

Need

Quick, high-performance measurement
of TLC samples

New

Shimadzu CS-920 microprocessor-based
automatic scanning densitometer

This new addition to our award winning series of TLC Scanners offers the following cost-saving features:

1) Accuracy—Unique zigzag scanning system and working curve linearizer ensure excellent quantitiveness with automatic background zeroing and background suppression in the ultraviolet and fluorescence ranges.

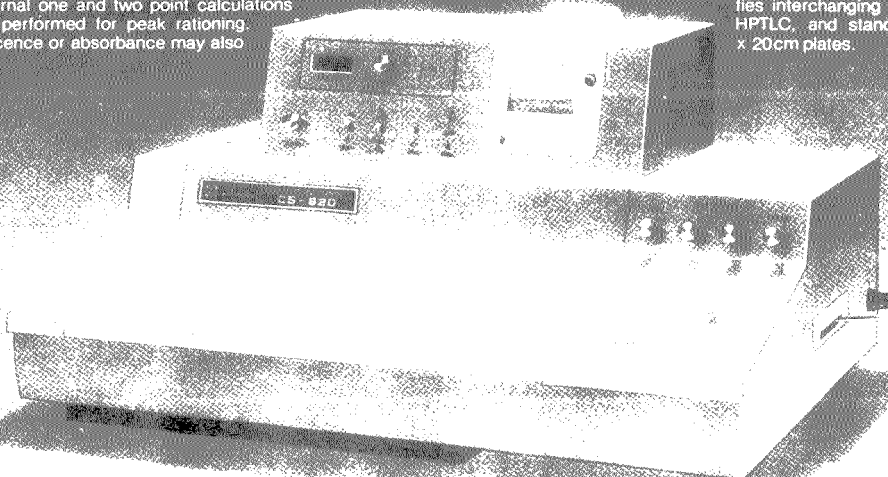
2) Concentrations—Immediately calculated and printed for each spot by a built-in microcomputer and printer. External and internal one and two point calculations can be performed for peak rationing. Fluorescence or absorbance may also

be read directly for best wavelength selection.

3) Speed—Automatic lane changing mechanism permits rapid automated analysis of numerous TLC spots. 30 samples can be measured in about 4 minutes.

4) Detection—Microcomputer automatically detects spots and calculates and prints each peak area. The computer also separates and determines fused spots automatically.

5) Adjustable programmable sample stage positioner simplifies interchanging of circular, HPTLC, and standard 20cm x 20cm plates.



• Write today for more information on these and other Shimadzu instruments.



SHIMADZU
SCIENTIFIC INSTRUMENTS, INC.

SHIMADZU SCIENTIFIC INSTRUMENTS, INC.

371-1725, 371-1726, 371-1727, 371-1728, 371-1729, 371-1730, 371-1731, 371-1732, 371-1733, 371-1734, 371-1735, 371-1736, 371-1737, 371-1738, 371-1739, 371-1740, 371-1741, 371-1742, 371-1743, 371-1744, 371-1745, 371-1746, 371-1747, 371-1748, 371-1749, 371-1750, 371-1751, 371-1752, 371-1753, 371-1754, 371-1755, 371-1756, 371-1757, 371-1758, 371-1759, 371-1760, 371-1761, 371-1762, 371-1763, 371-1764, 371-1765, 371-1766, 371-1767, 371-1768, 371-1769, 371-1770, 371-1771, 371-1772, 371-1773, 371-1774, 371-1775, 371-1776, 371-1777, 371-1778, 371-1779, 371-1780, 371-1781, 371-1782, 371-1783, 371-1784, 371-1785, 371-1786, 371-1787, 371-1788, 371-1789, 371-1790, 371-1791, 371-1792, 371-1793, 371-1794, 371-1795, 371-1796, 371-1797, 371-1798, 371-1799, 371-1800

SHIMADZU (EUROPA) GmbH

Postfach 1015, D-4970 Wesel 1, Germany, Tel. 05741-91-1, Telex 2519683P

SHIMADZU CORPORATION INTERNATIONAL MARKETING DIV.

3601 Central Expressway, Redwood City, CA 94063, U.S.A., Tel. 415-964-1000

JOURNAL OF LIQUID CHROMATOGRAPHY

Editor: DR. JACK CAZES Editorial Secretary: ELEANOR CAZES
P. O. Box 1440-SMS
Fairfield, Connecticut 06430

Editorial Board

E. W. ALBAUGH, *Gulf Research and Development Company, Pittsburgh, Pennsylvania*
K. ALTGELT, *Chevron Research Company, Richmond, California*
A. ASZALOS, *U.S. Food and Drug Administration, Washington, D. C.*
H. BENOIT, *Centre des Recherches sur les Macromolécules, Strasbourg, France*
W. BERTSCH, *University of Alabama, University, Alabama*
B. BIDLINGMEYER, *Waters Associates, Inc., Milford, Massachusetts*
P. R. BROWN, *University of Rhode Island, Kingston, Rhode Island*
J. A. CAMERON, *University of Connecticut, Storrs, Connecticut*
J. V. DAWKINS, *Loughborough University of Technology, Loughborough, England*
J. E. FIGUERUELO, *University of Valencia, Burjasot, Spain*
D. H. FREEMAN, *University of Maryland, College Park, Maryland*
R. W. FREI, *The Free University, Amsterdam, The Netherlands*
J. C. GIDDINGS, *University of Utah, Salt Lake City, Utah*
R. L. GROB, *Villanova University, Villanova, Pennsylvania*
E. GRUSHKA, *The Hebrew University, Jerusalem, Israel*
G. GUIOCHON, *Ecole Polytechnique, Palaiseau, France*
M. GURKIN, *E-M Science, Inc., Gibbstown, New Jersey*
A. E. HAMIELEC, *McMaster University, Hamilton, Ontario, Canada*
S. HARA, *Tokyo College of Pharmacy, Tokyo, Japan*
D. J. HARMON, *B. F. Goodrich Research Center, Brecksville, Ohio*
G. L. HAWK, *Zymark Corporation, Hopkinton, Massachusetts*
M. T. W. HEARN, *St. Vincent's School of Medical Research, Victoria, Australia*
E. HEFTMANN, *U.S. Department of Agriculture, Berkeley, California*
A. HEYRAUD, *Centre National de la Recherche Scientifique, France*
P. Y. HOWARD, *Micromeritics Instrument Corp., Norcross, Georgia*
H. J. ISSAQ, *Frederick Cancer Research Facility, Frederick, Maryland*
J. JANCA, *Institute of Analytical Chemistry, Brno, Czechoslovakia*
J. F. JOHNSON, *Institute of Materials Science - U. Conn., Storrs, Connecticut*
B. L. KARGER, *Northeastern University, Boston, Massachusetts*
P. T. KISSINGER, *Purdue University, West Lafayette, Indiana*
J. KNOX, *The University of Edinburgh, Edinburgh, Scotland*
J. C. KRAAK, *University of Amsterdam, Amsterdam, The Netherlands*
J. LESEC, *Ecole Supérieure de Physique et de Chimie, Paris, France*
B. MONRABAL, *Dow Chemical Iberica, S. A., Tarragona, Spain*
S. MORI, *Mie University, Tsu, Mie, Japan*
A. K. MUKHERJI, *Xerox Corporation, Webster, New York*
J. A. NELSON, *M. D. Anderson Hospital and Tumor Institute, Houston, Texas*
L. PAPA ZIAN, *American Cyanamid Corporation, Stamford, Connecticut*
V. PRETORIUS, *University of Pretoria, Pretoria, South Africa*
QIAN RENYUAN, *Institute of Chemistry, Beijing, People's Republic of China*

(continued)

JOURNAL OF LIQUID CHROMATOGRAPHY

Editorial Board *continued*

C. QUIVORON, *Ecole Supérieure de Physique et de Chimie, Paris, France*

F. M. RABEL, *Whatman, Inc., Clifton, New Jersey*

J. RIVIER, *The Salk Institute, San Diego, California*

C. G. SCOTT, *Hoffman-LaRoche, Inc., Nutley, New Jersey*

R. P. W. SCOTT, *Perkin-Elmer Corporation, Norwalk, Connecticut*

H. SMALL, *Dow Chemical Company, Midland, Michigan*

E. SOCZEWINSKI, *Medical Academy, Lubin, Poland*

E. STAHL, *Universität des Saarlandes, Saarbrücken, West Germany*

B. STENLUND, *Abo Akademi, Abo, Finland*

J. C. TOUCHSTONE, *Hospital of University of Pennsylvania, Philadelphia, Pennsylvania*

S. H. WONG, *University of Connecticut School of Medicine, Farmington, Connecticut*

JOURNAL OF LIQUID CHROMATOGRAPHY

December 1983

Aims and Scope. The journal publishes papers involving the application of liquid chromatography to the solution of problems in all areas of science and technology, both analytical and preparative, as well as papers that deal specifically with liquid chromatography as a science within itself. Included will be thin-layer chromatography and all modes of liquid chromatography.

Indexing and Abstracting Services. Articles published in *Journal of Liquid Chromatography* are selectively indexed or abstracted in:

● Analytical Abstracts ● ASCA ● BioSciences Information Service of Biological Abstracts (BIOSIS) ● Chemical Abstracts ● Current Contents/Life Sciences ● Current Contents/Physical and Chemical Sciences ● Engineering Index ● Excerpta Medica ● Journal of Abstracts of the All-Union Institute of Scientific and Technical Information of the USSR ● Physikalishe Berichte ● Science Citation Index

Manuscript Preparation and Submission. See the last page of this issue.

Subscription Information. *Journal of Liquid Chromatography* is published in fourteen numbers and two supplements in January, February, March (3 numbers), April, May, June (2 numbers), July, August, September (2 numbers), October, November, and December by Marcel Dekker, Inc., 270 Madison Avenue, New York, New York 10016. The subscription rate for Volume 6 (1983), containing fourteen numbers and two supplements, is \$298.00 per volume (prepaid). The special discounted rate for individual professionals and students is \$149.00* per volume. To secure this special rate, your order must be prepaid by personal check or may be charged to MasterCard or VISA. Add \$36.80 for surface postage outside the United States. For airmail to Europe, add \$72.32; to Asia, add \$91.52.

Mailing Address. Please mail payment with order to: Marcel Dekker Journals, P. O. Box 11305, Church Street Station, New York, New York 10249.

Copyright © 1983 by Marcel Dekker, Inc. All rights reserved. Neither this work nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, microfilming and recording, or by any information storage and retrieval systems without permission in writing from the publisher.

Permission to photocopy for internal or personal use or the internal or personal use of specific clients is granted by Marcel Dekker, Inc. for libraries and other users registered with the Copyright Clearance Center (CCC), provided that the stated fee is paid directly (per copy) to the CCC, 21 Congress Street, Salem, MA 01970. Special requests should be addressed to Marcel Dekker, Inc., Permissions Dept., 270 Madison Avenue, New York, New York 10016.

Contributions to this journal are published free of charge. Second-class mailing permit pending at New York, New York and at additional mailing offices.

***THIS REFLECTS A 50% DISCOUNT GIVEN TO INDIVIDUAL SUBSCRIBERS.**

Why select a column from Analytichem?

Because the greater the selection the greater the selectivity.

The new line of HPLC columns from Analytichem provides selectivity unequalled by anyone. The reason is simple. Analytichem offers a wider selection of phases than anyone.* Now you can choose the phase that is precisely suited to your particular application. These new columns, packed with our unique Sepralyte™ 5 μ m spherical media, set an unprecedented standard of chromatographic efficiency... regardless of the phase you select.

The performance of each new Analytichem column is fully

guaranteed and backed by the industry's strongest customer service and technical support teams. Our technical advisors have the training and hands-on experience to assist you in solving virtually any separation problem. Next time you're considering HPLC columns, be selective. Call Analytichem. You'll find the columns you need and the service you deserve.



Analytichem International
24201 Frampton Ave., Harbor City,
CA 90710, USA, (800) 421-2825.
In California (213) 539-6490
TELEX 664832 ANACHEM HRBO

*C18, C8, C2, Phenyl, Cyano, Amino, Diol, Silica, Quaternary Amine, Carboxylic Acid, Sulfonic Acid.

Circle Reader Service Card No. 110

JOURNAL OF LIQUID CHROMATOGRAPHY

Volume 6, Number 14, 1983

Special Section on Thin-Layer Chromatography

CONTENTS

- Fractionation into Components of a Mixture of Acidic Ninhydrin-Positive Compounds of Mouse Brain Extracts with Thin-Layer and Ion-Exchange Chromatography 2621**
P. Lähdesmäki and M. Timonen
- Analytical Separation of Reduced and Oxidized Forms of Glutathione from Amino Acid Mixtures by Overpressured Thin-Layer Chromatography 2635**
M. Ábrahám, B. Polyák, B. Szajáni, and L. Boross
- Isolation of Glycolipids from Blood Elements. 2647**
J. Pick, J. Vajda, and L. Leisztner
- Identification of Some Potential Antidiabetic Compounds on Impregnated Silica Gel G Plate as Their π -Complexes 2661**
R. Jain and D. D. Agarwal
- The Role of the Specific Surface Area of an Adsorbent in the Optimization of Mixture Separation Conditions in Thin-Layer Chromatography 2665**
J. K. Rozyło, I. Malinowska, and M. Pönieważ
- Determination of Biphenyl in Citrus Fruits by Quantitative Thin-Layer Chromatography 2679**
J. Sherma, P. J. Sielicki, Jr., and S. Charvat
- Use of Some Silver Salts of Aromatic Sulfonic Acids in Thin-Layer Chromatography 2687**
P. Ilinov and S. I. Dimov
- Characterization of Derivatives of Some Closely Related 1-Picolinoyl-3-methyl-4-(substituted)-pyrazoline-5-ones by Thin-Layer Chromatography . 2695**
D. R. Gupta and R. K. Arora
- Phospholipid Composition and Differentiation of Methanotrophic Bacteria. 2699**
L. V. Andreev and V. F. Galchenko

Regular Contributed Papers

- Modification of Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons Using Mixed Stationary Phases 2709**
S. A. Wise, L. C. Sander, and W. E. May

Aging of Pressure Sensitive Adhesives. II. Use of Multidetector SEC	2723
<i>D. J. P. Harrison, W. R. Yates, and J. F. Johnson</i>	
Determination of Uric Acid in Human Serum: Reversed-Phase Liquid Chromatography with Electrochemical Detection	2739
<i>K. Iriyama, M. Yoshiura, T. Iwamoto, T. Hosoya, H. Kono, and T. Miyahara</i>	
High Pressure Liquid Chromatographic Assay of Cefamandole in Serum Following Intravenous and Intraperitoneal Administration	2747
<i>R. E. Bawdon, K. J. Leveno, J. G. Quirk, F. G. Cunningham, and S. P. Guss</i>	
A Rapid High Performance Liquid Chromatographic Method for the Simultaneous Measurement of Six Tricyclic Antidepressants	2761
<i>J. S. Kiel, R. K. Abramson, S. L. Morgan, and J. C. Voris</i>	
Determination of Ritodrine in Plasma Using HPLC	2775
<i>P. Kuhnert, P. Erhard, A. Dixon, B. Kuhnert, and T. Gross</i>	
Analysis of Indenolol in Biological Fluids by High Performance Liquid Chromatography	2785
<i>S. A. Babhair, H. Y. Aboul-Enein, and S. El-Houfy</i>	
HPLC Analysis of Oligomeric Ethylene Glycol Mixtures via bis(2,4-Dinitrophenylation).	2797
<i>A. Warshawsky, N. Shoef, and A. Tishbee</i>	
Data Acquisition and Processing for High Speed Liquid Chromatography. . .	2809
<i>J. M. Anderson</i>	
Separation of Chlorinated Phenols by Isocratic High-Performance Liquid Chromatography on Reverse Phase Column	2829
<i>J. Nair, K. M. Munir, and S. V. Bhide</i>	
Announcements	2839
Liquid Chromatography News	2841
Liquid Chromatography Calendar.	2845
Author Index to Volume 6	2849
Subject Index to Volume 6	2857
Collective Contents to Volume 6	2881

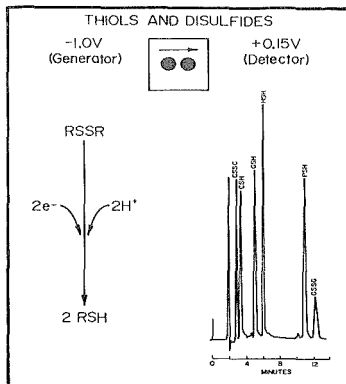
Liquid Chromatography / Electrochemistry

In 1974 BAS introduced the first commercial LCEC system. With over 5000 units installed and the largest research team to support them, it's not surprising that our nearest competitors are several generations behind us. Our thin-layer transducers lead the way with a wide selection of electrode materials in a variety of interchangeable configurations. The latest member of the family is the new LC4B/17 dual electrode amperometry package. Parallel and series amperometry can enhance selectivity, detection limits, speed and gradient compatibility. Let us show you how it's done, no one else can.

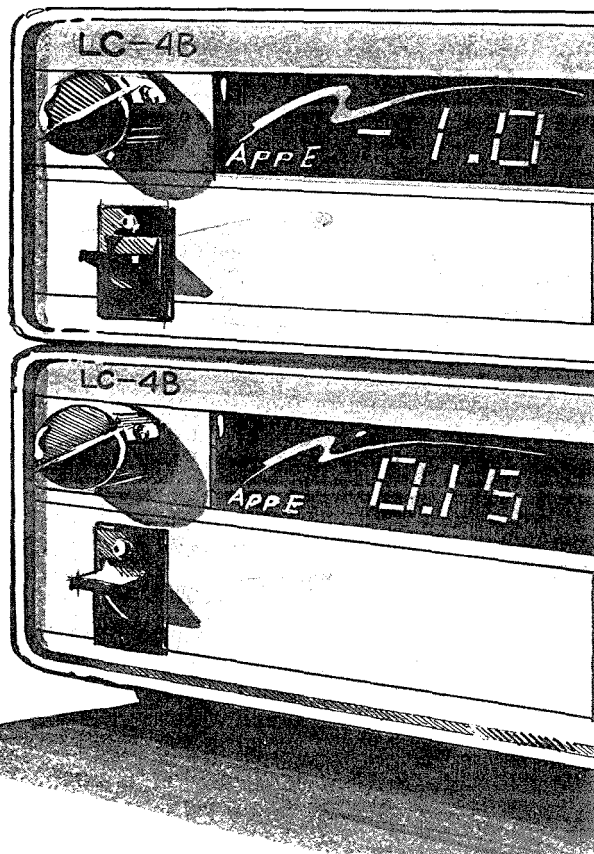
BAS

Purdue Research Park,
2701 Kent Avenue,
West Lafayette, IN. 47906
317-463-4527 TLX 276141

JAPAN: Tokai Irika Co. (Tokyo)
CANADA: Mandel Scientific
FRANCE: Biochrom s.a.r.l. (Vindelle)



Dual Electrode Detection: Standard mixture of Cystine, Cysteine, Glutathione, Homocysteine, Penicillamine, Glutathione Disulfide



THIN-LAYER CHROMATOGRAPHY

Edited by

HALEEM J. ISSAQ
NCI-Frederick Cancer Research Facility
Frederick, Maryland

and

JACK CAZES
Fairfield, Connecticut

This is a special section of *Journal of Liquid Chromatography*, Volume 6,
Number 14, 1983.

MARCEL DEKKER, INC. New York and Basel

**FRACTIONATION INTO COMPONENTS OF A MIXTURE OF ACIDIC
NINHYDRIN-POSITIVE COMPOUNDS OF MOUSE BRAIN EXTRACTS
WITH THIN-LAYER AND ION-EXCHANGE CHROMATOGRAPHY**

Pekka Lähdesmäki and Maritta Timonen
Department of Biochemistry, University of Oulu, SF-
90570, Oulu 57, Finland

ABSTRACT

A mixture of more than ten acidic or highly ionized ninhydrin-positive compounds of mouse brain extracts, occurring in ion-exchange chromatography from the beginning up to aspartic acid, was analyzed using ion-exchange and thin-layer chromatography standardized with known substances. These formed four peak groups in the chromatogram of an automatic amino acid analyzer. The first group contained cysteic acid, cysteine-sulfinic acid and phosphoserine, which could be separated from each others only by thin-layer chromatography. Only phosphoserine could be identified in the brain extract (about 0.14 mmol/kg brain wet weight), however. Taurine (4.1 mmol/kg) and phosphoethanolamine (1.05 mmol/kg) in the second group could be satisfactorily separated from each others after the hydrolysis of glycerylphosphoethanolamine (about 0.6 mmol/kg) and certain acidic peptides with 6 mol/l HCl. Hypotaurine (0.03 mmol/kg) and urea (6.6 mmol/kg) were completely overlapped in the third peak, but urea was decomposed in the hydrolysis with 6 mol/l HCl. The fourth group consisted of aspartic acid (2.1 mmol/kg). A number of low-molecular weight peptides also appeared in the chromatograms, above all in the phosphoserine and taurine peaks, but they were eliminated by the hydrolysis. They contained, however, some of the above-mentioned critical amino acids (phosphoserine, taurine and aspartic acid).

INTRODUCTION

Tissue extracts, and brain extracts in particular, contain a large number of acidic amino acids and certain other ninhydrin-positive compounds, which in the ion-exchange chromatography (e.g. in the automatic amino acid analyzers) can not be separated satisfactorily well from each others, but appear in two or three peaks in the chromatograms (1-6). This fraction (often called "taurine fraction") is a complex mixture of more than ten (see Ref. 1) acidic or highly ionized compounds of physiological importance, including taurine, phosphoserine, phosphoethanolamine, glycerylphosphoethanolamine, hypotaurine, cysteic and cysteinesulfinic acids, carbamylphosphate, urea and glucosaminephosphate. Moreover, the presence of a number of low-molecular weight peptides, including taurine and phosphoserine peptides, which are soluble in sulfosalicylic, trichloroacetic and perchloric acids used in the preparation of the tissue samples, has been reported (7,8).

Taurine (2-aminoethanesulfonic acid) is often the main constituent in the free amino acid pool, but how real are its concentration values calculated from the mixture of the most acidic amino compounds eluting in the ion-exchange chromatography just before aspartic and glutamic acids (5,7,9,10) ? For their strong ionization or having phosphoric acid groups or other extra acidic moieties, the amino acids of the "taurine fraction" as well as the peptides containing taurine and phosphoserine (7,8,11-15) behave similarly in the chromatography, in the ion-exchange chromatography in particular, and overlap each others. The first eluting acidic ninhydrin-positive compounds may thus distort seriously each others' quantitative determination. A good attempt

to clarify the chromatographic determination of the components of the taurine peak has recently been made by Kontro et al. (5) and Marnela (10).

Simple hydrolytic and chromatographic means, which for their part bring facilitation to the exact quantitative determination of the individual components of the "taurine fraction" are given here. The methods were standardized with pure amino acids and some peptides as well as with certain taurine and phosphoserine peptides prepared from calf brain synaptosomes (7). The results, although not yet definitive, will show that serious attention must be paid to the interpretation of the chromatograms regarding compounds eluting before aspartic acid.

MATERIAL AND METHODS

Whole brains of NMRI mice were used, but the sub-cellular fractions were prepared from calf brains. Newly removed brains were rinsed free of external blood clots, and the cortical layer from calf brains separated with a knife. The synaptosomes were prepared from the homogenates in cold 0.32 mol/l sucrose by the gradient centrifugation method of Whittaker et al. (16).

Tissue samples were homogenized in 5 % trichloroacetic acid (TCA), the precipitated proteins removed by centrifugation, and the TCA then removed by shaking with diethyl ether. The residual solution was lyophilized and the amino acids and peptides then dissolved in water. When 15 % perchloric acid (PCA) was used, the precipitable proteins were first removed by centrifugation and the supernatant then neutralized with KOH and the potassium perchlorate centrifuged. The neutral, protein-free extracts were lyophilized and the residue taken up in acidified water and used for chromatogra-

phic analysis. The extracts from the synaptosomes were prepared in a similar manner.

The amino acid samples were quantitatively analyzed in an automatic amino acid analyzer (Kontron Liquimat III), equipped with a two-channel peak integrator. Ion-exchange chromatography in separate short columns according to Garvin (17) also was used. Thin-layer chromatography was performed on silica gel (Kieselgel G) plates of thickness 0.25 mm. Two-dimensional chromatograms were developed with 70 % ethanol in water and 75 % phenol in water. The dried plates were sprayed with ninhydrin (0.5 % solution in butanol:acetic acid, 20:1, pH 3.3). The spots were developed for 10 min at 105 °C.

Two unknown hydrolyzable spots on the TLC plates from calf brain synaptosomes (7,9) were projected to the corresponding unsprayed plates, the silica gel scraped up and the peptides extracted with water and lyophilized. They were analyzed for the amino acid composition after the hydrolysis with 6 mol/l HCl, and used as such for studying the chromatographic mobility in an amino acid analyzer.

Commercial pure amino acids (E. Merck, Darmstadt and Fluka AG, Buchs, Basel, Switzerland) and γ -glutamyl-taurine (Chinoin Pharmaceuticals, Budapest) were used as standards.

RESULTS

As shown in Figure 1, the initial part of a chromatogram of the PCA extract of mouse brain, obtained with a modern "high resolution" automatic amino acid analyzer, contained more than ten different ninhydrin-positive compounds, which could not be separated satisfactorily well from each others. Using known amino acid

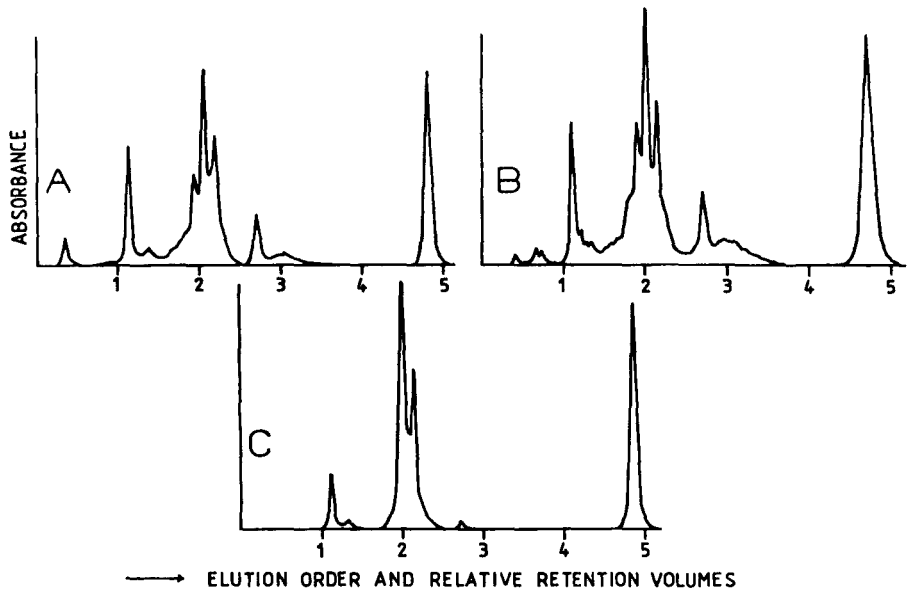


Figure 1. Initial parts of the ion-exchange chromatograms of the amino compounds of mouse brain extracts before (A) and after the hydrolysis with 2 mol/l (B) or 6 mol/l (C) HCl from a "high resolution" automatic amino acid analyzer (Kontron Liquimat III). For identification, see Figure 2.

or peptide samples, the retention volumes of 14 different compounds were determined (Figure 2). These formed four peak groups, which could be separated quite well from each others, but inside the groups the peaks of different compounds had almost the same retention volumes. The first group contained carbamylphosphate, cysteic acid, cysteinesulfinic acid, phosphoserine, γ -glutamyl-taurine and certain other taurine peptides (see Ref. 8). The second group contained taurine, phosphoethanolamine, glycerylphosphoethanolamine and certain phosphoserine and other acidic peptides (see Ref. 8), and the third

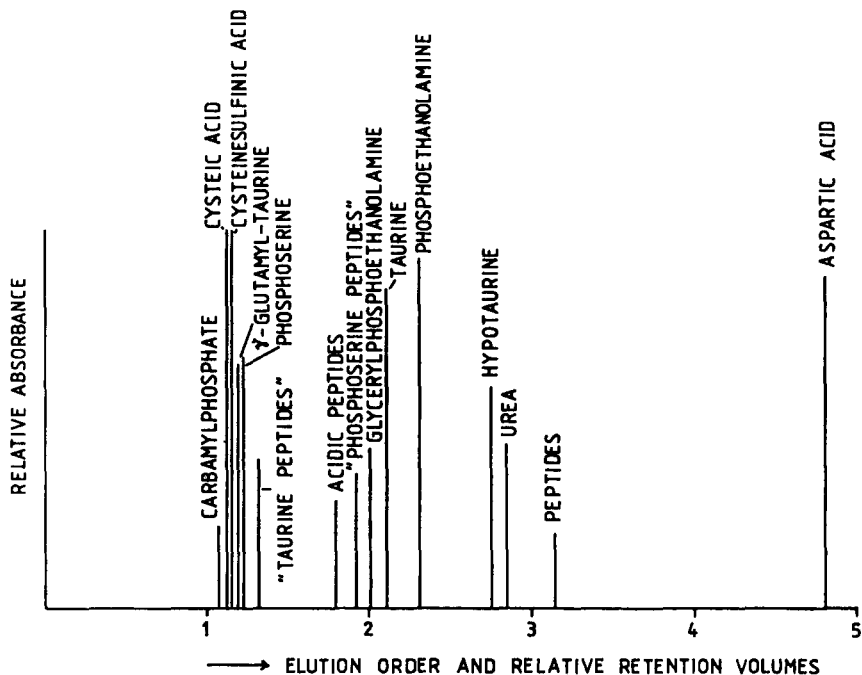


Figure 2. Retention volumes of 14 different acidic ninhydrin positive compounds analyzed with an automatic amino acid analyzer (Kontron Liquimat III) from standard amino acids, γ -glutamyl-taurine and certain other peptides prepared from calf brain synaptosomes. The height of the absorbance lines depicts approximate molar sensitivity of the ninhydrin reaction.

group hypotaurine, urea and again some synaptosomal peptides. The fourth group consisted of aspartic acid.

The concentrations of the amino compounds of the PCA extract of mouse brain, calculated from the chromatograms of Figure 1, are given in Table 1 before and after the hydrolysis with 2 and 6 mol/l HCl. Most of the peaks or peak groups of the brain extract could be identified with the standard samples (Figure 2), but very low concentrations of unknown compounds still ap-

TABLE I

Concentrations of the acidic ninhydrin-positive compounds of mouse brain after extraction with perchloric acid and after the hydrolysis with 2 and 6 mol/l HCl.

Compound	Concentrations mmol/kg wet weight		
	as such	2 M HCl	6 M HCl
unknown	0.01	-	-
unknown	-	0.02	-
Cysteic acid	} 0.25	} 0.18	} 0.14
Cysteinesulfinic acid			
Phosphoserine			
unknown	0.01	0.01	0.01
Peptides (ninhydrin-positive)	0.02	0.02	-
"Taurine peptides" (ninhydrin-negative)	-	0.27	-
Peptides (ninhydrin-positive)	0.55	0.50	-
"Phosphoserine peptides" (ninhydrin-negative)	-	0.21	-
Glycerylphosphoethanolamine	0.72	0.55	-
Taurine	4.10	4.90	4.88
Phosphoethanolamine	1.70	1.04	1.65
Hypotaurine	} 6.86	} 6.59	0.03
Urea			
Aspartic acid	2.06	6.87	7.50

Results are means of 5-6 experiments, S.D. being about 10 %. The order of substances refer to Figure 1. The concentrations of the peptides and unknown compounds were calculated using alanine as a standard.

peared both before and after the hydrolysis. Certain peptides or peptide groups appeared between the single amino acids. A part of these became visible not until after the hydrolysis with 2 mol/l HCl (for 30 min in a boiling water bath), which is known to hydrolyze the acyl groups from the amino group. This procedure converts the originally N-acetylated peptides ninhydrin-positive. All the peptides disappeared in the hydrolysis with 6 mol/l HCl. The amount of aspartic acid increased 3 fold in the hydrolysis with 2 mol/l HCl, and was derived apparently from the hydrolysis of N-acetylasp-

tate present in brain tissue (18), and still a little in the hydrolysis with 6 mol/l HCl, this aspartate being derived from the hydrolyzable peptides (4,9). The peaks of hypotaurine and urea were completely overlapped (Figure 2), but urea disappeared in the HCl hydrolysis, and a small amount of hypotaurine remained after the hydrolysis. As satisfied with the HCl treatments of the standards, hypotaurine, taurine, phosphoethanolamine, phosphoserine, cysteic acid and cysteinesulfinic acid did not be decomposed either in the 2 or 6 mol/l HCl solution.

The peaks of taurine, phosphoethanolamine and glycerylphosphoethanolamine appeared very close together (see also Ref. 5). A small decrease in the concentration of phosphoethanolamine due the hydrolysis with 2 mol/l HCl was observed, indicating that its original peak contained some hydrolyzable, ninhydrin-positive material. At the same time the amount of taurine increased slightly, but it is not known, what this increment in the taurine peak arises from, but probably from the large number of different acidic N-acetylaspartyl peptides (8), which are converted ninhydrin-positive by the hydrolysis with 2 mol/l HCl. Glycerylphosphoethanolamine was completely hydrolyzed to phosphoethanolamine, after which the determination of taurine and phosphoethanolamine was more reliable.

Cysteic and cysteinesulfinic acids and phosphoserine could not be separated from each others, but their common amount decreased significantly after the acid hydrolysis, indicating again some hydrolyzable material present in their original peak. In two-dimensional thin-layer chromatography, using 70 % ethanol and 75 % phenol in water, these critical amino acids could be separated very well from each others (Figure 3). Phosphoserine was

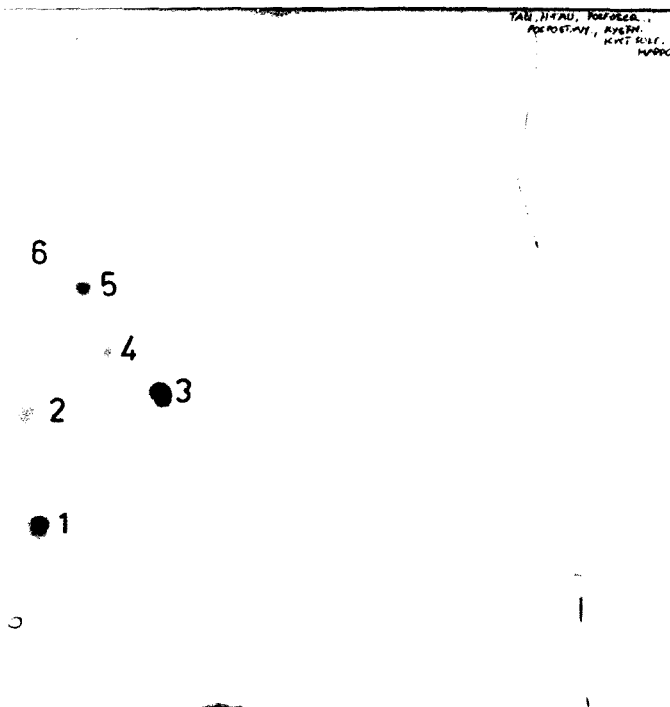


Figure 3. A two-dimensional thin-layer chromatogram of certain acidic amino acids, analyzed from a standard sample on 0.25 mm thick silica gel plates. The R_f values in brackets are $R_{fI}:R_{fII} = 70\% \text{ ethanol}: 75\% \text{ phenol in water}$. 1 = phosphoethanolamine (0.17:0.03), 2 = phosphoserine (0.40:0.01), 3 = hypotaurine (0.50:0.27), 4 = taurine (0.57:0.17), 5 = cysteinesulfinic acid (0.71:0.12), 6 = cysteic acid (0.73:0.07).

identified in the mouse brain extract (Figure 4), but no traces of cysteic or cysteinesulfinic acid were observed. The first peak group (phosphoserine-cysteic acid group, Figure 1-2) contained thus apparently only phosphoserine after the hydrolysis with 6 mol/l HCl.

The separation of taurine and phosphoethanolamine from each others was unsuccessful in the ion-exchange

1100000
hyd. 2A. HCl

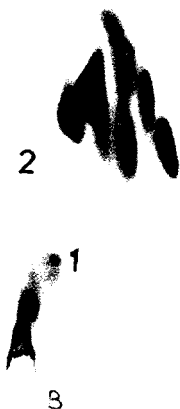


Figure 4. A two-dimensional thin-layer chromatogram of mouse brain extract, treated with 2 mol/l HCl for 30 min and run under identical conditions to that of Figure 3. 1 = probably phosphoethanolamine, 2 = probably phosphoserine.

chromatography by the method of Garvin (17, Table 2). Several other acidic amino acids came partially in the same fraction with these, but phosphoserine not. This old method is thus unsatisfactory for the determination of taurine, since it is contaminated by other acidic amino acids. More useful might be the o-phthalaldehyde-

TABLE II

Recovery percentages of taurine and certain other acidic compounds in ion-exchange chromatography performed according to the method of Garvin (17).

Compound	Recovery %
Taurine	100.0
Hypotaurine	26.2 \pm 5.0
γ -Glutamyl- <i>taurine</i>	27.4 \pm 15.2
Cysteic acid	22.6 \pm 18.1
Phosphoethanolamine	115.2 \pm 9.8
Phosphoserine	8.2 \pm 2.8

Results (means \pm S.D.) are from 3 determinations. The recovery value of taurine was taken as 100 and others expressed relative to this.

urea reaction of Gaitonde and Short (19), since it is more specific for taurine, even though performed after the passage of the tissue extract through the ion exchange resins, when there again is a possibility of contamination.

The acidic low-molecular weight peptides occurring in the chromatograms between the amino acids have been studied elsewhere (4, 7-9). They contained 5-6 different amino acids among which acidic amino acids, aspartic and glutamic acids, phosphoserine and taurine have a main role.

DISCUSSION AND CONCLUSIONS

The complicated composition of the fraction of the acidic ninhydrin-positive compounds in tissue extracts has been known for several years; at least 10-15 different amino acids or their derivatives have been reported in the ion-exchange chromatograms in two or three peak groups before aspartic and glutamic acids (see e.g. Ref. 1, p. 50-51). Numerous attempts have been made to divide

these mixtures of compounds into fractions of individual substances, but the problem is still largely the same: the most acidic compounds, being eluted in the volume of cysteic acid and taurine in particular, are completely overlapped.

The strongly acidic functional groups in the compounds dealt here make their pI values low. For instance the pK_a values of taurine are 1.5 and 8.74, giving the pI value of 5.12 for it (20). The situation is the same with most of the above-mentioned amino acids and peptides, which contain extra acidic groups. The free acidic groups in them seem to be the main chromatographic determinant in the ion-exchange chromatography, and for this reason several different compounds fall into the same fraction.

The present study led us to the conclusion that the direct analysis of a brain extract does not give correct results about the concentrations of the acidic amino acids. Better quantitative results can be obtained by comparing the analysis results after the hydrolysis of the sample with 2 and 6 mol/l HCl. In some cases qualitative thin-layer chromatography is essential for the identification of the amino acids.

ACKNOWLEDGEMENTS

This study was supported by the Science Research Council of the Academy of Finland and by Medica Oy Pharmaceuticals.

REFERENCES

1. Meister, A., Biochemistry of the Amino Acids, Vol. 1, Academic Press, New York, 1965.
2. Mangan, J. L. and Whittaker, V. P., The distribution of free amino acids in subcellular fractions of guinea-pig brain, Biochem. J., 98, 128, 1966.

3. Perry, T. L., Perry, K., Hansen, S., Diamond, S. and Mok, C., Regional distribution of amino acids in human brain obtained at autopsy, *J. Neurochem.*, 18, 513, 1971.
4. Lähdesmäki, P., Karppinen, A., Saarni, H. and Winter, R., Amino acids in the synaptic vesicle fraction from calf brain: Content, uptake and metabolism, *Brain Res.*, 138, 295, 1977.
5. Kontro, P., Marnela, K.-M. and Oja, S. S., Free amino acids in the synaptosome and synaptic vesicle fractions of different bovine brain areas, *Brain Res.*, 184, 129, 1980.
6. Kumpulainen, E., Karppinen, A., Marnela, K.-M. and Lähdesmäki, P., Subcellular distribution of intracerebrally injected taurine and certain other amino acids in mouse brain, in *The Effects of Taurine on Excitable Tissues*, Schaffer, S. W., Baskin, S. I. and Kocsis, J. J., eds., Spectrum Publications, New York, 1981, p. 135.
7. Lähdesmäki, P., Airaksinen, K., Vartiainen, M. and Halonen, P., Characterization of two synaptosomal peptides in calf brain, *Acta Chem. Scand.*, B 34, 343, 1980.
8. Marnela, K.-M. and Lähdesmäki, P., Mass spectral and hydrolytic determination of amino acid sequences in synaptosomal peptides from calf brain, *Neurochem. Res.*, 1983 (in press).
9. Lähdesmäki, P. and Winter, R., A peptide containing aspartic acid, glutamic acid and serine in calf brain synaptic vesicles, *Acta Chem. Scand.*, B 31, 802, 1977.
10. Marnela, K.-M., Automated ion-exchange chromatography in the detection of aspartylglucosaminuria, *J. Chromatogr.* 182, 409, 1980.
11. Reichelt, K. L. and Kvamme, E., Histamine-dependent formation of N-acetyl-aspartyl peptides in mouse brain, *J. Neurochem.*, 21, 849, 1973.
12. Reichelt, K. L. and Edminson, P., Biogenic amine specificity of cortical peptide synthesis in monkey brain, *FEBS Letters*, 47, 185, 1974.
13. Furka, Á., Sebestyén, F., Feuer, L., Horváth, A., Hercsel, J., Ormai, S. and Bányai, B., Isolation of γ -L-glutamyl-*taurine* from the protein free aqueous extract of bovine parathyroid powder, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 15, 39, 1980.
14. Török, K., Varga, V., Somogyi, J., Feuer, L. and Gulyás, J., Formation of γ -glutamyl-*taurine* in the rat brain, *Neurosci. Lett.*, 27, 145, 1981.
15. Lähdesmäki, P. and Timonen, M., Non-coded biosynthesis of N-acetylaspartyl peptides in mouse brain homogenates, *J. Liquid Chromatogr.*, 5, 989, 1982.

16. Whittaker, V. P., Michaelson, I. A. and Kirkland, R. J. A., The separation of synaptic vesicles from nerve ending particles ("synaptosomes"), *Biochem. J.*, 90, 293, 1964.
17. Garvin, J. E., A new method for the determination of taurine in tissues, *Arch. Biochem.*, 91, 219, 1960.
18. Tallan, H. H., Preparation and analysis of N-acetyl-L-aspartic acid, in *Methods in Enzymology*, Vol. 6, Colowick, S. P. and Kaplan, N. O., eds., Academic Press, New York, 1963, p. 562.
19. Gaitonde, M. K. and Short, R. A., Quantitative determination of taurine by an o-phthalaldehyde-urea reaction, *Analyst*, 96, 274, 1971.
20. Greenstein, J. P. and Winitz, M., *Chemistry of the Amino Acids*, Vol. 1, Wiley, New York, 1961.

ANALYTICAL SEPARATION OF REDUCED AND OXIDIZED FORMS OF
GLUTATHIONE FROM AMINO ACID MIXTURES BY OVERPRESSURED
THIN-LAYER CHROMATOGRAPHY

Magdolna Ábrahám, Béla Polyák, Béla Szajáni and László Boross
Department of Biochemistry, Attila József University
H-6726 Szeged, Középfasor 52, HUNGARY.

ABSTRACT

A new method has been developed for the separation of reduced and oxidized forms of glutathione from amino acid mixtures. The samples were spotted on Kieselgel plates and developed in phenol-water = 7:3 containing SDS. The separation was performed in pressurized ultramicro chamber. The running time was shorter than that in a normal chamber, decreasing the possibility of diffusion. In addition to the two forms of glutathione, nine different kinds of amino acids could be separated. The method affords a possibility for rapid analysis of two forms of glutathione both in the clinical and industrial practice.

INTRODUCTION

The tripeptide glutathione, γ -glutamylcysteinylglycine, has an important role in biochemical processes, such as the maintenance of the sulfhydryl state of cysteinyl side-chains of proteins, regulation of enzyme activity by disulphide interchange, detoxifying processes, removal of peroxides generated in the metabolism (1). Glutathione is present mainly in its reduced form in the tissues and the oxidized one is a lower concentration.

There are several chromatographic methods for determination of glutathione. It has been separated with two-dimensional paper chromatography using phenol and collidine as solvents, or one-dimensional chromatography using propanol-water mixture after blocking of the sulfhydryl groups with N-ethylmaleimid (2). Glutathione and its derivatives could be separated from other thiols and ninhydrin-positive compounds by HPLC analysis (3-4) or in amino acid analyser (5).

Murata and his coworkers (6) have published a procedure for the continuous production of glutathione from its constituent amino acids using immobilized whole cells of *Saccharomyces cerevisiae* entrapped in polyacrylamide gel. The cell membrane has become permeable for glutathione releasing it continually from the cells. An intensive amino acid synthesis accompanying the tripeptide production has been found.

The aim of the present study was the separation and determination of reduced (GSH) and oxidized glutathione (GSSG) in a biological mixture. The overpressured thin-layer chromatographic technique (OPTLC) reported by Thihák et al. (7-8) has been adopted. The method proved to be suitable for the separation of two forms of glutathione in phenol-water solvent system which has high viscosity.

MATERIALS AND METHODS

Chemicals for general use were analytical grade and purchased from Reanal Factory of Laboratorial Chemicals (Budapest, Hungary). Amino acid calibration mixture was purchased from BIO-RAD Laboratories (Richmond, California, USA), phenol was obtained from Loba Chemie (Wien, Austria) and it was vacuum distilled from magnesium. The Kieselgel 60 F₂₅₄ and HPTLC chromatoplates were purchased from Merck (Darmstadt, FRG).

The development of sheets was performed in Crompres¹⁰ pressurized ultramicro chamber obtained from Labor MIM (Budapest, Hungary). The concentration of glutathione was measured with Telechrom OE-974 videodensitometer, Chinoin Pharmaceutical Work, Ltd. (Budapest, Hungary).

EXPERIMENTS

Saccharomyces cerevisiae IFO 2044 cells were entrapped in polyacrylamide gel according to the method of Murata et al. (6).

For the production of glutathione the immobilized cells were incubated in a mixture containing 0.5 M glucose, 0.01 M MgCl₂, 0.02 M L-glutamate, 0.02 M L-cysteine, 0.02 M glycine and 0.1 M potassium phosphate buffer (pH 7.0), at 303 K.

The amino acids and glutathione were dissolved in 0.1 M potassium phosphate buffer (pH 5.5), their concentration varied from 1.7 to 2.3 mg/ml, each spot represents about 3-4 µg amino acid.

The chromatoplates were developed in phenol-water 7:3 (w/w) solvent system containing sodium dodecyl sulfate (SDS).

The concentration of SDS was varied between zero and one percentage. The chromatography was performed in Chrompres¹⁰, in linear arrangement, at 303 °K. The development required about 50 minutes with a longer migration distance, setting a filter paper stripe (20x13 cm) on the top of chromatoplates. The development was conducted until the solvent saturated the filter paper. The membrane pressure was 1.2 MPa. Flow rate was varied from $3.08 \times 10^{-3} \text{ cm}^3/\text{sec}$ to $2.75 \times 10^{-3} \text{ cm}^3/\text{sec}$, depending on the concentration of SDS in the solvent.

To remove the phenol from the layers, the plates were dried at 413 K, in -0.9 kp/cm^2 vacuum for 30 minutes.

The identification of amino acids happened with ninhydrin reagent sensitivised with polychromatic γ -collidine. The composition of staining reagent was the following:

50 ml solution A + 50 ml solution B + 2 ml γ -collidine. Solution A contained 2 g of ninhydrin in 100 ml acetone and solution B 1g of cupper acetate dissolved in 245 ml of deionized water, to which were added 5 ml of glacial acetic acid and 250 ml of acetone.

RESULTS AND DISCUSSION

The migrations of glutathione and different amino acids were investigated in phenol-water = 7:3 solvent system containing SDS in different concentrations, on Kieselgel 60 F₂₅₄ plates. Without SDS the GSSG has been separated from the neighbour amino acids, however the GSH has migrated close to the threonine and asparagine. The relative migration distances of glutathione and amino acids are shown in the Table I. The relative migration distances are referred to valine because

Table I.

Effect of SDS on the relative migration distances of glutathione and amino acids referred to valine in phenol-water = 7:3 solvent system containing SDS in different concentration.

	Relative migration distance		
	Phenol-water = = 7:3	Phenol-0.5% SDS = 7:3	Phenol-1% SDS = 7:3
Lys	0.036	0.119	0.245
Arg	0.094	0.238	0.570
GSSG	0.144	0.081	0.070
His	0.194	0.238	0.290
Asp	0.244	0.163	0.115
Cys ₂	0.244	0.325	-
Glu	0.300	0.238	0.200
Cys	0.344	0.263	0.245
Ser	0.344	0.344	0.355
Gly	0.444	0.431	0.475
Thr	0.544	0.538	0.570
Asn	0.569	-	0.570
GSH	0.575	0.519	0.410
Ala	0.650	0.656	0.675
Gln	0.781	0.794	0.810
Val	1.000	1.000	1.000
Tyr	1.081	1.088	1.110
Met	1.156	1.163	1.150
Leu	1.156	1.163	1.150
Ile	1.156	1.163	1.150
Met	1.156	1.163	1.150
Pro	1.206	1.250	1.150
Trp	1.256	1.250	1.200
Phe	1.256	1.313	1.200

of the longer migration distance. The following amino acids showed well defined spots: Lys, Arg, His, Glu, Gly, Ala, Gln, Val, Tyr and Pro.

The order of several components have changed in phenol-0.5% SDS = 7:3 solvent system. The migrations of GSH and GSSG, Asp, Glu and Cys have decreased and those of Lys, Arg, His have increased. His, Glu and Arg run with the same velocity. The relative migration distances are presented in the Table I. The oxidized form of glutathione could be separated again, but the reduced form migrated near the threonine.

In order to separate and quantify the GSH, the concentration of SDS has been enhanced. Developing the plates in phenol-1% SDS = 7:3, the two forms of glutathione have been separated from amino acids, the position of GSSG has not changed. The GSH was localized as a well separated spot under the glycine. The relative migration distances of the components are shown in the Table I. Beside the two forms of glutathione the following amino acids were separated: Asp, Glu, His, Ser, Gly, Ala, Gln, Val and Tyr. The localization of glutathione forms and amino acids both from standard mixture and biological samples are shown in Fig. 1. Lys and Cys migrated with the same velocity, and Asn, Thr and Arg could not be separated from each other in this solvent system.

Fig. 2 shows the densitometric calibration curve for the quantitative evaluation of GSH and GSSG. The most preferable range for the determination is about 1-10 μg GSH and 1-5 μg GSSG in a spot.

In biological samples the reduced glutathione is easily converted into its oxidized form in solution or during the development. To avoid this effect, the samples were bubbled

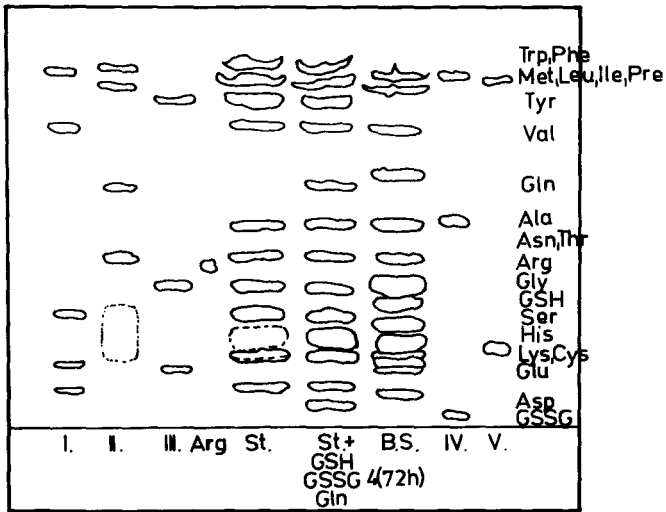


Fig. 1. Separation of reduced (GSH) and oxidized (GSSG) glutathione from amino acid mixture and biological sample on Merck 5548 HPTLC plate, in phenol-1% SDS=7:3 solvent system. Flow rate: $2.92 \times 10^{-3} \text{ cm}^3/\text{sec}$.

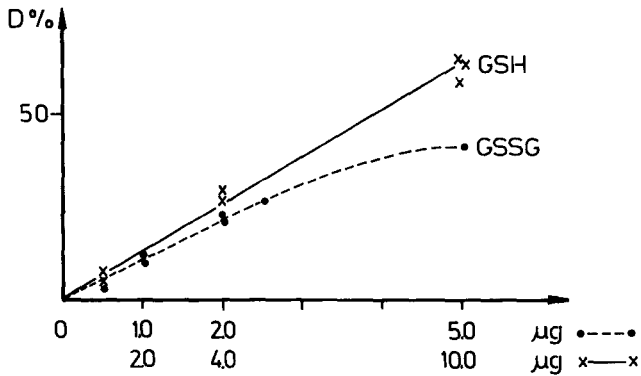


Fig. 2 Calibration curve for reduced and oxidized form of glutathione evaluated in videodensitometer.

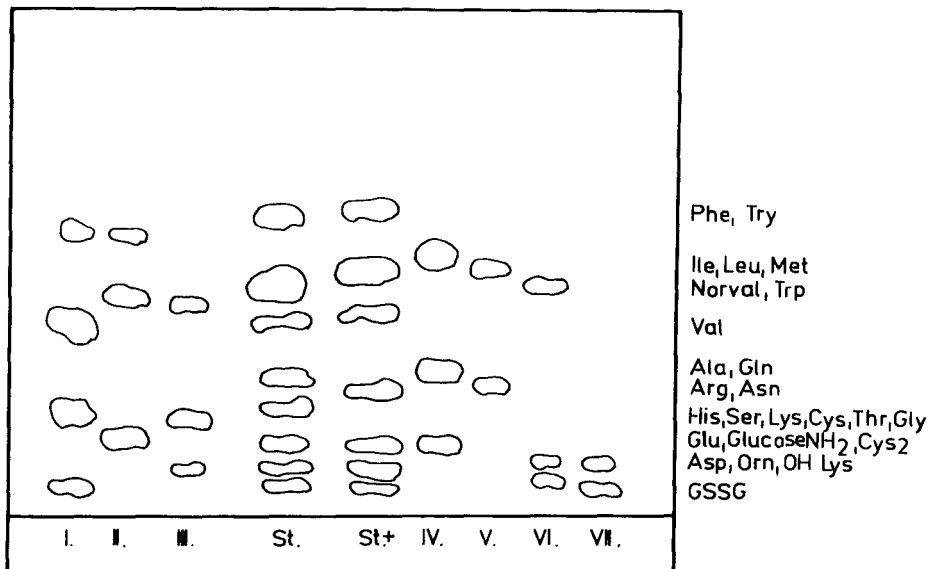


Fig. 3 Development of glutathione and amino acids in normal chamber on Merck 5554 Kieselgel 60 F₂₅₄ plate, in phenol-1% SDS solvent system. Running time: 9 hours.

with oxygen. By this way the total glutathione content might be determined quantitatively by videodensitometer.

These findings suggest that the two forms of glutathione can be separated from amino acid mixtures in phenol-1% SDS = 7:3 solvent system by OPTLC method. Comparing the development of samples in pressurized ultra-micro chamber with normal chamber (Fig. 3) it can be stated, that the previous method is suitable especially for solvent systems having a high viscosity. The slow migration can be reduced from 8-9 hours to 50 minutes in the case of our solvent system, decreasing the possibility of diffusion of components.

Development in phenol-water = 7:3 solvent system the reduced form of glutathione can not be separated from asparagine. The SDS changes the selectivity of the original solvent and the relative position of several components has changed. It is true especially for basic amino acids and GSH. Ahrland et al. (9) found that the titration curve of silica gel resembles that of a weakly acidic ion exchanger. A suppose is, that the detergent depletes the ion exchange capacity of layer and its partitional feature has been achieved. By this way the separation depends first of all on the polarity and size of different components.

According to our results a modified phenol-water solvent system containing SDS proved to be suitable for the separation of two forms of glutathione, especially for the oxidized form. The later showed a low migration velocity and could be localised as a well separated spot. On the basis of this phenomenon the application of OPTLC method might afford a possibility for rapid estimation of GSSG. By this way several amino acids, namely Asp, Glu, His, Ser, Gly, Ala, Gln, Val and Tyr could be separated from the glutathione forms and quantified, too.

The time of development was decreased from 9 hours to 50 minutes by application of OPTLC method, comparing it with the development in normal chamber. Reducing the running time, the possibility of diffusion was strongly limited. The oxidation of GSH in the biological samples prior to application might take possible the quantitative determination to escape the oxidation during the development. This method can be applied for rapid analysis of glutathione and amino acids in clinical and industrial practice.

Acknowledgement. We wish to thank Dr. H. Kalász for many helpful comments and suggestions.

REFERENCES

1. Akerboom, T.P.M. and Sies, H., Assay of Glutathione Disulfide and Glutathione Mixed Disulfides in Biological Samples, in: Methods in Enzymology, Vol. 77., Jacoby, W.B. ed., Acad. Press, New York-London-San Francisco, 1981. p. 373.
2. Patterson, J.W. and Lazarow, A., Determination of Glutathione, in: Methods of Biochemical Analysis. Vol. 2., Glick, D. ed., Intersci. Publ. New York, 1956. p. 259.
3. Reed, D.J., Babson, J.R., Beatty, P.W., Brodie, A.E., Ellis, W.W. and Potter, D.W., High-Performance Liquid Chromatography Analysis of Nanomole Levels of Glutathione, Glutathione Disulphide and Related Thiols and Disulphides. Anal. Biochem. 106. 55 (1980).
4. Murayama, K. and Kinoshita, T., Determination of Glutathione on High-Performance Liquid Chromatography using N-Chlorodansylamid (NCDA). Anal. Lett. 14. 1221 (1981).
5. Tabor, W.C. and Tabor, H., An Automated Ion-Exchange Assay for Glutathione. Anal. Biochem. 78. 543 (1977).
6. Murata, K., Tani, K., Kato, J. and Chibata, I., Glutathione Production by Immobilized *Saccharomyces cerevisiae* Cells Containing an ATP Regeneration System. Eur. J. Appl. Microbiol. Biotechnol. 11. 72 (1981).
7. Tyihák, E., Mincsovcics, E. and Kalász, H., New Planar Liquid Chromatographic Technique: Overpressured Thin-Layer Chromatography. J. Chromatog. 174. 75 (1979).
8. Tyihák, E., Székely, T.J. and Mincsovcics, E., Applicability of OPTLC in the Analysis of Various Substance Groups. Proceedings of the Second International Symposium on Instrumental High Performance Thin-Layer Chromatography, Interlaken, 1982.

9. Arhland, S., Grenthe, U.I. and Norén, B., The Ion Exchange Properties of Silica Gel. I. The Sorption of Na^+ , Ca^{2+} , Ba^{2+} , UO_2^{2+} , Gd^{2+} , Gd^{3+} , Zr (IV) + Nb, U(IV) and Pu(IV). II. Separation of Plutonium and Fission Products from Ir-radiated Uranium. Acta Chem. Scand. 14. 1059, 1077 (1960).

ISOLATION OF GLYCOLIPIDS FROM BLOOD ELEMENTS

J. Pick[✱], J. Vajda^{✱✱} and L. Leisztner^{✱✱}

✱ National Institute of Haematology & Blood Transfusion
H-1502 Budapest, P.O.B. 44., Hungary

✱✱ Institute of Forensic Science
H-1903 Budapest, P.O.B. 314/4., Hungary

ABSTRACT

Several chromatographic e.g. HPLC, HPTLC, etc. methods have been published in the literature for the separation, after sufficient pretreatment, of derivatized or non-derivatized glycolipid samples.

Our task is the extraction, isolation and separation of the glycolipids from different blood elements, followed by suitable fractionation methods, giving the lipid classes in sufficient purity and quantity for HPLC, HPTLC and OPTLC measurements and possibly further biochemical use.

We show the differences between the procedures commonly used and that developed in our laboratory.

The advantage of our method, which employs 3 cm long Brownlee Labs HPLC cartridges, is that it can be automated, it gives class fractionation of the lipid samples and as it is hardware compatible with HPLC equipment it can be used directly in a coupled column system for on line separation in the individual class. The development of this column coupling method for the fractionation of a given lipid class from the total lipid extract on an analytical column is under development.

Author to whom correspondence should be sent: Dr. L. Leisztner

INTRODUCTION

The sample preparation for most instrumental analytical methods is a time and labour consuming process. This is really true in the case of biological samples and HPLC, as the costly HPLC columns have to be protected from the various irreversibly or near irreversibly retained components of the biological sample. The use of short preparative columns for sample preparation is getting very common and is facilitated by the numerous commercial products available (Sep-Pak, Prep Flute, Bond Elute, Extrelute from Waters, Hamilton, Analytichem, Merck - to name a few - respectively). The problem with these is that they are suitable only for manual work (1,2), as they can not be installed in a chromatograph. We decided to develop a method suitable later for column coupling. The possibility of column coupling is necessary for our analysis, as the preparation of a very small sample can be performed with less wastage in that way.

