DECOMPOSITION (CHEMICAL INDEXES)

Confirmatory Tests for Aflatoxin B$_1$

By PETER JOHN ANDRELLOS and GEORGE R. REID (Division of Food Chemistry, Food and Drug Administration, 408 Atlantic Avenue, Boston, Mass. 02210)

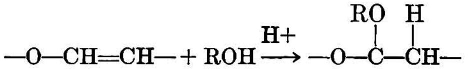
Three confirmatory tests have been devised to identify aflatoxin B$_1$. Portions of the isolated toxin are treated with formic acid-thionyl chloride, acetic acid-thionyl chloride, and trifluoroacetic acid, respectively, and aliquots of the three fluorescent reaction products are spotted on thin-layer chromatography plates. Standards treated with each of the three reagents, plus an untreated standard, are spotted on the same plate, and after development the spots are compared under ultraviolet light.

The aflatoxins are a group of fluorescent substances originally isolated from moldy peanut products contaminated by *Aspergillus flavus* (1–5). A number of procedures have been devised to detect and measure these toxic metabolites. However, additional confirmatory tests are still needed to differentiate unambiguously between the toxic compounds and other fluorescent materials that may be present in peanut products.

We have devised simple confirmatory tests for aflatoxin B$_1$, the principal component of aflatoxins. Each test is essentially independent of the others, but in the procedure described in this paper they are performed concurrently and the results compared for a confirmatory test. The isolated toxin undergoes three separate reactions with different reagents to form fluorescent compounds; separate portions of pure aflatoxin are also treated with each of the three reagents to serve as standards. The three reaction products and the three standards are spotted on a single thin-layer chromatography plate, together with a portion of pure, untreated aflatoxin as reference. The plate is developed and the spots of the reaction products are compared under ultraviolet light with the spots of the treated standards and the untreated reference for identification.

Experimental

The test is thought to be based on the ability of the olefinic linkage of an enol ether unit to react additively with a hydroxyl group under the catalytic influence of a strong acid (6). This adduct formation is a general, well-studied reaction that requires anhydrous conditions and is accompanied by few, if any, side reactions. The OR-group introduced is invariably bonded to the ether end of the double bond and the H-atom is attached to the other end, as shown:



$$\text{—O—CH=CH—} + \text{ROH} \xrightarrow{\text{H+}} \text{—O—}\overset{\text{RO}}{\overset{|}{\text{C}}}\text{—}\overset{\text{H}}{\overset{|}{\text{CH}}}\text{—}$$

Aflatoxin B$_1$ (see Fig. 1) contains such an enol ether unit in the furano ring (7), and this unsaturation is insulated from the main chromophore of the compound. Addition reactions at this isolated double bond should not, therefore, significantly alter the fluorescence spectrum of the original metabolite, but should alter its chromatographic behavior. By choosing the proper addition reactions and comparing the chromatographic behavior of the reaction products, the identity of aflatoxin B$_1$ can be confirmed.

We based our approach on the fact that a reaction medium composed of aflatoxin B$_1$ and a volatile strong acid dissolved in one of the lower boiling hydroxylic solvents could readily be evaporated to obtain a mixture consisting of the unreacted toxin and/or its reaction products. The residue could then be taken up in chloroform and applied directly to thin-layer chromatographic plates, developed, and examined visually.

After some investigation, we chose thionyl chloride for use as the acid catalyst for the addition reaction. Thionyl chloride reacts in comparatively small quantities with hydroxylic solvents to generate hydrogen chloride *in situ*. The low-boiling alcohols (methanol, ethanol, 2-propanol, and 1-butanol) were tried as solvents, but they gave erratic results. However, reproducible results could be obtained by using acetic acid and formic acid as hydroxylic reagents.

In the absence of thionyl chloride, these reactions were very slow. We assumed that this failure of the uncatalyzed addition was due to the inadequate acid strengths of formic and acetic acids. We therefore considered the possibility that trifluoroacetic acid (TFA), a very strong acid, would react with the mold metabolite without the addition of thionyl chloride. Experimental work confirmed this hypothesis and TFA was chosen as the reactant for the third confirmatory test.[1]

Confirmatory Test For Aflatoxin B_1

Reagents and Apparatus

(a) *Thionyl chloride.*—Clear colorless; commercial product is suitable.

(b) *Acetic acid.*—Glacial.

(c) *Formic acid.*—90% reagent grade.

(d) *Trifluoroacetic acid.*—Anhydrous (Eastman Kodak White Label was used). Caution: this compound is exceedingly hygroscopic; do not expose it to atmospheric moisture for prolonged periods.

(e) *Thin-layer chromatographic apparatus.*—20 × 20 cm plate, coated 250 μ thick with Silica Gel G-HR (may be obtained from Brinkmann Instruments, Inc., Long Island, N.Y.). Develop plate with methanol:chloroform (5:95, v/v).

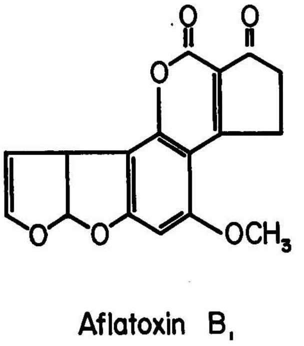


Fig. 1—Structure of aflatoxin B_1.

Extraction and Purification

Extract aflatoxin from the moldy food product by the procedure described previously (8, 9). To free the B_1 component from the other toxins and extraneous matter, separate it by preparatory thin-layer chromatography on silica gel, using methanol:chloroform (5:95, v/v) as the developer. In some cases a preliminary cleanup by column chromatography may be necessary. Scrape the isolated aflatoxin B_1 from the plate into a suitable container, and elute with methanol. Remove the silica gel by filtration, and the methanol by evaporation.

(This aflatoxin B_1 is pure enough for the test described here. At least 1 μg of chromatographically pure aflatoxin should be available for the three confirmatory tests, although we have occasionally been able to obtain positive results with about 0.25 μg.)

Dissolve the isolated aflatoxin B_1 in 300 μl chloroform, and divide it into three equal portions. Place one portion in each of three conical centrifuge tubes.

Determination

(a) *Acetic acid-thionyl chloride test.*—Add 0.2 ml glacial acetic acid and 1 drop clear, colorless thionyl chloride to one portion of the chloroform solution from *Extraction and Purification*, stopper the tube, and mix the contents by shaking until homogeneous. Let the mixture stand at room temperature for 5 minutes; then evaporate the solution just to dryness in a hot water bath under a stream of nitrogen, carefully keeping the solution and the residue in the very bottom of the tube. Carefully dissolve the residue in 30 μl chloroform. Transfer about half of this solution with a micro-syringe to a spot on the origin of the thin-layer chromatographic plate.

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[1] Although bromine almost instantly transformed crystalline aflatoxin B_1 to a substance that formed a single fluorescent spot on TLC, the action of bromine on crude aflatoxin B_1 isolated from mold extracts gave poor results. Efforts to improve this situation were unsuccessful.

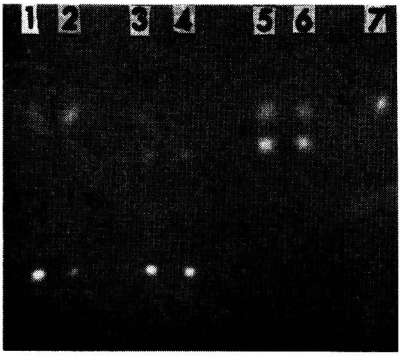

Fig. 2—**Example of positive confirmatory test for aflatoxin B_1. Spots are identified as follows, left to right: 1, trifluoroacetic acid (TFA) plus sample; 2, TFA plus crystalline B_1; 3, formic acid-thionyl chloride plus sample; 4, formic acid-thionyl chloride plus crystalline B_1; 5, acetic acid-thionyl chloride plus sample; 6, acetic acid-thionyl chloride plus crystalline B_1; 7, crystalline B_1.**

Treat 0.1 μg authentic aflatoxin B_1 with acetic acid and thionyl chloride in the same manner as the test sample. Spot on the origin line of the same thin-layer chromatographic plate about 2 cm from the sample spot.

(b) *Formic acid-thionyl chloride test.*—Proceed as for acetic acid-thionyl chloride, but use formic acid in place of acetic acid. Prepare a standard as described above, and spot both reaction products on the same thin-layer chromatographic plate, keeping all spots about 2 cm apart.

(c) *Trifluoroacetic acid (TFA) test.*—Treat the third portion of the chloroform solution from *Extraction and Purification* with 3 drops TFA and agitate the mixture for 60 sec. (If the solution has been prepared from extracted aflatoxin B_1, it often turns pink at this point.) Evaporate with gentle heating in a stream of nitrogen, dissolve the residue in chloroform, and spot it on the same thin-layer chromatographic plate with the formic and acetic acid samples and standards, in the same manner. Again prepare a standard from authentic aflatoxin B1, as described above.

(d) *Identification.*—Apply a spot of about 0.05 μg authentic aflatoxin B_1 was a reference standard on the same thin-layer chromatographic plate. (The final plate will contain seven spots at the origin.) Develop the chromatogram with methanol-chloroform (5:95, v/v) in an equilibrated, insulated tank. View the developed plate under long wavelength ultraviolet light. The plate may be photographed under ultraviolet illumination for a permanent record.

Discussion

Figure 2 shows an example of the results obtained when the proposed procedure was applied to aflatoxin B_1 extracted from a peanut meal. The acetic acid-thionyl chloride reaction products typically form two bright spots of about equal size and intensity. The R_f values of these spots are about 0.50 and 0.43, compared to 0.50 for authentic aflatoxin B_1. The formic acid-thionyl chloride reaction yields a single large bright spot with an R_f of about 0.18. The reaction with trifluoroacetic acid produces two spots, one with the same R_f as aflatoxin B_1 and the other with an R_f of about 0.20. Since these are the spots that have consistently appeared from these reactions, they can be considered characteristic.

It has been found that the tests are equally applicable to aflatoxin G_1.

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DISINFECTANTS

Resistance of *Pseudomonas* to Various Chemical Germicides

By A. BELOIAN and T. KOSKI (Pesticides Regulation Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.)

Studies were conducted to determine the range of susceptibility of *Pseudomonas* to seven samples of commercial contact germicides. Tests were made by the official phenol coefficient method, 5.001–5.009. Ranges of resistance for *Pseudomonas* strains varied with the germicides tested and the concentration of germicide used. Chlorine in water supply systems showed a resistance range of 45–150 ppm to *Pseudomonas* organisms. Comparative studies were also conducted, using the dilution test method, on various strains of *Pseudomonas* to determine the effectiveness of disinfectants; strain PRD-10 proved to be the test strain of choice for determining the validity of *Pseudomonicidal* claims.

It is commonly believed that bacteria of the genus *Pseudomonas* are highly resistant to chemical germicides. Thus, disinfectant manufacturers have been led into the belief that use of claims for efficacy against these organisms on the labels of their products serve as an aid to the public acceptance and promote sales.

Variations in sensitivity to antimicrobial chemicals of different strains of *Pseudomonas* have been recognized as factors of major importance in the control of microbiological deterioration (1). Interest in the genus *Pseudomonas* has been aroused recently by the implication of certain species in the destruction of monomolecular films of hexadecanol, a compound used in the suppression of evaporation from water reservoir surfaces in semiarid areas (2). In hospitals there has been a steady increase in the isolation of *Pseudomonas* from secondary infections in severely burned and terminal cancer patients, and this has led to an increased interest in germicides that may be effective in eliminating these bacteria.

A survey of the literature reveals that no studies have been made concerning variations in sensitivity of different strains of *Pseudomonas* to a representative spectrum of commercial disinfectants. This investigation was undertaken, therefore, to determine strain variation and sensitivity to germicides as a means for selecting a strain of this genus with uniformly high resistance to the various types of chemical germicides commonly sold as disinfectants; the strain will be used in the Microbiological Testing Laboratory of the Pesticides Regulation Division of the United States Department of Agriculture.

Susceptibility of *Pseudomonas* to Germicides

Ten strains of *Pseudomonas aeruginosa* and one strain of *Ps. malltophilia* were selected for this particular study following a preliminary screening in which the main criterion for acceptance was good growth in AOAC Nutrient Broth after 18–24 hours at 37°C. Monthly transfers of the cultures were carried on AOAC Nutrient Agar slants, Trypticase Soy Agar slants, and semi-solid Cystine Trypticase Agar stabs.

The latter medium appears to be superior to AOAC Nutrient Agar slants and Trypticase Soy Agar slants in sustaining viability of the various strains of *Pseudomonas* at refrigerator temperatures during extended periods of storage. Even more important, keeping characteristics for individual strains are better, with regard to dissociation and variations in such strain properties (pigment production, ability to grow in a variety of subculture media, and the like). Bennett, *et al.* (1) reported variations in the sensitivity of 15 *Pseudomonas* strains to germi-

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cides after 11 weeks of storage under refrigeration. Their cultures were carried in Nutrient Broth prepared with tap water and there is no indication that a solid medium was used for either primary propagation or storage. Thus, the relation of their findings to those made in studies where solid or semisolid media were employed is not completely clear. It has been further noted by Gainor and Williamson (3) that the resistance against aqueous iodine of a flocculent variant of *Ps. aeruginosa* was tenfold greater than the smooth phase of the same organism. Since these factors may be of importance to the continuing success of any germicidal test employing a strain of *Pseudomonas*, these investigations were planned to include a study on this phase of the problem; however, the results reported here cannot be differentiated on this basis because of the short time period involved.

Germicides were tested by the official phenol coefficient method (4). Working cultures in AOAC Nutrient Broth were found to give generally consistent responses up to 25 daily transfers.

The seven disinfectants selected for testing (see Table 1) were chosen for universality of use and included commercial products sampled and tested in the Pesticides Regulation Division Laboratories. Among these were a pure phenol, a synthetic phenol emulsion germicide, an iodophor, NF Saponated Solution of Cresol, a 10% aqueous preparation of alkyl (C_{12}, C_{14}, C_{16}) dimethyl benzyl ammonium chloride, a 5% quaternary alkali-salt egg wash, and a commercial sodium hypochloride. The hypochloride was standardized for available chlorine by the official arsenious oxide titration method (4).

Results and Discussion

The results are summarized in Table 1. Among the strains of *Ps. aeruginosa* the resistance to the pure chemical phenol ranged from a dilution of 1–60 to 1–85. This is not appreciably different from the ranges shown by strains of other species of gram-negative and gram-positive nonspore-forming bacteria in the test method employed and is in agreement with resistances found by other investigators (5, 6).

Resistances against the synthetic phenol emulsion and the iodophor were generally uniform for all 11 strains studied. A wider range of resistance is shown for the NF Saponated Solution of Cresol. Ostrolenk and Brewer (6) have reported killing *Ps. aeruginosa* with USP Saponated Cresol after 10 minutes of exposure at a dilution of 1–150.

Table 1. Range of susceptibility of *Pseudomonas* to samples of commercial contact germicides (maximum killing dilutions or lowest killing concentrations by phenol coefficient test; 10 minutes, 20°C)[a]

Pseudomonas Strain[a,b]	Phenol Diln	Synthetic Phenol Emulsion, Diln	Iodophor, 1.75% Available I_2		NF Saponified Solution Cresol Diln	Quaternary 10%		Quaternary Alkali-Salt, 5% (Egg-Wash)		Hypochlorite Concn, ppm Available Cl^-
			Diln	Concn, ppm		Diln	Concn, ppm	Diln	Concn, ppm	
PRD-1	1–85	1–550	1–250	70	1–150	1–1250	80	1–1000	50	60
PRD-2	1–85	1–600	1–250	70	1–150	1–1350	74	1–400	125	50
PRD-3	1–80	1–600	1–350	50	1–350	1–550	182	1–1000	59	60
PRD-6	1–80	1–500	1–350	50	1–150	1–1400	71	1–900	56	60
PRD-7	1–75	1–500	1–250	70	1–350	1–6000	17	1–1400	36	100
PRD-8	1–80	1–550	1–250	70	1–150	1–900	111	1–2200	23	70
PRD-9	1–60	1–600	1–250	70	1–150	1–950	105	1–2250	22	150
PRD-10	1–80	1–500	1–250	70	1–150	1–1050	95	1–1300	38	60
PRD-11	1–80	1–500	1–250	70	1–150	1–600	167	1–2200	23	45
PRD-12	1–80	1–450	1–350	50	1–150	1–750	133	1–2000	25	50
PRD-13	1–80	1–600	1–250	70	1–150	1–1300	77	1–1700	29	50

[a] *Official Methods of Analysis*, 9th Ed., 1960.

[b] All cultures identified as *Pseudomonas aeruginosa* except PRD-7 which was identified as *Ps. malltophilia.*

Iodophors have been reported effective in killing *Ps. aeruginosa* in laboratory studies at iodine concentrations of 10–12 ppm in 30 seconds (7). In other types of procedures activity of 50 ppm iodine, after 10 minutes of exposure at 20°C in water, increased to 1000 ppm in the presence of 10% horse serum.

The differences in resistance between the 10% aqueous quaternary and the 5% quaternary alkali-salt egg wash formulation were especially interesting. On the whole they show the quaternary alkali-salt formula to be from 2 to 4 times as active as the aqueous preparation against these organisms on the basis of the concentration of the quaternary salt present.

Variations in resistance between individual strains were most pronounced when testing against the 10% aqueous quaternary. As results with the strain of *Ps. malltophilia* (PRD-7) illustrate, we have found many exceptions to generalizations on the subject of resistance in this genus. This strain is highly susceptible to an aqueous quaternary (a dilution of 1–6000 of a 10% product or 17 ppm actual quaternary being effective), yet it exhibits high resistance to phenol and to available chlorine.

Aqueous quaternaries similar to that tested in these studies were reported to kill *Ps. aeruginosa* after 10 minutes of exposure at concentrations ranging from 100 ppm (5) to 250 ppm in distilled water, to 1000 ppm in the presence of 10% horse serum (8). Johns (7) has reported killing *Pseudomonas* in 30 seconds with quaternaries at a concentration of 100 ppm, but in the presence of 0.5% skim milk, 300 ppm was required. Strains of *Ps. aeruginosa* (Table 1) required dilutions as low as 1–550 or a concentration of approximately 200 ppm to kill. On the other hand, MacGregor and Elliker (9) have reported propagating *Ps. aeruginosa* repeatedly in media containing up to 2000 ppm of an aqueous quaternary to yield strains with high resistance to this germicide.

The range in resistance from 45 to 150 ppm of available chlorine is startling in view of the fact that organisms of the genus *Pseudomonas* are widely distributed in water supplies and great dependence is

Table 2. Statistical evaluation of the variation in sensitivity by *Pseudomonas aeruginosa* in 12 determinations against pure phenol[a]

	Pseudomonas Strain										
	PRD-1	PRD-2	PRD-3	PRD-6	PRD-7	PRD-8	PRD-9	PRD-10	PRD-11	PRD-12	PRD-13
	85	80	80	85	80	85	65	80	80	80	80
	85	85	80	90	75	85	75	85	85	90	75
	85	85	90	85	75	80	60	80	90	85	85
	90	95	90	80	75	80	55	90	80	80	85
	85	85	80	80	80	80	80	85	75	95	80
	90	85	75	95	75	85	60	80	80	85	85
	90	85	85	80	75	85	60	85	85	80	80
	85	90	85	80	80	85	55	80	80	85	80
	90	90	80	85	75	90	60	80	80	85	75
	90	85	90	90	90	80	60	85	80	80	85
	85	95	85	90	75	80	80	80	75	80	85
	90	85	80	80	75	80	60	75	80	80	80
Mean	88	87	83	85	78	83	65	82	81	84	81
Range	85–90	80–95	75–90	80–95	75–90	80–90	55–80	75–90	75–90	80–95	75–85
Std Dev.	2.6	4.5	5.8	8.1	4.5	2.7	8.6	4.0	4.2	4.8	3.8
Std Error	0.8	1.3	1.7	2.3	1.3	0.8	2.5	1.1	1.2	1.4	1.1

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[a] Results expressed as maximum killing dilution or lowest killing concentration by phenol coefficient test; 10 minutes, 20°C. PRD-7 identified as *Ps. malltophilia*.

placed on chlorine in the control of microorganisms in water supply systems.

The dilution tube method of testing employed in these studies has provided valid data for comparative purposes, but it should be emphasized that the effective concentrations or dilutions listed in Table 1 could not be expected to have significance in terms of practical use-dilutions or concentrations in all situations where these products might be recommended.

On the basis of the data in Table 1, strains PRD-3 and PRD-10 were selected as candidates for the test strain of choice for Pseudomonicidal testing in the existing AOAC methods to determine effectiveness of disinfectants. Strain PRD-3 possesses a pattern of resistance which appears most desirable when tested against various germicides. However, strain PRD-10, because of its consistently uniform behavior in the laboratory, was selected as the test strain. This selection was made following statistical analysis of data on 10 minute pure phenol killing dilution from 12 tests made on each strain. With strain PRD-10 a standard deviation of 4.0 and a standard error around the mean of 82 ± 1.1 are shown. Analysis of similar data for strain PRD-3 showed a standard deviation of 5.8 and a standard error of 83 ± 1.7. Data are presented in Table 2 for all test strains.

PRD-10 should be accepted as the test strain of choice for determining the validity of Pseudomonicidal claims. (The culture is being filed with the American Type Culture Collection.)

Studies on the effect of the media used in carrying stock cultures on strain stability will be continued for at least a year. The importance of this part of the study should not be underestimated, since the information in the literature and the observations reported here suggest that species of this genus have the ability to adapt themselves to wide changes in environment and that these adaptations probably include changes in resistance to chemicals.

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FLAVORS AND NON-ALCOHOLIC BEVERAGES

Determination of Compounds Related to Vanillin in Vanilla Extracts

By D. MORISON SMITH (Food and Drug Directorate, Tunney's Pasture, Ottawa, Ontario, Canada)

A method has been developed for the estimation of the quantities of vanillic acid, *p*-hydroxybenzoic acid, and *p*-hydroxybenzaldehyde in vanilla extracts. This method separates these compounds from each other and from vanillin by two-dimensional paper chromatography. An estimate of the quantity of each of these compounds is obtained by measuring the area of the colored spot produced on reacting the compounds with chromogenic reagents. The vanillin content was also determined by two different methods. *Vanilla planifolia* was found to have an unidentified phenolic component not present in *V. tahitensis.* The Tahiti vanillas contained over twice as much *p*-hydroxybenzoic acid as any other vanilla extracts.

The natural vanillin in vanilla beans is reported to be the result of the enzymatic hydrolysis and oxidation of glycosides during curing (1–3). In any combination of biochemical processes like these, small quantities of related compounds are also likely to occur. This has been the basis of similar work from this laboratory on other natural products (4, 5). The coproducts, in this case, should be present in a relatively constant ratio to the quantity of vanillin. The amounts of these substances can be used to show that an extract is weak because insufficient beans have been used, and will also indicate that additional vanillin has been added.

In general it should be possible to estimate the concentration of an extract by determining some analytical criterion, and establishing limits. In the past, in Canada, the standard for vanilla extract included limits for lead number and vanillin content (6), but these criteria are easily falsified. More recently, new analytical methods have been developed in the United States under the auspices of the Research Committee of the Flavoring Extract Manufacturers' Association (7, 8), some of which have been incorporated into *Official Methods of Analysis* of the AOAC (9). These are qualitative methods for detecting adulteration (10). The determination of the strength of extracts requires the complex results obtained from quantitative determinations such as organic acids (11) and amino acids (12). A quantitative measure of the phenolic compounds related to vanillin would be an additional criterion for this purpose. Independently of the work here, many of the phenolics in vanilla extract have been identified (1).

The present paper describes a method of analysis based upon the separation of the phenolic constituents related to vanillin by two-dimensional paper chromatography, and their estimation from measurements of the areas of the colored spots produced on the paper by chomogenic reagents. The method can be applied directly to commercial vanilla extracts.

Two-Dimensional Paper Chromatography

Sample Preparation

Moisture was determined on 10 g samples by the modification of the official azeotropic distillation method (13) using benzene:toluene (1:4, v/v) and distilling 4 hr. On the basis of this moisture determination, extracts were made of 7.5 g vanilla beans on the dry basis, which is equivalent to 10 g vanilla beans of 25% moisture. All extracts were made by the USP procedure, without sugar, described in *Official Methods of Analysis* (14). For com-

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parison, one sample was extracted according to the FEMA method (15), in a small-scale apparatus.

Apparatus and Reagents

(a) *Chromatographic equipment.*—American Medical Museum Jars, Size No. 11, or equivalent. (Optional—Universal type glass X-frames to hold papers (16).)

(b) *Solvents.*— (*1*) Isopropanol-ammonium hydroxide-water (8:1:1). (*2*) Benzene-acetic acid-water (125:72:3) plus extra water to cloud, depending on temperature.

(c) *Filter paper.*—Whatman No. 1, 8 × 8″ sheets.

(d) *Ultraviolet light sources.*— (*1*) Mineralight Short Wave Ultra-violet Model SL 2537 and (*2*) Mineralight Long Wave Ultra-violet Model SL 3660.

(e) *Chromogenic agents.*— (*1*) Diazotized *p*-nitroaniline: Solution A, 0.2% *p*-nitroaniline in 1*N* HCl; Solution B, 0.1*N* HCl; Solution C, 5% aqueous $NaNO_2$; Solution D, 20% aqueous K_2CO_3. Keep solutions A and B cold. Just before spraying, mix 5 ml A, 50 ml B, and 1 ml C.

(*2*) Hydrazine: 3% hydrazine sulfate in 0.4*N* HCl.

Procedure

Spot 30 μl one-fold vanilla, 5 μl at a time, at the lower left corner of 8 × 8″ Whatman No. 1 paper with the rough surface up and the machine direction going from left to right. Arrange for ascending paper chromatography in the jar with the original left vertical edge dipping into the first solvent. Seal lid with adhesive tape and run chromatogram until solvent front is about 1″ from the top of the paper. Remove papers from jar and dry in hood. Rotate 90° so that the original bottom edge dips into the second solvent. Seal lid and develop as before. Remove papers, dry in hood, and finally heat for 4 min. at 100°C in an oven (17). Observe papers under short wave ultraviolet light and mark dark, fluorescent, and phosphorescent spots. Mask the aldehyde spots by wedging the paper between two upright wooden blocks (8 × 10 × 1½″) so that only the aldehyde spots are between the blocks. Spray the exposed phenolic acid spots with diazotized nitroaniline (chromogenic agent 1, solutions A, B, and C), then spray with base (solution D). Dry papers, outline colored regions (rose pink for *p*-hydroxybenzoic acid and purple for vanillic acid), and determine areas of spots. Mask papers again, this time covering phenolic acid spots. Spray the exposed aldehyde spots with the hydrazine spray (chromogenic agent 2). Dry papers and observe under long wave ultraviolet light, marking the fluorescent areas (blue-white for *p*-hydroxybenzaldehyde and yellow for vanillin). Determine area of *p*-hydroxybenzaldehyde spot.

Make up three solutions containing all four compounds at levels which cover the range in composition expected in the vanillas to be examined. Spot these mixtures on separate papers and chromatograph with the papers for the sample vanillas, carrying them through the entire procedure detailed above. Use these results for areas of standard spots to construct calibration curves for the batch of samples whose chromatograms were all run at the same time.

Vanillin Determination

Vanillin was determined by two methods: the ultraviolet method for the determination of vanillin, which was recently adopted as official, first action as a screening method (18), and the Folin-Denis colorimetric procedure, **19.008–19.010** (19).

Results and Discussion

The method has been applied to 20 authentic vanilla extracts made from beans obtained from commercial sources.

Extraction Procedures.—The results are given in Table 1, Item 3, for the USP method (14) as compared with Table 1, Item 4, for the same bean extracted by the FEMA method (15). The quantity of these phenolic substances extracted did not appear to differ significantly within the limits of the methods. For this reason, the USP method was selected as the standard method. Furthermore, this method is the least efficient extraction which might be used in practice, and thus would delimit a lower level for extractables.

Chromatography.—Two-dimensional chromatography was used to give the required resolution without preliminary separation and extraction techniques which could alter the quantities of the various components to be detected. The solvent systems selected were modifications of those used for phenolic acids in urine (14, 20, 21). When the chro-

Table 1. Analytical results for vanillin and related compounds in the extract from vanilla beans

Item No.	Description of Beans		% Moisture[a]	Analysis, mg/100 ml				
	Provenance	Quality		Vanillin[b] by UV	Vanillin[b] by FD	Vanillic Acid[c]	p-Hydroxybenzaldehyde[c]	p-Hydroxybenzoic Acid[c]
	V. planifolia from:							
1	Mexico (10 g basis[d])	Manufacturing	7	18	90	10	5	2
2	Mexico (7.5 g dry basis[d])	Manufacturing	7	19	70	8	5	2
3	Mexico (USP extraction[d])	Cuts-poor	10	51	150	23	6	7
4	Mexico (FEMA extraction[d])	Cuts-poor	10	49	150	15	6	5
5	Mexico	Cuts-good	11	70	160	10	5	3
6	Madagascar	Second	26	216	330	23	13	4
7	Madagascar	Fourth	19	137	190	12	6	3
8	Madagascar	Manufacturing	18	175	260	15	10	4
9	Madagascar	Manufacturing	11	47	130	13	7	2
10	Comores (Shipper 1)	Third	29	122	190	9	13	2
11	Comores (Shipper 2)	Third	28	136	200	8	11	2
12	Comores	Cuts	10	115	190	10	10	7
13	Comores	Second	20	154	250	13	9	4
14	Uganda	Chopped	17	186	270	10	8	2
15	Uganda	Second	23	155	250	8	7	6
16	Uganda	Fourth	12	47	170	5	5	1
17	West Indies	Neat bundle	18	136	200	22	6	4
18	West Indies	Untidy bundle	10	20	80	15	2	2
19	Java	Short	18	71	150	8	8	4
20	Java	Long	17	63	150	8	10	2
21	Java	Grade A	5	142	220	11	7	2
	V. tahitensis from:							
22	Tahiti		31	54	170	5	9	30
23	Tahiti	White Label	33	60	180	4	8	16
24	Tahiti	White Label	32	60	180	4	4	20

[a] Determined by the AOAC method (13).

[b] UV: From the ultraviolet spectrum of the alkaline solution (23), average of 3 or more determinations; FD: determined by the Folin-Denis colorimetric procedure (24), average of 3, to the nearest 10 mg/100 ml.

[c] From a comparison of the spot areas; median of at least 4 determinations in all cases.

[d] Unless otherwise noted, all extractions were on the dry basis (7.5 g of beans on the dry basis to give 100 ml extract) by the USP procedure (14).

matograms were developed in ascending isopropanol-ammonium hydroxide-water, 3–4 hours (depending on the temperature) was required for the front to travel 6 inches. This system was found to separate the aromatic acids, vanillic and *p*-hydroxybenzoic acid, from the corresponding aldehydes, vanillin and *p*-hydroxybenzaldehyde. Formulas for these compounds are given in Fig. 1. Benzene-acetic acid-water, the developing solvents for the second direction, had to be used as a two-phase system. If chromatograms were developed in the organic layer alone, the small amount of water in it was removed in the initial stages of the development by the dry paper. The ascending liquid was apparently altered from the organic-aqueous phase, which would give the desired separation to a solely organic eluting phase and the components to be separated con-

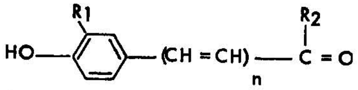

Fig. 1—Formula of vanillin and related compounds found in vanilla extract, where:

1. vanillin, $n = 0$, $R_1 = OCH_3$, $R_2 = H$.
2. vanillic acid, $n = 0$, $R_1 = OCH_3$, $R_2 = OH$.
3. ferulic acid, $n = 1$, $R_1 = OCH_3$, $R_2 = OH$.
4. *p*-hydroxybenzaldehyde, $n = 0$, $R_1 = H$, $R_2 = H$.
5. *p*-hydroxybenzoic acid, $n = 0$, $R_1 = H$, $R_2 = OH$.
6. *p*-coumaric acid, $n = 1$, $R_1 = H$, $R_2 = OH$.

gregated at the solvent front. To avoid this, a small amount of the aqueous layer was included in the developing solvent. Under these conditions, the developing front travelled 6 inches in 1½–2 hours and the monohydroxy and methoxyhydroxy compounds were separated.

Observation of the dried papers under a filtered short-wave ultraviolet light, maximum wavelength 254 mμ, showed various dark (quenching) and light (fluorescent) regions against the residual fluorescence of the paper. Figure 2 gives a diagrammatic representation of these areas. The transitory phosphorescence of vanillic acid (21) was observed when, after exposure of the spot to the ultraviolet, the lamp was moved rapidly aside. Originally it was intended to use the ultraviolet markings to indicate the areas corresponding to the compounds, in order to excise them, extract the compounds, and measure the ultraviolet spectrum. However, there was an upper limit on the amount of extract which could be separated chromatographically, and this limitation meant that there was an inadequate quantity of material for ultraviolet analysis. Furthermore, with this system in which no preliminary cleanup was used, other ultraviolet absorbing substances were found under most of the spots. Ultraviolet mapping was still useful for locating the spots which had to be masked in the multiple spraying.

Detection of Compounds.—Two chromogenic reagents were used: diazotized nitroaniline to give colored diazo dyes with the phenolic compounds, and hydrazine, which reacts with the aromatic aldehydes to give oximes which are colored or fluorescent when observed under ultraviolet light. The two sprays were applied sequentially to the same paper by using suitable masking arrangements. The spots revealed by these sprays are mapped in Figs. 3 and 4. It was necessary to develop and measure the diazo colors before using the hydrazine spray since the acid present in the latter spray interfered with the development of the diazo colors. The maps (Figs. 2, 3, and 4) show that there are regions corresponding to compounds characteristic of *V. tahitensis* which are not present in *V. planifolia* at levels detectable by these techniques. Characterization was considered definite only when a reference standard, developed on the same paper as an extract, met two criteria: First, there was an augmentation of the spot area to which it was attributed; second, the reference compound and the attributed spot showed the same colors when sprayed with

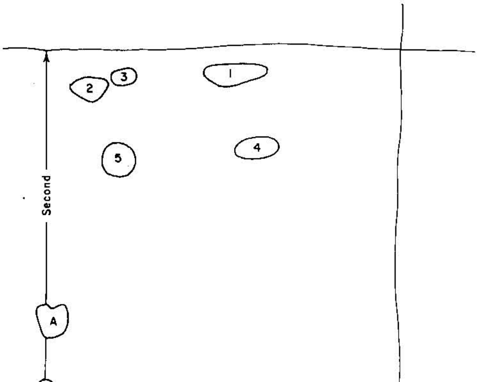

Fig. 2—Ultraviolet map of two-dimensional paper chromatogram of vanilla extracts. In this and the following diagrams of chromatograms, X marks the origin, the shaded circles in the corners represent the punch-holes for the pins in the glass frame, the arrows going up to the lines indicating the fronts show the direction, and "First" and "Second" indicate the order in which the solvents described in the methods were run. The areas outlined show up under the ultraviolet as follows: 1, vanillin, purple; 2, vanillic acid, royal blue fluorescence, white phosphorescence; 3, ferulic acid, present only in Tahiti beans, light blue fluorescence; 4, *p*-hydroxybenzaldehyde, dark; 5, *p*-hydroxybenzoic acid, when present in large enough quantities, as in Tahiti beans, dark, with a pale phosphorescence; A, unknown, in Tahiti beans, light blue fluorescence.

a variety of reagents. On this basis, only vanillin, vanillic acid, *p*-hydroxybenzaldehyde, and *p*-hydroxybenzoic acid were positively identified in all extracts. Ferulic acid and *p*-coumaric acid were present in extracts of Tahiti beans. When examined semiquantitatively, the ferulic acid content was between 5 and 10 mg/100 ml and the *p*-coumaric acid was present at less than 2 mg/100 ml. The formulas for these compounds are shown in Fig. 1 in a way which illustrates their similarity, and their R_f values are given in Table 2. These are the average R_f values obtained from at least seven separate chromatograms, and although there was some variation between papers, the spots were always in the same relative position to each other.

The most prominent spot, in addition to those already listed, is indicated on the map for *V. planifolia* (B, Fig. 3) as giving a rose color with diazotized nitroaniline at R_f 0.8 (isopropanol-aqueous ammonium hydroxide) and R_f 0.4 (benzene-aqueous acetic acid). This spot was not present in the extract of Tahiti beans. However, it was not coniferyl alcohol (1) for, on chromatographing, a commercial sample of coniferyl alcohol formed a purple substance with the diazotized nitroaniline, and had an R_f of 0.7 in isopropanol-aqeous ammonia and an R_f of 0.8 in the benzene-aqueous acetic acid solvent system. Also indicated on the maps for both types of beans is a yellow area (C, Figs. 3 and 4)

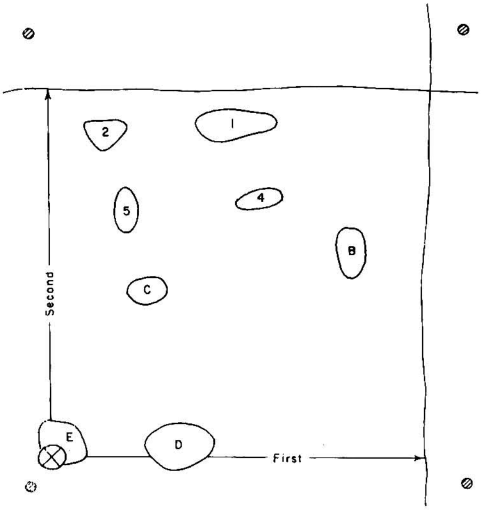

Fig. 3—Map of areas on two-dimensional paper chromatograms of *V. planifolia* extracts revealed by various sprays. 1, vanillin, purple changing to brown with diazotized *p*-nitroaniline (DpNA), yellow with the hydrazine reagent (H), and blue with the Folin-Denis reagents (F-D). 2, vanillic acid, purple with DpNA, blue with F-D. 4, *p*-hydroxybenzaldehyde, after spraying with H, blue-white under ultraviolet. 5, *p*-hydroxybenzoic acid, rose with DpNA, blue with F-D when present in large enough quantities. B, unknown, rose with DpNA, blue with F-D. C, unknown, yellow with DpNA, blue with F-D. D and E were also blue with F-D.

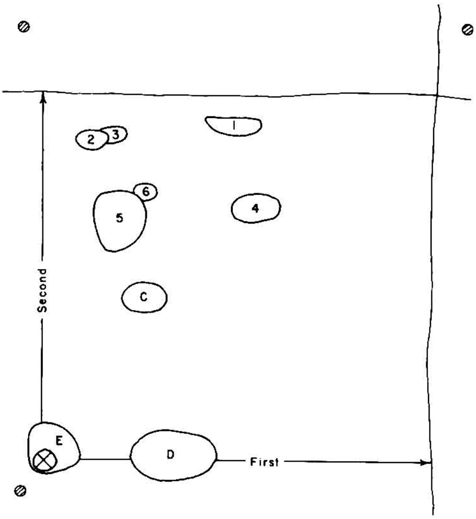

Fig. 4—Map of areas on two-dimensional paper chromatograms of *V. tahitensis* extracts revealed by various sprays. 1, vanillin, purple to brown with DpNA, yellow with H, and blue with F-D. 2, vanillic acid, purple with DpNA, blue with F-D. 3, ferulic acid, blue with DpNA. 4, *p*-hydroxybenzaldehyde, blue-white under ultraviolet after H. 5, *p*-hydroxybenzoic acid, rose with DpNA, blue with F-D. 6, *p*-coumaric acid, gray with DpNA. C, unknown, yellow with DpNA, blue with F-D. D and E, blue with F-D.

Table 2. R_f values for compounds in vanilla extract related to vanillin

	R_f in	
Name of Compound	Iso-propanol-Ammonia-Water (Solvent 1)	Benzene-Acetic Acid-Water (Solvent 2)
1. Vanillin	0.49	0.92
2. Vanillic Acid	0.16	0.89
3. Ferulic Acid	0.21	0.90
4. *p*-Hydroxybenzaldehyde	0.57	0.75
5. *p*-Hydroxybenzoic Acid	0.22	0.68
6. *p*-Coumaric Acid	0.26	0.76

which showed up on spraying with diazotized nitroaniline just below the region characteristic of *p*-hydroxybenzoic acid. This may be, in part, caffeic acid.

Estimation of the Compounds.—As can be seen from the maps in Figs. 3 and 4, obtained by using the procedures described above, the patterns for extracts of cured beans from *V. planifolia* and *V. tahitensis* differed both in the presence and absence of certain constituents and also in the size of the spots, that is, in the concentration of the chemicals which correspond to these spots. Furthermore, there was enough consistency in the pattern of the spots from the different samples of extract of a given species so that the maps could be drawn. In view of this it was anticipated that the patterns could be expressed numerically in terms of the quantities of the various components present. As mentioned previously, attempts to measure the concentration by extracting the spot and measuring the ultraviolet spectrum were unsuccessful. Since the components of immediate interest, that is, those related to vanillin in structure, were revealed by the spray system used, it was decided to use the areas of the colored spots as an indication of the concentration of the component concerned. For this determination, 30 μl of one-fold vanilla, when carried through the paper chromatographic procedure, gave well-resolved colored regions for all components.

For quantitative estimation, the areas of outlined colored spots were measured by placing graph paper under the sprayed chromatogram on a back-lighted surface and counting the squares corresponding to the outlined spot. Although concentrations could have been related to areas by calculation from one standard for a set of samples (22–24), it was felt that it would be more accurate to make a new calibration curve for each component each time a set of papers was run. A set was composed of samples from six vanillas and three standard mixtures of the components to be measured. These mixtures were selected to match the range of composition expected in the vanillas. This gave a three-point calibration curve for each component in the range desired. This curve of concentration versus area had a reasonable slope only up to about 20 μg of material in the spot. Above this limit, material seems to pile up on the spot to give increasingly dense colors. The lower limit corresponded to about 1 mg/100 ml of compound in the original extract, or less than 0.5 μg per spot. The vanillin was too concentrated to be determined by spot area, and it was measured separately.

In spite of the direct comparability between the reference areas and the spots to be measured, and in spite of other uniform procedures, the reproducibility of the determination was variable. Usually there were a number of fairly consistent results and a few outlying results. For this reason, median values are reported in Table 1, thus placing less emphasis on outlying values than an arithmetic average. The variations in individual determinations are illustrated in the profiles given in Fig. 5. The points represent the values obtained, and the bar is drawn to the median values tabulated in Table 1.

Determination of Vanillin.—The vanillin content was determined by the estimation of the ultraviolet peak height at 348 mμ of a suitably diluted alkaline solution of the vanilla (18, 25–27), and by the Folin-Denis colorimetric procedure, **19.008–19.010** (19). Both methods suffer from disadvantages as true measures of the vanillin content of a vanilla extract. The Folin-Denis method estimates the substances which can reduce the phosphomolybdotungstic acid to the blue complex under the conditions given. Vanillic and *p*-hydroxybenzoic acids are such substances. This was shown both by analyzing solutions of these compounds at the strength to be expected in vanillas and by spraying paper chromatograms of vanillas with the Folin-Denis reagents and alkali in the same sequence and for the same times as in the procedure referred to above.

Many other areas were observed, as well as the spots for vanillic and *p*-hydroxybenzoic acid, which became colored when the Folin-Denis reagents were applied. These areas are indicated in Figs. 3 and 4. In the case of the alkaline ultraviolet method, interfering substances may give erroneous values

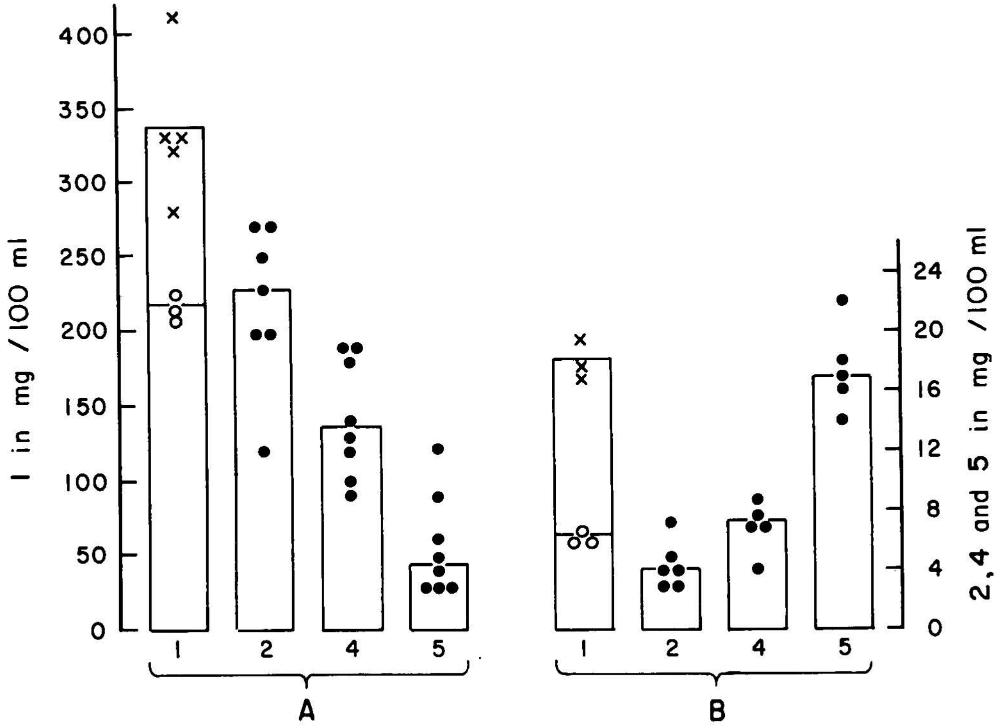

Fig. 5—Pattern of analytical values obtained for vanillin and related compounds for a *V. planifolia* extract (A) and for that from a *V. tahitensis* (B). 1, vanillin (scale on left hand side); o = individual values determined by ultraviolet method; x = individual values determined by colorimetric procedure. 2, vanillic acid; 4, p-hydroxybenzaldehyde; 5, p-hydroxybenzoic acid (scales on right hand side); dots represent individual values as determined from the area of the spots. Bars are drawn to the average or median values given in Table 1, Items 6 and 23.

to the background correction. A future paper will compare these methods rigorously.

The vanillin content in *V. planifolia* varies very greatly. The low figures reported in Table 1 may be due to poorly cured beans which had been subjected to mistreatment. There seems to be some correlation between a low grading, low moisture content, and low vanillin content, as indicated by comparing Items 6 and 7; 13, 11, 10, and 12; 15 and 16; and perhaps 17 and 18. The other components have a narrower range of variation, and there is only a slight indication that they might be present to a greater extent in the superior beans. The reproducibility of the USP extraction was gauged by repeating the extraction on a second sample of the same beans and analyzing the extract for vanillin by the ultraviolet method. In half the cases the results were identical, being within the limits of variation of the ultraviolet vanillin determination. In the remaining cases the variations were within 20% of the original figure.

The data given in Table 1 and in the profile in Fig. 5 definitely distinguish *V. planifolia* and *V. tahitensis*. When further samples are examined, it should be possible to set limits for the range to be expected for the vanillic and *p*-hydroxybenzoic acids, vanillin, and *p*-hydroxybenzaldehyde in genuine cured vanilla beans.

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TOTAL DIET STUDY

Pesticide Residues in Total Diet Samples

By S. WILLIAMS (Division of Food Chemistry, Food and Drug Administration, Washington, D.C. 20204)

Total diet samples collected since August 1962 were analyzed for the presence of chlorinated organic pesticides, parathion, and 2,4-D types of herbicides and plant growth regulators. Chlorinated pesticide residues were identified by paper, gas-liquid, thin-layer, and microcoulometric gas chromatography. Parathion residues were identified by polarography and confirmed by paper chromatography. Organophosphate pesticide residues were noted in a few samples by paper chromatography. No herbicides were detected in any samples.

The Food and Drug Administration began the periodic analysis of "Total Diet Samples" for pesticide residues in May 1961. These samples were representative of the types and amounts of food consumed by 16–19 year old boys. Pesticide analyses were run on composites of all foods and also on composites of the foods by class, viz., dairy products, fruits, leafy vegetables, etc.

Results on samples collected through August 1962 have been published (*This Journal,* **46**, 762 (1963)). This paper reports the findings on samples collected since that date.

As better and more sensitive procedures

Table 1. Part A. Chlorinated organic insecticide residues (ppm) in total diet samples collected in November 1962 and February 1963 found by paper chromatography (PC) and gas chromatography (GC)[a, b, c]

Sample No.	DDT p,p'-DDT		DDD $+o,p$-DDT		DDE		BHC		Heptachlor Epoxide		Lindane		Dieldrin		Kelthane		Methoxychlor		Perthane	
	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC
1				0.003^{e}		0.003^{e}						0.002^{e}		0.003^{e}						
2				0.004		0.006						0.002^{e}		0.002^{e}						
3																				
a[d]	T			0.003		0.006	0.01^{e}	0.005	0.01^{e}	0.004										
b	T	0.05		0.030		0.02				0.01^{e}										
c	0.01^{e}		0.004^{e}	0.01^{e}		0.03^{e}			0.01^{e}											
d	0.01^{e}	0.015			0.01^{e}	0.006					0.004^{e}	0.003								
e	0.004^{e}				T										0.08^{e}	0.05				
f, g	T																			
h								0.002												
4		0.02^{e}		0.008^{e}		0.007				T		0.001^{e}								
5				0.003		0.006														
6	0.01^{e}	0.008										0.001		T						
7	T																			
8														T						
9				0.02		0.004				T		0.002^{e}		0.003^{e}						
10	T			0.006		0.008														
11	T																			

[a] Samples 1–11 were purchased November 1962.

[b] Samples 1, 2, 12, and 13 were purchased in Atlanta; Samples 3, 4, 5, 14, 15, and 16, Baltimore; Samples 6, 7, 17, and 18, Minneapolis; Samples 8, 9, 19, and 20, St. Louis; Samples 10, 11, 21, and 22, San Francisco.

[c] T = Trace = Detectable but less than the lowest numerical value recorded in the column for the specific pesticide.

[d] The subgroups are the following categories: *a*, dairy products; *b*, meat, eggs, and fish; *c*, peanut butter and baked beans; *d*, ground bakery and cereal products; *e*, fruits and tomatoes; *f* and *g*, green leafy and smooth vegetables above the ground; *h*, vegetables produced in the soil.

[e] Indicates estimated value. Under PC, very rough approximations were made because of the limitations of the method. Under GC, visual comparisons, rather than mathematical calculations, between sample peaks and standard peaks were made because of the small size or indefinable nature of the peaks.

Table 1. Part B. Chlorinated organic insecticide residues (ppm) in total diet samples collected in November 1962 and February 1963 found by thin-layer chromatography (TLC) and gas chromatography (GC)[a, b, c]

Sample No.	DDT p,p'-DDT		DDD $+o,p$-DDT		DDE		BHC		Heptachlor Epoxide		Lindane		Dieldrin		Kelthane		Methoxychlor		Perthane	
	TLC	GC	TLC	GC	TLC	GC	TLC	GC	TLC	GC	TLC	GC	TLC	GC	TLC	GC	TLC	GC	TLC	GC
12	0.003	0.003	0.002			0.001		0.001						T			0.002			
13	0.002	0.005	0.002	0.001				0.002			0.002			T			0.002	T		
14	0.005								0.002		0.002	0.002					0.005		0.04	
15	0.005		0.002		0.009	0.009			0.005	T		0.002							0.009	
16[d]																				
1									0.001		T		0.001						0.005	
2	0.005		0.002						0.002		0.002	T		0.007						
3	0.015	0.03		0.006	0.008	0.005											0.005	T		
4			0.008																	
5[e]																				
6	0.005	0.009			0.008	0.001								0.002						
7	0.010		0.010																	
17	0.003	0.007		0.003	T	0.006					T	0.001		0.005						
18	0.003	0.007			T						T	0.001	T	0.005						0.006
19	0.003	0.006									T	0.001		0.004						0.006
20	0.004	0.019	0.002			0.003						T		0.022						0.006
21	0.002	T	0.002			0.001						T								
22	0.015	0.010		0.002	0.003	0.003					T	0.001		0.002						

[a] Samples 12–22 were purchased February 1963.

[b] Samples 1, 2, 12, and 13 were purchased in Atlanta; Samples 3, 4, 5, 14, 15, and 16, Baltimore; Samples 6, 7, 17, and 18, Minneapolis; Samples 8, 9, 19, and 20, St. Louis; Samples 10, 11, 21, and 22, San Francisco.

[c] T = Trace = Detectable but less than the lowest numerical value recorded in the column for the specific pesticide.

[d] The subgroups are the following categories: *1*, dairy products; *2*, grain and grain products; *3*, leafy vegetables; *4*, legumes; *5*, root vegetables; *6*, fruits; and *7*, meat, fish, and eggs.

[e] No chlorinated pesticides were detected by either gas chromatography or thin-layer chromatography.

Table 2. Chlorinated organic pesticide residues (ppm)

Sample	DDD (TDE)[c]			DDE[d]			p,p'-DDT			Heptachlor			Heptachlor Epoxide		
	MC	EC	TLC	MC	EC	TLC	MC	EC	TLC	MC	EC	TLC	MC	EC	TLC
August 1963															
A	0.001	0.001		0.004	0.006	[f]	0.005	0.007	<0.005	0.001	0.001	[f]	0.006	0.016	0.005
B (Pre)	0.003		0.004	0.02	0.016	0.01	0.03	0.029	0.01	0.001	0.002		0.004	0.004	0.003
B	0.009	0.002	0.005	0.02	0.025	>0.01	0.03	0.055	>0.01	0.002	0.002		0.007	0.008	0.005
C	0.007			0.008	0.002	0.01	0.009	0.007	0.005				0.004		0.001
D (Pre)		T		0.001	0.002		0.007	0.006	0.005						
D					T			T	0.003						
E,F,H (Pre)				T	T	T	0.004	0.003	0.004						
E		0.003		0.003	0.005		0.02	0.015	0.01						
F	0.003	0.002						0.003	0.003						
G (Pre)	0.014	0.013		0.012	0.009	0.007		0.003	0.007						
G		Int	Int		Int	Int		Int	Int						
H	0.006	0.003					0.002	0.003							
I (Pre)	0.008			0.006	0.005	0.005		0.013	0.01						0.001
I	0.01	0.016	0.005	0.006	0.005	0.005	0.006	0.003	0.005						
J	0.007			0.007	0.003	0.005	0.009	0.009	0.003		0.003	0.005	0.002		
K															
L															
Composite	0.004		0.003	0.07	0.002	0.003	0.009	0.005	0.003				0.006	0.002	0.005
November 1963															
A	0.001	0.002		0.001	0.004	0.007		0.004	0.001				0.01	0.040	>0.01
B	0.02	0.015	0.01	0.02	0.001	>0.01	0.04	0.003	>0.01				0.002	0.005	
C	0.006				0.002			0.002	0.007				0.002	0.002	
D	T			T	0.004		0.001	0.039	0.004						0.002
E	0.005						0.003	0.012	0.015						
F	0.004						0.007		0.002						
G	0.002		Int				T		Int						
H	0.01		0.001	0.003		0.006	0.007	0.011	0.01						
I	0.01	0.003					0.02								
J	0.002	0.003		0.002	0.005	0.002		0.007	0.002						
K	0.01	0.006		0.001	0.004		0.01	0.023	0.007				0.002	0.001	
L	0.005				0.003		0.008	0.004							
Composite	0.006	0.001		0.003	0.002		0.01	T		0.004	0.003	0.01	0.004	0.003	0.01
February 1964															
A	0.003	0.005		0.005	0.007	0.01	0.008	0.018	0.02				0.015	0.019	0.01
B	0.006	0.010	0.01	0.013	0.017	0.02	0.026	0.024	0.04				0.003	0.002	0.006
C	0.007	0.004	0.004	0.003	0.002	0.04	0.01	0.010	0.006				0.003	T	0.002
D	0.01	0.002	0.001	0.005	0.002		0.025	0.030	0.009				0.003	Int	0.002
E	0.038	0.018	0.01	0.02	Int		0.057	0.043	0.05						0.003
F	0.009	X[a]		T	X[a]	0.01	0.007	X[a]	0.01					X	0.002
G	0.008		Int				T		Int						
H	0.017	X[a]	0.01		X[a]	0.02	0.030	X[a]	0.015				0.001	X	0.004
I	0.019	X[a]	0.02	0.006	X[a]	0.02	0.042	X[a]	0.02				T	X	
J	0.01	0.006		0.007	0.007	0.01	0.016	0.007	0.01				T	0.001	0.002
K	0.01	0.012	0.007		0.003		0.015	0.010	0.006				0.009	0.002	0.002
L	0.004	X[a]		T	X[a]	0.01	0.004	X[a]	0.004				0.001	X	0.002
Composite	0.012	X[a]	0.006	0.009	X[a]	0.01	0.021	X[a]	0.006				0.006	X	0.004

MC = microcoulometric gas chromatography.
EC = electron capture gas chromatography.
TLC = thin-layer chromatography.
T = Trace.
Int = Interference.
Pre = Before preparation of food for consumption.

[a] Only positive results are reported. Most samples were analyzed by all three determinative steps. However, for the Feb. 196 samples, the electron capture gas chromatograph was not always available. "X" indicates samples not examined by EC.

in total diet categories and composite$^{a,\,b}$

BHC/Lindane[e]			Kelthane			Dieldrin			Endrin			Methoxychlor		
MC	EC	TLC	MC	EC	TLC	MC	EC	TLC	MC	EC	TLC	MC	EC	TLC
						August 1963								
0.002	0.001					0.004	0.006	0.004						0.005
0.004	0.002		0.009	0.005		0.003	0.003	0.001		T	T			
0.008	0.003		0.01	0.006		0.004	0.004	0.001		T	T			
0.01	0.009		0.05		>0.01				0.003					
							0.002							
							0.001				0.006			
						0.002	0.001		0.001	T	T			
							0.002			T	0.01			
						0.001	0.001							
	0.001	0.005					Int			Int				
							Int	Int		Int	Int			
						0.003	0.003	0.001			Int			
	T	0.001	0.05	0.035	0.01		T	0.001			0.004			
			0.03	0.02	0.01		T				T			
	0.003		0.02			0.009	0.012	0.005						
	T													
0.002	0.001		0.02				0.001	T						
						November 1963								
0.001						0.003	0.005							
						0.006	0.010							
0.006	0.017	0.01									0.004			
T	0.001									0.001				
0.001						0.001	0.002	<0.002			0.004			
	T										0.004			
0.002	T													
0.002							0.001	0.002			T			
T	T						0.001							
								0.002			0.002			
0.001	T					0.001	0.001							
						February 1964								
0.003	0.001	0.002				0.007	0.005	0.017			0.002			
0.004	0.002	0.002				0.003	0.001	0.004			0.001	0.002	T	0.004
0.008	0.003	0.005				T	T					0.005	T	0.004
T	T	0.001				T	0.001	0.003			0.001			
T	T	0.007					T			T				
T	X	0.001				T	X			X			X	
T	Int	Int												
0.003	X	0.002				T	X	0.001		X			X	
T	X	0.01					X			X			X	
0.01	0.006					0.005	0.007					<0.001		0.01
0.023	T	0.001					0.001							
	X						X			X			X	
0.003	X	<0.002					X	0.001		X			X	

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[b] In addition to the tabulated residues, the following pesticides were also found: Aug. 1963 group in "C", 0.05 ppm Perthane (MC); Nov. 1963 group in "E", 0.023 ppm *o,p*-DDT (EC); Feb. 1964 group in "C", 0.002 ppm aldrin (MC), "I" 0.007 ppm aldrin (MC); "J" T aldrin (MC), "K" 0.01 ppm aldrin (MC), Total Diet, T. Some unidentified peaks were also noted on the chromatograms. These may have been due to pesticides other than those sought.

