kg.

Quantitation by Thin Layer Chromatography

Combine 3 mL each of first and second chloroform extractions in 10 mL vial. Evaporate just to dryness in 80°C water bath under nitrogen stream. Redissolve residue in 200 μL benzene and place closed vial in ultrasonic bath for 30 s to ensure complete dissolution.

On TLC plate, 2 cm from bottom, spot standards and samples. Place plate in unsaturated tank with toluene–ethyl acetate–formic acid (5 + 4 + 1) TLC developing solvent. After development, let plate dry. Visualize ochratoxin A under longwave ultraviolet light and compare spot intensities. If necessary, respot samples and standards on another plate to obtain closer match between sample and standard spot intensities. Perform calculations according to AOAC (5). For confirmation, derivatization as proposed by Hunt et al. (10) may also be used.

Results and Discussion

Cleanup

Considering its crucial role in identification, sensitivity, and proper quantitation by TLC, our first efforts were directed to delineating the cleanup step. Clarifying agents were chosen because of their relative low cost and for being more rapid than most systems except for Sep-Pak or similar cartridges. Preliminary tests with ammonium sulfate were satisfactory, but it was clear from the start that it would not serve for all of our samples. Cupric carbonate was among other chemicals tried as a second clarifying agent. Its use yielded very poor recoveries (below 30%), but led us to try cupric sulfate in the belief that the cupric ion plays the active part in the clarifying process. The latter yielded complete recovery. Its action may result from its complexing properties, independent of whether it causes precipitation. By complexation, it might avoid partition of pigments and fluorescing compounds from the aqueous KCl–methanol phase into chloroform.

Ammonium sulfate and cupric sulfate were compared by assessing the extract behavior on TLC plate and minicolumn. For TLC, 2 characteristics are considered desirable: the absence of additional spots and elimination of streaking. For minicolumn chromatography, the desired result is the absence

Table 2. Recovery and reproducibility data for ochratoxin A added to some products

Product and clarifying agent	Ochratoxin added, µg/kg	Av. rec., %	CV,[a] %
Yellow corn meal + ammonium sulfate	400	86	21
	200	102	4
	80	111	17
	40	111	17
	10	89	18
Polished rice + cupric sulfate	400	93	14
	200	107	0
	80	133	0
	40	133	0
	10	107	0
Raw peanuts + cupric sulfate	400	107	0
	200	107	0
	80	133	0
	40	133	0
	10	160	0
Cassava flour + ammonium sulfate	400	98	16
	200	125	24
	80	133	0
	40	118	26
	10	107	0
Dried black beans + cupric sulfate	400	98	16
	200	107	0
	80	133	0
	40	133	0
	10	133	0

[a]Based on 3 determinations for each level.

of interfering coextractants at the silica gel/aluminum oxide interface. The results (Table 1) show that ammonium sulfate performs better in samples of corn and peanuts, and cupric sulfate is better in cassava and beans. Both perform well in polished rice, which hardly needs any cleanup.

For extraction, preliminary tests with pure methanol, methanol–water (1 + 1), and acetone–water (85 + 15) for ochratoxin A in corn exhibited poor recoveries (below 50%).

Minicolumn

For an ideal minicolumn, a tight band should be obtained and co-extractives should either elute or be located far from the mycotoxin band. A tight band is essential for sensitivity, and this is achieved when the mycotoxin is positioned at an interface of 2 adsorbents, which means the mycotoxin is unable to migrate in the second adsorbent. Several adsorbents in several combinations plus different developing solvents were tried. The silica gel/neutral aluminum oxide minicolumn in combination with toluene–ethyl acetate–acetic acid (50 + 48 + 1) gave the best results. Ochratoxin A exhibits a tight band and no interferences were observed in the extracts of the commodities tested. The detection limit was 80 µg/kg for all samples tested. We found Holaday's minicolumn (13) less sensitive probably because of spreading of the ochratoxin band on the minicolumn adsorbent (Florisil).

Recovery and Reproducibility Studies

The proposed quantitative method was evaluated by using spiked samples. Uncontaminated ground samples of yellow corn meal, raw peanuts, dried black beans, polished rice, and cassava flour were spiked to levels of 400, 200, 80, 40, and 10 µg ochratoxin A/kg by weighing a 50 g sample in a beaker and adding the desired amount of standard solution with a pipet. The standard solution was allowed to drop at different points on the center top of the mass. No mixing was done, to prevent standard solution from adhering to the walls of the container. The solvent from the standard solution was allowed to dry naturally overnight. The spiked sample was transferred to the blender jar and ochratoxin A was determined as described. The entire procedure was repeated 3 times for each level of spiking for each commodity. Quantitation was done by an observer who was unaware of the sample concentrations, and who matched spot intensity of ochratoxin A in sample with the closest standard spot.

Recoveries are shown in Table 2 together with reproducibility data. These results compare well with those of other methods that show equivalent recoveries (9) as well as against others which exhibit either much lower (14, 15) or variable (16, 17) recoveries. Recoveries were evaluated even at the detection limit which is not usually done.

The detection limit is 10 µg/kg for TLC, lower than for most methods described in the literature (9, 13, 14, 16–21) and equivalent to the most sensitive (1, 2). In this work, we also observed that the use of fluorescence enhancers such as aluminum chloride or sodium bicarbonate spray solutions or exposure to ammonium vapor actually decreased the fluorescence. For this reason, only the ochratoxin A natural fluorescence was used. The best detection limits observed so far were reported by researchers (1, 2) who also did not use fluorescence enhancers.

The time involved in the screening is 40 min and the whole procedure takes about 2½ h. The cost, moreover, is much lower than that of other methods that specify expensive reagents; reagents specified here are in common use.

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VETERINARY ANALYTICAL TOXICOLOGY

Liquid Chromatographic Determination of Strychnine in Stomach Contents

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A chloroform extract of stomach contents at basic pH is concentrated and then extracted with 0.1M phosphoric acid. The acid extract is chromatographed on a 10 cm reverse phase column, using 0.005M phosphate buffer (pH 3.0)–acetonitrile–tetrahydrofuran (750 + 135 + 115) containing 0.01M octanesulfonic acid at a flow rate of 1.0 mL/min for elution. Strychnine eluted in 7.3 min. Recoveries from spiked stomach contents averaged 92%. The method can be used without modification for other alkaloids.

Strychnine continues to be a relatively common cause of accidental and malicious poisoning in dogs (1) and, less frequently, in other animal species. The usual specimen submitted for laboratory analysis in such cases is the stomach contents.

Several methods for liquid chromatographic (LC) separation and determination of strychnine have been described (2–7). Most of these methods specify chloroform extraction at basic pH. When biological samples are analyzed, this results in co-extraction of most lipids present, which is undesirable because the injection of large amounts of apolar lipids on a reverse phase partition column or of polar lipids on an adsorption/normal phase column can adversely affect the LC separation. However, by re-extracting the chloroform extract with 0.1M aqueous H_3PO_4, many basic components can be selectively separated from the lipids. This has enabled development of a reverse phase LC method to quantitatively extract and determine strychnine from the stomach contents in suspected poisoning cases.

METHOD

Apparatus and Reagents

(a) *Liquid chromatographic system.*—Tracor 951 pump, Rheodyne 7125 valve injector with 20 μL loop, Tracor 970 variable wavelength detector set at 254 nm, and Perkin-Elmer Sigma 10 data station. Chromatographic separations were made on a Brownlee Labs 4.6 mm id × 10 cm MPLC RP-8 Spheri-5 analytical column protected by a NewGuard RP-8 column. Mobile phase was 0.005M sodium dihydrogen phosphate buffer (pH 3.0)–acetonitrile (May and Baker, Australia)–tetrahydrofuran (Ajax Chemicals, Australia) (750 + 135 + 115), containing 0.01M octanesulfonic acid sodium salt (Sigma Chemical Co., St. Louis, MO); flow rate 1.0 mL/min; temperature ambient.

(b) *Standard solution.*—Strychnine sulfate.$5H_2O$ (Sigma Chemical Co.), 10 μg/mL 0.005M sodium dihydrogen phosphate buffer (pH 3.0).

Preparation of Sample

Weigh 7.5 g stomach contents into 70 mL screw-cap Nalgene LPE bottle (Sybron/Nalge, Rochester, NY). Add 30 mL 0.2M NaOH and mix. Accurately add 30 mL chloroform and shake mechanically 10 min. Centrifuge at 3000 rpm for 15 min. Filter lower chloroform layer through Whatman 1PS paper and pipet 10 mL filtrate into 25 mL pear-shape flask. Evaporate to dryness and quantitatively transfer residue to glass-stopper reagent tube with 2.0 mL chloroform. Accurately add 2.0 mL 0.1M H_3PO_4 and shake on vortex-mixer 2 min. Centrifuge 5 min and use upper aqueous layer for analysis.

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Determination and Calculation

Inject 20 μL aliquots of both standard solution and sample. Use peak areas to calculate strychnine concentrations as shown:

$$\mu g\ strychnine/g = (A_u/A_s) \times (W_s/W_u) \times 0.39 \times D$$

where A_u and A_s = peak areas of sample and standard, respectively; W_s = μg strychnine sulfate/mL standard solution; W_u = g sample extracted; D = dilution factor; and 0.39 = factor for strychnine sulfate.$5H_2O$.

Results and Discussion

The detector response at 254 nm for strychnine was linear over the range tested (0–370 ng). The coefficient of variation was 0.9% for 6 consecutive 73 ng strychnine standard solution injections. The detection limit for strychnine, using the described analytical procedure, was 4.5 μg per sample (8). The average recovery of strychnine from stomach contents spiked at 7.0 μg/g was 92.0 ± 3.6%.

Figure 1 shows chromatograms from stomach contents with and without added strychnine. The large peak in the chromatogram was identified, by means of its retention time and ultraviolet spectrum, as tryptamine; this is often present

Table 1. Extraction of alkaloids from 0.2M NaOH into $CHCl_3$ (A) and from $CHCl_3$ into 0.1M H_3PO_4 (B)

Alkaloid	Extracted, %	
	A	B
Brucine	99	92
Colchicine	95	<1
Ergometrine	74	67
Eserine	87	52
Gramine	99	99
Papaverine	99	3
Piperine	99	<1
Reserpine	99	<1
Strychnine	99	99
Vincamine	99	75
Yohimbine	95	82

Table 2. Retention times of alkaloids

Alkaloid	Retention time, min
Colchicine	2.38
Brucine	4.93
Eserine	5.56
Ergometrine	6.63
Strychnine	7.28
Gramine	7.87
Papaverine	10.78
Vincamine	24.06
Yohimbine	31.77
Piperine	34.99
Reserpine	not eluted

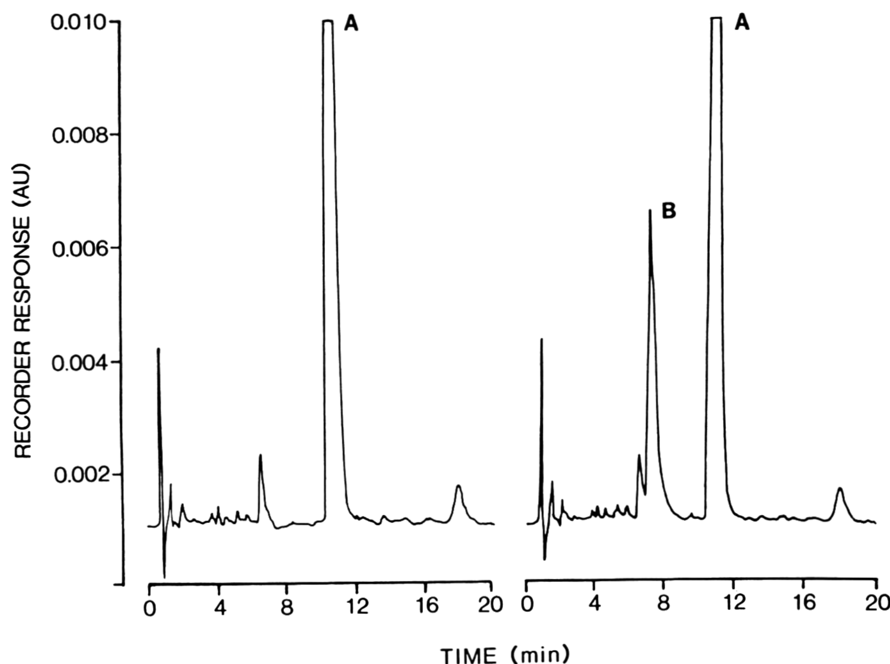

Figure 1. Chromatograms of (left) 20 μL injection of canine stomach content extract blank, equivalent to 25 mg sample; (right) 20 μL injection of canine stomach content extract spiked with 7.0 ppm strychnine: A, tryptamine; B, strychnine. Conditions are given under method.

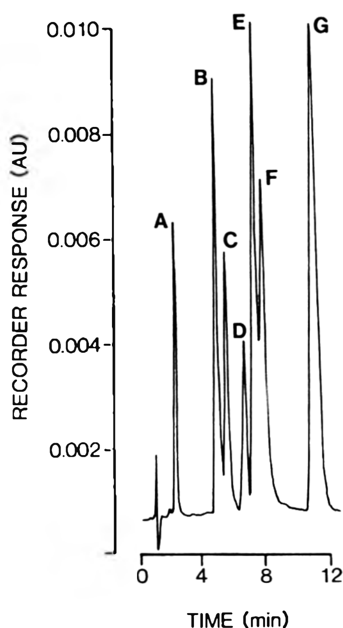

Figure 2. Chromatogram of standard alkaloids: A, colchicine; B, brucine; C, eserine; D, ergometrine; E, strychnine; F, gramine; G, papaverine. Conditions are given under method.

in stomach contents, being derived from the bacterial decarboxylation of tryptophan in the gastro-intestinal tract (9).

The double extraction method selectively extracts basic organic components; therefore, its usefulness for the extraction of other alkaloids was tested. The alkaloids (10 mg/L) were extracted from 0.2M NaOH into chloroform and then into 0.1M H_3PO_4; absorption measurements at 254 nm were used to calculate the proportions extracted (Table 1). The extraction procedure is clearly unsuitable for colchicine, papaverine, piperine, and reserpine; it should work very well with gramine and the other strychnos alkaloid brucine, and would be suitable to various extents for the other alkaloids tested.

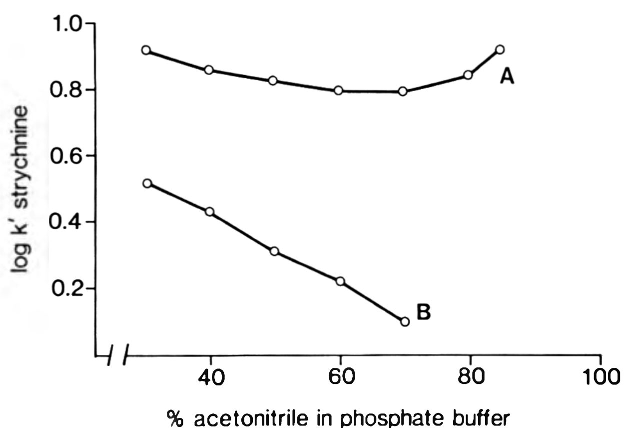

Figure 3. Dependence of log capacity factor for strychnine on acetonitrile content of eluant containing no (A) and 0.01M octanesulfonic acid (B).

Table 2 shows the retention times for the various alkaloids under the standard conditions used for strychnine, and Figure 2 shows the separation achieved. Brucine and strychnine gave baseline separation while gramine and strychnine showed a resolution of 0.97, which is adequate for quantitative analysis (10). The LC system showed 2500 theoretical plates for the strychnine peak, using the half peak height method (11).

Incorporation of octanesulfonic acid as counter-ion in the eluant had a marked effect on retention characteristics and resulted in linear dependence of log k' on organic solvent concentration (Figure 3). The octanesulfonic acid also increased resolution by reducing peak tailing. The eluant composition was optimized by varying the acetonitrile–tetrahydrofuran ratio while keeping the phosphate buffer and octanesulfonic acid concentrations constant at 75% and 0.01M, respectively.

The efficiency of the system was increased to 3500 theoretical plates by the additional incorporation of 0.001M tetrabutylammonium hydroxide in the eluant but this reduced the relative differences in retention times for most alkaloids. However, where other alkaloids do not interfere, the incor-

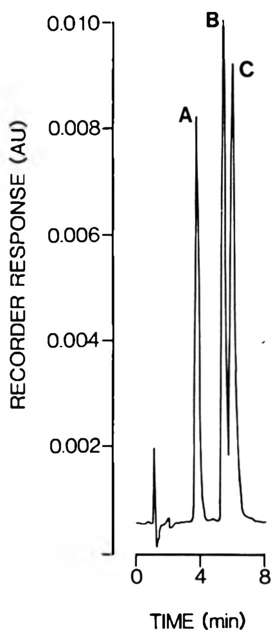

Figure 4. Chromatogram of brucine (A), strychnine (B), and gramine (C). Eluant: 0.005M phosphate buffer (pH 3.0)–acetonitrile–tetrahydrofuran (750 + 100 + 150) containing 0.01M octanesulfonic acid and 0.001M tetrabutylammonium hydroxide; flow rate 1.0 mL/min.

poration of both octanesulfonic acid and tetrabutylammonium hydroxide is advantageous (Figure 4).

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PESTICIDE FORMULATIONS

Normal Phase Liquid Chromatographic Determination of Pyrethrins in Formulations

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A liquid chromatographic method has been developed to quantitate pyrethrins in pesticide formulations. Samples were dissolved in tetrahydrofuran (THF) and injected onto an amino column with a solvent system of hexane–nonstabilized THF (90 + 10) at a flow rate of 1.5 mL/min. Detection was monitored at 240 nm and 0.4 AUFS. Total elution time was 7 min. Twelve products varying in concentration from 0.05 to 3.75% and formulated with numerous other ingredients were analyzed. Percent coefficients of variation ranged from 1.39 to 9.68 with the majority less than 5.00. Although piperonyl butoxide and *N*-octyl bicycloheptene dicarboximide (MGK 264) were not quantitated, neither interfered with the pyrethrin analysis.

Pyrethrins are a group of 6 closely related insecticides naturally occurring in the plant *Chrysanthemum cinerarieafolium*. They can be divided into 2 categories: *(1)* chrysanthemates comprised of pyrethrin I, cinerin I, and jasmolin I and *(2)* pyrethrates consisting of pyrethrin II, cinerin II, and jasmolin II. Of these 6, pyrethrins I and II predominate, followed by cinerins I and II and jasmolins I and II, respectively. Recently, the pyrethrins have been partially replaced by the synthetic pyrethroids, but the pyrethrins are still extensively used in household formulations for plants and pets.

The first method for the analysis of pyrethrin formulations was a titrimetric procedure (1) that is very nonspecific. It was not until the development of gas (2), thin layer (3), and liquid chromatographic (4, 5) procedures that the quantitation of pyrethrins became more specific. However, none of these methods can be used to analyze formulations that contain less than 0.1% pyrethrins (of which there are many).

This paper describes a modification of the LC procedure of Mourot et al. (6) for analyzing pyrethrin formulations ranging in concentrations of 0.05 to 3.75%. The synergists, piperonyl butoxide and *N*-octyl bicycloheptene dicarboximide, along with other active and inactive ingredients do not interfere.

METHOD

Apparatus and Reagents

(**a**) *Liquid chromatograph.*—Waters Associates (Milford, MA) 6000A pump, U6K injector, and M490 detector; Schoeffel variable wavelength UV detector (Westwood, NJ); and Omni-scribe recorder (Houston Instrument, Austin, TX). Operating conditions: injection volume, 5 μL; flow rate, 1.5 mL/min; wavelength, 240 nm; absorbance range, 0.4 AUFS; recorder setting, 10 mV; chart speed, 1.0 cm/min.

(**b**) *Chromatographic column.*—5 μm amino column (IBM Instruments, Danbury, CT) 25 cm × 4.5 mm id stainless steel.

(**c**) *Mobile phase.*—Hexane–nonstabilized tetrahydrofuran (THF) (90 + 10). Both solvents LC grade (Fisher Scientific, Fair Lawn, NJ).

(**d**) *Sample extraction solvent.*—Stabilized ACS grade THF (Fisher Scientific).

(**e**) *Standard solutions.—Pyrethrin stock standard solution.*—Weigh 112 mg 20% technical pyrethrin (Environmental Protection Agency, Research Triangle Park, NC) into 10 mL actinic volumetric flask and dilute to volume with stabilized THF. *Working standard solution.*—Take 2 mL pyrethrin stock standard and place in 25 mL actinic volumetric flask. Dilute to volume with stabilized THF. Actinic glassware is necessary because pyrethrins degrade readily in most types of light.

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Preparation of Sample

(**a**) *Liquids.*—Weigh sample equivalent to 4.5 mg pyrethrin into 25 mL actinic volumetric flask. Dissolve sample and dilute to volume with stabilized THF. Inject 5 μL into LC system.

(**b**) *Aerosols.*—Weigh sample equivalent to 4.5 mg pyrethrin into 25 mL actinic volumetric flask using the previous official method of sampling pressurized containers (7).

Determination

Inject standard, followed by 2 injections of sample, and finally another standard. Measure peak heights, average, and substitute into formula below:

$$\% \text{ Pyrethrins} = (H/H') \times (W'/W) \times \% \text{ purity std}$$

where H and H' = average peak heights of sample and standard, respectively; W' = g pyrethrin standard/25 mL; and W = g sample extracted. The % pyrethrins is calculated on the basis of one of the major peaks (pyrethrin I) of the pyrethrin extract.

Results and Discussion

The liquid chromatographic (LC) method developed to analyze pyrethrin formulations was a modification of the method of Mourot et al. (6). Both procedures are normal phase separations, but differ in the column, mobile phase, and wavelength used. Mourot et al. (6) used a silica column with a mobile phase of hexane–ethyl acetate (90 + 10) monitored at 254 nm, whereas the modified method used an amino column with a mobile phase of hexane–THF (90 + 10) monitored at 240 nm. These changes were made to provide more stability, quicker analyses, and increased sensitivity.

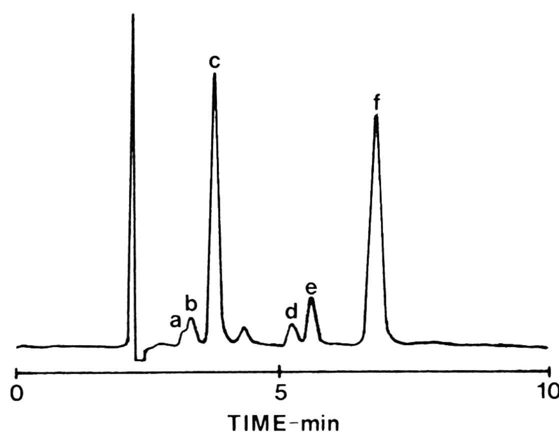

Figure 1. Liquid chromatogram of 20% pyrethrin technical standard: a, jasmolin I; b, cinerin I; c, pyrethrin I; d, jasmolin II; e, cinerin II; f, pyrethrin II.

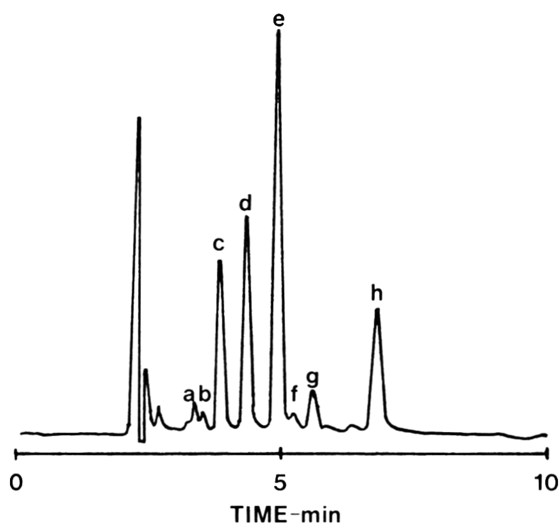

Figure 2. Liquid chromatogram of a pyrethrin formulation containing synergists: a, jasmolin I; b, cinerin I; c, pyrethrin I; d, piperonyl butoxide; e, MGK 264; f, jasmolin II; g, cinerin II; h, pyrethrin II.

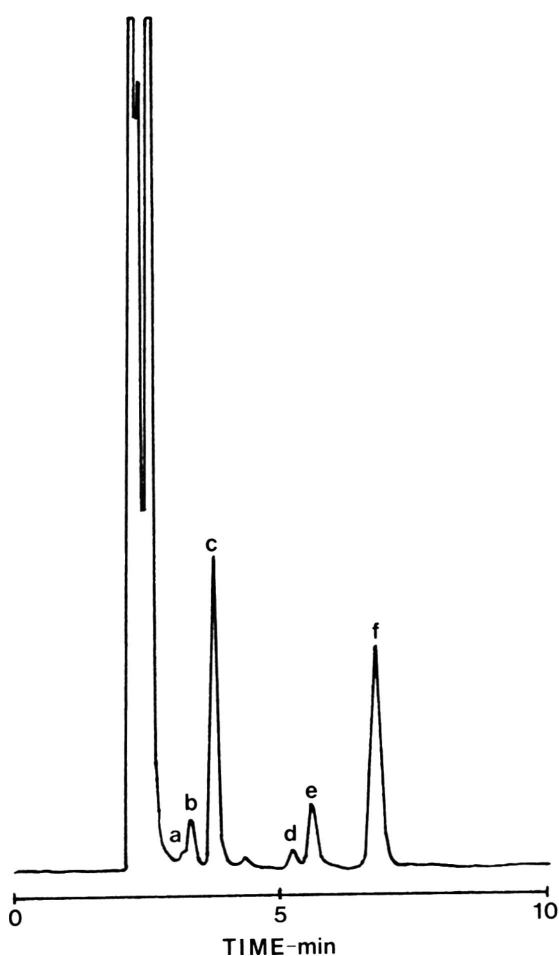

Figure 3. Liquid chromatogram of a pyrethrin formulation containing many emulsifiers and aromatic solvents: a, jasmolin I; b, cinerin I; c, pyrethrin I; d, jasmolin II; e, cinerin II; f, pyrethrin II.

A typical separation of a technical pyrethrin in standard and 2 formulations are shown in Figures 1–3. The 6 pyrethrin constituents are separated within 7 min (Figure 1). Chromatograms depicting the 2 samples were chosen to indicate where other active and inactive ingredients elute. For example, the formulation represented by Figure 2 contained the synergists piperonyl butoxide and *N*-octyl bicycloheptene dicarboximide; the other product (Figure 3) contained numerous solvents and emulsifiers. None of these ingredients interfere with the analysis of pyrethrins. The synergists elute after pyrethrin I while the inactive components come off before any of the pyrethrins.

Table 1. Comparison of calculation methods for pyrethrin formulations[a]

Sample	Form., %	Found, % (all 6)	Found, % (PyI)	Found, % (PyII)
Liquid	0.80	0.74	0.76	0.65
Liquid	0.80	0.75	0.78	0.67
Liquid	0.80	0.78	0.82	0.73
Aerosol	0.50	0.40	0.45	0.28
Liquid	0.33	0.30	0.31	0.25
Aerosol	0.20	0.19	0.20	0.17
Aerosol	0.18	0.19	0.18	0.16
Liquid	0.05	0.05	0.05	0.04
Pump	0.06	0.06	0.06	0.05
Pump	0.06	0.06	0.05	0.05
Aerosol	0.05	0.04	0.04	0.03

[a] All samples analyzed once.

There are no analytical standards available for individual pyrethrins, so quantitation must be based on a technical mixture, the purity of which must be accurately determined. To avoid the situation in which the technical standard and the technical material must come from the same source, one can use either the total peak height of the 6 pyrethrins or the height of pyrethrin I.

A comparison of 3 different calculation methods for quantitating pyrethrins in formulations is shown in Table 1. The agreement is good between the 6-pyrethrin peak technique and pyrethrin I peak procedure. However, when pyrethrin II (the other major pyrethrin peak) is used, the % pyrethrin values are lower. The peak height method for pyrethrin I was selected for use in this method because pyrethrin I shows more consistency than the other 5 pyrethrins in different sources of technical pyrethrins. Therefore, as long as one uses either all 6 constituents or pyrethrin I for the calculations, the values should be correct.

Samples for this study were chosen to give a representative sampling of pyrethrin formulations (Table 2). These products varied in the amount of pyrethrins and other pesticides along with the kind and amount of inactive ingredients.

The linearity of response for the pyrethrins was determined at 240 nm. The concentration of pyrethrins could be determined by direct comparison of peak heights for a sample and standard because they were shown to be linear over a wide concentration range. In fact if the sample concentration was higher or lower than the standard peak, a direct comparison still could be made.

To demonstrate the accuracy and reproducibility of this LC method, 12 formulations varying in pyrethrin concentrations from 0.05 to 3.75% were analyzed 5 times each (Table 2). For all but 2 samples, the percent coefficients of variation were below 5.0% and all were below 10.0%. The analyses were performed over a period of 5 days with 1 analysis per sample per day.

To further test this method, 4 formulations were analyzed using the reverse phase method of Bushway et al. (5) that was developed earlier (Table 3). The agreement between these formulations analyzed by 2 different methods is good, which further supports the validity of the normal phase procedure. Also peak purities were checked using absorbance ratios which agreed with the standards.

In conclusion, this LC method for the analysis of pyrethrins is simple, rapid, and precise, considering the complexity of the pyrethrins and their formulations.

Table 2. LC analysis of pyrethrin formulations[a]

Sample	Pyrethrins			Other active ingredients
	Form., %	Found, %	CV, %	
1. Liquid	0.80	0.79	2.18	1.1% rotenone
2. Liquid	0.80	0.79	2.08	1.1% rotenone
3. Liquid	0.80	0.82	1.39	1.1% rotenone
4. Aerosol	0.50	0.45	4.61	1.0% PBO,1.67% MGK 264
5. Liquid	3.75	3.97	1.68	3.75% PBO
6. Liquid	0.33	0.32	3.13	0.67% PBO,1.11% MGK 264
7. Aerosol	0.20	0.20	2.14	none
8. Aerosol	0.18	0.19	2.58	0.081% tetramethrin, 1.05% PBO
9. Liquid	0.05	0.05	3.63	0.10% PBO,0.17% MGK 264
10. Pump	0.06	0.05	2.46	0.12% PBO,0.20% MGK 264
11. Pump	0.06	0.06	5.39	0.20% PBO,0.20% MGK 264
12. Aerosol	0.05	0.04	9.68	0.09% PBO,0.15% MGK 264

[a]Means of 5 determinations for each sample.

Table 3. Comparison of 2 LC methods for pyrethrin formulations[a]

Sample	Form.,%	Normal phase		Reverse phase	
		Found, %	CV, %	Found, %	CV,%
Liquid	0.80	0.79	2.18	0.75	2.49
Liquid	0.80	0.79	2.08	0.77	2.38
Liquid	0.80	0.82	1.39	0.80	2.58
Aerosol	0.20	0.20	2.14	0.22	4.09

[a]Means of 5 determinations for each sample.

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Capillary Gas Chromatographic Determination of Pyrethrins in Low Level Formulations

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Low level pyrethrin formulations are extracted with tetrahydrofuran and determined via capillary gas chromatography with electron capture detection. Quantitation is based on the sum of peak heights of the 6 pyrethrin esters. The method was tested for precision, linearity, and recovery. Quadruplicate analysis of 5 formulations gave an average relative standard deviation of 3.3%. Linearity and recovery were excellent (correlation coefficient = 0.997, recovery = 101.2%). The data presented support the use of the method for formulations that cannot be analyzed by the AOAC official method.

The official AOAC method for the analysis of pyrethrins (1) is an excellent gas chromatographic (GC) technique which, unfortunately, is not applicable to shampoo products, some oil-containing products, or products containing less than 0.1% active ingredient. As the market for pyrethrins shifts to consumer uses, an increasing number of formulations fall into these categories. Liquid chromatographic methods (2, 3) may handle these types of samples but often require large sample weights which affect chromatography and thus distort the results. Regulatory laboratories often are forced to rely on the analysis of synergists in evaluating samples.

The response of an electron capture detector (ECD) to the 6 components of pyrethrins suggests that some of these limitations might be overcome. The sensitivity of the ECD should allow the use of less sample, which will ease the miscibility problems associated with shampoos, some aerosols, and other occasional formulations. This would also be beneficial in cases where a low level sample is available only in a limited amount. The selectivity of the ECD should allow the analysis of oil-diluted formulations that are impossible to analyze by the official method. The main difficulty in using the ECD to determine pyrethrin levels is that the detector very quickly becomes fouled and must be frequently cleaned. This problem can be overcome by making additional dilutions or by using capillary GC with split injection.

The official method provides fairly precise results despite basing quantitation on only 2 of the 6 components, cinerin I and pyrethrin I. Quantitation based on the 6 well resolved peaks generated by capillary gas chromatography may offset the imprecision inherent in the split injection technique. Thus, the combination of capillary GC and electron capture detection may yield a method comparable to the official method for these problem samples. This paper investigates this combination. The method reported here has been tested for linearity, precision, and recovery.

METHOD

Apparatus

(a) *Gas chromatograph.*—Varian Model 3700 equipped with split capillary injector and fritted insert with about 1 cm silanized glass wool loosely packed below the frit, Varian Model 8000 autosampler, and electron capture detector (Varian Associates, Palo Alto, CA 94303), or equivalent.

(b) *Chromatographic column.*—Durabond DB-1 0.25 μm thick bonded phase on fused silica, 15 m × 0.32 mm (Alltech Associates, Deerfield, IL 60015), or equivalent. Chromatographic conditions: temperatures: oven 190°C, detector 320°C, injector 270°C; carrier gas: helium at 70 cm/s, split ratio 30:1; make-up gas: nitrogen at 20 mL/min.

Reagents

(a) *Tetrahydrofuran.*—AR grade (Fisher Scientific, Fair Lawn, NJ 07410), or equivalent.

(b) *Analytical standard.*—Pyrocide 175 (McLaughlin Gormley King Co., Minneapolis, MN 55427).

Procedure

Weigh the equivalent of 5 mg standard (25 mg of 20%) into 50 mL volumetric flask and dilute to volume with tetrahydrofuran (THF). Dilute 10 mL to 50 mL with THF. Prepare samples by weighing the equivalent of 1 mg pyrethrins into 50 mL volumetric flask and diluting to volume with THF. Mix well and inject 1 μL. Bracket every sample with standards.

Quantitation

Figure 1 represents a typical chromatogram of a low level pyrethrin formulation. The 6 pyrethrin peaks are identified on the basis of the elution profile obtained by using the official method. Both this method and the official method use the same liquid phase and it is reasonable to assume that the elution orders would be the same. The resolution of capillary GC makes possible the use of all 6 pyrethrin peaks. The sum of these peak areas or heights is used in the equation:

$$\% \text{ Pyrethrin} = (R/R') \times (W'/W) \times (V/V') \times \% \text{ standard purity}$$

where R and R' = response for sample and standard, respectively; W and W' = weight of sample and standard, respectively; and V and V' = final volume of sample and standard, respectively. In the dilution scheme outlined above, $V = 50$ and $V' = 250$.

Method Evaluation

The precision of this method was tested by analyzing 5 low level samples in quadruplicate. Linearity was tested by chromatographing standards ranging from 4 to 40 μg/mL (0.2× to 2.0× target concentration). Recovery was evaluated by assaying 4 spiked formulation samples.

Results and Discussion

A typical chromatogram of a low level pyrethrin formulation is presented in Figure 1. All 6 esters are well resolved and elute within 10 min. Peaks corresponding to piperonyl butoxide and MGK 264 are also identified. The authors had originally intended to use this method to simultaneously assay these synergists, but found too much variation in the response of the ECD to these compounds. Injection of a series of standards indicated that inclusion of as many of the pyrethrin peaks as possible gave the best precision for quantitation (see Table 1). Occasionally, a formulation will give an offscale peak that coelutes with cinerin II; this peak must be neglected.

Table 2 presents the results of quadruplicate analyses of 5 low level pyrethrin formulation samples. Samples used in this study were selected to test the method as rigorously as possible. These samples included a low level aerosol, 2 pet shampoos, an oily insect repellent, and an aqueous pet spray. The

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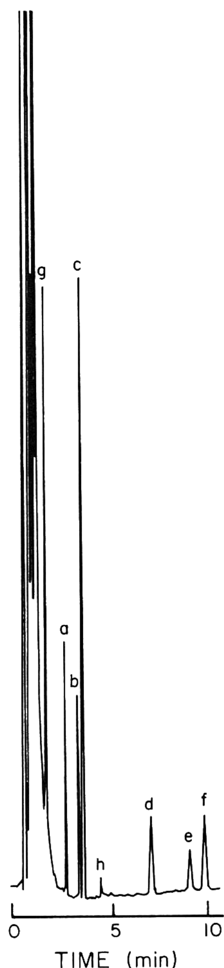

Figure 1. Chromatogram of diluted sample 84-27 (20 ng pyrethrin) identifying: (a) cinerin I, (b) jasmolin I, (c) pyrethrin I, (d) cinerin II, (e) jasmolin II, (f) pyrethrin II, (g) MGK 264, (h) piperonyl butoxide.

Table 1. Comparison of peaks used in pyrethrin quantitation

Peaks	Peak heights[a]	CV, %
Pyrethrin I	121.25 ± 3.30	2.7
Pyrethrins I + II	154.25 ± 4.99	3.2
All I pyrethrins	232.75 ± 2.99	1.3
Pyrethrin I + cinerin I	203.50 ± 2.65	1.3
All 6 peaks	307.25 ± 2.36	0.8

[a]Sum of peak heights, average ± standard deviation across 4 injections.

Table 2. Results of quadruplicate analysis of low level pyrethrin samples

Sample	Description	Found, %	Av. ± SD (% CV)
84-24	0.05% aerosol also containing 0.1% piperonyl butoxide, 0.166% MGK 264, and 0.5% Baygon	0.0492 0.0465 0.0450 0.0499	0.0477 ± 0.0023 (4.8)
84-27	0.06% oil diluted also containing 0.096% piperonyl butoxide, 0.2% MGK 264, and 0.4% MGK R326	0.0578 0.0588 0.0564 0.0550	0.0570 ± 0.0017 (2.9)
84-123	0.075% shampoo also containing 0.12% piperonyl butoxide and 0.25% MGK 264	0.0690 0.0693 0.0706 0.0675	0.0691 ± 0.0013 (1.8)
84-281	0.045% shampoo also containing 0.072% piperonyl butoxide and 0.15% MGK 264	0.0401 0.0368 0.0366 0.0366	0.0375 ± 0.0017 (4.5)
84-748	0.06% aqueous diluted also containing 0.48% piperonyl butoxide	0.0592 0.0571 0.0605 0.0598	0.0592 ± 0.0015 (2.5)

coefficients of variation (CV) ranged from 1.8 to 4.8% with an average of 3.3%. This is slightly larger than desired but should be expected for samples of this type. The 2 shampoos gave lower results than expected. Some cloudiness was observed in the extracts of these samples. It is possible that some of the pyrethrin in these samples was sequestered from the THF but it is also possible that these samples were underformulated.

Linearity was tested by chromatographing dilutions of standard ranging from 4 to 40 μg/mL (target standard = 20 μg/mL). Analysis of variance determined a regression coefficient of 0.994 and a correlation coefficient of 0.997. Recovery was evaluated by adding standard directly to 4 of the samples used in the study. The aerosol sample could not be accurately spiked. The results are given in Table 3. Recovery ranged from 99.8 to 104.2% with an average of 101.2%.

The accuracy of this method is difficult to assess. The official method is not applicable to samples used in this study. A comparison was made between the official method and this method, using flame ionization detection (FID) on higher level samples (Table 4). Four samples were analyzed in quadruplicate by each method. The capillary method gave lower results for 3 samples but also gave results closer to the guarantee for 3 of the samples. This may result from improved resolution of pyrethrins from other compounds in the sample.

The composition of "pyrethrins" is not stringently defined. It is also commonly reported that pyrethrins are succeptible to photolysis (4). Thus, a great deal of variation in the composition of pyrethrin formulations and standards would be expected. The authors have not found this to be the case. Five standards from different lots stored for different lengths of time all showed the same relative composition. Analysis of more than 40 formulations has revealed only slight variations in composition possibly due to a common source of pyrethrins for these samples. The effect on analysis of coupling these slight compositional variations with differences in ECD response factors of the individual pyrethrin esters is difficult to predict. The linearity and relative proportion of each ester was evaluated by chromatographing standard dilutions (4–40 μg/mL). The linearity of response to individual peaks was excellent. A change in relative proportions would be indicative of differences in response factors. No such change was observed. The use of all 6 pyrethrin peaks should minimize the effect of variation in composition.

The resolution, speed, ease, and ruggedness of capillary GC have made it very popular. Advocates accept the imprecision introduced by the split injection technique for their own uses, but hesitate to publish their findings. Various strategies to minimize this imprecision should be used whenever possible. The use of an autosampler is nearly essential. Packing silanized glass wool in the insert also proved helpful. The use of an internal standard is highly recommended (5). The authors did not use an internal standard in this study because its use resulted in worse precision. This could be due to variable splitting of the internal standard used. Peak heights were used in this study rather than areas due to a lack of an integrator capable of handling capillary peaks. It is not known whether areas would give better results than peak heights. The selection of the injection solvent is also important (5).

Table 3. Recovery of pyrethrin standard added to low level pyrethrin formulations

Sample	Found, %	Expected, %	Recovery, %
84-27	0.0784	0.0752	104.2
84-123	0.0822	0.0819	100.3
84-281	0.0490	0.0487	100.6
84-748	0.0921	0.0923	99.8

Table 4. Comparison between official AOAC method and capillary FID method

Sample	Sample type	AOAC results[a]	Capillary results[a]
84-163	1% In petroleum distillate	1.025 ± 0.0058 (0.6)	0.861 ± 0.017 (2.0)
84-251	0.15% flea mist	0.194 ± 0.0043 (2.2)	0.138 ± 0.0046 (3.3)
84-353	0.3% shampoo	0.187 ± 0.0040 (2.1)	0.224 ± 0.0049 (2.2)
84-790	1% in paraffinic oil	1.233 ± 0.0005 (0.4)	1.145 ± 0.0265 (2.3)

[a]Average (%) ± standard deviation (% relative standard deviation).

THF was used in this study because of the range of samples with which it is miscible, and its lack of effect on splitting. Use of methanol to dissolve some shampoos gave variable results due to splitting variations. Large sample weights required for some samples change the injection solvent and splitting will be affected.

It is hoped that the increased resolution of capillary GC will offset the problems introduced by split injection. Integration of well resolved peaks should be better than that of poorly resolved peaks. The chance of contaminants coeluting with the peaks of interest will also be less. Resolution of multi-peak samples will allow the use of more components in defining the sample.

The results presented here demonstrate the applicability of capillary GC to the analysis of low level pyrethrin formulations. This technique offers excellent linearity and recovery along with acceptable precision. This method may be used on the increasingly common samples that cannot be analyzed by the official method. This method is subject to variation when large sample weights are used, gives low results on some shampoo products, and is inferior to the official method for higher level formulations.

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Simultaneous Liquid Chromatographic Determination of Warfarin and Sulfaquinoxaline in Cornmeal-Based Rodenticide

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A method is reported for the simultaneous determination of warfarin and sulfaquinoxaline in a cornmeal-based commercial rodenticide by extraction of the 2 components with acetonitrile followed by liquid chromatographic separation. A reverse phase C-18 column is used with a simple buffered mobile phase (acetonitrile–water (50 + 50) 0.005M in heptanesulfonic acid sodium salt, pH 3.5) that elutes sulfaquinoxaline as a paired-ion and warfarin as an ion-suppressed non-ionic species. The 2 compounds are detected at 280 nm after separation and are quantitated using ethyl *p*-nitrobenzoate as an internal standard. The method compares favorably with existing methods of analysis for the 2 compounds in terms of accuracy and precision, and is superior to those methods in speed as well as specificity.

Warfarin (3-(acetonylbenzyl)-4-hydroxy-coumarin) is an anticoagulant used as the active ingredient in numerous commercial rodent baits. Many of these formulas also contain sulfaquinoxaline (2-(*p*-aminobenzenesulfonamide)quinoxaline) as a synergist. This substance acts as an antibacterial agent to inhibit the growth of organisms that produce vitamin K, an antidote to the effects of warfarin. Upon repeated feedings, these drugs cause fatal hemorrhage in rats and mice and, thus, help control rodent infestation. The common dosage level is 0.025% each of warfarin and sulfaquinoxaline.

Previous work on the analysis of warfarin alone in rodent baits includes spectrophotometric determination at 308 nm after extraction (1, 2) and after column chromatographic purification (3). Determinations of warfarin in matrices other than rodenticides have also been reported using UV spectrophotometry (4, 5), fluorometry (6), gas chromatography (GC) (7, 8), and GC-chemical ionization mass spectrometry (9). Liquid chromatographic procedures were described by Vessel and Shively (10) and Billings et al. (11), using external standards for quantitation. Procedures using internal standards have also been reported (12–14).

Sulfaquinoxaline alone has been determined in various matrices by colorimetry (15, 16) and by UV spectrophotometry after purification by thin layer chromatography (17, 18). Examples of analysis by LC have also been reported (19–21).

Methods for the simultaneous LC analysis of both warfarin and sulfaquinoxaline in rodenticide mixtures have been described by Trujillo (22) and Perez (23). Neither used an internal standard for quantitation, thereby making it difficult to achieve accurate and precise results. Moreover, the method of Trujillo was applied to the analysis of rodenticide concentrates and not the actual bait itself, while the method of Perez was concerned with baits prepared in the laboratory and not those commercially available.

The purpose of the research reported here was to develop an accurate, reproducible, rapid quantitative LC procedure for the simultaneous determination of warfarin and sulfaquinoxaline in a commercially available cornmeal-based rat bait.

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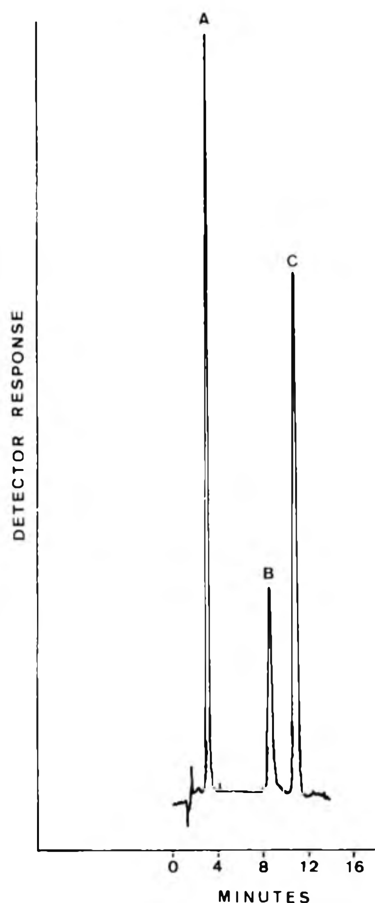

Figure 1. Typical chromatogram of an extract of a rodent bait. A, sulfaquinoxaline; B, warfarin; C, Ethyl p-nitrobenzoate (internal standard).

In addition, since the method necessarily involved multiple extraction and transfer steps, an internal standard was desired to aid in quantitation and to compensate for potential losses of the analytes during the procedure.

METHOD

Reagents and Standards

Acetonitrile, LC grade (Fisher Scientific); heptanesulfonic acid sodium salt and ethyl *p*-nitrobenzoate (both Eastman Kodak); and warfarin and sulfaquinoxaline (gift from E. R. Squibb) were used as received. Distilled water was purified to a conductivity of 0.055 microsiemens, using a Water-I Laboratory Water System (Gelman Sciences, Inc.).

Apparatus

To accurately determine weights of samples greater than 50 mg, use analytical balance, e.g., Sartorius Model 1601A. For weighing smaller samples, use suitable microbalance, e.g., Perkin-Elmer AD-2 Autobalance.

Set up liquid chromatograph suitable for use with stainless steel columns and capable of maintaining solvent flow of 1 mL/min and pressures up to 3000 psi. Acceptable apparatus consists of duplex minipump (Milton Roy, Laboratory Data Control), fixed-loop injector with 20 μL loop (Spectra-Physics Model HPSV-20), fixed wavelength (280 nm) UV detector (Laboratory Data Control Model 1205), and strip chart recorder (Fisher Series 5000 Recordall) operated at 10 mV. Use LC column 25 cm × 4.5 mm id packed with 5 μm octadecylsilane (Rainin Microsorb). Protect analytical column with a 2 cm × 2.6 mm id guard column (Upchurch Scientific) filled with 10 μm C-18 packing (Waters Associates).

Before injection, remove any solid material from all samples by using 13 mm Zetapor membrane disc filters with 0.45 μm pores (Rainin Instrument Co.).

Mobile Phase

Dissolve 1.1013 g heptanesulfonic acid sodium salt in 500 mL LC grade water. Mix with 500 mL acetonitrile and adjust pH to 3.5 with dilute HCl. Degas under vacuum before use.

Internal Standard Stock Solution

Dissolve exactly 6.6 mg ethyl *p*-nitrobenzoate in 500.0 mL acetonitrile to give final concentration of 0.0132 mg/mL.

Reference Standard Solution

Accurately weigh ca 1.5 mg warfarin reference standard and 1.5 mg sulfaquinoxaline reference standard and quantitatively transfer into 25 mL volumetric flask. Add 10 mL internal standard stock solution (IS solution), agitate to dissolve the powders, and dilute to volume with IS solution. Transfer exactly 1.00 mL of this solution into 10 mL volumetric flask and dilute to volume with IS solution.

Sample Preparation

Accurately weigh ca 0.625 g bait sample into 6 in. screw-cap test tube. Add 5.00 mL IS solution, cap tube, and shake on vortex mixer 1 min. Let mixture settle and transfer clear supernate to 25 mL volumetric flask. Repeat procedure 3 more times, each time adding supernate to flask. Wash residue twice with 2 mL IS solution and transfer washings to flask. Dilute to volume with IS solution and mix thoroughly.

Prior to injection onto liquid chromatograph, filter portion of this solution through 0.45 μm membrane filter.

LC Assay

Set instrument conditions so that flow rate is 1 mL/min at pressure of ca 2500 psig. Use suitable UV detector with wavelength fixed at 280 nm and detector operated at 0.04 AUFS. Set chart speed at 0.25 cm/min and recorder range at 10 mV. Make triplicate injections of 20 μL each of reference standard solution and sample solution and measure peak heights of sulfaquinoxaline, reference standard, and warfarin. Calculate sulfaquinoxaline and warfarin content of sample by using following equation:

$$\% \text{ Sulfaquinoxaline (or warfarin)} = (C_1R_2 \times 2.5)/(R_1 \times W)$$

where C_1 = concentration (mg/mL) of sulfaquinoxaline (or warfarin) in reference standard solution; R_1 = peak height ratio of sulfaquinoxaline (or warfarin) to internal standard in standard chromatogram; R_2 = peak height ratio of sulfaquinoxaline (or warfarin) to internal standard in sample chromatogram; W = weight of the sample (g).

Results and Discussion

Chromatographic Response

At the pH of the mobile phase, sulfaquinoxaline, a weak base, is protonated and forms an ion-pair with the counter-ion, heptane sulfonate. However, warfarin is weakly acidic, so its ionization is suppressed and it remains nonpolar, and can therefore interact effectively with the stationary phase. With the mobile phase and the experimental conditions described, the retention time for sulfaquinoxaline was 3.2 min, for warfarin 8.8 min, and for the internal standard 11.0 min. A typical chromatogram is shown in Figure 1.

To determine the linearity of the chromatographic response, calibration curves were run in which the internal standard

Table 1. Determination of warfarin in rat baits

Lot	LC		Modified AOAC		Corrected AOAC
	% of claim[a]	% RSD	% of claim	% RSD	% of claim
1	98.8	3.2	126.4	3.80	110.0
2	94.4	0.42	109.6	9.49	92.8
3	92.8	1.72	108.0	1.85	90.8
4	97.6	2.87	113.2	3.18	98.8
5	106.1	0.75	118.0	7.46	104.8
Overall	98.0	5.31	114.8	6.27	99.2

[a]Mean of 3 determinations. Label claim is 0.025%.

Table 2. Determination of sulfaquinoxaline in rat baits

Lot	LC		Modified AOAC		Corrected AOAC
	% of claim[a]	% RSD	% of claim	% RSD	% of claim
1	98.0	1.6	112.8	1.77	106.4
2	97.3	0.41	103.6	2.7	97.2
3	91.2	4.8	100.4	2.39	94.4
4	102.1	4.7	107.6	1.12	101.6
5	106.1	0.38	110.8	2.17	105.2
Overall	99.1	6.0	106.8	4.49	100.8

[a]Mean of 3 determinations. Label claim is 0.025%.

concentration was maintained constant while the sulfaquinoxaline or warfarin concentration was varied. The resulting responses for both calibration curves were linear (r = 0.99) over a range corresponding to quantities of the 2 analytes that would be obtained in the analysis of mixtures containing 50–150% of the label claim (0.0125–0.0375%). In both cases, the intercepts were essentially zero.

Extraction Procedure

To determine the optimum conditions for extraction of the active ingredients from the cornmeal-based rodenticide, a single sample was extracted sequentially with 5 mL portions of the solvent, and aliquots of the clear supernate from each step were injected onto the LC column. Most of each solute appeared in the first extraction; however, significant quantities were present in the second, third, and fourth portions, while the final extraction showed no solute peaks. As a result of these experiments, a final procedure was adopted which involved 4 extractions using 5 mL of solvent each.

The suitability of the extraction procedure was also tested by using the method of standard addition. Known weights of each of the analytes were added to samples and the quantity of standard material recovered by multiple extraction was determined by LC analysis. When the amounts of standard added were plotted against the amounts recovered, the plots were linear (r = 0.99) and the intercepts were essentially zero, thereby confirming the utility of the procedure.

Since the cornmeal base of the rat bait contains many potentially extractable substances that could interfere with the chromatography, a blank sample of cornmeal was extracted under the same conditions as the assay method. To eliminate the possibility that an interfering peak would be overlapped by the standard and thus be undetected, the extraction solvent contained no internal standard. The resulting chromatogram showed no peaks that would interfere with either of the analytes or the internal standard.

Reference Method

To provide a validation of the accuracy and precision of the proposed method, all samples were analyzed both by the LC procedure and by a spectrophotometric procedure. For sulfaquinoxaline, the reference method was the official AOAC assay, and for warfarin, a modified version of the official AOAC method was used. The modifications included addition of *n*-hexane to the ether extract of the sample before partitioning with aqueous sodium pyrophosphate, extraction of the aqueous phase with *n*-hexane instead of petroleum ether in the third step, preparation of warfarin reference standard in ethyl ether instead of in sodium pyrophosphate solution, and use of 1% sodium pyrophosphate solution as the blank for both standard and sample.

Analysis of Rat Baits

Five different lots of a cornmeal-based rat bait were analyzed by both methods. The results are shown in Tables 1 and 2, columns 2 through 5. Results are expressed as percent of label claim (0.025%) and all analyses were done in triplicate. By the LC method, the overall recovery of sulfaquinoxaline was 99.1% with a relative standard deviation (RSD) of 6.0%; for warfarin, 98.0% was recovered and the % RSD was 5.31. However, by the reference methods, recovery was 106.8% (% RSD = 4.49) and 114.8% (% RSD = 6.27) for sulfaquinoxaline and warfarin, respectively.

To determine the significance of the differences in the means obtained by the 2 methods, both paired t-tests and 2-tailed F-tests were performed. The F-test, which evaluates the hypothesis that the variances of the methods are the same, showed no significant difference between the variances at the 0.05 level. The t-test, which evaluates the hypothesis that the difference between the method means is zero, showed a significant difference at the 0.05 level.

The significant difference between the 2 method means, yet the lack of difference between their variances led to the supposition that the methodology itself could be the source of the errors. Because the AOAC methods are designed for the determination of a single analyte and not mixtures, it was suspected that some cross-interference, i.e., interference in warfarin analysis by co-extraction of some sulfaquinoxaline and vice versa, was taking place. Also, there was a strong possibility of interference from extraction of some material from the matrix. To test this hypothesis, the following experiments were run.

(a) Reference standard warfarin at an appropriate weight was carried through the assay for sulfaquinoxaline and the absorbance was measured at 545 nm. The absorbance corresponded to a 2.3% error in overestimating sulfaquinoxaline.

(b) Reference standard sulfaquinoxaline at an appropriate weight was carried through the warfarin assay and the absorbance was read at 308 nm. The measured absorbance corresponded to 3.5% error in overestimating warfarin.

(c) Two blank cornmeal samples at weights equivalent to those usually taken for the assay were carried through the separate pretreatment procedures for warfarin and sulfaquinoxaline and their absorbances were read at the corresponding analytical wavelengths. The absorbance values corresponded to errors in sulfaquinoxaline of 3.7% and in warfarin of 10.5%.

The results of the analysis of the rat baits, which have been corrected for the overall errors of 6% in sulfaquinoxaline and 14% in warfarin, are shown in Tables 1 and 2, column 6. The corrected values agree closely with those obtained by the LC method. To validate these results, F- and t-tests were run as before. Neither showed any significant difference between the means or variances at the 0.05 level.

In summary, an LC method has been developed for the simultaneous determination of warfarin and sulfaquinoxaline in a cornmeal-based rat bait. The method has been validated by comparing results obtained to those generated from identical samples analyzed by current official procedures for this type of bait material. Statistical evaluation using *t*- and *F*-tests to compare the means and variances, respectively, confirmed that the results produced were similar in accuracy and variability to those obtained by the current official methods. However, the proposed method has a great advantage in specificity, especially for mixtures, since neither component interferes with the determination of the other. In addition, it does not produce erroneously high results due to interferences from the sample matrix. Moreover, the LC method is significantly faster and more amenable to automated or robotized methods.

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Liquid Chromatographic and Gas Chromatographic Determination of *N*-Nitrosodiethanolamine in 2,4-D Diethanolamine Pesticide Formulations

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A method for the determination of *N*-nitrosodiethanolamine (NDElA) in diethanolamine formulations of 2,4-D herbicide has been developed. The NDElA fraction was isolated from the formulation by 2 disposable ion exchange columns followed by an extraction column to remove the acid and amine that might be deleterious to the analytical system and to concentrate the NDElA solution. The isolated fraction was then further purified on a silica gel disposable column and analyzed for NDElA by using a liquid chromatographic (LC) apparatus connected to an ultraviolet (UV) detector in series with a thermal energy analyzer (TEA) detector. NDElA was derivatized to the trimethylsilyl (TMS) derivative, the identity of which was confirmed by gas chromatography–mass spectrometry (GC-MS) and analyzed by gas chromatography with TEA detection (GC-TEA). The limit of detection of NDElA by LC-TEA and LC-UV is 1 ng and by GC-TEA 0.08 ng. These values correspond to 0.1 ppm relative to the active ingredient in the formulation. The recoveries for this analytical method averaged from 91 to 100% when the samples were spiked at the 0.30–3.47 ppm level. The specificity and reproducibility of these 3 methods of determination were compared; results suggest that NDElA was not generated during cleanup or in the GC injection port.

N-Nitrosodiethanolamine (NDElA) has been demonstrated to be carcinogenic in rats (1) and has been detected in cutting fluids (2), cosmetics (3), tobacco (4), and pesticides (5). It has also been found that NDElA can be formed easily by nitrosation of either di- or triethanolamine (6). Motoi et al. examined the formation of NDElA from diethanolamine in the presence of inorganic pigments (7).

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2,4-D, a broad leaf herbicide, is sold as the aqueous solution of the dimethylamine and as diethanolamine salts. The presence of *N*-nitrosodimethylamine in 2,4-D dimethylamine formulations used in Canada has been investigated in the Western Laboratory of Agriculture Canada (8), but no studies on NDElA in the diethanolamine formulations have been reported.

Most nitrosamine analyses have been concerned with residues in foods and human urine and have used such techniques as gas chromatography interfaced with thermal energy analyzer (GC-TEA) or liquid chromatography with thermal energy analyzer (LC-TEA) (9, 10). NDElA has also been analyzed in cosmetics by using GC-TEA preceded by the formation of a trimethylsilyl (TMS) derivative (11), and by using LC-TEA after ion exchange chromatographic separation of NDElA (12). The use of liquid chromatography and ultraviolet detection (LC-UV) to detect NDElA has also been described for cosmetics and compared with LC-TEA (13). Wolf et al. described analytical methods used to determine nitrosamines, other than NDElA contamination, in pesticide formulations at sub-ppm levels (14). To date, however, methods of extraction of NDElA from aqueous to organic phase and subsequent isolation from pesticide formulations have not been reported. NDElA, being a nonvolatile polar compound, soluble in water and partly soluble in acetone, methylene chloride, and methanol, is difficult to extract from aqueous pesticide formulations. Thus there is a need for a sensitive, specific, reproducible method for isolation, detection, and quantitation of NDElA in pesticide formulations.

The procedures described in this paper involve an anion exchange column to retain the 2,4-D acid, followed by a

cation exchange column to remove the diethanolamine. The aqueous solution is extracted with a CLIN ELUT column, thus avoiding the need for liquid-liquid extractions and any centrifugation of the resulting emulsions. The extract is then concentrated by evaporation, purified through a silica gel disposable column, and then analyzed by LC-TEA and LC-UV. The same extract is also derivatized to the silyl derivative and analyzed by GC-TEA. The specificity and reproducibility of these 3 methods are compared.

METHOD

Apparatus

(a) *Liquid chromatograph.*—Spectra-Physics (SP) (San Jose, CA) 8100 liquid chromatograph equipped with 25 cm × 4.6 mm Spheri-5™ cyano normal phase column (Brown-Lee Labs); SP 8110 autosampler; SP 8440 variable wavelength detector connected in series by pneumatic 3-way valve (Rheodyne Model 5302), which was controlled by Autochrom™ solenoid interface, to TEA detector Model 502A (Thermo Electron Corp., Waltham, MA); and SP 4200 dual-channel computer integrator equipped with SP 9600 Labnet™ computer. Operating conditions: mobile phase flow rate, 1 mL/min; UV wavelength, 234 nm; sensitivity, 0.02 AUFS; carrier gas, high purity argon, 100 mL/min; high purity oxygen, 45 mL/min; pyrolizer temperature, 600°C; vacuum, 1.2 torr; cold trap, dry ice–ethanol (50 + 50); attenuation, 8; chart speed, 0.5 cm/min.