The object of our investigations, the glycolipids of blood elements, are minor components of the cell membrane. The majority of glycosphingolipids are assumed to be present at the outer leaflet of plasma membranes and play very important biological roles. The functions of glycolipids (3,4) has been increasingly examined parallel with the methodological developments (5,6,7,8) of identification during the last few years.

At first only derivatized glycolipids were separated by HPLC (9) with UV detection, later non-derivatized glycolipids without on line detection (10) monitored by TLC runs. We presented non-derivatized glycolipid separation with refractive index (RI) detection (11), on line detection of these compounds was accomplished by Handa and Kushi (8) at 206 nm.

The importance of the separation of glycosphingolipids without derivatization is that the separated components can then be directly used for further biochemical, immunological and structural investigations.

EXPERIMENTAL

Reagents and materials. All the solvents used were HPLC grade purchased from E. Merck (Darmstadt, F.R.G.). The water emplo-

yed was prepared according to Gurkin's method (12), from water double distilled from glass after ion-exchange. DEAE Sephadex A-25 was obtained from Pharmacia Fine Chemicals AB (Uppsala, Sweden). Staining reagents were prepared from analytical grade chemicals. ODS-GU and SIL-GU cartridges were purchased from Brownlee Labs Inc. (Santa Clara, Calif., USA). The HPTLC plates used for monitoring HPLC fractions and purity checking were bought from E. Merck (Darmstadt, F.R.G.).

Total lipid extract was prepared from human lymphocytes.

Apparatus. Centrifuge: Janetzki K70 MLW (Leipzig, G.D.R.). Incubator: LP 507/1 Labor MIM (Esztergom, Hungary). Chromatographs: HP 1084 B with HP 79875 variable wavelength UV detector, Hewlett Packard (Palo Alto, Calif., USA), Beckman 112 solvent delivery module, Altex 156 differential refractometer, Beckman Instruments Inc. (Berkely, Calif., USA). Fraction collector: LKB Minirac 1700, LKB Produkter AB (Bromma, Sweden). Data system: HP 3354 Laboratory Automation System, Hewlett Packard (Avondale, Calif., USA).

Methods.

Total Lipid Extract (TLE) preparation. Lipid extraction was carried out similarly to the methods published previously (13, 14). The TLE was extracted from the liophilized cells at room temperature with mild sonication in chloroform : methanol mixtures in the ratios 2:1, 1:1, 1:2. The supernatant were collected together and dried in vacuo. The dried TLE was dissolved in chloroform : methanol : water = 30 : 60 : 8 mixture (100 ml) and the solution used for the isolation of the lipid classes described below.

Isolation of Acidic and Neutral Lipids (AcL, NF). The separation was performed on a ϕ 12 x 70 mm DEAE Sephadex A-25 column in the acetate form. The solution of TLE (100 ml) was applied to the column, was washed with 100 ml of the same solvent, then with 50 ml of methanol. The 250 ml solution issuing from the column was used after solvent evaporation (water traces were removed with benzene), for the fractionation of neutral lipids, dissolved in chloroform, as given later.

Acidic lipids were eluted with 50 ml of 0.25 M methanolic sodium acetate. This portion of the eluent was dried in vacuo

and incubated in 15 ml 0.1 M methanolic sodium hydroxide at 37°C for 2 hours to destroy the alkali labile phospholipids (PL). We have developed a two step chromatographic method for the recovery and isolation of acidic glycolipids. The first step is similar to the method using Sep-Pak C18 cartridges (?). After incubation the sample was dried in vacuo and the residue was dissolved in cold (4°C) HPLC grade water, neutralized very carefully to 4 < pH < 5 with 0.5 M HCl. The salt concentration was finally adjusted to 0.1 M by adding water. This sample solution was passed through an ODS-GU cartridge at a flow rate of 1.5 ml/min, the acidic glycolipids being collected on the column. After washing the cartridge with 30-50 ml of water, the glycolipids were eluted by 50 ml chloroform : methanol = 1 : 2 mixture.

Fractionation of Neutral Lipids. The neutral lipid portion derived from 2-8 x 10¹⁰ lymphocyte cells were taken up in 3 ml of dry chloroform and 5 - 30 µl aliquots were injected. Detection was at 254 : 600 nm, sample : reference wavelengths, attenuation 0.9 AUFS for the UV detector and 1 V attenuation FS for the RI detector. We employed the HP 1084 B IC equipped with UV and RI detectors coupled in series, with a fraction collector after the last detector.

Numerous elution profiles, given later, were tried, of which the selected chromatographic conditions were: flow 1 ml/min, gradient elution, solvent A was chloroform, B was methanol. The gradient started with 1 min. isocratic 100% A, followed by a linear increase to 100% B in 9 minutes, then a linear decrease to 100% A in 5 minutes. The run ended after further 5 minutes isocratic 100% A. This gradient, No.5, gave optimal results for our purposes. The separation was conducted at ambient temperature. In addition to on line detection the fractions collected during the run were also monitored by parallel TLC runs on silica plates. The empty fractions were discarded and those containing the same components (same subclass) united. Of the two chromatograms one was stained with 1 : 1 sulfuric acid : water, to visualize all of the components, giving also a specific red colour for cholesterol during heating. The other plate was stained with orcinol reagent, the neutral glycolipids appearing as purple spots.

RESULTS AND DISCUSSION

There are several methods for the separation of the total lipids, derived from biological sources such as cell membranes, in the literature. To have a comparable picture the commonly used methodologies have been summarized in flowcharts, shown in Figure 1. Comparing flowcharts 1, 2 and 3, it can be clearly seen that all these methods require a great amount of work, time and materials, especially high purity solvents. The trend of analysis of biological samples points to the importance of pretreatment and sample preparation (15). Because of the high cost of HPLC grade chemicals and analytical columns the use of guard and/or precolumns and rigorous sample pretreatment became more and more necessary, while decreasing the time and material requirements of the analysis was also needed.

A further requirement can be inherent in the samples, like in our case. The sample can be extremely small amount and unrepeatable (for example a lymphocyte sample of a leu-chemic patient before chemotherapy). For this reason the whole analytical process has to be precise, reproducibile and rugged.

The method developed in our laboratory for the separation of TLE into lipid classes in purity, adequate for further fractionation into separate lipid types, has the advantage of speed, automatebility and the possibility of development into an on line complete HPLC fractionation.

The first step in our method of TLE fractionation is the known DEAE Sephadex column chromatography for the isolation of neutral and acidic lipids. For the following steps of the separation we applied Brownlee Labs SIL-GU and ODS-GU cartridges.

Comparing the flowcharts 1, 2, 3 with 4 it can be seen (Figure 1) that the use of small cartridge columns for the class fractionation after the separation of acidic and neutral lipids is much simpler and more economic in terms of labour, solvents and time.

The acidic lipid purification with the ODS-GU cartridge described in the experimental part was checked for purity of the ganglioside class with HPTLC as shown in Figure 2. As can be seen on the chromatogram, only resorcinol positive compounds are present.

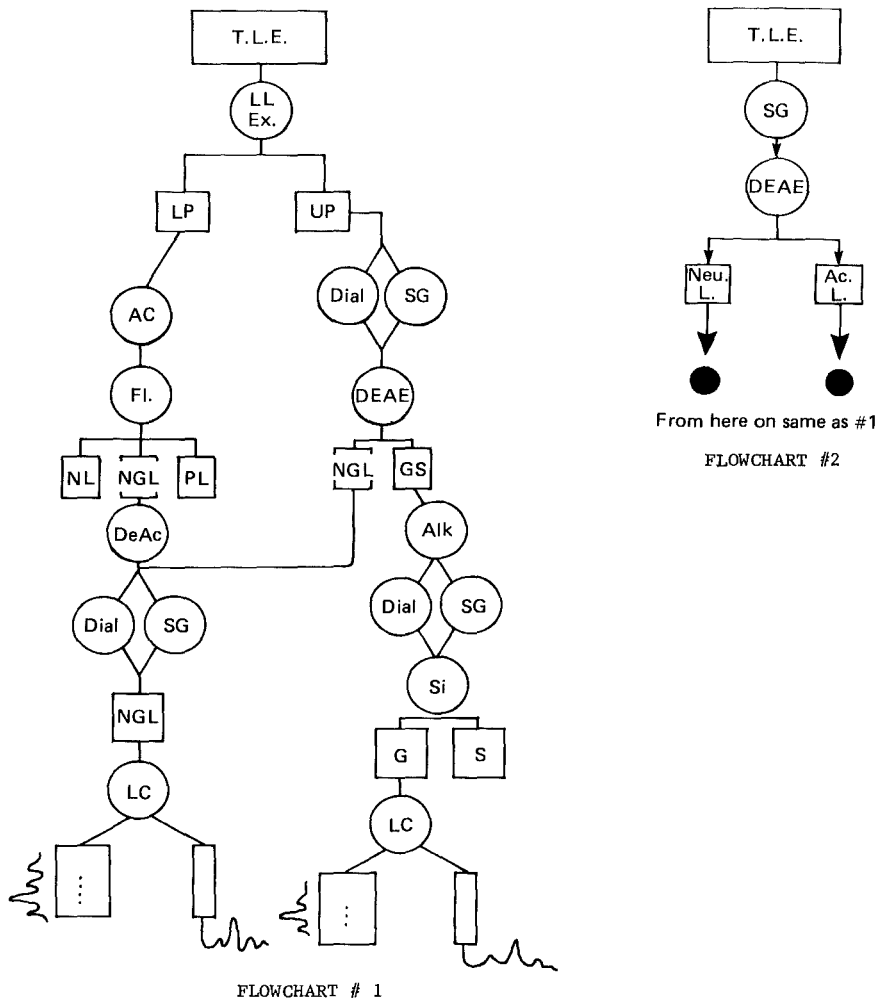
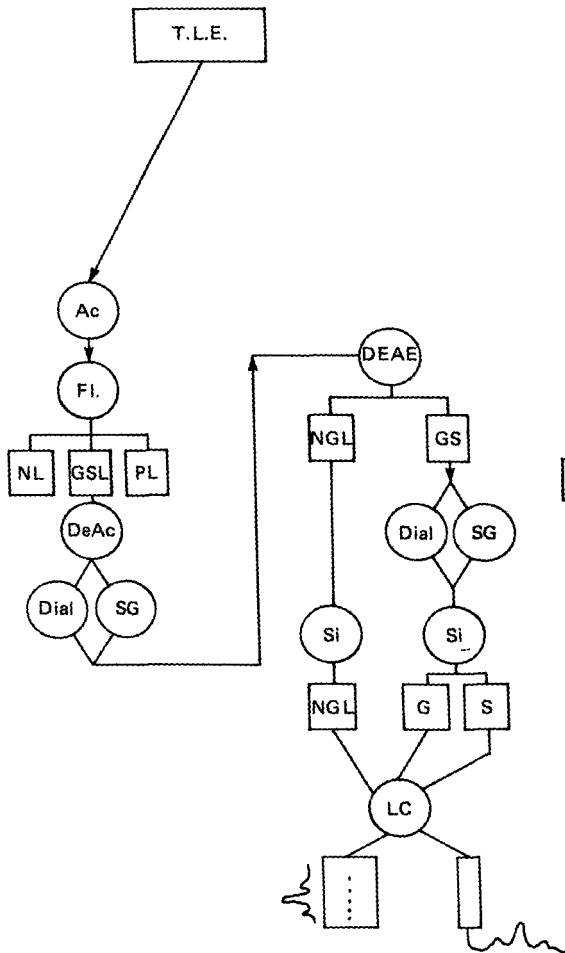


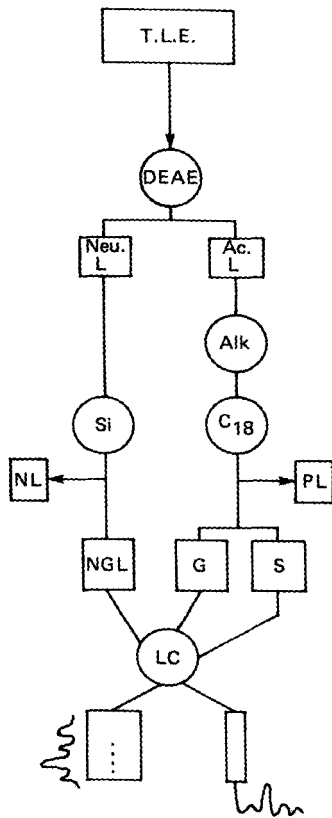
Figure 1. Possible work up routes of TLE.

Flowcharts #1 - 3 from literature data, #4 showing our method.

Symbols used: LL EX.: liquid-liquid extraction, LP: lower phase, UP: upper phase, Ac: acetylation, Fl: Florisil col.chrom., DeAc: deacetylation, Dial: dialysis, SG: Sephadex G col.chrom., G: gangliosides, S: sulphatides, Alk: alkalization, Si: silicic acid col.chrom., SI: STT-CU col.chrom., C₁₈: ODS-CU col.chrom.



FLOWCHART #3



FLOWCHART #4

Figure 1 - continued

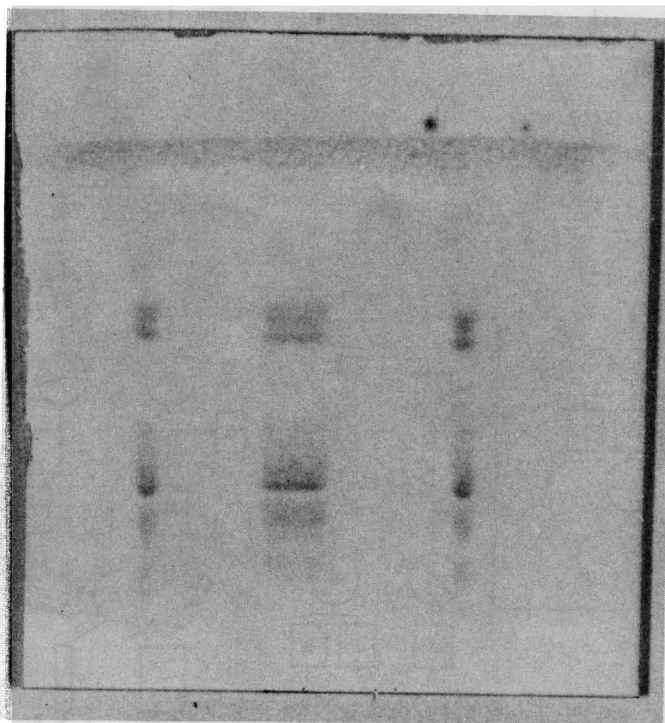


Figure 2. HPTLC separation of lymphocyte gangliosides to show their purity. Sample applications of 0.5, 1.5, 1.0 μ l of ganglioside solution of 8×10^9 lymphocytes in 1.0 ml chloroform : methanol = 1 : 2 .
Plate: 5 x 5 cm Merck HPTLC Si 60, developing system: CHCl_3 : CH_3OH : 0.025M KCl/ H_2O = 60 : 40 : 9 ,
staining with resorcinol-HCl, 100°C, 20 min.

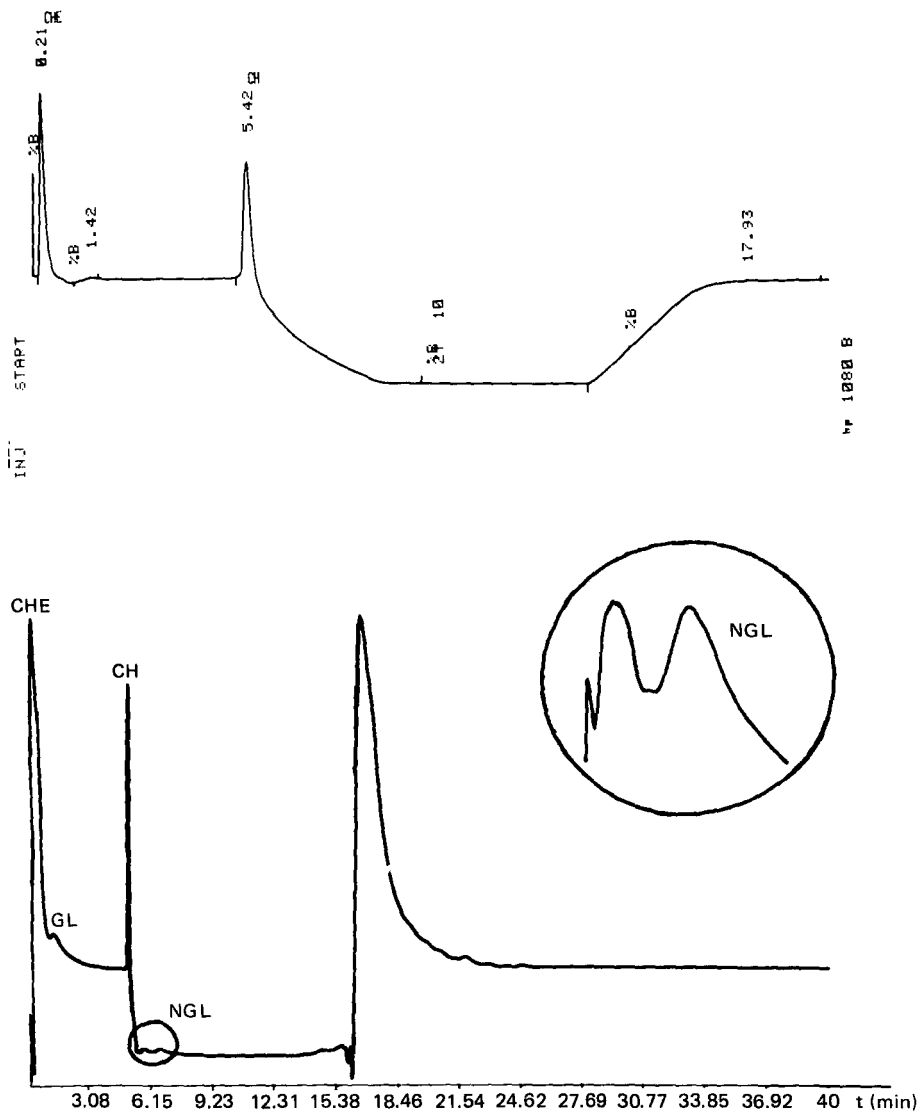
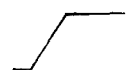





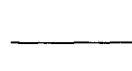


Figure 3. Chromatograms of lymphocyte neutral lipid fraction with elution profile No. 5.

1. UV detector signal, 2. RI detector signal.
 (CH): cholesterol esters, CH: cholesterol, NGL: neutral glycolipids.

Table 1. Elution profiles tested for the separation of neutral lipid classes on SIT-GU column, eluents; A:chloroform, B:methanol.

Gradient		SEGMENTS					Flow ml/min	No. of fractions
No.	profile	%B at start %B at end duration min.						
		1	2	3	4	5		
1		0 0 1	0 100 10	100 100 15	-	-	1.0	26
2		0 10 10	10 100 5	100 100 1	100 0 4	0 0 20	0.5	60
3		0 0 3	0 100 9	100 100 3	100 0 4	0 0 20	0.5	20
4		10 10 20	100 100 10	10 10 10	-	-	0.5	20
5		0 0 1	0 100 9	100 0 5	0 0 25	-	0.5	5
6		7 7 1	7 100 9	100 7 5	7 7 20	-	0.5	4
7		30 30 40	-	-	-	-	0.5	6

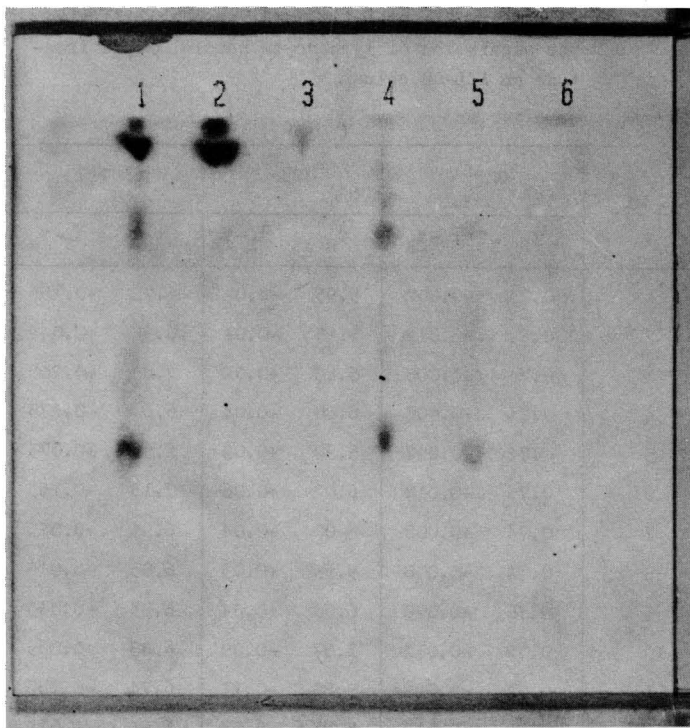


Figure 4. General monitoring plate of the separation of lymphocyte neutral lipid fraction on SIL-GU column.

Plate: 5 x 5 cm Merck HPTLC Si 60, developing system: $\text{CHCl}_3 : \text{CH}_3\text{OH} : \text{H}_2\text{O} = 65 : 25 : 4$, staining with $\text{H}_2\text{SO}_4 : \text{H}_2\text{O} = 1 : 1$, 100°C , 2 min.

Lanes: 1. TLE, 2., 3. neutral lipids (CHE, CH, glycerides etc.) 4., 5. neutral glycolipids, 6. background of solvents evaporated.

Table 2. Retention time stability and reproducibility of the separation of lymphocyte neutral lipid fraction on SII-CU column.

Run No.	CHE		CH		NGL	
	t_R	$\bar{t}_R - t_R$	t_R	$\bar{t}_R - t_R$	t_R	$\bar{t}_R - t_R$
1	0.73	-0.002	5.95	-0.01	6.72	-0.155
2	0.72	-0.012	5.95	-0.01	6.92	+0.045
3	0.74	+0.008	5.98	+0.02	7.08	+0.205
4	0.74	+0.008	6.00	+0.04	6.60	-0.275
5	0.74	+0.008	5.99	+0.03	6.95	+0.075
6	0.75	+0.018	6.02	+0.06	7.13	+0.155
7	0.74	+0.008	6.00	+0.04	6.95	+0.075
8	0.74	+0.008	5.99	+0.03	6.83	-0.045
9	0.76	+0.028	6.00	+0.04	6.83	-0.045
10	0.72	-0.012	5.87	-0.09	6.88	+0.005
11	0.71	-0.022	5.85	-0.11	6.74	-0.135
12	0.72	-0.012	5.83	-0.13	6.84	-0.035
13	0.72	-0.012	5.98	+0.02	6.79	-0.085
14	0.72	-0.012	5.99	+0.03	6.90	+0.025
15	0.76	+0.028	6.00	+0.04	6.97	+0.095
16	0.71	-0.022	6.01	+0.05	6.87	-0.005
\bar{t}_R	0.732		5.96		6.875	
SD	0.016		0.06		0.130	

The neutral lipid class fractionation was carried out on a SIL-GU column. The dried sample was dissolved in chloroform, as the use of chloroform-methanol mixtures for sample introduction interfered with the separation of cholesterol esters and cholesterol, although it dissolves the sample much better. After isocratic exploratory experiments, gradient runs were tried. The elution profiles tested and relevant data are summarized in Table 1. Of the several gradients tested for neutral lipid class fractionation, No.5, the best, was selected in the end. The corresponding chromatograms are shown in Figure 3. The aim was sufficient separation between classes and if possible, negligible separation in the classes themselves. This was to make possible the collection of small fractions for total recovery, or column coupling with the heart-cut method for analytical separation of the lipids of a selected class. As shown on a general monitoring plate (Figure 4) the class separation is adequate.

The stability and reproducibility of the method can be seen from Table 2, giving the retention times, their average values and standard deviation from 16 runs. It can also be seen from the table, that column performance is stable, there was no unidirectional drift in the \bar{t}_R-t_R data. Cholesterol esters and cholesterol retention times are from the UV signal, those for neutral glycolipids from the RI signal. The retention times are corrected for the delay caused by the connecting tubing between the detectors.

As shown by the standard deviations the reproducibility is very good. Numerous samples can be analysed on a small cartridge without any significant change in system behaviour, as can be seen in the random character of the variation of \bar{t}_R-t_R values.

ACKNOWLEDGEMENT. The authors thank NGUYEN ANH-TUAN MD PhD for his kind help in providing separated lymphocyte cells.

REFERENCES

1. M. Williams, R. McCluer, J. Neurochem., 35, 266 (1980).
2. S.K. Kundu, A. Suzuki, J. Chromatogr., 224, 249 (1981).
3. S-I. Hakomori, Annual Rev. Biochem., 50, 733 (1981).

4. H. Jiegand, *Advances in Neurochem.*, 4, 149 (1982).
5. A. Suzuki, S. Handa, T. Yamakawa, *J. Biochem.*, 82, 1185 (1977).
6. T. Yamazaki, A. Suzuki, S. Handa, T. Yamakawa, *J. Biochem.*, 83, 1101 (1979).
7. P. Hanfland, H. Egge, U. Dabrowski, D. Roelke, *J. Dabrowski, Biochemistry*, 20, 5310 (1981).
8. S. Handa, Y. Kushi, *Adv. Exp. Med. Biol.*, 152, 23 (1982).
9. R. McCluer, J. Evans, *J. Lipid Res.*, 14, 611 (1973).
10. K. Watanabe, Y. Arao, *J. Lipid Res.*, 22, 1020 (1981).
11. J. Vajda, J. Pick, L. Leisztner, *Spec. FEBS Meeting, Athens, Greece, 1982*.
12. M. Gurkin, J. Rippahn, *Int. Lab.*, *May/June*, 63 (1980).
13. R. Yu, R. Ledeen, *J. Lipid Res.*, 13, 680 (1972).
14. L. Svennerholm, P. Fredman, *Biochim. Biophys. Acta* 617, 97 (1980).
15. V. Tyengar, *Anal. Chem.*, 54, 554A (1982).

IDENTIFICATION OF SOME POTENTIAL ANTIDIABETIC COMPOUNDS
ON IMPREGNATED SILICA GEL G PLATE AS THEIR π -COMPLEXES

Rajeev Jain
School of Studies in Chemistry, Jiwaji University
Gwalior-474 011(INDIA)

and

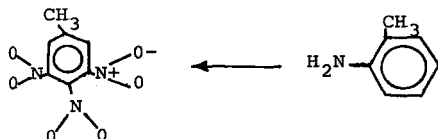
D.D. Agarwal
Institute De Recherches Sur La Catalyse-CNRS, 2-Avenue Albert
Einstein, F 69626 Villeurbanne(FRANCE)

ABSTRACT

A rapid and convenient procedure for the detection of potential antidiabetics as π complexes with aromatic amines is described. The method could be of immense value even in places where normal laboratory facilities are not available.

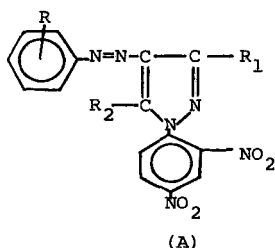
INTRODUCTION

The field of charge transfer complexes has been investigated recently by several chromatographic techniques. Unsaturated lipids have been resolved on columns of silica gel impregnated with silver nitrate (1). Thin-layer chromatography has been employed in the investigations of the complexes of silver with terpenes (2), glycerides (3) and silylic-propenylic isomers (4). The separation of the π - complexes of m-dinitrobenzene, 2, 4-dinitrotoluene, 2,4,5-trinitrotoluene and 2,4,6-trinitroanisole with some hydrocarbons on TLC plates has been carried out (5). Nitroaromatic compounds form π - complexes with aromatic donors (6) due to polarization of the nitro group. These π complexes involve hybrid structures with bedding of the filled Sp hybridized orbital on the nitrogen atom in the amine compound with the vacant p orbital on the nitrogen of the nitro group, e.g.



More recently it has been suggested that during the formation of 1:1 π complexes of the *m*-dinitrobenzene with various aminobenzoic acids only one nitro group plays role (7). The majority of the complexes are unstable. Solvent and adsorption forces, steric hindrance, temperature etc., weaken the charge transfer linkages and make their study difficult.

The present papers describes a simple and convenient method for the identification of trace amounts of 1-(2,4-dinitrophenyl)-3,5-dimethyl/diphenyl-4-arylazopyrazoles (A), which are well known as potential antidiabetic agents, as charge-transfer complexes with aromatic amines employing TLC technique. The resolved π complexes being highly coloured could be located easily on the chromatoplates. It was possible to identify as little as 1-2 μ g of these compounds as their π complexes.



where R represents various substituents, $R_1 = R_2 + \text{CH}_3$ or C_6H_5

EXPERIMENTAL

The TLC plates (thickness 0.5 mm) were prepared by spraying a slurry of a mixture of 50g of silica gel G+1 g of aromatic amine in 100 ml of distilled water and drying for 24 hrs at a constant temperature of 60°C. The compounds (I-XX)

TABLE - 1

Sl No.	R	R ₁	R ₂	Rf x 100				Detection limit (µg)
				A	B	A	B	
				Plain Gel	Silica G	Silica Toluidine	Gen C+p-	
I	H	CH ₃	CH ₃	44	52	36	46	2.0
II	2-CH ₃	CH ₃	CH ₃	55	49	29	40	1.5
III	3-CH ₃	CH ₃	CH ₃	37	50	32	32	2.0
IV	4-CH ₃	CH ₃	CH ₃	31	54	24	36	3.5
V	2-CI	CH ₃	CH ₃	21	28	20	29	2.0
VI	3-CI	CH ₃	CH ₃	22	34	21	23	1.5
VII	4-CI	CH ₃	CH ₃	25	35	16	19	1.5
VIII	2-OCH ₃	CH ₃	CH ₃	39	42	41	52	2.5
IX	3-OCH ₃	CH ₃	CH ₃	45	48	45	58	2.5
X	4-OCH ₃	CH ₃	CH ₃	46	51	50	54	1.5
XI	H	C ₆ H ₅	C ₆ H ₅	30	46	28	42	1.0
XII	2-CH ₃	C ₆ H ₅	C ₆ H ₅	27	40	23	38	1.5
XIII	3-CH ₃	C ₆ H ₅	C ₆ H ₅	29	38	20	28	2.0
IXV	4-CH ₃	C ₆ H ₅	C ₆ H ₅	32	32	18	31	2.0
XV	2-CI	C ₆ H ₅	C ₆ H ₅	09	16	07	19	1.5
XVI	3-CI	C ₆ H ₅	C ₆ H ₅	13	17	11	23	1.5
XVII	4-CI	C ₆ H ₅	C ₆ H ₅	15	20	13	16	2.0
XVIII	2-OCH ₃	C ₆ H ₅	C ₆ H ₅	26	23	31	45	1.5
XIX	3-OCH ₃	C ₆ H ₅	C ₆ H ₅	28	28	26	54	2.0
XX	4-OCH ₃	C ₆ H ₅	C ₆ H ₅	33	30	35	51	2.0

* For p-toluidine impregnated plates
a - Average of two identical runs

Solvent Systems: (A) Xylene (60/): Chloroform (30/): Acetone (10/).

(B) Benzene (55/): Chloroform (40/): Ethyl methyl ketone (5/)

Rate of Development: 15 cm in 40 min for plain silica gel plates and 15 cm in
50 min for p-toluidine impregnated silica gel plates.

in acetone (0.05/ w/v) were applied to the chromatographic plates using glass capillary and the chromatograms were eluted with various solvents systems but the best resolution was obtained with solvent systems A and B (Table-1).

After thorough research it was found that a mixture of p-toluidine and silica gel gave the most satisfactory separation. The results are presented in Table-1. For comparison the values on plain silica gel plates under similar conditions are also given. It can be seen that out of 20 pyrazoles only 12 can be separated on plain silica gel G plates. Further, it may be pointed out that the Rf-values are changed when a mixture of pyrazoles was applied.

RESULTS AND DISCUSSION

The resolution of π complexes was found to be governed by three factors (i) adsorbent (ii) nature of the π donor (aromatic amine) and (iii) irrigating solvent. In general the migration of these compounds were in relation to the effect of electron attracting or repelling groups present in them.

REFERENCES

1. Devries, D., Chem. Ind. (London), 1049 (1962).
2. Ikan, R., J. Chromatogr., 17, 591 (1965).
3. Barrett, C.B., Dablas, M.S.J. and Padley, Chem. Ind. (London), 1050 (1962).
4. Nanom M. and Martell, A., J. Chromatogr., 21, 349 (1966).
5. Neumann, M.F. and Jossang, P., J. Chromatogr., 14, 280 (1964).
6. Miller, R.E. and Jones-Mynne, W.F.K., J. Chem. Soc., 2375 (1959).
7. Tronov, B.V. and Syrneva, N.V., Izvest. Vysshikh Ucheb. Zavedenic, Khim. Khim. Technol., 4, 752 (1960).

THE ROLE OF THE SPECIFIC SURFACE AREA OF
AN ADSORBENT IN THE OPTIMIZATION OF MIXTURE
SEPARATION CONDITIONS IN THIN - LAYER
CHROMATOGRAPHY

Jan K.ROŻYŁO, Irena MALINOWSKA and
Małgorzata PONIEWAŻ

Institute of Chemistry, Maria Curie-Skłodowska
University, Pl. M.Curie-Skłodowskiej 3,
20-031 Lublin, POLAND.

ABSTRACT

The very important aspect of effect of the magnitude of specific surface area on R_M values obtained by using TLC method is presented. In experiments were performed on 4 adsorbents of different specific surface areas 50 - 500 m^2/g and with mixed binary mobile phases.

It is shown that R_M values of chromatographed substances aromatic hydrocarbons are lineary dependente upon the specific surface area of adsorbents for each composition of mobile phase. This relationship can be described by a straight line, with the parameters a and b that can be tabularized. These lines can be used to calculate the R_M values of chromatographed substances for any adsorbent if

is specific surface area is known. The illustrational comparison of experimentally obtained and theoretically predicted R_M values for different chromatographic system are presented.

INTRODUCTION

Adsorbents of different microporous structure (1-5) have been used for a long time in thin-layer chromatography for the separation of mixtures of various substances. The microporous structure of adsorbents is an important affecting optimization of the chromatographic process. The significance of the microporous structure of the adsorbent in the process of thin-layer chromatography was studied by Geiss (6) and Snyder (7). Practical possibilities of controlling the process of thin-layer chromatography were also indicated by Różyło (8,9). Attempts were also explain the physical-chemical significance of the value of the adsorbent specific surface area in thin-layer chromatography (10). Recent considerable interest in the role of the specific surface area of the adsorbent in thin-layer chromatography is due to growing importance of this method as a pilot technique for determining the optimum conditions of mixture separation on both analytical and preparative scales. The latter is widely applied for obtaining and purifying of natural origin in research laboratories, as well as in pharmaceutical and food industries, and also in the nature environment protection (11).

Linear relations between R_M values of substances for pure components of a mixed e.g. binary mobile phase $R_{M1,2}$

and the specific surface area of the adsorbent, as has already been observed - are not favourable enough to be applied in a routine process of optimization of separation conditions. Therefore, the present paper analyses the relations between the R_M values of substances and the specific surface area of the adsorbent. The values were investigated for identical concentrations of the mobile phase on adsorbents of different values of the specific surface area.

EXPERIMENTAL

Measurements were taken of the R_F (R_M) values of model substances obtained in the process of adsorption thin-layer chromatography on silica gels of different microporous structure, produced by MERCK (12):

pore diameter Å	specific surface area m ² /g
60	500
100	400
200	150
500	50

The chromatographed substances were certain polycyclic aromatic hydrocarbons showing neither electrodonor nor electroacceptor properties: naphthalene, β -methylnaphthalene, pyrene, chrysene, fluoranthene, diphenyl. This selection of model chromatographed substances aimed at eliminating possible additional intermolecular actions with the adsorbent surface and the components of the mobile phase which was composed of four mixed binary solvents: hexane - benzene, methylcyclohexane - benzene, benzene - methanol

and benzene - ethanol. The procedure of conducting the chromatographic process and substance detection were described in several earlier publications (1-4, 8, 13).

RESULTS AND DISCUSSION

It is apparent from the results presented in figures 1-4 that on silica gels of different specific surface area the R_M values of the chromatographed substance change in a regular manner. It turned out that there is a linear dependence of the R_M value on the specific surface of the adsorbent. This dependence can be presented from of a linear equation:

$$R_M = as + b \quad (1)$$

where s is the specific surface area of the adsorbent, and values "a" and "b" - parameters of the straight line.

As appears from the graphs, the R_M values obtained from the experiment are laid on a straight line drawn so as get the smallest deviation. Table 1-4 shows the $R_M = f(s)$ straight line parameters which were calculated and tabulated for particular concentrations of the mobile phase and the chromatographed substance.

Tables 1-4 show the $R_M = f(s)$ straight lines parameters, which were calculated and tabulated for some chosen concentrations of the mobile phase and the chromatographed substance. Tables 1 and 2 show the parameter "a" of the straight line whereas tables 3,4 - the parameters "b".

In the systems methylcyclohexane - benzene and he - benzene inactive mobile phase the "a" values decrease

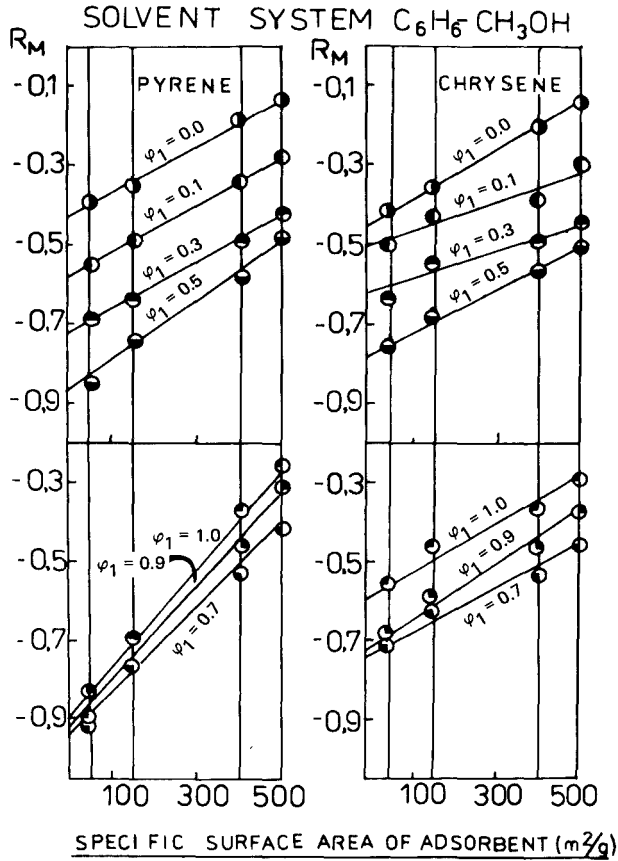


Fig. 1.

Linear relationship between R_M values and specific surface area of adsorbent. Solvent system: benzene-methanol. Points - experimental data, straight line - theoretical data, calculated from eq. 1.

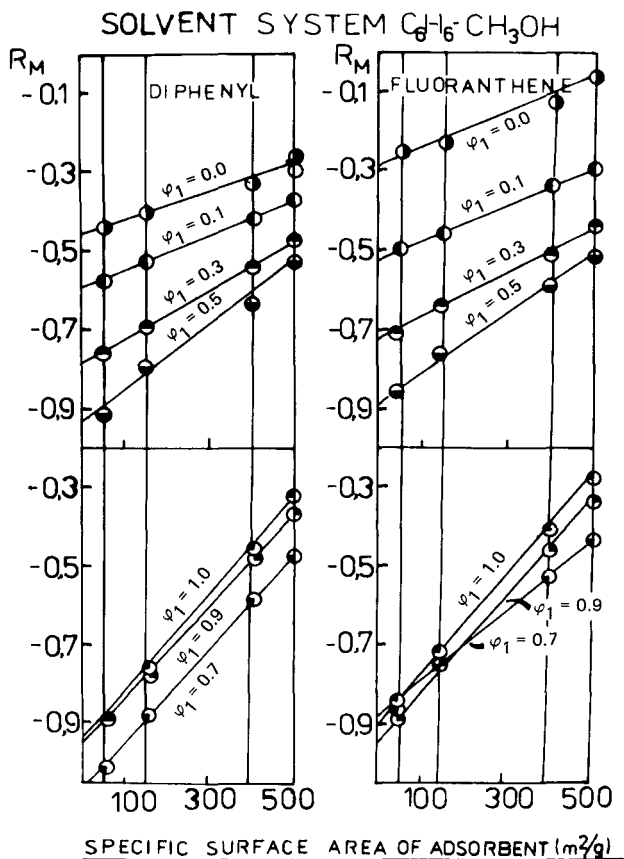


Fig. 2.

Linear relationship between R_M values and specific surface area of adsorbents. Points - experimental data, straight line - theoretical data calculated from eq.1.

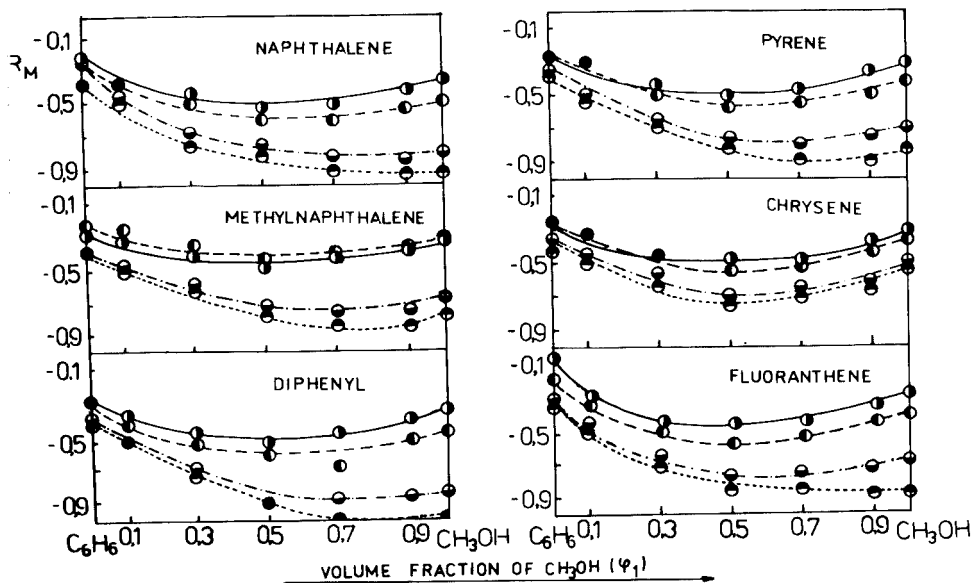


Fig.3. Relationship $R_M = f(\varphi_1)$. Points - experimental data, lines - theoretical data.

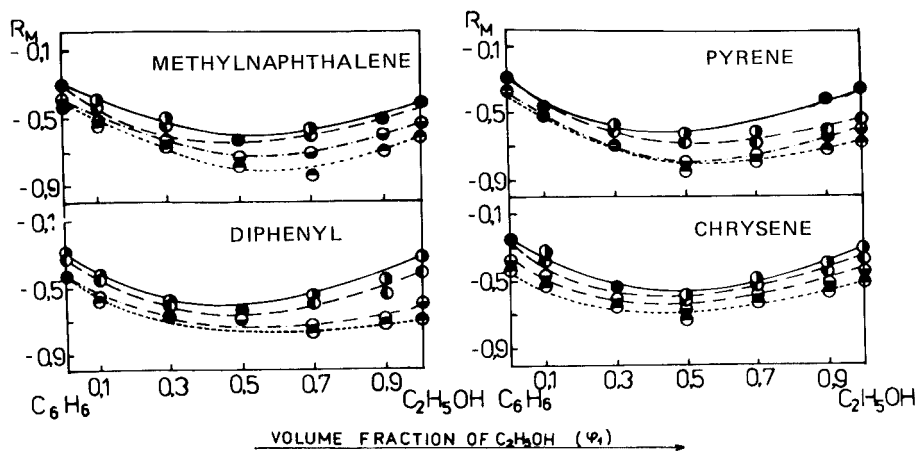


Fig.4. Relationship $R_M = f(\varphi_1)$.
 ○ — silica gel 60,
 ● — silica gel 100,
 ● — silica gel 200,
 ● — silica gel 500.

Table 1

Values of parameter "a" for inactive mobile phase.

Substance	concentration of benzene						
	mobile phase: methylcycloheksane - benzene						
	0.0	0.1	0.3	0.5	0.7	0.9	1.0
diphenyl	0.14	0.09	0.09	0.08	0.07	0.03	0.03
naphthalene	0.09	0.09	0.07	0.04	0.02	0.02	0.02
2,6-dimethyl-naphthalene	0.10	0.10	0.08	0.08	0.07	0.07	0.04
fluoranthene	0.09	0.07	0.05	0.03	0.02	0.02	0.03
anthracene	0.12	0.08	0.07	0.05	0.05	0.05	0.04
chrysene	0.11	0.10	0.09	0.08	0.08	0.07	0.03
pyrene	0.15	0.11	0.10	0.08	0.08	0.07	0.06
	mobile phase : heksane - benzene						
diphenyl	0.18	0.15	0.12	0.12	0.09	0.07	0.03
naphthalene	0.12	0.09	0.08	0.05	0.02	0.01	0.02
2,6-dimethyl-naphthalene	0.15	0.12	0.10	0.05	0.05	0.04	0.04
fluoranthene	0.12	0.12	0.11	0.05	0.04	0.04	0.03
anthracene	0.17	0.14	0.11	0.05	0.04	0.08	0.04
chrysene	0.16	0.15	0.12	0.06	0.08	0.06	0.03
pyrene	0.18	0.13	0.12	0.11	0.10	0.08	0.06

*Parameter "a" values are presented as $a \times 10^{-2}$

with the increase in the concentration of benzene which is the more active component of the mobile phase.

In the systems where the second component most active has the ability to formation of hydrogen bonds (benzene-methanol, benzene - ethanol), the slope of the straight line parameter "a" increase with the increasing concentration of alcohol. It is possible that this behaviour of the parameter "a" is to some extent influenced by the association of alcohol that occurs in such systems.

Table 2

Values of parameter "a" for active mobile phase

Substance	concentration of alcohol						
	mobile phase: benzene - Methanol						
	0.0	0.1	0.3	0.5	0.7	0.9	1.0
naphthalene	0.02	0.03	0.07	0.07	0.09	0.11	0.13
methylnaphthalene	0.02	0.05	0.05	0.07	0.10	0.11	0.12
chrysene	0.03	0.04	0.04	0.06	0.08	0.08	0.06
pyrene	0.06	0.06	0.06	0.07	0.11	0.11	0.12
diphenyl	0.03	0.04	0.06	0.09	0.15	0.16	0.15
fluoranthene	0.03	0.05	0.06	0.08	0.12	0.12	0.13
	mobile phase: benzene - ethanol						
methylnaphthalene	0.02	0.05	0.04	0.03	0.06	0.06	0.05
chrysene	0.03	0.05	0.02	0.05	0.03	0.04	0.04
pyrene	0.06	0.04	0.03	0.05	0.04	0.07	0.07
diphenyl	0.03	0.04	0.02	0.02	0.04	0.04	0.08

x parameter "a" values are presented as $ax10^{-2}$

Tables 3 and 4 contain the parameter "b" for considered systems. The parameter "b" may give information about the molecular action between mobile phase and chromatographed substance for the surface $s = 0$.

The straight lines described can be used for the calculation of the R_M values of substances on any adsorbent with a specific surface area. A series of arduous measurements can be avoided in this way. It is enough to take two measurements of possibly extreme R_M values in order to draw straight lines $R_M = f(s)$. When parameters "a" and "b" are known for two different substances, the separability of such mixture can be calculated with good approximation, with the use of the given mobile phase.

Table 3

Values of parameter "b" for nanactive mobile phase

Substance	concentration of benzene							
	mobile phase: methylcycloheksane - benzene							
	0.0	0.1	0.3	0.5	0.7	0.9	1.0	
diphenyl	-0.16	-0.22	-0.36	-0.45	-0.49	-0.48	-0.48	
naphthalene	-0.04	-0.14	-0.27	-0.32	-0.33	-0.35	-0.35	
2,6-dimethyl- naphthalene	-0.11	-0.22	-0.36	-0.42	-0.43	-0.47	-0.45	
fluoranthene	0.07	-0.05	-0.17	-0.31	-0.36	-0.38	-0.38	
anthracene	0.05	-0.07	-0.11	-0.30	-0.34	-0.38	-0.38	
chrysene	0.02	-0.28	-0.35	-0.42	-0.47	-0.48	-0.48	
pyrene	-0.16	-0.10	-0.37	-0.41	-0.43	-0.45	-0.47	
	mobile phase: heksane - benzene							
diphenyl	-0.14	-0.30	-0.43	-0.59	-0.58	-0.56	-0.48	
naphthalene	-0.15	-0.20	-0.28	-0.37	-0.36	-0.37	-0.37	
2,6-dimethyl- naphthalene	-0.15	-0.27	-0.41	-0.40	-0.45	-0.45	-0.45	
anthracene	0.01	-0.13	-0.30	-0.37	-0.39	-0.40	-0.40	
fluoranthene	0.02	-0.13	-0.30	-0.37	-0.39	-0.40	-0.40	
chrysene	-0.15	-0.17	-0.21	-0.42	-0.47	-0.47	-0.48	
pyrene	-0.07	-0.26	-0.40	-0.55	-0.50	-0.47	-0.47	

It results from the graphs 3 and 4 of dependencies $R_{M1.2} = f(\varphi_1)$ presented in figures 3 and 4, that and theoretically on the basis of the equation 1 are within the range of a permissible experimental error, i.e. that those theoretical R_M values are adequate representations of real R_M values.

The obtained results seem to suggest that it is possible to tabulate values "a" and "b" in order to calculate the dependence of $R_M = f(s)$. It has also been stated that there is a general dependence of the parameters of the straight

Table 4

Values of parameter "b" for active mobile phase

Substance	concentration of alcohol						
	mobile phase: benzene - methanol						
	0.0	0.1	0.3	0.5	0.7	0.9	1.0
naphthalene	-0.35	-0.53	-0.65	-0.81	-0.90	-0.94	-1.12
-methyl-naphthalene	-0.41	-0.53	-0.79	-0.86	-0.98	-0.99	-1.03
chrysene	-0.48	-0.51	-0.62	-0.79	-0.73	-0.72	-0.58
pyrene	-0.47	-0.57	-0.72	-0.85	-0.85	-0.87	-0.84
diphenyl	-0.48	-0.59	-0.79	-0.94	-1.06	-0.05	-1.13
fluoranthene	-0.38	-0.44	-0.64	-0.90	-0.89	-0.96	-0.92
	mobile phase: benzene - ethanol						
-methyl-naphthalene	-0.41	-0.63	-0.68	-0.80	-0.71	-0.71	-0.62
chrysene	-0.48	-0.53	-0.64	-0.73	-0.53	-0.58	-0.52
pyrene	-0.47	-0.52	-0.74	-0.86	-0.76	-0.76	-0.72
diphenyl	-0.48	-0.61	-0.79	-0.71	-0.75	-0.75	-0.73

line $R_M = as + b$ on the difference of the elution strenght of the components of the mobile phase, the surface on the adsorbent, occupied by the chromatographed substance and the molecular of the components of the mobile phase. This affords a wider range of possibilities of optimizing the thin-layer chromatography process.

The dependencies described above can undergo changes in the case of sieve effect leading to a non-linear from of equation 1. Consequently, the applicability of the obtained dependencies should be limited to the values of the specific surface 50 - 500 m²/g (14).

It seems that investigations presented above can be of great importance in transforming the chromatographic data

of thin-layer chromatography to the conditions of liquid column chromatography, due to frequent differences in the properties of the stationary phase in both methods, resulting from their nature.

REFERENCES

1. A.Waksmundzki, J.Różyło, *Annales UMCS, Lublin, Sec.AA*, 20, 93 (1965)
2. E.Stahl, *Dunnschichtchromatographie*, Springer, I-st and II-nd Eds. 1962, (1967)
3. A.Waksmundzki, J.K.Różyło, *Chem.Anal.*, 11, 101 (1968), 14, 1217 (1969); 15, 133 (1977); 16, 177 (1971).
4. A.Waksmundzki, J.K.Różyło, *J.Chromatog.*, 33, 90 (1968); 33, 96 (1968); 75, 55 (1973)
5. H.Halpaap, *J.Chromatog.*, 78, 63 (1973)
6. F.Ceiss, *Die Parameter der Dunnschichtchromatographie*, Vieweg, Braunschweig 1972, p. 89
7. L.R.Snyder, *Principles of Adsorption Chromatography*, Dekker, New York, 1968
8. J.K.Różyło, Interpretation and optimization of adsorption thin-layer chromatography process, in *Annales UMCS, Lublin 1975 (in Polish)*
9. J.K.Różyło, *Folia Soc. Scient. Acad Lublinensis*, 21, 71 (1979)
10. J.K.Różyło, J.A.Jaroniec, H.Kołodziejczyk, M.Jaroniec, *J of HRC and CC*, 2, 237 (1979)
11. M.E.Getz, *Paper and Thin-Layer Chromatography Analysis of Environmental Toxicants*, Heyden, London, 1980
12. *Catalog of Merck*, 1981

13. J.Ościk, J.K.Różyło, *Chromatographia*, 4, 516 (1971)

14. Ju.A.Eltekov, private communication.

DETERMINATION OF BIPHENYL IN CITRUS FRUITS BY QUANTITATIVE
THIN-LAYER CHROMATOGRAPHY

Joseph Sherma, Peter J. Sielicki, Jr., and Scott Charvat
Department of Chemistry
Lafayette College
Easton, PA 18042

ABSTRACT

Residues of the fungicide biphenyl in citrus fruits have been determined by direct scanning of spots on phosphor-impregnated high performance silica gel TLC plates under UV light. Biphenyl was separated from fruit tissue by steam liquid-liquid extraction. Recoveries from spiked samples ranged from 92-99% at 100, 50, and 10 ppm levels. The precision of the TLC determination and overall procedure are shown to be adequate for residue analysis.

INTRODUCTION

The official AOAC method for determination of residues of the fungicide biphenyl in citrus fruits is based on steam distillation, preparative thin layer chromatography (TLC), scraping and elution of biphenyl zones from the thin layer plate, and solution UV spectrometry at 218 nm (1,2). This paper reports a more rapid, simplified method for this analysis based on the direct scanning of the UV absorbance of biphenyl spots on phosphor-impregnated high performance silica gel layers. Precision (reproducibility) and accuracy (recovery) are at least comparable to the more laborious official method, and the specificity offered by the thin layer separation is retained.

EXPERIMENTAL

The preparation of citrus samples and biphenyl extraction using the SGA Scientific, Inc., No. JM-8590 lighter-than-water volatile oil trap were carried out as described in the official method (1,2) with the following exceptions. Extraction was performed for 3 hours at the two highest spike levels and for 4.5 hours at the lowest level. The solution was boiled vigorously during extraction and a rapid cooling-water flow was used in the condenser to prevent loss of biphenyl out of the top of the apparatus. The final heptane solution was freed from water by passing through Whatman phase separating paper (1PS) rather than a column of anhydrous Na_2SO_4 .

Samples were fortified by adding 1.00 ml of ethanolic spiking solution to 100 g of blended, peeled orange or lemon fruit or ground peel in the one liter round bottom flask. The spiking solution contained 1.00 g biphenyl per 100 ml for preparation of the 100 ppm sample, 0.500 g per 100 ml for the 50.0 ppm sample, and 0.100 g per 100 ml for the 10.0 ppm sample.

TLC was carried out on 20 x 10 cm Whatman HP-KDF high performance silica gel plates. These plates contained a fluorescent phosphor that was activated by 254 nm UV light, and were divided into nineteen lanes of 8 mm width. Plates were cleaned by pre-development with methanol-chloroform (1:1 v/v) and dried in a fume hood before use.

Biphenyl standard solutions were prepared in n-heptane at concentrations of 0.125, 0.250, 0.500, 1.00, 1.50, and 2.00 $\mu\text{g}/\mu\text{l}$. Standards and samples were applied to separate lanes, 2 cm up from the bottom of the plate, using disposable 4.00 μl Drummond microcap micropipets. After air drying, the layer was developed with n-heptane in a filter paper lined glass, rectangular HPTLC tank (Fotodyne) that had been pre-equilibrated with solvent for at least 10 minutes before inserting the plate.

The chromatogram was air dried in a hood and biphenyl spots were measured by scanning with a Kontes Model 800 fiber optics

densitometer equipped with a Hewlett Packard Model 3390A calculating integrator/recorder. Scanning was done in the single beam, transmission mode using the 254 nm shortwave-UV cobalt glass filter.

Percentage recovery was calculated by comparing the area of the sample zone to the area of the standard zone on the same plate representing the theoretical amount for 100% recovery. The final sample solutions were collected in 10.0 ml volumetric flasks. Sample volumes spotted and the theoretical weights representing 100% recovery were as follows for the three fortification levels: 100 ppm sample, 4.00 μ l, 4.00 μ g (4.00 μ l of the 1.00 μ g/ μ l standard); 50.0 ppm sample, 4.00 μ l, 2.00 μ g (4.00 μ l of the 0.500 μ g/ μ l standard); 10.0 ppm sample, 20.0 μ l, 2.00 μ g. Samples and standards were applied on adjacent lanes in duplicate, and the average area of the standard spots was compared to the two individual sample spot areas.

RESULTS AND DISCUSSION

Development with heptane provided tight, circular zones of biphenyl with an R_f value of ca. 0.5, which is within the optimum range for quantification by densitometry (3). Approximately 12 minutes was required for a 6 cm development distance. The spots were detected as dark, absorbing zones against a bright, fluorescent background (fluorescence quenching) when the plate was viewed under shortwave UV light. The zones were measured by scanning using the 254 nm filter over the densitometer light source.

To determine that analyses were being conducted within a linear calibration region, 0.5-8 μ g amounts of standard biphenyl were spotted in 4 μ l volumes, and scanned. Plots of peak area vs. weight had an average correlation coefficient (R) of 0.982 and a range of 0.991-0.967 (9 replicates). Recovery values were calculated by comparison of samples to single standard zones (4 μ g and 2 μ g), which were within the linear calibration range.

Reproducibility of the TLC determination was measured by spotting seven 2 μ g (4 μ l) spots in adjacent lanes and scanning

TABLE 1

Biphenyl Recovery from Orange Fruit

<u>Trial</u>	<u>Average Recovery</u>
<u>100 ppm Spike</u>	
1	97.9
2	95.1
3	94.9
<u>50.0 ppm Spike</u>	
1	93.5
2	96.5
3	92.4
4*	95.0
<u>10.0 ppm Spike</u>	
1	97.9
2**	97.9
3	97.7

* orange peel

** lemon fruit

the developed chromatogram. The relative standard deviation (coefficient of variation) of the peak areas was 2.76%, which is excellent precision considering the possible combined inconsistencies resulting from plate production, sample application, mobile-phase development, and scanning.

The recovery values obtained at the same three concentrations as used in the collaborative study (1) of the official method are summarized in Table 1. Only a limited number of

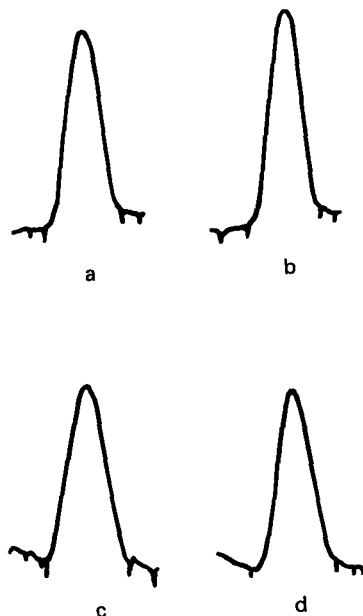


Figure 1. Densitometer scans of spots from duplicate 2.00 μg (4.00 μl) standards (a and b) and duplicate 20.0 μl extracts of the 10.0 ppm fortified lemon sample (c and d). Peak c represents 98.8% recovery and peak d 97.0%, compared to the average area of the standards.

Trials were performed on three sample types because the purpose of this research was not to confirm the applicability of the sample-preparation method but to demonstrate the efficacy of the TLC determination. All of the results in the table are for fortified peeled orange fruit, except the one value for orange peel and one for lemon fruit. The average recovery results range from 92.4 to 97.0%. The somewhat higher levels of recovery at the lowest level suggest that the longer extraction time may also be beneficial at the higher levels. The percentage difference between the two sample spots for the nine experiments averaged 2.81%, which is another indication of the satisfactory precision of the TLC determination. The agreement among the

trials within each concentration level illustrate the precision of the overall procedure. The results compare favorably both in accuracy and precision with those obtained in the collaborative study (1) of the official method. Recoveries above 100% at 100 ppm were not obtained, nor were recoveries proportional to the spiking level, as in this study (1).

A blank extraction of both fruit types was carried out, and chromatograms of these extracts contained no detectable spot at the R_F value of biphenyl. Therefore, correction of the data was not necessary. Figure 1 shows typical densitometer scans of duplicate sample and standard spots used to calculate the results in Table 1.

High performance layers were chosen after determining that biphenyl zones were less diffuse and darker than on either conventional silica gel or on C_{18} chemically bonded reversed phase layers. The latter were developed with methanol-water (85:15 v/v) to provide an R_F value of 0.26 for biphenyl. Pre-adsorbent layers could not be used because biphenyl spotted on the preadsorbent was not consistently detected after development, indicating either loss by volatilization or irreversible sorption in the spotting area.