[c] Gas chromatography did not resolve DDD from *o,p*-DDT and reported values include both.

[d] In TLC, *o,p*-DDT comes out at the same R_f as DDE and reported values include both.

[e] BHC and lindane are tabulated together. MC does not distinguish between the two. When both are present, none of the determinative steps accurately measures the relative amount of each.

[f] DDE and heptachlor not resolved by TLC on this plate. Total approx. 0.007 ppm.

have been developed, such as thin-layer chromatography, they have been used in the analysis of these samples. With thin-layer chromatography, residues of less than 0.001 ppm (less than 1 ppb) can be detected and identified. However, findings of less than 0.01 ppm are still subject to large percentage errors and should be considered as qualitative rather than quantitative. All results by paper chromatography and thin-layer chromatography are based on visual comparison of sample spots with standard spots and are only semiquantitative estimates.

Twenty different chlorinated pesticide residues would have been detected had they been present. They are:

DDT
DDD (TDE)
DDE
Lindane
BHC
Methoxychlor
Aldrin
Dieldrin
Endrin
Toxaphene
Strobane
Perthane
Chlordane
Heptachlor
Heptachlor epoxide
Kelthane
Chlorbenzide
Chlorobenzilate
PCNB
TCNB

Later samples were also checked for the 2,4-D type of herbicides and plant growth regulators.

The designations of the categories of the foods were changed for some of the latter samples. Because of these changes and alterations in some of the determinative steps, the results are reported in two parts (Tables 1 and 2).

Samples of November 1962 and February 1963 (Table 1A and 1B).—Each group consisted of 10 total diet samples and in each case 7 groups of the constituents included in one diet sample were analyzed. Pesticides in the November 1962 samples were determined and identified by paper chromatography and microcoulometric gas-liquid chromatography (Table 1A). The February 1963 samples were analyzed by thin-layer chromatography and microcoulometric gas chromatography (Table 1B).

Paper chromatographic tests for organophosphate types of pesticides were qualitative only and indicative of these compounds as a class (*Ibid.*, **45**, 393 (1962); **46**, 707 (1963)). Specific pesticides were not determined. Subgroups of Sample 3 (meat, eggs, and fish; fruits and tomatoes; green leafy and smooth vegetables above the ground; and vegetables produced in the soil) and of Sample 12 (dairy products, legumes, and fruits), purchased in Baltimore in November 1962 and February 1963, respectively, showed evidence of the presence of organophosphate insecticide residues.

Samples of August 1963, November 1963, and February 1964 (Table 2).—For the August 1963 samples, a special study was undertaken to determine what effect, if any, the preparation or processing for table use would have on the pesticide content of the various food categories. Thus, those foods which are usually prepared for table use were also analyzed before preparation. Since no significant difference was shown between the material analyzed as purchased and after preparation, this special effort was discontinued on later samples. Both values are reported in Table 2.

Starting with the August 1963 samples, the category designations were changed to: *a*, dairy products; *b*, meat, fish, and poul-

Table 3. Samples analyzed for parathion (0.04 ppm or greater) by the polarographic method of Gajan (*This Journal*, 46, 216 (1963)) with minor modifications[a]

Category	Samples		
	August 1963	November 1963	February 1964
A			
B			
C	0.05	0.05	
D			
E	0.23*	0.12	0.11
F			0.07
G	N.A.[b]	Int.[c]	Int.[c]
H	0.73* 0.83 (0.52)		
I	0.19*		
J	0.32	0.06	
K	0.2		0.03
L	0.36* (0.25)	0.20	
Composite	0.16	0.05	0.06

[a] Results marked * were further analyzed by paper chromatography (*This Journal*, **46**, 706 (1963)) to confirm the findings. The two results in parentheses, categories H and L of the August 1963 samples, were obtained by method **24.132.**

[b] N.A. = Not analyzed.

[c] Int. = Interference.

try; *c,* grains and cereal products; *d,* vegetables, potatoes; *e,* vegetables, leafy; *f,* vegetables, legumes; *g,* vegetables, roots; *h,* garden fruits; *i,* fruits; *j.* oils, fats, and shortenings; *k,* sugar and adjuncts; and *l,* beverages. These samples were analyzed for chlorinated organic pesticides, parathion, and 2,4-D-type herbicides and plant growth regulators.

Recoveries of parathion for the samples are recorded in Table 3.

No herbicides were detected in any of the samples. Recovery studies indicated that 2,4-D, 2,4-DB, 2,4,5-T, 2,4,5-TP, 2,3,6-TBA, MCP, and pentachlorophenol would have been detected if any had been present in concentration of 0.01 ppm or greater.

Total bromides were also determined in the composite of the February 1964 sample and were found to average 2.65 ppm.

Summary

The pesticide residues detected in the foods as prepared for consumption were at very low levels and did not differ significantly from the findings on earlier samples (*Ibid.,* **46,** 762 (1963)).

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Radioactivity in Total Diet Samples

By EDWIN P. LAUG and JAMES M. DIMITROFF (Division of Pharmacology, Food and Drug Administration, Washington, D.C. 20204)

Data have been compiled on the presence of strontinum-90 and cesium-137 in total diet samples. Strontium-90 and cesium-137 contents in the total diet have shown a steady increase in the 1963 samples, with the highest values concentrated during the fall of 1963.

Analyses of total diet samples for the presence of strontium-90 and cesium-137 have continued within the Food and Drug Administration. The amounts of radiation present in samples collected through November 1962 have been published (*This Journal,* **46,** 752 (1963)). Findings on samples since that time are recorded in this paper.

Since May 1963 all preparations of the diet and analyses for strontium-90 have been made in the districts, (procedures in the previous report, cited above), with duplicate check samples analyzed at the Washington laboratories. Four new stations were added to the network in May 1963: Boston, Seattle, Denver, and Dallas. All cesium-137 analyses were made in Washington.

Results on the analyses of total diet samples are shown in Table 1. Data back to May 1962 are included for comparison. A general picture emerges which depicts a steady rise in strontium-90 and cesium-137 in the total diet, with indications that peaking may have occurred some time during the fall of 1963. Consonance of cesium-137 and strontium-90, while not "tight," does indicate parallel trends.

The highest concentration of strontium-90 was found in the November sample from Atlanta. On the basis of a consumption of 3.76 kg of diet, the daily intake of strontium-90 is 71 pc (19 × 3.76). The Federal Radiation Council suggests 200 pc/day as the top of "Range II." "The Council believes, based on competent scientific advice, that any possible health risk which may be associated with exposures even many times

Table 1. Sr-90 and Cs-137 recoveries from total diet samples, pc/kg[a]

City	May 1962		Aug. 1962		Nov. 1962		Feb. 1963		May 1963		Aug. 1963		Nov. 1963		Feb. 1964		May 1964	
	Sr-90	Cs-137	Sr-90	Cs-137	Sr-90	Cs-137	Sr-90	Cs-137	Sr-90	Cs-137	Sr-90	Cs-137	Sr-90	Cs-137	Sr-90	Cs-137	Sr-90	Cs-137
Boston													13	99		119	7.0	108
Washington, D.C.	6.4	12	6.9	39	8.4	37	12	51	11	73	11	74	13	55	17	82		97
Atlanta	9.7	29	7.2	16	9.2	23	13	46	10	128	18	78	19	63	25	91		
Minneapolis	5.2	13	9.0	31	7.9	28	8.8	62	10	114	18	109	15	83	13	103		87
St. Louis	10	33	8.8	13	9.5	34	7.1	31	8.5		17	80	16		10	67		83
Denver									7.5	79	7.5	94	10	57	12	70	14	84
Dallas									13	100	10	68	13	44	13		14	56
Seattle									10	113	10	106	16	95	13	64	17	104
San Francisco	3.0	1	3.4	14	4.8	9	8.0	16	9.4	76	5.7	42	6.4	57	7	63	7	63

[a] Multiply by 3.76 kg for daily intake.

Table 2. Interlaboratory checks between Washington (W) and district (D) laboratories on Sr-90 occurrences in total diet samples, pc/kg

Districts	May 1963		August 1963		November 1963	
	W	D	W	D	W	D
Baltimore	10.6		11.9	10.3	13.6	12.1
Atlanta		10	20.4	16.2	16.6	20.5
Minneapolis	7.2	13.3	13.2	22	15	15.1
St. Louis		8.5	10.5	23		15.9
San Francisco	10.7	8.0	5.3	6.2		6.4
Dallas	10.7	15.7	8.3	12.1	14.9	10.9
Denver	6.9	8.2	10.1	4.8	10.2	9.8
Seattle	11.5	8.4	12.7	8.3	14.4	16.9
Boston						12.9

above the guide levels would not result in a detectable increase in the incidence of disease."

While the Council has made no determination with regard to cesium-137, all evidence at present indicates that a guide level 10 times that suggested for strontium-90 would be in an acceptable range. Reference to Table 1 shows that the daily cesium-137 intake is within the range of 2000 pc/day.

Table 2 is appended to show the results of interlaboratory checks between Washington and the districts.

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Total Diet Study: Carbohydrate Content and Methodology

By JAMES F. EHEART and BLANCHE S. MASON (Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. 20705)

Determinations were made of total and reducing sugars, sucrose, dextrin, and starch content of dry fat-free solids of the "total diet" samples previously reported by Food and Drug Administration. Samples represented differences in season, location, time of purchase, and store at which purchased. Mean dry fat-free solids contents did not differ significantly. Purchase periods and locations caused extreme variability of the sucrose content of the diets. Factor-effect variations among the other carbohydrate constituents were not considered nutritionally important. Reducing sugars, sucrose, and starch gave positive correlations with total sugars, while negative correlations were obtained for total sugars with dextrin and for reducing sugars with sucrose.

In an earlier publication (1), Food and Drug Administration personnel reported results of studies of fallout, nutrient, and pesticide content of composite diets selected as nutritionally adequate for boys 16 to 19 years old during a 14-day period ("total diet"). Beginning in May 1961, simulated samplings were made quarterly at each of the four different supermarket chain stores for those four periods with the largest variation in fresh fruit and vegetable supplies. In May 1962, in addition to Washington, D.C., the study was extended to include four other cities: Atlanta, Minneapolis, San Francisco, and St. Louis.

Methods of obtaining samples, preparing them, and grinding the selected ingredients into composite slurries, and the preservation by freezing of these slurries for subsequent analysis were given in the earlier publication (1). A composite sample was comprised of a slurry of all the ingredients making up a single market basket. On the basis of assays made on aliquots of these slurries, data were reported (1) for the following nutrients: vitamin A, thiamine, riboflavin, niacin, vitamin B_6, vitamin B_{12}, moisture, protein, fat, and ash.

Since samples were readily available, we decided to use this opportunity to obtain additional information on the chemical composition of these selected diet slurries. Assays have therefore been made in this laboratory for different carbohydrate constituents. The samples we used were portions of the original Food and Drug Administration composite slurries that had been frozen and stored.

Experimental

Since simulated food purchases were made at each of four different Washington, D.C. supermarket chain stores for four different seasonal purchase periods, the effects of these two factors on the carbohydrate content of the diet slurries were studied (4 supermarket chain stores × 4 seasonal purchase periods × 2 duplicates = 32 samples). The effects of location on the carbohydrate content of the diets were studied on the slurries whose ingredients were purchased in May 1962 at the following locations: Washington, D.C.; St. Louis, Mo.; and San Francisco, Calif. (3 locations × 2 duplicates = 6 samples). Each sample was analyzed for total and reducing sugars, sucrose, dextrin, and starch. All carbohydrate constituents have been calculated as glucose equivalents.

Clarification techniques and methods for total and reducing sugars and for glucose were essentially those described by Jacobs (2).

Analytical Procedures

Dry fat-free solids.—Dry approximately 100 g of diet slurry in a 1 × 7 × 9″ enamel tray, over $CaCl_2$, at 50–55°C in a forced air dehydrator for 48 hr and then in a 50–55°C vacuum oven for 48 hr or until constant in weight. Extract dry sample with ethyl ether for 16 hr in a Soxhlet extractor. Grind dry fat-free sample in an intermediate-size Wiley mill and store in a stoppered bottle at 0°F for dextrin and starch analyses.

Sugars.—Extract approximately 10 g of diet slurry in a ₸ 24/40 500 ml Erlenmeyer flask with 100 ml portions of 80% ethanol, each time refluxing, decanting, and filtering into a 500 ml volumetric flask, until a negative Molisch test is obtained. Three refluxings are required. Evaporate and clarify an appropriate aliquot of extract, and determine total sugars (invertase hydrolyzed + reducing) and reducing sugars by the Nelson-Somogyi copper sulfate-arsenomolybdate colorimetric procedure.

Extract, hydrolyze, and determine dextrin and starch by modifications of procedures used by the Southern Cooperative Nutrition Group (3) as described below.

Dextrin.—Transfer 1 g of dry fat-free solids to a 100 ml polyethylene centrifuge tube, add approximately 75 ml of 80% ethanol, and repeatedly stir, reflux, centrifuge, decant, and filter until a negative Molisch test is obtained. Three treatments are required. Discard the filtrate. Quantitatively transfer the residue on the filter paper to the same centrifuge tube with approximately 75 ml of 10% ethanol, protect with 0.5 ml of toluene, and refrigerate overnight. Extract dextrin from the residue with repeated stirring, centrifuging, decanting, and filtering with 10% ethanol until negative to the KI-I_2 test. A red substance indicates a positive test. (Reagent: 1.5 g KI + 0.5 g I_2/100 ml H_2O.) Three treatments are required. Quantitatively transfer the residue on the filter paper to the centrifuge tube with 95% ethanol, evaporate the ethanol, dry, and retain the residue for starch determination.

Evaporate ethanol from the 10% extract, cool, protect the solution with 0.5 ml toluene, add 2 ml 1% malt-diastase, and incubate overnight at 37°C. Boil solution for 15 min. in a covered beaker, cool, add 6 ml of concentrated HCl, autoclave at 8 psi for 105 min., cool, and neutralize with 20% NaOH, using a pH meter and a magnetic stirrer. Filter the extract into a 1 L volumetric flask, wash the paper with hot water until a negative Molisch test is obtained, and determine glucose on an appropriate aliquot.

Starch.—Quantitatively transfer the residue from the dextrin extraction to a 250 ml beaker with cold water, heat in a boiling water bath until thoroughly gelatinized (at least 1 hr), and cool to 35°C. Proceed with the same malt-diastase hydrolysis, HCl autoclaving, and NaOH neutralization steps used for dextrin. Filter the solution into a 250 ml volumetric flask and wash the residue with hot water until free from glucose (Molisch test). Clarify a 10 ml aliquot, filter into a 200 ml volumetric flask, wash the residue, and determine glucose on an appropriate aliquot.

Results

Means for carbohydrate content according to seasonal purchase period, supermarket chain store, and location are given in Table 1. Carbohydrate data are given on the dry fat-free basis and have been reduced by analysis of variance and Duncan's (4) multiple range test. Standard deviations and per cent coefficient of variation (CV) of the means are given. Significances of differences among constituents due to factor effects, with significances of their interactions, are also given.

Remarkable consistency was obtained for the mean dry fat-free solids content of the diet composites for all factor comparisons. This is indicated by the fact that no differences were significant.

Effects of Chain Store and Purchase Period.—Highly significant differences in results according to the chain store and purchase period were obtained for all carbohydrate fractions. A highly significant chain store × purchase period interaction was also obtained for each fraction, thereby indicating inconsistency of the data. Duplicate differences were significant only for dextrin, and these at the 5% level; these results indicate that the assay methods used were accurate and reliable.

Total sugar and starch contents were fairly consistent as indicated by CV values of 7.8 and 9.9%, respectively. Although differences by both chain store and purchase period are highly significant for total sugars and starch,

Table 1. Carbohydrate content of selected market basket diets of boys 16 to 19 years old: effects of supermarket chain store, seasonal purchase period, and location

Variable	Dry Fat-Free Solids[a], %	Carbohydrate Fractions on Dry Fat-Free Basis[b]				
		Total Sugars[c], %	Reducing Sugars[c], %	Sucrose by Difference × 0.95[c], %	Dextrin[c], %	Starch[c], %
Supermarket Chain Store[d]						
1	19.4	28.8A	19.5A	8.87A	3.47B	23.3C
2	19.1	25.7C	19.1A	6.35C	3.74AB	24.5B
3	19.1	26.8B	17.5C	8.85A	3.67AB	26.0A
4	19.0	26.7B	18.5B	7.64B	4.12A	23.3C
Seasonal Purchase Period[e]						
Spring	19.3	27.8A	16.1C	10.97A	3.79AB	24.1B
Summer	19.0	25.6C	21.4A	3.99D	3.54B	25.4A
Fall	19.2	26.8B	16.4C	9.87B	3.46B	26.2A
Winter	19.1	27.9A	20.6B	6.89C	4.20A	21.5C
Av.	19.1	27.0	18.6	7.93	3.75	25.3
Std Dev.	±0.3	±2.1	±3.0	±3.72	±0.63	±2.4
% Coeff. of Var.	1.6	7.8	16.1	46.9	16.8	9.9
Sig.: Chain	NS	**	**	**	**	**
Sig.: Period	NS	**	**	**	**	**
Sig.: C × P		**	**	**	**	**
Location[f]						
Washington	19.4	29.3A	19.7	10.28A	4.77	22.4
St. Louis	19.1	24.3B	21.4	2.59B	4.54	19.8
San Francisco	19.7	23.0C	20.7	2.27B	5.08	19.5
Av.	19.4	25.5	20.4	5.04	4.80	20.5
Std Dev.	±0.3	±3.3	±1.1	±4.22	±0.26	±1.8
% Coeff. of Var.	1.5	12.9	5.4	83.7	5.4	8.8
Sig.	NS	*	NS	**	NS	NS

[a] Chain store and purchase period: 4-sample means; location: single samples.
[b] Chain store and purchase period: 8-sample means; location: duplicate means.
[c] Data followed by different letters are significantly different (4).
[d] Washington, D.C.: Four different supermarket chain stores.
[e] Washington, D.C.: May, 1961, August 1961, November 1961, and February 1962.
[f] May 1962: Washington, D.C. (2-store means); St. Louis, Mo.; San Francisco, Calif.

these differences are not considered large enough to be of practical nutritional importance. The variabilities of reducing sugar and dextrin were quite similar, as indicated by CV values of 16.1 and 16.8%, respectively. Low reducing sugar values for Chain Store 3 and for the spring and fall purchase periods and high dextrin values for Chain Store 4 and for the winter purchase period probably account for most of the variability for these two fractions.

Sucrose content varied more than any other constituent, with an extremely high CV of 46.9%. More variation occurred among data by purchase period than among data by chain store. Significant differences were obtained among data for all four purchase periods and for three of the four chain stores (letters). The sucrose content of the samples purchased in the spring was the highest—275, 159, and 111% of the content of the summer, winter, and fall purchase period samples, respectively. Chain store value differences, although significant, are not considered large enough to be of nutritional importance.

Effects of Location.—Differences of sucrose and total sugar content of the diets at the three locations were statistically significant at the 1% and 5% levels, respectively. The large variability of sucrose (CV 83.7%) was due to the extremely high value at Washington, D.C. and the very low values at St. Louis and San Francisco. The sucrose content of the Washington, D.C. samples was approximately four times as much as those of the two other locations. Differences in sucrose content of the St. Louis and San Francisco samples were not significant (letters). Variation for total sugar (CV 12.9%) was not nearly as large as for sucrose (CV 83.7%). Significant differences in total sugar content occurred among the three locations (letters). Differences among means for reducing sugar, dextrin, and starch were not significant.

Results of these studies indicate the extreme variability of the sucrose content of the samples. The values for the spring period were much higher than those for the winter period and somewhat higher than values for fall and summer. The sucrose content of the Washington, D.C. samples was approximately fourfold more than those of the St. Louis and San Francisco samples. These extreme factor-effect variabilities of the sucrose content of the diets could well be considered of practical nutritional importance. Differences in sucrose content according to supermarket chain store, although significant, are not large enough to be considered nutritionally important. Factor-effect variations among the other carbohydrate fractions, although significantly different in most cases, also are not large enough to be considered of nutritional importance.

Correlation Coefficients. — To determine whether relationships occurred among the carbohydrate fractions in the samples, correlation coefficients (r) were calculated from the data for chain store and for purchase period (*see* Table 2). Highly significant positive r values were obtained for reducing sugars, sucrose, and starch with total sugars. Highly significant negative r values were obtained for total sugars with dextrin and for reducing sugars with sucrose. The other five correlations were not significant.

Table 2. Correlation coefficients (r) o carbohydrate fractions of selected market basket diets of boys 16 to 19 years old

Carbohydrate Fractions (Dry Fat-Free Basis)	r[a]
Total sugars:reducing sugars	0.64**
Total sugars:sucrose	0.63**
Total sugars:dextrin	−0.67**
Total sugars:starch	−0.32NS
Reducing sugars:sucrose	−0.86**
Reducing sugars:dextrin	0.09NS
Reducing sugars:starch	0.76**
Sucrose:dextrin	−0.31NS
Sucrose:starch	0.13NS
Dextrin:starch	−0.34NS

[a] N = 32, ** = significant at the 1% level.

Acknowledgments

The authors wish to express their appreciation to Food and Drug Administration personnel for furnishing the composite diet slurries used in this study.

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VITAMINS AND OTHER NUTRIENTS

Pyridoxine Determined Fluorometrically as Pyridoxal Cyanide Compound

By MARILYN MACARTHUR POLANSKY, RICHARD T. CAMARRA, and EDWARD W. TOEPFER (Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.)

Pyridoxine was oxidized with manganese dioxide to pyridoxal, which was converted to pyridoxal cyanohydrin by a reaction with potassium cyanide. Fluorescence readings were linearly related over a range of 0.005 to 0.5 μg of pyridoxine/ml. Recoveries of pyridoxine as pyridoxal were good and offered a method for the fluorometric determination of pyridoxine in aqueous solution.

Mixtures of pyridoxine, pyridoxal, and pyridoxamine in food extracts and in standard solutions can be quantitatively separated into individual fractions by column chromatography (1–3).

The addition of cyanide to pyridoxal, as described by Bonavita (4), greatly increased pyridoxal fluorescence, making it possible to develop a quantitative assay method for pyridoxal 5-phosphate in tissues. In this laboratory, pyridoxamine was fluorometrically determined by transamination to pyridoxal, followed by conversion to pyridoxal cyanohydrin (5). The reactions described thus far left pyridoxine as the missing link in the fluorometric procedure for the determination of the three vitamin B_6 components.

Studies by Hewston (6) confirmed earlier reports of both Snell, *et al.* (7) and Harris, *et al.* (8) that under controlled conditions considerable pyridoxal was formed from pyridoxine when oxidized with $KMnO_4$ or MnO_2. This paper reports results of studies on the quantitative oxidation of pyridoxine with MnO_2 to pyridoxal and its fluorometric determination as pyridoxal cyanohydrin.

METHOD

Reagents and Apparatus

(All chemicals were reagent grade.)

(a) *Pyridoxine standard stock solution.*—25 μg pyridoxine/ml. Add 30.40 mg USP pyridoxine.HCl reference standard to 1 L 1*N* HCl. 121.6 mg pyridoxine.HCl = 100 mg pyridoxine.

(b) *Pyridoxal standard stock solution.*—25 μg pyridoxal/ml. Add 30.45 mg pyridoxal.HCl (Nutritional Biochemical Corp.) to 1 L 1*N* HCl. 121.8 mg pyridoxal.HCl = 100 mg pyridoxal.

(c) *Manganese dioxide.*—Prepare as described by Mancera, *et al.* (9). Dissolve 15 g $MnSO_4$ in 200 ml H_2O, heat to 90°, and with continuous stirring add saturated $KMnO_4$ solution (about 15 g $KMnO_4$) until in slight excess. Continue stirring at 90° for 15 minutes, cool, and filter through a Büchner funnel. Wash MnO_2 with about 200 ml hot H_2O or until the filtrate is color-free; then wash with about 300 ml methanol. Dry to constant weight at 50°, screen through a 60-mesh sieve, and store in closed container.

(d) *Potassium phosphate.*—1.0*M*. Adjust to pH 7.4 with HCl. (Use pH meter with glass-calomel KCl electrodes for pH measurements and adjustments.)

(e) *Quinine Reference Standard.*—0.3 μg/ml quinine sulfate in 0.1*N* H_2SO_4.

(f) *Spectrophotofluorometer.*—Aminco-Bowman, as described by Bowman, *et al.* (10), equipped with an Osram xenon light source and a 1P21 photomultiplier tube (RCA). Standardize instrument at 0.03 sensitivity to read 50 with quinine sulfate reference standard.

Procedure

(Since vitamin B_6 is light-sensitive, all procedures were performed in a darkened laboratory with red lights as the source of illumination.)

Dilute pyridoxine and pyridoxal standard stock solutions with H_2O to contain 0.01–10 μg/ml. Transfer 1–20 ml aliquots to 150 ml beakers, make to 20 ml with H_2O, and adjust

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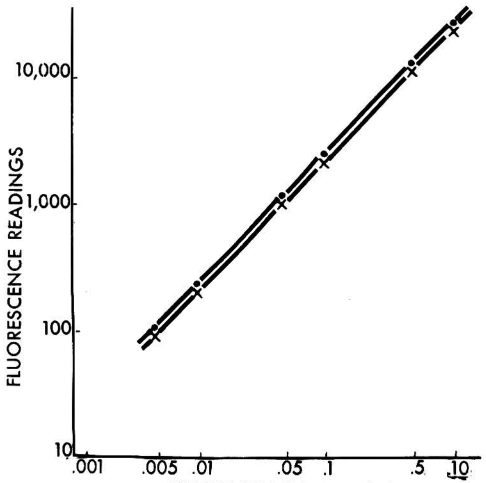

Fig. 1—**Fluorescence of pyridoxal cyanohydrin. from pyridoxal; x, from pyridoxine.**

to pH 5–6 with KOH or HCl. Add 0.1 g MnO_2, and stir continuously or shake on a rotary shaker for 30 min. at room temperature. Centrifuge, and decant supernatant. Wash MnO_2 residue with H_2O, centrifuge, and add washing to first supernatant portion. Discard residue. Add 10 ml 1.0*M* K_2HPO_4 and 2 ml 0.03*M* KCN to liquid portion, and adjust to pH 7.2–7.4. Maintain solution at 50°C for 2.5 hr, cool, adjust to pH 9.5 with 6*M* NH_4OH, and dilute to 100 ml. Filter solution, and read fluorescence at 435 mµ with an activation wavelength of 358 mµ.

Treat control pyridoxal in the same manner, except omit the MnO_2 oxidation step.

Results and Discussion

When 5 µg of pyridoxine was oxidized with MnO_2, stirred 5, 10, 15, 20, 30, and 60 minutes, and centrifuged, over 75% of the pyridoxine was oxidized after 5 minutes of stirring and over 90% after 10 minutes. Stirring for 30 minutes was selected for the routine procedure since maximum oxidation occurred by that time.

Relationships between the fluorescence readings and concentrations of pyridoxine (as pyridoxal cyanohydrin) were linear over the range of 0.005 to 0.5 µg/ml (Fig. 1). (The scale shown is logarithmic solely for convenience of illustration; curves plotted on regular coordinate paper were also linear.) The plotted data from pyridoxine regularly fell below the data from pyridoxal and indicated a slight loss due possibly to adsorption of the MnO_2 precipitate.

A study was made to determine recoveries of pyridoxine as pyridoxal cyanohydrin over the range 1–100 × 10^{-3} µg. The slightly lower recoveries of pyridoxine as pyridoxal cyanohydrin, when compared with recoveries of pyridoxal as pyridoxal cyanohydrin, were not found to be statistically significant.

The results of this study, in addition to those of our earlier study of the fluorometric determination of pyridoxamine, make possible the chemical determination of vitamin B_6 components in aqueous solutions. In the analyses of food extracts, problems concerning interfering fluorescences arise. This laboratory anticipates reporting future progress on a fluorometric procedure for the determination of pyridoxine, pyridoxal, and pyridoxamine in foods and like materials.

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PESTICIDES

Analysis of Insecticides in Aqueous Emulsions Used in Livestock Dips and Sprays: General Infrared Method

By FRED P. CZECH (Animal Disease Eradication Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. 20705)

An infrared spectrophotometric method can be widely applied as a general procedure for analysis of insecticides in aqueous emulsions used to eradicate ectoparasites in livestock. Analytical procedures are given for Delnav, Ciodrin, lindane, and toxaphene-lindane mixture. The method is rapid, simple, precise, versatile, generally free from interference, and unequivocally specific. It is recommended as a universal first approach to analysis of insecticides in animal dip and spray emulsions.

Many insecticides and insecticide mixtures are formulated as emulsifiable concentrates designed to be diluted with water and applied to livestock by means of large vat dips, power sprays, spray-dips, and other mechanical devices. To avoid injury to animals, yet destroy ectoparasites effectively, it is imperative that the amount of insecticide in these devices be kept homogeneously dispersed and within specific concentration limits. Frequently, simple and accurate quantitative field tests for insecticide content in aqueous emulsions are not available, and the field worker must rely upon the chemical laboratory to provide prompt analyses to check his field operations. The versatile infrared procedure described in this paper is expected to fill this need. Further, it is believed that the method is flexible enough to be extended to analysis of most insecticides in aqueous dip and spray emulsions.

In a previous paper (1), the author described a rapid infrared spectrophotometric procedure for analysis of livestock emulsion dips and sprays of toxaphene. The principles of this procedure can be employed for analysis of a variety of aqueous insecticide emulsions. Briefly, the insecticide is "salted-out" of aqueous emulsion into an appropriate organic solvent, and an infrared spectrum is taken of the solvent extract for quantitative determination of insecticide.

In comparison with chemical analytical procedures, the infrared method has the following advantages:

1. It is specific. Emulsion dips containing mixtures of insecticides, contaminants, toxic by-products, or unknown field dip or spray preparations may usually be qualitatively elucidated and frequently quantitatively estimated.
2. In addition, the infrared method is usually faster and less complex than chemical procedures, especially those requiring elaborate preliminary cleanup or other separation techniques.
3. The method is generally free from interference. The livestock dip or spray samples obtained from emulsifiable concentrate insecticide formulations investigated thus far have shown no interferences from proprietary solvents, emulsifiers, animal waste products, or other contaminants. Even the most filth-laden dip, spray, or spray-dip samples did not require any cleanup or separation other than simple centrifugation to remove the sediment and dirt from the solvent extract.

These advantages and, especially, the versatility and specificity of the infrared procedure indicate the method's usefulness as a general first approach to analysis of insecticides in animal emulsion dips and sprays.

Infrared procedures for vat dip and spray emulsions of Delnav (2,3-*p*-dioxanedithiol S,S-bis(O,O-diethyl phosphorodithioate) (Hercules Powder Company); Ciodrin (α-methylbenzyl 3-(dimethoxyphosphinyloxy)-cis-crotonate) (SD 4294, Shell Chemical Company); lindane (1,2,3,4,5,6-hexachlorocyclohexane, γ-isomer); and toxaphene-lindane mixtures, described below, illustrate the method's utility and versatility. These insecticides have been used for the control of ectoparasites on livestock.

Infrared Assay of Delnav, Ciodrin, Lindane, and Toxaphene-Lindane Mixture in Animal Emulsion Dips and Sprays

Reagents and Apparatus[1]

(**a**) *Carbon disulfide.*—Mallinckrodt Chemical Works, ACS grade.

(**b**) *Sodium chloride.*—Mallinckrodt Chemical Works, USP granular.

(**c**) *Delnav analytical standard.*—Certified assay (Hercules Research Center, Hercules Powder Company, Wilmington, Del.). Ingredients used in emulsion concentrates were obtained from William Cooper and Nephews, Inc., Chicago, Ill., and Thuron Industries, Dallas, Texas.

(**d**) *Ciodrin analytical standard.*—Purified 90% w/w. (Shell Chemical Company, New York.) Ingredients used in concentrate were obtained from William Cooper and Nephews, Inc.

(**e**) *Lindane analytical standard.*—99.7% source unknown. A 12.5% lindane emulsion concentrate was obtained from the Pearson-Ferguson Chemical Company, Kansas City, Mo.

(**f**) *Toxaphene plus lindane.*—45.00:2.00% (w/w). Emulsifiable spray concentrate (O. M. Franklin Serum Company, Denver, Colo.).

(**g**) *Separatory funnels.*—125 ml with Teflon stopcock.

(**h**) *Infrared spectrophotometer.*—Perkin-Elmer 237, or equivalent.

(**i**) *Matched absorption cells.*—Rock-salt windows, approximately 0.5 mm light path.

Preparation of Calibration Curves

Prepare standard curves for livestock dips and sprays from appropriate aqueous dilution of emulsion concentrates of accurately known insecticide content. Prepare synthetic concentrates from the insecticides, adjuvants, and diluents employed in the formulation under consideration. Alternatively, use proprietary emulsifiable concentrates of accurately known insecticide concentration.

Dilute the concentrate to a series of insecticide concentrations covering the range anticipated in field dip and spray samples. Because many emulsifiable concentrates do not form stable homogeneous emulsions it may be preferable to use one accurately known and well-mixed source of standard diluted concentrate. Obtain a series of concentrations of insecticide from the latter by final extraction into serial aliquots of the solvent used for infrared analysis. Determine the calibration curve by the same analytical procedure established for field samples.

Obtain a standard calibration curve by plotting the net absorbance, ΔA, of the characteristic absorption band, or bands, of the insecticide against concentration. The curve obtained should obey Lambert-Beer's law over the range of insecticide concentration anticipated in the animal dip and spray samples.

METHOD FOR DELNAV

Delnav has been used at a concentration of 0.15% (w/w) in livestock emulsion dips and sprays (2, 3). Technical grade Delnav is composed of 68–75% of the cis- and trans-isomers of 2,3-*p*-dioxanedithiol S,S-bis(O,O-diethyl phosphorodithioate).

Prepare calibration curves, as described above, from aqueous dilution of synthetic concentrates taken through the analytical procedure. Use the band peaks, at about 1099 cm^{-1} (about 9.10μ) and 1129 cm^{-1} (about 8.86μ), assumed to be characteristic for the cis- and trans-isomers of Delnav, respectively (2). Plot the sum of the differences in absorbance between the absorption maximum for each isomer and the "background" line at about 1230 cm^{-1} (about 8.13μ), after correcting for the influence in absorption of one isomer on the other, against Delnav concentration.

Use the corrections for the mutual effects of the two isomers on their respective absorbances given in reference 4. Apply a first correction for the relative absorptivities of the two isomers; the second correction for the mutual effects of the isomers on each other may usually be ignored, since this correction normally represents only about 0.5% of each isomer's net absorbance. Thus, the correction most often applied in the analysis of spray and dip samples is:

$$\Delta A = \Delta A^{\text{cis}}_{1099-1230\ \text{cm}^{-1}} + \Delta A^{\text{trans}}_{1129-1230\ \text{cm}^{-1}} \times 1.42$$

With the parameters used, the calibration curve is linear to beyond 0.3% Delnav.

Determination

Transfer a 50.00 ml aliquot of livestock insecticide emulsion sample to a 125 ml separa-

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[1] Mention of specific sources of supply does not imply endorsement over similar products not mentioned by name.

tory funnel equipped with Teflon stopcock. Add 15.00 ml CS_2 and 14–17 g NaCl. Shake the mixture vigorously for a full 60 sec. and let stand until it separates. Withdraw the lower CS_2 layer and transfer to the 0.5 mm cell, (i). With the matched reference cell filled with CS_2 in the reference beam, take a spectrum of the unknown sample at slow scan speed (about 57 wavenumbers per minute).

Calculation

Read Delnav concentrations from the calibration curve or calculate it from the slope of this curve. Since Delnav concentration is obtained from a calibration curve constructed from an emulsion concentrate containing a known amount of Delnav, no correction need be made for technical Delnav (68–75% actual Delnav). If any samples contain Delnav concentrations falling above the linear portion of the calibration curve, take them through the procedure again and extract with a larger aliquot of CS_2. Adjust calculation for any change in sample-extract ratio.

METHOD FOR CIODRIN

Ciodrin has been used in livestock sprays and spray-dips at concentrations of 2, 1, 0.35, and 0.1 to 0.25%, etc., depending upon the ectoparasite under attack (3). The following procedure is designed for emulsion spray-dips in the range 0.1–0.3% by weight. However, the method may be adapted for higher concentrations of Ciodrin merely by appropriately increasing the quantity of extracting solvent employed.

Obtain a calibration curve from standards, as previously outlined, in the range from 0 to 0.3000% Ciodrin by weight. Take the absorption maximum with approximate band center at 910 cm^{-1} (10.99μ) as characteristic for Ciodrin (7). Plot the difference in absorbance, ΔA, between the band peak at 910 cm^{-1} and "background" line, or absorption minimum, at approximately 935 cm^{-1} (10.70μ) against Ciodrin concentration to obtain a useful calibration curve. Do not use the baseline method of determining ΔA for emulsion sprays, since it results in almost twice the error of the "background" line method.

Determination

Use the same analytical procedure for Ciodrin in emulsion sprays and dips as for Delnav, except use a ratio of 10 parts (50.00 ml) spray sample to 4 parts (20.00 ml) CS_2 extractant for Ciodrin concentrations up to 0.3%; a ratio of 10 parts sample to 8 parts extractant for concentration ranges from 0.3 to 0.6%; 10 parts sample to 12 parts extractant for concentrations from 0.6 to 0.9%; 10 parts sample to 16 extractant for 0.9 to 1.2%; and so forth. To improve the reproducibility of the procedure from about 97.9 to 98.2%, perform a second extraction with a smaller aliquot of CS_2 and combine this with the first extract (cf. *Results*). Incorporate this change in procedure in the calibration curve.

With very dirty spray-dip samples, centrifuge after the extraction of Ciodrin in order to separate filth and sediment from CS_2. Place a small salt-saturated aqueous layer on top of the CS_2 extract to prevent evaporation during centrifugation.

Calculation

Obtain the Ciodrin concentration directly from the calibration curve or calculate it from the slope of this curve. The sample-extract ratio should be considered in computation.

METHOD FOR LINDANE

Lindane has been used at concentrations up to 0.06% in aqueous emulsions applied to livestock (3). The procedure described is designed for dip and spray concentrations from 0 to 0.10% lindane.

Obtain a calibration curve from a synthetic or a proprietary concentrate of known lindane content taken through the analytical procedure. Subtract the absorption minimum at about 715 cm^{-1} (about 14.0μ) from the γ-isomer's characteristic absorption peak (9) at 687 cm^{-1} (14.56μ), and plot the resulting ΔA against lindane concentration. Under the conditions employed in this procedure, the curve is linear beyond 0.1% lindane.

Determination and Calculation

The lindane procedure is the same as that for Delnav. Read lindane concentration from the calibration curve or calculate it from the slope of this curve.

TOXAPHENE AND LINDANE

Simultaneous Determination of Toxaphene and Lindane in Livestock Emulsion Sprays and Dips

Mixtures of insecticides are frequently employed for the treatment of livestock. Infrared absorption spectroscopy offers a direct, simple, and rapid method for the analysis of such mixtures. Chemical procedures are not suitable for direct assay of multi-component chlorinated hydrocarbon insecticide formulations; components must first be isolated by laborious and time-consuming separation techniques.

A combination of toxaphene and lindane has been used in aqueous livestock emulsion sprays. The mixed insecticide is prepared in a ratio of 4.5 parts toxaphene to 0.2 parts lindane, and an aqueous spray is commonly used in the range of about 0.1–0.3% toxaphene plus 0.004–0.013% lindane, respectively. The following procedure is intended to cover this range.

Calibration curves for toxaphene (1) and lindane have been previously prepared. These same curves, with the corrections given under *Calculation,* may be applied to analysis of the combined insecticides.

Determination

Use the same procedure as that for Delnav, except use 10.00 ml CS_2 to extract the mixed insecticide.

Calculation

Read toxaphene content directly from the calibration curve or calculate it from the slope of this curve. Multiply the result by 2 to account for the difference in extraction solvent used (10.00 ml CS_2 vs. 5.00 ml CCl_4), and correct for the ratio of the densities of the mixed insecticide concentrate to the toxaphene concentrate. The density ratio varies with the nature of the emulsifiers and solvents used in the concentrates, but it is usually in the range of 1.13 toxaphene-lindane concentrate/1.23 toxaphene concentrate.

Obtain lindane concentration from the calibration curve previously prepared, or from a calibration curve based on the mixed insecticide formulation under consideration. Obtain lindane concentration derived from the former curve by correction as follows:

Lindane concentration (w/w%) = $\Delta A_{687-715\ cm^{-1}}$ × slope × ratio of extracting solvent × density ratio × correction factor; where ratio of extracting solvent = 10.00 ml CS_2/15.00 ml CS_2, density ratio = specific gravity of mixed insecticide concentrate/specific gravity of lindane concentrate, and correction factor is for influence of toxaphene upon 687 cm^{-1} absorption band (approximately 0.59 for the mixed insecticide containing 0.4500% toxaphene).

The last correction factor was empirically determined by comparing the absorptivities of 0.0050–0.0250% lindane alone with 0.1000% serial concentrations of toxaphene in the range from 0.2000 to 0.6000% superimposed upon lindane. The average corrections ranged from 0.5763 to 0.5999, respectively, with an interpolated value of 0.5881 for the influence of 0.4500% toxaphene upon lindane. It is believed the spectroscopist may confidently use this correction within the indicated limits of lindane-toxaphene concentration.

Results

Delnav

To quantitatively extract Delnav from livestock emulsion sprays and dips, a "salting-out" agent must be applied. The effectiveness of combined carbon disulfide extraction and sodium chloride "salting-out" was checked by infrared analysis of a second extraction in which 2.00 ml of carbon disulfide was used on a series of 10 filth-laden dip samples. No evidence for the presence of Delnav in the second extracts could be obtained, and the extraction efficiency of the first extract was assumed to be 100%.

The standard deviation for a series of 11 analyses on a single sample of filthy cattle dip was ± 1.59% (Table 1).

Figure 1-A is a spectrum of Delnav analytical standard containing 48% trans- and 26% cis-isomer. The accompanying infrared spectrum (Fig. 1-B) of an extremely dirty Delnav sheep dip emulsion taken through the above procedure is illustrative of the method's general freedom from interference. The characteristic absorption bands are sharp, well-defined, and free from any intru-

Table 1. Precision of the infrared method for livestock Delnav emulsion dips and sprays

$\Delta A_{cis+trans}$	% Delnav (from curve)[a]
0.202	0.1472
0.200	0.1457
0.205	0.1494
0.197	0.1435
0.199	0.1450
0.205	0.1494
0.204	0.1486
0.202	0.1472
0.195	0.1421
0.202	0.1472
0.198	0.1443

[a] Std Dev.: ±0.00233% Delnav = 1.59% deviation.

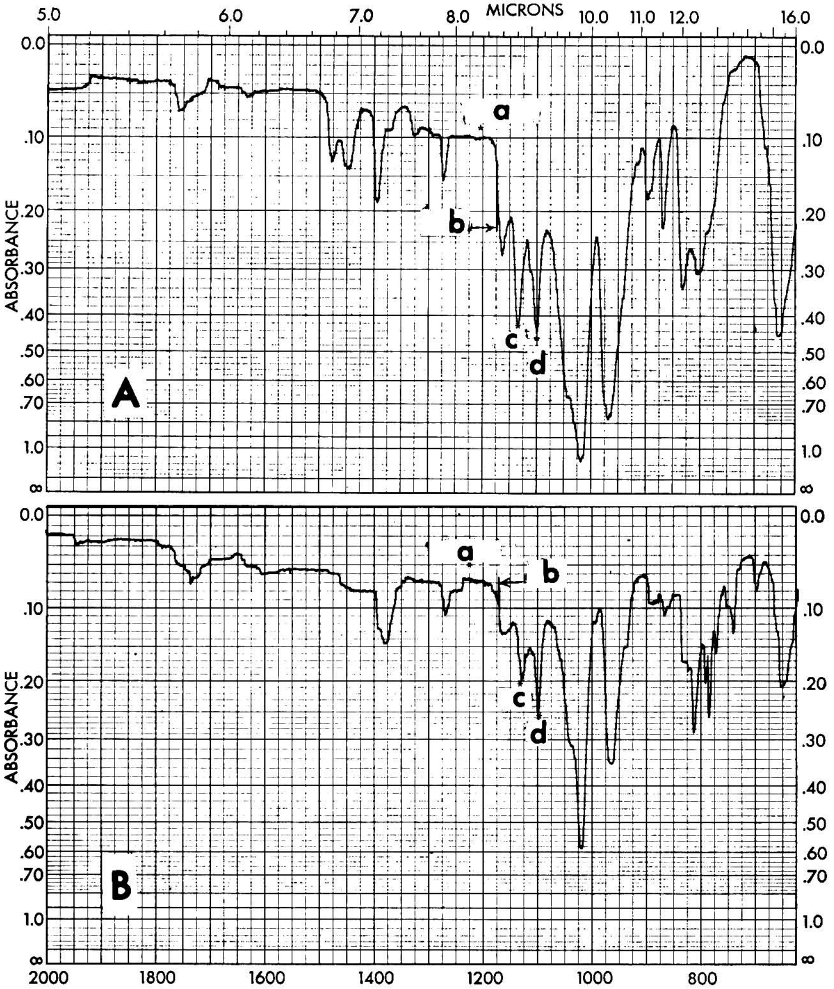

Fig. 1—**Infrared spectrum of Delnav. A. Analytical standard containing 48% trans- and 26% cis-isomers. Scan taken of capillary film between NaCl plates; reference beam attenuated. (a, "background"; b, filter; c, trans-; d, cis-). B. Extract obtained from a filth-laden, aqueous sheep dip emulsion prepared from a 30% Delnav emulsifiable concentrate. IR cell path, 0.511 mm. Delnav concn, 0.132 ± 0.002% (w/w). (a, "background"; b, filter; c. trans-; d, cis-).**

sion from absorption due to animal waste-products or to components of the insecticide formulation. There is no guarantee that future formulations or other dip samples will be free from interferences. However, such interferences will be immediately detected by this versatile infrared technique, and judicious alterations in analytical procedure can then be made.

The infrared method agrees very favorably with the colorimetric chemical procedure of C. L. Dunn (5, 6). Table 2 compares results obtained by the two methods on dip samples specially chosen for their filthiness. In contrast to the infrared procedure, the chemical method requires elaborate separation technique and equipment, involves complex reaction parameters, and is generally more subject to error, more time-consuming, and tedious.

Normally, a single sample can be taken through the infrared method in less than 20 minutes. Ten samples may be run in an hour. This procedure should be the method of choice for Delnav emulsion dips and sprays.

Ciodrin

Carbon disulfide alone will not completely extract Ciodrin; a "salting-out" agent must be employed. Extraction efficiency was checked by infrared investigation of a 2.00 ml second extract with carbon disulfide on 10 repeated analyses of a single sample of very dirty cattle spray-dip emulsion (Table 3). The extraction efficiency of the first extract is about 99.4%. By combining the first and second carbon disulfide extracts, the standard deviation of the method may be improved from about ± 2.1% to ± 1.8%. It was felt that such a small improvement in error did not warrant the addition of a second solvent extraction to the procedure. A third extract contained no detectable Ciodrin.

The procedure is more rapid than that for Delnav.

A spectrum of analytical standard Ciodrin is given in Fig. 2-A. As is illustrated by the infrared spectrum (Fig. 2-B) of a filth-laden spray-dip sample taken through the analytical procedure, the aqueous emulsions of Ciodrin investigated thus far have been free from interferences.

Alternative analytical procedures for Ciodrin, such as the measurement of acetylcholinesterase inhibition (8), are nonspecific, much more complex, and have relatively poor precision compared to the infrared method. It is the method of choice for animal dips and sprays.

Lindane

The efficiency of the first carbon disulfide extract was checked by a second 2.00 ml extraction on a series of eight filth-laden dip samples. Evidence for the presence of lindane in these second extracts could not be obtained. The efficiency of the first extract was, therefore, assumed to be 100%. For quantitative extraction, a "salting-out" agent must be employed.

A series of eight repeated analyses on a single sample of very dirty sheep dip yielded a standard deviation of ± 3.03% (Table 4). The relatively high error was believed due to the poor stability of the aqueous emulsion and the intrinsic variation of the absorptivity for the 687 cm^{-1} band of lindane.

Table 2. Comparison of some representative results obtained by the colorimetric method of Dunn (5, 6) and the infrared method for Delnav

Sample	Colorimetric Method[a]	Infrared Method
3–4	0.066 ± 0.002	0.068 ± 0.001
3–8	0.106 ± 0.003	0.115 ± 0.002
3–13	0.139 ± 0.004	0.149 ± 0.002
4–1	0.163 ± 0.005	0.171 ± 0.003
4–2	0.159 ± 0.005	0.168 ± 0.003
4–3	0.157 ± 0.005	0.165 ± 0.003
5–5	0.202 ± 0.006	0.224 ± 0.004
5–6	0.236 ± 0.007	0.262 ± 0.004

[a] Average of 3 results.

Once again, interferences from formulation, animal waste-products, or other sources have not been a problem (Fig. 3).

The infrared method is much simpler and less time-consuming than chemical procedures for lindane. Although the precision is somewhat less than desired, it is entirely adequate for application to rapid analysis of field samples of lindane emulsions. A minimum of 10 samples may be analyzed in an hour.

Table 3. Error analysis of the infrared analytical method for Ciodrin

	% Ciodrin in First Extract	% Ciodrin in Second Extract	Total % Ciodrin	% Total Ciodrin in Second Extract
	0.2876	0.0007	0.2883	0.24
	0.2756	0.0021	0.2777	0.76
	0.2778	0.0018	0.2796	0.64
	0.2767	0.0020	0.2787	0.72
	0.2822	0.0014	0.2836	0.49
	0.2855	0.0010	0.2865	0.35
	0.2876	0.0007	0.2883	0.24
	0.2811	0.0012	0.2823	0.43
	0.2822	0.0014	0.2836	0.49
	0.2712	0.0030	0.2742	1.09
Mean:	0.2808	0.0015	0.2823	0.55[a]
Std Dev.:	±0.0059 = ±2.09% deviation		±0.0052 = ±1.83% deviation	

[a] To obtain the average per cent of first extract efficiency, subtract this number from 100.00.

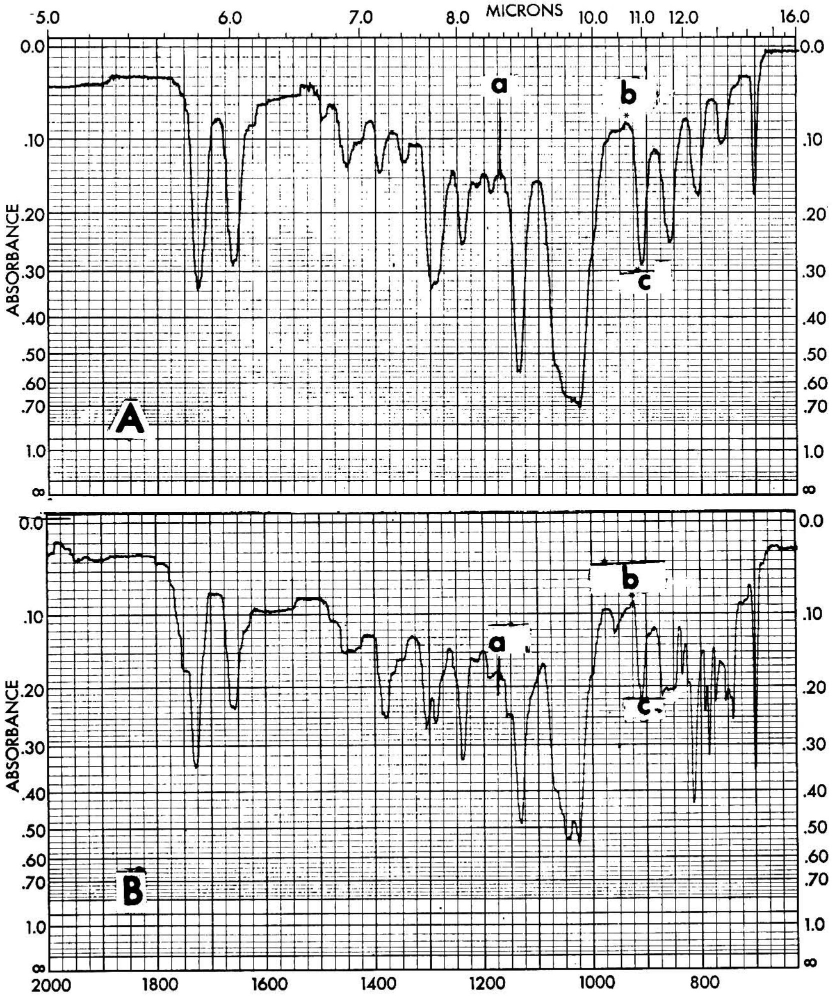

Fig. 2—Infrared spectrum of Ciodrin. A. Analytical standard, purified, 90% (w/w). Conditions same as Fig. 1A. (a, filter; b, 935 cm^{-1}; c, 910 cm^{-1}). B. Extract derived from a dirty, aqueous cattle spray-dip emulsion of Ciodrin. Dip prepared from 14.40% Ciodrin proprietary spray-dip formulation. Sample contained 0.183 ± 0.004% (w/w) Ciodrin. Cell path, 0.511 mm. (a, filter; b, "background"; c, 910 cm^{-1})

Toxaphene and Lindane

Extraction efficiency was checked, as before, by a second 2.00 ml extract of dip sample. The average recovery for both toxaphene and lindane in the first carbon disulfide extract was greater than 99% in all cases of a series of 12 filthy sheep dip samples with varying insecticide concentration.

Preliminary data indicate that the error in infrared analysis of toxaphene-lindane mixtures is approximately the same as the previously reported analytical error of these insecticides measured individually.

The infrared method for simultaneous determination of lindane and toxaphene is rapid, simple, and accurate. An infrared spectrum is given in Fig. 4 to illustrate the method's general freedom from interference. The procedure may doubtless be extended to the determination of other mixed insecticides (10).

Discussion

The successful application of the infrared procedure to the assay of aqueous emulsions of insecticides depends primarily upon freedom from interferences in insecticide absorption bands obeying Lambert-Beer's law.

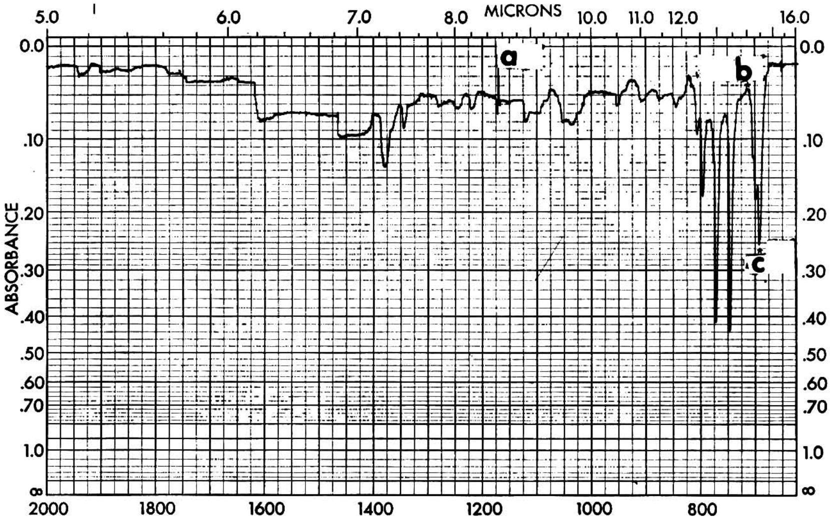

Fig. 3—Infrared spectrum of filth-laden, aqueous lindane sheep dip emulsion. Cell path, 0.511 mm. Dip sample contained 0.0467 ± 0.0014% (w/w) lindane. (a, filter; b, 715 cm^{-1}; c, 687 cm^{-1})

Interferences from animal sources are not a problem. So long as the emulsifiable concentrate does not contain materials that interfere with characteristic insecticide absorption bands, the concentrate itself (cf. Ref. 1), as well as the aqueous emulsion derived from it, is amenable to infrared analysis. Thus, the prospect for successful application of the infrared method to aqueous emulsions may readily be determined by examining a qualitative infrared spectrum of the insecticide concentrate being investigated. Moreover, interferences from formulation sources do not necessarily preclude success, since corrections may be applied when the interferences are constant or predictable.

Table 4. Precision of the infrared method for livestock lindane emulsion dips and sprays

$\Delta A_{687-715\ cm^{-1}}$	% Lindane (from curve)[a]
0.289	0.0642
0.294	0.0653
0.271	0.0602
0.288	0.0640
0.291	0.0647
0.297	0.0660
0.279	0.0620
0.294	0.0653

[a] Std Dev.: ±0.00194% lindane = ±3.03% deviation.

The choice of extracting solvent depends primarily upon the infrared absorption characteristics of the insecticide under investigation. The solvent should not interfere with the infrared bands used for determination of the insecticide. Alternatively, a solvent that does not meet this criterion but has a superior extraction efficiency may be chosen. In this event, the solvent should be completely evaporated after extraction and the resulting insecticide residue taken up in another solvent free from interference at the infrared bands of interest. The solvent chosen should have a very low aqueous solubility. It should have a constant extraction efficiency; that is, aided by "salting-out," it should extract all insecticide from aqueous emulsion, or it should extract a reproducible percentage of the insecticide present in a given time interval.

To date, sodium chloride has been found to be most useful for "salting-out" to completely extract the insecticide. Other salts have been tried for this purpose (1) and it is not unlikely that one of them may be more effective than sodium chloride under certain conditions. The analyst is urged to experiment to obtain optimal insecticide recovery in the first extract.