(b) *Gas chromatograph–mass spectrometer.*—Finnigan Model 9610 gas chromatograph with J & W Model II capillary on-column injector and Finnigan 4500 mass spectrometer with pulsed positive ion negative ion chemical ionization and Townsend discharge ionization. GC column: 15 m × 0.32 mm id DB-1 (J & W Scientific, Rancho Cordova, CA) with precolumn extended ca 0.6 m from injector. Data acquisition and MS control: Finnigan 2300 INCOS data system. GC operating conditions: column temperature, 85°C for 0.1 min, then 5°/min to 150°C; head pressure, 3 psi; carrier gas, helium UHP. Mass spectrometer source pressure: 0.48 torr. Ionization mode: (*1*) methane (UHP) positive chemical ionization (MPCI): temperature, 140°C; emission current, 0.34 mA, 54 eV (Figure 1A); (*2*) methane negative chemical ionization (MNCI): temperature, 100°C, emission current 0.34 mA, 50 eV (Figure 1B); (*3*) oxygen (UHP) negative chemical ionization (ONCI) with Townsend discharge mode: temperature, 100°C; discharge current 70 μA, voltage 1.5 kV (Figure 1C).

(c) *Gas chromatograph– thermal energy analyzer.*—Hewlett-Packard Model 5710A gas chromatograph with Model 18740B capillary column controller interfaced to TEA detector (Model 502A, Thermo Electron Corp.) and equipped with Spectra-Physics SP4200 computing integrator. GC column: 30 m × 0.315 mm DB-5 0.25 μm fused silica capillary column (J & W Scientific). Operating conditions: column oven temperature, 90 to 160°C at 8°/min; head pressure, 15 psi; injection port, 200°C; pyrolizer temperature, 500°C with CTR™ gas stream filter; vacuum 0.22 torr; attenuation, 8; chart speed, 0.5 cm/min; oxygen, 15 mL/min. Injection technique: splitless purge delay time, 20 s septum purge flow, 0.5 mL/min; injection volume, 3 μL.

(d) *Extraction tube.*—CLIN ELUT™ columns unbuffered (part No. 1020, Analytichem International, Harbor City, CA).

(e) *Ion exchange columns.*—5 mL anion exchange material AG1-X8 (100–200 mesh) chloride ion packed with water in disposable 11 mL polypropylene Econo-Columns (Bio-Rad Laboratories) and 0.8 × 4 cm prefilled Econo-Columns packed with 100–200 mesh cation exchange resin AG50W-*X*8 (H^+) in water (Bio-Rad Laboratories).

(f) *Adsorbent column.*—Baker-10 SPE™ silica gel, 6 mL disposable columns (J. T. Baker Chemical Co.).

(g) *Sample filtering unit.*—MILLEX-SR, 0.5 μm filter unit for nonaqueous solutions (Millipore Corp., Bedford, MA), and 5 mL FORTUNA® disposable, sterilized, nonpyrogenic, and all-polypropylene/polyethylene syringes (Aldrich Chemical Co., Inc.).

(h) *UV lamp.*—General Electric G15T8 germicidal, 15 watts.

Reagents

(a) *Solvents.*—Butanol, reagent grade; chloroform, LC grade; all others, glass distilled.

(b) *Standard stock solution.*—104 mg NDElA in 10 mL acetone (Thermo Electron Corp.).

(c) *Analytical standard.*—Dilute appropriate aliquot of stock solution to 10 mL with 25% butanol in methylene chloride to give final concentrations of 0.1, 0.3, 0.5, 1.0, 2.0 ng/μL, respectively.

(d) *Mobile phase.*—Methanol–hexane–methylene chloride (5 + 60 + 35).

(e) *N-Nitrosation inhibitor.*—Sodium azide solution. Weigh 10 g sodium azide (reagent grade) in 500 mL glass-distilled water (purified with Milli-Q2 system).

(f) *Silylating agent.*—*N,O*-Bis-(trimethylsilyl)-acetamide (BSA), specially purified grade (Pierce Chemical Co.).

Extraction and Cleanup

Measure 1 mL formulation and mix with 1 mL sodium azide solution, and *N*-nitrosation inhibitor to prevent artificial formation, in test tube. Quantitatively transfer mixture with 10 mL water to drained anion exchange column connected in series with cation exchange column which elutes directly to extraction tube. Wait 3 min for eluted sample to be adsorbed onto dry column. Place 500 mL round-bottom flask under column. Elute NDElA with 20 mL 10% acetone in ethyl acetate, wait 1 min to ensure efficient extraction of NDElA between the 2 aliquots, then elute again with 230 mL of the same solution. Add 0.5 mL butanol to eluate to prevent complete evaporation of solvent, and concentrate to 0.5–1 mL on rotary evaporator at 36°C. Quantitatively transfer concentrated solution to silica gel Baker-10 column, prewashed with 5 mL methanol and then 5 mL chloroform, using 15 mL chloroform and elute NDElA with 10 mL 10% methanol in chloroform. Discard first 15 mL eluate and collect remainder in 15 mL centrifuge tube. Proceed to either LC determination or preparation of TMS derivative.

LC Determination

Add 0.5 mL butanol to centrifuge tube and evaporate solvents under nitrogen to 0.5 mL. Dilute residue to 2 mL with methylene chloride and then filter through sample filtering unit. Transfer aliquots to sealed vials for LC analysis.

Inject 50 μL NDElA aliquots into LC apparatus using autosampler. Record peak areas and retention times with SP 4200 computing integrator. Quantitate with external standard:

$$\text{NDElA (ppm of active ingredient)} = (A/A') \times (W'/W) \times \text{purity of std}$$

where A and A' = area response of sample and standard, respectively; W and W' = weight (μg) of standard, and 2,4-D sample calculated from amount of 2,4-D (% w/w) contained in 2,4-D diethanolamine sample as determined by LC-UV analysis.

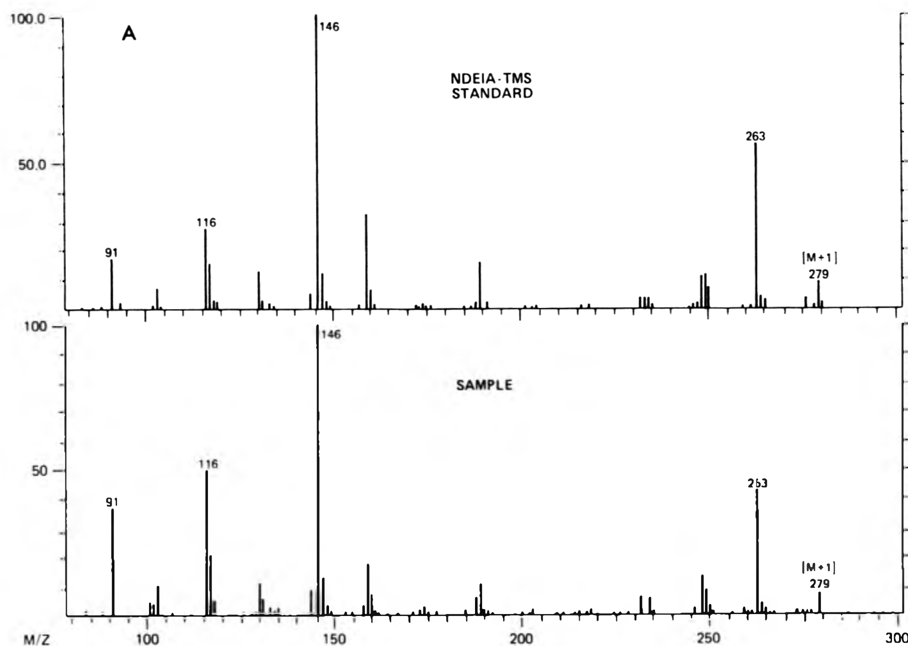

Figure 1A. Mass spectral comparison of NDEIA-TMS derivative standard and sample using methane positive chemical ionization.

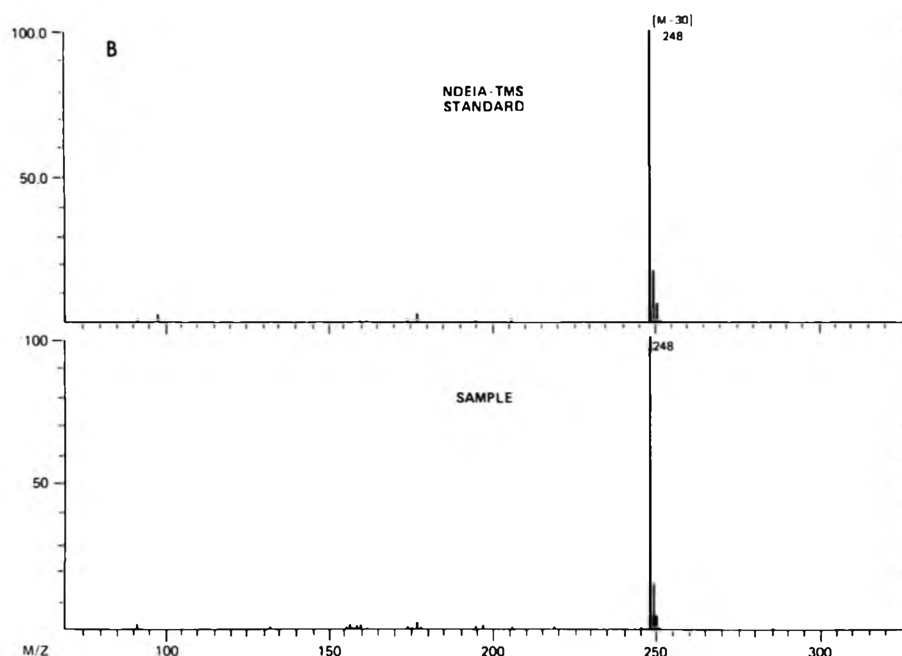

Figure 1B. Mass spectral comparison of NDEIA-TMS derivative standard and sample using methane negative chemical ionization.

Preparation of TMS Derivative

Evaporate NDEIA extract in 15 mL centrifuge tube under nitrogen to dryness. Add 0.5 mL BSA reagent, shake, stopper, and heat 30 min at 70°C (9). Cool to room temperature and dilute to 2 mL with acetone. To prepare standard used for GC quantitation, add 200 μL standard stock solution in 15 mL centrifuge tube, evaporate to dryness, and prepare silyl derivatives as above. Proceed to GC determination within 24 h.

GC Determination

Inject 3 μL aliquot of NDEIA silyl derivative into GC apparatus. Quantitate NDEIA concentrations using external standard, and calculate as described in *LC Determination*.

Ultraviolet Confirmatory Test

Transfer 1 mL NDEIA extract to quartz cell, and place within several inches of UV source. After exposure for 2 h, re-analyze extract by LC-TEA. Disappearance of peak corresponding to NDEIA is good evidence of its identity.

Gas Chromatographic–Mass Spectrometric Confirmation

Using conditions under instrumental apparatus, inject 3 μL aliquot of standard NDEIA silyl solution within 24 h of preparation and obtain mass spectrum using the 3 modes of ionization described. Repeat with silylated sample solution, and compare mass spectrum with that of standard for confirmation.

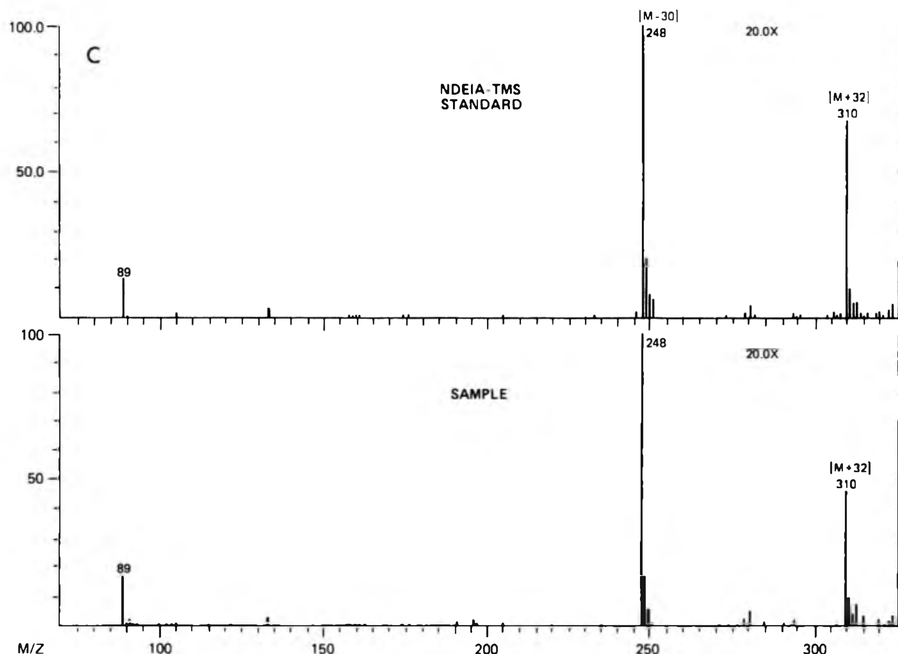

Figure 1C. Mass spectral comparison of NDEIA-TMS derivative standard and sample using oxygen negative chemical ionization using Townsend discharge.

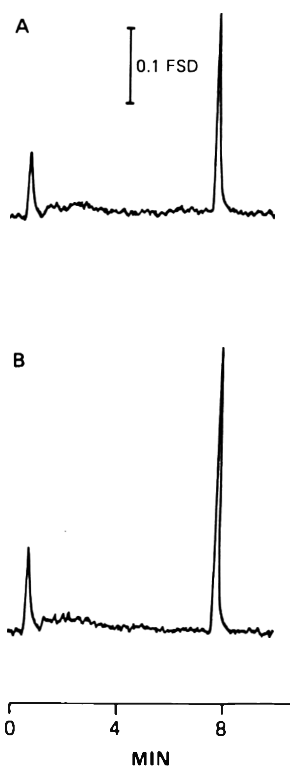

Figure 2. LC chromatogram showing 26 ng NDEIA standard: A, UV detector; B, TEA detector.

Results and Discussion

Initial attempts to clean up the formulation were carried out by centrifuging the 2,4-D acid precipitated by addition of HCl. The filtrate was purified through a Sep-Pak C_{18} cartridge and transferred directly to the extraction column. The NDEIA extract was eluted, concentrated, and transferred to a silica gel Baker-10 column. These procedures appeared to be long and tedious, so they were replaced by 2 ion exchange columns, the anion exchange column to retain the acid and then the cation exchange column to retain diethanolamine. The entire aqueous extract along with the wash solution was transferred to the extraction column. The NDEIA was isolated from water by eluting the extraction column containing the aqueous extract with 10% acetone in ethyl acetate, and was then concentrated and purified on a silica gel disposable column. The elution of NDEIA standards spiked in water from the column gave recoveries of 100%. The same study (standards spiked in acetone) with a Baker-10 silica gel column gave 100% recovery from 10% methanol in chloroform. Low recoveries (0–30%) were noticed only when NDEIA was evaporated to dryness on the rotary evaporator, presumably due to the adsorption of NDEIA on glassware surfaces (15). This was minimized by adding butanol to compete for adsorption sites and to prevent NDEIA from evaporating to dryness.

Initially, an analytical 5 μm silica gel column was used for LC analysis. Nonreproducibility of retention time of NDEIA necessitated changing to a bonded cyano column. The mobile phase conditions were very similar to those used by Motoi et al. (7) at a flow rate of 1 mL/min. NDEIA gave a sharp peak at a reproducible retention time of 8.7 min for TEA detection and 8.1 min for UV detection (Figure 2). The difference in these 2 retention times is due to the dead volume between the detectors.

The same cleanup procedures were conducted on the reagent blank, giving NDEIA levels below the limits of detectability. Samples of the diethanolamine before and after the prescribed cleanup procedures were also analyzed for NDEIA by using LC-TEA determination, and 0.1 ppm NDEIA was detected in both cases. These results demonstrate that NDEIA was not generated during the analytical preparation.

Recovery studies were carried out by the described method on 15 samples by spiking NDEIA directly into the formulation and shaking well before adding the other ingredients for extraction and cleanup. Recovery efficiencies were determined by calculating the fraction (%) of spiked amount recovered. The lowest detectable amount found in some samples was 0.10 ppm and these samples were used for spiking at a

Table 1. Percent recoveries of NDEIA from fortified formulations, using LC-TEA

Sample	Original level, ppm	Fortified level, ppm	Found, ppm	Recovery, %
1	0.10	0.35	0.46	102.0
2	0.10	0.35	0.42	90.0
3	0.10	0.35	0.42	92.0
4	0.10	0.35	0.40	87.0
Av.			0.42	92.7
SD			0.02	6.5
Range			0.40–0.46	87.0–102.0
5	0.10	0.87	0.86	87.0
6	0.10	0.87	0.83	84.0
7	0.10	0.87	0.99	102.0
Av.			0.89	91.0
SD			0.08	9.6
Range			0.83–0.99	84.0–102.0
8	0.8	1.73	2.32	88.0
9	0.8	1.73	2.31	85.0
10	0.8	1.73	2.46	96.0
11	0.8	1.73	2.51	99.0
Av.			2.40	92.0
SD			0.10	6.6
Range			2.31–2.51	85.0–99.0
12	0.1	3.47	3.12	87.0
13	0.1	3.47	4.09	115.0
14	0.1	3.47	3.67	100.0
15	0.1	3.47	3.50	98.0
Av.			3.59	100.0
SD			0.40	11.5
Range			3.12–4.09	87.0–115.0

Table 2. Level (ppm) of NDEIA relative to active ingredient found in 2,4-D diethanolamine formulations

Sample	NDEIA, ppm		
	LC-TEA	GC-TEA	UV
1	0.7	0.8	0.8
2	0.8	1.1	1.0
3	0.9	0.8	0.9
4	0.7	0.7	0.7
5	0.8	1.0	1.2
6	1.0	1.0	1.4
7	10.2	9.3	15.7
8	1.4	1.1	1.7
9	0.1	0.2	0.1
10	0.1	0.2	0.1

low level. Recovery studies were conducted at levels of 0.35, 0.87, 1.73, and 3.47 ppm, using LC-TEA determination. Results are shown in Table 1. Recoveries averaged from 92.7 ± 6.5% for 0.35 ppm to 100.0 ± 11.5% for 3.47 ppm.

The NDEIA extracts were converted to TMS derivatives by reaction with BSA followed by GC-TEA determination. The results are summarized in Table 2. The absence of TEA response from LC-TEA determination of all sample extracts after the silylation reaction indicates the completion of the reaction. Fan and Fine (16) have shown that nitrosamine can be generated in the injection port of a gas chromatograph under suitable conditions. Table 2 shows similar results from LC-TEA and GC-TEA analysis of the same samples, thus suggesting that NDEIA was not generated in the GC injection port in the present work.

Based on the criterion that the minimum detectable amount (MDA) of a compound gives a recorder response twice the baseline noise, the MDA for both LC-TEA and LC-UV detections was 1 ng. With the starting sample volume of 1 mL, active ingredient concentration of 48% as determined by LC-UV and the final NDEIA extract volume of 2 mL of which 50 μL was analyzed, this MDA corresponds to 0.1 ppm NDEIA relative to 2,4-D weight in the initial sample. The MDA for GC-TEA detection was 0.08 ng. Since only a 3 μL aliquot was analyzed, this MDA also corresponds to 0.1 ppm NDEIA.

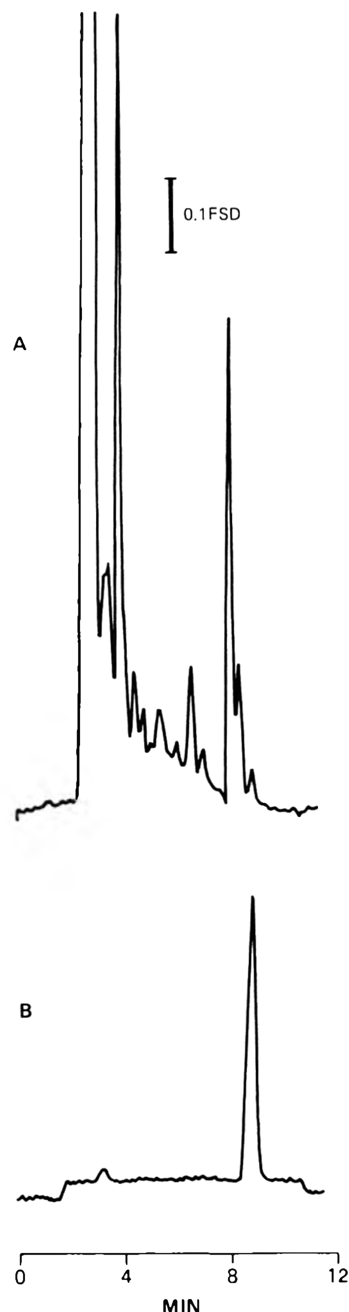

Figure 3. Typical LC chromatogram of sample 8 containing 1.4 ppm NDEIA: A, UV detector; B, TEA detector.

Table 2 outlines the results obtained from the analysis of 10 samples. Two samples gave 0.1 ppm on both LC-UV and LC-TEA detection, while 8 samples gave 0.7 ppm of NDEIA or higher. Samples 6, 7, and 8 had been stored in the laboratory for at least 2 years, which may have affected the level. The results are usually comparable for all 3 methods of determination. The UV detection results are sometimes higher because of some interference with retention time very close to that of the NDEIA. Figure 3 shows 2 chromatograms of sample 8 and supports this interpretation. The chromatogram from UV detection (Figure 3A) shows NDEIA peak at 8.1 min and an unknown peak at 8.4 min whereas the TEA trace (Figure 3B) shows a single peak at 8.7 min. Sample 8 was irradiated and re-analyzed by LC. Figure 4B which is the TEA chromatogram of the irradiated sample 8 shows the

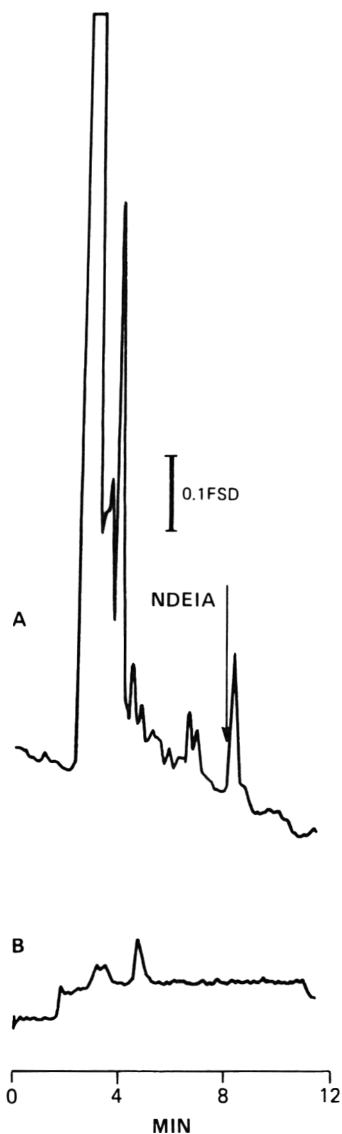

Figure 4. LC chromatogram of sample 8 after UV irradiation: A, UV detector; B, TEA detector.

absence of NDEIA at 8.7 min, while Figure 4A which is the UV trace of the irradiated sample 8 shows the absence of NDEIA at 8.1 min but the continued presence of the unknown peak at 8.4 min. Figure 2 represents 26 ng of NDEIA standard at a retention time of 8.1 min for UV (2A) and 8.7 min for TEA detection (2B).

Two combined extracts of sample 8 as well as the stock NDEIA solution were subjected after silylation to mass spectral analysis using instrumental apparatus described. The presence of NDEIA in the sample extracts was confirmed by comparing the CI mass spectrum of the derivatized sample with that of the standard NDEIA-TMS derivative. Both mass spectral fragmentation patterns of sample and standard were identical in the 3 modes of chemical ionization used. Figure 1A shows the MPCI mode with (M + 1) at 279, Figure 1B shows the MNCI with (M − 30) at 248, and Figure 1C shows ONCI mode with (M + 32) at 310. Figure 5 represents the typical GC-TEA chromatograms of silylated NDEIA standard (5A) and sample solution (5B).

The reproducibility of the LC-TEA, LC-UV, and GC-TEA detection systems was also compared by injecting a standard 7 times, giving corresponding relative standard deviations of 12, 2, and 11.5%. Initially, the LC-TEA results were highly variable. This was corrected by raising the vacuum from 0.45

Figure 5. Typical GC-TEA chromatogram of NDEIA-TMS derivative: A, 0.78 ng standard; B, sample 6.

to 1.2 torr by increasing the flow of the carrier gas and of the oxygen. The reproducibility of the GC-TEA detection could also be improved by using the auto injection technique.

In conclusion, the described method for LC-TEA-UV analysis has been demonstrated to give specific, reliable, and reproducible results for NDEIA in 2,4-D diethanolamine formulations at a level of detection of 0.1 ppm. Since the UV detector offers the same sensitivity as the TEA detector, it can be used with a considerable degree of confidence as an inexpensive alternative to TEA for monitoring the levels of NDEIA. Where the results are above the acceptance level, confirmation by LC-TEA will be desirable to eliminate the possibility of any false positive interferences. Similar results are also obtained when samples are derivatized to trimethylsilyl derivative and analyzed by GC-TEA. Since the TEA Model 543 could be interfaced with a gas chromatograph,

whereas LC requires the more costly TEA Model 502A, this GC-TEA combination provides a less expensive alternative but with the same specificity as LC-TEA detection.

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We thank Narine Gurprasad and Walter Miles for their helpful suggestions and efforts in obtaining the mass spectral data of the silylated derivative of *N*-nitrosodiethanolamine; N. P. Sen, Health and Welfare Canada, for helpful suggestions; and W. P. Cochrane for making this project possible.

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DRUG RESIDUES IN ANIMAL TISSUES

Determination of Monensin Sodium Residues in Beef Liver Tissue by Liquid Chromatography of a Fluorescent Derivative

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Monensin sodium does not have an ultraviolet (UV) absorbance above 220 nm, and therefore cannot be detected by liquid chromatography (LC) with a UV detector. A method was developed in which monensin residues are extracted from beef liver tissue, acetylated, partitioned, and reacted with 9-anthryldiazomethane to form a fluorescent derivative for quantitation by LC. The reliable level of sensitivity is 50 ppb, but 15 ppb can be detected. Recoveries ranged between 71 and 96% with an average of 83.5%.

The antibiotic monensin is used as a coccidiostat in chickens and increases feed efficiency and weight gain in beef cattle. Methods have been published for the determination of monensin sodium in animal feeds, premixes, and tissues. In 1967, Donoho and Kline (1) introduced a bioautography method for monensin in chicken tissue. A microbiological plate assay of monensin in chicken rations was introduced in 1970 by Kline et al. (2), and in 1973, Golab et al. (3) reported a colorimetric method. We recently developed a bioautography method for the semiquantitation of monensin sodium in animal feed (4) and tissue (5).

Monensin sodium has a complex chemical structure, has a molecular weight of 692, and does not have ultraviolet (UV) absorbance above 220 nm. These factors contribute to the fact that monensin cannot be detected by liquid chromatography (LC) under standard operating conditions.

We have modified an acetylation technique as developed by Chamberlin and Agtarap (6), in which the hydroxy groups on the monensin sodium from animal tissue are acetylated. The acetylated monensin is then reacted with 9-anthryldiazomethane (ADAM) (7) to form the fluorescent derivative monensin-9-anthryldiazomethane. The ADAM derivative is determined by LC with use of a fluorometric detector to obtain better sensitivity. The level of quantitation is 50 ppb and the detection limit, although not always consistent, is 15 ppb. The method was tested by extracting monensin residues from spiked beef liver tissue. Recoveries ranged between 71 and 96% or 1.6 and 2.1 μg. In addition, animal feed containing monensin and liver tissue from sheep given the feed, obtained from the Division of Veterinary Medical Research, Center for Veterinary Medicine (CVM), Beltsville, MD, were tested. The reaction time and stability of the ADAM reagent were also tested.

METHOD

Reagents

(a) *Solvents.*—Pyridine, methanol, ethanol, methylene chloride, hexane, and petroleum ether, distilled-in-glass (Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442); anhydrous ethyl ether, reagent grade (American Scientific Products, McGaw Park, IL 60085).

(b) *Acetic anhydride.*—Distilled-in-glass (Burdick & Jackson Laboratories, Inc.).

(c) *Alumina.*—80–200 mesh (Fisher Scientific Co., Fair Lawn, NJ 07410), for 75 mm column.

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(d) *Sephadex LH-20.*—Particle size 25–100 μm (Pharmacia Fine Chemicals, Piscataway, NJ 08854), for 75 mm column.

(e) *Silica gel.*—Bio-Sil A, particle size 100–200 mesh (Bio-Rad Laboratories, Richmond, CA 94804), for 5 mm column.

(f) *9-Anthraldehyde.*—Technical, 90% (remainder anthracene) (Aldrich Chemical Co., Inc., Milwaukee, WI 53233).

(g) *Potassium permanganate.*—(Merck & Co., Rahway, NJ 07065).

(h) *Hydrazine hydrate.*—85% (Aldrich Chemical Co).

(i) *Manganous sulfate.*—Tetrahydrate (Van Walters and Rogers, Denver, CO 80239).

(j) *LC eluants.*—Eluant A, UV acetonitrile (J. T. Baker Chemical Co., Phillipsburg, NJ 08865); eluant B, water–acetonitrile (9 + 1). Filter both acetonitrile and water through 0.5 μm Millipore filter (Millipore Corp., Bedford, MA 01730) before use.

(k) *Monensin sodium reference standard.*—Label declaration, 960 μg/mg (Eli Lilly Laboratories, Greenfield, IN 46140). (*1*) *Stock solution.*—1000 μg/mL. Weigh 10.4 mg monensin sodium (or, if different label declaration, weigh amount to give 1000 μg/mL), transfer to 10 mL volumetric flask, and dilute to volume with anhydrous methanol. Store in dark, cool place. Discard after 30 days. (*2*) *Intermediate solution.*—10 μg/mL. Pipet 1.0 mL stock solution (*1*) into 100 mL volumetric flask and dilute to volume with methanol–water (8 + 2). Prepare fresh before each use. (*3*) *Working solutions.*—2.5 and 5.0 μg/mL. Pipet 2.5 and 5.0 mL of intermediate solution (*2*) into individual 10 mL volumetric flasks and dilute both to volume with methanol–water (8 + 2). Prepare fresh before each use.

Apparatus

(a) *Tissue homogenizer.*—Tissumizer (Tekmar Co., Cincinnati, OH 45222).

(b) *Chromatographic tubes.*—20 × 300 mm, fitted with coarse porosity fritted glass disk and Teflon stopcock for Sephadex LH-20 column; 20 × 200 mm Chromaflex tube (Kontes Co., Vineland, NJ 08300), for alumina column; 15 mm disposable Pasteur pipet (Fisher Scientific Co., Pittsburgh, PA 15219), for 5 mm silica gel column.

(c) *Rotary evaporator.*—Rotavapor R110 vacuum evaporator (Brinkmann Instruments, Inc., Westbury, NY 11590), water bath temperature 48–50°C.

(d) *Receiving flasks.*—100 mL pear-shape flask and 10 mL concentrator tubes, with ₸ 19/22 ground glass stoppers (K-570050, Kontes Co.).

(e) *Analytical evaporator.*—N-EVAP (Organomation Associates, Inc., Northborough, MA 01532), water bath temperature 48–50°C with nitrogen flowing into each tube.

(f) *Liquid chromatograph.*—Hewlett-Packard Model 1084B with automatic injector and 79850B LC terminal (Hewlett-Packard, Englewood, CO 80110).

(g) *Liquid chromatography columns.*—200 × 4.6 mm id, stainless steel column packed with RP-C_8 or RP-C_{18}, 5 μm particle size (Hewlett-Packard); direct connect guard column

with Packer fitting, packed with pellicular C_{18} (Alltech Associates, Inc., Deerfield, IL 60015). Operating conditions: Flow rate 1.0 mL/min; 80% B; eluant temperature 40°C; chart speed 0.50 in./min; injection 20 μL. UV detector external signal; sample wavelength 340 nm; reference wavelength 600 nm; 0.0008 AU/cm; slope sensitivity 0.10 μA. Gradient program: 0–7 min, 80% B; area rejection, 2000; 13–20 min, 90% B; stop. Mobile phase isocratic.

(h) *Fluorescence detector.*—Model FS-950 LC fluorometer (Kratos/Schoeffel Instruments, Westwood, NJ 07675). Operating conditions: wavelength drive FSA 339 GM 970 monochromator with automatic overload reset control, excitation at 365 nm, 418 nm emission filter, and sensitivity at 0.10.

(i) *Syringes.*—For preparation of samples for LC analysis: 2 mL hypodermic reusable glass Luer-Lok syringe (V.W.R. Scientific Inc., Denver, CO 80239); SP Varipet, 10 mL with metal Luer-Lok tip-syringe type variable volume transfer pipet (American Scientific Products, Denver, CO 80239).

Synthesis of 9-Anthraldehyde Hydrazone (7)

Dissolve 8.8 g 9-anthraldehyde in 150 mL absolute ethanol, add 8.5 g 85% hydrazine hydrate, and stir at room temperature 3 h. After 10 min the color will change from yellow to dark yellow. Filter off solid product and dry under vacuum. Recrystallize from ethanol to give red-yellow crystals of 9-anthraldehyde hydrazone, mp 124–126°C (yield 90%).

Synthesis of Activated Manganese Dioxide (8, 9)

Dissolve 1110 g manganous sulfate (tetrahydrate) in 1.5 L water and add simultaneously with 1170 mL 40% NaOH during 1 h to a hot stirred solution of 960 g potassium permanganate in 6 L water. Manganese dioxide precipitates as a fine brown solid soon after the start. Continue stirring for an additional 1 h and then collect solid by centrifugation. Wash with water until washings are colorless. Dry solid in 100–120°C oven and grind to fine powder before use (yield 920 g).

Preparation of 9-Anthryldiazomethane (ADAM) (10)

Weigh 0.220 g 9-anthraldehyde hydrazone and place in Erlenmeyer flask. Add 100 mL anhydrous ethyl ether and shake to dissolve crystals. Add 0.8 g activated manganese dioxide and 0.6 mL ethanol (saturated with potassium hydroxide). Stir vigorously 30 min with magnetic stirrer. Filter off manganese dioxide, using microfiber glass filter, and collect filtrate in 150 mL pear-shape flask. Wash manganese dioxide on filter with 20 mL anhydrous ether and add wash to flask. Evaporate to reduce volume. Transfer to actinic 100 mL volumetric flask and dilute to volume with anhydrous ethyl ether. Store in refrigerator in the dark. *Note:* Discard after 30 days.

Column Preparation

Prepare alumina and Sephadex LH-20 columns as previously described (4). Prepare silica gel column by placing glass wool plug at base of 15 mm Pasteur pipet. Add silica gel to height of 5 mm from top of glass wool plug. Tap gently and add more silica gel if necessary. Prepare just before use. Do not restrict flow. Silica gel should be oven-dried overnight at 110°C.

Sample Preparation

Weigh 10 g frozen tissue into 50 mL round-bottom glass centrifuge tube, add 20 mL methanol–water (8 + 2), and homogenize tissue for 2–3 min. Wash homogenizer blade with 5 mL methanol-water and add wash to tube. Centrifuge homogenate 15 min at 1500–1800 rpm. Decant supernate directly onto alumina column and elute into 250 mL separatory funnel. Repeat extraction by adding 25 mL methanol–water (8 + 2) to homogenate, mix, shake tube for 15 min on automatic shaker, and centrifuge 15 min at 1500–1800 rpm. Decant supernate directly onto alumina column and elute. Wash alumina column with additional 100 mL methanol–water (8 + 2). Collect eluates and wash in 250 mL separatory funnel containing 100 mL 5% NaCl. Shake vigorously and let mixture stand 5 min. Partition monensin into three 30 mL portions of methylene chloride; after addition of each 30 mL, shake separatory funnel for 1 min and let methylene chloride layer separate. Drain lower layer into 100 mL pear-shape flask. Combine methylene chloride portions and evaporate to dryness, using rotary evaporator with water bath at 48–50°C (4).

Dissolve residue in 1.0 mL methanol–water (8 + 2) and transfer to Sephadex LH-20 column. Let solution just enter bed of column. Begin collecting eluates immediately. Wash flask with two 3.5 mL portions of methanol–water (8 + 2). Let each portion enter bed of column. Wash with additional 10 mL portion of solvent, and discard total 18 mL eluate. Add additional 10 mL portion of solvent and collect this fraction, which should contain monensin, in 10 mL concentrator tube. Evaporate to dryness in analytical evaporator, with water bath at 48–50°C and nitrogen flowing into tube. Process blank control and spiked sample equivalent to 250 ppb monensin sodium reference standard with each set of experimental samples.

Preparation of Acetylated Monensin

Dissolve residue in concentrator tube with 1.0 mL methylene chloride and transfer to 1.0 mL crimp-top glass vial. Evaporate to dryness under gentle flow of nitrogen in N-EVAP analytical evaporator with water bath at 48–50°C. Wash tube with 1.0 mL methylene chloride, 0.5 mL methanol, and final 1.0 mL methylene chloride, evaporating each volume to dryness. Add 100 μL pyridine and 100 μL acetic anhydride to monensin residue. Stopper vial with metal crimp top before gently swirling mixture until residue is dissolved. Allow reaction to proceed overnight at room temperature.

Partition acetylated monensin into petroleum and ethyl ether as follows: Transfer acetylated monensin into 125 mL separatory funnel containing 35 mL distilled water. Use additional 5 mL to wash vial. Add 40 mL petroleum ether and shake mixture for 1.0 min. Decant aqueous phase into second 125 mL separatory funnel and save petroleum ether fraction. Add 40 mL anhydrous ethyl ether, shake 1.0 min, discard aqueous phase, and combine both ethers into one separatory funnel. Shake to mix. Add 20 mL 10% HCl, shake 1.0 min, and discard aqueous phase. Add two 20 mL portions of water, shake 1.0 min after each portion has been added, and discard aqueous phase. Add 40 mL saturated NaCl, shake 1.0 min, and discard aqueous phase. Filter ethers through glass microfiber filter containing 20–30 g Na_2SO_4; wash Na_2SO_4 with additional 10–15 mL petroleum ether and collect total volume in 100 mL pear-shape flask. Evaporate total volume, using N-EVAP with water temperature at 48–50°C. Dissolve residue in 1.0 mL methanol (dried over Na_2SO_4) and transfer to graduated 10 mL conical glass tube. Wash flask with three 2 mL portions of methanol. Add washings to tube and evaporate contents to near dryness under nitrogen, with water temperature at 48–50°C.

Table 1. Recovery data for 10 sheep liver tissue samples, each spiked with 2.2 ppm monensin sodium, for quantitation by LC

Sample[a]	Area counts	Rec., %[b]	Retention time, min
Blank			
1	7264	79	14.16
2	8262	89	14.07
3	8622	93	14.09
4	6567	71	14.18
5	8880	96	14.16
6	8140	88	14.01
7	7493	81	13.98
8	6568	71	14.07
9	7677	83	14.07
10	7770	84	14.13
Ref. std rec.	8788	95	14.12
Ref. std	9250		
Mean		83.5	
SD		8.4096	
CV, %		10.07	

[a]Blank, beef liver tissue without monesin; samples 1–10, 2.2 ppm. Reference standard recovery sample, 2.2 μg monensin sodium/mL carried through procedure starting with 5% NaCl–methylene chloride partition; reference standard, 2.2 μg monensin sodium/mL taken directly from dilution flask and acetylated. An injection volume of 20 μL was used to obtain results as close to 50 ppb as possible.
[b]Percent recoveries based on LC determination, assuming 100% recovery of 44 ppb reference standard.

Preparation of Monensin-9-Anthryldiazomethane from Diacetyl Monensin

Add ethanol (dried over Na_2SO_4) to 1.0 mL in conical centrifuge tube to dissolve residue. Add 0.5 mL ADAM, mix, and stopper tube. Allow mixture to react 30 min in dark place (e.g., cabinet drawer). To stop reaction, evaporate to dryness under nitrogen, with water bath at 48–50°C.

Isolation of Derivatized Monensin

Prepare a silica gel column. Do not restrict flow throughout cleanup. Elute column with 5–6 mL hexane to wet silica gel.

Dissolve monensin-ADAM residue in 1.0 mL hexane and transfer to silica gel column. Wash tube with additional 9.0 mL hexane, elute through column, and discard eluate. Wash tube with 10 mL hexane–methylene chloride (1 + 1), add to column, and discard eluate. Add 10 mL hexane–methylene chloride (2 + 8) to column, followed by 10 mL methylene chloride, and discard each eluate. (Elution to this point can be hastened by using safety pipetting bulb to apply pressure.)

Add 1.0 mL methanol to column, elute without pressure, and discard eluate. Add additional 1.0 mL methanol to column and collect eluate in 1.0 mL crimp-top vial. Seal with metal crimp top for storage until eluate is injected into liquid chromatograph.

Results and Discussion

Recovery determinations were performed by using 10 g portions of blank frozen beef liver tissue spiked with 2.2 μg monensin sodium. Ten spiked samples, a blank control, and 2 reference standards were analyzed simultaneously. Recoveries ranged between 71 and 96%, with an average of 83.5% (Table 1). All data are based on results obtained from 20 μL injections of test samples and reference standards. The unknown test samples were compared with reference standards equivalent to 50 ppb.

The detection limit was determined to be 15 ppb; data were obtained by making serial dilutions of a beef liver tissue sample spiked with 7.5 ppm monensin. The sensitivity, determined by using beef liver tissue spiked with 2.5 ppm monensin, is 50 ppb. The pure monensin derivative showed linearity between 40 and 200 ppb. In all tests, 20 μL injections were used.

Sheep liver tissue samples were obtained from an experiment conducted by CVM in which animal feed containing 20 g monensin/ton, 0.3–0.6% calcium, and 0.3–0.6% sodium phosphate was used. Results of the analysis showed that the addition of calcium and sodium phosphate to the diets fed to the sheep did not affect the retention of monensin residues. The treated sheep liver tissue samples were negative for control blank tissue spiked with 2.5 ppm monensin residues when compared with control blank tissue spiked with 2.5 ppm monensin reference standard; recoveries for spiked tissue samples (20 μL injections) were 94–96%. Further studies are needed to determine whether the presence of calcium and sodium phosphate in animal feed interferes with the derivatization of monensin.

Bile samples from horses administered [^{14}C]monensin were tested by using this method; results showed interference and poor peak integrations. The presence of monensin residues was confirmed in 2 of 3 samples analyzed by bioautography (5). The third sample showed several zones of inhibition, but the zones were not at the same R_f as the monensin reference standard. Two horse fecal samples, one wet and one dry, were also tested; recoveries were 94.3 and 65.2% of the declared concentrations of 5.30 and 6.89 μg/mL, respectively. These samples were obtained from a study conducted by the Agricultural Research Service, U.S. Department of Agriculture, Fargo, ND.

The acetylation of monensin sodium was confirmed by mass spectroscopy (MS), infrared spectroscopy (IR) and XE-fast atom bombardment (FAB) (Figure 1). Data obtained by MS, IR, and LC suggest that monensin had been derivatized by the ADAM reagent, but confirmation was inconclusive because of the lack of the MS mass range capacity to confirm the molecular weight of this large compound. The pathway of monensin sodium, diacetyl monensin, and the suggested monensin-9-anthryldiazomethane derivative is shown in Figure 2. The synthesis of 9-anthraldehyde hydrazone and 9-anthryldiazomethane was accomplished, and the results were confirmed by MS (Figure 3). The ADAM reagent can now be obtained from Sigma Chemical Co., St. Louis, MO, but was not commercially available when our work was performed.

The reaction time of the ADAM derivatizing agent was tested over a period of 2.5 h at 15 min intervals. The 30 min reaction time gave the best results when the Hewlett-Packard 1084B liquid chromatograph with a Kratos fluorometer attachment was used. The ADAM reagent was also tested for stability and it was determined to be stable for 30 days. To confirm the technique, samples were analyzed on both Hewlett-Packard and Waters liquid chromatographs and results were comparable.

Other antibiotics, classed as ionophores, have been tested for interference against monensin and none interfered when analyzed by thin layer chromatography, bioautography, or as derivatized compounds. The results of this work are being incorporated into a multiresidue method for the determination of ionophore residues in beef liver tissue.

Acknowledgments

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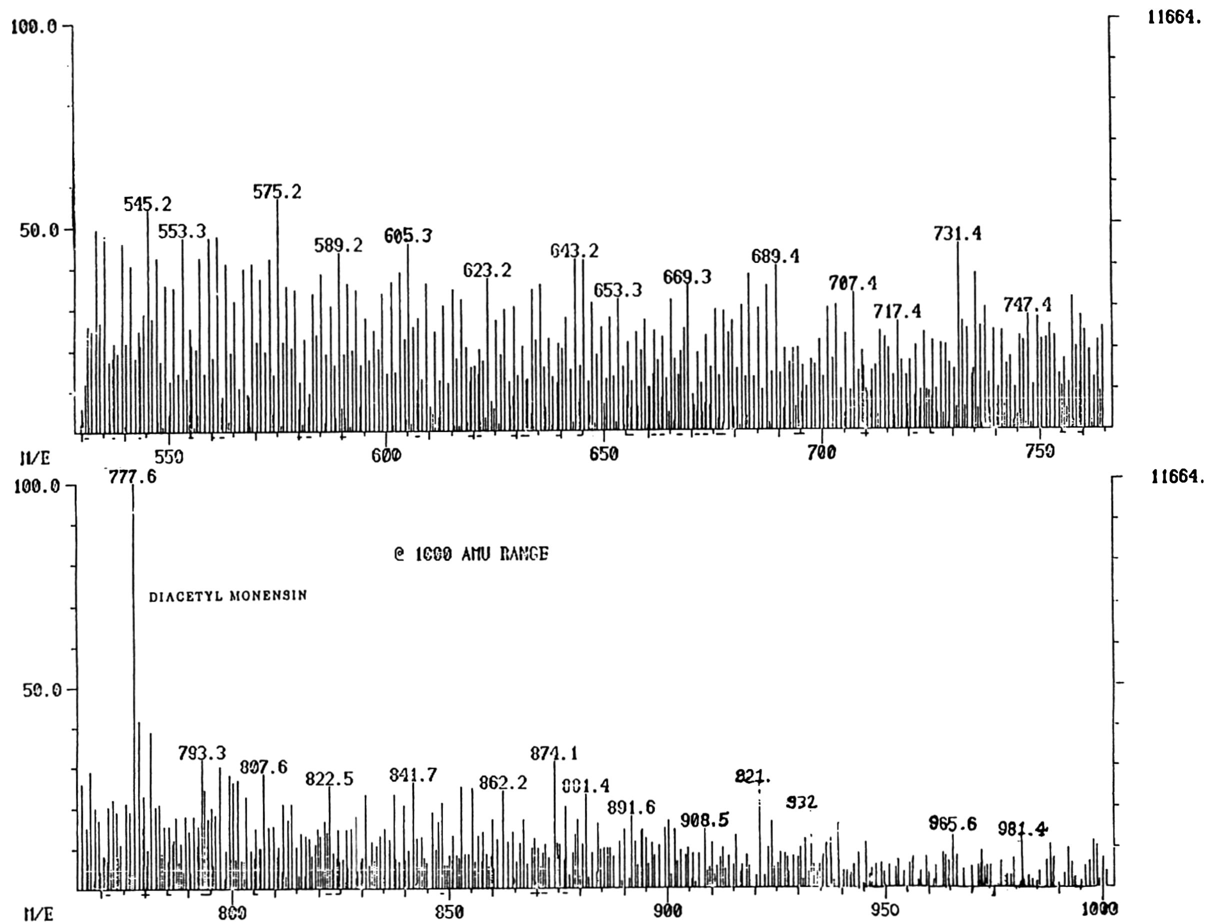

Figure 1. XE-FAB mass spectrum of 9-anthryldiazomethane monensin. Peak at mass 777.6 identified as diacetylated monensin.

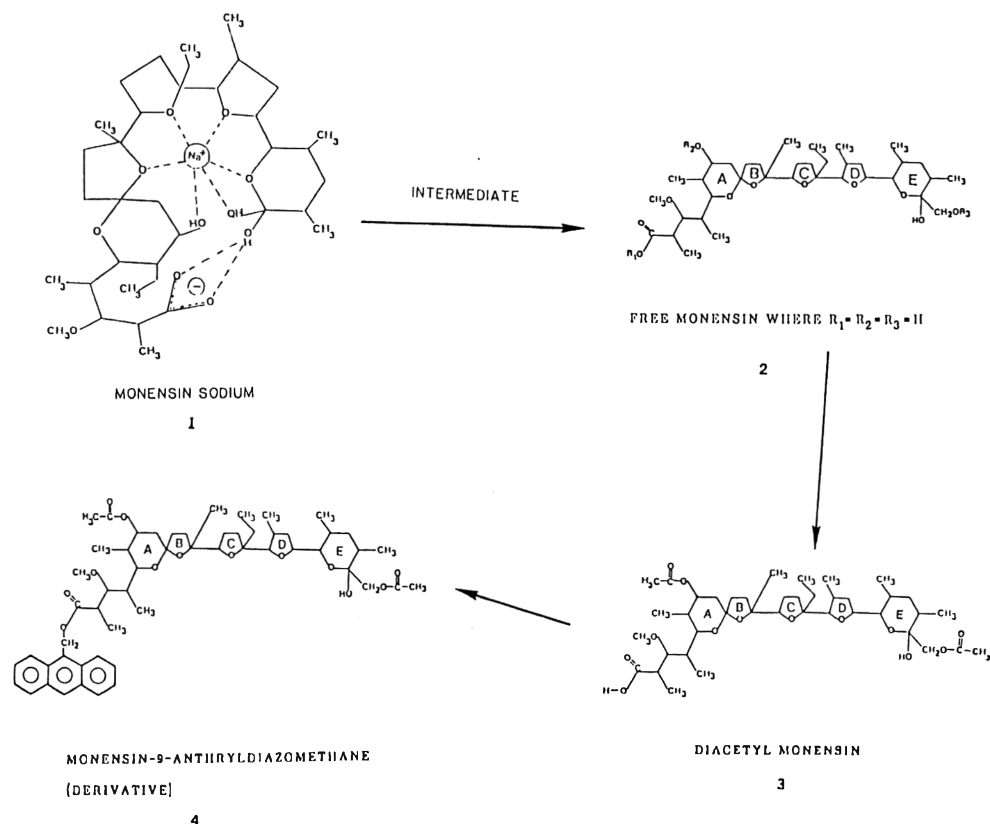

Figure 2. Derivatization pathway of monensin sodium to fluorescent derivative. (1) Monensin sodium molecule; (2) free monensin as an intermediate where each *R* group is a hydrogen; (3) monensin acetylated in pyridine–acetic acid at room temperature to form diacetyl monensin; (4) diacetyl monensin reacted 30 min in the dark with ADAM to form monensin-9-anthryldiazomethane.

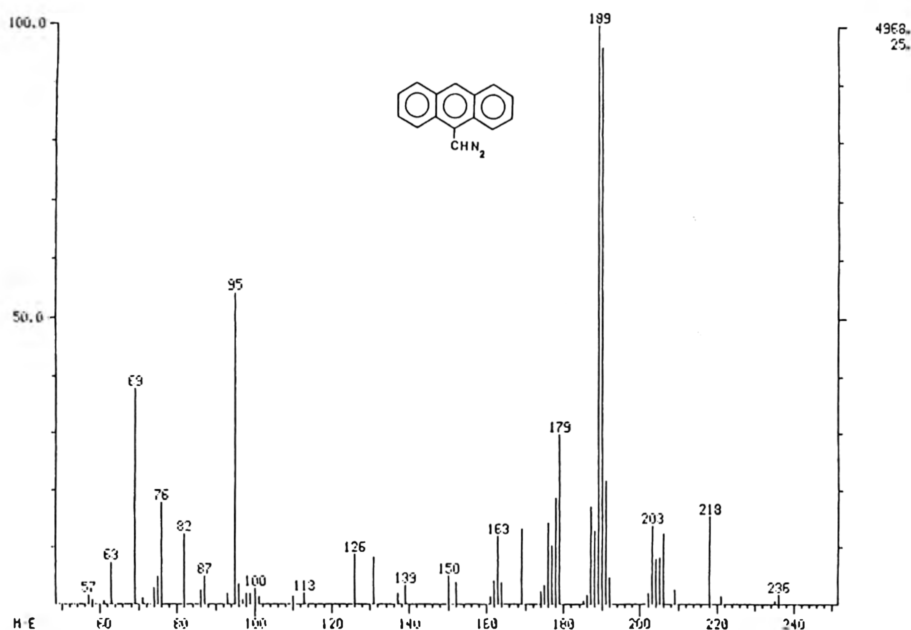

Figure 3. Mass spectrum confirmation of the synthesis of 9-anthryldiazomethane (ADAM) derivatizing agent.

bile and fecal samples from horses; and Ronald K. Mitchum, NCTR, FDA, for performing the XE-FAB mass spectrum of 9-anthryldiazomethane monensin.

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FOOD ADULTERATION

Gas Chromatographic-Mass Spectrometric Detection of Adulteration of Natural Lime Oils

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Distilled and expressed lime oils were characterized by glass capillary gas chromatography. Components present at concentrations greater than 0.1% were identified by mass spectrometry. Expressed oils contained less than 1% monoterpene alcohols, whereas distilled oils contained more than 10%. Expressed oils contained sabinene and 4 times as much beta-pinene as distilled oils. Adulteration was detected by finding component area percents and/or their ratios to be outside the 95% confidence intervals established for pure oils.

The Tariff Schedules of the United States (TSUS) (1) list rates of duty applicable to each imported commodity. To assess the proper rate of duty on importations of lime oil, it is necessary to identify the product and detect any adulteration. Pure lime oil, whether expressed, extracted, or distilled, is classified under the provision for "Essential Oils." However, the addition of any substance to the oil, even a natural substance, requires classification under the provision for "Artificial Mixtures of Aromatic and Odoriferous Substances," usually at a significantly higher rate of duty. To tell whether a sample is adulterated, the Customs analyst must, therefore, identify the constituents of lime oil, and determine their normal concentration ranges and the quantitative relations that these ranges have with respect to each other.

The purpose of this work is to establish 95% confidence intervals for the concentration of characteristic components in expressed and distilled lime oils and to use these data in a logical scheme to determine whether adulteration has taken place.

METHOD

Apparatus and Reagents

(a) *GC column.*—Scientific Glass Engineering Inc., Austin, TX. 30 m × 0.5 mm id SCOT glass capillary column containing Carbowax 20M stationary phase.

(b) *Gas chromatograph-mass spectrometer (GC/MS).*—Perkin-Elmer Model 3920 equipped with flame ionization detector, and capillary injector system, providing 30:1 injection split ratio, and interfaced via jet separator with Hitachi RMU-6L single focusing, low resolution, magnetic sector mass spectrometer. Chromatographic operating conditions: injector temperature, 225°C; detector temperature, 250°C; MS interface temperature, 250°C; initial column temperature, 60°C, held for 4 min, then programmed at 2°/min to 200°C and held for 16 min; helium carrier gas linear flow 19 cm/s; detector hydrogen and air flows 40 and 550 mL/min, respectively. MS operating conditions: ionization voltage, 70 eV; ionization chamber temperature, 200°C; target current, 50 μamp; accelerating voltage 3200 V; source pressure during GC/MS runs, 5×10^{-6} torr.

(c) *Chromatographic data system.*—Hewlett-Packard 3353B laboratory automation system.

(d) *Essential oils.*—Imported samples submitted for Customs examination and reference samples from Citrus and Allied Essences, Floral Park, NY 11001; Polarome, Inc., New York, NY 10013; and Aroma Resources Inc., New York, NY 10001.

(e) *Essential oil components.*—Limonene, gamma-terpinene, linalool, linalyl acetate (IFF Inc., Hazlett, NJ 07730); terpinolene, *cis*-beta-ocimene (Colgate Palmolive Co., Piscataway, NJ 08854); alpha-farnesene, *trans*-alpha-bergamotene, beta-elemene, alpha-cedrene, valencene, beta-caryophyllene, beta-terpineol, bisabolene (Roure-Bertrand-Dupont, Teaneck, NJ 07666); alpha-pinene, beta-pinene, camphene, myrcene, geraniol, citral (neral and geranial), 1,8-cineole, para-cymene, fenchyl alcohol, nerol (Aldrich Chemical Co., Milwaukee, WI 53233); alpha-terpinene (PCR Research Chemicals Inc., Gainesville, FL 32602); alpha-terpineol (Eastman Kodak Co., Rochester, NY 14650); 4-terpineol (Applied Science Laboratories Inc., State College, PA 16801). Neryl acetate was prepared by acetylation of nerol with acetic anhydride.

Procedure

(a) *Gas chromatography/mass spectroscopy.*—Prepare a 1% solution of each available known essential oil component by dissolving 10 μL component in 990 μL previously prepared stock solution of 1% linalool in chloroform. Inject 0.3 μL into GC system after first opening splitter valve for 30 s under described chromatographic conditions. Close injector splitter valve 30 s after injection. Record and measure retention time and peak area for each component using GC data system. Calculate relative retention time (RRT) with respect to linalool and use these values routinely for evaluation of lime oil samples. Reinject 1 μL into GC system as described. After chloroform peak elutes, open mass spectrometer inlet valve and increase source pressure to 5×10^{-6} torr. Record mass spectrum of component whenever total ion monitor curve reaches a maximum.

Inject 0.2 μL of each neat essential oil as described for individual components, except open MS inlet valve immediately after injector splitter valve is closed. Record and measure spectra retention times, and peak areas as described. Chromatographic peaks were judged to be identified by MS when the 10 largest MS fragments in spectrum matched those in spectrum recorded at same RRT during single component runs, or, in case of sabinene, recorded in *Eight Peak Index of Mass Spectra* (2).

(b) *Statistical analysis.*—Ninety-five percent confidence intervals for average peak area percents and peak area percent ratios were calculated using the normal distribution (3) and confirmed by Student's *t*-test or the *Q*-test.

Results and Discussion

The characterization of pure lime oil, especially distilled lime oil, is a complex problem. Many chemical reactions that occur during steam distillation of the crushed fruit alter the original composition of the oil produced by the plant. For instance, expressed lime oil contains significant amounts of sabinene and monoterpene aldehydes, while distilled lime oils contain no sabinene and much lower concentrations of the aldehydes and beta-pinene. On the other hand, the distilled oil contains several monoterpene alcohols, such as alpha-

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terpineol, and much higher levels of para-cymene and terpinolene than does the expressed oil. These differences in composition, due simply to differences in processing, must be recognized in order to properly classify the imported product.

The reactions that cause changes in the qualitative and quantitative composition of lime oil depend on variables such as time elapsed between crushing the fruit and distillation of the juice, pH of the crushed fruit, and duration of the distillation (4–8). The Customs analyst has little, if any, information concerning these parameters when evaluating an imported product. In addition, lime oil may be derived from 2 plant species, *Citrus aurantifolia* and *Citrus latifolia* ("Persian") (4). The former is used primarily for distilled oils and the latter primarily for expressed oils. Although, most of the components of imported lime oils are the same, regardless of species, some changes in their concentrations may be expected because of differences in growing conditions, production variables, and possibly, country of origin.

Several excellent publications are available containing more or less complete qualitative and quantitative analyses of lime oils. Guenther's (4) classical work describes production methods, physical characteristics, and types of adulteration, but gives an incomplete listing of the components and their concentrations. Several more recent works (5–8) have reviewed the current commercial production methods and the chemical changes that they effect on the lime and its oil. Since 1970, gas chromatographic (GC) and mass spectrometric (MS) data (5–15) have been published, which give the identities and concentrations of trace components of these oils. Even the nonvolatile, but highly characteristic, psoralens (16) have been characterized by liquid chromatography (LC). Finally, Shaw (17) has recently reviewed the whole subject of citrus oil quantitative analysis, including lime oils. However, none of these authors have provided the statistical evaluation of concentrations required to decide whether a particular sample is truly an unadulterated lime oil.

During this study, 55 lime oil samples, including 13 reference samples, were examined. Of the samples, 31 (3 reference samples) were distilled oils, 5 (3 reference samples) were expressed oils, 10 (5 reference samples) were concentrated distilled oils, 2 (2 reference samples) were concentrated expressed oils, and 7 were found to be adulterated or formulated oils by evaluation of the data acquired by application of the procedure described. Of the 7 found to be adulterated or formulated, 4 had been claimed to be pure lime oils.

Typical chromatograms of distilled and expressed oils are presented in Figures 1 and 2. The 95% confidence intervals for the peak area percent (95% area range) of 25 selected components of pure unconcentrated distilled and expressed lime oils are presented in Table 1. The area observed for these 25 peaks accounts for more than 90% of the total area recorded in the chromatograms of the pure expressed and distilled lime oils studied. The 95% area range was confirmed by application of Student's t-test (3) for distilled oils or the Q-test (3) for expressed oils. Several other components, such as 1,4-cineole, alpha-4-dimethylstyrene, beta-elemene, and several isomers of farnesene, were also identified, but their 95% area ranges were so wide or their chromatographic purity so questionable that they were not included in Table 1. Twenty-two of the 25 components listed in Table 1 were identified by comparing relative retention times and mass spectra with those of authentic standards. Peak 4 (sabinene) was identified by comparing its mass spectrum with that in Aldermaston (2). Peaks 14 and 21 gave the same spectra in each of the lime products analyzed, but could not be identified more completely by comparison with Aldermaston (2) or with our own user libraries. Table 1 lists the components in order of increasing RRT with respect to linalool. The peak area percents for the 7 samples believed to be adulterated or formulated oils are also presented.

The chromatographic peak area percent data acquired for all reference and imported samples examined during this study were evaluated using 3 increasingly selective criteria to establish characteristics for products derived from natural fruit sources in order to identify, in turn, those oils that would be considered adulterated. The first approach was a broad qualitative evaluation of the chromatograms. The curves were visually searched for the presence of any recorded peaks exceeding 0.5% of the total area, which were not generally present in the lime oils studied. The absence of minor peaks was not considered significant because the components represented may have been removed or reduced below the limits of detection during a legitimate production process, e.g., by distillation or by extraction. This simple visual examination of the chromatogram was sufficient to distinguish samples 6 and 7 as adulterated or formulated.