CONCLUSION

The above results illustrate that the official AOAC analytical method for biphenyl residues in fruit can be improved by replacing the scraping and elution of TLC zones and solution UV spectrometry by in situ measurement of zones directly on the thin layer plate. The changes, along with the ability to analyze multiple samples at the same time under identical conditions and to process standards in parallel, result in greater convenience and saving of time without loss of accuracy or precision. The revised method is applicable to any samples that can be successfully analyzed by the AOAC method.

REFERENCES

1. Corneliussen, P. E., J. Assoc. Off. Anal. Chem., 50(4), 934 (1967).
2. Horwitz, W., ed., Official Methods of Analysis of the Association of Official Analytical Chemists, 13th edition, 1980, Sections 29.067-29.074.
3. Fried, B. and Sherma, J., Thin Layer Chromatography - Techniques and Applications, Marcel Dekker, Inc., 1982, p. 142.

USE OF SOME SILVER SALTS OF AROMATIC SULFONIC ACIDS IN THIN-
LAYER CHROMATOGRAPHY

Peter Ilinov and S. I. Dimov
Institute of Nutrition, Medical Academy,
Department of Biochemistry,
15, Dimitar Nestorov St.,
1431 Sofia,
Bulgaria

SUMMARY

Some silver salts of aromatic sulfonic acids were used for thin-layer impregnation. The chromatographic properties of these salts were examined by separation of fatty acid methyl esters with different degrees of saturation as well as of their cis- and trans-isomers. Benzene sulfonic acid silver salt was more thoroughly investigated. An attempt was made to prove the effect of the anion of the different silver salts on the R_f values of a model mixture of fatty acid methyl esters.

INTRODUCTION

Silver nitrate has long been used for thin-layer separation of compounds according to the degree of saturation (1, 2, 3,

4, 5). Silver perchlorate and fluoroborate (6) as well as silver oxide (7), thallos nitrate (8) and silver sulfamate (9) have also been suggested for this purpose. Silver sulfamate has proven to be very suitable for separation of fatty acid cholesterol esters. The metallic ion (Ag, Pt, Pd, Tl) is thought to form a π -complex with compounds having double bonds. The stability of this complex depends on the number, type, geometry and position of the double bonds in the molecule of the compound. All this is used in certain chromatographic methods for separation of compounds according to the degree of saturation.

This paper deals with the possibility of use silver salts of aromatic sulfonic acids in thin-layer chromatography.

EXPERIMENTAL

Silver salts of the following aromatic acids were prepared:

Table 1

1. Benzenesulfonic acid (HO_3SAr)
 2. 4-Methylbenzenesulfonic acid
 3. 2,5-Dimethylbenzenesulfonic acid
 4. 1-Naphtalenesulfonic acid
 5. 2-Naphtalenesulfonic acid
 6. 4-Hydroxybenzenesulfonic acid
 7. 3-Carboxy-4-hydroxybenzenesulfonic acid
 8. Sulfamic acid
- Silver nitrate

All salts were prepared in a similar manner. The aqueous solution of the corresponding acid was neutralized with freshly precipitated silver oxide at room temperature. The silver salt crystallized out either on storing at room temperature or

on evaporation under vacuum. The concentration of the stock solutions was chosen according to the solubility of the silver salt to be obtained. For example, 6 g of benzenesulfonic acid was dissolved in 100 ml water and freshly precipitated silver oxide was added to the solution until complete neutralization. The consumed silver oxide was equivalent to about 7 g silver nitrate. The yield of silver benzenesulfonate was close to the theoretical one (97%). Silver benzenesulfonate represents leaf-like crystals with a melting point of more of 250 C which are very soluble in water and ethanol. Since some of the silver salts of the aromatic sulfonic acids are more slightly soluble in water, water and acetonitrile (4:1 v/v) were used as a solvent.

The silver salt of benzenesulfonic acid was first synthesized by Freund (1861) but by a different procedure.

Chromatographic System: 0,4 g (about 1,5 mmoles) of benzenesulfonic acid silver salt was dissolved in 5,5-6,0 ml distilled water. A mixture of 1,0 g Silica Gel G and 1,0 g Silica Gel HR (Merck) were slurried with this solution. Five glass plates (19 x 4 cm) were uniformly coated by means of spreader. Thickness is about 0,25 to 0,30 mm. The chromatographic plates were allowed to air-dry and kept in dry and dark until use. The model mixture of fatty acid methyl esters was prepared using lipid standard for chromatography (no 189-1, Sigma, St. Louis, Mo, USA) to which trans-oleic acid methyl ester and trans, trans-linoleic acid methyl ester were added. The concentration of the fatty acid methyl esters was about 2 mg/ml except for the trans, trans-linoleic acid methyl ester which was 1,0 to 1,3 mg/ml. Hexane was used as a solvent. From this solution 2 μ l dropwise or 20-25 μ l in a way of band was applied on the start.

Solvent System: Hexane-pentane-diethylether-acetic acid (100:30:6:3 v/v). Development time - about 30 min. Length of run - about

12 cm. Chamber volume/mobile phase volume ratio was 30:1. The development was carried out in a closed chamber at room temperature. Separation improved if a precooled plate was placed in the chamber.

After development of the chromatograms and evaporation of the solvent the plates were sprayed with 20% solution of sulfuric acid in water-methanol (3:2 v/v) and dried in an oven at 120 C for 10 min. The chromatograms were then placed over a heated (200 C) metallic plate for 10 min. Unsaturated fatty acids appeared as black spots on a white background. To obtain more intensive darkening of the spots of the saturated acids they were sprayed again with the sulfuric acid solution and the chromatograms were reheated. The spots could be successfully measured by densitometry.

RESULTS AND DISCUSSION

Fig.1 shows two chromatograms developed on a silver nitrate- and silver benzenesulfonate-impregnated layer. The silver ion concentration and all other conditions of development of the chromatograms were the same. The chromatograms demonstrate that silver benzenesulfonate allows for the separation of the critical pair cis-oleic acid/trans, trans-linoleic acid, which could not be achieved with silver nitrate only. The limit of detection was of the order of 0,8 to 1,0 μg for unsaturated acids and about 5,0 μg for saturated acids. The chromatographic plates impregnated with silver benzenesulfonate were more resistant to light and to mechanical damage as compared to those with silver nitrate. The Table 2 summarizes the R_f values obtained on a thin-layer impregnated with AgNO_3 and AgO_3SAr :



Fig. 1. Chromatography of the fatty acid methyl esters on silver nitrate and silver benzenesulfonate (AgO_3SAr) impregnated silica-gel layers:

1. all cis-trienoic esters
2. all cis-dienoic esters
3. cis-monoenoic esters
4. all trans-dienoic esters
5. trans-monoenoic esters
6. saturated esters

Table 2

$R_f \times 100$ values of some fatty acid methyl esters (mean \pm SD)

Impregnated layer with	all cis-trienoic esters	all cis-dienoic esters	cis-monoenoic esters	trans-dienoic esters	trans-monoenoic esters	saturated
AgNO_3	17 \pm 5	25 \pm 5	37 \pm 6	-	44 \pm 8	58 \pm 4
AgO_3SAr	21 \pm 3	28 \pm 5	36 \pm 6	40 \pm 5	47 \pm 6	67 \pm 4

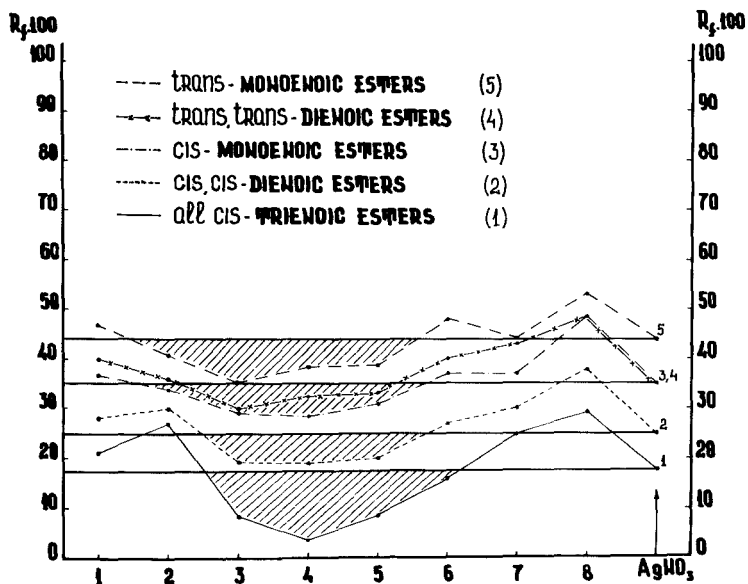


Fig. 2. $R_f \times 100$ values of 5 different fatty acid methyl esters. For numbers 1-8 see Table 1. The solid horizontal lines correspond to the R_f values obtained on AgNO_3 -layer. The shaded areas express a greater stability of the π -complex, which can be attributed to the different chemical nature of silver salt anion.

Here again the silver ion concentration (about 1,5 mmoles) in the layer and the conditions of development of the chromatograms were the same. From Fig.2 it is clear that the stability of the π -complex depends not only on the number and geometry of the double bonds but also on the corresponding acid residue (anion) of the silver salt. This suggests that besides silver nitrate other silver salts could also be used for thin-layer separation of fatty acid methyl esters.

CONCLUSION

The proposed silver benzenesulfonate proved to be very suitable for separation of fatty acids with different degree of saturation as well as of their cis- and trans-isomers on thin-layer chromatographic plates. This chromatographic procedure could be used as a rapid and easy-to-perform method for control of the process of producing hydrogenated oils or for control of foods containing partially hydrogenated oils. The use of densitometry allows for the quantitative evaluation of the results.

REFERENCES

1. Barrett, C.B., M.S.J. Dallas and F.B. Padley (1962)
Chem. Ind. (London), 1050-1051
2. Vries de B. and G. Yurriens (1963) Fette Seifen 65, 725-727
3. Morris, L.J. (1966) J. Lipid Res. 7, 717-732
4. Morris, L.J., R.T. Hollman and K. Fontelb (1961)
J. Lipid Res. 2, 68-
5. Carreau, J.P. and J. Raulin (1965) Rev. Franc. de
Corp. Gras. 12, 87-89

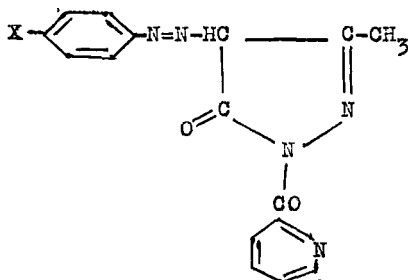
6. Morris, L.J., D.M. Wharry and E.W. Hammond (1967)
J. Chromatogr. 31, 69-75
7. Tabak, S., and M.R.M. Verzola (1970) J. Chromatogr.
51, 52, 334-338, 500-504
8. Baines, D.A. and R.A. Jones (1970) J. Chromatogr.
47, 130-132
9. Ilinov, P., (1979) Lipids 14, 598-600

CHARACTERIZATION OF DERIVATIVES OF SOME CLOSELY RELATED
1-PICOLINOYL-3-METHYL-4-(SUBSTITUTED) PYRAZOLINE-5-ONES
BY THIN LAYER CHROMATOGRAPHY

D.R. Gupta and R.K. Arora
Department of Chemistry, University of Roorkee
Roorkee-247667, (U.P.) India

INTRODUCTION

The antitubercular activity associated with pyrazoline-5-ones (1), picolinic acid hydrazide (2) and the antibacterial activity of sulphonamides (3) prompted us to undertake the synthesis of 1-picolinoyl-3-methyl-4-(substituted)-pyrazoline-5-ones (see formula) as possible new biological active agents. In view of their medicinal importance, it is thought worthwhile to carry out a TLC separation of these compounds.



where X-represents different substituents (Table 1)

Table - 1

Sl. No.	X	$R_f \times 100$	
		A	B
1	$-\text{So}_2\text{NH}_2$	37	36
2	$-\text{So}_2\text{NH} \cdot \text{C} \begin{array}{l} \text{NH} \\ \text{NH}_2 \end{array}$	49	-
3	$-\text{So}_2\text{NH} \begin{array}{c} \text{---} \\ \text{N} \\ \text{---} \end{array}$	59	-
4	$-\text{So}_2\text{NH} \begin{array}{c} \text{---} \\ \text{N} \\ \text{---} \end{array}$	74	83
5	$-\text{So}_2\text{NH} \cdot \text{Co} \cdot \text{CH}_3$	76	77
6	$-\text{So}_2\text{NH} \cdot \begin{array}{c} \text{---} \\ \text{N} \\ \text{---} \end{array}$	68	60
7	$-\text{So}_2\text{NH} \begin{array}{c} \text{---} \\ \text{N} \\ \text{---} \end{array}$	71	73
8	$-\text{So}_2\text{NH} \begin{array}{c} \text{---} \\ \text{N} \\ \text{---} \end{array}$	40	71
9	2-Cl	12	14
10	3-Cl	24	23
11	4-Cl	27	32
12	2-OCH ₃	20	17
13	3-OCH ₃	61	60
14	4-OCH ₃	13	25
15	4-Br	43	77

Rate of development
for 15 cms in min

45

45

A = Chloroform:methanol (90:10)

B = Benzene :ethyl acetate (60:40)

EXPERIMENTAL

All the derivatives of pyrazoline-5-one were synthesized in this laboratory by usual methods (4). TLC plates (0.5 m.m. thick) were prepared from silica gel G (Sisco) by means of Stahl's applicator. The plates were activated at 60°C for 24 hrs. All the compounds were recrystallized prior to their use. The pure organic compounds were dissolved in hot glacial acetic acid and were applied to the chromatoplates with the help of a glass capillary and the spots were allowed to air dry. The chromatograms were developed at constant temperature (30±2°) with different solvent systems. The chamber used for the development was saturated with vapours of the solvent mixture before carrying out the separation. A mixture of chloroform:methanol (90:10) and benzene:ethyl acetate (60:40) was found to give the best results. As these compounds gave coloured spots, no visualiser was therefore employed. The R_f values of the compounds were determined and recorded in table 1. The results were found fairly reproducible within the limits of experimental error.

ACKNOWLEDGEMENT

One of us (R.K.A.) is grateful to U.G.C., India for financial assistance.

REFERENCES

1. S. Chandra, Bull. Chem. Soc. Japan, 40, 2422(1967)
2. H. Herbert Fox and John T. Gibas, J. Org. Chem., 17, 1653-60, (1952).
3. A. Burger, Medicinal Chemistry, Part I (Wiley Interscience, Virginia), 255, (1970).
4. L. Knorr, Ber., 17, 546(1884).

PHOSPHOLIPID COMPOSITION
AND DIFFERENTIATION OF METHANOTROPHIC
BACTERIA

L.V.Andreev and V.F.Galchenko
Institute of Biochemistry and Physio-
logy of Microorganisms, USSR Academy
of Sciences, 142292 Pushchino Moscow
Region USSR

ABSTRACT

A combination of TLC and extraction of native cells on the layer of sorbent was used for analysis of phospholipid composition of bacteria. The method is suitable for rapid routine differentiation of genera and species of obligate methanotrophic bacteria.

INTRODUCTION

During the past decade bacterial taxonomy has undergone essential changes which are reflected in the 8th edition of Bergey's Manual of Determinative Bacteriology (1). It was largely due to the use of modern chemotaxonomic techniques which become currently accepted in systematics of bacteria (2-3).

All organisms contain polar lipids, and their analyses often enable one to elaborate classification systems of microorganisms (3). Presently,

researchers pay a special attention to rapid and qualitative methods of analysis which allow the study of a great number of strains and their rapid preliminary identification (express-diagnostics).

Methanotrophic bacteria attract an increasing attention due to their practical importance. However, the taxonomy of the organisms still needs further investigation. So far the questions of methanotroph classification have been tackled with help of conventional methods of bacterial taxonomy. The elaboration of progressive chemotaxonomical approaches in the taxonomy of methanotrophic bacteria is an urgent task.

Previously it was shown (4) that methanotroph types (groups) differ in their phospholipid and fatty acid composition. A more detailed analysis of fatty acids of a large group of methanotrophic bacteria (68 strains) allowed the identification of these organisms to genera and species (5). Such an analysis revealed also the differences in the phospholipid composition of different genera and types of methanotrophs (6).

In the present work the possibility of the express-diagnostics of obligate methanotrophic bacteria based on thin-layer chromatography of their phospholipids.

MATERIALS AND METHODS

Materials

Thin-layer glass plates, 20 x 20 cm (DC-Fertigplatten Kieselgel 60 F 254 from E.Merk, Darmstadt, FRG), were used. Phosphatidic acid (PA), phosphatidyl glycerol (PG), diphosphatidyl glycerol (DPG), phosphatidyl choline (PC), phosphatidyl ethanolamine

(PE), monomethyl- (PMME) and dimethylphosphatidyl ethanolamine (PDME) were obtained from Supelco (USA). Agar was purchased from Difco (USA). Solvents and mineral salts were from Khimreaktiv (USSR).

Methods

Methanotrophic bacterial cultures were grown on mineral salts medium containing (g/l distilled water): KNO_3 , 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; CaCl_2 , 0.02; $\text{Na}_2\text{HPO}_4 \cdot 5\text{H}_2\text{O}$, 1.5; KH_2PO_4 , 0.7; (mg/l): EDTA, 5.0; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.03; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2; $\text{CuCl}_2 \cdot 5\text{H}_2\text{O}$, 0.1; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02; Na_2MoO_4 , 0.03; pH 6.9 ± 0.2 . The medium was sterilized at 121°C (1 atm) for 1 h. Phosphates were sterilized separately, cooled and added to the other components. The medium was solidified with agar (2%).

The cultures were grown in test tubes with solid medium or in 0.7 l - flasks with 100 ml liquid medium. Flasks were sealed with rubber stoppers. A mixture of methane and air (1:1) was added through two glass tubes in the stoppers with cotton filters. For better aeration the flasks were shaken on a rotor shaker (140 ppm). In case of a solid medium the bacteria were grown in vacuum dissicators filled with a methane - air mixture (1:1).

Use was made of pure cultures of obligate methanotrophs of the culture collection of IBPhM, USSR Academy of Sciences. Strains OB3b and 5 were courteously provided by Prof. Wittenbury (University of Warwick, Coventry, UK); and strain Texas, by Prof. Quayle (University of Sheffield, Sheffield, UK).

For thin-layer chromatography the cell suspension was washed with saline (0.15 M NaCl) to a final concentration of 5 to 15 $\mu\text{g}/\text{ml}$ of dry weight. 10 μl of the cell suspension was applied onto a silica gel

layer to obtain an evenly coated region of 5 x 5 mm, Then the plate was dried under nitrogen stream, and the procedure was repeated. The cells were extracted on the layer by a chloroform - methanol mixture (1:2). To do so, the plates were developed in the solvent mixture, the start line being 2-3 cm above the line of application of the cell suspension. This was coupled with the extraction of lipids from the cells and their movement along the layer. After this procedure was 5-6 times repeated the extracted lipids were concentrated in the form of a narrow strip at the front line of the extractant. Between two subsequent extractions the solvent was removed by drying under nitrogen stream. Then the silica gel layer with the extracted cells was removed by a scalpel.

The phospholipids were separated in corresponding solvents. For unidimensional chromatography the solvent system containing chloroform, methanol, acetone, acetic acid a water (10:3:8:2:1) was used. Two-dimensional chromatograms were obtained using the solvent systems of chloroform-methanol - 26% NH_4OH solution (65:25:4 the first separation) and chloroform-acetone-methanol-acetic acid-water (8:2:8:2:1, the second separation). To visualize the lipid spots, the plates after separation were sprayed with 10% CuSO_4 solution in 8% orthophosphate acid and heated for 10 min at 175-200°C. (7).

RESULTS AND DISCUSSION

Fig. 1A and 1B present the unidimensional chromatograms of phospholipids of methanotrophic bacteria of different genera and types. The chromatogram 1B was developed once in the solvent system to a length of 12 cm, whereas the chromatogram 1A was primarily

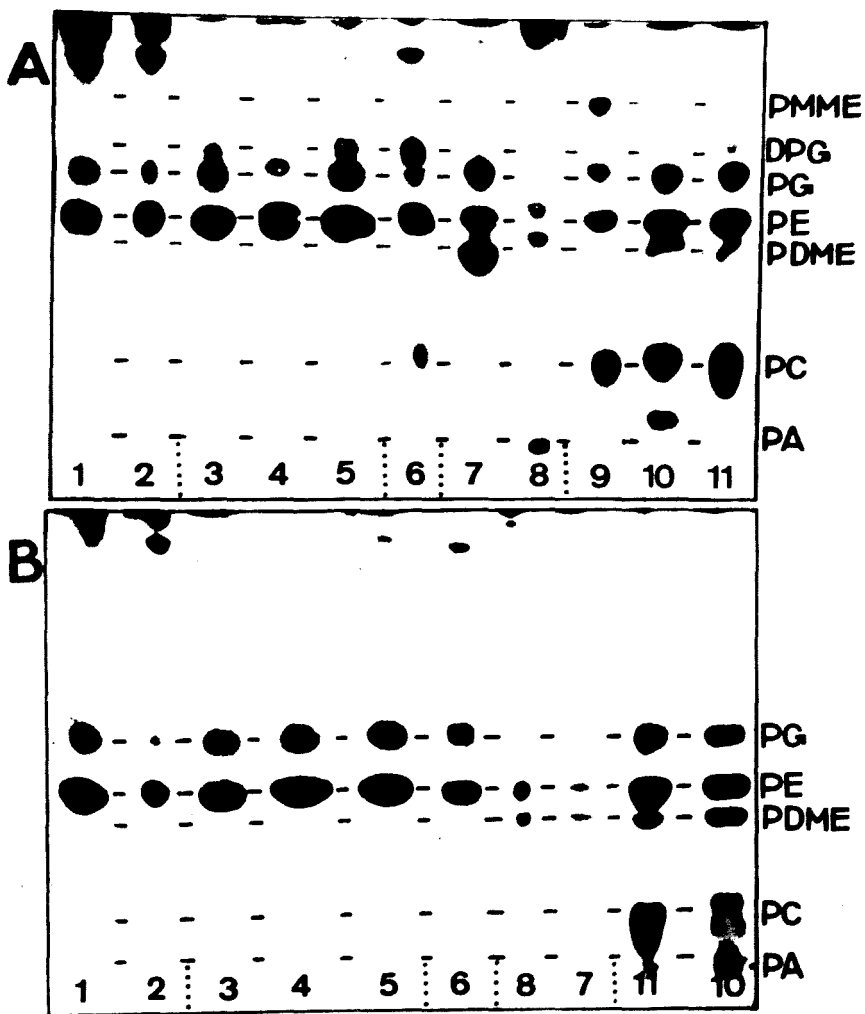


FIGURE 1. One-dimensional chromatograms of phospholipids of obligate methanotrophic bacteria grown in liquid media (A) and on solid media (B).

- (1) *Methylomonas methanica* 12; (2) *Mm.albus* B88; (3) *Methylobacter vinelandii* 87; (4) *Mb.bovis* 89; (5) *Mb.chroococcum* 90; (6) *Methylococcus capsulatus* "Texas"; (7) *Methylosinus trichosporium* OB3b; (8) *Ms.sporium* 5; (9) *Methylocystis minimus* 82; (10) *Mcs.parvus* OBBP; (11) *Mcs.echinoides* 2.

developed to a length of 6 cm and then to 12 cm in the same solvent system.

To obtain accurate results, each bacterial cultures was three times analyzed at different cell concentrations, since it was taken into consideration that the treatment with a mixture of chloroform and methanol may result in the release of some substances, e.g. polyoxybutyrate which dissolve phospholipids and thus affect their motility. Such a process looks like a reverse-phase TLC variant. Fig. 1A illustrates such a distorted chromatogram (sample 10). As is seen in the case with *Methylocystis parvus* OBBP this effect results in the flattening of the phosphatidyl choline zone. After the second development of the chromatogram in an alkaline solvent system this component moves upward and distorts the dimethyl phosphatidyl ethanolamine zone form (Fig. 1A, sample 10). However, it does not influence the qualitative analysis.

Comparing the phospholipid composition of bacteria grown on solid and in liquid media, one can see that in the latter case the chromatograms are far more informative. Nevertheless, in both cases the methanotrophs may be divided into two groups.

All tested strains of methanotrophic bacteria using a serine pathway (II type) contain the methylated phosphatidyl ethanolamine derivatives, mono-methyl phosphatidyl ethanolamine or, as in the case of *Methylocystis minimus* 82, dimethyl phosphatidyl ethanolamine, and phosphatidyl choline. The said phospholipids are not contained in the lipid pool of methanotrophs with ribulose monophosphate cycle (I type).

Methylococcus capsulatus is an exception to this rule. As is seen on Figures 1B and 2C, the bacteria of this species have small amounts of phospho-

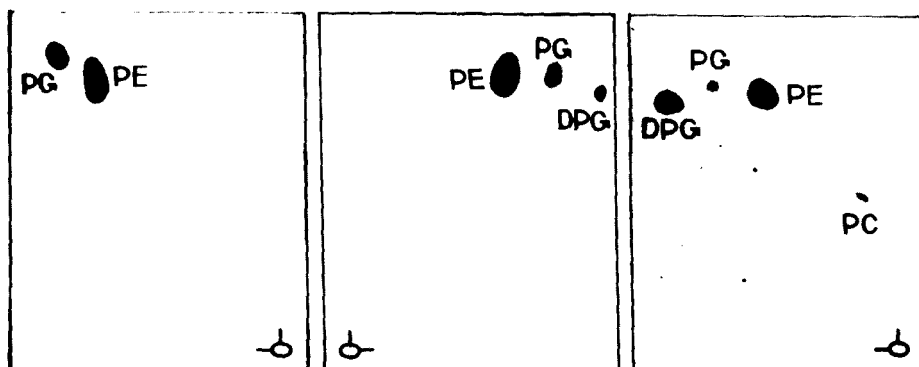


FIGURE 2. Two-dimensional chromatograms of phospholipids of obligate methanotrophic bacteria. (A) *Mm.methanica* 12; (B) *Mb.vinelandii* 87; (C) *Mc.capsulatus* "Texas".

tidyl choline. *Methylococcus capsulatus* is a I type methylotroph and uses a ribulose monophosphate part-way of methane carbon assimilation. But as it was shown (8) this organism displays some characteristics of II type methanotrophs (e.g. possesses some activities of key enzymes of the serine cycle of methane carbon assimilation). This must have somehow affected its phospholipid composition as well: like the II type methanotrophs, *Methylococcus capsulatus* has phosphatidyl choline.

Based on the differences in the phospholipid spectra the methanotrophs within the said groups (types) can be differentiated to genera. For example, *Methylomonas* differs from *Methylobacter* by the absence of diphosphatidyl glycerol whose content in the latter is substantial. *Methylobacter* can easily be differentiated from *Methylococcus* by the ratio of diphosphatidyl glycerol and phosphatidyl glycerol. In

the former this proportion is far less than 1, whereas in Methylococcus capsulatus the content of phosphatidyl glycerol is lower than of diphosphatidyl glycerol and their ratio varies from 1.5 to 2.0 (Fig.1A). The differences in diphosphatidyl glycerol and phosphatidyl glycerol proportions are clearly seen on the two-dimensional chromatograms (Fig.2).

In the I type methanotrophs grown on a solid medium no difference in the phospholipid contents are observed. On the chromatograms only two phospholipids are seen: phosphatidyl glycerol and phosphatidyl ethanolamine (Fig.1B).

The phospholipid composition permits also to differentiate the representatives of the II type methanotrophs. Methylosinus bacteria contain small amounts of phosphatidyl choline, which is one of the major components in Methylocystis.

When using the phospholipid composition for differentiation of methanotrophic bacteria, purity of cultures is a must, because even slight contamination, e.g. with molds, results in the unproportional increase of the phosphatidyl choline content.

REFERENCES

1. Buchanan R.E., Gibbons N.E., Bergey's Manual of Determinative Bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore, 1974.
2. Lechevalier M.P., Lipids in bacterial taxonomy - a taxonomist's view, CRC Crit.Rev.Microbiol., 5, 109, 1977.
3. Minnikin D.E., Goodfellow M., In: Microbial Classification and Identification, ed.M.Goodfellow a. R.G.Board, Acad. Press, London, 189, 1980.
4. Makula R.A., J.Bacteriol., 134, 771, 1978.

5. Andreev L.V., Galchenko V.F., Doklady Akad.Nauk SSSR, 239, 1645, 1978.
6. Andreev L.V., Galchenko V.G., II All-Union Conference "Lipid Biosynthesis and Metabolism in Microorganisms", Abstracts, Moscow, 31, 1979.
7. Touchstone J.C., Levin C.C., Dobbins M.F., Carter P.J., HRC and CC, 4, 423, 1981.

REGULAR CONTRIBUTED PAPERS

MODIFICATION OF SELECTIVITY IN REVERSED-PHASE LIQUID
CHROMATOGRAPHY OF POLYCYCLIC AROMATIC HYDROCARBONS
USING MIXED STATIONARY PHASES

Stephen A. Wise^{*}, Lane C. Sander, and Willie E. May
Organic Analytical Research Division
Center for Analytical Chemistry
National Bureau of Standards
Washington, DC 20234

ABSTRACT

Monomeric and polymeric C₁₈ materials provide significantly different selectivities for polycyclic aromatic hydrocarbons (PAH) in reversed-phase liquid chromatography. Selectivity factors vary in a regular manner with respect to surface concentration of C₁₈ groups on different C₁₈ columns. In this study, we investigated the feasibility of "customizing" a C₁₈ column to provide an intermediate selectivity by mixing 5- μ m polymeric C₁₈ material from two different lots with high and low C₁₈ surface concentrations. Polymeric C₁₈ materials from different production lots were mixed in ratios of 30/70, 50/50, and 70/30 (w/w). Selectivity factors for these columns were found to be similar to those predicted by the linear addition of the selectivities of the two individual phases. The PAH selectivities on these mixed columns were also found to be comparable to data obtained from coupled short columns of appropriate lengths each containing one of these different C₁₈ materials. These studies indicate that columns of specific selectivity can be prepared by either mixing two different C₁₈ materials or by coupling columns containing each of these different phases. The use of mixed phase columns is illustrated for the analysis of a fraction containing five condensed ring PAH isomers (molecular weight 278) isolated from an air particulate sample.

^{*}To whom correspondence should be addressed.

INTRODUCTION

Monomeric and polymeric C₁₈ materials provide significantly different selectivities for polycyclic aromatic hydrocarbons (PAH) in reversed-phase liquid chromatography (1,2). Polymeric C₁₈ phases generally provide larger selectivity factors (α) than monomeric C₁₈ phases for most planar PAH solutes. One polymeric C₁₈ material investigated has a very low surface area (~ 90 m²/g for the underivatized silica) which provides a high C₁₈ surface coverage and a unique selectivity for PAH separations when compared to monomeric C₁₈ phases and to other polymeric C₁₈ phases prepared on higher surface area silica (300-400 m²/g). Recently, we reported that selectivity differences for PAH on different C₁₈ columns from various manufacturers were related to the monomeric or polymeric nature of the chemically bonded layer and to the surface concentration of the C₁₈ layer (2). In addition, several different lots of a polymeric C₁₈ phase from one manufacturer were studied with respect to C₁₈ surface coverage and selectivity factors for PAH. In this study (2) the selectivity factors for selected PAH (relative to benzo[a]pyrene) on the different polymeric columns were found to vary linearly as the surface concentration of the C₁₈ groups varied. As an extension of these studies, we investigated the feasibility of "customizing" a C₁₈ column to provide an intermediate selectivity by physically mixing two polymeric C₁₈ materials from different production lots (i.e., lots with high and low C₁₈ surface coverage). The selectivities of these "mixed" phase columns were compared to that obtained by coupling two short columns (in appropriate lengths) each containing one of these same C₁₈ materials.

EXPERIMENTAL

A liquid chromatograph with a fixed wavelength UV detector at 254 nm and an autosampler were used for all liquid chromatographic measurements. HPLC grade acetonitrile and water were used as the mobile phase. PAH standards were obtained from several sources as previously reported (2). Columns and bulk packing material (~ 2 g)

from two different bonding production lots (lots 11 and 15) of polymeric C₁₈ material (5- μ m) and one lot (lot 17) of monomeric C₁₈ material (5- μ m) were obtained from the manufacturer. The lot numbers were arbitrarily assigned by the authors and correspond to those in the previous study (2). The manufacturer indicated that lot 11 had a low carbon loading, whereas lot 15 had a high carbon loading. Both the polymeric and monomeric C₁₈ materials used in this study utilized the same low surface area silica support (90 m²/g).

The columns (25 cm x 4.6 mm i.d.) containing the mixtures of lots 11 and 15 [70/30, 50/50, and 30/70 (w/w) lot 11/lot 15] were prepared by the manufacturer. Short columns (12.5 cm x 4.6 mm i.d.) containing packing from lots 11 and 15 were also prepared by the manufacturer.

Specific surface area and percent carbon measurements on the chemically modified C₁₈ materials were determined and used to calculate the surface concentrations of the C₁₈ ligands on the silica as described previously (2).

The mobile phase mixtures were pre-mixed and allowed to equilibrate to room temperature prior to use. All retention data were obtained under isocratic and isothermal conditions (27 °C). Retention times were obtained from injections of acetonitrile solutions of each compound. Capacity factors, k' , were determined from $k' = \frac{(t-t_0)}{t_0}$, where t is the retention time of the solute and t_0 is the unretained time which was determined from an injection of acetone in a mobile phase of acetonitrile. Selectivity factors (α) for each compound were calculated from $\alpha = k'/k'_{\text{BaP}}$, where k'_{BaP} is the capacity factor for benzo[a]pyrene.

RESULTS AND DISCUSSION

In gas chromatography Laub *et al.* (3) and Kong *et al.* (4) have reported the use of mixed liquid phases to optimize the separation of selected PAH solutes. In liquid chromatography Glajch and

Kirkland (5) recently reported mixing LC packing materials with different chemically bonded functionalities (i.e., C₈, CN, and phenyl) to optimize the separation of phenylthiohydantoin derivatives of 20 amino acids. Ogan and Katz (6) evaluated the retention and selectivity characteristics of several PAH on columns with different proportions of C₂ bonded material mixed with C₁₈ materials and found the contribution of the individual phases to be additive.

In a recent study (2) seven different production lots of a polymeric 5- μ m C₁₈ material were found to have C₁₈ surface concentrations varying from 4.3 – 8.2 μ moles/m². The selectivity factors for PAH solutes on these columns varied linearly as the C₁₈ surface concentration increased. Two lots of material from this previous study were selected to evaluate the feasibility of mixing C₁₈ materials with high and low surface concentrations to obtain columns with intermediate selectivities. The physical characteristics of these two lots of polymeric C₁₈ material are summarized in Table 1. The materials from lots 11 and 15 were rejected by the manufacturer's normal criteria for selectivity in the separation of PAH, i.e., insufficient resolution of benz[a]anthracene and chrysene on the low coverage material and insufficient resolution of dibenz-

TABLE 1. Physical Characteristics of Different C₁₈ Materials

Column Lot ^a	Surface Area ^b (m ² /g)	Percent Carbon ^c (wt% \pm 1s)	Surface Concentration ^d (μ mol/m ²)	k' BaP ^e
11 (polymeric)	61.0	7.5 \pm 0.2	5.7 \pm 0.2	3.1
15 (polymeric)	52.4	9.3 \pm 0.1	8.2 \pm 0.1	4.9
17 (monomeric)	53.4	4.0 \pm 0.1	3.1 \pm 0.1	0.9

^aLot numbers were arbitrarily assigned by authors as in ref. 2.

^bDetermined by BET.

^cFour samples, uncertainty is $\pm 1\sigma$.

^dCalculated from equation 2 in ref. 2.

^e85/15 acetonitrile/water (v/v) as mobile phase.

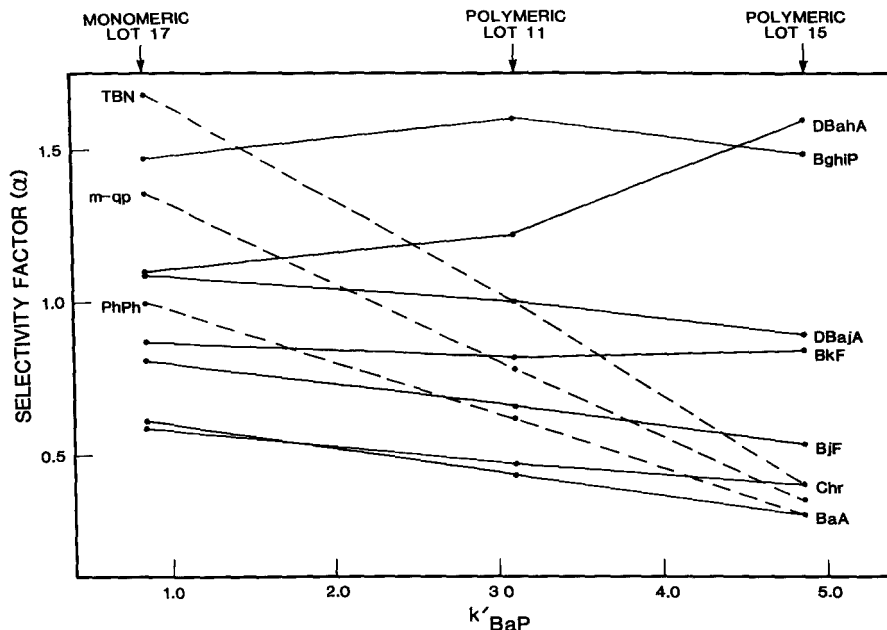


FIGURE 1: Selectivity factors (α), relative to benzo[*a*]pyrene, for selected PAH on three columns containing a monomeric C_{18} material (lot 17) and two polymeric C_{18} materials (lots 11 and 15). BaA = benz[*a*]anthracene, Chr = chrysene, BjF = benzo[*j*]fluoranthene, BkF = benzo[*k*]fluoranthene, DBajA = dibenz[*a,j*]anthracene, BghiP = benzo[*ghi*]perylene, DBahA = dibenz[*a,h*]anthracene, TBN = 1,2:3,4:5,6:7,8-tetrabenzonaphthalene, PhPh = phenanthro[3,4-*c*]phenanthrene, and m-qp = *m*-quinquephenyl.

[*a,h*]anthracene and benzo[*ghi*]perylene on the high coverage material. Data are also included in Table 1 for a monomeric C_{18} phase prepared on the same low surface area silica ($90 \text{ m}^2/\text{g}$) as the two polymeric C_{18} materials.

Selectivity factors for several PAH solutes on these three C_{18} columns are plotted in Figure 1 as a function of k'_{BaP} . As noted previously (2) and as shown in Table 1, k'_{BaP} values are related to the C_{18} surface concentrations. This linear relationship

of k'_{BaP} and surface coverage is valid, however, only when C_{18} phases prepared on silica of the same surface area are compared. In the previous study (2) data for columns from five different lots of the polymeric material and from a monomeric column were plotted in a similar manner as in Figure 1. However, the monomeric column in the previous study was prepared on a high surface area silica ($\sim 300 \text{ m}^2/\text{g}$) with 13% carbon resulting in a k'_{BaP} value of ~ 5.5 . The monomeric column in the present study was prepared on a low surface area silica ($90 \text{ m}^2/\text{g}$) with a 4% carbon loading resulting in a k'_{BaP} value of 0.9. As shown in Figure 1, when the selectivity factors are plotted as a function of k'_{BaP} , the linear trends observed for the polymeric columns can generally be extrapolated back to the monomeric column. This behavior suggests that for some types of PAH solutes (particularly nonplanar solutes such as TBN and PhPh in Figure 1) selectivity is dependent only on the extent of C_{18} surface coverage (when phases prepared on silicas with similar surface areas are compared) rather than the monomeric or polymeric nature of the C_{18} phase.

Mixed phase columns were prepared by combining material from lots 11 and 15 in proportions of 70/30, 50/50, and 30/70 (w/w) lot 11/lot 15. The selectivity factors (α) for several PAH are plotted in Figure 2 as a function of k'_{BaP} values obtained for columns from lots 11 and 15. The values on the abscissa for the mixed phase columns (70/30, 50/50, and 30/70 lots 11/15) were determined from the α values of the PAH. The data in Figure 2 indicate that columns of intermediate selectivities can be prepared by mixing the two phases of different selectivity. The selectivities of these mixed phase columns were found, however, to be slightly different from those predicted by the actual mixture proportions, i.e., predicted k'_{BaP} values of 3.62, 3.97, and 4.32 compared to actual values of 3.84, 4.10, and 4.38 for the 70/30, 50/50, 30/70 lot 11/15 mixtures, respectively. The actual and predicted values are in better agreement as the proportion of lot 15 (high coverage) increases.

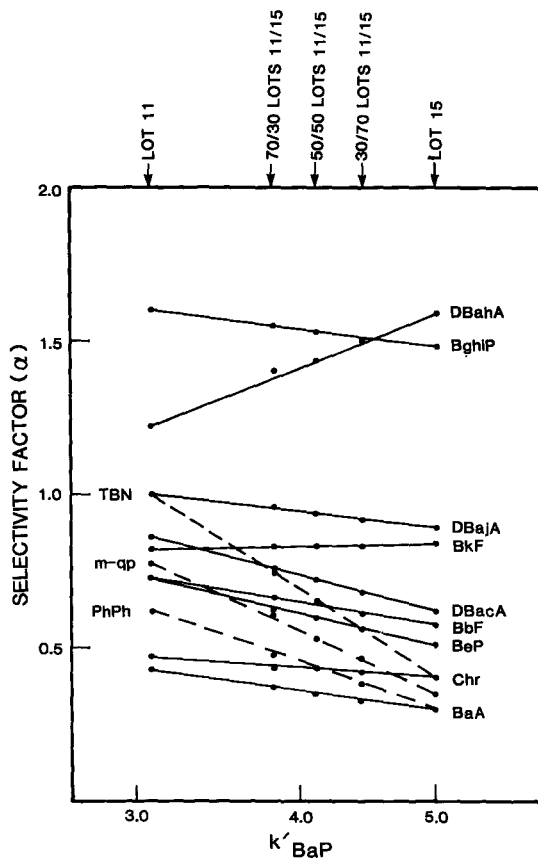


FIGURE 2: Selectivity factors (α), relative to benzo[*a*]pyrene, for selected PAH on polymeric C_{18} columns from mixtures of two different lots. BeP = benzo[*e*]pyrene, BbF = benzo[*b*]fluoranthene, DBacA = dibenz[*a,e*]anthracene. See Figure 1 for additional compound identification.

Differences in column selectivity for seven PAH solutes are illustrated in Figure 3. Six columns were compared: the monomeric phase, the high and low coverage polymeric phases, and three columns consisting of mixtures of the two polymeric phases. Phenanthro[3,4-*c*]phenanthrene (no. 2) and 1,2:3,4:5,6:7,8-tetra-benzonaphthalene (no. 4) are nonplanar solutes for which the selectivity factors vary significantly as the C_{18} surface concentration varies (see Figure 2). These differences in selectivity are discussed in detail elsewhere (2). On the monomeric column the phenanthro[3,4-*c*]phenanthrene and the 1,2:3,4:5,6:7,8-tetrabenzonaphthalene have longer retention times relative to the planar PAH than on the polymeric columns. Since the selectivity factors for these two solutes are very sensitive to changes in the C_{18} surface coverage, they have been used as an empirical test to evaluate the monomeric or polymeric characteristics of C_{18} columns by comparing their selectivity factors relative to a planar PAH solute (7). For the selected solutes, the columns from lots 11 and 15 did not separate all seven solutes. However, a mixed phase column of 70% lot 11 and 30% lot 15 provided the appropriate selectivity to achieve separation of all of these solutes. These studies indicate that columns of specific selectivity can be prepared by mixing C_{18} phases of different selectivities.

The selectivity factors obtained for the 50/50 lot 11/lot 15 mixed column were compared with data obtained by coupling two short columns (12.5 cm each), one containing material from lot 11 and one containing material from lot 15. The results shown in Table 2 indicate that short columns of appropriate lengths packed with materials from different lots can be coupled to achieve selectivities similar to those obtained by physically mixing the different packing materials. Thus, the chromatographer could have a collection of short C_{18} columns of different selectivities which could be coupled together in various combinations to achieve the necessary selectivity for a particular separation.

To illustrate the potential use of mixed phase columns of different selectivities, a fraction containing five condensed ring

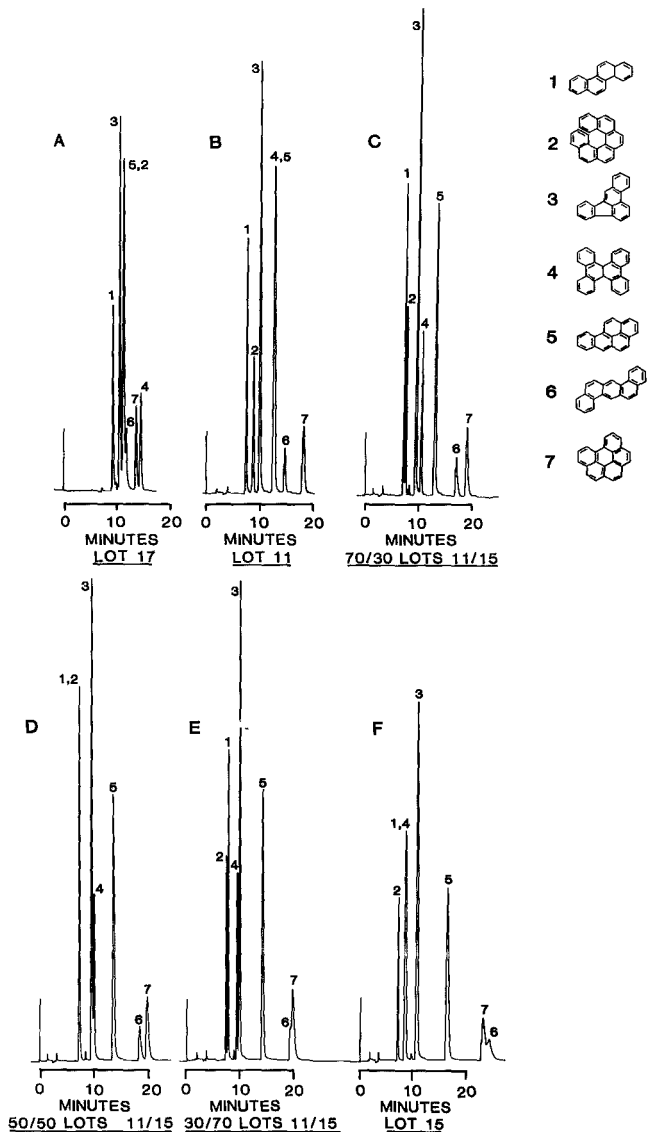


FIGURE 3: Reversed-phase LC separation of selected PAH on a monomeric C₁₈ column from lot 17 (A) and on polymeric C₁₈ columns from lot 11 (B) and lot 15 (F) and mixtures of these two lots [70/30 (C), 50/50 (D), and 30/70 lot 11/15 (E)]. Compound identification: (1) chrysene, (2) phenanthro[3,4-*c*]phenanthrene, (3) benzo[*b*]fluoranthene, (4) 1,2:3,4:5,6:7,8-tetrabenzonaphthalene, (5) benzo[*a*]pyrene, (6) dibenz[*a,h*]anthracene, and (7) benzo[*ghi*]perylene.

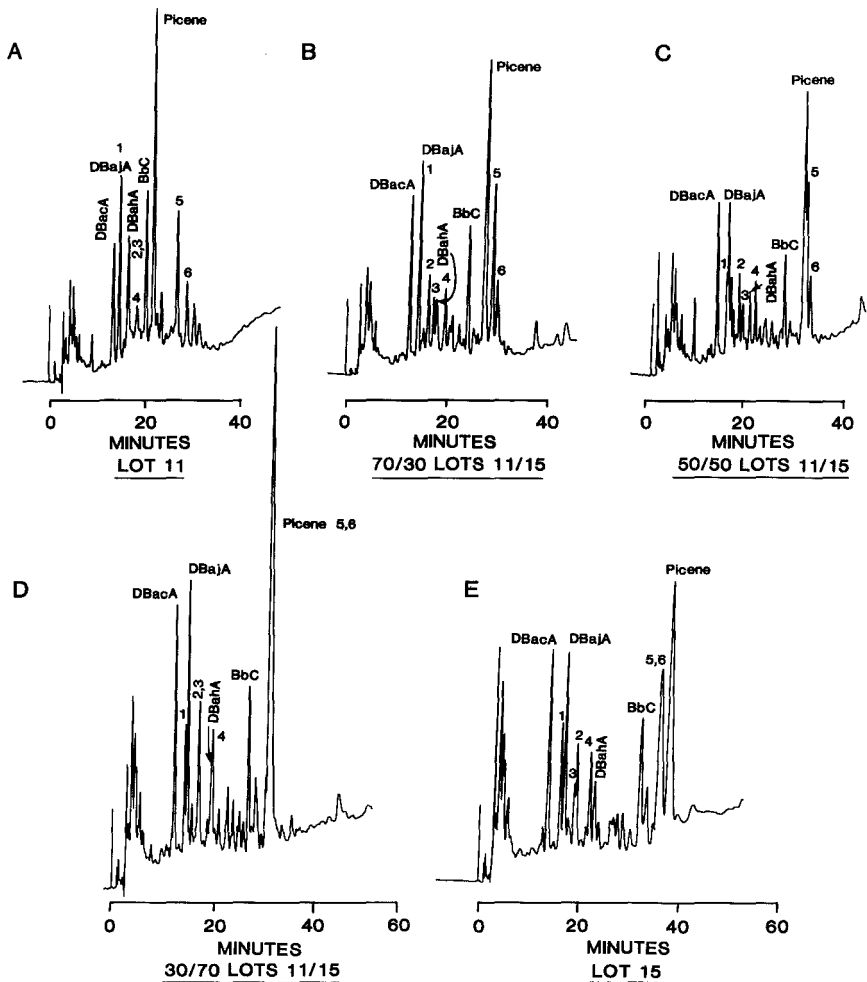


FIGURE 4: Reversed-phase LC separation of five condensed ring PAH fraction (isolated from an air particulate extract) on C_{18} columns of different selectivities (A) lot 11, (B) 70/30 lot 11/15, (C) 50/50 lot 11/15, (D) 30/70 lot 11/15, and (E) lot 15. Peak identification: DBacA = dibenz[α , α]anthracene, DBajA = dibenz[α , j]anthracene, DBahA = dibenz[α , h]anthracene, BbC = benzo[b]chrysene, and no. 6 = coronene. Peaks 1-5 are unknown.

TABLE 2. Selectivity Factors (α) for Selected PAH on Mixed vs. Coupled Columns

	<u>50/50 Lot 11/15</u>	
	<u>Mixed</u>	<u>Coupled</u>
<i>m</i> -Tetraphenyl	0.288	0.291
Phenanthro[3,4- <i>c</i>]phenanthrene	0.436	0.438
9,10-Diphenylanthracene	0.454	0.459
<i>m</i> -Quinquephenyl	0.557	0.564
Tetrabenzonaphthalene	0.676	0.676
Dibenz[<i>a,c</i>]anthracene	0.728	0.730
Dibenz[<i>a,j</i>]anthracene	0.939	0.941
Dibenz[<i>a,h</i>]anthracene	1.42	1.43

PAH (molecular weight of 278) and coronene, which was isolated from an air particulate sample as described previously (8), was separated on columns from lots 11 and 15 and on the three mixed phase columns. This fraction contains coronene and five PAH isomers of molecular weight 278 which were identified by gas chromatography-mass spectrometry (GC-MS) and LC with fluorescence detection as dibenz[*a,c*]anthracene, dibenz[*a,j*]anthracene, dibenz[*a,h*]anthracene, benzo[*b*]chrysene, and picene. In addition, at least one unidentified PAH isomer of molecular weight 278 was confirmed by GC-MS.

The LC analyses of this fraction on the five different columns are shown in Figure 4. All of these chromatograms were obtained under the same chromatographic conditions. The five identified PAH isomers of molecular weight 278 were separated on each of the five columns. However, the unknown peaks (nos. 1-5) and coronene coelute with each other and/or with the identified peaks on some of the columns. These chromatograms illustrate the advantage of having several columns of differing selectivities for the analysis of complex environmental PAH mixtures.

CONCLUSIONS

The complexity of PAH mixtures often necessitates the modification of column selectivity to obtain the separation of particular constituents. Two methods for achieving a particular selectivity for PAH in reversed-phase LC have been described in this paper, i.e., physically mixed C₁₈ sorbents of different selectivities and coupled columns each containing a different C₁₈ sorbent. Both of these methods provide a selectivity which is intermediate to that of the individual C₁₈ materials and which is related to the proportions of each material. These two approaches allow the chromatographer to "customize" a column with an optimized selectivity for the separation of the components of interest. In practice the coupled column approach provides the most flexibility for preparing columns of a specific selectivity. With the availability of several short columns containing C₁₈ materials with greatly differing selectivities, a particular intermediate selectivity can be achieved readily by coupling the appropriate columns.

ACKNOWLEDGMENTS

The authors thank Barry Diamondstone for providing the percent carbon determinations and Kervin Harrison for preparing the mixed phase columns.

REFERENCES

1. Wise, S. A., Bonnett, W. J., Guenther, F. R., and May, W. E. A Relationship Between Reversed-Phase C₁₈ Liquid Chromatographic Retention and the Shape of Polycyclic Aromatic Hydrocarbons, J. Chromatogr. Sci., **19**, 457, 1981.
2. Wise, S. A. and May, W. E., Effect of C₁₈ Surface Coverage on Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons, Anal. Chem., **55**, 1479, 1983.
3. Laub, R. J., Roberts, W. L., and Smith, C. A., Use of Mixed Phases for Enhanced Gas-Chromatographic Separation of Polycyclic Aromatic Hydrocarbons. II. Prediction of Retentions with Open-Tubular Columns of High Efficiency with Liquid Crystal Solvents, in Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate, Cooke, M. and Dennis, A. J., Eds., Battelle Press, Columbus, OH, 1981, p. 287.

4. Kong, R. C., Lee, M. L., Tominaga, Y., Pratap, R., Iwao, M., Castle, R. N., and Wise, S. A., Capillary Column Gas Chromatographic Resolution of Isomeric Polycyclic Aromatic Sulfur Heterocycles in a Coal Liquid, J. Chromatogr. Sci., 20, 502, 1982.
5. Glajch, J. L. and Kirkland, J. J., Optimization of Selectivity in Liquid Chromatography, Anal. Chem., 55, 319A, 1983.
6. Ogan, K. and Katz, E., Retention Characteristics of Several Bonded-Phase Liquid Chromatography Columns for Some Polycyclic Aromatic Hydrocarbons, J. Chromatogr., 188, 115, 1980.
7. Sander, L. C. and Wise, S. A., Synthesis and Characterization of Polymeric C₁₈ Stationary Phases for Liquid Chromatography, submitted to Anal. Chem.
8. Wise, S. A., Bonnett, W. J., and May, W. E., Normal- and Reversed-Phase Liquid Chromatographic Separations of Polycyclic Aromatic Hydrocarbons, in Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Bjørseth, A. and Dennis, A. J., Eds., Battelle Press, Columbus, OH, 1980, p. 791.

AGING OF PRESSURE SENSITIVE ADHESIVES. II:
USE OF MULTIDETECTOR SEC

Daniel J. P. Harrison*, W. Ross Yates⁺
and Julian F. Johnson
Department of Chemistry
and
Institute of Materials Science
University of Connecticut
Storrs, CT 06268

ABSTRACT

The use of multiple detector size exclusion chromatography is described for the study of aging of pressure sensitive adhesive films based on styrene-isoprene-styrene block copolymers. In this preliminary investigation of thin films, room temperature oxidation resulted in the formation of an easily detected carbonyl chromophore. While the chromophore concentration was monitored with a UV detector on the chromatograph, the molecular weight distribution was measured with a differential refractometer. Several important general implications of this combination of detectors in SEC are described.

INTRODUCTION

The first paper in this series (1) described initial efforts to elucidate the nature of aging changes in the commercially important styrene-isoprene-styrene (SIS) block copolymers, especi-

* Present Address: Research Laboratories, Eastman Kodak Co.,
Bldg. 82, Kodak Park, Rochester, NY 14450

+ Present Address: The Kendall Company, Lexington Laboratories,
17 Hartwell Avenue, Lexington, MA 02173

ally as they are incorporated in pressure sensitive adhesives (PSA). The use of multi-detector size exclusion chromatography (SEC) has proven extremely valuable in this study, and several aspects of its application will be described herein.

The use of SEC for the analysis of polymers has been widely reviewed (2-5). Since SEC is primarily used to determine the molecular weight distribution (MWD) of polymers, it has found extensive use in study of polymer degradation, many such applications of which have been discussed by Abbàs (6). Relative changes in MWD are very readily observed, and with extreme care useful information can also be obtained on the kinetics of oxidation chain scission.

The usefulness of SEC for the analysis of random and block copolymers has been increased on occasion by using two or more detectors (7,8,9,10). In these studies, typically one detector (usually ultraviolet or infrared) is used to monitor the concentration of the comonomers, while a second detector (refractive index or light scattering) monitors molecular weight. Harmon and Folt (7) used such a technique to measure the comonomer ratio in SBR. In a similar study, Stojanez et al. (8) investigated both random and block styrene/butadiene copolymers. In the latter, the copolymer composition was determined by SEC, infrared spectroscopy and nuclear magnetic resonance spectroscopy, and although results for the block copolymers were in very good agreement, the random SBR results varied somewhat. This was attributed to a change in the UV extinction coefficient of styrene, depending upon the sequence length in the random copolymers.

Dual detector (UV/RI) SEC has also been used to determine the distribution of olefinic linkages in elastomers (11). In some elastomers, small amounts of unsaturated monomers are incorporated for crosslinking, and to estimate the concentration of these external double bonds, Anderson reacted double bonds in the elastomer with 2,4-dinitro-benzene sulfonyl chloride, thus forming a chromophore with a high extinction coefficient at 254 nm. While the SEC separated the "stained" elastomer by molecular size, the

RI detector measured the total concentration of polymer and the UV detector provided the concentration of external double bonds. Thus, the distribution of external double bonds as a function of MW was determined.

In the oxidative chain scission of polyisoprene, a similar effect is obtained. According to Shelton et. al. (12), the end group residues after the chain scission of polyisoprene are typically aldehydes and ketones. However, approximately 50% of these residues are α,β -unsaturated carbonyls, which are known to have an extremely high extinction coefficient at about 237 nm (13). It is expected that the polyisoprene mid-block in SIS will behave in an analogous manner. It is the use of a combination of UV and RI detectors in SEC for studying aging in SIS formulated PSA's which is investigated in this study.

It should be pointed out that multiple detector SEC has received some criticism by Bressau (14), who contends that the connecting tubing and fittings between detectors not only changes the elution volume but also alters the shape of the chromatogram. In his experiments, 20-50 cm capillary tubing and high dead volume fittings were used. By shortening tubing lengths and using only low dead volume fittings, these problems can be minimized.

EXPERIMENTAL

The SEC used in these experiments consisted of a Waters 6000A solvent delivery system with a model U-6 sample injector, 4 μ -Styragel columns (10^5 , 10^4 , 10^3 , and 500\AA), a Varian model UV-50 variable wavelength UV detector, and a Waters Model R401 RI detector. These detectors were connected in series, with the RI following the UV detector. Valco low dead volume fittings were used in all connections. The tubing length between the UV and RI detectors was ~ 25 cm and the capillary ID was .05 cm. The UV flow through cell was cylindrical (1mm x 6mm) to minimize mixing. The solvent was freshly distilled unstabilized THF, and the flow rate was $1.5 \text{ cm}^3/\text{min}$. The instrument was calibrated with narrow MWD

polystyrene standards (Pressure Chemical), but since the Mark-Houwink constants were not known for the SIS block-copolymers used in these experiments, data are reported as a function of elution volume.

Rubber solutions (20% SIS by weight) were mixed for 24 hours in the dark on a gentle wrist action shaker, using nitrogen-degassed toluene (reagent grade) as the solvent. SIS was from a commercial lot of Kraton 1107 (Shell Chemical Company). The resulting solutions were stored in the dark under argon until used.

Solutions were coated with a Gardner Ultra Film Applicator on 25- μ m polyethyleneterephthalate films taped to glass plates. The coatings were first dried in the dark at 20-25°C for one hour under a continuous, slow nitrogen purge. They were then heated to 40°C in a 3300-Pa vacuum for 4 hours. During the heating cycle, a small nitrogen leak was provided to the drying system to sweep away any residual toluene or oxygen. It is likely that most of the butylated hydroxytoluene (BHT) incorporated in the rubber manufacturing process will be removed in this vacuum drying step.

UV spectra of polymers in unstabilized THF were recorded with a Beckman Model 25 spectrophotometer.

RESULTS

In order to determine the optimum wavelength for the UV detector, UV spectra of both polystyrene and polyisoprene in THF were recorded. While the polyisoprene can only be detected below 232 nm, the polystyrene can be detected below 232 nm, and also between 250 and 270 nm. Since it was found that the RI detector responded well to solutions of either polystyrene or polyisoprene, the UV detector was operated at 259 nm, the absorption maximum for polystyrene in that region of its spectrum. It should be noted that UV spectra of the polymer solutions cannot be recorded below \sim 224 nm due to a maximum at \sim 220 for THF. Further considerations of detector wavelength will be discussed later.