There is no guarantee that the analysis of all emulsifiable insecticide formulations will be possible with the infrared method as it

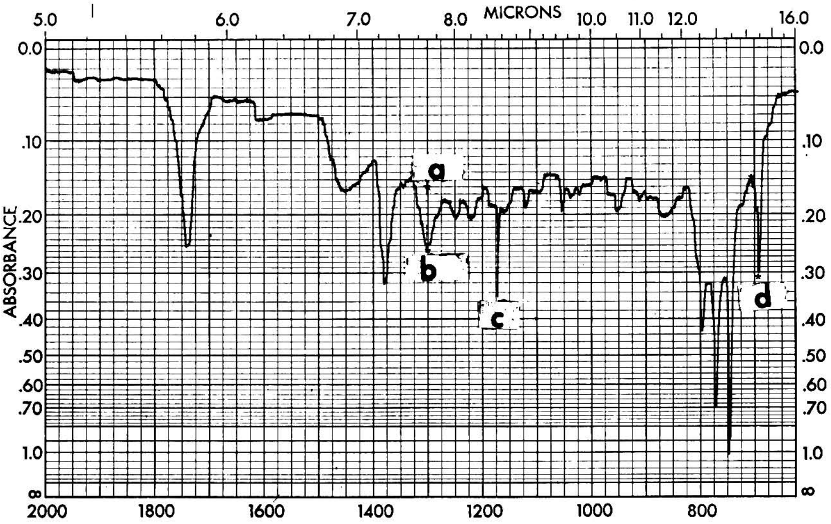

Fig. 4—**Infrared spectrum of mixed, filth-laden, aqueous toxaphene-lindane sheep dip emulsion prepared from a 45.00% toxaphene + 2.00% lindane emulsion concentrate. Cell path, 0.511 mm. Dip contained 0.321% toxaphene and 0.013% lindane. (a, baseline; b, toxaphene; c, filter; d, lindane)**

is presented. Separation or cleanup procedures may have to be used. However, because of the advantages of simplicity, speed, excellent precision, versatility, general freedom from interference, and unqualified specificity, the method should always be considered as a general first approach to the analysis of insecticides in emulsifiable concentrates and in aqueous livestock emulsion dips and sprays.

Acknowledgments

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The technical assistance of G. B. Clark, George Evans, and M. D. Mack is also gratefully acknowledged.

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Gas Chromatographic Analysis of Insect Attractants: Trimedlure, Medlure, Siglure, Butyl Sorbate, Cue-lure, Methyleugenol

By MORTON BEROZA and RAFAEL SARMIENTO (Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. 20705)

Means of analyzing trimedlure, medlure, siglure, butyl sorbate, cue-lure, and methyleugenol by gas chromatography have been devised to ensure the procurement of high quality attractants needed for the detection of insect pests, especially those invading noninfested areas. The methods permit the estimation of the 4 *trans* isomers present in commercially produced trimedlure and medlure, and of the *cis* and *trans* isomers of siglure. Commercially available butyl sorbate gives a single sharp peak and is supposed to be the *trans, trans* isomer. Cue-lure and methyleugenol each give a single peak.

The use of chemical insect attractants to detect infestations of harmful species before they become established in noninfested areas is now a validated procedure. For example, trimedlure (1) is produced commercially for use in traps (2) that are placed around international ports of entry to forestall an accidental importation of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). This pest was eradicated from Florida in 1957 by Federal and State workers, and reinfestations of the insect occurring in 1962 and 1963 were quickly spotted and eradicated. U.S. Department of Agriculture officials estimate that $9,000,000 in eradication costs were saved by these early detections.

The procurement of highly attractive chemicals (3) is essential to such programs. This paper gives details of the gas chromatographic procedures that were devised to analyze and check the quality of insect attractants, trimedlure, medlure, siglure, butyl sorbate, cue-lure, and methyleugenol, all of which have been or are in commercial production. Chromatograms of the attractants are given in Fig. 1. Their chemical formulas are shown in Fig. 2. All are male attractants. The determination of gyplure, the sex attractant of the gypsy moth, by gas chromatography has been described by Jones and Jacobson (4).

An F & M Scientific Corp. (Avondale, Pa.) Model 609[1] flame ionization gas chromatograph was used in the present work.

Trimedlure

This chemical (*tert*-butyl 4(or 5)-chloro-2-methylcyclohexanecarboxylate) can exist in 8 isomeric forms. However, in its commercial production at least 85% of the acid intermediate possesses the *trans* configuration of the methyl and the carboxyl group, so that only 4 isomeric forms are obtained in appreciable quantity. The 4 isomers may be separated sufficiently (solid line, Fig. 1A) to permit their estimation based on areas under the peaks. Peak heights give a rough estimation of isomeric content. Conditions for the analysis follow:

Column: 15 ft × 3/16″ o.d. copper tubing.

Stationary phase: 7% RC Polymeric BGA (Polyadipate, mol. wt about 2500, Rubber Corp. of America, Hicksville, N.Y.).

Support: 60–80 mesh Gas Chrom P (Applied Science Laboratory, State College, Pa.).

Carrier gas: nitrogen at 30 ml/min.

Column temperature: 120°C.

Detector block temperature: 255°C.

Sample: 2 μl of 1% solution of trimedlure in carbon disulfide.

Retention times (min.): Isomer 1, 29.9; 2, 32.3; 3, 33.5; 4, 36.1.

Trimedlure decomposed in part when injected into the usual injection port of the gas chromatograph, no matter how low the temperature of the injection port was set. The dotted lines of Fig. 1A show the extra peaks due to decomposition. By injecting

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[1] Mention of a proprietary product does not necessarily imply endorsement of this product by the USDA.

the sample directly on the column, such decomposition was avoided. The major peaks of the decomposition moiety between 9 and 12 minutes coincide with the retention times of *trans* and *cis*-*tert*-butyl 6-methyl-3-cyclohexenecarboxylate (9.9 and 10.3 min., respectively), which are dehydrochlorination products of trimedlure. The decomposition peak at 42.8 minutes appears to be the trimedlure acid (which could arise from the loss of isobutylene), since the retention times agree.

The determination of isomer content is important for two reasons. First, at least one of the isomers, *3*, is unattractive, so its content should be minimized. Second, two of the components, *3* and *4*, are solids. A solid is undesirable because it crystallizes in the cotton dental-roll wick used to hold the lure in the Steiner trap and does not constantly migrate to the surface of the wick by capillary action, as does a liquid when the lure vaporizes. The volatilization of the crystallized solid inside the wick is greatly depressed and the clogged wick cannot accept much lure when it is to be recharged. As would be expected, crystallization of the solid isomers from trimedlure is most troublesome during cold weather. This problem is now being studied through the use of the above-described gas chromatographic procedure.

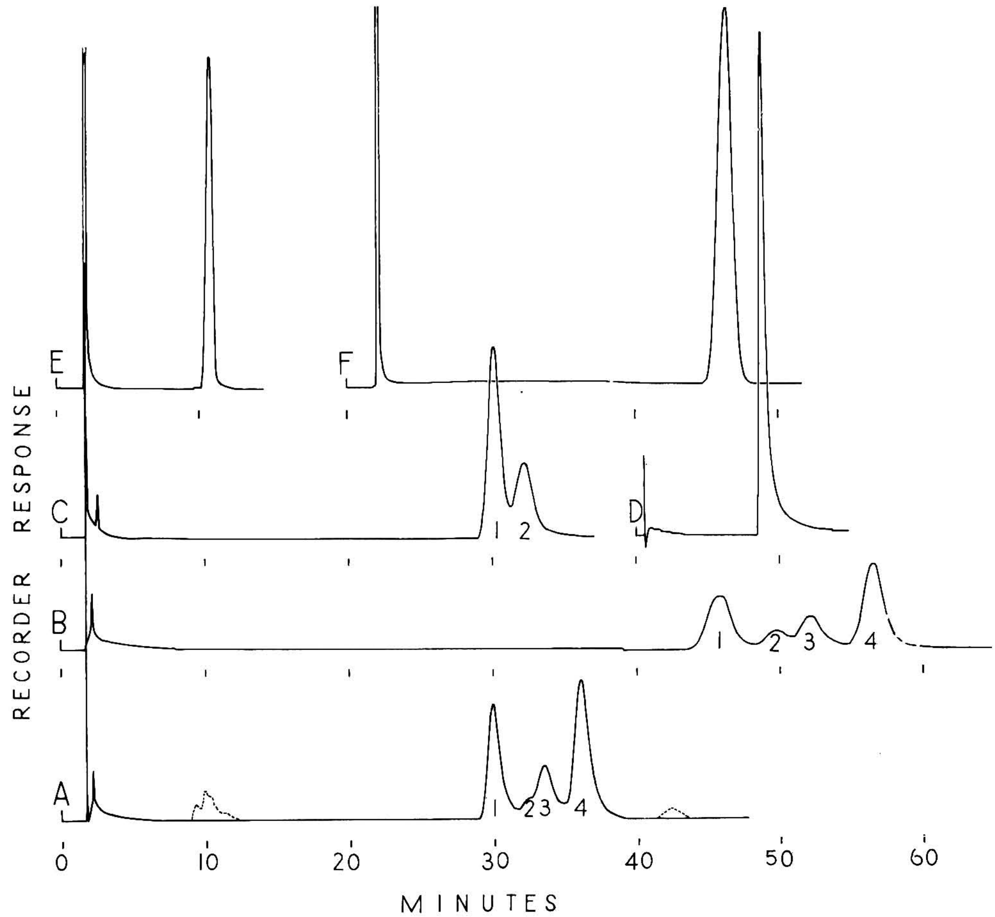

Fig. 1—Chromatograms of insect attractants. Conditions of analysis given in text. First peak(s) is solvent. *A*, Trimedlure; solid line: injected directly on column; dotted lines: extra peaks arising when trimedlure is introduced through regular injection port. *B*, Medlure. *C*, Siglure; 1, *trans*; 2, *cis*. *D*, Cue-lure. *E*. Butyl sorbate. *F*, Methyleugenol.

Medlure

The foregoing procedure may also be used to determine the isomer content of medlure (*sec*-butyl analog of trimedlure (1)). The ratio of its isomers parallels that for trimedlure. Retention times (min.): Isomer 1, 45.8; 2, 49.8; 3, 52.1; 4, 56.5. See Fig. 1B. Medlure and siglure (5) are also Mediterranean fruit fly attractants, but are less effective than trimedlure.

Siglure

To determine the amount of *cis* and *trans* isomers of siglure (*sec*-butyl 6-methyl-3-cyclohexenecarboxylate (6)) the above procedure is utilized with one exception: the column temperature is set at 100°C. See Fig. 1C. Retention times (min.): *trans* 1, 30.2; *cis* 2, 32.2. Several preparations analyzed by the previous infrared procedure and the present gas chromatographic method agreed within 2%. The present method is much more convenient and undoubtedly less subject to error.

Butyl Sorbate

Butyl sorbate attracts the European chafer, *Amphimallon majalis* (Razoumowsky), which infests limited areas of New York, New Jersey, and Connecticut. It has been eradicated from West Virginia. The location of this pest of turf, grasslands, and grain crops is being monitored by traps baited with butyl sorbate (7), and efforts to prevent its spread are being exercised by our Plant Pest Control Division. Butyl sorbate may exist in 4 isomeric forms. According to Merck's Index (7th Ed.) commercial sorbic acid is the *trans,trans* isomer, and presumably the ester is likewise this isomer. Butyl sorbate gives a sharp single peak (Fig. 1E) by the gas chromatographic procedure described below:

Column: 12 ft × ¼″ o.d. copper tubing.

Stationary phase: Carbowax 20M (Union Carbide Corp.).

Support: 60–80 mesh Gas Chrom P (Applied Science Laboratory).

Carrier gas: nitrogen at 60 ml/min.

Column temperature: 165°C.

Injection port and detector block temperature: 250°C.

Sample: 1 μl of 1:1 acetone-butyl sorbate.

Retention time: 10.6 min.

Methyleugenol

Methyleugenol is a powerful attractant for the oriental fruit fly, *Dacus dorsalis* Hendel (8, 9). Under the conditions described above for butyl sorbate, methyleugenol (1-allyl-3,4-dimethoxybenzene) has a retention time of 26.4 minutes (Fig. 1F).

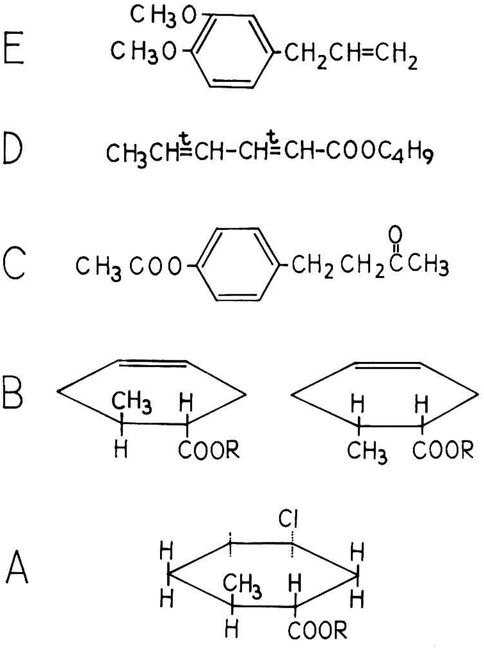

Fig. 2—Chemical formulas of insect attractants. *A*, Trimedlure [R = $-C(CH_3)_3$]. Medlure [R = $-CH(CH_3)CH_2CH_3$]. Chlorine may occupy one of dotted lines; other bonds occupied by hydrogen. *B*, Siglure [R = $-CH(CH_3)CH_2CH_3$]; left, *trans*; right, *cis*. *C*, Cue-lure. *D*, Butyl sorbate. *E*, Methyleugenol.

Cue-lure

Cue-lure attracts the melon fly, *Dacus cucurbitae* Coquillett (10). The lure did not pass through a variety of ordinary packed columns at all. The following procedure, based on the low-substrate loaded columns (11) used for highly sensitive steroids, was found suitable (Fig. 1D):

Column: 8 ft × ¼″ o.d. copper tubing.

Stationary phase: 0.3% SE-30 methyl silicone gum polymer (General Electric Company).

Support: 80–120 mesh siliconized glass beads.

Carrier gas: nitrogen at 60 ml/min.

Column temperature: 120°C.

Injection port: 225°C.

Detector block: 275°C.

Sample: 2 μl of 1% solution cue-lure in carbon disulfide.

Retention time: 8.8 min.

Before analyses were begun, 15 μl of hexamethyldisilazane was injected into the column. The first sample or two injected on the column produces weak peaks, but subsequent runs with equivalent amounts give good peaks of the same size. Accordingly, when this analysis is started, the first two runs should be discarded. Although peak tailing is a defect, it is believed that the analysis does give a good measure of homogeneity because an appreciable amount of impurity would probably separate from cue-lure or distort its smooth curve. The method is advanced as the best available at this time, but a better procedure would be most welcome.

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FISH AND OTHER MARINE PRODUCTS

Protein Typing of Some Authentic Fish Species by Disc Electrophoresis

By VIVIAN M. MANCUSO (Food and Drug Administration, 222 U.S. Customhouse Bldg., New Orleans, La. 70130)

Disc electrophoresis has been used to identify protein types of authentic fish species. Patterns are given for 22 varieties of fish, representing members of the Snapper, Grouper, and Weakfish families.

Fish of various species differ sharply in total protein content and distribution of the fractions. The identification of fish species by chemical means is of primary importance in the case of fillet fish, where most physical means of identification are no longer present. The application of electrophoresis (1) to the fractionation of fish proteins has been used in this laboratory on 22 different fish species. An authentic[1] library of protein patterns of these fish species has been prepared by using a disc electrophoresis appa-

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[1] Authentic fish samples have been identified by Louisiana State Department of Commerce, Division of Wildlife and Fisheries.

ratus and polyacrylamide gels (2). Protein typing of five varieties of fish by disc electrophoresis has previously been reported (3).

The starch gel method (4) previously used in this laboratory required approximately 9 hours, and the apparatus was designed to handle only three samples per run. The disc apparatus presently in use can handle eight times as many samples in less time. Resolutions of protein bands were found to be more distinct with the polyacrylamide gels. These gels are more durable than starch gels and can be conveniently stored in tubes of 7.5% acetic acid or dried out and reconstituted at a later date.

METHOD

Apparatus and Reagents

The Canalco Model 12 Disc Electrophoresis with a 300 A power supply was used. The power source had a maximum of 180 ma. The electrophoresis range was 5–60 ma; the destain range 5–180 ma.

Pre-mixed chemicals provided with the Model 12 apparatus (600; Unit Chemical Kit #600 P) were used in this study.

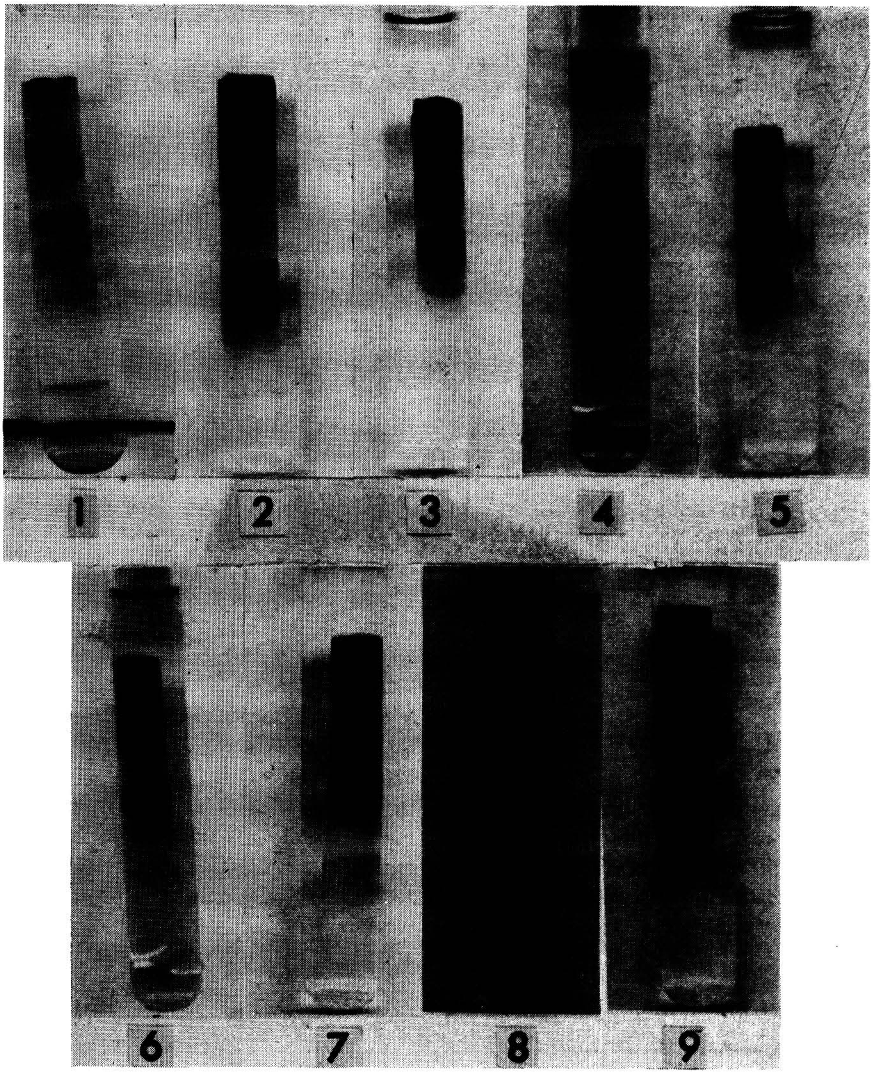

Fig. 1—Electrophoretic gel patterns of fish of the Snapper Family. *1*, Red or American; *2*, Lane; *3*, White; *4*, Bastard; *5*, Yellow Eye; *6*, Mexican; *7*, Mangrove; *8*, Mexican Green Tail; and *9*, Hambone.

Sample Preparation

Fillet fish samples, and blend 50 g fillet with 75 ml water for 2 min. Centrifuge in 50 ml centrifuge tubes for about 20 min. at 1500 rpm. Filter supernatant into 50 ml glass-stoppered flask. Use 40 μl supernatant for column run. (Patterns from the same protein extract stored frozen did not change within 3 months.)

Gel Preparation

Detailed procedures for gel preparation are given in the Canalco operating manual (2).

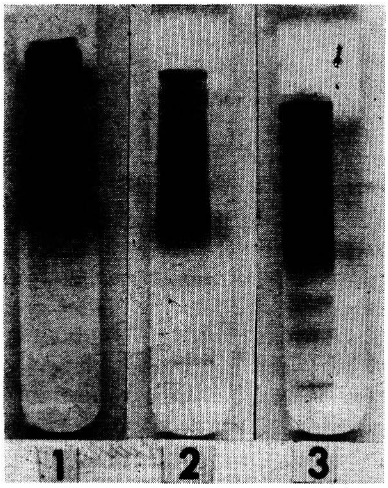

Fig. 2—Electrophoretic gel patterns of fish of the Grouper Family. 1, Red; 2, Kitty Mitchell; and 3, Scamps.

The polyacrylamide gel column is composed of three layers: the upper large-pore gel containing the sample ions in which electrophoretic concentration of these ions is initiated; the middle large-pore gel (spacer gel) in which electrophoretic concentration of the sample ions is completed; the lower small-pore gel in which electrophoretic separation occurs (5). Fill capped tubes with prepared lower gel and diluted upper gel.

Sample gel.—To a small test tube add 100 μl concentrated upper gel, 40 μl prepared protein extract, and 60 μl distilled water. Shake test tube well and let settle. When polymerization of spacer gel is complete, add sample gel to fill tube. Let sample gel photopolymerize for not longer than an hour from the time of preparing spacer gel.

Remove base caps from tubes (columns) and position in apparatus with sample end up. Perform the electrophoresis (2), using 5 ma current per column. Set polarity of electrodes so that sample ions migrate to the lower bath (positive electrode). (With bromphenol blue as tracking dye, the dye front is represented as a thin blue line, which migrates into gels, overtaking and passing the protein species which concentrate behind the dye.) Run all samples for about 35 minutes or until the tracking dye front is about ¼" from end of column.

When electrophoresis is complete, remove the gels from their tubes by rimming under water. (Rimming is difficult unless the tube

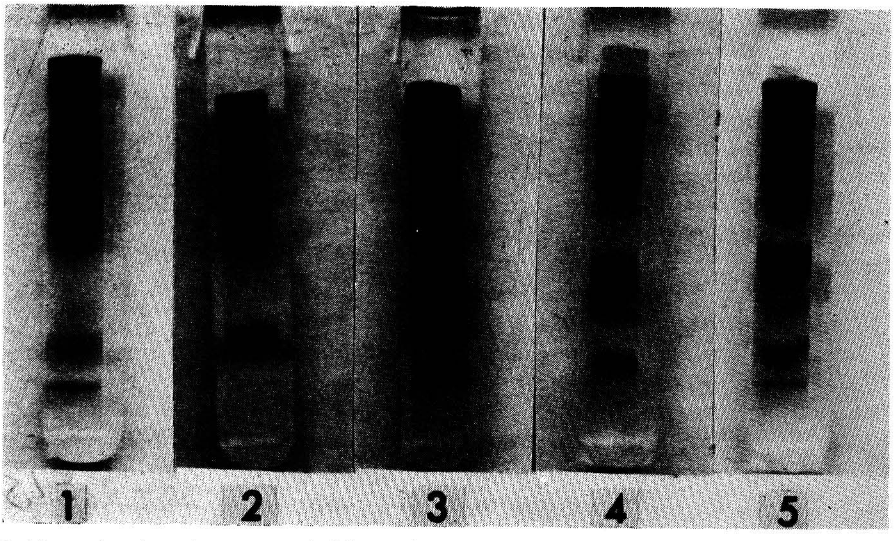

Fig. 3—Electrophoretic gel patterns of fish of the Weakfish Family. 1, Sand Trout; 2, White or Silver Trout; 3, Speckled Trout or Spotted Weakfish; 4, Redfish or Red Drum; and 5, Black Drum.

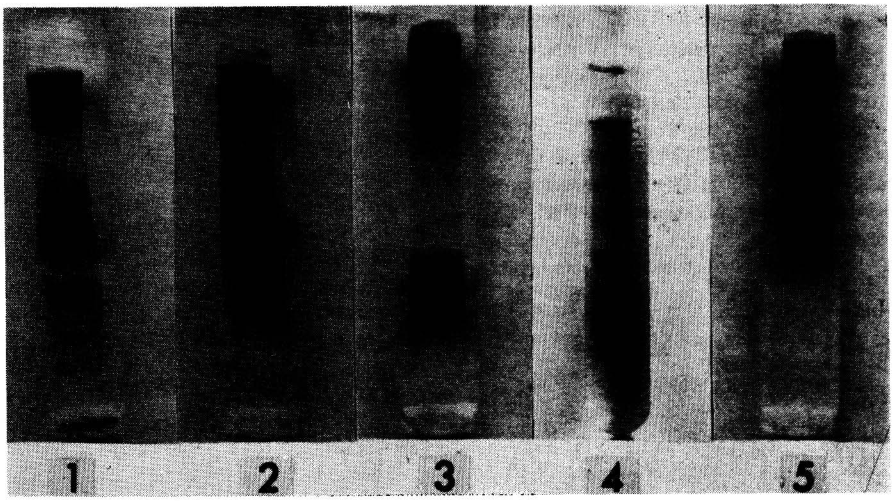

Fig. 4—Electrophoretic gel patterns formed by *1*, Perch; *2*, Pollock; *3*, Flounder; *4*, Hake or Whiting; and *5*, Striped or Black Mullet.

is perfectly clean and extreme care is taken.) Place gel in a destain tube "front" end downward (sample end upward). Discard the flimsy sample gel. Fix and stain the gels for at least 1 hr with Amido Schwartz dye in acetic acid. Destain (3) at 15 ma of current per tube, and store in 7.5% acetic acid. Visually compare the finished tubes with authentics.

Results

This procedure has been carried out on 22 varieties of fish. An entire run takes 7 hours to complete. Figures 1–3 show representative samples of the families Snapper, Grouper, and Weakfish, respectively. Figure 4 shows patterns of various other species of fish.

As an interlaboratory test, six unknown fish extracts were run in duplicate. The analyst properly identified all unknowns by comparison with the reference gel patterns of authentics.

Acknowledgment

The author wishes to thank A. T. Sturdivant for his participation in this study.

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PESTICIDE RESIDUES

Gas Chromatography with Microcoulometric Detection for Pesticide Residue Analysis

By JERRY BURKE and WENDELL HOLSWADE (Division of Food Chemistry, Food and Drug Administration, Washington, D.C. 20204)

Conditions are given for the most efficient operation of the microcoulometric gas chromatograph as a multiple detection technique for residues of chlorinated pesticides. Conditions are as follows: Column, aluminum, 6′ long by 4.5 mm i.d., packed with 10% DC 200 silicone fluid (12,500 cst) on 80/90 mesh Anakrom ABS (conditioned 1–5 days at 250°C), 210°C column temperature, and 120 ml/min. N_2 flow.

Chromatography of certain pesticides is improved by injection of cleaned up crop extracts. Columns with a minimum number of bends are superior to coiled columns. Chromatography is not improved by using glass columns. Relative retention times and instrument sensitivity are tabulated for 87 chlorinated and 26 thio pesticide chemicals. Theoretical chloride recoveries are given for 14 compounds and sulfur recoveries for 3 compounds. Theoretical recoveries indicate that the MCGC system is linear for a particular pesticide when it is present above some minimum quantity. Sample cleanup is essential for consistently accurate and reproducible results. The general level of "maximum sensitivity" of this multiple detection technique is approximately 0.01 ppm. The specificity of the detection system (for Cl^-, Br^-, I^- or sulfur) is the instrument's greatest virtue.

Gas chromatography with microcoulometric detection (MCGC) is one of the multiple detection systems used in our laboratories for the determination of residues of chlorinated pesticides in foods. The technique was first reported by Coulson, *et al.* (1–3). This laboratory reported its observations on the use of MCGC for chlorinated pesticide residue analysis in 1962 (4). Since that time improvements in the MCGC system, including the use of a more efficient GLC column, and additional knowledge gained through use of this analytical tool have made obsolete much of the data reported in our previous publications (4, 5). The purpose of this paper is to present data that are important in the efficient use of MCGC for qualitative and quantitative analysis in either routine or research investigations, and a few general comments and observations on the use of MCGC for residue analysis.

Equipment

A Micro Tek Model 2503 R gas chromatograph with a Dohrmann Model S-100-combustion unit and Model C-100 microcoulometric detection system was used in the present work. Titration cells, Dohrmann Models T-200-S and T-200-P (6, 7), were used to measure chlorinated and thio pesticides, respectively. The recorder was a 1 millivolt Minneapolis-Honeywell, equipped with a Disc integrator.

GLC Column and Operating Conditions

The columns were aluminum, 6 ft long × 4.5 mm i.d. packed with 10% DC 200 silicone fluid (12,500 cst) on 80/90 mesh Anakrom ABS. A column temperature of 210°C and carrier gas flow rate of 120 ml N_2/min. proved to be the best operating conditions when using this multiple detection technique for the analysis of chlorinated pesticide residues. Through experimentation with column temperatures and flow rates, these conditions were found most effective, if both resolution and total retention time are considered, when the following mixture is chromatographed: lindane, heptachlor, aldrin, heptachlor epoxide, *o,p′*-DDT, and *p,p′*-DDT. Conditions that produce the best chromatography of this mixture are most

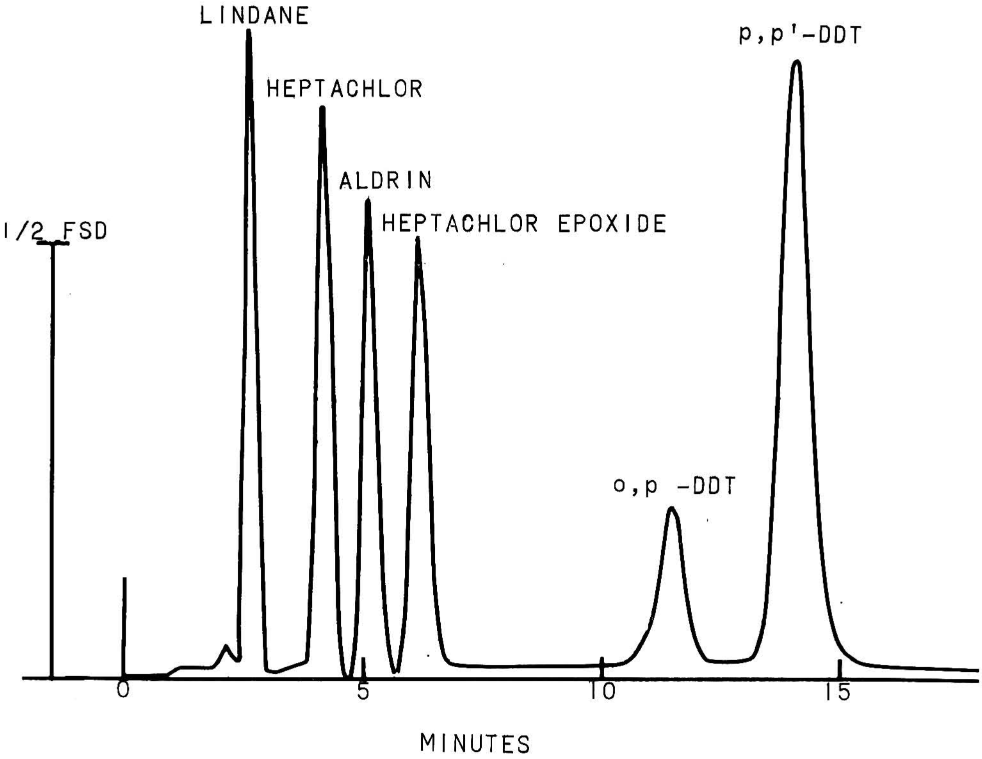

Fig. 1—MCGC of 1 μg each lindane, heptachlor, aldrin, heptachlor epoxide; and 5 μg technical DDT. Column: 10% DC 200 (12,500 cst) on 80/90 Anakrom ABS; column temp., 210°C; flow rate, 120 ml/min.; sensitivity, 64 ohms.

suitable for pesticide compounds frequently encountered in residue analysis. Figure 1 is a typical chromatogram of 1 μg each (5 μg of technical DDT) of the above mixture at the specified operating conditions. The retention time of *p,p'*-DDT is approximately 14 minutes. Injection temperature was 240°C, sensitivity 64 ohms, and chart speed ½ in./min.

The column packing material was prepared in 20 g batches by slurrying 18 g of Anakrom ABS with 2 g of DC 200 silicone fluid (12,500 cst) dissolved in about 100 ml $CHCl_3$ in a 300 ml Morton flask. The solvent was removed by a flash evaporator at about 50°C with vacuum, and the slurry was stirred intermittently by occasionally switching on the rotator. One end of the straight aluminum column was plugged with pyrex glass, and the column was filled with a minimum of vibration followed by lightly tapping the plugged end on the floor to completely settle the packing material. Care was taken at all stages of column preparation to prevent fracturing the solid support. The other end of the column was then plugged with glass wool. Approximately 40 ml (9.5–10 g) of the packing material is required to fill the column.

After the columns were filled, they were formed into an M-shape by making three 180° bends over a 2″ cylinder at distances of 18, 36, and 54″ from one end of the column. Better chromatography was obtained from the M-shaped columns than from those which were coiled. It is thought that particles of the solid support are fractured during coiling, thus exposing uncoated and "active" areas that may cause a column to be unsatisfactory for anlytical work. Very few bends are involved with the M-shaped column and only rarely is it necessary to discard a new column of this shape.

Glass columns, both coiled and U-shaped, were examined. There was no improvement in chromatography over that obtained when aluminum was used. When insufficiently cleaned up extracts were injected into the glass columns, poor results (degradation, low recovery, loss of resolution) were evident as rapidly as with aluminum columns. Glass was also fragile and inconvenient to handle.

Anakrom ABS solid support produced a more efficient GLC column for chlorinated pesticide analysis than Chromosorb P did. A column packed with 10% DC 200 on 80/90 mesh Anakrom ABS had 1680 theoretical plates compared to 800 for a column of 20% DC 200 on 30/60 Chromosorb P. The *p,p'*-DDT peak was used for calculations of theoretical plates. Certain pairs of compounds that appeared as single peaks or were hard to differentiate on the Chromosorb P column were resolved sufficiently by the Anakrom column for identification (e.g., *o,p'*-DDT and *p,p'*-DDD) and in some instances quantitative measurement (Perthane and *p,p'*-DDD; aldrin and Telodrin). Small quantities are more distinguishable on the Anakrom column, especially late eluters, because peaks are sharper.

Columns must be conditioned to obtain best performance with regard to both column bleed and satisfactory chromatography. Column bleed has not been a problem. Conditioning is done for relatively long periods at about 250°C, which is well above the operating temperature. Column performance continues to improve for about 4 days with heat conditioning at 250°C. It is desirable to condition a new column at about 250°C and 100 ml/min. gas flow for at least over the weekend. Best results are obtained after the column has been in use for some time. Heptachlor and DDT usually do not chromatograph well (low response) on a new column. Injection (vent open) of about 1–3 mg of these (and other) pesticides generally remedies this situation. Other compounds, including endrin, Perthane, methoxychlor, Kelthane, and Tedion, do not chromatograph well until the column is thoroughly conditioned. This thorough conditioning is usually accomplished through chromatography of crop extracts. Endrin may not exhibit satisfactory chromatography until the column has been used 2–3 weeks for residue analysis. Observations on the gas chromatography of endrin on the DC 200/Anakrom column and various methods used to improve the chromatography of this compound have been reported (8, 9).

Qualitative Analysis

A single analysis by gas chromatography does not unequivocally identify the material under study. The retention time is the basis for gas chromatographic identification. It is readily seen that two or more compounds, with any given column and conditions, could have retention times which are the same or so nearly the same that their respective chromatographic bands overlap. In such cases positive identification is difficult or impossible. A gas chromatographic identification would be strengthened considerably if it could be based on results obtained from two different GLC columns that elute the compounds in different sequence. Such columns have not as yet been developed.

Various other techniques may be employed to reduce the number of conflicting responses in a GLC analysis, for example, use of a detector that is specific for the compound or type of compound being investigated. Column chromatography and liquid-liquid partitioning can be used to isolate pesticide residues from coextracted substances. Column chromatography prior to GLC can be used to separate compounds with conflicting retention times. Chemical methods may be used to aid in differentiating between compounds with conflicting retention times, for example, by decomposing one and leaving the other unchanged, changing them chemically, or making derivatives. Thin-layer or paper chromatography may yield information helpful in establishing the identity of a compound shown to be present by GLC. In addition to these techniques, any spray history of the sample may provide valuable information for the analyst.

Since the number of chlorinated pesticides that could conceivably be present as residues is quite large, the analyst should have access to retention data on the compounds he is likely to encounter. Retention times vary with slight changes in column temperature and gas flow rate. They are not precisely reproducible from one column to another, even with the same type of packing. When related to the retention time of a reference compound and expressed as a relative value, however, retention "times" are reproducible from column to column (with the same type of packing) and do not change markedly with slight variations in column temperature or flow rate. GLC retention times of chlorinated pesticides expressed as relative values have previously been reported (4). A tabulation of retention times relative to the retention time of aldrin obtained when using a 6 ft × 4.5 mm i.d. column packed with 20% DC Hi Vac silicone grease on 30/60 mesh Chromosorb P in the MCGC system has proved very useful as an aid to qualitative analysis of pesticide residues and in research investigations. The values reported originally have been found to be valid, for the column and conditions specified, in more than 3 years of use. The more efficient GLC column described above —10% DC 200 on 80/90 mesh Anakrom ABS, 6 ft × 4.5 mm i.d.—does not change the elution sequence of the compounds, but the retention times and their relative values are different. Table 1 lists the retention times relative to aldrin of 87 chlorinated pesticide chemicals. Table 2 lists the retention times relative to sulphenone of 26 thio pesticide chemicals. These values were calculated from retention times measured from the point of injection. Although neither of these tabulations is a complete listing of the chlorinated and thio pesticide chemicals, they should aid the analyst in making a tentative identification.

The microcoulometric detection systems, halogen or sulfur, are specific for the respective atoms. Responses caused by other types of compounds have not been obtained. Microcoulometric GLC analysis is often used to check residues indicated to be present by GLC and less specific but very sensitive electron capture detection.

Occasionally nonhalogenated solvents contain materials that cause a response, e.g., a small peak at the beginning of the chromatogram. This may be eliminated by venting during the first minute after injection. Redistilled petroleum ether and acetonitrile are

Table 1. Relative retention times of 87 chlorinated pesticides

Column: 10% DC 200 (12,500 cst) on 80/90 Anakrom ABS, 6 ft × 4.5 mm i.d.

Column temp., 210°C; flow rate, 120 ml/min.; injection temp., 240°C

Detector: Microcoulometric (halogen)

Pesticide	Relative Retention Time
Dichloral urea	0.15
Trithion, thiol-sulfoxide	0.15, 1.78, 1.89
Ethylene dichloride	0.16
Chlorpicrin	0.17
Trithion, sulfoxide	0.17, 1.78, 2.46, *4.54*[a]
Ethylene dibromide	0.18
p-Dichlorobenzene	0.18
Monuron	0.18
Diuron	0.19
Neburon	0.19
Ethide	0.19
DDT (tech.)	0.35, 1.69, 2.21, *2.70*[a]
TCNB	0.36
Methyl ester, 2,4-D	0.36
CIPC	0.37
Vegadex	0.44
BHC (tech.)	*0.44*, 0.52[a]
Isopropyl ester, 2,4-D	0.44
Simazine	0.45
α-BHC	0.46
Propazine	0.47
Hexachlorobenzene	0.49
β-BHC	0.50
Methoxychlor (tech.)	0.50, 2.87, *3.90*[a]
Lindane	0.52
PCNB	0.54
δ-BHC	0.55
Dichlone	0.57
Pentachlorophenol	0.60
Tetraiodoethylene[b]	0.63
Isobutyl ester, 2,4-D	0.63
Isopropyl ester, 2,4,5-T	0.65
n-Butyl ester, 2,4-D	0.69
Ronnel	0.77
Heptachlor	0.81
Chlorthion	0.99
Kelthane (tech.)	*0.99*, 3.82[a]
Aldrin[c]	1.00
n-Butyl ester, 2,4,5-T	1.05
Telodrin	1.09
o,p'-DDD olefin	1.12

(Continued)

Table 1. (*Continued*)

Pesticide	Relative Retention Time
Dyrene	*1.15*, 1.38, 1.61, 1.87, 2.23[a]
Captan	1.16
Sulphenone	1.19
Heptachlor epoxide	1.20
Chlorbenside	1.25
p,p'-DDD olefin	1.34
Propylene glycol butyl ether ester, 2,4-D	1.34, 3.12
γ-Chlordane	1.35
Perthane olefin	1.35
o,p'-DDE	1.37
Thiodan	*1.44*, 1.91[a]
Ovex	1.46
β-Chlordane	1.46
Methyl ester, *p,p'*-DDA	1.48
Butoxy ethanol ester, 2,4-D	1.50
p,p'-DDE	1.69
Iso-octyl ester, 2,4-D	1.70, *1.98*[a]
Dieldrin	1.72
o,p'-DDD	1.72
Aramite	1.76, 1.84
Endrin	*1.90*, 2.09[a]
Ethyl hexyl ester, 2,4-D	1.77
Trithion, thiol	1.89
Trithion, thiol-sulfone	1.89, 2.54, *3.23*[a]
Perthane	1.91
Chlorbenzilate	2.07
Dilan	2.05, 2.40, *2.86*[a]
p,p'-DDD	2.12
o,p'-DDT	2.21
Iso-octyl ester, 2,4,5-T	2.28, *2.62*, *3.08*, 3.60[a]
Butoxy ethanol ester, 2,4,5-T	2.32
Kepone	2.35
Prolan	2.40
Bulan	2.40, *2.89*[a]
Trithion	2.44
p,p'-Methoxychlor olefin	2.52
p,p'-DDT	2.70
p,p'-Methoxychlor	3.90
Tedion	4.22
Trithion, sulfone	4.30
Chlordane (tech.)	0.52, 0.66, 0.74, 0.82, 0.96, 1.14, *1.36*, 1.50, 2.3[a]
Strobane	0.91, 1.07, 1.24, 1.44, 1.63, *2.07*, 2.35, 2.66, 3.17, 3.77[a]
Toxaphene	1.29, 1.45, 1.55, 1.87, *2.17*, 2.45, *2.85*, *3.36*, 4.03, 4.52[a]
BEP ester, 2,4,5-T (tech.)	0.66, 1.06, 2.32, 2.72, 3.85, *5.23*[a]
BEP ester, 2,4-D (tech.)	0.71, 1.21, 1.53, 1.81, *3.51*, 8.5[a]
Co-Ral	7.6

Chlorinated phenoxy acids and their salts do not chromatograph.

(*Continued*)

[a] When more than one peak is present, the major peak(s) is italicized.

[b] Not chlorinated.

[c] Aldrin reference: retention time approximately 5.5 minutes.

used in the extraction and cleanup methods to keep solvent peaks to a minimum. Petroleum ether extracts of polyethylene have been found to contain significant quantities of several halogenated compounds that chromatograph and cause a coulometric response which would interfere with an analysis. Plastic containers for solvents should not be used in the residue laboratory.

Sensitivity and Quantitative Analysis

The detector response (peak area) to a given quantity of a chlorinated or thio pesticide depends on the amount of chlorine or sulfur in the molecule and on the efficiency with which the compound passes through the chromatographic system. Response cannot always be predicted on the basis of chlorine or sulfur content alone. As previously mentioned, efficient chromatography of several compounds may not be achieved until the column is well conditioned. Table 3 lists the approximate quantity of pesticide necessary to give a one-half scale recorder deflection at a sensitivity of 64 ohms. These data were obtained from a well conditioned column operating at maximum efficiency. Detector sensitivity can be doubled (128 ohms) without an appreciable increase in the noise level; the 256 ohm sensitivity (4 times as sensitive as 64 ohms) can frequently be used depending on the stability of the individual MCGC system. Rarely can the 512 ohm sensitivity be effectively utilized. The 128 ohm sensitivity is used in most of our work.

The MCGC system described here (T-200 series titration cell and column of 10% DC 200 silicone fluid on 80/90 mesh Anakrom ABS) gives about a twofold increase in effective sensitivity over the older system (T-100 series titration cell and 20% DC Hi Vac silicone grease or DC 200 silicone fluid on 30/60 mesh Chromosorb P). The column presently used elutes the pesticides in more

Table 2. Relative retention times of 26 thio pesticides

Column: 10% DC 200 (12,500 cst) on 80/90 Anakrom ABS, 6 ft × 4.5 mm i.d.

Column temp., 210°C; flow rate, 120 ml/min.; injection temp., 240°C

Detector: Microcoulometric (sulfur)

Pesticide	Relative Retention Time
Trithion, sulfoxide	0.13, 1.52, 1.61, 2.10, *3.90*[a]
Trithion, thiol	0.13, *1.61*[a]
Ethion	0.16, *1.83*[a]
Thiono Systox	0.27
Systox	0.27, *0.37*[a]
Delnav	*0.41*, 0.67[a]
Diazinon	0.44
Dimethoate	0.51
Methyl parathion	0.60
Ronnel	0.65
Malathion	0.71
Trithion, thiol-sulfone	0.72, 1.61, *2.77*[a, c]
Parathion	0.78
Chlorthion	0.81
Captan	0.95
Sulphenone[b]	1.00
Ovex	1.23
Thiodan	*1.23*, 1.64[a]
Aramite	1.48, 1.58
Trithion, thiol-sulfoxide	1.53, 1.62, 2.03[a, c]
Trithion	2.10
EPN	3.15
Trithion, sulfone	3.62
Tedion	3.67
Guthion	3.75
Co-Ral	6.55

[a] When more than one peak is present, the major peak(s) is italicized.

[b] Sulphenone reference: retention time, approximately 5.9 minutes.

[c] Large response immediately after injection.

Table 3. Approximate sensitivity of GLC with microcoulometric detection to chlorinated and thio pesticides

Column: 10% DC 200 (12,500 cst) on 80/90 Anakrom ABS, 6 ft × 4.5 mm i.d.

Column temp., 210°C; flow rate, 120 ml/min.; injection temp., 240°C

Detectors: T-200-S (halogen); T-200-P (sulfur)

Detector sensitivity: 64 ohms[a]

Pesticide	Approx. Quantity (µg) for 1/2 FSD[b]
Halogen Detection	
Dichloral urea	1.5
Trithion, thiol-sulfoxide	150
Ethylene dichloride	1
Chloropicrin	1
Trithion, sulfoxide	60
Ethylene dibromide	0.75
p-Dichlorobenzene	0.75
Monuron	1.5
Diuron	2
Neburon	2
Ethide	1.5
TCNB	1
Methyl ester, 2,4-D	4
CIPC	3.5
Vegadex	4
BHC (tech.)	1.25
Isopropyl 2,4-D	1.5
Simazine	4
α-BHC	0.75
Propazine	5
Hexachlorobenzene	0.4
β-BHC	0.75
Methoxychlor (tech.)	7.5
Lindane	0.75
PCNB	0.75
δ-BHC	0.75
Dichlone	2.25
Pentachlorophenol	7.5
Tetraiodoethylene	8
Isobutyl ester, 2,4-D	2
Isopropyl ester, 2,4,5-T	2
n-Butyl ester, 2,4-D	2.5
Ronnel	2.5
Heptachlor	1
Chlorthion	2
Kelthane (tech.)	15
Aldrin	1
n-Butyl ester, 2,4,5-T	2
Telodrin	1
o,p'-DDD olefin	5
Dyrene	25
Captan	3.5
Sulphenone	8
Heptachlor epoxide	1
Chlorbenside	3.5
p,p'-DDD olefin	2
Propylene glycol butyl ether ester, 2,4-D	6
γ-Chlordane	1.5
Perthane olefin	6
o,p'-DDE	2
Thiodan	3
Ovex	5
β-Chlordane	1
Methyl ester, *p,p'*-DDA	7

(*Continued*)

Table 3. *(Continued)*

Pesticide	Approx. Quantity (μg) for 1/2 FSD[b]
Butoxy ethanol ester, 2,4-D	5
DDT (tech.)	4
p,p′-DDE	2.5
Iso-octyl ester, 2,4-D	9
Dieldrin	1.75
o,p′-DDD	2
Aramite	15
Endrin	3
Ethyl hexyl ester, 2,4-D	10
Trithion, thiol	24
Trithion, thiol-sulfone	150
Perthane	4.5
Chlorobenzilate	7
Dilan	15
p,p′-DDD	2.5
o,p′-DDT	3.5
Iso-octyl ester, 2,4,5-T	10
Butoxy ethanol ester, 2,4,5-T	5
Kepone	3
Prolan	11
Bulan	10
Trithion	8
p,p′-Methoxychlor olefin	8
p,p′-DDT	3.5
p,p′-Methoxychlor	7
Tedion	8
Trithion, sulfone	30
Chlordane (tech.)	10
Strobane	18
Toxaphene	22
BEP ester, 2,4,5-T (tech.)	30
BEP ester, 2,4-D (tech.)	18
Co-Ral	25
Sulfur Detection	
Trithion, sulfoxide	16
Trithion, thiol	4
Ethion	1
Thiono Systox	1
Systox	0.75
Delnav	4
Diazinon	1
Dimethoate	0.75
Methyl parathion	2
Ronnel	2.5
Malathion	0.75
Trithion, thiol-sulfone	60
Parathion	1.5
Chlorthion	1
Captan	6
Sulphenone	2.5
Ovex	3.5
Thiodan	10
Aramite	10
Trithion, thiol-sulfoxide	60
Trithion	3
EPN	17
Trithion, sulfone	6
Tedion	20
Guthion	20
Co-Ral	6

[a] Sensitivity can be doubled (128 ohms) without appreciable increase in noise level.
[b] Approximate quantity necessary to cause a 1/2 full scale recorder deflection.

compact chromatographic bands that result in sharper and more distinct recorded chromatographic peaks. The T-200 titration cell has a shorter time constant and lower noise level than the T-100 cell, contributing to a greater effective sensitivity.

Quantitative determinations are made by comparing the peak area of the sample (after identification) with the peak area of a standard of approximately the same quantity. Areas may be determined by automatic integration or triangulation, base × height/2. Standard solutions in a concentration range of 10–100 μg pesticide/ml iso-octane are convenient for most work.

It is difficult to assign a maximum sensitivity to the MCGC technique in terms of ppm pesticide residue. This varies with the individual pesticide, the amount of sample injected, the product from which the pesticide was extracted, the degree to which the sample has been cleaned up, and the operating efficiency and stability of the individual MCGC system. The injection system is designed to receive large-volume injection; injection volumes of 50–100 μl are common. This volume may represent the equivalent of 10–25 g of the original sample. Certain pesticides that cause a high chloride response and are eluted in narrow chromatographic bands can be determined at the 0.005 ppm level. Generally speaking, however, at levels below 0.01 ppm the determination is not made routinely.

Because a large sample injection is necessary for detection of low level residues, the purified extract must be concentrated to a small volume, generally 1 ml or less and frequently to less than ½ ml. Quite commonly the final step of the concentration is the evaporation of the solvent (to a small volume or to dryness) in a gentle stream of clean dry air or nitrogen. Experiments in our laboratory indicate that significant losses of pesticide can occur in this step, particularly at volumes below 1 ml, and that large losses occur if the solution is taken to dryness (10). Several variations in this technique of solution concentration all resulted in losses of pesticide. One method was found that would allow rapid concentration to volumes of 0.2–0.5 ml with good recovery

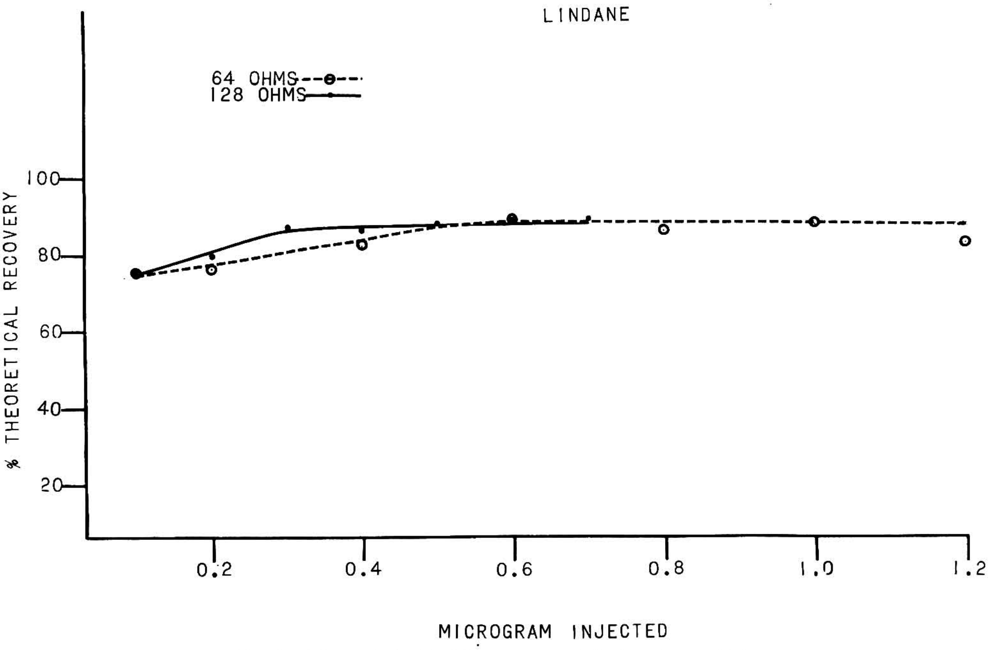

Fig. 2—Theoretical chloride recovery of lindane by MCGC.

of pesticide. According to our usual practice, a Kuderna-Danish concentrator was used in the normal manner to bring the solution volume to 5–10 ml. The calibrated collection tube was then fitted with a micro Snyder column and evaporation was continued until the desired volume was reached (11). The apparatus was allowed to cool and the solvent vapor condensate was permitted to drain back into the tube before removing the column. The minimum volume attainable with this method is about 0.2 ml. The micro Snyder column, now available commercially, is used routinely in our laboratories for this evaporation step.[1]

The coulometric titration of halogen (Cl^-, Br^-, or I^-) and SO_2 proceeds according to Faraday's law, which allows for the calculation of the quantity of halogen or sulfur being titrated (12). Knowing the quantity of the particular compound injected, its halogen or sulfur content, instrument sensitivity, recorder sensitivity, chart speed, and peak area, it is possible to determine the theoretical recovery of the material through the MCGC system. Not all pesticides pass through the MCGC system with a high percentage of theoretical chloride or sulfur recovery. However, the recoveries are constant (the system is linear) when the pesticide is present above some minimum quantity. A plot of theoretical recovery as the ordinate versus quantity injected as the abscissa will show the linear range of the instrument and the recovery level for the compound in question. Figures 2–9 are such plots for lindane, heptachlor, aldrin, heptachlor epoxide, *p,p'*-DDT, dieldrin, endrin, and toxaphene. Various quantities of these pesticides were chromatographed at two sensitivity settings, 64 ohms and 128 ohms. The sensitivity ranges, column, and operating conditions were all used as in actual residue analysis. Concentrations of standard solutions were such that the desired quantity was always injected as a 10 μl volume from a 50 μl syringe. Peak areas were determined by a Disc integrator.

Table 4 gives the average theoretical recovery obtained from 14 chlorinated pesticides and 3 sulfur-containing pesticides. These averages were determined from values taken from the constant recovery portion of the plot of theoretical recovery versus quantity injected. The averages were de-

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[1] **Micro Snyder column, sketch No. F-1284-A; Kontes Glass Co., Vineland, N.J.**

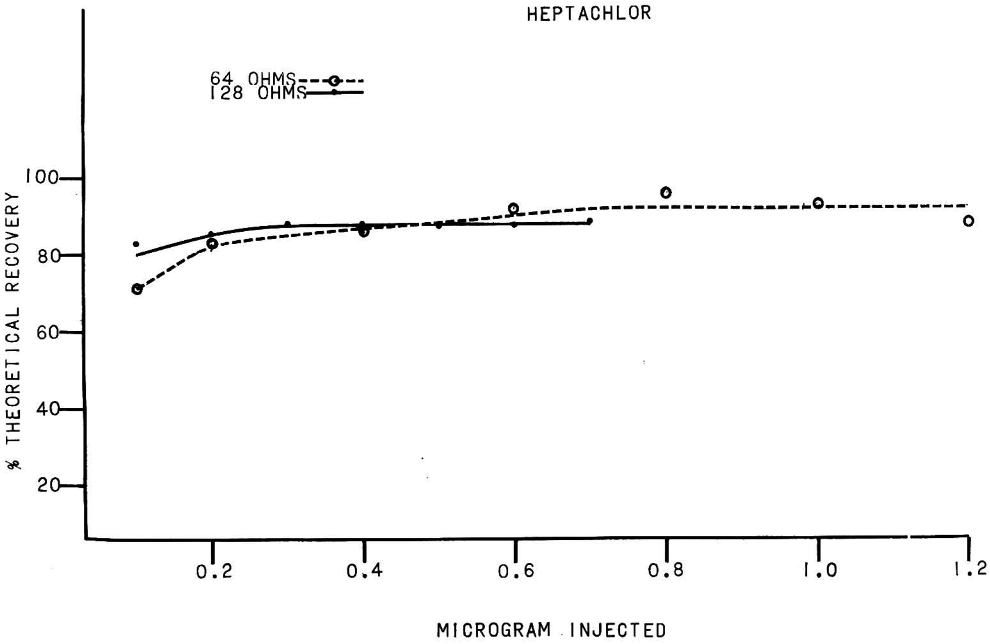

Fig. 3—**Theoretical chloride recovery of heptachlor by MCGC.**

termined on a minimum of five values within the quantity limits indicated. The lower quantity is the level below which negative deviation from linearity occurs.

Generally speaking, a linear response is obtained when quantities of pesticide present are above about 0.5 μg. Below this level there is a negative deviation from linearity. This varies with the compound; heptachlor epoxide shows a linear response down to 0.1

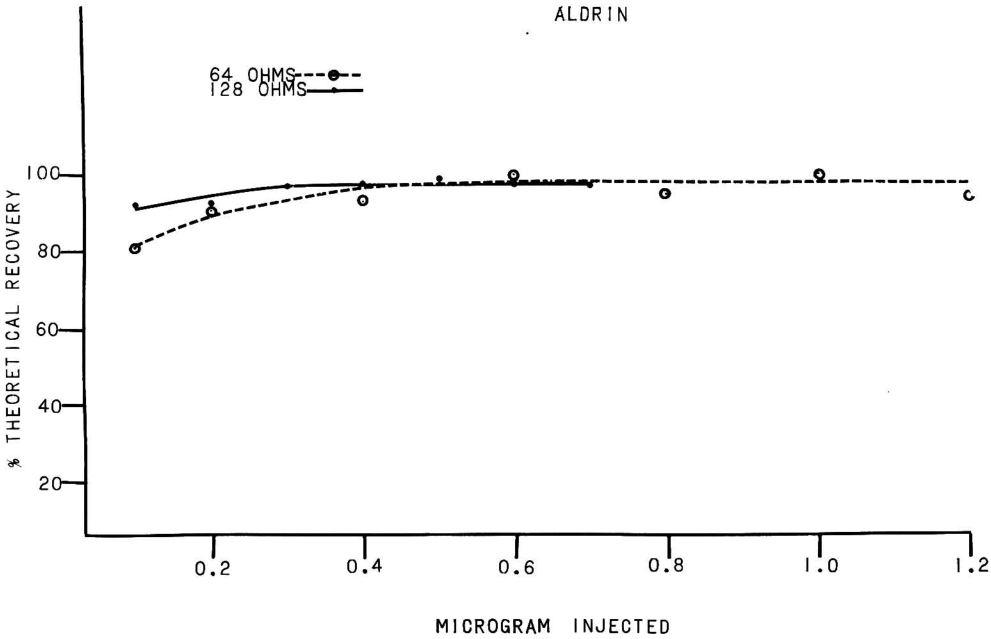

Fig. 4—**Theoretical chloride recovery of aldrin by MCGC.**

Table 4. Recovery of pesticides through MCGC system

Column: 10% DC 200 (12,500 cst) on 80/90 Anakrom ABS

Column temp., 210°C; flow rate, 120 ml/min.