Superficially, sample 6 appeared to be a mixture of both distilled and expressed lime oils because it contained both sabinene (0.4%) and alpha-terpineol (8.2%), along with a typical amount of limonene. However, the chromatogram had several unidentified peaks in the linalool region (near RRT = 1.000), which were not seen in any other lime oil chromatogram. Sample 7 appeared superficially to be an expressed oil. Its chromatogram exhibited sabinene at 1.7%, high concentrations of alpha- and beta-pinene, and low concentrations of para-cymene, terpinolene, and alpha-terpineol. However, a series of late eluting peaks (RRT > 1.5) were observed that were probably cinnamates on the basis of the accompanying aroma.

The second step in the evaluation procedure involved the consideration of the 95% area range established for the 25 representative peaks, each of which exceeded 0.1% of the total area. Area percent ranges have been relied on as the primary means for determining the purity of essential oils in similar studies of cold pressed lemon (18), lavender (19, 20), spike lavender, lavendin, and bitter orange tree oils (21). However, in the case of the lime oils, the standard deviations of the mean of the area percent of the components are considerably greater than those previously encountered, many exceeding 50%, leading to broader 95% area ranges.

Despite this problem, the peak area statistics from Table 1 clearly distinguish distilled and expressed lime oils. With respect to the expressed oils, the distilled oils exhibit decreased concentrations of alpha- and beta-pinenes, sabinene (0%), neral, and geranial. On the other hand, the distilled oils contain higher concentrations of terpinolene, para-cymene, and the monoterpene alcohols. These differences are to be expected on the basis of the well documented reactions known to take place during the distillation of the highly acidic juice (5, 7, 8, 11). Therefore, the final composition of the oils depends not so much on growing conditions as on the production conditions, such as the pH of the juice, the time interval between crushing the fruit and the start of distillation, and the duration of the distillation. Product 4 can be recognized as an artificial mixture or formulated oil, using the broad qualitative examination followed by peak area percent analysis. In product 4, which is believed to be formulated as a distilled oil, the most significant and obvious deviations from the natural product profile are the high levels of limonene and alpha-terpinene. In addition, the peak areas of 17 of the 25 selected components are outside the 95% confidence intervals established for distilled lime oil.

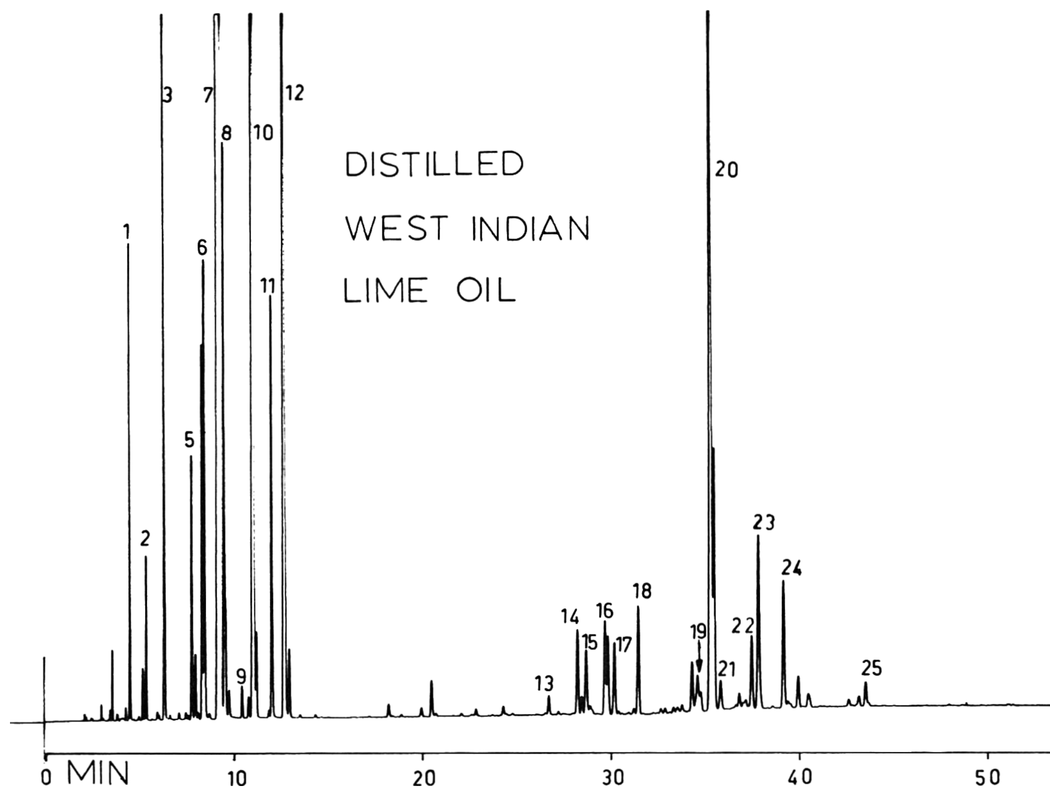

Figure 1. Chromatogram of distilled West Indian lime oil. See Table 1 for identification of peak numbers.

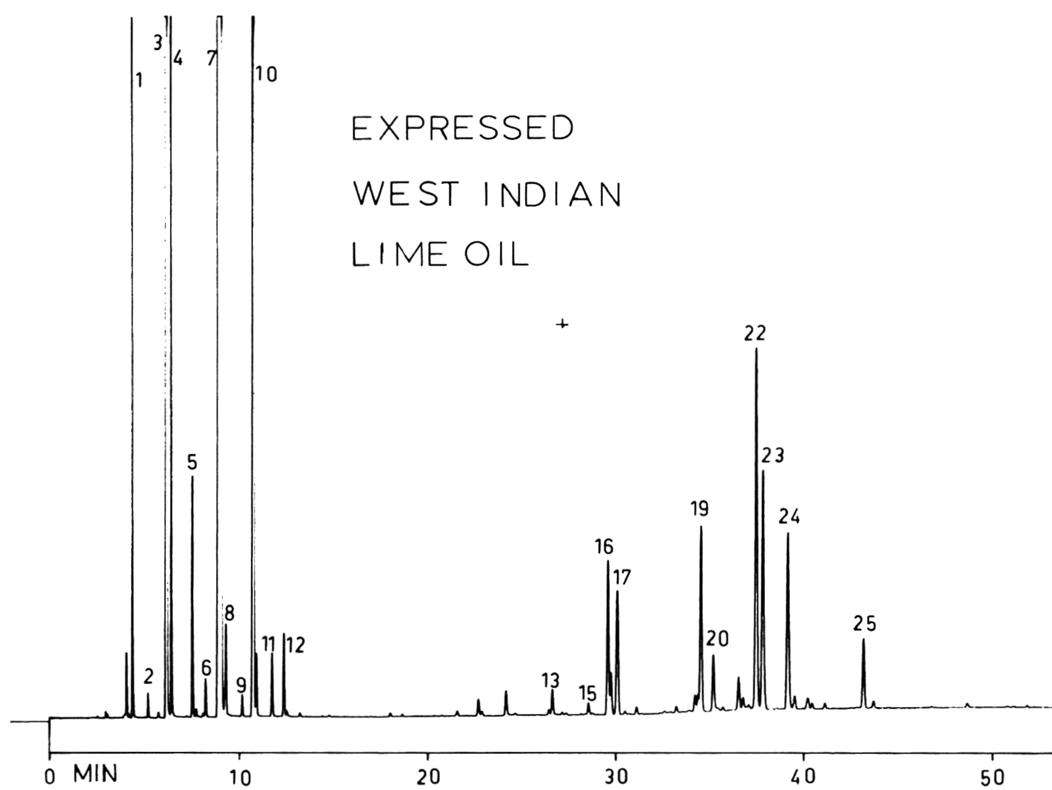

Figure 2. Chromatogram of expressed West Indian lime oil. See Table 1 for identification of peak numbers.

Table 1. Composition of pure and adulterated lime oils, based on area percent of the chromatographically separated components

			95% Area range		Suspect oils						
Peak	Identity	RRT	Distilled	Expressed	1	2	3	4	5	6	7
1	alpha-pinene	0.269	1.3– 1.9	2.7– 3.1	3.1	1.3	1.4	0.6	0.1	1.3	2.4
2	camphene	0.315	0.4– 0.6	0 – 0.5	0.8	0.5	0.9	0.1	ND[e]	0.3	0.1
3	beta-pinene	0.367	1.7– 5.3	4.3–23.0	3.3	4.5	4.9	1.5	0.2	4.1	9.4
4	sabinene	0.379	ND	0.4– 4.2	0.1	ND	ND	ND	ND	0.4	1.7
5	myrcene[a]	0.429	0.7– 1.1	0.8– 1.6	1.0	1.0	1.0	1.0	0.1	0.5	1.1
6	alpha-terpinene[b]	0.459	0.8– 1.1	0.1– 0.4	2.2	3.3	2.5	3.7	0.4	1.3	0.2
7	limonene	0.495	42.8–52.3	43.7–56.2	44.0	41.0	38.0	64.0	4.1	46.0	64.0
8	1,8-cineole	0.504	1.8– 3.0	0.3– 0.5	2.5	3.5	4.1	4.9	0.5	2.3	ND
9	*cis*-beta-ocimene	0.553	0.2– 0.4	0.1– 0.3	0.3	0.3	0.3	ND	ND	ND	ND
10	gamma-terpinene	0.559	6.8–11.6	2.5–16.6	9.8	12.0	6.4	1.1	0.9	4.2	6.8
11	para-cymene	0.593	1.5– 7.5	0.1– 1.3	3.2	2.4	4.0	6.3	0.3	3.8	0.7
12	terpinolene	0.615	2.9– 7.5	0.5– 0.7	5.6	11.0	7.5	0.7	0.7	2.2	0.4
13	linalool	1.000	0.2– 0.3	0.1– 0.3	0.2	ND	0.1	0.2	ND	1.1	1.7
14	$C_{10}H_{16}6. H_2O$[c]	1.049	0.4– 1.0	ND	0.9	0.8	0.5	0.4	ND	0.8	ND
15	fenchyl alcohol	1.062	0.5– 0.9	0 – 0.4	0.6	1.0	0.4	0.7	34.0	0.8	ND
16	bergamotene & 4-terpineol	1.092	0.6– 1.4	0.7– 1.3	0.7	2.8	0.7	0.1	0.5	2.4	0.4
17	beta-caryophyllene	1.116	0.4– 0.8	0.1– 0.9	0.4	0.3	0.2	ND	ND	0.6	0.2
18	beta-terpineol	1.191	0.5– 1.0	ND	0.9	1.0	0.9	0.9	51.0	0.9	ND
19	neral	1.212	0.2– 0.3	0.5– 1.0	0.3	0.2	0.3	0.6	ND	0.4	ND
20	alpha-terpineol	1.230	6.1– 9.1	0.1– 0.4	9.4	6.0	7.8	9.4	1.3	8.2	0.6
21	$C_{15}H_{24}$[c]	1.255	0.3– 0.4	ND	ND	ND	0.3	0.2	ND	0.3	0.2
22	geranial	1.284	0.2– 0.4	0.3– 4.9	0.2	0.3	0.4	ND	ND	4.2	1.5
23	bisabolene & neryl acetate	1.298	1.3– 2.1	1.5– 2.3	1.5	0.7	0.6	ND	0.5	2.7	0.6
24	geranyl acetate[d]	1.321	0.6– 1.4	0.1– 1.8	1.0	0.4	0.4	0.3	0.3	1.7	0.3
25	geraniol	1.432	0.2– 0.7	0 – 0.2	0.2	1.4	1.3	0.4	0.5	2.8	0.1

[a] A small peak at m/z 138 in the mass spectrum indicates that a compound of the empirical formula $C_{10}H_{18}$ coelutes.
[b] A small peak at m/z 154 in the mass spectrum indicates that a terpenoid ether of the empirical formula $C_{10}H_{18}O$, possibly 1,4-cineole, coelutes.
[c] These 2 compounds could not be further characterized, but they help to distinguish distilled and expressed oils.
[d] Small peaks at m/z 204 and m/z 189 in the mass spectrum indicate that a small amount of a sesquiterpene hydrocarbon of empirical formula $C_{15}H_{24}$ coelutes.
[e] Not detected.

The data obtained for products 1, 2, 3, and 5 were not considered sufficient at this stage to determine their status. Assuming from its monoterpene alcohol content that product 1 is formulated as a distilled oil, it differs statistically from the pure oil only in its content of alpha-pinene, camphene, and sabinene. Products 2 and 3 had lower neryl acetate/bisabolene values than either expressed or distilled lime oil, and the 2 products were higher in geraniol, terpinolene, 1,8-cineole, and alpha-terpinene than even the distilled oil. However, the relative standard deviations (RSD) observed for the area percents of these components are high, and there were no instances in which a component of products 2 and 3 was outside the 95% area range when the RSD was low. Finally, although 19 of the 25 selected components are grossly out of the 95% area range of expressed or distilled lime oil for product 5, the presence of 2 large peaks, corresponding to fenchyl alcohol and beta-terpineol, accounting for more than 85% of the total peak area, suggested that product 5 might be some type of concentrated natural product.

To discriminate further, a third approach to the analysis was developed: the establishment of 95% confidence intervals for *ratios* of certain peak area percents. This step magnifies small differences in peak area percents without a proportional increase in the RSD, since the RSD of the ratio does not involve the direct product of the standard deviations of the peak area percents. The maximum RSD of an average peak area percent ratio of 2 components, x and y, is given by the equation (3):

$$RSD = \sqrt{(s_x/A_x)^2 + (s_y/A_y)^2}$$

where s is the standard deviation calculated assuming the normal distribution and A is the average peak area percent. The total number of such ratios which may be calculated from a chromatogram of 25 peaks, eliminating reciprocals and ratios such as A_x/A_x, is 300. These ratios are intrinsic properties of the oils studied, just as refractive index and density are. Thus, even if many ratio intervals overlap when comparing 2 types of oil, such as expressed lemon and expressed lime, or when trying to confirm adulteration in an oil, only 1 discrepancy is enough to confirm a difference. Therefore, it is usually necessary to calculate only a few of the possible ratios. Using these derived data, problems presented by oils that are only slightly outside the established ranges, and possible admixtures of expressed lime and cold pressed lemon oils, can be addressed. A small number of the possible ratios are displayed in Table 2. These data were obtained by calculating the average peak area percent ratio, A_x/A_7, where A_x is the average peak area percent of one of the numbered peaks listed in Table 1 divided by the average peak area percent of peak 7 (limonene), for distilled lime oil, expressed lime oil, expressed lemon (18) oil, and the remaining 4 questionable oils. The equation was then applied to calculate the RSD and the 95% confidence intervals for ratios found in the known oils (95% ratio range).

Seven of the 8 tabulated ratios are outside the corresponding 95% ratio range when suspect product 1 (nominally a distilled oil) is compared with distilled lime oil. Similarly, 5 of the 8 ratios are outside the interval for suspect product 2, and 6 of the 8 ratios are outside the interval for suspect product 3. These results explicitly confirm earlier suspicions that products 1, 2, and 3 are adulterated.

Strictly speaking, 8 of 8 ratios are outside the range for suspect product 5. However, this product may be a concentrated oil, and the ratio set, developed in Table 2, may not be applicable, because all of the data is referenced to limonene, which is present in very low concentration in product

Table 2. Ratios of peak area percents of some components with respect to limonene for some pure and suspected adulterated oils

	95% Ratio range			Suspect oils			
	Lime oil		Lemon oil				
Peak	Distilled	Expressed	Expressed[a]	1	2	3	5
1	0.031–0.039	0.049–0.067	0.031–0.035	0.068	0.032	0.037	0.024
2	0.010–0.012	0.001–0.011	0.001–0.002	0.018	0.012	0.023	0
5	0.017–0.021	0.015–0.033	0.021–0.023	0.023	0.024	0.026	0.024
8	0.046–0.056	0.005–0.011	0	0.080	0.085	0.110	0.122
18	0.016–0.018	0	0	0.019	0.023	0.024	12.4
20	0.147–0.173	0.001–0.007	0.003–0.003	0.210	0.150	0.210	0.317
21	0.006–0.008	0	0	0	0	0.008	0
23	0.033–0.039	0.028–0.048	0.004–0.005	0.034	0.016	0.122	0.122

[a]Calculated from the area percent data reported by Prager and Miskiewicz (18).

Table 3. Composition of some concentrated lime oils, based on area percent of chromatographically separated components

Peak	(Germany) Extracted distilled	(Mexico) Folded distilled	(Mexico) Folded distilled	(W. Indies) 2-Fold distilled	(W. Indies) 4-Fold distilled	(W. Indies) 5-Fold distilled	(W. Indies) 4-Fold expressed	("Persian") 4-Fold expressed	Suspect product 5
1	0.9	ND[a]	0.2	0.3	ND	0.2	ND	ND	0.1
2	0.4	ND	ND	0.1	ND	ND	ND	ND	ND
3	2.2	0.3	0.6	1.9	0.1	0.7	0.2	ND	0.2
4	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	0.7	ND	0.3	0.5	ND	0.1	ND	ND	0.1
6	1.5	ND	1.1	1.1	0.3	0.4	ND	ND	0.4
7	28.0	18.0	28.0	34.0	10.0	14.0	6.2	15.0	4.1
8	3.7	1.0	1.9	2.1	0.9	1.0	0.3	0.4	0.5
9	ND	ND	0.1	0.2	ND	ND	ND	ND	ND
10	5.2	3.0	6.8	8.7	4.6	4.6	3.7	17.0	0.9
11	3.4	4.9	5.9	2.3	1.4	1.4	0.5	0.8	0.3
12	2.6	3.3	6.6	10.0	4.9	6.8	0.3	1.6	0.7
13	0.3	ND	0.3	0.3	0.5	0.5	0.6	0.8	ND
14	0.6	2.3	2.2	1.0	2.0	1.5	ND	ND	ND
15	0.7	0.3	0.3	1.1	1.0	1.5	0.6	0.4	34.0
16	1.0	ND	2.2	2.0	5.1	4.1	9.1	7.6	0.5
17	0.6	2.0	1.1	0.8	1.7	1.8	5.8	3.1	ND
18	0.7	2.2	1.7	1.8	3.0	2.8	0.4	0.3	51.0
19	1.2	0.4	ND	0.7	1.1	1.2	0.7	0.5	ND
20	6.6	32.0	0.2	18.0	35.0	32.0	ND	0.6	1.3
21	0.3	0.3	0.9	0.4	1.2	0.8	ND	0.2	ND
22	3.8	0.9	0.3	0.9	2.1	1.7	14.0	9.5	ND
23	2.1	0.7	0.2	1.9	0.6	4.9	15.0	11.0	0.5
24	0.8	ND	2.9	1.5	3.9	3.5	9.3	3.3	0.3
25	0.7	0.2	0.9	2.5	1.2	5.2	0.4	0.5	0.5

[a]Not detected.

5. Therefore, the A_{15}/A_{18} ratio was calculated because peak 15 (fenchyl alcohol) and peak 18 (beta-terpineol) represent the major components of product 5. The 95% ratio range for A_{15}/A_{18} is 0.791 to 1.08 for distilled lime oil, whereas the ratio is 0.667 for suspect product 5. The A_{15}/A_{18} ratio observed for 7 authentic lime oil samples, concentrated by various procedures and to varying degrees, ranged between 0.136 and 1.5. However, the area percent values for fenchyl alcohol (peak 15) and beta-terpineol (peak 18) in these commercial concentrates (Table 3) are less than 6% of the values observed for suspect product 5. Therefore, product 5 could not be derived solely from natural lime oil and is believed to be a formulated product.

It has been observed that cold-pressed (expressed) lemon oil and expressed lime oil are very similar in composition. However, a comparison of the ratio data for cold pressed lemon oil derived from the area percent values of Prager and Miskiewicz (18) with the data derived here for expressed lime oil distinguishes absolutely between these lemon and lime oils. Thus, while the chromatograms of these 2 oils appear to be virtually identical under visual qualitative comparison, and even careful comparison of the 95% area ranges shows that only 2 of the ranges for the 18 components measurable for both oils are mutually exclusive, the oils can be readily distinguished by the fact that 3 of the 8 displayed 95% ratio ranges are different (Table 2).

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CHEMICAL CONTAMINANTS MONITORING

Permethrin and Its Two Metabolite Residues in Seven Agricultural Crops

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Metabolite residues of permethrin are not reported in the literature for most agricultural crops. This paper reports residues of permethrin and its 2 metabolites (dichlorovinyl acid and metaphenoxybenzyl alcohol) in 7 different agricultural crops (Chinese cabbage, spinach, asparagus, raspberries, green peas, turnip roots, and turnip greens). Permethrin residues declined approximately 85% within 7 days after treatment in all crops. In most cases, the acid metabolite residues peaked at 3 days, and declined after that. Translocation of residues into turnip roots was very slight; the average was less than 0.05 ppm for permethrin and alcohol metabolite residues and none was detected for the acid metabolite residue. Permethrin residues in the turnip greens averaged approximately 2 ppm for the 0.112 kg ai/ha treatment, and 6 ppm for the 0.224 kg ai/ha treatment.

Permethrin [*cis,trans*-3-phenoxyphenylmethyl-3-(2,2-dichloreothenyl)-2,2-dimethylcyclopropanecarboxylate] is a broad spectrum pesticide with low mammalian toxicity. It has a high insecticidal activity against a wide variety of pests on agricultural crops. The use of permethrin has been increasing in the last few years because of its low toxicity and its increased lethal ability against insects over a longer period of time. Natural pyrethrums and a few of the synthetic pyrethroids disappear rapidly from plant material, which makes the longer lasting effectiveness of permethrin a more valuable tool for insect control.

Papadopoulou-Mourkidou et al. (1) developed a new residue method using infrared spectroscopy and studied the persistence of permethrin on lettuce over a 24 h period. They found 1.0 ppm *cis,trans*-permethrin on the leaf. Reichel and coworkers (2) developed a procedure for determining permethrin residues in animal tissues by using gas chromatography. They confirmed their results with mass spectroscopy. Braun and Stanek (3) achieved 0.005 ppm sensitivity for celery and animal tissues. Harris et al. (4, 5) found that 79–98% of the permethrin residues on asparagus dissipated within 7 days after treatment. However, on celery grown on microplots, permethrin residues had declined 33% in the 70 g ai/ha treatment, and 63% in the 2 × treatment at the end of 7 days. Wong et al. (6) studied the glycosyl conjugates of the alcohol and acid metabolites in french bean cotyledon tissue cultures by using mass spectral methods of identification.

Registration for the use of pesticides on agricultural crops in the United States requires residue data on the parent compound and its toxic metabolites (40 CFR 180.378). The need arose for additional data on residues (by performing degradation studies or by analyzing harvest samples) of permethrin and its dichlorovinyl acid and metaphenoxybenzyl alcohol metabolites with the increased use of permethrin insecticide. Very few metabolite residues of permethrin are reported in the literature. This paper reports these residues on a wide variety of crops.

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This paper reports the results of research only. Mention of a commercial product does not constitute an endorsement by the USDA.

Experimental

Reagents and Apparatus

(a) *Solvents*.—All solvents were redistilled in an all-glass system.

(b) *Insecticide standard solutions*.—Prepare separate solutions of 100 μg/mL of *cis-trans*-permethrin, dichlorovinyl acid metabolite, and metaphenoxybenzyl alcohol metabolite (FMC, Richmond, CA) in methylene chloride. Store solutions in refrigerator. Dilute solutions as needed.

(c) *Gas chromatograph*.—Hewlett-Packard 5840A, equipped with electron capture detector. Operating conditions: temperatures (°C)—injector 250, column 210, detector 300. Column: glass, 4 ft × 3/16 in. packed with, for permethrin residue, 3% QF-1 on 100–120 mesh GCQ; for alcohol and acid metabolite residue, 3% OV-101 on 100–200 mesh GCQ. Column gas: 5% methane in argone at flow rate of 60–70 mL/min. (All 3 standards are eluted from column within 10 min.)

Field Treatment

Ambush® or Pounce® emulsifiable concentrate was applied as a water-based spray (in addition, a wettable powder formulation was used in the green pea experiment) at the rate of 0.112 kg ai/ha or 0.224 kg ai/ha, except for spinach (0.056 kg ai/ha) and raspberries (0.224 and 0.44 kg ai/ha). Turnips and Chinese cabbage were sprayed at 1 week intervals for 8 applications, and residue samples were taken immediately after the last application, up to 7 days later. Green peas and spinach received one application, and were sampled immediately after treatment, up to 7 days later. The asparagus was treated 5 times at the fern stage of growth during the summer, with the residue samples being taken the following spring (ca 5 months later). Raspberry plants were treated twice at a 2 week interval, with residue samples taken at 0 h and 3, 5, and 7 days after treatment.

Sampling Procedure and Preparation

Residue samples were randomly selected from each replicated pot with a sampling interval ranging from immediately after treatment to as long as 200 days after treatment. Most residue samples were taken from 0 to 7 days after treatment. Samples (2.2 kg) from each replicate of each treated plot and 4.4 kg from untreated plots were taken for analysis and immediately frozen. The samples were ground, while frozen, in a Buffalo® chopper, and mixed thoroughly; 50–100 g subsamples were weighed and returned to the freezer until analyzed (raspberries were not ground before subsampling).

Extraction, Cleanup, and Analysis

Samples were blended with methylene chloride and transferred to a beaker, anhydrous Na_2SO_4 was added, and the mixture was filtered through Na_2SO_4. Turnips and asparagus required the addition of 1.5 mL concentrated HCl for efficient extraction of all the residue. An aliquot was then extracted with 0.1N NaOH, washed with water, and filtered through Na_2SO_4 (marked as A, which contains permethrin and the alcohol metabolite).

Table 1. Recovery of permethrin and its 2 metabolites (metaphenoxybenzyl alcohol and dichlorovinyl acid) added to 7 nontreated control crops before extraction

Crop	Average recovery, % (± SD)[a]		
	Permethrin	Alcohol metab.	Acid metab.
Chinese cabbage	97.5 ± 15	98.9 ± 13	97.3 ± 5
Spinach	87.4 ± 12	85.6 ± 14	79.4 ± 15
Asparagus	99.0 ± 8	82.4 ± 14	79.9 ± 7
Raspberries	69.6 ± 4	95.0 ± 25	82.6 ± 13
Green peas	103.3 ± 13	81.2 ± 19	85.6 ± 13
Turnip	89.3 ± 14	102.8 ± 15	93.3 ± 15
Turnip greens	105.5 ± 17	104.8 ± 14	87.5 ± 16

[a] 5–10 determinations each.

To the combined water wash and hydroxide solution, 5N HCl was added to make the solution acid; the solution was extracted with methylene chloride and filtered through Na_2SO_4 (marked as B, which contains the acid metabolite).

The A solution was evaporated on a rotary evaporator (40°C bath), pentane was added, and the mixture was chromatographed through Bakers 0536 aluminum oxide. The column was washed with pentane, and the permethrin residue was eluted with 1.5% ethyl ether in pentane (marked as A); the column was further washed with 2 and 5% ethyl ether in pentane. The alcohol was then eluted with 10% ethyl ether in pentane (marked as AA). A different batch of aluminum oxide required a prewash of 5% ethyl ether in pentane, and then pentane. The elution pattern was then changed, with permethrin eluting from the column in the first pentane wash and the alcohol metabolite eluting with the 5% ethyl ether in pentane and continuing into the 10% ethyl ether in pentane eluate.

The A solution was evaporated on the rotary evaporator and diluted to volume with hexane for GC analysis of permethrin residues.

The AA and B solutions were evaporated on the rotary evaporator, and the alcohol and acid metabolites were derivatized by the method of George et al. (7).

The asparagus samples required slight modifications in the cleanup procedures. After being separated and extracted with hydroxide, the A and B solutions were shaken with 1% mercuric chloride solution (8), washed with water, dried with Na_2SO_4, and derivatized. A separate aliquot was analyzed for the permethrin residues, following the original procedure.

Results and Discussion

Recovery of permethrin and its dichlorovinyl acid and metaphenoxybenzyl alcohol metabolites from fortified control samples of each of the 7 crops is shown in Table 1. Equal amounts of the 3 standards were added to control samples, ranging from 0.01 to 5.00 ppm, depending on residues found in the sample. The average recovery from the 7 crops for permethrin ranged from 69.6 to 105.5%, from 81.2 to 104.8% for the alcohol metabolite, and from 79.4 to 97.3% for the acid metabolite. No detectable residue was found in nontreated control samples representing the same amount of plant material used in recoveries and samples for any of the 7 crops. Lower limits of detection using this methodology were 0.01 ppm permethrin and 0.05 ppm for acid and alcohol metabolites. Minimum results are reported as none detected (no indication of residue) or as below the minimum sensitivity of the method (residue present, but less than the accuracy of the method).

No detectable permethrin residue was found in asparagus spears that had been treated at the fern stage the previous summer (Table 2). The alcohol metabolite residue was less than the minimum sensitivity of the method (<0.05 ppm), while the acid metabolite residue ranged from 0.13 ppm for the 0.112 kg treatment to 0.05 ppm for the 0.224 kg treatment. Harris et al. (4) found 0.14 ppm permethrin residue with a 0.07 kg treatment, and 0.48 ppm with a 0.14 kg treatment for samples taken immediately after spraying; the residues declined to 0.03 and 0.01 ppm, respectively, 7 days later. No metabolite residues were reported.

Residues of permethrin and the 2 metabolites in green peas (vines and pods) declined approximately 20% in the first day after treatment, 50% in 3 days, and 85% in 7 days. There was no significant difference in total residue found or in the loss of residue between the wettable powder formulation and the emulsifiable concentrate formulation.

To determine the effect of various growing conditions on the loss of permethrin residues, turnip plants were treated in Vincennes, Indiana, during the summer months and in Weslaco, Texas, during the early winter months. Permethrin residues in turnip greens from Vincennes gave very erratic results, while the Weslaco samples showed a decline of 78% within 3 days after treatment, with no further loss in residue 5 days after treatment. Very little permethrin was translocated to the roots. The average residue in roots was less than 0.1 ppm from both locations. Little or no alcohol or acid metabolite residues were found in these samples. More acid than alcohol metabolite residue was found in the turnip greens. The alcohol metabolite residue was at the lower limits of detection of the method (<0.05 ppm), while the acid metabolite residues ranged from none detected in the day 1 sample to 0.61 ppm in one of the day 3 samples. The acid residues peaked at the day 3 sampling, and declined after that. Belanger and Hamilton (9) reported permethrin residues in onions and carrots, both root crops. They found 0.03 ppm in onion and none detected in carrots, when the soil had been treated with a granular formulation of the insecticide at 0.56 kg ai/ha, and 0.06 ppm and none detected, respectively, for the 1.12 kg treatment. Although the method of application is different than what we report, these results are similar to residues found in turnip roots. They did not report residues of the metabolites.

Initial residues of permethrin in Chinese cabbage taken immediately after treatment ranged from 0.29 ppm for the 0.112 kg ai/ha treatment to 0.94 ppm for the 2× treatment. These residues declined approximately 80% in 3 days. Residues of the 2 metabolites were less than the minimum sensitivity of the method (<0.05 ppm). These residues do not follow the same pattern reported for (head) cabbage (7). They reported zero hour permethrin residue at 1.34 ppm, the alcohol metabolite residue at 1.41 ppm, and the acid metabolite at approximately the same rate as those reported in this paper for Chinese cabbage.

Residues of permethrin in spinach ranged from 1.61 ppm for the 0.056 kg ai/ha treatment to 2.17 ppm for the 2× treatment. These residues declined approximately 50% in 3 days and 80% in 7 days after treatment. Relatively high metabolite residues were detected in the spinach samples. Belanger and Hamilton (9) reported permethrin residues in lettuce (another leafy vegetable) at 0.03 and 0.57 ppm for the soil application treatment of 0.56 and 1.12 kg ai/ha.

MacPhee et al. (10) found no permethrin residues in blueberries when Ambush® WP was applied as a foliar spray at the rate of 0.4 kg ai/ha to blueberry plants. Permethrin residues on raspberries (Table 2) ranged from 0.30 ppm for the 0.224 kg ai/ha treatment to 0.70 ppm for the 0.448 kg treatment, when sampled 1 day after application. In 7 days, these residues had declined approximately 57% from the first day. MacPhee and coworkers did not report metabolite residues.

Table 2. Average residues of permethrin and its 2 metabolites (metaphenoxybenzyl alcohol and dichlorovinyl acid) in 7 crops at various sampling intervals and treatment rates

Rate, kg ai/ha	Sampling interval, days	Average residue, ppm[a]		
		Permethrin	Alcohol metab.	Acid metab.
		Asparagus		
0.112 EC	ca 200	ND[b]	ND	0.13 SD ± 0.09
0.224 EC	ca 200	ND	<0.05[c]	0.05 SD ± 0.05
		Green Peas		
0.112 EC	0 h	0.98 SD ± 0.06	0.60 SD ± 0.03	0.94 SD ± 0.10
	1	0.70 SD ± 0.11	0.56 SD ± 0.07	0.61 SD ± 0.25
	3	0.59 SD ± 0.06	0.39 SD ± 0.07	0.28 SD ± 0.06
	7	0.19 SD ± 0.02	0.13 SD ± 0.03	0.14 SD ± 0.04
0.112 WP	0 h	1.15 SD ± 0.54	0.76 SD ± 0.34	1.21 SD ± 0.44
	1	1.00 SD ± 0.87	0.48 SD ± 0.42	0.98 SD ± 0.85
	3	0.50 SD ± 0.43	0.31 SD ± 0.29	0.33 SD ± 0.34
	7	0.21 SD ± 0.16	0.15 SD ± 0.07	0.18 SD ± 0.31
		Turnip Roots (Vincennes, IN)		
0.112 EC	1	0.09 SD ± 0.03	ND	ND
	3	0.01 SD ± 0.01	0.23 SD ± 0.16	ND
	5	0.03 SD ± 0.03	ND	ND
0.224 EC	1	0.04 SD ± 0.02	ND	ND
	3	0.05 SD ± 0.02	ND	0.08 SD ± 0.16
	5	0.08 SD ± 0.04	ND	ND
		Turnip Roots (Weslaco, TX)		
0.112 EC	1	0.04 SD ± 0.02	0.06 SD ± 0.08	ND
	3	0.02 SD ± 0.01	ND	ND
	5	0.02 SD ± 0.01	<0.05	ND
0.224 EC	1	0.06 SD ± 0.04	<0.05	ND
	3	0.04 SD ± 0.01	ND	ND
	5	0.02 SD ± 0.01	<0.05	ND
		Turnip Greens (Vincennes, IN)		
0.112 EC	1	1.79 SD ± 1.23	<0.05	0.18 SD ± 0.13
	3	1.70 SD ± 1.40	<0.05	0.38 SD ± 0.13
	5	2.22 SD ± 0.75	0.13 SD ± 0.04	0.27 SD ± 0.06
0.224 EC	1	7.35 SD ± 1.18	<0.05	ND
	3	5.98 SD ± 0.51	<0.05	0.33 SD ± 0.20
	5	3.85 SD ± 0.93	ND	0.20 SD ± 0.04
		Turnip Greens (Weslaco, TX)		
0.112 EC	1	6.33 SD ± 1.66	<0.05	ND
	3	1.43 SD ± 0.24	ND	0.43 SD ± 0.09
	5	1.66 SD ± 0.20	0.07 SD ± 0.06	0.44 SD ± 0.17
0.224 EC	1	6.69 SD ± 0.67	<0.05	<0.05
	3	1.43 SD ± 0.53	<0.05	0.61 SD ± 0.35
	5	1.32 SD ± 0.40	0.05 SD ± 0.04	0.32 SD ± 0.20
		Chinese Cabbage		
0.112 EC	0 h	0.29 SD ± 0.18	<0.05	<0.05
	1	0.29 SD ± 0.18	<0.05	<0.05
	3	0.05 SD ± 0.03	<0.05	<0.05
0.224 EC	0 h	0.94 SD ± 1.03	<0.05	<0.05
	1	0.32 SD ± 0.20	ND	<0.05
	3	0.20 SD ± 0.16	<0.05	<0.05
		Spinach		
0.056 EC	0 h	1.61 SD ± 0.08	1.42 SD ± 0.30	1.26 SD ± 0.06
	1	1.15 SD ± 0.13	1.39 SD ± 0.08	0.94 SD ± 0.04
	3	0.79 SD ± 0.30	0.67 SD ± 0.29	0.68 SD ± 0.26
	7	0.29 SD ± 0.13	0.26 SD ± 0.13	0.22 SD ± 0.11
0.112 EC	0 h	2.17 SD ± 0.13	2.11 SD ± 0.45	1.82 SD ± 0.16
	1	1.73 SD ± 0.09	1.64 SD ± 0.43	1.49 SD ± 0.18
	3	1.02 SD ± 0.19	0.90 SD ± 0.29	0.89 SD ± 0.05
	7	0.44 SD ± 0.16	0.52 SD ± 0.30	0.39 SD ± 0.25
		Raspberries		
0.224 EC	1	0.30 SD ± 0.17	<0.05	<0.05
	3	0.29 SD ± 0.09	<0.05	ND
	5	0.26 SD ± 0.04	<0.05	<0.05
	7	0.13 SD ± 0.02	<0.05	ND
0.448 EC	1	0.70 SD ± 0.27	<0.05	ND
	3	0.66 SD ± 0.31	<0.05	<0.05
	5	0.33 SD ± 0.16	<0.05	<0.05
	7	0.20 SD ± 0.07	<0.05	ND

[a]Results have been corrected for average recovery found. Results are an average of 4 replicated plots.
[b]ND = none detected.
[c]Lower limit of sensitivity for the method: permethrin: 0.01 ppm; alcohol metab.: 0.05 ppm; acid metab.: 0.05 ppm.

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Pesticides, Selected Elements, and Other Chemicals in Infant and Toddler Total Diet Samples, October 1979–September 1980

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The U.S. Food and Drug Administration (FDA) conducts Total Diet Studies to determine the dietary intake of selected pesticides, industrial chemicals, and elements (including radionuclides). These studies involve the retail purchase and analysis of foods representative of the diets of infants, toddlers, and adults. The individual food items are separated into a number of food groups, each of which is analyzed as a composite. This report summarizes the results for infant and toddler Total Diet samples collected in 10 cities between October 1979 and September 1980. The average concentration, range of concentrations, and calculated average daily intake of each chemical found are presented by food group. The average daily intakes of the chemicals are similar to those found in the several preceding years and generally are within acceptable limits. The results for samples collected during the same period that represent the adult diet are reported separately.

Total Diet Studies (also referred to as Market Basket Programs) have been conducted by the U.S. Food and Drug Administration (FDA) since the early 1960s. These studies are a part of FDA's surveillance of the food supply and are designed to measure the dietary intake of selected pesticides, industrial chemicals, and elements (including radionuclides), to identify problems and trends in the intake of these chemicals, and to determine the source of unusual residues. These studies also provide an additional check on the effectiveness of U.S. regulations concerning the levels of chemical contaminants in foods.

The Total Diet Study initially involved determination of the intake of selected chemical contaminants only in the adult diet. Since 1974, the diets of infants (6 months old) and toddlers (2 years old) also have been analyzed. Levels of chemicals in the diet are determined by collecting and analyzing samples (subsequently referred to as market basket samples) from retail markets in urban areas throughout the continental United States. These samples represent the typical 14-day diet. Approximately 120 individual food items, including drinking water, are collected for each market basket sample; the infant diet consists of about 50 of these foods, and the toddler diet includes about 110. Regional variations in food consumption patterns are reflected in slight differences among the foods collected and consumption values used for the Northeast, South, West, and North Central regions. The choice of foods collected for the market basket samples and their consumption values are based on a food consumption survey conducted by the U.S. Department of Agriculture in 1965.

After collection, the samples are sent to FDA's Kansas City District laboratory for preparation and analysis. Where appropriate, the foods are prepared in the manner in which they usually are consumed. Individual food items from the

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Table 1. Fiscal Year 1980 infant and toddler Total Diet sample collections

Sample collection area	Geographic region	Date of collection
Cleveland, OH	North Central	10/79
Boston, MA	Northeast	11/79
Omaha, NE	North Central	2/80
Baton Rouge, LA	South	2/80
New York, NY	Northeast	4/80
Sacramento, CA	West	4/80
Wilmington, DE	South	6/80
Pueblo, CO	West	7/80
Louisville, KY	South	8/80
Boise, ID	West	9/80

Table 2. Average composition of the infant and toddler Total Diet[a]

	Av. wt, g/day (% of Total Diet)			
Food group	Infant		Toddler	
Drinking water	260	(19)	354	(23)
Whole milk	614	(45)	500	(32)
Other dairy products & dairy substitutes	147	(11)	68	(4)
Meat, fish, & poultry	49	(4)	124	(8)
Grain & cereal products	35	(3)	114	(7)
Potatoes	5	(<1)	33	(2)
Vegetables	94	(7)	74	(5)
Fruit & fruit juices	137	(10)	139	(9)
Oils & fats	4	(<1)	14	(1)
Sugar & adjuncts	12	(1)	30	(2)
Beverages	22	(2)	96	(6)
Total	1379	(100)	1546	(100)

[a]The average composition of the Total Diet varies slightly from year to year depending on the number of market baskets collected in each region. In Fiscal Year 1980, 2, 3, 3, and 2 baskets were collected in the Northeast, South, West, and North Central regions, respectively. The sum of values for percent composition does not equal 100 because of rounding.

Table 3. Average concentration (ppm) and average daily intake (µg/day) of pesticides and industrial chemicals in the infant diet by food group[a]

Chemical	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
BHC, alpha					
Average concn		0.0003	0.0003	0.0001	
No. positive	0	9	6	2	0
Range of concn		T	T-0.001	T	
Average intake		0.203	0.0255	0.0040	
Captan					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Carbaryl					
Average concn					
No. positive	0	NA	NA	NA	0
Range of concn					
Average intake					
Chlorobenzilate					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Chlorpropham					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
DDE					
Average concn		0.0003	0.0001	0.0014	
No. positive	0	5	1	7	0
Range of concn		T-0.001	T	T-0.005	
Average intake		0.152	0.0213	0.0798	
Diazinon					
Average concn				0.0005	
No. positive	0	0	0	1	0
Range of concn				0.005	
Average intake				0.0320	
Dicloran					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Dicofol					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Dieldrin					
Average concn		0.0003	0.0003	0.0003	
No. positive	0	6	5	5	0
Range of concn		T-0.001	T	T	
Average intake		0.227	0.0291	0.0119	
Endosulfan II					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Endosulfan sulfate					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Ethion					
Average concn			0.0002		
No. positive	0	0	1	0	0
Range of concn			0.002		
Average intake			0.0104		
Fonofos					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					

[a]The average concentration is based on all composites analyzed and is expressed in ppm. The number of positives is indicated as NA in cases in which the analytical methodology used was not capable of determining that chemical. The range of concentrations is for those composites in which the chemical was detected and is expressed in ppm. The average daily intake is based on all composites analyzed and is expressed in µg/day. Detections of chemicals whose identity was confirmed but which were present at concentrations below the limit of quantitation were reported as trace (T). The limit of quantitation varies with the chemical and type of food. When trace level findings were reported, the analyst included an estimate of the amount of the chemical present; these estimates were used in calculation of the average concentration and average daily intake.

Table 3. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0	0	0	0	0	17 T-0.001 0.233
0	0.0013 1 0.013 0.119	0	0	0	0	1 0.013 0.119
0	0	0	NA	0.050 1 0.50 0.489	0	1 0.50 0.489
0	0	0.0002 1 0.002 0.0275	0	0	0	1 0.002 0.0275
0.0466 4 0.035-0.289 0.191	0.0002 1 0.002 0.0141	0	0	0	0	5 0.002-0.289 0.205
0	0.0001 2 T-0.001 0.0137	0	0.0006 2 0.002-0.004 0.0122	0	0	17 T-0.005 0.279
0.0006 2 0.001-0.005 0.0020	<0.0001 1 T 0.0027	0	0	0	0	4 T-0.005 0.0367
0.0033 2 0.009-0.024 0.0377	0	0.0002 2 T-0.002 0.0304	0.0007 1 0.007 0.0142	0	0	5 T-0.024 0.0823
0	0	0	0	0	0.0003 1 0.003 0.0062	1 0.003 0.0062
0.0001 1 0.001 0.0013	0	0	0.0001 2 T 0.0031	0	0	19 T-0.001 0.272
0	0	<0.0001 1 T 0.0055	0	0	0	1 T 0.0055
0.0003 1 0.003 0.0040	0	0.0002 1 0.002 0.0275	0	0	0	2 0.002-0.003 0.0315
0	0	0	0	0	0	1 0.002 0.0104
0	0	0	0.0014 1 0.014 0.0285	0	0	1 0.014 0.0285

Table 3. (continued)

Chemical	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
Heptachlor epoxide					
Average concn		0.0002	0.0001	<0.0001	
No. positive	0	6	3	1	0
Range of concn		T	T	T	
Average intake		0.136	0.0099	0.0026	
Hexachlorobenzene					
Average concn		<0.0001	<0.0001	0.0001	
No. positive	0	3	3	3	0
Range of concn		T	T	T	
Average intake		0.0183	0.0023	0.0064	
Lindane					
Average concn			<0.0001	<0.0001	0.0001
No. positive	0	0	2	1	1
Range of concn			T	T	0.001
Average intake			0.0051	0.0015	0.0050
Malathion					
Average concn					0.0236
No. positive	0	0	0	0	9
Range of concn					0.006-0.044
Average intake					0.907
Methoxychlor					
Average concn		0.0001	0.0007		
No. positive	0	1	1	0	0
Range of concn		T	0.007		
Average intake		0.0451	0.0888		
Octachlor epoxide					
Average concn		<0.0001			
No. positive	0	3	0	0	0
Range of concn		T			
Average intake		0.0263			
Parathion					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Pentachloroaniline					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Pentachloroanisole					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Pentachlorobenzene					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Pentachlorophenol					
Average concn				0.0004	0.0007
No. positive	0	0	0	1	1
Range of concn				T	T
Average intake				0.0207	0.0190
Pentachlorothioanisole					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Perthane					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Phosalone					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Quintozene					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					

Table 3. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0	0	0.0001 2 T 0.0031	0	0	12 T 0.152
0	0	0	0.0009 2 0.002-0.007 0.0183	0	0	11 T-0.007 0.0453
0	0	0	0	0	0	4 T-0.001 0.0116
0	0.0001 1 0.001 0.0091	0	0.0320 1 0.320 0.651	0	0	11 0.001-0.320 1.57
0	0	0	0	0	0	2 T-0.007 0.134
0	0	0	0	0	0	3 T 0.0268
0	<0.0001 1 T 0.0046	0.0001 2 T 0.0193	0	0	0	3 T 0.0239
0	0	0	0.0050 2 0.007-0.043 0.102	0	0	2 0.007-0.043 0.102
0	0	0	0.0011 2 0.003-0.008 0.0224	0	0	2 0.003-0.008 0.0224
0	0	0	0.0013 2 0.003-0.010 0.0265	0	0	2 0.003-0.010 0.0265
0	0	0	0.0021 2 T-0.016 0.0427	0.0007 1 T 0.0007	0	5 T-0.016 0.0831
0	0	0	0.0012 2 0.003-0.009 0.0244	0	0	2 0.003-0.009 0.0244
0	0	0.0005 1 0.005 0.0688	0	0	0	1 0.005 0.0688
0	0	0.0008 1 T 0.101	0	0	0	1 T 0.101
0	0	0	0.0008 2 0.003-0.005 0.0163	0	0	2 0.003-0.005 0.0163

Table 3. (continued)

Chemical	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
Tecnazene					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Tetrachloroaniline					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Tetrachloroanisidine					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Tetrachlorothioanisole					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Toxaphene					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Tri-n-butyl phosphate					
Average concn					0.0086
No. positive	0	NA	NA	NA	3
Range of concn					0.003-0.080
Average intake					0.422
Tris(2-chloroethyl) phosphate					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
No. of chemicals detected	0	7	8	8	4
Total residues detected	0	33	22	21	14

Table 3. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0.0007 1 0.007 0.0044	0	0	0	0	0	1 0.007 0.0044
0.0005 1 0.005 0.0031	0.0001 1 T 0.0055	0	0	0	0	2 T-0.005 0.0086
0.0002 1 0.002 0.0013	0	0	0	0	0	1 0.002 0.0013
<0.0001 1 T 0.0001	0	0	0	0	0	1 T 0.0001
0	0	0	0.0086 2 T-0.036 0.175	0	0	2 T-0.036 0.175
0	0	0	NA	NA	NA	3 0.003-0.080 0.422
0	0	0.0002 1 0.002 0.0287	0	0	0	1 0.002 0.0287
9	7	8	14	2	1	36
14	8	10	25	2	1	150

Table 4. Average concentration (ppm) and average daily intake (µg/day) of pesticides and industrial chemicals in the toddler diet by food group[a]

Chemical	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
BHC, alpha					
Average concn		0.0003	0.0016	0.0004	
No. positive	0	9	10	7	0
Range of concn		T	T-0.003	T-0.001	
Average intake		0.159	0.115	0.0524	
Carbaryl					
Average concn					
No. positive	0	NA	NA	NA	0
Range of concn					
Average intake					
Chlordane					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Chlorobenzilate					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
2-Chloroethyl linoleate					
Average concn				0.0059	
No. positive	0	0	0	1	0
Range of concn				0.059	
Average intake				0.745	
2-Chloroethyl palmitate					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Chlorpropham					
Average concn					0.0003
No. positive	0	0	0	0	1
Range of concn					0.003
Average intake					0.0301
Chlorpyrifos					
Average concn				<0.0001	0.0001
No. positive	0	0	0	1	1
Range of concn				T	0.001
Average intake				0.0051	0.0100
DCPA					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
DDE					
Average concn		0.0003	0.0015	0.0030	
No. positive	0	5	8	9	0
Range of concn		T-0.001	T-0.004	0.001-0.007	
Average intake		0.131	0.111	0.365	
DDT					
Average concn				0.0002	
No. positive	0	0	0	1	0
Range of concn				0.002	
Average intake				0.0253	
Diazinon					
Average concn				0.0006	0.0001
No. positive	0	0	0	3	1
Range of concn				T-0.003	0.001
Average intake				0.0692	0.0100
Dicloran					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Dicofol					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					

[a]The average concentration is based on all composites analyzed and is expressed in ppm. The number of positives is indicated as NA in cases in which the analytical methodology used was not capable of determining that chemical. The range of concentrations is for those composites in which the chemical was detected and is expressed in ppm. The average daily intake is based on all composites analyzed and is expressed in µg/day. Detections of chemicals whose identity was confirmed but which were present at concentrations below the limit of quantitation were reported as trace (T). The limit of quantitation varies with the chemical and type of food. When trace level findings were reported, the analyst included an estimate of the amount of the chemical present; these estimates were used in calculation of the average concentration and average daily intake.

Table 4. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0	0	0	0.0019 9 T-0.006 0.0576	0	35 T-0.006 0.384
0	0	0.005 1 T 0.479	NA	0	0	1 T 0.479
0	0	0	0.0001 1 T 0.0008	0	0	1 T 0.0008
0	0	0.0012 2 0.004-0.008 0.115	0	0	0	2 0.004-0.008 0.115
0	0	0	0.0766 2 0.160-0.606 1.23	0	0	3 0.059-0.606 1.98
0	0	0	0.0056 2 0.019-0.037 0.0900	0	0	2 0.019-0.037 0.0900
0.2929 7 0.032-1.65 9.91	0	0	0	0	0	8 0.003-1.65 9.94
0	0	0	0	0	0	2 T-0.001 0.0151
0	<0.0001 1 T 0.0036	0	0	0	0	1 T 0.0036
0	0	0	0.0008 5 0.001-0.002 0.0105	0	0	27 T-0.007 0.618
0	0	0	0	0	0	1 0.002 0.0253
0.0006 2 0.001-0.005 0.0146	0.0003 3 T-0.001 0.0214	0	0.0016 3 T-0.014 0.0297	0.0001 1 T 0.0024	0	13 T-0.014 0.147
0.0009 1 0.009 0.0354	0	0.0093 8 T-0.035 1.32	0	0	0	9 T-0.035 1.36
0	0	0.0004 1 0.004 0.0676	0	0	0	1 0.004 0.0676

Table 4. (continued)

Chemical	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
Dieldrin					
Average concn		0.0003	0.0016	0.0017	
No. positive	0	6	8	9	0
Range of concn		T-0.001	T-0.004	T-0.004	
Average intake		0.160	0.105	0.202	
Endosulfan I					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Endosulfan II					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Endosulfan sulfate					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Ethion					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
2-Ethylhexyl diphenyl phosphate					
Average concn				0.0291	
No. positive	0	0	0	2	0
Range of concn				0.030-0.261	
Average intake				3.21	
Fonofos					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Heptachlor epoxide					
Average concn		0.0002	0.0007	0.0009	
No. positive	0	6	8	8	0
Range of concn		T	T-0.002	T-0.003	
Average intake		0.0962	0.0501	0.116	
Hexachlorobenzene					
Average concn		<0.0001	0.0003	0.0002	
No. positive	0	3	9	7	0
Range of concn		T	T	T	
Average intake		0.0148	0.0197	0.0257	
Lindane					
Average concn				0.0001	
No. positive	0	0	0	3	0
Range of concn				T	
Average intake				0.0160	
Malathion					
Average concn			0.0001		0.0224
No. positive	0	0	1	0	10
Range of concn			T		T-0.045
Average intake			0.0031		2.63
Methidathion					
Average concn					
No. positive	NA	NA	NA	NA	0
Range of concn					
Average intake					
Methoxychlor					
Average concn		0.0001	0.0011		
No. positive	0	1	2	0	0
Range of concn		T	0.002-0.009		
Average intake		0.0304	0.0571		
Octachlor epoxide					
Average concn		<0.0001	0.0001	0.0003	
No. positive	0	3	3	5	0
Range of concn		T	T	T-0.001	
Average intake		0.0185	0.0088	0.0349	
Pentachloroaniline					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					

Table 4. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0.0001 2 T 0.0048	0.0016 4 T-0.013 0.140	0	0.0017 7 T-0.005 0.0233	0	0	36 T-0.013 0.635
0	0.0001 2 T-0.001 0.0091	0	0	0	0	2 T-0.001 0.0091
0	0.0004 2 T-0.004 0.0302	0	0	0	0	2 T-0.004 0.0302
0.0002 1 0.002 0.0090	0.0009 1 0.009 0.0579	0	0	0	0	2 0.002-0.009 0.0669
0	0	0.0006 4 T-0.002 0.0715	0	0	0	4 T-0.002 0.0715
0	0	0	0	0.0330 1 0.330 1.00	0	3 0.030-0.330 4.21
0	0	0	0.0009 2 0.003-0.006 0.0099	0	0	2 0.003-0.006 0.0099
0	0	0	0.0004 5 T-0.001 0.0054	0	0	27 T-0.003 0.268
0	0	0	0.0020 10 0.001-0.004 0.0281	0	0	29 T-0.004 0.0883
0	0	0	0	0.0029 8 T-0.009 0.0916	0	11 T-0.009 0.108
0	0.0001 1 0.001 0.0090	0	0.0431 8 0.002-0.200 0.549	0.0002 1 0.002 0.0056	0	21 T-0.200 3.20
0	0	0.0002 1 0.002 0.0271	NA	NA	NA	1 0.002 0.0271
0	0	0.0020 1 0.020 0.271	0	0	0	4 T-0.020 0.359
0	0	0	<0.0001 1 T 0.0004	0	0	12 T-0.001 0.0626
0	0	0	0.0077 10 0.003-0.024 0.107	<0.0001 1 T 0.0011	0	11 T-0.024 0.108

Table 4. (continued)

Chemical	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
Pentachloroanisole					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Pentachlorobenzene					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Pentachlorophenol					
Average concn			0.0014	0.0032	0.0021
No. positive	0	0	3	5	2
Range of concn			T	T	T-0.014
Average intake			0.0798	0.396	0.238
Pentachlorothioanisole					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Perthane					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Quintozene					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
TDE					
Average concn				0.0002	
No. positive	0	0	0	1	0
Range of concn				0.002	
Average intake				0.0253	
Tecnazene					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Tetrachloroaniline					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Tetrachloroanisidine					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Tetrachlorothioanisole					
Average concn					
No. positive	0	0	0	0	0
Range of concn					
Average intake					
Toxaphene					
Average concn				0.0050	
No. positive	0	0	0	1	0
Range of concn				0.050	
Average intake				0.525	
Tri-n-butyl phosphate					
Average concn					0.0158
No. positive	0	NA	NA	NA	4
Range of concn					0.003-0.104
Average intake					1.81
No. of chemicals detected	0	7	9	15	6
Total residues detected	0	33	52	63	19

Table 4. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0	0	0.0023 10 0.001-0.004 0.0334	0	0	10 0.001-0.004 0.0334
0	0	0	0.0029 10 0.001-0.006 0.0414	0	0	10 0.001-0.006 0.0414
0	0	0	0.0093 9 T-0.024 0.135	0.0026 3 T-0.013 0.0814	0	22 T-0.024 0.930
0	0	0	0.0033 10 0.001-0.006 0.0477	0.0001 1 T 0.0011	0	11 T-0.006 0.0488
0	0	0.0026 1 0.026 0.466	0	0	0	1 0.026 0.466
0	0	0	0.0032 10 T-0.014 0.0514	0	0	10 T-0.014 0.0514
0	0	0	0	0	0	1 0.002 0.0253
0.0007 1 0.007 0.0275	0	0	0	0	0	1 0.007 0.0275
0.0005 1 0.005 0.0196	0	0	0	0	0	1 0.005 0.0196
0.0002 1 0.002 0.0079	0	0	0	0	0	1 0.002 0.0079
<0.0001 1 T 0.0008	0	0	0	0	0	1 T 0.0008
0	0	0	0.0426 6 T-0.150 0.569	0	0	7 T-0.150 1.09
0	0	0	NA	NA	NA	4 0.003-0.104 1.81
9	7	8	18	8	0	42
17	14	19	111	25	0	353

Table 5. Daily intake per unit of body weight (µg/kg of body weight/day) of pesticides and industrial chemicals in Fiscal Years 1977–1980[a]

Chemical	FAO/WHO Acceptable Daily Intake	Infant				Toddler			
		FY 1977	FY 1978	FY 1979	FY 1980	FY 1977	FY 1978	FY 1979	FY 1980
BHC (total)		0.037	0.037	0.034	0.029	0.035	0.034	0.035	0.036
BHC, alpha		0.031	0.034	0.033	0.028	0.025	0.029	0.029	0.028
BHC, beta		ND	ND	ND	ND	0.002	ND	ND	ND
Lindane (BHC, gamma)	10	0.006	0.003	0.001	0.001	0.008	0.005	0.006	0.008
Captan	10	ND	ND	ND	0.015	ND	ND	ND	ND
Carbaryl	10	<0.001	0.088	ND	0.060	ND	0.050	0.049	0.035
Chlordane (total)	1	0.001	0.010	<0.001	0.003	0.005	0.032	0.003	0.005
Chlordane		<0.001	0.005	ND	ND	<0.001	0.024	ND	<0.001
Octachlor epoxide		0.001	0.005	<0.001	0.003	0.005	0.008	0.003	0.005
Chlorobenzilate	20	ND	ND	ND	0.003	ND	ND	ND	0.008
2-Chloroethyl linoleate		ND	0.061	ND	ND	ND	0.418	0.198	0.145
2-Chloroethyl palmitate		ND	0.008	ND	ND	ND	0.015	0.009	0.007
Chlorpropham		0.051	0.007	0.060	0.025	0.225	0.034	0.238	0.726
Chlorpyrifos	10	ND	ND	0.002	ND	ND	ND	0.007	0.001
DCPA		ND	0.020	0.002	ND	ND	<0.001	<0.001	<0.001
DDT (total)	5	0.102	0.091	0.113	0.034	0.548	0.104	0.090	0.049
DDE		0.100	0.088	0.110	0.034	0.332	0.088	0.089	0.045
DDT		0.001	0.003	ND	ND	0.048	0.013	ND	0.002
TDE		0.001	ND	0.003	ND	0.168	0.003	0.001	0.002
Diazinon	2	0.014	0.002	0.002	0.004	0.007	0.007	0.004	0.011
Dicloran	30	0.013	0.016	0.127	0.010	0.085	0.106	0.088	0.099
Dicofol	25	ND	ND	ND	0.001	ND	ND	0.006	0.005
Dieldrin	0.1	0.041	0.045	0.048	0.033	0.042	0.039	0.036	0.046
Endosulfan (total)	8	ND	<0.001	0.013	0.005	0.002	0.015	0.074	0.008
Endosulfan I		ND	ND	0.001	ND	<0.001	0.005	0.037	0.001
Endosulfan II		ND	<0.001	0.005	0.001	0.001	0.006	0.037	0.002
Endosulfan sulfate		ND	<0.001	0.007	0.004	0.001	0.004	ND	0.005
Endrin	0.2	ND	<0.001	ND	ND	ND	ND	<0.001	ND
Ethion	1	0.001	0.003	0.002	0.001	0.001	0.004	0.003	0.005
2-Ethylhexyl diphenyl phosphate		ND	ND	ND	ND	ND	ND	ND	0.307
Fenitrothion	1	ND	<0.001	ND	ND	ND	0.002	ND	ND
Fenthion	1	ND	ND	ND	ND	ND	<0.001	ND	ND
Fonofos		ND	ND	0.002	0.003	0.001	0.001	0.001	0.001
Heptachlor epoxide	0.5	0.013	0.023	0.021	0.019	0.018	0.019	0.018	0.020
Hexachlorobenzene		0.038	0.012	0.010	0.006	0.022	0.015	0.008	0.006
Malathion	20	0.064	0.331	0.126	0.191	0.209	0.299	0.259	0.234
Methidathion	5	NA	NA	NA	ND	NA	NA	NA	0.002
Methoxychlor		0.007	0.029	0.016	0.016	0.002	0.008	0.015	0.026
Nonachlor, trans		ND	ND	ND	ND	0.002	ND	ND	ND
Parathion	5	0.011	0.005	0.002	0.003	<0.001	0.003	0.002	ND
Pentachloroanisole		0.001	0.004	0.003	0.003	0.003	0.007	0.003	0.002
Pentachlorobenzene		0.001	0.005	0.008	0.003	0.003	0.005	0.004	0.003
Pentachlorophenol		ND	ND	0.009	0.010	0.006	ND	0.003	0.068
Perthane		ND	0.012	0.026	0.008	ND	ND	ND	0.034
o-Phenylphenol	20	ND	ND	0.061	ND	ND	ND	0.086	ND
Phosalone	6	0.012	0.037	0.086	0.012	0.043	0.014	0.008	ND
Polychlorinated biphenyls		0.025	0.011	ND	ND	0.030	0.099	ND	ND
Quintozene (total)	7	0.008	0.016	0.035	0.017	0.014	0.011	0.013	0.016
Quintozene		0.001	0.005	0.003	0.002	0.003	0.003	0.001	0.004
Pentachloroaniline		0.005	0.004	0.022	0.012	0.008	0.004	0.008	0.008
Pentachlorothioanisole		0.002	0.007	0.010	0.003	0.003	0.004	0.004	0.004
Ronnel		ND	ND	ND	ND	ND	<0.001	ND	ND
Tecnazene	10	0.001	<0.001	0.008	0.001	0.003	0.001	0.028	0.002
Tetrachloroaniline		<0.001	ND	0.003	0.001	<0.001	ND	0.010	0.001
Tetrachloroanisidine		<0.001	<0.001	0.003	<0.001	0.001	<0.001	0.010	0.001
Tetrachloroanisole		<0.001	ND	ND	ND	<0.001	ND	ND	ND
Tetrachlorobenzene		0.002	ND	<0.001	ND	0.001	0.008	0.001	ND
Tetrachlorothioanisole		<0.001	ND	<0.001	<0.001	<0.001	ND	0.001	<0.001
Toxaphene		0.068	0.088	0.072	0.021	0.044	0.059	0.050	0.080
Tri-n-butyl phosphate		NA	NA	NA	0.051	NA	NA	NA	0.132
Tris(2-chloroethyl) phosphate		ND	ND	0.016	0.004	ND	ND	0.009	ND

[a]The FAO/WHO ADIs (36) are expressed here in µg/kg of body weight/day. The intake is presented as a total in cases in which the ADI is expressed as the sum of related chemical residues. The intake is indicated as ND in cases in which the chemical was not detected but could have been if it had been present. It is listed as NA in cases in which the analytical methodology used was not capable of determining that chemical. The intakes for Fiscal Years 1977-1979 were reported previously (15-17). Because trace level findings were assigned a value of zero in calculation of intakes prior to Fiscal Year 1978, the intakes shown for Fiscal Year 1977 may be slightly lower than if they had been calculated using an estimate of the level for trace findings.

market basket sample are separated into 11 groups (e.g., grain and cereal products), and the foods in each group are blended (in amounts proportional to the weight consumed) to yield a homogeneous mixture (composite). The composites are analyzed for selected pesticides (organochlorine, organophosphorus, chlorophenoxy acids, carbaryl, and *o*-phenylphenol), industrial chemicals (including polychlorinated biphenyls), and elements (arsenic, cadmium, lead, mercury, selenium, and zinc) (1–9). The elemental analyses also include selected minerals and radionuclides; the results of these analyses are presented elsewhere (10 and 11, respectively). The Total Diet Studies are conducted to determine levels of chemical contaminants in foods prepared for consumption rather than to enforce tolerances and other regulatory limits on raw agricultural commodities. Therefore, the analytical procedures used are modified to permit quantitation at levels that are 5–10 times lower than those used in FDA programs for enforcement of tolerances (a greater equivalent sample weight is submitted to the final determinative step). For example, the limit of quantitation in the Total Diet Studies is about 0.002 ppm for heptachlor epoxide and 0.01 ppm for cadmium (12). The identity of each organic chemical reported is confirmed by an alternative method of identification (thin layer chromatography, element-selective gas chromatographic detection, mass spectrometry, *p*-values, derivatization, etc.). For each market basket, recovery analyses are conducted for selected composite/analyte combinations to assure acceptable analytical performance.