It was found that when solutions taken from thin SIS films were analyzed on this SEC, the UV and RI results were quite

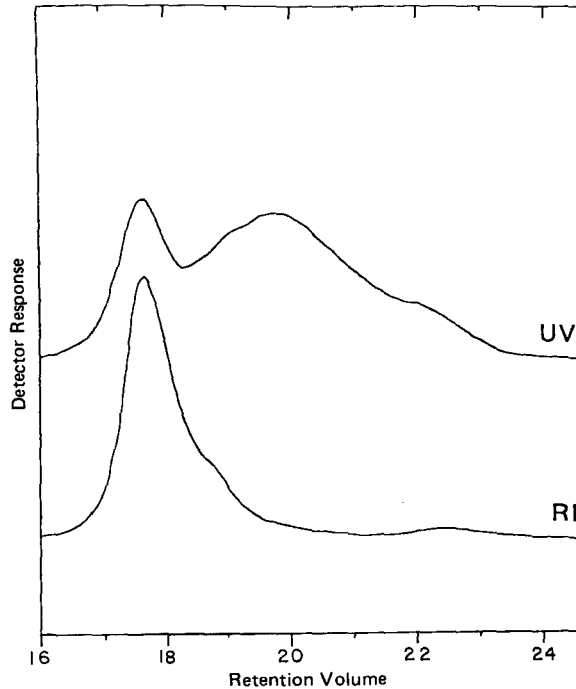


FIGURE 1: SEC chromatograms of unaged 4 μm SIS Films. The UV detector wavelength was fixed at 259 nm and the retention volume was recorded in ml.

different. As seen in Figure 1, the RI detector indicates that very little degradation has occurred to this 0.16 mil unaged SIS film. This molecular weight distribution, is typical of unaged SIS block copolymers. The large peak at 17.8 min ($\sim 2.5 \times 10^5$ amu) represents the SIS tri-block molecules, while the shoulder at 18.8 min ($\sim 1.3 \times 10^5$ amu) results from SI diblock fragments which did not couple in synthesis. The small peak at 22.5 min ($\sim 10^4$ amu) is polystyrene (PS) homopolymer which terminated prior to anionic polymerization with isoprene. For a general discussion of the synthesis of these block copolymers see Dreyfuss, et al. (15).

On the other hand the UV detector response in Figure 1 appears to indicate that substantial degradation of tri-block SIS

TABLE 1

SEC Fraction Location

<u>Fraction</u>	<u>Retention Volume (ml)</u>
1	16.0 - 17.8
2	17.8 - 18.5
3	18.5 - 19.2
4	19.2 - 20.8
5	20.8 - 21.5
6	21.5 - 24.0

molecules to di-block SI fragments has occurred, if one views the response in the usual way as an indication of MWD. Since the RI response is unmistakably MWD, it would appear that very little degradation to the SIS film has actually occurred, and that the small amount of degraded material must contain a chromophore whose molar extinction coefficient (ϵ) is substantially greater than that of polystyrene at 259 nm, thus yielding a large UV response in this region.

In order to determine the chemical moiety responsible for the very large absorbance at 259 nm, a 3.8 μ m thick film of SIS was dissolved in THF and fractionated in the SEC. Six fractions were collected, two from the SIS region, one from between the SIS and the SI peaks and three from the SI to the PS region (Table 1). UV spectra (220-290nm) were recorded for each of these fractions and the results are summarized in Figure 2. It was observed that fractions 1 and 2 are composed largely of polystyrene and polyisoprene, as expected. Fraction 3 shows some traces of polystyrene as well as a broad shoulder centered at 240 nm on the large polystyrene/polyisoprene absorption. In fractions 4, 5, and 6, very little polystyrene is observed at 260 nm while the shoulder observed in fraction 3 is now resolved into a peak which has its maximum at approximately 237 nm.

The Woodward-Fieser rules for carbonyl compounds (13), predict that a substituted α,β -unsaturated carbonyl will strongly

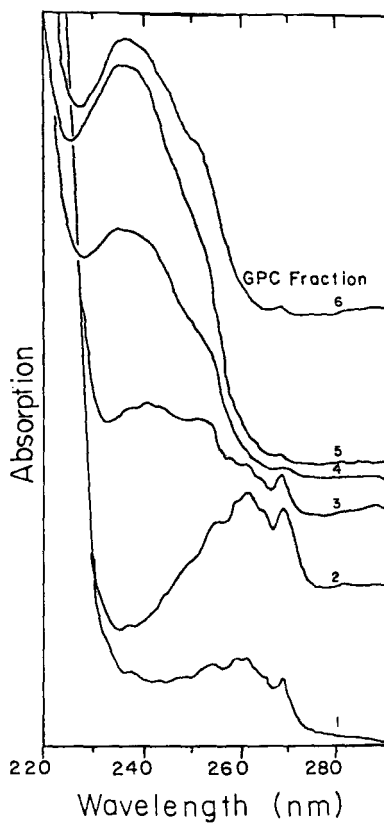


FIGURE 2: Ultraviolet spectra of SEC fractions in THF. The retention volume range of each fraction is shown in Table 1.

absorb between 220 and 250 nm. It has been shown by Shelton et al. (12) that such compounds are formed in the oxidative chain scission of polyisoprene. While low MW compounds like 2-butenone are a volatile product of this oxidative chain scission, other α,β -unsaturated carbonyls may exist as end groups on the isoprene chain after scission.

In addition to this maximum of 237 nm, a strongly absorbing shoulder at \sim 250 nm is also observed in fraction 3-6. This is probably a similar residue with a slightly different structure. This shoulder extends well beyond 259 nm and thus is responsible for the interference observed in the UV chromatogram (Figure 1), since its extinction coefficient may be as much as an order of magnitude greater than that of PS at this wavelength. Thus, while the RI detector shows very little material in the SI to polystyrene region of the chromatogram, a small amount of material with a high extinction coefficient produces a large UV detector response at 259 nm.

As a result of this finding, SEC chromatograms of 2.5- μ m SIS films were recorded with the variable wavelength UV detector at 237 nm. A comparison of UV chromatograms at 237 nm, 226 nm and 259 nm is shown in Figure 3. At 226 nm both polystyrene and polyisoprene absorb strongly, while the α,β -unsaturated carbonyl residue absorbs only moderately. The SI and SIS peaks thus resemble those found in the RI response, and are strongly related to MWD in the polymer; there is however some interference from the strongly-absorbing carbonyl group, as seen by comparison with the RI chromatogram in Figure 1. The SEC chromatogram run at 237 nm is very sensitive to both polystyrene and PI. The chromatogram recorded at 259 nm is less sensitive to the carbonyl group, and moderately sensitive to the polystyrene, however, the carbonyl group still dominates.

These results point out a potential problem from using a fixed wavelength UV detector alone to study MWD in polymers susceptible to oxidation. If only the UV detector at 254 nm had been used in this study, as an example, the results would have

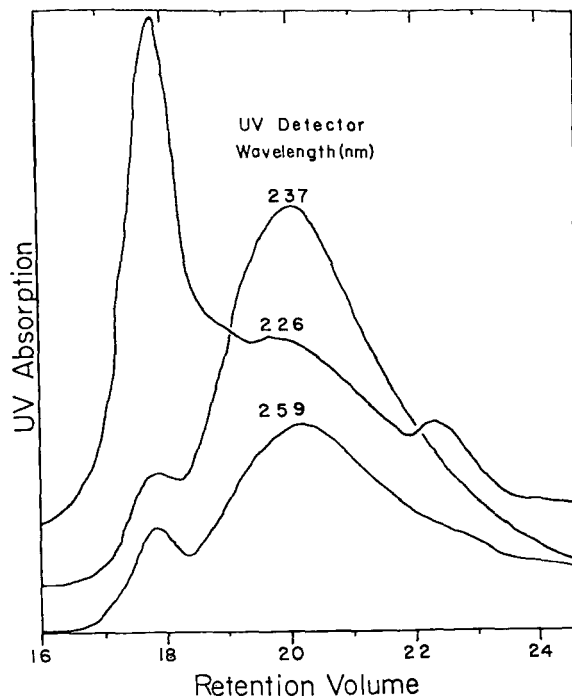


FIGURE 3: SEC chromatograms of 2.5 μm SIS films using a variable wavelength UV detector at 259, 237 and 226 nm.

been very misleading. Data collected at 254 nm would lead one to believe that extensive degradation of SIS tri-block to SI diblock had occurred, while in fact very little occurred, and the small amount of degradation which did occur was accompanied by formation of a product with a very large extinction coefficient. Information obtained simultaneously from UV (226 nm) and RI detectors prevent this misinterpretation. Thus, whenever possible, it is desirable to use both detectors simultaneously when studying unsaturated polymers under conditions of degradation.

From this information it seems that this is a very sensitive method of detecting carbonyl groups that result from the oxidative chain scission of polyisoprene. However, in order for this tech-

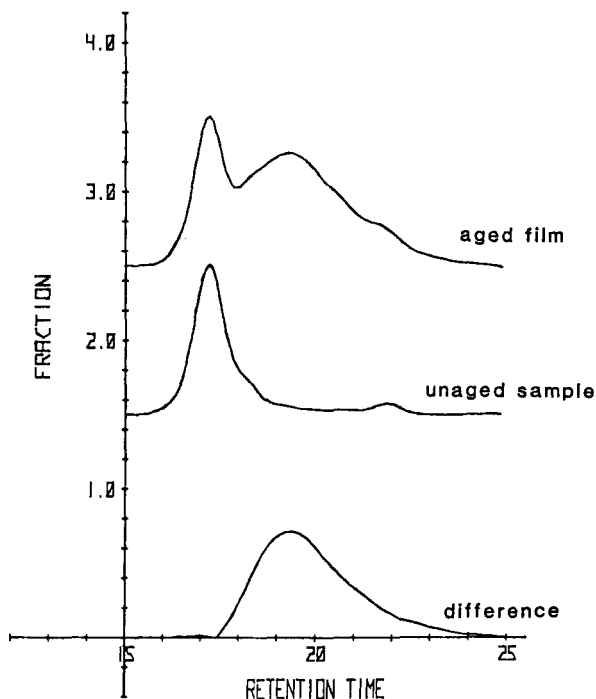


FIGURE 4: SEC difference chromatograms using an UV detector fixed at 237 nm. Normalization of the chromatograms was achieved through dividing the detector response by the response at the SIS peak (16.8 ml). The unaged sample was taken from a bulk SIS lot. The aged film was coated from the same lot and had a dry thickness of 8.6 μm . It was aged in air for seven hours at 80°C.

nique to be at least semi-quantitative, a method of normalization must be designed. Using the information obtained from both RI and UV detectors, we have found that the concentration of SIS molecules in a given sample is essentially constant, for short aging times at temperatures no greater than about 80°C. Also, since this anionically polymerized polymer has a very narrow MWD, one can achieve normalization by dividing each point on the chromatogram by the SIS peak height.

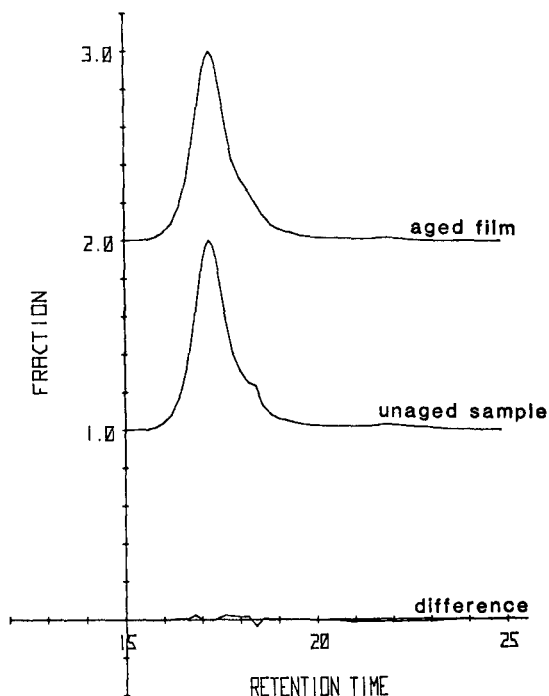


FIGURE 5: SEC difference chromatograms using a RI detector. Normalization of the chromatograms was achieved through dividing the detector response by the response at the SIS peak (16.8 ml). The unaged sample was taken from a bulk SIS lot. The aged film was coated from the same lot and had a dry thickness of 8.6 μm . It was aged in air for seven hr. @ 80°C.

Once the data have been normalized, changes in the carbonyl content can be followed with aging time. In order to facilitate such an experiment, a difference chromatogram is determined by subtracting the chromatogram of an uncoated and unaged sample from one of a coated and/or aged sample, so long as each chromatogram was previously normalized to the SIS peak height. The area under the resulting, difference chromatogram represent the carbonyl content in the sample being investigated. If the film volumes are standardized to 1cm x 1cm x the film thickness, the relative

concentration of carbonyl groups in a film can be calculated from the area under the difference chromatogram. Using such a standard sampling volume, the relative concentration of carbonyl groups in films of varying thickness can be compared.

Figure 4 illustrates the subtraction procedure used. In this case, the chromatogram of SIS bulk polymer is subtracted away from one of an aged (7 hr, 80°C, Air) 8.6 μm SIS film. The area under the difference chromatogram is then calculated and used as a measure of the carbonyl content in the specimen. To illustrate the soundness of this technique, figure 5 shows the subtraction of the RI chromatogram of SIS bulk from one of SIS film aged seven hours at 80°C in air. The difference chromatogram shows that the two films are essentially equivalent, with no appreciable mass change of SIS to SI having occurred.

DISCUSSION

Although SEC is well suited for studies of polymer degradation, detector choice is crucial. In the case of thin SIS block copolymers films a fixed UV detector at 254 nm can give misleading results due to interference from carbonyl bands. By using both a variable wavelength UV detector and an RI detector, the extent of oxidation is easily followed. Further, if the UV detector is set to a wavelength at which the degradation product absorbs, substantial information on the degradation process can be obtained. Such a method has been developed for studying the oxidation of thin SIS block copolymers films used in PSA's. The method measures the formation and/or deterioration of carbonyl groups which absorb at 237 nm. Such groups are typically found as chain end residues after oxidative scission (12).

Further improvement of such experiments could be made by using a UV spectrometer which is capable of quickly scanning (1-2 sec.) the flowing eluent from the SEC (16). This would yield a separate UV spectrum for each segment of the chromatogram. Thus typical components could easily be identified.

In the SIS films examined in this study, the presence of small amounts of oxidative chain scission products had a dramatic effect on the ultraviolet detection of these materials. It is believed that surface oxidation of these films was responsible for this effect. It has been shown by Chang et al. (17) that polybutadiene (PB) films exposed for short periods to room temperature air showed a dramatic increase in adhesive bond strength to other identical films. It was believed that surface oxidation was responsible for this effect. Polyisoprene also undergoes such surface oxidation. However, since chain scission predominates over cross-linking, no covalent bonds are formed across the adhesive interface, as in the case of PB, and no increase in adhesive strength is realized.

The films studied in these experiments were very thin ($\sim 2.5 \mu\text{m}$) and the surface to bulk ratio was sufficiently high to allow the detection of these materials. Other methods such as multiple internal reflectance infrared spectroscopy (MIR/IR) proved too insensitive to detect the surface oxidation in these films. However, in SIS films aged for several hours at 95°C MIR/IR was found to be very useful. (1) The results of those experiments were found to be in agreement with the FTIR studies of Shelton (12).

Future studies will further characterize surface oxidation in SIS films. These data will then be used to develop a model of the influence of surface oxidation on the aging of SIS based PSA's.

ACKNOWLEDGMENTS

One of us, D.H. wishes to acknowledge the support of Avery International Corporation which provided a research fellowship for these investigations.

REFERENCES

1. Harrison, D.J.P., Johnson, J.F. and Yates, W.R., Aging of Pressure Sensitive Adhesives I: Stability of Styrene-Isoprene-Styrene Block Copolymer Films at 95°C , Polym. Eng. Sci., 22(14), 865, 1982.

2. Cazes, J., XXIX. Gel Permeation Chromatography, *J. Chem. Ed.*, 43(7) and 43(8), 1966.
3. Ouano, A.C., Barrall II, E.M. and Johnson, J. F., Gel Permeation Chromatography, Chapt. 6 in Polymer Molecular Weights, Slade, P.E. and Jenkins, L.T., eds., Marcel Dekker, New York, 1975.
4. Abbott, S.D., Size Exclusion Chromatography in the Characterization of Polymers, *Am. Lab.*, 9(8), 41, 1977.
5. Gaylor, V.F. and James, H.L., Gel Permeation Chromatography (SEC), *Anal. Chem.*, 50(5), 29R, 1978.
6. Abbās, K.B., Molecular Characterization of Degraded Polymers, in Liquid Chromatography of Polymers and Related Materials II, Cazes, J. and Delamare, X., eds., Marcel Dekker, New York, 1980, pp. 123-142.
7. Harmon D.J. and Folt, V.L., Multidetectors for Analysis of the Composition of Copolymers as a Function of Molecular Size Distribution by Steric Exclusion Chromatography, *Rub. Chem. Tech.*, 46(2), 449, 1973.
8. Stojanov, C.H., Shirazi, Z.H., and Audu, T.O.K., Contribution to the Determination of Chemical Composition of Copolymers by Means of GPC, *Pak. J. Sci. Ind. Res.*, 20(3), 161, 1977.
9. Tung, L.H., Block Copolymer Molecular Weight by GPC, *J. Appl. Polym. Sci.*, 24, 953, 1979.
10. Gallot, Z., Utilization of Multidetector Systems for Gel Permeation Chromatography, in Liquid Chromatography of Polymers and Related Materials II, Cazes, J. and Delamare, X., eds. Marcel Dekker, New York, 1980, pp. 113-122.
11. Anderson, J.N., The Distribution of Olefinic Linkages in Elastomers, *J. Appl. Polym. Sci.*, 18, 2819, 1974.
12. Shelton, J.R., Pecsok, R.L. and Koenig, J.L., FTIR Studies of the Uninhibited Oxidation of Elastomers, *ACS Symp. Ser.*, 95, 75, 1979.
13. Silverstein R.M., Bassler, G.C. and Morrill, T.C., Spectroscopic Identification of Organic Compounds, 4th ed., John Wiley and Sons, New York, 1981.

14. Bressau, R., Problems in Multiple Detector of GPC Eluents, in Liquid Chromatography of Polymers and Related Materials II, Cazes, J. and Delamare, X., eds. Marcel Dekker, New York, 1980, pp. 73-93.
15. Dreyfuss, P. and Fetters, L.J., Elastomeric Block Polymers, *Rub. Chem. Tech.*, 53, 729, 1980.
16. Saitoh, K. and Suzuki, Multiwavelength Detection for Liquid Chromatography with a Repeat-Scanning Ultraviolet-Visible Spectrophotometer, *Anal. Chem.*, 51(11), 1683, 1979.
17. Chang, R.J., Gent, A.N., Hsu, C.C. and Sehgal, K.C., Bonding Elastomer Layers Together by a Surface Oxidation Reaction, *J. Appl. Polym. Sci.*, 25, 163, 1980.

DETERMINATION OF URIC ACID IN HUMAN SERUM:
REVERSED-PHASE LIQUID CHROMATOGRAPHY
WITH ELECTROCHEMICAL DETECTION

Keiji Iriyama, Masahiko Yoshiura, Takeo Iwamoto, *Tatsuo Hosoya,
*Hideo Kono, and *Tadashi Miyahara

Division of Biochemistry, Central Research Laboratory,
and *2nd Department of Internal Medicine,
The Jikei University School of Medicine,
3-25-8, Nishi-Shinbashi, Minato-ku, Tokyo 105, Japan.

ABSTRACT

A method for the simultaneous determination of uric acid in human serum by reversed-phase high-performance liquid chromatography with electrochemical detection has been developed. Human serum (0.5 ml) was mixed with 0.5 ml of 0.2 N perchloric acid solution and the mixture was centrifuged at 3,000 g for 20 min. An aliquot (10 μ l) of the supernatant (deproteinized human serum) was injected into the chromatographic system employed in this study. The assay limit for quantitation was about 10 pg for uric acid. Complete separation of uric acid was achieved in about 8 min under the present chromatographic conditions.

INTRODUCTION

As described elsewhere (1), high-performance liquid chromatography (HPLC) may be an obvious candidate as a reference method for the determination of serum uric acid. Different separation principles and detectors have been used for determining uric acid (UA) in serum by aid of HPLC. Ion-exchange columns have been

used, coupled with ultraviolet detection (2) or electrochemical detection (3). Reversed-phase high-performance liquid chromatography (RPHPLC) has been also used with ultraviolet detection (4). Recently, we have developed a method for the determination of UA and catecholamines in rat serum and brain by RPHPLC with electrochemical detection (5).

The object of this paper is to report a simple, rapid, selective and highly sensitive method for the determination of UA in human serum by RPHPLC with electrochemical detection (ECD).

MATERIALS AND METHODS

UA was purchased from Wako Pure Chemicals, Tokyo, Japan. All other chemicals used in this study were the same ones as used in our previous report (6). All buffers and aqueous solutions were prepared with glass-distilled deionized water.

Serum samples prepared from healthy adult men were stored at -80°C until use. Standards were prepared by appropriately diluting a stock UA solution (1mg/ml) with water. The stock solution was prepared as follows. An aliquot (0.05 ml) of 0.05 N NaOH was dropped into 10 mg of UA for dissolving it completely because of its low solubility in pure water. A 0.1 N HCl (9.95 ml) was added into the alkaline UA solution.

Samples were analyzed using a reversed-phase partition mode of HPLC. A JASCO-HPLC, TRIROTAR III, was used throughout this

work. The instrument was fitted with an electrochemical detector (Model ECP-1, Kotaki Inc., Funabashi, Chiba, Japan). A Finepak SIL C18 column (\varnothing 4.6 x 250 mm, JASCO, Tokyo, Japan) was used for the separation of UA. The column temperature was always kept at 35°C. The mobile phase was 0.2 M phosphate buffer (KH_2PO_4 - H_3PO_4 , pH 2). The flow rate was 0.5 ml/min. Ten microliters of each sample was injected into the RPHPLC-ECD system. The electrochemical detector was set at +800 mV vs. the silver/silver chloride reference electrode, as Pachela et al. (2) reported that the onset potential of UA was about +330 mV vs. the silver/silver chloride reference electrode under their electrochemical conditions and also that an electrochemical detector was set at +800 mV. Fig. 1 shows the standard curve for high-performance liquid chromatographic determination of UA under the present chromatographic conditions. As shown here, the minimum detectable quantity is about 10 pg for UA. UA was quantitated by comparing the peak height in the respective chromatogram with value from a standard curve.

RESULTS AND DISCUSSION

Recently, we have found (5) that alumina can adsorb UA as well as catecholamines and also that the adsorption of UA onto alumina is not always quantitative. These findings suggest that the so-called alumina treatment procedure recently optimized (6) is not useful for extraction and preliminary purification of UA in biological materials. Therefore, we aimed to develop a pretreatment in

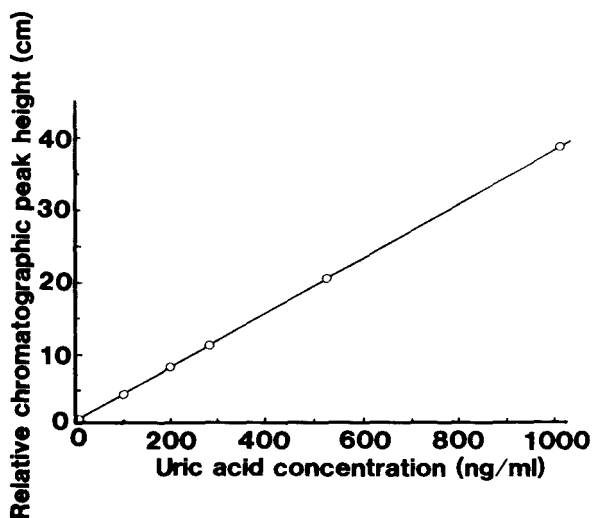


Figure 1. Calibration curve for uric acid under the present chromatographic conditions.

extracting procedure for UA of biological materials. In addition, we have found (5) that concentrations of UA in rat serum and brain are greater than those of any other electrochemically active components in both biological materials. From the above observation, we assumed that simply deproteinized serum might be able to be injected into the RPHPLC-ECD system for the determination of UA in serum without any interferences.

Deproteinization of human serum was achieved as follows: mix vigorously 0.5 ml of human serum with an equal volume of 0.2 M perchloric acid solution, and then centrifuge at 3,000 g for 20 min. An aliquot (10 μ l) of the simply deproteinized serum was injected into the RPHPLC-ECD system.

Figure 2 shows such a reversed-phase high-performance liquid chromatogram. Peak X in the chromatogram has been found to be



Figure 2. The typical reversed-phase high-performance liquid chromatogram, obtained by injecting 10 μl of the deproteinized human serum into the RPHPLC-ECD system. Ten microliters of the serum was injected into a column (Finepak SIL C18, JASCO) by using a microsyringe. The eluent was 0.2 M phosphate buffer ($\text{KH}_2\text{PO}_4\text{-H}_3\text{PO}_4$, pH 2.0). The column temperature was maintained at 35°C . The flow rate was 0.5 ml/min. Eluate from RPHPLC was electrochemically monitored by aid of an electrochemical detector under the potentiostatic condition (+800 mV vs. Ag/AgCl). The sensitivity of the detector was set at 64 nA full scale. For further explanations, see the text.

UA after the further co-elution of a mixture of UA and the deproteinized serum. As shown in Fig. 2, the method for the determination of UA in serum, which has been developed in this study, is not subject to interferences in other methods. The phosphotungstic acid method is subject to interference resulting from endogenous

nonurate chromogens, nutrients, and drugs (7). Urikase methods based on spectrophotometry are also not without problems, in that the specificity of the absorbance measurement is less than that of the enzyme, and several interferences have been noted (8). Content of UA in 0.5 ml of healthy human serum, as shown in Fig. 2, was found to be 21.6 μg (43.2 $\mu\text{g}/\text{ml}$). Since the sample preparation did not involve any transfer, the value for UA amount might be nearly absolute, indicative of an endogenous quantity.

As described above, we have developed a practical method for the determination of UA in human serum by RPHPLC with ECD. In addition, most recently, we have developed a method for the determination of xanthine and hypoxanthine by RPHPLC with ECD (Iwamoto, Yoshiura, and Iriyama, to be published in *Jikeikai Med. J.* with any other related experimental results). Xanthine, hypoxanthine, and UA are themselves produced either as a result of the breakdown of cellular material in toto, the turnover of nucleic acids in the cells, or as a result of the intermediary metabolism of various purine nucleotide derivatives. Modern biochemical investigators have found purine metabolism in general to be of great theoretical interest, and in addition to the problem there is hope the study of this metabolic system will provide answers to a number of ancillary enigmas of biochemistry. The biochemical and clinical importance of UA in gout and several other disease states was discussed by Balis (9) and Glynn et al. (10). As described by Glynn et al. (10), previous epidemiologic studies of UA have been limited by a cross-sectional design which precludes a determination of factors predictive changes in UA levels. Furthermore, some studies have not controlled for the

health status and drug intake of their populations. We believe that the well-known separation power of RPHPLC, combined with current state of the art in electrochemical monitoring will circumvent some of the problems presently encountered in the analysis of UA of biological and clinical importance. Because of its simplicity and applicability to small sample volumes, this method is useful in basic biomedical research. For example, we have determined concentrations of UA in rat tissues (e.g. brain, heart, stomach, and ren) according to the procedures developed in this study (Yoshiura, Iwamoto, and Iriyama, to be published elsewhere). This method can be applied for studying the change of UA levels in mammalian tissues.

REFERENCES

1. Ingebresten, O.C., Borgen, J., and Farstad, M., "Uric acid determinations: reversed-phase liquid chromatography with ultraviolet detection compared with kinetic and equilibrium adaptations of the uricase method". *Clin. Chem.*, 28, 496-498, 1982.
2. Pachla, L.A., Kissinger, P.T., Yu, L., Watson, F., Pragay, D., Chilcote, M.E., Weiner, L.M., and Pennick, B.R., "Measurement of serum uric acid by liquid chromatography", *Clin. Chem.*, 25, 1847-1852, 1979.
3. Milner, J.A. and Perkins, E.G., "Determination of uric acid in biological fluids by high-pressure liquid chromatography", *Anal. Biochem.*, 88, 560-565, 1978.
4. Brown, N.D., Kintzios, J.O., and Koetitz, S.E., "Determination of hypoxanthine, xanthine, and uric acid in biological fluids by ion-pair high-performance liquid chromatography", *J. Chromatogr.*, 177, 170-173, 1979.
5. Iwamoto, T., Yoshiura, M., and Iriyama, K., "Uric acid determination: reversed-phase liquid chromatography with electrochemical detection", *Jikeikai Med. J.*, 30, in press.
6. Iriyama, K., Yoshiura, M., and Iwamoto., "Determination of catecholamines in rat tissues by high-performance liquid chromatography with electrochemical detection", *Jikeikai Med. J.*, 30, 35-44, 1983.

7. Geisinger, K.R., Batsakis, J.G., and Bauer, R.C., "Serum uric acid", *Amer. J. Clin. Pathol.*, 72, 330-336, 1979.
8. Young, D.S., Pestaner, L.C., and Gilberman, V., "Effects of drugs on clinical laboratory tests", *Clin. Chem.*, 21, 1D-432D, 1975.
9. Balis, M.E., "Uric acid metabolism in man", *Adv. Clin. Chem.*, 18, 213-246, 1976.
10. Glynn, R.J., Canpion, E.W., and Silbert, J.E., "Trends in serum uric acid levels", *Arthr.Rheum.*, 26, 87-93, 1983.

HIGH PRESSURE LIQUID CHROMATOGRAPHIC ASSAY OF CEFAMANDOLE IN
SERUM FOLLOWING INTRAVENOUS AND INTRAPERITONEAL ADMINISTRATION

Roger E. Bawdon, Ph.D., Kenneth J. Leveno, M.D.
J. Gerald Quirk, M.D., Ph.D., F. Gary Cunningham, M.D.,
and Stephen P. Guss, M.S.

From the Department of Obstetrics and Gynecology
The University of Texas Health Science Center
Southwestern Medical School
5323 Harry Hines Boulevard
Dallas, Texas 75235

ABSTRACT

Following cesarean section 102 women were treated with cefamandole by either perioperative intravenous administration or intraperitoneal irrigation. High-pressure liquid chromatographic (HPLC) methods for the quantitation of the low serum levels of cefamandole following intraperitoneal lavage were developed. The antibiotic was assayed in the serum using a standard microbiological assay and two types of reverse phase column technology for HPLC. The two HPLC systems were almost identical in performance. Both HPLC methods were at least 10-fold more sensitive than the microbiological assay. The correlation between the three methods was 0.9739. The half-life of cefamandole was 37 min, which was not significantly different from the half-life of the drug in serum of non-pregnant women. The peak serum levels were 47.6 ± 36.8 $\mu\text{g/ml}$ and 1.98 ± 1.5 $\mu\text{g/ml}$ for the intravenous and intraperitoneal methods of administration, respectively.

INTRODUCTION

Intrauterine intraperitoneal (IU/IP) irrigation and intravenous (IV) administration of antibiotics have been used effectively as prophylaxis in high-risk cesarean section patients (1,2). Rudd and associates used IU/IP lavage with cefamandole nafate to reduce post-cesarean section endometritis (2). Thirty patients received antibiotic via IU/IP irrigation and none developed endometritis. The reported serum levels of cefamandole using IU/IP lavage were frequently less than 0.5 $\mu\text{g/ml}$. Although the microbiological assay may be capable of measuring these low levels, the coefficient of variation of the assay has been very large (3). The purpose of this investigation was to develop a phase extraction procedure for cefamandole and to develop sensitive high-pressure liquid chromatographic (HPLC) assays for the determination of the wide range of levels of cefamandole anticipated in serum subsequent to IU/IP lavage and IV administration of the drug. Two types of column technology were compared, the conventional C_{18} μ -Bondapak steel column, and the new Radial Compression System (RCM-Z) containing a C_{18} μ -Bondapak compressible column. Serum levels were then determined following both IU/IP irrigation and the IV administration of the drug. In addition, the half-life of cefamandole was calculated from these data to determine if physiologic changes associated with pregnancy affected the clearance of the drug from these women (4).

MATERIALS AND METHODS

Patients

One hundred and two women at high-risk for infection following cesarean section were entered into this study. Informed consent was obtained. The patients received either 2 g of cefamandole IV over a 20 min infusion or 2 g of cefamandole in 800 ml of saline as an IU/IP lavage of the uterus and pelvis commencing at repair of the uterine incision. Using a bulb syringe, the irrigant was applied and simultaneously suctioned as follows: 200

ml to the uterine incision before closure, 200 ml to the bladder flap and closed uterine incision, 200 ml to the colic gutters with the patient in reverse Trendelenburg position, and 200 ml to the subfascial and subcutaneous space. Blood samples were obtained at 30, 60, and 120 min following both the IU/IP irrigation and IV administration of the drug. The serum was separated and frozen at -20°C until assayed.

Microbiological Methods

The serum concentration of cefamandole was measured by the agar diffusion technique (5). The test organism was Bacillus subtilis ATCC 6633. All specimens were assayed in duplicate.

HPLC Assay

The serum concentration of cefamandole was measured by modification of an existing HPLC assay for other penicillin and cephalosporin antibiotics (6,7). The HPLC analysis was performed on an extract of 0.5 ml of serum. The sera were extracted using a two phase extraction procedure. Acetonitrile and dichloromethane were used in the phase extraction which eliminates dilution of the specimens. A 50 μl aliquot was injected into the HPLC. The HPLC system was an ALC/GPC Model 204 liquid chromatograph attached to a Model 710 B Waters Intelligent Sample Processor (WISP), (Waters Associates, Milford, Mass.). All analyses were performed utilizing a 30 cm μ -Bondapak C_{18} steel column (Waters Associates, Milford, Mass.). The eluate was monitored at 254 μm with a Waters 440 detector. The detector sensitivity was 0.10 absorbance units (AU) full scale for the analysis of serum extracts from patients receiving intravenous cefamandole, and 0.01 AU for analysis of serum extracts from patients receiving IU/IP irrigation with cefamandole. Peaks were recorded on a 10 mv chart recorder (Houston Instruments, Houston, Texas) at a chart speed of 0.5 cm/min. The mobile phase consisted of 0.1 M sodium phosphate (85%) and acetonitrile (15%) at a pH of 6.0. The flow rate was

3.0 ml/min. Prior to use the mobile phase was degassed by filtration with a 0.45 μm FHUP filter (Millipore Corp., Bedford, Mass.).

In addition, assays were performed using the radial compression Z module (RCM-Z) (Waters Assoc., Milford, Mass) equipped with a column 10 cm long with an inside diameter of 8 mm containing a 10 μC_{18} packing which should produce a more rapid and efficient separation than the 30 cm C_{18} steel column. The mobile phase for this system consisted of 0.1 M sodium phosphate (83%) and acetonitrile (17%) at a pH of 6.0. The flow rate was 4.0 ml/min. Prior to use the mobile phase was degassed by filtration as described previously.

Standard curves for both HPLC assays of cefamandole in serum were generated by extracting and assaying normal human serum spiked with cefamandole ranging from 0 to 100 $\mu\text{g/ml}$. In addition, three serum specimens containing an unknown quantity of cefamandole were assayed with the patients' specimens each time the assay procedure was performed (the quantities were unknown to the person performing the assay). The controls and the unknowns were prepared by the addition of a stock solution of cefamandole to serum with a microliter syringe (Hamilton Co., Reno, Nevada). Between-batch and within-batch recoveries were determined throughout the study using the spiked controls prepared as mentioned previously.

Statistics

Friedman's Chi-square nonparametric analysis was used to compare the three assay methods. Correlation coefficients for the three methods were done using multiple least squares linear regression. Comparison of the between-batch and within-batch data were done by linear least squares regression.

RESULTS

A chromatogram of the separation of cefamandole is depicted in Figure 1. Chloramphenicol, vancomycin, aminoglycosides, penicillins, cephalosporins or theophylline were without effect on

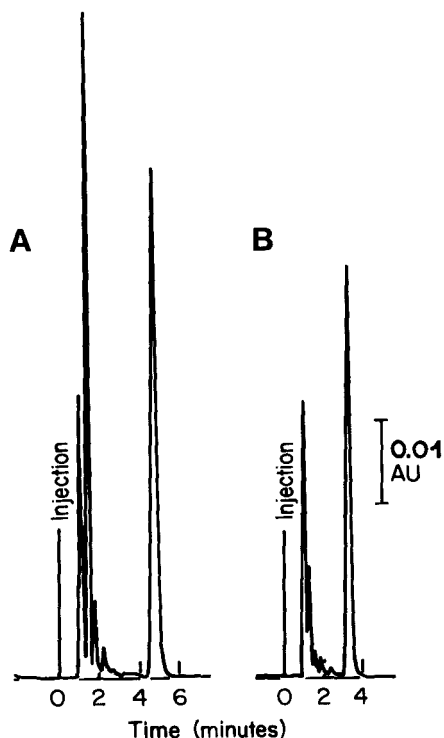


FIGURE 1. HPLC chromatograms of serum extracts of cefamandole (A) normal pooled human serum containing 47.5 μ g/ml of cefamandole using a standard μ -Bondapak C_{18} steel column; (B) the same serum specimens using the Waters Radial Compression System (RCM-Z) equipped with a C_{18} column.

the separation when added to the specimens. Between-batch and within-batch reproducibility studies showed recoveries of at least 84% for the wide range of levels found using the two different methods of administering cefamandole. The within-batch and between-batch reproducibility data are depicted in Tables 1 and 2 and the correlation among the three methods of assay are shown in Table 3. When standard curves were prepared for quantitation of the low IU/IP irrigation levels, recoveries of greater than 90%

TABLE 1.
Results of Within-Batch Reproducibility With the C₁₈ Column and the RCM-Z Module.

Cefamandole Concentration ($\mu\text{g/ml}$)	C ₁₈			Average % Recovery			RCM-Z			Average % Recovery	
	\bar{x}	SD	CV %	Recovery			\bar{x}	SD	CV %		
	n				n						
1.0	9	0.84	0.05	5.95	84.0	8	0.90	0.04	4.44	90.0	
5.0	7	4.93	0.18	3.65	98.6	6	4.76	0.17	3.45	95.2	
24.4	9	23.80	0.74	3.11	97.9	10	24.5	1.00	4.08	100.0	

\bar{x} , mean; SD, standard deviation; CV, coefficient of variation.

TABLE 2.
Results of Between-Batch Reproducibility With the C₁₈ Column and the RCM-Z Module.

Cefamandole Concentration ($\mu\text{g/ml}$)	C ₁₈			Average % Recovery			RCM-Z			Average % Recovery	
	\bar{x}	SD	CV %	Recovery			\bar{x}	SD	CV %		
	n				n						
1.0	5	0.87	0.13	14.83	87.0	4	0.83	0.10	12.05	83.0	
5.0	5	4.57	0.49	10.72	91.4	4	4.77	0.38	7.97	95.4	
24.4	3	24.28	0.88	3.62	99.5	4	23.74	1.45	6.11	97.3	

\bar{x} , mean; SD, standard deviation; CV, coefficient of variation.

TABLE 3.

The Comparison of Concentration of Cefamandole by the Microbiological, the C₁₈ Column and RCM-Z HPLC Methods.

Method of Administration	ASSAY		
	Microbiological	HPLC RCM-Z	HPLC C ₁₈
IP	3.40	3.95	3.10
IP	2.60	2.23	1.70
IP	6.20	5.38	4.50
IP	4.50	2.72	2.30
IP	0.80	0.74	0.45
IP	2.95	2.80	2.10
IP	1.30	1.10	1.10
IP	0.60	0.76	0.45
IP	7.50	5.90	5.10
IP	3.60	5.10	4.20
IP	2.80	3.20	2.80
IP	1.30	1.00	0.86
IV	34.00	46.30	44.10
IV	15.20	21.00	18.60
IV	4.50	6.90	5.80
IV	26.50	21.90	17.10
IV	13.50	10.50	7.70
IV	4.30	3.50	2.80
IV	0.74	0.81	0.86
IP*	ND	0.31	0.67
IP*	ND	0.14	0.51
IV	46.00	34.90	33.80
IV	21.00	18.10	17.20
IV	4.20	5.10	3.90
IV	34.00	31.40	26.10
IV	14.00	13.60	11.00
IV	2.90	4.30	3.70
IV	53.00	55.50	45.40
IV	31.80	25.90	23.50
IV	102.00	100.50	106.90
IV	15.80	20.20	19.30
IV	47.50	37.00	36.00
IV	18.50	26.00	25.00

* Indicates that microbiological assay could not detect the presence of cefamandole in these specimens.

The correlation matrix for the above assays are as follows.

Microbiological to RCM-Z = 0.9792
 Microbiological to C₁₈ = 0.9739
 RCM-Z to C₁₈ = 0.9934

IP = Intraperitoneal.

IV = Intravenous.

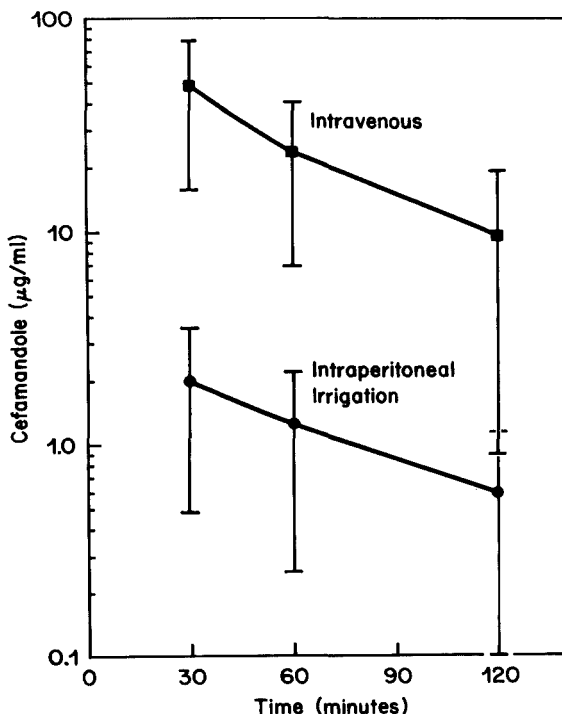


Figure 2. Semi-logarithmic plot of serum levels following the intravenous or intrauterine administration of 2 grams of cefamandole. Each time represents the mean and standard deviation. Forty-five or more values were used to achieve each point.

were obtained (data not shown). All methods were accurate and specific for cefamandole. The limit of detection for cefamandole by HPLC was $< 0.05 \mu\text{g/ml}$. The limit of the microbiological assay was approximately $0.50 \mu\text{g/ml}$. Therefore the concentration of cefamandole in some specimens could not be measured by the microbiological assay method. The regression coefficient ($Y = \alpha + \beta X$; where X and Y represent the dependent and independent variables, β is the regression coefficient in the population and α is the value of Y when X is 0) for the comparisons of the RCM-Z method to the

TABLE 4.
 Serum Concentration of Cefamandole Following Intravenous Infusion and Intrauterine Irrigation at Cesarean Section.

Administration	No. of Patients	30 Min.	60 Min.	120 Min.
IV Mean	53	** 47.6 ± 31.80	23.67 ± 16.80	9.54 ± 9.67
Range		(15.0 - 165.6)	(3.5 - 80.9)	(0* - 34.7)
IU/IP irrigation Mean	49	1.98 ± 1.50	1.24 ± 0.99	0.58 ± .5.7
Range		0.1 - 5.1	(0.05 - 2.6)	(0 - 2.3)

** µg/ml ± standard deviation.

* Less than 0.01 µg/ml of cefamandole.

microbiological assay method, the C₁₈ steel column method to the microbiological assay method, and the RCM-Z to the C₁₈ steel column assay method was $0.51 = 0.953X$, $-0.87 + 0.957X$, and $1.56 + 0.984X$, respectively.

Friedman's Chi-square test indicated a significant difference in the comparison of the microbiological data with the HPLC C₁₈ steel column data ($p < .001$). However, since these data had a very high correlation coefficient for all three methods, we do not feel that this significance was of practical importance.

Table 4 depicts the serum levels at the times collected. The serum levels decreased from 47.6 $\mu\text{g/ml}$ at 30 min to 9.54 $\mu\text{g/ml}$ at 120 min for the IV dose. The serum levels from the patients receiving cefamandole by IU/IP irrigation decreased from 1.98 $\mu\text{g/ml}$ at 30 min to 0.58 $\mu\text{g/ml}$ at 120 min. The disposition of cefamandole through the two hours is shown on Figure 2. The half-life for both methods of administration was 37 min.

DISCUSSION

The RCM-Z system provided a more rapid analysis of cefamandole than the standard C₁₈ steel column. The C₁₈ was slightly more sensitive than the RCM-Z as depicted in Figure 1. The decreased sensitivity of the RCM-Z is attributed to its shorter length and larger diameter. The C₁₈ steel column has a lower flow rate, thus requiring less mobile phase for completion of the assay. Both HPLC assay methods worked well for the quantitation of cefamandole at the very low serum levels measured in this study.

In this report, IU/IP irrigation with cefamandole resulted in serum levels that were 4.5% of an equivalent IV dose. Although the cefamandole administered by lavage was present in the peritoneal cavity only momentarily, average serum concentrations of 1.98 $\mu\text{g/ml}$ were present 30 minutes following irrigation. The serum concentrations measured in patients receiving cefamandole by IU/IP irrigation were 1.5 to 3 times higher than levels reported in two previous studies (1,8). This likely reflects idiosyncrasies in

the method of irrigation used as well as differences in the duration that the drug was in contact with the peritoneum before it was removed by suction. A second less likely explanation is that the microbiological assays used in the previous reports were less sensitive than the assays used in the present report. The sensitivity and precision of the microbiological assay probably decreases substantially at the low levels of cefamandole in the IU/IP lavage. Since the previous studies reported much lower cefamandole levels, this lack of sensitivity may also explain the discrepancy in results. Following either IU/IP irrigation or IV administration of cefamandole, large variations in serum concentration were measured in these women. We attributed this wide deviation to large differences in volume distribution in these patients who varied greatly in weight but who were all treated with a standard dose of the drug.

The serum half-life for cefamandole in non-pregnant individuals has been shown to be 34 minutes following an intravenous dose and 60 minutes following an intramuscular dose (9). In the present study a serum half-life of cefamandole of 37 minutes was measured in patients following either IU/IP irrigation or IV administration of the drug. It is well established that the glomerular filtration rate (GFR) increases during pregnancy, returning to normal only gradually over several days. It has been suggested that this change in renal function may affect the half-life of several drugs (4,5). From the data presented in this report, it would appear that pregnancy associated changes in GFR do not affect the renal excretion of cefamandole in patients treated with the drug in the peripartum period. The low but significant levels of cefamandole measured in the serum following rapid IU/IP irrigation likely reflects the rapid uptake of the drug across a large, well-vascularized space. Several reports have documented the efficacy of peritoneal lavage with antimicrobials at the time of cesarean section (1,2,8). The decreased incidence of postoperative infection in patients treated with IU/IP irrigation of cefamandole may be the result of either the

local bactericidal action of the drug or the result of a low but significant systemic level of cefamandole obtained in these patients. The results of this investigation justify the evaluation of the mean inhibitory concentrations of cefamandole for the bacteria recovered in these high-risk patients undergoing cesarean section.

ACKNOWLEDGEMENTS

We thank Dr. Joan Reisch for the statistical analysis on these data.

This work was supported in part by a grant from Eli Lilly and Co., Indianapolis, Indiana.

REFERENCES

1. Matthews, M.D., Prevention of Endomyometritis Using Antibiotic Irrigation during Cesarean Section., *Obstet. Gynecol.*, 60:413, 1982.
2. Rudd, E.G., Long, W.H., and Dillon, M.B., Febrile Morbidity Following Cefamandole Nafate Intrauterine Irrigation During Cesarean Section., *Am. J. Obstet. Gynecol.*, 141:12, 1981.
3. Rudrik, J.T., Bawdon, R.E., Determination of Penicillinase-Resistant Penicillins in Serum Using High-Pressure Liquid Chromatography, *J. Liquid Chromatogr.*, 4:1525, 1981.
4. Davidson, J.M., and Hytten, F.E., Glomerular Filtration During and After Pregnancy, *J. Obstet. Gynecol. Brit. Commonw.*, 81:588, 1974.
5. Griffith, R.S., Black, H.R., Brier, G.L., and Wolny, J.D., Cefamandole: In Vitro and Clinical Pharmacokinetics., *Antimicrob. Agents.*, 10:814, 1976.
6. Bawdon, R.E., Cunningham, F.G., Quirk, J.G., and Roark, M.L., Maternal and Fetal Pharmacokinetics of Moxalactam Given Intrapartum, *Am. J. Obstet. Gynecol.*, 144:546, 1982.
7. Dajani, A.S., Thirumoothi, M.C., Bawdon, R.E., Buckley, J.A., Pfeiffer, M., VanHarken, D.R., and Smyth, R.D., Pharmacokinetics of Intramuscular Ceforanide in Infants, Children, and Adolescents, *Antimicrob. Agents Chemother.*, 21:282, 1982.
8. Duff, P., Gibbs, R.S., Jorgensen, J.H., and Alexander, G., The Pharmacokinetics of Prophylactic Antibiotics Administered

by Intraoperative Irrigation at the Time of Cesarean Section,
Obstet. Gynecol., 60:409, 1982.

9. Fong, I.W., Ralph, E.D., Engelking, E.R., and Kirby, W.M.M.,
Clinical Pharmacology of Cefamandole as Compared With
Cephalothin., Antimicrob. Agents Chemother., 9:65, 1976.

A RAPID HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE
SIMULTANEOUS MEASUREMENT OF SIX TRICYCLIC ANTIDEPRESSANTS

Jeffrey S. Kiel^{1,2}, Ruth K. Abramson¹, Stephen L. Morgan^{2*} and
John C. Voris³

¹William S. Hall Psychiatric Institute, P. O. Box 202, Columbia, SC 29201

²Department of Chemistry, University of South Carolina, Columbia, SC 29208

³Dept. of Pharmacy Practice, University of South Carolina, Columbia, SC 29208

ABSTRACT

A reversed-phase high pressure liquid chromatographic procedure has been developed for the quantitation of the concentration of six different tricyclic antidepressants in the plasma of patients undergoing routine drug therapy. Plasma samples were extracted into a 97:3 hexane:isoamyl alcohol solution and then extracted back into dilute acid. A Supelcosil C-8 column with 5 micron packing was employed in combination with an acetonitrile/phosphate buffer/diethylamine mobile phase. At an optimized mobile phase pH of 7.22, baseline separation of all six tricyclic antidepressants plus the internal standard was achieved within 8 minutes. UV detection at 254 nm resulted in limits of detection of 2.5 µg/L for each drug. The potential interferences from 13 different benzodiazepines and neuroleptics was investigated. Five of the 13 parent drugs and three metabolites were found to interfere with this tricyclic antidepressant assay.

INTRODUCTION

The growing practice of monitoring the concentration of tricyclic antidepressants (TCAs) in plasma from patients undergoing antidepressant therapy has required many clinical laboratories to introduce procedures for quantitation of the common antidepressant drugs (1,2). Therapeutic plasma levels of antidepressants are usually in the µg/L range and a number of concurrently administered medications may possibly interfere with the analysis (3,4). These considerations have resulted in many laboratories using high-pressure liquid chromatography (HPLC) for the plasma monitoring of the six common drugs: doxepin, desmethyldoxepin, imipramine, desipramine, amitriptyline and nortriptyline.

Several authors have published normal-phase chromatographic methods for the separation of one or more TCAs (5-14); however, only Sutheimer has accomplished baseline separation of all six drugs listed above (15). Sutheimer's method is based on the earlier work of Vandemark et al. (6) in which hydrophilic endogenous components were found to cause background interference. Sutheimer did not apply the method to patient samples and, further, presented no information on interferences, precision, or the extraction procedure.

Reversed-phase HPLC techniques have less interference from plasma extracts and have achieved more rapid chromatography of a few TCAs (16-29). Kabra et al. (19) described a procedure providing baseline resolution of all six drugs listed above; however, the retention time for the last eluting component was about 13 min. Bannister et al (21) described a rapid system for the automatic extraction and quantitation of TCAs in plasma samples but routine use required two chromatographic runs with two different standards because all TCAs could not be simultaneously separated in a single run.

Before chromatographic analysis, TCAs must be extracted from the plasma samples. Several liquid-solid and liquid-liquid extraction techniques have been published (3). The liquid-solid techniques have required a second liquid-liquid extraction (28) or evaporation step to concentrate the samples (13). The liquid-liquid extraction methods have required long mechanical shaking times (19), freezing steps for the isolation of the organic phase (11), evaporation of solvent to concentrate the samples (7), or an elaborate autoextraction system not available to many clinical laboratories (21). All of these techniques are time consuming. Additionally, the use of an evaporation step results in increased variability and requires two internal standards (10).

In this paper we report the development of a reversed-phase HPLC method for the simultaneous quantitation of six TCAs in the plasma from patients undergoing routine antidepressant therapy.

EXPERIMENTAL

Apparatus

A Waters HPLC system (Waters Associates, Milford, Ma.) equipped with a Model 441 UV absorbance detector (operated at 254 nm), a Model 6000A solvent-delivery system, a Model 710 autoinjector, a Model 720 integrator and a Model 730 System Controller was employed. A Supelcosil C-8 reversed-phase column (4.6 x 250 mm with 5 μ m packing), fitted with a C-8 guard column (4.6 x 50 mm with 40 μ m packing) was used (Supelco, Inc., Bellefonte, Pa.).

The mobile phase used for routine analysis was prepared by mixing 53.3 volumes of acetonitrile, 45.1 volumes deionized water, 1 volume diethylamine, and 0.4 volumes 85% phosphoric acid. The pH was adjusted to 7.2 by dropwise addition of phosphoric acid or sodium hydroxide. Other mobile phase combinations were prepared as needed. All mobile phases were degassed by ultrasonic vibration prior to use. Column mobile phase flow rate was 2.0 mL/min.

Reagents

HPLC grade hexane, acetonitrile, and isopropanol, and reagent grade potassium carbonate, diethylamine, and isoamyl alcohol were obtained from Fisher Scientific Co. (Atlanta, Georgia). A 25% solution of potassium carbonate containing 0.1% diethylamine was prepared and stored at room temperature. A 97:3 mixture of hexane:isoamyl alcohol was stored in a glass container at room temperature.

Standards

A standard stock solution of tricyclic antidepressant drugs contained 1 g/L of each of the following pure drugs was prepared in isopropanol: doxepin (Pfizer Laboratories), desmethyldoxepin (Pfizer Laboratories), imipramine (USV Laboratories), desipramine (USV Laboratories), amitriptyline (Merck, Sharp & Dohme Laboratories), and nortriptyline (Merck, Sharp & Dohme Laboratories). This stock standard was stored at -15°C.

A stock internal standard containing 1 g/L of Loxapine (American Cyanamid Co.) was prepared in isopropanol. This solution was stored at -15°C.

A tricyclic working standard (100 µg/L) was made by a 10⁴-fold dilution of the TCA stock standard. To prevent adsorption of the TCAs onto the glass container, diethylamine was added to a final concentration of 0.1%. The tricyclic working solution was stored at 4°C.

The working internal standard was prepared by a 10³-fold dilution of the stock internal standard to a final concentration of 1 mg/L with diethylamine added (0.1%); this solution was stored at 4°C.

Samples

Patient blood samples were collected in Dark Blue stoppered Vacutainers (Bectin-Dickinson, Rutherford, N.J.) and centrifuged within 2 hours of sampling to obtain the plasma. The plasma was then stored at -15°C in a clean Dark Blue Vacutainer tube until analyzed.

Sample Preparation

Two mL of plasma was transferred to a 15 x 150 mm borosilicate glass disposable test tube. Into this tube was added 100 μ L of working internal standard, 250 μ L of the 25% potassium carbonate solution, and 5 mL of the 97:3 hexane:isoamyl alcohol solution. Each tube was vortexed rapidly for 30 s and then centrifuged for 3 min at 500 x g to break the emulsion. The aqueous layer was removed by aspiration and the organic layer was transferred to a 15 mL glass conical centrifuge tube. A 100 μ L aliquot of 0.25 mol/L HCl was added and the tube vortexed rapidly for 30 s. The organic layer was discarded and 50 μ L of the aqueous layer was injected onto the column.

Quantitation

The quantity of each drug in a sample was calculated by determining the ratio of the peak absorbance of that tricyclic drug to that of the working internal standard. The concentration was then calculated by comparison with a TCA standard curve generated with each run.

RESULTS

Chromatography

The pH of the mobile phase was found to be a crucial factor in obtaining baseline separation of the six TCAs plus the internal standard. Over the pH range of 3.9 to 7.8 the retention times of the solutes showed considerable variation (Figure 1). At the low pH extreme several of the drugs coelute. Doxepin and nortriptyline reversed in elution order at pH 7. Amitriptyline and loxapine (the internal standard) also reversed in elution order at high mobile phase pH (pH 7.8). The optimal regions of chromatographic separation were determined from the retention data of Figure 1 using the "window diagram" technique popularized by Laub and Purnell (30) and applied to reverse-phase HPLC by Deming and Turoff (31). Third-order models were fitted to the retention data for each of the seven individual peaks. These fitted equations were then employed to calculate the relative retention ratios for all pairs of peaks (21 different pairs in this case) over the pH range investigated. These results are plotted in Figure 2. Regions of best separation for the worst separated pair of peaks are darkened in as "windows" in Figure 2. The highest window occurs at pH 7.22 where the worst separated pairs show a relative retention of about 1.10. At this pH all seven components are completely resolved as shown in the chromatogram of Figure 3.

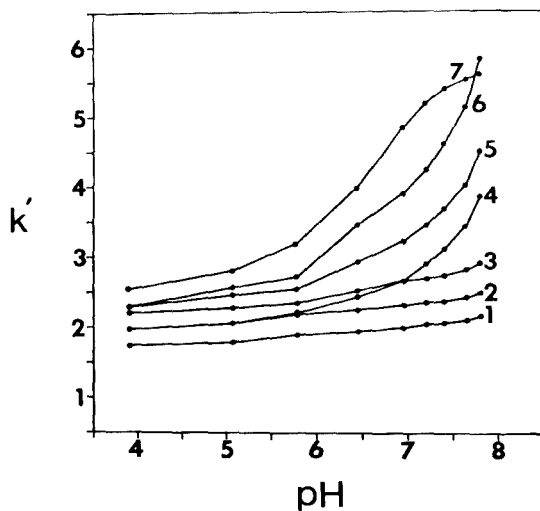


FIGURE 1. The relationship between k' and pH for the seven solutes (1 = desmethyldoxepin, 2 = desipramine, 3 = nortriptyline, 4 = doxepin, 5 = imipramine, 6 = amitriptyline, 7 = loxapine (internal standard)).

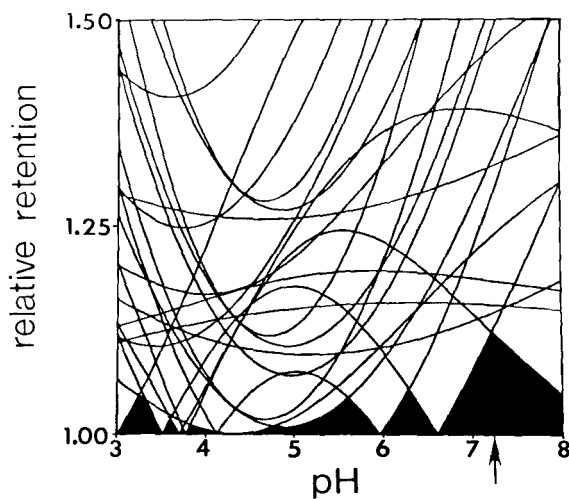


FIGURE 2. Window diagram of relative retention as a function of pH for all 21 pairs of tricyclic antidepressants. The location of the optimum pH is marked by an arrow.

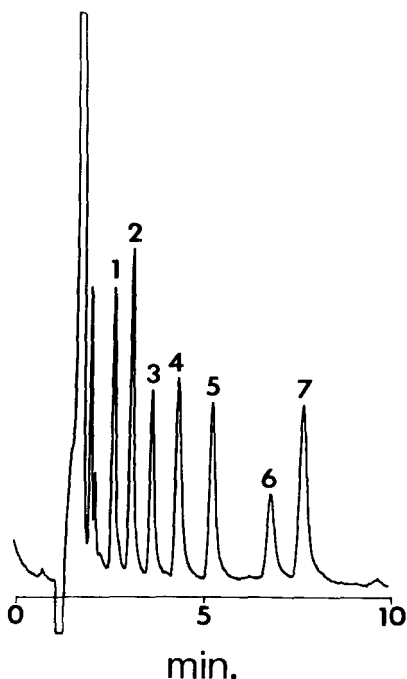


FIGURE 3. Chromatogram of a spiked plasma standard extract containing 100 $\mu\text{g/L}$ of each of the tricyclic antidepressants. Peak identities: 1 = desmethyldoxepin, 2 = desipramine, 3 = nortriptyline, 4 = doxepin, 5 = imipramine, 6 = amitriptyline, 7 = loxapine (internal standard).