	Average % Theoretical Recovery		Range (μg)[a]	
Pesticide	128 ohms	64 ohms	128 ohms	64 ohms
Chloride Detection[b]				
Lindane	88	87	0.3– 0.7	0.6– 1.2
BHC	78	80	0.4– 1.5	0.4– 3.0
Heptachlor	88	92	0.3– 0.7	0.6– 1.2
Aldrin	98	97	0.3– 0.7	0.4– 1.2
Heptachlor epoxide	97	97	0.1– 0.7	0.4– 1.2
p,p'-DDT	86	88	1.5– 3.5	2.0– 6.0
p,p'-DDD	89	91	0.4– 2.0	0.8– 4.0
p,p'-DDE	88	90	0.4– 2.0	0.8– 4.0
p,p'-Methoxychlor	78	81	1.5– 5.0	3.0–10.0
Dieldrin	95	95	0.4– 1.5	0.8– 3.0
Endrin	83	88	0.2– 1.5	1.2– 3.0
Thiodan	93	94	0.1– 2.0	0.2– 4.0
Tedion	90	88	0.2– 5.0	0.4–10.0
Toxaphene	90	95	4.0–20.0	8.0–40.0
Sulfur Detection[c]				
Thiodan	76	70	0.6– 3.0	0.8– 6.0
Tedion	90	88	0.5– 5.0	1.0–10.0
Parathion	74	71	0.4– 2.0	0.4– 4.0

[a] Average determined in this region of constant recovery. Lower quantity indicates level below which negative deviation from linearity occurs.
[b] T-200-S titration cell.
[c] T-200-P titration cell.

μg, but *p,p'*-DDT shows a deviation from linearity at about 1.5 μg. The linear region is extended to lower quantities by use of the 128 ohms sensitivity. The system is linear between these two sensitivity settings; the same quantity of pesticide causes twice the response at 128 ohms that it does at 64 ohms.

There may be a number of causes for the negative deviation from linearity; however, these will not be discussed here. It is sufficient to say that if the analyst is aware of this fact and always compares peaks to be measured with standard peaks of approximately the same size, good quantitative results will be obtained down to minimum detectable quantities.

In a similar experiment, theoretical chloride recoveries were determined on various quantities of five pesticides; lindane, heptachlor, aldrin, heptachlor epoxide, and *p,p'*-DDT. The different quantities were obtained by varying the volume injected from a 10 μl syringe. Large positive deviations from linearity were obtained with injection volumes of 1 and 2 μl, indicating "over injections." A 10 μl syringe is also difficult to manipulate during injection into the Micro Tek-Dohrmann MCGC equipment. The syringe is easily damaged and injections are difficult to reproduce. One μg amounts of the 5 pesticides were injected in volumes of 10, 25, 50, and 100 μl from a 50 and 100 μl syringe. Peak areas of all 4 curves were reproduced to within 3 or 4% of each other.

In another experiment a skilled operator made 10 injections of 10 μl, each containing 1 μg of lindane, from a 50 μl syringe. Average deviation of individual peak areas (measured by a Disc integrator) was ± 0.7% of the mean area. The maximum deviation from the average area was 3.1%.

Injection volumes of less than 10 μl should not be used in quantitative work. Volumes of 10 μl should be injected from a 50 μl syringe because a 10 μl syringe is difficult to handle properly with this injection system. Samples and standards need not be injected in the same volume but the two volumes should be more nearly equal than 100 μl for the sample and 10 μl for the standard, or vice versa. It is important that the quantity of pesticide standard used for quantitative analysis be close to the quantity of pesticide in the sample.

Cleanup

If consistently accurate and reproducible qualitative and quantitative analyses are to be made and if the MCGC is to operate continuously in an efficient and reliable manner, the GLC analysis must be preceded by a sample cleanup step. Ideally the total elimination of material coextracted with the pesticide is desirable. However, with many products, total elimination is difficult to

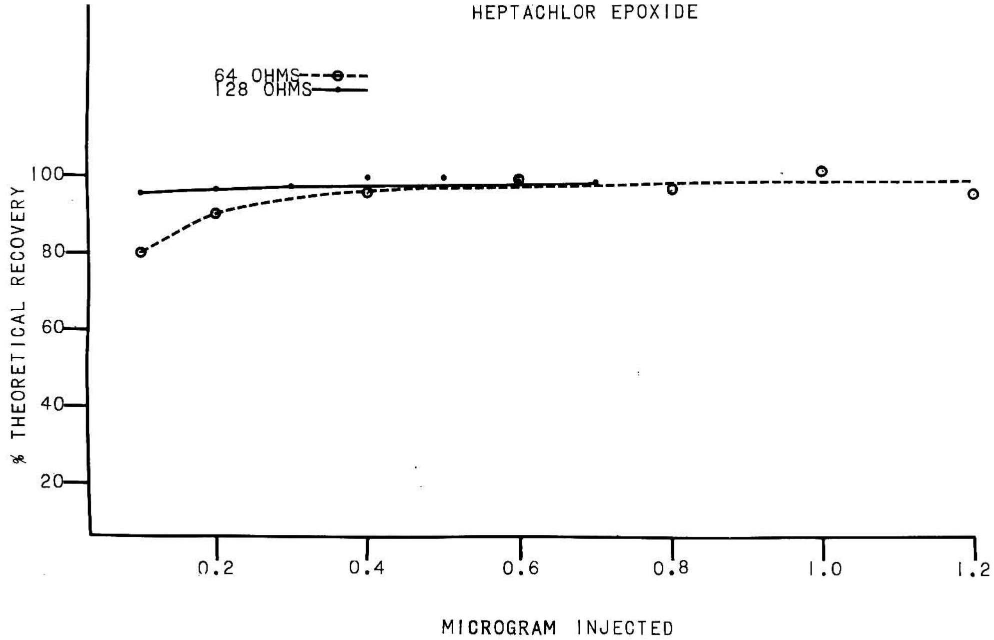

Fig. 5—Theoretical chloride recovery of heptachlor epoxide by MCGC.

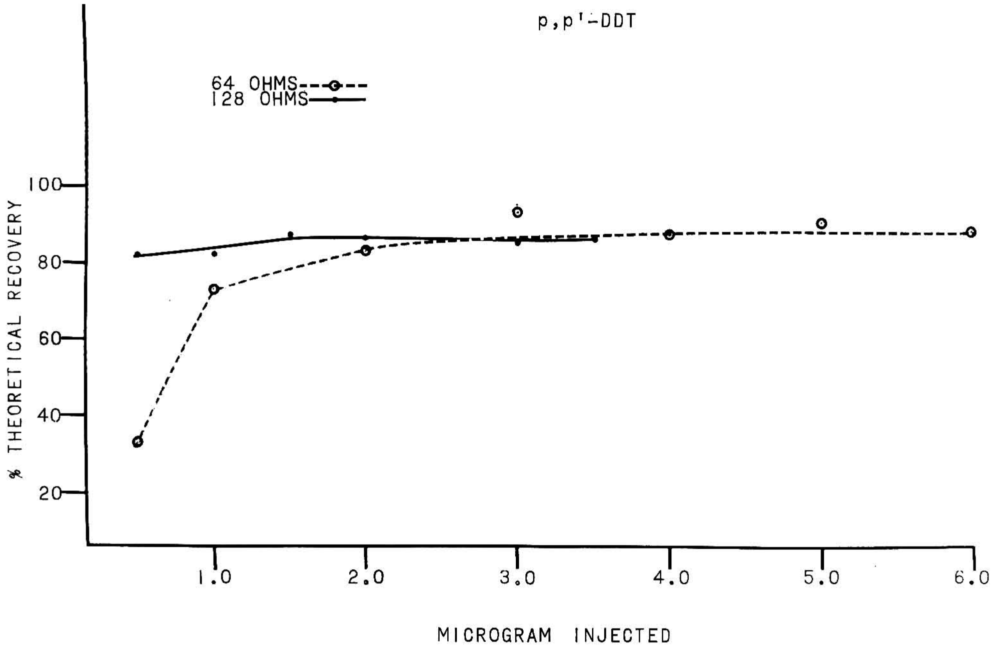

Fig. 6—Theoretical chloride recovery of *p,p'*-DDT by MCGC.

achieve and some coextracted material will remain in the final cleaned up extract. This material must be kept to a minimum because the coextracted material is deleterious to the GLC column and injections representing large sample quantities are required when determining residues at low levels.

It is sometimes difficult to thoroughly

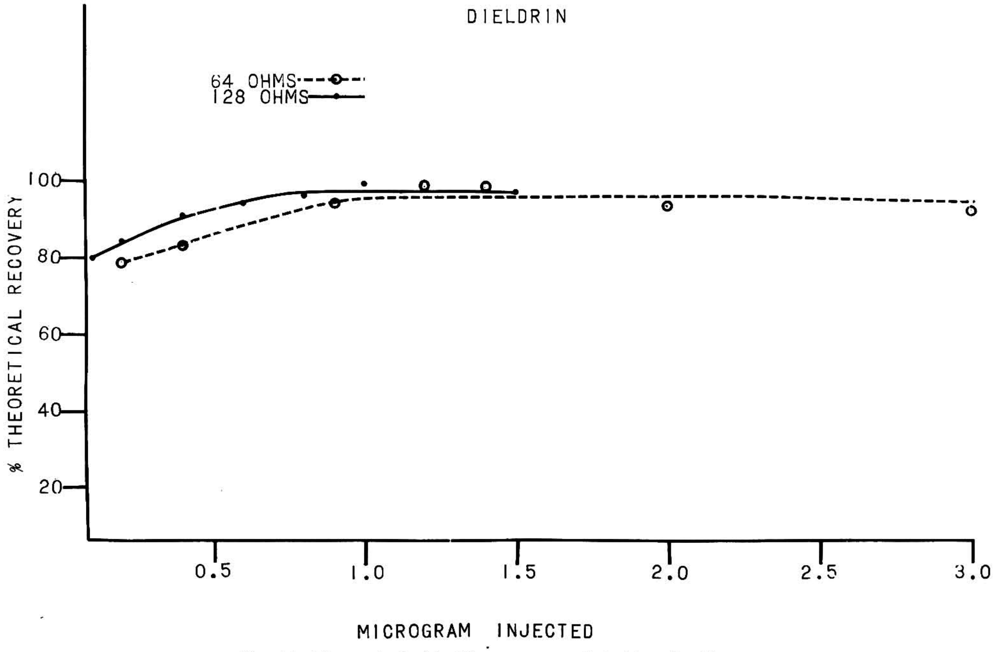

Fig. 7—Theoretical chloride recovery of dieldrin by MCGC.

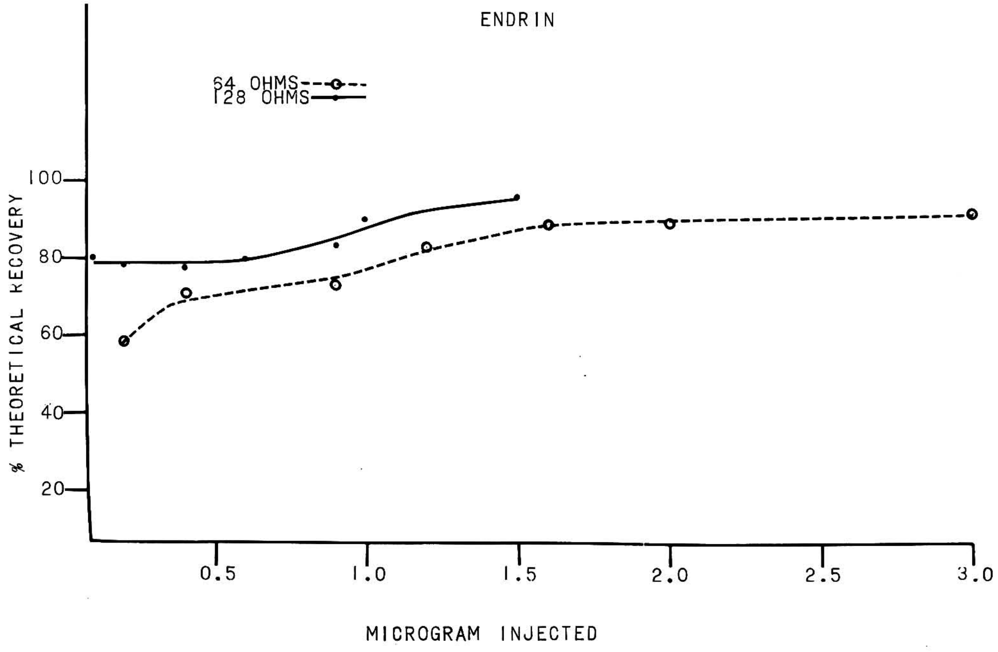

Fig. 8—Theoretical chloride recovery of endrin by MCGC.

clean up extracts of materials containing large amounts of fats or oils, e.g., dairy products, animal tissue, cottonseed meal, fish oils, etc. The oily material that may remain when such an extract is concentrated to a fraction of a milliliter will almost always cause irreversible damage if injected into a GLC column. The effect may show in a

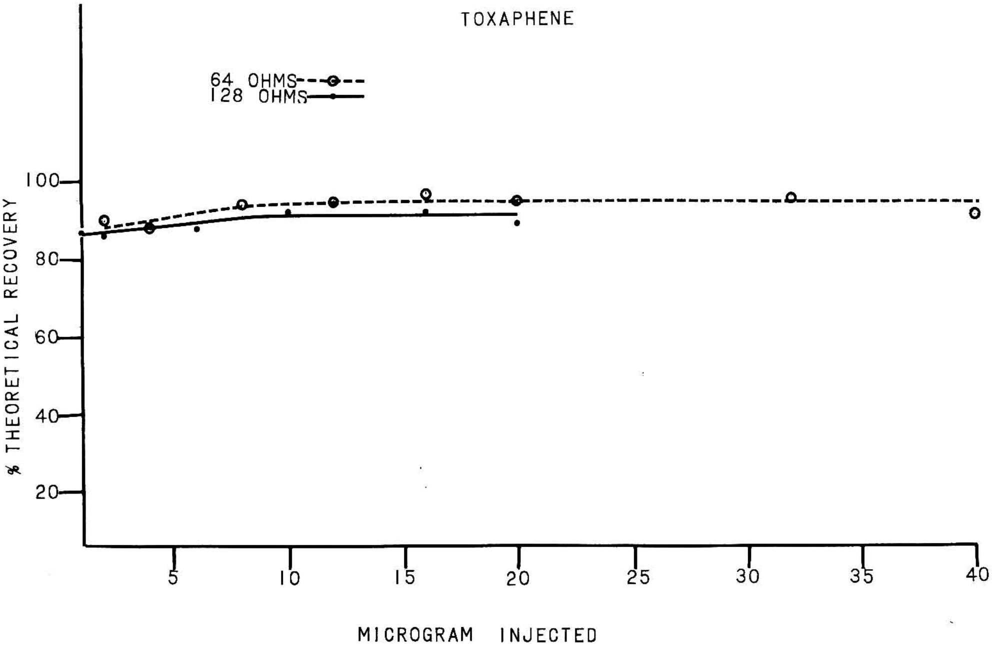

Fig. 9—**Theoretical chloride recovery of toxaphene by MCGC.**

variety of ways: degradation of certain pesticides (notably DDT), poor resolution, or low recoveries.

We believe that the degradation of *p,p'*-DDT on a GLC column which has been contaminated with this type of oily material results in the formation of *p,p'*-DDD. The formation of *p,p'*-DDD from *p,p'*-DDT, brought about by other means, has been reported (13, 14).

Waxy or colored coextracted material may also damage the GLC column; however, their effect is not so readily apparent as is that from oily material. Small amounts of colored matter (presumably carotenes) do not seem to harm the column; however, the injection of any extract that is not well cleaned up should be avoided. The user of the MCGC must determine if individual samples are suitable for analysis. In some instances, it will be necessary to reject or perform further cleanup on some samples prior to analysis.

The injection system of the Micro Tek-Dohrmann equipment is designed so that a Vycor tube may be inserted into the injection block. This tube, in which nonvolatile material accumulates, can be periodically removed for replacement or cleaning. The tube is easily cleaned by flaming off the accumulated material. This insert protects both the injection block area and the column from the damaging deposits and eliminates the troublesome need for cleaning the block. Retention of oily material is better and protection of the GLC column is increased if a cylinder of platinum gauze, 2 inches long, is placed inside the Vycor tube. We recommend the use of the Vycor tube at all times.

In our laboratories the extraction and cleanup procedures of Mills and his group are used for the preparation of a wide variety of food products for MCGC analysis for residues of chlorinated pesticides (15–23). In many cases it is not necessary to follow the Florisil column cleanup step with acetonitrile partitioning, MgO-Celite column chromatography, alkali saponification, or acid-Celite column chromatography. This decision must be based on consideration of the condition of individual samples at this point in the method.

At this time one of our MCGC units contains a very efficient column (same as described above) that has been in continuous use for approximately one year. Samples derived from a wide variety of products (including several hundred milk samples) have been analyzed. "Unclean" extracts and those which contain oily material are not chromatographed.

Small amounts of coextracted material have not been found to cause a detector response to either halogen or sulfur, except from a few crops. A small halogen response at the beginning of the chromatography has been obtained from injections of large quantities of cabbage and radish extract. A very large sulfur response has been obtained from injections of large quantities of cabbage and onion extract. A response is usually obtained at the beginning of the chromatogram when extracts of fish oil or animal tissue are injected. However, to avoid this detector response, it is general practice to vent the column effluent to atmosphere during the first 1–2 minutes of the chromatography via the system provided on the instrument. Injection of rather large weight equivalents (10–25 g) of sample causes no more than a slightly raised recorder baseline, which (if it does occur) returns to zero within about 20 minutes.

Summary

Our experience with the MCGC system for the analysis of chlorinated pesticide residues in food indicates that the specificity of the detector (for Cl^-, Br^-, and I^- or sulfur) is this instrument's greatest virtue. Because of this specificity the MCGC is quite frequently used to check for the presence of an organohalogen indicated by some other nonspecific or semi-specific means. If gas chromatography is to be consistently efficient, attention to certain detail is necessary, i.e., care in column preparation and conditioning, satisfactory removal of plant and animal extractives from samples, proper maintenance of titration cell including periodic replating of silver anodes in the halogen detector and fresh (weekly) electrolyte for the sulfur detector, and the analyst's familiarity with the equipment and the technique.

We suggest the following GLC column and operating conditions if this technique is to be used as a multiple detection system for residues of organohalogen pesticides. Column: Aluminum, 6 ft × 4.5 mm i.d., filled with 10% DC 200 silicone fluid (12,500 cst) on 80/90 mesh Anakrom ABS. The column should be formed with a minimum of bending to conform with oven dimensions and should be conditioned at least over the weekend, preferably 4–5 days. Several pesticides may not chromatograph satisfactorily until the column has been in use for about 2–3 weeks. Injections of milligram quantities of pesticides and cleaned up crop or milk extracts may enhance conditioning. Conditions are as follows: column temperature, 210°C; carrier gas flow rate, 120 ml nitrogen per min. (water-pumped or pre-purified N_2); injection temperature, 240°C; chart speed, ½ in./min.

When the instrument is operated under these conditions, the relative retention times tabulated for 87 chlorinated pesticide chemicals and 26 thio pesticide chemicals are valid (Tables 1 and 2) and should aid the analyst in establishing tentative identification.

Sample cleanup is necessary for consistently accurate and reproducible results from GLC analysis. Column life will be long (6 months to 1 year) and overall operation will be less troublesome. The methods of Mills and his coworkers (15–23) provide satisfactory extraction and cleanup of a variety of food products. The pesticides recoverable are listed in the methods.

Linearity data show that the MCGC system is linear except at quantities below about 0.5 μg of pesticide where negative deviations occur. The extent of the deviation and the level at which it begins may vary with the pesticide. The level of theoretical chloride recovery may appear low for certain compounds. Various reasons have been advanced for this behavior, but at present the cause is not certain. However, this "low" recovery does not impair the analysis (except that the sensitivity towards these materials is reduced) if the recovery level is constant, since quantitative measurements are made by comparisons with a standard. The standard should be chromatographed immediately before or after the sample (after identification) and its peak size should approximate that of the sample peak.

Maximum sensitivity (ppm) depends a great deal on the smoothness with which the particular MCGC system operates and the extent of sample cleanup. Although realizing that in many cases smaller quantities can be measured, and that the level will vary with the pesticide and food product in question, we feel that 0.01 ppm is a general level of maximum sensitivity. At lower levels we are faced with a reduction in both quantitative and qualitative accuracy.

In conclusion we reiterate our belief that the greatest need in chlorinated pesticide analysis by gas chromatography is for another column that would efficiently chromatograph a large number of pesticides in an entirely different sequence from that obtained with the silicone liquid phases now used in most laboratories.

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A Cold Bath and Stirring Assembly for Low Temperature Precipitation of Fat

By K. A. McCULLY and W. P. McKINLEY (Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada)

A cold bath and stirring assembly are illustrated for use in the precipitation of fat at a low temperature. This precipitation of fat is useful as a cleanup procedure for the determination of chlorinated pesticide residues.

Low temperature precipitation of fat has been used as a cleanup procedure in the determination of chlorinated pesticide residues in fats and oils (1–3).

Figure 1 shows diagrams of the cold bath and stirring assembly that are used in this laboratory for the precipitation of fats. Details for the various parts of the apparatus are shown in Figs. 2 and 3. A brief description of the apparatus has been reported previously (3).

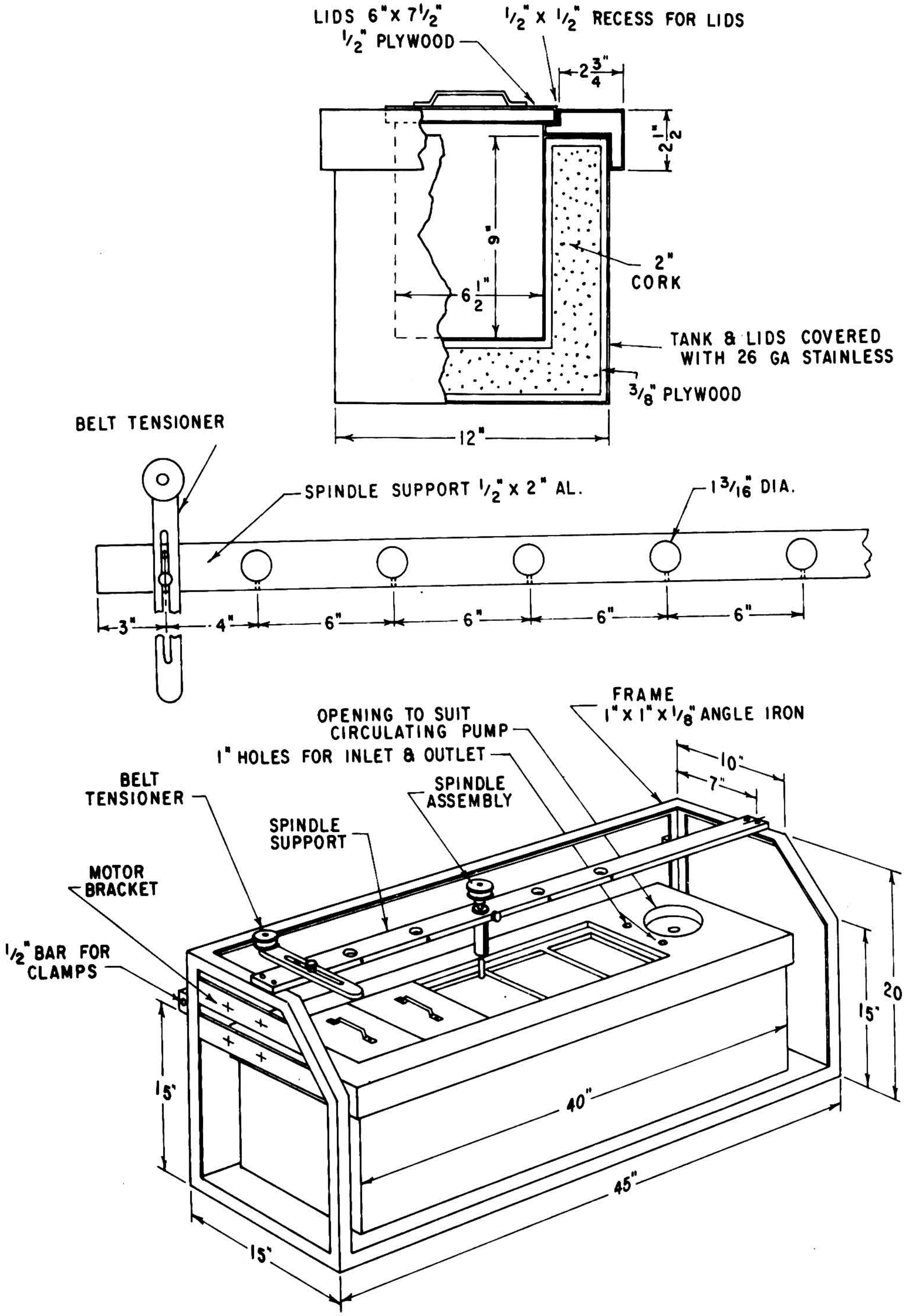

Fig. 1—Diagrams of the cold bath and stirring apparatus used for the low temperature precipitation of fat.

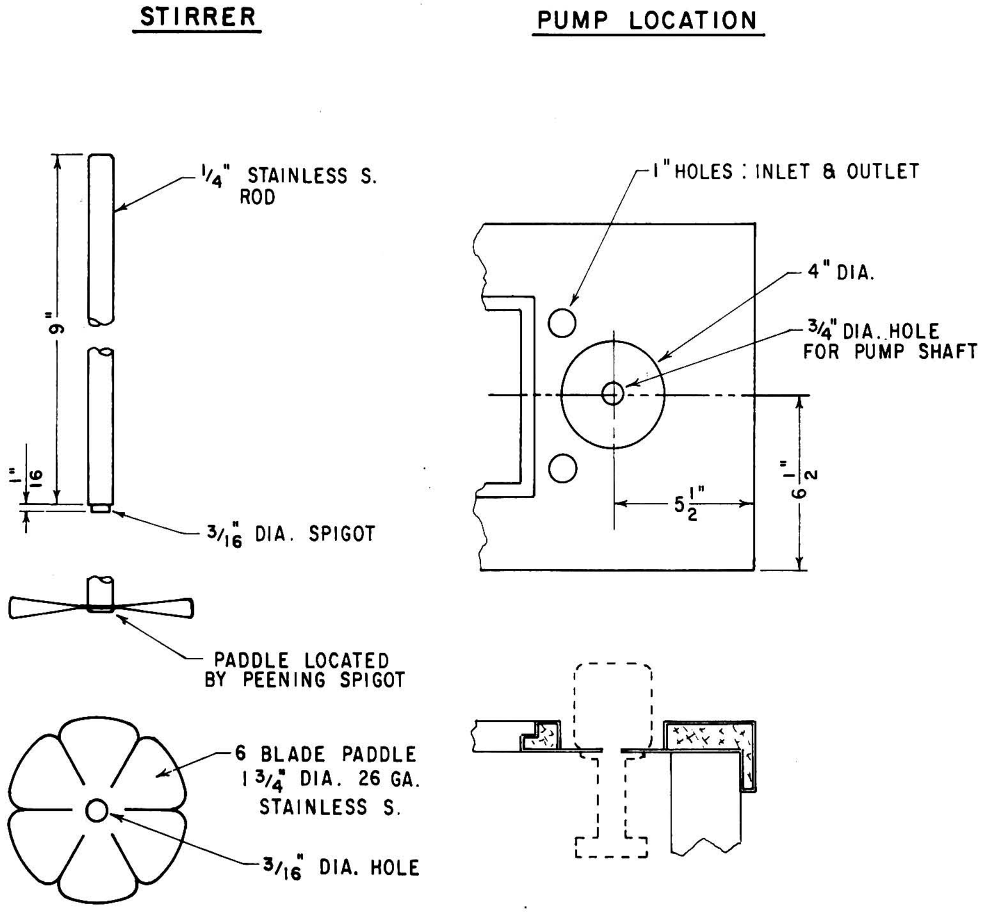

Fig. 2—Diagrams of a stainless steel stirrer and the location of the circulating pump.

The double-walled bath was constructed of 3/8″ plywood and was insulated with 2″ sheet cork (Fig. 1). The box was lined, both inside and outside, with 26 gauge sheet stainless steel. The cover of the bath was provided with openings to accommodate 5 sample flasks at one time. One end of the cover was provided with an opening to accommodate a circulating pump (Wilkens-Anderson Co., Chicago) and two 1″ holes, one for the pump outlet and the other for the return to the bath (Fig. 2).

The pump was attached with the motor above and the pump below the cover to prevent solvent vapors from gaining access to the motor. Methanol was used as the cooling liquid rather than acetone since the latter solvent attacks the pump more readily than methanol. The circulating pump is used to circulate the methanol, cooled to −70°C with Dry Ice, through the jacketed funnels which are used to remove the precipitated fat.

The funnels, 150 ml capacity with medium porosity fritted discs, were fitted with ₮ vacuum adapters.

A frame of 1 × 1 × 1/8″ angle iron was constructed around the outside of the bath. At one end of the frame a bracket was attached to hold a motor (1/8 H.P.) to drive the stirrers (Fig. 2) by means of a nylon belt. An aluminum bar 1/2 × 2″ was attached to the top of the frame to support the stirrer spindles. This bar (Fig. 1) was provided with holes to support 5 spindles and a belt tensioner (Fig. 3). A 1/2″ bar was attached to the back of the frame to hold the clamp holders. Ordinary laboratory

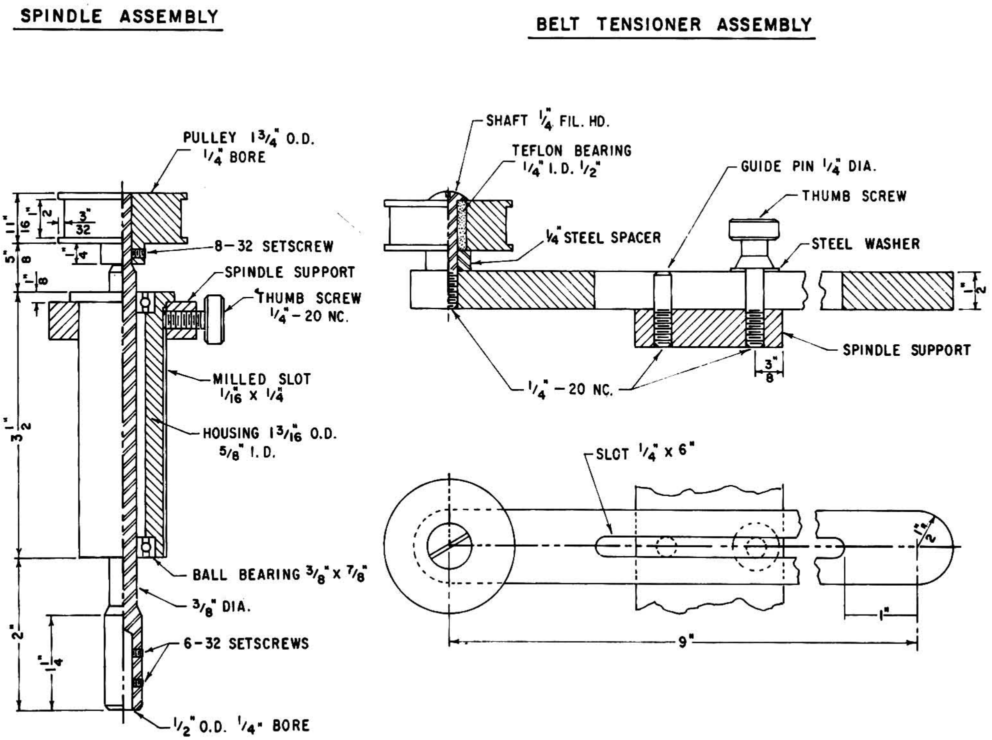

Fig. 3—**Diagrams of the spindle and belt tensioner assemblies.**

clamps and clamp holders were used to hold the sample flasks.

The apparatus has been used successfully in this laboratory for cleanup prior to analysis for 12 of the common chlorinated pesticides (lindane, heptachlor, aldrin, Telodrin, heptachlor epoxide, *p,p'*-DDE, dieldrin, *o,p'*-DDT, Rhothane, *p,p'*-DDT, endrin, methoxychlor) in a variety of fats and oils (3). Extracts from the low temperature fat precipitation procedure are suitable for analysis by gas phase chromatography with electron capture detection and by thin layer chromatography.

Acknowledgments

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Cleanup Processes for Pesticide Residue Analysis

By W. P. McKINLEY, D. E. COFFIN, and K. A. McCULLY (Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada)

At least six cleanup procedures developed for the isolation of chlorinated hydrocarbon pesticides are described. Four procedures for organophosphate pesticide cleanup in plant extracts are also given. A combination of methods is suggested for a broad scope organophosphate pesticide cleanup technique.

A pesticide residue method is not complete unless it includes with the means of detecting and measuring the residue an extraction and cleanup procedure, which should be broad enough in scope to be applicable to a wide range of biological materials and to a number of pesticides and their toxic degradation products and metabolites. The usefulness of any residue method is completely dependent on the efficiency of the extraction and cleanup stages of the procedure.

Paper chromatography is a very useful technique for the screening of samples for pesticide residues. The mobility of a compound in a paper chromatographic system is directly proportional to the partition coefficient between mobile and immobile phases. If too large a quantity of extraneous material, particularly fat or wax, is present in the extract, the partition coefficient and consequently the mobilities and resolution of the components may be altered.

Gas phase chromatographic techniques have similar problems of altered mobilities and resolution due to extraneous material. Also, these materials may adhere to the interior of certain detectors with a resultant loss in sensitivity. These problems are enhanced due to the required repetitive use of columns and detectors. Some extraneous components in the extract often alter the background level considerably and some may give responses which complicate or interfere with the interpretation of results.

The bioassay techniques which are utilized for quantitative analyses generally require rigorous cleanup procedures. Brine shrimp and mosquito larvae require aqueous media during testing. If any fat or wax remains in the extract, the fat soluble pesticides remain in the fat and do not contact the test organism. Contact testing with the housefly, fruit fly, and other insects is not as useful on extracts containing large quantities of extraneous material as when cleaned extracts are employed.

Spectrophotometric methods usually give high blanks, unless the major portion of the extraneous material is removed prior to color development. These materials interfere either by asborbing light of the wavelength used for the quantitative measurement or by reacting with the reagents to produce a product which absorbs at this wavelength. Extraneous materials may also interfere by decreasing either the production or persistence of the measurable product of the pesticide and the reagents.

Much of the chronic toxicity data is obtained by adding the pesticide to the animal's feed. If the major metabolites of the compound in question are tested for chronic toxicity, they are usually added separately to the diet. The food consumed by humans may contain several combinations of pesticides and metabolites, and there is little knowledge available concerning the effects of various combinations of pesticides and their metabolites which form on the plants or in the animals used for food. The complexity of this problem is illustrated in Fig. 1 of a previous paper, which shows the persistence of oxidative metabolites of Trithion on lettuce (1). With efficient cleanup procedures applicable to metabolites, as well as parent pesticides, the chemist could determine the quantity of each component in a food which had been treated under normal field conditions. Chronic toxicity studies on this type of treated food might give a differ-

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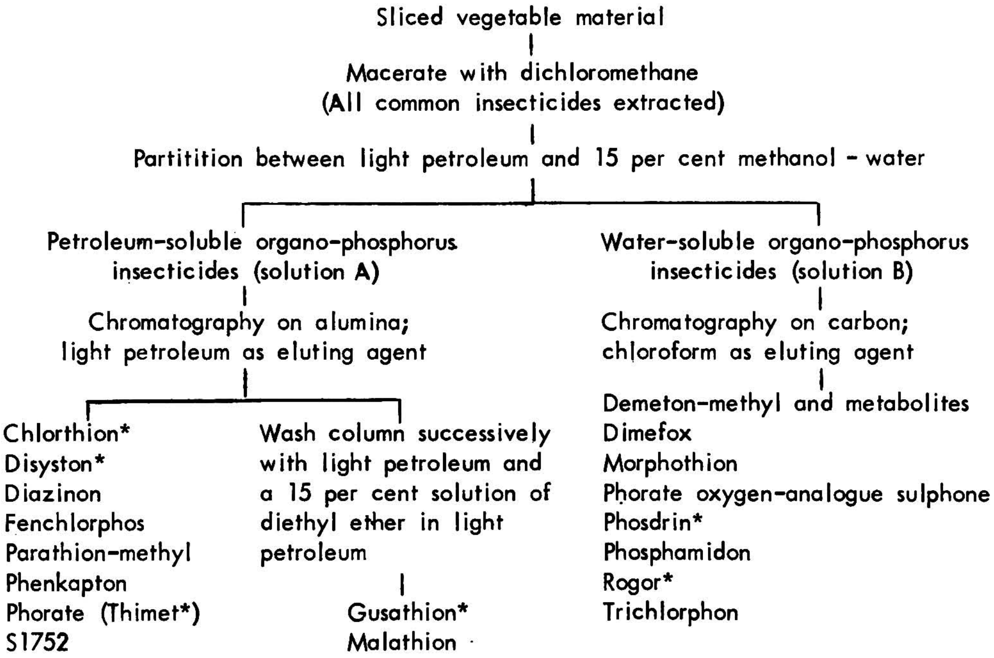
Presented to V International Pesticides Congress, London, July 1963.



* Trade name.

Fig. 1—A scheme for the extraction and cleanup of plant materials prior to the analyses of organophosphate residues (5).

ent picture for some pesticides than those obtained by the addition of the pesticide directly to the animal's feed.

The ideal cleanup procedure would be one applicable to all types of pesticides and biological extracts. Because of the complexity of the problem, there is not now, and probably never will be, such a procedure. However, there are several cleanup procedures which are applicable to a number of different pesticides and to different types of biological extracts.

PROCEDURES

1. *Saponification*

Most of the chlorinated hydrocarbon pesticides have a great affinity for fats and oils; there are no tolerances for them in materials such as milk fat. The isolation of very low levels of these chemicals from fat requires the cleanup of large samples of fat without loss of the pesticide. Analytical methods must have sufficient sensitivity to measure the amount of residue which the pharmacologist considers to be an insignificant level. If it can be assumed that 0.05 ppm of a chlorinated pesticide in the fat of milk is the pharmacologically insignificant level, the residue chemist must remove quantitatively 5 parts of the pesticide from 100,000,000 parts of fat before measuring the residue present. This can be accomplished quite readily by saponification of the fat followed by extraction of the pesticide from an aqueous solution of the soap, but for a general method there are certain limitations.

Aldrin, dieldrin, and endrin are usually stable to saponification and this procedure is used for cleanup prior to the phenyl azide colorimetric measurement of these compounds. However, not all the chlorinated pesticides are stable to the strong alkali used in saponification. The isomers of DDT are converted to the corresponding isomers of DDE and large amounts of *p,p'*-DDE cannot be resolved from dieldrin on many of the columns used in gas phase chromatography. The *p,p'* and *o,p* isomers of DDT and the metabolite *p,p'*-DDE cannot be measured separately after saponification. The *o,p*-DDE is not normally found in animal fats. Certain other chlorinated pesticides such as DDD and Perthane are also altered by saponification. With most of the paper chromatographic and spectrophotometric procedures the extraneous nonsaponifiable portion of the sample must be removed by a further cleanup technique prior to analyses, which makes the procedure long and laborious.

2. *Oxidation*

Oxidation has been used in a number of laboratories as a partial cleanup procedure. Blinn and Gunther (2) used an acidified potassium dichromate, oxidative cleanup step in a method for the determination of DDT in milk and butter extracts. Buckley and Colthurst (3) determined parathion residues in tomato extracts after peroxide treatment to destroy plant pigments. Total phosphorus and total chloride methods have been used by a number of workers after column cleanup. Chemagro Corporation (4) used a wet combustion procedure after column cleanup to destroy the remaining extraneous material and to convert the phosphorus in the pesticide to orthophosphate. Laws and Webley (5) used essentially the same wet combustion procedure after cleanup of plant material on either active carbon for the water-soluble or alumina for the petroleum ether-soluble organophosphate pesticides. One procedure used by Shell Chemical Corporation (6) for chlorinated hydrocarbon pesticides was the concentration of the eluate from a chromatographic column in chlorine-free white oil. The sample was then vaporized and burned in a stream of air. The oxidation was completed by passing the sample over quartz particles at a temperature of 950–1000°C. The combustion products were absorbed on sodium carbonate and titrated amperometrically with standard silver nitrate.

Lisk (7) modified the Schöniger (8, 9) combustion flask for the safe combustion of plant and animal tissue. This procedure has been used by Lisk and co-workers for the determination of chlorinated pesticide residues (7); mercury in apples (10); arsenic, bromide, manganese, and nickel in plant material (11); selenium in oats; and carbon in organic soils (12). The Lisk procedure has been used successfully on column eluates (1, 13) in this laboratory for the quantitative evaluation of organophosphorus pesticide residues from plant material. In this procedure eluates from columns are concentrated and subjected to paper chromatographic procedures. The area of paper occupied by the pesticide is cut out and ashed by the combustion flask procedure, and the phosphate content is determined. Total phosphorus determinations on cleaned up extracts of plant material have been much more satisfactory than anticipated. The crop blank depends on the cleanup procedure employed. In this laboratory the blank has been reduced to a level below 0.1 ppm expressed as Systox. Reagent blanks vary from laboratory to laboratory but, with care in the cleaning of the glassware and the use of wicks for ignition with a low phosphorus content, the reagent blanks should be less than 0.5 ppm expressed as Systox. This reagent blank is constant; thus, low levels of organophosphates may be measured. The use of an infrared lamp for ignition requires the use of dark papers to absorb sufficient heat for ignition. These dark papers usually contribute substantially to the reagent blank but this is not a problem as long as the blanks are constant. Dental cottons may be used, if the sample is ignited electrically (14) or with an open flame. Certain types of dental cottons contain low levels of phosphorus and they lend themselves to extraction procedures which will remove phosphorus compounds if they are present. As has been stated already, the reagent blank can be corrected readily as long as it is constant. The cleanup for the reduction of the crop blank is the most important part of this procedure.

3. *Sulfonation*

Extracts may be chromatographed through a column, consisting of a hexane slurry of 30 g of Celite, 9 ml of concentrated sulfuric acid, and 9 ml of fuming sulfuric acid (15). This column was designed to retain 5 g of fat. The technique is useful for chlordane, heptachlor, and several other pesticides but it is not applicable to all the commonly used chlorinated pesticides. The technique must be handled carefully because of the use of fuming sulfuric acid.

Schechter and co-workers (16, 17) used a method for DDT determinations which required shaking a hexane solution of rendered fat with a mixture of concentrated and fuming sulfuric acids. The hexane solution was then washed with a potassium bicarbonate-potassium permanganate solution. The potassium permanganate was used to remove the sulfur dioxide produced by the reaction of acid and fat. Whether or not this material had any effect on cleanup was not mentioned. The extract was treated with dispersed sodium to split the chlorine from the pesticide, and quantitative measurements were made by titrating the chloride with an automatic titrator. Recoveries for DDT, toxaphene, chlordane, heptachlor, and heptachlor epoxide were excellent. Lindane and methoxychlor recoveries were between 85 and 102%. Dieldrin and 2,4-D ethyl ester recoveries were 76 and 68%, respectively.

This procedure shows promise as a general cleanup technique for chlorinated hydrocarbon pesticides in fats but it should be applied to other residues and the clean extract should be analyzed by paper and gas phase chromatography to test its applicability.

4. Acetone Precipitation

Fairing and Warrington (18) used acetone at −15°C to separate methoxychlor from the unsaponifiable portion of fat and waxes. A procedure was developed in this laboratory consisting of precipitation of 100 g of fat at 5°C followed by two precipitations at −70°C (19). This removed most of the fat but the samples were not free enough from pigments and fat to allow them to be analyzed by paper chromatography. The extracts were evaporated and cleaned up further on a Florisil column; benzene was used for elution. Table 1 shows the percentage removal of the fat from a number of different types of animal fat extracts and the recovery of DDT, DDD and DDE from this column. Aldrin, dieldrin, and most other chlorinated pesticides require 0.5% acetone or acetonitrile in benzene for quantitative elution; these eluates are usually not clean enough for paper chromatography.

Table 1. Efficiency of removal of fat by acetone precipitation + florisil chromatography and recovery of added pesticides (19)

Type of Fat	Purification, %	Recovery of Added Pesticide, % DDT	DDD	DDE
Butter	99.98	91–96	80–89	100–109
Beef	99.98	91–102		
Pork	99.98	86–95		
Mutton	99.99	78–86		
Human	99.93	77–86		99–103

Partitioning of the fat between acetonitrile and *n*-hexane followed by elution with acetone at −70°C from a column of purified Darco G60 carbon and Solka Floc, a crude wood cellulose, gave a clean eluate from 50 g of butter oil (20). Eluates from this column have been analyzed by paper chromatography, by gas phase chromatography equipped with an electron capture detector, and by bioassay employing mosquito larvae without interference from fat or pigments.

Low temperature precipitation was our approach to the problem of developing a general method of analysis applicable to a large number of different types of extracts containing fats or waxes, one which allowed for the simultaneous analyses of a number of chlorinated hydrocarbon pesticides in one operation. However, the method (20) was too cumbersome for routine work.

Recently the method for fats has been streamlined (21). Continuous stirring of the fat while precipitation at −70°C was taking place allowed the volume of solvent to be reduced considerably. The use of jacketed, sintered glass filter funnels cooled to −70°C, as illustrated in that publication, prevented melting of the fat during filtration. A pad of the purified Darco G60—Solka Floc mixture in the filter allowed for a much better cleanup, and the use of a solvent consisting of 5% benzene in acetone improved the

Table 2. Purification of fats and oils and recovery of added pesticides after cleanup by precipitation at −70°C from benzene + acetone (1 + 19) and filtration at −70°C through a Darco G60-Solka Floc mixture (21)

Fat or Oil	Purification,	Recovery, %			
		Lindane	Heptachlor Epoxide	Dieldrin	p,p′-DDT
Corn oil	99.93	88	87	92	101
Cottonseed oil	99.88	89	98	97	105
Rapeseed oil	99.94	83	85	90	96
Butterfat	97.43	62	83	72	79
Margarine	99.87	87	94	92	92
Beef fat	99.93	81	86	101	103
Mutton fat	99.90	82	88	99	102

recovery of the pesticides. Table 2 shows the percentage removal of the fat and recovery of pesticides from fat and oil extracts. A gas phase chromatographic chart for chlorinated pesticides recovered from corn oil by this procedure was also given (21).

5. *Partitioning Between Immiscible Solvents*

Jones and Riddick (22) used a partitioning system of *n*-hexane and acetonitrile as a means of isolating pesticides from biological extracts. Burchfield and Storrs (23) used a system of N,N-dimethylformamide and *n*-hexane for the same purpose. The hexane removed the major portion of the fat in both of these procedures, while the pesticide remained in the other phase. In the partitioning of a fat sample, large volumes of solvent must be used or the fat becomes the predominant solvent and poor recoveries of the pesticides are obtained. This has been illustrated by Kaufman and Jackson (24) who applied the Jones and Riddick (22) procedure prior to analyses by the microcoulometric gas phase chromatograph. The recoveries for most of the commonly used pesticides ranged from 30.8% for aldrin to 56.5% for methoxychlor. The recoveries for BHC and lindane were 76 and 68.4%, respectively. Table 3 shows a comparison of the efficiency of removal of plant extractives and recovery of DDT by these two partition systems when combined with elution from a Florisil column (25). These two cleanup techniques were also compared with acetone precipitation of the waxes followed by Florisil column cleanup. There was little difference in the efficiency of cleanup among the three methods; however, the recovery of DDT from apples and lettuce illustrated the difference in recoveries of the pesticides by the three procedures. Apple extracts which were high in wax showed poor recoveries by the N,N-dimethylformamide-*n*-hexane system and only fair recoveries by the acetonitrile-*n*-hexane system. The acetone precipitation system gave good recoveries for both apple and lettuce extracts.

6. *Column Chromatography*

A. *Chlorinated Hydrocarbon Pesticides.*— The most widely used material on the North American continent as a column material for cleanup is Florisil (a synthetic magnesium silicate prepared by the Floridin Co., Tallahassee, Florida, U.S.A.). This material is activated by the producer at three different

Table 3. Removal of extraneous material from plant extracts and the recovery of DDT by three different procedures (25)

Cleanup Procedures	Apple Extract		Lettuce Extract	
	Removal (%)	Recovery (%)	Removal (%)	Recovery (%)
Acetonitrile-*n*-hexane + Florisil chromatography	99.8	63–80	99.8	92–97
N,N-dimethylformamide-*n*-hexane + Florisil chromatography	99.9	30–51	100.0	54–68
Acetone precipitation + Florisil chromatography	99.4	91–97	98.3	92–96

temperatures. Therefore, the required temperature of activation must be specified. This material was used by Ordas and co-workers (26) for the cleanup of alfalfa extracts prior to heptachlor residue analysis. It has been used widely by the laboratories of the U.S. Food and Drug Administration for several years and is their general method of cleanup for chlorinated hydrocarbon pesticides. It is now referred to as the "Mills procedure" (27). The Canadian Food and Drug Laboratories have used essentially the same procedure combined with acetonitrile-*n*-hexane partitioning for a number of years (28). Several analysts have found that different batches of Florisil have acted differently in retention of certain pesticides and extraneous fat. Moddes (29) made a study of activation conditions of Florisil and attempted to standardize the procedure of preparation of the material. Most laboratories at present obtain Florisil that has been activated at approximately 650°C (1200°F), heat it again at 130°C for 5 hours, and store it in a jar with a tight fitting cover.

Moats (30) used Florisil alone for cleanup of 2 g samples of butter fat and was able to chromatograph the eluates with 35% dimethylformamide as the immobile phase and trimethylpentane as the mobile phase. He used as much as 100 g of Florisil in his columns and eluted with methylene chloride in petroleum ether. Mills and co-workers (31) used Florisil as a cleanup for 100 g samples of food containing 2% or less of fatty constituents. The material was extracted with acetonitrile and the pesticides were partitioned into *n*-hexane after the addition of water and sodium chloride. The hexane phase was passed through a Florisil column and the pesticides eluted with 200 ml of 6% ethyl ether in petroleum ether followed by 200 ml of 15% ethyl ether in petroleum ether. Both these fractions were analyzed by gas phase chromatography using microcoulometric measurement of the chlorine which was split from the molecule after passage through the column. The pesticides eluted with 6% ethyl ether in petroleum ether could usually be analyzed by paper chromatography but those eluted with 15% ethyl ether in petroleum ether were cleaned further on a magnesium oxide-Celite column. Eluates from this column which were not free from oily materials were saponified with 2% alcoholic NaOH and the resulting material was partitioned between water and petroleum ether.

The acetone precipitation technique has been combined with Florisil columns for plant extracts using benzene for elution of DDT, DDD, DDE, and methoxychlor (25). This technique was also applicable to fat extracts containing DDT, DDD, and DDE (19). To recover dieldrin it was necessary to elute with benzene containing 0.5% acetone or acetonitrile. These solvents eluted some extraneous material which interfered on paper chromatograms. These extraneous materials could be removed efficiently on a Darco G60—Solka Floc column. This latter column has been used alone for a number of different plant materials and is being studied as a possible replacement for the Florisil column as a general cleanup technique for chlorinated pesticides. Acetonitrile extracts of most plant materials are cleaned up efficiently with this column as the only cleanup. All solvents and column materials must be purified prior to use. Hexane will elute most of the commonly used chlorinated hydrocarbon pesticides, and acetone is being investigated as an eluting solvent for the remainder of the group.

The extracts from this column have been analyzed by paper and gas phase chromatography.

B. *Organophosphate Pesticides.*—Most of the cleanup procedures described have been developed for the isolation of chlorinated hydrocarbon pesticides. The organophosphate pesticides, in general, do not appear to leave residues in animal fats; therefore the general types of cleanup procedures are for plant extracts. It is not possible to deal with all the cleanup procedures developed for individual organophosphorus pesticides, so comments will be restricted to four procedures which have been investigated by different workers as general cleanup procedures.

The wood cellulose-Darco G60 carbon column referred to earlier in this discussion has been investigated as a possible cleanup technique for the analyses of plant extracts for organophosphorus pesticide residues (32). This column, using acetone as the eluting solvent, was efficient for the removal of extraneous material from extracts of apples, lettuce, cabbage, and oranges.

The main criticism of this technique was that some compounds were converted to other compounds and some were not eluted with acetone. Of the compounds studied, Diazinon, Di-Syston, malathion, Rogor, Systox and Thimet were recovered quantitatively. Ethion, Guthion, Trithion, and Co-Ral were converted to other substances. EPN, Nemacide, and parathion were not recovered. The wood cellulose must be extracted prior to use and all solvents redistilled. This column is one of the most efficient cleanup procedures in use and is applicable to a number of organophosphorus pesticides and chlorinated hydrocarbons as discussed earlier in this article. However, before its usefulness as a general cleanup procedure is maximum, it is necessary to overcome the problem of losses and degradation of some of the organophosphorus pesticides.

Laws and Webley (5) described a procedure for the extraction of organophosphorus pesticides from plant material and for their separation into water-soluble and petroleum-soluble groups (Fig. 1). The separation into these two groups was accomplished by partitioning between light petroleum and 15% methanol in water. The water-soluble group was cleaned up on a carbon column with chloroform as the eluting solvent. The petroleum ether fraction was chromatographed on aluminum oxide, with petroleum ether as the eluting solvent. To elute malathion and Gusathion (Guthion) a mixture of 15% diethyl ether in petroleum ether was required. The quantitative recoveries were determined by measuring the total phosphorus after wet combustion. Crop blank values never exceeded the equivalent of 0.10 ppm of insecticide and the recoveries for most of the compounds from the column were quite satisfactory. There appeared to be losses of some of the compounds added to cabbage either during extraction or partitioning, or by decomposition in the presence of the plant material.

Getz (33) investigated the absorption characteristics of several charcoals and reported that Norit A, when treated with an ethanolic solution of hydrochloric acid, gave consistent results from one lot to another. The plant material was extracted with acetonitrile by the procedure of Moddes and Cook (34), and the pesticides were eluted from a column consisting of a layer of silica gel, a layer of a mixture of Celite and Norit A charcoal, and a second layer of silica gel. The pesticides were eluted with 100 ml of 25% chloroform in ethyl acetate followed by 100 ml of chloroform. Benzene was substituted for chloroform for the recovery of Guthion. The method was used successfully for the recovery of malathion, parathion, Diazinon, Guthion, Systox, and Trithion from extracts of kale. The cleaned up extracts were analyzed by paper chromatography, fly bioassay, and acetylcholinesterase inhibition.

Thornburg (35) described a procedure which was used by Hoskins and co-workers (36) for the isolation of a number of chlorinated hydrocarbon and some organophosphorus pesticides from several types of plant extracts. This procedure utilized polyethylene-coated alumina columns and aqueous acetonitrile as the eluting solvent.

The method employed (37) in our laboratory consists of extraction of the pesticides with acetonitrile and cleanup of the extract by elution from polyethylene-coated alumina with 40% acetonitrile in water, partitioning between chloroform and water, and elution from magnesol with chloroform. Parathion, methyl parathion, EPN, and their oxygen analogues were eluted, resolved by paper chromatography, and measured quantitatively, after hydrolysis to *p*-nitrophenol (38). Recoveries varied from 84 to 100% when standards were added to lettuce, apples, and strawberries. The procedure is applicable for quantities of 2–20 μg.

A total phosphorus procedure following oxidation in a Schöniger flask has been employed to study the recovery of a number of other organophosphorus pesticides (37). The following pesticides have been recovered (80–107%) from the cleanup procedure and chromatographed prior to combustion: Bayer 29492, Bayer 29493, Ciodrin, Co-Ral, DDVP, Delnav, Diazinon, Dibrom, dimethoate, Di-Syston, EPN, EPN oxon, Ethion, ethyl Guthion, Guthion, Imidan, malathion, malaoxon, menazon, meta-Systox (thiol isomer), methyl parathion, methyl paraoxon, Nemacide (VC-13), parathion, paraoxon, Phosdrin, ronnel, Ruelene, sumithion, sumioxon, Systox (thiol and thiono isomers), thiol-Systox sulfoxide, thiol-Systox sulfone, Thimet, Trithion, Trithion sulfoxide, Trithion sulfone, Trithion-oxygen analogue, Trithion-oxygen analogue sulfoxide, Trithion-oxygen analogue sulfone, Zytron. The sulfoxide of the thiol isomer of Systox and the sulfoxide of the oxygen analogue of Trithion were held on the magnesol column but the column may be eluted with acetone to remove pigments and then eluted with methanol to remove these two sulfoxides. TEPP and Schradan could not be recovered by this procedure and phosphamidon, menazon, and the oxygen analogues of dimethoate and Diazinon were eluted simultaneously with the acetone and the pigments.

A satisfactory cleanup procedure for organophosphorus pesticides with a broad scope would appear to be an acetonitrile extraction technique followed by a partitioning into the water-soluble and petroleum ether-soluble fractions as described by Laws and Webley (5). The water-soluble group may be cleaned up on a carbon column (5, 33) and a procedure utilizing polyethylene-coated alumina (37, 38) could be used for the petroleum ether-soluble group.

7. *Procedures Without Cleanup*

Regardless of the efficiency of any of the cleanup procedures used, this step in an analytical method is the most laborious, and a technique which can do away with cleanup would solve many problems. Neutron activation analysis appears to be a possible answer. The procedure has been used by Schmitt and Zweig (39) for the analyses of chlorinated pesticides in butter. The sample was subjected to a neutron flux for a definite time and then was analyzed by examining the gamma-ray spectrum with a multichannel gamma-ray spectrometer. The method was extremely sensitive but did not distinguish one chlorinated pesticide from the other.

Resolution of tissue esterases after the addition of plant extracts containing organophosphorus pesticides by electrophoresis showed promise in studies by McKinley and Read (40) and by Braux, Dormal, and Thomas (41) but, again, further study is necessary before this technique can be used routinely.

Summary

Some of the general types of cleanup techniques applicable to either a group of chlorinated hydrocarbons or organophosphorus pesticides have been discussed. There are many other cleanup procedures which are excellent for their intended purposes, but it was not possible to cover all of them in this discussion.

Collaborative studies of some of these methods is urgently needed. In order to study procedures with the broadest scope some of the existing methods should be combined prior to collaborative study. This can be done successfully only if some of the best analytical chemists in the field are given the job. The collaborative chemist must have sufficient time to do a complete and comprehensive job if the complex problem of cleanup is to be solved.