Results for the infant and toddler market basket samples and the adult market basket samples collected between June 1964 and September 1979 were reported previously (13–17 and 18–33, respectively). This report presents the data for the infant and toddler market basket samples collected and analyzed between October 1979 and September 1980 (Fiscal Year 1980). The results for the adult market basket samples collected during the same period are reported separately (34).

Results

Ten infant and toddler market basket samples were collected and analyzed (Table 1). The average contribution from each of the 11 food groups to the total diet is listed in Table 2 and is based on the 10 market basket samples collected (2, 3, 3, and 2 baskets from the Northeast, South, West, and North Central regions, respectively).

Pesticides and Industrial Chemicals

The pesticides and industrial chemicals found are listed by food group in Table 3 for the infant diet and Table 4 for the toddler diet. The average concentration (ppm) of the chemical, based on all 10 market baskets, is listed along with the number of findings and range of concentrations for composites in which the chemical was identified. The concentration values are not corrected for recovery. Residues whose identities were confirmed but which were present at levels below the limit of quantitation are reported as trace. The limit of quantitation varies with the chemical and type of food. In cases where residues were reported at the trace level, the analyst included an estimate of the amount of the chemical present. These estimates were used in calculation of the average concentration.

Tables 3 and 4 also show the average daily intake of each chemical by food group (μg/day). The daily intake of a chemical is determined by multiplying the concentration of the chemical in each food group by the weight of that food group consumed in a day and then adding the intakes determined for each food group. The estimates reported by the analyst for trace level findings were used in calculation of the average daily intake.

Table 5 shows the daily intake per unit of body weight (μg/kg of body weight/day) of the pesticides and industrial chemicals found in the infant and toddler Total Diet. The average body weight is taken to be 8.2 kg (18 lb) for infants and 13.7 kg (30 lb) for toddlers. The intakes reported previously for Fiscal Years 1977–1979 are presented for comparison (15–17).

The daily intakes per unit of body weight determined in the Total Diet Studies may be compared with the Acceptable Daily Intakes (ADIs) proposed by the United Nations' Food and Agriculture Organization and the World Health Organization (FAO/WHO) (35). The ADI is the maximum daily intake of a chemical which, during a lifetime, appears to be without appreciable risk. ADIs are shown in Table 5 for chemicals for which they have been established (36). The ADI is expressed as an aggregate for chemicals that are related as a result of manufacturing impurities or degradation of the pesticide (e.g., the DDT group).

Elements

The average concentration, number and range of concentrations of positive findings, and average daily intake of the 6 selected elements are listed by food group in Table 6 for the infant diet and Table 7 for the toddler diet. The values are not corrected for recovery. Trace level findings were treated the same as those for the organic chemicals in calculating the average concentrations and average daily intakes. The average daily intakes calculated for these elements in Fiscal Years 1977–1980 are presented in Table 8 for comparison with their acceptable intake limits. For cadmium and mercury, the Provisional Tolerable Daily Intake (PTDI) is listed. The PTDIs were derived from the FAO/WHO Provisional Tolerable Weekly Intakes (PTWIs), which are maximum acceptable intakes that appear to be without appreciable risk (37). The PTWIs were proposed for adults and may not apply to infants and toddlers. The maximum acceptable intake of lead listed in Table 8 was proposed by the FDA and covers all sources, including air and pica (38).

The Total Diet analyses determine total arsenic (organic and inorganic). Although a number of studies have been conducted, no maximum acceptable intake has been agreed on for total arsenic. However, the FAO/WHO has estimated a PTDI for inorganic arsenic of 2 μg/kg of body weight (39); this PTDI was proposed for adults and may not apply to infants and toddlers.

Selenium and zinc are essential nutrients. The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) proposed for selenium by the National Research Council of the National Academy of Sciences is presented for comparison with the calculated intake (40, p. 178). The Recommended Dietary Allowance (RDA) for zinc proposed by the National Research Council also is listed (40, Appendix).

Discussion

Pesticides and Industrial Chemicals

In the infant diet, pesticides and/or industrial chemicals were found most frequently in the whole milk and oils-fats groups. Thirty-six different pesticides and industrial chemicals (including degradation products) were identified in the infant diet. The greatest variety of chemicals was found in the oils-fats and potato groups (14 and 9 chemicals, respectively).

Table 6. Average concentration (ppm) and average daily intake (µg/day) of selected elements in the infant diet by food group[a]

Element	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
Arsenic[b]					
Average concn				0.003	0.026
No. positive	0	0	0	2	7
Range of concn				T-0.02	0.02-0.06
Average intake				0.168	0.759
Cadmium					
Average concn	0.0039	0.0097	0.0028	0.0106	0.0328
No. positive	3	4	4	5	10
Range of concn	T-0.026	T-0.060	T-0.018	T-0.040	0.014-0.052
Average intake	1.14	6.75	0.674	0.579	1.13
Lead					
Average concn	0.023	0.004	0.022	0.038	0.087
No. positive	5	2	7	9	10
Range of concn	0.02-0.09	0.02	T-0.07	0.02-0.12	0.04-0.33
Average intake	7.63	2.96	2.44	2.04	2.50
Mercury					
Average concn					0.0004
No. positive	0	0	0	0	1
Range of concn					T
Average intake					0.0078
Selenium					
Average concn	0.002	0.008	0.009	0.080	0.196
No. positive	1	3	3	10	10
Range of concn	T	T-0.03	T-0.05	T-0.15	0.04-0.33
Average intake	0.879	4.95	1.60	4.20	7.12
Zinc					
Average concn	0.03	3.76	5.25	20.3	19.6
No. positive	2	10	10	10	10
Range of concn	0.1-0.2	3.3-4.3	3.1-6.9	10.6-35.2	9.1-33.4
Average intake[c]	0.01	2.34	0.74	1.05	0.65

[a]The average concentration is based on all composites analyzed and is expressed in ppm. The range of concentrations is for those composites in which the element was detected and is expressed in ppm. The average daily intake is based on all composites analyzed and is expressed in µg/day. Detections below the limit of quantitation were reported as trace (T). The limit of quantitation varies with the element. When trace level findings were reported, the analyst included an estimate of the amount of the element present; these estimates were used in calculation of the average concentration and average daily intake.
[b]Calculated as arsenic trioxide (As_2O_3).
[c]Expressed in mg/day.

Table 6. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0.001 1 T 0.091	0	0	0.005 1 0.05 0.005	0	11 T-0.06 1.02
0.0204 7 0.015-0.044 0.127	0.0133 9 T-0.033 1.36	0.0025 3 T-0.021 0.346	0.0101 2 0.027-0.074 0.206	0.0053 5 T-0.033 0.0754	0.0003 1 T 0.0062	0.0003 53 T-0.074 12.4
0.026 5 0.02-0.11 0.124	0.064 9 0.02-0.19 6.46	0.064 9 0.02-0.12 8.70	0.003 2 T-0.02 0.061	0.014 5 0.02-0.04 0.238	0.014 3 0.02-0.10 0.313	66 T-0.33 33.5
0	0	0.0002 1 T 0.0275	0	0.0007 2 T 0.0082	0.0003 1 T 0.0062	5 T 0.0497
0	0.002 1 T 0.199	0	0	0.002 1 T 0.026	0	29 T-0.33 19.0
1.72 7 0.5-4.8 0.01	3.84 10 2.9-5.3 0.37	0.76 10 0.4-1.0 0.10	5.18 2 22.0-29.8 0.11	0.26 8 0.1-0.4 <0.01	0.07 5 0.1-0.2 <0.01	84 0.1-35.2 5.38

Table 7. Average concentration (ppm) and average daily intake (μg/day) of selected elements in the toddler diet by food group[a]

Element	Drinking water	Whole milk	Other dairy & dairy substitutes	Meat fish & poultry	Grain & cereal products
Arsenic[b]					
Average concn				0.105	0.009
No. positive	0	0	0	8	3
Range of concn				0.03-0.32	0.02-0.04
Average intake				13.8	1.02
Cadmium					
Average concn	0.0039	0.0097	0.0027	0.0053	0.0265
No. positive	3	4	5	4	9
Range of concn	T-0.026	T-0.060	T-0.010	T-0.022	0.015-0.068
Average intake	1.44	4.49	0.206	0.680	3.06
Lead					
Average concn	0.023	0.004	0.025	0.028	0.056
No. positive	5	2	7	10	10
Range of concn	0.02-0.09	0.02	0.02-0.09	0.02-0.05	0.03-0.07
Average intake	8.68	1.95	1.68	3.53	6.26
Mercury					
Average concn			0.0001	0.0029	0.0008
No. positive	0	0	1	7	4
Range of concn			T	T	T
Average intake			0.0052	0.397	0.0869
Selenium					
Average concn	0.002	0.008	0.021	0.194	0.236
No. positive	1	3	5	10	10
Range of concn	T	T-0.03	T-0.07	0.12-0.27	0.12-0.36
Average intake	0.818	4.25	1.34	23.2	26.8
Zinc					
Average concn	0.03	3.76	6.08	32.9	11.2
No. positive	2	10	10	10	10
Range of concn	0.1-0.2	3.3-4.3	4.7-8.4	27.6-38.2	6.7-14.6
Average intake[c]	0.01	1.89	0.40	4.05	1.30

[a]The average concentration is based on all composites analyzed and is expressed in ppm. The range of concentrations is for those composites in which the element was detected and is expressed in ppm. The average daily intake is based on all composites analyzed and is expressed in μg/day. Detections below the limit of quantitation were reported as trace (T). The limit of quantitation varies with the element. When trace level findings were reported, the analyst included an estimate of the amount of the element present; these estimates were used in calculation of the average concentration and average daily intake.
[b]Calculated as arsenic trioxide (As_2O_3).
[c]Expressed in mg/day.

Table 7. (continued)

Potatoes	Vegetables	Fruit & fruit juices	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0	0	0.006 1 0.06 0.109	0	0	12 0.02-0.32 14.9
0.0333 10 0.015-0.068 1.12	0.0102 7 0.010-0.022 0.739	0.0030 4 T-0.022 0.333	0.0441 10 0.017-0.088 0.619	0.0078 8 T-0.023 0.231	0.0022 3 T-0.011 0.146	67 T-0.088 13.1
0.032 7 0.02-0.11 0.895	0.105 10 0.04-0.17 7.96	0.064 10 0.02-0.15 9.11	0.016 7 T-0.04 0.221	0.037 9 0.02-0.09 1.12	0.017 5 T-0.10 1.95	82 T-0.17 43.4
0.0006 1 T 0.0269	0	0	0.0009 4 T 0.0123	0.0006 2 T 0.0174	0.0006 2 T 0.0419	21 T 0.588
0	0.010 4 T 0.775	0.002 1 T 0.192	0.012 3 T-0.06 0.175	0.002 1 T 0.056	0	38 T-0.36 57.6
2.66 10 0.5-5.2 0.09	5.80 10 4.1-11.2 0.43	0.88 10 0.6-1.4 0.12	14.5 10 8.9-21.4 0.20	3.63 10 1.8-8.1 0.11	0.14 8 0.1-0.4 0.01	100 0.1-38.2 8.61

Table 8. Average daily intake (μg/day) of selected elements in Fiscal Years 1977–1980[a]

Element	Acceptable intake limit	Infant FY 77	Infant FY 78	Infant FY 79	Infant FY 80	Toddler FY 77	Toddler FY 78	Toddler FY 79	Toddler FY 80
Arsenic[b]	—[c]	5	2	5	1	24	18	23	15
Cadmium	57–72[d]	6	6	4	12	8	11	9	13
Lead	100–150[e]	24	25	28	34	29	35	46	43
Mercury	43[d]	1	<1	<1	<1	1	1	<1	1
Selenium	10–80[f]	15	18	15	19	46	52	45	58
Zinc[g]	3–10[h]	4	5	5	5	8	9	8	9

[a]The average daily intakes for Fiscal Years 1977–1979 were reported previously (15–17).
[b]Calculated as arsenic trioxide (As_2O_3).
[c]No maximum acceptable intake has been agreed on for total arsenic (organic and inorganic). However, the FAO/WHO (39) has estimated a PTDI for inorganic arsenic of 2 μg/kg of body weight (calculated as arsenic trioxide, this is equivalent to 22 μg/day for an 8.2 kg person and 36 μg/day for a 13.7 kg person); the PTDI was proposed for adults and may not apply to infants and toddlers.
[d]Provisional Tolerable Daily Intake (PTDI) calculated from the Provisional Tolerable Weekly Intake (PTWI) proposed by the FAO/WHO (37); this limit was proposed for adults and may not apply to infants and toddlers.
[e]The maximum intake proposed by the FDA for lead from all sources, including air and pica, is 100 μg/day for infants up to 6 months old and 150 μg/day for children 6 months to 2 years old (38).
[f]The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) proposed by the National Research Council of the National Academy of Sciences is 10–40 μg/day for infants up to 6 months old, 20–60 μg/day for infants 6 months to 1 year old, and 20–80 μg/day for children 1–3 years old (40).
[g]All zinc intake values are expressed in mg/day.
[h]The Recommended Dietary Allowance (RDA) proposed by the National Research Council of the National Academy of Sciences is 3 mg/day for infants up to 6 months old, 5 mg/day for infants 6 months to 1 year old, and 10 mg/day for children 1–3 years old (40).

The food groups of the toddler diet in which pesticides and industrial chemicals were found most frequently were oils-fats, meat-fish-poultry, and other dairy products. Forty-two pesticides and industrial chemicals were identified in the toddler diet. The oils-fats and meat-fish-poultry groups displayed the greatest variety of residues (18 and 15 chemicals, respectively).

An additional procedure for the determination of organophosphorus chemicals (2) was introduced into the Total Diet Studies in Fiscal Year 1980. Use of this procedure resulted in the identification of 2 previously unreported chemicals (methidathion and tri-*n*-butyl phosphate) that may well have been present in earlier years but which would not have been identified. The number of pentachlorophenol identifications was substantially increased from previous years and may have resulted from the use of a new analytical procedure for chlorophenoxy acids beginning in mid-Fiscal Year 1979 (3).

The daily intakes per unit of body weight of the pesticides and industrial chemicals for Fiscal Years 1977–1980 (Table 5) appear to be relatively constant. None of the intakes for Fiscal Year 1980 approached the FAO/WHO ADI; dieldrin was closest, at 33 and 46% of the ADI in the infant and toddler diets, respectively.

Elements

No trends are apparent in the average daily intakes of the 6 selected elements for Fiscal Years 1977–1980 (Table 8). The Fiscal Year 1980 intakes of cadmium and mercury were well below the PTDI; as mentioned earlier, the PTDIs were proposed for adults and may not apply to infants and toddlers. The intakes of lead in the infant and toddler diets represented 22 and 29%, respectively, of the maximum acceptable intakes proposed by FDA for lead from all sources.

The intakes of total arsenic in the infant and toddler diets were 1 and 15 μg/day, respectively, calculated as arsenic trioxide. As mentioned previously, no maximum acceptable intake has been agreed on for total arsenic. The PTDI estimated by the FAO/WHO for inorganic arsenic is 2 μg/kg of body weight (calculated as arsenic trioxide, this is equivalent to 22 μg/day for an 8.2 kg person and 36 μg/day for a 13.7 kg person); the PTDI was proposed for adults and may not apply to infants and toddlers.

The intakes calculated for selenium were slightly less than the ESADDI for the infant diet and within the range of the ESADDI for the toddler diet. The intake of zinc calculated for the infant diet was the same as the RDA, whereas the intake calculated for the toddler diet was slightly lower than the RDA.

Conclusion

The dietary intakes determined in the Fiscal Year 1980 Infant and Toddler Total Diet Study are similar to those found in the several preceding years and are generally within the acceptable limits outlined earlier. The same approach for sample collection and analysis was continued in the Fiscal Year 1981 Infant and Toddler Total Diet Study.

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Pesticides, Selected Elements, and Other Chemicals in Adult Total Diet Samples, October 1979–September 1980

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The U.S. Food and Drug Administration (FDA) conducts Total Diet Studies to determine the dietary intake of selected pesticides, industrial chemicals, and elements (including radionuclides). These studies involve the retail purchase and analysis of foods representative of the diets of infants, toddlers, and adults. The individual food items are separated into a number of food groups, each of which is analyzed as a composite. This report summarizes the results for adult Total Diet samples collected in 20 cities between October 1979 and September 1980. The average concentration, range of concentrations, and calculated average daily intake of each chemical found are presented by food group. The average daily intakes of the chemicals are similar to those found in the several preceding years and are within acceptable limits. The results for samples collected during the same period that represent the diets of infants and toddlers are reported separately.

Total Diet Studies (also referred to as Market Basket Programs) have been conducted by the U.S. Food and Drug Administration (FDA) since the early 1960s. These studies are a part of FDA's surveillance of the food supply and are designed to measure the dietary intake of selected pesticides, industrial chemicals, and elements (including radionuclides), to identify problems and trends in the intake of these chemicals, and to determine the source of unusual residues. These studies also provide an additional check on the effectiveness of U.S. regulations concerning the levels of chemical contaminants in foods.

Table 1. Fiscal Year 1980 adult Total Diet sample collections

Sample collection area	Geographic region	Date of collection
Rochester, NY	Northeast	10/79
Springfield, IL	North Central	10/79
San Jose, CA	West	11/79
Baltimore, MD	South	11/79
Binghamton, NY	Northeast	12/79
Fort Wayne, IN	North Central	1/80
San Diego, CA	West	2/80
Beaumont, TX	South	2/80
Trenton, NJ	Northeast	3/80
Rockford, IL	North Central	3/80
Portland, OR	West	4/80
Lakeland, FL	South	4/80
Erie, PA	Northeast	5/80
Minneapolis, MN	North Central	6/80
Salt Lake City, UT	West	6/80
Nashville, TN	South	6/80
Pittsburgh, PA	Northeast	7/80
Milwaukee, WI	North Central	8/80
Montgomery, AL	South	8/80
Richmond, VA	South	8/80

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The Adult Total Diet Study involves determination of the intake of selected chemical contaminants in the diet of 16- to 19-year-old males, who generally consume more food than other age groups and, on this basis, are considered to have the highest dietary intake of chemical contaminants. Levels of chemicals in the diet are determined by collecting and analyzing samples (subsequently referred to as market basket samples) from retail markets in urban areas throughout the continental United States. These samples represent the typical 14-day diet. Approximately 120 individual food items, including drinking water, are collected for each market basket sample. Regional variations in food consumption patterns are reflected in slight differences among the foods collected and consumption values used for the Northeast, South, West, and North Central regions. The choice of foods collected for the market basket samples and their consumption values are based on a food consumption survey conducted by the U.S. Department of Agriculture in 1965.

After collection, the samples are sent to FDA's Kansas City District laboratory for preparation and analysis. Where appropriate, the foods are prepared in the manner in which they usually are consumed. Individual food items from the market basket sample then are separated into 12 food groups (e.g., leafy vegetables), and the foods in each group are blended (in amounts proportional to the weight consumed) to yield a homogeneous mixture (composite). The composites are analyzed for selected pesticides (organochlorine, organophosphorus, chlorophenoxy acids, carbaryl, and *o*-phenylphenol),

Table 2. Average composition of the adult Total Diet[a]

Food group	Av. wt, g/day	% (by wt) of Total Diet
Dairy products	758	26
Meat, fish, & poultry	262	9
Grain & cereal products	419	14
Potatoes	159	5
Leafy vegetables	56	2
Legume vegetables	74	3
Root vegetables	32	1
Garden fruits	74	3
Fruits	220	8
Oils & fats	73	3
Sugar & adjuncts	82	3
Beverages (including water)	701	24
Total	2910	(100)

[a]The average composition of the Total Diet varies slightly from year to year depending on the number of market baskets collected in each region. In Fiscal Year 1980, 5, 6, 4, and 5 baskets were collected in the Northeast, South, West, and North Central regions, respectively. The sum of values for percent composition does not equal 100 because of rounding.

industrial chemicals (including polychlorinated biphenyls), and elements (arsenic, cadmium, lead, mercury, selenium, and zinc) (1–9). The elemental analyses also include selected minerals and radionuclides; the results of these analyses are presented elsewhere (10 and 11, respectively). The Total Diet Studies are conducted to determine levels of chemical contaminants in foods prepared for consumption rather than to enforce tolerances and other regulatory limits on raw agricultural commodities. Therefore, the analytical procedures used are modified to permit quantitation at levels that are 5–10 times lower than those used in FDA programs for enforcement of tolerances (a greater equivalent sample weight is submitted to the final determinative step). For example, the limit of quantitation in the Total Diet Studies is about 0.002 ppm for heptachlor epoxide and 0.01 ppm for cadmium (12). The identity of each organic chemical reported is confirmed by an alternative method of identification (thin layer chromatography, element-selective gas chromatographic detection, mass spectrometry, *p*-values, derivatization, etc.). For each market basket, recovery analyses are conducted for selected composite/analyte combinations to assure acceptable analytical performance.

Since 1974, FDA also has collected and analyzed market basket samples representing the diets of infants and toddlers. Results for the adult market basket samples and the infant and toddler market basket samples collected between June 1964 and September 1979 were reported previously (13–28 and 29–33, respectively). This report presents the data for the adult market basket samples collected and analyzed between October 1979 and September 1980 (Fiscal Year 1980). The results for the infant and toddler market basket samples collected during this period are reported separately (34).

Results

Twenty adult market basket samples were collected and analyzed (Table 1). The average contribution from each of the 12 food groups to the total diet is listed in Table 2 and is based on the 20 market basket samples collected (5, 6, 4, and 5 baskets from the Northeast, South, West, and North Central regions, respectively).

Pesticides and Industrial Chemicals

The pesticides and industrial chemicals found are listed for each food group in Table 3 along with the average concentration (ppm) of the chemical, based on all 20 composites analyzed, and the number of findings and range of concentrations for composites in which the chemical was identified. The concentration values are not corrected for recovery. Residues whose identities were confirmed but which were present at levels below the limit of quantitation are reported as trace. The limit of quantitation varies with the chemical and type of food. In cases where residues were reported at the trace level, the analyst included an estimate of the amount of the chemical present. These estimates were used in calculation of the average concentration.

Table 3 also shows the average daily intake of each chemical by food group (μg/day). The daily intake of a chemical is determined by multiplying the concentration of the chemical in each food group by the weight of that food group consumed in a day and then adding the intakes determined for each food group. The estimates reported by the analyst for trace level findings were used in calculation of the average daily intake.

Table 4 shows the daily intake per unit of body weight (μg/kg of body weight/day) of the pesticides and industrial chemicals found in the adult Total Diet. The average body weight of 16- to 19-year-old males is taken to be 69.1 kg (152 lb). The intakes reported previously for Fiscal Years 1977–1979 are presented for comparison (26–28).

The daily intakes per unit of body weight determined in the Total Diet Studies may be compared with the Acceptable Daily Intakes (ADIs) established by the United Nations' Food and Agriculture Organization and the World Health Organization (FAO/WHO) (35). The ADI is the maximum daily intake of a chemical which, during a lifetime, appears to be without appreciable risk. ADIs are shown in Table 4 for chemicals for which they have been established (36). The ADI is expressed as an aggregate for chemicals that are related as a result of manufacturing impurities or degradation of the pesticide (e.g., the DDT group).

Elements

The average concentration, number and range of concentrations of positive findings, and average daily intake are listed by food group in Table 5 for the 6 selected elements. The values are not corrected for recovery. Trace level findings were treated the same as those for the organic chemicals in calculating the average concentration and average daily intake. The average daily intakes calculated for these elements in Fiscal Years 1977–1980 are presented in Table 6 for comparison with their acceptable intake limits. For the toxic elements cadmium, lead, and mercury, the Provisional Tolerable Daily Intake (PTDI) is listed. The PTDIs were derived from the FAO/WHO Provisional Tolerable Weekly Intakes, which are maximum intakes that appear to be without appreciable risk (37).

The Total Diet analyses determine total arsenic (organic and inorganic). Although a number of studies have been conducted, no maximum acceptable intake has been agreed on for total arsenic. However, the FAO/WHO has estimated a PTDI for inorganic arsenic of 2 μg/kg of body weight (138 μg/day for a 69.1 kg person) (38).

Selenium and zinc are essential nutrients. The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for selenium and the Recommended Dietary Allowance (RDA) for zinc proposed by the National Research Council of the National Academy of Sciences are presented for comparison with the calculated intakes (39).

Discussion

Pesticides and Industrial Chemicals

Pesticides and/or industrial chemicals were found most frequently in the meat-fish-poultry and oils-fats groups. The beverage, legume vegetable, and root vegetable groups had the fewest residues. Fifty-four different pesticides and industrial chemicals (including degradation products) were identified. The greatest variety of chemicals was found in the fruit and garden fruit groups (20 and 19 chemicals, respectively).

An additional procedure for the determination of organophosphorus chemicals (2) was introduced into the Total Diet Study in Fiscal Year 1980. Use of this procedure resulted in the identification of 4 previously unreported chemicals (demeton-S sulfone, dimethoate, methidathion, and tri-*n*-butyl phosphate) that may well have been present in earlier years but which would not have been identified. The number of pentachlorophenol identifications was substantially increased from previous years and may have resulted from the use of a

Table 3. Average concentration (ppm) and average daily intake (µg/day) of pesticides and industrial chemicals by food group[a]

Chemical	Dairy products	Meat fish & poultry	Grain & cereal products	Potatoes	Leafy vegetables	Legume vegetables
BHC, alpha						
Average concn	0.0006	0.0006	<0.0001			
No. positive	19	16	1	0	0	0
Range of concn	T-0.001	T-0.003	T			
Average intake	0.441	0.156	0.0162			
BHC, delta						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Captan						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Carbaryl						
Average concn						0.002
No. positive	NA	NA	0	0	0	1
Range of concn						T
Average intake						0.172
Chlordane						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Chlorobenzilate						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
2-Chloroethyl linoleate						
Average concn		0.0145	0.0071			0.0011
No. positive	0	2	1	0	0	1
Range of concn		0.140-0.150	0.142			0.023
Average intake		3.84	3.03			0.0792
2-Chloroethyl palmitate						
Average concn			0.0011			
No. positive	0	0	1	0	0	0
Range of concn			0.022			
Average intake			0.469			
Chlorpropham						
Average concn			0.0007	0.1135	0.0006	
No. positive	0	0	2	18	1	0
Range of concn			0.002-0.013	0.004-0.672	0.012	
Average intake			0.307	18.0	0.0348	
Chlorpyrifos						
Average concn			0.0001			
No. positive	0	0	1	0	0	0
Range of concn			0.002			
Average intake			0.0435			
DCPA						
Average concn					0.0016	
No. positive	0	0	0	0	4	0
Range of concn					T-0.017	
Average intake					0.0920	
DDE						
Average concn	0.0009	0.0048		0.0005	0.0017	
No. positive	9	18	0	4	10	0
Range of concn	T-0.006	T-0.024		T-0.006	0.001-0.010	
Average intake	0.626	1.28		0.0847	0.0954	
DDT						
Average concn		0.0008		<0.0001	0.0002	
No. positive	0	7	0	1	2	0
Range of concn		T-0.006		0.001	0.002-0.003	
Average intake		0.219		0.0079	0.0137	
Demeton-S sulfone						
Average concn					0.0028	
No. positive	NA	NA	0	0	1	0
Range of concn					0.056	
Average intake					0.146	

[a]The average concentration is based on all composites analyzed and is expressed in ppm. The number of positives is indicated as NA in cases in which the analytical methodology used was not capable of determining that chemical. The range of concentrations is for those composites in which the chemical was detected and is expressed in ppm. The average daily intake is based on all composites analyzed and is expressed in µg/day. Detections of chemicals whose identity was confirmed but which were present at concentrations below the limit of quantitation were reported as trace (T). The limit of quantitation varies with the chemical and type of food. When trace level findings were reported, the analyst included an estimate of the amount of the chemical present; these estimates were used in calculation of the average concentration and average daily intake.

Table 3. (continued)

Root vegetables	Garden fruits	Fruits	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0.0008 2 0.001-0.016 0.0615	0	0.0003 1 0.006 0.0222	0.0011 17 T-0.003 0.0935	0	56 T-0.016 0.790
0	0.0003 1 0.006 0.0217	0	0	0	0	1 0.006 0.0217
0	0	0.0002 1 0.004 0.0422	0	0	0	1 0.004 0.0422
0	0.002 1 T 0.193	0.005 2 T-0.05 1.11	NA	0	0	4 T-0.05 1.48
0	0.0002 1 0.003 0.0107	0	0	0	0	1 0.003 0.0107
0	0	0.0002 1 0.004 0.0469	0	0	0	1 0.004 0.0469
0	0	0	0.0925 6 0.050-0.942 6.70	0	0	10 0.023-0.942 13.6
0	0	0	0.0047 4 0.006-0.045 0.338	0	0	5 0.006-0.045 0.807
0	0.0002 1 0.004 0.0145	0.0007 2 0.007-0.008 0.159	0	0	0	24 0.002-0.672 18.5
0	<0.0001 1 T 0.0025	0	0	0	0	2 T-0.002 0.0460
0.0001 3 0.001 0.0050	0.0006 4 0.001-0.008 0.0476	0	0	0	0	11 T-0.017 0.145
0.0010 6 0.001-0.013 0.0309	0.0002 3 0.001-0.002 0.0185	0	<0.0001 1 T 0.0028	<0.0001 1 0.001 0.0042	0	52 T-0.024 2.14
0	0	0	0	0	0	10 T-0.006 0.241
0	0	0	NA	NA	NA	1 0.056 0.146

Table 3. (continued)

Chemical	Dairy products	Meat fish & poultry	Grain & cereal products	Potatoes	Leafy vegetables	Legume vegetables
Diazinon						
Average concn		0.0003	0.0003	0.0002	0.0004	<0.0001
No. positive	0	4	7	2	5	1
Range of concn		T-0.003	T-0.001	T-0.003	T-0.002	T
Average intake		0.0775	0.110	0.0295	0.0209	0.0018
Dicloran						
Average concn				0.0010	0.0007	
No. positive	0	0	0	5	2	0
Range of concn				0.002-0.008	0.004-0.011	
Average intake				0.167	0.0388	
Dicofol						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Dieldrin						
Average concn	0.0007	0.0033	<0.0001	0.0001		
No. positive	13	19	1	2	0	0
Range of concn	T-0.003	T-0.036	0.001	T-0.001		
Average intake	0.549	0.860	0.0213	0.0127		
Dimethoate						
Average concn						
No. positive	NA	NA	0	0	0	0
Range of concn						
Average intake						
Endosulfan I						
Average concn					0.0005	
No. positive	0	0	0	0	4	0
Range of concn					0.001-0.006	
Average intake					0.0274	
Endosulfan II						
Average concn				0.0003	0.0005	
No. positive	0	0	0	1	3	0
Range of concn				0.006	0.002-0.005	
Average intake				0.0476	0.0284	
Endosulfan sulfate						
Average concn				0.0002	0.0008	
No. positive	0	0	0	1	4	0
Range of concn				0.004	0.002-0.006	
Average intake				0.0318	0.0452	
Ethion						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
2-Ethylhexyl diphenyl phosphate						
Average concn		0.0985				
No. positive	0	1	0	0	0	0
Range of concn		2.0				
Average intake		25.8				
Fenitrothion						
Average concn			0.0001			
No. positive	0	0	2	0	0	0
Range of concn			0.001-0.002			
Average intake			0.0608			
Fonofos						
Average concn			0.0001			
No. positive	0	0	1	0	0	0
Range of concn			0.002			
Average intake			0.0409			
Heptachlor epoxide						
Average concn	0.0003	0.0009				
No. positive	12	16	0	0	0	0
Range of concn	T-0.002	T-0.006				
Average intake	0.244	0.229				
Hexachlorobenzene						
Average concn	0.0001	0.0004				
No. positive	8	15	0	0	0	0
Range of concn	T	T-0.002				
Average intake	0.0683	0.108				
Lindane						
Average concn	<0.0001	0.0002				
No. positive	1	8	0	0	0	0
Range of concn	T	T-0.001				
Average intake	0.0112	0.0496				

Table 3. (continued)

Root vegetables	Garden fruits	Fruits	Oils & fats	Sugar & adjuncts	Beverages	All groups
0.0001 2 0.001 0.0029	0.0001 2 0.001 0.0075	<0.0001 1 T 0.0085	0.0001 3 T-0.001 0.0099	0.0001 2 0.001 0.0081	0	29 T-0.003 0.277
0	0	0.0063 5 0.014-0.058 1.39	0	<0.0001 1 0.001 0.0040	0	13 0.001-0.058 1.60
0	0	0.0038 9 0.002-0.024 0.828	0	0	0	9 0.002-0.024 0.828
<0.0001 1 T 0.0006	0.0012 11 T-0.004 0.0852	<0.0001 1 T 0.0032	0.0001 2 0.001-0.002 0.0105	0	0	50 T-0.036 1.54
0	0.0001 1 0.002 0.0077	0.0001 1 0.003 0.0352	NA	NA	NA	2 0.002-0.003 0.0429
0	0.0010 9 T-0.004 0.0759	0.0005 5 T-0.005 0.113	0	0	0	18 T-0.006 0.216
0	0.0013 10 0.001-0.008 0.0952	0.0018 7 T-0.017 0.378	0	0	0	21 T-0.017 0.549
0	0.0005 5 0.001-0.004 0.0367	0.0008 5 0.001-0.007 0.170	0	0	0	15 0.001-0.007 0.284
0.0008 1 0.016 0.0263	0	0.0003 3 0.001-0.003 0.0658	0	0	0	4 0.001-0.016 0.0921
0	0	0	1.34 4 1.3-12.0 99.7	0	0	5 1.3-12.0 126
0	0	0	0	0	0	2 0.001-0.002 0.0608
0	0	0	0.0015 2 T-0.030 0.107	<0.0001 1 0.001 0.0040	0	4 T-0.030 0.152
0	0	0	<0.0001 1 T 0.0018	0	0	29 T-0.006 0.475
0	0	0	0.0015 14 T-0.015 0.108	0.0001 4 T 0.0045	0	41 T-0.015 0.289
0	0.0004 2 T-0.007 0.0268	0	0	0.0013 16 T-0.008 0.107	0	27 T-0.008 0.195

Table 3. (continued)

Chemical	Dairy products	Meat fish & poultry	Grain & cereal products	Potatoes	Leafy vegetables	Legume vegetables
Linuron						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Malathion						
Average concn		0.0001	0.0299			
No. positive	0	1	20	0	0	0
Range of concn		0.002	0.009-0.079			
Average intake		0.0264	12.5			
Methidathion						
Average concn						
No. positive	NA	NA	0	0	0	0
Range of concn						
Average intake						
Methoxychlor						
Average concn	0.0005	0.0003				
No. positive	3	2	0	0	0	0
Range of concn	0.002-0.006	0.003-0.004				
Average intake	0.376	0.0909				
Nonachlor, trans						
Average concn		<0.0001				
No. positive	0	1	0	0	0	0
Range of concn		T				
Average intake		0.0078				
Octachlor epoxide						
Average concn	0.0001	0.0004				
No. positive	3	10	0	0	0	0
Range of concn	T-0.001	T-0.002				
Average intake	0.0751	0.112				
Parathion						
Average concn			<0.0001		0.0004	<0.0001
No. positive	0	0	1	0	3	1
Range of concn			0.001		T-0.006	0.001
Average intake			0.0203		0.0204	0.0037
Parathion-methyl						
Average concn					0.0002	
No. positive	0	0	0	0	1	0
Range of concn					0.005	
Average intake					0.0126	
Pentachloroaniline						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Pentachloroanisole						
Average concn		<0.0001				
No. positive	0	1	0	0	0	0
Range of concn		T				
Average intake		0.0052				
Pentachlorobenzene						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Pentachlorophenol						
Average concn		0.0084	0.0006			
No. positive	0	12	3	0	0	0
Range of concn		T-0.074	T			
Average intake		2.20	0.275			
Pentachlorothioanisole						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Perthane						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
o-Phenylphenol						
Average concn						
No. positive	NA	NA	0	0	0	0
Range of concn						
Average intake						

Table 3. (continued)

Root vegetables	Garden fruits	Fruits	Oils & fats	Sugar & adjuncts	Beverages	All groups
0.0005 1 0.010 0.0164	0	0	0	0	0	1 0.010 0.0164
0	0	0.0002 4 T-0.001 0.0351	0.0167 13 0.003-0.080 1.22	0.0031 6 T-0.037 0.256	0	44 T-0.080 14.0
0	0	0.0003 3 0.002-0.003 0.0743	NA	NA	NA	3 0.002-0.003 0.0743
0	0	0	0	0	0	5 0.002-0.006 0.467
0	0	0	0	0	0	1 T 0.0078
0	0	0	0	0	0	13 T-0.002 0.187
0	0.0001 2 T-0.001 0.0057	0.0001 1 0.003 0.0320	0	0	0	8 T-0.006 0.0821
0	0	0	0	0	0	1 0.005 0.0126
0	0.0001 1 0.003 0.0109	0	0.0083 14 T-0.127 0.585	0.0004 4 T-0.006 0.0320	0	19 T-0.127 0.628
0	0	0.0001 1 0.002 0.0235	0.0012 13 T-0.009 0.0872	<0.0001 2 T 0.0032	0	17 T-0.009 0.119
0	0	0	0.0018 9 T-0.019 0.130	0.0001 2 T-0.001 0.0052	0	11 T-0.019 0.135
0	0	0	0.0032 8 T-0.026 0.228	0.0003 1 T 0.0240	0	24 T-0.074 2.73
0	0	0	0.0027 14 T-0.031 0.191	0.0001 3 T 0.0060	0	17 T-0.031 0.197
0	0	0.0004 1 0.008 0.0844	0	0	0	1 0.008 0.0844
0	0	0.005 1 T 1.06	NA	0	0	1 T 1.06

Table 3. (continued)

Chemical	Dairy products	Meat fish & poultry	Grain & cereal products	Potatoes	Leafy vegetables	Legume vegetables
Phosalone						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
Polychlorinated biphenyls						
Average concn		0.002				
No. positive	0	2	0	0	0	0
Range of concn		T				
Average intake		0.523				
Quintozene						
Average concn						
No. positive	0	0	0	0	0	0
Range of concn						
Average intake						
TDE						
Average concn		0.0001				
No. positive	0	3	0	0	0	0
Range of concn		T				
Average intake		0.0262				
Tecnazene						
Average concn				0.0005		
No. positive	0	0	0	1	0	0
Range of concn				0.010		
Average intake				0.0792		
Tetrachloroaniline						
Average concn				0.0002		
No. positive	0	0	0	1	0	0
Range of concn				0.004		
Average intake				0.0317		
Tetrachloroanisidine						
Average concn				<0.0001		
No. positive	0	0	0	1	0	0
Range of concn				T		
Average intake				0.0048		
Tetrachlorothioanisole						
Average concn				<0.0001		
No. positive	0	0	0	1	0	0
Range of concn				T		
Average intake				0.0040		
Toxaphene						
Average concn					0.0025	0.0030
No. positive	0	0	0	0	1	2
Range of concn					0.050	0.030
Average intake					0.154	0.243
Tri-n-butyl phosphate						
Average concn			0.0068			
No. positive	NA	NA	9	0	0	0
Range of concn			0.003–0.031			
Average intake			2.85			
No. of chemicals detected	8	18	13	12	13	5
Total residues detected	68	138	50	38	41	6

Table 3. (continued)

Root vegetables	Garden fruits	Fruits	Oils & fats	Sugar & adjuncts	Beverages	All groups
0	0	0.0011 1 0.022 0.258	0	0	0	1 0.022 0.258
0	0	0	0	0	0	2 T 0.523
0	0.0003 1 0.007 0.0253	0	0.0010 14 T-0.005 0.0685	<0.0001 2 T 0.0020	0	17 T-0.007 0.0958
0	0	0	0	0	0	3 T 0.0262
0	0	0	0	0	0	1 0.010 0.0792
0	0	0	0	0	0	1 0.004 0.0317
0	0	0	0	0	0	1 T 0.0048
0	0	0	0	0	0	1 T 0.0040
0	0.0077 2 0.077-0.078 0.575	0	0.0010 1 T 0.0711	0	0	6 T-0.078 1.04
0	0	0	NA	NA	NA	9 0.003-0.031 2.85
6	19	20	18	14	0	54
14	60	55	124	62	0	656

Table 4. Daily intake per unit of body weight (µg/kg of body weight/day) of pesticides and industrial chemicals in Fiscal Years 1977–1980[a]

Chemical	FAO/WHO Acceptable Daily Intake	FY 1977	FY 1978	FY 1979	FY 1980
BHC (total)		0.015	0.012	0.015	0.014
BHC, alpha		0.011	0.009	0.010	0.011
BHC, beta		<0.001	0.001	0.001	ND
BHC, delta		<0.001	<0.001	ND	<0.001
Lindane (BHC, gamma)	10	0.004	0.002	0.004	0.003
Captan	10	0.031	0.008	0.005	0.001
Carbaryl	10	ND	0.016	0.016	0.021
Chlordane (total)	1	0.004	0.004	0.004	0.003
Chlordane		0.001	0.001	ND	<0.001
Octachlor epoxide		0.003	0.003	0.004	0.003
Chlorobenzilate	20	ND	ND	ND	0.001
2-Chloroethyl caprate		ND	0.009	ND	ND
2-Chloroethyl laurate		ND	0.056	ND	ND
2-Chloroethyl linoleate		0.078	0.228	0.079	0.197
2-Chloroethyl myristate		ND	0.023	ND	ND
2-Chloroethyl palmitate		0.010	0.023	0.005	0.012
Chlorpropham		0.310	0.144	0.300	0.268
Chlorpyrifos	10	ND	0.005	0.005	0.001
DCPA		0.001	0.001	0.001	0.002
DDT (total)	5	0.046	0.070	0.093	0.034
DDT		0.006	0.008	0.004	0.003
DDE		0.039	0.061	0.087	0.031
TDE		0.001	0.001	0.002	<0.001
DEF		ND	ND	<0.001	ND
Demeton-S sulfone		NA	NA	NA	0.002
Diazinon	2	0.006	0.004	0.010	0.004
Dicloran	30	0.056	0.033	0.030	0.023
Dicofol	25	0.004	0.005	0.007	0.012
Dieldrin	0.1	0.023	0.017	0.016	0.022
Dimethoate	20	NA	NA	NA	0.001
Endosulfan (total)	8	0.010	0.011	0.010	0.015
Endosulfan I		0.003	0.003	0.003	0.003
Endosulfan II		0.003	0.003	0.002	0.008
Endosulfan sulfate		0.004	0.005	0.005	0.004
Endrin	0.2	<0.001	<0.001	ND	ND
Ethion	1	0.018	0.001	0.005	0.001
2-Ethylhexyl diphenyl phosphate		ND	ND	ND	1.82
Fenitrothion	1	ND	ND	ND	0.001
Fenthion	1	ND	<0.001	ND	ND
Fonofos		ND	0.001	<0.001	0.002
Heptachlor epoxide	0.5	0.007	0.008	0.006	0.007
Hexachlorobenzene		0.002	0.004	0.003	0.004
Leptophos		0.002	ND	ND	ND
Linuron		NA	NA	NA	<0.001
Malathion	20	0.154	0.142	0.265	0.203
Methidathion	5	NA	NA	NA	0.001
Methoxychlor		0.008	0.007	0.003	0.007
Nitrofen		ND	ND	<0.001	ND
Nonachlor, trans		0.002	0.001	<0.001	<0.001
Parathion	5	0.002	0.004	0.002	0.001
Parathion-methyl	1	0.001	ND	ND	<0.001
Pentachloroanisole		<0.001	0.001	0.001	0.002
Pentachlorobenzene		0.001	0.002	0.002	0.002
Pentachlorobenzonitrile		ND	<0.001	ND	ND
Pentachlorophenol		0.001	ND	0.006	0.040
Perthane		<0.001	0.005	0.003	0.001
o-Phenylphenol	20	0.004	0.038	0.046	0.015
Phosalone	6	0.001	0.017	0.003	0.004
Polychlorinated biphenyls		0.016	0.027	0.014	0.008
Quintozene (total)	7	0.002	0.003	0.005	0.013
Quintozene		0.001	0.001	0.001	0.001
Pentachloroaniline		0.001	0.001	0.003	0.009
Pentachlorothioanisole		<0.001	0.001	0.001	0.003
Ronnel		ND	ND	0.001	ND
Tecnazene	10	0.035	0.005	0.001	0.001
Tetrachloroaniline		0.005	0.002	<0.001	<0.001
Tetrachloroanisidine		0.003	0.001	0.001	<0.001
Tetrachloroanisole		<0.001	ND	ND	ND
Tetrachlorobenzene		0.005	<0.001	ND	ND
Tetrachlorothioanisole		ND	0.001	<0.001	<0.001
Toxaphene		0.080	0.107	0.003	0.015
Tri-n-butyl phosphate		NA	NA	NA	0.041
Vinclozolin		ND	ND	0.003	ND

[a]The FAO/WHO ADIs (36) are expressed here in µg/kg of body weight/day. The intake is presented as a total in cases in which the ADI is expressed as the sum of related residues. The intake is indicated as ND in cases in which the chemical was not detected but could have been if it had been present. It is listed as NA in cases in which the analytical methodology used was not capable of determining that chemical. The intakes for Fiscal Years 1977-1979 were reported previously (26-28). Because trace level findings were assigned a value of zero in calculation of intakes prior to Fiscal Year 1978, the intakes shown for Fiscal Year 1977 may be slightly lower than if they had been calculated using an estimate of the level for trace findings.

new analytical procedure for chlorophenoxy acids beginning in mid-Fiscal Year 1979 (3). Linuron was reported for the first time as a result of the characterization of a previously unidentified analytical response.

The daily intakes per unit of body weight of the pesticides and industrial chemicals for Fiscal Years 1977–1980 (Table 4) appear to be relatively constant. None of the intakes for Fiscal Year 1980 approached the FAO/WHO ADI; dieldrin was closest, at 22% of its ADI.

Elements

No trends are apparent in the average daily intakes of the 6 selected elements for Fiscal Years 1977–1980 (Table 6). The Fiscal Year 1980 intakes of cadmium, lead, and mercury were well below their PTDIs. The intake of total arsenic was 63 μg/day, calculated as arsenic trioxide. As mentioned previously, no maximum acceptable intake has been agreed on for total arsenic. The PTDI estimated by the FAO/WHO for inorganic arsenic is 2 μg/kg of body weight (for a 69.1 kg person, this is equivalent to 182 μg/day, calculated as arsenic trioxide). The intake calculated for selenium was within the range of the ESADDI. The intake calculated for zinc was slightly higher than the RDA.

Conclusion

The dietary intakes determined in the Fiscal Year 1980 Adult Total Diet Study are similar to those found in the several preceding years and are within the acceptable limits outlined earlier. The same general approach for sample collection and analysis was continued in the Fiscal Year 1981 Adult Total Diet Study.

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Table 5. Average concentration (ppm) and average daily intake (µg/day) of selected elements by food group[a]

Element	Dairy products	Meat fish & poultry	Grain & cereal products	Potatoes	Leafy vegetables	Legume vegetables
Arsenic[b]						
Average concn		0.204	0.022	0.001	0.001	0.001
No. positive	0	20	15	1	1	1
Range of concn		0.04-0.52	T-0.06	0.02	0.02	T
Average intake		53.5	9.02	0.159	0.050	0.041
Cadmium						
Average concn	0.0034	0.0083	0.0246	0.0373	0.0342	0.0056
No. positive	8	13	20	20	20	8
Range of concn	T-0.023	T-0.062	0.016-0.034	0.014-0.073	0.011-0.128	T-0.070
Average intake	2.55	2.19	10.3	5.92	1.92	0.443
Lead						
Average concn	0.010	0.042	0.048	0.018	0.016	0.180
No. positive	8	20	19	12	11	20
Range of concn	0.02-0.05	T-0.14	0.02-0.21	0.02-0.08	0.02-0.05	0.04-0.36
Average intake	7.52	11.0	19.8	2.86	0.878	13.4
Mercury						
Average concn		0.0122	0.0021	0.0006	0.0001	0.0010
No. positive	0	19	9	3	1	2
Range of concn		T-0.033	T	T	T	T
Average intake		3.21	0.888	0.103	0.0092	0.0733
Selenium						
Average concn	0.014	0.204	0.174	0.010	0.008	0.007
No. positive	8	20	19	6	3	5
Range of concn	T-0.06	T-0.52	0.06-0.39	T-0.06	T-0.08	T-0.07
Average intake	10.4	53.5	72.9	1.51	0.478	0.551
Zinc						
Average concn	4.56	31.1	8.91	3.56	1.97	8.08
No. positive	20	20	20	20	20	20
Range of concn	3.2-5.9	26.9-38.0	7.1-13.7	1.5-5.7	1.4-2.9	4.9-17.1
Average intake[c]	3.44	8.15	3.73	0.57	0.11	0.59

[a]The average concentration is based on all composites analyzed and is expressed in ppm. The range of concentrations is for those composites in which the element was detected and is expressed in ppm. The average daily intake is based on all composites analyzed and is expressed in µg/day. Detections below the limit of quantitation were reported as trace (T). The limit of quantitation varies with the element. When trace level findings were reported, the analyst included an estimate of the amount of the element present; these estimates were used in calculation of the average concentration and average daily intake.
[b]Calculated as arsenic trioxide (As_2O_3).
[c]Expressed in mg/day.

Table 5. (continued)

Root vegetables	Garden fruits	Fruits	Oils & fats	Sugar & adjuncts	Beverages	All groups
0.006				0.001		
2	0	0	0	1	0	41
T-0.11				0.02		T-0.52
0.189				0.081		63.0
0.0225	0.0137	0.0010	0.0090	0.0046	0.0023	
19	16	3	14	12	6	159
T-0.082	T-0.068	T-0.014	T-0.023	T-0.015	T-0.020	T-0.128
0.719	1.02	0.240	0.652	0.378	1.56	27.9
0.054	0.111	0.042	0.009	0.030	0.008	
17	20	16	5	17	5	170
0.02-0.21	0.03-0.34	T-0.13	T-0.05	0.02-0.06	0.02-0.07	T-0.36
1.73	8.18	9.40	0.647	2.46	5.00	82.9
	0.0003	0.0001	0.0019	0.0004	0.0001	
0	1	1	7	3	1	47
	T	T	T	T-0.004	T	T-0.033
	0.0250	0.0320	0.143	0.0329	0.110	4.63
0.007	0.006	0.001			0.002	
4	4	1	0	0	2	72
T-0.05	T-0.05	T			T	T-0.52
0.197	0.438	0.106			1.31	141
2.37	2.70	0.84	4.52	1.97	0.48	
20	20	20	20	20	20	240
1.6-3.6	1.5-5.2	0.6-1.5	2.9-5.4	0.9-5.0	0.2-1.8	0.2-38.0
0.08	0.20	0.18	0.33	0.16	0.33	17.9

Table 6. Average daily intake (μg/day) of selected elements in Fiscal Years 1977–1980[a]

Element	Acceptable intake limit	FY 1977	FY 1978	FY 1979	FY 1980
Arsenic[b]	—[c]	72	59	62	63
Cadmium	57–72[d]	37	31	32	28
Lead	429[d]	79	95	82	83
Mercury	43[d]	6	3	5	5
Selenium	50–200[e]	110	156	152	141
Zinc[f]	15[g]	18	17	18	18

[a]The average daily intakes for Fiscal Years 1977–1979 were reported previously (26–28). Intake data for selenium and zinc were presented previously in a report covering the Total Diet Study analyses for minerals (10). The intakes reported here are based on a diet providing 3900 kcal/day, whereas those presented in the previous report were calculated based on a 2850 kcal/day diet. The diet of 3900 kcal/day was selected to show the maximum exposure to toxic residues, whereas the report using the diet of 2850 kcal/day focuses on nutritional adequacy.
[b]Calculated as arsenic trioxide (As_2O_3).
[c]No maximum acceptable intake has been agreed on for total arsenic (organic and inorganic). However, the FAO/WHO (38) has estimated a PTDI for inorganic arsenic of 2 μg/kg of body weight (for a 69.1 kg person, this is equivalent to 182 μg/day calculated as arsenic trioxide).
[d]Provisional Tolerable Daily Intake (PTDI) calculated from the Provisional Tolerable Weekly Intake (PTWI) proposed by the FAO/WHO (37).
[e]Estimated Safe and Adequate Daily Dietary Intake (ESADDI) proposed by the National Research Council of the National Academy of Sciences (39).
[f]All zinc intake values are expressed in mg/day.
[g]Recommended Dietary Allowance (RDA) proposed by the National Research Council of the National Academy of Sciences (39).

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FLAVORS

Liquid Chromatographic Determination of Vanillin and Related Flavor Compounds in Vanilla Extract: Cooperative Study

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Vanillin and related flavor compounds present in vanilla extract were determined by reverse phase liquid chromatography. Results compared favorably with the official AOAC spectrophotometric method. Compounds such as *p*-hydroxybenzaldehyde and ethyl vanillin known to interfere in the official method were resolved from vanillin. The method was evaluated by 6 industrial laboratories under the aegis of the Technical Committee of the Flavor and Extract Manufacturers Association. The method is suggested for AOAC collaborative study.

The principal flavoring component in vanilla extract is vanillin. Vanillin develops as a result of fermentation during the sun-curing process to which vanilla beans are subjected after picking. The variety of bean and the quality of curing dictate the amount of vanillin produced and hence the quality of the finished extract.

Climatic conditions and curing practices vary with the source of origin and greatly affect the quality of the beans. Poor extracts containing low vanillin levels do not command high prices which, in some cases, leads to economic adulteration. Adulteration by the addition of ethyl vanillin or bourbonal is recorded as early as 1874 (1).

Jurgens (2) showed a correlation between the 4-hydroxybenzaldehyde and vanillin contents in beans originating in Madagascar. He proposed that this ratio could be used to identify the geographic origin of beans in an extract.

In 1982, Herrmann and Stockli reported a method for the control of vanilla products on the basis of identification and quantitation of 4-hydroxybenzyl alcohol, aldehyde, and acid, and vanillyl alcohol, vanillin, ethyl vanillin, and coumarin (3).

Wallace in 1983 (4) identified, by liquid chromatography (LC), several other phenolic compounds including syringaldehyde and acetovanillone produced during the manufacture of vanillin from pulp mill effluents.

The current AOAC method (5) for determination of vanillin in the presence of ethyl vanillin is time-consuming and is not frequently used. The method most often used by the flavor industry determines vanillin only, which is quantitated by absorbance of an alkaline solution of the extract at 348 nm. Both ethyl vanillin and *p*-hydroxybenzaldehyde interfere at this wavelength.

The presently reported LC method was developed with the following objectives: (*1*) elimination of interferences in the AOAC method for vanillin; (*2*) quantitation of additional components present in vanilla extracts; (*3*) use of standard LC apparatus; (*4*) minimal analyst involvement; and (*5*) rapid analysis time. Member laboratories of the Flavor and Extract Manufacturers Association (FEMA) agreed to supply representative samples of commercially available extracts and to analyze these samples by the proposed method. The intent of this study was to document the capability of the method in laboratories which routinely assess the quality of flavoring extracts.

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Experimental

Apparatus and Reagents

(**a**) *Liquid chromatograph.*—Meeting the following analytical capabilities: flow rate 2.5 mL/min, UV detection at 254 nm fixed wavelength.

(**b**) *Column.*—Stainless steel, 25 cm × 4.6 mm, packed with totally porous irregular particles, C_8 packing, 10 μm size (LiChrosorb, E. Merck, or equivalent).

(**c**) *Solvents.*—LC grade methanol, LC grade water, 95% nondenatured ethanol, 40% nondenatured ethanol, and glacial acetic acid.

(**d**) *Mobile phase.*—Methanol–acidified water (10 + 90) pumped at 2.50 mL/min. Acidify water by adding 10 mL glacial acetic acid per 800 mL.

(**e**) *Sample filters.*—0.45 μm, alcohol-compatible.

(**f**) *Standards.*—Vanillin (Matheson Coleman and Bell), 4-hydroxybenzoic acid (Kodak), 4-hydroxybenzaldehyde (Kodak), vanillic acid (Kodak).

Standard Preparation

Weigh 1.2000 g vanillin, 0.0800 g vanillic acid, 0.0200 g 4-hydroxybenzoic acid, and 0.0600 4-hydroxybenzaldehyde into 100 mL volumetric flask and dilute to volume with 95% ethanol. Dilute 10 mL aliquot to 100 mL with 40% ethanol, and inject 10 μL into chromatograph.

Sample Preparation

If vanillin content is less than 0.3 g/100 mL extract, filter and inject 10 μL. If content is above 0.3 g/100 mL, dilute extract to below that level with 40% ethanol, filter, and inject 10 μL; record appropriate dilution factor.

Discussion

Ethyl vanillin, present in some imitation vanilla extracts, elutes at 2.2 relative to vanillin. 4-Hydroxybenzaldehyde, 4-hydroxybenzoic acid, and vanillic acid are also adequately resolved under the specified conditions (Figure 1).

The equipment used with the exception of the C_8 column is standard equipment for most LC work. Detection at 254 nm is adequate, although sensitivity is greater at 280 nm for vanillin and ethyl vanillin; however, the response for *p*-hydroxybenzoic acid is reduced. The response factor for vanillin at 280 nm is nearly 4 times that at 254 nm, while the response factor for *p*-hydroxybenzoic acid decreases by nearly 600%. Vanillin, however, is present in most extracts at 50–75 times the level of *p*-hydroxybenzoic acid and, therefore, minor changes in the response of vanillin are not as significant as for *p*-hydroxybenzoic acid. The trade-off was not considered appropriate, given the initial intent to use standard equipment.

The use of a C_8 vs C_{18} column was preferred because a greater number of samples could be analyzed without loss of column resolution. In both this laboratory and others using the method on a routine basis, the resolution of *p*-hydroxybenzaldehyde and vanillic acid decreases measureably over

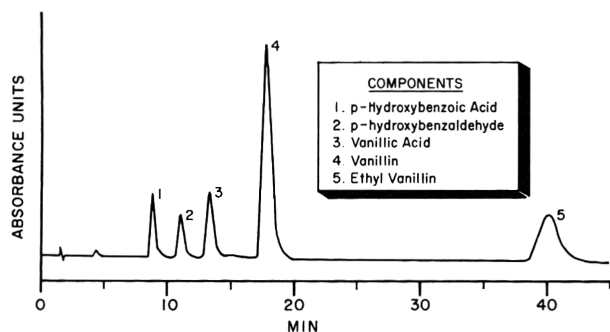

Figure 1. Chromatogram of working standard for 1-fold extracts.

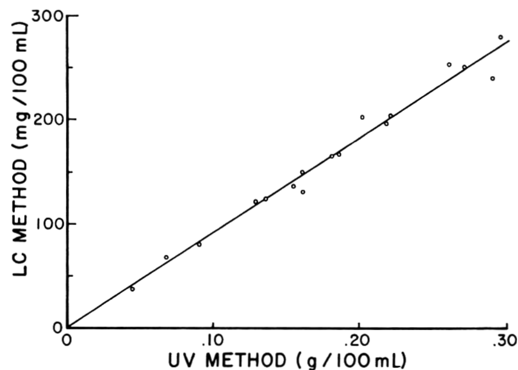

Figure 2. Comparison of vanillin content of vanilla extracts determined by AOAC-UV method and proposed LC method.