An amine modifier was employed to reduce peak tailing of the solutes. Diethylamine was chosen since it proved effective in improving peak symmetry at pH 7.2. The relatively high concentration of diethylamine (1%) not only improved peak shape, but also improved selectivity among solutes when compared with dibutylamine (Figure 4).

Precision, Accuracy and Recovery

The precision of analysis for a pooled serum sample spiked with 100 $\mu\text{g/L}$ of each of the TCAs is shown in Table 1. The percent relative standard deviation ranged from 3.2 to 4.0%.

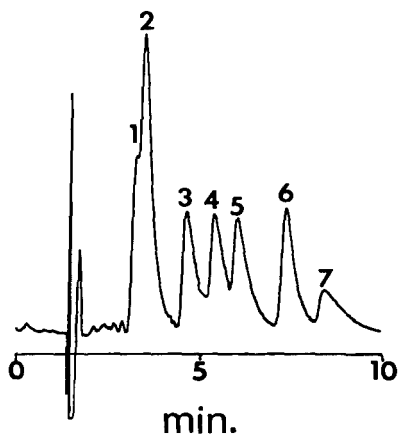


FIGURE 4. Chromatogram of tricyclic antidepressants with 1% dibutylamine as the amine modifier in the mobile phase. Peak identities as in Figure 3.

TABLE 1. Precision of the Method

	<u>%RSD</u> ^a	<u>Mean</u> ^b
Desmethyldoxepin	3.2	101.8
Desipramine	3.6	100.6
Nortriptyline	3.4	102.3
Doxepin	3.8	102.5
Imipramine	3.8	103.8
Amitriptyline	4.0	104.3

^a n = 25 measurements; ^b µg/L

TABLE 2. Analytical Recovery of Tricyclic Drugs from Pooled Serum

<u>Drug</u>	<u>% Recovery</u> ^a
Desmethyldoxepin	53.8
Desipramine	48.8
Nortriptyline	50.9
Doxepin	54.6
Imipramine	54.3
Amitriptyline	50.0

^a concentration, 100µg/L

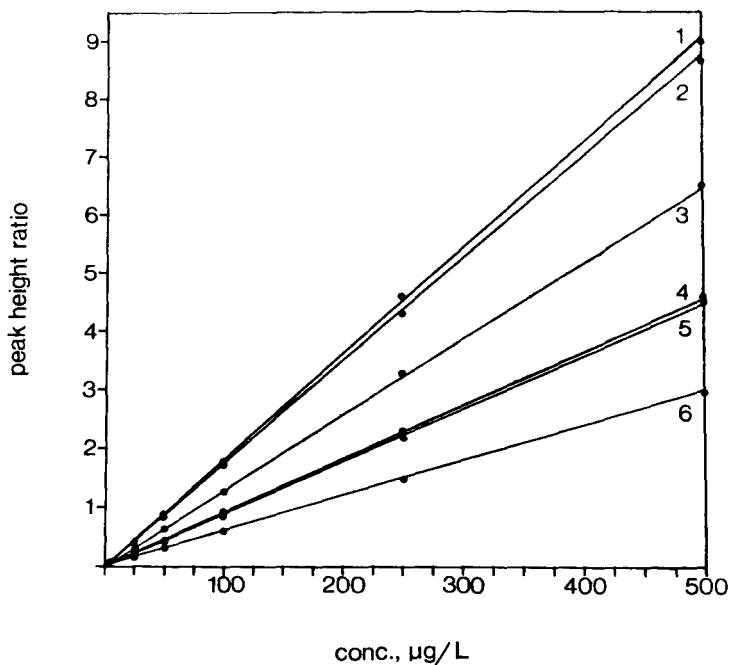


FIGURE 5. Calibration curves (1 = desmethyldoxepin, 2 = desipramine, 3 = nortriptyline, 4 = doxepin, 5 = imipramine, 6 = amitriptyline).

In Figure 5, a calibration curve is presented for each of the drugs over a concentration range of 2.5 to 500 $\mu\text{g/L}$. Using this technique, however, TCA concentrations up to 1000 $\mu\text{g/L}$ can be measured without dilution.

The two stage liquid-liquid extraction can be completed within eight minutes. The percent recovery (Table 2) for these TCAs ranged from 48.8 to 54.6%.

Interference

Table 3 lists the k' of the TCAs and some additional psychotropic drugs which are often concurrently administered to patients receiving TCA therapy. To identify whether or not the antipsychotic drugs or metabolites interfered with the measurement of TCAs, plasma samples from patients receiving standard neuroleptic drug therapy were assayed. Each of the benzodiazepines was tested for interference using pooled human plasma

TABLE 3. k' for Tricyclic Drugs and Other Common Psychiatric Drugs and Metabolites

<u>Tricyclic Antidepressants</u>	<u>k'</u>
Desmethyldoxepin	2.50
Desipramine	2.95
Nortriptyline	3.45
Doxepin	4.18
Imipramine	5.00
Amitriptyline	6.41
Loxapine ^a	7.18
<u>Benzoiazepines</u>	<u>k'</u>
Desmethylchloridiazepoxide	2.06
Oxazepam	2.44
Chlordiazepoxide	2.53
Desmethyldiazepam	3.93
Diazepam	5.20
<u>Neuroleptics</u>	<u>k'</u>
Molindone	<2.00
Trifluoperazine	<2.00
Perphenazine	<2.00
Fluphenazine	2.67
Haloperidol	3.34
Thiothixene	3.39
Thioridazine metabolites	<2.00-4.14 ^b
Chlorpromazine	8.23
Chlorpromazine metabolite (1)	3.02
Chlorpromazine metabolite (2)	3.65

^a internal standard; ^b several unresolved peaks

spiked with drug standards since the metabolites of these compounds were available.

Among the benzodiazepines, three drugs were found to cause interference with this assay. Diazepam interferes with the measurement of imipramine, and the desmethyl metabolite of diazepam interferes with the measurement of doxepin. The third benzodiazepine, chlordiazepoxide, interferes with the analysis of desmethyldoxepin.

Of the group of neuroleptic drugs tested, five were found to cause interference: Haloperidol, thiothixene, fluphenazine, chlorpromazine and thioridazine. Haloperidol and thiothixene elute with retention times which conflict with nortriptyline. Fluphenazine interferes with the measurement of desmethyldoxepin.

Although the parent compounds of chlorpromazine and thioridazine do not interfere with the measurement of TCAs, the metabolites do. Chlorpromazine metabolites interfere with the measurement of both desipramine and nortriptyline. The metabolites of thioridazine provide the greatest problems in this assay. In the plasma from a patient whose thioridazine treatment had been discontinued for seven days prior to the time the plasma sample was obtained, sufficient levels of thioridazine metabolites were still present to cause interference in the measurement of all six TCAs.

DISCUSSION

In this report, a rapid and sensitive HPLC technique is described for the simultaneous measurement of six tricyclic antidepressants (doxepin, desmethyldoxepin, imipramine, desipramine, amitriptyline, and nortriptyline) in human plasma. A simple extraction technique is used to prepare the samples for analysis by reversed-phase chromatography. The liquid-liquid extraction requires no evaporation step, small extraction volumes, and vortexing instead of mechanical shaking. The addition of the diethylamine to the potassium carbonate buffer prevents adsorption of the TCAs to the extraction glassware. These steps improve the reproducibility of the extraction procedure, with typical % RSDs for the complete assay ranging from 3.2 to 4.0% at a plasma concentration of 100 µg/L. Plasma samples can be prepared for chromatography within eight minutes.

Using this reversed-phase HPLC technique, the six tricyclic drugs and internal standard all elute within eight min. Thus, a total of only 16 minutes are required for both extraction and chromatography. Improvements in peak symmetry and selectivity were obtained by using the amine modifier diethylamine. The optimum mobile phase pH (7.22) was determined by conducting experiments over the pH range 3.9 to 7.8 and by plotting a window diagram of relative retention for each pair of peaks versus pH. Loxapine,

which provides acceptable extraction and chromatographic characteristics based on the structural similarities to the TCAs, was chosen as the internal standard.

It has been suggested that the use of a mobile phase pH above 7.0 in combination with an amine modifier may reduce the life of a silica based column by dissolving the silica (16). The method described in this report has been used for several years in both a routine clinical laboratory and in a research setting. Several thousand injections can be made on a single column before performance deteriorates. Degradation is primarily due to a buildup of lypophilic compounds on the column surface and is not due to the silica base of the column dissolving. The use of a guard column greatly extends the analytical column life by slowing lypophilic contamination.

Psychiatrists use TCAs as the drugs of choice for the treatment of affective disorders. TCA therapy is also sometimes combined with concurrent administration of other psychotropic agents. An evaluation, using the plasma from patients receiving some of these psychotropic drugs, showed limited interference from the parent compounds of three neuroleptics (thiothixene, haloperidol, and fluphenazine) and two benzodiazepines (diazepam and chlordiazepoxide). In addition, the metabolites of one of the benzodiazepines (diazepam) and two neuroleptics (chlorpromazine and thioridazine) caused interference in the assay.

This sensitive and rapid HPLC technique has been effectively applied for two years in a tricyclic drug monitoring program for both inpatients and outpatients at a major medical facility. Studies involving the pharmacokinetics and drug metabolism of TCAs are in progress.

ACKNOWLEDGMENT

This work was supported in part by NIH Biomedical Research Support Grant No. S07-RR-05815, University of South Carolina. Assistance from Dr. Sharon R. Batey, College of Pharmacy, University of South Carolina, and William S. Hall Psychiatric Institute, is gratefully acknowledged.

REFERENCES

- (1) Risch, S. C., Huey, L. Y., and Janowsky, D. S., *J. Clin. Psych.*, 40, 4-16 (1979).
- (2) Risch, S. C., Huey, L. Y., and Janowsky, D. S., *J. Clin. Psych.*, 40, 58-59 (1979).
- (3) Scoggins, B. A., Maguire, K. P., Norman, T. R., and Burrow, G. D., *Clin. Chem.*, 26, 5-17 (1980).

- (4) Scoggins, B. A., and Maguire, K. P., *Clin. Chem.*, 26, 805-815 (1980).
- (5) Watson, I. D., and Steward, M. J., *J. Chromatogr.*, 132, 155-159 (1977).
- (6) Vandemark, F. L., Adams, R. F., and Schmidt, G. J., *Clin. Chem.*, 24, 87-91 (1978).
- (7) Sonsalla, P. K., Jennison, T. A., and Finkle, B. S., *Clin. Chem.*, 28, 457-461 (1982).
- (8) van den Berg, J. H. M., De Ruwe, H. J. J. M., and Deelder, R. S., *J. Chromatogr.*, 138, 431-436 (1977).
- (9) Dixon, R., and Martin, D., *Res. Commun. Chem. Pathol. Pharmacol.*, 33, 537-545 (1981).
- (10) Sonsalla, P. K., Jennison, T. A., and Findle, B. S., *Clin. Chem.*, 28, 1401-1402 (1982).
- (11) Godbillon, J., and Gauton, S., *J. Chromatogr.*, 204, 303-311 (1981).
- (12) Watson, I. D., and Steward, M. J., *J. Chromatogr.*, 134, 182-186 (1977).
- (13) Bidlingmeyer, B. A., Korpi, J., and Little, J. N., *Chromatographia*, 15, 83-85 (1982).
- (14) De Ridder, J. J., Koppens, P. C. J. M., and Van Hal, H. J. M., *J. Chromatogr.*, 143, 281-287 (1977).
- (15) Sutheimer, C., *Chromatogr. Newslett. (Perkin-Elmer)*, 7, 38-39 (1979).
- (16) Proelss, H. F., Lohmann, H. J., and Miles, D. G., *Clin. Chem.*, 24, 1948-1953 (1978).
- (17) Kraak, J. C., and Bijster, P., *J. Chromatogr.*, 143, 499-512 (1977).
- (18) Jensen, K. M., *J. Chromatography*, 183, 321-329 (1980).
- (19) Kabra, P. M., Mar, N. A., and Marton, L. J., *Clin. Chem. Acta*, 111, 123-132 (1981).
- (20) Burke, D., and Sokoloff, H., *J. Pharm. Sci.*, 69 (2), 138-140 (1980).
- (21) Bannister, S. J., Van de Wal, S., Dolan, J. W., and Snyder, L. R., *Clin. Chem.*, 27, 849-855 (1981).
- (22) Bock, J. L., Giller, E., Gray, S., and Jatlow, P., *Clin. Pharmacol. Ther.*, 609-616 (1982).
- (23) Breutzmann, D. A., and Bowers, L. D., *Clin. Chem.*, 27, 1907-1911 (1981).
- (24) Wong, S. H. Y., McCauley, T., and Kramer, P. A., *J. Chromatogr.*, 226, 147-154 (1981).
- (25) Fekete, J., del Castilho, P., and Kraak, J. C., *J. Chromatogr.*, 204, 319-327 (1981).
- (26) Feece, P. A., and Zacest, R., *J. Chromatogr.*, 163, 310-314 (1979).

- (27) Suckow, R. F., and Cooper, T. B., *J. Pharm. Sci.*, 70, 257-261 (1981).
- (28) Thoma, J., and Bondo, P., *Analytichem International*, brochure no. 1 TCA 1001 (1979).
- (29) Koteel, P., Mullins, R. E., and Gadsden, R. H., *Clin. Chem.*, 28, 462-466 (1982).
- (30) Laub, R. J., and Purnell, J. H., *J. Chromatogr.*, 112, 71-79 (1975).
- (31) Deming, S. N., and Turoff, M. L. H., *Anal. Chem.*, 50, 546-548 (1978).

DETERMINATION OF RITODRINE IN PLASMA
USING HPLC

Paul Kuhnert, Penny Erhard, Andrew Dixon,
Betty Kuhnert and Thomas Gross

Perinatal Clinical Research Center
Case Western Reserve University
Cleveland Metropolitan General Hospital
3395 Scranton Rd., Cleveland, Ohio 44109

ABSTRACT

An HPLC instrument coupled with an electrochemical detector was used to determine ritodrine (erythro-p-hydroxy- α -[1-[(p-hydroxyphenethyl)-amino]ethyl] benzyl alcohol hydrochloride) at nanogram levels in serum. Extraction of ritodrine was accomplished using a multistep ethyl acetate procedure, and the mobile phase consisted of acetonitrile, ammonium acetate, glacial acetic acid, and a counterion. The stationary phase was a Biophase ODS 5 μ m column at ambient temperature. Nalbuphine hydrochloride (Nubain®) was used as an internal standard to quantitate the ritodrine levels of pregnant patients receiving ritodrine. The procedure's linearity for both ritodrine standards and spiked plasma samples was demonstrated. The precision of the assay was found to be 3.4% at 20 ng/ml ritodrine. The minimum detectable concentration, with a signal-to-noise ratio of 6, was determined to be 0.31 ng per 50 μ l injected, corresponding to a concentration of 0.6 ng/ml plasma. The sensitivity, precision, and reproducibility of the assay were all found to be acceptable for determining ritodrine in patient serum.

INTRODUCTION

Premature birth is the leading cause of neonatal mortality and morbidity(1). Ritodrine hydrochloride (Yutopar®) is the first product in the United States approved as tocolytic agent for the management of premature labor. However, little information is available describing its effective serum levels, pharmacokinetics, and side effects in pregnant women and neonates.

The only analytical technique available for determining ritodrine in serum is radioimmunoassay, a technique that requires the development of a specific antiserum(2). Therefore, the purpose of this study was to develop a high performance liquid chromatography (HPLC) procedure that would obviate the need for an antiserum and still provide a high level of sensitivity. Coupling HPLC with electrochemical detection (EC) provided a high level of sensitivity and precision, as well selectivity, for the analysis of ritodrine in serum. HPLC/EC methods similar in nature have been used to analyze tricyclic antidepressants(3).

MATERIALS AND METHODS

Reference Compounds

Reference ritodrine hydrochloride (Yutopar®) was obtained from the Merrell Dow Research Center (Cincinnati, Ohio). The internal standard, nalbuphine hydrochloride (Nubain®), was donated by Endo Laboratories, Inc. (Garden City, New York).

Apparatus

An HPLC instrument (Perkin-Elmer Model 3B) coupled with an electrochemical detector (Bioanalytical Systems Model 4B) and a data terminal (Perkin-Elmer Model Sigma 10B) was used for determining ritodrine. The electrochemical detector was equipped with a glassy-carbon electrode; the chromatography column was a Biophase ODS 5 μm column. A Rheodyne injector (model 7125) was used with a 50 μl loop. The samples were centrifuged with an International centrifuge (model HN) and the samples were mixed in a Vortex shaker (model Vortex-Genie).

Chromatography Conditions

The mobile phase consisted of acetonitrile, ammonium acetate, and water (20/10/70) with 3mM ion-pair reagent (1-heptanesulfonic acid sodium salt) at pH 3.7. The ammonium acetate buffer (10x) contained 400mM ammonium acetate, 2.1M glacial acetic acid and water. Each liter of mobile phase contained 12ml of stock ion-pair solution (5.52gm ion-pair salt, 10ml glacial acetic acid, diluted to 100ml with water). The mobile phase was recirculated at a flow rate of 1ml/min.. The Biophase ODS 5 μm column was used at ambient temperature; the carbon electrode at 0.95 volts (vs. Ag/AgCl).

Treatment of Glassware

All test tubes and centrifuge tubes were surface treated with PROSIL-28 (PCR Research Chemicals, Inc.) an organosilane surface

treating agent. The procedure used was that recommended by the product's manufacturer.

Extraction Procedure

One ml of plasma was added to 1.0 ml of distilled water (Type 1, one megaohm) and 1.0 ml of 0.6 M K_2CO_3 in a 15 ml conical centrifuge tube with screw cap(3); this resulted in a pH of 9.4.. Then 8.0 ml of ethyl acetate was added. The tube was then mechanically shaken for 5 minutes, and then centrifuged at 2000 rpm for 15 minutes. The organic layer was transferred to a 15 ml conical centrifuge tube with a screw cap containing 1.2 ml of 0.1 M HCl. The tube was again shaken for 5 min. and centrifuged at 2000 rpm for 10 minutes. The top layer was aspirated to waste. Five-tenths ml of 0.6 M K_2CO_3 and 1.0 ml ethyl acetate was added (pH 9.4) and the tube was again shaken and centrifuged as mentioned above. The ethyl acetate layer was transferred to a 12 ml conical test tube and evaporated under a stream of purified nitrogen to dryness at room temperature. The residue was reconstituted in 300 μ l mobile phase and 50 μ l of the sample was injected into the liquid chromatograph.

RESULTS

LC Chromatograms

A chromatogram of ritodrine in the mobile phase (10 ng/ml) is shown in Figure 1. The retention time of the ritodrine was

approximately 9.33 min.; the contaminant came from the reagents but it was not further identified since it did not interfere with the procedure. Also shown in Figure 1 is a chromatogram from a plasma extracted sample; the concentration of ritodrine in this sample was also 10 ng/ml. The retention time of the internal standard, nalbuphine, was 13.58 min..

Standard Curves

The linearity for both rithodrine standards and plasma extracted samples over a range of 0-50ng/ml is demonstrated in Figure 2. The minimum detectable concentration was 0.6ng/ml in plasma, which yielded a signal-to-noise ratio of 6; this corresponds to 0.31 ng/ml per 50 μ l injected.

Oxidation Potential

The oxidation potential for ritodrine (+0.95 volts) was optimized by plotting peak height versus applied potential. The appropriate range for the applied potential was first determined by Bioanalytical Systems of West Lafayette, Indiana.

Mobile Phase

Methanol was first used in the mobile phase; however, satisfactory results were not obtained. This led us to experiment with an acetonitrile solvent system. The mobile phase described in the methods section was found to be satisfactory after experimenting with the various solvent variables.

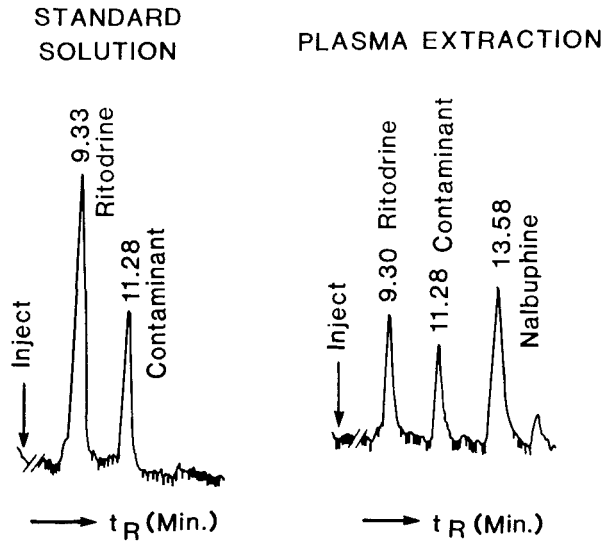


FIGURE 1 LC of Ritodrine Hydrochloride

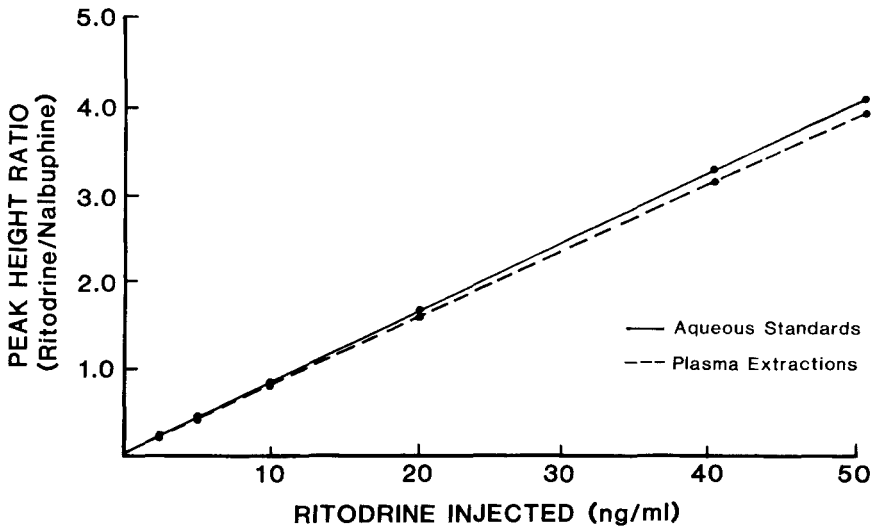


FIGURE 2 Linearity of Ritodrine Standards Using LCEC

TABLE I
Recovery of Ritodrine Added to Plasma

Tissue	Number	Recovery %	SD	CV%
Plasma (1 ml)	10	80.1	1.2	2.5%

TABLE II
Assay Precision for Plasma Ritodrine

Conditions	Concentration (ng/ml)	Mean \pm SD	CV%
Separate Extractions (n=12)	20	19.6 \pm 0.7	3.4

Recovery

The recovery of ritodrine was determined using spiked plasma samples (25ng/ml). The results are shown in Table 1. The mean recovery was 80.1% with a coefficient of variation of 2.5%. The extraction solvent used (ethyl acetate) was arrived at by experimenting with the solvents used in our laboratory for drug extraction (see Discussion).

Precision

The analytical precision at a plasma concentration of 20ng/ml was found to be excellent. The coefficient of variation was 3.4% (Table 2).

DISCUSSION

A radioimmunoassay technique for analyzing ritodrine was not used by us because of the difficulty in producing a suitable antiserum. Instead, an HPLC/EC technique was developed that gave a level of sensitivity (0.6ng/ml plasma) comparable to that reported by Gandar et al. (0.3ng/ml plasma)(2) using radioimmunoassay. We found the HPLC/EC technique to be sufficiently sensitive for analyzing ritodrine in pregnant women receiving the drug to prevent premature labor.

Since our lab does not have cyclic voltammetry, the oxidation potential for ritodrine was determined by Bioanalytical Systems of West Lafayette, Indiana. They found the optimum potential to be approximately +900 mv with a glassy carbon electrode. The potential used in our method was +950 mv; this value was arrived at experimentally and was within the appropriate range suggested by the voltammogram produced by Bioanalytical Systems.

A considerable amount of experimentation was done to determine which solvent system would provide the best results. The first system tried was that used by Merrell Laboratories, the previous manufacturer of ritodrine. Their mobile phase contained methanol, water, sodium-n-heptal sulphinate, and ammonium acetate. The solvent system we report here, using acetonitrile, etc., gave us good sensitivity and precision.

Several organic extraction solvents were tried before one was found that was satisfactory. Among those tried were: acetonitrile, methyl-t-butyl ether, diethyl ether, and ethyl acetate. Ethyl acetate gave an extraction efficiency of 80%; however, adjustment of

the pH as described in the procedure section is vital to obtain this efficiency (personal communication with Dr. Lan K. Wong of the University of Pittsburgh). The extraction procedure followed was that described by Suckow and Cooper with the exception of using ethyl acetate and adjustment of the pH.

Overall, the sensitivity, precision, and reproducibility of the assay were all found to be acceptable for determining ritodrine in the serum of pregnant mothers.

REFERENCES

1. Caritis, S.N., Edelstone, D.I., and Mueller-Heubach, E.: Pharmacologic inhibition of preterm labor. *Am. J. Obstet. Gynecol.* 133 :557-580, 1979.
2. Gandar, R., de Zoeten, L.W., and van der Schoot, J.B.: Serum level of ritodrine in man. *Eur. J. Clin. Pharmacol.* 17 :117-122, 1980.
3. Suckow, R.F., and Cooper, T.B.: Simultaneous determination of imipramine, desipramine, and their 2-hydroxy metabolites in plasma by ion-pair reverse-phase high-performance liquid chromatography with amperometric detection. *J. Pharm. Sci.* 70 :257-261, 1981.

Analysis of Indenolol In Biological Fluids
By High Performance Liquid Chromatography.

S.A. Babhair*, H.Y. Aboul-Enein and Sami El-Houfy,
Departments of Pharmaceutics and Pharmaceutical Chemistry,
College of Pharmacy, King Saud University,
Riyadh, Saudi Arabia.

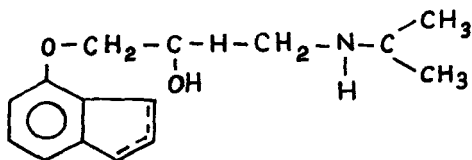
ABSTRACT

The analysis of indenolol in plasma and urine is described. The method involves extraction of the drug from plasma or urine using chloroform at basic pH. The separation was performed on CN column using methanol and 0.01M potassium dihydrogen phosphate solution 50:50. The efficiency of extraction was 97%. Minimum detectable amount by fluorescence was 20 ng/ml.

INTRODUCTION

Indenolol (1) a 1-(7-indenyloxy)-3-isopropylamino-2-propanol a relatively new beta-blocking agent is prescribed for the treatment of angina, cardiac arrhythmias and hypertension. Previous methods of analysis involved gas-liquid-chromatography, non-aqueous potentiometry, U.V. spectrophotometry and HPLC in dosage forms (1,2). The present study reports a simple method for the analysis of the drug in biological fluids using HPLC.

* To whom correspondence should be addressed.

**1**EXPERIMENTALApparatus

The chromatographic equipment consisted of Model 6000A Pump with Fluorescence detector model 420-C and 420-E from Water Associates (Bedford, Massachusetts, USA). The signal output was displayed on a Philipps PM 8251 single-pen recorder.

Chromatographic System

A 3.9ID X 30 cm commercially available stainless steel CN column made by chemically bonding, a cyano group to PORASIL at 9% w/w was used (Waters Associates). Mobile phase consisted of methanol and 0.01M KH_2PO_4 (50:50). The mixture was degassed for 5 minutes by filtration: Flow rate was 1.5 ml/min. and detector gain was 64.

Reagents

Standard solutions were made by dissolving indenolol in the mobile phase. Methanol and chloroform (spectral grade) were obtained from Merck (61 Darmstadt Germany) and KH_2PO_4 (analytical grade) was obtained from BDH (Poole, England). Authentic sample (labelled purity 99.2% w/w) of indenolol hydrochloride was obtained from Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan.

Standard Curve

20 mg of indenolol hydrochloride was dissolved in 200 ml of distilled water. From this stock solution a series of dilutions were

made ranging from 2 $\mu\text{g}/\text{ml}$ to 20 $\mu\text{g}/\text{ml}$. 25 μl of these solutions were injected onto the column in triplicate, the peak height was measured and plotted versus the concentration injected. The results are shown in Figure 1.

Extraction from the urine

Urine samples were collected from an apparently healthy adult male. In each run, various amount of the stock solution was added to 2 ml of urine giving final concentration ranging from 166 to 833 $\mu\text{g}/\text{L}$. Extraction was performed by adding 0.5 ml of 25% ammonia solution and the sample was then extracted with 5 ml of chloroform. The sample was then centrifugated for 10 minutes at 2500 r.p.m. The Chloroform layer was transferred and evaporated at 60° using water bath. The residual was dissolved in 2 ml of the mobile phase and 25 μl of this solution was injected in duplicate.

Extraction from plasma

Plasma was obtained from a whole citrated human blood which was then centrifugated for 10 minutes at 2000 r.p.m. and then pippted to another tube. In each run, various amounts of the stock solution was added to one ml of plasma giving final concentrations ranging also from 166 to 833 $\mu\text{g}/\text{L}$. The same procedure for urine was followed except that 0.1 ml of 25% ammonia solution was added instead of 0.5 ml.

RESULTS AND DISCUSSION

A simple method for the analysis of indenolol in biological fluids was developed using HPLC and fluorescence detector. A typical graph of the results when peak height was plotted versus concentration injected from both urine and plasma are shown in Figures 2 and 3 respectively. Typical chromatograms using this method are shown in Figures 4 and 5. The retention time for the drug was 4.2 min. The method is

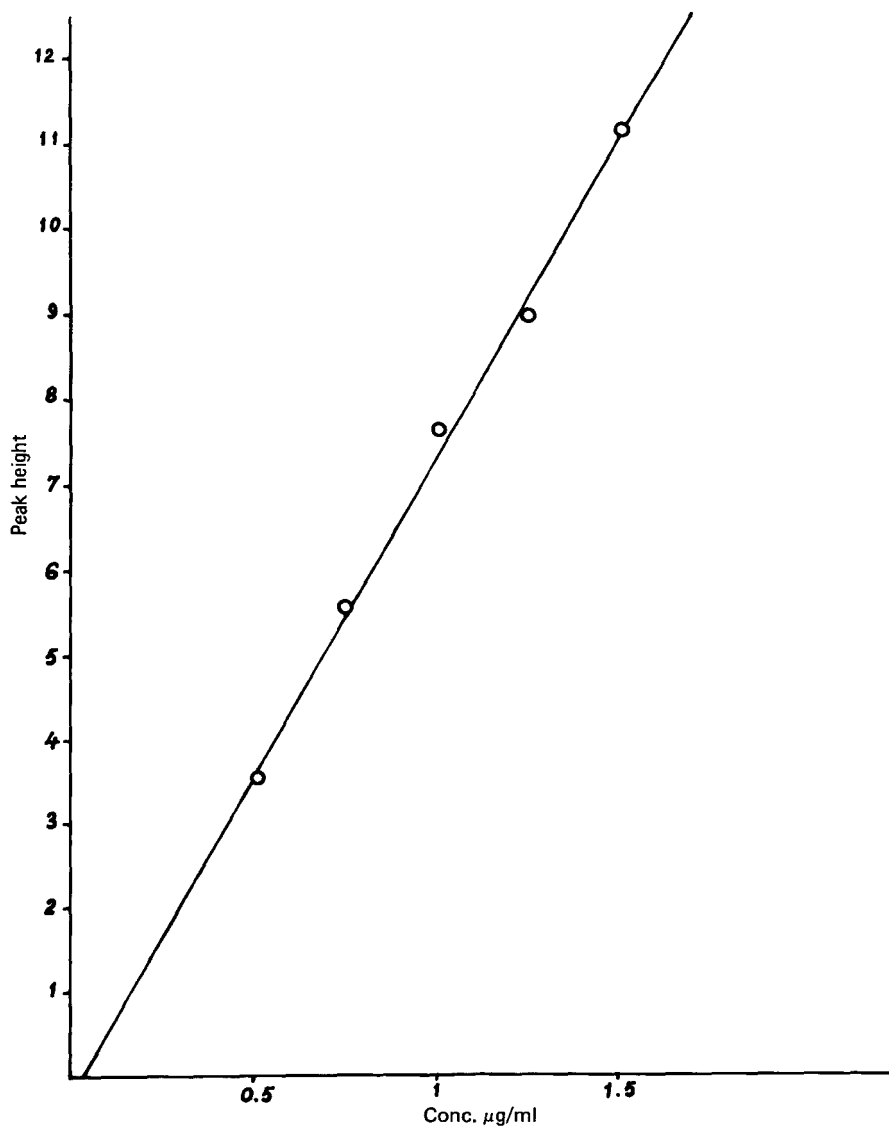


Fig. 1: Standard curve of indenolol when the peak height was plotted versus the Conc. injected.

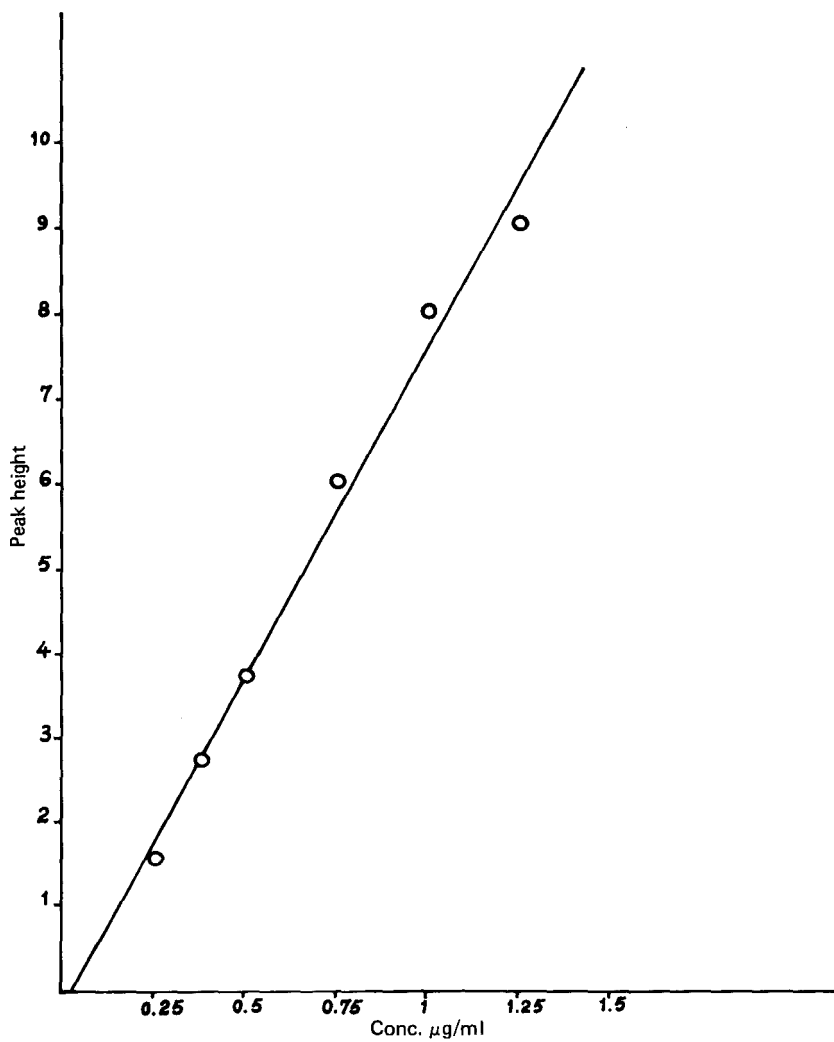


Fig. 2: Standard curve of indenolol extracted from plasma.

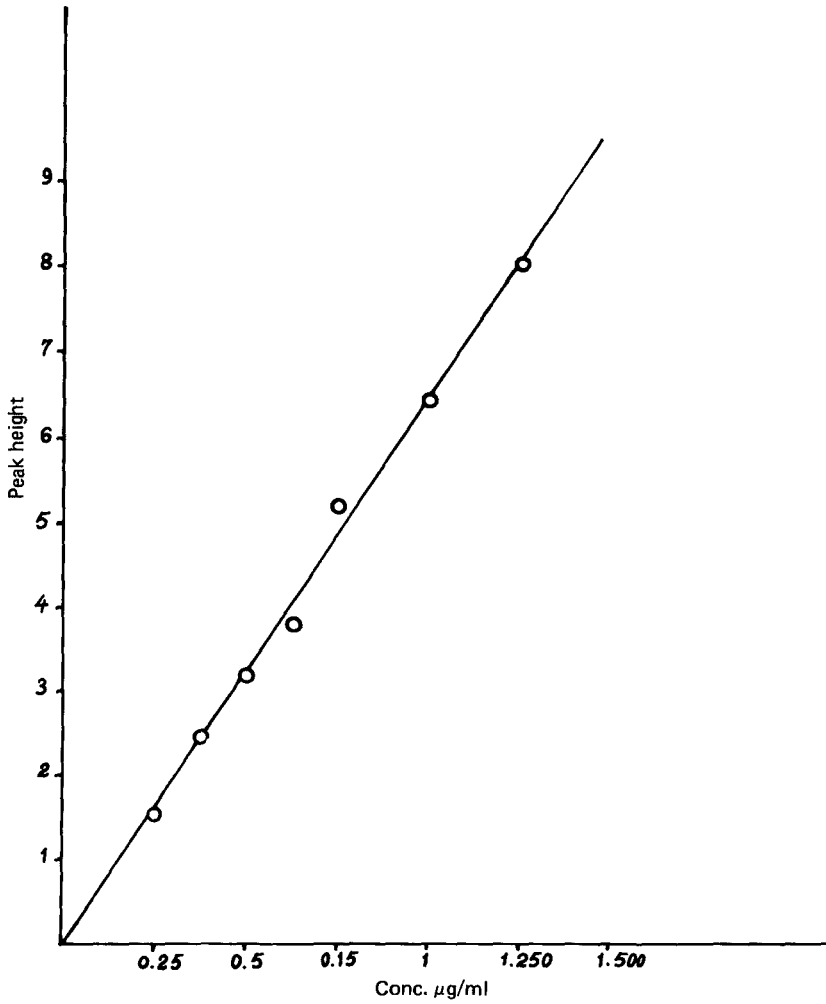


Fig. 3: Standard curve of indenolol extracted from urine.

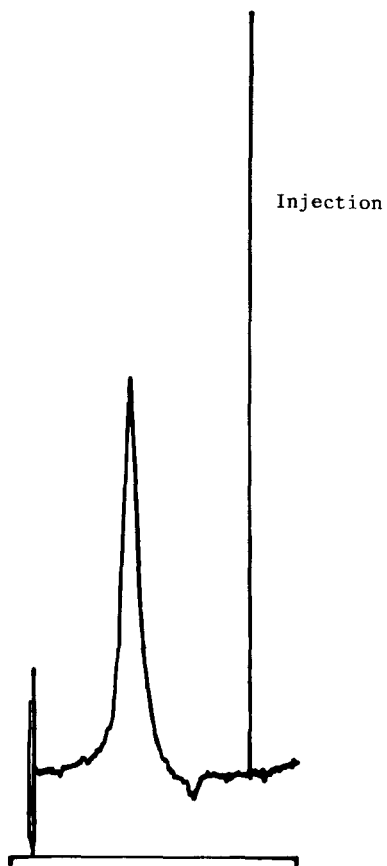


Fig. 4: A typical chromatogram of indenolol when 25 μ l of the drug was injected.

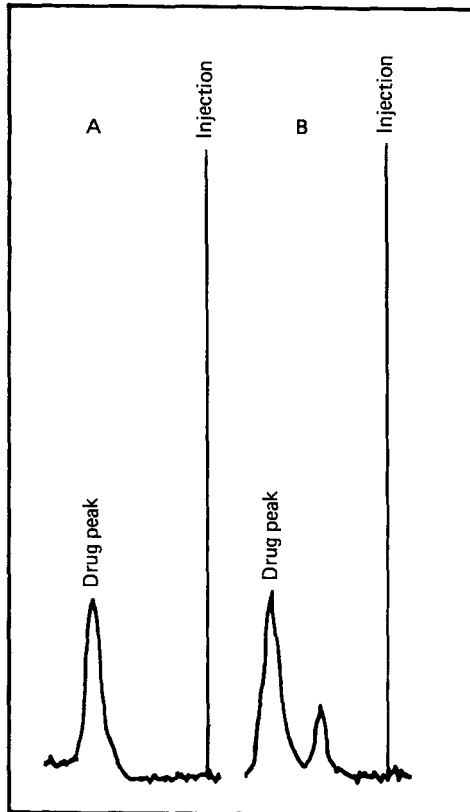


Fig. 5: Typical chromatograms of Indenolol extracted from Plasma (A) and Urine (B)

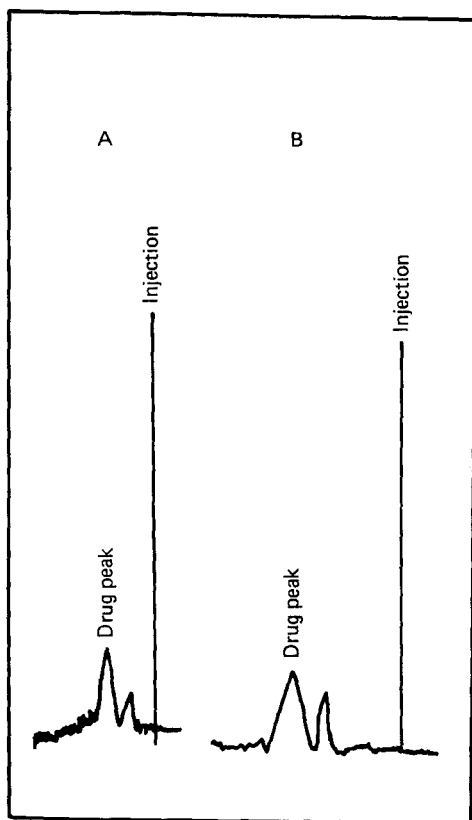


Fig.6: Typical chromatograms when 20 $\mu\text{g/L}$ of Indenolol extracted from Plasma (A) and Urine (B)

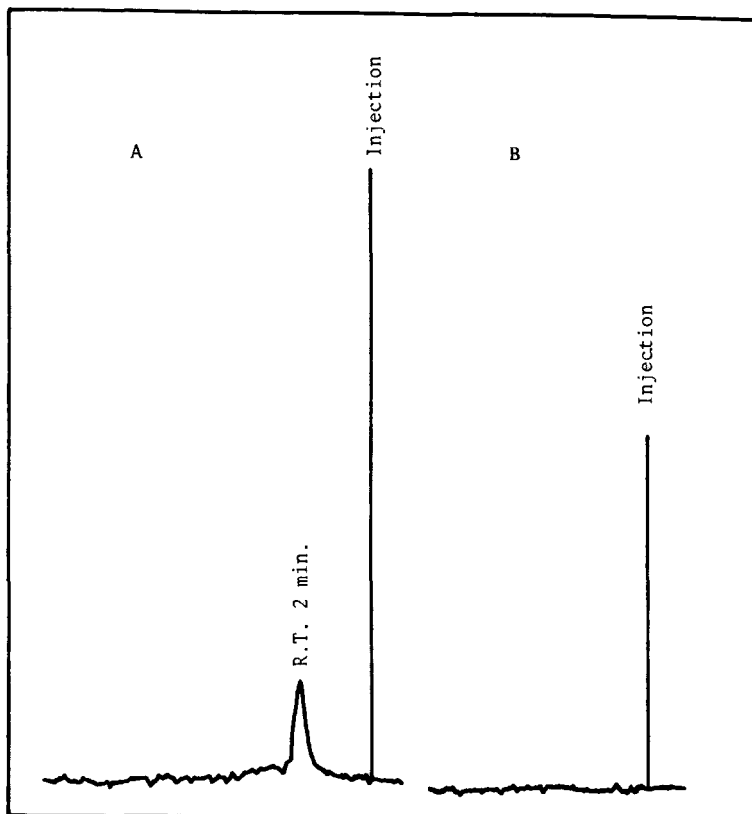


Fig. 7: Typical chromatograms when blank Urine (A) and blank Plasma (B) were extracted, and then injected onto the column.

very sensitive and concentrations as low as 20 $\mu\text{g/L}$ ($S/N > 2$) can be detected (Figure 6). Peak height was linearly correlated to the drug concentration for the standard curves, urine and plasma with correlation coefficients of 0.995, 0.994 and 0.994. Intercepts of -0.35, -0.4, -0.1 and slopes of 0.007, 0.0066 and 0.0076 respectively. The interference from other plasma constituents was minimal and the recovery of the drug from the plasma or urine using this method of extraction was 97%. The sensitivity of the method can be considerably improved by dissolving the residual after evaporating the chloroform in 500 μl or even 100 μl of the mobile phase and injecting 50 or 75 μl of the reconstituted solution onto the column (Figure 7).

REFERENCES

1. The Central Research Laboratory, Yamanuchi Pharmaceutical Co., Ltd., 1-k-8m Azauswa, Atabashi-ku, Tokyo, Japan.
2. Mohamed E. Mohamed, H.Y. Aboul-Enein, Salim A. Babhair and Sami El-Hofi. J. of Liquid Chromatography, 6(4), 715-723 (1983).

HPLC ANALYSIS OF OLIGOMERIC ETHYLENE GLYCOL MIXTURES
VIA BIS(2,4-DINITROPHENYLATION).

Abraham Warshawsky*, Nurit Shoef and Arieh Tishbee
Department of Organic Chemistry,
Weizmann Institute of Science, Rehovot, Israel.

ABSTRACT

Bis(2,4-dinitrophenylation) of oligomeric ethylene glycols of the formula $\text{HO}-(\text{CH}_2\text{CH}_2\text{O})_n\text{-H}$ ($n=4-16$) to the corresponding $\text{DNP-O}(\text{CH}_2\text{CH}_2\text{-O})_n\text{-DNP}$ (where DNP stands for 2,4-dinitrophenyl) provides chromophoric derivatives, which are separated chromatographically on HPLC column.

The bis(2,4-dinitrophenyl) glycols are stable in presence of triethylamine, but undergo ethanolysis in presence of hydroxide ions. The quantitative removal of the DNP groups allows an integrated scheme to pure glycols from commercially available polyethylene glycol mixtures, by bis(2,4-dinitrophenylation), chromatographic separation, end-group removal, using HPLC of the bis-(2,4-dinitrophenyl) glycols for purity monitoring.

INTRODUCTION

Ethylene glycol oligomers are important starting materials in the synthesis of macrocyclic ethers (1-5). Their special solubility and chain folding properties are extensively studied (6-8). Considerable attention is also given to the use of oligoglycols as liquid polymeric supports in automatic liquid phase peptide synthesis (9).

Our interest in facile synthetic methods to pure glycols has risen in the course of studies on the synthesis of polymeric crown (10) and pseudocrown ethers (11). This has also led us to develop a highly sensitive and accurate analytical method based on conversion of the glycols to bis(2,4-dinitrophenyl derivatives) (12), coupled with High Performance Liquid Chromatography Analysis (HPLC), which is reported in this paper.

Synthesis of Bis(2,4-Dinitrophenyl) Glycols: Chromophoric Ligands for HPLC Analysis.

The bis(2,4-dinitrophenylation) of glycols converts the water-soluble glycols, to hydrophobic derivatives incorporating high-extinction coefficient chromophores, which can be readily resolved by chromatographic methods using spectroscopic monitoring. The synthesis of bis(2,4-dinitrophenyl) glycols (abbreviated bis-DNP-glycols), the product composition are to be described elsewhere (13). The lower glycols (tri-to-penta glycol) yield exclusively the bis-DNP derivatives after 24 hours reaction. The reactivity of the glycol decreases as chain length increases, and longer reaction times are needed to convert the higher glycols to the bis-DNP derivatives.

High Performance Liquid Chromatography Analysis of Polyethylene Glycol Oligomer Mixtures.

The resolution of a prepared mixture of tetra, penta, hexa, hepta and octa glycols (as the bis-2,4-dinitrophenyl derivatives)

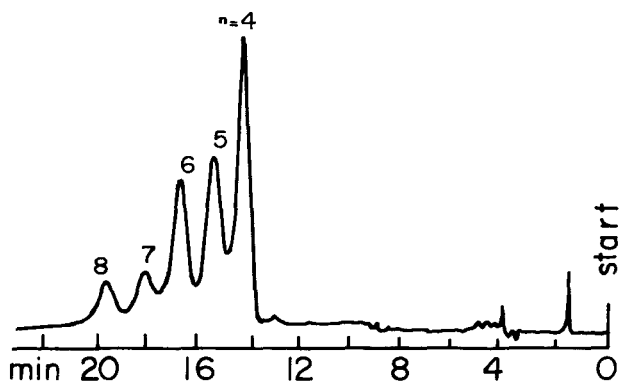


FIGURE 1
HPLC analysis of a prepared mixture of pure bis(2,4-dinitrophenyl) derivatives of tetra to octa glycol.

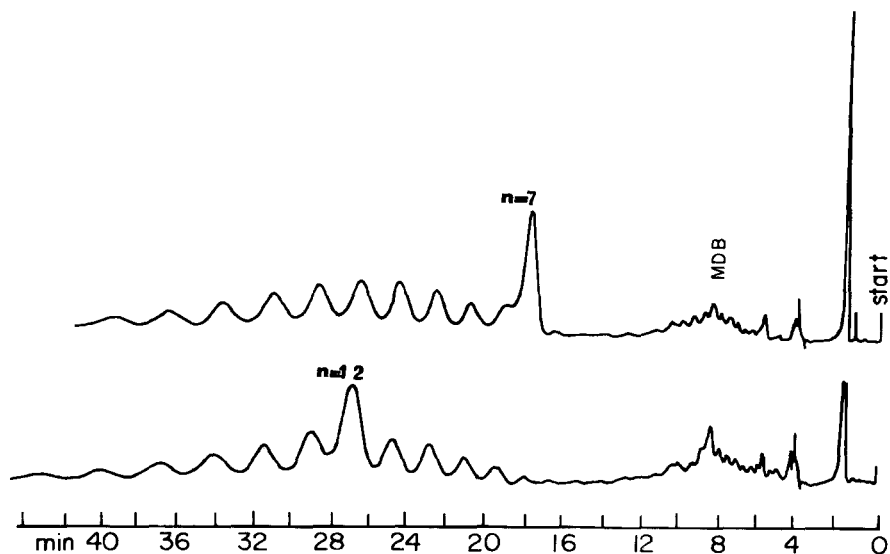


FIGURE 2

HPLC analysis of a mixture of bis(2,4-dinitrophenyl)polyethylene glycol 600, spiked with: (a) bis(2,4-dinitrophenyl)hepta glycol and (b) bis(2,4-dinitrophenyl)octa glycol.

on a nucleosil C_6H_5 (7 μ m) column is shown in Fig. 1. Next, the chromatogram of the bis(2,4-dinitrophenylated) mixture of polyethylene glycol (PEG)-600 is shown in Fig. 2. Bis(2,4-dinitrophenyl) heptaethylene glycol and bis(2,4-dinitrophenyl) dodecaethylene glycol were added as internal markers.

Under the above conditions, resolution between the bis(2,4-dinitrophenyl) derivatives of ethylene glycols from diglycol to pentaethylene glycol is possible. The resolution of crude bis(2,4-dinitrophenylated) mixtures of polyethylene glycols (product of Fluka, Switzerland) are shown in Fig. 3. (Fig. 3A - PEG-200; Fig. 3B - PEG-300; Fig. 3C - PEG-400; Fig. 3D - PEG-600). Each chromatogram contains 2,4-dinitrophenol, 2,4-dinitrofluorobenzene (2,4 DNFB, $R_t=6.0$ min), then a band of closely packed mono (2,4-dinitrophenyl) glycol homologes (abbreviated MDB, for mono-DNP-BAND). Then a band of the bis(2,4-dinitrophenyl) glycols, assigned by numerals identical with the integer n in $HO(CH_2CH_2O)_nH$.

The identification of the components in Fig. 3 was through comparison with prepared mixtures of the pure glycols, such as a mixture of tetra to octa glycols shown in Fig. 1. Alternatively, samples were spiked with a known component, as shown in Fig. 2.

HPLC Purity Monitoring in Chromatographic Separation of Bis(2,4-Dinitrophenyl) Oligoethylene Glycol Mixtures.

Bis(2,4-dinitrophenyl) glycols are very sensitive to nucleophilic substitution by ethoxide in the system $NaOH/EtOH/CHCl_3$ [the reaction being completed within 5 minutes at $80^\circ C$ (13)], and produce a mixture of liberated glycols and 2,4-dinitrophenol. This

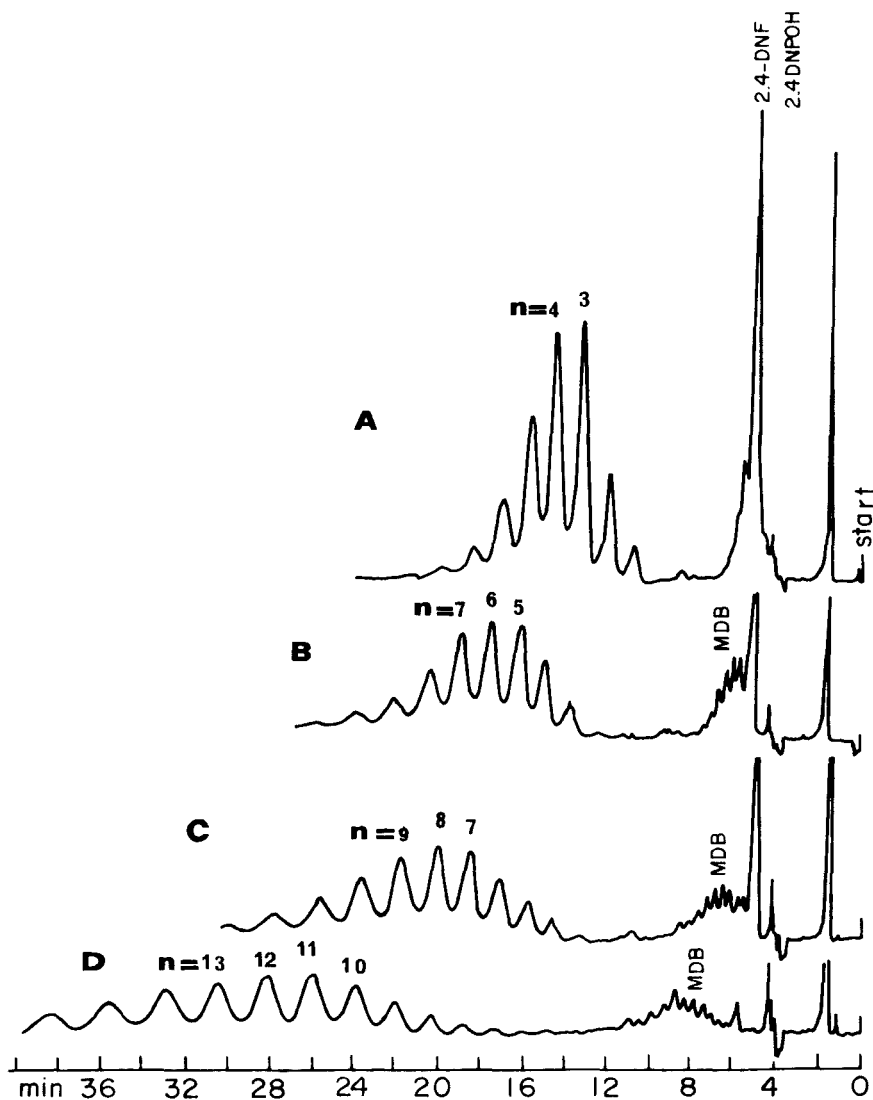


FIGURE 3

HPLC analysis of bis(2,4-dinitrophenyl) derivatives of polyethylene glycol mixtures: (a) PEG-200; (b) PEG-300; (c) PEG-400; (d) PEG-600. Numbers identify with n in $\text{HO}-(\text{CH}_2\text{CH}_2\text{O})_n-\text{H}$.

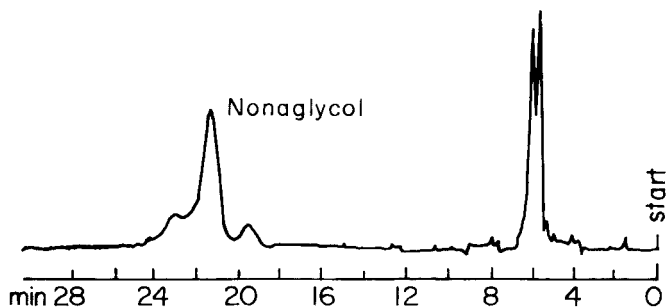


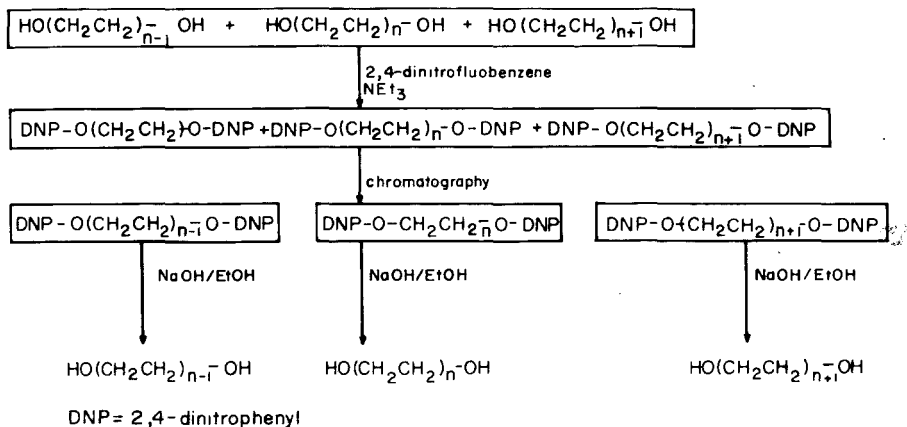
FIGURE 4

HPLC analysis of nonaglycol with octa glycol and decaglycols as impurities: PEG-300, starting material.

property allows the design of an integrated scheme leading from mixtures of polyethylene glycols, via bis-(2,4-dinitrophenylation) chromatographic separation and removal of the 2,4-dinitrophenyl protecting group to pure glycols (see scheme 1). The HPLC method described in this work allows this scheme by ensuring purity monitoring and control of the chromatographic separation procedure. From the composition of the commercial PEG-mixtures (see Fig. 3) it follows that PEG-200 is a source of tri, tetra and penta glycols, PEG-300 and PEG-400 are a source for penta to deca glycols and PEG-600 a source for octa to hexadeca glycols. Fig. 4 shows enriched nonaglycol obtained from PEG-300, and Fig. 5 shows enriched fractions of the Bis-(2,4-dinitrophenyl) derivatives of the higher glycols obtained from PEG-600 using scheme 1. Further chromatographic separation yields pure glycols.

In conclusion, in the present paper we have presented a highly sensitive HPLC method for the resolution and identification of

Scheme no. 1 : pure glycol via bis-(dinitrophenilation)of glycol mixtures, chromatographic separation and deblocking



oligoethylene glycols in synthetic samples or in their commercial admixtures, resulting from anionic polymerization of ethylene oxide. In addition, we have elaborated a method to pure oligo-ethyleneglycols ($n=4-16$) from commercially available polyethylene glycol mixtures by bis(2,4-dinitrophenylation) chromatographic separation and end-group removal. The HPLC analysis of the bis-DNP glycols enables purity monitoring of the glycols obtained by either route.

Making available high purity glycols will contribute to the synthesis of ultra pure crownethers, and will make evaluation of thermodynamic and biological measurements of various physical properties of crownethers more significant.

Although analysis of higher than hexadeca glycol was not attempted, the method should be applicable to higher glycols.