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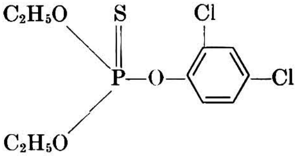
Colorimetric Determination of Nemacide and Ronnel in Animal Tissues

By H. V. CLABORN and M. C. IVEY (Entomology Research Division, U.S. Department of Agriculture, Kerrville, Texas)

A colorimetric method is described for determining Nemacide® and ronnel in the body tissues of sheep and cattle. After extraction and suitable cleanup, the insecticide is hydrolyzed and the resulting 2,4-dichlorophenol or 2,4,5-trichlorophenol is steam distilled. The phenols are reacted with 4-aminoantipyrine; the colored compound is extracted into a nitromethanepyridine mixture and the absorbance determined at 490 mμ. Recoveries of 88–95% are possible when known amounts of Nemacide are added to the tissues. The method will detect residues as small as 0.05 ppm of Nemacide, and preliminary tests indicate that it is equally sensitive for the determination of ronnel.

The development of a reliable and sensitive method for determining micro amounts of Nemacide® (*O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate) and 2,4-dichlorophenol, a likely metabolite, in the body tissues of sprayed animals was required for a residue study of Nemacide planned by the Kerrville, Texas laboratory of the Entomology Research Division, U.S. Department of Agriculture. Nemacide has the following structure:



A suitable method was developed based upon the hydrolysis of Nemacide and subsequent determination of the 2,4-dichlorophenol produced, by the method described by Gottlieb and Marsh (1). This is a colorimetric method in which the phenolic compound is reacted with 4-aminoantipyrine in an alkaline oxidizing medium to give a red compound. The method is described for Nemacide and 2,4-dichlorophenol, but it is equally applicable to the determination of ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) and 2,4,5-trichlorophenol.

The color reaction is very sensitive but is reliable only if the pH is accurately controlled. The chlorophenols are quite volatile and their solutions cannot be evaporated to dryness without excessive losses. Conversion to the sodium salts to prevent volatilization results in excess alkali, which adversely affects the subsequent color reaction. Therefore, it was found convenient to use a micro steam distillation to transfer the phenol to the buffer solution for color development. This distillation also served very effectively as a cleanup step. An apparatus used for micro Kjeldahl distillation worked remarkably well for the distillation of 2,4-dichlorophenol and 2,4,5-trichlorophenol.

The sensitivity of the color reaction was improved by extracting the colored compound with a mixture of nitromethane and pyridine. The pyridine acts as a stabilizer for the color (2). The volume of solvent may be adjusted to the size of the cuvette used.

After the initial extraction, natural substances in the tissues that interfered with the determination of Nemacide had to be removed. The steps for removal included an extraction into nitromethane, removal of the solvent, passage through a column of Attaclay, saponification, and steam distillation. Interfering substances in the phenol determination were removed from all the tissues, except fat, by steam distilling the chopped sample, collecting the phenol, and partitioning it into acetonitrile from 20% sodium hydroxide. This reaction, which was described by Elliot, *et al.* (3) as a cleanup method for *p*-nitrophenol, was found to work equally well for 2,4-dichlorophenol and 2,4,5-trichlorophenol. Fat samples were first extracted with benzene; the phenol was partitioned into 20% sodium hydroxide, and then into acetonitrile. The phenols are present in the acetonitrile as their ammonium salts. The acetonitrile was removed by distillation and evaporation, and the residue was acidified and steam distilled in the micro still.

Naturally occurring phenols do not interfere in the Nemacide procedure. The absorbance reading from a control sample is no higher than the reading from the reagents alone, which ranges from 0.062 to 0.067 units for a 16 mm light path. The cleanup procedure for 2,4-dichlorophenol in fat is less effective, and control samples give readings that range from 0.015 to 0.020 units above the reagent blank. Readings from control samples of other tissues are approximately half those from control samples of fat.

METHODS

Reagents

(a) *Solvent.*—Benzene, acetone, nitromethane, *n*-hexane, acetonitrile, and pyridine. Redistill before use.

(b) *Ethylene glycol.*—Reagent grade.

(c) *Borax solution.*—Dissolve 3.79 g borax in 100 ml water to produce a 2% solution of $Na_2B_4O_7$.

(d) *4-Aminoantipyrine.*—4.5%. Prepare daily. Weigh desired amount, dissolve in water, and add an equal weight of Norit-A charcoal. Mix and filter. Store in a dark place.

(e) *Potassium ferricyanide solution.*—13.7%. Store in an amber bottle away from bright light. Prepare fresh weekly.

(f) *Hydrolysis mixture.*—Mix 40 ml 20% KOH solution in 60 ml ethylene glycol. Prepare fresh for each use.

(g) *Adsorbent mixture.*—Mix 2 parts Celite with 3 parts Attaclay. Keep in a moisture-proof container. Calibrate a column to determine at which point the insecticide is eluted.

(h) *Nitromethane-pyridine solution.*—Dilute 10 ml pyridine to 100 ml with nitromethane. Make fresh daily.

Apparatus

(a) *Chromatographic tubes.* — Shell design; 20 mm i.d. (Scientific Glass Apparatus Co., Cat. No. JD-4030 or equivalent).

(b) *Waring Blendor.*—With 1000 ml glass jars.

(c) *Steam distillation apparatus.*—(Scientific Glass Apparatus Co., Cat. No. JD-3170 or equivalent) provided with a heating mantle and a variable autotransformer.

(d) *Centrifuge.*—Provided with 200 ml bottles and 125 ml separatory funnels.

(e) *Micro distillation apparatus.*—Kjeldahl (Scientific Glass Apparatus Co., Cat. No. JM-4250 or equivalent) provided with a variable autotransformer.

(f) *Filter tubes.*—25 × 150 mm.

(g) *Spectrophotometer.*—Beckman Model B or equivalent, with 19 × 105 mm round cuvettes.

Standard Curve for Nemacide

Prepare a standard solution of Nemacide in *n*-hexane containing 10 μg/ml. Pipet aliquots of the standard solution, ranging from 0 to 6 ml, into 200 ml Erlenmeyer flasks. Carefully evaporate the solvent; then add 10 ml of the hydrolysis mixture, and reflux for 1 hr. Add 5 ml water through the condenser, and then transfer the mixture to the micro-distillation apparatus with 12 ml water. Add 10 ml of the borax solution to a 125 ml separatory funnel calibrated at 70 ml, and place the funnel in position as a receiver. Add 3 ml of the 12*N* sulfuric acid solution to the distillation flask, immediately close the stopcock, and start the distillation. Collect 60 ml distillate at a rate of 5 ml/min., add to it 2.7 g borax, and shake to dissolve. Add 2 ml of the 4-aminoantipyrine and mix; then add 2 ml potassium ferricyanide solution, mix, and let the color develop for 5 min. Add 15 ml of the nitromethane-pyridine mixture. Shake vigorously for 1 min. Drain the nitromethane layer into a cuvette and centrifuge for 5 min. at 3000 rpm. If the solutions are turbid, place the cuvettes in hot water for 30 sec. Read at 490 mμ against a reference solution of nitromethane-pyridine. Subtract the reagent blank and plot a standard curve from the net readings. The curve follows Beer's law well beyond these concentrations.

Standard Curve for 2,4-Dichlorophenol

Prepare a standard solution of 2,4-dichlorophenol in acetone containing 10 μg/ml. Pipet aliquots of the standard solution ranging from 0 to 5 ml into 50 ml beakers. Add 5 ml 0.5*N* sodium hydroxide and evaporate the solvent by blowing a gentle stream of air over the beakers while heating on a hot plate. Transfer the residue to the micro-distillation apparatus with 22 ml water. Add 3 ml 12*N* sulfuric acid to the still, and proceed with the distillation and color development as described for Nemacide. Plot a standard curve for 2,4-dichlorophenol.

Table 1. Absorbance readings at 490 mμ for various amounts of 2,4-dichlorophenol, Nemacide, 2,4,5-trichlorophenol, and ronnel

μg	2,4-Dichlorophenol	Nemacide	2,4,5-Trichlorophenol	Ronnel
10	0.231	0.120	0.199	0.112
20	0.475	0.221	0.415	0.238
30	0.711	0.347	0.633	0.339
40	0.960	0.456	0.840	0.461
50	1.201	0.591	——	0.580
60	——	0.704	——	0.699

Absorbance readings obtained from different amounts of Nemacide, 2,4-dichlorophenol, ronnel, and 2,4,5-trichlorophenol are shown in Table 1.

Extraction and Determination of Nemacide

Fat, muscle, and heart.—Blend a 10–25 g sample in a blender with 20 g sodium sulfate and 150 ml benzene for 3 min. Transfer the mixture to a 600 ml beaker, stir in 1 g Celite, and filter through a folded filter into a 500 ml glass-stoppered Erlenmeyer flask. Wash the blender and residue with 200 ml benzene. Reduce the volume of benzene to 25 ml by distillation through a Snyder column. Remove the last traces of solvent with an aspirator while heating the solution on a steam bath.

Table 2. Per cent recoveries of 2,4-dichlorophenol, Nemacide, or ronnel added to samples of body tissues

Tissue	Nemacide[a]	2,4-Dichlorophenol[b]	Ronnel[c]
Omental fat	88	96	90
Renal fat	91	95	—
Muscle	92	83	89
Heart	95	93	87
Liver	84	90	90
Kidney	91	85	85
Brain	87	79	89
Spleen	94	79	—

[a] 50 μg added to each sample.
[b] 30 μg added to each sample.
[c] 60 μg added to each sample.

Add 50 ml nitromethane to the fatty residue and heat to boiling. Insert the glass stopper and shake vigorously, frequently releasing pressure. Decant into a 200 ml centrifuge bottle, spin 5 minutes at 3000 rpm, and chill in an ice bath to solidify the fat. Puncture the fat layer and decant the solvent through a plug of cotton into a 200 ml Erlenmeyer flask. Melt the fat in the centrifuge bottle and repeat the extraction twice, using 50 ml nitromethane previously heated to boiling in the original flask. Chill the combined extracts in a deep freeze for 1–2 hours; if a precipitate forms, filter through a plug of cotton into a 300 ml Erlenmeyer flask and wash with cold nitromethane. Remove the solvent by distillation through a Snyder column to a volume of 5 ml; then remove the last traces of nitromethane by adding and evaporating three 25 ml portions of hexane. To the dry residue, add 10 ml hexane, stopper, and hold for chromatography. Prepare a chromatographic column by adding, in order, a plug of glass wool, 10 cm adsorbent, 1 cm sodium sulfate, and a plug of glass wool. Wet the column with hexane; then transfer the sample to it with an additional 50 ml hexane. Change the receiver and elute with 130 ml hexane at the rate of 200 ml/hr. Concentrate the eluate to 5 ml by distillation through a Snyder column; then evaporate carefully to dryness. After removal of the solvent, add 10 ml of the hydrolysis mixture and proceed as described for the standard curve.

Liver, kidney, brain, and spleen.—Extract these tissues in the same manner as above, except use acetone in place of benzene. Condense the acetone extract to 60 ml by distillation through a Snyder column. Transfer to a 500 ml separatory funnel with 15 ml acetone, and then add 300 ml distilled water. Add 75 ml nitromethane, swirl gently, and let the layers separate. Filter the lower layer through a plug of cotton into a 300 ml Erlenmeyer flask. Repeat the extraction with 2 more 70 ml portions of nitromethane. Place the combined extracts in the deep freeze for 1–2 hr; if a precipitate forms, filter through a plug of cotton and wash flask and filter with cold nitromethane. Proceed from this point through the chromatographic cleanup and color development as described for fat, muscle, and heart.

Extraction and Determination of 2,4-Dichlorophenol from Tissues

Muscle, heart, liver, kidney, spleen, and brain.—Place a 10 g sample of chopped tissue in the reaction flask of the steam distillation apparatus. With the steam generator on and the receiver in place, add 20 ml 12*N* sulfuric acid to the reaction flask and quickly connect the system. Boil the acid in the reaction flask for 15 minutes with as little distillation as possible; then remove the heating mantle and increase the flow of steam until about 20 ml condensate is added to the reaction flask. Replace the heating mantle and adjust the steam flow so that 60 ml distillate is collected in 20–25 minutes. The distillate is collected in a 125 ml separatory funnel containing 10 ml 20% sodium hydroxide solution. Lower the receiver and disconnect the apparatus. Wash the distillate with ether and with *n*-hexane, and drain into a beaker. Evaporate to a volume of 35 ml by heating gently on a hot plate under a stream of dry, filtered air. Transfer to a 125 ml centrifuge separatory funnel, using 15 ml 50% sodium hydroxide to make the transfer and to increase the concentration of sodium hydroxide to 20%. Add 50 ml acetonitrile, and shake vigorously for 2 min. Centrifuge for 5 min. at 1250 rpm, discard the aqueous phase, and wash the acetonitrile with 50 ml sodium hydroxide and then with 25 ml *n*-hexane. Transfer the acetonitrile to a 150 ml beaker and evaporate the solvent almost to dryness on a hot plate under a jet of dry filtered air. Complete the evaporation with air alone. Transfer the residue to the micro-distillation apparatus with 27 ml water, place the receiver in position, add 3 ml 12*N* sulfuric acid, and start the steam distillation. Proceed as for standard curve.

Fat.—Extract a 10–25 g sample of fat with benzene by the procedure described for the extraction of Nemacide from fat. Transfer the benzene to a separatory funnel, and shake vigorously with 45 and 5 ml aliquots of 20% sodium hydroxide. Combine the sodium hydroxide extracts in a 125 ml centrifuge separatory funnel and extract with an equal volume of acetonitrile. Discard the aqueous phase. From this point follow the procedure described above for the other body tissues.

Discussion

Recoveries of Nemacide, 2,4-dichlorophenol, and ronnel from the various body tissues are shown in Table 2. The method for V-C 13 Nemacide will detect residues as small as 0.05 ppm in all tissues and the method for 2,4-dichlorophenol will detect residues down to 0.10 ppm in the fat and 0.05 ppm in the other tissues. The sensitivity of the method for residues in the fat is reduced because of the higher blank.

The method described is lengthy and time consuming. However, all of the steps used are necessary to remove interfering substances and obtain good recoveries. The use of the nitromethane-pyridine mixture to extract and stabilize the colored compound adds greatly to the sensitivity and reliability of the method. This step might be recommended as a valuable addition to any method in which the aminoantipyrine-phenol reaction is used. Although nitromethane was used in this method, if it is not available, nitroethane may be used instead.

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Procedure for Extraction and Cleanup of Plant Material Prior to Determination of Organophosphate Residues

By D. E. COFFIN, and G. SAVARY (Food and Drug Directorate, Tunney's Pasture, Ottawa, Ontario, Canada)

A procedure has been developed for the extraction and cleanup of plant samples prior to the determination of organophosphate residues. It involves acetonitrile extraction, elution of the extract from polyethylene-coated alumina with slightly acidified 40% acetonitrile solution, partitioning of recovered material from water into chloroform, and final elution of the organophosphates from Magnesol with successive portions of chloroform, acetone, and methanol.

Quantitative recoveries (80–107%) by phosphorus determination after paper chromatography of 41 organophosphate standards were obtained from the cleanup procedure. Eight others either were not completely recovered or were recovered at a stage that was not applicable to the determination of residues in plant material. Quantitative recoveries (80–100%) of 25 of these organophosphate compounds were obtained after extraction and cleanup from samples of lettuce.

Many extraction and cleanup procedures have been employed for residues of organophosphorus pesticides on plant materials. Most of them have been developed for specific purposes, and very few generally applicable procedures are available. Laws and Webley (1) outlined a scheme for extraction and cleanup of organophosphate residues in plant material. They separated the compounds into water-soluble and petroleum-soluble groups. The water-soluble group was cleaned up by elution from carbon with chloroform and the petroleum-soluble group by elution from alumina with light petroleum. Recoveries of 65–98% were obtained for 18 compounds added to cabbage. No mention was made of the applicability of the cleaned up extracts to paper chromatographic or other separation and identification techniques. Getz (2) obtained extracts suitable for paper chromatography by a procedure involving acetonitrile extraction, partitioning from water into ethyl acetate, and elution from a Norit A-Celite-silica gel column with ethyl acetate and chloroform. This technique has been shown to be applicable to subsequent separation and identification of a number of organophosphorus compounds, but no recovery data have been published. MacRae and McKinley (3) extracted plant materials with acetone and benzene, and eluted the residues from a Darco G60-Solka Floc column with acetone. The resultant extracts were suitable for paper chromatographic separation, but certain compounds were not recovered and others were altered during cleanup.

This paper describes an extraction and cleanup procedure for organophosphate residues in plant materials. Data are presented to show the recovery of 49 of these compounds from the cleanup procedure, and the recovery of 25 of these compounds from plant materials.

Materials and Methods

Chromatographic Systems

Whatman No. 1 paper, 8½ × 8½″, is used for all three systems.

(a) Immobile phase: 4% (v/v) light mineral oil in peroxide-free ether. Mobile phase: absolute ethanol + acetone + water (1+1+2 v/v).

(b) Immobile phase: 4% (v/v) light mineral oil in peroxide-free ether. Mobile phase: acetone + water (1+9 v/v).

(c) Immobile phase: 15% propylene glycol in absolute ethanol. Mobile phase: cyclohexane + benzene (6+4 v/v).

Reagents

(Redistill all solvents.)

(a) *Polyethylene coated alumina.* — (Kensington Scientific Corp., Berkeley, Calif.)

(b) *Magnesol.*—Industrial regular (Canada Colours and Chemicals Ltd., Toronto, Ontario).

(c) *Beef liver homogenate* (4).—Homogenize 1 g beef liver with 9 ml water. Freeze to store. Dilute with 30 ml water just prior to use.

(d) *Chromogenic reagent* (4).—Prepare fresh just prior to use. (*1*) Dissolve 25 mg 1-naphthylacetate in 10 ml absolute ethanol. (*2*) Dissolve 50 mg Azoene Fast Blue RR salt in 20 ml water and add 20 ml BDH buffer (pH 7.0). Add solution (*1*) to solution (*2*).

(e) *Iodoplatinate reagent* (3).—Dissolve 1 g platinic chloride in 10 ml water and add to a solution of 10 g KI in 250 ml water. Prepare the working solution by mixing 1 volume of this stock solution with 6 volumes of water.

(f) *Vanillin-sulfuric acid* (5).—Dissolve 5 g vanillin in 100 ml concentrated H_2SO_4.

(g) *Acid molybdate reagent* (6).—To 60 ml water add 10 ml 1*N* HCl, 25 ml 4% ammonium molybdate solution, and 5 ml 72% perchloric acid.

(h) *Isobutyl-benzene.*—Mix equal volumes of isobutyl alcohol and thiophene-free benzene.

(i) *Ethanolic sulfuric acid.*—Dissolve 20 ml concentrated H_2SO_4 in 480 ml absolute ethanol.

(j) *Stannous chloride.*—Dissolve 10 g $SnCl_2 \cdot 2H_2O$ in 25 ml concentrated HCl and store in a glass-stoppered brown bottle. This solution is stable for several months. Dilute 1 ml of this stock solution to 200 ml with 1.0*N* H_2SO_4 solution immediately prior to use.

(k) *p-Nitrophenol.*—Dissolve 25 mg *p*-nitrophenol in 25 ml water.

Apparatus

(a) *Flash evaporator.*—(Buchler Instruments, N.Y.)

(b) *Chromatographic columns.*—Glass tube 2.5 cm o.d. by 35 cm long, with a 150 ml reservoir at the top and a medium-sintered glass filter plate at the bottom. The plate leads into a drip tube surrounded by a 24/40 inner member of a glass joint. This outer tube is equipped with a side arm for attachment to vacuum.

(c) *Oxygen flask ignitor.* — (Arthur H. Thomas Co., Philadelphia, Pa.)

(d) *Ultraviolet light.* — Hanovia Mercury Arc, 5 amp lamp. (Hanovia Chemical Company, Newark, N.J.)

Standards

(a) *Organophosphates.* — Approximately 2 mg/ml solutions of each of the organophosphates in chloroform.

(**b**) *Phosphorus.*—Prepare from oven-dried KH_2PO_4 and dilute to give 1 μg phosphorus/ml.

Extraction and Cleanup

Homogenize a 50 g sample of plant material with 100 ml acetonitrile for 3 minutes. Centrifuge at 1500 rpm for 10 minutes and decant the supernatant through a glass wool plug. Homogenize the residue with 100 ml acetonitrile for 2 minutes, centrifuge, and decant the supernatant as for the first extraction. Evaporate the combined decantates in a flash evaporator at 35°C to completely remove the acetonitrile (complete when water droplets appear in the neck of the evaporator and frothing ceases in the distilling flask). Measure the volume of aqueous solution remaining and add enough acetonitrile to make a 40% acetonitrile solution. Add 1 ml 1*N* HCl, mix thoroughly, and transfer to a column containing 15 g polyethylene-coated alumina. Adjust the vacuum to collect the effluent at the rate of 5–10 ml per minute. Wash the distilling flask with two 20 ml portions of a solution containing 1 ml 1*N* HCl in 100 ml 40% acetonitrile solution. Add each portion to the column when the level of solution closely approaches the top of the polyethylene-coated alumina. Add 60 ml of the 40% acetonitrile solution and continue collecting the effluent until the column is dry. Remove the acetonitrile from the effluent in the flash evaporator at 35°C. Transfer the aqueous residue to a 250 ml separatory funnel. Wash the flask and extract the aqueous solution four times with 50 ml portions of chloroform. Add the chloroform extracts to a column containing a layer of 30 g anhydrous sodium sulfate over 5 g Magnesol. Attach the column to a collecting flask and to vacuum. Wash the column three times with 25 ml portions of chloroform. Evaporate the chloroform effluent in the flash evaporator at 35°C, until the volume is reduced to 1–2 ml. Transfer this solution to a 10 ml volumetric flask and dilute to 10 ml with chloroform. After chloroform elution, add 50 ml acetone to the column. Discard the acetone effluent, add 100 ml methanol, collect the methanol effluent, and evaporate to dryness at 35°C. Transfer the residue to a 10 ml volumetric flask, using small portions of chloroform.

Separation and Identification

Concentrate suitable aliquots of the cleaned up extracts under a stream of nitrogen and transfer to paper chromatograms, using chloroform to complete the transfer. Develop the chromatograms in the appropriate systems, dry, and employ one of the following procedures for identification of the organophosphates present.

(**a**) The enzymatic detection technique using beef liver homogenate, 1-naphthylacetate, and Azoene Fast Blue RR salt as described by McKinley and Johal (4).

(**b**) The iodoplatinate detection technique as described by MacRae and McKinley (3).

(**c**) Spray with vanillin-H_2SO_4 reagent and leave overnight for color development (5).

(**d**) Spray with the acid molybdate reagent, heat at 80°C for 2 minutes, and expose to ultraviolet light for 30 minutes (6).

Cut areas corresponding to the organophosphates out of the chromatograms and estimate the amount of organophosphate present by determination of total phosphorus.

Phosphorus Determination

Place 10 ml 7.5*N* nitric acid solution in a 1000 ml Schöniger flask. Wrap the sample in black paper and place in the platinum holder. Flush the flask with oxygen, insert the platinum holder, tighten a screw-clamp on the top of the flask, and ignite the sample, using an infrared lamp. After ignition, shake the flask and contents thoroughly, cool, release the screw-clamp, and transfer the contents to a 50 ml beaker, using small portions of water to rinse the flask and the platinum holder. Evaporate to approximately 0.5 ml on a hot plate, cool, add 3 drops *p*-nitrophenol indicator, and then add 7.5*N* ammonium hydroxide solution dropwise until a yellow substance appears. Add 1*N* nitric acid dropwise until the yellow substance disappears. Transfer the solution to a 10 ml glass-stoppered graduate cylinder and make the volume to 5 ml. Add 5 ml isobutylbenzene solution and 1.7 ml molybdate reagent. Stopper the graduate and shake vigorously for 15 seconds. Allow the phases to separate. Pipet a 3 ml sample of the upper isobutylbenzene layer into a 5 ml volumetric flask and wash the pipet with 1–1.5 ml ethanolic-H_2SO_4 solution, adding the washings to the 5 ml volumetric flask. Add 0.1 ml stannous chloride solution, dilute to volume with ethanolic-H_2SO_4 solution, and mix well. Read the absorbance of the solution at 625 mμ. Determine the phosphorus content of the sample by comparison with phosphorus standards containing up to 2.5 μg phosphorus and processed in the same manner as the samples.

Table 1. Common names and chemical names of organophosphorus compounds

Common or Trade Name	Chemical Name
Bayer 29492	0,0-diethyl 0-(3-methyl-4-methylthio) phenyl phosphorothioate
Bayer 29493	0,0-dimethyl 0-(3-methyl-4-methylthio) phenyl phosphorothioate
Ciodrin	0,0-dimethyl 0-(alpha-methylbenzyloxycarbonyl-1-methyl) vinyl phosphate
Co-Ral	0,0-diethyl 0-(3-chloro-4-methyl) coumarin-7-yl phosphorothioate
DDVP	0,0-dimethyl 0-2,2-dichlorovinyl phosphate
Delnav	2,3-p-dioxane S,S-bis (0,0-diethyl phosphorodithioate)
Diazinon	0,0-diethyl 0-(2-isopropyl-4-methyl) pyrimid-6-yl phosphorothioate
Diazinon-oxygen analog	0,0-diethyl 0-(2-isopropyl-4-methyl) pyrimid-6-yl phosphate
Dibrom	0,0-dimethyl 0-(1,2-dibromo-2,2-dichloro) ethyl phosphate
Dimethoate	0,0-dimethyl S-(N-methylcarbamoyl) methyl phosphorodithioate
Dimethoate-oxygen analog	0,0-dimethyl S-(N-methylcarbamoyl) methyl phosphorothioate
Dipterex	0,0-dimethyl (1-hydroxy-2,2,2-trichloro) ethyl phosphonate
Di-Syston	0,0-diethyl S-(2-ethylthio) ethyl phosphorodithioate
EPN	0,0-diethyl 0-4-nitrophenyl phenylphosphonothioate
EPN oxon	0,0-diethyl 0-4-nitrophenyl phenylphosphonate
Ethion	0,0,0′,0′-tetraethyl S,S^1-methylene bisphosphorodithioate
Guthion	0,0-dimethyl S-(4-oxo-1,2,3-benzotriazin-3-yl) methyl phosphorodithioate
Guthion-ethyl	0,0-diethyl S-(4-oxo-1,2,3,-benzotriazin-3-yl) methyl phosphorodithioate
Imidan	0,0-dimethyl S-(phthalimido) methyl phosphorodithioate
Malathion	0,0-dimethyl S-(1,2-dicarbethoxy) ethyl phosphorodithioate
Malaoxon	0,0-dimethyl S-(1,2-dicarbethoxy) ethyl phosphorothioate
Mecarbam	0,0-diethyl S-(N-ethoxycarbonyl-N-methylcarbamoyl) methyl phosphorodithioate
Menazon	0,0-dimethyl S-(4,6-diamino-s-triazin-2-yl) methyl phosphorodithioate
Metasystox I	0,0-dimethyl S-(2-ethylthio) ethyl phosphorothioate
Methyl parathion	0,0-dimethyl 0-4-nitrophenyl phosphorothioate
Methyl paraoxon	0,0-dimethyl 0-4-nitrophenyl phosphate
Nemacide VC-13	0,0-diethyl 0-2,4-dichlorophenyl phosphorothioate
Parathion	0,0-diethyl 0-4-nitrophenyl phosphorothioate
Paraoxon	0,0-diethyl 0-4-nitrophenyl phosphate
Phosdrin	0,0-dimethyl 0-(2-methoxycarbonyl-1-methyl) vinyl phosphate
Phosphamidon	0,0-dimethyl 0-(2-chloro-2-diethylcarbamoyl-1-methyl) vinyl phosphate
Ronnel	0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate
Ruelene	0-methyl 0-(2-chloro-4-tertiary butyl) phenyl N-methyl phosphoramidate
Schradan	octamethyl pyrophosphoramide
Sumithion	0,0-dimethyl 0-(3-methyl-4-nitro) phenyl phosphorothioate
Sumioxon	0,0-dimethyl 0-(3-methyl-4-nitro) phenyl phosphate
Systox-thiono isomer	0,0-diethyl 0-(2-ethylthio) ethyl phosphorothioate
Systox-thiol isomer	0,0-diethyl S-(2-ethylthio) ethyl phosphorothioate
Systox-thiol sulfoxide	0,0-diethyl S-(2-ethylsulfinyl) ethyl phosphorothioate
Systox-thiol sulfone	0,0-diethyl S-(2-ethylsulfonyl) ethyl phosphorothioate
TEPP	tetraethyl pyrophosphate
Thimet	0,0-diethyl S-(ethylthio) methyl phosphorodithioate
Trithion	0,0-diethyl S-(4-chlorophenylthio) methyl phosphorodithioate
R 1472	0,0-diethyl S-(4-chlorophenylthio) methyl phosphorothioate
R 1776	0,0-diethyl S-(4-chlorophenylsulfonyl) methyl phosphorodithioate
R 1777	0,0-diethyl S-(4-chlorophenylsulfonyl) methyl phosphorothioate
R 1988	0,0-diethyl S-(4-chlorophenylsulfinyl) methyl phosphorodithioate
R 1990	0,0-diethyl S-(4-chlorophenylsulfinyl) methyl phosphorothioate
Zytron	0-methyl 0-2,4-dichlorophenyl N-isopropylphosphoramidothioate

Results and Discussion

The waxy materials and some of the pigments from acetonitrile extracts of plant material were retained on polyethylene-coated alumina after elution with slightly acidified 40% acetonitrile solution. Other materials, especially the red pigments of ripe apples, cranberries, raspberries, and strawberries, remained in the aqueous phase during partitioning, while the organophosphorus compounds were extracted into chloroform. At this stage, sufficient materials were present in the chloroform solution to interfere with the subsequent paper chromatographic separation of the organophosphates. Chloroform elution from Magnesol removed many of the organophosphates, while most of the extraneous plant material was retained. Acetone was employed to elute the pigments and other plant material from the Magnesol prior to elution with methanol to remove certain organophosphorus compounds. The chloroform and methanol eluates were suitable for paper chromatographic separation and phosphorus determination. The acetone eluate usually contained sufficient extraneous material to interfere with paper chromatographic separation of organophosphate residues.

All organophosphate determinations were performed after paper chromatographic separation to eliminate any impurities present in the organophosphate standards and to determine if alteration of the compounds occurred during the extraction or the cleanup. The common or trade names and the corresponding chemical names of the 49 organophosphorus compounds are shown in Table 1. This table illustrates the great differences in the chemical nature of the compounds involved in this study.

A known amount of each of these 49 organophosphorus compounds was added to 50 ml of 40% acetonitrile solution and then subjected to the cleanup procedure, beginning at the addition of 1 ml 1*N* HCl to the solution. The chloroform, acetone, and methanol eluates were retained and portions of each were chromatographed in the appropriate systems. Areas corresponding to the organophosphates were cut out of the chromatograms and analyzed for total phosphorus. Data on the paper chromatographic separation, detection procedures, and recoveries of these 49 compounds from cleanup and paper chromatographic separation are shown in Table 2. The three paper chromatographic systems were selected so that R_f values of 0.10 to 0.90 could be obtained for practically all of the organophosphorus compounds. Menazon and Schradan could not be detected on paper chromatograms by the iodoplatinate or enzymatic detection techniques. Menazon appeared as an orange spot when left overnight after it had been sprayed with 5% vanillin in concentrated H_2SO_4. Schradan was identified on chromatograms only by detection of the phosphorus in the molecule.

Recoveries of 39 of these compounds ranging from 80 to 107% were obtained in the chloroform eluates. Three compounds were quantitatively recovered in the acetone and two sulfoxides in the methanol eluates. Schradan and TEPP were not recovered; Dipterex was partially recovered in the chloroform eluate; and the oxygen analog (7) of dimethoate was partially recovered in the acetone eluate. While a 93% recovery was obtained for mecarbam, it appeared in both the chloroform and acetone eluates. TEPP could not be recovered at any stage of the procedure. It would appear that Schradan was retained on the Magnesol column, since a recovery of 95% of this compound was obtained at the partition stage of the procedure. The low recoveries of Dipterex and the oxygen analog (7) of dimethoate were apparently due to unfavorable partition of these compounds between water and chloroform. Similar recoveries were obtained by extraction of aqueous solutions of these compounds with chloroform.

From the preceding results, it is evident that this procedure is not applicable to the quantitative determination of Schradan, TEPP, Dipterex, and the oxygen analog of dimethoate. Furthermore the procedure is not applicable to the measurement of residues of mecarbam, menazon, phosphamidon, and the oxygen analog of Diazinon, because the acetone eluate generally contains plant materials which interfere with paper

Table 2. Paper chromatographic characteristics and recovery of organophosphorus compounds after cleanup and paper chromatographic separation procedures

Compound	Chromatographic System[a]	Detection	Added (μg)	Recovered (%)[b]		
				Chloroform	Acetone	Methanol
Bayer 29492	a	I[c]	43.0	91 ± 5		
Bayer 29493	a	I	49.4	90 ± 2		
Ciodrin	b	E[d]	39.0	93 ± 2		
Co-Ral	a	EB[e]	62.5	94 ± 3		
DDVP	c	E	24.4	98 ± 4		
Delnav	a	I	56.9	80 ± 5		
Diazinon	a	EB	39.8	92 ± 2		
Diazinon-oxygen analog	b	E	33.7	0	95 ± 3	
Dibrom	b	E	30.5	98 ± 7		
Dimethoate	b	I	48.1	96 ± 2		
Dimethoate-oxygen analog	c	E	40.7	0	48 ± 2	0
Dipterex	c	EN[f]	34.7	42 ± 2	0	0
Di-Syston	a	I	52.8	96 ± 5		
EPN	a	EB	64.5	94 ± 5		
EPN oxon	b	E	41.9	98 ± 5		
Ethion	a	I	36.0	86 ± 2		
Guthion	b	I	76.8	86 ± 4		
Guthion-ethyl	b	EB	34.0	96 ± 5		
Imidan	b	I	42.0	97 ± 7		
Malathion	b	EB	56.1	94 ± 5		
Malaoxon	c	E	27.0	107 ± 4		
Mecarbam	b	I	45.2	34 ± 2	59 ± 2	0
Menazon	a	V[g]	36.9	0	99 ± 3	0
Metasystox I	c	I	44.0	102 ± 7		
Methyl parathion	a	EB	56.3	90 ± 4		
Methyl paraoxon	b	E	59.7	99 ± 3		
Nemacide VC-13	a	EB	37.5	80 ± 4		
Parathion	a	EB	60.4	90 ± 4		
Paraoxon	b	E	40.6	98 ± 2		
Phosdrin	c	E	20.7	96 ± 2		
Phosphamidon	c	E	47.2	0	96 ± 4	
Ronnel	a	EB	35.1	91 ± 7		
Ruelene	c	EB	30.9	98 ± 5		
Schradan	c	P[h]	35.9	0	0	0
Sumithion	b	EB	37.3	101 ± 6		
Sumioxon	b	E	32.1	90 ± 4		
Systox-thiono	a	EB	50.3	90 ± 5		
Systox-thiol	b	E	34.5	89 ± 4		
Systox-thiol sulfoxide	c	I	48.0	0	0	96 ± 4
Systox-thiol sulfone	c	E	45.6	98 ± 3		
TEPP	c	E	38.2	0	0	0
Thimet	a	I	47.7	90 ± 6		
Trithion	a	EB	58.6	81 ± 2		
R 1472	b	E	34.4	87 ± 2		
R 1776	a	EB	30.0	105 ± 2		
R 1777	b	E	62.3	99 ± 6		
R 1988	a	EB	49.0	102 ± 5		
R 1990	b	E	35.9	0	0	105 ± 4
Zytron	b	EB	32.9	102 ± 8		

[a] Designation a, b, c refers to chromatographic systems listed in *Materials and Methods*.
[b] Each value is the mean and standard deviation calculated from 4 determinations.
[c] I—iodoplatinate detection.
[d] E—enzymatic detection.
[e] EB—enzymatic detection after bromination.
[f] EN—enzymatic detection after treatment in NH_3 atmosphere (4).
[g] V—vanillin-H_2SO_4 detection.
[h] P—phosphorus detection.

chromatographic separation. It would appear that these extraction and cleanup techniques would be applicable to the determination of plant residues of the other 41 organophosphorus compounds.

A known amount of each of 25 of these

Table 3. Recovery after extraction, cleanup, and paper chromatographic separation of organophosphorus compounds added to lettuce

Compound	Added (µg)	Recovered[a]	
		µg	%
Ciodrin	39.0	35.8	92
Diazinon	39.8	35.0	88
Dimethoate	48.1	44.0	94
Di-Syston	52.8	48.9	93
EPN	64.5	56.4	87
EPN oxon	41.9	41.8	100
Ethion	36.0	29.8	83
Guthion	76.8	64.7	84
Methyl parathion	56.3	49.0	87
Methyl paraoxon	59.7	54.0	90
Nemacide VC-13	37.5	30.0	80
Parathion	60.4	52.8	87
Paraoxon	40.6	38.0	93
Phosdrin	20.7	19.2	93
Systox-thiol isomer	34.5	30.8	89
Systox-thiono isomer	50.3	45.8	91
Systox-thiol sulfoxide[b]	48.0	47.5	99
Systox-thiol sulfone	45.6	44.5	98
Thimet	47.7	40.3	85
Trithion	58.6	43.7	81
R 1472	34.4	29.7	86
R 1776	30.0	29.9	100
R 1777	62.3	60.2	96
R 1988	49.0	48.5	99
R 1990[b]	35.9	34.8	97

[a] Each value is the average of 4 determinations.

[b] Recovered in methanol eluate from magnesol.

compounds was added to lettuce immediately before extraction. Recoveries of these compounds after extraction, cleanup, and paper chromatographic separation are shown in Table 3. The 80–100% recoveries obtained illustrate the adequacy of this procedure for the extraction and recovery of organophosphate residues from plant material. Similar recoveries of some of these compounds have been obtained from apples, cabbage, cranberries, potatoes, and strawberries. The procedure has been applied to other plant materials such as apricots, carrots, celery, cherries, grapes, peaches, pears, plums, and raspberries with seemingly adequate cleanup, but no recovery data have been obtained from these materials.

Table 4. Recovery after extraction, cleanup, and paper chromatographic separation of organophosphorus compounds from lettuce as determined by two methods of analysis

Organophosphate	Added (µg)	Recovered (%)[a]	
		Measured as *p*-Nitrophenol	Measured as Phosphorus
EPN	64.5	87	87
EPN oxon	41.9	99	100
Methyl parathion	56.3	85	87
Methyl paraoxon	59.7	92	90
Parathion	60.4	87	87
Paraoxon	40.6	94	93

[a] Each value is average of 4 determinations.

The recoveries from lettuce of EPN, methyl parathion, parathion, and their oxons had previously been determined from paper chromatograms by measurement of the *p*-nitrophenol produced by alkaline hydrolysis (8). The recoveries of these compounds as determined by the two methods of analysis are shown in Table 4. Many of the compounds have been recovered at levels down to 0.1 ppm. These results indicate a very close agreement between the two methods and the adequacy of the cleanup for more than one type of determinative procedure.

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A New Colorimetric Method for Determination of Residues of Guthion and Ethyl Guthion and Their Oxygen Analogs

By J. R. W. MILES (Entomology Laboratory, Research Branch, Canada Department of Agriculture, Chatham, Ontario)

A new and rapid colorimetric method of analysis for residues of Guthion and Ethyl Guthion and their oxygen analogs is presented. It is based on the direct coupling of the Guthion series with N-(1-naphthyl) ethylenediamine dihydrochloride in the presence of acetic acid and hydrochloric acid to produce a purple solution with absorption maximum at 556 mμ. The reaction is complete in less than 5 min. and the method is sensitive to 5 μg of Guthion. The oxygen analogs are separated on a Florisil column, the parent insecticides are eluted by chloroform, and the oxygen analogs are then eluted with acetone-chloroform mixture and determined separately. Recoveries from crops ranged from 78 to 97%.

Several analytical methods have been published for assessing residues of Guthion (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3 (4*H*)-ylmethyl) phosphorodithioate). Giang and Schechter (1) hydrolyzed Guthion with acid; the formaldehyde formed was distilled and determined colorimetrically with chromotropic acid. Wollenberg and Schrader (2) described a qualitative test involving coupling with phenyl-1-naphthylamine in acid solution. This test was modified to a quantitative determination by Kawashiro and Takeuchi (3). Bates (4) utilized cathode-ray polarography. Meagher, *et al.* (5) used alkaline hydrolysis of Guthion to form anthranilic acid, which was then diazotized and coupled with N-(1-naphthyl) ethylenediamine dihydrochloride to produce a colored complex. Cox (6) described the collaborative testing of the Meagher method on a number of crops and commented on the length of the procedure. George (7) increased the sensitivity of the Meagher method by extracting the colored product into chloroform.

The object of this study was to develop a rapid and reliable colorimetric micromethod for the separate analysis of Guthion and its oxygen analog (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorothiolate) or "Oxyguthion." The rapid method of Kawashiro and Takeuchi (3) gave high chemical blanks despite attempts to purify the phenyl-1-naphthylamine. Studies conducted at this laboratory showed that Guthion and Ethyl Guthion and their oxygen analogs would couple directly with N-(1-naphthyl) ethylenediamine dihydrochloride in dilute acetic acid (containing hydrochloric acid) to give a blue-violet solution with absorption maximum at 556 mμ. This reaction was developed into the quantitative procedure described herein.

Method for Guthion Residues on Apples.—The method as described in *Preparation of Standard Curve* is applicable to chloroform extracts (tumbled) from Delicious and McIntosh apples without prior cleanup. Some plant waxes separate out when the solution is cooled after the reaction; these are filtered off before the absorbance of the clear filtrate is measured. Recoveries, compared to the standard curve, ranged from 82 to 89% for Guthion, and 87 to 91% for Oxyguthion. As there is negligible loss of these insecticides during the evaporation of the solvent, this apparent loss must be due to interference by the waxes in the color-forming reaction. The complete cleanup described below eliminates the waxes and pigments and the final colored solutions are clear, requiring no extra filtration after the reaction.

METHOD

Reagents

(**a**) *Chloroform.*— Reagent grade; or USP purified by passing through a column of Florisil.

(**b**) *Dilute acetone.*—Mix 1 volume acetone with 4 volumes of distilled H_2O.

(c) *Attaclay-Celite.*—Mix in the ratio of 2 g Attaclay to 1 g Celite 545. (Attaclay available from Minerals and Chemicals Phillipp Corp., 1141 Essex Turnpike, Menlo Park, N.J., or, in Canada, Drew Brown Ltd., 50 Titan Rd., Toronto 18, Ont. Celite 545 available from the Johns-Manville Co., 22 E. 40th St., New York 16, N.Y., or Port Credit, Ont.)

(d) *Fibra-Flo 11C.*—A Celite-asbestos filter aid available from Johns-Manville.

(e) *Florisil, 60/100 mesh.*—The Floridin Co., Tallahassee, Fla. Most batches of Florisil have been satisfactory as received from the supplier, that is, the Guthion is completely eluted by the 50 ml of chloroform as described in *Cleanup*. However, one batch of Florisil was more retentive and required the addition of 4% distilled water to bring it to the same activity as the other batches. New batches should be calibrated by eluting 50 μg analytical grade Guthion through 10 g Florisil with chloroform, collecting 10 ml fractions, and developing the color as in *Preparation of Standard Curve*. The Guthion should preferably be in the first four fractions.

(f) *Acetone-chloroform.*—Mix 1 volume acetone with 1 volume chloroform.

(g) *Dilute acetic acid.*—Mix 1 volume glacial acetic acid with 4 volumes distilled water.

(h) *Coupling reagent.*—0.5% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride in dilute acetic acid (reagent **g**). Store in refrigerator.

(i) *Hydrochloric acid.*—6*N*.

Apparatus

(a) *Beckman Model DU spectrophotometer.*—With 1.0 cm square cells.

(b) *Large test tubes.*—Quickfit MF24/3/6, 150 mm long, available from the Fisher Scientific Co.

(c) *Chromatographic tubes.*—15 mm i.d., 400 mm long, with coarse porosity disc sealed in. Special No. C4189, Scientific Glass Apparatus Co., Inc., 100 Lakewood Terrace, Bloomfield, N.J.

(d) *Filter funnel.*—Buchner type (40 mm coarse sintered glass disc) with vacuum adapter. "Home-made," similar to Scientific Glass Apparatus JM-5370, with lower joint to fit neck of 125 ml separatory funnel.

Preparation of Standard Curve

Transfer aliquots of standard solution containing 4–40 μg Guthion (or Ethyl Guthion or their oxygen analogs) in chloroform or acetone to a series of large test tubes and evaporate the solvent. Add 5 ml of the coupling reagent (**h**) and 1 ml 6*N* HCl. Mix the contents by swirling and place the test tubes in a boiling water bath for 5 min. Cool the tubes to room temperature, transfer the contents to 10 ml volumetric flasks with a few ml dilute acetic acid (**g**), and make to volume with dilute acetic acid. Measure the absorbances of the colored solutions at 556 mμ.

Extraction

Macerate a 100 g sample of crop for 3 min. in a quart size blender with 200 ml chloroform. Pour the mixture into a funnel (150 mm diameter), the stem of which is plugged with glass wool, and collect the liquid in a suitable flask or bottle. Separate this liquid mixture by pouring it into a second funnel (120 mm diameter), fitted with a 24 cm diameter filter paper and containing 100 g anhydrous sodium sulfate, and collect the clear chloroform extract in a storage bottle.

Cleanup Procedure

Transfer an aliquot of the chloroform extract to a 50 ml Erlenmeyer flask and evaporate the solvent. Take up the residue in 10 ml acetone. Add 40 ml H_2O, 1 ml concentrated HCl, and 1 g Attaclay-Celite mixture (**c**). Stir and filter (with vacuum) through the filter funnel with a few mm overlay of Fibra-Flo 11C into a 125 ml separatory funnel. Complete the transfer with two rinses of dilute acetone (**b**). Extract with 25 ml chloroform by shaking for 1 min., and drain lower layer into a 125 ml Erlenmeyer flask. Repeat the extraction with two 15 ml portions of chloroform. Evaporate the combined extracts to dryness. Take up in a few ml of chloroform and chromatograph through 10 g Florisil (**e**). Elute the Guthion (or ethyl homolog) with

Table 1. Stability of colored products from Guthion and Oxyguthion reacted as in procedure

Min. after Reaction	Absorbance at 556 mμ	
	40 μg Guthion	40 μg Oxyguthion
10	0.470	0.511
15	0.470	0.511
30	0.471	0.512
45	0.472	0.513
60	0.473	0.515
90	0.472	0.514
120	0.472	0.514

50 ml chloroform into a large test tube. Elute the oxygen analog with 50 ml of the acetone-chloroform mixture (**f**). Evaporate the solvents from the test tubes and develop the color as in *Preparation of Standard Curve*.

Results and Discussion

Accuracy

The colored solutions obtained from Guthion and Oxyguthion by this reaction are very stable, as shown in Table 1. Samples of Guthion determined as described in *Preparation of Standard Curve* agree within ± 1%. Results of the analyses of 50 μg of analytical grade Guthion carried through the complete procedure agree within ± 2%.

Recoveries

Analytical grade Guthion (50 μg) carried through the complete cleanup procedure gave recoveries from 95 to 98%. The recovery of Oxyguthion (50 μg) was from 95 to 96%. No significant improvement in recovery was obtained by increasing the number of chloroform extractions in the cleanup procedure beyond the three recommended.

If the analyst does not wish to separate the Guthion and Oxyguthion they may be eluted simultaneously from the Florisil by 50 ml of the acetone-chloroform mixture (**f**). A mixture of 25 μg of Guthion and 25 μg of Oxyguthion gave recovery of 94.5% when analyzed by using this modification.

Recoveries from fortified crop extracts ranged from 78.0 to 96.7% and are shown in Table 2. Fortification of Guthion was at the level of the Canadian tolerance for these crops. Difficulties occurred in the analysis of onions due to variable oily turbidities in the final colored solution.

Apparent Guthion and Oxyguthion, respectively, in untreated check samples were as follows: McIntosh apples, < 0.01 and 0.04 ppm; cabbage, 0.02 and 0.08 ppm; and tomatoes, 0.11 and 0.16 ppm. These crops were purchased from a supermarket and their spray histories were unknown. As Guthion might have been used in their production, the actual check values may be even lower than shown. However, these values are satisfactory for an insecticide with tolerance at the 1–2 ppm level.

Table 2. Recoveries from crops

Crop	Guthion Added (ppm)	% Recovery	Oxyguthion Added (ppm)	% Recovery
Macerated Samples				
Apples	2	80.7	1.0	95.0
Cabbage	1	78.2	1.0	81.2
Tomatoes	1	79.3	1.0	87.8
Tumbled Samples				
Delicious apples	1	91.4	—	—
	2	89.1	—	—
	3	85.8	—	—
Green tobacco	40	94.0	10	85.0
McIntosh apples	2	85.5	1	96.7
McIntosh apples	2[a]	78.0	1[b]	93.5

[a] Determined as Ethyl Guthion.
[b] Determined as Oxyethylguthion.

Sensitivity

The method is very sensitive; 7 μg yields a product with net absorbance of 0.100. Beer's law is obeyed up to 50 μg. The reaction appears to be an acid hydrolysis with simultaneous coupling of the N-(1-naphthyl) ethylenediamine, since no color is obtained if the HCl is omitted from the procedure. The reaction is specific for the benzotriazinyl group of the Guthion series of compounds. No interference occurs when milligram quantities of carbaryl, demeton, Diazinon, dimethoate, Di-Syston, EPN, malathion, parathion, phorate, schradan, or trichlorphon are carried through the procedure.

Acknowledgments

Thanks are due to W. W. Sans of this laboratory for assistance and suggestions in the development of the chromatographic separation of Guthion and its oxygen analog.

Analytical grade samples of Guthion and Ethyl Guthion and their respective oxygen analogs were supplied by Chemagro Corp., Kansas City, Mo.

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COSMETICS

Analysis of Lipsticks. II. Chromatographic Separation of Castor Oil-Oleyl Alcohol Mixtures

By FREDERICK C. GROSS and SYLVAN H. NEWBURGER (Division of Color and Cosmetic Chemistry, Food and Drug Administration, Washington, D.C. 20204)

Absorption chromatography with silica gel as the adsorbent was used to separate castor oil-oleyl alcohol mixtures, alone or in the presence of a lipstick base. Results show that oleyl alcohol can be eluted with isopropyl ether-isooctane (65+35) and castor oil with ethyl ether-isooctane (10+30). Identification was made through the infrared spectra of the residues from the eluates.

An earlier paper on lipstick analysis described the isolation and identification of either castor oil or oleyl alcohol (1). These two materials are isolated in one fraction by this procedure; however, no method was developed for the further analysis of such fractions.

In recent years, absorption chromatography with silica gel has been used for the analysis of glycerides and glycol esters (2, 3). This work suggested that the technique might also be of value in analyzing mixtures of oleyl alcohol and castor oil. The subsequent investigation in this laboratory with silica gel as an adsorbent showed that oleyl alcohol could be eluted with isopropyl ether-isooctane (65 + 35) and castor oil with ethyl ether-isooctane (70 + 30). Identification was made through the infrared spectra of the residues from the eluates.

When our investigation was almost complete, an interesting paper by Rosen (4) was published. This paper described a general method for the chromatographic separation of nonionic surface-active agents and related materials. Dr. Rosen, however, did not investigate the problem of castor oil-oleyl alcohol mixtures.

METHOD

Reagents

(a) *Isopropyl ether.*—(Alcohol-free), b.p. 67–69°C. Treat to remove peroxides by passing the ether through a column of alumina in a ¾″ i.d. glass chromatograph tube with a constricted tip. Eighty g alumina (Merck #71707) will purify about 700 ml isopropyl ether. Use the isopropyl ether on the same day it is purified.

(b) *Silica gel.*—Davison Grade 922.

(c) *"Isooctane" (2,2,4-trimethylpentane).*—Eastman #2396.

(d) *Ethyl ether.*—Anhydrous (reagent grade). If ether contains peroxides, treat as described in (a) for isopropyl ether.

(e) *Benzene.*—(Thiophene-free). Reagent grade.

Chromatographic Equipment

(a) Glass chromatograph tube 24″ long by ¾″ i.d. with an ungreased stopcock, preferably Teflon, and a constricted tip 3″ long by 3/16″ i.d. Insert glass wool plug in bottom of column and in the tip.

(b) Brass tamper with diameter a little smaller than that of chromatograph tube.

(c) Pressure gauge, source of air pressure, rubber tubing, glass elbow, and rubber stopper to fit glass chromatograph tube.

Determination

Thoroughly mix 25 g silica gel with 15 ml benzene and add to the glass chromatograph tube in about three equal portions. Tamp each portion well before adding the succeeding one.

Transfer a sample, not exceeding 200 mg, to the column, using a total of 25 ml benzene for dissolving and transferring the sample.

Elute the column with 200 ml isopropyl ether-isooctane (65 + 35) at a rate of 4–5 ml/min., regulating the flow by air pressure. Collect the eluate in a tared 250 ml beaker. Change receivers (another tared beaker) and continue to elute with 25 ml more of the same solvent mix. Reserve these two eluates containing any oleyl alcohol which may be present; label them Eluate 1.

Continue to elute the column with 200 ml ethyl ether-isooctane (70 + 30) at a rate of 4–5 ml/min., regulating the flow by air pressure. Collect the eluate in a tared 250 ml beaker. Change receivers (another tared beaker) and elute with 25 ml additional solvent mix. Reserve these two eluates containing any castor oil which may be present; label them Eluate 2.

Evaporate Eluate 1 and Eluate 2 on the steam bath under a gentle stream of air. Dry the residues at 105°C in an oven, cool, and weigh. The residues of Eluate 1 are oleyl alcohol; those of Eluate 2 are castor oil. Obtain IR film spectra of these residues and compare them with standard IR film spectra of castor oil and oleyl alcohol.

Results and Discussion

The analyses of a number of known mixtures of castor oil and oleyl alcohol are presented in Table 1. The results represent only the first 200 ml of each eluate, as the residues from the additional 25 ml eluates were insignificant. Note that when the oleyl alcohol used in the experiments was chromatographed separately, 1.5% appeared in the ethyl ether-isooctane eluate. An infrared spectrum of this fraction indicated the presence of some unknown impurity in the oleyl alcohol. When castor oil, in turn, was chromatographed separately, 0.5–1.0% of the sample appeared in the isopropyl ether-isooctane eluate. It is surmised that this fraction is a minor component of the complex mixture that comprises castor oil.

Qualitative examination of the infrared spectra of all the castor oil eluate residues fails to show any cross-contamination with oleyl alcohol. However, the spectrum of oleyl alcohol is such that small percentages of oleyl alcohol would not be observed in the presence of castor oil.

The infrared spectra of the oleyl alcohol residues from the 90% alcohol mixture indi-

Table 1. Analysis of mixtures of oleyl alcohol and castor oil (9 experiments)

	1	2	3	4	5	6	7	8	9
				Oleyl Alcohol					
Sample composition, g	0.095[a]	0.094	0.164	0.021	0.037	0.201	0	0	0.021
Recovery, g	0.090	0.092	0.159	0.025	0.037	0.192	0.001[d]	0.002[d]	0.020
%	95	98	97	119	100	96	—	—	95
				Castor Oil					
Sample composition, g	0.081[b]	0.115	0.037	0.191	0.188	0	0.195	0.192	0.176
Recovery, g	0.082	0.111	0.034	0.183	0.181	0.003[c]	0.183	0.186	0.172
%	101	97	92	96	96	—	94	97	98

[a] Oleyl alcohol: A technical grade of oleyl alcohol was distilled under reduced pressure. The fraction boiling at 170–200°C (15–20 mm) was used in the investigation.

[b] Castor oil: For experiment numbers 8 and 9 a USP castor oil was used. In all other experiments the castor oil was a product of B. R. Elk & Co., Inc.

[c] Residue from oleyl alcohol recovered in ethyl ether-isooctane (70 + 30).

[d] Residue from castor oil recovered in isopropyl ether-isooctane (65 + 35).

cated no cross-contamination; those from the 50% mixtures indicated only a trace contaminant; and those from the 10% alcohol mixtures clearly indicated the presence of glycerides.

Table 2. Analysis of lipstick base containing oleyl alcohol and USP castor oil (3 experiments)

	1	2	3
Oleyl Alcohol			
Sample composition, g	0.012	0.130	0.062
Recovery, g	0.014	0.127	0.056
%	117	98	90
Castor Oil			
Sample composition, g	0.124	0.016	0.064
Recovery, g	0.116	0.019	0.059
%	94	119	92
Lipstick Base			
Sample composition, g	0.128[a]	0.146	0.127
Recovery, g	0.125	0.143	0.129
%	98	98	102

[a] Lipstick base contains the following ingredients:

Beeswax USP White Snowflake Brand	2.65 g
Paraffin Wax USP (Med.)—Fisher Scientific	2.51 g
Ceresin Wax (white)—J. T. Baker	2.66 g
Candelilla Wax Pure Refined—International Wax	2.51 g
Carnauba Wax W-30 Yellow—Fisher Scientific	2.52 g

Table 2 presents the results obtained from a lipstick base containing both oleyl alcohol and castor oil. An initial separation of the oleyl alcohol-castor oil mixture was made by the procedure described in *This Journal,* **41**, 435 (1958). However, the reagents used in this investigation required that the ratio of *n*-heptane to Celite be changed from 9 ml/10 g to 7 ml/10 g. Of the 1.2 mg blank associated with this procedure, 0.6 mg was arbitrarily subtracted from both the oleyl alcohol and castor oil residues.

The infrared spectra of the recovered fractions (Table 2) were similar to those described for the analysis of oleyl alcohol-castor oil mixtures (Table 1).

Two lipstick base samples were analyzed on silane-treated Celite columns in the usual manner (1) except that 14 ml of *n*-heptane was used instead of 18 ml. Samples of 0.1269 g and 0.1266 g gave isopropyl ether extracts of 5.2 and 5.3 mg, respectively. The 5.3 mg residue was eluted from the silica gel column, giving a residue of 4.1 mg in the first eluate, isopropyl ether-isooctane (65 + 35), and 2.2 mg in the second eluate, ethyl ether-isooctane (70 + 30).

Commercial lipsticks will undoubtedly contribute similar residues from the lipstick base. Unfortunately, with a lipstick of unknown composition, there is no procedure for determining this residue. The recoveries listed in Table 2 for oleyl alcohol and castor oil include these residues.

Conclusions

Although not completely quantitative, this method has been satisfactorily employed in analyzing mixtures of castor oil and oleyl alcohol both alone and in the presence of a typical lipstick base.

It is probable that similar techniques could be applied to the analysis of other mixtures of oxygenated compounds.

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FOOD ADDITIVES

Application of Thin-Layer Chromatography to the Quantitative Estimation of Antioxidants: BHA, BHT, PG, and NDGA

By MADHU R. SAHASRABUDHE (Food and Drug Directorate, Tunney's Pasture, Ottawa, Ontario, Canada)

A procedure is described for the quantitative estimation of BHA, BHT, PG, and NDGA in lard. The antioxidants are extracted from *n*-hexane solutions of the fat with 80% ethyl alcohol and acetonitrile, separated by two-dimensional thin-layer chromatography using benzene and acetonitrile, and estimated quantitatively by colorimetric methods after extraction of the spots. A special flask for concentration and a suction tube assembly for spot pickup are described. Recoveries of the antioxidants from lard were in the following ranges: BHA, 98–101%; BHT, 96–101%; PG, 90–101%; NDGA, 82–98%. The advantages of the method over others in use are that (*1*) all four antioxidants can be estimated in one aliquot of fat, (*2*) PG and NDGA can be estimated when present together in mixtures, and (*3*) the isomers of BHA can be effectively separated.