Table 1. Linearity data at 254 nm for naturally occurring compounds in vanilla extract

p-Hydroxybenzoic acid:							
μg Injected	0	0.103	0.172	0.343	0.686	1.715	3.430
Area[a]	0	4,065	6,930	13,825	28,221	69,989	147,321
p-Hydroxybenzaldehyde:							
μg Injected	0	0.190	0.316	0.632	1.264	3.160	6.320
Area[a]	0	2,412	4,166	8,690	17,674	44,621	94,091
Vanillic acid:							
μg Injected	0	0.260	0.433	0.866	1.732	4.330	8.660
Area[a]	0	5,150	8,633	17,556	35,447	89,250	188,471
Vanillin:							
μg Injected	0	3.709	6.181	12.362	24.724	61.810	123.62
Area[a]	0	22,928	38,223	76,993	156,024	389,331	814,826

[a]Average of triplicate injections.

Table 2. Results for *p*-hydroxybenzoic acid (mg/100 mL) in selected commercial extracts

	Laboratory								
Sample	1	2-A[a]	2-B	3	4	5	6	$\bar{x}$	S
1	3	2	2	3	2	2	2	2.3	0.49
2	3	3	3	2	2	2	2	2.4	0.53
3	3	3	3	2	3	2	2	2.6	0.54
4	26	24	27	30	26	19	20	24.6	3.90
5	2	1	2	2	1	1	1	1.4	0.54
6	1	1	1	1	1	1	1	1.0	—
7	3	3	4	3	5	3	3	3.4	0.79
8	3	3	3	3	3	2	3	2.9	0.38
9	7	6	8	4	6	4	5	5.7	1.50
10	9	9	10	12	8	6	8	8.9	1.86
11	2	2	2	2	2	2	2	2.0	—
12	4	4	4	4	3	3	3	3.6	0.53
13	3	3	3	3	3	2	3	2.9	0.38

[a]Two analysts from same laboratory submitted results.

Table 3. Results for *p*-hydroxybenzaldehyde (mg/100 mL) in selected commercial extracts

	Laboratory								
Sample	1	2-A[a]	2-B	3	4	5	6	$\bar{x}$	S
1	11	11	11	12	11	10	11	11.0	0.58
2	10	10	9	11	11	9	9	9.9	0.90
3	12	12	11	11	12	11	11	11.4	0.53
4	109	110	87	120	109	102	87	103.4	12.39
5	3	3	3	3	3	3	3	3.0	—
6	11	12	10	12	13	11	12	11.6	0.98
7	12	12	11	12	18[b]	11	11	11.5	0.55
8	12	12	11	13	13	12	12	12.1	0.69
9	26	24	27	24	27	24	24	25.1	1.46
10	34	34	34	49[b]	35	33	32	33.7	1.03
11	17	16	15	19	18	16	18	17.0	1.41
12	15	15	13	17	16	14	14	14.9	1.35
13	13	12	11	13	13	12	12	12.3	0.76

[a]Two analysts from the same laboratory reported results.
[b]Rejected from statistical compilation on basis of Dixon's test.

Table 4. Results for vanillic acid (mg/100 mL) in selected commercial extracts

Sample	Laboratory							$\bar{x}$	S
	1	2-A[a]	2-B	3	4	5	6		
1	10	8	9	10	9	8	8	8.9	0.90
2	10	9	9	9	9	9	8	9.0	0.58
3	12	10	11	10	11	10	9	10.4	0.98
4	117	102	100	120	109	99	79	103.7	13.66
5	4	4	4	5	4	4	3	4.0	0.58
6	6	6	5	6	6	5	5	5.6	0.53
7	15	13	13	14	20[b]	13	12	13.3	1.03
8	14	12	12	14	13	12	12	12.7	0.95
9	23	18	21	18	20	19	16	19.3	2.29
10	36	30	33	36	32	30	429[b]	32.8	2.71
11	11	9	10	11	10	9	9	9.9	0.90
12	18	15	15	17	18	15	14	16.0	1.63
13	13	11	11	13	12	11	11	11.7	0.95

[a]Two analysts from same laboratory submitted results.
[b]Rejected from statistical compilation on basis of Dixon's test.

Table 5. Results for vanillin (g/100 mL) by LC and by AOAC-UV methods in selected commercial extracts

Sample	Laboratory							$\bar{x}$	S	CV, %	AOAC-UV
	1	2-A[a]	2-B	3	4	5	6				
1	0.149	0.146	0.146	0.168	0.166	0.147	0.150	0.153	0.010	6.27	0.163
2	0.160	0.155	0.154	0.157	0.153	0.155	0.155	0.156	0.002	1.47	0.174
3	0.163	0.158	0.157	0.160	0.178	0.159	0.162	0.162	0.007	4.44	0.183
4	1.438	1.436	1.210	1.530	1.534	1.430	1.258	1.405	0.126	8.94	1.629
5	0.024	0.028	0.030	0.033	0.027	0.026	0.025	0.028	0.003	11.25	0.029
6	0.207	0.200	0.180	0.218	0.226	0.203	0.203	0.205	0.015	7.09	0.219
7	0.165	0.161	0.160	0.164	0.167	0.161	0.161	0.163	0.003	1.61	0.189
8	0.171	0.166	0.163	0.179	0.179	0.167	0.171	0.171	0.006	3.65	0.193
9	0.326	0.316	0.306	0.322	0.354[b]	0.318	0.305	0.316	0.008	2.69	0.355
10	0.467	0.454	0.432	0.471	0.483	0.457	0.448	0.459	0.017	3.63	0.522
11	0.185	0.183	0.176	0.194	0.195	0.180	0.204	0.188	0.010	5.16	0.204
12	0.198	0.195	0.183	0.204	0.216	0.197	0.197	0.199	0.010	5.01	0.225
13	0.183	0.178	0.158[b]	0.190	0.190	0.178	0.180	0.183	0.006	3.07	0.201

[a]Two analysts from same laboratory submitted results.
[b]Rejected from statistical compilation on basis of Dixon's test.

several months of use on a C_{18} column, while no loss was observed on a C_8 column. Costs for the 2 types of columns are similar.

The majority of samples including several multi-fold extracts are simply filtered and injected, requiring only several moments of analyst time. The high polarity of the mobile phase retains many organic compounds common to the extract until flushed with greater than 50% methanol, thereby allowing run times under 20 min for pure vanilla extracts and less than 40 min for artificial extracts containing ethyl vanillin. In addition, this method was readily adaptable to automation.

Applicability of the AOAC UV method for vanillin ranges up to or slightly above the quantity present in a 1-fold extract. Many extracts available commercially contain vanillin levels above this range and require further dilution. The LC method proposed here not only minimizes dilutions, but also has a greater linear range.

Over the intended range, the detector response was linear when the μg/injection was compared with peak area (Table 1). Applying a linear least squares fit, the correlation coefficient was 0.99 or better for each of the 4 components. The maximum quantity of vanillin injected corresponds to an extract containing 1.24 g/100 mL, or a 5-fold extract.

At this laboratory, 17 extract samples were analyzed by both the current UV method (5) and the LC procedure set forth in this paper (Figure 2). Comparison of the results confirms the earlier statement that *p*-hydroxybenzaldehyde present in extracts interferes when the AOAC procedure is used.

Thirteen samples of commercially available extracts were analyzed by 6 FEMA membership laboratories (Tables 2–5). At the request of the participating laboratories, all samples were diluted to a 1-fold level and analyzed. This is in keeping with standard practices in the extract industry. The samples were also evaluated by one laboratory using the AOAC vanillin procedure (Table 5).

Statistical analysis of the data showed an average coefficient of variation of 7.3% for vanillin. The range of the data may be due to the extended time over which the samples were analyzed (9 months), the use of only single determinations, the dilution of samples to 1-fold, and the lack of familiarity with the method.

This method provides an additional means of determining vanillin content in vanilla extract and offers potential as a useful tool for evaluation of the overall authenticity of extracts. It also has the capability to quantitatively determine the presence of related flavor compounds.

Based on the data presented above, we recommend a collaborative study under AOAC guidelines be initiated.

Acknowledgments

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Co.; and Robert Breyer, Norda, Inc. These individuals kindly provided standards and samples of vanilla extract, and participated in the collaborative testing. All of this work was done under the guidance of the Technical Committee of the Flavor and Extract Manufacturers Association during the 1983–84 term.

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FRUITS AND FRUIT PRODUCTS

Spectral Characteristics of Florida Orange Juice and Orange Pulpwash. Collaborative Study

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Visible and ultraviolet absorption and room temperature fluorescence excitation and emission spectra, obtained from alcoholic solutions of Florida orange juice (OJ) and orange pulpwash (PW), were used to characterize the collaborative study samples. The absorbance sum at 443, 325, and 280 nm and absorbance 443/325 nm ratio were used to estimate the percent total citrus material (TCM), OJ, PW, and dilution of the samples. Six laboratories participated in the study. Known samples of OJ and PW were given to each laboratory for familiarization and instrumental verification. Ten unknown samples (various combinations of OJ and PW) were analyzed by each laboratory. Three duplicate and 2 "closely matched pair" sample set combinations were reported. The results revealed that the method successfully detected the differences in sample formulations. The UV/vis and fluorescence spectrophotometric method has been adopted official first action for detecting adulteration of Florida orange juice with orange pulpwash.

Adulterations of citrus juices have progressed from simple dilutions with water, sugar, and acids to sophisticated methods using adulterants designed to obscure product purity. Juice adulteration appears to be a world-wide economic and regulatory problem. Numerous reports (1–3) have revealed extensive adulteration of citrus juices from retail markets in Europe and the United States. A microbiological method (4) has been developed to help detect adulteration, by dilution, of orange juice. Stable carbon isotope ratio analysis for detecting illegal addition of high fructose corn syrup to honey (5) and apple juice (6) has been applied to orange juice (7).

Orange juice may also be adulterated by the addition of orange pulpwash (water extraction of soluble fruit solids from orange pulp). The present paper reports a collaborative study on the use of visible and ultraviolet absorption and fluorescence excitation and emission to qualitatively detect orange pulpwash addition to orange juice (3). Currently, no other methods are available for this determination.

Collaborative Study

The results of 6 participating laboratories are reported. The Associate Referee results are not included. Each laboratory received a known sample each of orange juice and orange pulpwash (with absorption and fluorescence spectra) for familiarization and instrumental verification. Ten unknown samples were then analyzed by each laboratory for total citrus material (TCM), orange juice (OJ), and pulpwash (PW), and dilution. Materials were supplied as 3 duplicate sample combinations and 2 "closely matched pair" sample combinations. All samples were reconstituted to 11.8° Brix and kept frozen until ready for sample preparation. Two pints of USI absolute alcohol were also provided for sample preparation.

Submitted for publication August 25, 1983.
The recommendations of the Associate Referee, D. R. Petrus, were approved by the General Referee and the Committee on Foods II and were adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1986) **69**, March issue.

Adulteration of Processed Florida Orange Juice UV/Vis and Fluorescence Spectrophotometric Method First Action

Principle

Orange juice produced or packed in Florida cannot contain pulpwash solids. Adulteration by diln and/or addn of pulpwash is qualitatively detected by comparison of UV/vis absorption and fluorescence excitation and emission spectra, by measuring sum of A at 443, 325, and 280 nm, and by ratios of A at 443/325 nm.

Apparatus

(a) *UV/vis spectrophotometer.*—Coleman Model 124 UV/vis recording spectrophtr (Perkin-Elmer Corp.) or equiv. with capability to scan from 600 to 200 nm with spectral bandwidth of 2 nm, and interfaced to recorder having scale expansion capabilities.

(b) *Fluorescence spectrophotometer.*—Farrand Mark I ratio spectrofluorometer or equiv. instrument stdzd with solid Pyrex std with excitation at 315 nm and emission at 355 nm, range setting 0.3, sample-ratio switch on sample, and fluorescence intensity adjusted with gain to 0.180 μamp.

Orange Juice and Pulpwash Standards

Authentic frozen concentrated orange juice (FCOJ) and pulpwash samples with ref. UV/vis and fluorescence spectra may be obtained from State of Florida Dept. of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850. Use authentic products and ref. spectra for method familiarization and to verify instrumental performance. Reconstitute FCOJ and pulpwash stds to 11.8° Brix as in **22.026.**

Reagents

Alcohol.—Absolute EtOH (U.S. Industrial Chemicals or equiv.).

Preparation of Sample

Concentrated orange juice.—Reconstitute com. frozen concd orange juice according to product label directions, using graduated cylinder for vol. measurements; mix thoroly, and det. total sol. solids (°Brix) as in **22.026.** Dil. reconstituted samples further with equal vol. of H_2O. Take 5 mL of this sample, dil. to 50 mL with absolute EtOH, and place in dark at room temp. for <24 h. Following storage, centrf. sample at 1500 rpm for 5 min to sep. flocculent ppt from serum (soln). Use serum directly for UV/vis spectrophotometry; mix equal vols of serum and 90% EtOH for fluorescence characterization.

Single-strength orange juice.—Det. total sol. solids (°Brix) of single-strength juice by **22.026.** Prep. single-strength juice sample by dilg thoroly mixed juice with equal vol. of H_2O. From this point, prepn procedure is same as for concd orange juice, viz., "Take 5 mL of this sample . . .".

Analysis

(a) *UV/vis analysis*.—Transfer aliquot of clear serum into 10 mm quartz cuvet; scan (spectral bandwidth of 2 mm) serum from 600 to 200 nm at 60 nm/min; use appropriate *A* range settings for different portions of spectrum: 600–370 nm, 0–0.5 absorbance; 370–300 nm, 0–2 absorbance; 300–200 nm, 0–5 absorbance. Record sample *A* at 443, 325, and 280 nm and sum (434 + 325 + 280); also calc. and record 443/325 nm *A* ratio. Multiply *A* sum of sample by 1.085 (ratio of 12.8/11.8° Brix) to give adjusted absorbance sum (AAS). AAS value is required to relate results to data in JAOAC **63**, 1317(1980), which were obtained during Florida's 12.8° Brix reconstitution requirement.

$$1.085 \times (443 + 325 + 280)_{sample} = \text{AAS}$$

(b) *Fluorescence analysis*.—Det. fluorescence excitation and emission spectra of EtOH-dild serum sample at room temp. by using following parameters:

Excitation, nm	*Emission, nm*	*Range setting*
340	423	0.1
302	353	0.1
290	343	0.1
283	333	0.1
270	333	0.1
230	310	0.03

Fluorescence spectra are qual. and confirmatory in combination with UV/vis spectra. Fluorescence spectra confirm adulteration of orange juice by pulpwash addn or H_2O diln.

Interpretation

To distinguished types of adulteration, description of spectral shifts and intensity is outlined:

Adulteration by dilution:

(a) *UV/vis spectra*.—Simple diln causes noticeable shift of 223 nm peak to shorter wavelength; dild sample will register peak in region extending from 223 to 206 nm; diln causes adjusted *A* (AAS) to occur near or below lower end of distribution range for authentic samples (2.002–2.992; 2.410 ± 0.164).

(b) *Fluorescence spectra*.—Diln causes reduction in intensity of 340 nm (excitation) and 423 nm (emission) peaks; nondild orange juice samples show fluorescence intensity of 0.06–0.07 for both these wavelengths.

Adulteration by addition of pulpwash:

(a) *UV/vis spectra*.—Pulpwash addn causes the following: reduction in 443/325 nm ratio, normal range of OJ is 0.09–0.23 (0.144 ± 0.026); increased AAS; lack of resolution for 443 and 425 nm peaks; increased resolution of 280 nm peak; shift in 223 nm peak toward 230 nm; $A > 1.0$ for 325 nm peak strongly indicates adulteration. Fluorescence excitation and emission characterization of orange juice and pulpwash:

Excitation, nm	*Emission, nm*	*Range setting*	*Excitation spectra*	
			Orange juice	*Pulpwash*
340	423	0.1	strong	much stronger
302	353	0.1	inflection	strong max.
290	343	0.1	strong max.	min. or inflection
283	333	0.1	strong max.	min.
270	333	0.1	inflection	shoulder or max.
230	310	0.03	weak	weaker

Adulteration by addition of pulpwash and by dilution:

(a) *UV/vis spectra*.—Adulteration causes the following: decrease in 443/325 nm ratio and lack of resolution of 443 nm peak; *A* at 325 and 280 nm (indicative of polyphenols and flavonoids) may appear normal because of combined effects of pulpwash addn and diln; increased resolution of 280 nm peak; pulpwash addn alone causes upward shift in 223 nm peak toward 230 nm; diln alone causes shift downward toward 206 nm; suspected pulpwash-contg sample with 223 nm peak indicates diln also.

(b) *Fluorescence spectra*.—Dild, pulpwash-added juice shows deformities in excitation-emission curves (see table).

Complete substitution of pulpwash for orange juice:

(a) *UV/vis spectra*.—Adulteration causes the following: AAS ranges from 2.617 to 4.991 (3.781 ± 0.473); 443/325 ratio ranges from 0.017 to 0.112 (0.048 ± 0.020); poor resolution of 443 and 425 nm peaks; absorbance at 325 and 280 nm is stronger and more resolved than for OJ.

(b) *Fluorescence spectra*.—See table on differences between orange juice and pulpwash; see also JAOAC **63**, 1317(1980).

Results and Discussion

The participating laboratories were asked to qualitatively evaluate the absorption and fluorescence spectra, obtained of each sample, for the presence of PW by entering Yes, No, or ?. Seventy-eight percent of the samples were correctly evaluated by absorption and 67% by fluorescence characteristics. If a question mark was assumed to indicate a positive response, then 95% of the samples were correctly evaluated by absorption and 80% by fluorescence. Laboratory familiarization with the method should improve the qualitative evaluations.

Laboratory instrumental variations indicated that absorbance sum (at 443, 325, and 280 nm) and ratio (at 443/325 nm) values required a correction factor for interlaboratory comparisons. This was accomplished by the following expressions:

$$\pm \text{ Av. } A \text{ sum correction} = [(\Sigma\text{known OJ} - \Sigma\text{DOC OJ}) + (\Sigma\text{known PW} - \Sigma\text{DOC PW})]/2$$

where Σ is the laboratory absorbance sum of the known OJ and PW, and DOC is Department of Citrus absorbance sum. And

$$\pm \text{ Av. } A \text{ ratio correction} = [(R \text{ known OJ} - R \text{ DOC OJ}) + (R \text{ known PW} - R \text{ DOC PW})]/2$$

where R is the laboratory absorbance ratio of the known and DOC is Department of Citrus absorbance ratio.

Collaborative reported absorbance sum and ratio data were corrected and %TCM, OJ, PW, and dilution were calculated. Corrected results are presented in Tables 1, 2, and 3. Each laboratory reported 3 duplicate sample combinations: samples 1 and 6, 3 and 10, and 5 and 9. Each laboratory also reported 2 "closely matched pair" sample combinations: samples 2 and 8, and samples 4 and 7.

The AOAC committee statistician provided the following evaluations: Dixon's outlier test and Youden's rank test were applied and no aberrant values were detected, nor was a pronounced systematic error indicated for any laboratory.

Statistical evaluations of percent TCM data are presented in Table 4. One-way analysis of variance revealed a significant difference ($P < 0.05$) among laboratory means (reproducibility) of sample combination 1 and 6. The *F*-max. test detected no significant difference ($P > 0.05$) among repeatability measures (variability within laboratories) for percent recovery data. A 2-way analysis of variance on percent recovery data revealed a significant difference ($P < 0.01$) among laboratory means and sample means. No significant ($P > 0.05$) interaction was detected.

Statistical evaluations of percent OJ data are presented in Table 5. No significant difference ($P > 0.05$) was found among

Table 1. Collaborative determination of percent total citrus material[a]

Lab.	Sample 1, 6	Sample 2	Sample 3, 10	Sample 4	Sample 5, 9	Sample 7	Sample 8
1	85	101	109	98	95	97	100
	84		108		94		
2	88	105	115	100	99	98	101
	84		111		103		
3	78	92	99	88	90	93	96
	74		107		104		
4	89	112	102	107	104	96	105
	84		107		107		
5	83	105	107	101	95	104	106
	90		118		107		
6	104	111	111	106	98	93	98
	96		125		96		

[a] 1 and 6 = 80% TCM; 2 = 100%; 3 and 10 = 100%; 4 = 90%; 5 and 9 = 100%; 7 = 90%; 8 = 100%.

Table 2. Collaborative determination of percent orange juice[a]

Lab.	Sample 1, 6	Sample 2	Sample 3, 10	Sample 4	Sample 5, 9	Sample 7	Sample 8
1	57	83	0	40	100	31	83
	54		0		94		
2	54	80	0	30	100	24	82
	46		0		100		
3	51	80	0	20	90	20	84
	43		0		100		
4	50	85	0	39	100	15	83
	43		0		100		
5	51	70	0	35	100	36	87
	63		0		100		
6	53	76	0	31	100	23	85
	54		0		100		

[a] 1 and 6 = 50% OJ; 2 = 80%; 3 and 10 = 0%; 4 = 30%; 5 and 9 = 100%; 7 = 25%; 8 = 85%.

Table 3. Collaborative determination of percent pulpwash[a]

Lab.	Sample 1, 6	Sample 2	Sample 3, 10	Sample 4	Sample 5, 9	Sample 7	Sample 8
1	27	17	100	58	0	66	17
	30		100		0		
2	34	20	100	70	0	74	18
	38		100		0		
3	27	20	100	67	0	73	16
	31		100		0		
4	39	15	100	67	0	81	17
	41		100		0		
5	31	30	100	67	0	68	13
	28		100		0		
6	51	24	100	76	0	70	15
	42		100		0		

[a] 1 and 6 = 30% PW; 2 = 20%; 3 and 10 = 100%; 4 = 60%; 5 and 9 = 0%; 7 = 65%; 8 = 15%.

Table 4. Statistical evaluation for recovery of percent total citrus material

Statistic	Samples 1 and 6 (80%)	Samples 3 and 10 (100%)	Samples 5 and 9 (100%)	Samples 2 and 8 (100%)	Samples 4 and 7 (90%)
Mean%	86.6	109.9	99.3	102.7	98.4
Reproducibility	8.15	7.05	5.57	5.96	5.65
CV, %	9.4	6.4	5.6	5.8	5.7
Repeatability	3.77	5.94	5.55	4.58	5.24
CV, %	4.4	5.4	5.6	4.5	5.3
Average % recovery	108.2	109.9	99.3	102.7	109.4
Reproducibility	10.19	7.05	5.57	5.96	6.29
CV, %	9.4	6.4	5.6	5.8	5.7
Repeatability	4.72	5.94	5.55	4.58	5.82
CV, %	4.4	5.4	5.6	4.5	5.3

Table 5. Statistical evaluation for recovery of percent orange juice

Statistic	Samples 1 and 6 (50%)	Samples 3 and 10 (0%)	Samples 5 and 9 (100%)	Samples 2 and 8 (80 & 85%)	Samples 4 and 7 (30 & 25%)
Mean %	51.6	0.0	98.7	81.5	28.7
Reproducibility	5.77	0.0	3.37	4.94	7.46
CV, %	11.2	0.0	3.4	6.1	26.0
Repeatability	5.25	0.0	3.37	4.94	6.37
CV, %	10.2	0.0	3.4	6.1	22.2
Average % recovery	103.2	0.0	98.7	98.7	103.9
Reproducibility	11.55	0.0	3.37	5.60	27.04
CV, %	11.2	0.0	3.4	5.7	26.0
Repeatability	10.50	0.0	3.37	5.60	22.45
CV, %	10.2	0.0	3.4	5.7	21.6

Table 6. Statistical evaluation for recovery of percent pulpwash

Statistic	Samples 1 and 6 (30%)	Samples 3 and 10 (100%)	Samples 5 and 9 (0%)	Samples 2 and 8 (20 & 15%)	Samples 4 and 7 (60 & 65%)
Mean %	34.9	100.0	0.0	18.5	69.8
Reproducibility	7.68	0.0	0.0	4.94	5.58
CV, %	22.0	0.0	0.0	26.7	8.0
Repeatability	3.35	0.0	0.0	4.94	4.77
CV, %	9.6	0.0	0.0	26.7	6.8
Average % recovery	116.4	100.0	0.0	105.8	111.6
Reproducibility	25.61	0.0	0.0	24.36	8.75
CV, %	22.0	0.0	0.0	23.0	7.8
Repeatability	11.19	0.0	0.0	24.36	7.13
CV, %	9.6	0.0	0.0	23.0	6.4

Table 7. Statistical evaluation for recovery of percent dilution[a]

Statistic	Samples 1 and 6 (20%)	Samples 2 and 8 (0%)	Samples 4 and 7 (10%)
Mean %	13.8	0.0	2.8
Reproducibility	7.38	0.0	3.74
CV, %	53.6	0.0	135.9
Repeatability	3.20	0.0	2.14
CV, %	23.3	0.0	77.9
Average % recovery	68.8	0.0	27.5
Reproducibility	36.88	0.0	37.37
CV, %	53.6	0.0	135.9
Repeatability	16.01	0.0	21.41
CV, %	23.3	0.0	77.9

[a]Recovery = % dilution/% dilution found by collaborators × 100%.

Table 8. Coefficients of determination—calculated vs actual values

Lab.	r^2 % OJ	r^2 % PW
1	0.986	0.998
2	0.997	0.988
3	0.986	0.993
4	0.981	0.973
5	0.966	0.989
6	0.997	0.958
All labs	0.983	0.980

laboratory means for any sample combination. An overall view revealed the repeatability measure decreased as the sample value increased. The variability among laboratories and within laboratories was greatest for sample combination 4 and 7 (closely matched pairs). No significant difference ($P > 0.05$) was found among laboratory means or among sample means, tested by a 2-way analysis of variance, on percent recovery data.

Statistical evaluations of percent PW data are presented in Table 6. One-way analysis of variance revealed a significant difference ($P < 0.01$) only among laboratory means for sample combination 1 and 6. The variability within laboratories tends to decrease as the sample value increases. The *F*-max. test revealed a significant difference ($P < 0.05$) within laboratories for percent recovery of "closely matched pair" sample combinations 2 and 8, and 4 and 7. Variability among laboratories is greater for sample combination 2 and 8 than for combination 4 and 7. Two-way analysis of variance detected no significant difference ($P > 0.05$) among laboratory means or sample means of percent recovery data sets.

Statistical evaluations of percent dilution data are presented in Table 7. One-way analysis of variance revealed a significant difference ($P < 0.05$) among laboratory means for sample combination 1 and 6 (duplicates), and 4 and 7 (closely matched pairs). The *F*-max test revealed no significant difference ($P > 0.05$) within laboratories.

Regression analysis of calculated vs actual values of % OJ and % PW are presented in Table 8. Individual laboratory data produced very high coefficients of determination. All laboratory data also produced very high and significant ($P < 0.01$) coefficients of determination for % OJ and % PW, indicating 98% of the variation in actual OJ and PW values was explained by the calculated values.

The overall results of the study indicate good agreement with known formulated sample values. The method successfully detected the differences in sample formulations. Addition of pulpwash to orange juice is illegal (3) regardless of the amount added. Currently, this is the only method available for its detection.

Recommendations

Laboratories using the method should become familiar with the absorption and fluorescence characteristics of orange juice and orange pulpwash. Laboratories must obtain orange juice and orange pulpwash, with spectra, for instrument verification and standardization from the Florida Department of Citrus. It is recommended that this visible and ultraviolet absorption and fluorescence excitation and emission method be adopted official first action.

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DRUGS

Amperometric Determination of Phenazopyridine Hydrochloride in a Flowing Stream at the Glassy Carbon Electrode

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A flow-injection method is described for the determination of phenazopyridine hydrochloride, based on electrochemical oxidation at the glassy carbon electrode. The suggested method is highly specific and can be used to determine phenazopyridine HCl in the presence of most drugs commonly found in pharmaceutical dosage forms or administered therapeutically. Applying a constant potential of +950 mV vs Ag/AgCl/3.5M KCl reference electrode, the calibration curve was linear in the 1–30 μg/mL range, with minimum detectability of 0.2 ng (signal-to-noise ratio 2). Good accuracy and precision were obtained when the method was applied to some dosage forms containing phenazopyridine HCl. Although automation was not used in this study, an automated system could be incorporated because the method uses the technique of continuous analysis in a flowing stream.

Phenazopyridine, 2,6-diamino-3-phenylazopyridine, is a widely prescribed urinary antiseptic and analgesic. It has been analyzed by diverse methodologies, including spectrophotometry (1–3), coulometry (4), gravimetry (5), partition chromatography (6), and ion-exchange chromatography (7). All of these methods lack specificity, sensitivity, or both.

Phenazopyridine was reported to be reduced at the mercury pool electrode with a half-wave potential of −400 mV vs SCE, and this fact has been the basis of the coulometric procedure for its quantitation (4). There appear to be no data in the literature on electrochemical oxidation of this drug.

Flow-injection analysis is a relatively new analytical process which shows considerable potential for high-speed, precise analysis of discrete samples. However, the application of this technique in pharmaceutical analysis was not reported until Shah and Stewart (8–10) described its use to determine some pharmaceutical compounds.

Our report describes amperometric determination of phenazopyridine HCl in a flowing stream, based on oxidation at the glassy carbon electrode. The proposed method detects the drug in the 1–30 μg/mL range with good accuracy and precision, and is applicable to the analysis of phenazopyridine HCl in dosage forms.

Experimental

Apparatus

Voltammograms were obtained using a conventional 3-electrode system, consisting of a glassy carbon working electrode, a platinum auxiliary electrode, and a Ag/AgCl/3.5M KCl reference electrode. All potentials were recorded against this reference electrode. Cyclic voltammograms were obtained using a Bioanalytical Systems Model CV-1A potentiostat, with output monitored by a digital oscilloscope (Nicolet Instrument Corp., Model 200). Subsequent hard copies were obtained from an X-Y recorder (Houston Omnigraphic recorder, Model 200). A 3-electrode, wall-jet type electrochemical cell constructed in our laboratory was used for flow-injection analysis.

Mobile phase was pumped through the cell at a fixed flow rate of 1 mL/min. Samples were manually injected with a microsyringe (B-D, 1 cm, tuberculin syringe, Becton Dickinson and Co.) into an electrically activated sample injector (Valco Instruments Inc., Houston, TX) equipped with a 50 μL injection loop. The pump (Varian HPLC, 8500), injector, and electrochemical cell were connected with HPLC stainless steel tubing and fittings. Potentials were monitored with a Data Precision Digital Multimeter, Model 935. Cell currents were recorded at ambient temperature with a strip chart recorder (Fisher Scientific recorder, Series 5000).

Chemicals

Phenazopyridine HCl powder and dosage forms were provided by Hoffmann-La Roche, Nutley, NJ. All other chemicals were commercially available and were used as received.

Sample Preparation

A stock solution containing 1.0 mg phenazopyridine HCl/mL was prepared (1 + 1) in methanol–aqueous pH 7 phosphate buffer containing 2% sodium acetate. Further dilutions of stock solution were made to provide working standards in the 1–30 μg/mL range.

Procedure

Pump 1 + 1 mixture of methanol–aqueous pH 7 phosphate buffer containing 2% sodium acetate through the electrochemical cell at a flow rate of 1.0 mL/min. Inject aliquots of the drug solution in the same solvent mixture into the flowing stream, and measure the current with an applied potential of +950 mV vs Ag/AgCl/3.5M KCl reference electrode.

To determine whether other drugs commonly found in dosage forms with phenazopyridine interfere with the assay by altering the current flow of the drug or through oxidation at the glassy carbon electrode, the following study was performed: Prepare individual solutions (0.1 mg/mL) of sulfamethoxazole, sulfisoxazole, nitrofurantoin, oxytetracycline, ampicillin sodium, tobramycin, amikacin sulfate, neomycin sulfate, gentamycin sulfate, kanamycin sulfate, acetylsalicylic acid, paracetamol (acetaminophen), vancomycin HCl, atropine sulfate, and hyoscyamine sulfate. Accurately pipet aliquots of these solutions and use them to prepare various mixtures containing the individual drugs in the 1–30 μg/mL range with the phenazopyridine HCl concentration kept at 15 μg/mL. Assay each mixture for phenazopyridine HCl content.

The data obtained were then compared to those of a pure solution of phenazopyridine HCl to calculate the degree of interference, if any, at the various concentration levels of the added drugs.

Analysis of Solid Dosage Forms

Grind 20 tablets containing phenazopyridine HCl and dissolve by sonication with the same solvent mixture. Filter the resulting solution and dilute the filtrate to the 1–30 μg/mL range. Assay an aliquot of the diluted solution for phenazopyridine HCl content as described above.

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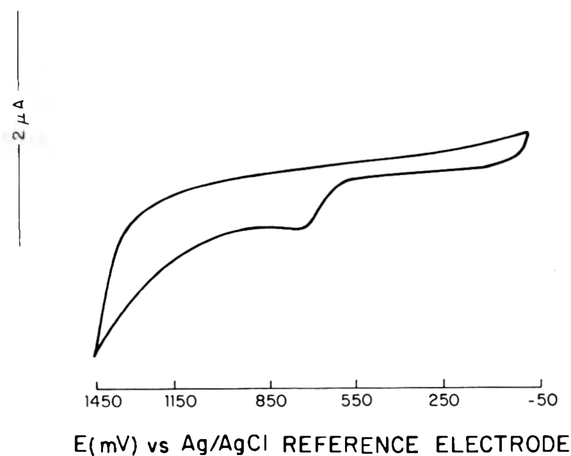

Figure 1. Cyclic voltammogram of phenazopyridine HCl (1×10^{-3}M) in 1 + 1 methanol–aqueous 0.01M phosphate buffer pH 7 containing 2% sodium acetate. Scan rate 500 mV/s.

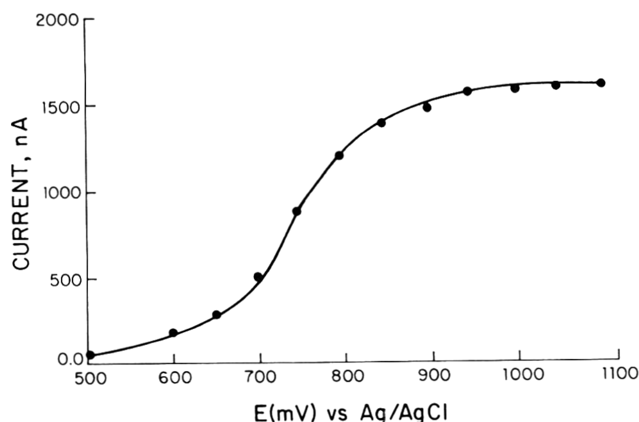

Figure 2. Hydrodynamic voltammogram of phenazopyridine HCl (20 μg/mL) in 1 + 1 methanol–aqueous 0.01M phosphate buffer pH 7 containing 2% sodium acetate. Flow rate 1.0 mL/min.

Results and Discussion

The presence of the aromatic amino groups suggested that phenazopyridine can be oxidized at positive potentials, by analogy to the behavior of various substituted phenolic and aromatic amine species (11). Oxidative cyclic voltammetric scan was initiated at 5.0 mV to switch potential of +1450 mV. Figure 1 shows the cyclic voltammogram of phenazopyridine HCl (10^{-3}M) at the glassy carbon electrode in methanol–0.01M pH 7 phosphate buffer (1 + 1). The voltammogram indicates that phenazopyridine exhibits an oxidative wave (at E_p = +750 mV), and the electrode process is completely irreversible because no cathodic wave was observed in the reverse scan mode.

Figure 2 shows the hydrodynamic voltammogram of phenazopyridine HCl (20 μg/mL) when subjected to electrochemical oxidation at the glassy carbon electrode. A potential of +950 mV was selected for the amperometric determination because it represented the least potential on the plateau at which maximum drug sensitivity could be obtained.

At the optimum electrode potential of +950 mV vs Ag/AgCl/3.5M KCl reference electrode, a phenazopyridine HCl calibration curve was obtained in the 1–30 μg/mL range. Linear regression analysis of the calibration data gave typical slope, intercept, and correlation coefficient of 77.2, −1.40, and 0.9997 (n = 12), respectively. To estimate the reproducibility of the electrode response by the amperometric method, 6 replicate injections were made at phenazopyridine HCl concentrations of 5, 10, 15, and 20 μg/mL. Mean peak currents of 385.5 ± 1.72, 772.3 ± 1.92, 1160 ± 1.84, and 1550 ± 1.90, respectively, were obtained. The precision of these measurements is expressed by relative standard deviations of 0.44, 0.25, 0.16, and 0.19%, respectively.

Interference studies were performed to establish the specificity of the method for phenazopyridine HCl in the presence of other drugs that might be found in its combination dosage forms, or administered in the same therapeutic situation (12). Nitrofurantoin, ampicillin sodium, amikacin sulfate, tobramycin, kanamycin sulfate, vancomycin HCl, atropine sulfate, hyoscyamine sulfate, and acetylsalicylic acid did not interfere in the assay of phenazopyridine, even at 10-fold concentration (Table 1). Gentamycin sulfate, doxycycline, paracetamol, and sulfa drugs showed appreciable interference when their concentrations were equal to or greater than the phenazopyridine level. This would indicate that these interfering compounds would have to be separated from phenazopyridine HCl before amperometric determination.

Application of the proposed method to the assay of phenazopyridine HCl in commercial dosage forms was then studied. After sample preparation and dilution to the μg/mL calibration range, the drug solutions were analyzed, and the concentration in each dosage form was calculated using the slope and intercept values obtained from linear regression analysis of the phenazopyridine HCl calibration curve. The results in Table 2 indicate that phenazopyridine HCl content in dosage forms can be conveniently determined by the amperometric method described here with good accuracy and precision. Also, there is good agreement between the results obtained by using the proposed method and those by the official USP method (1). By using the parameters established for the assay, phenazopyridine samples can be injected into the flowing stream at the rate of 60 samples/h (Figure 3). The sensitivity of the assay, based on signal-to-noise ratio of 2, is 0.2 ng of the drug. Electrode fouling was not encountered in the case of phenazopyridine, because the concentration of this analyte would be much smaller (>10μM) and the swift

Table 1. Determination of phenazopyridine HCl in synthetic drug mixtures

	Concentration, μg/mL[a]			
Compound	5.0	10.0	20.0	30.0
Nitrofurantoin	99.3 ± 1.02	99.4 ± 0.72	100.2 ± 0.87	101.0 ± 1.05
Atropine sulfate	99.5 ± 0.53	99.5 ± 0.49	100.5 ± 0.30	100.9 ± 0.42
Amikacin	99.7 ± 0.19	99.6 ± 0.37	99.9 ± 0.39	100.2 ± 0.51
Tobramycin	99.5 ± 0.15	99.5 ± 0.31	101.1 ± 0.33	100.3 ± 0.32
Kanamycin sulfate	99.3 ± 0.27	99.3 ± 0.52	100.5 ± 0.61	100.8 ± 0.33
Atropine sulfate	99.4 ± 0.42	98.7 ± 0.55	100.8 ± 0.43	101.3 ± 0.72

[a]Concentration of each drug in mixtures that also contained 10 μg/mL of phenazopyridine HCl. Results are mean percent recovery ± SD of phenazopyridine HCl in drug mixture, quadruplicate determinations of each mixture.

Table 2. Determination of phenazopyridine HCl in tablets

Product components	Phenazopyridine declared/tablet	Recovery,[a] %	
		Proposed method	Official method (1)
Phenazopyridine HCl	100 mg	99.43 (0.51)	100.2 (0.29)
Phenazopyridine HCl	200 mg	99.10 (0.67)	99.7 (0.80)
Phenazopyridine HCl, hyoscyamine HCl, atropine sulfate, scopolamine HBr	100 mg	101.5 (0.39)	100.1 (0.41)
Phenazopyridine HCl, hyoscine HBr, barbital sodium	150 mg	101.7 (0.49)	100.15 (0.50)

[a]Each result is the average of 8 separate determinations. Values in parentheses are coefficients of variation (%).

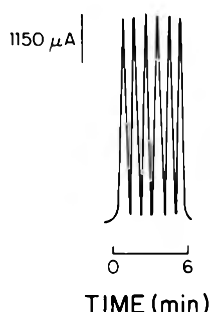

Figure 3. Replicate injections of phenazopyridine HCl (15 μg/mL) into the flowing system at a rate of 60 samples/h.

linear flow rate of eluant across the face of the electrode would provide some degree of mechanical cleaning.

The amperometric method described here has several principal advantages that should make it useful in meeting the need for a fast, reliable procedure for phenazopyridine. High sensitivity, simplicity, good reproducibility, use of inexpensive equipment, and no need for reagents help make the method ideal for routine analysis.

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Titrimetric and Spectrophotometric Determination of Acetylenic Hypnotics, Using Brominating Agents

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Titrimetric and spectrophotometric titration methods are described for the quantitative determination of acetylenic hypnotics ethchlorvynol, ethinamate, and meparfynol carbamate as pure substances and in dosage forms. The methods involve the use of different brominating agents. A known excess of the reagent is added and, after the specified time, the residual reagent is determined iodometrically. These procedures permit semimicro determination (1–20 mg) of the drug. Recoveries ranged from 98.48 ± 0.76 to 102.74 ± 2.60%. The procedures have been successfully applied to pharmaceutical dosage forms; the results agree well with those for compendial methods.

Ethchlorvynol, ethinamate, and meparfynol carbamate are monosubstituted acetylene derivatives which are used as sedative and hypnotic drugs (1). The official methods (2, 3) specify addition of excess silver nitrate reagent and titration of the equivalent liberated acid. Among the methods used for determination of these acetylenes, either as pure drug substance or in biological media, are titrimetry (4, 5), colorimetry (6), spectrophotometry (7–10), nuclear magnetic resonance spectrometry (11), spectrofluorometry (10), and gas chromatography (12–15).

The proposed methods involve the use of 1,3-dibromo-5,5-dimethylhydantoin, *N*-bromosuccinimide, and potassium bromate/potassium bromide solution as titrants. These reagents have been used to determine a considerable number of compounds in our laboratories, especially compounds of pharmaceutical interest (16–25).

The reaction conditions were thoroughly studied and the molar ratio of the reaction was calculated.

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METHOD

Apparatus and Reagents

(a) *Spectrophotometer.*—Pye Unicam SP 1800 equipped with matched pair of 1 cm quartz cells.

(b) *1,3-Dibromo-5,5-dimethylhydantoin.*—Prepare according to method described by Burry et al. (26). Prepare 5 × 10^{-3}M solution by dissolving 1.43 g pure crystalline powder in 1 L water.

(c) *N-Bromosuccinimide.*—1 × 10^{-2}M. Prepare by dissolving 1.78 g freshly crystallized powder in 1 L water.

(d) *Potassium bromate/potassium bromide.*—5 × 10^{-3}M solution. Prepare by dissolving 0.15 g potassium bromate and 0.75 g potassium bromide in 1 L water. Standardize these brominating agents iodometrically by adding potassium iodide solution in the presence of HCl, and titrating the liberated iodine with sodium thiosulfate solution, using starch as indicator.

Table 1. Molar ratios and times of reaction between acetylenic hypnotics and brominating agents

	Dibromohydantoin		N-Bromosuccinimide		Bromate/bromide	
Compound	Time, min	Molar ratio	Time, min	Molar ratio	Time, min	Molar ratio
Ethchlorvynol	30	1:1	30	1:2	5 60	1:1 1:2
Ethinamate	90	1:1	90	1:2	30	1:1
Meparfynol carbamate	90	1:1	90	1:2	30	1:1

(e) *Sodium thiosulfate*.—Prepare 2×10^{-2}M solution by dissolving 4.96 g in 1 L water.

(f) *Potassium iodide*.—4% (w/v) in water.

(g) *Hydrochloric acid*.—10% (w/v) in water.

(h) *Starch mucilage*.

(i) *Drugs*.—Ethchlorvynol (BP grade, Abbott Laboratories Ltd, Queensborough, Kent, UK). Ethinamate (NF grade, CID Laboratories, Giza, Egypt). Meparfynol carbamate (Latéma Laboratories, 11, bis rue Bolzae, Paris 8, France). Prepare each drug in 10% HCl to contain 1 mg/mL. The purity of these drugs was established by applying official methods (2, 3); drug purity was ≥ 98% of the active ingredients present.

Procedure A: Analysis of Pure Samples—Titrimetry

Add an aliquot (1–20 mg) of the acetylenic compound to a known volume of 0.005M dibromohydantoin or 0.01M *N*-bromosuccinimide or 0.005M bromate/bromide solution in a glass-stopper Erlenmeyer flask. Shake mixture occasionally and, after the specified time (Table 1), add 10 mL 4% potassium iodide solution. Titrate the liberated iodine with 0.02M sodium thiosulfate solution (V_2). Carry out a blank experiment in the same manner (V_1). Calculate the amount of drug from the equation:

$$\text{Drug (mg)} = [(V_1 - V_2)\, MR]/N$$

where V_1 = volume of sodium thiosulfate consumed in blank titration (mL); V_2 = volume of sodium thiosulfate consumed in experiment (mL); M = relative molecular mass of drug; R = molarity of brominating agent; and N = number of moles of brominating agent per mole of sample.

Procedure B: Spectrophotometric Titration

Transfer known volumes of solutions containing 1–5 mg acetylenic compound to each of ten 25 mL volumetric flasks. Add brominating solution to each succeeding flask in increasing increments differing by 1 mL, followed by sufficient 10% HCl to bring volume to 25 mL. Measure absorbance at $\lambda_{378\ nm}$ against 10% HCl. Plot absorbance, A, against volume V. Two straight lines are obtained; their intersection corresponds to the equivalence point.

Procedure C: Analysis of Commercial Dosage Forms

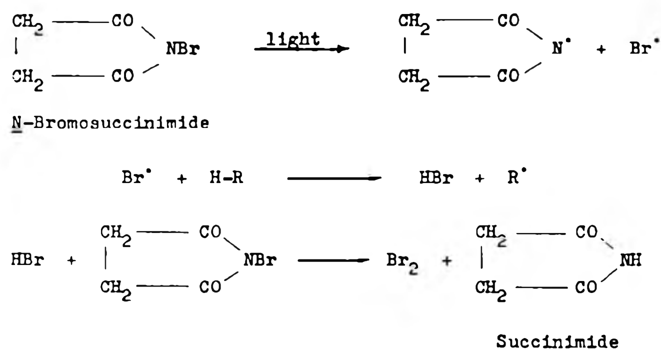
Transfer an accurately weighed amount of powdered tablets, contents of capsules, or measured volume of drops equivalent to 100 mg drug to 100 mL volumetric flask, add 50 mL 10% HCl, and then shake thoroughly. Dilute the mixture with HCl and filter if necessary. Analyze an aliquot of this solution according to procedure A or B.

Results and Discussion

The percentage recoveries of these acetylenic compounds and their dosage forms are summarized in Tables 2 and 3. The results obtained by the proposed methods were compared with those obtained by the official methods (2, 3) and were in good agreement. The results were subjected to significance tests (27) to calculate the Student's *t*-test and variance ratio (Table 2). The tests showed no significant differences between the results of the 2 procedures, and proved the results to be satisfactorily valid. However, the proposed method has the advantage that it can be used to determine semimicro quantities, 1–20 mg, of the acetylenic compounds; not less than 100 mg is required for assay by the official procedures (2, 3).

Direct titration of the studied compounds with the organic brominating agents, in neutral or acid medium, and with methyl red, methyl orange, amaranth, and indigotin indicators, was not practicable. This may be because the indicators were more easily attacked by the brominating agent than were the investigated drugs, or because of a rather decreased reaction velocity. Therefore, we studied the indirect titration reactions in neutral and acidic media with the different brominating agents. This was successful when the medium was made acidic with 10% HCl or H_2SO_4.

N-Bromosuccinimide provides bromine in a slow, steady-state concentration (28). The formation of a low concentration of bromine from *N*-bromosuccinimide can reasonably be accompanied by the ionic reaction with hydrogen bromide as follows:



The liberated bromine from 2 moles of *N*-bromosuccinimide adds at the double bond in olefins (28, 29).

Ethinamate and meparfynol carbamate form 1-cyclo-hexenyl-methyl ketone and (1-methyl-1-propenyl)methyl ketone, respectively, when treated with acids (9, 10). These compounds contain an olefine linkage which adds bromine that originates from *N*-bromosuccinimide (Br) or dibromohydantoin (2Br) or bromate/bromide (2Br), with the molar ratio shown in Table 1.

For ethchlorvynol, the molar ratio is 1:2, which could be explained by the fact that one mole adds to the olefinic bond and the other to the acetylenic bond in the compound 3-ethyl-2-penten-4-ynal, which is the hydrolyzed product of ethchlorvynol in acids (9, 30).

Addition of the brominating agents to the studied compounds shows absorption spectra of 250–290 nm. So, the

Table 2. Comparison of compendial and proposed methods for acetylenic hypnotics

Compound	Amt taken, mg	Dibromohydantoin Found, mg	Dibromohydantoin Rec.,[a] %	N-Bromosuccinimide Found, mg	N-Bromosuccinimide Rec.,[a] %	Bromate/bromide Found, mg	Bromate/bromide Rec.,[a] %	Compendial method (2,3) Rec., %
Indirect Titration								
Ethchlorvynol	1	1.0122	101.22	1.0122	101.22	1.0122	101.22	103.4
	2	2.0244	101.22	2.0022	100.11	2.0244	101.22	102.7
	5	4.9885	99.77	5.061	101.22	5.013	100.26	100.6
	10	9.905	99.05	10.050	100.50	9.905	99.05	98.5
	15	15.762	105.08	14.8935	99.29	14.8215	98.81	99.4
	20	21.038	105.19	19.882	99.41	19.882	99.41	100.9
Mean ± SD[b]			101.92 ± 2.63		100.29 ± 0.85		99.99 ± 1.07	100.92 ± 1.88
Student's t-test			0.32(2.23)[c]		0.36(2.23)		0.62(2.23)	
Variance ratio			1.96(5.05)[d]		4.91(5.05)		3.08(5.05)	
Ethinamate	1	1.0032	100.32	1.0032	100.32	1.0199	101.99	102.4
	2	1.9646	98.23	1.9506	97.53	2.0064	100.32	102.8
	5	5.058	101.16	4.849	96.98	5.016	100.32	102.2
	10	9.823	98.23	10.200	102.00	9.865	98.65	99.1
	15	14.7136	98.09	15.4005	102.67	15.3825	102.55	99.95
	20	19.688	98.44	19.60	98.00	20.378	101.89	102
Mean ± SD			99.08 ± 1.32		99.58 ± 2.43		100.95 ± 1.46	101.41 ± 1.51
Student's t-test			1.99(2.23)		0.71(2.23)		0.36(2.23)	
Variance ratio			1.31(5.05)		2.59(5.05)		1.07(5.05)	
Meparfynol carbamate	1	1.059	105.9	0.9884	98.84	0.9884	98.84	98.2
	2	2.0474	102.37	1.9768	98.84	1.9768	98.84	98.9
	5	4.9065	98.13	4.9285	98.57	4.942	98.84	98.6
	10	9.9546	99.546	9.814	98.14	9.813	98.13	100.3
	15	15.1125	100.75	15.375	102.5	14.8725	99.15	101.7
	20	20.016	100.08	19.734	98.67	19.416	97.08	100.7
Mean ± SD			101.13 ± 2.72		99.26 ± 1.61		98.48 ± 0.76	99.73 ± 1.38[e]
Student's t-test			0.45(2.23)		0.36(2.23)		1.55(2.23)	
Variance ratio			3.92(5.05)		1.37(5.05)		3.24(5.05)	
Spectrophotometric Titration								
Ethchlorvynol	1	1.0303	103.03	1.0305	103.05	1.0324	103.24	103.4
	2	2.0262	101.31	2.0262	101.31	2.0282	101.40	102.7
	3	2.9877	99.59	3.042	101.40	2.9499	98.33	100.6
	4	4.122	103.05	4.1104	102.76	4.1668	104.17	98.5
	5	5.9375	104.75	4.9835	99.67	5.162	103.24	99.4
								100.9
Mean ± SD			102.35 ± 1.96		101.64 ± 1.35		102.08 ± 2.32	100.92 ± 1.88
Student's t-test			0.64(2.26)[c]		0.41(2.26)		0.63(2.26)	
Variance ratio			1.095(6.26)[d]		1.94(6.26)		1.53(6.26)	
Ethinamate	1	1.0157	101.57	1.0132	101.32	1.045	104.5	102.4
	2	2.0314	101.57	2.0264	101.32	2.095	104.5	102.8
	3	3.0498	101.66	3.0699	102.33	3.018	100.60	102.2
	4	4.0756	101.89	4.1772	103.43	3.9712	99.28	99.1
	5	5.2165	104.33	5.066	101.32	5.2415	104.83	99.95
								102
Mean ± SD			102.20 ± 1.20		101.94 ± 0.94		102.74 ± 2.60	101.41 ± 1.51
Student's t-test			0.67(2.26)		0.49(2.26)		0.46(2.26)	
Variance ratio			1.59(6.26)		2.58(6.26)		2.99(6.26)	
Meparfynol carbamate	1	1.0336	103.36	0.9884	98.84	0.9884	98.84	98.2
	2	3.0242	101.21	2.0474	102.37	2.0474	102.37	98.9
	3	3.0147	100.49	3.0663	102.21	3.0852	102.84	98.6
	4	3.89	97.25	4.0644	101.61	4.0576	101.44	100.3
	5	5.134	102.68	5.134	102.68	5.0305	100.61	101.7
								100.7
Mean ± SD			100.99 ± 2.39		101.54 ± 1.56		101.22 ± 1.58	99.73 ± 1.38[e]
Student's t-test			0.51(2.26)		1.39(2.26)		1.13(2.26)	
Variance ratio			3.01(6.26)		1.29(6.26)		1.33(6.26)	

[a]Mean recovery for 3 determinations.
[b]Standard deviation.
[c]Tabulated t (at $P = 0.05$) related to the official methods.
[d]Tabulated variance ratio (at $P = 0.05$).
[e]As in *British Pharmacopoeia* (2) for ethchlorvynol.

Table 3. Determination of acetylenic hypnotics in various dosage forms[a]

Preparation	No. of detns	Dibromohydantoin Amt found, mg	Dibromohydantoin Rec., %	N-Bromosuccinimide Amt found, mg	N-Bromosuccinimide Rec., %	Bromate/bromide Amt found, mg	Bromate/bromide Rec., %	Compendial method (2, 3) No. of detns	Compendial Amt found, mg	Compendial Rec., %
Indirect Titration Method										
Ethchlorvynol capsules, 500 mg	9	507.9	101.58 ± 2.20	516.55	103.31 ± 1.91	510.95	102.19 ± 2.93	8	510.90	102.18 ± 2.00
Ethinamate tablets, 500 mg	9	520.4	104.08 ± 1.51	521.1	104.22 ± 0.97	518.3	103.66 ± 0.84	8	504.65	100.93 ± 1.39
Meparfynol carbamate drops, 20%	9	20874	104.37 ± 1.42	20756	103.78 ± 1.87	20568	102.84 ± 1.41	8	20036	100.18 ± 1.25
Meparfynol carbamate tablets, 300 mg	9	313.2	104.4 ± 0.84	308.19	102.73 ± 1.96	309.24	103.08 ± 0.71	8	307.53	102.51 ± 0.70
Spectrophotometric Titration Method										
Ethchlorvynol capsules, 500 mg	8	515.99	103.198 ± 2.48	508.6	101.72 ± 2.16	510.95	102.19 ± 0.23	8	510.90	102.18 ± 2.00
Ethinamate tablets, 500 mg	8	501.35	100.27 ± 3.49	512.1	102.42 ± 1.58	519.35	103.87 ± 1.36	8	504.65	100.93 ± 1.39
Meparfynol carbamate drops, 20%	8	20284	101.42 ± 2.84	20299	101.495 ± 1.97	20338	101.69 ± 3.34	8	20036	100.18 ± 1.25
Meparfynol carbamate tablets, 300 mg	8	302.76	100.92 ± 2.57	302.31	100.77 ± 1.48	302.31	100.77 ± 1.70	8	307.53	102.51 ± 0.70

[a]Mean recovery ± standard deviation.

excess brominating agent is measured at $\lambda_{378\,nm}$, which is that of bromine, without interference.

The results of quantitative assays (Tables 2 and 3) clearly demonstrate the utility of indirect and spectrophotometric titration methods for analysis of the drug compounds and commercial dosage forms. The validity of the method for pharmaceutical preparations as well as the effect of interference was demonstrated by assaying authentic samples containing the drug and common additives and excipients, e.g., lactose, magnesium stearate, and starch. The percent recovery was satisfactory.

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Synthesis, Identification, and Acute Toxicity of α-Benzylphenethylamine and α-Benzyl-*N*-Methylphenethylamine. Contaminants in Clandestine Preparation of Amphetamine and Methamphetamine

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Amphetamine samples obtained from clandestine laboratories often contain other by-product amines. The most common of these are the α-benzylphenethylamine derivatives. This article reports the independent synthesis of reference samples of these amines, spectrophotometric and chromatographic methods for their identification, as well as acute toxicological studies. The utility of the analytical methods is demonstrated by the analysis of several amphetamine samples containing these α-benzylphenethylamines.

Amphetamine and methamphetamine continue to be 2 of the more frequently encountered drugs produced in clandestine laboratories. Drugs produced in clandestine laboratories often contain impurities resulting from incomplete reactions and inadequate purification of intermediates and final synthetic products.

Because of U.S. Federal control of the synthetic precursor for amphetamine and methamphetamine, phenylacetone (methyl benzyl ketone, phenyl-2-propanone, or P-2-P), one of the most attractive methods of obtaining phenylacetone is preparation from phenylacetic acid. The base-catalyzed condensation of phenylacetic acid and acetic anhydride results in the production of phenylacetone and a second ketone, dibenzylketone (1,3-diphenyl-2-propanone). Thus, the reductive amination of these ketones by using the Leuckart procedure not only produces amphetamine and methamphetamine but also α-benzylphenethylamine and α-benzyl-*N*-methylphenethylamine, respectively (1).

Knowledge of impurities in clandestine drug synthesis is important for several reasons. One important reason is the potential for additional harmful effects produced by the impurity. Information concerning synthetic methods used to produce the drug, including chemicals and equipment, can also be obtained. In addition, the presence or absence of impurities can aid in identifying drug samples of common origin and in distinguishing samples of legitimate and illicit manufacture. Impurities contained in clandestinely manufactured methamphetamine are well documented (2). Ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and combined gas chromatography/mass spectrometry (GC-MS) have been used to identify the impurities (1, 2).

Liquid chromatography (LC) has proven to be an effective method for detection and separation of pharmaceutical preparations and biological samples. LC is a nondestructive technique which allows the analyst to recover the sample following the analysis and perform other confirmatory tests such as thin layer chromatography (TLC), gas chromatography (GC), IR and UV spectrophotometry, and mass spectrometry (MS). Many pharmaceutically important amines such as methamphetamine and amphetamine have low molar absorptivities in the UV region. In addition to the problem of UV detectability, amphetamine and methamphetamine exhibit poor chromatographic properties in many cases. Derivatization procedures (3–5) are often used to enhance the UV absorptivity and to improve the chromatographic properties of these compounds. The structural elucidation of low molecular weight primary and secondary amines as phenylisothiocyanate (PIT) derivatives by using IR, MS, and NMR has been reported (6). Phenylisothiocyanate derivatives have also been used in LC to enhance detectability and chromatography (7). Phenylisothiocyanate derivatization procedures are characterized by their relative ease and result in stable, nonhygroscopic derivatives.

The purpose of this paper is to report the results of using phenylisothiocyanate derivatives for the determination of the primary and secondary amines amphetamine, methamphetamine, α-benzylphenethylamine, and α-benzyl-*N*-methylphenethylamine in clandestinely produced samples. Also reported are the results of acute toxicity studies in mice for the above amines.

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Experimental

Reagents and Chemicals

Samples of *d,l*-amphetamine sulfate and *d,l*-methamphetamine hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO. LC grade methanol, hexane, acetonitrile, and phenylisothiocyanate (reagent grade) were obtained from Fisher Scientific Co., Fair Lawn, NJ; 1,3-diphenyl-2-propanone (practical) was obtained from Eastman Kodak Co., Rochester, NY. The water used in the chromatographic mobile phases was double distilled. All other reagents and chemicals were obtained from their representative manufacturers and used without further purification. Illicit samples containing amphetamine, α-benzylphenethylamine, methamphetamine, and α-benzyl-*N*-methylphenethylamine were submitted to the Alabama Department of Forensic Sciences by various Alabama law enforcement agencies.

Instrumentation

The liquid chromatograph consisted of a Waters Associates (Milford, MA) Model 6000A pump, Model U6K injector, Model 440 UV detector with dual wavelength accessory operated at 254 and 280 nm, and Houston Instrument (Austin, TX) OmniScribe dual pen recorder. IR spectra were recorded on a Perkin Elmer (Norwalk, CT) Model 1500 Fourier transform infrared spectrophotometer (FTIR). NMR spectra (^1H) were determined in $CDCl_3$ solution using a Varian T-60A spectrometer (Varian Instruments, Palo Alto, CA) with an internal standard of tetramethylsilane. Elemental analyses (C, H, and N) were performed by Atlantic Microlab Inc., Atlanta, GA.

Chromatographic Procedures

All separations were carried out on a 30 cm × 3.9 mm id μBondapak C_{18} column (Waters Associates) with a water–acetonitrile–acetic acid (49 + 50 + 1) or water–methanol–acetic acid (39 + 60 + 1) mobile phase. The analytical column was preceded by a 7 cm × 2.1 mm id guard column dry-packed with CO:Pell ODS (Whatman Inc., Clifton, NJ). The mobile phase flow rate was 1.5 mL/min and the UV detector was operated at 2.0 AUFS. Sample solutions were prepared

in LC grade acetonitrile and all separations were made at ambient temperature. Absorbance ratios were calculated from the average peak height measurements of a minimum of 3 injections for each compound.