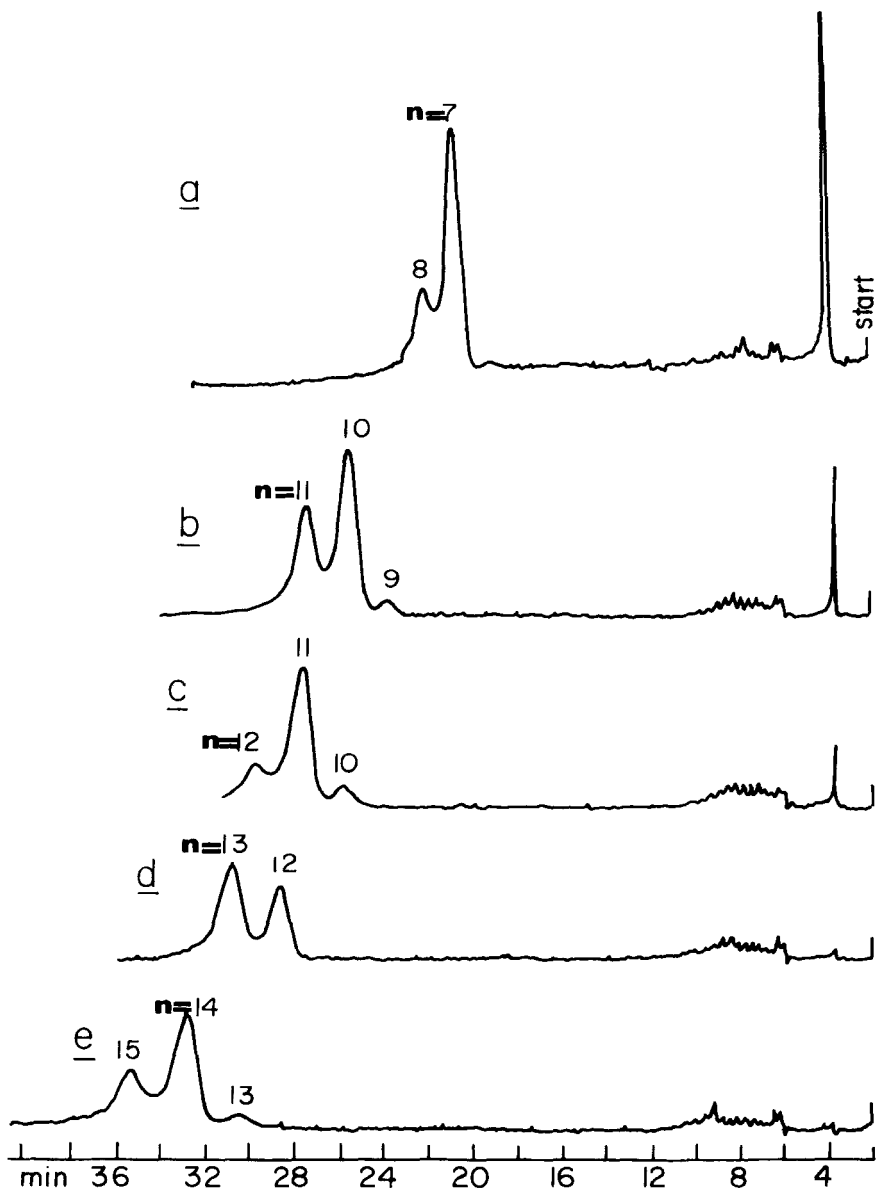


FIGURE 5

HPLC analysis of chromatographically separated bis(2,4-dinitrophenyl) derivatives of: (a) hepta, octa; (b) nona, deca, undeca; (c) deca, undeca, dodeca; (d) dodeca, trideca and (e) trideca, tetradeca and pentadeca glycols. PEG-600, as starting material.

EXPERIMENTALPreparation of Bis(2,4-Dinitrophenyl) Derivatives.

The glycol (13.0 mmole) is dissolved in 30 ml distilled acetone, and 4.7 ml (66 mmoles) of triethylamine added, followed by 5.0 g (26.4 mmoles) of 2,4-dinitrofluorobenzene (A.R). The solution is allowed to stand at room temperature for 24-48 hours in the case of the lower glycols (up to decaglycol), and up to 7 days with the higher glycols.

After evaporating the acetone, the product is taken in 100 ml CHCl_3 , washed with 2x50 ml in HCl, and then with water to neutral pH. The solution is dried on MgSO_4 , and the CHCl_3 removed by distillation. Chromatographic separation allows isolation of the bis(2,4-dinitrophenyl) derivatives in 45-76% yields (13).

High Performance Liquid Chromatography Analysis.

The HPLC analysis was performed on a Waters Associates Model 244 HPLC using a self-packed Nucleosil (-7 μm) C_6H_5 column of 250 by 4.6 mm, at room temperature. Several solvent elution systems were tried: (a) 20% dioxane in CHCl_3 ; (b) 20% dioxane + 1% isopropanol in CHCl_3 ; (c) 30% H_2O in CH_3OH . The last system was found to be superior and all the results reported here refer to the last system. Flow rate of 1 ml/min and chart speed 0.5 cm/min. Sample concentration in CH_2Cl_2 : 10^{-4}M . Sample size: 5-20 μl , UV detector - 254 nm. All the HPLC chromatograms of the crude bis(2,4-dinitrophenyl) glycols contain 2,4-dinitrofluorobenzene (2,4-DNFB) and 2,4-dinitrophenol (2,4-DNPOH). In the case of the 2,4-dinitrophenylated polyethylene glycol mixtures a band corres-

ponding to the mono-2,4-dinitrophenyl derivatives is presented and is designated as MDB.

ACKNOWLEDGEMENTS

The support of the U.S.-Israel Binational Foundation for this work through grant number 1943 is gratefully acknowledged. We thank Dr. Steve Weiner for suggesting the HPLC column.

REFERENCES

1. Pedersen, C.J., J. Amer. Chem. Soc., 89, 7017 (1967).
2. Lehn, J.M., in Structure and Bonding, J.D. Dunitz, ed. Springer-Verlag, Berlin, Vol. 16, 1973.
3. Cram, D.J. and Cram, J.M., Acc. Chem. Res., 11, 8 (1978).
4. Newkome, G.R., Kawato, T., Fronczek, F.R. and Benton, W.H., J. Org. Chem., 45, 5423-25 (1980).
5. Ikeda, I., Yamamura, S., Nakatsuji, Y. and Okahara, M., J. Org. Chem., 45, 5355-58 (1980).
6. Taylor, L.D. and Cerankowski, L.D., J. Polymer Sci., 13, 2551 (1975).
7. Marshall, A., Mobbs, R.H. and Booth, C., Euro. Polymer. J., 16, 881-5 (1980), and references therein.
8. Panayotov, I.M., Dimov, D.K., Tsvetanov, Ch.B, Stoebov, V.V. and Skorokhodov, S.S., J. Polymer Sci., Polymer Chem. Edit., 18, 3050-67 (1980).
9. Rajasekharan-Pillai, V.N., Mutter, M., Bayer, E. and Gatfield, I., J. Org. Chem., 45, 5364-70 (1980), and references cited therein.
10. a) Kahana, N. and Warshawsky, A., preprints, 2nd North American Chemical Congress, San Francisco, August 25-29, 1980.
b) Warshawsky A. and Kahana, N., J. Amer. Chem. Soc., 104, 2663 (1982).

11. Warshawsky, A., Kalir, R., Berkowitz, H. and Patchornik, A., J. Amer. Chem. Soc., 101, 4249 (1979).
12. Derivatization of alcohols, see for example: D.J. Edwards, in Handbook of Derivatives for Chromatography, Blau, K. and King, G., (Eds.), Chap. 10, Heyden, London, 1978.
13. Warshawsky, A. and Shoef, N., submitted for publication, (1983).

DATA ACQUISITION AND PROCESSING
FOR HIGH SPEED LIQUID CHROMATOGRAPHY

J. M. Anderson
Alltech Associates
2051 Waukegan Road
Deerfield, IL 60015

SUMMARY

The effect of sampling rate and digital smoothing on data acquired from high speed liquid chromatography (HSLC) is explored. The amount of data required per peak is determined from the precision of area, height, and retention time measurements. The correct sampling rate is related mathematically to column characteristics and operating conditions. The effect of a modified moving average digital smoothing routine on peak width and height is investigated. Digital smoothing functions are shown to behave similarly to analog noise filters. The merits of raw data storage and post analysis processing are discussed in light of the short analysis times in HSLC and the decreased cost of computer memory.

INTRODUCTION

Short HPLC columns filled with 3 micron particles, when operated at high flow rates, are capable of performing many separations 5 - 50 times faster than with conventional HPLC columns, with little or no loss of resolution. Specialized or modified HPLC equipment has been developed to take full advantage of this technique. The high efficiency and low volume of the

peaks generated in high speed liquid chromatography (HSLC) require reduced volume in injectors, connecting tubing, and detector flow cells.(1) Detector electronics must also be modified to cope with the sharp, relatively high frequency peaks. Noise filtering networks must have low time constants so as not to distort peak shape and thereby decrease resolution.(1-3) In a similar fashion, the data handling system employed for HSLC must be carefully designed to accurately represent the chromatographic data in a digital format without significantly distorting peak shape.

This paper describes a data handling system for use with HSLC based on the Apple II computer. The effects of sampling rate and digital smoothing routines on chromatographic data are investigated. The use of low cost RAM memory for raw data retention is described.

EXPERIMENTAL

CHROMATOGRAPHY

A Gilson Model 303 Pump (Gilson Medical Electronics, Middleton, WI, USA) in conjunction with an LDC Mark III Pulse Dampener (LDC, Riviera Beach, FL, USA) was used as the solvent delivery system. Samples were injected with a Rheodyne 7410 internal loop injector fitted with either a 2 or 5 ul sample loop (Rheodyne, Cotati, CA, USA). Separations were performed on 100mm x 4.6mm columns packed with 3 micron RoSiL C18

DA (Alltech Associates, Deerfield, IL, USA). Tubing used to connect the column to the injector and detector was 1/16" OD x .004" ID (Alltech Associates, Deerfield, IL, USA). A Kratos 773 UV-Visible Detector (Kratos Analytical Instruments, Westwood, NJ, USA), equipped with a 0.5 ul flow cell, was used to monitor column effluent at 254 nm. The square wave rise time for the detector's noise filter was set at 100 msec. Samples and mobile phases were as noted with each figure.

DATA SYSTEM

An Apple II Plus with 48K RAM memory was used as the host processor. Peripherals consisted of a single floppy disk drive with controller and an Apple DOT Matrix Printer (All obtained from Alltech Associates, Deerfield, IL, USA). Analog to digital (A/D) conversion and data integration was done with an Analytical Computers' Chromcard (Analytical Computers, Elmhurst, IL, USA) supplied with an optional 128K RAM memory expansion card.

The Chromcard consists of a printed circuit board that fits into one of the empty slots in the back of the Apple and receives the analog signal from the detector's recorder output. The accompanying software digitizes incoming data with 12 bit precision at up to 20 Hz. The chromatogram is displayed in real time on the Apple's CRT, using the high resolution graphics mode.

The incoming data is smoothed using 3, 5 or 9 points and is fitted to a quadratic curve. The first and second derivatives are calculated to define the onset of the peak, the position of valleys between peaks, and the proper peak end point. User defined values for slope sensitivity, minimum peak height and minimum peak area are used to discriminate against noise and other artifacts. Raw data points are stored in RAM memory for subsequent re-analysis or for transfer to disk for permanent storage.

Once all peaks have been defined and raw areas calculated, retention times are compared with expected values supplied by the operator. If experimental values match expected values within a user defined window, peak names are assigned. Raw peak areas are then normalized, compared with an internal standard (if desired), and multiplied by response factors prior to generation of the final report.

The final report lists all chromatographic conditions, together with operator name, date, and sample identification. Each peak is listed with retention time, area, area %, normalized area and concentration. A typical report is shown in Figure 1. The chromatogram may then be reproduced on the line printer in a dot-matrix format, as shown in Figure 2.

DATA ACQUISITION AND PROCESSING

2813

DATE 06/22/83 OPERATOR DSB SAMPLE EXPT1 SIZE 20 ULITERS
 METHOD N1.M METHOD DESCRIPTION NUCLEOTIDES

COLUMN DESCRIPTION:
 COLUMN ID 8 MM OD PACKING C 18 REVERSE TYPE RADIAL PAK
 MOBILE PHASE 50 MM AMM/PHOS PH6 FLOW RATE 1 ML/MIN PRESSURE 500 PSI

OPERATING CONDITIONS:
 INITIAL TEMP ENDING TEMP TEMP RATE
 DETECTOR TYPE UV 258NM DETECTOR SENS 0.2 DETECTOR TEMP

PK#	COMPOUND NAME	TIME	HT	WIDTH	AREA	AREA%	NORM	CONC
1	GTP	5.18	7.48	14.38	9472	14.88	0.	
2	GDP	5.67	10.90	20.88	17709	27.82	0.	
3	GMP	7.51	17.79	23.06	36467	57.29	0.	

FIGURE 1

DOT MATRIX REPRODUCTION
 OF CHROMATOGRAM

DATE : 06/22/83

OPERATOR : HNV

SAMPLE : EXPT1

SIZE : 20 ULITERS



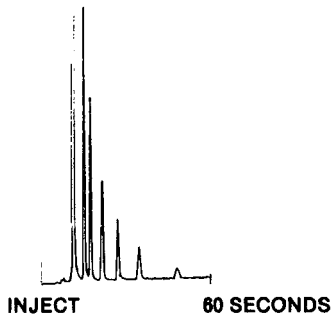
FIGURE 2

RESULTS AND DISCUSSION

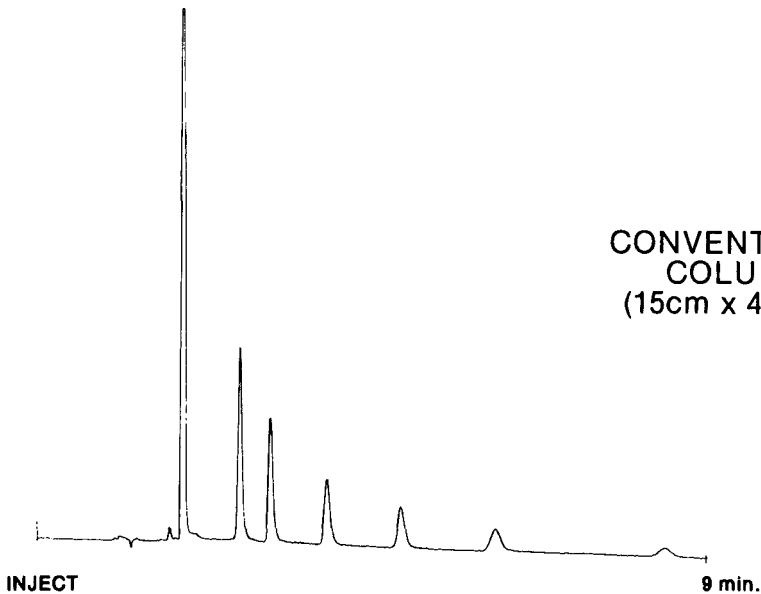
The extremely narrow peaks generated by HSLC present a unique problem to chromatography data systems. These narrow peaks are a result of the high efficiency of HSLC columns, coupled with the speed at which peaks elute. The speed arises from the use of short columns (30 - 150 mm) and operation at high mobile phase velocities. A comparison of HSLC with conventional HPLC is shown in Figure 3. The conventional column (150 mm, 5 micron) separates seven components in just under 9 minutes. The HSLC column (100 mm, 3 micron) separates the same mixture in just under 50 seconds, with little or no loss of resolution. The amount of information available from both chromatograms is the same, but the time base has been decreased almost twelvefold. The data system, to maintain the same integrity of information, must digitize the chromatogram twelve times faster, requiring higher sampling rates. Simultaneously, the peaks shift to a higher frequency, increasing the effect of digital smoothing routines on the peak shape.

A comparison may be made between these digital effects and their analog counterparts. Faster sampling rates correspond to faster strip chart recorder speeds. Improved digital smoothing routines parallel analog noise filters with shorter time constants.

CONVENTIONAL vs. HIGH SPEED HPLC



HIGH SPEED
COLUMN
(10cm x 4.6mm)



CONVENTIONAL
COLUMN
(15cm x 4.6mm)

FIGURE 3 Conventional Column, 15cm x 4.6mm P/W RoSiL C18 HL 5 micron. High Speed Column 10cm x 4.6mm P/W RoSiL C18 HL 3 micron. Mobile Phase 75/25 Acetonitrile/Water. 1.0ml/min for Conventional Column, 3.0ml/min for High Speed Column. Sample - Anilides.

SAMPLING RATE

The sampling rate determines the interval between consecutive examinations of the analog signal. The speed of the analysis and the shape of the peaks determine the sampling rate required--the sharper the peaks, the greater the sampling rate. The sampling rate should be fast enough to provide an adequate digital representation of the peak profile. On the other hand, excessive sampling rates will generate large quantities of data. If raw data is being stored, this rapidly fills the computer's memory.

The sampling rate required for a given peak can be related to chromatographic parameters using Equation (1).

$$(1) \quad W = \frac{4 V_0 (1 + k^1)}{Q N^{\frac{1}{2}}}$$

W = Peak Width (at Base)

V₀ = Column Void Volume

Q = Volume Flow Rate

N = Column Efficiency

k¹ = Capacity Factor

If the peak duration (W) is known, it is simple to calculate the sampling rate, using Equation (2).

$$(2) \quad F = \frac{C Q N^{\frac{1}{2}}}{4 V_0 (1 + k^1)}$$

F = Sampling Rate

C = The Number of Data Points Desired per Peak

Inspection of Equation (2) reveals that sampling rate increases with increased flow rate (Q), increased efficiency (N), and decreased column void volume (V_0). All three of these occur in HSLC. Smaller particles result in higher efficiencies; shorter columns have smaller void volumes, and shallow H/U curves for small particles allow operation at higher flow rates.

Choosing the correct value for C depends on the precision required from subsequent calculations. In most cases area, height, and retention time are extracted from raw peak data. Examining the precision of area, height, and retention time at various sampling rates may be used to determine the correct value for C .

The Chromcard system allows A/D conversion rates of 20 Hz, 10 Hz, 2 Hz, 1 Hz, and 0.5 Hz. For the high speed separation shown in Figure 4, A/D conversion rates of 20 Hz, 10 Hz, and 2 Hz were examined. Ten runs were collected at each rate. The earliest eluting peak (phenol) is the sharpest of the five, hence presenting the most demanding case to the data handling system. The mean values for area, height, and retention time, along with the relative standard deviation (RSD) for each quantity are shown in Table 1.

The mean values for retention time, peak height and peak area are nearly identical when calculated from data collected at 10 Hz and 20 Hz. As expected, the precision obtained from data collected at 20 Hz is slightly

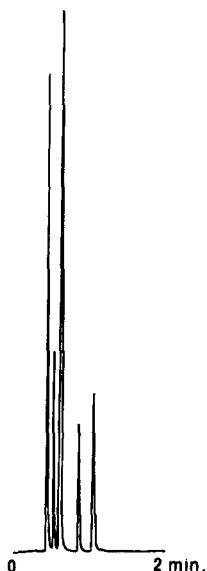


FIGURE 4 Column 10cm x 4.6mm P/W RoSiL C18 HL 3 micron. Mobile Phase 75/25 Acetonitrile/Water. Flow 3.0ml/min 3000 PSIG. Peak 1, Phenol; Peak 2, Benzaldehyde; Peak 3, N,N-Diethyl-m-Toulamide; Peak 4, Toluene; Peak 5, Ethyl Benzene.

TABLE 1

Integrator Precision at Various A/D Conversion Rates*

Sampling Rate (Points/Sec.)	Mean Value/% RSD		
	Retention Time (Seconds)	Peak Area	Peak Height
20	21.83 (0.771)	1522.55 (1.329)	2712.108 (1.271)
10	21.84 (0.418)	1536.92 (1.512)	2622.113 (1.497)
2	21.95 (1.293)	1623.90 (16.38)	1901.200 (12.34)

*10 runs at each rate. Data for Peak 1, phenol.

better, although the maximum RSD for 10 Hz is only 1.512%. This is acceptable precision for most applications. When the sampling rate is dropped to 2 Hz, however, the mean values for peak height and peak area differ greatly from those obtained at 10 and 20 Hz. The precision at the 2 Hz rate also becomes unacceptable (RSD>12%).

This information suggests that the number of points collected at the 10 Hz and 20 Hz rates provides an adequate data base for integration with good precision. Table 2 displays the number of data points obtained experimentally at each sampling rate, along with the theoretical value obtained by solving Equation (2) for C.

TABLE 2

Number of Data Points Collected at Various A/D Conversion Rates

<u>Sampling Rate (Points/Second)</u>	<u>Number of Data Points</u>	
	<u>Experimental*</u>	<u>Calculated**</u>
20	19.6	16.4
10	10.1	8.2
2	3.2	1.6

* Average values for 10 runs. Data for Peak 1, phenol.

** Calculated using $C = \frac{4 F V_0(1 + k^1)}{Q N^2}$, from Eqn (2).
N = 11,300 plates.

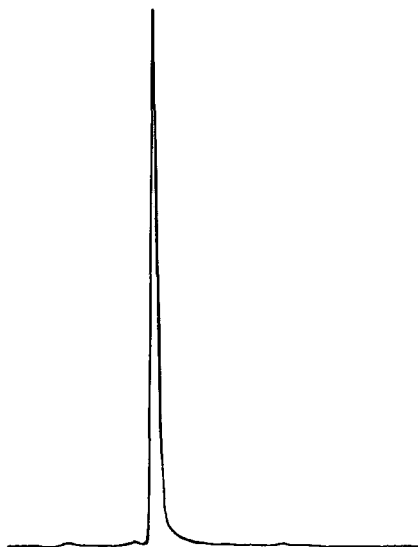


FIGURE 5 Phenol Peak Profile

The lack of correlation between the experimental and calculated values may be explained by the non-Gaussian shape of the phenol peak. The efficiency value used in calculating C by Equation (2) was obtained using bandwidth at 50% of peak height. This method assumes a Gaussian peak shape, and as Figure 5 reveals, the phenol peak has a significant tail. This leads to an inflated value for N and a reduced value for C . Nonetheless, Equation (2) provides a reasonable method for choosing sampling rate. This data indicates that a C value of 10 - 20 points/peak (minimum) will provide acceptable precision.

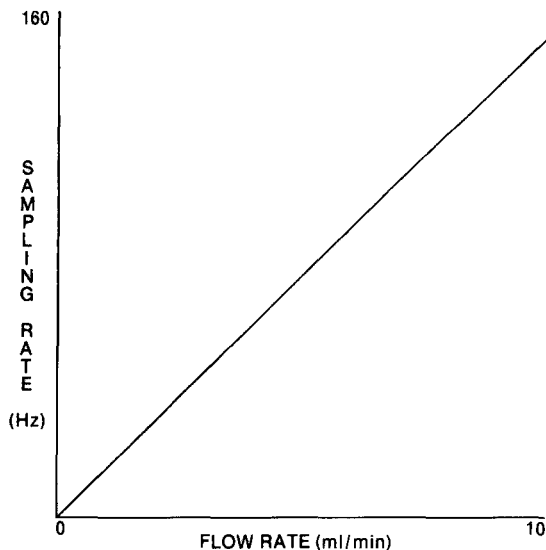


FIGURE 6 Sampling Rate vs. Flow Rate

For a typical 3 micron HSLC column 100 mm long with 12,000 plates and a V_0 of 0.6 ml, then substituting in Equation (2) with $C = 20$, yields Equation (3).

$$(3) \quad F = \frac{15.22 Q}{(1 + k^1)}$$

A peak eluting at the void volume ($k^1 = 0$) will require the fastest sampling rate. For HSLC with 4.6mm columns, flow rates of up to 6 ml/min may be encountered. Figure 6 displays the plot of sampling rate vs. flow rate, for a peak eluting at the void volume. For a flow rate of 6 ml/min, sampling rates of

close to 100 Hz may be required to generate 20 data points. Chromcard's current maximum sampling rate of 20 Hz should be adequate for most HSLC and for all conventional HPLC. However, to provide adequate precision and accuracy for the fastest analyses, a system with higher sampling rates would be desirable.

DIGITAL SMOOTHING

Most commercial LC detectors incorporate some type of analog noise filter as part of the signal electronics. The purpose of these filters is to eliminate noise at frequencies higher than that of the chromatographic peaks. Most of these filters can be characterized by their time constant. The time constant should be high enough to remove short term noise without distorting the peak shape. If a 5% increase in bandwidth due to noise filters is deemed acceptable, then the time constant should not exceed 32% of the peak's bandwidth.(3)

Haddad, et al., quantifies the effects of RC filters on chromatographic efficiency and resolution.(2) As the peak's bandwidth decreases, the effect of the noise filters on the peak shape increases. Analog noise filters for HSLC have been designed with small time constants so as not to significantly degrade peak shape.

Chromatography data systems usually include software for smoothing digitized data. The purpose is similar to that of analog noise filters--to remove

spurious signals from chromatographic data. The simplest of these numeric noise suppression methods is the moving average. The values of several consecutive data points are summed and divided by the number of points collected. This average is used for the value of the middle point in the array. The oldest point is dropped; a new point is added, and the process is repeated for the new array. In this manner, a "window" moves through the data, assigning a value for the middle data point in the window based on the past and future points in the array. The number of points used in the average (the size of the window) determines the extent of the smoothing. The length of the smoothing array can be qualitatively compared with the magnitude of the time constant used in analog filters. The more points used in the smoothing function, the greater the distortion of peak shape. As the peak's bandwidth becomes smaller, the effect of the moving average on the peak shape becomes more significant.

The software used with Chromcard uses a modified moving average as described by Savitsky and Golay.⁽⁴⁾ The software operates on a 3, 5, or 9 point window. Instead of calculating a simple average of the data points, the data in the array is fitted to a quadratic curve ($y = ax^2+bx+c$) using a least squares approximation. A quadratic curve fit is superior to a simple

moving average in that the quadratic function can be fitted more correctly to a rapidly changing signal such as a chromatographic peak. The result is better smoothing with less distortion.

The effect of the size of the window on peak shape is shown in Table 3. Using a 3, 5, or 9 point window, data from the phenol peak (Fig. 4) was acquired and smoothed with a quadratic curve fit. Peak height and peak bandwidth (at 50%) were measured for the smoothed data. Efficiency was calculated using the 1/2 height method.

Inspection of bandwidth and peak height values in Table 3 demonstrates that digital smoothing affects peak shape in a manner similar to analog noise filters. If the smoothing function begins to affect signals in the same frequency range as the chromatographic peaks, then the apparent bandwidth will increase, decreasing efficiency. Peak height will decrease, affecting

Table 3

Effect of Digital Smoothing on Peak Width and Height

<u>Points Used in Smoothing Routine</u>	<u>Peak Height</u>	<u>Peak Width (50%)</u>	<u>Efficiency (Plates)</u>
3	3529	6.750	12,100
5	3526	6.750	12,100
9	3385	7.125	11,000

sensitivity. In the case of the quadratic curve fit program applied in this work, no decrease in performance due to digital smoothing occurred for the 3 or 5 point windows. Using 9 points, however, reduced measured efficiency by 8.5% and peak height by 4.1%.

Unlike analog noise filters, whose characteristics are fixed by the value and nature of their electronic components, digital smoothing functions may take on almost any characteristic. The virtually unlimited ability to manipulate data through software makes this so. The Savitsky-Golay procedure, for example, may be modified to fit the raw data to virtually any mathematical function. In the future, it may be possible to dispense completely with analog signal processing in favor of more powerful, versatile digital methods.

When applying digital smoothing to chromatographic data, care must be taken to avoid distortion of peak shape. When the frequency of the peaks becomes closer to that of the noise to be eliminated, such as in HSLC, the design of the digital smoothing becomes increasingly important.

RAW DATA STORAGE

Chromatography data systems may be categorized according to the way they treat raw data. So called "on-the-fly" systems process data as it is received from the A/D converter, determining when peaks occur and

performing integration as the analysis takes place. Raw data values are discarded after they are processed. Small microprocessor based data systems usually have been limited to operation in this mode due to the high cost of solid state memory and the limited addressing capacity of 8 bit processors. In some cases, raw peak data can be stored in a small solid state memory buffer, then transferred to disc when the buffer is filled. The buffer is then filled with new data until the buffer once again requires "dumping" to disc. Unfortunately, transfer of data to a typical floppy disc can take up to one full second, during which time data acquisition must be interrupted. This places a severe limitation on sampling rate.

Larger computers with increased memory capacity have normally been employed for peak processing with retention of raw data. The major advantage of raw data retention is the ability to reanalyze the data under an alternate set of conditions. Peak processing parameters may be modified after the analysis to cope with unforeseen changes in the incoming data. For example, peak threshold and minimum area parameters can be adjusted to eliminate an unexpected peak from calculations.

In 1980 Reese predicted that data systems would be developed to operate with raw data retention as the cost of memory decreased.(5) Since that time, advances in

small personal computers have made fairly sophisticated computing power available at modest costs. Relatively inexpensive, high volume, solid state memory has also been developed making it possible to retain a fair amount of raw data with a microprocessor based system.

HSLC is an ideal candidate for systems of this nature since the amount of data generated is relatively small. Although the amount of data per unit time is high in HSLC due to the high sampling rates, the analyses themselves are typically quite short. In most cases, the entire separation takes place in less than ten minutes.

The data system employed in this work was equipped with a 128K RAM memory expansion card for the purpose of

Table 4

Data Storage Capacity at Various Sampling Rates*

<u>Sampling Rate (Hz)</u>	<u>Approximate Storage Capacity (Minutes)</u>
100	10
75	13.34
50	20
40	25
20	50
10	100

*Based on 128K system.

raw data storage. Table 4 shows the amount of raw chromatographic data (in minutes) that may be stored with 128K memory at various sampling rates. Even at sampling rates of 100 Hz, ten minutes of raw data may be stored in a 128K buffer. This is certainly adequate for even the most demanding HSLC.

CONCLUSION

With the advent of HSLC, the components of the LC system have been critically reviewed and modified to cope with the narrow, high speed peaks produced by HSLC columns. The data system must be viewed in the same light. Sampling rates should be adjusted to produce a minimum of 10 - 20 points per peak. Digital smoothing programs should be carefully evaluated to insure that no artificial decreases in performance are introduced. With the advent of low cost RAM memory to complement microcomputers, data processing with retention of raw data is possible.

REFERENCES

1. Cunico, R. L., Varian Instrument Applications, 16 (2), (1982) p. 9.
2. Haddad, P. R., Keating, R. W., and Low, G. K. C., J. of Liquid Chromatogr., 5, (1982) p. 853.
3. Kucera, P., J. of Chromatogr., 198, (1980), pg. 93.
4. Savitzky, A., and Golay, M., Anal. Chem., 36, (1964), pg. 1627.
5. Reese, C. E., J. of Chromatogr. Sci., 18, (1980), pg. 249.

SEPARATION OF CHLORINATED PHENOLS BY ISOCRATIC
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON
REVERSE PHASE COLUMN

J. Nair, K.M. Munir and S.V. Bhide
Carcinogenesis Division
Cancer Research Institute
Tata Memorial Centre
Parel, Bombay-400 012
India.

ABSTRACT

A method is described for isocratic high-performance liquid chromatographic separation of chlorinated phenols using methanol : phosphate buffer - pH 7.20 (50:50) solvent system. 13 out of 15 congeners of chlorophenols and phenol have been separated in 32 minutes at a flow rate of 0.8 ml per minute. The system is found to be useful for the separation of chlorophenols extracted from mouse liver fed with hexachlorocyclohexane.

INTRODUCTION

Hexachlorocyclohexane (HCH) is a widely used pesticide in developing countries and is reported to be carcinogenic in mice by many laboratories (1 - 3). We in our laboratory were interested in finding out the profile of the chlorophenols which are possible metabolites of HCH in mouse liver - the target tissue. Among the various separation techniques, high-performance liquid chromatography (HPLC) with reverse phase column is found to be very effective for

the separation of chlorophenols in recent years. McLeod and Laver (4) have reported an isocratic system for the separation of 13 out of 19 congeners of chlorophenols using two reverse phase columns and acetonitrile : phosphate buffer - pH 9.2. Smit et al. (5) have separated 11 congeners of chlorophenols using tetrahydrofuran : perchloric acid - pH 3.0 solvent system. Ugland et al. (6) have successfully resolved 18 congeners of chlorophenols using a 30 minute linear gradient from methanol : phosphate buffer - pH 4.0 (56 : 44 to 80 : 20) as mobile phase. Lores et al. (7) separated several chlorophenols using methanol : acetonitrile : phosphate buffer - pH 4.0 (40:14:46) and acetonitrile : phosphate buffer (50:50).

The present communication reports an isocratic HPLC separation of chlorophenols using a solvent system consisting of methanol : phosphate buffer - pH 7.20 (50:50). Separation of 13 congeners of chlorophenols out of 15 and phenol is achieved by this system with a single C₁₈ reverse phase column. The system has been successfully used to separate chlorophenols extracted from mouse liver fed with HCH. The clean up procedure of the chlorophenols from the liver and the chromatographic parameters like retention time, response factor are discussed.

MATERIALS AND METHODS

HPLC was carried out using a Waters Associates (Milford, Massachusetts, U.S.A.) HPLC system fitted with a model 6000 A solvent delivery system, μ Bondapak C₁₈ reverse phase column (30 cm X 0.29 cm), U6K universal injector and a model 440 absorbance

detector fitted with 254 nm filter. Chromatograms were recorded on Omniscribe (Houston Instruments, Austin, Texas) strip-chart recorder.

The chlorophenols were obtained from Aldrich Chemical Co. and were purified before use, if necessary. Stock solutions were prepared by dissolving 4-10 mg of the compounds in 5 ml of methanol. The stock solutions were diluted in methanol singly or in mixture so as to get desired standard concentrations.

Distilled E. Merck (India) G.R. grade methanol and triple distilled water from all glass apparatus were used for preparing solvent system.

Solvent System : Two stock solutions of 50 mM dipotassium hydrogen phosphate and 50 mM of potassium dihydrogen phosphate were prepared from AnalaR grade reagent and stored at 4 °C. The stock solutions were mixed and diluted appropriately to make a buffer of 0.5 mM strength and pH 7.20 ± 0.02. Running solvent was prepared by mixing methanol and phosphate buffer (50:50) after filtering through appropriate Millipore filters.

Sample Preparation : Livers were collected from male Swiss mice (8-week-old) fed with a diet containing 500 ppm HCH continuously for 2 months. Livers were minced and the chlorophenols were extracted from it by alkaline and acid hydrolysis followed by distillation and extraction in toluene as described by Sackmauerova-veningerova et al. (8). Instead of 15 ml, 3 X 50 ml of toluene was used. The combined toluene extract was then concentrated to dryness using rotary vacuum evaporator at 60 °C. The residue was taken in 2 ml

benzene and cleaned up using Sephadex QAE Q-25-120 anion exchanger (Sigma) as described by Renberg (9). The final benzene solution was concentrated to dryness in a stream of nitrogen at 40 °C. The residue was then taken in 0.5 ml of running solvent and filtered before injection.

Procedure : C₁₈ μ Bondapak column was first washed with methanol for 15 minutes and then with running solvent for 60 minutes at a rate of 2 ml per minute. The flow rate was then adjusted to 0.8 ml per minute. Suitable aliquots of standard mixture and the samples were injected (1-5 μ l) by Hamilton syringe.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of standard chlorophenols from a mixture using the present system. 13 out of 15 congeners of chlorophenols and phenol can be well separated. At a flow rate of 0.8 ml per minute 32 minutes were required to elute all chlorophenols under the present experimental condition. 2-chlorophenol eluted with 2,3,4,5-tetrachlorophenol while 2,4-dichlorophenol eluted with 2,3,4-trichlorophenol. The column required a minimum of 60 minutes washing for getting constant retention time of the chlorophenols.

The pH of the running solvent was very critical in the elution pattern of the chlorophenols. Change in pH caused change in the retention time and co-elution of several congeners. We have tried the separation with methanol : water (50:50) and methanol : phosphate buffer (50:50) ranging from pH 6 - 8. We found that pH 7.20 \pm 0.02 was the optimum pH at which maximum separation of 13 congeners of chlorophenols and phenol was obtained. Methanol : water and methanol : phosphate buffer - pH < 6 caused

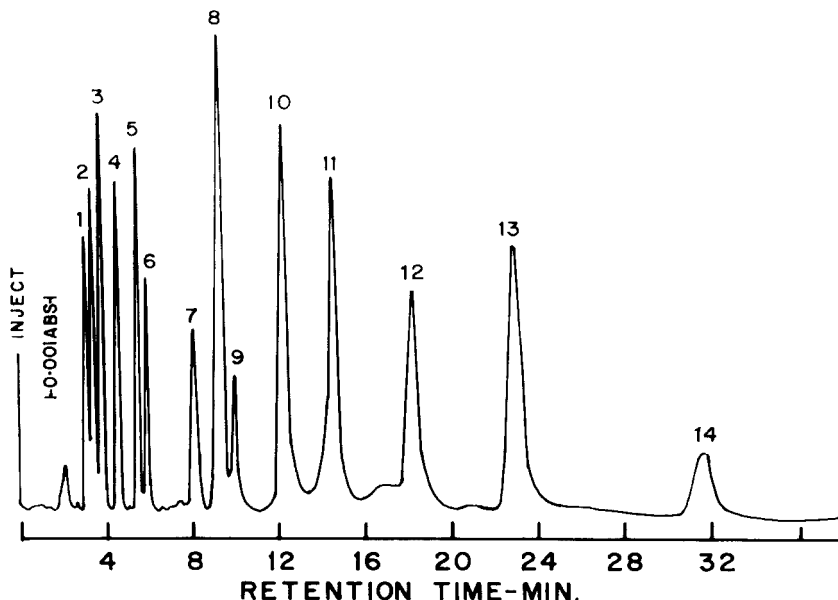


FIGURE 1. HPLC profile of the Chlorophenols and Phenol (Refer Table 1 for the Congeners of Chlorophenols).

poor resolution of the later eluting congeners. Although separation of chlorophenols have been reported using solvent with $\text{pH} > 8$ (4,10) we have not tried solvent system with higher pH as it might damage the reverse phase.

The retention time, response factor (peak area/concentration) and the minimum detectable amounts of chlorophenols at 0.01 AUFS (254 nm) are given in Table 1. Pentachlorophenol has the maximum response factor followed by tetrachlorophenols, trichlorophenols and dichlorophenols. Phenol and monochlorophenols have the least response factor. It can be noted that ng of chlorophenols can be detected at 0.01 AUFS.

Table 1. Retention Time, Response Factor and Minimum Detectable Amounts of Chlorophenols at 0.01 AUFS (254 nm)

Peak No.	Phenols	Retention Time, Min.	Response Factor *	Minimum Detectable amount, ng.
1.	2,3,5,6-Tetrachloro-	3.00	72.85	5.0
2.	2,3,6-Trichloro-	3.26	67.40	5.0
3.	Pentachloro-	3.66	86.65	4.0
4.	2,4,6-Trichloro-	4.34	58.19	9.0
5.	2,6-Dichloro-	5.34	23.10	16.0
6.	Phenol	5.82	7.14	35.0
7.	2-Chloro-and 2,3,4,5-Tetrachloro-	8.00 8.00	4.70 77.65	40.0 7.0
8.	2,3,5-Trichloro-	9.08	37.90	20.0
9.	4-Chloro-	9.90	4.72	45.0
10.	2,4,5-Trichloro-	12.08	46.76	9.0
11.	2,4-Dichloro-and 2,3,4-Trichloro-	13.40 13.40	14.30 43.65	25.0 9.0
12.	3,4-Dichloro-	17.08	7.19	45.0
13.	3,5-Dichloro-	22.74	11.54	40.0
14.	3,4,5-Trichloro-	31.40	30.42	25.0

* Peak area $\text{cm}^2/\text{mg} \times 10^{-3}$, calculated from three concentrations.

Fig. 2 shows the HPLC profile of the chlorophenols extracted from mouse liver fed with HCH. It can be seen that 2,6-dichlorophenol was the major chlorophenol in the sample. Apart from this, 2,3,5,6-, 2,3,4,5-tetrachlorophenols, 2,3,6-, 2,4,6-, 2,3,5-trichlorophenols and pentachlorophenol were present to a lesser extent. Alkaline and acid hydrolysis

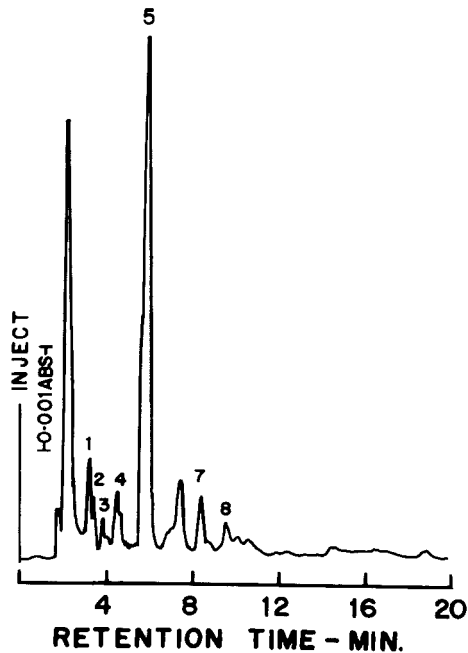


FIGURE 2. HPLC Profile of the Chlorophenols Extracted from HCH Treated Mouse Liver (Refer Table 1 for the Congeners of Chlorophenols).

followed by distillation and solvent extraction is reported to give good recovery of the chlorophenols from the biological samples (8). However, we found that some unknown compounds interfered with separation of chlorophenol if the samples were injected directly without QAE Sephadex ion exchange clean-up.

The present system is useful for separating most of the congeners of chlorophenols from a mixture. It can be used for estimating chlorophenols in biological samples where they are present as degraded products

of chlorinated pesticides. The method may further be useful for the quantitation of chlorophenols in environmental samples.

ACKNOWLEDGEMENT

The authors are thankful to the Government of India for the award of a Fellowship to one of them (K.M.M.).

REFERENCES

1. Nigam, S.K., Bhatt, D.K., Karnik, A.B., Thakore, K.N., Aravinda Babu, K., Lakkad, B.C., Kashyap, S.K. and Chatterjee, S.K., Experimental Studies on Insecticides Commonly Used in India, *J.Cancer Res. Clin. Oncol.*, 99, 143, 1981.
2. Ito, N., Hanahochi, M., Sugimara, S., Shirai, T., Fukushima, S. and Nagasaki, H., Reversibility and Irreversibility of Liver Tumour in Mice Induced by γ -Isomer of 1,2,3,4,5,6-Hexachlorocyclohexane, *Cancer Res.*, 36, 2227, 1976.
3. Nagasaki, H., Marugami, M., Tomi, S., Mega, T. and Ito, N., Development of Hepatoma in Mice Treated with Benzenehexachloride, *Gann*, 62, 431, 1971.
4. McLeod, H.A. and Laver, G., Separation of Chlorinated Phenols by Reverse Phase High-Performance Liquid Chromatography at an Alkaline pH, *J. Chromatogr.*, 244, 385, 1982.
5. Smit, H.C., Lub, T.T. and Vloon, W.J., Application of Correlation High-Performance Liquid Chromatography to the Reverse Phase Separation of Traces Chlorinated Phenols, *Anal. Chim. Acta*, 122, 267, 1980.
6. Uglund, K., Lundanes, E., Greibrokk, T. and Bjorseth, A., Determination of Chlorinated Phenols by High-Performance Liquid Chromatography, *J. Chromatogr.*, 213, 83, 1981.
7. Lores, E.M., Edgerton, T.R. and Moseman, R.F., Method of Confirmation of Chlorophenols in Human Urine by LC with an Electrochemical Detector, *J. Chromatogr. Sci.*, 19, 466, 1981.

8. Sackmauerova-Veningerova, M., Uhnak, J., Szokolay, A. and Kocan, A., Identification of Chlorinated Phenols as Degradation Products of Chlorinated Pesticides in Biological Materials, *J. Chromatogr.*, 205, 194, 1981.
9. Renberg, L., Ion-exchange Technique for the Determination of Chlorinated Phenols and Phenoxy Acids in Organic Tissue, Soil and Water, *Anal. Chem.*, 46, 459, 1974.
10. Lee, D.P., Reversed - Phase HPLC from pH 1 to 13, *J. Chromatogr. Sci.*, 20, 203, 1982.

ANNOUNCEMENT

The Biological Research Center of the Hungarian Academy of Sciences, the Chromatography Committee of the Hungarian Academy of Sciences, and the Hungarian Chemical Society announce a conference entitled

"ADVANCES IN LIQUID CHROMATOGRAPHY"

consisting of two parts:

-Fourth Annual American-Eastern European Symposium on Liquid Chromatography,

-International Symposium on Thin-Layer Chromatography with Special Emphasis on Overpressured Layer Chromatography (OPLC).

The conference will include invited papers, oral presentations, and posters in all fields of liquid chromatography and will be held in Szeged, Hungary on September 10-14, 1984.

All correspondence concerning the conference should be directed to:

-Dr. Huba Kalasz, Department of Pharmacology, Semmelweis University of Medicine, P.O.Box 370, H-1445 Budapest, Hungary

-Dr. E. Tyihak, Research Institute for Plant Protection, P.O. Box 102, H-1525 Budapest, Hungary.

ANNOUNCEMENT

14th NORTHEAST REGIONAL ACS MEETING

June 10-14, 1984

Fairfield University
Fairfield, ConnecticutSymposia

Applications of Molecular Orbital Theory	Chem'l Separations & Identifications
Chem'l Education Towards the 21st Century	The Future of the US Comodity & Specialty Chemicals Industry
Chemical Technicians	Hazardous Waste Management
Computers in Chemistry	Medicinal Chemistry
Organometallic Chemistry	Physical Organic Chemistry
Synthetic Organic Chemistry	Water Soluble Polymers

General Sessions

Analytical Chemistry, Biochemistry, Environmental, Inorganic Chemistry, Physical Chemistry.

The deadline for abstracts is January 15, 1984. Abstracts should be submitted to Miss Linda Somma, P.O.Box 2559, Waterbury, CT, 06725.

LC NEWS

FAST FLOW ION EXCHANGE GELS offer significant improvements in processing speed and throughput for large scale use. The gels are derived from DEAE- and CM-Sepharose gels via modification of the cross-linking that results in improved mechanical strength. Pharmacia Fine Chemicals, Inc., JLC/83/14, 800 Centennial Avenue, Piscataway, NJ, 08854, USA.

ALKYLPHENONE HPLC INTERNAL STANDARD KIT consists of a family of neutral compounds whose retention volumes will not be affected by solvent modifiers or acidic/basic buffers. Due to their wide solubility range, they can be used with polar and non-polar solvents. Pierce Chemical Co., JLC/83/14, P.O.Box 117, Rockford, IL, 61105, USA

STRONG ANION EXCHANGE COLUMNS FOR HPLC are prepared by bonding an organic quaternary nitrogen via Si-C bonds to spherical silica. The columns are said to resist deterioration in aqueous, polar and low pH mobile phases. Phase Separations, Inc., JLC/83/14, River View Plaza, 16 River Street, Norwalk, CT, 06850, USA.

IMPROVED BIOPOLYMER YIELDS are claimed for an ion exchange 25 liter column bed capable of absorbing up to 2 kg of biopolymer. According to the manufacturer, packing and separation can be accomplished in a single shift. The column is made of transparent PVC to afford direct view of the column bed, allowing early detection of bed cracking. Whatman Chemical Separation, JLC/83/14, 9 Bridewell Place, Clifton, NJ, 07014, USA.

REACTIVATION SOLUTION RESTORES SILICA COLUMNS following water-deactivation during normal phase LC. The three-component solution provides a cost-saving treatment which avoids a lengthy series of multiple solvent washes and reduces down time. Burdick & Jackson Labs., LC/83/14, 1953 S. Harvey Street, Muekegon, MI, 49442, USA.

DIGITAL MICROPIPETTOR is a positive displacement device with a digital display, is easily set and secured at a precise volume. Sample volumes are unaffected by surface tension, viscosity, vapor pressure or density. No calibration for each reagent is necessary. Labindustries, JLC/83/14, 620 Hearst Avenue, Berkeley, CA, 94710, USA.

REVERSED-PHASE ODS COLUMNS are constructed of stainless steel with a highly polished interior surface. They are packed with Spherisorb ODS-II, a spherical silica of very narrow particle size distribution. It is coated with a C18 function and is fully end capped. Columns are guaranteed to deliver 50,000 to 80,000 plates per meter. HPLC Specialties, JLC/83/14, P. O. Box 484, Edmond, OK, 73083, USA.

PHENOLS IN WATER are determined by isocratic mode LC. A large volume of water is injected onto a reversed-phase column, and eluted with a 40:60 acetonitrile/water mobile phase. Detection is at 200 nm down to less than 0.001 ppm. Pye Unicam Ltd., JLC/83/14, York Street, Cambridge CB1 2PX, England.

ULTRA-RESOLUTION IN GPC ANALYSIS is offered by a new generation of GPC columns for polymer analysis and testing. They are available in 500, 1,000, 10,000, and 100,000 angstroms exclusion limits as well as a mixed-bed column that provides ultra-resolution from less than 100 to well over 20 million Daltons. They are available packed in any of several commonly used GPC solvents and in many polymer solvents not generally available with GPC columns. Jordi Associates, Inc., JLC/83/14, 397 Village Street, Millis, MA, 02054, USA.

PROTEIN ANALYSIS LC is described in a recent brochure. Four separation mechanisms are used--ion exchange, gel filtration, reverse phase, and hydroxyapatite fractionation. The methods allow identification and quantitation of components that might not be amenable to any single mechanism. Bio-Rad Labs, JLC/83/14, 2200 Wright Avenue, Richmond, CA, 94804, USA.

AUTOMATED TLC SAMPLE APPLICATOR consists of a control unit and the actual applicator. The control unit is a Z-80 microprocessor-based device that holds 8 sample application modes, five of which are user programmable. Sample volumes from 100 nL to 20 microl may be applied. Applied Analytical Industries, JLC/83/14, Route 6, Box 55, New Hanover Air Park, Wilmington, NC, 28405, USA.

THIN-LAYER CELL FOR ELECTROCHEMICAL DETECTION allows placement of the auxilliary electrode both downstream and across from the working electrode. A highly polished stainless steel top extends cell life, permits compatibility with new "high speed" columns, and allows for connection of low dead volume fittings for use with micro columns. Bioanalytical Systems, Inc., JLC/83/14, 1205 Kent Avenue, Purdue Research Park, West Lafayette, IN, 47906, USA.

LIQUID PROCESSING UNIT can be used to feed most analytical instruments. It performs all of the crucial sample pickup, mixing and dispensing operations. A pair of syringes whose plungers are driven by a stepper motor and precision ball lead screws, a mixing chamber, and a hand-held control unit are the principal working parts. A computer program directs all operations. Processing

parameters such as time, volume, ratios, and increments are entered thru the control unit and become a part of an individual routine program. Hamilton Company, JLC/83/14, P. O. Box 10030, Reno, NV, 89510, USA.

PREPARATIVE SPHERICAL SUPPORTS are said to provide resolution and loading characteristics approaching those of microparticulate columns. They are available in 30 micron particles for ion exchange, reversed phase, and hydrophobic interaction chromatography of proteins. SynChrom, Inc., JLC/83/14, P.O.Box 110, Linden, IN, 47955, USA.

LC CALENDAR

1983

DECEMBER 6-7 and 8-9: Electrofocusing and Electrophoresis Workshop, Los Angeles, CA, USA. Contact: Workshop Registrar, LKB Instruments, Inc., 9319 Gaither Road, Gaithersburg, MD, 20877, USA.

1984

JANUARY 19-20: Workshop: "Low Dispersion Liquid Chromatography," The Free University of Amsterdam, The Netherlands. Contact: LDLC Workshop Office, Dept. of Analytical Chem., The Free University, DeBoelelaan 1083, 1081 HV Amsterdam, The Netherlands.

FEBRUARY 12-16: 14th Australian Polymer Symposium, Old Ballarat Travel Inn, Ballarat, Australia, sponsored by the Polymer Div., Royal Australian Chemical Inst. Contact: Dr. G. B. Guise, RACI Polymer Div., P. O. Box 224, Belmont, Victoria 3216, Australia.

FEBRUARY 20-22: International Symposium on HPLC in the Biological Sciences, Regent Hotel, Melbourne, Australia. Contact: The Secretary, Int'l Symposium on HPLC in the Biological Sciences, St. Vincent's School of Medical Research, 41 Victoria Parade, Fitzroy 3065, Victoria, Australia.

APRIL 8-13: National ACS Meeting, St. Louis, MO. Contact: Meetings, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

MAY 20 - 26: 8th Intl. Symposium on Column Liquid Chromatography, New York Statler Hotel, New York City. Contact: Prof. Cs. Horvath, Yale University, Dept. of Chem. Eng., P. O. Box 2159, Yale Stn., New Haven, CT, 06520, USA.

JUNE 18-21: Symposium on Liquid Chromatography in the Biological Sciences, Ronneby, Sweden, sponsored by The Swedish Academy of Pharmaceutical Sciences. Contact: Swedish Academy of Pharmaceutical Sciences, P. O. Box 1136, S-111 81 Stockholm, Sweden.

AUGUST 26-31: National ACS Meeting, Philadelphia, PA. Contact: Meetings, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

SEPTEMBER 10-14: Advances in Liquid Chromatography, including the 4th Annual American-Eastern European Symposium on LC and the Int'l Symposium on TLC with Special Emphasis on Overpressured Layer Chromatography, sponsored by the Hungarian Academy of Sciences' Chromatography Committee & Biological Research Center and the Hungarian Chemical Society, in Szeged, Hungary. Contact: Dr. H. Kalasz, Dept. of Pharmacology, Semmelweis University of Medicine, P.O.Box 370, H-1445 Budapest, Hungary, or Dr. E. Tyihak, Research Inst. for Plant Protection, P.O.Box 102, H-1525 Budapest, Hungary.

OCTOBER 1-5: 15th Int'l. Sympos. on Chromatography, Nuremberg, West Germany. Contact: K. Begitt, Ges. Deutscher Chemiker, Postfach 90 04 40, D-6000 Frankfurt Main, West Germany.

DECEMBER 16-21: International Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, sponsored by the Chemical Inst. of Canada, Chemical Soc. of Japan, and the American Chem. Soc. Contact: PAC CHEM '84, International Activities Office, American Chem. Soc., 1155 Sixteenth St., NW, Washington, DC, 20036, USA.

1985

FEBRUARY 11-14: Polymer 85, Int'l Symposium on Characterization and Analysis of Polymers, Monash University, Melbourne, Australia, sponsored by the Polymer Div., Royal Australian Chemical Inst. Contact: Polymer 85, RACI, 191 Royal Parade, Parkville Victoria 3052, Australia.

APRIL 28 - MAY 3: 189th National ACS Meeting, Miami Beach. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

JULY 1-5: Ninth International Symposium on Column Liquid Chromatography, sponsored by the Chromatography Discussion Group and by the Royal Society of Chemistry's Chromatography & Electrophoresis Group, Edinburgh, Scotland. Contact: Prof. J. H. Knox, 9th ISCLC Secretariat, 26 Albany Street, Edinburgh, EH1 3QH, Great Britain.

SEPTEMBER 8-13: 190th National ACS Meeting, Chicago. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA

1986

APRIL 6-11: 191st National Am. Chem. Soc. Mtng., Atlantic City, NJ. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

SEPTEMBER 7-12: 192nd National Am. Chem. Soc. Mtng., Anaheim, Calif. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA

1987

APRIL 5-10: 193rd National Am. Chem. Soc. Mtng., Denver, Colo. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

AUGUST 30 - SEPTEMBER 4: 194th National Am. Chem. Soc. Mtng., New Orleans, LA. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

The Journal of Liquid Chromatography will publish announcements of interest to liquid chromatographers in every issue of the Journal. To be listed in the LC Calendar, we will need to know: Name of the meeting or symposium, sponsoring organization, when and where it will be held, and whom to contact for additional details. You are invited to send announcements to Dr. Jack Cazes, Editor, Journal of Liquid Chromatography, P. O. Box 1440-SMS, Fairfield, CT, 06430, USA.

AUTHOR INDEX TO VOLUME 6

A

- Abbott, S. R., S77
Abell, C. W., 1407
Aboul-Enein, H. Y., 715, 2785
Ábrahám, M., 2635
Abramson, R. K., 2761
Abusteit, E. O., 725
Ackerly, R. A., 1239
Adell, A., 527
Adlard, M. W., 2019
Agarwal, D. D., 2661
Agrawal, S., 1535
Akintonwa, A., 1513
Alba, D., 2411
Allard, M., 2501
Allison, L. A., 1785
Anderson, J. M., 2809
Andreev, L. V., 2699
Ansari, G. A. S., 1407
Appendino, G., 543
Armstrong, D. W., 1, 23, 95
Arora, R. K., 2695
Artigas, F., 527
Asnis, G. M., 1111
Atha, D. H., 229
Atkins, T. M., 1171
Aubert, J. H., S219
Aucouturier, P., 301
Ayers, C. R., 1265
- B
- Babhair, S. A., 715, 2785
- Baker, J. K., 1319
Barstow, L. E., 1591
Baust, J. G., 1139
Bawdon, R. E., 2747
Beers, P. C., 179
Beier, R. C., 917
Belliaro, F., 543, 551
van Bennekom, W. P., 1849
Bennett, J. C., 249
Bennett, L., 1193
Bergquist, S., 559
Berkowitz, S. A., 1359
van Bezoooyen, J. A. G., 1849
Bhatia, A. B., 1475, 1693
Bhide, S. V., 2829
Bhown, A. S., 249
Bidlingmeyer, B., 605, 1015, 2109
Bisagni, E., 939
Bishara, R. H., 1171, 1221
Blais, D., 939
Blakely, R. D., 1873
Boehm, R. E., 1
Boiron, M., 939
Bojarski, J., 73
Bond, A. M., 1799
Bopp, R. J., 2209
Boquet, S., 301
Boross, L., 2635
Bos, P., 1745
Boudoulas, O., 2033
Boymel, J. L., 1183
Braselton, W. E., Jr., 861
Brisson, A. M., 301
Britton, J., Jr., 627
Bueding, E., 1291

Note: Numbers with an "S" prefix indicate entries appearing in Supplements 1 and 2 of Volume 6.