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and nordihydroguaiaretic acid (NDGA) are the four antioxidants most commonly used in foods. Anglin, Mahon, and Chapman (1) described methods which have been widely used for quantitative estimation of the above antioxidants in mixtures. In their method, PG and NDGA were extracted with 72% ethyl alcohol from fat dissolved in petroleum ether or carbon tetrachloride, while BHA and BHT were steam distilled. The only limitation of the method was that PG and NDGA could not be estimated in mixtures.

Lundberg and Halvorson (2) reported that a number of antioxidants including BHA, PG, and NDGA could be quantitatively recovered from fat by extraction with 80% ethyl alcohol. Mahon and Chapman (3) found 72% alcohol more suitable for the extraction of BHA, PG, and NDGA since it excludes tocopherol. Under these conditions BHT is only partially extracted. Our preliminary experiments indicated that acetonitrile effectively removed BHT from a glyceride oil in *n*-hexane solutions.

Qualitative separation of the antioxidants by thin-layer chromatography (TLC) has been used successfully for pure antioxidant mixtures (4, 5). However, lipid materials associated with antioxidants interfere in the determination. Quantitative estimation by TLC depends upon (*1*) effective extraction of the antioxidants from the fat, (*2*) separation of the antioxidants by TLC, and (*3*) quantitative recovery from TLC plates.

The present paper describes a method for determination of BHA, BHT, propyl gallate, and NDGA in all combinations.

METHODS

Apparatus

As TLC requires concentrated solutions for effective application, it is desirable to evaporate the solvent. A special evaporation flask was devised for the purpose (Fig. 1 A).

For the recovery of the antioxidants from TLC plates a suction tube assembly was devised (Fig. 1 B).

Reagents

(a) *Silica Gel-G.*—(According to Stahl.)

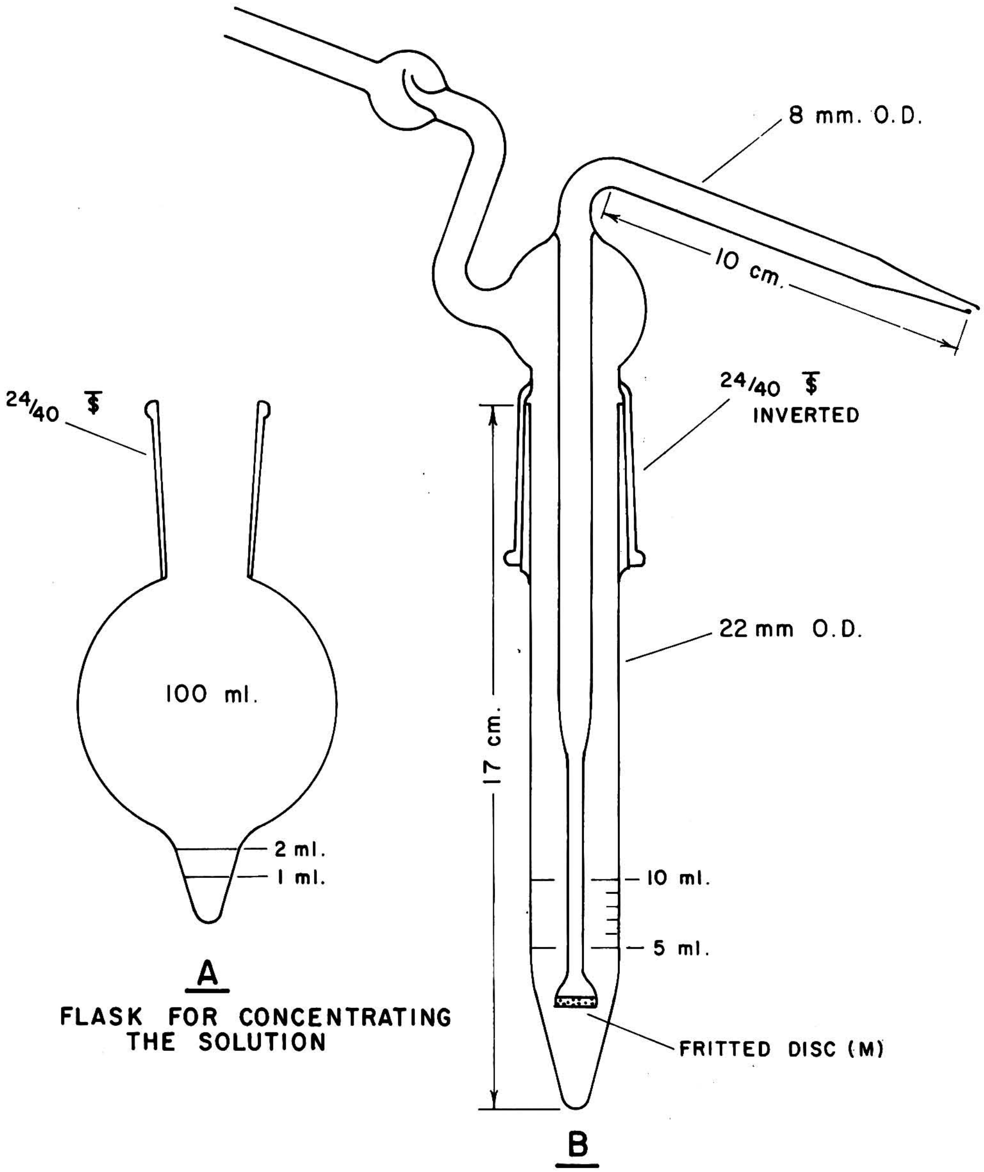
(b) *Benzene.*—Reagent grade.

(c) *Acetonitrile.*—Freshly distilled.

(d) *2,6-Dichloroquinonechloroimide* (CQC).—0.1% in absolute ethyl alcohol spray reagent.

(e) *Ethyl alcohol.*—Distill commercial absolute alcohol in an all-glass apparatus with 0.1% KOH and 0.1% $KMnO_4$. Take the distillate as 100% and dilute to 80%, 50%, and 25% (v/v).

(f) *Ferrous sulfate.*—0.04% $FeSO_4.7H_2O$ in distilled water. (Prepare daily.)



A FLASK FOR CONCENTRATING THE SOLUTION

B SUCTION ASSEMBLY FOR SPOT PICK-UP

Fig. 1—A, evaporation flask for concentration of the antioxidant solutions. B, suction assembly for spot pickup.

(g) *Ferric chloride.*—0.2% $FeCl_3.6H_2O$ in distilled water.

(h) *Sodium carbonate-bicarbonate buffer.*—5.3% anhydrous sodium carbonate and 4.2% sodium bicarbonate in distilled water.

(i) *2,2′-Bipyridine reagent (BiP).*—Dissolve 200 mg 2,2′-bipyridine in 1 ml ethyl alcohol and dilute to 100 ml with distilled water. If 2,2′-bipyridine is discolored and sticky, it should be purified as described by Mahon and Chapman (3).

(j) *2,6-Dichloroquinonechloroimide (CQC) reagent.*—Dissolve 10 mg CQC in 100 ml ethyl alcohol. Prepare fresh daily.

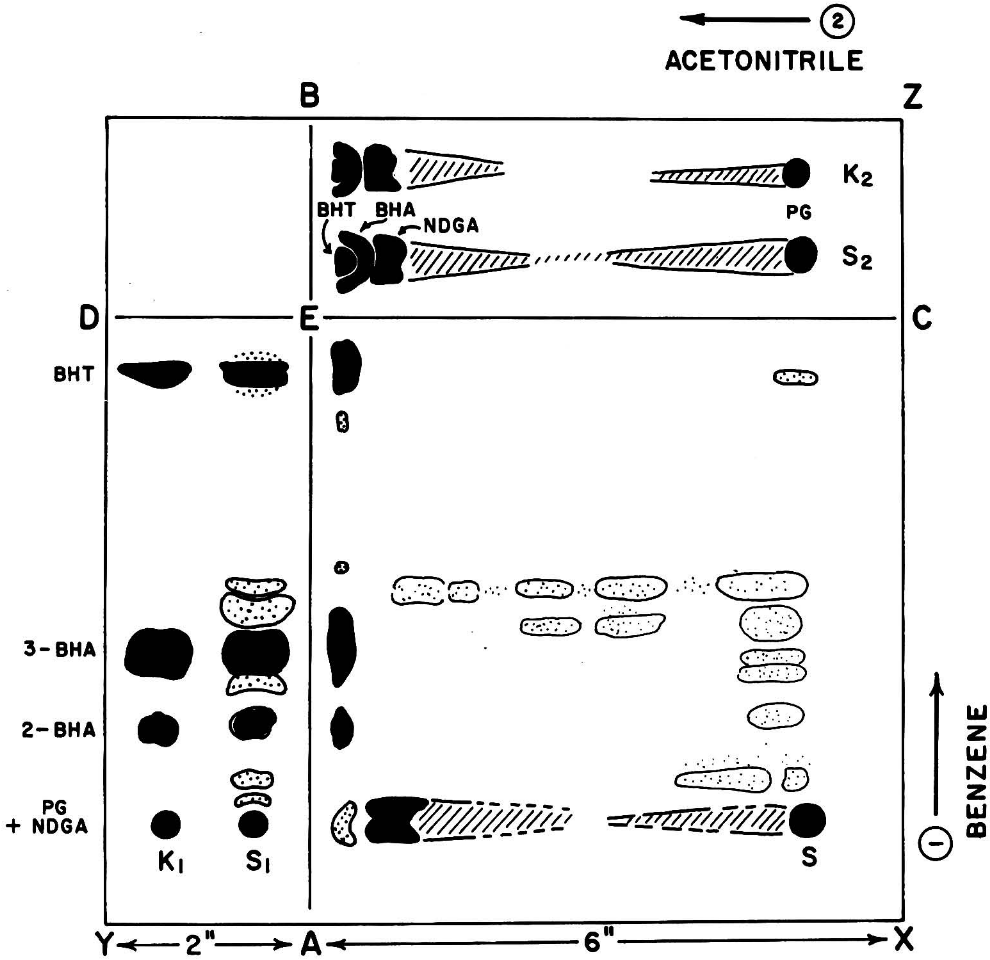


Fig. 2—Diagram of a TLC plate drawn from an actual tracing. Solid black spots are the antioxidants. Streaks obtained with PG and NDGA are shown as shaded areas. Dotted spots and areas represent lipid material. These spots become visible in iodine vapor. In the method, BHA and BHT spots in the area XAEC are not visible. On spraying with CQC the antioxidants are seen as different colored spots, viz., BHA, blue; BHT, yellow; NDGA, purple; and PG, greyish purple.

(k) *Borax buffer*.—2% $Na_2B_4O_7.10\ H_2O$ in distilled water.

(l) *Ammonium acetate*.—2.5% in distilled water.

Determination

Extraction of antioxidants.—Dissolve 10 g lard in 100 ml *n*-hexane in a separatory funnel; extract with four 25 ml portions of 80% ethyl alcohol by inverting the funnel 5 times for each extraction. Then, extract with eight 25 ml portions of acetonitrile in the same manner. After each extraction, transfer the clear lower layer to the evaporation flask (Fig. 1 A) and remove the solvent on a flash evaporator. During evaporation the flask is partially immersed in a water bath maintained at 40 ± 2°C.

When all the solvent is removed, dissolve the residue carefully in a few drops of absolute ethyl alcohol. Care should be taken to wash the sides of the flask. Bring final volume to 2 ml in the graduated projection of the flask.

Thin-layer chromatography.—Any equipment for the preparation of 8 × 8″ TLC plates coated with Silica Gel-G (250 μ) is suitable.

Mark off the glass plates which are coated with Silica Gel-G as shown in Fig. 2. Apply 20, 40, and 60 μl of the sample on separate plates at S and S_1. A 50 μl syringe is suitable. Spot a standard mixture of the antioxidants on each of these plates at K_1 and develop in

Table 1. Outline of the procedure for colorimetric determinations of antioxidants

Procedure	3-BHA	2-BHA	BHT	PG	NDGA
1. Place 5 ml solvent in suction tube	50% alcohol	50% alcohol	100% alcohol	50% alcohol	25% alcohol
2. Wash tube with 2 ml solvent	50% alcohol	50% alcohol	25% alcohol	2.5% NH_4 acetate	25% alcohol
3. Make up volume to 10 ml	50% alcohol	50% alcohol	water	2.5% NH_4 acetate	25% alcohol
4. Add reagents to develop color	2 ml Borax 2 ml CQC	2 ml BiP 2 ml $FeCl_3$	2 ml BiP 2 ml $FeCl_3$	1 ml $FeSO_4$	1 ml $FeSO_4$ 1 ml carb.-bicarb. buffer
5. Reaction time	15 min.	30 min.	30 min. IN DARK	10 min.	10 min.
6. Add *n*-butanol	5 ml	5 ml	5 ml	—	—
7. Read absorbance on a suitable spectrophotometer or colorimeter	620 mμ	515 mμ	522 mμ	515 mμ	515 mμ

benzene until the solvent front is about 2 cm from the line CD. After air drying, apply 50 μl sample and standard mixture at S_2 and K_2, respectively. Develop the plates in acetonitrile and again air dry. Cover the area XAEC with paper and spray the exposed surfaces, AEDY and CEBZ, with CQC reagent. Locate the antioxidant spots in the area XAEC with reference to the standards.

Recovery of antioxidants from the TLC plate.—Place 5 ml appropriate solvent (Table 1) in the tube (Fig. 1 B) and apply suction. By properly positioning the tip end, suck the silicic acid layer into the inner tube. To obtain a consistent area of the silicic acid layer, cut a plastic sheet into rings with an inner diameter of 1″. Place the ring on the plate to enclose the area of the spot and pick up all silicic acid inside the area. Immerse the tip in 2 ml of the appropriate solvent to wash the silicic acid onto the fritted disc. Dissolve the antioxidant by applying mild suction alternately on the tip and suction ends. (Five alternate applications of suction were found to be sufficient.) Remove the inner tube and treat the antioxidant solution with appropriate reagents for *Colorimetric analysis.* Rinse the inner tube carefully with up to 1 ml of appropriate solvent.

Colorimetric analysis.—The methods were essentially the same as described by Anglin, Mahon, and Chapman (1). A Beckman DU spectrophotometer was used in this study. The analytical procedure is outlined in Table 1.

Calculations.—Standard *K* values (absorbance per microgram of the antioxidant): *K* values for each antioxidant were calculated by spotting 20, 40, and 60 μg of the individual antioxidant on a Silica Gel-G plate. The spots were extracted and developed in the manner described earlier. This was considered necessary, as some of the reactions involved are influenced by the pH of the medium (6). However, recoveries of the antioxidants from the plate as compared to direct estimation were in the range of 98–100%. The respective concentrations of the antioxidant in the aliquot of fat used are obtained by dividing the observed absorbance by the *K* value.

Example (BHA):

Sample (fat):	10 g	Absorbance (BHA)	0.108
Final volume in evaporation flask:	2 ml	K BHA 620:	0.0043
Spot application (TLC):	0.05 ml		

$$\% \text{ BHA} = \frac{0.108 \times 2 \times 100}{0.0043 \times 0.05 \times 10 \times 10^6} = 0.01004$$

Table 2. Recovery of antioxidants

Antioxidant Added to Lard, %		Antioxidant Found, %	Recovery, %
		Individual	
BHA[a]	0.005	0.0044 (3-BHA) + 0.0006 (2-BHA)	100
	0.010	0.0091 (3-BHA) + 0.0010 (2-BHA)	101
	0.020	0.0180 (3-BHA) + 0.0017 (2-BHA)	98
3-BHA	0.010	0.0102	102
	0.020	0.0196	98
2-BHA	0.010	0.0098	98
	0.020	0.0200	100
BHT	0.005	0.0048	96
	0.010	0.0101	101
	0.020	0.0197	98
PG[b]	0.005	0.0051	102
	0.010	0.0090	90
NDGA[b]	0.005	0.0040 (+0010)[c]	100
	0.010	0.0082 (+0013)[c]	95
		Mixtures	
1. PG	0.010	0.0092 (+0.0006)[c]	98
NDGA	0.010	0.0086 (+0.0011)[c]	97
2. 3-BHA	0.010	0.0101	101
2-BHA	0.010	0.0098	98
3. BHA[a]	0.007	0.0063 (3-BHA) + 0.006 (2-BHA)	98
BHT	0.007	0.0068	97
PG[b]	0.005	0.0047	94
NDGA[b]	0.005	0.0048	96
4. BHA[a]	0.010	0.0089 (3-BHA) + 0.0011 (2-BHA)	101
		0.0088 (3-BHA) + 0.0010 (2-BHA)	99
		0.0090 (3-BHA) + 0.0010 (2-BHA)	100
BHT	0.010	0.0098	98
		0.0097	97
		0.0099	99
PG[b]	0.007	0.0069	98
		0.0068	97
		0.0069	98
NDGA[b]	0.007	0.0068	97
		0.0067	96
		0.0068	97

[a] Commercial BHA is a mixture of 3-BHA and 2-BHA. Each isomer is determined separately.

[b] PG and NDGA often streak on TLC. These spots and streaks are visible on the plate without spraying and can be effectively picked up.

[c] Figures in parentheses account for the streaks obtained with PG and NDGA.

Results and Discussion

The four antioxidants were added to 100 g samples of antioxidant-free lard, individuaally as well as in mixtures. The amounts used, shown in Table 2, were in the range permitted in fats and oils. Results of analysis show recoveries of BHA and BHT in the ranges 98–101% and 96–102%, respectively. PG and NDGA gave streaks, obviously because of the impurities associated with the antioxidants. The spots and streaks of these antioxidants are visible without spraying and the entire area can be picked up effectively to give recoveries up to 98%. Reproducibility of the method was tested and found satisfactory by applying a mixture of the four antioxidants (Mixture 4, Table 2) on three separate plates.

Commercial BHA is a mixture of 3-*tert*-butyl-4-hydroxyanisole (3-BHA) and 2-*tert*-butyl-4-hydroxyanisole (2-BHA) and may contain from 5 to 37% of 2-BHA. 3-BHA is more effective as an antioxidant than 2-BHA (7, 8). Chapman and Mahon reported a method for the estimation of isomers in commercial BHA (9) based on relative absorbances of the mixture obtained with 2,6-dichloroquinonechloroimide-borax reagent and ferric chloride-bipyridine reagent. The proportions of the two were calculated by difference. In the present investigation it was found that the isomers can be quantitatively separated by thin-layer chromatography. BHA used in this study contained 10.5% 2-BHA (Table 2).

The advantages of the above method are that (*1*) all four antioxidants can be estimated in one aliquot of the fat, (*2*) PG and NDGA can be estimated when present together in mixtures, and (*3*) the isomers of BHA can be effectively separated. The data demonstrate that this is a decided improvement over accepted methods of analysis of antioxidants.

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The evaporation flask and the suction assembly were fabricated in the Food and Drug Laboratory by R. G. Campbell.

BHA, BHT, and PG used in the study were obtained from Eastman Chemical Products, Inc.; NDGA was a commercial product of Wm. Stange & Co.

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A Simple Method for the Detection of Benzo[a]pyrene in Smoked Foods

By C. GENEST and D. M. SMITH (Food and Drug Directorate, Tunney's Pasture, Ottawa, Ontario, Canada)

Benzo[a]pyrene can be used as an index of carcinogens in smoke. A simple and rapid method has been developed for determining this substance. The dried food was extracted with *n*-hexane, the hexane was then extracted with dimethyl sulfoxide, and the polynuclear hydrocarbon was displaced from this solvent into benzene by water. The benzene extract was reduced to a small volume and an aliquot was spotted on a thin-layer chromatographic plate beside spots of a standard benzo[a]pyrene solution. The plate was chromatographed with 2,2,4-trimethyl pentane:benzene (97:3). When observed under ultraviolet light at 3660 Å, 0.002 μg of pure benzo[a]pyrene could be found and the addition of 0.01–0.05 ppm to unsmoked foods could be detected. No smoked food examined contained this level of benzo[a]pyrene.

Usually smoke for food processing is produced on the North American continent by one of three methods: burning dampened sawdust, burning dry sawdust, or by friction. These processes are carried out at the same time as the food is heated at 75–90°F (cold smoking process) or at 170–180°F (hot smoking process) (1, 2). The following effects have been claimed to result from such treatment: drying; giving a finish to the skin; imparting desirable flavor and odor; impregnating outer portions of the meat with constituents of smoke which can exert a preservative action or act as antioxidants in the fat; reducing microbial level present in meat. The time required for smoking varies according to the type of smoking process used and the kind of food product to be smoked.

It has already been recognized that smoking chamber soot contains carcinogenic polynuclear hydrocarbons (3, 4). In the late 1950's smoked food products such as fish and sausage were investigated by Russian and British workers (5–9) for the possible presence of polynuclear hydrocarbons. They suspected that these chemical compounds caused a higher than normal incidence of malignant tumors in the human alimentary tract (10–12). In these investigations of smoked food products, levels of 0.0001–0.05 ppm of benzo[a]pyrene have been reported to be present. The quantity of this carcinogen did not depend significantly on the smoking process used.

Benzo[a]pyrene was used for recovery studies because it is the most commonly reported carcinogen in smoke. The usual analytical procedure for the determination of benzo[a]pyrene and other polynuclear hydrocarbons involved saponification of 1 kg of the material to be analyzed (9, 13, 14). The unsaponifiable residue was extracted from this alkaline solution into an organic solvent, frequently with the formation of emulsions. This procedure was rather lengthy and it gave variable results. For these reasons another analytical approach was sought by which minute amounts of benzo[a]pyrene could be detected in a smaller sample of a food product without having to use the saponification step and column cleanup procedure (15–17). The problem was, essentially, to find a solvent for polynuclear hydrocarbons which did not dissolve fat.

Recently dimethyl sulfoxide has been reported to be a superior extraction solvent for polynuclear hydrocarbons (18, 19). The extraction procedure using this solvent for petroleum waxes was modified and was then applied to a variety of food products. This procedure gave a concentrated extract which could be used directly for chromatography. The thin-layer technique (20) was found to be superior to paper chromatography (21–

23) in that large quantities of an extract could be applied to one spot, whereas the lengthy paper chromatographic procedure requires that a number of chromatograms have to be eluted and the combined eluates have to be redeveloped (19, 24). The spot corresponding to the benzo[a]pyrene was detected on the thin-layer chromatogram simply by its fluorescence under ultraviolet light, thus meeting the requirement that the equipment for this determination should be readily available.

METHOD

Apparatus

(a) *Adjustable thin-layer chromatographic equipment.*—E.g., the Desaga apparatus according to Stahl (25).

(b) *Actinic glassware.*

(c) *Micrometer syringe outfit.*—E.g., the "Agla" Brand of Burroughs Wellcome & Co., London.

(d) *Ultraviolet lamp.*—Long wave, 3660 Å.

(e) *Suction tubes.*—With built-in filtering device (26).

(f) *Spectrophotofluorometer.*—The Aminco-Bowman instrument was used in this work.

Reagents

(a) *Dimethyl sulfoxide.*—Reagent grade.

(b) *n-Hexane.*—Redistilled before use.

(c) *Benzene.*—Redistilled before use.

(d) *Benzo[a]pyrene.*—Standard solution, 1 mg/L in 2,2,4-trimethylpentane.

(e) *Silica Gel G.*—Brinkmann Co., N.Y.

(f) *2,2,4-Trimethylpentane.*—Redistilled before use.

Extraction

The extraction procedures used depend on the product examined.

(a) *Extraction of benzo[a]pyrene from smoked cheese.*—Grind 100 g smoked cheese in a meat grinder and put into a 250 ml beaker. Leave beaker on a warm waterbath overnight and dissolve melted-out fat in *n*-hexane. Wash cheese several times with *n*-hexane. Dry cheese in vacuum desiccator over phosphorus pentoxide for approximately 2–3 days. As soon as cheese is dry, break it up into small pieces and transfer these pieces into a Soxhlet extraction thimble. Extract residual oil into *n*-hexane. Combine *n*-hexane extracts and reduce to 100–150 ml. Place this extract into a 1 L separatory funnel and shake it three times with 50–75 ml portions of dimethyl sulfoxide. Combine dimethyl sulfoxide layers and add the same amount of benzene. To this mixture add 400 ml distilled water (some heat is generated upon dilution with water). Displace dimethyl sulfoxide into water by shaking and remove upper benzene layer. Run benzene solution through a bed of anhydrous sodium sulfate until clear. Reduce this dry solution to 5 ml on steam bath under a stream of nitrogen.

(b) *Extraction of benzo[a]pyrene from wieners.*—Grind 100 g wieners in Waring Blendor. Spread the ground meat on a flat dish and dry in vacuum oven at 105°C for 4 hours. Transfer the dried pieces to a thimble, extract in Soxhlet apparatus with *n*-hexane, and reduce volume of extracts to 100–150 ml. Proceed as described under (a), beginning "Place this extract . . . ".

(c) *Extraction of benzo[a]pyrene from kippers.*—Grind 100 g whole kippers in Waring Blendor. Dry the ground fish on a flat dish in vacuum oven at 105°C for 4 hours. Extract the dried material with *n*-hexane in a Soxhlet apparatus. Reduce the *n*-hexane extract to 100–150 ml, place in a separatory funnel, and shake it three times with 50–75 ml dimethyl sulfoxide. Break emulsion by centrifuging each individual shakeout. Remove lower layer from centrifuge bottle by suction and combine the three layers. Add the same amount of benzene to the dimethyl sulfoxide in a separatory funnel and add 400 ml distilled water. Displace dimethyl sulfoxide into water by shaking, and filter upper benzene layer through folded filter paper before running it through a bed of anhydrous sodium sulfate. Reduce clear benzene solution to 5 ml in a stream of nitrogen on steam bath.

Thin-layer Chromatography

Mix 75 g Silica Gel G with 150 ml distilled water in a Waring Blendor for 45 sec. Pour slurry into applicator with the scale set to give layers 0.75 mm thick. Prepare the layers on 20 × 20 cm glass plates and allow to stand at room temperature for 20 minutes. Activate the plates for 1 hour at 100°C, and store in storage cabinet supplied with drying agent. Apply 20, 40, 60, 80, and 100 μl of the final benzene extract through the micrometer syringe at the starting line 2.5 cm from the bottom edge of the plate. This is done by spotting 1 μl quantities, and drying the spot each time with a specimen dryer from underneath. Also apply the same size portions of the standard benzo[a]pyrene solution on the same plate. Develop the thin-layer chromatogram with 3% benzene in 2,2,4-trimethylpentane until the solvent front is 14 cm from the baseline. Remove plate from solvent, dry in air, and repeat the developing procedure twice more in the same direction. Dry plate after third run for 1 minute and examine plate immediately, while still wet, in UV light at 3660 Å (R_f about 0.3) for royal blue fluorescent spot. Outline area of fluorescence and remove layer by suction into the suction tube which is filled with benzene. Elute the Silica Gel G caught on the filter with benzene and evaporate eluate to dryness in a stream of nitrogen on the steam bath. Keep residue for further investigation.

Recovery Studies

Add 1 ml, 5 ml, and 10 ml of the standard solution of benzo[a]pyrene (1 μg/ml) to 100 g portions of the unsmoked products, processed cheddar cheese, wiener emulsion, and herring to give samples with 0.1, 0.05, and 0.01 ppm of the benzopyrene. Dry the mixture and extract according to the same procedure as described above. Investigate aliquots of 20–100 μl of the final extracts by thin-layer chromatography, and elute spots for spectrophotofluorometric investigation.

Confirmation by Spectrophotofluorometry

Dissolve residue from Silica Gel G eluate in 1 ml 2,2,4-trimethylpentane and measure fluorescence in Aminco-Bowman spectrophotofluorometer. Determine characteristic fluorescence at 410 mμ when the solution is activated at 380 mμ (27, 28).

Results and Discussion

Addition of the benzo[a]pyrene, at the three stated levels, to 100 g of the three food products gave the following results: In all cases, 0.1 and 0.05 ppm of the carcinogen were detected; the lowest level of 0.01 ppm could be detected only in wieners. As in all chromatographic procedures, the limits of detectability are dictated by the nature and quantity of interfering substances. In this case, these factors and the viscosity of the final solution set an upper limit of 100 μl on the amount that could be spotted. The effect of background interferences was reduced by developing the plate three times consecutively in the same direction. In the recovery studies the royal blue fluorescent spot of benzo[a]pyrene showed up between lighter blue fluorescent areas which were also present in extracts from cheddar cheese, wiener emulsion, and herring without added benzo[a]pyrene. None of the smoked food products contained the polynuclear hydrocarbon at or above the stated recovery levels. It was noticed, however, that the benzo[a]pyrene used as a standard either was not chemically pure or decomposed during chromatography. The compound resolved frequently into two spots: one was the original spot, the other had an orange fluoroescence (28). On rerunning the eluted spot on a thin-layer chromatogram, another whitish blue fluorescent spot of lower R_f appeared which indicated that oxidation of the compound may have occurred during elution (19, 29). This decomposition did not seem to take place as long as the benzopyrene was in the oil phase, and not exposed to air. The procedure was carried out in subdued light. If other polynuclear hydrocarbons are suspected to be present in the extract, cellulose acetate thin-layer plates with ethanol-toluene-water as the mobile solvent can be utilized to identify them individually (28).

The particular advantages of the above mentioned procedure are its simplicity and rapidity. Minute amounts of benzo[a]pyrene can be detected on one thin-layer chromatogram without additional cleanup steps. The presence of certain interfering substances present naturally in foods limits the detectability. Recoveries of added benzo[a]pyrene were satisfactory.

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EXTRANEOUS MATERIALS

Filth in Fruit Paste

By HELEN H. WHEELER (Food and Drug Administration, 518 Federal Office Bldg., San Francisco, Calif. 94102)

A two-step procedure for examining fig paste for light filth and heavy filth has been investigated. The first step is a modification of method 36.052, using a kerosene-water flotation treatment to separate light filth elements. The second step consists of treating the residual material from flotation with Na_4EDTA and screening on a No. 140 sieve to recover heavy filth elements, along with light filth not separated by flotation. Studies indicate that the proposed kerosene-water flotation gives superior recovery of light filth and heavy filth, and the method is recommended for adoption as official, first action.

A practical and reliable method is needed for determining various insects and other filth contaminating fruit paste. Preliminary investigations of methods for filth in straight Calimyrna fig paste were reported last year (1). The paste was boiled with Versene (Na_4EDTA) solution, screened onto a No. 140 mesh sieve, and washed with a forceful spray of water; the residue was finally transferred to filter paper for microscopic examination. The method gave good recovery of insects and rodent hairs but was time-consuming.

In an attempt to shorten the time required for microscopic examination, the screened residue of digested fig paste was treated by kerosene-water flotation (2) and the "light" filth was separated. "Heavy" insects, such as maggots, remained in the trap flask.

Experimental

Additional studies showed that good recovery of light filth was obtained by boiling the fig paste with water for about 30 minutes, screening onto a No. 140 sieve and washing with hot water, transferring the residue on the screen to a 2 L Wildman flask, and extracting with kerosene. The trap flask residue was then examined for additional insects, such as maggots, by screening onto a No. 140 sieve, transferring the fig material to a beaker and boiling with Versene solution, again screening the mixture, and washing with a very forceful spray of hot water. Most of the seeds were eliminated by transferring the final residue on the screen to filter papers by decantation, and the papers were examined at 30×. Microscopic examinations of digested and screened trap flask residues showed very few light insects. This examination measured the completeness of the separation of light insects by the kerosene extraction step.

Portions of fig paste with added light and heavy insects were examined to see whether handling and treatment resulted in loss. Beetle larvae and maggots less than 1 mm long were not completely recovered. Maggots less than 1–1.5 mm long isolated from whole dried figs were altered when boiled 5 minutes in very dilute Na_4EDTA; some became swollen and others were collapsed and empty.

Attempts to adapt the procedure for fly eggs and maggots in tomato products, **36.064(a)** and **(b)**, were unsuccessful under the conditions employed.

Another laboratory of the Food and Drug Administration compared methods **36.052**, **36.053** (2), and a modified version of **36.052** which included sieving prior to kerosene flotation (3). Consecutive 300 g portions were cut from a 50 lb carton of Turkish fig paste, kneaded by hand, and divided into three 100 g portions for analysis by each of the three methods listed. Each of seven analysts prepared and examined the filter papers from several sets of determinations. Thus, the same analyst prepared and read the plates for a complete set; in this way individual variation was eliminated.

Results (Table 1) show that recoveries of whole insects or equivalent by method **36.053** are lower than those by either of the other two methods, and that the combined sieving flotation procedure gives better recovery.

Table 1. Statistical survey on insect recovery from fig paste (3)

Method	Whole or Equivalent Insects[a] Found: Mean	Median	No. of 100 g Portions Examined
36.052	14.7	15	49
36.053	6.3	6	35
36.052, modified	21.9	21	49

[a] Dried fruit beetle adult, adult head, larvae, larval heads, larval head capsules; moth larvae, larval heads, larval head capsules; Drosophila pupa, maggots, and maggot mouthhooks; ant heads; and one sawtooth grain beetle larval head.

Earlier studies of method **36.053** were reported by Tilden (4), who observed that castor oil isolated many of the insect parts, but that a second trapping with gasoline yielded significantly more. In addition, she noted that time, degree, and manner of agitating the oil-and-paste mixture, as well as the physical state of the sample, were important factors influencing the number of insect parts that could be recovered by the oil, and that the gasoline probably acts only as a sweeping agent.

Collaborative Study

A collaborative study of methods for filth in fig paste was initiated to compare the efficiencies of methods **36.052**, **36.053**, and a procedure for light and heavy insects described below.

Test samples of sufficiently homogeneous filth content were needed as the basis of comparison. Mechanical mixing of commercial fig paste, diluted with water to increase plasticity, appeared feasible, and trial mixes of straight Calimyrna paste were made in a Hobart Model A 120 mixer with wire whip attachment (Hobart Manufacturing Co., Troy, Ohio). The quantity of water added, mixing time, and speed were varied to observe the effect of different conditions.

Collaborative test samples were prepared from 3200 g fig paste and 2560 ml water; these quantities were suitable for the size and shape of the Hobart equipment and stable to separation of liquid. Preliminary warming on the steam bath and mixing at low speed in the Hobart aided absorption of water. After initial blending, the sample was mixed at high speed for 1.5 hours; at 15 minute intervals mixing was stopped, the bowl and beater were scraped, and top and bottom layers were intermixed by hand. Samples of 180 g of treated paste, equivalent to 100 g of original material, were weighed into glass jars with screw caps, labeled, and frozen without addition of preservative.

Three batches of treated paste were prepared from a 52 lb carton of Turkish fig paste. Portions were cut from the eight corners and from the center of the block for each batch. Three batches of treated, domestic Adriatic paste were prepared in the same way. Each batch was identified as an individual sample.

Triplicate portions of the initial batch of treated Turkish paste, equivalent to 100 g of original material, were examined by the procedure for light and heavy filth described below. Duplicate portions of treated Adriatic paste were similarly examined. The insect counts varied, but the range was accepted as inherent to the conditions. The Hobart blending treatment influenced the subsequent behavior of the paste; improved dispersion of fig paste was obtained.

Eight laboratories participated in the collaborative test program. Each collaborator was sent three jars from each of the six treated batches of pastes. Collaborators were asked to examine these triplicate portions by methods **36.052**, **36.053**, and the procedure for light and heavy insects described below, and to summarize results on supplied report forms. The original directions to collaborators in the procedure below were: Pour hot mixt. portionwise (ca 1/3 at time) on a No. 140 sieve, washing each portion using hot H_2O from household type spray device, and with the sieve set in a pan, to allow the fig tissue to be completely immersed in H_2O and agitated by tilting and shaking the screen. Drain the H_2O from the screen and pan, and repeat washing step two more times. They were also asked to evaluate the methods with respect to time required, simplicity of operation, difficulties encountered, relative apparent efficiencies of recovery of light filth, deficiencies of the methods, and any suggestions for improvement.

METHOD

Reagent

Kerosene.—Deodorized kerosene.

Determination

Weigh 100 g fig paste into 1–2 L beaker. Add ca 400 ml H_2O. Heat to boiling, stirring to disintegrate lumps and prevent scorching. Boil mixt. at least 10 min. (30–60 min. if necessary for max. settling of fig tissue during flotation treatment). Pour hot mixt. portionwise (ca 1/3 at time) onto No. 140 sieve. Wash each portion with hot H_2O from household spray device, agitating and tilting to insure that any lumps of tissue are completely dispersed. Drain H_2O from screen and pan, and wash twice more. Transfer residue on screen to 2 L Wildman trap flask. Continue portionwise washing and transfer of residue on screen to trap flask; rinse beaker washings thru screen, and finally add screen rinsings to trap flask.

Light filth.—Add cold H_2O to trap flask until vol. of mixt. is ca 900 ml. Add 35 ml kerosene and treat as in **36.003(a)**, filling flask with deaerated H_2O (let stand at room temp. to deaerate). Ext. again with 25 ml kerosene. Examine filter papers at 30× magnification as in **36.003(f)**(*1*) and (**g**), thoroly wetting papers with glycerine-alcohol (1 + 1), **36.003(c)**(*2*).

Heavy filth.—Empty remaining trap flask contents and flask rinsings onto No. 140 sieve. Wash fig material on screen with hot H_2O from spray device to remove kerosene as completely as possible. Transfer material on screen and screen rinsings to 1 L beaker used for weighing, and add hot H_2O to ca 400 ml. Heat to boiling, and boil 15 min., adding 10% Versene (Na_4EDTA) soln portionwise to keep mixt. at pH 8 (measure with Hydrion paper).

Table 2. Filth examinations of treated fig paste samples (equal to 100 g original paste)

	No. Insect Equivalents Found[a]			No. Found,[a] Omitting Mandibles		
	Method **36.052**	Method **36.053**	Proposed Method	Method **36.052**	Method **36.053**	Proposed Method[b]
Turkish Fig Paste						
Mean	34.0	15.4	30.4[b], 5.0[c]	20.7	12.9	19.8
Median	27	13	31, 5	21	11.5	21
No. of Examinations	20	21	21, 24	23	24	24
Adriatic Fig Paste						
Mean	7.2	5.4	6.2, 7.2	6.4	5.6	5.4
Median	6.5	5	5.5, 6	6	5	4
No. of Examinations	18	18	18, 21	21	21	21

[a] Defined as whole insects and heads, adult or other stages; larval cast skins and head capsules; paired mouthhooks valued at 1; split mouthhooks and mandibles, 1/2.
[b] Light insect examination.
[c] Heavy insect examination.

Transfer hot mixt. in portions onto No. 140 sieve, washing between addns with forceful stream of hot H_2O, from spray device, to prevent screen clogging. Continue forceful washing until residue on screen consists of seeds and fibrous fig tissue, along with filth elements.

Transfer residue on screen and screen rinsings to 1 L beaker used for weighing. Add H_2O to ca 300 ml, swirl, and quickly decant suspended fig tissue and filth elements onto ruled filter paper in Hirsch funnel, retaining seeds in beaker as much as possible. Replenish H_2O in beaker and repeat decantation, changing filter papers. (Four papers may be required.) After all tissue has been decanted from seeds, wash seeds thoroly, using three 200 ml portions H_2O, swirling, decanting, and filtering washings, retaining seeds in beaker. Discard seeds. To det. adequacy of washing, examine seeds microscopically after washing. Examine filter papers at 30× magnification for heavy filth elements, wetting papers thoroly with glycerine-alcohol (1 + 1).

Results and Discussion

Table 2 summarizes the data reported by the eight collaborating laboratories on the three different methods. Results are given as number of insect equivalents, defined as whole adults, pupae, and larvae; heads of adults, pupae, and larvae; entire larval cast skins; larval head capsules; and paired mouthhooks of maggots, each valued as 1, with single mandibles or split mouthhooks valued as 1/2.

The average number of insects was lower by method **36.053** than by the other two methods. Data on striated hairs, not included in Table 2, showed that method **36.053** yielded no striated hairs; efficiency of the other two methods was about equal for striated hairs.

The number of insect equivalents found by a given method in a particular sample varied greatly among the collaborators, partly because of differences in mandible counts. For example, by method **36.052**, the mandible counts for the treated Turkish fig paste samples ranged from 5 to 104. Excluding mandible counts, the variation was still wide, possibly because some small larvae and larval heads were overlooked.

Five of the eight collaborating laboratories evaluated the apparent efficiencies of the three methods for filth recovery. All five laboratories rated method **36.053** as the least efficient. Three laboratories considered method **36.052** as slightly more efficient than the proposed method for light insects. Method **36.052** was regarded as the simplest procedure by three collaborators. Seven of

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This report of the Associate Referee was presented at the Seventy-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 14–17, 1963, at Washington, D.C.

Table 3. Filth examinations of untreated Turkish fig paste (100 g portions)

	No. of Insect Equivalents Found			No. Found, Omitting Mandibles		
	Light Type	Heavy Type	Total	Light Type	Heavy Type	Total
			Method 36.052			
In oil layer	34.5			26		
In trap flask residue	29.5	14		11	14	
Total	64	14	78	37	14	51
% light insects[a]	54			70		
			Method 36.053			
In oil layer	19.5			13		
In trap flask residue	23	14.5		10	14.5	
Total	42.5	14.5	57	23	14.5	37.5
% light insects[a]	46			56		
			Proposed Method			
In oil layer	58			27		
In trap flask residue	2	15.5		0	15.5	
Total	60	15.5	75.5	27	15.5	42.5
% light insects[a]	97			100		

[a] % light insects found in oil layer compared to total light insects.

the eight participants stated that the proposed method for heavy insects was very time-consuming, and two of them questioned the value of the procedure because of the few insects recovered in their examinations.

Two collaborators discussed the effect of the Hobart pretreatment of the collaborative samples on the relative efficiencies of the three methods. Collaborator N felt that the essential difference between method **36.052** and the proposed method for light filth was the introduction of a sieve, which aided the disintegration-boiling step. He stated that, with the proposed method, the sieve did not accomplish anything because the fig paste had already been disintegrated in the pretreatment. Moreover, pretreatment gave better results with method **36.053** than would be otherwise expected.

Collaborator M noted that all fig paste check samples were completely dispersible in cold water and sufficiently dilute so as not to gel or agglomerate on boiling. With untreated fig paste, filth elements became precipitated or coated with fig material upon boiling as prescribed in method **36.052**, and recovery results were therefore fictitiously high for treated paste samples.

The following experiment corroborated these observations: Three 100 g portions of untreated fig paste were taken from the remainder of the test block of Turkish paste. The three portions were examined by each of the three methods. Conditions and manipulations were kept as uniform as possible. All trap flask residues were examined by the proposed procedure for heavy insects. Table 3 gives the number of insect equivalents and the number, excluding mandible counts by type, light or heavy, found by each method, at the different stages of the examinations.

Conclusion

The collaborative program to determine the relative efficiencies of methods **36.052**, **36.053**, and the proposed methods showed that method **36.053** was inferior to the other two methods, and the efficiency of method **36.052** compared to the proposed method was dependent on pretreatment of the fig paste. Method **36.052** as written does not provide for pretreatment, and the instructions, "Continue boiling ca 10 min., or until material is disintegrated," are not specific enough for fig paste to insure optimum recovery of insects.

Recommendations

It is recommended—

(1) That method **36.053** for insect heads in fig paste and fig slices be deleted.

(2) That the proposed methods for light filth and heavy filth in fig paste be adopted as official, first action.

(3) That studies be continued.

Acknowledgments

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The recommendations of the Associate Referee were approved by the General Referee and by Subcommittee E and were adopted by the Association. See *This Journal,* 47, 133 (1964).

FRUITS AND FRUIT PRODUCTS

Calculation of Ratio of Fruit to Sugar in Mixtures Where Ratio Is Unknown

By CHARLES H. COLEMAN (Laboratory Division, Directorate of Technical Operations, Hq. Defense Subsistence Supply Center, 1819 West Pershing Rd., Chicago, Ill. 60609)

Jam and jelly manufacturers commonly use fruits packed with sugar when they are unable to secure fresh fruits locally or in season (1). The ratio of fruit to sugar is given to the manufacturer by the producer of the mixture. This information commonly takes the form "2 × 1," "3 × 1," etc., which means pounds of fruit to one pound of sugar. For example, frozen strawberries packed with sugar and labeled "2 × 1" would have fruit to sugar in the ratio of two pounds of fruit to one pound of sugar. The manufacturer uses this information to calculate the pounds of mixture needed to comply with Federal specifications (2), which require 45 pounds of fruit to 55 pounds of sugar.

The ratio of fruit to sugar, although often unknown, is also useful to the analyst determining end item conformance. A jam or jelly which appears deficient in fruit content, based on a comparison of analytical results with average authentic constants, may actually meet Federal requirements if the fruit used has lower constants. The analyst determines whether the fruit has lower constants by analyzing a sample of the fruit used in the end item. The values obtained must be adjusted to reflect the actual amount of fruit contained in the fruit-sugar mixtures. Adjustments may be made by determining the approximate fruit content (3) or by using the following equation to calculate the ratio of fruit to sugar.

R = Ratio = lb fruit/1 lb sugar.
Sm = % Soluble solids of mixture (4).
Sf = Soluble solids of fruit (use soluble solids of fruit ingredient; if not known, use average soluble solids of authentic fruits (5)).
$R \times Sf/100$ = lb Soluble solids contributed to mixture by fruit.
$1 \times 100\%/100$ = lb Soluble solids contributed to mixture by sugar.
$R + 1$ = Total lb of mixture.
$(R \times Sf/100 \times 100)/(R + 1)$ = % Soluble solids contributed to mixture by fruit.
$(1 \times 100\%/100 \times 100)/(R + 1)$ = % Soluble solids contributed to mixture by sugar.
$[(RSf)/(R + 1)] + [(100)/(R + 1)] = Sm$.
$R = (100 - Sm)/(Sm - Sf)$.

Example using equations:

Question: What is the fruit-to-sugar ratio of an unknown strawberry-sugar mixture? The soluble solids of the mixture is 38.67% (4).

$Sm = 38.67\%$.
$Sf = 8.0\%$ (5).
R = Ratio = lb fruit to 1 lb sugar.
$R = (100 - Sm)/(Sm - Sf) = (100 - 38.67)/(38.67 - 8.0) = (61.33/30.67) = 2.$

Answer: The fruit to sugar ratio is 2:1 or "2 × 1." The per cent of fruit in mixture = 2/3 × 100 = 66 2/3%.

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MEAT AND MEAT PRODUCTS

A Method for the Routine Determination of Corn Sirup in Prepared Meat Products

By LESTER HANKIN and ALPHONSE F. WICKROSKI (The Connecticut Agricultural Experiment Station, New Haven, Conn.)

A method has been devised for the determination of corn sirup added to processed meat products. The method is based on the quantitative determination of dextrin added to corn sirup. The dextrins are enzymatically hydrolyzed by α-amylase and β-amylase, and maltose is calculated as the difference in CuO_2 found by copper reduction between a treated and an untreated aliquot. A correction factor was devised to determine the average amount of dextrin in corn sirup by testing a number of commercial sirups for their dextrin content and subjecting the data to statistical analysis. With this equation the method is applicable to a wide range of sirups. The method also permits the estimation of dextrose added to meats in excess of that included as one of the components of corn sirup.

Prepared meat products, such as frankfurts, often contain corn sirup as one of the optional ingredients. Other nutritive materials commonly added include skim milk powder (nonfat dry milk), dextrose, sucrose, cereal, and flour. Corn sirup is prepared commercially by hydrolysis of corn starch and contains dextrose, maltose, dextrin, and polysaccharides intermediate between maltose and dextrin. Corn sirups are designated as having a specific DE value or dextrose equivalent; the higher the DE value the greater the degree of hydrolysis. The meat industry generally uses corn sirups in the range of 36–43 dextrose equivalent, and these corn sirups may be purchased as a liquid, a dried powder, or granules.

It is claimed that corn sirup improves color retention in meat, acts as a moisture conditioner, adds to the body of the product, and provides some sweetness. The dextrins help bind the meat particles together and also act as an emulsifying agent.

With meat products containing both corn sirup and skim milk powder, the analysis for lactose by the AOAC yeast method (1) gives difficulty. It has been suggested that the yeast fails to ferment all of the reducing components in the corn sirup, and consequently inflated values for lactose are obtained. The dextrin portion of the corn sirup is probably the source of the trouble (2). A method has been described for the determination of lactose in the presence of corn sirup, based on an enzymatic hydrolysis of all carbohydrates except lactose (3). This method overcomes the difficulties encountered with the AOAC yeast method.

Since the analysis for food ingredients is of continuing interest in this laboratory, a method was sought for the routine determination of corn sirup added to meat products. The procedure described below is based on the determination of the dextrins added to meat products. The dextrins are enzymatically hydrolyzed, and the increase in reducing sugars is measured and calculated as maltose. Available methods for determination of dextrins in the presence of other sugars (4) are lengthy and are not suitable for routine testing.

Connecticut has no statute regulating the amount of corn sirup which may be added to meat, but Federal Regulations specify a maximum of 2% (5).

Experimental

Reagents

(a) *Acetate buffer, pH 4.8.*—Mix 4.5 ml water, 2.0 ml 1*M* acetic acid (57.8 ml glacial acetic acid/L) and 3.5 ml 1*M* sodium acetate (82 g anhydrous sodium acetate/L).

(b) *Enzymes.*—α-Amylase, type III, bacterial, crude from *B. subtilis*; and β-amylase, crude from sweet potato. (Both may be obtained from Sigma Chemical Co., St. Louis, Mo.)

(c) *Dextrin for internal standard.*—A 1% water suspension of bacteriological dextrin. (Obtainable from Nutritional Biochemicals Corp., Cleveland, Ohio.)

(d) *Sodium chloride solution.*—Analytical reagent grade; 0.5% solution in water.

(e) *Toluene.*—Analytical reagent grade.

Determination

Enzyme suspension.—Triturate together in water enough α-amylase and β-amylase for all samples to be run at one time. Use 50 mg α-amylase and 5 mg β-amylase for each 40 mg corn sirup expected in sample aliquot. (See *Internal Standard* for standardization of enzyme activity, if necessary.)

For 4 samples and the internal standard, triturate 250 mg α-amylase and 25 mg β-amylase in water. Transfer to a 25 ml volumetric flask, dilute to volume, and shake well. Use 5 ml for each test. Stir the enzyme suspension *continuously* while pipetting.

Preparation of meat sample.—As described by Hankin and Wickroski (3). Place 20 g sample in 200 ml volumetric sugar flask, add small amount of water, and break up by agitation. Add about 100 ml water and warm on steam bath 30 min., shaking frequently. Cool to room temperature. Add 4 ml HCl and dilute to volume, using bottom of fat layer as meniscus. Add 10 ml 20% phosphotungstic acid solution, mix well, and let stand few minutes. Filter through moist filter paper (centrifugation is recommended but is not indispensable). Measure exactly 160 ml filtrate into a 200 ml volumetric flask, and neutralize to pH 4.8–5.0 (chlorophenol red indicator). Use this solution to determine reducing sugars before and after enzyme treatment. (The size of aliquot taken depends on the expected amount of corn sirup; generally 25 ml (equivalent to 2 g meat) is convenient, but smaller or larger aliquots (up to 40 ml) may be used.)

Enzyme treatment.—Pipet the following into bottle or flask (100 ml glass bottles with screw caps are satisfactory): 25 ml test solution, 1 ml buffer, (a), 1 ml sodium chloride solution, (d), and enzyme suspension containing 50 mg α-amylase and 5 mg β-amylase. Dilute to 50 ml with water, add 2 drops toluene, (e), stopper, and shake well. Incubate 18–20 hr at 35°C in a bacteriological incubator.

Internal standard.—Test 40 mg dextrin by method described in *Enzyme treatment;* omit *Preparation of meat sample.*

If all dextrin is hydrolyzed to maltose, a theoretical value of 52.9 mg for Cu_2O should be obtained. Use this theoretical value, divided by mg Cu_2O actually obtained from 40 mg dextrin, as correction factor in final calculations. (A correction factor between 0.70 and 1.30 is considered satisfactory. If the correction factor is outside this range, adjust the amount of enzyme used by testing different levels of α-amylase with a constant level of 5 mg β-amylase on 40 mg dextrin samples until theoretical Cu_2O value of 52.9 mg is approached. This adjustment may be necessary for each new bottle of enzyme purchased.)

Reducing sugar determination.—Determine reducing sugars by Munson-Walker method, **29.038** and **29.039** (1), on entire contents of bottle after enzyme treatment, and also determine total reducing sugars on untreated aliquot of same size (control).

Calculations

Per cent corn sirup in sample.—Calculate maltose (difference in mg Cu_2O between untreated and enzyme-treated aliquot) by use of the Munson-Walker tables. Multiply maltose value by correction factor obtained from dextrin internal standard. Multiply corrected maltose value by 0.95 to obtain mg dextrin (Y in formula below). Substitute Y value in following formula to obtain mg corn sirup in sample aliquot:

$$X = (Y - 3.8795)/0.3250$$

Example: 25 ml prepared meat solution taken for enzyme treatment (represents 2 g original meat sample):

	mg
Cu_2O in 25 ml aliquot, untreated	43.6
Cu_2O in enzyme-treated aliquot	59.6
Difference	16.0
Maltose equivalent, 16.0 mg Cu_2O (from table)	11.2
Multiplied by correction factor, 1.01	11.3
Multiplied by 0.95	10.7

Substituted in formula: $X = (10.7 - 3.8795)/0.3250 = 20.9$ mg corn sirup in 2 g meat sample = 1.05% corn sirup in sample.

Per cent added dextrose in sample.—From total amount of Cu_2O found in untreated aliquot, subtract mg Cu_2O due to lactose (if present), and subtract mg Cu_2O due to dextrose, maltose, and other carbohydrates. (Amount of Cu_2O for various levels of corn sirup is found in Table 3. Any significant difference in mg Cu_2O is attributable to added dextrose.) Calculate dextrose value from Munson-Walker tables and convert to per cent added dextrose.

Results and Discussion

Enzyme Hydrolysis

β-Amylase hydrolyzes the nonreducing end of the polysaccharide chain to maltose. However, the reduction in size is not as rapid as with α-amylase, which hydrolyzes the polysaccharide by attacking internal linkages in random fashion. The action of α-amylase is not as specific as that of β-amylase and results in formation of both maltose and dextrose units (6). β-amylase is included in the proposed method, so that the major end product will be maltose. This has been established by hydrolyzing bacteriological dextrin and calculating the results obtained as maltose. Good agreement was found.

The method described in this paper is based on the ability of these two enzymes to hydrolyze the dextrin in the corn sirup to maltose. Since meat processors generally use corn sirup in the range 36–43 DE, about 60% of the material is either dextrin or carbohydrates larger in molecular size than maltose. If processors use a corn sirup high in DE, they are essentially adding large amounts of commercial dextrose. If they use a corn sirup with a DE lower than 30, they are essentially adding only partially hydrolyzed corn starch, and most of this material acts like starch itself. The proposed method is based on this premise, and corn sirups collected from meat processors in this area were all in the range 36–43 DE.

Tests have shown that hydrolysis of dextrin was 85–90% complete in 6 hours at 35°C with 50 mg of α-amylase and 5 mg of β-amylase. Shorter incubation times gave a lower degree of hydrolysis. Increasing the amount of enzyme and maintaining a 6 hour incubation period did not give the expected increase to 100% hydrolysis. In any event, the use of excessively large amounts of enzyme is to be avoided. Thus, the 18–20 hour incubation period not only assures complete hydrolysis but is also convenient.

Consistent theoretical yields of maltose from dextrin under the test conditions have been achieved. Various combinations of amounts of α-amylase and β-amylase were tested, and it was found that increasing the β-amylase made very little difference. Although we have used 50 mg of α-amylase in our studies, the amount needed may vary with enzymes from different sources and suppliers. The use of an internal standard in each test is necessary to provide both the essential correction factor and a safety factor to indicate loss of enzyme activity during storage. The internal standard also permits enzyme activity to be standardized between laboratories.

The amylases used in this test should be selected carefully. Amylases from malt are to be avoided, since they may contain maltases as contaminants, which would hydrolyze the dextrins to dextrose and give higher values than theoretical. In addition, it has been noted (3) that crude maltase possesses some glucose oxidase activity which, if present, would lead to erratic results.

A pH of 4.8 is recommended for these enzymes (7). Both maltose (8) and ascorbic acid (9) inhibit β-amylase. We have tested these compounds and have observed no detrimental effects. Nitrate ion can inhibit α-amylase (10) by causing a shift in optimum pH. Preliminary testing showed that the concentration of nitrate ion in solutions from prepared meat samples is too low to alter or influence the degree of hydrolysis of dextrin. Sodium chloride is used in the incubation mixture because chloride ion is an activator for α-amylase (10).

Analysis of Corn Sirup

Thirteen samples of corn sirup, some solid and some liquid, were tested at 10, 20, and 30 mg (dry weight basis) by the method to obtain the average amount of dextrin present. The data were subjected to an analysis of variance (Table 1), and an equation was derived for the straight line by the method of least squares (11). From this equation, $X = (Y - 3.8795)/0.3250$, the amount of corn sirup present in the test sample can be estimated. In the equation Y represents mg of dextrin found by analysis and calculation. The slopes of the lines for each individual corn sirup differed slightly but not significantly. This was expected, since not all of the corn sirups were manufactured by the same process, and different amounts of the components (dextrose, maltose, and dextrin) were present. However, the line obtained for each corn sirup, as well as the average line obtained by the method of least squares, shows no curvature, indicating that the analysis is a straight line function (Table 1).

Table 1. Analysis of variance of data on 13 corn sirup samples

	Difference	Sum of Squares	Mean Squares
Between samples	12	56.277	4.690
Combined slopes	1	274.625	274.625
Curvature	1	0.046	0.046
Slope × sample	12	11.990	0.999
Samples × curvature	12	5.765	0.480
Total	38	348.704	9.176
C_m	1	4201.616	4201.616

$b = 0.3250$
$Y = 10.3795$
$a = 3.8795.$
$X = (Y - 3.8795)/0.3520.$

When a value of high accuracy for corn sirup is needed for an individual sample, it is recommended that an actual sample of the corn sirup under test be collected, and 10, 20, and 30 mg portions (dry weight basis) be analyzed by the method. After determining the amount of dextrin at each level, obtain the equation for the best fit of the line by the method of least squares (11). This equation can be used to calculate the corn sirup potency of the sample. (Sample preparation may be omitted and the material assayed directly.)

The proposed method saves considerable time and effort because the sample preparation is the same as that described for determining lactose (3). Since many meat products contain both lactose and corn sirup, an aliquot of the same solution can be used for both tests.