Synthesis of α-Benzylphenethylamine (1,3-Diphenyl-2-Propylamine)

This amine was prepared using a modification of the method of Borch et al. (8). A 10.0 g (0.05 mole) portion of 1,3-diphenylacetone was dissolved in about 200 mL methanol and the resulting solution was added to 500 mL 3-necked flask fitted with a reflux condenser and a magnetic stirrer. To the stirring solution was added 30 g ammonium acetate and 3.0 g (0.05 mole) of sodium cyanoborohydride. The resulting mixture was stirred at ambient temperature for 48 h. The solution was then made strongly basic with saturated sodium hydroxide and subjected to gentle reflux for 2 h. The volatiles were removed under vacuum evaporation and the resulting residue was suspended in 100 mL water and extracted with chloroform (3 × 100 mL). The extracts were combined, dried over magnesium sulfate, and evaporated to yield a yellow oil. The oil was distilled under vacuum (1.3 mm Hg) to yield a colorless liquid, bp 136–138°C. Infrared and nuclear magnetic resonance spectra (^1H) were consistent with structure. Elemental analysis (C, H, N): theory, %C = 85.26, %H = 8.11, %N = 6.63; found, %C = 85.16, %H = 8.13, %N = 6.57.

The hydrochloride salt was prepared by dissolving about 3 g of the free base in 25 mL dry diethyl ether followed by the dropwise addition of a solution of HCl in ether. The resulting precipitate was collected by filtration and recrystallized from a 10:1 mixture of isopropyl ether and ethanol to yield white crystals, mp = 199–201°C. Elemental analysis (C, H, N): theory, %C = 72.71, %H = 7.32, %N = 5.65; found, %C = 72.74, %H = 7.34, %N = 5.63.

Synthesis of α-Benzyl-N-Methylphenethylamine (N-Methyl-1,3-Diphenyl-2-Propylamine)

A 5 g sample of 1,3-diphenylacetone was dissolved in 50 mL methylamine (40% aqueous solution) contained in a 250 mL 3-neck flask fitted with a reflux condenser and magnetic stirrer. The solution was warmed to ca 80°C for 1 h followed by the portion-wise addition of 2 g sodium borohydride over a period of 20 min. The resulting mixture was stirred for an additional hour. Then 100 mL of 3N sodium hydroxide was added and the mixture refluxed gently for 2 h. The solution was cooled and extracted with chloroform (3 × 50 mL), and the combined extracts were dried over magnesium sulfate. The solvent was evaporated to yield a reddish oil which was distilled under vacuum (1.1 mm Hg) to yield a colorless liquid, bp 129–131°C. Infrared and nuclear magnetic resonance (^1H) spectra were consistent with structure. Elemental analysis (C, H, N): theory, %C = 85.28, %H = 8.50, %N = 6.22; found, %C = 85.12, %H = 8.54, %N = 6.17.

The hydrochloride salt was prepared as previously described and recrystallized from isopropyl ether to yield white crystals, mp = 192–194°C. Elemental analysis (C, H, N): theory, %C = 73.40, %H = 7.70, %N = 5.35; found, %C = 73.47, %H = 7.71, %N = 5.31.

Formation of Phenylisothiocyanate Derivatives

The phenylisothiocyanate (PIT) derivatives were prepared by extraction of ca 10 mg amine or amine combination from dilute sodium hydroxide into hexane or chloroform (2 × 20 mL). Phenylisothiocyanate (10 μL) was added to the organic layer and the organic layer was evaporated to dryness under a stream of air. For LC studies, the residues were dissolved in acetonitrile. For IR studies, the PIT derivatives were recrystallized from hexane.

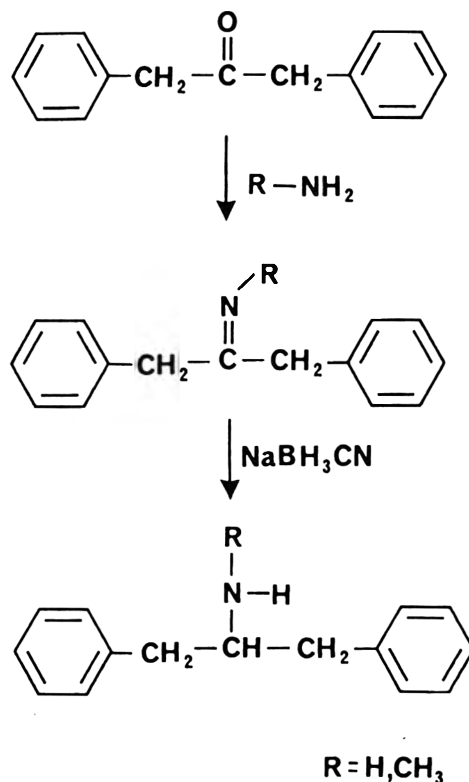
Acute Toxicity Studies

The relative toxicity of the amphetamines and the corresponding α-benzylphenethylamines were compared by determining the lethal dose 50 (LD_{50}) and the convulsive dose 50 (CD_{50}) for each of the 4 compounds. These studies were conducted using male ICR Swiss mice obtained from Southern Animal Farms, Prattville, AL.

Food and water were available to the animals ad libitum. The compounds were administered intraperitoneally (IP) as the HCl salts (except amphetamine sulfate) in normal saline solution. Three dosing levels were used with 6 animals per dose. Mortality was determined after 24 h for the LD_{50} measurement and convulsions were defined as a mixture of tonic-clonic type seizures of 3 to 5 s duration in the CD_{50} study. A computer program of the Litchfield and Wilcoxon method (9) was used to calculate the LD_{50} and CD_{50} values with upper and lower limits at the 0.05 level of significance for each compound.

Results and Discussion

Samples of the desired α-benzyl analogs of amphetamine and methamphetamine were prepared from 1,3-diphenyl-2-propanone (1,3-diphenylacetone) by a reductive amination procedure. The general outline for the synthetic method is shown in Scheme I.



Scheme I

The *N*-methyl analog was prepared by condensation of the ketone with methylamine to form the Schiff base followed by $NaBH_4$ reduction to yield the corresponding amine. In the case of the primary amine, ammonium acetate was used as the nitrogen source and $NABH_3CN$ was

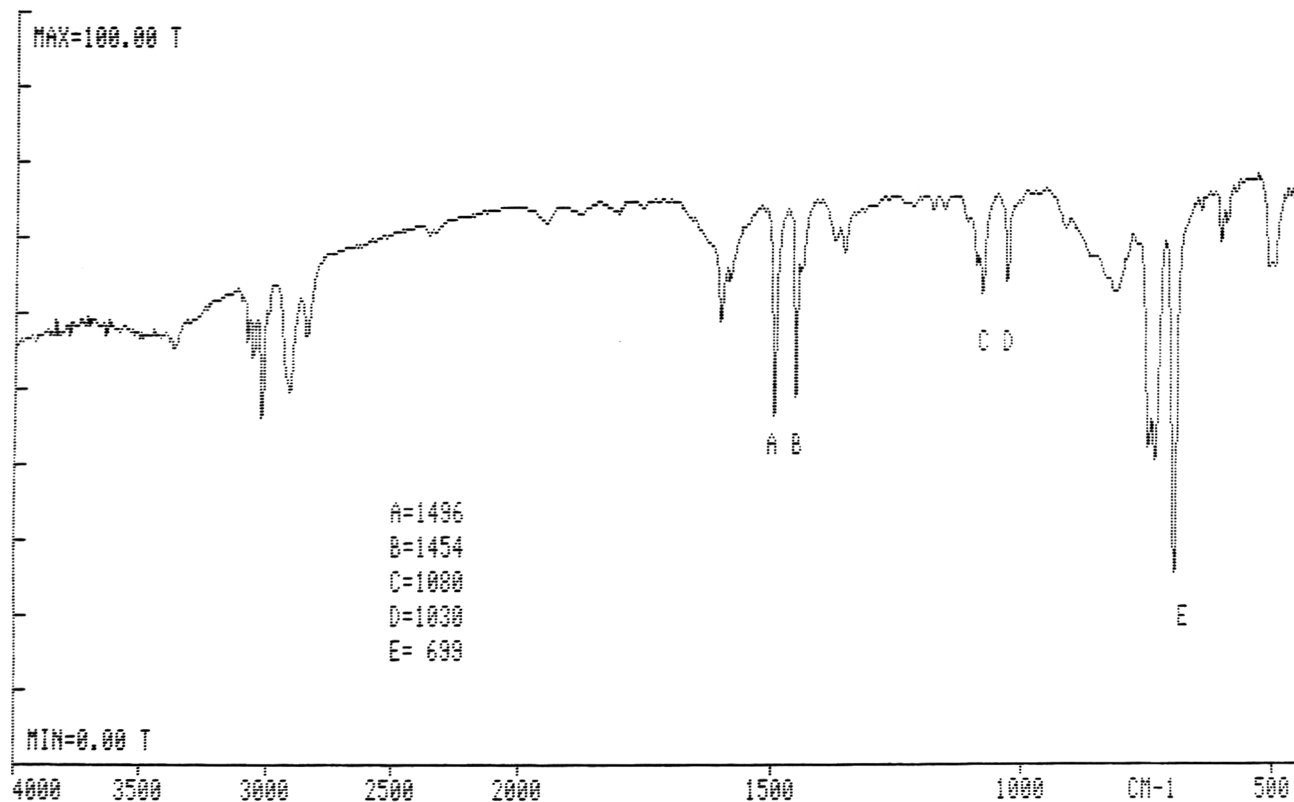


Figure 1. IR spectrum of α-benzylphenethylamine (base).

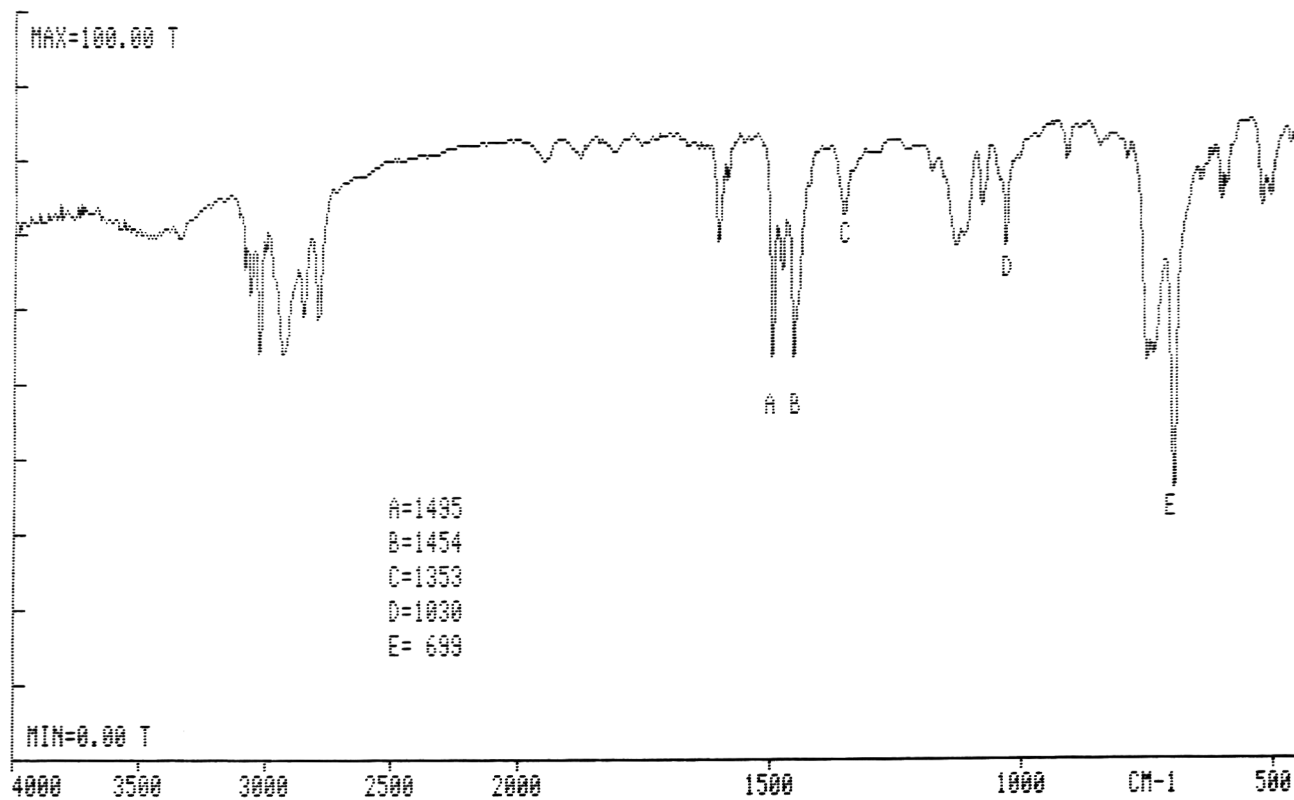


Figure 2. IR spectrum of α-benzyl-*N*-methylphenethylamine (base).

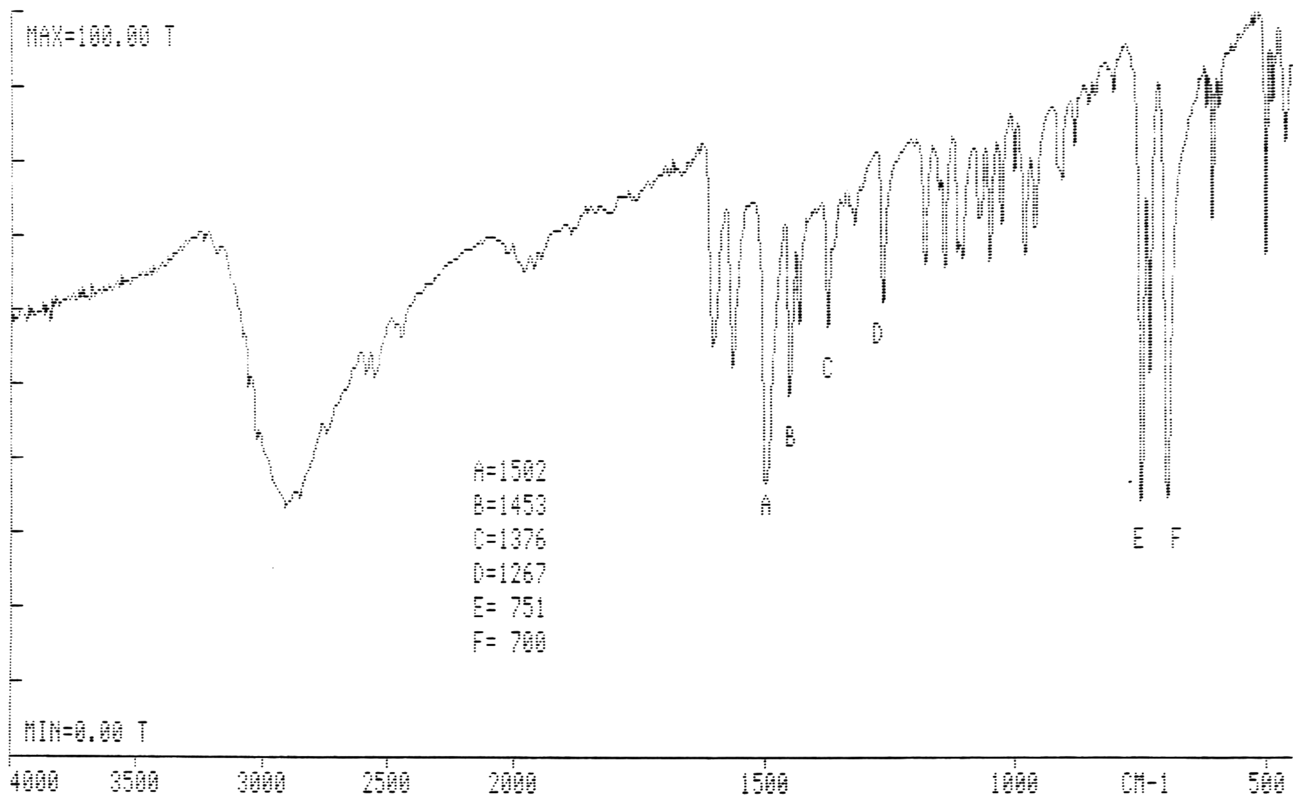


Figure 3. IR spectrum of α-benzyl-*N*-methylphenethylamine hydrochloride.

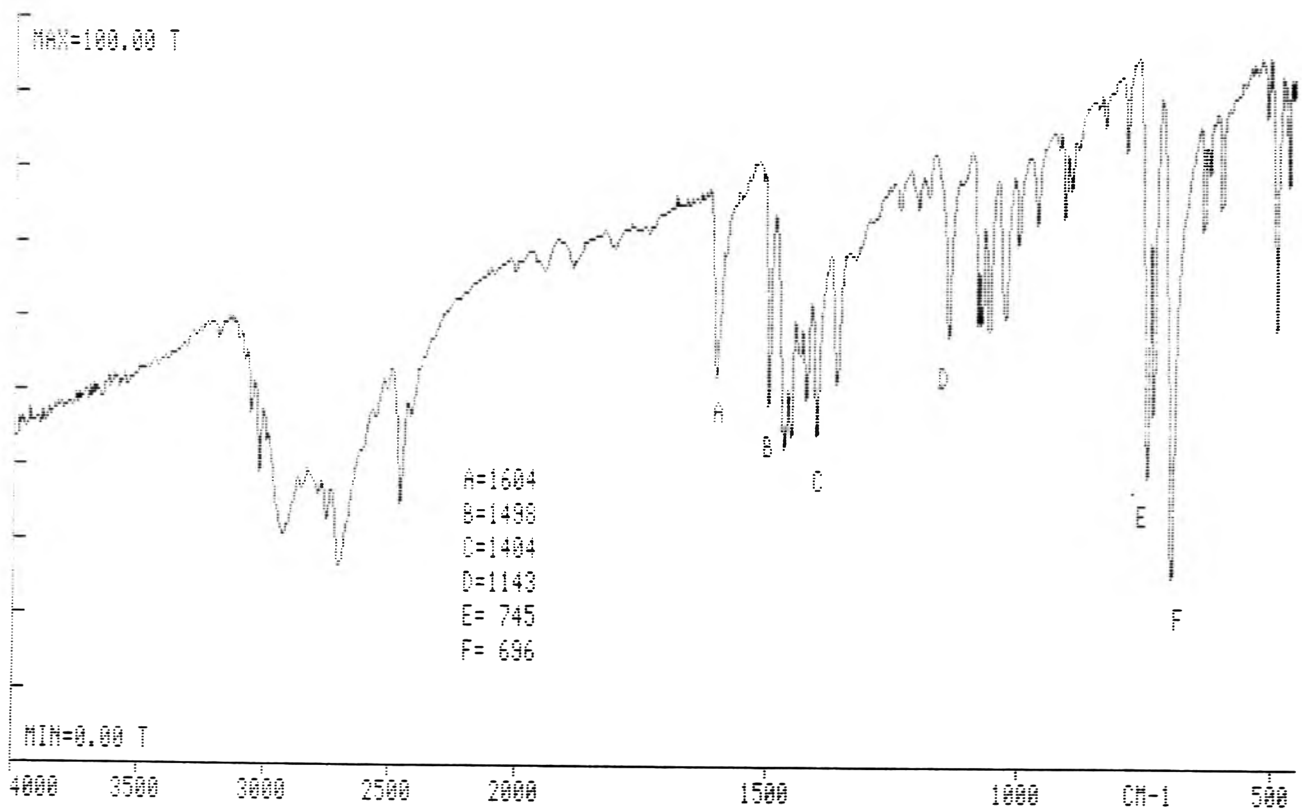


Figure 4. IR spectrum of α-benzylphenethylamine hydrochloride.

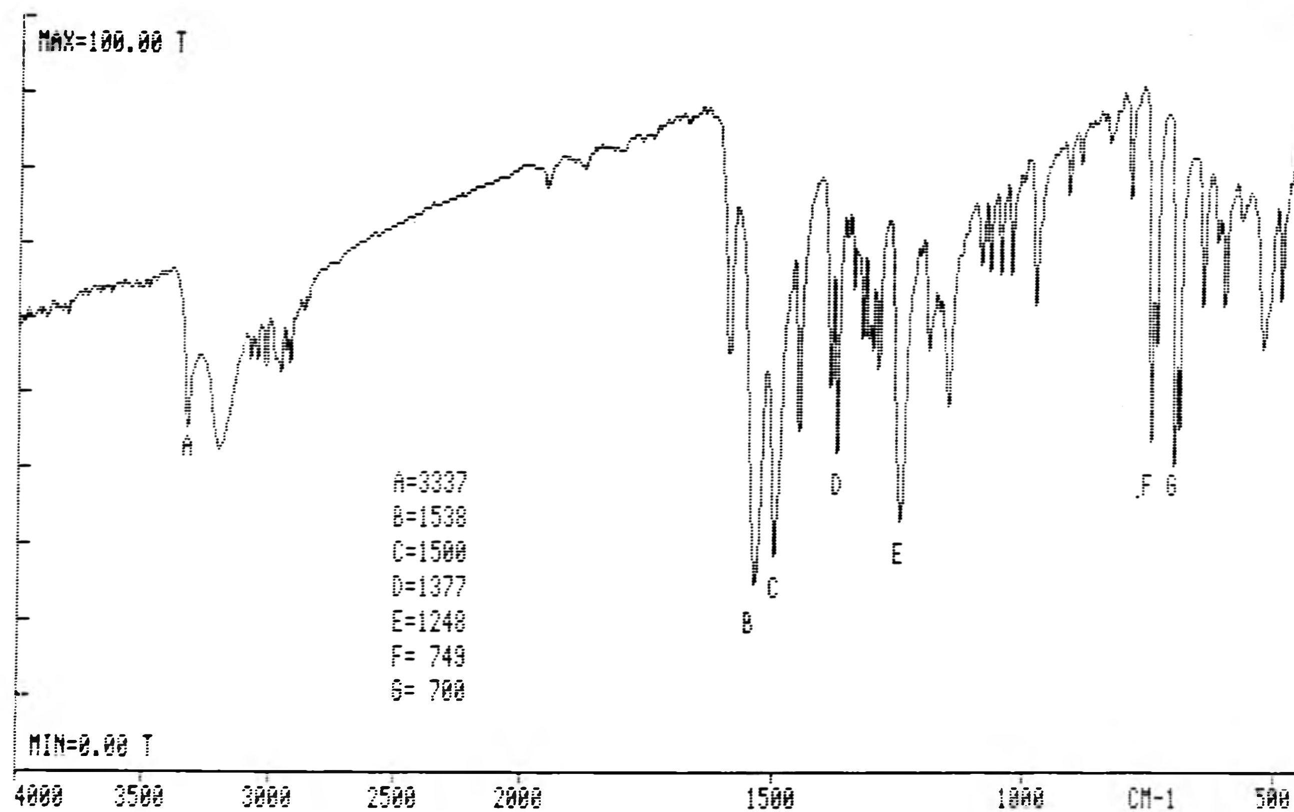


Figure 5. IR spectrum of *d,l*-amphetamine phenylisothiocyanate.

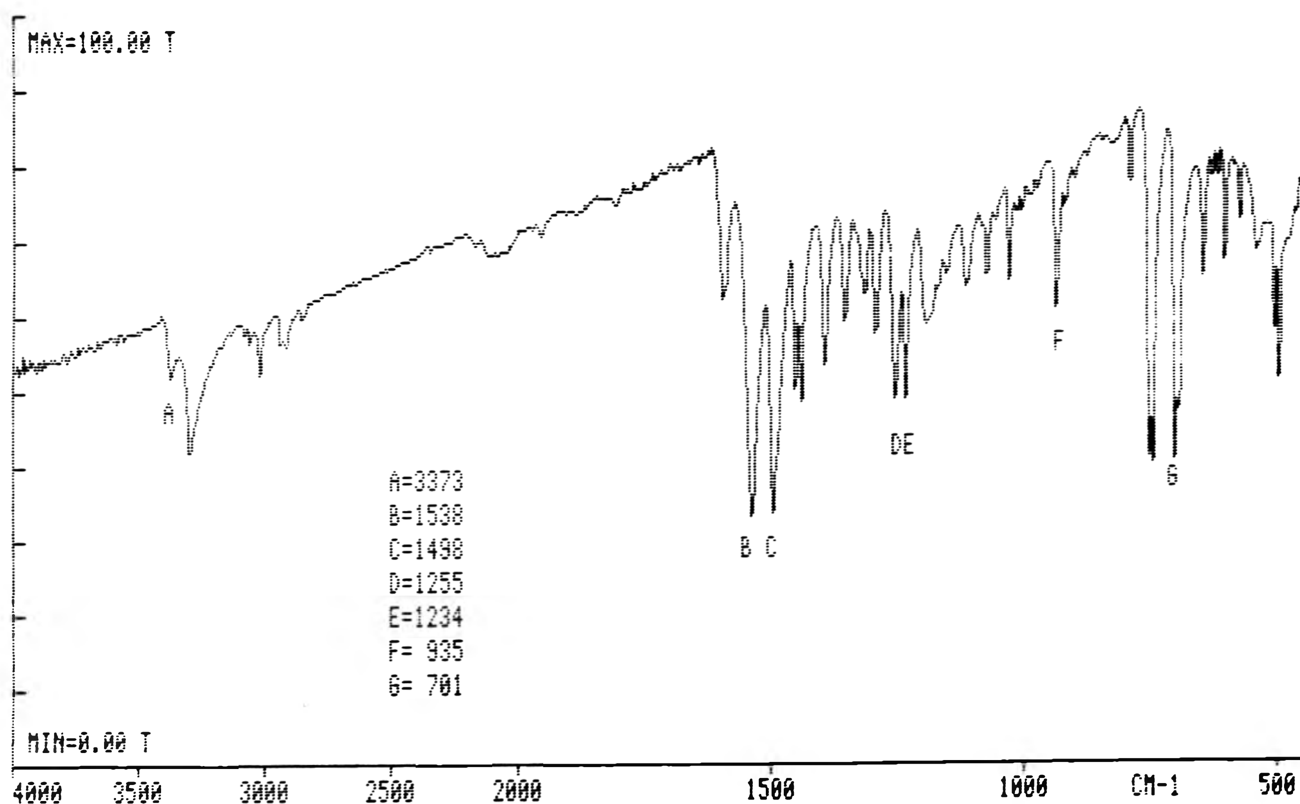


Figure 6. IR spectrum of α-benzylphenethylamine phenylisothiocyanate.

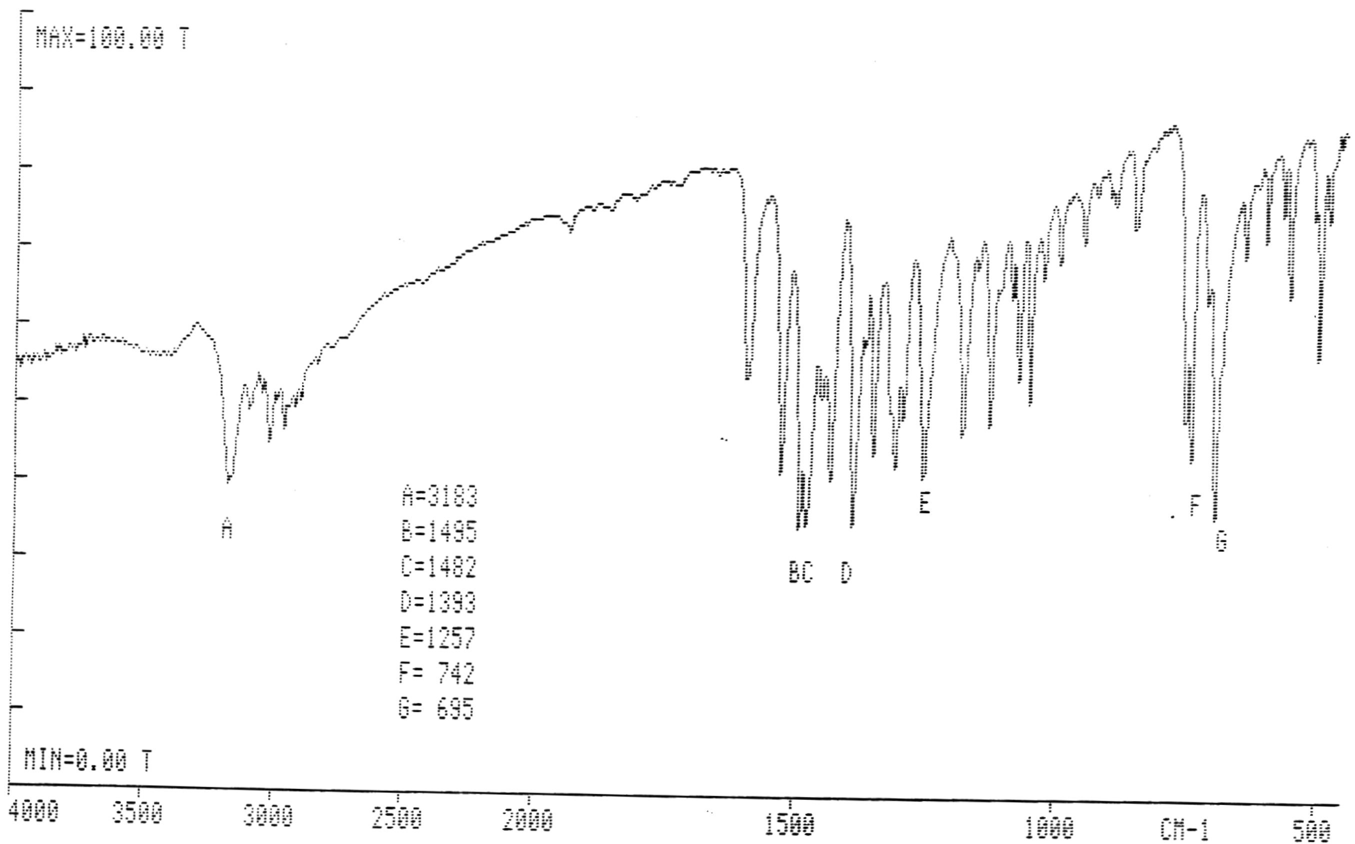


Figure 7. IR spectrum of *d,l*-methamphetamine phenylisothiocyanate.

MAX=100.00 T

A

A=3225
B=1483
C=1397
D=1257
E=1068
F= 744
G= 700

B C D E F G

MIN=0.00 T

4000 3500 3000 2500 2000 1500 1000 CM-1 500

Figure 8. IR spectrum of α-benzyl-*N*-methylphenethylamine phenylisothiocyanate.

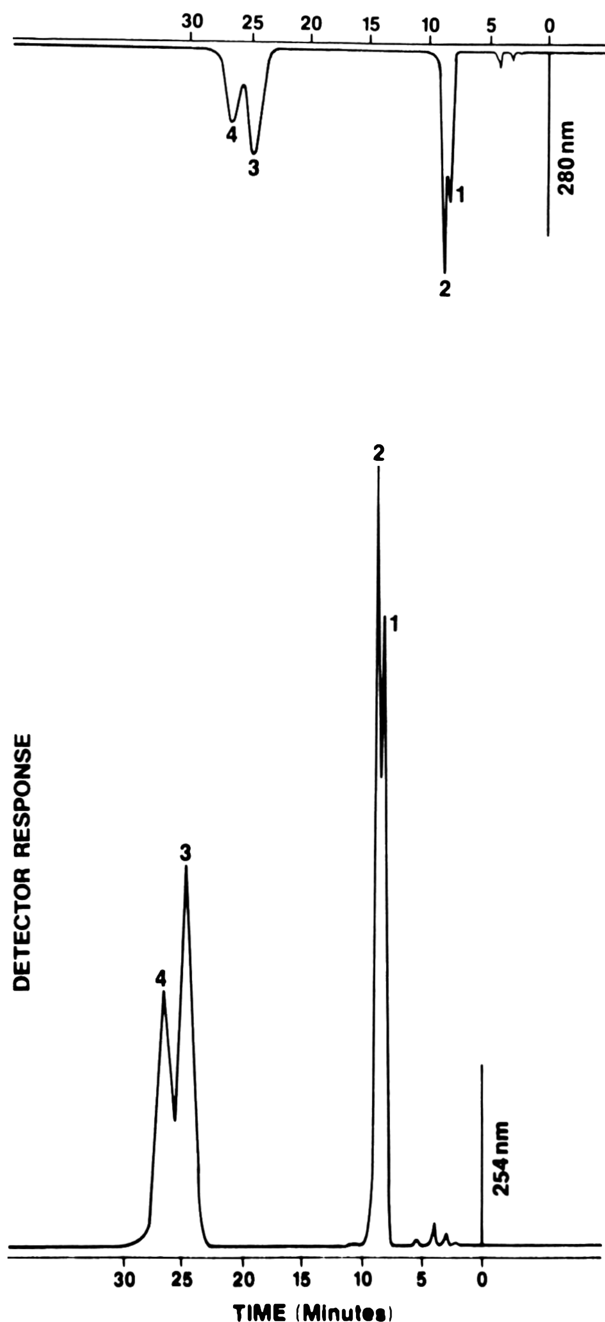


Figure 9. LC separation of PIT derivatized amines using solvent system 1. Peaks: 1, *d,l*-methamphetamine; 2, *d,l*-amphetamine; 3, α-benzyl-*N*-methylphenethylamine; 4, α-benzylphenethylamine.

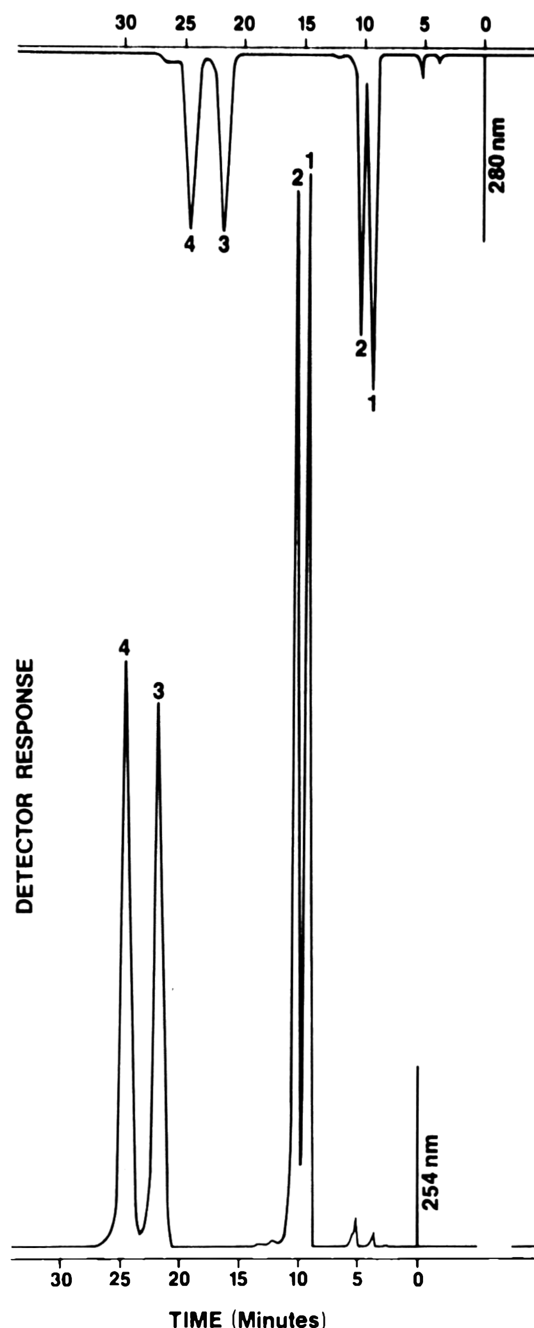


Figure 10. LC separation of PIT derivatized amines using solvent system 2. Peaks: 1, *d,l*-amphetamine; 2, *d,l*-methamphetamine; 3, α-benzylphenethylamine; 4, α-benzyl-*N*-methylphenethylamine.

used as the reducing agent according to the method of Borch et al. (8). The advantage of this procedure lies in the selectivity of the reducing agent. $NaBH_3CN$ is a selective reagent for reducing imines (Schiff-bases) and under the reaction conditions does not reduce the ketone. The (BH_3CN) reduction of aldehydes, ketones, and imines is pH dependent and proper control of the pH allows for selectivity in these competing reactions. Negligible reduction of aldehydes and ketones occurs under neutral conditions in water or methanol (8). Thus, the desired amine is obtained even though the immonium intermediate is the unfavored species in the ketone-imine equilibrium. The secondary alcohol reduction product of 1,3-diphenylacetone is not obtained from $NaBH_3CN$ at pH 7. The $NaBH_3CN$ procedure can also be used to prepare the *N*-methyl analog. The product amines were purified by vacuum distillation and the hydrochloride salts were prepared in the usual manner. Elemental analysis (C, H, N) data consistent with structure were obtained for both the free bases and the HCl salts. The NMR (^1H) spectra were consistent with the assigned structure.

After α-benzylphenethylamine and α-benzyl-*N*-methylphenethylamine were synthesized, the infrared spectra of the 2 compounds were determined by FTIR. Since the free bases of the 2 amines were oils, the infrared spectra were prepared by placing the oils as a thin film on a blank KBr disk. The infrared spectrum of α-benzylphenethylamine base is shown in Figure 1 and the spectrum of α-benzyl-*N*-methyl-phenethylamine base is shown in Figure 2. The infrared spectra of the hydrochloride salts of the 2 amine compounds were also prepared. Figure 3 illustrates the spectrum of α-benzyl-

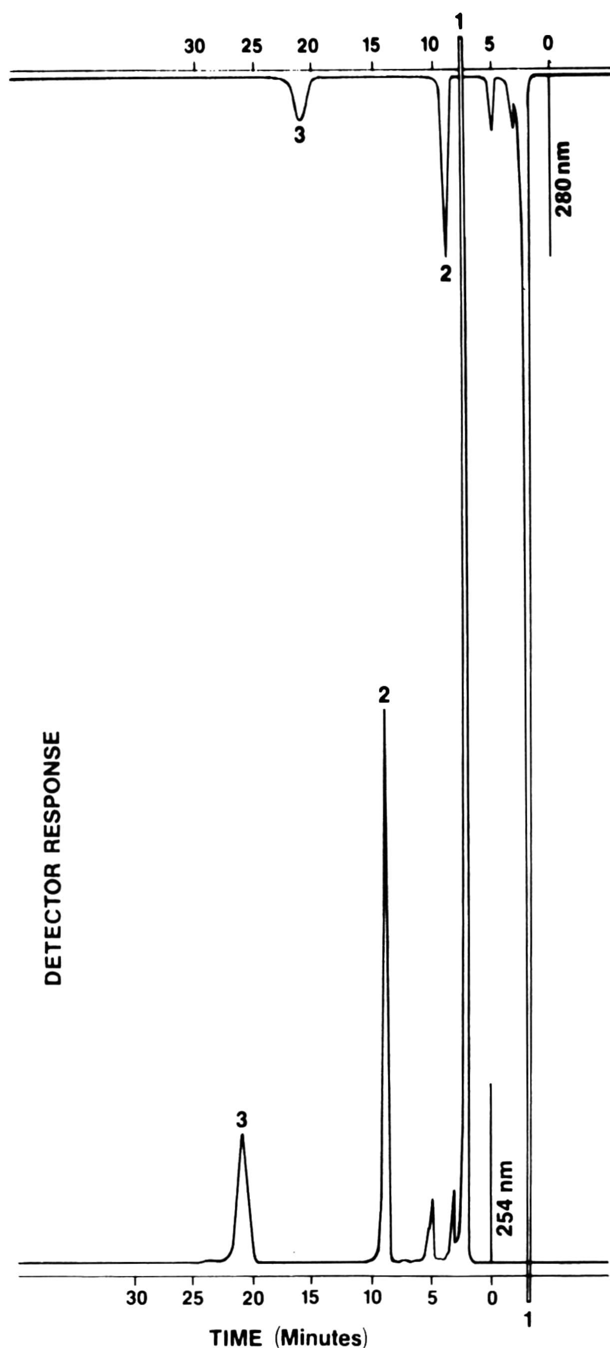


Figure 11. LC separation of PIT derivatized components of a "minibennie" tablet identified as containing 1, caffeine, 2, *d,l*-amphetamine, and 3, α-benzylphenethylamine. Solvent system 2.

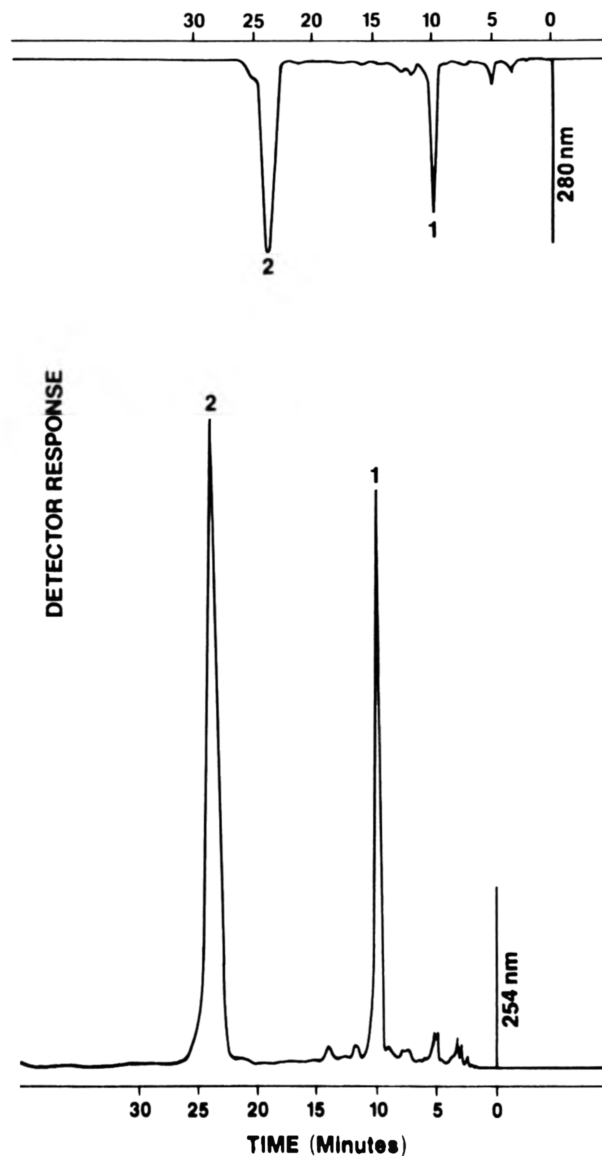


Figure 12. LC separation of PIT derivatized powder from a clandestine laboratory. Peaks: 1, *d,l*-methamphetamine; 2, α-benzyl-*N*-methylphenethylamine. Solvent system 2.

N-methylphenethylamine hydrochloride and Figure 4 shows the infrared spectrum of α-benzylphenethylamine hydrochloride. These spectra are readily distinguishable from the corresponding amphetamines.

The IR studies for the PIT derivatives of the amines were conducted by preparing KBr disks from the recrystallized compounds. Figure 5 shows the spectrum of *d,l*-amphetamine PIT and Figure 6 the spectrum of α-benzylphenethylamine PIT. The spectra are considerably different in the region from 1500 to 400 cm^{-1} as expected, while the PIT absorption bands dominate both spectra in the 1700–1500 cm^{-1} region. Figure 7 is the spectrum of *d,l*-methamphetamine PIT and Figure 8 the spectrum of α-benzyl-*N*-methylphenethylamine PIT. The marked differences between Figures 7 and 8 are obvious. Furthermore, the absence of the N–H absorption in the 3300 to 3400 cm^{-1} range for both Figures 7 and 8 show the ease of IR differentiation between PIT derivatives of primary and secondary amines.

Reference samples of the amine-PIT derivatives were prepared and used to develop a liquid chromatographic procedure for screening amphetamine and/or methamphetamine samples suspected of containing their respective α-benzyl analogs. Adequate separation of the 4 amine-PIT derivatives was achieved using a reverse phase chromatographic system consisting of a C_{18} stationary phase and either of 2 solvent systems. Solvent system 1 was a mixture of methanol–water–acetic acid (60 + 39 + 1) and solvent system 2 was a mixture of acetonitrile–water–acetic acid (50 + 49 + 1). Figure 9 shows the separation of the 4 amine-PIT derivatives in solvent system 1 and Figure 10 illustrates the separation of the same compounds in solvent system 2. A comparison of Figures 9 and 10 clearly shows enhanced resolution in the acetonitrile mobile phase (solvent system 2) with essentially equivalent analysis time. A second interesting point of comparison between the chromatograms in Figures 9 and 10 is the elution order reversal between the 2 amphetamines and the 2 α-benzyl compounds. For each set of compounds the primary

Table 1. Chromatographic data for PIT derivatives of amphetamines and α-benzylphenethylamines

Solvent system[a]	Drug	Elution time, min	Absorbance ratio (A_{254}/A_{280})
1	Amphetamine	9.0	3.498
1	Methamphetamine	8.2	4.156
1	α-Benzylphenethylamine	26.4	3.315
1	α-Benzyl-*N*-methylphenethylamine	24.8	3.583
2	Amphetamine	9.0	3.111
2	Methamphetamine	10.0	3.776
2	α-Benzylphenethylamine	21.4	2.979
2	α-Benzyl-*N*-methylphenethylamine	24.2	3.330

[a]Solvent system 1, methanol–water–acetic acid (60 + 39 + 1); solvent system 2, acetonitrile–water–acetic acid (50 + 49 + 1).

Table 2. Acute toxicological evaluation of amphetamine, methamphetamine, α-benzylphenethylamine, and α-benzyl-*N*-methylphenethylamine in male mice

$C_6H_5-CH_2-CH(CH_2R)-NH-R'$

Compound[a]	LD_{50}[b]	CD_{50}[c]
R = R' = H *d,l*-Amphetamine SO_4	91.14 mg/kg (86.55–95.97)[d]	88.35 mg/kg (83.56–94.41)[d]
R = H, R' = CH_3 *d,l*-Methamphetamine HCl	57.19 mg/kg (52.51–62.28)	56.96 mg/kg (51.29–61.73)
R = C_6H_5, R' = H α-Benzylphenethylamine HCl	63.25 mg/kg (60.95–65.62)	45.59 mg/kg (42.23–49.23)
R = C_6H_5, R' = CH_3 α-Benzyl-*N*-methylphenethylamine HCl	78.20 mg/kg (73.69–82.98)	54.09 mg/kg (50.86–57.53)

[a]The drugs were given IP in saline solution at 3 dose levels with 6 animals per dose.
[b]Lethal dose 50.
[c]Convulsive dose 50.
[d]Values in parentheses are the lower and upper limits of significance at the 0.05 level.

amine PIT derivatives elute first in Figure 10 while the *N*-methyl analogs elute first in Figure 9. However, the amphetamines show much lower capacity factors than the α-benzyl compounds in both reverse phase separations.

In addition to comparison of elution times, further proof of the identity of the individual amines may be obtained from a comparison of the ratio of absorbances at 254 and 280 nm (A_{254}/A_{280}). Baker et al. (10) have used this ratio to determine the identity of drugs having similar elution characteristics in an LC system. The elution times and absorbance ratios for the 4 amine-PIT derivatives are given in Table 1. The absorbance ratios for the compounds are similar because the PIT-moiety common to all the derivatives is a strong chromophore. In all cases the secondary amine-PIT derivatives have higer A_{254}/A_{280} ratios than do the corresponding primary amine-PIT.

Figure 11 demonstrates the separation and detection of the PIT derivatized contents of a "minibennie" tablet identified as containing caffeine, amphetamine, and α-benzylphenethylamine. Caffeine does not form the PIT derivative and thus is unchanged by the derivatization reaction. Figure 12 illustrates the separation and detection of a PIT derivatized sample of gummy brown powder removed from a baking dish discovered at the scene of a suspected clandestine methamphetamine laboratory. Positive identification was achieved by comparison of elution times and absorbance ratios with known compounds. Further identification of the peaks in this sample was achieved by collecting the chromatographic effluent, rendering the effluent basic with sodium hydroxide, and extracting with chloroform. The chloroform was evaporated to dryness under a stream of air and the resulting residue was recrystallized from hexane. The infrared spectrum of the residue corresponding to each peak was determined and compared with previously prepared known spectra.

The acute toxicity of the α-benzylamines was compared to their amphetamine counterparts by determining the lethal dose (LD_{50}) and convulsive dose (CD_{50}) in mice. The compounds were administered intraperitoneally (IP) in saline. Lethality was determined at 24 h after dosing and convulsions were defined as tonic-clonic seizures of at least 3 s duration. The data for amphetamine and methamphetamine were determined using the racemic mixture (*d,l*-pair), not an individual enantiomer. Table 2 shows that both α-benzyl compounds have lower LD_{50} values than does *d,l*-amphetamine. The LD_{50} for *d,l*-methamphetamine is the lowest of the 4 compounds studied. In the course of the lethality studies it was noted that the animals treated with the α-benzyl compounds exhibited very different nonlethal symptoms compared with animals given the corresponding amphetamines. In doses well below the LD_{50}, animals treated with the α-benzyl compounds exhibited marked convulsive episodes characterized by clonic and tonic seizures. This observation prompted an investigation of the CD_{50} values for these compounds. Table 2 shows that the CD_{50} values for the α-benzyl compounds are significantly lower than the LD_{50} for the same compound and less than the corresponding amphetamine. The CD_{50} observed for the amphetamines is essentially equivalent to the LD_{50}. The CD_{50} study indicates that the α-benzyl compounds cause greater CNS stimulation at the brain stem and cord levels as noted by the clonic and tonic seizures. These convulsions were not seen with the primary cortical stimulants amphetamine and methamphetamine except at or near LD_{50} dosing levels. This limited acute animal toxicity study certainly points out the potential danger of street drugs containing substantial amounts of the stimulant/convulsant α-benzylphenethylamines.

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Fluorometric and Spectrophotometric Determination of Pirbuterol Hydrochloride in Authentic and Dosage Forms

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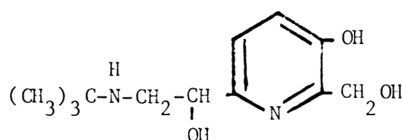
Pirbuterol hydrochloride has been assayed in alkaline medium by using a fluorometric method to measure fluorescence intensity at 372 nm with excitation at 310 nm and by the ΔA method at 242 nm. The linearity ranges are 0.5–4 μg/mL and 10–50 μg/mL, respectively. An authentic pirbuterol HCl sample was analyzed by nonaqueous potentiometric titration using 0.1N perchloric acid, and the results were compared with those for fluorometric and ΔA methods. The mean percent recoveries for the authentic sample were 98.72 ± 1.13 and 99.24 ± 0.85, respectively. When applied to commercial capsules containing 10 mg and 15 mg each, the fluorometric method gave mean percent recoveries of 101.11 ± 1.05 and 98.12 ± 0.93; the ΔA method gave mean percent recoveries of 100.57 ± 0.83 and 97.80 ± 0.75, respectively.

Pirbuterol is a relatively new bronchodialater (1) which is orally effective in patients with reversible chronic broncospastic disease (2, 3). Falkner and McIlhenny (4) determined the plasma levels of pirbuterol in humans and animals by a gas chromatographic-mass spectrometric method using a trimethylsilylation (TMS) derivatization technique and an external standard accordingly. Pharmacokinetic properties of pirbuterol after sublingual administration to dogs were investigated by Constantine et al. (5). The purpose of the present study is to establish reliable analytical methods for the determination of pirbuterol hydrochloride in pharmaceutical formulations.



Pirbuterol

Since pirbuterol HCl is a pyridine derivative with a phenolic group, its acidic solutions when rendered alkaline undergo spectral changes with regard to intensity of absorption and absorption maxima. Thus the ΔA spectrophotometric method (6) can be applied with the advantage of eliminating irrelevant absorption that is insensitive to change of pH.

Pirbuterol HCl alkaline solution, unlike phenols, exhibits strong fluorescence intensity, and accordingly a fluorometric method has been elaborated for the analysis of pirbuterol HCl. The fluorometric method, which is more sensitive than the ΔA method, can be used as a basis for quantitation of pirbuterol HCl in biological fluids after a suitable extraction procedure.

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Experimental

Reagents and Materials

Authentic pirbuterol HCl (Batch No. 10, 234-22-1F) and commercial capsules (Exirel®), 10 mg and 15 mg, were donated by Pfizer, Inc., Groton, CT, and Pfizer Taito Co., Tokyo, Japan, respectively.

Analytical grade H_2SO_4 (0.1N) and NaOH (0.1N) were used as solvents in the fluorometric and spectrophotometric experiments. For nonaqueous potentiometric work, potassium hydrogen phthalate and glacial acetic acid were analytical grade; perchloric acid and mercuric acetate were general purpose reagents.

Apparatus

Perkin-Elmer 650-10S fluorescence spectrophotometer, Varian DMS 90 double beam UV-Vis spectrophotometer, and 1 cm quartz cuvets were used for the fluorometric and spectrophotometric measurements. The nonaqueous potentiometric titrations were performed with an automatic potentiograph Model E576 (Metrohm, Herisau, Switzerland) and a combined glass-calomel electrode.

Standard and Sample Preparation

Stock solution for the standard was prepared fresh daily by dissolving about 50 mg authentic pirbuterol HCl, accurately weighed, in water in a 50 mL calibrated flask. Standard solutions were prepared by diluting the stock solution.

For commercial capsules, 20 capsules were weighed, emptied, and contents were saved. Then the empty shells were cleaned with a gentle stream of air. The weight of the clean capsules was subtracted from the gross weight to obtain the net fill weight, which was divided by 20 to obtain the net fill weight per capsule.

For each type of capsule (10 and 15 mg), an amount of powder containing 50 mg pirbuterol HCl was accurately weighed and quantitatively transferred into a 50 mL calibrated flask. Thirty mL water was added, and the mixture was shaken ≥30 min and allowed to settle. Water was added to volume. The mixture was finally shaken and then filtered through Whatman No. 40 paper, 11 cm diameter. The first 2–3 mL of filtrate was rejected.

A. Nonaqueous Potentiometric Determination of Authentic Pirbuterol HCl

Three samples, each containing about 80 mg pirbuterol HCl, were accurately weighed. Each sample was quantita-

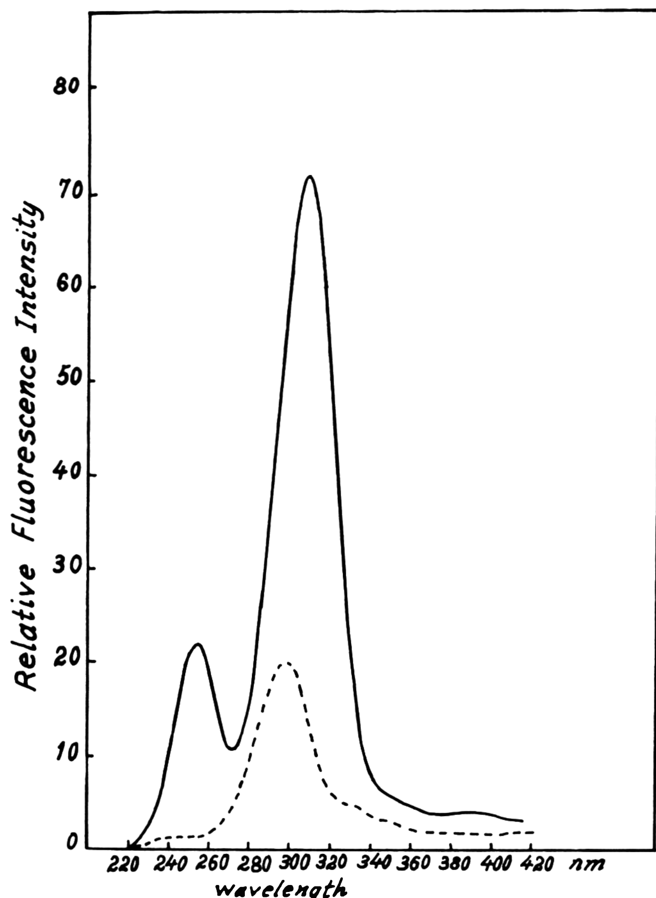

Figure 1. Relative excitation spectra of pirbuterol in 0.1N NaOH (—) and in 0.1N H_2SO_4 (---).

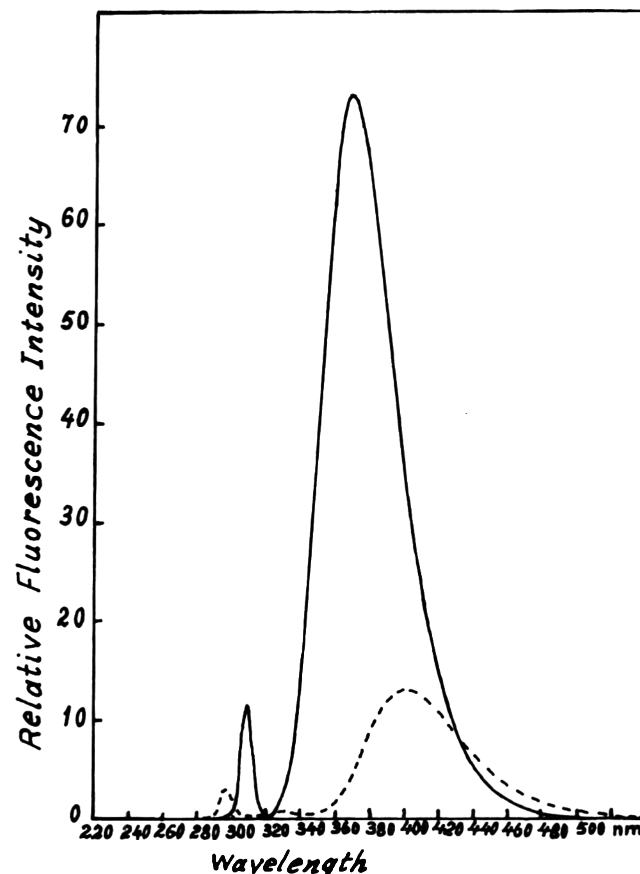

Figure 2. Relative emission spectra of pirbuterol in 0.1N NaOH (—) and in 0.1N H_2SO_4 (---).

tively transferred into a 100 mL beaker, and about 25 mL glacial acetic acid and 15 mL 5% w/v mercuric acetate in glacial acetic acid were added. The potentiometric titration curve for each sample was recorded using the potentiograph. The volume of titrant (0.1N perchloric acid in glacial acetic acid) at the end point was estimated graphically by the method of parallel tangents (7).

The percentage of pirbuterol HCl in each sample was calculated from the following expression:

$$\% \text{ pirbuterol hydrochloride} = \frac{V \times F \times 15.6605}{\text{weight sample, mg}} \times 100$$

where V = mL 0.1N perchloric acid at the end point; F = the factor of the standard acid; and 15.6605 = mg pirbuterol HCl chemically equivalent to 1 mL 0.1N perchloric acid.

The mean percentage of 3 determinations was taken as the purity of authentic pirbuterol HCl.

B. *Fluorometric Method*

A volume of 10 mL of the standard stock solution (1 mg/mL) was pipetted into a 100 mL calibrated flask and 0.1N NaOH was added to give a final solution of 0.1 mg pirbuterol HCl/mL. Using 0.1N NaOH as solvent, serial dilutions of this final solution were prepared containing 50–400 μg/100 mL. Fluorescence intensity was measured at 372 nm using the excitation wavelength 310 nm and 0.1N NaOH as blank. A calibration curve was plotted.

A volume of 10 mL of the sample solution was pipetted into a 100 mL calibrated flask and 0.1N NaOH was added to give a final solution of pirbuterol HCl of 0.1 mg/mL nominal concentration. From this final solution, 6 samples were prepared containing 50–400 μg pirbuterol HCl/100 mL. The fluorescence intensity was measured for each sample solution under the same experimental conditions used for the standard. The concentrations of the sample solution were calculated from the calibration curve, and the percentage of the active ingredient was computed. Alternatively, the percentage of the drug can be obtained from the following expression:

$$\frac{F_t}{F_s} \times 100$$

where F_t and F_s are the fluorescence intensities for the test and standard solutions, respectively.

C. *ΔA Method*

Into two 100 mL calibrated flasks, two 20 mL portions were transferred from the sample solution. One flask labeled (a) and the other labeled (b) were diluted to volume with 0.1N H_2SO_4 and 0.1N NaOH, respectively. The absorbances, A_b and A_a, of the diluted sample solutions were measured at 242 nm against a proper blank. The difference $\Delta A = A_b - A_a$ was calculated and the concentration of pirbuterol HCl was computed from the linear equation or an equivalent standard curve. The latter was established as above using solutions of final concentrations 10, 20, 25, 30, 40, and 50 μg/mL prepared from the standard stock solution.

Results and Discussion

Figures 1 and 2 show the relative excitation and emission spectra of pirbuterol in acidic and alkaline media; the fluorescence in alkaline medium is appreciably greater. Preliminary investigations indicated that fluorescence intensity was a linear function of the concentration over the range 0.02–4 μg/mL. However, under the experimental conditions

Table 1. Fluorometric and spectrophotometric determination of pirbuterol HCl

Compound	Method	% Found ± SD (n = 6)
Authentic pirbuterol HCl	nonaqueous potentiometric titration	99.2
	fluorometry	98.72 ± 1.13
	ΔA method	99.24 ± 0.85
10 mg Capsules	fluorometry	101.11 ± 1.05
	ΔA method	100.57 ± 0.83
15 mg Capsules	fluorometry	98.12 ± 0.93
	ΔA method	97.80 ± 0.75

Table 2. Absorbance ($A_{1\,cm}$) readings for several dilutions of pirbuterol HCl stock solution over 48 h

	Solvent: 0.1N H_2SO_4 λ_{max} 294 nm			Solvent: 0.1N NaOH λ_{max} 242 nm		
Concn, µg/mL	Start	24 h	48 h	Start	24 h	48 h
10	0.308	0.296	0.298	0.390	0.395	0.385
20	0.608	0.580	0.584	0.796	0.782	0.782
30	0.900	0.880	0.878	1.162	1.169	1.167
40	1.182	1.160	1.158	1.545	1.568	1.532
50	1.450	1.445	1.425	1.888	1.888	1.882

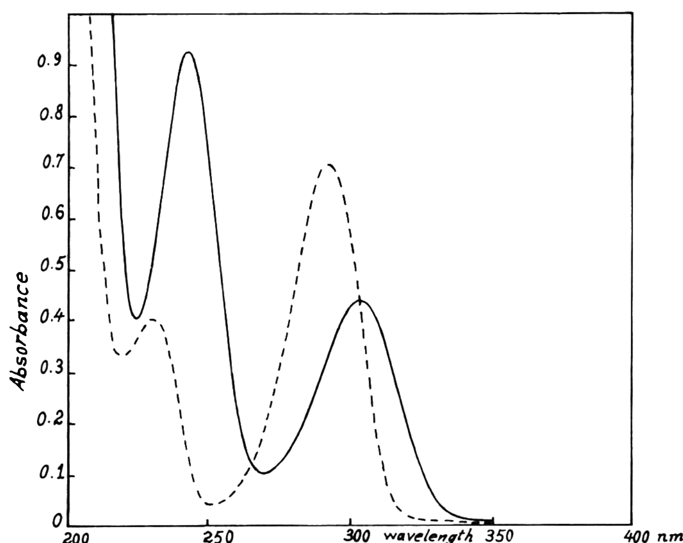

Figure 3. Absorption spectra of pirbuterol in 0.1N NaOH (—) and in 0.1N H_2SO_4 (---).