Bui, K. H., 1
 Busby, T. F., 229

C

Cahill, W. R., Jr., 1645
 Caronia, J. P., 1673
 Carr, S. M., 1617
 Casey, M. L., 55
 Chang, E. S., 291
 Chapman, G. E., 215
 Charvat, S., 2679
 Chue, M., 1333
 Ciegler, A., 833
 Clement, A., 1705
 Colgan, S., 605, 1015, S125
 Conkerton, E. J., S105
 Cooper, T. B., 1111
 Corbin, F. T., 725
 Cornelius, T. W., 249
 Courtois, Ph., 301
 Critcher, E. C., 343, 2043
 Crowther, J. B., 1673
 Cuddy, K. K., 2383
 Cunliffe, B., 1123
 Cunningham, F. G., 2747
 Cusanovich, M. A., 1591
 Czapifiska, K. L., 63

D

Daigle, D. J., S105
 Dark, W. A., 325
 Davis, K. L., 1997
 Decazes, J. M., 575
 Degen, G. H., 725
 Dellacherie, E., 2543
 Del-Razo, L. Ma., 845
 Di Bussolo, J. M., 2353
 Dimov, S. I., 2687
 Ding, X.-D., 2173
 Dixon, A., 2775
 Dkiert, L., 73
 Dobbins, M. F., 179
 Dong, M. W., 2353
 Dorsey, J. G., 511
 Dousset, I., 575
 Downie, R. H., 2557
 Doyle, M. J., 2141

Dreux, C., 939
 Duffield, A. M., 311

E

Earl, R. A., 1591
 Eckers, C., 2383
 Edasery, J. P., 1997
 Edholm, L.-E., 559
 Eggers, H. M., 2141
 Eggers, N. J., 1955
 Ehrlich, K. C., 833
 El-Hofi, S., 715
 El-Houfy, S., 2785
 Elmer, G. W., 585
 Erhard, P., 2775
 Evans, M. E., S153

F

Faddoul, A., 2501
 Farooqui, W. U., 109
 Fasching, C. E., 2513
 Ferrell, W. J., 2033
 Fleckenstein, L., 1523
 Forastieri, H., 229
 Fourtillan, J. B., 301
 Frei, R. W., 1745
 Frick, D. A., 445
 Friday, D., 1139
 Fritz, R. R., 1407

G

Gabriel, R. F., 647
 Galchenko, V. F., 2699
 Gandhi, J. S., 1153
 Gant, J. R., 2353
 Garg, M., 973
 Garrison, J. L., 343, 2043
 Gause, E. M., 1523
 Gelpf, E., 527
 Girardie, J., 2501
 Gisselbrecht, C., 939
 Glöd, B. K., 1823, 1837
 Gnanasambandan, T., 1777
 Goldwasser, J. M., 2433
 Goto, J., 1977

- Goto, M., 1907 I
 Gourley, R. E., 1375
 Gourmel, B., 939
 Grego, B., 199, 215
 Grenblatt, G. A., 917
 Grinstead, G., 1099
 Grob, R. L. (book review), 2129
 Gross, T., 2775
 Gupta, D. R., 2695
 Gupta, V. K., 145
 Guss, S. P., 2747
- H
- Haddad, P. R., 311
 Hadj-Mohammadi, M. R., 511
 Hagberg, C.-E., 655
 Halsall, H. B., 2141
 Hamielec, A. E., S179
 Hanai, T., 1081
 Harmenberg, J., 655
 Harrison, D. J. P., 2723
 Hartwick, R. A., 1673
 Hassan, M. M. A., 1491
 Hayes, D. K., 993
 Hearn, M. T. W., 199, 215
 Heftmann, E., 281
 Heineman, W. R., 2141
 Hemans-Lokkerbol, A. C. J., 1849
 Hendren, D. J., 367
 Henion, J. D., 2383
 Hepler, B. R., 2275
 Hernandez, O., 1475, 1693
 Hester, R. D., 1037
 Holt, G., 2019
 Hong, J.-S., 871
 Hoogewijs, G., 2521
 Hoogvliet, J. C., 1849
 Hosoya, T., 2739
 Howard, C. C., 1897
 Hsu, S., 627
 Hubert, J., 1081
 Hunter, E., 249
 Hunter, I. R., 281
 Hurst, W. J., 927, 2067
 Hutchens, D. E., 1037
 Hwang, P. T. R., 743, 1513
- Ichinoki, S., 2079
 Ilinov, P., 2687
 Ingham, K. C., 229
 Iqbal, M., 959
 Iriyama, K., 2739
 Ishigaki, M., 1065
 Ishii, D., 1907
 Issaq, H. J., 259, 825, 1213, 1375
 Iwamoto, T., 2739
 Izod, T., 605
- J
- Jaffe, H., 993
 Jahnová, V., 1559
 Jain, R., 2661
 James, H., 1139
 Janča, J., 1559
 Jandel, A.-S., 1395
 Janini, G. M., 259
 Jaroniec, M., 81
 Jauge, P., 845
 Jinno, K., 1065, 1631
 Johnson, D. C., 1727
 Johnson, J. F., 2723
 de Jong, H. C., 1745
 de Jong, J., 2255
 Judd, R. C., 1421
 Justice, J. B., Jr., 1873
- K
- Kamlesh, 145
 Kapeghian, J., 1319
 Karlesky, D., 471
 Keddington, J., 1785
 Kemula, W., 1823, 1837
 Khan, M. A., 959
 Khanna, J. M., 907
 Kieboom, A. J., 1123
 Kiefer, B. A., 927
 Kiel, J. S., 2761
 Kim, C., 907
 King, R., 605, 1015
 Kissinger, P. T., 1863, 2311
 Klassen, R., 895
 Kneifel, H., 1395

Knox, J. H., S1
 Kono, H., 2739
 Korte, K., 55
 Kfestan, L., 1759
 Kreuzig, F., 1227
 Krull, I. S., 605, 1015, 2109,
 2173, S125
 Kuhnert, B., 2775
 Kuhnert, P., 2775
 Kumari, K., 973
 Kumari, V., 155
 Kuo, C.-Y., 667
 Kutner, W., 1823, 1837

L

Laakso, J. T., 453
 Lähdesmäki, P., 2621
 Larsson, A., 655
 Leach, S. J., 199
 Lee, L. S., 833
 Lee, R. E., Jr., 1139
 LeGrand, R., 1099
 Leisztner, L., 2647
 Leveno, K. J., 2747
 Levin, S. S., 179
 Lim, C. K., 375, 887, 1969
 Lin, J.-T., 1987
 Lipari, F., 425
 Loubinoux, B., 1705
 Low, G. K. C., 311
 Lugari, M. T., 1073
 Lunte, C. E., 1863
 Lurie, I. S., 1617

M

McCormack, J. J., 1239
 McEntire, J. E., 1099
 McGovern, E. P., 1523
 MacGregor, J. F., S179
 Malihi, F. B., 667
 Malinowska, I., 81, 2665
 Mangia, A., 1073
 Mangold, D. J., 1523
 Manning, D. L., 705
 Markowski, W., 63
 Martin, D. A., 2465
 Martin, R. A., Jr., 927, 2067
 Martínez, E., 527

Marty, M., 939
 Maskarinec, M. P., 705
 Masoud, A. N., 1291
 Massano, G., 551
 Massart, D. L., 2521
 Matula, T. I., 2557
 May, W. E., 2709
 Meinhofer, J., 647
 Meinsma, D. A., 3211
 Meira, G. R., 2411
 Melbin, G., 1603
 Meulemans, A., 575
 Meyer, M. C., 743, 1513
 Michael, A. C., 1873
 Michalewsky, J., 647
 Mietling, S. W., 871
 Minasian, E., 199
 Mineo, L. C., 1175
 Miner, D. J., 2209
 Mitchell, M., 1441
 Miyahara, T., 2739
 Modai, A., 575
 Mohamed, M. E., 715, 1491
 Mohler, J., 575
 Morgan, S. L., 2761
 Mori, S., 813, S61
 Morita, T., 2093
 Mueller, H., S1
 Munir, K. M., 2829
 Murray, G. M., 931
 Muschik, G. M., 259, 825
 Myers, R. D., 343, 2043

N

Nabi, S. A., 109, 777
 Nair, J., 2829
 Nambara, T., 1977
 Neill, D. B., 1873
 Neue, U., 605, 1015, 2109
 Neuzil, E., 2501
 Newburger, J., 1333
 Newhart, A., 2109
 Newman, R. A., 1239
 Nieminen, E. H., 453
 Nilsson-Ehle, I., S251
 Niño, J. A., 1523
 Noggle, F. T., Jr., 2005

O

Ogawa, T., 1385
 Ohashi, S., 271, 755
 Óscik, J., 81
 Otto, M., 685

P

Pahuja, S. L., 127
 Pakuts, A. P., 2557
 Parker, C. E., 725
 Patel, N. T., 1407
 Pearson, J. D., 497, 1441
 Pei, P., 627
 Peng, G. W., 1499
 Penlidis, A., S179
 Perez, R. L., 353
 Perkins, E. G., 367
 Peterson, L. R., 2513
 Pick, J., 2647
 Pietrzyk, D. J., 1645
 van der Poel, C. J., 1849
 Pohl, C. A., 1577
 Polta, J. A., 1727
 Polyák, B., 2635
 Ponieważ, M., 2665
 Poppe, H., 2231
 Potter, N. M., 805
 Prasad, C. V. C., 951
 Prasad, V. K., 1949
 Provder, T., 667
 Purdy, W. C., 2275

Q

Quirk, J. G., 2747
 Qureshi, M., 165, 1249

R

Rabel, F. M., 2465
 Radzik, D. M., 2311
 Rammell, C. G., 1123
 Rankin, N. E., 1591
 Rao, K. N., 951
 Rao, R. A. K., 109, 777
 Rathore, H. S., 973
 Rawat, J. P., 959

Reena, 139
 Regnier, F. E., 497, 1441
 Reid, T. W., 127
 Rempel, R. P., 585
 Rideout, J. M., 887, 1969
 Rigglin, R. M., 1897
 Ring, G. J. F., 401
 Rocklin, R. D., 1577
 Rogers, M. E., 2019
 Rojas, R., 1139
 van Rooijen, H. W., 2231
 Ross, R. M., 1265
 Rożyło, J. K., 2665
 Rudin, A., 2433

S

Saarinen, L. H., 453
 Sacco, D., 2543
 Sacco, T., 543
 Saint-Joly, C. M., 1955
 Sakai, M., 1385
 Sakurai, E., 1907
 Sander, L. C., 2709
 Santasania, C. T., 2109
 Sato, T., 1631
 Saunders, G., 2019
 Savory, J., 1265
 Schroeder, L. R., 401
 Seki, T., 1131
 Sepaniak, M. J., 931
 Sethi, B. M., 165, 1249, 1253
 Shah, V. P., 1949
 Shamsa, F., 1977
 Sharma, R. S., 199
 Sharma, S. D., 165, 1249, 1253
 Sharma, T. R., 1253
 Sheeley, R. M., 927
 Sheldon, J. W. S., 887
 Shelly, D. C., 471
 Sherma, J., 95, 1175, 1183, 1193,
 2679
 Shoef, N., 2797
 Shoup, R. E., 1785
 Shulman, S. D., 35
 Siddiqi, W. A., 777
 Siddiqui, Z. M., 109
 Sielicki, P. J., Jr., 2679
 Siemion, C. C., 765
 Singh, V. P., 155
 Singhal, R. P., 2095

- Skibic, M. J., 2209
 Sleckman, B. P., 95, 1175
 Small, H. (book review), 1547
 Smith, B. J., 1265
 Smith, R. L., 1645
 Smoll, D. B., 2095
 Snyder, K. P., 2067
 Soczewinski, E. (book review), 1549
 Sood, V. K., 1499
 Souter, R. W., 1221
 Srivastava, S. P., 139, 145
 Stahr, J. M., 123
 Statton, R. A., 401
 Stavchansky, S., 1333
 Stavric, B., 895
 Stine, G. Y., 23
 Suckow, R. F., 1111, 2195
 Suñol, C., 527
 Suzuki, T., S61
 Swarin, S. J., 425
 Swynnerton, N. F., 1523
 Szajáni, B., 2635
- T
- Takeuchi, Y., 755
 Tallman, D. E., 2157
 Tawakkol, M. S., 1491
 Thind, P. S., 1153
 Thomas, B. R., 2055
 Timonen, M., 2621
 Tirrell, M., S219
 Tishbee, A., 2797
 Tjaden, U. R., 2255
 Tokunaga, Y., 271, 755
 Touchstone, J. C., 179
 Towles, D. G., 1171
 Tran, K. C., 1081
 Trofanek, A., 1759
 Truex, C. R., 367
 Tusa, J., S77
 Tusell, J. J., 527
- U
- Unger, K. K., S1
 Upadhyay, R. K., 155
- V
- Vajda, J., 2647
 Varshney, K., 1535
 Varshney, K. G., 1535
 Vemulapalli, G. K., 1777
 Verlangieri, A., 1319
 Visek, W. J., 367
 Voigtman, E., 1275
 Voogt, W. H., 1745
 Voris, J. C., 2761
- W
- Wada, A., S61
 Wages, S. A., 1873
 Waki, H., 271
 van der Wal, S., S37
 Walker, M., 1475, 1949
 Wallace, G. G., 1799
 Wallace, J., 1333
 Ward, J. L., 511
 Warner, I. M., 471
 Warshawsky, A., 2797
 Wegscheider, W., 685
 Wehmeyer, K. R., 2141
 Weisshaar, D. E., 2157
 Westerink, B. H. C., 2337
 Wilke, D. L., 861
 Wills, M. R., 1265
 Wilson, G. S., 1591
 Wilson, W. E., 871
 Winefordner, J. D., 1275
 Wise, S. A., 2709
 Wong, J. L., 1935
 Wright, D. J., 383
 Wright, D. S., 2141
 Wright, J. E., 2055
- X
- Xie, K.-H., 605, 1015, 2109, S125
- Y
- Yamaguchi, Y., 375, 1131
 Yamazaki, M., 2079

Yang, R.-K., 1997
Yates, W. R., 2723

Yoshiura, M., 2739
Young, R. S., 1591

SUBJECT INDEX TO VOLUME 6

A

- 2-AAF. See 2-Acetylaminofluorene (2-AAF)
- 2-Acetylaminofluorene (2-AAF), in laboratory diets, determination of by HPLC, 367-373
- Acetylated cellulose, separation of amino acids on RP-TLC plates of, 95-108
- Acids
- HPLC determination of for oenology, S153-S158
 - See also Arachidonic acid; Aromatic acids; Ascorbic acid; Barbitoric acid; Dehydroascorbic acid; Iodoamino acid; Organic acids
- Acrylic latexes, determination of gel content of by SEC, 667-683
- Adenine, in cacao products, identification of by HPLC, 927-930
- Adhesives, pressure sensitive, use of multiple detector SEC in determination of aging in, 2723-2737
- Adrenaline, dansyl derivatives of, chemiluminescence detector for trace determination of, 1603-1616
- Adsorbent, role of surface area of, in TLC, 2665-2677
- Adsorption behavior
- of metal ions in aqueous HCL systems using stannic arsenate layers, determination of by TLC, 1253-1264
 - of solvents with molecules of different polarity, 63-71
- Adsorption effects, in GPC, 1385-1394
- AEC. See Anion exchange chromatography (AEC)
- Aflatoxins, HPLC determination of for oenology, S167
- Aging, of pressure sensitive adhesives, use of multiple detector SEC in determination of, 2723-2737
- Air pollutants. See Amines; Formaldehyde; Isocyanates
- Alcohols, polymeric permanganate oxidation of, in HPLC with solid phase reactor, 2109-2127
- Aldehydes
- determination of, by RP-HPLC with fluorescence detection, 425-444
 - polymeric permanganate, oxidation of in HPLC with solid phase reactor, 2109-2127
- n-Alkyl chain length. See Chain length, n-alkyl
- Aluminum, in alloys and silicate rocks, quantitative determination of after cation-exchange separation on zirco-

Note: Numbers with an "S" prefix indicate entries appearing in Supplements 1 and 2 of Volume 6.

- [Aluminum]
 nium(IV) phospho and silico arsenates, 1535-1545
- Aluminum hydroxide, separation of organic acids on papers impregnated with, 973-981
- α -Amanitin, in serum, separation of, by RP-HPLC, 551-558
- Amine metabolites, automated analysis of brain extracts for, by HPLC, 871-886
- Amine modifiers, determination of salbutamol by RP-HPLC with, 1955-1967
- Amines
 analysis of impurities in, by RP-HPLC using binary and ternary solvent mixtures, 311-323
 carcinogenic, determination of in work atmosphere by RP-HPLC, 453-469
 in beer, determination of, by RP-HPLC with laser fluorescence, 931-938
 separation of, on papers impregnated with zinc silicate, 959-972
See also Biogenic amines; Biogenic amine metabolites
- Amino acid mixtures, separation of glutathione from, by TLC, 2635-2645
- Amino acids
 chemiluminescence detector for trace determination of, 1603-1616
 conversion of in membrane reactor, HPLC in combination with dansylation technique for monitoring, 1395-1405
 electrochemical detection of, at platinum electrode in alkaline chromatographic effluent, 1727-1743
 HPLC determination of for oenology, S167-S168
 separation of by TLC on stannic tungstate, 109-122
 separation of on RP-TLC plates, 95-108
- Amino acid sequence, of 12K fragment of avian sarcoma virus structural protein p27, determination of using HPLC, 249-258
- Amino bonded phase column. See Column, amino bonded phase
- Amino coumarin dyes. See Dyes, amino coumarin
- S-2-(3-Aminopropylamino)ethyl phosphorothioate. See Radioprotective drug
- Amperometric detection
 pulsed, determination of carbohydrates by AEC, with, 1577-1590
See also Coulometric-amperometric detection
- Analgesics, in serum and urine, determination of by LCEC, 2311-2335
- Androgen acetates, separation of by HPLC, 1987-1995
- Animal feed, analysis of diazepam in, by HPLC, 2557-2564
- Anion-exchange chromatography (AEC), with pulsed amperometric detection, determination of carbohydrates by, 1577-1590
- Anion-exchange extraction, assay of azocillin and mezlocillin by HPLC with, 2513-2520
- Anionic surfactants. See Surfactants, anionic, cationic, and nonionic
- Antibiotics
 in biological fluids, analysis of by HPLC, S251-S293
See also Azocillin; Mezlocillin; Penicillins; Piperacillin
- Anticonvulsant bensodiazepine. See Clonazepam
- Antidiabetic compounds, potential, identification of by TLC, 2661-2664
- Arachidonic acid, separation of by one-dimensional TLC, 55-62
- Arbaprostil, separation of by HPLC, 1499-1511

- Arene oxides, K-region, of diastereoisomeric glutathione adducts, separation of by RP-HPLC, 1693-1704
- Aromatase, hypothalamic, rapid assay for, using HPLC, 861-870
- Aromatic acids, chromatographic behavior of on macroporous ion-exchange resin, 1081-1097
- Aromatic isocyanates. See Isocyanates, aromatic
- Aromatic sulfonic acids. See Sulfonic acids, aromatic
- Arsenates, zirconium(IV) phospho and silico, quantitative determination of iron and aluminum in alloys and silicate rocks after cation-exchange separation on, 1535-1545
- Ascorbic acid, determination of by RP-HPLC, 1319-1332
- Automated analyses, of brain extracts for dopamine, serotonin, and amine metabolites by HPLC, 871-886
- Avian sarcoma virus, amino acid sequence of 12K fragment of structural protein p27 of, determination of using HPLC, 249-258
- Azocillin, assay of by HPLC with anion-exchange extraction, 2513-2520
- B
- Bacteria, analysis of phospholipid composition of, by TLC, 2699-2707
- Barbituric acid, derivatives of, correlation of chromatographic parameters for, with modified valence connectivity index, 73-80
- Beer, determination of amines in by RP-HPLC with laser fluorescence, 931-938
- Biogenic amine metabolites, determination of by micro-HPLC with dual electrochemical detection, 1907-1925
- Biogenic amines
gradient elution of, in ion-pair RP-HPLC with electrochemical and fluorometric detection, 2255-2274
HPLC determination of for oenology, S159-S160
- Biological fluids
analysis of antibiotics in, by HPLC, S251-S293
analysis of drugs in, by HPLC using flow programming, 1949-1954
analysis of indenolol in, by HPLC, 2785-2795
analysis of phospholipids in, using TLC, 179-192
determination of chloroquine and desethylchloroquine in, by HPLC, 1513-1522
determination of reserpine and trichlormethiazide in, by micro-HPLC, 2383-2409
- Biological samples
determination of pharmaceuticals in, by LCEC, 2209-2230
determination of α -tocopherol in by HPLC, 1123-1130
- Biopterin, in tissue samples, determination of using ion-pair RP-HPLC with electrochemical detection, 1863-1872
- Biphenyl, determination of in citrus fruits, by TLC, 2679-2685
- 2,2'-Bipyridine complexes, separation of nickel and iron complexes as, with ion pair HPLC, 1073-1080
- Blocking agent, β -adrenergic. See Indenolol hydrochloride
- Blood
determination of chlormethiazole in by RP-HPLC, 907-916
See also Biological fluids
- Blood elements, isolation of glycolipids from, for liquid chromatography, 2647-2660

- Bonded phases, octyl and diphenyl, separation of protein and peptides by HPLC on, 2465-2499
- Borohydride/silica reductions. See Reductions, borohydride/silica
- Brain indoleamine metabolites, of tryptophan, fluorometric determination of by ion pair HPLC, 527-541
- Brain tissue
analysis of trace amounts of catecholamines in, by LCEC, 2337-2351
See also Mouse brain
Bricanyl. See Terbutaline
- Buffers, effect of on retention behavior of ionogenic species in RP-HPLC, 684-704
- C
- Cacao products, identification of adenine in by HPLC, 927-930
- Cadmium hydroxide, separation of organic acids on papers impregnated with, 973-981
- Caffeine metabolites, separation of by HPLC, 895-906
- Calibration, dispersion-compensated, for GPC, 401-424
- Carbamates, separation of using TLC on plates impregnated with zinc acetate, 139-143
- Carbohydrates
determination of by AEC with pulsed amperometric detection, 1577-1590
evaluation of eluent recycling and column life for HPLC analysis of, 1139-1151
- Carbon electrodes. See Electrodes, carbon
- Carbon packings
application of, S29-S31
chromatographic studies on, S14-S29
preparation of, S4-S13
use of in HPLC, S1-S36
- Carbonyl compounds
HPLC determination of for oenology, S160-S161
on-line HPLC derivatizations via borohydride/silica reductions for, 1015-1036
polymeric reductions for, by RP-HPLC, solid phase derivatization reactions in, 605-626
- Carboxylic acids, low molecular weight, in water, determination of by RP-HPLC with conductivity detection, 705-714
- Catecholamines
high-speed analysis of, using LCEC, 2335-2373
trace amounts of in brain tissue, analysis of by LCEC, 2337-2351
- Cation-exchange separation, on zirconium(IV) phospho and silico arsenates, quantitative determination of iron and aluminum in alloys and silicate rocks after, 1534-1545
- Cationic surfactants. See Surfactants, anionic, cationic and nonionic
- Cefamandole, in serum, determination of by HPLC, 2747-2759
- Cefoperazone, in plasma, urine, and CSF, microassay for by RP-HPLC, 743-754
- Cell extracts, analysis of deoxythymidine in, by RP-HPLC, 655-666
- Cellulose. See Acetylated cellulose
- Cephalosporin C, separation of by ligand exchange chromatography, 2543-2556
- Cerebrospinal fluid
determination of piperacillin in by RP-HPLC, 575-583
microassay for cefoperazone in by RP-HPLC, 743-754
- β -Chain, of hemoglobins, separation of tryptic peptides of by RP-HPLC, 199-213

- Chain length, n-alkyl, influence of on protein retention, resolution, and recovery in RP-HPLC, 497-510
- Chelates, nickel and iron, separation of as 2,2'-bipyridine complexes with ion pair HPLC, 1073-1080
- Chemiluminescence detector, for trace determination of fluorescent compounds, 1603-1616
- Chemotherapy agents, platinum-derived, dual electrode LCEC for, 2173-2194
- Chlormethiazole, in blood, determination of by RP-HPLC, 907-916
- 1-m-Chlorophenylpiperazine, in plasma, determination of by LCEC, 2195-2208
- Chloroquine, in biological fluids, determination of by HPLC, 1513-1522
- Chromatographic behavior
of aromatic acids on macroporous ion-exchange resin, 1081-1097
of metal ions on TBP impregnated silica gel-G layers, 165-177
- Chromatographic effluent, alkaline, electrochemical detection of amino acids with platinum electrode in, 1727-1743
- Chromatographic mobility, of tetrabutylammonium and related thiazoles, relationship of to structure, 1221-1226
- Chromatographic papers
aluminum and cadmium hydroxide, separation of organic acids on, 973-981
ferric phosphate, separation of drugs on, 1153-1162
zinc silicate, separation of amines on, 959-972
- Chromatographic parameters, for barbituric acid derivatives, correlation of with modified valence connectivity index, 73-80
- Chromatography (book review), 2129-2130
- Chromatography discussion groups (announcement), 2131
- Chromatotron, isolation of glycoalkaloids with and HPLC, 281-289
- Citrus fruits, determination of biphenyl in, by TLC, 2679-2685
- Clonazepam
determination of in human plasma by HPLC, 301-310
and metabolites, separation of by RP-HPLC, 585-598
- H-Coal liquids, fractionation of by polyvinylacetate GPC, 1935-1948
- Cocoa beans, determination of iodine in by anion HPLC, 2067-2077
- Column life, evaluation of for HPLC analysis of carbohydrates, 1139-1151
- Columns
amino bonded phase, study of polynuclear aromatic hydrocarbons on, 471-495
comparative evaluation of for analysis of drugs of forensic interest by ion-pair RP-HPLC, 1617-1630
high efficiency, for HPLC, 765-775
microbore. See High performance (pressure) liquid chromatography (HPLC), micro
short, containing ODS particles, separation of polycyclic aromatic hydrocarbons by HPLC, using, 1375-1383
tin(IV)-iodophosphate, synthesis and ion exchange properties of, 777-794
twin phase, ion exchange, and reverse phase, separation of organic acid in plant tissue by HPLC with, 1705-1716
- Complex formation, of monomeric phosphorus oxoacids with sodium ion in DEC, 755-763

Computers

- control of HPLC by, hardware for, 2095-2108
- use of for data acquisition and analysis for SEC, 1037-1063
- Conductivity detection, determination of low molecular weight carboxylic acids by RP-HPLC with, 705-714
- Corticosteroids, detection of by fluorimetric method using post-column derivatization with glycinamide, 1131-1138
- Cotton bracts, determination of lacinilene C 7-methyl ether in, by HPLC, 917-926
- Coulometric-ampereometric detection, thyroid hormone assay using, 2275-2310
- Crystalline quartz detector, for HPLC, 1275-1289
- Cyclopentylphenol, separation of isomers of by TLC, 1171-1174
- Cytochrome C-derived peptides. See Peptides, cytochrome-C derived

D

- Dansylation technique, in combination with HPLC for monitoring amino acid conversion in membrane reactor, 1395-1405
- Data acquisition and analysis, computer assisted, for SEC, 1047-1063
- DEC. See Donnan exclusion chromatography (DEC)
- Degradation, of tetrahydroisoquinolines, determination of effect of temperature on, by HPLC, 343-352
- Dehydroascorbic acid, determination of by RP-HPLC, 1319-1332
- Densitometry
 - determination of pulegone in He-deoma pulegioides and peppermint oil by TLC with, 1175-1182
 - RP-TLC with, quantitation of herbicides by, using Bratton-Marshall detection reagent, 1183-1192
- Deoxynivalenol. See Vomitoxin
- Deoxyribonucleosides, separation of by RP-HPLC using tetrabutylammonium hexafluorophosphate, 1673-1691
- Deoxythymidine, in cell extracts, analysis of by RP-HPLC, 655-666
- Derivatization
 - determination of penicillins by HPLC with, 2019-2031
 - in HPLC, solid phase reactions for, S125-S151
 - on-line HPLC, via borohydride/silica reductions for carbonyl compounds, 1015-1036
 - post-column, with glycinamide, detection of corticosteroids by fluorimetric method using, 1131-1138
- Derivatization reactions
 - polymer supported, in HPLC, S137-S139
 - solid phase, in polymeric reductions for carbonyl compounds by RP-HPLC, 605-626
- Desethylchloroquine, in biological fluids, determination of by HPLC, 1513-1522
- Detection reagent
 - Bratton-Marshall, quantitation of herbicides by RP-TLC with densitometry, using, 1183-1192
 - comparison of for lipid and phospholipid detection and densitometry on silica gel and C₁₈ thin layers, 1193-1211
- Detection systems. See Post-column reactors
- Diastereoisomeric glutathione
 - separation of adducts of K-region arene oxides of, by RP-HPLC, 1693-1704
 - separation of styrene oxide adducts of, by HPLC, 1475-1489

- Diastereoisomeric impurities. See Impurities, enantiomeric and diastereoisomeric
- Diazepam, in animal feed, analysis of by HPLC, 2557-2564
- Differential refractometer (RI), for quantitative analysis in HPLC, effect of temperature on response of, 813-824
- Digital smoothing, effect of on HSLC, 2809-2828
- Digoxin, detection of by tensametry in RP-HPLC, 1745-1758
- Diphenyl bonded phase, separation of proteins and peptides by HPLC on, 2465-2499
- Dispersion-compensated calibration. See Calibration, dispersion compensated
- Dithiolthiones, determination of by HPLC, 1275-1289
- Donnan exclusion chromatography (DEC)
 complex formation of monomeric phosphorus oxoacids with sodium ion in, 755-763
 elution behavior of organic acids in, 271-280
- Dopamine, automated analysis of brain extracts for by HPLC, 871-886
- Drift, in electrochemical detectors with solid electrodes, 2231-2254
- Drugs
 in biological fluids, analysis of by HPLC using flow programming, 1949-1954
 of forensic interest, analysis of by ion-pair RP-HPLC, comparative evaluation of columns for, 1617-1630
 separation of, on ferric phosphate papers, 1153-1162
 standardized analysis of by ion-pair HPLC, 2521-2541
See also Pharmaceuticals; Radioprotective drug; Sulpha drugs
- Dual electrochemical detection. See Electrochemical detection, dual
- Dual electrode LCEC, for platinum-derived chemotherapy agents, 2173-2194
- Dyes, amino coumarin, purification of by RP-HPLC, 951-958
- E
- Eastern analytical symposium (announcement), 397-398
- Ecdysteroids, of boll weevil, determination of by HPLC, 2055-2066
- Electrochemical detection
 of amino acids at platinum electrode in alkaline chromatographic effluent, 1727-1743
 assay of MHPG by HPLC with, 1997-2003
 determination of bipterin in tissue samples using ion-pair RP-HPLC with, 1863-1872
 determination of phenylenediamines by HPLC with, 1897-1905
 determination of ritodrine in plasma by HPLC with, 2775-2783
 determination of uric acid in human serum by RP-HPLC with, 2739-2746
 determination of voltammetry in striatum by HPLC with, 1873-1896
 dual, determination of biogenic amine metabolites by micro-HPLC with, 1907-1925
 ion-pair partition RP-HPLC with, gradient elution of biogenic amines in, 2255-2274
 leak-tight glassy carbon electrodes for, 1849-1861
 separation of thyromimetic iodoamino acids by gradient elution RP-HPLC with, 511-526
 simultaneous determination of metals in zinc sulfate plant electrolyte using RP-HPLC with, 1799-1822

- [Electrochemical detection]
See also High performance (pressure) liquid chromatography (HPLC), with electrochemical detection; Liquid chromatography with electrochemical detection (LCEC)
- Electrochemical detectors, with solid electrodes noise and drift in, 2231-2254
- Electrodes
 carbon
 composite, for LCEC, 2157-2172
 leak-tight glassy, for electrochemical detection, 1849-1861
 platinum, in alkaline chromatographic effluent, electrochemical detection of amino acids with, 1727-1743
 solid, noise and drift in electrochemical detectors with, 2231-2254
- Electrokinetic detection
 of quaternary ammonium ion-pairs of volatile fatty acids in RP-HPLC, 1837-1848
 of volatile fatty acids in RP-HPLC, 1823-1835
- Eluent recycling, evaluation of for HPLC analysis of carbohydrates, 1139-1151
- Elution behavior, of organic acids in DEC, 271-280
- Elution volumes, effect of flow rate on in SEC, S219-S249
- Emulgen 911
 removal of from solubilized microsomes by HPLC, 1459-1473
See also Surfactants
- Enantiomeric impurities. See Impurities, enantiomeric and diastereoisomeric
- Engine coolants, determination of sodium tolyltriazole and sodium mercaptobenzothiazole in by HPLC, 805-812
- Enzyme immunoassay. See Immunoassay, enzyme
- Ephedrine. See Amines
- Epimeric guaianolides. See Guaianolides, epimeric
- Ethylene glycol, oligomeric mixtures of, analysis of by HPLC, 2797-2807
- Excretion patterns, of urinary phenolic compounds, determination of by HPLC, 375-381
- F
- Fast Fourier transform (FFT), correction of instrumental broadening in SEC by, 2411-2431
- Fatty acids, volatile
 electrokinetic detection of, in RP-HPLC, 1823-1835
 quaternary ammonium ion-pairs of, electrokinetic detection of in RP-HPLC, 1837-1848
- Fenitrothion, determination of in pesticides by HPLC, 353-365
- Fermentations, process control of by HPTLC, 1227-1238
- Ferric phosphate, separation of drugs on papers impregnated with, 1153-1162
- FFF. See Field-flow fractionation (FFF)
- FFT. See Fast Fourier transform (FFT)
- Field-flow fractionation (FFF)
 sedimentation-flotation focusing, in channels with modulated cross-sectional permeability, theory of, 1559-1576
See also Fractionation
- Flavonoids, analysis of, by HPLC, S105-S118
- Flow programming, analysis of drugs in biological fluids by HPLC using, 1949-1954
- Flow rate, effect of on elution volumes in SEC, S219-S249
- Flow-through detector, polarographic, 1759-1776
- Fluorescence detection
 determination of 6 β -hydroxycortisol in urine by HPLC with, 1977-1985

- [Fluorescence detection]
determination of formaldehyde by RP-HPLC with, 425-444
determination of reserpine in plasma by liquid chromatography with, 1111-1122
- Fluorimetric method, using post-column derivatization with glycinamide, detection of corticosteroids by, 1131-1138
- Fluorometric detection, ion-pair partition RP-HPLC with, gradient elution of biogenic amines in, 2255-2274
- Formaldehyde, determination of by RP-HPLC with fluorescence detection, 425-444
- Fractionation
of polymers by RP-TLC, 1-22
See also Field-flow fractionation
- Fractogel. See Gel permeation chromatography (GPC), polyvinylacetate
- G
- Gel content, of acrylic latexes, determination of by SEC, 667-683
- Gel permeation chromatography (GPC)
dispersion-compensated calibration for, 401-424
polyvinylacetate, fractionation of H-coal liquids by, 1935-1948
solvation and adsorption effects in, 1385-1394
use of Mark-Houwink constants in, 2433-2463
- Glutamic acid decarboxylase, microassay for, using ion-exchange TLC, 127-137
- Glutathione
separation of from amino acid mixtures by TLC, 2635-2645
See also Diastereoisomeric glutathione
- Glycinamide, detection of corticosteroids by fluorimetric method using post-column derivatization with, 1131-1138
- Glycoalkaloids, isolation of with chromatotron and HPLC, 281-289
- Glycolipids, isolation of from blood elements for liquid chromatography, 2647-2660
- GPC. See Gel permeation chromatography (GPC)
- Gradient elution, of biogenic amines, in ion-pair RP-HPLC with electrochemical and fluorometric detection, 2255-2274
- Guaianolides, epimeric, separation of by preparative HPLC, 543-550
- H
- Hardware, for computer controlled HPLC, 2095-2108
- Hedeoma pulegioides, determination of pulegone in by TLC with densitometry, 1175-1182
- Hemoglobins, separation of tryptic peptides of β -chain of by RP-HPLC, 199-213
- Herbicides, urea, carbamate, and anilide, quantitation of by RP-TLC with densitometry using Bratton-Marshall detection reagent, 1183-1192
- hGH. See Human growth hormone (hGH)
- High performance (pressure) liquid chromatography (HPLC)
analysis of antibiotics in biological fluids by, S251-S293
analysis of carbohydrates by, evaluation of eluent recycling and column life for, 1139-1151
analysis of diazepam in animal feed by, 2557-2564
analysis of flavonoids by, S105-S118
analysis of indanolol in biological fluids by, 2785-2795

- [High performance (pressure) liquid chromatography (HPLC)]
analysis of oligomeric ethylene glycol mixtures by, 2797-2807
analysis of pentazocine and tripeleennamine in combination by, 2005-2017
with anion-exchange extraction, assay of azocillin and mezlocillin by, 2513-2520
automated analyses of brain extracts for dopamine, serotonin, and amine metabolites by, 871-886
carbon as packing material in, S1-S36
combined with dansylation technique for monitoring amino acid conversion in membrane reactor, 1395-1405
combined with ^{125}I -labeled peptide mapping, separation of proteins of Neisseria gonorrhoeae by, 1421-1439
computer controlled, hardware for, 2095-2108
crystalline quartz, laser-excited fluorescence, photoacoustic, and two-photon photoionization detector for, 1275-1289
detection of mycoplasma contamination in lymphoblastoid cultures by, 1099-1110
determination of 2-acetylaminofluorene in laboratory diets by, 367-373
determination of amino acid sequence of 12K fragment of avian sarcoma virus structural protein p27 using, 249-258
determination of boll weevil ecdysteroids and juvenile hormones by, 2055-2066
determination of cefamandole in serum by, 2747-2759
determination of chloroquine and desethylchloroquine in biological fluids by, 1513-1522
determination of clonazepam in human plasma by, 301-310
determination of dithiolthiones by, 1291-1317
determination of effect of temperature on degradation of tetrahydroisoquinolines by, 343-352
determination of excretion patterns of urinary phenolic compounds by, 375-381
determination of indenolol hydrochloride by, 715-723
determination of iodine in milk and milk chocolate by, 2067-2077
determination of lacinilene C 7-methyl ether in cotton bracts by, 917-926
determination of N-methyl-2-pyrrolidone in water by, 445-452
determination of metribuzin and metabolites in plant tissue by, 725-742
determination of naloxone hydrochloride by, 1491-1497
determination of organics in high purity water, by, 2565-2570
determination of radioprotective drug by, 1523-1534
determination of sodium tolyltriazole and sodium mercaptobenzothiazole in engine coolants by, 805-812
determination of stability of promethazine hydrochloride by, 1333-1344
determination of α -tocopherol in biological specimens, by, 1123-1130
determination of verapamil and norverapamil in serum by, 887-893
effect of temperature on response of RI for quantitative analysis in, 813-824
with electrochemical detection assay of MHPG by, 1997-2003
determination of phenylenediamines by, 1897-1905
determination of ritodrine in plasma by, 2775-2783

- [High performance (pressure) liquid chromatography (HPLC), with electrochemical detection]
determination of voltammetry in striatum by, 1873-1896
See also Electrochemical detection; Liquid chromatography with electrical detection (LCEC)
with fluorescence detection, determination of 6 β -hydroxycortisol in urine by, 1977-1985
identification of adenine in cacao products by, 927-930
ion-pair. See Ion-pair high performance liquid chromatography
isolation of glycoalkaloids with chromatotron and, 281-289
isolation of individual peptides from thymus fraction by, 647-654
micro
determination of reserpine and trichlormethiazide in biological fluids by, 2382-2409
with dual electrochemical detection, determination of biogenic amine metabolites by, 1907-1925
evaluation of for separation of styrene oligomers, 1631-1643
use of for monitoring reaction processes, 1065-1071
on octyl and diphenyl bonded phases, separation of proteins and peptides by, 2465-2499
in oenology, S153-S158
on-line derivatizations in, via borohydride/silica reductions for carbonyl compounds, 1015-1036
post column reactors in, S37-S59
preparation of high efficiency columns for, 765-775
preparative
separation of epimeric guaianolides by, 543-550
separation of lubricating base oil into hydrocarbon fractions by, 627-645
purification of human platelet monamine oxidase B by, 1407-1419
rapid assay for hypothalamic aromatase using, 861-870
removal of Emulgen 911 from solubilized microcomes by, 1459-1473
separation and characterization of cytochrome C-derived peptides by, 1591-1601
separation of androgen acetates by, 1987-1995
separation of arbaprostil by, 1499-1511
separation of caffeine metabolites by, 895-906
separation of diastereoisomeric glutathione adducts of styrene oxide by, 1475-1489
separation of organic acid in plant tissue by, 1705-1716
separation of polycyclic aromatic hydrocarbons by, using short columns containing ODS particles, 1375-1383
separation of proteins of locust neurosecretory endings by, 2501-2512
separation of shale oil by, 325-342
simultaneous determination of warfarin, sulphaquinoxaline, and fenitrothion in pesticides by, 353-365
size exclusion. See Size exclusion chromatography (SEC)
solid phase reactions for derivatization in, S125-S151
with solid phase reactor, polymeric permanganate oxidations of alcohols and aldehydes in, 2109-2127
use of in combination with TLC for determination of tiazofurin in serum, 1239-1247
using flow programming, analysis of drugs in biological fluids by, 1949-1954

- High performance thin layer chromatography (HPTLC), process control of fermentations by, 1227-1238
- High speed liquid chromatography (HSLC), effect of sampling rate and digital smoothing on, 2809-2828
- HPLC. See High performance (pressure) liquid chromatography (HPLC); Reversed phase high performance liquid chromatography (RP-HPLC)
- HPTLC. See High performance thin layer chromatography (HPTLC)
- Human growth hormone (hGH), and its 20k dalton variant, separation of tryptic peptides of by RP-HPLC, 215-228
- Human plasma, determination of clonazepam in by HPLC, 301-310
- Hungarian chemical society (announcement), 2839
- Hydrocarbons
 polynuclear aromatic, study of on amino bonded phase column, 471-495
See also Shale oil
- Hydrocarbon fractions, separation of lubricating base oil into by preparative HPLC, 627-645
- Hydrochloric acid (HLC) systems, using stannic arsenate layers, adsorption behavior of metal ions in, determined by TLC, 1253-1264
- Hydronamic chromatography (HDC), separation of particle suspensions by, S179-S217
- 6 β -Hydroxycortisol, in urine, determination of by HPLC with fluorescence detection, 1977-1985
- I
- Immunoassay, enzyme, LCEC of phenol and NADH for, 2141-2156
- Impurities, enantiomeric and diastereoisomeric, in amines, analysis of using RP-HPLC, 311-323
- Indenolol, in biological fluids, analysis of by HPLC, 2785-2795
- Indenolol hydrochloride, and pharmaceutical formulation, determination of by HPLC, 715-723
- Indoleamine metabolites. See Brain indoleamine metabolites
- Indole derivative, antineoplastic, determination of by RP-HPLC, 939-949
- Infrared spectroscopy. See Spectroscopy, micro infrared
- Inorganic ions, separation of using TLC on plates impregnated with sulphadiazole, 145-153
- Insect hormones, analysis of by radial compression separation system, 291-299, 1725
- Instrumental broadening, in SEC, correction of by FFT, 2411-2431
- Instrumental spreading, Tung's equation for, in SEC, S61-S75
- Interfacial tension, role of in RP-HPLC, 1777-1784
- Iodine, in milk and milk chocolate, determination of by anion HPLC, 2067-2077
- Iodoamino acids, thyromimetic, separation of by gradient elution RP-HPLC with electrochemical detection, 511-526
- Ion-exchange chromatography (IEC) fractionation of acidic ninhydrin-positive compounds of mouse brain by, 2621-2634
 separation of thiol compounds by, 1785-1798
- Ion-exchange high performance (pressure) liquid chromatography, separations by, using high content organic modifier mobile phase, 825-831

- Ion-exchange materials. See Chromatographic papers
- Ion-exchange properties, of tin (IV)-iodophosphate columns, 777-794
- Ion exchangers, in analytical chemistry (book review), 1547
- Ion-exchange thin layer chromatography, microassay for glutamic acid decarboxylase using, 127-137
- Ion-pair high performance (pressure) liquid chromatography fluorometric determination of tryptophan and brain indoleamine metabolites by, 527-541
separation of nickel and iron chelates as 2,2'-bipyridine complexes with, 1073-1080
standardized analysis of drugs by, 2521-2541
- Ionogenic species, retention behavior of in RP-HPLC, effect of buffers on, 685-704
- Iron, in alloys and silicate rocks, quantitative determination of after cation-exchange separation on zirconium(IV) phospho and silico arsenates, 1535-1545
- Isocyanates, aromatic, determination of in work atmosphere by RP-HPLC, 453-469
- Isomers, of cyclopentylphenol, separation of by TLC, 1171-1174
- J
- Juvenile hormones, of boll weevil, determination of by HPLC, 2055-2066
- L
- Laboratory diets, determination of 2-acetylaminofluorene in by HPLC, 367-373
- Lacinilene C 7-methyl ether, in cotton bracts, determination of by HPLC, 917-926
- Laser fluorescence, determination of amines in beer by RP-HPLC with, 931-938
- Laser-excited fluorescence detector, for HPLC, 1275-1289
- Latexes. See Acrylic latexes
- LCEC. See Liquid chromatography with electrochemical detection (LCEC)
- Ligand exchange chromatography, separation of cephalosporin C by, 2543-2556
- Lincomycin, diagnostic toxicological analysis of using TLC, 123-126
- Linear thin layer chromatography. See Thin layer chromatography (TLC), linear
- Lipids, detection of by RP-TLC with densitometry, 1193-1211
- Liquid chromatography
isolation of glycolipids from blood elements for, 2647-2660
with fluorescence detection, determination of reserpine in plasma by, 1111-1122
- Liquid chromatography calendar, 197-198, 399-400, 601-603, 799-803, 987-991, 1165-1169, 1347-1351, 1553-1556, 1721-1724, 1931-1934, 2137-2140, 2379-2381, 2573-2575, 2845-2847, S121-S123
- Liquid chromatography directory, 2579-2619
- Liquid chromatography news, 193-195, 395-396, 599-600, 795-797, 983-985, 1163-1164, 1345-1346, 1551-1552, 1717-1719, 1927-1929, 2133-2136, 2375-2377, 2841-2843, S119-S120
- Liquid chromatography with electrochemical detection (LCEC)
analysis of trace amounts of catecholamines in brain tissue by, 2337-2351
carbon composite electrodes for, 2157-2172

[Liquid chromatography with electrochemical detection LCEC)]
 determination of analgesics in serum and urine by, 2311-2335
 determination of pharmaceuticals in biological samples by, 2209-2230
 determination of trazodone and 1-m-chlorophenylpiperazine in plasma by, 2195-2208
 dual electrode, for platinum-derived chemotherapy agents, 2173-2194
 high speed analysis of phenols and catecholamines using, 2335-2373
 of phenol and NADH for enzyme immunoassay, 2141-2156
 quantitative analysis of terbutaline in human plasma by, 559-574
See also Electrochemical detection; High performance (pressure) liquid chromatography (HPLC), with electrochemical detection
 Lymphoblastoid cultures, detection of mycoplasma contamination in by HPLC, 1099-1110
 Lynestrenol, detection of by ten-sammetry in RP-HPLC, 1745-1758

M

Manufacturers. See Liquid chromatography directory
 Mark-Houwink constants, use of in GPC, 2433-2463
 Metabolite, of trazodone. See 1-m-Chlorophenylpiperazine
 Metal chelates. See Chelates, nickel and iron
 Metal ions
 chromatographic behavior of on TBP impregnated silica gel-G layers, 165-177
 in HCL systems using stannic arsenate layers, adsorption behavior of, determined by TLC, 1253-1264
 3d transition, analysis of using TLC, 155-164
 quantitative separation of by TLC, 1249-1252
 Metals
 heavy, in water, determination of by RP-HPLC, 2079-2093
 simultaneous determination of in zinc sulfate plant electrolyte using RP-HPLC with electrochemical and spectrophotometric detection, 1799-1822
 3-Methoxy-4-hydroxythylene glycol (MHPG), assay of by HPLC with electrochemical detection, 1997-2003
 N-Methyl-2-pyrrolidone, in water, determination of by HPLC, 445-452
 Metribuzin, determination of and metabolites in plant tissue by HPLC, 725-742
 Mezlocillin, assay of by HPLC with anion-exchange extraction, 2513-2520
 MHPG. See 3-Methoxy-4-hydroxythylene glycol (MHPG)
 Microassay, for glutamic acid decarboxylase, using ion-exchange TLC, 127-137
 Microbore column HPLC. See High performance (pressure) liquid chromatography (HPLC), micro
 Micro-HPLC. See High performance (pressure) liquid chromatography (HPLC), micro
 Micro technique, HPLC, for monitoring reaction processes, 1065-1071
 Microsomal incubations, rat liver, quantitation of clonazepam from, 585-598
 Microsomes, solubilized, removal of Emulgen 911 from, by HPLC, 1459-1473
 Milk, determination of iodine in by anion HPLC, 2067-2077

- Mobile phase
 high content organic modifier, separations by ion-exchange HPLC using, 825-831
 mixed, parameters characterizing TLC systems with, 81-93
 selection of for optimal separation in RP-HPLC, 259-269
- Monoamine oxidase B, human platelet, purification of by HPLC, 1407-1419
- Monomeric phosphorus oxoacids, complex formation of with sodium ion in DEC, 755-763
- Mouse brain, fractionation of acidic ninhydrin-positive compounds of, by TLC and IEC, 2621-2634
- Mycoplasma, detection of contamination by in lymphoblastoid cultures by HPLC, 1099-1110
- N
- NADH. See Nicotinamide adenine dinucleotide (NADH)
- Naloxone hydrochloride, determination of by HPLC, 1491-1497
- Neuropeptides, invertebrate, analysis of by RP-HPLC, 993-1013
- Neurosecretory endings, of locust, separation of proteins of by HPLC, 2501-2512
- Nicotinamide adenine dinucleotide (NADH), LCEC of for enzyme immunoassay, 2141-2156
- Neisseria gonorrhoeae, separation of proteins of, by HPLC combined with ¹²⁵I-labeled peptide mapping, 1421-1439
- Ninhydrin-positive compounds, acidic, of mouse brain, fractionation of by TLC and IEC, 2621-2634
- Noise, in electrochemical detectors with solid electrodes, 2231-2254
- Nonionic detergent. See Emulgen 911
- Nonionic surfactants. See Surfactants, anionic, cationic and nonionic
- Noradrenaline, dansyl derivatives of, chemiluminescence detector for trace determination of, 1603-1616
- Norverapamil, determination of by HPLC, 887-893
- Nucleosides. See Deoxythymidine
- O
- Octyl bonded phase, separation of proteins and peptides by HPLC on, 2465-2499
- ODS particles, separation of polycyclic aromatic hydrocarbons by HPLC, using short columns containing, 1375-1383
- Oenology, HPLC in, S153-S158
- Oil, lubricating base, separation of into hydrocarbon fractions by preparative HPLC, 627-645
- Oligomeric ethylene glycol mixtures. See Ethylene glycol, oligomeric mixtures of
- One-dimensional thin layer chromatography, separation of prostanoids and arachidonic acid by, 55-62
- Optical detection, HPLC advances in, S77-S104
 new techniques in, S99-S103
- Optical detectors, HPLC, impact of new technology on, S79-S99
- Organic acids, elution behavior of in DEC, 271-280
- Organic acids
 in plant tissue, separation of by HPLC with twin phase, ion exchange, and reverse phase column, 1705-1716
 separation of on papers impregnated with aluminum and cadmium hydroxides, 973-981
- Organics, in high purity water, determination of, by HPLC, 2565-2570
- Oxidations
 polymeric permanganate, of alcohols and aldehydes in HPLC with solid phase reactor,

- [Oxidations]
2109-2127
via non-polymeric, solid supported reagents, in HPLC, S135-S137
- P
- Packing material, use of carbon as in HPLC, S1-S36
- Paraxanthine. See Caffeine metabolites
- Particle suspensions, separation of by SEC and HDC, S179-S217
- PCR. See Post-column reactors (PCR)
- Penicillins, determination of by HPLC with derivatization, 2019-2031
- Pentacarboxylic porphyrins, from uroporphyrinogen III, isolation and characterization of by RP-HPLC, 1969-1976
- Pentazocine, in combination with tripeleennamine, analysis of by HPLC, 2005-2017
- Pentobarbital, analysis of by RP-HPLC, 2033-2041
- Peppermint oil, determination of pulegone in by TLC with densitometry, 1175-1182
- Peptides
cytochrome C-derived, separation and characterization of, by HPLC, 1591-1601
influence of structure on retention of small chain peptides on reverse stationary phases, 1645-1671
isolation of from thymus extract by HPLC, 647-654
separation of by HPLC on octyl and diphenyl bonded phases, 2465-2499
See also Neuropeptides; Tryptic peptides
- Peptide mapping, ¹²⁵I-labeled, combined with HPLC, separation of proteins of Neisseria gonorrhoeae by, 1421-1439
- Permeability, modulated cross-sectional, sedimentation-flotation focusing FFF with, 1559-1576
- Pesticides, wheat-based, simultaneous determination of warfarin, sulphaquinoxaline, and fenitrothion in, by HPLC, 353-365
- Pharmaceuticals
in biological samples, determination of by LCEC, 2209-2230
See also Drugs; Radioprotective drug; Sulpha drugs
- Phenol, LCEC of for enzyme immunoassay, 2141-2156
- Phenolic compounds
HPLC determination of for oenology, S161-S167
urinary, determination of excretion patterns of by HPLC, 375-381
- Phenols
chlorinated, separation of by isocratic RP-HPLC, 2829-2837
high-speed analysis of, using LCEC, 2335-2373
- Phenylenediamines, determination of by HPLC with electrochemical detection, 1897-1905
- Phospholipid composition, of bacteria, analysis of by TLC, 2699-2707
- Phospholipids
detection of by RP-TLC with densitometry, 1193-1211
saturated and unsaturated, in biological fluids, analysis of using TLC, 179-192
- Photoacoustic detector, for HPLC, 1275-1289
- Photoionization detector, two-photon, for HPLC, 1275-1289
- Phytotoxic terpene. See Pulegone
- Piperacillin, determination of in plasma and cerebrospinal fluid by RP-HPLC, 575-583
- Plant tissue
determination of metabolites of metribuzin in, by HPLC, 725-742

- [Plant tissue]
separation of organic acids in
by HPLC with twin phase, ion
exchange, and reverse phase
column, 1705-1716
- Plasma
detection of reserpine in by
liquid chromatography with
fluorescence detection,
1111-1122
determination of piperacillin in
by RP-HPLC, 575-583
determination of trazodone and
1-m-chlorophenylpiperazine
in, by LCEC, 2195-2208
determination ritodrine in, by
HPLC with electrochemical
detection, 2775-2783
microassay for cefoperazone in
by RP-HPLC, 743-754
See also Biological fluids
- Platinum, dual electrode LCEC for
chemotherapy agents derived
from, 2173-2194
- Polarity, effect of on adsorption
behavior of solvents, 63-71
- Polarographic flow-through detec-
tor, 1759-1776
- Polycyclic aromatic hydrocarbons
modification in selectivity in
RP-HPLC for determination
of, 2709-2721
separation of by HPLC, using
short columns containing
ODS particles, 1375-1383
- Polymers
fractionation of by RP-TLC, 1-22
synthetic, determination of spe-
cific refractive index of
during SEC, 1359-1373
- Polynuclear aromatic hydrocarbons.
See Hydrocarbons, polynu-
clear aromatic
- Poly(styrene). See Polymers
- Post-column reactors (PCR)
in HPLC, S37-S59
design of, S38-S47
reaction types in, S47-S54
- Promethazine hydrochloride, deter-
mination of stability of by
HPLC, 1333-1344
- Prostaglandins
separation of by linear TLC,
1265-1272
See also Arbabrostil
- Prostanoids, separation of by
one-dimensional TLC, 55-62
- Protein-ligand interactions, char-
acterization of by SEC, 229-
248
- Protein-protein interactions,
characterization of by SEC,
229-248
- Proteins
of locust neurosecretory end-
ings, separation of by HPLC,
2501-2512
of Neisseria gonorrhoeae, sepa-
ration of by ¹²⁵I-labeled
peptide mapping and HPLC,
1421-1439
retention, resolution, and re-
covery of, influence of n-
alkyl chain length on in
RP-HPLC, 497-510
separation of by HPLC on octyl
and diphenyl bonded phases,
2465-2499
- Pseudoephedrine. See Amines
- Pulegone, in Hedeoma pulegioides
and peppermint oil, deter-
mination of by TLC with
densitometry, 1175-1182
- Pulsan. See Indenolol hydrochlor-
ide
- Pulsed amperometric detection.
See Amperometric detection,
pulsed
- Pyrazoline-5-ones, characteriza-
tion of derivatives of by
TLC, 2695-2697
- Pyrrrolidone. See N-Methyl-2-pyr-
rolidone
- Q
- Quantitative analysis, in HPLC,
effect of temperature on
response of RI in, 813-824
- Quantitative separation, of metal
ions, by TLC, 1249-1252

- Quaternary ammonium ion-pairs, of volatile fatty acids, electrokinetic detection of, in RP-HPLC, 1837-1848
- R
- Radial compression separation system, analysis of insect hormones by, 291-299, 1725
- Radiochromatogram analysis instrumentation, review of, 35-53
- Radiolabeled TLC plates. *See* Radiochromatogram analysis instrumentation
- Radioprotective drug, determination of by HPLC, 1523-1524
- Reaction processes, micro-HPLC technique for monitoring, 1065-1071
- Reagents, solid supported, in HPLC, S131-S133
- Reductions
borohydride/silica, for carbonyl compounds, on-line HPLC derivatizations via, 1051-1036
via non-polymeric, solid supported reagents, in HPLC, S133-S135
- Refractometer, Waters R401 differential, determination of refractive index of synthetic polymers during SEC, using, 1359-1373
- Reserpine
in biological fluids, determination of by micro-HPLC, 2382-2409
in plasma, detection of by liquid chromatography with fluorescence detection, 1111-1122
- Resin, macroporous ion-exchange, chromatographic behavior of aromatic acids on, 1081-1097
- Retention behavior, of ionogenic species in RP-HPLC, effect of buffers on, 685-704
- Reversed phase high performance (pressure) liquid chromatography (RP-HPLC)
with amine modifiers, determination of salbutamol by, 1955-1967
analysis of deoxythymidine in cell extracts by, 655-666
analysis of impurities in amines by, using binary and ternary solvent mixtures, 311-323
analysis of invertebrate neuropeptides by, 993-1013
analysis of pentobarbital by, 2033-2041
comparative evaluation of columns for analysis of drugs of forensic interest by, 1617-1630
with conductivity detection, determination of low molecular weight carboxylic acids by, 705-714
detection of lynestrenol and digoxin by tensametry in, 1745-1758
detection of vomitoxin using, 833-843
determination of antineoplastic indole derivative by, 939-949
determination of ascorbic acid and dehydroascorbic acid by, 1319-1332
determination of chlormethiazole in blood by, 907-916
determination of heavy metals in water by, 2079-2093
determination of piperacillin in plasma and cerebrospinal fluid by, 575-583
determination of stability of tetrahydro- β -carbolines by, 2043-2053
determination of urinary uric acid by, 845-860
effect of buffers on retention behavior of ionogenic species in, 685-704
with electrochemical detection, determination of uric acid in human serum by, 2739-2746
with electrochemical and spectrophotometric detection, simultaneous determination

- [Reversed phase high performance (pressure) liquid chromatography (RP-HPLC)]
of metals in zinc sulfate plant electrolyte using, 1799-1822
electrokinetic detection of quaternary ammonium ion-pairs of volatile fatty acids in, 1837-1848
electrokinetic detection of volatile fatty acids in, 1823-1835
with fluorescence detection, determination of formaldehyde by, 425-444
fractionation of tRNA by, 1441-1457
gradient elution, separation of thyromimetic iodoamino acids by, 511-526
influence of n-alkyl chain length on protein retention, resolution, and recovery in, 497-510
ion-pair, with electrochemical detection, determination of bipterin in tissue samples using, 1863-1872
ion-pair partition, with electrochemical and fluorometric detection, gradient elution of biogenic amines in, 2255-2274
isocratic, separation of chlorinated phenols by, 2829-2837
isolation and characterization of pentacarboxylic porphyrins from uroporphyrinogen III by, 1969-1976
with laser fluorescence, determination of amines in beer by, 931-938
microassay for cefoperazone in plasma, urine, and CSF by, 743-754
mobile phase selection for optimal separation in, 259-269
modification of selectivity of in determination of polycyclic aromatic hydrocarbons by, 2709-2721
purification of amino coumarin dyes by, 951-958
role of interfacial tension in, 1777-1784
separation of α -amanitin in serum by, 551-558
separation of clonazepam and metabolites by, 585-598
separation of deoxyribonucleosides by, using tetrabutylammonium hexafluorophosphates, 1673-1691
separation of diastereomeric glutathione adducts of K-region arene oxides by, 1693-1704
separation of thiol compounds by, 1785-1798
separation of tryptic peptides of β -chain of hemoglobins by, 199-213
separation of tryptic peptides of hGH and its 20k dalton variant by, 215-228
separations of uroporphyrin somers by, 383-394
simultaneous determination of aromatic isocyanates and carcinogenic amines in work atmosphere by, 453-469
simultaneous measurement of tricyclic antidepressants by, 2761-2773
solid phase derivatization reactions in polymeric reductions of carbonyl compounds by, 605-626
Reversed phase thin layer chromatography (RP-TLC)
with densitometry, quantitation of herbicides by using Bratton-Marshall detection reagent, 1183-1192
polymer fractionation by, 1-22
separation and quantitation of anionic, cationic, and non-ionic surfactants by, 23-33
separation of amino acids on plates of, 95-108
See also Thin layer chromatography (TLC)

- Reversed phase thin layers, silica gel and C₁₈, comparison of detection reagents for lipid and phospholipid detection and densitometry on, 1193-1211
- Reverse stationary phases, influence of structure on retention of on, 1645-1671
- RI. See Differential refractometer (RI)
- Ritodrine, in plasma, determination of by HPLC with electrochemical detection, 2775-2783
- tRNA, fractionation of by RP-HPLC, 1441-1457
- Rodenticide baits. See Pesticides, wheat-based
- RP-HPLC. See Reversed phase high performance (pressure) liquid chromatography
- RP-TLC. See Reversed phase thin layer chromatography (RP-TLC)
- S
- Salbutamol, determination of by RP-HPLC with amine modifiers, 1955-1967
- Salsolinol. See Tetrahydroisoquinolines
- Sampling rate, effect of on HSLC, 2809-2828
- SEC. See Size exclusion chromatography (SEC)
- Sedimentation-flotation focusing FFF. See Field-flow fractionation (FFF), sedimentation-flotation focusing
- Selectivity, in RP-HPLC, modification of in determination of polycyclic aromatic hydrocarbons, 2709-2721
- Serotonin, automated analysis of brain extracts for by HPLC, 871-886
- Serum
determination of analgesics in by LCEC, 2311-2335
- determination of cefamandole in, by HPLC, 2747-2759
- determination of tiazofurin in, by HPLC and TLC, 1239-1247
- determination of verapamil and norverapamil in, by HPLC, 887-893
- human, determination of uric acid in, by RP-HPLC with electrochemical detection, 2739-2746
- separation of α -amanitin in, by RP-HPLC, 551-558
- See also Biological fluids
- Shale oil
separation of by HPLC, 325-342
- See also Hydrocarbons
- Silica gel, C₁₈, separation of amino acids on RP-TLC plates of, 95-108
- Silica gel-G layers, TBP impregnated, chromatographic behavior of metal ions on, 165-177
- Silver salts, of aromatic sulfonic acids, use of in TLC, 2687-2694
- Size exclusion chromatography (SEC)
characterization of protein-protein and protein-ligand interactions by, 229-248
- computer assisted data acquisition and analysis for, 1047-1063
- correction of instrumental broadening in, by FFT, 2411-2431
- determination of gel content of acrylic latexes by, 667-683
- determination of specific refractive index increment of synthetic polymers during, 1359-1373
- effect of flow rate on elution volumes in, S219-S249
- multiple detector, use of in determination of aging of pressure sensitive adhesives, 2723-2737
- separation of particle suspensions by, S179-S217

- [Size exclusion chromatography (SEC)]
Tung's instrumental spreading equation in, S61-S75
- Size fractionation, of H-coal liquids by polyvinylacetate GPC, 1935-1948
- Slaframine, diagnostic toxicological analysis of using TLC, 123-126
- Small chain peptides, influence of peptide structure on retention of on reverse stationary phases, 1645-1671
- Sodium ion, complex formation of monomeric phosphorus oxoacids with, in DEC, 755-763
- Sodium mercaptobenzothiazole, in engine coolants, determination of by HPLC, 805-812
- Sodium tolyltriazole, in engine coolants, determination of by HPLC, 805-812
- Solid phase reactions, for derivatization in HPLC, S125-S151
- Solid phase reactor, polymeric permanganate oxidations of alcohols and aldehydes in HPLC with, 2109-2127
- Solid supported reagents. See Reagents, solid supported
- Solvation effects, in GPC, 1385-1394
- Solvent mixtures, binary and ternary, analysis of impurities in amines by RP-HPLC using, 311-323
- Solvents, with molecules of different polarity, adsorption behavior of, 63-71
- Specific refractive index increment, of synthetic polymers during SEC, using Waters R401 differential refractometer, 1359-1373
- Spectrophotometric detection, simultaneous determination of metals in zinc sulfate plant electrolyte using RP-HPLC with, 1799-1822
- Spectroscopy, micro infrared, combined with TLC, 1213-1220
- Stability, of tetrahydro- β -carbonylines, determination of by RP-HPLC, 2043-2053
- Stannic arsenate layers, adsorption behavior of metal ions in HCL systems using, determination of by TLC, 1253-1264
- Stannic tungstate, separation of amino acids by TLC on, 109-122
- Striatum, determination of voltammetry in, by HPLC with electrochemical detection, 1873-1896
- Structure
of peptides, influence of on retention of small chain peptides on reverse stationary phases, 1645-1671
of tebuthiuron and related thiazidizoles, relationship of to chromatographic mobility, 1221-1226
- Styrene oligomers, evaluation of micro-HPLC for separation of, 1631-1643
- Styrene oxide, separation of diastereoisomeric glutathione adducts of, by HPLC, 1475-1489
- Sugars, HPLC determination of for oenology, S158-S159
- Sulfonic acids, aromatic, use of silver salts of, in TLC, 2687-2694
- Sulpha drugs, separation of inorganic ions using TLC on plates impregnated with, 145-153
- Sulphaquinoxaline, determination of in pesticides by HPLC, 353-365
- Surface area, of adsorbent, role of in TLC, 2665-2677
- Surfactants, anionic, cationic, and nonionic, separation and quantitation of by RP-TLC, 23-33
- Synthesis properties, of tin(IV)-iodophosphate columns, 777-794