To test the validity of the method, sam-

Table 2. Determination of the amount of corn sirup in prepared frankfort samples[a]

Sample No.	Sample Contains:			Found		Meat in Aliquot Tested, g
	% Corn Sirup	% Skim Milk Powder	% Dextrose	% Corn Sirup	% Recovery	
1	–	–	–	0	–	2
2	–	–	–	0	–	2
3	–	3.5	–	0	–	2
4	–	3.5	2.0	0	–	2
5	1.0	–	–	0.88	88.0	2
6	1.0	–	–	1.15	115.0	3.2
7	2.0	–	–	1.90	95.0	2
8	3.0	–	–	2.99	99.7	2
9	3.0	–	–	3.29	109.7	1.36
10	4.0	–	–	3.53	88.3	2
11	4.0	–	–	3.88	97.0	1
12	2.0	1.75	–	2.05	102.5	2
13	2.0	1.75	–	1.85	92.5	2
14	2.0	1.75	–	2.02	101.0	2
15	2.0	1.75	–	1.68	84.0	2
16	2.0	1.75	–	2.47	123.2	2
17	2.0	3.5	–	2.01	100.3	2
18	2.0	3.5	–	1.67	83.5	2
19	2.0	3.5	–	1.67	83.5	2
20	2.0	3.5	–	1.51	75.5	2
21	2.0	5.0	–	1.85	92.5	2
22	3.0	3.5	–	2.99	100.0	2
23	5.0	3.5	–	4.99	99.8	2
24	2.0	3.5	–	1.61	80.5	2
25	2.0	3.5	1.0	1.59	79.5	2
26[b]	2.0	3.5	–	1.90	95.0	2
				Av.[c]	94.8 ± 2.5%	

[a] All samples contained frankforts (20 g equivalent) except Nos. 2 and 3. No. 2 contained ground beef and No. 3 contained no meat sample.

[b] Sample contains 2.0% sucrose.

[c] Samples 1–4 not included in average.

ples of frankforts known to contain no added carbohydrates were prepared with various combinations of skim milk powder, corn sirup, dextrose, and sucrose to represent commercial products and were analyzed by the method. Table 2 shows the composition of the samples and analytical results. Different corn sirups were used to survey the range of such materials used in meat processing. In most cases, 25 ml aliquots, representing 2 g of original meat sample, were used; where the amount of corn sirup added was high or low, aliquots of other sizes were taken.

Satisfactory results were obtained for all the test combinations (Table 2). The average recovery of 94.8 ± 2.5% was well within the limits expected for routine analysis. There were a few high and a few low percentage recoveries for the corn sirups which deviated most widely from the average value obtained when the sirups were checked for dextrin content. It should again be noted that we are dealing with an average value for corn sirup, and some degree of accuracy must be sacrificed.

The first four lines in Table 2 represent tests not included in the over-all percentage recovery. These tests were made to show that there is no blank due to any of the reagents used or to a meat sample that contained no added carbohydrates, and that lactose is not affected by the enzyme. The addition of sucrose or dextrose to samples containing corn sirup had no effect on the test. It had previously been determined that the amylases do not hydrolyze sucrose or dextrose, a result confirmed by Table 2.

Determination of Dextrose

Many meat products contain added dextrose as well as corn sirup. Since both dextrose and maltose are components of corn sirup, there is some uncertainty in estimating added dextrose on a routine basis.

However, added dextrose can be estimated from the data obtained in the determination of corn sirup and of lactose. The procedure is relatively straightforward; from the total amount of Cu_2O obtained from an aliquot of the untreated meat solution, the amount of Cu_2O due to lactose (if present) in the same size aliquot is subtracted. Then, the amount of Cu_2O due to dextrose, maltose, and other carbohydrates in the corn sirup is subtracted. Table 3 was prepared by using values for dextrose and maltose in corn sirup as published in manufacturers' technical specification sheets and calculated for amount of Cu_2O from the Munson-Walker tables. When these data were averaged, corn sirups used in meat manufacturing processes were found to contain 17.2% dextrose and 13.2% maltose.

Table 3. Calculation of amount of Cu_2O due to dextrose, maltose, and other carbohydrates added to meat as components of corn sirup[a]

% Corn Sirup Found	mg Corn Sirup in 2 g Aliquot of Test Soln	Total mg Cu_2O in 2 g Aliquot of Test Soln[b]
0.6	12	20.8
0.8	16	23.5
1.0	20	26.0
1.2	24	28.4
1.4	28	30.6
1.6	32	33.5
1.8	36	35.1
2.0	40	36.8
2.2	44	39.4
2.4	48	43.2
2.6	52	45.7
2.8	56	47.5
3.0	60	50.1
3.2	64	51.5
3.4	68	54.3
3.6	72	56.6
3.8	76	58.9
4.0	80	61.3

[a] Additional values may be interpolated from the above table or calculated from the Munson-Walker tables based on average of 17.2% dextrose and 13.2% maltose in corn sirup plus 13.1 mg Cu_2O added to each value obtained as amount of Cu_2O due to carbohydrates other than dextrose and maltose.

[b] Includes 13.1 mg Cu_2O calculated as amount contributed by carbohydrates in corn sirup other than dextrose and maltose.

Values for Cuprous Oxide

The reducing properties of carbohydrates of longer chain length than maltose contribute some Cu_2O to the total. The value of 13.1 mg of Cu_2O (Table 3) for these carbohydrates was calculated from data obtained from analyses of samples shown in Table 4. The difference between the Cu_2O attributable to dextrose and maltose in corn sirup and the Cu_2O needed to give a theoretically perfect recovery of dextrose was calculated. The average value obtained from samples containing 0.5–2.0% of corn sirup was 13.1 mg. Although the amount of Cu_2O varies at each level, an average value is considered to be adequate for routine determinations.

The values for per cent corn sirup found in the sample and the amount of Cu_2O assigned to this amount of sirup are shown in Table 3. By calculation, a 2 g aliquot of

Table 4. Recovery of dextrose added to prepared frankfort samples also containing dextrose added as a component of corn sirup

Sample Contains[a]				
% Dextrose	% Skim Milk Powder	% Corn Sirup	% Dextrose Found	% Recovery
0.5	3.5	2.0	0.44	88.0
1.0	3.5	2.0	0.91	91.0
1.5	3.5	2.0	1.54	102.7
2.0	3.5	2.0	1.98	99.0
2.5	–	0.5	2.43	97.2
1.5	–	1.0	1.70	113.3
1.0[b]	–	2.0	0.97	97.0
2.0	–	3.0	2.06	103.0
				Av. 98.9 ± 2.8%

[a] Equivalent of 20 g frankfort per sample.

[b] Sample contains 1.0% sucrose.

an unknown sample yields 55.0 mg of Cu_2O from lactose and 1.6% from corn sirup. The total amount of Cu_2O for the 2 g untreated aliquot was 146.0 mg. The calculations are as follows:

	mg
Total Cu_2O	146.0
Cu_2O due to lactose	55.0
Difference	91.0
Cu_2O due to all carbohydrates in 1.6% corn sirup found in sample (Table 3)	33.5
Cu_2O due to added dextrose in 2 g aliquot	57.5

57.5 mg Cu_2O = 24.6 mg dextrose (Munson-Walker tables) = 1.23% added dextrose in sample.

A series of test samples was prepared, each containing corn sirup and dextrose; to some of these, skim milk powder and sucrose were also added. Excellent recoveries were obtained on the amount of added dextrose (Table 4).

By using the enzyme method for lactose (3), the proposed method for corn sirup, the proposed method for calculation of added dextrose, and the usual inversion methods for sucrose, the entire carbohydrate content of meat products may easily be determined under routine conditions.

Acknowledgments

We wish to thank Anheuser-Busch, Inc., American Maize Products Company, Clinton Corn Processing Company, Corn Products Company, A. E. Staley Manufacturing Company, and Union Starch and Refining Company for providing corn sirup samples; and inspectors of the Connecticut Department of Consumer Protection who collected corn sirup samples from meat processors in Connecticut. We are extremely grateful to Dr. C. I. Bliss, Station Biometrician, for help with statistical analysis of the data.

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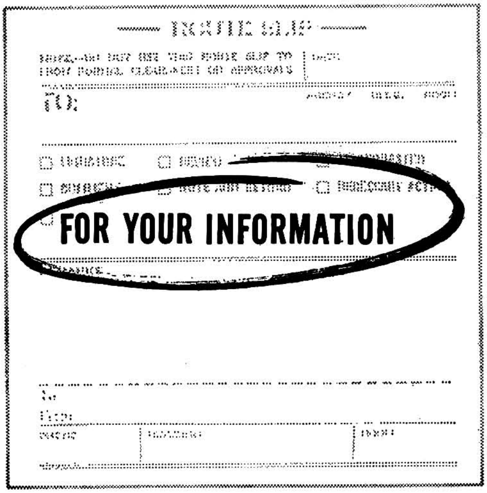
This paper was presented at the Seventy-eighth Annual Meeting of the Association of Official Agricultural Chemists, Oct. 19–22, 1964, at Washington, D.C.

CORRECTIONS

This Journal, **47**, 540 (1964), "A Standard Test for Efficacy of Germicides and Acceptability of Residual Disinfecting Activity in Swimming Pool Water," by L. F. Ortenzio and L. S. Stuart, p. **542**, *Operating Technique,* 1st para., lines 2–3; 2nd para., lines 8–10.	Change to read: "Add ca 1.5–3.0 ml $0.15M$ KH_2PO_4 **(e)**(*1*) and 0.5 ml $0.15M$ K_2HPO_4 **(e)**(*2*), and dilute to 900 ml. Change to read: "No. ml $0.001N$ sodium thiosulfate × 0.1773 = ppm residual available chlorine. This result represents the available chlorine at zero time in the test."
Ibid., **47**, 577 (1964), "Sterol Acetate Test for Foreign Fats in Dairy Products," by James H. Cannon, p. 577, right col., line 9.	Change to read: ". . . butter containing 10%, 2%, and 5% added oleomargarine, respectively."



FOR YOUR INFORMATION

Journal of the Association of Public Analysts

Contents for Volume 2, No. 2, of the Journal of the Association of Public Analysts, are as follows:

Analytical and technical contributions: R_f Values of Some Non-Permitted Synthetic Water-Soluble Colouring Matters, by D. Pearson and A. B. Chaudhri; Some R_f Values of Permitted Synthetic Water-Soluble Colouring Matters, by D. Pearson; Determination of Residual Chlorine in Swimming Bath Waters, by H. Amphlett Williams; The Determination of Sorbic Acid in Dried Prunes and Prunes in Syrup, by W. Carr and G. A. Smith; The Determination of Reserpine in Tablets, by P. F. G. Boon. Miscellaneous Contributions: Imported Tomato Products; Bell's 'Sale of Food and Drugs,' by E. C. Wood; Availability of Polenske Flasks.

Dr. McDonald Resigns as General Referee on Sugars

Dr. Emma J. McDonald, National Bureau of Standards, has resigned as AOAC General Referee on Sugars. She succeeded Dr. C. F. Snyder in this capacity in 1957, and had previously held Associate Refereeships on other aspects of sugar analysis. Dr. McDonald has been awarded the Industry Fellowship of the Cane Sugar Refining Research Project at the U.S. Department of Agriculture's Southern Regional Research Laboratory in New Orleans.

IN MEMORIAM

William L. Hill (1899–1964)

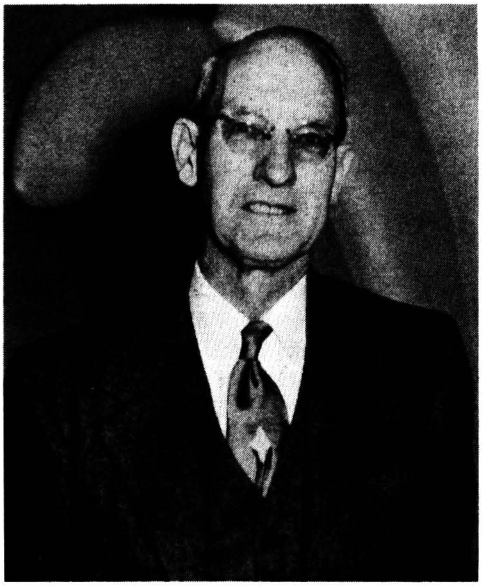
Mr. William L. Hill, AOAC Fellow and Director of the Department of Agriculture's U.S. Fertilizer Laboratory, died suddenly on July 17 after a heart attack at his home in Hillandale, Maryland.

Mr. Hill, affectionately known to his colleagues as Bill or Willie, served the Association for 35 years as a member of various committees and as an Associate Referee. Twenty of his many research reports and papers have been published in the AOAC Journal. His passing is a real loss to the Association, which valued his support and participation and always welcomed his sense of humor and lighter touch that enlivened many of our meetings.

Mr. Hill was born in 1899 in Chilhowie, Virginia. He was a graduate of Milligan College, Tennessee, where he served for several years as professor of physics and chemistry. He later obtained his M.S. in chemistry from the University of Virginia.

In 1928 Mr. Hill came to Washington and entered the Government service as a chemist in the former Bureau of Chemistry and Soils of the Department of Agriculture. He gained prominence both in this country and abroad for his studies of phosphate technology, phosphate utilization by plants, and fertilizer process development. He was made chief of Fertilizer Investigations Research Branch in 1959 and Director of the U.S. Fertilizer Laboratory in 1961.

Mr. Hill's active career included membership in the American Association for the Advancement of Science, the American Chemical Society, the Geochemical Society, the Geological Society, the Mineralogical Society of America, the Association of Official Agricultural Chemists, and the Association of American Fertilizer Control Officials.



Henry Albert Halvorson (1885–1964)

Henry A. Halvorson, a past president of the AOAC, passed away on June 24, 1964, at Williston, North Dakota at the age of 79.

Mr. Halvorson was born in Minneapolis, Minn. on January 17, 1885. He received his Bachelor of Science Degree in Chemistry with honors in 1911 from the University of Minnesota. Prior to his appointment in 1919 as Director and Chief Chemist for the then newly established Division of Feed Control in the Minnesota Department of Agriculture, he had been an assistant chemist for the Great Northern Railway; served for six years as first assistant chemist in the Food Laboratory of the Minnesota Dairy and Food Division; State Food and Drug Analyst for the Montana State Board of Health, at Bozeman, Mont.; and assistant chemist in the Food and Drug Administration, St. Louis, Mo. He held the position of Director in the Minnesota Department of Agriculture until his retirement on December 31, 1954.

Immediately after assuming the responsibilities in the Minnesota Department of Agriculture, Mr. Halvorson became active in the workings of the Association of Official Agricultural Chemists, American Association of Feed Control Officials, and later in the American Association of Fertilizer Control Officials. He held many assignments as referee and committee member of the AOAC and was its president in 1951.

He was also a member of the American Chemical Society, the American Association of Cereal Chemists, and the American Association for the Advancement of Science, in which organization he was elected a Fellow in 1937.

He and his associates have published, individually or jointly, approximately 225 bulletins, addresses, technical articles, and scientific papers.

Dr. Etheredge Resigns as General Referee on Feeds

Dr. M. P. Etheredge, Mississippi State University, has resigned as AOAC General Referee on Feeds, an assignment he has held since 1950. Dr. Etheredge has served the AOAC in numerous capacities as Associate Referee, General Referee, and member of various committees. He was president of the Association in 1957.

Dr. Haller Retires

H. L. Haller, who celebrated his 70th birthday on August 15, retired from the U.S. Department of Agriculture after more than 39 years as an outstanding public servant.

In 1919, Dr. Haller joined the Department as an Associate Chemist and, with the exception of an assignment with the Rockefeller Institute for Medical Research in New York City from 1923 to 1929, he has devoted his scientific and administrative abilities to the U.S. Department of Agriculture. He is recognized both here and abroad for his leadership and achievements in research on agricultural chemicals and the control of agricultural pests. Over 200 publications and 43 patents bear his name. In recent years, he has served as administrator of research programs and as consultant to a number of Government interagency committees and international organizations on agricultural chemicals, food additives, standards, and laws, and on the Common Market.

Dr. Haller has been honored by the USDA with a Distinguished Service Award, by the American Chemical Society with the Hillebrand Prize and the Charles F. Spencer Award, and by the War Department with a Certificate of Merit

Dr. Quackenbush Relinquishes Duties as State Chemist

Dr. Forrest W. Quackenbush, who for the last 21 years has been head of Purdue University's Department of Biochemistry and Indiana State Chemist, has requested that he be relieved of his administrative duties in order to devote full time to research and teaching.

In addition to his administrative duties, Dr. Quackenbush has maintained international standing in the area of fats and other lipids by his teaching and research in that field. He is currently investigating the role of fats and cholesterol in atherosclerosis and pathways of biosynthesis of the lipids.

As State Chemist, Dr. Quackenbush has been responsible for administering five state laws regulating the sale and distribution of feeds, fertilizers, seeds, plant growth substances, and herbicides. The aggregate value of the products distributed annually in Indiana by these industries is about $300 million.

He has been active in control work both regionally and nationally and served as president of the Association of Official Agricultural Chemists in 1963.

FDA Inspectors Receive Training at Rhode Island

The first university training course on drug manufacture for Federal drug inspectors was announced today by the Food and Drug Administration, U.S. Department of Health, Education, and Welfare, and the University of Rhode Island.

The course was held in the University's new $2,000,000 Fogarty Health Science Building, named in honor of Representative John E. Fogarty of Rhode Island. The building contains a modern pilot plant with the latest drug manufacturing equipment and is the newest such facility in the country.

The faculty provided by URI will include Dr. Sanford M. Bolton, associate professor of pharmacy; Dr. John DeFeo, professor of pharmacology and chairman of that department; Dr. Robert J. Gerraughty, associate professor and chairman of the Pharmacy Department; Dr. George E. Osborne, professor of pharmacy; Dr. James C. Price, assistant professor of pharmacy; Dr. Charles I. Smith, associate professor of pharmaceutical chemistry; Dr. Leonard R. Worthen, associate professor of pharmacognosy and public health; and Dean H. W. Youngken.

William W. Goodrich, Head of the Food and Drug Division, Office of General Counsel, U.S. Department of Health, Education, and Welfare, and others from FDA will lecture on the Kefauver-Harris Drug Amendments of 1962 and the various regulations governing manufacturing practice, new drugs, investigational new drugs, factory inspections, and related provisions.

Fellows of the AOAC

Forty-one scientists have been selected as Fellows of the Association of Official Agricultural Chemists, and will be presented with certificates at the Annual Meeting of the AOAC this October. The Committee on Fellows, with K. L. Milstead, Food and Drug Administration, as chairman, chose the forty-one from among present and past members of the AOAC who have given ten years or more of outstanding service to the Association.

Established by the Executive Committee of the AOAC in October 1961, the honorary status of Fellow of the AOAC is a means of recognizing meritorious service to the Association, particularly in development of methods of analysis for the variety of foods, drugs, beverages, cosmetics, agricultural materials, and allied products studied by the AOAC.

The first group of Fellows, announced this year, included all living ex-presidents of the Association and several scientists who have now retired from active work.

The certificate will read: "In recognition of outstanding professional contributions toward the development of analytical methods which are important to agriculture and public health."

The list of Fellows and their special fields of scientific contribution are as follows:

E. J. Benne (Plants)
Fred C. Blanck (Foods)
A. L. Brandon (Malt Beverages)
Jonas Carol (Drugs)
Ross A. Chapman (Preservatives)
Paul A. Clifford (Pesticide Residues)
Henry A. Davis (Fertilizers)
M. P. Etheredge (Feeds)
Harry J. Fisher (Drugs)
J. Fitelson (Flavors)
O. W. Ford (Fertilizers)
Guy G. Frary (Dairy Products)
J. J. T. Graham (Pesticides)
E. L. Griffin (Pesticides)
H. A. Halvorson (Vitamins)
H. H. Hanson (Feeds)
Henri D. Haskins (Fertilizers)
William L. Hill (Fertilizers)
Fred Hillig (Decomposition)
William Horwitz (Dairy Products)
K. D. Jacob (Fertilizers)
Henry A. Lepper (Foods)
George Marsh (Drugs)
A. P. Mathers (Alcoholic Beverages)
Richard T. Merwin (Drugs in Feeds)
K. L. Milstead (Foods)
V. E. Munsey (Cereals)
S. H. Newburger (Cosmetics)
Clyde L. Ogg (Microchemistry)
Robert A. Osborn (Foods)
F. W. Quackenbush (Fertilizers)
William F. Reindollar (Preservatives)
A. H. Robertson (Dairy Products)
Oswald Schreiner (Trace Elements)
John B. Smith (Fertilizers)
Carl F. Snyder (Sugars)
Al Steyermark (Microchemistry)
Peter J. Valaer (Alcoholic Beverages)
Frank A. Vorhes (Foods)
L. S. Walker (Feeds and Fertilizers)
C. O. Willits (Microchemistry)

ANNOUNCEMENTS

Feeds:

Ernest A. Epps, Jr., State Department of Agriculture and Immigration, P.O. Box 589, Baton Rouge, La., has been appointed as General Referee on Feeds to replace M. P. Etheredge, Mississippi State University, State College, Miss.

Plants:

Ronald J. Moquin, Food and Drug Administration, Detroit 7, Mich., has been appointed as Associate Referee on Polarographic Methods.

George R. Smith, Nova Scotia Department of Agriculture and Marketing, Truro, Nova Scotia, Canada, has resigned as Associate Referee on Calcium and Magnesium.

Nuts and Nut Products:

John P. Dempster, Food and Drug Administration, Denver 2, Colo., has been appointed as Associate Referee on Composition (Ash, Fat, Fiber, and Water).

Robert K. Munns, Food and Drug Administration, Denver 2, Colo., has been appointed as Asociate Referee on Hydrogenated Oils in Peanut Butter.

Eggs and Egg Products:

Leonard J. Stauffer, Food and Drug Administration, Buffalo 2, N.Y., has been appointed as Associate Referee on Updating Analytical Constants for Egg Content of Food.

Hazardous Substances:

Robert C. Bieneman, Food and Drug Administration, Baltimore 1, Md., has been appointed as Associate Referee on Pressure Generation in Containers.

Pesticides:

Ronald L. Andersen, Pacific Northwest Division, Reichhold Chemicals, Inc., P.O. Box 1482, Tacoma 1, Wash., has been appointed as Associate Referee on Pentachlorophenol (PCP).

Cosmetics:

Milda J. Bauza, Food and Drug Administration, Detroit 7, Mich., has been appointed as Associate Referee on Cold Permanent Waves.

Julius C. Prinzo, Food and Drug Administration, Philadelphia 6, Pa., has been appointed as Associate Referee on Deodorants and Antiperspirants.

Vegetable Drugs:

Charlotte Brunner, Food and Drug Administration, Washington 25, D.C., has been appointed as Associate Referee on Digitoxin and Related Glycosides.

Coffee and Tea:

Eleanor B. Archie, Food and Drug Administration, Philadelphia 6, Pa., has been appointed as Associate Referee on Moisture in Coffee and Tea.

Dairy Products:

Richard J. Ronk, Food and Drug Administration, Kansas City 6, Mo., has been appointed as Associate Referee on Comparison of FAO and AOAC methods.

Gelatin, Dessert Preparations, and Mixes:

James A. Barnett, Food and Drug Administration, Denver 2, Colo., has been appointed as General Referee on Gelatin, Dessert Preparations, and Mixes.

Cacao Products:

Armen V. Barooshian, Food and Drug Administration, Boston 9, Mass., has been appointed as Associate Referee on Pectic Acid.

Food Additives:

Elias B. Boyce, Food and Drug Administration, Boston 9, Mass., has been appointed as Associate Referee on Cheese Spreads (Gums).

Earl B. Detwiler, Food and Drug Administration, Washington 25, D.C., has been appointed as Associate Referee on Flexible Barrier Materials to replace E. P. Ragelis, of the same address.

Howard R. Smith, Food and Drug Administration, Washington 25, D.C., has been appointed as Associate Referee on Fumaric Acid in Foods.

John E. Wiskerchen, Food and Drug Administration, Minneapolis 1, Minn., has been appointed as Associate Referee on Sodium Lauryl Sulfate.

Miscellaneous Drugs:

James Myrick, Food and Drug Administration, St. Louis 1, Mo., has been appointed as Associate Referee on Benzocaine.

Jean A. Herbert, Food and Drug Administration, Detroit 7, Mich., has been appointed as Associate Referee on Glycols and Related Compounds.

John D. Ogger, Food and Drug Administration, Detroit 7, Mich., has been appointed as Associate Referee on Moisture in Drugs (Karl Fischer Method).

Carolyn A. Andres, Food and Drug Administration, Denver 2, Colo., has been appointed as Associate Referee on Qualitative Tests.

Phosphated Insecticides and Miticides:

Kermit M. Floyd, Food and Drug Administration, Atlanta 9, Ga., has been appointed as Associate Referee on Gas Chromatography (Coulometric Titrimeter).

W. Oscar Landen, Food and Drug Administration, Atlanta 9, Ga., has been appointed as Associate Referee on Gas Chromatography (Electron Capture).

Radioactivity:

Richard Velten, R. A. Taft Sanitary Engineering Center, 4676 Columbia Pkwy., Cincinnati 26, Ohio, has been appointed as Associate Referee on Iodine-131 to replace Conrad Straub of the same address.

Chlorinated Insecticides and Miticides:

Ronald E. Joyce, Food and Drug Administration, Buffalo 2, N.Y., has been appointed as Associate Referee on Chlorobenzilate.

Edna Elizabeth Roquemore, Food and Drug Administration, Atlanta 9, Ga., has been appointed as Associate Referee on Dichlorodiphenyldichloroethane (DDD, TDE).

Martin Eidelman, Food and Drug Administration, Washington 25, D.C., has been appointed as Associate Referee on Chlorinated Pesticides in Fats and Oils.

John R. Wessel, Food and Drug Administration, Baltimore 1, Md., has been appointed as Associate Referee on Heptachlor and Heptachlor Epoxide.

Preservatives and Artificial Sweeteners:

David J. Winters, Food and Drug Administration, Cincinnati 2, Ohio, has been appointed as Associate Referee on Fluorides.

Theodore J. Wazenski, Food and Drug Administration, Philadelphia 6, Pa., has been appointed as Associate Referee on Microbiological Tests for Preservatives.

Trean Korbelak, Food and Drug Administration, Philadelphia 6, Pa., has been appointed as Associate Referee on Paper and Thin Layer Chromatography of Artificial Sweeteners.

Decomposition and Filth in Foods (Chemical Indexes):

Paul McMillan Ward, Food and Drug Administration, Kansas City 6, Mo., has been appointed as Associate Referee on Acids.

David C. Holland, Food and Drug Administration, Denver 2, Colo., has been appointed as Associate Referee on Rancidity in Nuts.

Gin Farn, Food and Drug Directorate, Tunney's Pasture, Ottawa, Ontario, Canada, has resigned as Associate Referee on Uric Acid in Foods.

Fruits and Fruit Products:

Billy F. Hopkins, Food and Drug Administration, Los Angeles 15, Calif., has been appointed as Associate Referee on Isoascorbic Acid (Antioxidant) in Fruit Purees.

Fungicides, Fumigants, and Miscellaneous:

Paul E. Corneliussen, Food and Drug Administration, Minneapolis 1, Minn., has been appointed as Associate Referee on Biphenyl.

Terryl J. Farrell, Food and Drug Administration, Los Angeles 15, Calif., has been appointed as Associate Referee on Cyanides.

William G. Martin, Food and Drug Administration, Denver 2, Colo., has been appointed as Associate Referee on Sodium Monofluoroacetate.

Extraneous Materials:

Paris M. Brickey, Jr., Food and Drug Administration, Washington 25, D.C., has been appointed as Associate Referee on Cacao Products.

Richard Ruybal, Food and Drug Administration, Denver 2, Colo., has been appointed as Associate Referee on Cereal Grains, Cereal Products, and Confectionery.

Robert F. Brown, Food and Drug Administration, Boston 10, Mass., has been appointed as Associate Referee on Mushrooms.

Ramona S. Dorcas, Food and Drug Administration, Baltimore 1, Md., has been appointed as Associate Referee on Radiographic Examination of Spices and Food Seeds.

Metals and Other Elements:

David J. Winters, Food and Drug Administration, Cincinnati 2, Ohio, has been appointed as Associate Referee on Fluorine.

Robert J. Jakubiec, Food and Drug Administration, Detroit 7, Mich., has been appointed as Associate Referee on Lead.

Sugar and Sugar Products:

James B. Peterson, U.S. Customs Laboratory, New Orleans, La., has been appointed as General Referee on Sugar and Sugar Products.

Analytical Biology:

Sue Ellen Hays, Food and Drug Administration, Boston 9, Mass., has been appointed as Associate Referee on Filth in Dried Mushrooms.

BOOK REVIEWS

Food Chemicals Codex. Justin L. Powers, Director. Publication 1143, National Academy of Sciences-National Research Council, Washington, D.C., 1963. LC Cat. Card No. 63-65355. Loose-leaf binding. Part I, 114 pp. Part II, 48 pp. Part III, 49 pp. Index. Price $25.00, subscription basis.

Publication of the *Food Chemicals Codex* is an event of great significance in regulatory work. Although the Codex does not now have legal status, its importance will probably be considered comparable to that of the *United States Pharmacopeia.* Its function is to publish organized, national standards for food-grade chemicals and chemicals used in the processing of food.

As stated in the Preface, "The aim of the Codex is to define a substantial number of food-grade chemicals in terms of minimum identity and purity specifications based on the elements of safety and good manufacturing practice. It is expected that these quality standards will be acceptable to food processors as procurement and acceptance specifications and to primary manufacturers of chemicals as release specifications. It is hoped that they will be recognized in an appropriate manner by government food law enforcement agencies as adequate for defining food-grade chemicals."

The Codex is under the responsibility of the Food Protection Committee of the National Academy of Sciences-National Research Council, and its preparation is directed by Justin L. Powers. It is being issued over a period of two and a half years in eight to ten parts in loose-leaf form, on a subscription basis. Three parts, totaling 218 pages, have been issued to date. The completed Codex will eventually be published as a case-bound book.

Format of the *Food Chemicals Codex* is somewhat similar to that of the USP. A series of monographs is supplemented by sections on general tests and apparatus. The monographs are headed by a title (usually the common name of the chemical) and include the formula, the molecular weight, a description of the physical form of the material, identification and/or specifications, and tests. Specifications typically include assay, loss on drying, and limits of impurities. Tests vary, of course, according to the particular chemical, but in general they include assay, directions for packaging and storage, and a statement of the functional use of the chemical in foods, as well as one or more specific tests. The type format and the arrangement of the material are easy to read and follow, and the monographs have been carefully prepared.

As succeeding parts are issued, they will be announced in *This Journal.*

HELEN L. REYNOLDS

Antibiotic Producing Microscopic Fungi. By V. I. Bilai. Elsevier Publishing Co., New York, 1963. vii + 215 pp. Price $11.00.

This book was written by the Soviet scientist, V. I. Bilai. However, unless one is already familiar with Dr. Bilai, he can only infer that he is Russian. The cover sheets give no information about him; a statement of his affiliations would provide the reader with a background from which to evaluate his information as compared with that of the Western scientists.

Antibiotics produced by the following genera are described in separate chapters: Penicillin, Aspergillus, Cephalosporium, Trichoderma, Trichothecium, Alternaria, and Fusarium. One chapter briefly discusses antibiotics produced by miscellaneous genera such as Chaetomium, Gliocladium, Stereum, and Ustilago. These chapters provide useful information on chemical, antimicrobial, and toxicological properties of the antibiotics produced.

There is a final and very interesting chapter on the antibiotic properties of the toxins of fungi and the possibilities of their use, ranging from the treatment of malignant tumors to the inhibition of the tobacco mosaic virus.

The text does not include the Strepto-

SUBSCRIPTION RENEWAL

Subscriptions to the Journal of the Association of Official Agricultural Chemists expire with the December Issue. Volume 48 will appear in six numbers: February, April, June, August, October, and December. The price is $15.00 ($16.00, foreign).

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mycetes. While these organisms may not be true fungi, they are so closely related that one would expect them to be included. With the exception of penicillin, most of the commonly used antibiotics such as the tetracyclines, streptomycin, chloramphenicol, erythromycin, and novobiocin are produced by Streptomycetes.

Regardless of this omission, the text contains information of considerable value to scientists working in the general fields of antibiotics as well as to mycologists interested in the activities of the fungi.

AMIEL KIRSCHBAUM

Composition of Foods. Raw, Processed, Prepared. By Bernice K. Watt and Annabell L. Merrill. Agriculture Handbook No. 8. U.S. Department of Agriculture. For sale by Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Revised December 1963. 190 pp. Price $1.50.

Agriculture Handbook No. 8 is well known to nutritionists, home economists, and food chemists as a primary source of composition data on familiar foods. This edition covers 2500 primary entries with a number of subentries. New tables include information on selected fatty acids, cholesterol, and magnesium content of food. The difficulties involved in compiling and revising such extensive compilations are so immense that the authors in the Department of Agriculture can only be congratulated in continuing this most useful compilation, which should be available to all food chemists.

WILLIAM HORWITZ

The Pollution of Water by Detergents. By J. Prat and A. Giraud. O.E.C.D. publication No. 16.601/March 1964. 86 pp., paper bound. 244 bibliographic references. Available in English or French. Free of charge, upon request to the Organization for Economic Co-operation and Development, 2 rue André Pascal, Paris (16ème).

This publication is a review summarizing and analyzing the many studies published to date on the pollution of water by synthetic detergents of the anionic type. The book is divided into eight parts: general remarks, nuisances resulting from the use of detergents, toxicity to man and animals, determination of detergents in water, ability to undergo biological degradation, measures taken or envisaged by the public authorities, ways of combating nuisances caused by the pollution of water by detergents, conclusions, and economic considerations. In the course of their review of present knowledge, the authors have attempted to bring out the different aspects which might require further investigation.

DRUGS

Infrared Spectra of Some Compounds of Pharmaceutical Interest

By OSCAR R. SAMMUL, WILSON L. BRANNON, and ALMA L. HAYDEN (Division of Pharmaceutical Chemistry, Food and Drug Administration, Washington, D.C. 20204)

This compilation of infrared absorption spectra is intended as a supplement to the spectra of the USP and NF Reference Standards published in *This Journal,* **45,** 797 (1962). This second group is composed of new and nonofficial drugs, USP and NF items, solvents, and reagents.

In general the samples were prepared and the spectra were recorded by the techniques and under the conditions which were described in the earlier publication. Most of the solids were observed in potassium halide disks after recrystallization from various solvent systems. The spectra of some compounds were obtained in carbon disulfide, tetrachloroethylene, and chloroform solutions in 1 mm NaCl cells. Other compounds were studied as films, paraffin oil or hexachlorobutadiene mulls, and vapors. The spectra of the vapors were obtained in a 10 cm gas cell, at room temperature and reduced pressure.

The spectra are arranged in general alphabetical order and the experimental conditions, namely, crystallization solvent(s) and infrared medium, are given in Table 1. These spectra and those included in the earlier publication have been incorporated in the Termatrex System for data storage and retrieval (Jonkers Business Machines Corporation). Numbers following the conditions for each compound in the table are Termatrex code numbers.

Acknowledgments

The technical assistance of Stephen R. Seater, J. Donald Weber, Alden H. Reine, and Ruth Kessler, and the editorial assistance of Marie C. Talley and Helen L. Reynolds are greatly appreciated.

The authors gratefully acknowledge the compounds and spectra which were contributed by Millard Maienthal, Llewellyn H. Welsh, and others of this Division.

Table 1. Drugs and their spectral conditions

Name	No.	Conditions	Retrieval No.	Page No.
Acacia	208	Direct KBr	0032	925
Acetic Acid	209	Film	1042	925
Acetone	213	Vapor	1031	926
Acetophenazine Dimaleate	214	EtOH; KBr	8200	926
Acetophenetidine	210	$CHCl_3$; KBr	4087	925
N-Acetyl-*d*-amphetamine	212	C_6H_6-petr ether; KBr	8233	925
N-Acetyl-*dl*-amphetamine	211	C_6H_6-petr ether; KBr	8232	925
Acetyl Salicylic Acid	215	Direct KBr	3018	926
N^4-Acetylsulfanilamide	216	Me_2CO; KBr	4062	926
Akineton Hydrochloride	217	Direct KCl	8108	926
Aluminum Acetate	218	Direct KBr	1020	927
Alvodine Base	219	Direct KBr	8107	927
dl-Amidon Hydrochloride	220	$CHCl_3$; KCl	4061	927
Aminoacetic Acid	228	EtOH-H_2O; KBr	2051	929
2-Amino-5-chlorobenzophenone	222	Direct KBr	4070	927
4-Amino-6-chloro-*m*-benzene-disulfonamide	221	Direct KBr	4084	927
Aminophylline	223	Direct KBr	8137	928
p-Aminosalicylic Acid	224	H_2O; KBr	4059	928

(*Continued*)

Table 1. *(Continued)*

Name	No.	Conditions	Retrieval No.	Page No.
Ammonium Chloride	225	Direct KCl	0014	928
Amobarbital	229	C_6H_6; KBr	8230	929
d-Amphetamine Hydrochloride	226	Direct KBr	4094	928
n-Amyl Alcohol	230	Film	1041	929
n-Amyl Alcohol	231	CS_2 solution	1052	929
Amylene Hydrate	227	Vapor	1026	928
Amyl Metacresol	232	Film	3021	929
delta-5-Androstene-3-beta-17-beta-diol	234	Direct KBr	6055	930
delta-5-Androstene-3-beta-ol-17-one	236	CS_2 solution	6054	930
delta-4-Androstene-3,17-dione	237	CS_2 solution	6052	930
delta-4-Androstene-3,17-dione	235	Direct KBr	6053	930
Angiotensin Amide	238	Direct KBr	8213	931
Anhalamine Hydrochloride	239	Direct KCl	7020	931
Anhalonine Hydrochloride	240	Direct KCl	8124	931
Ansolysen Bitartrate	242	Direct KBr	8106	931
Anthracene	241	Direct KBr	3051	931
Anthralin	243	$CHCl_3$ solution	3048	932
Anthrone	233	$CHCl_3$-C_7H_{16} (1:3); KBr	3052	930
Antipyrine	244	EtOH-C_7H_{16} (1:3); KBr	8138	932
Apomorphine Hydrochloride	245	Direct KCl	8139	932
Aprobarbital	246	EtOH-H_2O (1:1); KBr	8182	932
Azaphenothiazine	249	EtOH-C_7H_{16} (1:3); KCl	8189	933
Aspirin Anhydride	247	EtOH-H_2O (95:5); KBr	3019	932
Atropine	248	Direct KBr	7012	933
Barbital	250	H_2O; KBr	8195	933
Bentyl Analog	251	EtOH-C_7H_{16} (1:3); KCl	2041	933
Benzaldehyde	253	Film	3043	934
Benzoic Acid	252	CS_2 solution	3023	933
Benzoic Acid	254	Direct KBr	3038	934
Benzoin	255	H_2O-Me_2CO; KBr	3039	934
Benzphetamine Hydrochloride	258	Direct KCl	4073	935
Benzthiazide	259	Direct KBr	8172	935
Benztropine Methanesulfonate	260	Direct KBr	8196	935
Benzyl Alcohol, Redistilled	256	CS_2, C_2Cl_4 solutions	3045	934
Benzyl Alcohol, Redistilled	257	Film	3044	934
Betamethasone	261	Direct KBr	6071	935
Betazole Hydrochloride	262	Mull	8167	935
d-Brompheniramine Maleate	263	Direct KBr	8104	936
Bunamiodyl	264	EtOH-C_7H_{16} (1:3); KBr	4066	936
Butabarbital	265	CH_3OH-C_7H_{16} (1:3); KBr	8212	936
Butethamine Hydrochloride	266	EtOH; KCl	4063	936
n-Butyl Alcohol	267	Vapor	1027	936
sec-Butyl Alcohol	268	Vapor	1028	937
Butylated Hydroxytoluene	269	CS_2, C_2Cl_4 solutions	3030	937
Caffeine	270	Direct KBr	8087	937
Calcium Cyanamide	271	Direct KBr	0010	937
Camphor	272	CS_2, C_2Cl_4 solutions	1036	937
Camphor-10-sulfonic Acid	273	EtOAc; KBr	2019	938
Caramiphen Ethanedisulfonate	274	Me_2CO-C_7H_{16} (1:1); KBr	2021	938
Carbimazole	275	EtOH; KBr	8102	938
Carbocaine Hydrochloride	276	Direct KCl	8155	938
Carbon Disulfide	292	Vapor	2055	941
Cardrase	277	Me_2CO-C_7H_{16} (1:3); KBr	8108	938
Castor Oil	278	Film	0023	939
Cellulose	279	Direct KBr	1010	939
Chlordantoin	280	CS_2, C_2Cl_4 solutions	8204	939
Chlorhexidine Dihydrochloride	281	Direct KCl	4042	939
Chloroprocaine Hydrochloride	282	EtOH-C_7H_{16} (1:3); KCl	4041	939
Chlorpropamide	283	EtOH-C_7H_{16} (1:3); KBr	4037	940
Cholesterol	284	CS_2,C_2Cl_4 solutions	6076	940

(Continued)

Table 1. *(Continued)*

Name	No.	Conditions	Retrieval No.	Page No.
Cholesterol	285	Et_2O; KBr	6072	940
Cinchonidine	288	C_6H_6; KBr	7035	941
Cinchonine	289	C_6H_6; KBr	7036	941
Citric Acid	286	C_3H_7OH; KBr	1023	940
Codeine	287	CS_2, C_2Cl_4 solutions	7027	940
Codeine	293	CS_2; KBr	7014	942
Codeine Phosphate	294	EtOH-H_2O (9:1); KBr	7028	942
Corn Oil	290	Film	0035	941
meta-Cresol	291	Film	3040	941
Cyclothiazide	295	EtOH-C_7H_{16} (1:3); KBr	8205	942
Cyproheptadine Hydrochloride	296	Me_2CO-C_7H_{16} (1:3); KCl	8109	942
Dequadin Chloride	297	CH_3OH-C_7H_{16}-C_3H_7OH (0.5:0.5:3.0); KCl	8113	942
Dequalinium Chloride	298	Direct KCl	8110	943
Desoxycorticosterone Acetate	299	CS_2 solution; $CHCl_3$	6032	943
Desoxycorticosterone Trimethylacetate	300	CS_2, C_2Cl_4 solutions	6077	943
Desoxyephedrine Hydrochloride	301	Direct KCl	4060	943
Dexamethasone	302	Me_2CO-isoC_8H_{18} (1:3); KBr	6038	943
Dexamethasone Acetate	303	Me_2CO-isoC_8H_{18} (1:3); KBr	6036	944
Dextrochlorpheniramine Maleate	304	Direct KBr	8099	944
Dextrose, Anhydrous	306	Direct KBr	1014	944
Diacetylchondocurarine Iodide	305	(H_2O with added KI) KI	7038	944
N,O^3-Diacetylphenylephrine	313	C_6H_6; KBr	4093	946
N,O-Diacetyl-*p*-hydroxyamphetamine	307	CCl_4; KBr	4057	944
Dibenzothiophene	308	EtOH-C_7H_{16} (1:3); KBr	8210	945
Dihydrochlorothiazide	309	EtOH-C_7H_{16} (1:3); KBr	8118	945
1,8-Dihydroxyanthraquinone	314	$CHCl_3$ solution	3049	946
Dilabron Methanesulfonate	310	EtOH-MeOH-isoC_8H_{18} (1:1:5); KBr	4043	945
Dimethoxinate Hydrochloride	312	Direct KCl	8112	945
p,p-Dimethoxydiphenylacetylene	315	Glacial CH_3COOH; KBr	3050	946
Dimethpyrindene Maleate	318	Direct KBr	8174	947
Dimethylaminopropyl-*d*-camphidine Dimethyl Sulfate	311	Direct KBr	7024	945
3,5-Dimethyl-2-nitroanisole	316	Film	4095	946
3,5-Dimethyl-4-nitroanisole	317	EtOH-H_2O; KBr	4101	946
Diphenylhydantoin	319	Direct KBr	8148	947
Diphenylpyraline Hydrochloride	320	Direct KCl	8100	947
Dromostanolone Propionate	321	EtOH-isoC_8H_{18} (1:9); KBr	6066	947
Dynacaine Hydrochloride	322	Direct KCl	7025	947
Ectylurea	323	$CHCl_3$-C_7H_{16} (2:3); KBr	2024	948
Enovid	324	CS_2 solution	6057	948
Enovid	325	EtOH-C_7H_{16} (1:3); KBr	6056	948
Ephedrine Sulfate	326	C_3H_7OH-Et_2O-H_2O-isoC_8H_{18} (4.5:5.0:0.5:40.0); KBr	7011	948
Equilenin	328	EtOH; KBr	6059	949
Equilin	327	EtOH; KBr	6058	948
Erythritol Tetranitrate	329	$CHCl_3$; KBr	2032	949
Erythromycin Propionate	330	Et_2O-isoC_8H_{18} (0.3:3.7); KBr	2022	949
beta-Estradiol	331	Direct KBr	6060	949
Estradiol Dipropionate	332	CS_2, C_2Cl_4 solutions	6079	949
Ethanol	333	Vapor	1022	950
Ether	334	Vapor	1025	950
Ethinyl Estradiol-3-benzoate	335	MeOH; KBr	6062	950
Ethinyl Nortestosterone	336	EtOH-C_7H_{16} (1:3); KBr	6037	950
Ethyl Nitrate	337	CS_2 solution	2038	950
2-Ethyl Thioisonicotinamide	338	EtOH-C_7H_{16} (1:7); KBr	8176	951
Ethynyl Estradiol-3-methyl ether	339	EtOH-C_7H_{16} (1:5); KBr	6035	951

(Continued)

Table 1. (*Continued*)

Name	No.	Conditions	Retrieval No.	Page No.
Etryptamine Acetate	340	EtOH-C_7H_{16} (1:3); KBr	8175	951
Eugenol	341	Film	3025	951
Ferric Sodium Ethylenediamine Tetraacetate	342	Direct KBr	2026	951
Fluocinolone Acetonide	343	Direct KBr	6068	952
5-Fluorouracil	344	Direct KBr	8165	952
Fluoxymesterone	345	EtOAc-C_7H_{16} (1:1); KBr	6034	952
Fluphenazine Dihydrochloride	346	EtOH-C_7H_{16} (1:3); KCl	8236	952
Fluphenazine Dihydrochloride	347	Direct KCl	8157	952
Flurandrenolone	348	$CHCl_3$-C_7H_{16} (1:7); KBr	6075	953
Formamide	349	Film	2030	953
Gantanol	350	Direct KBr	8178	953
Gitogenin	351	EtOH; KBr	6063	953
Glycerin	352	Film	1009	953
Glycopyrrolate	353	Direct KBr	8199	954
Griseofulvin	354	EtOH-C_7H_{16} (1:3); KBr	8119	954
Guaiacol	355	Film	3026	954
1,3-Hexachlorobutadiene	356	Film	1038	954
Hexachlorophene	357	CS_2, C_2Cl_4 solutions	3032	954
Hexadimethrine Bromide	358	Direct KBr	2023	955
Hexestrol	359	Direct KBr	3022	955
Hexetidine	360	Film	8116	955
Hexocyclium	361	$CHCl_3$-C_7H_{16} (1:3); KBr	8209	955
Hexylethyl Barbituric Acid	362	Direct KBr	8149	955
Hydrocortamate	364	Direct KBr	6039	956
Hydrocortisone-21-phosphate Disodium Salt	365	Direct KBr	6040	956
Hydroflumethiazide	366	Direct KBr	8117	956
17-Hydroxydesoxycorticosterone	367	Direct KBr	6064	956
3-Hydroxymercuri-2-methoxysuccinimido-propane Theophyllinate	368	Direct KBr	9501	957
2-Hydroxy-2-phenylethylcarbamate	369	EtOH-isoC_8H_{18} (1:10); KBr	4049	957
Hyoscine Hydrobromide	363	Direct KBr	7018	956
Ipodate Calcium	371	Direct KBr	4071	957
Ipodate Sodium	372	Direct KBr	4072	957
Iso-octane	370	Film	1053	957
Isopregenone	373	Direct KBr	6073	958
Isopropamide Iodide	374	C_6H_6-MeOH-isoC_8H_{18} (1:1:10); KI	4044	958
Isosorbide Dinitrate	375	Et_2O; KBr	2037	958
Isuprel Ethanesulfonate	376	Direct KBr	4046	958
Kanamycin Sulfate	377	Direct KBr	2027	958
Lactose	378	Direct KBr	1013	959
Levarterenol Bitartrate	379	Direct KBr	4015	959
Librium base	380	CS_2, C_2Cl_4 solutions	8169	959
Librium Hydrochloride	381	Direct KCl	8170	959
Linoleic Acid	383	Film	1050	960
Lophophorine Hydrochloride	382	Direct KCl	7019	959
Mannitol Hexanitrate	384	Direct KBr	2034	960
Menadione	385	CS_2, C_2Cl_4 solutions	3047	960
Menthol	386	$CHCl_3$ solution	1006	960
Mephenoxalone	387	Me_2CO-C_6H_{14} (1:3); KBr	8171	960
Meprobamate	388	H_2O; KBr	2031	961

(*Continued*)

Table 1. (*Continued*)

Name	No.	Conditions	Retrieval No.	Page No.
Mescaline Hydrochloride	389	Direct KCl	4039	961
Mescaline Sulfate	390	Direct KBr	7040	961
Meta-Butethamine Hydrochloride	409	EtOH; KCl	4064	965
Metaxalone	391	$\mathrm{Me_2CO}$; KBr	8177	961
Methalamic Acid	392	iso$\mathrm{C_3H_7OH}$-$\mathrm{C_7H_{16}}$ (1:3); KBr	4076	961
Methanol	393	Vapor	1032	962
Methaqualone	394	Direct KBr	8198	962
Metharbital	395	EtOH; KBr	8039	962
Methenamine Mandelate	396	Direct KBr	8129	962
Methenamine Undecylenate	397	Melt	8094	962
Methocarbamol	398	EtOH-$\mathrm{C_7H_{16}}$ (0.4:0.6); $\mathrm{C_7H_{16}}$ (1:5); KBr	4047	963
Methoxyflurane	399	Film	1035	963
p-Methoxythiobenzaldehyde	410	Direct KBr	4096	965
Methsuximide	407	EtOH; KBr	8231	964
Methyl Acetate	400	Vapor	1033	963
6-alpha-Methyl-17-acetoxy-progesterone	401	Direct KBr	6041	963
Methyldopate Hydrochloride	411	Direct KCl	4103	965
6-Methylhydrocortisone Acetate	402	EtOH-iso$\mathrm{C_8H_{18}}$-iso$\mathrm{C_8H_{18}}$ (0.2:0.5:5.0); KBr	6082	963
3-Methyl-4-nitroanisole	412	EtOH-$\mathrm{H_2O}$; KBr	4097	965
3-Methyl-3-pentanol Carbamate	403	EtOH (30%); KBr	2025	964
6-Methyl-prednisolone	404	*n*$\mathrm{C_3H_7OH}$-iso$\mathrm{C_8H_{18}}$-iso$\mathrm{C_8H_{18}}$ (0.2:0.8:5.0); KBr	6042	964
Methyl Testosterone	405	$\mathrm{CS_2}$, $\mathrm{C_2Cl_4}$ solutions	6047	964
Metopirone	406	Direct KBr	8179	964
Mikedimide	408	EtOH-iso$\mathrm{C_8H_{18}}$-iso$\mathrm{C_8H_{18}}$ (0.2:0.8:5.0); KBr	8128	965
Neomycin Undecylenate	414	$\mathrm{CHCl_3}$-$\mathrm{C_7H_{16}}$ (1:3); KBr	0020	966
Nialamide	415	Direct KBr	8130	966
Nicotinic Acid	416	Mull	8168	966
p-Nitrofluorobenzene	413	Film	4100	966
Nitroglycerine	417	$\mathrm{CS_2}$ solution	2033	966
Nitromethane	418	Vapor	2045	967
19-Nor-delta-4-androstene-17-beta-ol-3-one-beta-phenylpropionate	419	Direct KBr	6045	967
Norethisterone Acetate	420	EtOH-iso$\mathrm{C_8H_{18}}$ (1:10); KBr	6044	967
Nostal	421	EtOH-$\mathrm{H_2O}$; KBr	8184	967
Olive Oil	422	Film	0038	967
Orphenadrine Citrate	423	Direct KBr	4050	968
Orphenadrine Hydrochloride	424	Direct KCl	4048	968
Oxyphencyclimine Hydrochloride	426	EtOH-$\mathrm{C_7H_{16}}$ (1:3); KBr	8154	968
Oxytetracycline	425	Mull	4069	968
Palmitic Acid	427	$\mathrm{CS_2}$ solution	1045	968
Palmitic Acid	428	$\mathrm{CS_2}$; KBr	1044	969
Parabromdylamine Maleate	429	Direct KBr	8089	969
Paraffin Oil	430	Film	0025	969
Peanut Oil	483	Film	0033	980
Pentaerythritol Tetranitrate	432	EtOH-$\mathrm{Me_2CO}$; KBr	2035	969
Pentamethylene Tetrazole	431	Direct KBr	8153	969
Pentobarbital	433	$\mathrm{C_6H_6}$-$\mathrm{C_7H_{16}}$; KBr	8152	970
Pentobarbital	434	$\mathrm{C_2Cl_4}$ solution	8147	970
Perphenazine	435	Direct KBr	8090	970
Persantin	436	iso$\mathrm{C_3H_7OH}$-$\mathrm{C_7H_{16}}$ (1:4); KBr	8097	970
Phenazocine Hydrobromide	437	Direct KBr	8125	970
Phendimetrazine Bitartrate	438	iso$\mathrm{C_3H_7OH}$-$\mathrm{C_7H_{16}}$ (1:3); KBr	8201	971

(*Continued*)

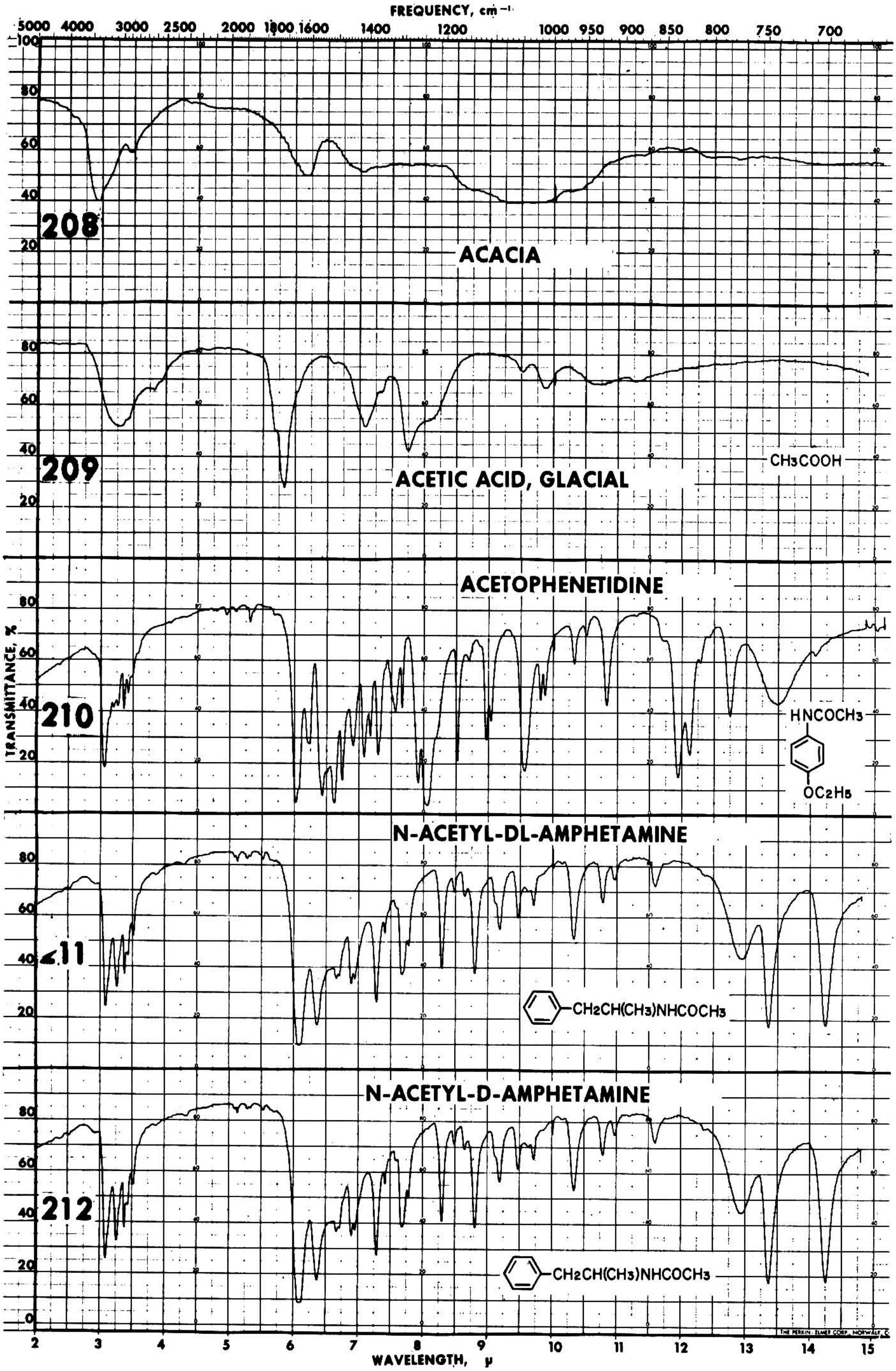
Table 1. (*Continued*)