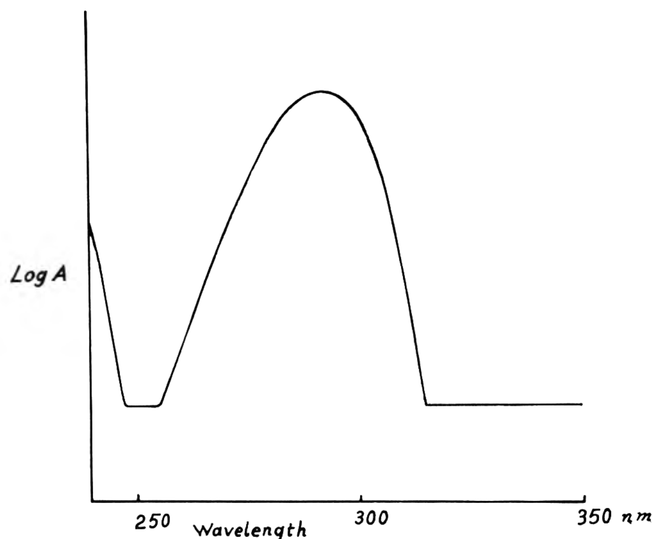

Figure 4. Log A vs λ, 50 µg/mL authentic pirbuterol HCl in 0.1N H_2SO_4. Maximum log A reading = 0.155.

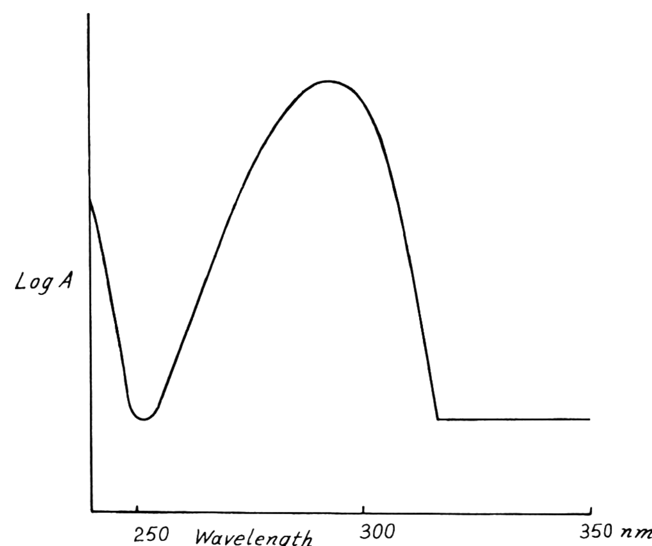

Figure 5. Log A vs λ, 40 µg/mL sample solution in 0.1N NaOH. Maximum log A reading = 0.069.

described, the concentration range adopted was 0.5–4 µg/mL. Above 4 µg/mL the inner-filter effect builds up and consequently linearity no longer holds. Below 0.02 µg/mL the solvent interference becomes appreciable.

The mean percent recovery of 6 determinations of an authentic sample was 98.72 ± 1.13 (Table 1). When applied to the 10 and 15 mg capsules, the fluorometric method gave mean percent recoveries of 101.11 ± 1.05 and 98.12 ± 0.93, respectively.

Figure 3 shows the absorption spectra of pirbuterol hydrochloride in 0.1N H_2SO_4 and 0.1N NaOH. A bathochromic shift from 230 to 242 nm can be observed when the medium changes from acidic to alkaline. The difference at 242 nm, which coincides with maximum ΔA curve, can be used for the analysis of pirbuterol HCl to correct for interference that may be present and which is insensitive to change of pH.

To check the stability of pirbuterol HCl, the stock solution was kept in the dark at room temperature (25°C). Absorbance readings were taken over a period of 48 h for concentrations ranging from 10 to 50 µg/mL in 0.1N H_2SO_4 and 0.1N NaOH. From Table 2, it can be deduced that the stock solution probably deteriorates gradually with time. Consequently, only freshly prepared solutions of pirbuterol HCl are recommended for use.

The existence of UV-absorbing impurities, as a result of synthesis or for other reasons, causes distortion of the UV absorption spectrum. Figures 4 and 5 represent log A vs λ curves for authentic pirbuterol HCl and sample in 0.1N H_2SO_4. The 2 curves can be superimposed, suggesting the absence of interference by UV-absorbing impurities.

As with all spectrophotometric measurements, substantial errors arise when absorbances lie outside a satisfactory range. Thus, the concentration range of choice was 10–50 µg/mL. The calculated molar absorptivity (ε) was 1.1×10^4 L $mole^{-1}$ cm^{-1} in alkaline medium at 242 nm compared with 9.1×10^3 L $mole^{-1}$ cm^{-1} in acidic medium at 294 nm.

Table 3. *F*- and *t*-Tests: statistical analysis

Compound	Method	No. of experiments	Variance ratio, $F_{0.05}$-test[a]	Student's $t_{0.05}$-test[a]
10 mg capsules	fluorometry	6	1.60 (5.05)	2.21 (2.23)
	ΔA method	6		
15 mg capsules	fluorometry	6	1.54 (5.05)	1.47 (2.23)
	ΔA method	6		

[a]Calculated levels; significant levels in parentheses.

The linear relationship between ΔA and concentration (C, µg/mL) was described by the equation $\Delta A = 3.02 \times 10^{-2} C - 0.003$; the correlation coefficient, r, was 0.995 at 95% confidence limits.

The mean percent recovery of 6 determinations for an authentic sample was 99.24 ± 0.85. For the ΔA method, when applied the 10 and 15 mg capsules, the mean percent recoveries (6 determinations) were 100.57 ± 0.83 and 97.80 ± 0.75, respectively (Table 1).

According to the *F*- and *t*-tests (Table 3), there is no significant difference between the fluorometric and ΔA methods with regard to precision and accuracy. This implies that the 2 methods are suitable for quantitation of pirbuterol HCl; the fluorometric method is the more sensitive.

Acknowledgments

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TECHNICAL COMMUNICATIONS

Determination of Fat in Vegetable Foods

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The fat in vegetable foods—tree nuts, peanuts, sunflower seeds, avocado, and olives—can be determined volumetrically by acid digestion of the material and separation of the fat. The assay can be performed conveniently by using the equipment developed for fat determination of milk (Gerber method). The results agree well with those obtained by Soxhlet extraction. The advantages of using the Gerber method for vegetable foods are simplicity, speed, low operation cost, and elimination of the use of inflammable solvents.

The determination of fat is one of the most frequently encountered analyses of food. The test, which serves as a basis for determining payment (milk), for defining harvesting time (avocado), for processing (soybean, corn, sunflower seeds, etc.), or for nutritional information (nuts, peanuts) often requires rapid and accurate results. The conventional and almost universal method for fat determination in solid foods is based on solvent extraction, usually in ether or petroleum ether. Occasionally, hydrolysis or drying of the product must precede the extraction. The whole procedure requires from a few hours to several days (1). Faster volumetric methods were developed for determination of fat in fluid milk products. Two basically similar tests are in use: the Gerber and Babcock methods (2).

The Gerber method is official in most European countries and in other parts of the world, while in the United States, the Babcock test is widely used and the Gerber test is used only in certain states. In the Gerber method, 11 mL milk followed by 1 mL isoamyl alcohol are added to a Gerber glass butyrometer containing 10 mL sulfuric acid (specific gravity 1.82). A lock-stopper is inserted and the bottle is shaken until the curd dissolves and the content is homogeneous. The butyrometer is centrifuged 4 min, submerged to the top of the graduated stem in 63 ± 2°C water for 5 min, and the fat content (%) is read.

In addition to fluid milk products, the Gerber test can be used for the assay of fat in cheese, cream, condensed milk, and ice cream, provided special butyrometers with both ends open are used. The very similar Babcock method, with various digestion reagents, has been applied satisfactorily to fish products (3), and the Gerber method has been applied to sausages (4).

We found that the speed, simplicity, and low expense of operation of the Gerber method can be extended to the analysis of numerous foods of vegetable origin: macadamia nuts and pecans, avocados, olives, peanuts, and sunflower seeds.

METHOD

Apparatus and Reagents

(a) *Butyrometers.*—K. Schneider & Co., Ltd, Zurich, Switzerland. Two kinds of glassware were used: cheese butyrometers (Van Gulik type) with 2 openings, graduated from 0 to 40% fat; and condensed-milk butyrometers, graduated from 0 to 20% fat. Both types were equipped with glass beakers with holes to fit 3 g cheese samples (Figure 1).

(b) *Centrifuge.*—Model K-100, used for Gerber method, allows butyrometers to be spun in the swing-out bushing, and has 30 min timer and thermostatically-controlled heater to maintain temperature at 65 ± 1°C. When fully loaded, centrifuge can produce, within 2 min, a relative centrifugal force of 350 ± 50 × g at outer end of large stopper (K. Schneider & Co., Ltd).

(c) *Water bath.*—Thermostatically controlled to maintain temperature of 65 ± 2°C, provided with stand to hold butyrometers in vertical position.

(d) *Sulfuric acid.*—d^{20} = 1.615 g/mL, 70% aqueous (m/m). Acid should be colorless or not darker than pale amber and should not contain any impurity to affect determination.

(e) *Homogenizer.*—Ultra-Turrax, equipped with 18 mm shaft (Janke & Kunkel KG, Switzerland).

Sample Preparation

Samples were either finely grated (macadamia nuts, pecans, peanuts, unripe avocados) or ground in a blender to a fine powder (soybeans, sunflower seeds, peanuts). Ripe fruits, like avocados and olives, were homogenized to a paste, which was freeze-dried for Soxhlet extraction.

Procedure

Weigh into separate butyrometer glass beakers, samples prepared as described above. Insert glass beakers with rubber stoppers into large opening of each butyrometer, and secure tightly. Gently shake butyrometers to loosen samples to avoid formation of lumps, which impede digestion. Add 12 mL sulfuric acid through narrow opening of each butyrometer positioned vertically in stand, and then place stand with butyrometers into warm water bath. Shake butyrometers every 10–15 min until complete digestion (see Table 1). When liquid mass appears homogeneous, fill butyrometers with acid to

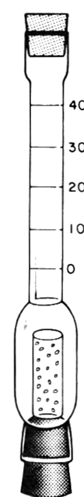

Figure 1. Gerber butyrometer.

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Table 1. Experimental conditions for oil determination in vegetable foods by using Gerber method

Sample	Butyrometer: Graduation, %	Butyrometer: Scale precision, %[a]	Butyrometer: Code[a]	Digestion: Temp., °C[b]	Digestion: Time, min	Sample wt, g	Factor[c]
Avocado	0–20	± 0.1	103–20	55	30	2.0	2.5
Macadamia nuts	0–40	± 0.25	238–40	65	120	1.5	2
Pecans	0–40	± 0.25	238–40	65	120	1.5	2
Peanuts	0–40	± 0.25	238–40	65	120	1.5	2
Olives	0–20	± 0.1	103–20	65	120	1.5	3.3
Sunflower seeds	0–40	± 0.25	238–40	65	120	1.5	2

[a]Described in catalog for Gerber equipment (K. Schneider & Co., Ltd).
[b]In 70% sulfuric acid (m/m), used for all assays.
[c]% oil = oil reading × factor.

Table 2. Linear regression of oil determination in some vegetable foods, according to Soxhlet extraction and Gerber methods

Sample	Linear equation, $Y = b + aX$[a]	r, calc.	r, tab.[b]	df[c]	SD of B[d]	SE of B[e]	Mean, %: Soxhlet	Mean, %: Gerber
Avocado	$Y = 0.57 + 0.989X$	0.926	0.607	26	0.081	0.0066	13.60	13.17
Peanuts	$Y = 2.15 + 0.962X$	0.957	0.597	27	0.055	0.0030	48.95	48.64
Pecans	$Y = 0.36 + 0.991X$	0.966	0.607	26	0.050	0.0025	61.23	61.41
Macadamia nuts	$Y = -1.81 + 1.022X$	0.914	0.490	44	0.068	0.0047	76.55	76.67

[a]Y = Soxhlet, X = Gerber.
[b]Tabulated r (correlation coefficient) at 99.9% and at given degrees of freedom.
[c]Degrees of freedom.
[d]Standard deviation of butyrometer reading.
[e]Standard error of butyrometer reading.

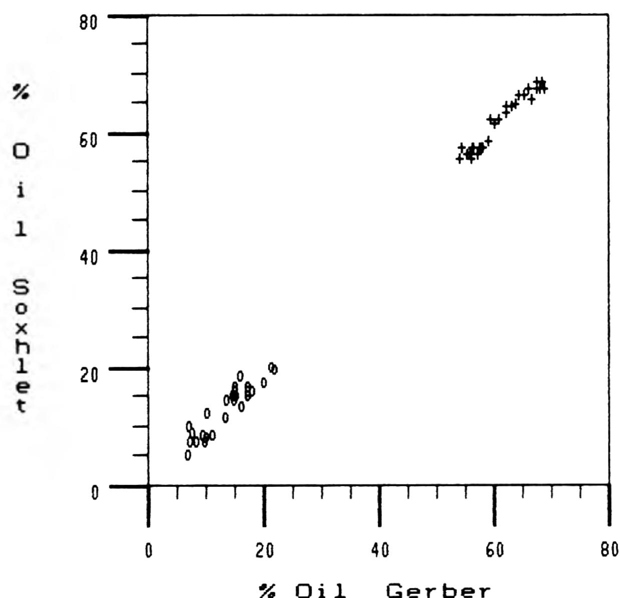

Figure 2. Relationship between oil content of avocados and pecans, determined by Soxhlet extraction and Gerber method (o = avocados; + = pecans).

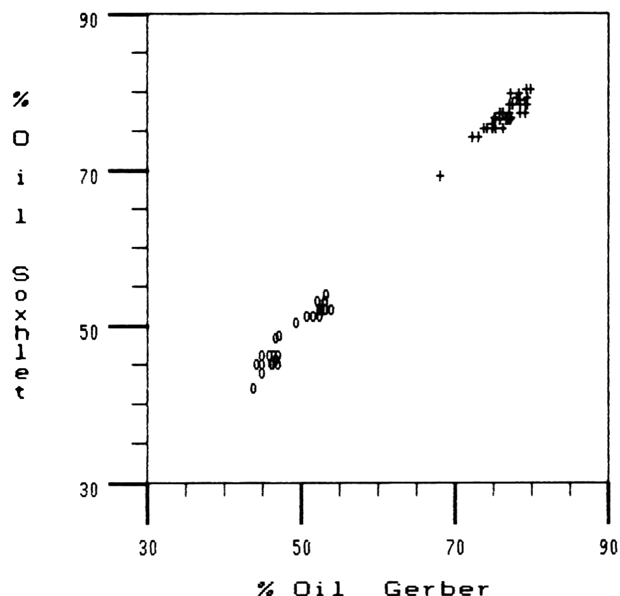

Figure 3. Relationship between oil content of macadamia nuts and peanuts, determined by Soxhlet extraction and Gerber method (o = peanuts; + = madadamia nuts).

15% and 30% graduation marks in 20% and 40% butyrometers, respectively. Plug narrow openings of butyrometers with stoppers. Place butyrometers in heated centrifuge and spin 15–20 min. Remove butyrometers from centrifuge and place them in water bath for 3–5 min.

Read height of column in graduated neck by subtracting reading obtained at bottom from that obtained at top of fat column (lower meniscus). Slightly move large stopper in or out to bring bottom of fat column, with minimum movement of column, to nearest graduation mark. Multiply reading by factor given in Table 1 to obtain fat content in g fat/100 g sample. The optimal assay conditions for various foods are summarized in Table 1.

Results and Discussion

In all these determinations, the assays had perfect appearances: the color of the fat column was colorless to straw-yellow; the ends of the fat column were clearly and sharply determined; the fat was free of specks and sediments.

The difference between the results of 2 determinations carried out simultaneously did not exceed a value corresponding to half the smallest scale division. The results obtained by the present assay were compared with those secured by the alternative method of Soxhlet extraction with petroleum ether for macadamia nuts, peanuts, sunflower seeds, olives, avocados, and soybeans. About 30 samples of each food were submitted to parallel assays, and the results agreed within

the limits of the experimental error. The experimental error is the product of scale precision of the butyrometer times the multiplication factor. The fat content of the materials tested ranged from 5% in some unripe avocado samples (others reached 22%) to 80% in macadamia nuts. Fat contents of peanuts (44–54%), pecans (54–69%), and macadamia nuts (68–80%) are within this range.

A linear regression of the analytical data was performed for each of the foods tested, and the results are summarized in Table 2. The statistical analysis shows no significant difference between the Soxhlet extraction (the dependent variable = Y) and the Gerber method (independent variable = X). All the data were plotted from the same regression analysis, which is presented in Figures 2 and 3. It should be noted that the spread of results for the same food is caused by the different varieties tested. Ten different varieties of avocados, 3 peanut varieties, and 2 pecan and macadamia nut varieties were tested.

We failed to define the experimental conditions for fat determination in soybeans by the present method. In this case, although fat was well separated from other components, erratic, irreproducible, and consistently lower results were obtained. We assumed that acid digestion and the total release of fat in this material are difficult to achieve. The use of more concentrated sulfuric acid, higher temperature, or longer digestion time did not solve the problem.

Preliminary results indicate good correlation between the Gerber and Soxhlet extraction methods for olives (28–33% fat) and sunflower seeds (44–46% fat).

The advantages of using the Gerber method in vegetable foods are simplicity, speed, low cost of operation, and elimination of the use of inflammable solvents.

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Comparison of Ether and Chloroform for Soxhlet Extraction of Freeze-Dried Animal Tissues

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Chloroform was a satisfactory replacement for ether in solvent extraction for lipid determination in freeze-dried animal tissues, although values obtained were not identical. Fat content of tissues used in this study ranged from less than 10 to more than 95%. Chloroform has the great advantage of being fireproof, but proper fume hoods should be used. An antifoaming agent should be added to chloroform, both in the Soxhlet apparatus and when it is redistilled.

Extraction with nonpolar solvent is the only satisfactory method for determination of total lipid in most solids. Normally, the loss of weight of an extracted sample is measured. Alternatively, one may recover the extract by evaporating the solvent, although volatiles such as terpenes may be lost in this way. Woodward et al. (1) measured the change in density of the solvent.

Complete extraction is best achieved by repeated washing with pure solvent; the first automatic apparatus for doing this was designed in Germany by Franz von Soxhlet (2). The Soxhlet extractor is still used today, though other designs such as those of Goldfisch or Butt extract more quickly and may be more suitable on a semimicro or micro scale. If the lipids are to be characterized further, extraction with chloroform–methanol such as described by Folch et al. (3) or Bligh and Dyer (4) is the more usual approach.

The standard extraction solvent for crude fat determination has for many years been diethyl ether. More recently, petroleum ether (which is mainly pentane) has been used as an alternative. These are still recommended solvents (5, 6). Probably diethyl ether was chosen in the nineteenth century because at that time it was an inexpensive solvent, made by reacting ethanol with sulfuric acid. Only later, with the development of the oil industry, did paraffins such as petroleum ether become economically advantageous.

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A disadvantage of diethyl ether and pentane is that they are highly flammable and easily make explosive mixtures with air. Ether in addition is liable to form peroxides which can detonate without warning. For shipping ether, a small percentage of ethanol or butylated hydroxytoluene is usually added because these reducing agents decompose peroxides. Peroxides usually are not a danger in the ether extraction of plant or animal tissues because these tissues contain enough unsaturated lipids (such as glycerides of oleic or linoleic acids) to destroy peroxides.

Laboratory safety would be improved if a nonflammable solvent could be used for fat extraction. In the oilseed industry many solvents, flammable and otherwise, have been tried (7, 8). Papers on studies comparing different solvents on duplicate analytical samples of animal tissue may have been published, but the authors have so far failed to find them. We therefore decided to compare ether, a standard solvent, with chloroform, which is nonflammable, in Soxhlet extraction. Chloroform was chosen because it is already widely used as a solvent, it is more stable than carbon tetrachloride, and its price per liter is about the same as that of ether. Chloroform is carcinogenic (metabolized to phosgene in the liver) but with proper fume hoods and careful handling it is not dangerous. It was used in hospitals for many years as a general anesthetic.

Ether is not without biological hazard. It causes cellular abnormalities in yeast (9), inhibits multiplication of hamster lung fibroblasts by causing abnormal mitosis (10), and damages DNA in *Escherichia coli* (11). DNA damage was caused by "aged" ether but not by the freshly distilled solvent, suggesting that peroxides rather than ether itself were responsible (11), and this may also apply to the yeast and fibroblasts.

Experimental

Tissues of sheep were dried, ground, and extracted with ether or chloroform in large Soxhlet extractors (Corning Glass

Table 1. Percentage extractable lipid from body tissues

		Body tissue of Dorset rams							
		Carcass		Non-carcass		Omental and mesenteric fat		Muscle	
Animal[a]	Duplicate	Ether	Chloroform	Ether	Chloroform	Ether	Chloroform	Ether	Chloroform
1	1	73.38	72.82	26.30	25.59	98.39	98.11	11.61	11.48
	2	73.82	73.07	26.10	24.30	98.24	98.36	10.90	11.59
2	1	46.61	47.00	32.17	30.39	96.30	95.92	20.53	22.25
	2	46.80	46.83	31.32	29.50	92.75	95.46	22.23	21.80
3	1	67.87	66.59	44.02	42.71	97.88	98.04	22.41	22.45
	2	66.30	65.77	44.08	43.10	96.24	97.57	21.81	22.08
4	1	32.76	32.98	29.02	26.77	96.98	98.61	15.96	17.11
	2	31.16	33.01	29.78	26.85	97.76	98.68	15.50	17.74
5	1	70.19	71.18	39.97	37.85	97.88	98.23	10.63	11.26
	2	70.40	70.85	40.14	38.02	98.30	98.13	8.60	10.98
6	1	55.56	56.07	37.84	37.05	98.17	98.68	13.85	15.98
	2	55.62	56.12	38.03	36.76	97.76	98.41	13.26	16.38
7	1	32.13	30.88	32.90	34.21	95.58	94.54	15.50	19.28
	2	32.37	31.63	32.93	33.42	94.51	96.47	21.47	21.66
8	1	72.00	74.07	33.49	30.99	95.73	97.25	15.76	16.93
	2	72.50	73.10	32.36	32.71	97.72	97.03	17.23	17.08
9	1	48.97	49.14	25.15	25.29	97.08	98.06	10.76	12.13
	2	48.81	49.73	24.98	24.22	98.45	97.58	10.37	10.73
10	1	46.63	47.54	32.58	32.20	96.25	93.73	22.74	24.03
	2	47.57	48.01	32.25	31.80	96.33	96.62	24.44	22.36
11	1	39.93	39.72	35.29	34.10	95.57	96.83	14.73	15.59
	2	40.07	39.91	35.46	33.98	96.90	97.51	15.04	14.35
12	1	25.61	25.32	25.73	25.62	92.11	93.32	20.43	20.16
	2	25.68	26.66	25.39	25.58	91.35	92.82	21.20	21.64
13	1	61.66	61.48	30.88	27.45	92.56	93.50	18.92	16.83
	2	61.67	61.64	30.22	27.36	90.67	91.81	18.31	19.37
14	1	44.54	45.06	15.65	11.69	98.07	98.65	9.33	9.82
	2	44.71	44.63	14.52	11.41	97.65	98.33	9.81	9.63
15	1	15.65	11.54	27.42	27.27	98.23	98.42	16.83	15.51
	2	14.52	12.10	28.01	27.23	97.72	98.29	15.88	16.83
16	1	25.04	25.20	26.80	24.05	97.77	98.40	11.11	8.87
	2	25.75	25.19	26.36	24.68	98.19	98.05	11.34	11.67
17	1	38.74	38.48	31.30	27.78	97.60	98.19	8.61	12.31
	2	38.40	38.80	29.48	27.67	97.98	98.20	9.21	11.64
18	1	39.71	40.05	40.21	39.77	98.09	98.04	10.17	10.85
	2	39.86	38.64	39.93	39.17	97.54	97.39	8.18	12.00
19	1	45.10	43.38	30.25	31.09	97.66	97.45	7.67	9.22
	2	46.27	43.27	32.59	30.29	97.89	98.09	5.77	6.66
20	1	38.01	37.90	34.36	33.83	98.55	97.64	13.25	14.88
	2	38.49	38.48	34.60	32.70	98.43	97.98	12.65	14.04
21	1	63.40	63.98	32.18	32.45	92.61	96.12	7.91	10.80
	2	63.99	64.26	31.99	32.38	95.50	94.37	7.65	9.24
22	1	62.44	61.28	29.88	26.66	97.23	97.68	18.08	18.89
	2	61.60	63.81	30.11	27.45	97.66	96.10	16.84	18.55
23	1	58.41	59.15	50.37	46.93	97.06	96.85	14.76	14.41
	2	58.28	59.72	49.78	46.85	97.52	97.64	13.71	14.83

[a]Each tissue came from a different set of animals.

Works, Corning, NY). Samples were obtained from sheep ranging in live weight from 14 to 65 kg and represented omental and mesenteric fat (adipose tissue), skeletal muscle, carcass, and non-carcass tissues. Sheep were the same as those used in an experiment described by Firth (12). "Carcass" means one side of the carcass as prepared commercially, and "non-carcass" means all other parts of the animal, excluding contents of digestive tract and bladder which are not considered parts of the body. Carcass and non-carcass materials were cut into pieces and ground 7 times through an industrial meat grinder (Paul Autio, Astoria, OR). Samples were freeze-dried, and then ground with dry ice in a Wiley mill (Model 2; Arthur H. Thomas Co., Philadelphia, PA). From the other side of each carcass, several individual muscles were taken, freeze-dried, and ground dry in a Waring Blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, CT). Fat from the mesentery and kidney depots was freeze-dried and cut into small pieces by knife.

Samples were held in groups of 17 per extractor for one week. Extraction was done only during business hours, to allow supervision. Thus, extraction proceeded for about 40 working hours, with sample chambers full of solvent overnight. Previous experience in this laboratory had shown that animal tissues high in fat (adipose tissue, carcass) require extraction times of this length. The 2–5 g samples were held in porous clay (alundum) thimbles by glass wool. Solvents were distilled to remove fat when their appearance darkened or became cloudy. Ether was not treated with sodium to keep it anhydrous. With low levels of sugars normally found in animal tissues, traces of water in ether would not noticeably change results by extracting sugars or other non-lipid constituents.

When thimbles were taken out of the extractors they were stood on paper towels in a fume hood to dry before being put in desiccators. If a stain was left on the towel, extraction was not complete and the thimbles were returned to the apparatus for 3 days. This seldom happened, but was more likely to occur with ether than with chloroform.

Results and Discussion

Twenty-three samples of each of the 4 tissue groups were extracted in duplicate in each solvent, giving a balanced data

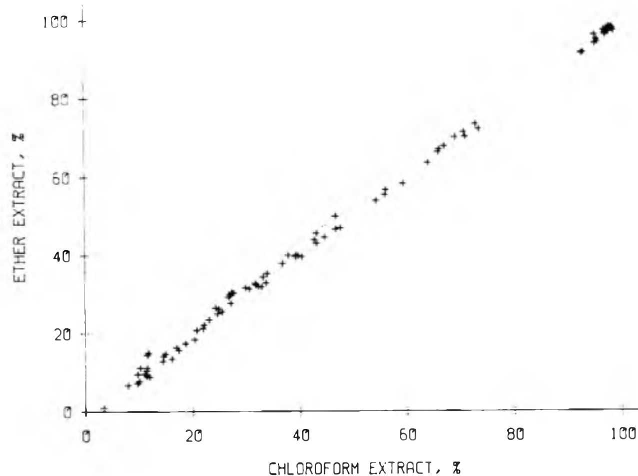

Figure 1. Means of duplicate samples extracted with each solvent.

set. The samples were chosen at random, except that an attempt was made to include animals from all parts of the weight range. Results are listed in Table 1 and plotted in Figure 1. Table 2 shows mean values for each tissue and solvent, and repeatabilities (intraclass correlations) as described by Snedecor and Cochran (13).

For each tissue type, ether extract percentage was regressed on chloroform extract percentage to obtain an equation to predict ether values from chloroform values:

$$\text{Ether extract} = \text{intercept} + \text{slope} \times (\text{chloroform extract})$$

Table 3 lists the intercepts and slopes. Had the solvents given identical results, the intercepts would be 0 and the slopes would be 1.

High r^2 values indicate closely clustered data points which were well fitted by the least-squares line. As a check, the residuals from the regressions were plotted against the independent variable. The plots appeared random, with correlations of 0.32 or less, suggesting that the model was adequate.

The means and standard deviations for the intercepts and slopes were used to test whether the latter differed significantly from 0 and 1. The intercept for muscle was the only measure in this test, which was significantly different from its "null" value as expected from the null hypothesis. Ether gave an intercept for muscle which was 2 percentage units lower than that given by chloroform. This was against expectation because ether, which is more polar than chloroform, extracts polar lipids more readily and should give values the same as or higher than those of chloroform. Apart from this discrepancy, it appeared that chloroform and ether gave similar results.

Individual data values provided enough degrees of freedom to estimate the variance for each solvent. Thus we were able to answer the question: Do the 2 solvents show about the same variability in extract values, or does one give results more variable than the other? This was tested from the ratio of the solvent variances, since this ratio will follow the F-distribution if the original data are Gaussian. (A histogram of the residuals from the regression showed an approximately Gaussian distribution.) No significant difference could be shown. The highest standard deviations for any tissue were 1.17 and 0.97 for ether and chloroform, respectively.

Because the extract values for both solvents were random variables, the orthogonal regression approach described by Cramer (14) and Waldo et al. (15) was more appropriate. In orthogonal regression the squares of deviations at right angles to the regression line are minimized, rather than the squares

Table 2. Mean and repeatability for each tissue and solvent

Tissue	Solvent	Mean, %	Repeatability**
Carcass	ether	48.02	0.9991
	chloroform	47.96	0.9989
Non-carcass*	ether	32.27	0.9938
	chloroform	30.86	0.9955
Fat*	ether	96.66	0.8031
	chloroform	97.03	0.8335
Muscle*	ether	14.42	0.9453
	chloroform	15.30	0.9558

*Means significantly different at $P < 0.05$ with standard errors of the difference of 0.25 (non-carcass), 0.14 (fat), and 0.23 (muscle). For carcass the SED was 0.22.
**Intraclass correlation (see reference 13).

of the vertical (Y) deviations as in conventional regression. That is, the shortest distance between an observation and the line is the quantity whose square is minimized. Regression coefficients calculated by this method, which avoided the assumption that chloroform values were fixed and known without error, are given in Table 4. No method was available for calculating standard deviations for these coefficients, but the r^2 values were the same as for the conventional regression since they are functions of the X and Y values only. Orthogonal regression would be the method of choice for converting values between solvents.

Perhaps the most searching test for differences between 2 analytical methods is the paired t-test on values for the same samples by each method. The differences between solvents for all tissues combined and for carcass tissue separately were nonsignificant ($P > 0.6$). For non-carcass, fat, and muscle the differences were significant ($P < 0.05$). As shown in Table 2, the chloroform value for non-carcass was lower by 1.41 percentage units, and for fat and muscle the chloroform value was higher by 0.37 and 0.88 percentage units, respectively. These differences, although small, mean that chloroform and ether values cannot directly replace each other. But in any given study the conclusions reached about treatment differences should not be affected by which solvent was used (Figure 1). We conclude that chloroform is a satisfactory replacement for ether for Soxhlet extraction of animal tissues.

Disadvantages of Chloroform

Chloroform has a higher boiling point than does ether (62°C against 35°C) and much of it will condense before reaching the condenser as used in official methods unless the middle part of the apparatus is insulated. We used Fiberglas as sold for insulating buildings (Owens-Corning Co.).

When a solvent contains large amounts of fat it is liable to foam when it boils. Chloroform was worse than ether in this respect and in preliminary trials sometimes foamed suddenly and violently. We used stone boiling chips (Hengar Granules, Arthur H. Thomas Co., Philadelphia, PA). Chips made of compressed carbon, although excellent for water solutions, were not dense enough for chloroform which has a specific gravity of 1.48, and were lifted from the bottom by convection currents. Film strength can be reduced by long-chain alcohols. 1-Octanol is a standard additive and is miscible with chloroform (its boiling point is 194°C). Silicones and sulfonated oils are available, which probably work in the same way. We used a silicone anti-foam spray (ref. 1130-D, Arthur H. Thomas Co.). Use of a nonvolatile additive raises the possibility that the additive may increase the extracted dry weight of the samples, introducing a bias. This would be particularly so if the additive collected preferentially on the samples or thimbles, rather than remaining uniformly distributed in the

Table 3. Conventional regression coefficients

Tissue	Intercept	SD of intercept	Slope	SD of slope	r^2
Carcass	1.26	0.81	0.979	0.015	0.995
Non-carcass	2.15	1.1	0.975	0.035	0.974
Fat	−10.7	7.8	1.11	0.080	0.901
Muscle	−2.19**	0.66	1.07	0.043	0.967

** $P < 0.01$.

solvent. We do not believe that such a bias is likely because the nonvolatile additive would not evaporate from the heated flask along with the solvent, and therefore would not reach the sample chamber. Furthermore, the small differences between solvents were not always in the same direction. Thus it is unlikely that the nonvolatile additive we used during chloroform extraction affected the results.

When thimbles were removed from the extractor, time was required for residual solvent to evaporate from them. More time was required for this with chloroform than with ether. Four hours in a gentle draft in a fume hood was adequate.

Acknowledgment

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Table 4. Orthogonal regression coefficients

Tissue	Intercept	Slope
Carcass	1.14	0.981
Non-carcass	1.75	0.988
Fat	−17.4	1.18
Muscle	−2.47	1.09

Re-Evaluation of Selectivity of Malachite Green Test for Presence of Sulfite in Mechanically Deboned Poultry

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A study was undertaken to determine whether free chlorine or sulfide anions could yield positive results for the malachite green test in meat samples when sulfite was not present. Malachite green solution was added to a mechanically deboned poultry (MDP) sample spiked with either chlorine or sulfide ions. The presence of chlorine in MDP samples at any concentration tested did not yield a positive reaction for the malachite green test. When sulfide ions were introduced into MDP samples, positive results were observed at 40 μg sulfide ion/g MDP and above. Because aqueous solutions of sulfide at the levels mentioned have a relatively high pH, it was necessary to determine whether the pH or the presence of the sulfide ions had caused this positive reaction to the malachite green test. After a comparison of a solution of sulfide, and a solution of a matching pH, it was observed that the sulfide present caused the positive reaction to the malachite green test.

Sodium sulfite has been used to enhance the appearance of red meat even though the product may be in a decomposed or decomposing state. The use of such compounds to artificially enhance the appearance of red meat has been considered a misleading practice (1), and adverse reactions to sulfited foods have also been investigated (2).

To detect the presence of sulfite in foods, the dye malachite green has been used. In the presence of sulfite, malachite green undergoes a reaction to yield its colorless leuco form (3).

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The selectivity of the malachite green test was questioned when several mechanically deboned poultry samples were found to be positive for the presence of sulfite by this procedure, but when the Monier-Williams test was performed on the same meat samples, sulfite did not appear to be present. Chloride and sulfide were investigated as possible interfering anions because solutions of these compounds also cause malachite green to undergo a reaction, forming its leuco analog. Because a chlorinated water washing procedure is to be used to prevent bacterial growth on poultry products, chloride ions appear to be the most likely anions to cause false positive results for the malachite green test.

Experimental

The malachite green test procedure used on poultry samples spiked with chlorine or sulfide can be found in secs **20.129–20.130,** *Official Methods of Analysis* (14th Ed., 1984, AOAC). Part of the experiment also involved measuring the pH change due to the presence of sulfide anions in an aqueous solution, using standard instrumental methods.

Results and Discussion

Chlorine solutions with concentrations as high as 5% available chlorine were thoroughly mixed into a mechanically deboned poultry (MDP) sample, as described in the experimental section. The treated meat samples yielded the same

results as the unspiked meat samples in that both retained their bluish-green coloring caused by the dye indicator. When malachite green was added to a chlorine solution, however, the color disappeared. These results seem to indicate that chlorine may have a high tendency to chemically bind to sites in a MDP sample, causing the substance of interest to be inaccessible for a reaction with malachite green.

An attempt was also made to investigate the chemical interference of sulfide in the malachite green test. Initial experiments indicated that sulfide in aqueous solutions caused the decolorization of malachite green. Because sulfide solutions tend to have high pH values, it was necessary to determine whether hydroxide ions or sulfide ions were causing the conversion of this dye to its leuco form. A 100 ppm solution of sulfide ions, on the average, had a pH of 11.1. When 30 mL distilled water was adjusted to this pH with 0.1N NaOH, the addition of several drops of malachite green turned the solution blue. This solution was compared with a solution of distilled water with the same volume of malachite green added. The adjusted pH solution appeared to be somewhat decolorized when compared with the solution of malachite green and water. The 100 ppm sulfide solution remained clear even after larger amounts of the dye indicator were added. The results lead to the conclusion that the sulfide anions contributed to a much greater extent to the decolorization of the malachite green dye than did the hydroxide ions. MDP samples were treated with varying volumes of sulfide solution, which made the content of this anion in the meat sample either 40, 80, or 120 ppm. All samples spiked at these levels displayed the characteristic decolorization of the malachite green dye, while the unspiked MDP samples remained blue. To observe any effect that decomposition may have on the malachite green test, untreated MDP samples were left at room temperature for 2 days. Even though the presence of mercaptans were readily apparent, these meat samples appeared to remain bluish-green when malachite green solution was added.

According to these results, the malachite green test is not specific for sulfite, because of this indicator's tendency to react with sulfide ions. Because malachite green does not convert to its leuco form in the presence of the most frequently occurring interfering substance in meat samples, namely, chlorine, results of this test may still be valid in a majority of cases.

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Photodegradation of Sulfa Drugs by Fluorescent Light

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Thin layer chromatographic and liquid chromatographic procedures were used to show that sulfonamides containing a heterocyclic amine moiety and free N^1 acidic hydrogen will photodegrade under fluorescent light in model systems containing riboflavin. The photodegradation product was characteristic of the drug. In-depth studies on sulfamethazine showed that the drug also photodegraded in the presence of lumichrome and flavin mononucleotide; the rate of photodegradation depended on the photosensitizer and its concentration. Crude polar liver extracts sensitized the photodegradation of sulfamethazine, but to a degree less than expected on the basis of reported riboflavin content of livers. It is recommended that procedures for quantitating sulfa drugs and their metabolites be performed in subdued lighting and/or that amber or low actinic vessels be used to prevent losses due to photochemical reactions.

In the course of studies on the development of procedures for sulfa drugs and their metabolites in swine and chicken livers (1, 2), unexplained losses of the drugs and/or metabolites occasionally occurred during the isolation procedure. Preliminary investigations into the source of these decreased values led to the observation that sulfa drugs are susceptible to photochemical reactions by fluorescent light in the presence of polar extracts of liver tissues. Since previous studies have demonstrated that vitamin B_2 (riboflavin) catalyzes photochemical reactions in a variety of biological materials (3, 4) and liver contains relatively large amounts of this vitamin (5), studies on the susceptibility of sulfa drugs to photochemical reactions by fluorescent light in model systems were initiated. This communication reports the results of studies on a number of sulfa drugs and alerts the analyst to a potential problem in analyzing for these drugs in tissue samples.

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Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Experimental

Irradiation

Aqueous methanol solutions of sulfa drugs or their derivatives, 1 mL in 60 × 17 mm screw-cap flint glass vials, were exposed to fluorescent light (250 ft-candles) for 5–60 min at ambient temperature in the presence of riboflavin (J. T. Baker Chemical Co., Phillipsburg, NJ 08865), flavin mononucleotide (FMN) sodium salt (Sigma Chemical Co., St. Louis, MO 63178), or lumichrome (Pfaltz and Bauer, Stamford, CT 06902). The light source consisted of two 15 watt cool white fluorescent bulbs (Westinghouse F15T8/cw) positioned 15.25 cm above the sample vials which were placed in a horizontal position.

Thin Layer Chromatography (TLC) Studies

Approximately 75–100 μg drug and 35 μg riboflavin in 1 mL 50% aqueous methanol was exposed to fluorescent light for 60 min. Following exposure, 1–2 μL samples were subjected to TLC on 2.5 × 10.0 cm glass plates with 250 μm layer of silica gel G (Analtech, Newark, DE 19711). Plates were developed to 0.5 cm initially, dried in a hot air oven at 50°C, and redeveloped to 5 cm. Developing solvents: (a) chloroform–ethyl acetate–methanol (5 + 5 + 1), and (b) chloroform–methanol (1 + 1) or upper layer of butanol–acetic acid–water (4 + 1 + 5). Sulfa drugs, N^1-methyl derivatives, and photodegradation products containing a primary aromatic amine were visualized with the Bratton-Marshall (B-M) spray reagent previously described (6). N^4-Acetyl com-

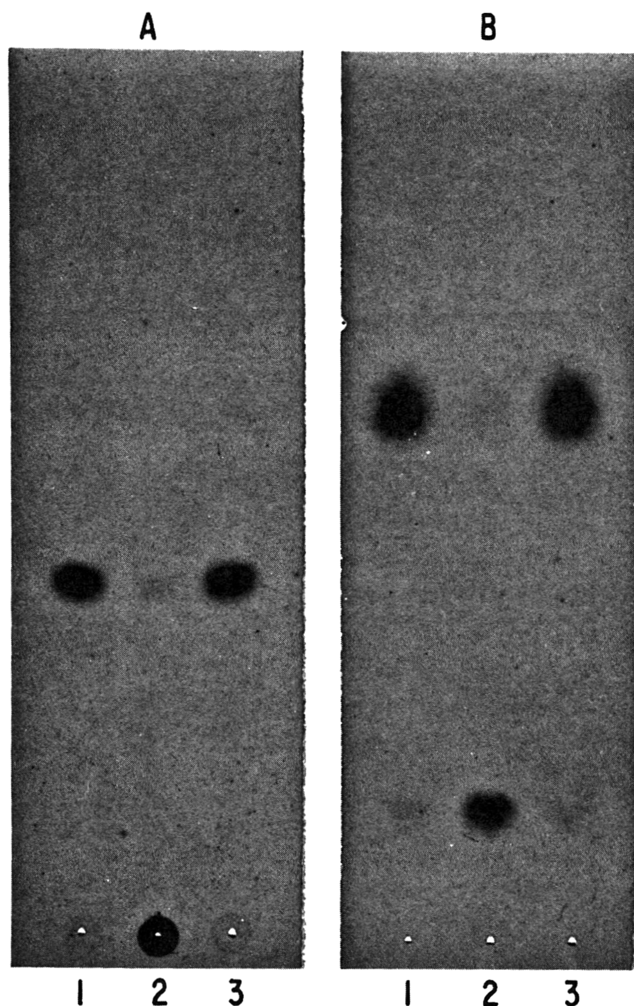

Figure 1. TLC chromatograms of 1, sulfamethazine + riboflavin control; 2, sulfamethazine + riboflavin exposed to 250 ft-candles fluorescent light for 1 h; and 3, sulfamethazine exposed to 250 ft-candles fluorescent light for 1 h. Solvent systems: A, chloroform–ethyl acetate–methanol (5 + 5 + 1); B, upper layer *N*-butanol–acetic acid–water (4 + 1 + 5).

pounds were visualized with the *N*-chlorination procedure of Schwartz and Sherman (7). N^4-Glucopyranosylsulfamethazine and its photodegradation product were visualized with the B-M spray reagent following acid hydrolysis (1). Photodegradation was considered positive when the color intensity of the parent compound obviously decreased and/or an additional spot(s) appeared within 1 h of light exposure.

Liquid Chromatography (LC) Studies

A solution of 3.54 μg sulfamethazine and 3.6 μg riboflavin or 2.3 μg lumichrome in 1 mL methanol–water (30 + 70) was exposed to fluorescent light as described above; 3.54 μg sulfamethazine in 1 mL aqueous methanol solution served as a control. At predetermined intervals, 50 μL samples were subjected to LC. Sulfamethazine remaining in the exposed solutions was determined by comparing peak heights at the appropriate retention time. The LC system consisted of an Altex Model 100 A pump (Altex Scientific, Inc., Berkeley, CA 94710) connected to a Schoeffel Model SF 770 Spectroflow variable wavelength detector operated at 254 nm; Altex Model 210 sampling valve with a 50 μL loop; 25 cm × 4.6 mm id column of 5 μm Alltech C_{18} (Alltech Associates, Deerfield, IL 60015); mobile phase methanol–water (30 + 70); flow rate 1 mL/min. Retention times: sulfamethazine 8.8 min; riboflavin 15.0 min; lumichrome 55.8 min.

Polar Liver Extracts

An aliquot of 5 g frozen, ground swine or chicken liver was shaken 45 min (Ika-Vibrax VXR shaker, Tekmar Co., Cincinnati, OH 45222) with 20 mL water, methanol, or acetone in 50 mL polypropylene screw-cap centrifuge tube. The samples were centrifuged at 3000 rpm for 5 min. The aqueous supernate was recovered and filtered through a plug of glass wool. The methanol and acetone supernates were recovered and evaporated to near dryness at 50°C under a stream of nitrogen. The residues were taken up in 15 mL water and filtered. The aqueous suspensions were passed through a 5 cm column of Duolite ES-863 resin (Diamond Shamrock Corp., Cleveland, OH 44114) prepared as previously described (1). Preliminary studies showed that riboflavin and FMN were readily removed from aqueous solutions by the resin. The columns were washed with 15 mL water and the flavins eluted with 5 mL methanol. The effluents were extracted with 5 mL hexane and evaporated to near dryness. One mL of 50% aqueous methanol containing 54 μg *S*-methazine was added and the samples were exposed to fluorescent light for 1 h. Following exposure, 2 μL samples were subjected to TLC.

Results and Discussion

The role of riboflavin in the photodegradation of sulfa drugs by fluorescent light in model systems is demonstrated by the TLC results in Figure 1, in which there is a concurrent loss of sulfamethazine and appearance of a polar B-M positive compound. The appearance of a singular sulfamethazine photodegradation product is further illustrated by the LC tracings in Figure 2. As determined by TLC, as little as 0.1 μg riboflavin/mL solution was found to sensitize the photochemical reaction and the rate of photodegradation of sulfamethazine was demonstrated to be a function of the riboflavin concentration and light intensity. The degradation of sulfamethazine in the presence of 3.6 μg riboflavin/mL solution was a first-order type reaction, where $K(s^{-1}) = 1.86 \times 10^{-3}$. An equimolar concentration of lumichrome, shown by LC to be the major photodegradation product of riboflavin in these studies, also catalyzed sulfamethazine photodegradation but at a slower rate $[K(s^{-1}) = 4.72 \times 10^{-4}]$. Significantly, lumichrome is not further degraded in the process.

A compilation of the results on a variety of sulfa drugs and drug derivatives in aqueous methanol solutions subjected to fluorescent light for 1 h in the presence of riboflavin is as follows:

Compounds Photodegraded

Sulfamethazine	Sulfaquinoxaline
Sulfamerazine	Sulfaethoxypyridazine
Sulfadiazine	Sulfabromomethazine
Sulfapyridine	N^4-Acetylsulfamethazine
Sulfathiazole	N^4-Acetylsulfamerazine
Sulfadimethoxine	N^4-Glucopyranosylsulfamethazine

Compounds Not Photodegraded

Sulfaguanidine	Sulfanitran
Sulfanilamide	N^1-Methylsulfamethazine
Sulfabenz	N^1-Methylsulfamerazine

With the exception of sulfathiazole, which photodegraded to several compounds, all photodegraded drugs resulted in one product, characteristic of the drug. All sulfa drugs and derivatives gave negative results in the absence of riboflavin, and degradation stopped on removal of the light source. An analysis of these results suggests that the sulfonamide must contain a heterocyclic amine moiety and free N^1 acid hydro-

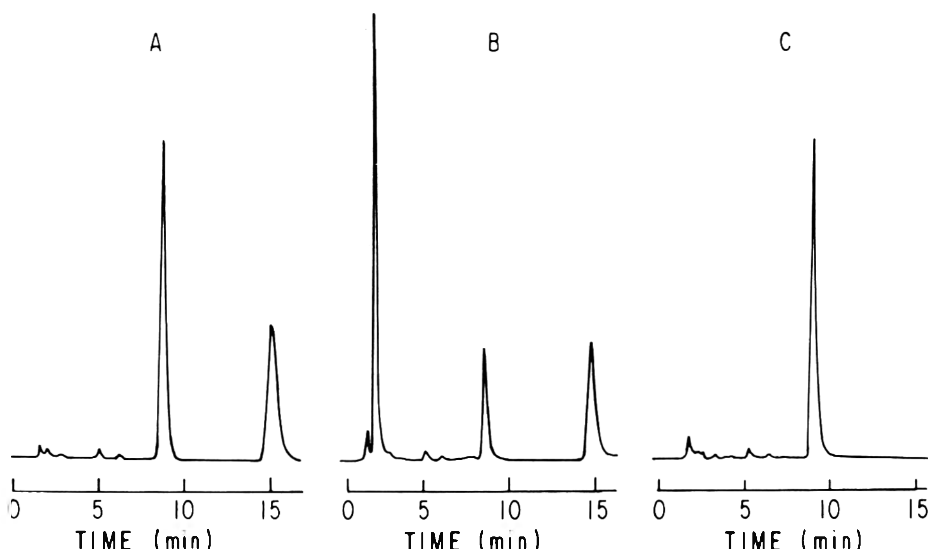

Figure 2. LC chromatograms of A, sulfamethazine + riboflavin control; B, sulfamethazine + riboflavin exposed to 250 ft-candles of fluorescent light for 15 min; and C, sulfamethazine exposed to 250 ft-candles of fluorescent light for 15 min. Retention times: photodegradation product, 2.3 min; sulfamethazine, 8.8 min; and riboflavin, 15 min.

gen on the $-SO_2NH-$ linkage to undergo photodegradation. The results are unaffected by substituents on the N^4 amino group.

TLC of polar liver extracts, spiked with sulfamethazine and exposed to fluorescent light for 1 h, showed the presence of the same photodegradation product as observed in model systems of sulfamethazine. The extent of the photodegradation was not as extensive as expected on the basis of reported riboflavin levels of swine and chicken livers (5). Spiking the tissue extracts with an additional 7.2 μg riboflavin increased the amount of photodegradation but not to the same extent observed in model systems, indicating the relatively crude extracts contained compounds that inhibited or competed with the photodegradation of sulfamethazine. Riboflavin appeared to be photodegraded more readily to lumichrome in the liver extracts which may explain, in part, the slower rate of photodegradation compared with the model system. A further explanation in chicken livers may lie in the observations of Reyes et al. (8) that 85% of riboflavin in chicken liver is present as FMN, which was shown by TLC of model systems to sensitize the photodegradation at a rate comparable to lumichrome.

In conclusion, the results reported herein indicate that it is necessary for the analyst to take precautionary measures to protect liver extracts in which riboflavin, FMN, or lumichrome and sulfa drugs and/or their metabolites are present to prevent losses due to photodegradation as the result of fluorescent light. Protecting such solutions from direct natural light is self-evident. It is recommended that analyses be conducted in subdued lighting and/or that solutions be maintained in protective amber or low-actinic vessels.

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Inventory of IDF/ISO/AOAC Adopted Methods of Analysis for Milk and Milk Products: 1985 Update

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This inventory is intended to provide a complete catalog of adopted methods of analysis prepared by IDF (International Dairy Federation), ISO (International Organization for Standardization) (ISO/TC 34/SC 5), and AOAC for milk and milk products. At the request of the FAO/WHO Committee on the Code of Principles for Milk and Milk Products, the 3 bodies have been cooperating for many years in the preparation of the methods of analysis needed to support the compositional standards associated with the Code of Principles. The present list includes not only these methods, but also indicates standard or adopted methods issued separately by the 3 organizations, and also methods under study by these organizations.

The inventory is intended for use by the 3 organizations, by FAO/WHO (Code of Principles and the Codex Committees on Methods of Analysis and Sampling), and others (for example, the Inter-Agency meeting). Different users will have different requirements; accordingly, the inventory includes 2 lists, one classified on the basis of the products to which the methods apply (Table 1), and the other classified on the property or component being examined or analyzed (in alphabetical order) (Table 2).

The designations "reference" and "routine" in relation to the methods have not been included. Similarly, the classification scheme for methods devised by the Codex Committee on Methods of Analysis and Sampling has not been applied. On the other hand, it has been indicated whether a method has undergone an interlaboratory study to establish its precision.

Although work has not been completed, there are plans to compile an appendix, for use by IDF, ISO, and AOAC, listing those methods still required for the Code of Principles Standards.

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[2]Present address: ISO/TC 34/SC 5, RIKILT, Bornsesteeg 45, 6708 PD Wageningen, The Netherlands.

Key to Symbols and Abbreviations
(Symbols for units and well known abbreviations, for example, TLC = thin layer chromatography, not included)

Column	Symbol/abbrev.	Explanation
Description/principle	RMP	Reichert-Meissl-Polenske value
	BDI	Bureau of Dairy Industries, USA
Collab. study	+	method has been subjected to interlaboratory study (Youden and Steiner and/or ISO 5725 statistics)
	–	Interlaboratory study not appropriate or method has not been subjected to a fully rigorous interlaboratory study
FAO/WHO	B4:1967 (example)	Standard B4 associated with the Code of Principles concerning Milk and Milk Products, published 1967
	19th.App. IX-F (example)	Appendix IX-F to the report of the 19th session of the Committee of Government Experts on the Code of Principles concerning Milk and Milk Products
	CCEI	Codex Committee on Edible Ices
	submitted 1982	method in question was submitted to the Code of Principles Committee at the 1982 session (20th) but did not appear in an appendix to the report of the meeting
IDF	6A:1969 (example)	IDF Standard 6A, published 1969
	(rev.)	standard under revision by a joint IDF/ISO/AOAC group of experts
	Q 983/E (example)	Questionnaire 983/E latest draft
IDF, ISO, and AOAC	IDF/ISO/AOAC Group E 33 (example)	method is under consideration by joint IDF/ISO/AOAC Group E 33
ISO	1740:1980 (example)	ISO Standard 1740, published 1980
	DIS	Draft International Standard
	DP	Draft Proposal
AOAC	OMA	Official Methods of Analysis, 13th edition (1980); 14th edition (1984)
	16.237 (example)	Chapter 16, paragraph 237 in OMA
	not	method referred to is not the same as those listed in the IDF and ISO columns

Table 1. IDF/ISO/AOAC inventory of standard and adopted methods of sampling and analysis for milk and milk products, arranged by product (published or in progress)

Product	Property/component	Description/principle	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Acid casein	ash (fixed)	gravimetric, after incineration at 825°C	–	18th, App. IX-C	89:1979	5544:1978		
Ammonium caseinate	ash (fixed)	gravimetric, after incineration at 825°C	–	18th, App. IX-C	89:1979	5544:1978		
	ammonium					IDF/ISO/AOAC Group E 11		
Baby foods based on dried milk	colony count at 30°C	poured plates (yeast extract, tryptone glucose, skimmed milk)	–			IDF/ISO/AOAC Group E 22		
Butter	acid value; free fatty acid index	titrimetric	+	B4:1967	6A:1969	1740:1980	16.237–16.239	16.211–16.213
	aflatoxin M_1	TLC	+				26.090	26.090
	butter stirrer		–				44.002	44.002
	casein, ash, salt	oven <500°C, Cl/gravimetric	+				16.234	16.208
	chloride	titrimetric (Mohr)	+	B8:1967	12A:1969 (rev.)	1738:1980	16.235	16.209
	color additives	qualitative	+				16.244	16.218
	contaminating organisms	colony count at 30 and 20°C on carbohydrate-free medium	–		30:1964			
	critical temp. of dissolution	thermometric	+				16.241–16.243	16.215–16.217
	DDT residues	GC + identification	+				29.084	29.097
	fat	gravimetric (water, solids-not-fat, and fat content on one test portion)	+	B9:1978	80:1977	3727:1977	16.232	16.206
	fat	gravimetric, direct extraction	+				16.233	16.207
	filth	mold count	+				44.022–44.024	44.018–44.020
	lactic acid	spectrophotometric	+				16.245	16.219
	mold	filtration	+		94:1980	DIS 6611	*not* 44.194	
	organoleptic attributes	sensory evaluation	–		99:1981			
	pH value (of serum)	electrometric	–		104A:1984	7238:1983		
	phosphatase, residual	photometric, 610 nm filter	+				16.256	16.230
	preservatives	various methods	+				16.254	16.228
	refractive index (butterfat)	refractometric	+	B5:1967	7A:1969	1739:1975	16.240	16.214
	sampling		–	B1:1982	50A:1980 (rev.)	707:1985	16.012–16.014, 16.229	16.012–16.014, 16.203
	sediment	vacuum filtration	+				44.021	44.024
	solids-not-fat	gravimetric, on solvent extract	–		11:1960 (rev.)			
	solids-not-fat	gravimetric, on solvent extract (water, solids-not-fat, and fat)	+	B9:1978	80:1977 (rev.)	3727:1977	16.231	
	solids-not-fat	gravimetric (rapid method)	+		Q 185/E	DP 8851		
	total solids (moisture)	gravimetric, drying at 102°C	–		10:1960			
	total solids (water)	gravimetric, drying at 102°C (water, solids-not-fat, and fat on one test portion)	+	B9:1978	80:1977 (rev.)	3727:1977	16.231	16.205
	total solids (water)	gravimetric, heating (rapid method)	+		Q 185/E	DP 8850		
	volatile acids	distillation, chromatographic separation	+				16.253	16.227
	volatile fatty acids	sterol acetate melting point (RMP value)	+				16.236	16.210
	water dispersion	indicator paper comparison	–		112:1962	DIS 7586		
	water-insoluble fatty acids	gravimetric	+				16.246	16.220
	water-insoluble fatty acids + butyric acid	chromatographic	+				16.247–16.251	16.221–16.225
Butter, cream, cheese, milk	pesticide residues *(see also organochlorine pesticides)*	GC + identification	+				29.001–29.028	29.001–29.028

Butterfat	iodine value	titrimetric (Wijs)	+				28.023	28.020
	iodine value	titrimetric with Na thiosulfate (Wijs)	–		8:1959			
Butteroil *(see also Milkfat products)*	water	titrimetric (Karl Fischer)	–		23:1964 (rev.)			
	fat	gravimetric (calculation from solids-not-fat and water)	–		24:1964			
Calf rennet and adult bovine pepsin	chymosine and bovine pepsin	clotting time, after chromatographic separation	–		110:1982 (rev.)			
Casein	filth	microscopic, after sieving	+				44.027	44.022
	phosphatase, residual	colorimetric and spectrophotometric	+				16.127–16.129	—
Caseinate *(see Ammonium caseinate)*								
Caseins *(see also Acid casein, Rennet casein)*	acidity (free acidity)	titrimetric, on aqueous extract	–	19th App. IX-F	91:1979	5547:1978		
Caseins and caseinates	ash (fixed ash)	gravimetric, after incineration at 825°C	–	19th App. IX-D	89:1979	5544:1978		
	colony count at 30°C	poured plates (yeast extract, tryptone glucose, skimmed milk)	–		Q 883/E	DP 8198		
	extraneous matter		–			IDF/ISO/AOAC Group E 11		
	fat	gravimetric (Schmid-Bondzynski-Ratzlaff)	+		Q 3183/E	DIS 5543		
	lactose	photometric, with phenol and H_2SO_4	–	19th App. IX-A	106:1982	5548:1980		
	nitrate and nitrite	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	+		120:1984	DP 8195		
	nitrogen (protein)	titrimetric, Kjeldahl	–	19th App. IX-E	92:1979	5549:1978		
	pH value	electrometric measurement	–		115:1982	5546:1979		
	scorched particles	comparison with standard disks, after filtering		submitted 1982	107:1982	5739:1983		
	total solids (water)	gravimetric drying at 102°C	–	19th App. IX-B	78B:1980 (rev.)	5550:1978		
	whey protein		–			IDF/ISO/AOAC Group E 11		
Cheese	chloride	titrimetric	+				16.272	
	ash	furnace <550°C	+				16.267	16.241
	chloride	indicating-strip method	+				16.273	16.244
	chloride	potentiometric titrimetric	+	B18:1978	88:1979	5943:1978		—
	citric acid	gravimetric	+				16.291	16.262
	citric acid	qualitative test	+				16.290	16.261
	citric acid	photometric	+	B13:1972	34B:1971	2963:1974	16.292–16.296	
	collection of sample		–				16.257	16.231
	color additives	several methods	+				16.277	16.248
	DDT residues	TLC	+			IDF/ISO/AOAC Group E 12	29.109	29.097
	dehydroacetic acid	qualitative	+				20.069–20.070	20.052–20.053
	enzymes (residual)		–			IDF/ISO/AOAC Group E 32		
	examination of fat *(see also volatile fatty acids)*	RMP value, sterol acetate melting point	+				16.286	16.257
	extraneous materials	filtration	+				44.024–44.027	44.020–44.023
	fat	butyrometric (Van Gulik)	–			3433:1975		
	fat butyrometers	requirements (Van Gulik)	–			3432:1975		
	moisture *(see also total solids)*	distillation	+				16.261–16.264	16.235–16.238
	moisture	forced draft oven ± 130°C, screening	+				16.260	16.234
	moisture	microwave oven	+				16.265–16.266	16.239–16.240
	moisture	vacuum oven 100°C	+				16.259	16.233
	monofluoro acid residues	qualitative	+				29.148–29.150	29.140–29.142

Table 1. (continued)

Product	Property/component	Description/principle	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
	natamycin	photometric or LC, after extraction	–			IDF/ISO/AOAC Group E 43		
	nisin		–			IDF/ISO/AOAC Group E 43		
	nitrate	(range 2–15 mg/kg)	–			IDF/ISO/AOAC Group E 8		
	nitrate and nitrite	>1 ppm NO_3, Cd reduction method	+				16.278–16.283	16.249–16.254
	nitrate and nitrite	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	+	B19:1978	84A:1984	4099:1984		
	nitrogen (protein)	titrimetric, Kjeldahl				IDF/ISO/AOAC Group E 27		
	nitrogen (protein)	titrimetric, Kjeldahl	+				16.274	16.245
	organoleptic attributes	sensory evaluation	–		IDF group D6c			
	phosphatase, residual	photometric, 610 nm	+				16.304–16.306	16.275–16.277
	phosphatase, residual	qualitative, screening	+				16.307	16.278
	phosphorus	photometric, molybdate-ascorbate	–	B12:1972	33B:1982	2962:1984		
	sampling		–	B1:1982	50A:1980	707:1985	16.015–16.018	16.015–16.018
	sediment	vacuum filtration	+				44.021	44.024
	sorbic acid	oxidation	+				16.303, 20.115	16.274, 20.098
	tartaric acid	qualitative	+				16.287	16.258
	tartaric acid	quantitative, titrimetric	+				16.288–16.289	16.259–16.260
	titanium	spectrophotometric	+				16.275	16.246
	total solids	gravimetric drying at 102°C	+	20th App. VI-D	4A:1982	5534:1985	all methods differ	
Cheese (cold-pack)	acrylonitrile	gas chromatographic	+				21.A01–21.A05	
Cheese (pasteurized, stabilized)	phosphatase activity	photometric, with Gibbs reagent on phenol liberated	–		53:1969			
Cheese and processed cheese	chloride	potentiometric titration	+	B18:1978	88:1979	5943:1978	16.268–16.271	
	citric acid	photometric, with pyridine and acetic acid	+	B13:1972	34B:1971	2963:1974	16.292–16.296	16.321–16.325
	fat	gravimetric (Schmid-Bondzynski-Ratzlaff)	+	B3:1967	5A:1969	1735:1975	16.284	16.313
	phosphorus	photometric, molybdate-ascorbate	–	B12:1972	33B:1982	2962:1984		
	total solids	gravimetric, drying at 102°C	+	20th App. IV-D	4A:1982	5534:1985	all methods differ	
Cheese (powders)	*Salmonella*	membrane filter	+				46.A06–46.A11	
Chocolate frozen desserts	alginates	confirmation, qualitative	+				16.326	16.297
Cond. milk, sweetened	ash	furnace 525°C (2 methods)	+				16.204	16.184
	lactic acid	spectrophotometric	+				16.202	16.182
	lactose	gravimetric	+				16.207	16.187
	nitrogen (protein)	titrimetric, Kjeldahl	+				16.206	16.186
	sucrose	inversion and polarimetry	+	B14:1972	35:1966	2911:1976	16.208–16.209	16.188–16.189
	total solids	gravimetric drying at 102°C	–	20th App. VI-C	15A:1982	DIS 6734	*not* 16.203	*not* 16.183
Cond. and evap. milk	sampling		–		50A:1980	707:1985	16.008–16.009	16.008–16.009
Cottage cheese	gelatin	qualitative	+				16.302	16.273
Cream	ash	furnace <550°C	+				16.173	16.153
	Babcock cream test bottle		–				16.177(a)	16.157(a)
	collection of sample		–				16.164	16.144
	color additives	qualitative	+				16.181	16.161
	DDT residues	TLC				IDF/ISO/AOAC Group E 12		
	DDT residues	TLC	+				29.109	29.097
	fat	Babcock	+				16.177–16.178	16.157–16.158

	fat	gravimetric (Roese-Gottlieb)	+	B15:1973	16B:1984	DIS 2450	16.176	16.156
	filth	microscopic, after filtration	+				44.022	44.019
	gelatin	qualitative	+				16.179	16.159
	lactic acid	spectrophotometric	+				16.167	16.147
	lactose	gravimetric	+				16.175	16.155
	nitrogen (protein)	titrimetric, Kjeldahl	+				16.174	16.154
	phosphatase, reactivated and residual	differential test for phosphatase activity	+				16.185	16.165
	phosphatase, residual	photometric, 610 nm filter	+				16.183–16.184	16.163–16.164
	preservatives	various methods	+				16.180	16.160
	sediment	vacuum filtration	+				44.021	44.024
	total solids	gravimetric drying at 102°C	+	20th App. VI-B	21A:1982	DIS 6731	16.171	
	volatile acids	chromatographic	+				16.170	16.150
	water-insoluble fatty acids	gravimetric	+				16.168	16.148
Dairy plant surfaces	sampling	microbiological	–		121:1984	DP 8066		
Dried milk	acidity (titratable acidity)	titrimetric, on dissolved product	–		81:1981	6092:1980		
	acidity (titratable acidity)	titrimetric, on dissolved product	–	19th App. IX-H	86:1981	6091:1980		
	ammonium		–			IDF/ISO/AOAC Group E 9		
	ash	furnace <550°C	+				16.216	16.196
	citric acid	gravimetric	+				16.221	16.201
	density	bulk density	–			IDF/ISO/AOAC Group E 2		
	fat	gravimetric	+	B2:1967	9B:1984	DIS 1736	16.219-16.220	16.199-16.200
	flecks		–			IDF/ISO AOAC Group E 2		
	heat class (heat number)	calculation, from Kjeldahl N of precipitated casein plus some denatured serum protein	–		114:1982	DIS 6735		
	insolubility index	dissolution and determination of insoluble residue	–		Q1283/E	DIS 8156		
	lactic acid and lactates	enzymatic	+		69A:1984	DIS 8069		
	neutralizers	calculation (from titratable acidity) and confirmation of Na, K, Ca content	+		102:1981			
	nitrate	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction (rapid method)	+		118:1984	DP 8151		
	nitrate and nitrite	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after reduction	+		95A:1984	6736:1982		
	organoleptic attributes	sensory evaluation	–		99:1981			
	protein denaturation	(extra-low heating)	–			IDF/ISO/AOAC Group E 17		
	protein denaturation	(heat classification)	–			IDF/ISO/AOAC Group E 17		
	sampling		–	B1:1982	50A:1980	707:1985	16.210	16.190
	sodium, potassium and calcium	flame photometric	+		119:1984	DIS 8070		
	staphylococci, coagulase-positive	detection, after incubation at 37°C (Giolitti and Cantoni, Baird-Parker)	–		60A:1978			
	Staphylococcus aureus	colony count at 37°C	–		Q1285E	DP8869		
	suitability for cheesemaking		–			IDF/ISO/AOAC Group E 17		
	total solids (water)	gravimetric, drying at 102°C	–		26:1964			
Dried milk (enriched)	vitamin A	LC, colorimetry	+			IDF/ISO/AOAC Group E 46		
Dried milk (instant)	dispersibility and wettability	dissolution and determination of solids dissolved, wetting time	–		87:1979			
Dried milk and dried milk products	sampling		–	B1:1982	50A:1980	707:1985	16.010–16.011	16.010–16.011
Dried milk borers	sampling instrument	requirements	–		71:1973 (rev.)			