T

- Tebuthiuron, separation of by TLC, 1221-1226
- Temperature
 effect of on degradation of tetrahydroisoquinolines, determination of by HPLC, 343-352
 effect of on response of RI for quantitative analysis in HPLC, 813-824
 effect of on stability of tetrahydro- β -carbolines, determination of by RP-HPLC, 2043-2053
- Tensammetry, in RP-HPLC, detection of lynestrenol and digoxin by, 1745-1758
- Terbutaline, quantitative analysis of in human plasma with LCEC, 559-574
- Tetrabutylammonium hetaerons, separation of deoxyribonucleosides by RP-HPLC using, 1673-1691
- Tetrahydro- β -carbolines, effect of temperature on stability of, determinations of by RP-HPLC, 2043-2053
- Tetrahydroisoquinolines, effect of temperature on degradation of, determination of by HPLC, 343-352
- Tetrahydropapaveroline. See Tetrahydroisoquinolines
- Theobromine. See Caffeine metabolites
- Theophylline. See Caffeine metabolites
- Thiadiazoles, separation of by TLC, 1221-1226
- Thin layer chromatography (TLC)
 advances in (book review), 1549-1550
 analysis of phospholipid composition of bacteria by, 2699-2707
 analysis of saturated and unsaturated phospholipids in biological fluids using, 179-192
 analysis of 3d transition metal ions using, 155-164
 characterization of derivatives of pyrazoline-5-ones by, 2695-2697
 combined with micro infrared spectroscopy, 1213-1220
 with densitometry, determination of pulegone in Hedeoma pulegioides and peppermint oil by, 1175-1182
 determination of adsorption behavior of metal ions in aqueous HCL systems using stannic arsenate layers by, 1253-1264
 diagnostic toxicological analysis of licomycin and slaframine using, 123-126
 fractionation of acidic ninhydrin-positive compounds of mouse brain by, 2621-2634
 identification of potential antidiabetic compounds by, 2661-2664
 linear, separation of prostaglandins by, 1265-1272
 with mixed mobile phase, parameters characterizing, 81-93
 quantitative, determination of biphenyl in citrus fruits by, 2679-2685
 quantitative separation of metal ions by, 1249-1252
 role of surface area of adsorbent in, 2665-2677
 separation of amino acids by, on stannic tungstate, 109-122
 separation of carbamates using, on plates impregnated with zinc acetate, 139-143
 separation of glutathione from amino acid mixtures by, 2635-2645
 separation of inorganic ions using, on plates impregnated with sulpha drugs, 145-153
 separation of isomers of cyclopentylphenol by, 1171-1174
 separation of tebuthiuron and related thiadiazoles by, 1221-1226

- [Thin layer chromatography (TLC)]
 use of in combination with HPLC for determination of tiazofurin in serum, 1239-1247
 use of silver salts of aromatic sulfonic acids in, 2687-2694
See also One-dimensional thin layer chromatography; Reversed phase thin layer chromatography
- Thiol compounds, liquid chromatographic behavior of, 1785-1798
- Thymus extract, isolation of individual peptides from, by HPLC, 647-654
- Thyroid hormone, assay of using coulometric-amperometric detection, 2275-2310
- Tiazofurin, in serum, determination of by HPLC and TLC, 1239-1247
- Tin(IV)-iodophosphate columns.
See Columns, tin(IV)-iodophosphate
- Tissue samples, determination of biopterin in using ion-pair RP-HPLC with electrochemical detection, 1863-1872
- α -Tocopherol, in biological specimens, determination of by HPLC, 1123-1130
- Toxicology, diagnostic, application of TLC to, 123-126
- Trace determination, of fluorescent compounds, chemiluminescence detector for, 1603-1616
- Trazodone, in plasma, determination of by LCEC, 2195-2208
- Trichlormethiazide, in biological fluids, determination of by micro-HPLC, 2382-2409
- Tricyclic antidepressants, simultaneous measurement of, by RP-HPLC, 2761-2773
- Tripelennamine, in combination with pentazocine, analysis of by HPLC, 2005-2015
- Tryptic peptides, of β -chain of hemoglobins, separation of by RP-HPLC, 199-213
- Tryptic peptides, of hGH and its 20k dalton variant, separation of by RP-HPLC, 215-228
- Tryptophan, fluorometric determination of by ion pair HPLC, 527-541
- U
- Uric acid
 in human serum, determination of by RP-HPLC with electrochemical detection, 2739-2746
 urinary, determination of by RP-HPLC, 845-860
- Urine
 determination of analgesics in by LCEC, 3211-3235
 determination of 6 β -hydroxycortisol in, by HPLC with fluorescence detection, 1977-1985
 microassay for cefoperazone in by RP-HPLC, 743-754
See also Biological fluids
- Uroporphyrin isomers, separation of by RP-HPLC, 383-394
- Uroporphyrinogen III, isolation and characterization of pentacarboxylic porphyrins from, by RP-HPLC, 1969-1976
- V
- Valence connectivity index, modified, correlation of chromatographic parameters for barbituric acid derivatives with, 73-80
- Verapamil, determination of by HPLC, 887-893
- Voltammetry, in striatum, determination of by HPLC with electrochemical detection, 1873-1896
- Vomitoxin, detection of using RP-HPLC, 833-843

W

Warfarin, determination of in pesticides by HPLC, 353-365

Water

determination of heavy metals in by RP-HPLC, 2079-2093

determination of N-methyl-2-pyrrolidone in, using HPLC, 445-452

high purity, determination of organics in, by HPLC, 2565-2570

Wines. See Oenology

Z

Zinc acetate, separation of carbamates using TLC on plates impregnated with, 139-143

Zinc silicate, separation of amines on papers impregnated with, 959-972

Zinc sulfate plant electrolyte, simultaneous determination of metals in using RP-HPLC with electrochemical and spectrophotometric detection, 1799-1822

COLLECTIVE CONTENTS TO VOLUME 6

Volume 6, Number 1, 1983

Special Issue on Thin-Layer Chromatography

Practice, Mechanism and Theory of Reversed Phase TLC Polymer Fractionation	1
<i>D. W. Armstrong, K. H. Bui, and R. E. Boehm</i>	
Separation and Quantitation of Anionic, Cationic, and Nonionic Surfactants by TLC	23
<i>D. W. Armstrong and G. Y. Stine</i>	
A Review of Radiochromatogram Analysis Instrumentation	35
<i>S. D. Shulman</i>	
Separation of Prostanoids by One-Dimensional Thin-Layer Chromatography	55
<i>K. Korte and M. L. Casey</i>	
Effect of Diluent on the Structure of the Adsorption Layer in Systems of the Type Diluent + Polar Solvent-Silica	63
<i>W. Markowski and K. L. Czapińska</i>	
Evaluation of Modified Valence Molecular Connectivity Index for Correlations of Chromatographic Parameters	73
<i>J. Bojarski and L. Ekiert</i>	
Thermodynamic Approach to TLC with Mixed Mobile Phase. Determination of Parameters Characterizing TLC Systems	81
<i>J. Oscik, M. Jaronec, and I. Małinowska</i>	
Chromatography of Amino Acids on Reversed Phase Thin Layer Plates	95
<i>J. Sherma, B. P. Sleckman, and D. W. Armstrong</i>	
Thin-Layer Chromatographic Separations of Amino Acids on Stannic Tungstate	109
<i>S. A. Nabi, W. U. Farooqui, Z. M. Siddiqui, and R. A. K. Rao</i>	

Some Advances in Application of TLC to Diagnostic Toxicology	123
<i>H. M. Stahr</i>	
A Simple Microassay for Glutamic Acid Decarboxylase by Ion Exchange Thin-Layer Chromatography	127
<i>S. L. Pahuja and T. W. Reid</i>	
TLC Separation of Some Carbamates on Metal Salt Impregnated Layers	139
<i>S. P. Srivastava and Reena</i>	
Thin Layer Chromatographic Separation of Some Inorganic Ions on Sulpha Drug Impregnated Layers	145
<i>S. P. Srivastava, Kamlesh, and V. K. Gupta</i>	
Chromatographic Analysis of Some 3d Metal Complexes	155
<i>V. Kumari, R. K. Upadhyay, and V. P. Singh</i>	
Chromatographic Behaviour of 48 Metal Ions on TBP Impregnated Silica Gel-G Layers	165
<i>M. Qureshi, B. M. Sethi, and S. D. Sharma</i>	
Analysis of Saturated and Unsaturated Phospholipids in Biological Fluids	179
<i>J. C. Touchstone, S. S. Levin, M. F. Dobbins, and P. C. Beers</i>	
Liquid Chromatography News	193
Liquid Chromatography Calendar	197

Volume 6, Number 2, 1983

A Comparative Study of the Separation of the Tryptic Peptides of the β-Chain of Normal and Abnormal Hemoglobins by Reversed Phase High Performance Liquid Chromatography	199
<i>E. Minasian, R. S. Sharma, S. J. Leach, B. Grego, and M. T. W. Hearn</i>	
Separation and Assignment of the Tryptic Peptides of Human Growth Hormone (hGH) and the 20K Dalton hGH Variant by Reversed Phase High Performance Liquid Chromatography.	215
<i>M. T. W. Hearn, B. Grego, and G. E. Chapman</i>	
Characterization of Protein-Protein and Protein-Ligand Interactions by High Performance Size Exclusion Chromatography	229
<i>K. C. Ingham, T. F. Busby, D. H. Atha, and H. Forastieri</i>	

- Exclusive Use of High Pressure Liquid Chromatography for the Determination of the Complete Amino Acid Sequence of the 12K Fragment of Avian Sarcoma Virus Structural Protein p27 249**
A. S. Bhowan, J. C. Bennett, T. W. Cornelius, and E. Hunter
- A Graphic Representation of Binary Mobile Phase Optimization in Reversed-Phase High Performance Liquid Chromatography 259**
H. J. Issaq, G. M. Muschik, and G. M. Janini
- Elution Behavior of Organic Acids in Donnan Exclusion Chromatography . . . 271**
Y. Tokunaga, H. Waki, and S. Ohashi
- Isolation of Glycoalkaloids with the Chromatotron and Their Determination by High-Performance Liquid Chromatography. 281**
I. R. Hunter and E. Heftmann
- Analysis of Insect Hormones by Means of a Radial Compression Separation System. 291**
E. S. Chang
- High-Performance Liquid Chromatographic Determination in Human Plasma of a Anticonvulsant Benzodiazepine: Clonazepam. 301**
S. Bouquet, P. Aucouturier, A. M. Brisson, Ph. Courtois, and J. B. Fourtillan
- The Analysis of Enantiomeric and Diastereoisomeric Mixtures of Ephedrine and Pseudoephedrine Using Reversed-Phase High Performance Liquid Chromatography of Nickel Dithiocarbamate Complexes 311**
G. K. C. Low, P. R. Haddad, and A. M. Duffield
- Shale Oil Separation by High Performance Liquid Chromatography 325**
W. A. Dark
- Degradation Characteristics of Two Tetrahydroisoquinolines at Room and Body Temperatures: HPLC Determination with Electrochemical Detection. 343**
R. D. Myers, J. L. Garrison, and E. C. Critcher
- Simultaneous Determination of Warfarin, Sulphaquinoxaline and Fenitrothion in Wheat-Based Rodenticide Baits by High Pressure Liquid Chromatography 353**
R. L. Perez
- A Simple and Rapid Method for the Determination of 2-Acetylaminofluorene in Laboratory Diets 367**
E. G. Perkins, D. J. Hendren, C. R. Truex, and W. J. Visek

Liquid Chromatographic Determination of Excretion Patterns of Urinary Phenolic Compounds	375
<i>Y. Yamaguchi</i>	
High Performance Liquid Chromatography of Uroporphyrin Isomers	383
<i>J. M. Rideout, D. J. Wright, and C. K. Lim</i>	
Liquid Chromatography News	395
The Eastern Analytical Symposium — A Report	397
Liquid Chromatography Calendar	399

Volume 6, Number 3, 1983

Dispersion-Compensated Calibration for Gel Permeation Chromatography: Theory and Utilization for Direct and Universal Calibration	401
<i>G. J. F. Ring, R. A. Stratton, and L. R. Schroeder</i>	
Determination of Formaldehyde and Other Aldehydes by High Performance Liquid Chromatography with Fluorescence Detection	425
<i>S. J. Swarin and F. Lipari</i>	
High Performance Liquid Chromatographic Determination of N-Methyl-2-pyrrolidone in Water	445
<i>D. A. Frick</i>	
Simultaneous Determination of Aromatic Isocyanates and Some Carcinogenic Amines in the Work Atmosphere by Reversed-Phase High-Performance Liquid Chromatography	453
<i>E. H. Nieminen, L. H. Saarinen, and J. T. Laakso</i>	
A Study of Polynuclear Aromatic Hydrocarbons on an Amino Bonded Phase Liquid Chromatographic Column in the Normal and Reversed Phase	471
<i>D. Kariesky, D. C. Shelly, and I. M. Warner</i>	
The Influence of Reversed-Phase n-Alkyl Chain Length on Protein Retention, Resolution and Recovery: Implications for Preparative HPLC . . .	497
<i>J. D. Pearson and F. E. Regnier</i>	
Rapid Separation and Determination of Thyromimetic Iodoamino Acids by Gradient Elution Reverse Phase Liquid Chromatography with Electrochemical Detection	511
<i>M. R. Hadj-Mohammadi, J. L. Ward, and J. G. Dorsey</i>	
Fluorometric Determination of Tryptophan and Its Brain Indoleamine Metabolites by Ion-Pair HPLC	527
<i>A. Adell, J. M. Tusell, F. Artigas, E. Martínez, C. Suñol, and E. Gelpí</i>	

Preparative HPLC Separation of Epimeric Guaianolides	543
<i>F. Belliardo, T. Sacco, and G. Appendino</i>	
Determination of α-Amanitin in Serum by HPLC.	551
<i>F. Belliardo and G. Massano</i>	
Quantitative Analysis of Terbutaline (Bricanyl[®]) in Human Plasma with Liquid Chromatography and Electrochemical Detection Using On-Line Enrichment	559
<i>S. Bergquist and L.-E. Edholm</i>	
Determination of Piperacillin in Plasma and Cerebrospinal Fluid by High Performance Liquid Chromatography.	575
<i>A. Meulemans, J. Mohler, J. M. Decazes, I. Dousset, and A. Modai</i>	
Separation of Clonazepam and Five Metabolites by Reverse Phase HPLC and Quantitation from Rat Liver Microsomal Incubations	585
<i>R. P. Rimmel and G. W. Elmer</i>	
Liquid Chromatography News	599
Liquid Chromatography Calendar.	601

Volume 6, Number 4, 1983

Solid Phase Derivatization Reactions in HPLC Polymeric Reductions for Carbonyl Compounds.	605
<i>I. S. Krull, K.-H. Xie, S. Colgan, U. Neue, T. Izod, R. King, and B. Bidlingmeyer</i>	
Hydrocarbon Type Separation of Lubricating Base Oil in Multigram Quantity by Preparative HPLC.	627
<i>P. Pei, J. Britton, Jr., and S. Hsu</i>	
Isolation of Thymosin α_1 from Thymosin Fraction 5 by High Performance Liquid Chromatography	647
<i>T. F. Gabriel, J. Meienhofer, and J. Michalewsky</i>	
Reversed-Phase High Performance Liquid Chromatography (HPLC) of Nucleosides in Cell Extracts with Special Reference to Deoxythymidine	655
<i>J. Harmenberg, A. Larsson, and C.-E. Hagberg</i>	
Determination of Gel Content of Acrylic Latexes by Size Exclusion Chromatography	667
<i>F. B. Malihi, C.-Y. Kuo, and T. Provder</i>	
Buffer Choice for Tuning the Selectivity in Reverse Phase Chromatography . .	685
<i>M. Otto and W. Wegscheider</i>	

Determination of Low Molecular Weight Carboxylic Acids in Water by HPLC with Conductivity Detection.	705
<i>D. L. Manning and M. P. Maskarinec</i>	
High-Performance Liquid Chromatographic Determination of Indenolol Hydrochloride and Its Pharmaceutical Formulation	715
<i>M. E. Mohamed, H. Y. Aboul-Enein, S. Babhair, and S. El-Hofi</i>	
The Determination of Metribuzin and Its Metabolites by High Pressure Liquid Chromatography	725
<i>C. E. Parker, G. H. Degen, E. O. Abusteit, and F. T. Corbin</i>	
High-Performance Liquid Chromatographic Micro-Assay for Cefoperazone in Human Plasma, Urine and CSF	743
<i>P. T. R. Hwang and M. C. Meyer</i>	
Complex Formation of Monomeric Phosphorus Oxoacids with Sodium Ion in Donnan Exclusion Chromatography.	755
<i>Y. Tokunaga, Y. Takeuchi, and S. Ohashi</i>	
Preparation of High Efficiency Columns for High Performance Liquid Chromatography	765
<i>C. C. Siemion</i>	
A Comparative Study of the Synthesis and Ion Exchange Properties of Iodophosphates of Tin(IV), Zirconium(IV) and Iron(III): Separations of Metal Ions on Tin(IV)-iodophosphate Columns	777
<i>S. A. Nabi, R. A. K. Rao, and W. A. Siddiqi</i>	
Liquid Chromatography News	795
Liquid Chromatography Calendar	799

Volume 6, Number 5, 1983

Determination of Sodium Tolyltriazole and Sodium Mercaptobenzo-thiazole in Engine Coolants	805
<i>N. M. Potter</i>	
Influence of Temperature on the Response of Differential Refractometer for Quantitative Analysis in High-Performance Liquid Chromatography	813
<i>S. Mori</i>	
Ion Exchange High Performance Liquid Chromatography Separations Using a High Content Organic Modifier Mobile Phase	825
<i>H. J. Issaq and G. M. Muschik</i>	

A Simple, Sensitive Method for Detection of Vomitoxin (Deoxynivalenol) Using Reversed Phase, High Performance Liquid Chromatography	833
<i>K. C. Ehrlich, L. S. Lee, and A. Ciegler</i>	
Urinary Uric Acid Determination by Reversed-Phase High Pressure Liquid Chromatography	845
<i>P. Jauge and L. Ma. Del-Razo</i>	
Rapid Assay of Hypothalamic Aromatase Using High Performance Liquid Chromatography	861
<i>D. L. Wilke and W. E. Braselton, Jr.</i>	
Automated HPLC Analysis of Tissue Levels of Dopamine, Serotonin, and Several Prominent Amine Metabolites in Extracts from Various Brain Regions	871
<i>W. E. Wilson, S. W. Mietling, and J.-S. Hong</i>	
Determination of Verapamil and Norverapamil in Serum by High-Performance Liquid Chromatography	887
<i>C. K. Lim, J. M. Rideout, and J. W. S. Sheldon</i>	
HPLC Separation of Theophylline, Paraxanthine, Theobromine, Caffeine and Other Caffeine Metabolites in Biological Fluids	895
<i>R. Klassen and B. Stavric</i>	
Determination of Chlormethiazole in Blood by High Performance Liquid Chromatography	907
<i>C. Kim and J. M. Khanna</i>	
A Reevaluation of Endogenous Levels of Lacinilene C 7-Methyl Ether in Bracts of Genus <i>Gossypium</i>	917
<i>G. A. Greenblatt and R. C. Beier</i>	
The Identification of Adenine in Cacao Products.	927
<i>B. A. Kiefer, R. M. Sheeley, W. J. Hurst, and R. A. Martin, Jr.</i>	
HPLC Laser Fluorometric Determination of Amines in Beer	931
<i>G. M. Murray and M. J. Sepaniak</i>	
High Performance Liquid Chromatography of an New Antineoplastic: (1-γDiethyl amino propyl amino)-5-methyl-dipyrido [4,3-b] [3,4-f] Indole (BD 40)	939
<i>D. Blais, B. Gourmel, M. Marty, C. Gisselbrecht, E. Bisagni, M. Boiron, and C. Dreux</i>	
The Purification of Amino Coumarin Dyes by Reverse Phase High Performance Liquid Chromatography	951
<i>C. V. C. Prasad and K. N. Rao</i>	

Chromatography on Papers Impregnated with Zinc Silicate: A New Adsorbent for Qualitative and Quantitative Separation of Amines.	959
<i>J. P. Rawat, M. Iqbal, and M. A. Khan</i>	
Chromatographic Studies of 34 Organic Acids on Papers Impregnated with Hydroxides of Aluminum and Cadmium.	973
<i>H. S. Rathore, K. Kumari, and M. Garg</i>	
Liquid Chromatography News	983
Liquid Chromatography Calendar.	987

Volume 6, Number 6, 1983

Analysis of Invertebrate Neuropeptides by RP-HPLC	993
<i>H. Jaffe and D. K. Hayes</i>	
Solid Phase Derivatizations in HPLC: Borohydride/Silica Reductions for Carbonyl Compounds.	1015
<i>I. S. Krull, S. Colgan, K.-H. Xie, U. Neue, R. King, and B. Bidlingmeyer</i>	
Computer Assisted Data Acquisition and Analysis for Size Exclusion Chromatography	1037
<i>D. E. Hutchens and R. D. Hester</i>	
Direct Monitoring of Reaction Processes with Micro-HPLC Technique.	1065
<i>K. Jinno and M. Ishigaki</i>	
HPLC of Metal Chelates: Separation of Ni^{II} and Fe^{II} as 2,2'-Bipyridine Complexes with Ion-Pair Chromatography	1073
<i>A. Mangia and M. T. Lugari</i>	
Chromatographic Behavior of Aromatic Acids on Macroporous Ion-Exchange Resin.	1081
<i>T. Hanai, K. C. Tran, and J. Hubert</i>	
Detection of Mycoplasma Contamination in Lymphoblastoid Cultures by a Simple, HPLC Method	1099
<i>J. E. McEntire, R. LeGrand, and G. Grinstead</i>	
An Improved Method for the Determination of Reserpine in Plasma Using Liquid Chromatography with Fluorescence Detection	1111
<i>R. F. Suckow, T. B. Cooper, and G. M. Asnis</i>	
Determination of Alpha-Tocopherol in Biological Specimens by High-Performance Liquid Chromatography	1123
<i>C. G. Rammell, B. Cunliffe, and A. J. Kieboom</i>	

New Fluorimetric Detection Method of Corticosteroids after High-Performance Liquid Chromatography Using Post-Column Derivatization with Glycinamide	1131
<i>T. Seki and Y. Yamaguchi</i>	
An Evaluation of Eluent Recycling and Column Life for HPLC Analysis of Carbohydrates.	1139
<i>R. E. Lee, Jr., D. Friday, R. Rojas, H. James, and J. G. Baust</i>	
Analytical Applications of Ion-Exchange Materials. V. Chromatographic Separation of Drugs on Ferric Phosphate Papers	1153
<i>P. S. Thind and J. S. Gandhi</i>	
Liquid Chromatography News	1163
Liquid Chromatography Calendar	1165

Volume 6, Number 7, 1983

Special Section on Thin-Layer Chromatography

The Separation of 2-, 3- and 4-Cyclopentylphenol by Thin-Layer Chromatography	1171
<i>T. M. Atkins, D. G. Towles, and R. H. Bishara</i>	
Determination of Pulegone in <i>H. pulegioides</i> and Peppermint Oil by Thin Layer Chromatography with Densitometry	1175
<i>B. P. Sleckman, J. Sherma, and L. C. Mineo</i>	
Densitometric Quantitation of Urea, Carbamate, and Anilide Herbicides on C₁₈ Reversed Phase Thin Layers Using Bratton-Marshall Detection Reagent.	1183
<i>J. Sherma and J. L. Boymel</i>	
Comparison of Reagents for Lipid and Phospholipid Detection and Densitometric Quantitation on Silica Gel and C₁₈ Reversed Phase Thin Layers.	1193
<i>J. Sherma and S. Bennett</i>	
A Combined Thin-Layer Chromatography/Micro Infrared Method	1213
<i>H. J. Issaq</i>	
Structure and TLC Mobility Relationships for Tebuthiuron and Related Thiadiazoles	1221
<i>R. W. Souter and R. H. Bishara</i>	
Routine HPTLC in Industrial Analysis: Process Control of Fermentations . .	1227
<i>F. Kreuzig</i>	

- A TLC-HPLC Method for Determination of Tiazofurin (NSC 286193) in Serum** 1239
C. C. Ackerly, R. A. Newman, and J. J. McCormack
- Quantitative Separation of Zr^{4+} from La^{3+} and Ce^{3+} ; W^{6+} from Cr^{3+} , Mo^{6+} and VO^{2+} by Thin Layer Chromatography** 1249
M. Qureshi, B. M. Sethi, and S. D. Sharma
- Prediction of K_{sp} from R_f Values: Thin Layer Chromatography of 47 Metal Ions on Stannic Arsenate in Aqueous Hydrochloric Acid Systems.** . . . 1253
S. D. Sharma, T. R. Sharma, and B. M. Sethi
- Rapid Separation of Prostaglandins by Linear High Performance Thin Layer Chromatography.** 1265
B. J. Smith, R. M. Ross, C. R. Ayers, M. R. Wills, and J. Savory

Regular Contributed Papers

- A Crystalline Quartz, Laser-Excited Fluorescence, Photoacoustic, and Two-Photon Photoionization Detector for HPLC.** 1275
E. Voigtman and J. D. Winefordner
- High Performance Liquid Chromatography of Dithiolthiones.** 1291
A. N. Masoud and E. Bueding
- Determination of Ascorbic Acid and Dehydroascorbic Acid in Blood Plasma Samples.** 1319
J. K. Baker, J. Kapeghian, and A. Verlangieri
- High Pressure Liquid Chromatographic Determination of Promethazine Hydrochloride in the Presence of Its Thermal and Photolytic Degradation Products: A Stability Indicating Assay** 1333
S. Stavchansky, J. Wallace, M. Chue, and J. Newburger
- Liquid Chromatography News** 1345
- Liquid Chromatography Calendar.** 1347

Volume 6, Number 8, 1983

- Reader Survey** 1353
- Specific Refractive Index Increment Measurements on Macromolecules Using a Waters R401 Differential Refractometer** 1359
S. A. Berkowitz
- High Performance Liquid Chromatography Separations Using Short Columns Packed with Spherical and Irregular Shaped ODS Particles** 1375
H. J. Issaq and R. E. Gourley

Solvation and Adsorption Effects in Gel Permeation Chromatography	1385
<i>T. Ogawa and M. Sakai</i>	
Rapid Amino Acid Analysis in Biotechnology: Determination of Alanine and Aspartic Acid	1395
<i>H. Kneifel and A.-S. Jandel</i>	
Purification of Human Platelet Monoamine Oxidase B by High Performance Liquid Chromatography	1407
<i>G. A. S. Ansari, N. T. Patel, R. R. Fritz, and C. W. Abell</i>	
¹²⁵I-Labeled Peptide Mapping and High Performance Liquid Chromatography ¹²⁵I-Peptide Separation of Protein I of Four Strains of <i>Neisseria gonorrhoeae</i>.	1421
<i>R. C. Judd</i>	
High-Performance Liquid Chromatography of T-RNA	1441
<i>J. D. Pearson, M. Mitchell, and F. E. Regnier</i>	
Removal of Nonionic Detergent Emulgen 911 from Solubilized Microsomes by HPLC.	1459
<i>O. Hernandez and K. K. Kohli</i>	
HPLC Separation of the Diastereomeric Glutathione Adducts of Styrene Oxide	1475
<i>O. Hernandez, A. V. Bhatia, and M. Walker</i>	
Determination of Naloxone Hydrochloride in Dosage Form by High-Performance Liquid Chromatography	1491
<i>M. S. Tawakkol, M. E. Mohamed, and M. M. A. Hassan</i>	
Liquid Chromatographic Assay of Arbacaprostil.	1499
<i>G. W. Peng and V. K. Sood</i>	
Simultaneous Determination of Chloroquine and Desethylchloroquine in Blood, Plasma, and Urine by High-Performance Liquid Chromatography	1513
<i>A. Akintonwa, M. C. Meyer, and P. T. R. Hwang</i>	
HPLC Assay for S-2-(3-Aminopropylamino)ethyl Phosphorothioate (WR 2721) in Plasma	1523
<i>N. F. Swynnerton, E. P. McGovern, D. J. Mangold, J. A. Niño, E. M. Gause, and L. Fleckenstein</i>	
Quantitative Determination of Iron and Aluminum in Some Alloys and Silicate Rocks After a Cation Exchange Separation on Zirconium(IV) Phospho and Silico Arsenates.	1535
<i>K. G. Varshney, S. Agrawal, and K. Varshney</i>	
Book Reviews.	1547

Liquid Chromatography News	1551
Liquid Chromatography Calendar	1553

Volume 6, Number 9, 1983

Lest We Exaggerate	1557
Sedimentation-Flotation Focusing Field-Flow Fractionation in Channels with Modulated Cross-Sectional Permeability. I. Theoretical Analysis . . .	1559
<i>J. Janča and V. Jahnová</i>	
Determination of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection	1577
<i>R. D. Rocklin and C. A. Pohl</i>	
HPLC Separation and Characterization of Synthetic and Native Cytochrome C Derived Peptides	1591
<i>N. E. Rankin, R. A. Earl, R. S. Young, L. E. Barstow, M. A. Cusanovich, and G. S. Wilson</i>	
A Chemiluminescence Detector for Trace Determination of Fluorescent Compounds	1603
<i>G. Mellbin</i>	
Comparison of Various Columns for the High-Speed HPLC Analysis of Drugs of Forensic Interest	1617
<i>I. S. Lurie and S. M. Carr</i>	
Evaluation of Micro-HPLC and Various Stationary Phases in Reversed-Phase Mode for the Separation of Styrene Oligomers	1631
<i>K. Jinno and T. Sato</i>	
The Influence of Peptide Structure on the Retention of Small Chain Peptides on Reverse Stationary Phases	1645
<i>D. J. Pietrzyk, R. L. Smith, and W. R. Cahill, Jr.</i>	
Reversed-Phase Separation of the Major Deoxyribonucleosides and Their Mononucleotides Using Tetrabutylammonium Hexaerons	1673
<i>J. P. Caronia, J. B. Crowther, and R. A. Hartwick</i>	
HPLC Separation of Diastereomeric Adducts of Glutathione with Some K-Region Arene Oxides	1693
<i>O. Hernandez, A. B. Bhatia, and M. P. Walker</i>	
Separation of Organic Acids in Plant Tissue by HPLC with a Twin Phase, Ion Exchange and Reverse Phase, Column	1705
<i>A. Clement and B. Loubinoux</i>	

COLLECTIVE CONTENTS TO VOLUME 6	2893
Liquid Chromatography News	1717
Liquid Chromatography Calendar	1721
Erratum	1725

Volume 6, Number 10, 1983

Special Issue on Liquid Chromatography/Electrochemistry. Part 1

The Direct Electrochemical Detection of Amino Acids at a Platinum Electrode in an Alkaline Chromatographic Effluent	1727
<i>J. A. Polta and D. C. Johnson</i>	
Tensammetric Detection in High Performance Liquid Chromatography. Application to Lynestrenol and Some Cardiac Glycosides	1745
<i>H. C. de Jong, W. H. Voogt, P. Bos, and R. W. Frei</i>	
A New Polarographic Flow-Through Detector	1759
<i>A. Trojánek and L. Křestán</i>	
Role of Interfacial Tension in Reverse Phase Liquid Chromatography	1777
<i>G. K. Vemulapalli and T. Gnanasambandan</i>	
Liquid Chromatographic Behavior of Biological Thiols and the Corresponding Disulfides	1785
<i>L. A. Allison, J. Keddington, and R. E. Shoup</i>	
Simultaneous Determination of Cadmium, Cobalt, Copper, Lead, Mercury and Nickel in Zinc Sulfate Plant Electrolyte Using Liquid Chromatography with Electrochemical and Spectrophotometric Detection	1799
<i>A. M. Bond and G. G. Wallace</i>	
Electrokinetic Detection in Reversed Phase High Performance Liquid Chromatography. I. Volatile Fatty Acids	1823
<i>W. Kemula, B. K. Głod, and W. Kutner</i>	
Electrokinetic Detection in Reversed Phase High Performance Liquid Chromatography. II. Quaternary Ammonium Ion-Pairs of Some Volatile Fatty Acids	1837
<i>W. Kemula, B. K. Głod, and W. Kutner</i>	
Fabrication and Characterisation of Leak-Tight Glassy Carbon Electrodes, Sealed in Glass Employing Silicon Coating, for Use in Electrochemical Detection	1849
<i>J. C. Hoogvliet, J. A. G. van Bezooyen, A. C. J. Hermans-Lokkerbol, C. J. van der Poel, and W. P. van Bennekom</i>	

Determination of the Oxidized and Reduced Forms of Biopterin in Tissue Samples	1863
<i>C. E. Lunte and P. T. Kissinger</i>	
Interpretations of Voltammetry in the Striatum Based on Chromatography of Striatal Dialysate	1873
<i>J. B. Justice, Jr., S. A. Wages, A. C. Michael, R. D. Blakely, and D. B. Neill</i>	
High Performance Liquid Chromatographic Determination of Phenylenediamines in Aqueous Environmental Samples	1897
<i>R. M. Riggin and C. C. Howard</i>	
Dual Electrochemical Detection of Biogenic Amine Metabolites in Micro High-Performance Liquid Chromatography	1907
<i>M. Goto, E. Sakurai, and D. Ishii</i>	
Liquid Chromatography News	1927
Liquid Chromatography Calendar	1931

Volume 6, Number 11, 1983

Polyvinylacetate Gel Permeation Chromatography of H-Coal Liquids and Model Compounds. Comparison with Rigid Gel, Reversed Phase and Normal Phase Chromatography	1935
<i>J. L. Wong</i>	
Application of Flow Programming in the Analysis of Drugs and Their Metabolites in Biological Fluids	1949
<i>V. P. Shah, M. A. Walker, and V. K. Prasad</i>	
The Effect of Amine Modifiers on the Chromatographic Behavior of Salbutamol on Reversed Phase Chemically Bonded Silica Gel.	1955
<i>N. J. Eggers and C. M. Saint-Joly</i>	
HPLC Isolation and Characterization of Pentacarboxylic Porphyrins Derived from Uroporphyrinogen III	1969
<i>C. K. Lim and J. M. Rideout</i>	
Studies on Steroids. CLXXXII. Determination of 6β-Hydroxycortisol in Urine by High-Performance Liquid Chromatography with Fluorescence Detection.	1977
<i>J. Goto, F. Shamsa, and T. Nambara</i>	
High Performance Liquid Chromatography of Androgen Acetates.	1987
<i>J.-T. Lin</i>	

A Simple Assay of 3-Methoxy-4-hydroxyphenylethyleneglycol in Cerebrospinal Fluid by High Performance Liquid Chromatography	1997
<i>R.-K. Yang, J. P. Edasery, and K. L. Davis</i>	
Liquid Chromatographic Analysis of Pentazocine and Tripeleennamine in Combination.	2005
<i>F. Taylor Noggle, Jr.</i>	
High-Performance Liquid Chromatographic Determination of Penicillins following Derivatization to Mercury-Stabilized Penicillenic Acids	2019
<i>M. E. Rogers, M. W. Adlard, G. Saunders, and G. Holt</i>	
Liquid Chromatographic Analysis of Pentobarbital	2033
<i>W. J. Ferrell and O. Boudoulas</i>	
Determination by High Performance Liquid Chromatography of Stability of Tetrahydro-β-carbolines at Different Ambient Temperatures	2043
<i>R. D. Myers, J. L. Garrison, and E. C. Critcher</i>	
Boll Weevil: Determination of Ecdysteroids and Juvenile Hormones with High Pressure Liquid Chromatography.	2055
<i>J. E. Wright and B. R. Thomas</i>	
The Determination of Iodine in Milk and Milk Chocolate by Anion HPLC . .	2067
<i>W. J. Hurst, K. P. Snyder, and R. A. Martin, Jr.</i>	
Simultaneous Determination of Heavy Metals in Water by High Performance Liquid Chromatography after Solvent Extraction of Heavy Metals as Hexamethylenedithiocarbamate Chelates	2079
<i>S. Ichinoki, T. Morita, and M. Yamazaki</i>	
Hardware for Microprocessor Controlled HPLC: Interfacing of an Interval Timer and Interrupt Controller to the "S100 Bus System".	2095
<i>D. B. Smoll and R. P. Singhal</i>	
Solid Phase Derivatizations in HPLC: Polymeric Permanganate Oxidations of Alcohols and Aldehydes in HPLC-SPR.	2109
<i>K.-H. Xie, C. T. Santasania, I. S. Krull, U. Neue, B. Bidlingmeyer, and A. Newhart</i>	
Book Review	2129
Announcement	2131
Liquid Chromatography News	2133
Liquid Chromatography Calendar.	2137

Volume 6, Number 12, 1983

Special Issue on Liquid Chromatography/Electrochemistry. Part II

- Liquid Chromatography with Electrochemical Detection of Phenol and NADH for Enzyme Immunoassay 2141**
K. R. Wehmeyer, M. J. Doyle, D. S. Wright, H. M. Eggers, H. B. Halsall, and W. R. Heineman
- Carbon Composite Electrodes for Liquid Chromatography/Electrochemistry: Optimizing Detector Performance by Tailoring the Electrode Composition 2157**
D. E. Tallman and D. E. Weisshaar
- Dual Electrode Liquid Chromatography-Electrochemical Detection (LCEC) for Platinum-Derived Cancer Chemotherapy Agents 2173**
X.-D. Ding and I. S. Krull
- A Simultaneous Determination of Trazodone and Its Metabolite 1-m-Chlorophenylpiperazine in Plasma by Liquid Chromatography with Electrochemical Detection 2195**
R. F. Suckow
- Practical Aspects of LC/EC Determinations of Pharmaceuticals in Biological Media 2209**
D. J. Miner, M. J. Skibic, and R. J. Bopp
- Noise and Drift Phenomena in Amperometric and Coulometric Detectors for HPLC and FIA 2231**
H. W. van Rooijen and H. Poppe
- Gradient Elution of Biogenic Amines and Derivatives in Reversed Phase Ion-Pair Partition Chromatography with Electrochemical and Fluorometric Detection 2255**
U. R. Tjaden, J. de Jong, and C. F. M. van Valkenburg
- Use of a Dual Coulometric-Amperometric Detection Cell Approach in Thyroid Hormone Assay 2275**
B. R. Hepler and W. C. Purdy
- Determination of Common Analgesics in Serum and Urine by Liquid Chromatography/Electrochemistry 2311**
D. A. Meinsma, D. M. Radzik, and P. T. Kissinger
- Analysis of Trace Amounts of Catecholamines and Related Compounds in Brain Tissue: A Study Near the Detection Limit of Liquid Chromatography with Electrochemical Detection 2337**
B. H. C. Westerink

COLLECTIVE CONTENTS TO VOLUME 6 2897

High-Speed LC Analysis Using Electrochemical Detection 2353
J. M. Di Bussolo, M. W. Dong, and J. R. Gant

Liquid Chromatography News 2375

Liquid Chromatography Calendar 2379

Volume 6, Number 13, 1983

Special Section – Liquid Chromatography Directory

Practical Microbore Column HPLC: System Development and Drug Applications 2383
C. Eckers, K. K. Cuddy, and J. D. Henion

Instrumental Broadening Correction in Size Exclusion Chromatography through Fast Fourier Transform Techniques 2411
D. Alba and G. R. Meira

Analysis of Block and Statistical Copolymers by Gel Permeation Chromatography: Estimation of Mark-Houwink Constants 2433
J. M. Goldwasser and A. Rudin

Protein Separations on Octyl and Diphenyl Bonded Phases 2465
F. M. Rabel and D. A. Martin

Separation of the Proteins of Locust Neurosecretory Endings with HPLC 2501
J. Girardie, M. Allard, A. Faddoul, and E. Neuzil

High-Pressure Liquid Chromatographic Assay of Azlocillin and Mezlocillin with an Anion-Exchange Extraction Technique 2513
C. E. Fasching and L. R. Peterson

Development of a Standardized Analysis Strategy for Basic Drugs, Using Ion-Pair Extraction and High-Performance Liquid Chromatography. IV. Application to Solid Pharmaceutical Dosage Forms 2521
G. Hoogewijs and D. L. Massart

Ligand Exchange Chromatography of Cephalosporin C on Polystyrene Resins Containing Copper Complex of Lysine Derivatives 2543
D. Sacco and E. Dellacherie

A Rapid HPLC Analysis of Diazepam in Animal Feed 2557
A. P. Pakuts, R. H. Downie, and T. I. Matula

Degradation of High Purity Water on Storage	2565
<i>R. Gabler, R. Hegde, and D. Hughes</i>	
Letter to the Editor	2571
Liquid Chromatography Calendar	2573
Liquid Chromatography Directory	2579

Volume 6, Number 14, 1983

Special Section on Thin-Layer Chromatography

Fractionation into Components of a Mixture of Acidic Ninhydrin-Positive Compounds of Mouse Brain Extracts with Thin-Layer and Ion-Exchange Chromatography	2621
<i>P. Lähdesmäki and M. Timonen</i>	
Analytical Separation of Reduced and Oxidized Forms of Glutathione from Amino Acid Mixtures by Overpressured Thin-Layer Chromatography	2635
<i>M. Ábrahám, B. Polyák, B. Szajani, and L. Boross</i>	
Isolation of Glycolipids from Blood Elements	2647
<i>J. Pick, J. Vajda, and L. Leisztner</i>	
Identification of Some Potential Antidiabetic Compounds on Impregnated Silica Gel G Platea as Their π-Complexes	2661
<i>R. Jain and D. D. Agarwal</i>	
The Role of the Specific Surface Area of an Adsorbent in the Optimization of Mixture Separation Conditions in Thin-Layer Chromatography	2665
<i>J. K. Rożyło, I. Malinowska, and M. Pönieważ</i>	
Determination of Biphenyl in Citrus Fruits by Quantitative Thin-Layer Chromatography	2679
<i>J. Sherma, P. J. Sielicki, Jr., and S. Charvat</i>	
Use of Some Silver Salts of Aromatic Sulfonic Acids in Thin-Layer Chromatography	2687
<i>P. Ilinov and S. I. Dimov</i>	
Characterization of Derivatives of Some Closely Related 1-Picolinoyl-3-methyl-4-(substituted)-pyrazoline-5-ones by Thin-Layer Chromatography	2695
<i>D. R. Gupta and R. K. Arora</i>	
Phospholipid Composition and Differentiation of Methanotrophic Bacteria	2699
<i>L. V. Andreev and V. F. Galchenko</i>	

Regular Contributed Papers

Modification of Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons Using Mixed Stationary Phases	2709
<i>S. A. Wise, L. C. Sander, and W. E. May</i>	
Aging of Pressure Sensitive Adhesives. II. Use of Multidetector SEC	2723
<i>D. J. P. Harrison, W. R. Yates, and J. F. Johnson</i>	
Determination of Uric Acid in Human Serum: Reversed-Phase Liquid Chromatography with Electrochemical Detection	2739
<i>K. Iriyama, M. Yoshiura, T. Iwamoto, T. Hosoya, H. Kono, and T. Miyahara</i>	
High Pressure Liquid Chromatographic Assay of Cefamandole in Serum Following Intravenous and Intraperitoneal Administration	2747
<i>R. E. Bawdon, K. J. Leveno, J. G. Quirk, F. G. Cunningham, and S. P. Guss</i>	
A Rapid High Performance Liquid Chromatographic Method for the Simultaneous Measurement of Six Tricyclic Antidepressants	2761
<i>J. S. Kiel, R. K. Abramson, S. L. Morgan, and J. C. Voris</i>	
Determination of Ritodrine in Plasma Using HPLC	2775
<i>P. Kuhnert, P. Erhard, A. Dixon, B. Kuhnert, and T. Gross</i>	
Analysis of Indenolol in Biological Fluids by High Performance Liquid Chromatography	2785
<i>S. A. Babhair, H. Y. About-Enein, and S. El-Houfy</i>	
HPLC Analysis of Oligomeric Ethylene Glycol Mixtures via bis(2,4-Dinitrophenylation).	2797
<i>A. Warshawsky, N. Shoef, and A. Tishbee</i>	
Data Acquisition and Processing for High Speed Liquid Chromatography. . .	2809
<i>J. M. Anderson</i>	
Separation of Chlorinated Phenols by Isocratic High-Performance Liquid Chromatography on Reverse Phase Column	2829
<i>J. Nair, K. M. Munir, and S. V. Bhide</i>	
Announcements	2839
Liquid Chromatography News	2841
Liquid Chromatography Calendar.	2845
Author Index to Volume 6	2849
Subject Index to Volume 6	2857

Volume 6, Supplement 1, 1983

Prospects for Carbon as Packing Material in High-Performance Liquid Chromatography	1
<i>J. H. Knox, K. K. Unger, and H. Mueller</i>	
Post-Column Reaction Detection Systems in HPLC	37
<i>Sj. van der Wal</i>	
A Simplified Solution of Tung's Instrumental Spreading Equation in Size-Exclusion Chromatography	61
<i>S. Mori, T. Suzuki, and A. Wada</i>	
Recent Advances in HPLC Optical Detection	77
<i>S. R. Abbott and J. Tusa</i>	
Analysis of Flavonoids by HPLC	105
<i>D. J. Daigle and E. J. Conkerton</i>	
Liquid Chromatography News	119
Liquid Chromatography Calendar	121

Volume 6, Supplement 2, 1983

Solid Phase Reactions for Derivatization in HPLC (HPLC-SPR)	125
<i>K-H. Xie, S. Colgan, and I. S. Krull</i>	
High Performance Liquid Chromatography in Oenology	153
<i>M. E. Evans</i>	
Hydrodynamic and Size Exclusion Chromatography of Particle Suspensions — An Update	179
<i>A. Penlidis, A. E. Hamielec, and J. F. MacGregor</i>	
Flow Rate Dependence of Elution Volumes in Size Exclusion Chromatography: A Review	219
<i>J. H. Aubert and M. Tirrell</i>	
High-Performance Liquid Chromatography for Analyses of Antibiotics in Biological Fluids	251
<i>I. Nilsson-Ehle</i>	

keep in touch with the latest . . .

ADVANCES
IN
chromatography

edited by **J. CALVIN GIDDINGS**, *University of Utah, Salt Lake City*
ELI GRUSHKA, *The Hebrew University of Jerusalem, Israel*
JACK CAZES, *Fairfield, Connecticut*
PHYLLIS R. BROWN, *University of Rhode Island, Kingston*

VOLUME **21**

March, 1983 360 pages, illustrated

VOLUME 21

CONTENTS

High-Performance Liquid Chromatography/
Mass Spectrometry, (HPLC/MS)
David E. Games

High Performance Liquid Affinity Chroma-
tography
*Per-Olof Larsson, Mågnus Glad, Lennart
Hansson, Mats-Olle Mansson, Sten Ohlson,
and Klaus Mosbach*

Dynamic Anion-Exchange Chromatography
R.H.A. Sorel and A. Hulshoff

Capillary Columns in Liquid Chromatography
Daido Ishii

Droplet Counter-Current Chromatography
Kurt Hostettmann

Chromatographic Determination of Copoly-
mer Composition
Sadao Mori

High-Performance Liquid Chromatography
of K Vitamins and Their Antagonists
Martin J. Shearer

Problems of Quantitation in Trace Analysis
by Gas Chromatography
Josef Novák

MARCEL DEKKER, INC.
270 MADISON AVENUE, NEW YORK, N.Y. 10016
(212) 696-9000

ADVANCES IN **chromatography**

THE EXPLOSIVE GROWTH of chromatography has made it difficult for any single person to maintain a coherent view of progress in the field. Individual investigators trying to preserve even a moderate awareness of advances must rely upon responsible surveys, rather than attempting to read the avalanche of original research papers.

This continuing series presents critical, current reviews, written by noted authorities, of the most important developments of chromatographic science. The wealth of literature, even in specialized areas of chromatography, is so great that in these superb volumes recognized experts separate hard-core advances from the overwhelming mass of supporting evidence and data. The authors summarize and develop self-contained evaluations of various facets and trends in research, putting their subject matter into perspective.

Volume 21, like its predecessors, provides up-to-the-minute coverage of topics of contemporary interest, including

- **quantitation in trace analysis by GC, by the renowned expert, Josef Novák**
- **HPLC/MS instrumentation and applications, one of the fastest growing chromatographic techniques**
- **the use of capillary columns in LC, an approach that lowers costs, speeds analyses, and affords ultra high resolution**
- **droplet counter-current chromatography, a newly developed partition chromatography method**
- **chromatographic determination of copolymer composition, expanding the range of chromatographic applications**

These volumes are of utmost interest to all researchers who need to use separation techniques effectively—especially analytical, organic, and physical chemists; biochemists; biomedical researchers; clinical chemists; polymer scientists; environmental scientists; as well as scientists in academia, government, hospitals, and industry, both in research and quality control.

INSTRUCTIONS FOR PREPARATION OF MANUSCRIPTS FOR DIRECT REPRODUCTION

Journal of Liquid Chromatography is a bimonthly publication in the English language for the rapid communication of liquid chromatographic research.

Directions for Submission

One typewritten manuscript suitable for direct reproduction, carefully inserted in a folder, and two (2) copies of the manuscript must be submitted. Since all contributions are reproduced by direct photography of the manuscripts, the typing and format instructions must be strictly adhered to. Noncompliance will result in return of the manuscript to the authors and delay its publication. To avoid creasing, manuscripts should be placed between heavy cardboards and securely bound before mailing.

Manuscripts should be mailed to the Editor:

Dr. Jack Cazes
Journal of Liquid Chromatography
P. O. Box 1440-SMS
Fairfield, Connecticut 06430

Reprints

Owing to the short production time for articles in this journal, it is essential to indicate the number of reprints required upon notification of acceptance of the manuscript. Reprints are available in quantities of 100 and multiples thereof. For orders of 100 or more reprints, twenty (20) free copies are provided. A reprint order form and price list will be sent to the author with the notification of acceptance of the manuscript.

Format of Manuscript

1. The general format of the manuscript should be as follows: title of article; names and addresses of authors; abstract; and text discussion.

2. Title and Authors: The entire title should be in capital letters and centered on the width of the typing area at least 2 inches (5.1 cm) from the top of the page. This should be followed by three lines of space and then by the names and addresses of the authors in the following way (also centered):

A SEMI-AUTOMATIC TECHNIQUE FOR THE
SEPARATION AND DETERMINATION OF
BARIUM AND STRONTIUM IN SURFACE WATERS
BY ION EXCHANGE CHROMATOGRAPHY AND
ATOMIC EMISSION SPECTROMETRY

F. D. Pierce and H. R. Brown
Utah Biomedical Test Laboratory
520 Wakra Way
Salt Lake City, Utah 84108

3. Abstract: Three lines below the addresses, the title ABSTRACT should be typed (capitalized and centered on the page). This should be followed by a single-spaced, concise, abstract comprising less than 10% of the length of the text of the article. Allow three lines of space below the abstract before beginning the article itself.

4. Text Discussion: Whenever possible, the text discussion should be divided into such major sections as INTRODUCTION, MATERIALS, METHODS, RESULTS, DISCUSSION, ACKNOWLEDGMENTS, and REFERENCES. These major headings should be separated from the text by two lines of space above and one line of space below. Each heading should be in capital letters, centered, and underlined. Secondary headings, if any, should be flush with the left margin, underscored, and have the first letter of all main words capitalized. Leave two lines of space above and one line of space below secondary headings.

5. Paragraphs should be indented five (5) typewriter spaces.

6. Acknowledgment of collaboration, sources of research funds, and address changes for an author should be listed in a separate section at the end of the paper.

7. References (including footnotes) in the text will be numbered consecutively by numbers in parentheses. All references (and footnotes) should then be aggregated in sequence at the end of the communication. No footnotes should be shown at the bottom of pages. The reference list follows immediately after the text. The word REFERENCES should be capitalized and centered above the reference list. It should be noted that all reference lists should contain initials and names of all authors; *et al.* will not be used in reference lists. Abbreviations of journal titles and styles of reference lists will follow the American Chemical Society's Chemical Abstracts List of Periodicals. References should be typed single-spaced with one line space between each reference.

8. Each page of manuscript should be numbered lightly at the bottom of the sheet with a light blue pencil.

9. Only standard symbols and nomenclature approved by the International Union of Pure and Applied Chemistry should be used.

10. Any material that cannot be typed, such as Greek letters, script letters, and structural formulae, should be drawn carefully in black India ink (do not use blue ink).

Typing Instructions

1. The manuscript must be typewritten on good quality white bond paper measuring approximately 8½ x 11 inches (21.6 cm x 27.9 cm). Do not use Corrasible bond or its equivalent. The typing area of the article opening page, including the title, should be 5½ inches wide by 7 inches deep (14 cm x 18 cm). The typing area of all other pages should be no more than 5½ inches wide by 8½ inches deep (14 cm x 21.6 cm).

2. In general, the chapter title and the abstract, as well as the tables and references, are typed single-spaced. All other text discussion should be typed 1½-line spaced, if available, or double-spaced. Prestige elite characters (12 per inch) are recommended, if available.

3. It is essential to use black typewriter ribbon (carbon film is preferred) in good condition so that a clean, clear impression of the letters is obtained. Erasure marks, smudges, creases, etc., may result in return of the manuscript to the authors for retyping.

4. Tables should be typed as part of the text but in such a way as to separate them from the text by a three-line space at both top and bottom of each table. Tables should be inserted in the text as close to the point of reference as possible, but authors must make sure that one table does not run over to the next page, that is, no table may exceed one page. The word TABLE (capitalized and followed by an Arabic number) should precede the table and be centered on the page. The table title should have the first letters of all main words in capitals. Titles should be typed single-spaced. Use the full width of the type page for the table title.

5. Drawings, graphs, and other numbered figures should be professionally drawn in black India ink (do not use blue ink) on separate sheets of white paper and placed at the end of text. Figures should not be placed within the body of the text. They should be sized to fit within the width and/or height of the type page, including any legend, label, or number associated with them. Photographs should be glossy prints. A typewriter or lettering set should be used for all labels on the figures or photographs; they may not be hand drawn. Captions for the pictures should be typed single-spaced on a separate sheet, along the full width of the

type page, and preceded by the word FIGURE and a number in arabic numerals. All figures and lettering must be of a size to remain legible after a 20% reduction from original size. Figure numbers, name of senior author, and arrow indicating "top" should be written in light blue pencil on the back or typed on a gummed label, which should be attached to the back of the illustration. Indicate approximate placement of the illustrations in the text by a marginal note in light blue pencil.

6. The reference list should be typed single-spaced although separated from one another by an extra line of space. Use Chemical Abstract abbreviations for journal titles. References to journal articles should include (1) the last name of all author(s) to any one paper, followed by their initials, (2) article title, (3) journal, (4) volume number (underlined), (5) first page, and (6) year, in that order. Books should be cited similarly and include (1) author, surname, first and middle initials, (2) title of book, (3) editor of book (if applicable), (4) edition of book (if any), (5) publisher, (6) city of publication, (7) year of publication, and (8) page reference (if applicable). E.g., Journals: Craig, L. C. and Konigsber, W., Use of Catechol Oxygenase and Determination of Catechol, *Chromatogr.*, 10, 421, 1963. Books: Albertsson, P. A., *Partition of Cell Particles and Macromolecules*, Wiley, New York, 1960. Article in a Book: Walter, H., *Proceedings of the Protides of Biological Fluids, XVth Colloquim, Peeters., H., eds., Elsevier, Amsterdam, 1968, p. 367.*

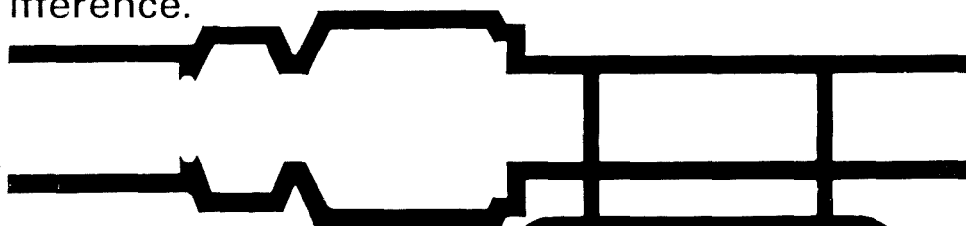
Custom packing HPLC columns has become our specialty. Any length, several ID's (including .2mm) and almost any commercially available packing material may be specified. We'll supply the column others won't.

With each column, you will receive the original test chromatogram plus a vial of the test mixture. Our advanced technology and computer testing is your assurance of a quality product.

When custom packing and testing is your special concern, we make the difference.

**Each
one
is
our
special
concern**

**CUSTOM
PACKED
HPLC
COLUMNS**



For further information contact:

ALLTECH ASSOCIATES, INC.
2051 Waukegan Road
Deerfield, Illinois 60015
312/948-8600

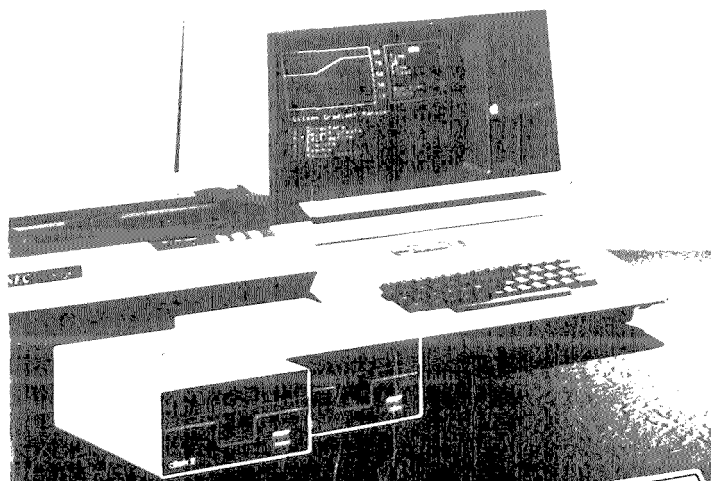
Specifications

*The way
you want it!*

ALLTECH ASSOCIATES

GILSON HPLC

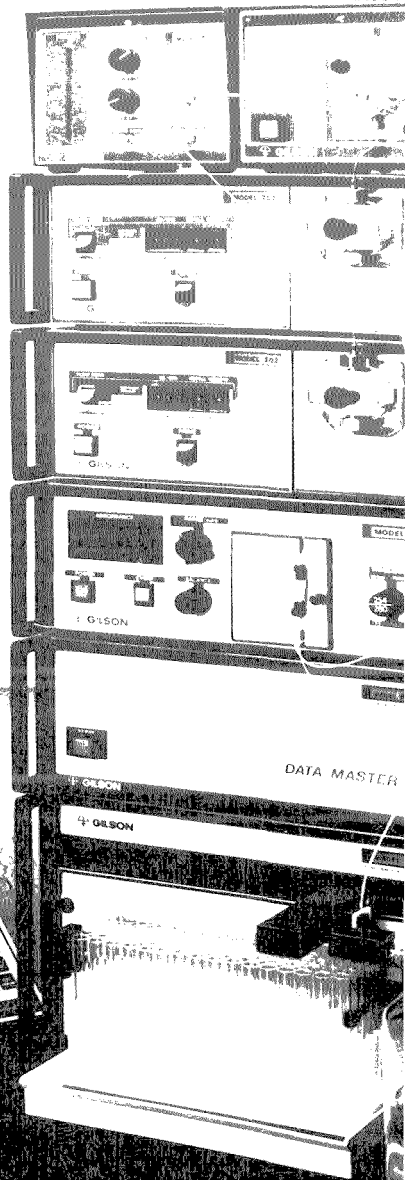
adds up to
the right system
at the right price.



Going with Gilson can add up to this. Gilson Gradient System with Apple-base gradient Controller, Data-Master for peak analysis and programmable 201 fraction collector.

...all for less than \$21,000.*

Modularity makes it work. Our add-on approach lets you match the right system to your application. And expansion through hardware and software additions will enhance your system's capabilities without costly duplication. Let Gilson modularity work for you. Contact us for complete information.



GILSON
INTERNATIONAL

Box 27, 3000 W. Beltline, Middleton, WI 53562
Telephone (608) 836-1551

B.P. No. 45, 95400, Villiers-le-Bel, France
Telephone (3) 990.54.41

*U.S. shipments only