Table 1. (continued)

Product	Property/component	Description/principle	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Dried milk, dried whey	coliforms	MPN at 30°C (brilliant green lactose bile)	–		64:1971 (rev.)	DIS 5541/2		
Dried milk, dried whey, lactose	colony count at 30°C	poured plates (yeast extract, tryptone glucose, skimmed milk)	–		109:1982	DP 7924		
Dried milk, dried whey, dried buttermilk, dried butter serum	fat	gravimetric (Roese-Gottlieb)	+	B2:1967	9B:1984	DIS 1736	16.219–16.220	16.199–16.200
Dried milk, nonfat dry milk, malted milk	moisture *(see also total solids)*	ventilated oven 100°C	+				16.212	16.192
	nitrogen (protein)	dye-binding, spectrophotometric 480 nm	+				16.214	16.194
	nitrogen (protein)	titrimetric, Kjeldahl	+				16.213	16.193
	lactic acid	spectrophotometric	+				16.222	16.202
	Salmonella	membrane filter	+				46.A06–46.A11	
	sulfur amino acids	ion exchange chromatographic	+				43.A08–43.A13	
Dry skim milk	alkalinity of ash	titrimetric	+				16.217	16.197
Edible ices	density	immersion	+	CCEI	Q 1480/E	DP 6738	16.310	16.281
	total solids	gravimetric, drying at 102°C	+		70:1972	3728:1977	*not* 16.313	
Edible ices and ice-mixes (milk-based)	fat	gravimetric (Roese-Gottlieb)	+	submitted 1982	116:1983	7328:1984	16.316	16.287
	fat	gravimetric (Weibull-Berntrop)	–		Q3283/E	DIS 8262/2		
Evap. milk, unsweetened	preservatives	various methods	+				16.198	16.178
	albumin *(see also whey protein)*	titrimetic, Kjeldahl in filtrate of 16.194, 16.174	+				16.195	16.175
	ash	furnace <550°C	+				16.190	16.170
	casein	titrimetic, Kjeldahl, ppt after acid-washing	+				16.194	16.174
	color additives	qualitative	+				16.199	16.179
	gelatin	qualitative	+				16.197	16.177
	lactic acid	spectrophotometric	+				16.188	16.168
	lactose	polarimetric or gravimetric	+				16.196	16.176
	lead	AAS	+				16.191	16.171
						IDF/ISO/AOAC Group E 15		
	lead	spectrometric	+		Q2085/E	DP 6733		
	nitrogen (protein)	titrimetric, Kjeldahl	+				16.193	16.173
	tin	AAS	+				25.A01–25.A04	
						IDF/ISO/AOAC Group E 15		
	total solids	gravimetric at 102°C	+	20th App. VI-B	21A:1982	DIS 6731	16.189	
Evap. (unsweetened) and cond. (sweetened) milks	fat	gravimetric (Roese-Gottlieb)	+	B7:1967	13B:1984	DIS 1737	16.205, 16.192	16.185, 16.172
Fermented milks	coliforms	MPN at 30°C (brilliant green lactose bile)	–		65:1971 (rev.)			
	contaminating organisms	colony count at 30 and 20°C on carbohydrate-free medium	–		66:1971			
Fluid milk products	beta-lactam (see also penicillin)	qualitative color reaction	+				16.152–16.157	—
Ice cream	separation of fat from ice cream	extraction + detection of fats	+				16.317	16.288

Ice cream and frozen desserts	color additives	qualitative	+			16.328	16.299
	gums	infrared	+			16.319–16.325	16.290–16.296
	lactic acid	spectrophotometric	+			16.318	16.289
	nitrogen (protein)	dye-binding	+			16.315	16.286
	nitrogen (protein)	titrimetric, Kjeldahl	+			16.314	16.285
	phosphatase, residual	photometric, 610 nm filter	+			16.329	16.300
	total solids	oven 100°C	+			16.313	16.284
Ice cream and milk ices	coliforms	colony count at 30°C and MPN (brilliant green lactose bile)	–	62:1971 (rev.)	DIS 5541/2		
	colony count at 30°C	poured plates (yeast extract, tryptone, glucose, skimmed milk)	–	61:1971 (rev.)			
Ice-mixes *(see Edible Ices)*							
Infant foods (milk-based, less than 5% starch)	fat	gravimetric (Roese-Gottlieb)	+	Q 384/E	DIS 8381		
Infant foods (other types)	fat	gravimetric (Weibull-Berntrop)	+	Q 3283/E	DIS 8262/1		
Infant formula (milk-based)	vitamins, minerals	spectrometric	+			43.A21–43.A40	
Light cream, skim, whole milk	phosphatase, residual	qualitative, screening			IDF/ISO/AOAC Group E 51		
	phosphatase, residual	qualitative, screening	+			16.123–16.124	16.127–16.128
Milk	acidity	titrimetric	+			16.023	16.023
	added water *(see also freezing point)*	refractometric (acetic serum)	+			16.093	16.907
	added water	refractometric (copper serum)	+			16.094	16.098
	added water, freezing point	osmometric, vapor pressure	+			16.101–16.104	16.105–16.108
	albumin *(see also whey protein)*	titrimetric, Kjeldahl in filtrate of 16.047	+			16.050	—
	antibiotics *(see also penicillin)*	many methods	–		IDF/ISO/AOAC Group E 47		
	ash	furnace < 550°C	+			16.035	16.035
	ash (minerals)	automated methods	–		IDF/ISO/AOAC Group E 29		
	automated methods *(see also individual components)*	statistics, overall accuracy and calibration	–	Q 983/E	DP 8196		
	calcium	titrimetric, after oxalate precipitation	–	36:1966			
	casein	titrimetric, Kjeldahl in filtrate and in milk	+			16.048–16.049	16.048–16.049
	casein	titrimetric, Kjeldahl N on acetic acid precipitate	+	29:1964		16.047	16.047
	cesium 137	gamma-ray spectroscopy	+			48.025	48.025
	citric acid	gravimetric	+			16.024–16.026	16.024–16.026
	collection of sample		–			16.019–16.020	16.019–16.020
	colony count at 30°C	plate loop inoculation	–	Q 984/E	DP 8553		
	colony count at 30°C	poured plates (yeast extract, tryptone, glucose, skimmed milk)	–	100:1981	DIS 6610		
	color additives	qualitative	+			16.109	16.113
	correction table for specific gravity *(see also density)*		–			52.023	52.023
	crude fat or extract	ether extraction	+			7.064	7.059
	DDT residues	TLC		75B:1983	DIS 3890	29:001	
	DDT residues	colorimetric	+			29.109	29.097
	ethylenethiourea residues	GC	+			29.119	29.112
	extraneous matter	sediment	+			16.110	16.114
	fat				IDF/ISO/AOAC Group E 29		
	fat	automated, Foss Electric, Mark III, and UFM 100	+			16.068–16.072	16.063–16.067
	fat	Babcock	+			16.065–16.066	16.060–16.061
	fat	butyrometric (Gerber)	–		2446:1976		

Table 1. (continued)

Product	Property/component	Description/principle	Collab. study
	fat	gravimetric (Roese-Gottlieb)	+
	fat	IR (automated method)	−
	fat butyrometers	requirements (Gerber)	−
	fat, protein, lactose, total solids	automated	+
	freezing point *(see also added water)*	cryoscopic (thermistor)	+
	freezing point	cryoscopic, Hortvet	+
	hydrogen peroxide	qualitative	+
	hypochlorites, chloramines	qualitative	+
	iodide		−
	lactic acid	spectrophotometric	+
	lactose	enzymatic	+
	lactose	gravimetric	+
	lactose	infrared	
	lactose	infrared	+
	lactose	polarimetric	+
	lactose	titrimetric, with chloramine T and KI	−
	lead		
	lead	anodic stripping voltammetry	+
	nitrogen (protein)	dye-binding (Orange 12)	+
	nitrogen (protein)	dye-binding (Amido Black)	+
	nitrogen (protein)	infrared	+
	nitrogen (protein)	titrimetric, Kjeldahl	+
	penicillin *(see also antibiotics)*	zones of inhibition of *Bacillus stearothermophilus* (disk assay)	+
	penicillins	affinity quantitative	+
	penicillins		
	penicillins	qualitative disk II	+
	penicillins	quantitative disk *(Bacillus stearothermophilus)*	+
	penicillins	quantitative overnight method	+
	penicillins	zones of inhibition of *Bacillus subtilus* (field disk assay)	+
	phosphatase, reactivated and residual	differential test for phosphatase activity	+
	phosphatase, residual	colorimetric or spectrophotometric, 650 nm	+
	phosphatase, residual	dialysis, spectrophotometric, 550 nm	+
	phosphatase, residual (raw milk)	photometric, 610 nm	
	phosphorus	photometric, NH_4 phosphomolybdate	−
	preservatives, formaldehyde, benzoic or salicylic acid	various methods	+
	protein-reducing substances	spectrophotometric	+
	psychrotrophic organisms	colony count at 6, 5°C, 10 days	−
	psychrotrophic organisms	colony count at 21°C, 25 h	−
	quaternary ammonium compounds	qualitative and quantitative methods	+
	somatic cell count	automatic counter	+
	specific gravity *(see also density)*	pycnometer or std hydrometer	
	strontium-89 and strontium-90	ion exchange	+
	total solids	infrared	+
	total solids	lactometer	+
	total solids	microwave, IR (automated methods)	−

FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
B6:1967	1B:1983	1211:1984	16.064	16.059
		IDF/ISO/AOAC Group E 29		
	105:1981	488:1983		
		IDF/ISO/AOAC Group E 29		
			16.083–16.092	16.078–16.097
submitted 1982	108:1982	DIS 5764	16.096–16.100	16.100–16.104
			16.095	16.099
			20.083	20.066
			16.107	16.111
		IDF/ISO/AOAC Group E 15		
			16.027–16.031	16.027–16.031
			16.059–16.063	—
			16.057	16.057
		IDF/ISO/AOAC Group E 29		
			16.058	16.058
			16.055–16.056	16.055–16.056
	28:1974			
		IDF/ISO/AOAC Group E 15		
			25.101	25.080
			16.037	16.037
	98:1980	5542:1984	16.042–16.045	16.042–16.045
		IDF/ISO/AOAC Group E 29		
			16.046	16.046
	20:1962 (rev.)	DP 8968	16.036	16.036
	57:1970 (rev.)		16.140–16.145	16.131–16.136
			16.130–16.134	—
		IDF/ISO/AOAC Group E 47		
			16.146–16.151	—
			16.135–16.139	—
			16.163	16.142
			16.158–16.162	16.137–16.141
			16.125–16.126	16.129–16.130
			16.121–16.122	16.125–16.126
			16.116–16.120	16.120–16.124
			16.112–16.114	16.116–16.118
	42:1967 (rev.)			
			16.106	16.110
			16.051–16.054	16.051–16.054
	101:1981	DIS 6730		
	Q1384/E	DP 8552		
			20.077–20.090	20.094–20.107
			16.022	16.022
			16.021	16.021
			48.016–48.024	48.016–48.024
			18.034	18.034
			18.033	18.033
		IDF/ISO/AOAC Group E 29		

	total solids	gravimetric, drying at 102°C	+	20th App. VI-B	21A:1982	DIS 6731	16.032	
Malted and choc. malted milk	casein	titrimetric, Kjeldahl	+				16.215	16.195
Malted milk	fat	gravimetric, Roese-Gottlieb	+				16.218	16.198
Milk and foodstuffs containing milk products	lactose (in the presence of other reducing substances)	photometric, after enzymic formation	–	19th App. IX-G	79:1977	DP 5765		
Milk and liquid milk products	sampling		–	B1:1982	50A:1980	707:1985	16.001–16.018	16.001–16.018
Milk and milk powder, buttermilk and buttermilk powder, whey and whey powder	phosphatase activity	color comparison of *p*-nitrophenol	–		82:1978	DP 6090		
Milk and milk powder, buttermilk and buttermilk powder	phosphatase activity	photometric, with Gibbs reagent on phenol liberated	–		63:1971	3356:1975		
Milk and milk products	aflatoxin M_1		–			IDF/ISO/AOAC Group E 33		
	coliforms	colony count at 30°C and MPN (brilliant green lactose bile)	–		73:1974 (rev.)	DIS 5541/2 and DIS 5541/1		
	copper	AAS	–			IDF/ISO/AOAC Group E 15		
	copper	photometric, diethyldithiocarbamate	+	submitted 1982	76A:1980	5738:1980	25.066–25.071	25.038–25.043
	Enterobacteriaceae		–			IDF/ISO/AOAC Group E 32		
	Escherichia coli		–			IDF/ISO/AOAC Group E 32		
	fat	gravimetric (other than Roese-Gottlieb)	–		Q 3285/E	DIS 8262/3		
	inhibitors		–			IDF/ISO/AOAC Group E 47		
	iron	AAS	–			IDF/ISO/AOAC Group E 15		
	iron	photometric with bathophenanthroline	–	submitted 1982	103:1981	DIS 6732		
	lactose	galactosidase	–			IDF/ISO/AOAC Group E 6		
	lactose	glucose oxidase	–			IDF/ISO/AOAC Group E 6		
	lactose	LC	–			IDF/ISO/AOAC Group E 6		
	lead (canned, liq.) (see evap. milk)							
	lead (canned, liq.)	AAS				IDF/ISO/AOAC Group E 15		
	nitrogen/protein conversion	calculation	–			IDF/ISO/AOAC Group E 27		
	organochlorine pesticides	many methods	+		75B:1983	DIS 3890	29.001–29.028	29.001–29.028
	organoleptic attributes	sensory evaluation (general)	–		99:1981	5495 (methodology)		
	polychlorinated biphenyls (PCBs)	many methods	–		Q 2483/E	DP 8260		
	reproducibility and repeatability	statistics	–		Q 2385/E	5725:1981		
	Salmonella	pre-enrichment, enrichment on selective media, recognition, confirmation	–		93:1980	DIS 6785		
	sample preparations and dilutions	preparation for microbiological examination			IDF122:1984	DP 8261		
	scorched particles, milk and milk products, caseins and caseinates	visual comparison with standard disks, after filtering		submitted 1982	107:1982	5739:1983		
	sampling	attributes sampling schemes	–	20th App. VI-A	113:1982	DP 5583		
	sampling	general sampling techniques	–	B1:1982	50A:1980 (rev.)	707:1985	16.001–16.005	16.001–16.005
	sampling	variable sampling schemes	–		Q 1083/E	DP 8197		
	staphylococci, thermonuclease	color zones on toluidine blue 0-DNA medium	–		83:1978			
	yeasts and molds	chloramphenicol agar, colony count at 25°C, 4 days	–		94:1980 (rev.)	DIS 6611		
	zinc		–			IDF/ISO/AOAC Group E 15		

Table 1. (continued)

Product	Property/component	Description/principle	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Milk chocolate	milk protein					IDF/ISO/AOAC Group E 53		
	milk protein	titrimetric, Kjeldahl	+				13.047	13.047
	milkfat	calculation from RMP value	+				13.039–13.046	13.039–13.046
Milk products	vitamin E	TLC isolation, colorimetric	+				43.129	43.088
Milk, butter	lipolytic organisms	colony count at 30°C, sugar-free medium, Victoria blue indicator	–		41:1966			
Milk, cream and evaporated milk	total solids	gravimetric, drying at 102°C	+	20th App. VI-B	21A:1982	DIS 6731	16.032, 16.171, 16.189	16.032, 16.151, 16.169
Milk, cream, buttermilk, evap. milk	gelatin	qualitative	+				16.105	16.109
Milk, dried milk, cheese	aflatoxin M_1	fluorodensitometric, after chromatographic separation	+		111:1982 (rev.)	DP 7923	26.095, 26.090	26.090
Milk-based foods, milk products (special cases) (see Infant foods)								
Milk-based infant foods with little starch	fat	gravimetric (Roese-Gottlieb)	–		Q 384/E	DP 8381		
Milkfat	foreign fat	GC of sterois	+	B17:1978	54:1979	3594:1976	28.100	28.089
	foreign fat	phytosteryl acetate test	+	B16:1978	32:1965 (rev.)	3595:1976	28.096	28.085
	foreign fat	TLC of steryl acetates	–		38:1966 (rev.)			
	volatile fatty acids	titrimetric, after saponification and distillation	–		37:1966			
Milkfat (anhydrous)	peroxide value	photometric, $FeCl_3$, NH_4CNS	+	B20:1978	74:1974	3976:1977	*not* 28.025	*not* 28.022
Milkfat and other milk products	free fatty acids	BDI, auto-analyzer	–			IDF/ISO/AOAC Group E 39		
	free fatty acids	titration, colorimetry, GC	–			IDF/ISO/AOAC Group E 39		
Milkfat products	foreign fat	GC of sterols	+				28.104	28.093
	solids-not-fat	calcn of fat	–		Q 185/E	DP 8852		
Milkfat, milk products	BHA		–			IDF/ISO/AOAC Group E 43		
	BHT		–			IDF/ISO/AOAC Group E 43		
	TBHQ		–			IDF/ISO/AOAC Group E 43		
Milk powder (see dried milk)								
Multicomponent food (milk products)	cholesterol	GC	+				43.283	43.229
Nonfat dry milk	*N*-nitrosodimethylamine	GC	+				16.223–16.228	—
Pasteurized milk	coliforms	MPN at 30°C (brilliant green lactose bile)	–		40:1966			
Pepsin *(see calf rennet)*								
Processed cheese	lactose	polarimetric	+				16:297	16:268
	nitrogen (protein)	titrimetric, Kjeldahl	–		25:1964			

Processed cheese products	ash	gravimetric, after incineration at 550°C	–		27:1964			
	phosphate emulsifying agents	calculation	–		51:1969			
	citrate emulsifying agents	calculation	–		52:1969			
Raw milk	coliforms	colony count at 30°C (violet red bile)	–		39:1966 (rev.)	DIS 5541/1		
	psychrotrophic organisms	colony count at 21°C, 25 h	–		Q 1384/E	DP 8552		
	psychrotrophic organisms	colony court at 6.5°C, 10 days	–		101:1981	DIS 6730		
Raw, whole, mixed herd milk	fat	automated				IDF/ISO/AOAC Group E 29		
	fat	automated	+				16.074–16.082	16.069–16.077
Rennet (*see calf rennet*)								
Rennet casein, and caseinates	ash	gravimetric, after incineration at 825°C	–	19th App. IX-C	90:1977	5545:1978		
Skimmed milk, whey, buttermilk	fat	gravimetric (Roese-Gottlieb)	+	submitted 1982	22A:1983	7208:1984		
Soft curd cheese	gums	qualitative	+				16.298–16.301	16.269–16.272
Sterilized milk	sampling and sample preparation	specific sampling technique	–		48:1969 (rev.)			
	stability (ethanol)	observation of precipitation on mixing with ethanol	–		48:1969 (rev.)			
Sweetened condensed milk	fat	gravimetric (Roese-Gottlieb)	+	B7:1967	13B:1984	DIS 1737	16:192	
	lactic acid	spectrophotometric	+				16.202	
	lactose	gravimetric	+				16.207	
	lead					IDF/ISO/AOAC Group E 15		
	nitrogen (protein)	titrimetric, Kjeldahl	+				16.274	
	sucrose	inversion and polarimetry	+	B14:1972	35:1966	2911:1976	16.208–16.209	
	total solids		+	20th App. VI-C	15A:1982	DIS 6734	*not* 16.203	
Whey cheese	fat	gravimetric (Roese-Gottlieb)	+	B10:1973	59:1970 (rev.)	1854:1972 (rev.)	16.285	16.256
	nitrate and nitrite	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	+		96:1980	6739:1982		
	total solids (dry matter)	gravimetric, drying at 88°C	–	B11:1970	58:1970	2920:1974		
Whey powder	nitrate and nitrite	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	+		97A:1984	DIS 6740		
Whole dry, nonfat milk	*Salmonella*	isolation in selective broth	+				46.115–46.119	46.054–46.058
Yogurt	benzoic acid	LC, capillary GC	–			IDF/ISO/AOAC Group E 43		
	Lactobacillus bulgaricus	identification	–			IDF/ISO/AOAC Group E 44		
	sorbic acid	LC, capillary GC				IDF/ISO/AOAC Group E 43		
	Streptococcus thermophilus		–			IDF/ISO/AOAC Group E 44		
	sulfite	LC, capillary GC	–			IDF/ISO/AOAC Group E 43		
	total solids		–			IDF/ISO/AOAC Group E 5		
	yogurt organisms	total count at 37°C (acidified MRS and M17 media)	–		117:1983	DIS 7889		

Table 2. IDF/ISO/AOAC inventory of standard and adopted methods of sampling and analysis for milk and milk products, arranged by component (published or in progress)

Property/component	Description/principle	Product	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Acid value and free fatty acid index	titrimetric	butter	+	B4:1967	6A:1969 (rev.)	1740:1980	16.237–16.239	16.211–16.213
Acidity	titrimetric	milk	+				16.023	16.023
	titrimetric	cheese	+				16.276	16.247
Acidity (free)	titrimetric, on aqueous extract	caseins	–	19th App. IX-F	91:1979	5547:1978		
Acidity (titratable)	titrimetric, on dissolved product	dried milk	–		81:1981	6092:1980		
	titrimetric, on dissolved product	dried milk	–	19th App. IX-H	86:1981	6091:1980		
Acids (citric, fatty)								
Acrylonitrile	gas chromatographic	cheese (cold pack)	+				21.A01–21.A05	
Added water *(see also freezing point)*	refractometric (acetic serum)	milk	+				16.093	16.097
	refractometric (copper serum)	milk	+				16.094	16.098
Added water, freezing point	osmometric, vapor pressure	milk	+				16.101–16.104	16.105–16.108
Additives *(see individual additives)*								
Aerobic count *(see colony count at 30°C)*								
Aflatoxin M_1		milk and milk products	–			IDF/ISO/AOAC Group E 33		
	fluorodensitometric, after chromatographic separation	milk, dried milk, cheese	+		111:1982	DP 7923	26.095, 26.090	26.090
	TLC	butter	+				26.090	26.090
Albumin *(see also whey protein)*	titrimetric, Kjeldahl	evap. milk, unsweetened	+				16.195	16.175
	titrimetric, Kjeldahl	milk	+				16.050	16.050
Alginates	confirmation, qualitative	chocolate frozen desserts	+				16.326	16.297
Alkalinity of ash	titrimetric	dry skim milk	+				16.217	16.197
Ammonium		ammonium caseinate	–			IDF/ISO/AOAC Group E 11		
		dried milk	–			IDF/ISO/AOAC Group E 9		
Antioxidants *(see BHA, BHT, TBHQ)*								
Antibiotics *(see also Penicillin)*	many methods	milk	–			IDF/ISO/AOAC Group E 47		
Ash	furnace < 550°C	milk	+				16.035	16.035
	furnace < 550°C	cheese	+				16.267	16.241
	furnace < 550°C	cream	+				16.173	16.153
	furnace < 550°C	dried milk	+				16.216	16.196
	furnace < 550°C	evap. milk, unsweetened	+				16.190	16.170
	furnace 525°C (2 methods)	cond. milk, sweetened	+				16.204	16.230
	gravimetric, after incineration at 550°C	processed cheese products	–		27:1964			
	gravimetric, after incineration at 825°C	acid casein	–	18th App. IX-C				
	gravimetric, after incineration at 825°C	rennet casein and caseinates	–	19th App. IX-C	90:1977	5545:1978		
Ash (fixed ash)	gravimetric, after incineration at 825°C	caseins and caseinates	–	19th App. IX-D	89:1979	5544:1978		
Ash (minerals)	automated methods	milk	–			IDF/ISO/AOAC Group E 29		

Automated methods *(see also individual components)*	statistics, overall accuracy and calibration	milk	–		Q 983/E	DP 8196		
Babcock cream test bottle		cream	+				16.177(a)	16.157(a)
Benzoic acid	LC, capillary GC	yogurt	–			IDF/ISO/AOAC Group E 43		
Beta-lactam *(see also Penicillin)*	qualitative color reaction	fluid milk products	+				16.152–16.157	—
BHA		milkfat, milk products	–			IDF/ISO/AOAC Group E 43		
BHT		milkfat, milk products	–			IDF/ISO/AOAC Group E 43		
Butter stirrer		butter	–				44.002	44.002
Calcium	titrimetric, after oxalate precipitation	milk	–		36:1966			
Calcium (dried milk) *(see Sodium)*								
Casein	titrimetric, Kjeldahl, ppt after acid-washing	evap. milk, unsweetened	+				16.194	16.174
	titrimetric, Kjeldahl in filtrate and in milk	milk	+				16.048–16.049	16.048–16.049
	titrimetric, Kjeldahl	malted and choc. malted milk	+				16.215	16.195
	titrimetric, Kjeldahl N on acetic acid precipitate	milk	+		29:1964		16.047	16.047
Casein, ash, salt	oven < 500°C, Cl^- gravimetric	butter	+				16.234	16.208
Cesium-137	gamma-ray spectroscopy	milk	+				48.025	48.025
Chloride	indicating-strip method	cheese	+				16.273	16.244
	potentiometric titration	cheese and processed cheese	+	B18:1978	88:1979	5943:1978	16.268–16.271	
	titrimetric	cheese	+				16.272	—
	titrimetric (Mohr)	butter	+	B8:1967	12A:1969 (rev.)	1738:1980	16.235	16.209
Cholesterol	GC	multicomponent food (milk products)	+				43.283	43.229
Chymosine and bovine pepsin	clotting time, after chromatographic separation	calf rennet and adult bovine pepsin	–		110:1982			
Citrate emulsifying agents	calculation	processed cheese products	–		52:1969			
Citric acid	gravimetric	cheese	+				16.291	16.292
	gravimetric	dried milk	+				16.221	16.201
	gravimetric	milk	+				16.024–16.026	16.024–16.026
	photometric, with pyridine and acetic anhydride	cheese and processed cheese	+	B13:1972	34B:1971	2963:1974	16.292–16.296	16.321–16.325
	qualitative test	cheese	+				16.290	16.261
Coagulase-positive staphylococci *(See Staphylococci)*								
Coliforms	colony count at 30°C (violet red bile)	raw milk	–		39:1966			
	colony count at 30°C and MPN (brilliant green lactose bile)	ice cream and milk ices	–		62:1971 (rev.)			
	colony count at 30°C and MPN (brilliant green lactose bile)	milk and milk products	–		73:1974 (rev.)	DIS 5541/ 1&2		
	MPN at 30°C (brilliant green lactose bile)	dried milk, dried whey	–		64:1971 (rev.)			
	MPN at 30°C (brilliant green lactose bile)	fermented milks	–		65:1971 (rev.)			
	MPN at 30°C (brilliant green lactose bile)	pasteurized milk	–		40:1966			

Table 2. (continued)

Property/component	Description/principle	Product	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Collection of sample		cheese	–				16.257	16.231
		cream	–				16.164	16.144
		milk	–				16.019–16.020	16.019–16.020
Colony count at 30°C	poured plates (yeast extract, tryptone glucose, skimmed milk)	baby foods based on dried milk	–			IDF/ISO/AOAC Group E 22		
	poured plates (yeast extract, tryptone glucose, skimmed milk)	caseins and caseinates	–		Q 883/E	DP 8198		
	poured plates (yeast extract, tryptone glucose, skimmed milk)	dried milk, dried whey, lactose	–		109:1982	DP 7924		
	poured plates (yeast extract, tryptone glucose, skimmed milk)	ice cream and milk ices	–		61:1971 (rev.)			
	poured plates (yeast extract, tryptone glucose, skimmed milk)	milk	–		100:1981	DIS 6610		
	plate loop inoculation	milk	–		Q 984/E	DP 8553		
Color additives	qualitative	butter	+				16.244	16.218
	qualitative	ice cream and frozen desserts	+				16.328	16.299
	qualitative	milk	+				16.109	16.113
	qualitative	cream	+				16.181	16.161
	qualitative	evap. milk, unsweetened	+				16.199	16.179
	several methods	cheese	+				16.277	16.248
Contaminating organisms	colony count at 30 and 20°C on carbohydrate-free medium	butter	–		30:1964			
	colony count at 30 and 20°C on carbohydrate-free medium	fermented milks	–		66:1971			
Copper	AAS	milk and milk products	–			IDF/ISO/AOAC Group E 15		
	photometric, diethyldithiocarbamate	milk and milk products	+	submitted 1982	76A:1980	5738:1980	25.066–25.071	25.038–25.043
Correction table for specific gravity *(see also Density)*		milk	–				52.023	52.023
Critical temp. of dissolution	thermometric	butter	+				16.241–16.243	16.215–16.217
Crude fat or extract	ether extraction	milk	+				7.064	7.059
DDT residues	GC + identification	butter	+				29.109	29.097
	TLC	cheese				IDF/ISO/AOAC Group E 12		
	TLC	cheese	+				29.109	29.097
	TLC	cream				IDF/ISO/AOAC Group E 12		
	TLC	cream	+				29.109	29.097
	TLC	milk				IDF/ISO/AOAC Group E 12		
	TLC	milk	+				29.109	29.097
Dehydroacetic acid	qualitative	cheese	+				20.069–20.070	20.052–20.053
Density	bulk density	dried milk	–		Q 2585/E	DP 8967		
	immersion	edible ices	+	CCEI	Q 1480/E	DP 6738	16.310	16.281
Dilutions *(see Sample preparation)*								
Dispersibility and wettability	dissolution and determination of solids dissolved, wetting time	dried milk (instant)	–		87:1979			

Dry matter *(see total solids)*								
Emulsifying agents *(see Citrate, phosphate)*								
Enterobacteriaceae		milk and milk products	–			IDF/ISO/AOAC Group E 32		
Enzymes (residual)		cheese	–			IDF/ISO/AOAC Group E 32		
Enzymic activity *(see Phosphatase)*								
Escherichia coli		milk and milk products	–			IDF/ISO/AOAC Group E 32		
Ethylenethiourea residues	GC	milk	+				29.119	29.112
Examination of fat *(see also Volatile fatty acids)*	RMP value, sterol acetate melting point	cheese	+				16.286	16.257
Extraneous materials	filtration	cheese	+				44.024–44.027	44.020–44.023
		caseins and caseinates	–			IDF/ISO/AOAC Group E 11		
	sediment	milk	+				16.110	16.114
Fat	automated, Foss Electric, Mark III and UFM 100	milk	+			IDF/ISO/AOAC Group E 29	16.068–16.072	16.063–16.067
	automated, Technicon Instr.	raw, whole, mixed herd milk	+			IDF/ISO/AOAC Group E 29	16.074–16.082	16.069–16.087
	Babcock	milk	+				16.065–16.066	16.060–16.061
	Babcock	cream	+				16.177–16.178	16.157–16.158
	gravimetric (Roese-Gottlieb)	ice cream and frozen desserts	+				16.316	16.287
	butyrometric (Gerber)	milk	–			2446:1976		
	butyrometric (Van Gulik)	cheese	–			3433:1975		
	gravimetric (calculation from solids-not-fat and water)	butteroil (milkfat products)	–		24:1964			
	gravimetric (Roese-Gottlieb)	cream	+	B15:1973	16B:1984	DIS 2450	16.176	16.156
	gravimetric (Roese-Gottlieb)	dried milk, dried whey, dried buttermilk	+	B2:1967	9B:1984	DIS 1736	16.219–16.220	16.199–16.200
	gravimetric (Roese-Gottlieb)	edible ices and ice-mixes	+	submitted 1982	116:1983	7328:1984	16.287	16.258
	gravimetric (Roese-Gottlieb)	evap. (unsweetened) and cond. (sweetened) milk	+	B7:1967	13B:1984	DIS 1737	16.205, 16.192	16.231, 16.172
	gravimetric (Roese-Gottlieb)	milk	+	B6:1967	1B:1983	1211:1984	16.064	16.059
	gravimetric (Roese-Gottlieb)	milk-based infant foods with little starch	–		Q 384/E	DP 8381		
	gravimetric (Weibull)	milk-based infant foods (other types)	+		Q 3283/E	DIS 8262/1		
	gravimetric (Roese-Gottlieb)	skimmed milk, whey, buttermilk	+	submitted 1982	22A:1983	7208:1984		
	gravimetric (Roese-Gottlieb)	whey cheese	+	B10:1973	59:1970 (rev.)	1854:1972 (rev.)	16.285	16.256
	gravimetric (Schmid-Bondzynski-Ratzlaff)	caseins and caseinates	+		Q 3183/E	DIS 5543		
	gravimetric (Schmid-Bondzynski-Ratzlaff)	cheese and processed cheese	+	B3:1967	5A:1969 (rev.)	1735:1975	16.284	16.313
	gravimetric (water, solids-not-fat, and fat content on one test portion	butter	+	B9:1978	80:1977	3727:1977	16.232	16.206
	gravimetric (Weibull)	milk-based foods, milk products (special cases)	+ +		Q 3283/E	DP 8262/3		
	gravimetric (Weibull)	edible ices and ice mixes	+		Q 3283/E	DP 8262/2		
	gravimetric, direct extraction	butter	+				16.233	16.207
	gravimetric (Roese-Gottlieb)	malted milk	+				16.218	16.196
	IR (automated method)	milk	–			IDF/ISO/AOAC Group E 29		
Fat butyrometers	requirements (Gerber)	milk	–		105:1981	488:1983		
	requirements (Van Gulik)	cheese	–			3432:1975		

Table 2. (continued)

Property/component	Description/principle	Product	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Fat, foreign *(see Foreign fat)*								
Fat, protein, lactose, total solids	automated, Irma, Milko-Scan, Multispec	milk	+			IDF/ISO/AOAC Group E 29	16.083–16.092	16.078–16.097
Fatty acids *(see Free fatty acids, Volatile fatty acids)*								
Filth	filtration	butter	+				44.022–44.024	44.018–44.020
	microscopic, after filtration	cream	+				44.022	44.019
	microscopic, after sieving	casein	+				44.027	44.022
Fixed ash *(see Ash)*								
Flecks		dried milk	–			IDF/ISO/AOAC Group E 2		
Foreign fat *(see also milkfat in mixtures)*	GC of sterols	milkfat	+	B17:1978	54:1979	3594:1976	28.100	28.089
	GC of sterols	milkfat products	+				28.104	28.093
	phytosteryl acetate test	milkfat	+	B16:1978	32:1965 (rev.)	3595:1976	28.096	28.085
	TLC of steryl acetates	milkfat	–		38:1966 (rev.)			
Free acidity *(see Acidity)*								
Free fatty acid index *(see Acid value)*								
Free fatty acids	BDI, auto-analyzer	milkfat and other milk products	–			IDF/ISO/AOAC Group E 39		
	titration, colorimetry, GC	milkfat and other milk products	–			IDF/ISO/AOAC Group E 39		
Freezing point *(see also Added water)*	cryoscopic (thermistor)	milk	+	submitted 1982	108:1982	DIS 5764	16.096–16.100	16.100–16.104
Freezing point *(see also Added water)*	cryoscopic, Hortvet	milk	+				16.095	16.099
Gelatin	qualitative	cottage cheese	+				16.302	16.273
	qualitative	ice cream and frozen desserts	+				16.327	16.298
	qualitative	milk, cream, buttermilk, evap. milk	+				16.105	16.109
	qualitative	cream	+				16.179	16.159
	qualitative	evap. milk, unsweetened	+				16.197	16.177
Gums	infrared	ice cream and frozen desserts	+				16.319–16.325	16.290–16.296
	qualitative	soft curd cheese	+				16.298–16.301	16.269–16.272
Heat class (heat number)	calculation, from Kjeldahl N of precipitated casein plus some denatured serum protein	dried milk	–		114:1982	DIS 6735		
Hydrogen peroxide	qualitative	milk	+				20.083	20.066
Hypochlorites, chloramines	qualitative	milk	+				16.107	16.111
Impurities *(see Extraneous matter, Flecks, Scorched particles)*								
Inhibitors		milk and milk products	–			IDF/ISO/AOAC Group E 47		
Insolubility index	dissolution and determination of insoluble residue	dried milk	–		Q 1283/E	DIS 8156		
Iodide		milk	–			IDF/ISO/AOAC Group E 15		

Iodine value	titrimetric (Wijs)	butterfat	+				28.023	28.020
	titrimetric with Na thiosulfate (Wijs)	butterfat	–		8:1959			
Iron	AAS	milk and milk products	–			IDF/ISO/AOAC Group E 15		
	photometric with bathophenanthroline	milk and milk products	–	submitted 1982	103:1981 (rev.)	DIS 6732		
Lactic acid	spectrophotometric	butter	+				16.245	16.219
	spectrophotometric	cond. milk, sweetened	+				16.202	16.182
	spectrophotometric	cream	+				16.167	16.147
	spectrophotometric	dried milk, nonfat dry, malted milk	+				16.222	16.202
	spectrophotometric	evap. milk, unsweetened	+				16.188	16.168
	spectrophotometric	ice cream and frozen desserts	+				16.318	16.289
	spectrophotometric	milk	+				16.027–16.031	16.027–16.031
Lactic acid and lactates	enzymatic	dried milk	–		69A:1984	DIS 8069		
Lactobacillus bulgaricus (see also Yogurt)	identification	yogurt	–			IDF/ISO/AOAC Group E 44		
Lactose	galactosidase	milk and milk products	–			IDF/ISO/AOAC Group E 6		
	glucose oxidase	milk and milk products	–			IDF/ISO/AOAC Group E 6		
	gravimetric	cond. milk, sweetened	+				16.207	16.187
	gravimetric	cream	+				16.175	16.155
	gravimetric	milk	+				16.057	16.057
	LC	milk and milk products	–			IDF/ISO/AOAC Group E 6		
	infrared	milk				IDF/ISO/AOAC Group E 29		
	infrared	milk	+				16.058	16.058
	photometric, with phenol and H_2SO_4	caseins and caseinates	–	19th App. IX-A	106:1982	5548:1980		
	enzymatic	milk	+				16.059–16.063	—
	polarimetric	milk	+				16.055–16.056	16.055–16.056
	polarimetric	processed cheese	+				16.297	16.296
	polarimetric or gravimetric	evap. milk, unsweetened	+				16.196	16.176
	titrimetric, with chloramine T and KI	milk	–		28:1974			
Lactose (in the presence of reducing substances)	photometric, after enzymic formation of NADPH	milk and foodstuffs containing milk products	–	19th App. IX-G	79:1977 (rev.)	DP 5765		
Lead		milk and milk products (canned, liquid)	–			IDF/ISO/AOAC Group E 15		
	spectrometric	evap. milk unsweetened, canned	+		Q 2085/E	DP 6733 IDF/ISO/AOAC Group E 15		
	AAS	evap. milk, unsweetened	+				16.191	16.171
	AAS	milk and milk products, (canned, liquid)	–			IDF/ISO/AOAC Group E 15		
		milk	–			IDF/ISO/AOAC Group E 15		
	anodic stripping voltammetry	milk	+				25.101	25.080
Lipolytic organisms	colony count at 30°C, sugar-free medium, Victoria blue indicator	milk, butter	–		41:1966			
Mass per unit volume *(see Density)*								
Milk protein	titrimetric, Kjeldahl	milk chocolate	+			IDF/ISO/AOAC Group E 53	13.047	13.047
Milkfat	calculation from RMP value	milk chocolate	+				13.039–13.046	13.039–13.046

Table 2. (continued)

Property/component	Description/principle	Product	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Moisture *(see also Total solids)*	distillation	cheese	+				16.261–16.264	16.235–16.238
	forced draft oven ± 130°C, screening	cheese	+				16.260	16.234
	microwave oven	cheese	+				16.265–16.266	16.239–16.240
	vacuum oven 100°C	cheese	+				16.259	16.233
	ventilated oven 100°C	dried milk, nonfat dry milk, malted milk	+				16.212	16.192
Mold	mold count	butter	+		94:1980	DIS 6611	44.194	
Monofluoro acid residues	qualitative	cheese	+				29.148–29.150	29.140–29.142
Mycotoxins *(see Aflatoxin M_1)*								
Natamycin	photometric or LC, after extraction	cheese	–			IDF/ISO/AOAC Group E 43		
Neutralizers	calculation (from titratable acidity) and confirmation of Na, K, Ca content	dried milk	+		102:1981			
Nisin		cheese	–			IDF/ISO/AOAC Group E 43		
Nitrate	(range 2–15 mg/kg)	cheese	–			IDF/ISO/AOAC Group E 8		
	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction (rapid method)	dried milk	+		118:1984	DP 8151		
Nitrate and nitrite	>1 mg/kg NO_3, cadmium reduction method	cheese	+				16.278–16.283	16.249–16.254
	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	caseins and caseinates	+		120:1984	DP 8195		
	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCi after cadmium reduction	cheese	+	B19:1978	84A:1984	4099:1984		
	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	dried milk	+		95A:1984	6736:1982		
	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	whey cheese	+		96:1980	6739:1982		
	photometric, with sulfanilamide and *N*-1-naphthylethylenediamine HCl after cadmium reduction	whey powder	+		97A:1984	DIS 6740		
Nitrogen (protein)	dye-binding (Amido Black)	milk	+		98:1980 (rev.)	5542:1984	16.042–16.045	16.042–16.045
	dye-binding (Orange 12)	milk	+				16.037	16.037
	dye-binding	ice cream and frozen desserts	+				16.315	16.286
	dye-binding, spectrophotometric, 480 nm	dried milk, nonfat dry milk, malted milk	+				16.214	16.194
	infrared	milk				IDF/ISO/AOAC Group E 29		
	infrared	milk	+				16.046	16.046
	titrimetric, Kjeldahl	caseins and caseinates	–	19th App. IX-E	92:1979	5549:1978		
	titrimetric, Kjeldahl	cheese				IDF/ISO/AOAC Group E 27		
	titrimetric, Kjeldahl	cheese	+				16.274	16.245
	titrimetric, Kjeldahl	cond. milk, sweetened	+				16.206	16.186
	titrimetric, Kjeldahl	cream	+				16.174	16.154
	titrimetric, Kjeldahl	dried milk, nonfat dry milk, malted milk	+				16.213	16.193

	titrimetric, Kjeldahl	evap. milk, unsweetened	+				16.193	16.173
	titrimetric, Kjeldahl	ice cream and frozen desserts	+				16.314	16.285
	titrimetric, Kjeldahl	milk	+		20:1962 (rev.)	DP 8968	16.036	16.036
	titrimetric, Kjeldahl	processed cheese	–		25:1964			
Nitrogen/protein conversion	calculation	milk and milk products	–			IDF/ISO/AOAC Group E 27		
N-Nitrosodimethylamine	GC	nonfat dry milk	+				16.223–16.228	—
Organochlorine pesticides	many methods	milk and milk products	+		75B:1983	DIS 3890	29.001–29.028	29.001–29.028
Organoleptic attributes	sensory evaluation	butter	–		99:1981			
	sensory evaluation	cheese	–		IDF group D6c			
	sensory evaluation	dried milk	–		99:1981			
	sensory evaluation (general)	milk and milk products	–		99:1981	5495 (methodology)		
Overrun *(see Density)*								
Oxidizing substances *(see Peroxide value)*								
PCBs *(see Polychlorinated biphenyls)*								
Penicillin *(see also Antibiotics)*	zones of inhibition of *Bacillus stearothermophilus* (disk assay)	milk	+		57:1970 (rev.)		16.140–16.145	16.131–16.136
	zones of inhibition of *Bacillus subtilus* (field disk assay)	milk	+				16.158–16.162	16.137–16.141
	affinity quantitative	milk	+				16.130–16.134	—
		milk				IDF/ISO/AOAC Group E 47		
	qualitative disk II	milk	+				16.146–16.151	—
	quantitative disk *(Bacillus stearotherm.)*	milk	+				16.135–16.139	—
	quantitative overnight method	milk	+				16.163	16.142
Pepsin *(see Chymosin)*								
Peroxide value	photometric, $FeCl_3$, NH_4CNS	milkfat (anhydrous)	–	B20:1978	74:1974	3976:1977	*not* 28.025	*not* 28.022
Pesticide residues *(see also Organochlorine pesticides)*	GC + identification	butter, cream, cheese, milk	+				29.001–29.028	29.001–29.028
pH value	electrometric measurement	caseins and caseinates	–	submitted 1982	115:1982	5546:1979		
pH value (of serum)	electrometric	butter	–		104:1981	7238:1983		
Phosphatase activity	color comparison of *p*-nitrophenol	milk and milk powder, buttermilk and buttermilk powder, whey and whey powder	–		82:1978	DP 6090		
	photometric, with Gibbs reagent on phenol liberated	cheese (pasteurized, stabilized)	–		53:1969			
	photometric, with Gibbs reagent on phenol liberated	milk and milk powder, buttermilk and buttermilk powder, whey and whey powder	–		63:1971	3356:1975		
Phosphatase, reactivated and residual	differential test for phosphatase activity	cream	+				16.185	16.165
	differential test for phosphatase activity	milk	+				16.125–16.126	16.137–16.141

Table 2. (continued)

Property/component	Description/principle	Product	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Phosphatase, residual	colorimetric and spectrophotometric	casein	+				16.127–16.129	—
	colorimetric or spectrophotometric 650 nm	milk	+				16.121–16.122	16.125–16.126
	dialysis, spectrophotometric 550 nm	milk	+				16.116–16.120	16.120–16.124
	photometric, 610 nm	butter	+				16.256	16.230
	photometric, 610 nm	raw milk	+				16.112–16.114	16.116–16.118
	photometric, 610 nm	cheese	+				16.304–16.306	16.275–16.277
	photometric, 610 nm	cream	+				16.183–16.184	16.163–16.164
	photometric, 610 nm	ice cream and frozen desserts	+				16.329	16.300
		cheese	+				16.307	16.278
	qualitative, screening	light cream, skim, whole milk				IDF/ISO/AOAC Group E 51		
			+				16.123–16.124	16.127–16.128
Phosphate emulsifying agents	calculation	processed cheese products	–		51:1969			
Phosphorus	photometric, molybdate-ascorbate	cheese and processed cheese products	–	B12:1972 (rev.)	33B:1982 (rev.)	2962:1984		
	photometric, NH_4 phosphomolybdate	milk	–		42:1967 (rev.)			
Polychlorinated biphenyls (PCBs)	many methods	milk and milk products	–		Q 2483/E	DP 8260		
Potassium *(see Sodium)*								
Preservatives	various methods	evap. milk, unsweetened	+				16.198	16.178
	various methods	butter	+				16.254	16.228
	various methods	cream	+				16.180	16.160
Preservatives, formaldehyde, benzoic, or salicylic acid	various methods	milk	+				16.106	16.110
Protein *(see Whey protein, Nitrogen)*								
Protein denaturation	(extra-low heating)	dried milk	–			IDF/ISO/AOAC Group E 17		
	(heat classification)	dried milk	–			IDF/ISO/AOAC Group E 17		
Protein reducing substances	spectrophotometric	milk	+				16.051–16.054	16.051–16.054
Proteins, functional properties			–			IDF/ISO/AOAC Group E 53		
Psychotrophic organisms	colony count at 6.5°C, 10 days	milk	–		101:1981	DIS 6730		
	colony count at 21°C, 25 h (rapid method)	raw milk	–		Q 1384/E	DP 8552		
Quaternary ammonium compounds	qualitative and quantitative methods	milk	+				20.077–20.090	20.094–20.107
Refractive index (butterfat)	refractometric	butter	+	B5:1967	7A:1969	1739:1975	16.240	16.214
Repeatability *(see Reproducibility)*						IDF/ISO/AOAC Group E 30		
Reproducibility and repeatability	statistics	milk and milk products	–		Q 2385/E	5725:1981		
Salmonella	isolation in selective broth	whole dry, nonfat dry milk	+				46.115–46.119	46.054–46.058
Salmonella	pre-enrichment, enrichment on selective media, recognition,	milk and milk products	–		93:1980	DIS 6785		
	membrane filter	cheese powder, dry milk					46.A06	
Salt *(see Chloride)*								
Sampling		butter	–				16.012–16.014, 16.229	16.012–16.014, 16.203

		cheese	–				16.015–16.018	16.015–16.018
		condensed and evap. milk	–				16.008–16.009	16.008–16.009
		dried milk	–				16.210	16.190
		dried milk and dried milk products	–				16.010–16.011	16.010–16.011
		milk and liquid milk products (except evap. and sweetened cond.)	–				16.006–16.007, 16.019	16.006–16.007, 16.019
	attributes sampling schemes	milk and milk products	–	20th App. VI-A	113:1982	DP 5583		
	general sampling techniques	milk and milk products	–	B1:1982	50A:1980 (rev.)	707:1985	16.001–16.005	16.001–16.005
	microbiological	dairy plant surfaces	–		121:1984	DIS 8086		
	variable sampling schemes	milk and milk products	–		Q 1083/E	DP 8197		
Sampling and sample preparation	specific sampling technique	sterilized milk	–		48:1969 (rev.)			
Sampling instrument	requirements	dried milk borers	–		71:1973 (rev.)			
Sample preparation and dilutions	preparation for microbiological examination	milk and milk products	–		122:1984	DP 8261		
Scorched particles	visual comparison with standard disks, after filtering	caseins and caseinates		submitted 1982	107:1982	5739:1983		
Sediment	vacuum filtration	butter	+				44.021	44.024
	vacuum filtration	cheese	+				44.021	44.024
	vacuum filtration	cream	+				44.021	44.024
Separation of fat from ice cream	extraction + detection of fats	ice cream	+				16.317	16.288
Sodium, potassium, and calcium	flame photometric	dried milk	+		119:1984	DIS 8070		
Solids-not-fat	gravimetric, on solvent extract	butter	–		11:1960 (rev.)			
	calculation of fat	milkfat products	–		Q 185/E	DP 8852		
	gravimetric, on solvent extract (water, solids-not-fat, and fat on one test portion)	butter	+	B9:1978	80:1977 (rev.)	3727:1977	16.205	16.185
	gravimetric	butter	+		Q 185/E	DP 8851		
Solubility (dried milk) *(see Insolubility)*								
Somatic cell count	automatic counter	milk	+				16.022	16.022
Sorbic acid	LC, capillary GC	yogurt	–			IDF/ISO/AOAC Group E 43		
	oxidation	cheese	+				16.303, 20.115	16.274, 20.098
Specific gravity *(see also Density)*	pycnometer or std hydrometer	milk	–				16.021	16.021
Stability (ethanol)	observation of precipitation on mixing with ethanol	sterilized milk	–		48:1969 (rev.)			
Staphylococci, coagulase-positive	detection, after incubation at 37°C (Giolitti and Cantoni, Baird-Parker)	dried milk	–		60A:1978	DP 5944		
Staphylococci, thermonuclease	color zones on toluidine blue O-DNA medium	milk and milk products	–		83:1978	DP 8870		
Staphylococcus aureus	colony count at 37°C	dried milk	–		Q 1285/E	DP 8869		
Sterol, β sito-acetate *(see foreign fat)*								
Streptococcus thermophilus		yogurt	–			IDF/ISO/AOAC Group E 44		

Table 2. (continued)

Property/component	Description/principle	Product	Collab. study	FAO/WHO	IDF	ISO	AOAC (14th Ed.)	AOAC (13th Ed.)
Strontium-89 and strontium-90	ion exchange	milk	+				48.016–48.024	48.016–48.024
Sucrose	inversion and polarimetry	cond. milk, sweetened	+	B14:1972	35:1966	2911:1976	16.208–16.209	16.188–16.189
Suitability for cheesemaking		dried milk	–			IDF/ISO/AOAC Group E 17		
Sulfite	LC, capillary GC	yogurt	–			IDF/ISO/AOAC Group E 43		
Tartaric acid	qualitative	cheese	+				16.287	16.258
	quantitative, titrimetric	cheese	+				16.288–16.289	16.259–16.260
TBHQ		milkfat, milk products	–			IDF/ISO/AOAC Group E 43		
Thermonuclease (see *Staphylococci*)								
Tin	(quercetine)	milk and milk products	–			IDF/ISO/AOAC Group E 15		
	AAS		+				25.A01–25.A04	
Titanium	spectrophotometric	cheese	+				16.275	16.246
Titratable acidity *(see Acidity)*								
Total count *(see Colony count at 30°C)*								
Total solids		yogurt	–			IDF/ISO/AOAC Group E 5		
	gravimetric, drying at 102°C	cheese and processed cheese	+	20th App. VI-D	4A:1982	5534:1985	all methods differ	
	gravimetric, drying at 102°C	cond. milk, sweetened	–	20th App. VI-C	15A:1982	DIS 6734	*not* 16.203	*not* 16.183
	gravimetric, drying at 102°C	edible ices	–		70:1972 (rev.)	3728:1977		
	gravimetric, drying at 102°C	milk, cream, and evaporated milk, unsweetened evap.	+	20th App. VI-B	21A:1982	DIS 6731	16.032, 16.171, 16.189	16.032, 16.151, 16.169
	infrared	milk	+				16.034	16.034
	lactometer	milk	+				16.033	16.033
	microwave, IR (automated methods)	milk	–			IDF/ISO/AOAC Group E 29		
	oven 100°C	ice cream and frozen desserts	+				16.313	16.284
Total solids (dry matter)	gravimetric, drying at 88°C	whey cheese	–	B11:1970	58:1970	2920:1974		
Total solids (moisture)	gravimetric, drying at 102°C	butter	–		10:1960			
Total solids (water)	gravimetric, heating (rapid method)	butter	–			IDF/ISO/AOAC Group E 5		
	gravimetric, drying at 102°C	caseins and caseinates	–	19th App. IX-B	78B:1980 (rev.)	5550:1978		
	gravimetric, drying at 102°C	dried milk	–		26:1964			
	gravimetric, drying at 102°C (water, solids-not-fat, and fat on one test portion)	butter	+	B9:1978	80:1977 (rev.)	3727:1977	16.231	16.205
Vegetable fat *(see Foreign fat)*								
Vitamins, minerals	spectrophotometric	infant formula	+				43.A21–43.A40	
Vitamin A	LC, colorimetry	dried milk (enriched)	+			IDF/ISO/AOAC Group E 46		
Vitamin E	TLC isolation, colorimetric	milk products	+				43.129	43.088
Volatile acids	chromatographic	cream	+				16.170	16.150
	distillation, chromatographic separation	butter	+				16.253	16.227
Volatile fatty acids	sterol acetate melting point	butter	+				16.236	16.210
	titrimetric, after saponification and distillation	milkfat	–		37:1966			

Water *(see also Total solids)*	titrimetric (Karl Fischer)	butteroil (milkfat products)	–	23:1964 (rev.)			
Water dispersion	indicator paper comparison	butter	–	112:1982	DIS 7586		
Water-insoluble fatty acids	gravimetric gravimetric	cream butter	+ +			16.168 16.246	16.148 16.220
Water-insoluble fatty acids + butyric acid	chromatographic	butter	+			16.247–16.251	16.221–16.225
Wettability *(see Dispersibility)* Whey protein		caseins and caseinates	–		IDF/ISO/AOAC Group E 11		
Yeasts and molds	chloramphenicol agar, colony count at 25°C, 4 days	milk and milk products	–	94:1980 (rev.)	DIS 6611		
Yogurt organisms	total count at 37°C (acidified MRS and M17 media)	yogurt	–	117:1983	DIS 7889		
Zinc		milk and milk products	–		IDF/ISO/AOAC Group E 15		

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A N N O U N C I N G

A New Handbook on How to Set Up or Improve Laboratory Quality Assurance Programs

Quality Assurance Principles for Analytical Laboratories

by Frederick M. Garfield

former Assistant Administrator
U.S. Drug Enforcement Administration

This handbook provides essential information needed to design, document, implement, or improve a laboratory quality assurance program . . . A program that can enable you to document the credibility of your laboratory's analytical data.

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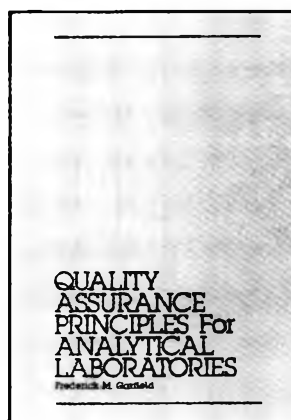

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