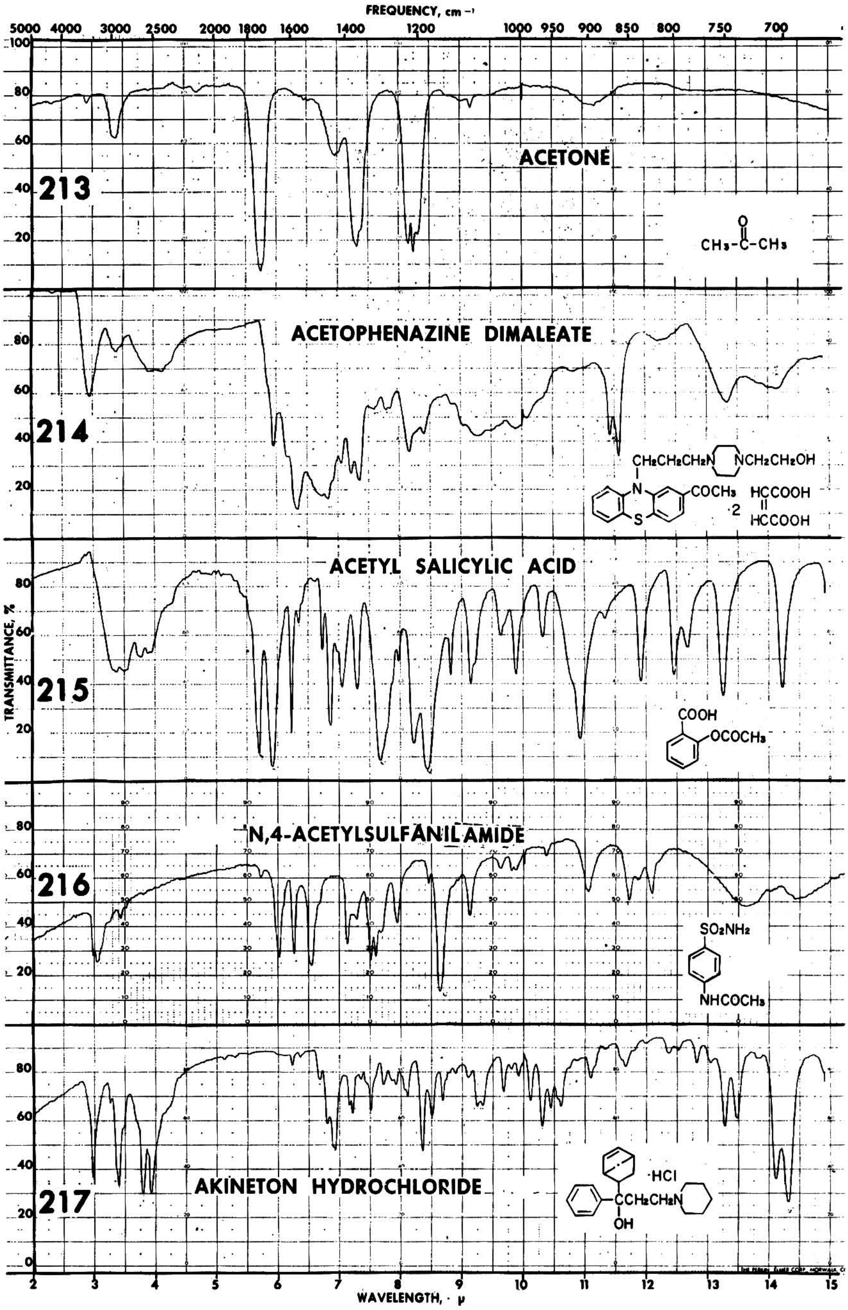
Name	No.	Conditions	Retrieval No.	Page No.
Phenformin Hydrochloride	439	isoC_8H_{18}-C_3H_7OH-isoC_8H_{18} (0.6:0.4:5.0); KCl	4036	971
Pheniramine	440	CS_2, C_2Cl_4 solutions	8211	971
Phenobarbital	441	C_6H_6-C_7H_{16}; KBr	8140	971
Phenobarbital	442	$CHCl_3$; KBr	8173	971
Phenobarbital	443	C_6H_6-MeOH (1:1); KBr	8187	972
Phenobutiodil	444	CS_2, C_2Cl_4 solutions	3031	972
Phenobutiodil	445	Direct KBr	3035	972
Phentolamine Methanesulfonate	446	Mull	8190	972
Phenylephrine Hydrochloride	447	nC_3H_7OH-isoC_8H_{18} (0.4:0.6); KCl	4035	972
Phenylpropanolamine Hydrochloride	448	EtOH-isoC_8H_{18} (1:3); KCl	4033	973
Phenyl-tert-butylamine Hydrochloride	449	CH_3OH-C_6H_6-isoC_8H_{18}-C_8H_{16} (0.2:0.2:0.6:5.0); KCl	4068	973
Phenylramidol Hydrochloride	450	C_3H_7OH-isoC_8H_{18} (1:3); KCl	8093	973
Phenylramidol Salicylate	451	Direct KBr	8115	973
Physostigmine	452	$CHCl_3$; KBr	7017	973
Picric Acid	453	EtOH; KBr	4065	974
Piperidolate	454	$CHCl_3$-isoC_8H_{18}-C_8H_{18} (0.5:0.5:5.0); KBr	8098	974
Placidyl	455	Film	1016	974
Poldine Methosulfate	456	Direct KBr	8088	974
Polyethylene Glycol tert-Dodecylthioether	457	C_2Cl_4 solutions	2042	974
Polythiazide	458	Direct KBr	8197	975
Potassium Aluminum Sulfate	478	Direct KBr	0039	979
Potassium Aspartate	459	Direct KBr	2028	975
Potassium Bromate	460	Direct KBr	0015	975
Potassium Chlorate	461	Direct KBr	0027	975
Potassium Iodate	462	Direct KBr	0028	975
Potassium Perchlorate	463	Direct KBr	0018	976
Potassium Periodate	464	Direct KBr	0019	976
Potassium Sulfate, Anhydrous	479	Direct KBr	0030	979
Potassium Warfarin	465	Direct KBr	8096	976
Potato Starch	466	Direct KBr	1015	976
Pregnenolone Acetate	467	EtOAc-isoC_8H_{18}-isoC_8H_{18} (0.5:0.5:5.0); KBr	6043	976
Preludin	468	Direct KBr	8091	977
Probarbital	469	EtOH-H_2O; KBr	8185	977
Procaine Hydrochloride	470	Direct KCl	4051	977
Progesterone	471	CS_2, C_2Cl_4 solutions	6049	977
Proketazine Maleate	472	EtOH-C_7H_{16} (2:3); KBr	8206	977
1,2-Propanediol Acetate	473	Film	1004	978
Propanol	474	Vapor	1029	978
1-(4-hydroxyphenyl)-1-Propanone	475	nC_3H_7OH-C_7H_{16}-nC_7H_{16} (0.2:0.8:4.0); KBr	3017	978
Propionyl Erythromycin Lauryl Sulfate	476	nC_3H_7-isoC_8H_{18}-isoC_8H_{18} (0.2:0.8:3.0); KBr	2020	978
1-Propoxyphene-2-naphthalene Sulfonate Monohydrate	480	Direct KBr	4034	979
1-Propoxyphene-N-oxide-hydrochloride	477	Direct KCl	4032	978
Prothipendyl Hydrochloride	481	nC_3H_7OH-isoC_8H_{18}-isoC_8H_{18} (0.2:0.8:4.0); KCl	8095	979
Pyridine	482	Film	4099	979
Quinethazone	484	Direct KBr	8180	980
Quinidine	485	$CHCl_3$ solution	7013	980
Salicylic Acid	486	Direct KBr	3020	980
Sandoptal	487	EtOH-H_2O; KBr	8186	980

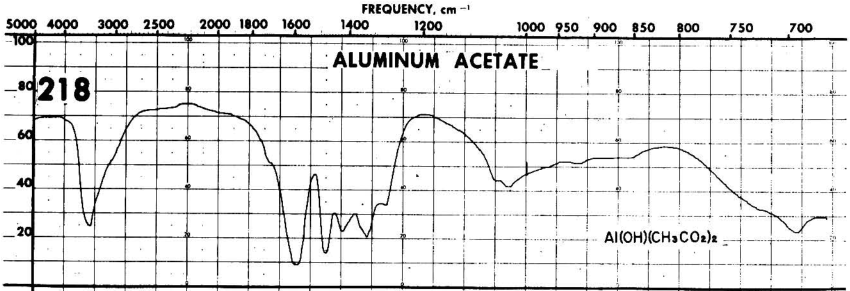
(*Continued*)

Table 1. (*Continued*)

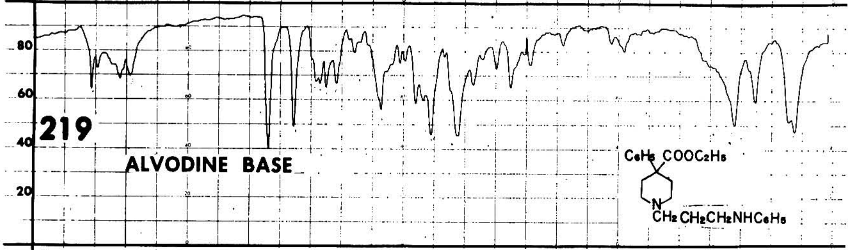
Name	No.	Conditions	Retrieval No.	Page No.
Scopolomine	488	CS_2 solution	7015	981
Secobarbital Sodium	489	Direct KBr	8150	981
Sitosterols	490	CS_2, C_2Cl_4 solutions	0036	981
beta-Sitosterol	491	CS_2, C_2Cl_4 solutions	6083	981
beta-Sitosterol	492	Et_2O; KBr	6084	981
Sodium Diacetate	503	Direct KBr	1019	984
Sodium Lauryl Sulfate	493	Direct KBr	2039	982
Sodium Nitrate	494	Direct KBr	0012	982
d-Sorbitol	495	EtOH; KBr	1017	982
Soya Sitosterols	496	CS_2, $CHCl_3$ solutions	0024	982
Soybean Oil	504	Film	0034	984
Sparteine Sulfate	497	Direct KBr	8127	982
Spironolactone	498	Me_2CO-isoC_8H_{18} (1:4); KBr	6050	983
Stanozolol	499	$CHCl_3$-*n*C_7H_{16} (1:3); KBr	6081	983
Starch	500	Direct KBr	1011	983
Stearic Acid	505	CS_2 solution	1043	984
Stearic Acid	506	CS_2; KBr	1046	984
Stearic Acid	501	Direct KBr	1007	983
Stigmasterol	502	CS_2, C_2Cl_4 solutions	6051	983
Succinyl Choline Picrate	507	H_2O, KBr	4098	984
Sucrose	508	Direct KBr	1012	985
Sulfachloropyridazine	509	Direct KBr	8122	985
Sulfamethazole	510	EtOH-isoC_8H_{18}-isoC_8H_{18} (1:4:5); KBr	8133	985
Sulfaphenazole	511	EtOH-isoC_8H_{18}-isoC_8H_{18} (2:8:5); KBr	8132	985
Sulfobromophthalein Sodium	512	Direct KBr	4053	985
Taractan	513	Direct KBr	8181	986
Tenuate	514	Direct KCl	4056	986
Testosterone	515	isoC_8H_{18}; KBr	6074	986
Testosterone	516	CS_2, C_2Cl_4 solutions	6080	986
Testosterone Propionate	517	CS_2, C_2Cl_4 solutions	6078	986
Tetrachloroethylene	518	Film	1018	987
Tetrachloroethylene	519	Liquid cell, 1 mm	1039	987
Thalidomide	520	Direct KBr	8126	987
Theobromin	521	EtOH, KBr	7016	987
Thiethylperazine Dimaleate	522	Direct KBr	8191	987
Thioguan(ine)	523	Direct KBr	8183	988
Trans-Stilbene	525	EtOH; KBr	3041	988
Thioridazine Hydrochloride	526	Direct KCl	8161	988
Thonzonium Bromide	524	Direct KBr	8134	988
Thymol	527	Sublimed (vacuum); KBr	3029	988
Tofranil	528	EtOH-C_7H_{16} (1:3); KBr	8121	989
O^3,O^4-*n*-Triacetyl-1-epinephrine	529	Me_2CO-Et_2O; KBr	4058	989
O^3,O^4-*n*-Triacetyl-1-norepinephrine	530	Me_2CO-C_6H_5Me; KBr	7023	989
Triburon Chloride	531	Direct KCl	2044	989
Trichlorocarbanilide	532	MeOH-C_6H_6-isoC_8H_{18} (0.5:0.5:5.0); KBr	4079	989
Trichlormethiazide	533	EtOH-isoC_8H_{18}-isoC_8H_{18} (0.2:0.8:5.0); KBr	8160	990
Triethylamine	538	CS_2 solution (redistilled)	2053	991
Triflupromazine	534	CS_2, C_2Cl_4 solutions	8141	990
Triflupromazine Hydrochloride	535	Direct KCl	8142	990
3,5,3′-Triodothyropropionic Acid	536	Direct KBr	4054	990
Triparanol	537	isoC_3H_7OH; KBr	4088	990
Uracil Mustard	539	Direct KBr	8166	991
Urea	540	Direct KBr	2052	991
Vanillic Acid Diethylamide	541	Direct KBr	4055	991
Xanthiol Hydrochloride	542	Direct KCl	8207	991


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
208
ACACIA
209
ACETIC ACID, GLACIAL
CH3COOH
ACETOPHENETIDINE
210
HNCOCH3
OC2H5
N-ACETYL-DL-AMPHETAMINE
211
CH2CH(CH3)NHCOCH3
N-ACETYL-D-AMPHETAMINE
212
CH2CH(CH3)NHCOCH3
TRANSMITTANCE, %
100 80 60 40 20 0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ
THE PERKIN-ELMER CORP., NORWALK, C.

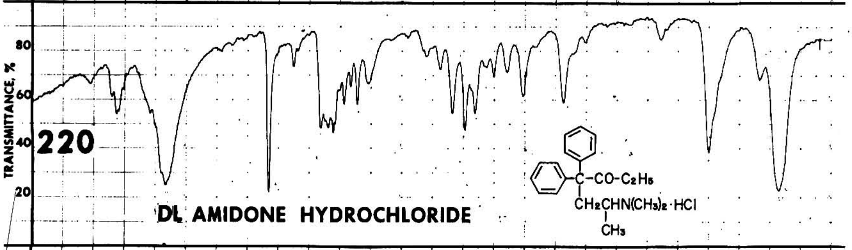

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
100
80
60
40
20
0
TRANSMITTANCE, %
213
ACETONE
CH₃-C(=O)-CH₃
214
ACETOPHENAZINE DIMALEATE
CH₂CH₂CH₂N NCH₂CH₂OH
N
S
COCH₃
·2
HCCOOH
HCCOOH
215
ACETYL SALICYLIC ACID
COOH
OCOCH₃
216
N,4-ACETYLSULFANILAMIDE
SO₂NH₂
NHCOCH₃
217
AKINETON HYDROCHLORIDE
·HCl
CCH₂CH₂N
OH
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



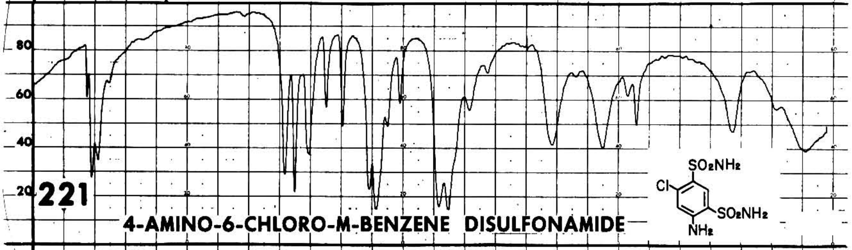
218 ALUMINUM ACETATE



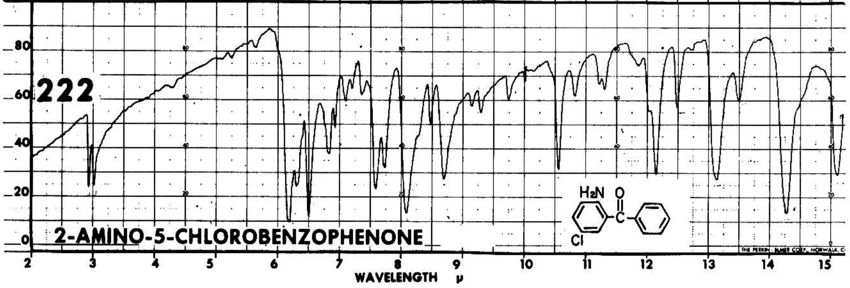
219 ALVODINE BASE



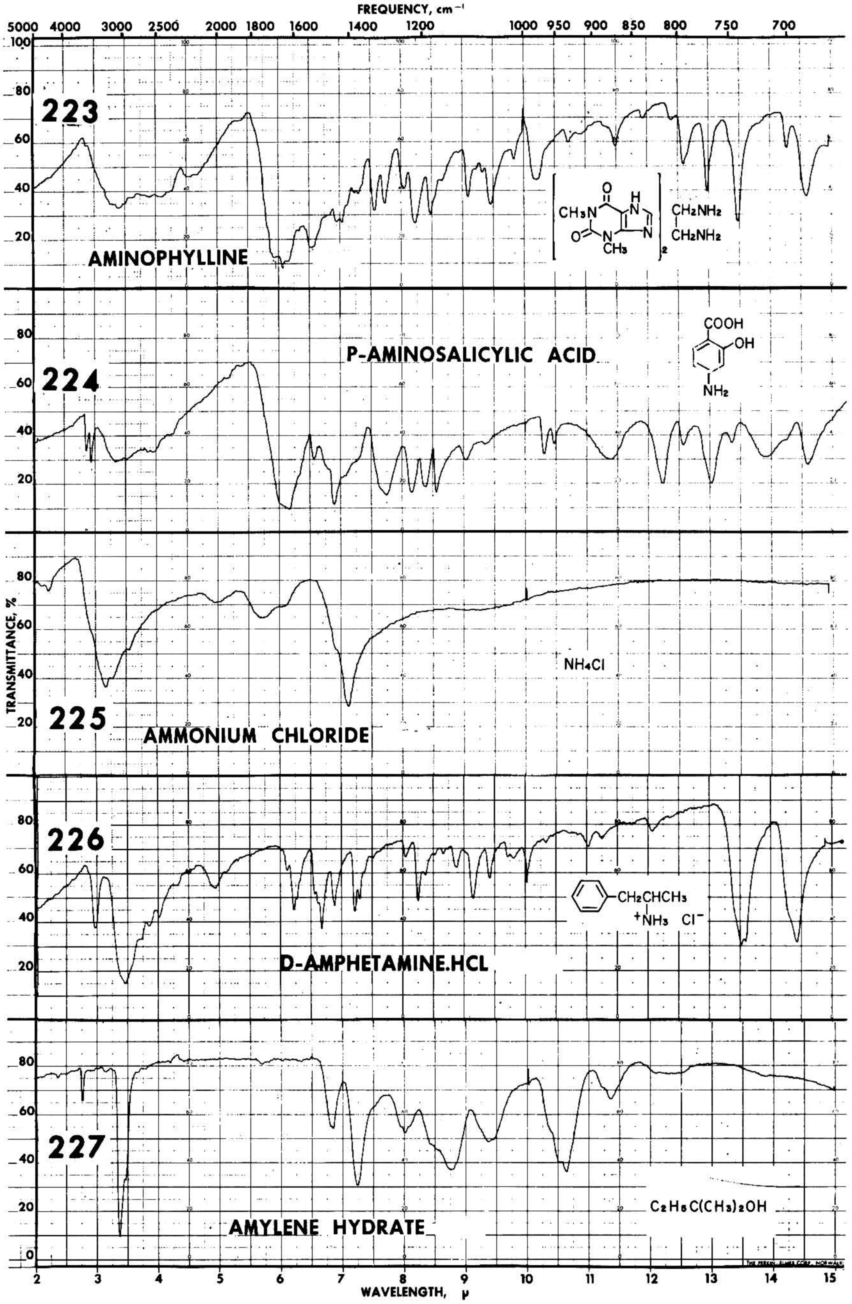
220 DL AMIDONE HYDROCHLORIDE

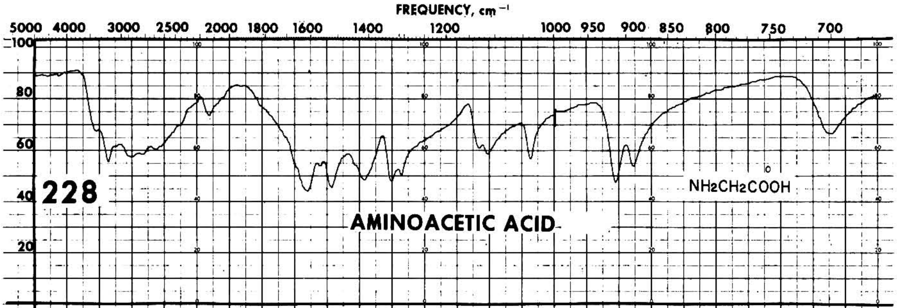


221 4-AMINO-6-CHLORO-M-BENZENE DISULFONAMIDE



222 2-AMINO-5-CHLOROBENZOPHENONE

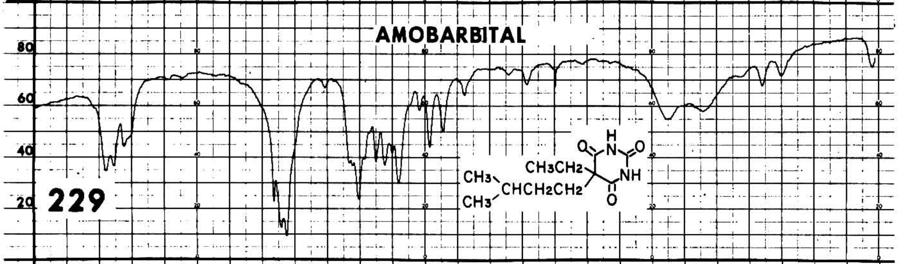

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100 80 60 40 20 0
223
AMINOPHYLLINE
CH3N O N H N N CH3 O CH2NH2 CH2NH2 2
224
P-AMINOSALICYLIC ACID
COOH OH NH2
225
AMMONIUM CHLORIDE
NH4Cl
226
D-AMPHETAMINE.HCL
CH2CHCH3 +NH3 Cl⁻
227
AMYLENE HYDRATE
C2H5C(CH3)2OH
THE PERKIN-ELMER CORP. NORWALK
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, µ



228

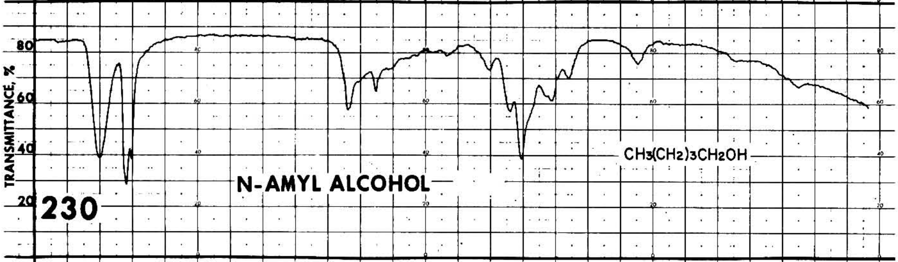
AMINOACETIC ACID

NH_2CH_2COOH



229

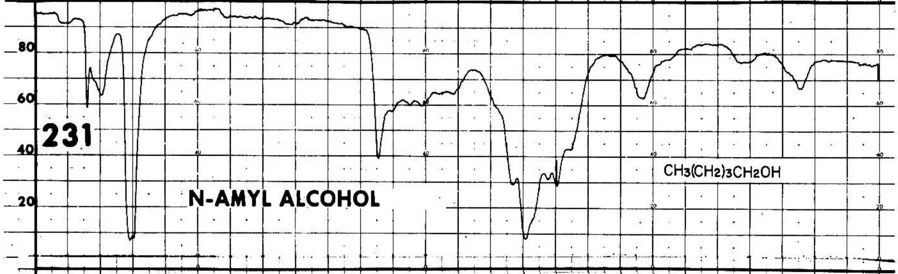
AMOBARBITAL



230

N-AMYL ALCOHOL

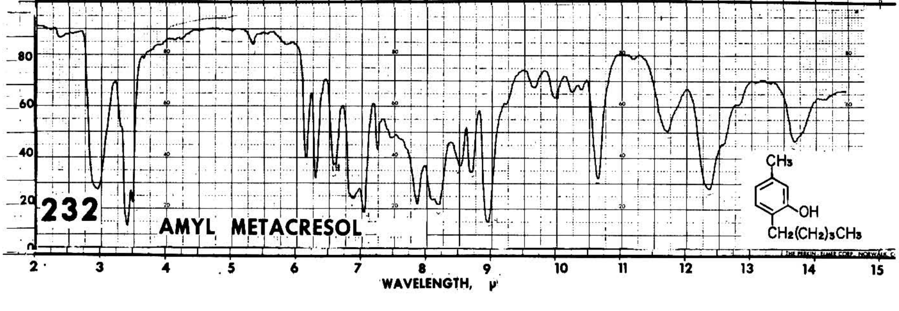
$CH_3(CH_2)_3CH_2OH$



231

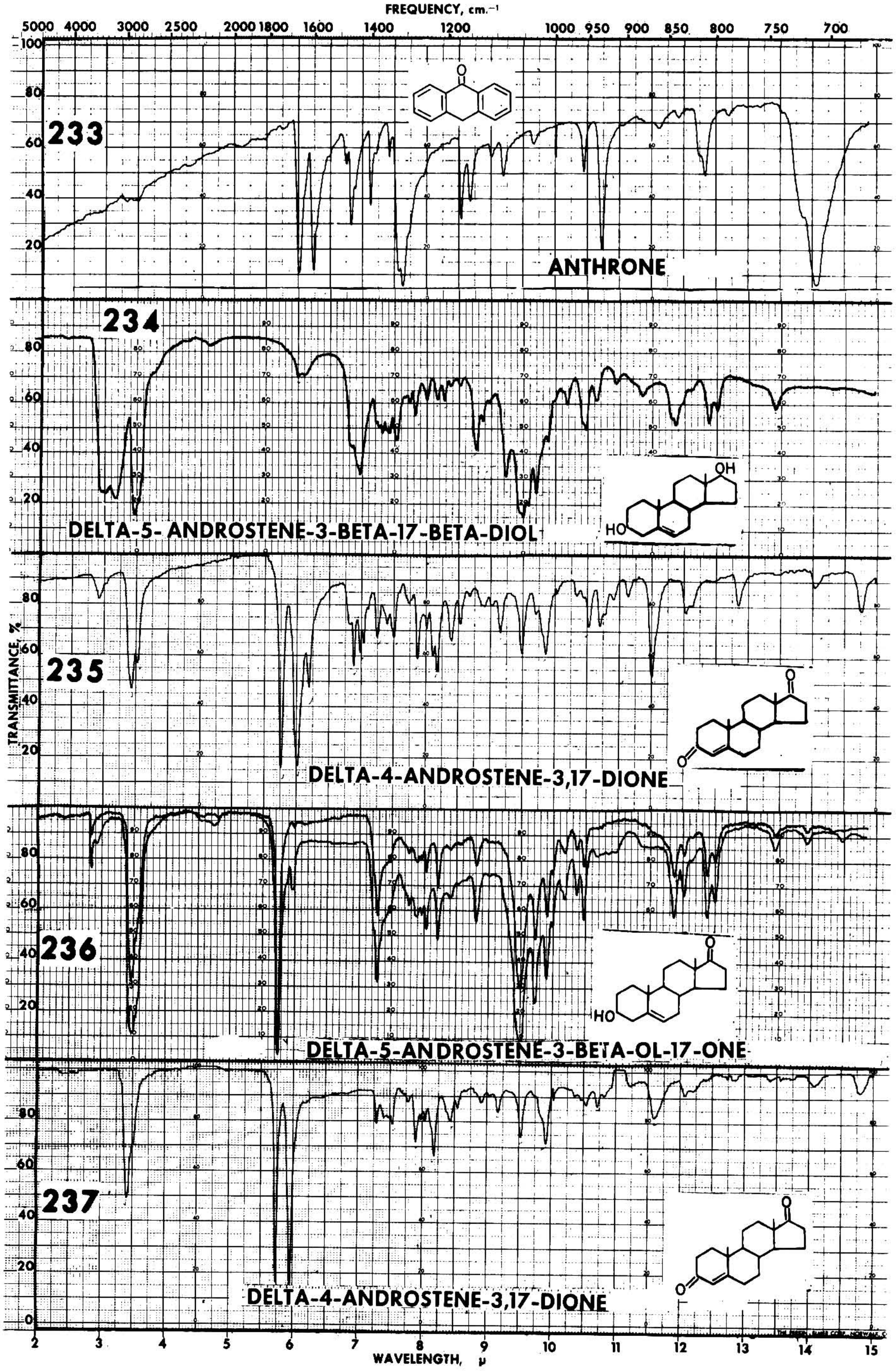
N-AMYL ALCOHOL

$CH_3(CH_2)_3CH_2OH$



232

AMYL METACRESOL



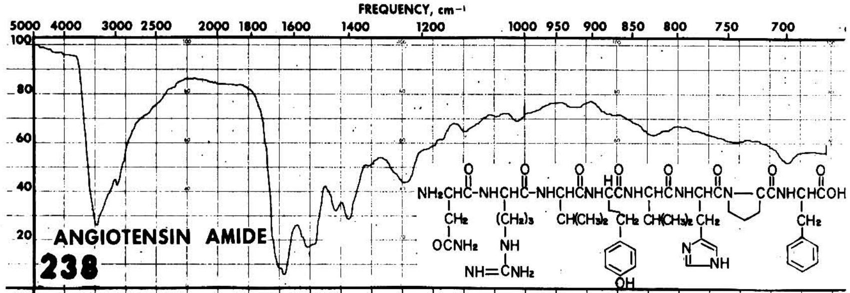
233 ANTHRONE

234 DELTA-5- ANDROSTENE-3-BETA-17-BETA-DIOL

235 DELTA-4-ANDROSTENE-3,17-DIONE

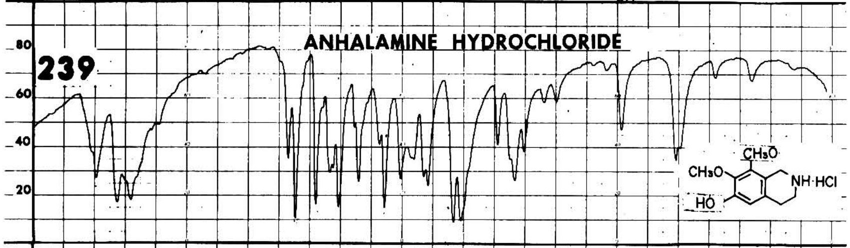
236 DELTA-5-ANDROSTENE-3-BETA-OL-17-ONE

237 DELTA-4-ANDROSTENE-3,17-DIONE



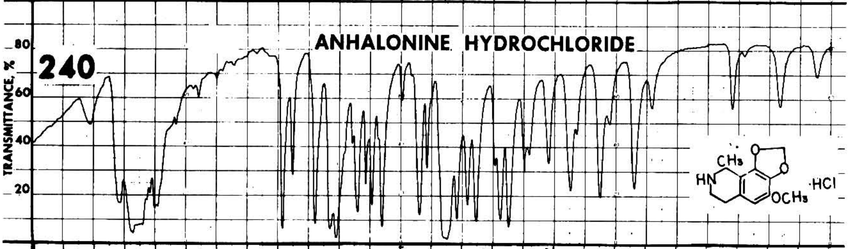
238

ANGIOTENSIN AMIDE



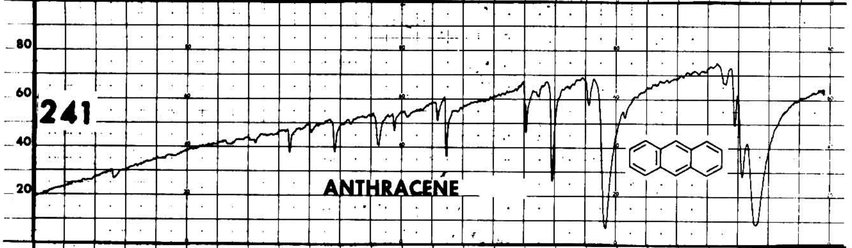
239

ANHALAMINE HYDROCHLORIDE



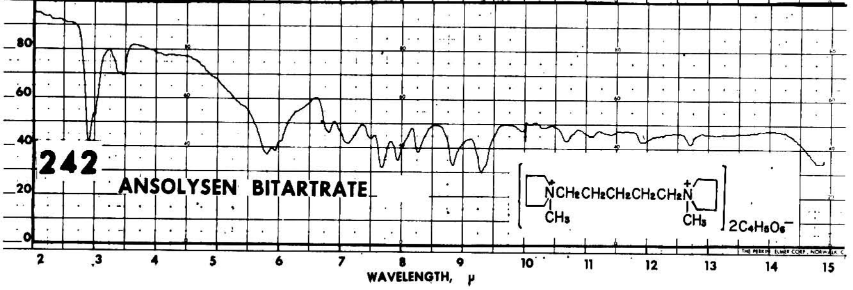
240

ANHALONINE HYDROCHLORIDE



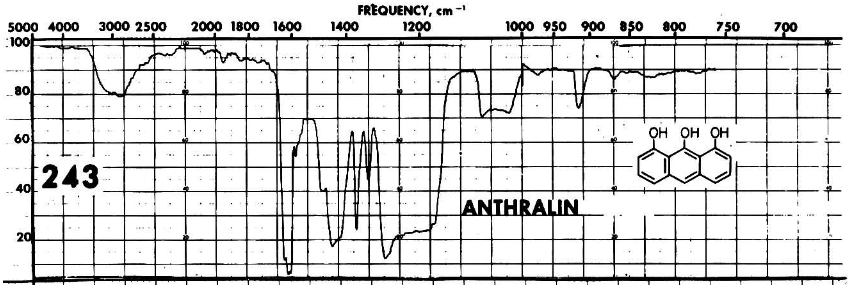
241

ANTHRACENE

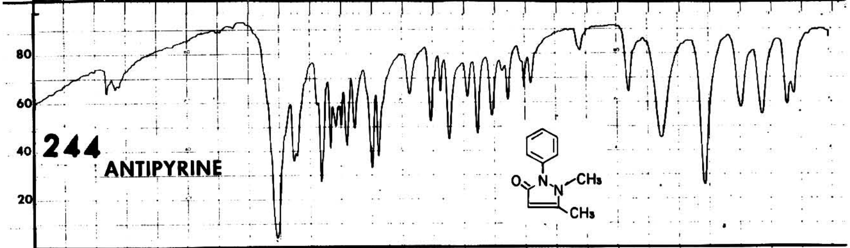


242

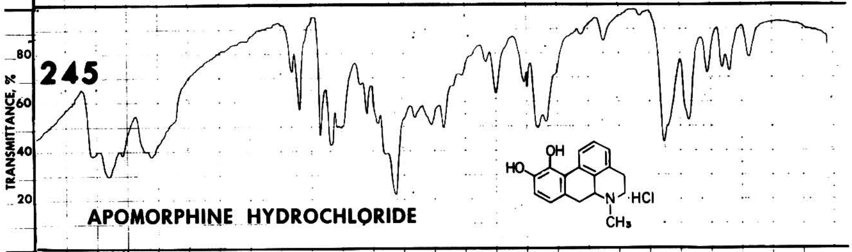
ANSOLYSEN BITARTRATE



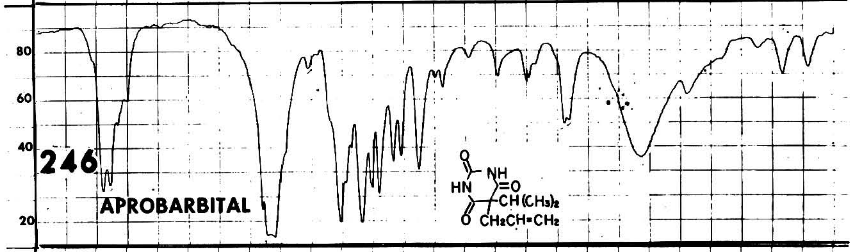
243 ANTHRALIN



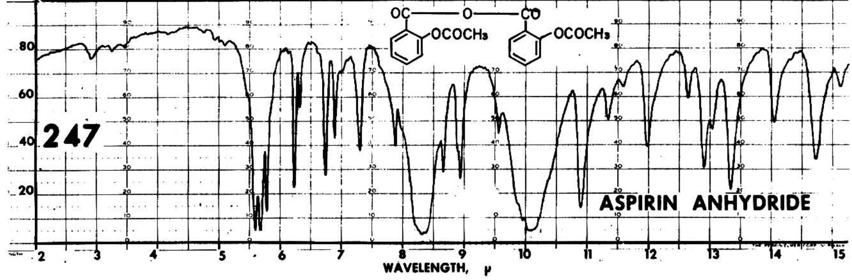
244 ANTIPYRINE



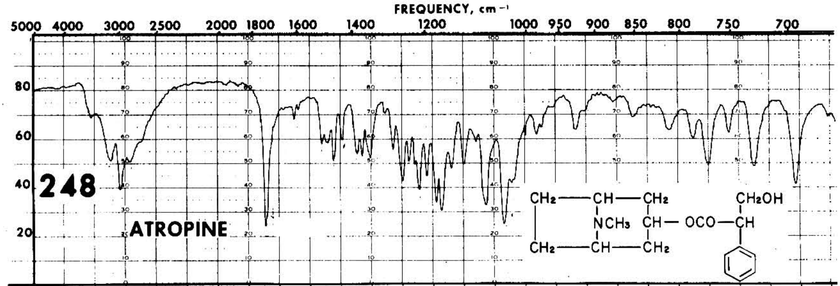
245 APOMORPHINE HYDROCHLORIDE



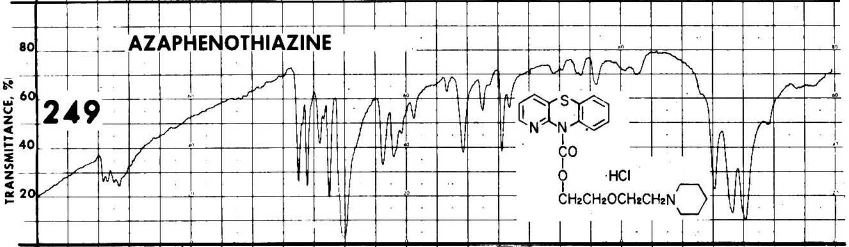
246 APROBARBITAL



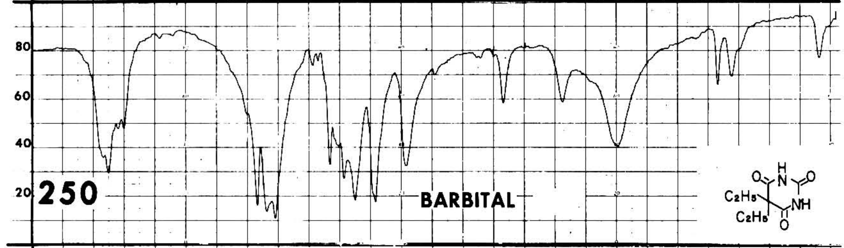
247 ASPIRIN ANHYDRIDE



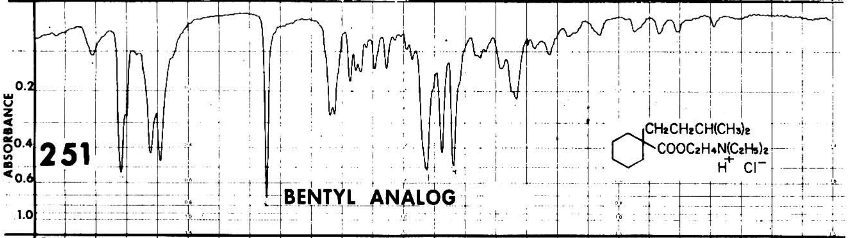
248 **ATROPINE**



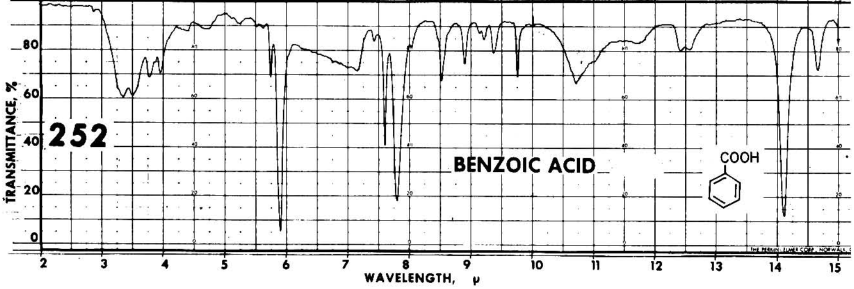
249 **AZAPHENOTHIAZINE**



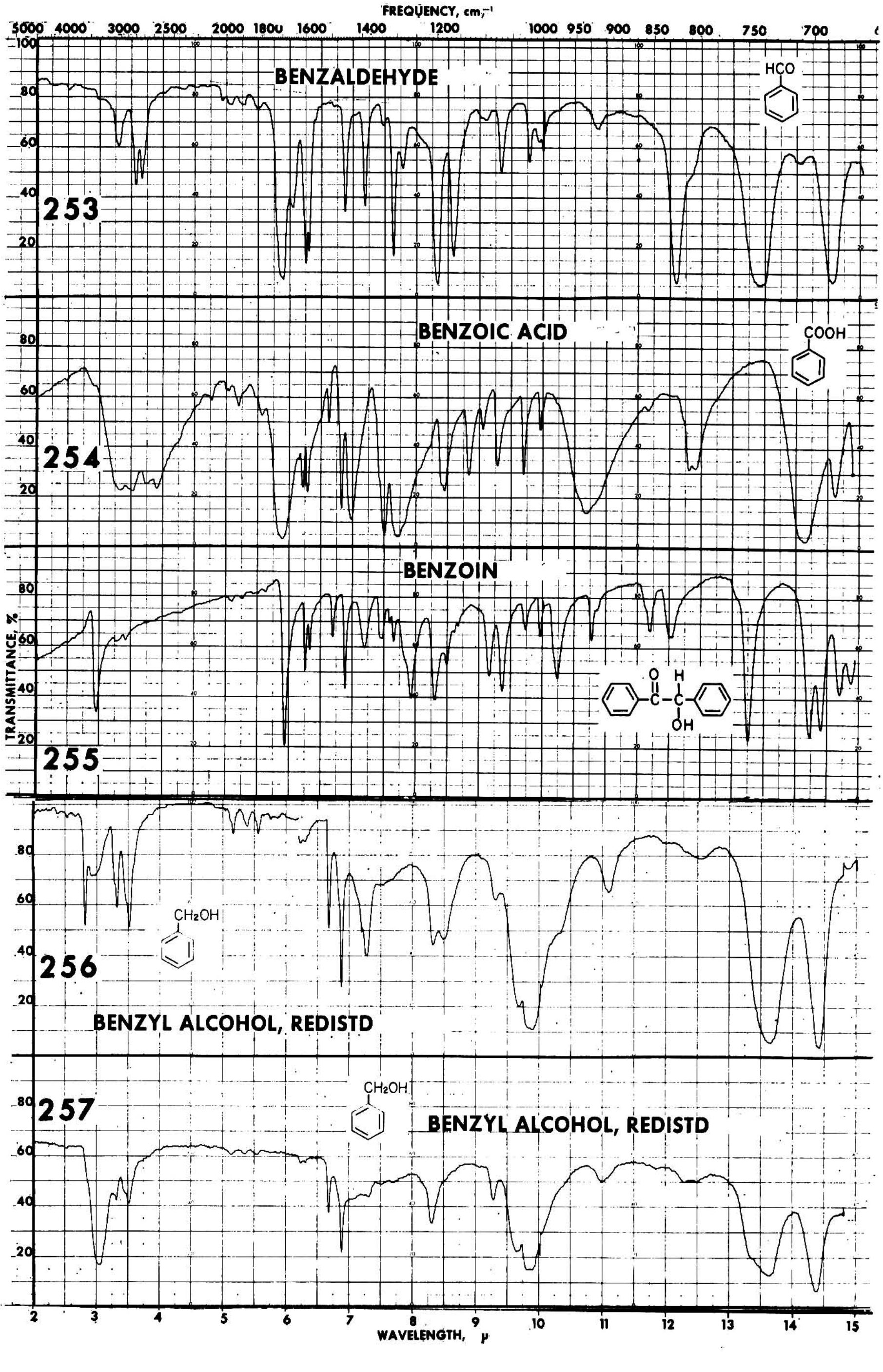
250 **BARBITAL**

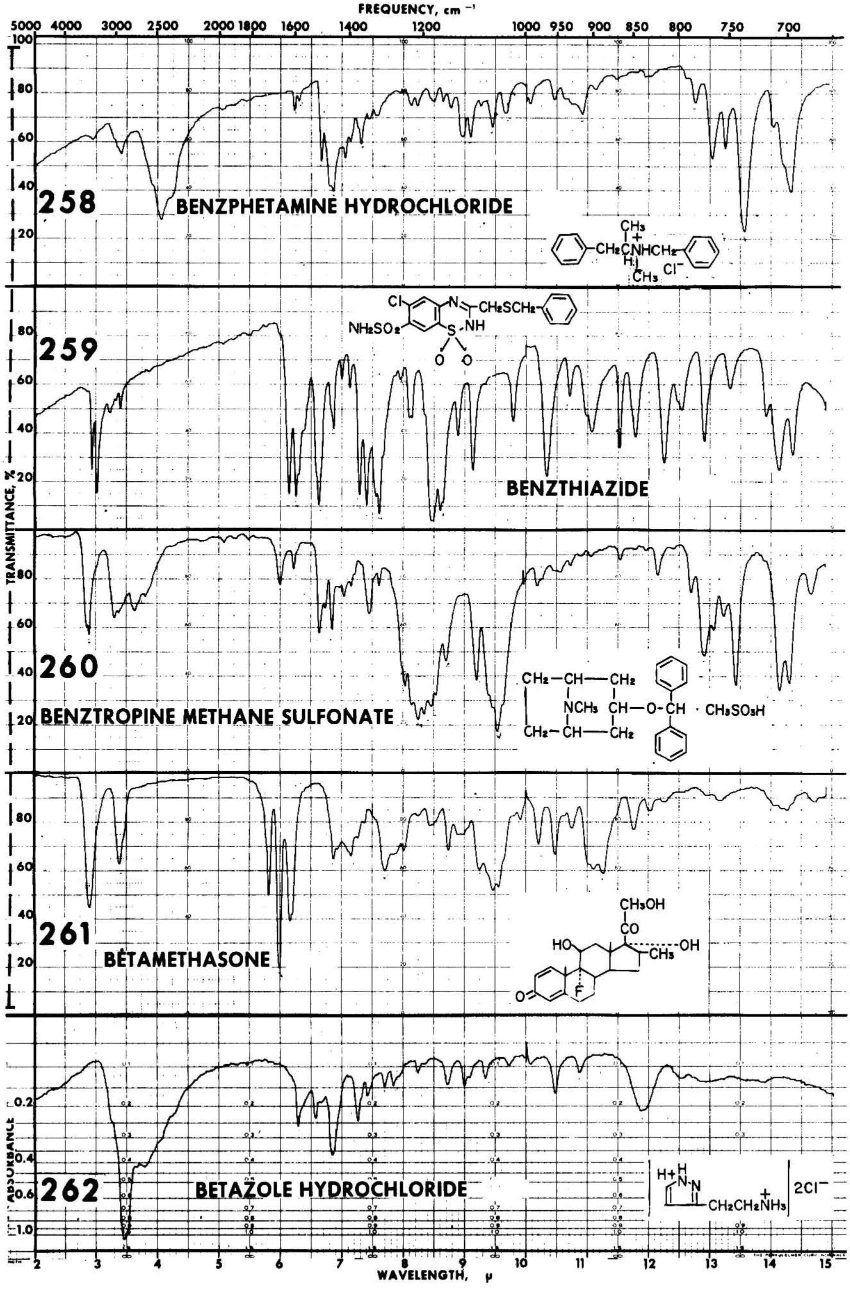


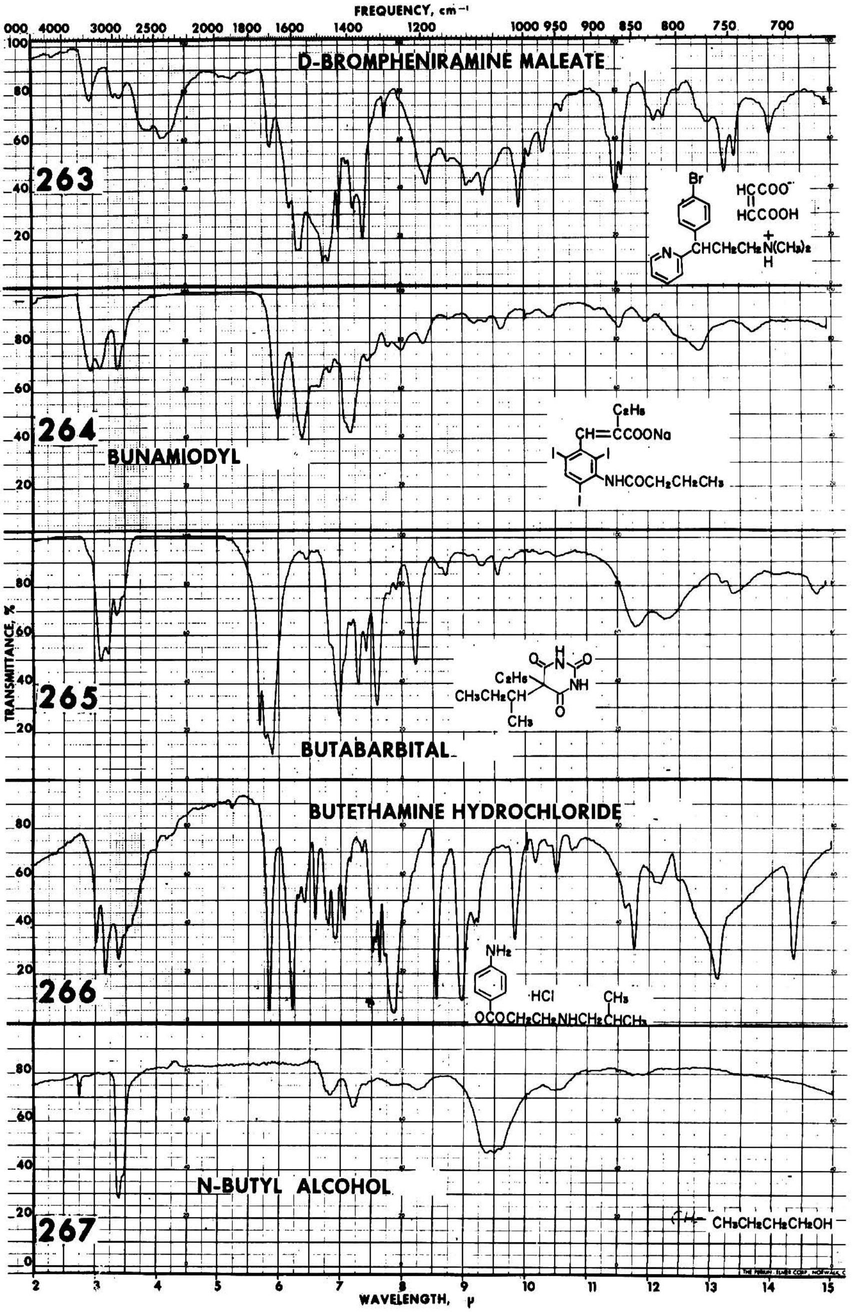
251 **BENTYL ANALOG**

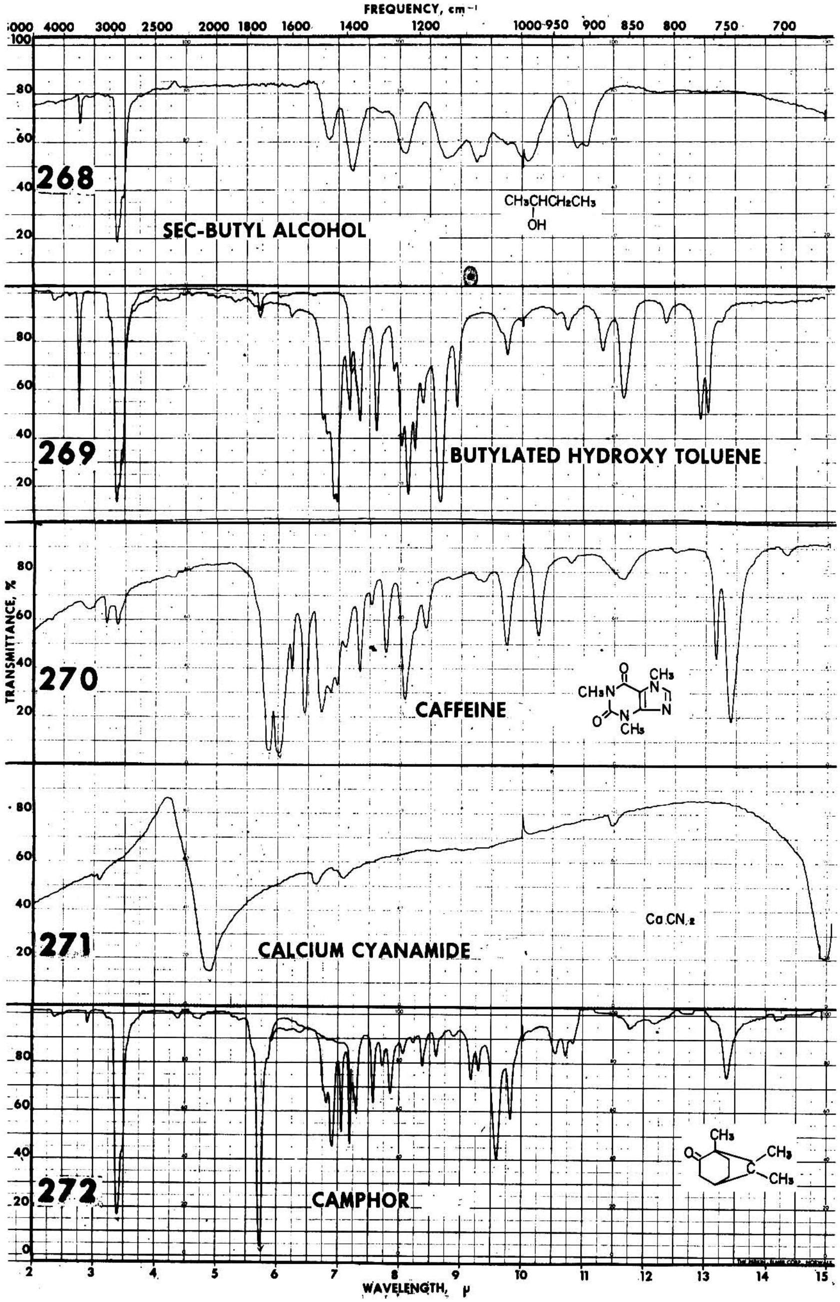


252 **BENZOIC ACID**


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
BENZALDEHYDE
HCO
253
BENZOIC ACID
COOH
254
BENZOIN
255
O H
C–C
OH
TRANSMITTANCE, %
100 80 60 40 20
CH2OH
256
BENZYL ALCOHOL, REDISTD
257
CH2OH
BENZYL ALCOHOL, REDISTD
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100
80
60
40
20
258
BENZPHETAMINE HYDROCHLORIDE
CH₃ CH₂CNHCH₂ H CH₃ Cl⁻
259
BENZTHIAZIDE
Cl N CH₂SCH₂ NH₂SO₂ S NH O O
260
BENZTROPINE METHANE SULFONATE
CH₂—CH—CH₂ NCH₃ CH—O—CH · CH₃SO₃H CH₂—CH—CH₂
261
BETAMETHASONE
CH₂OH CO HO CH₃ OH O F
ABSORBANCE
0.2
0.4
0.6
1.0
262
BETAZOLE HYDROCHLORIDE
H+ H N N CH₂CH₂NH₃ + 2Cl⁻
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm⁻¹
000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
263
D-BROMPHENIRAMINE MALEATE
Br
HCCOO⁻
HCCOOH
CHCH₂CH₂N(CH₃)₂
H
N
264
BUNAMIODYL
C₂H₅
CH=CCOONa
I
NHCOCH₂CH₂CH₃
265
BUTABARBITAL
O
H
N
NH
C₂H₅
CH₃CH₂CH
CH₃
TRANSMITTANCE, %
266
BUTETHAMINE HYDROCHLORIDE
NH₂
·HCl
CH₃
OCOCH₂CH₂NHCH₂CHCH₃
267
N-BUTYL ALCOHOL
CH₃CH₂CH₂CH₂OH
100
80
60
40
20
0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



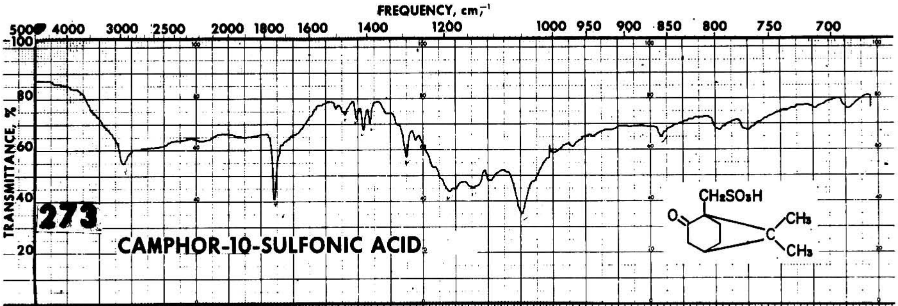
268 SEC-BUTYL ALCOHOL

269 BUTYLATED HYDROXY TOLUENE

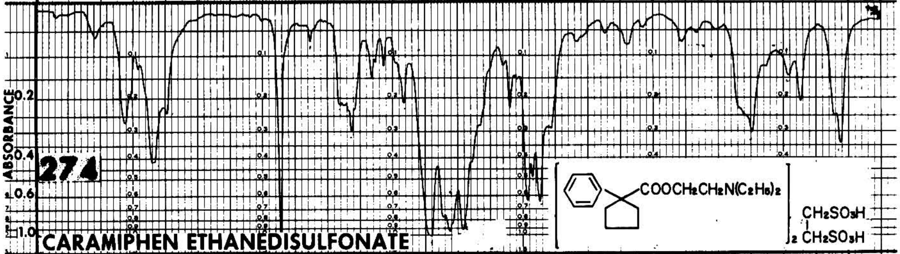
270 CAFFEINE

271 CALCIUM CYANAMIDE

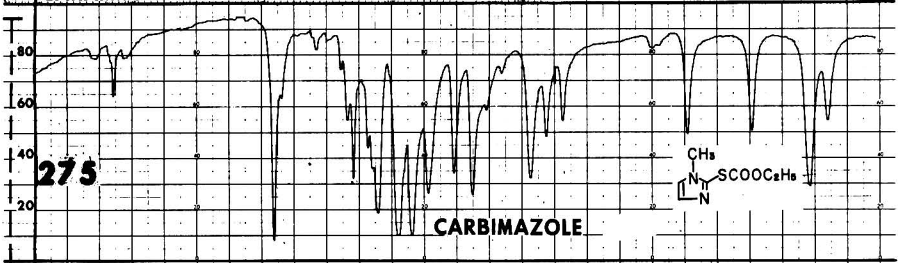
272 CAMPHOR



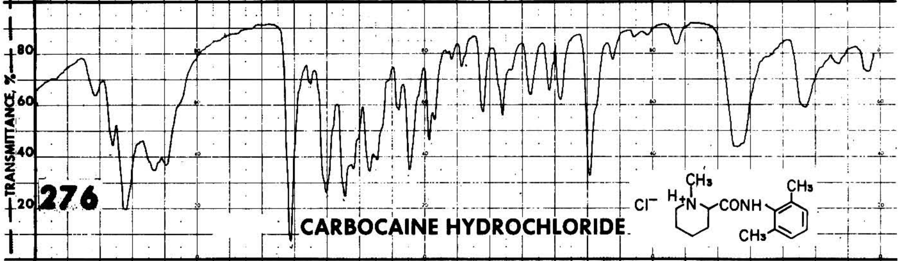
273 CAMPHOR-10-SULFONIC ACID



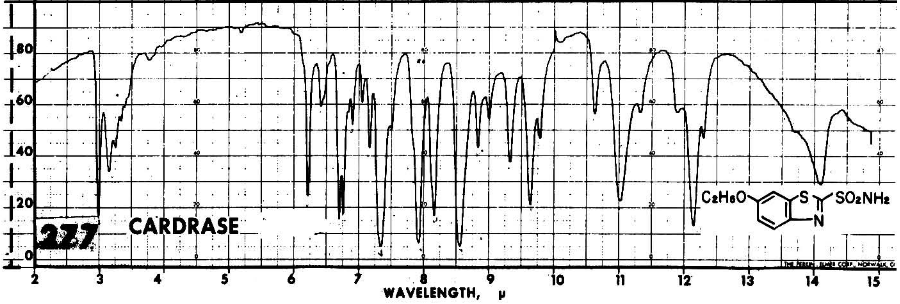
274 CARAMIPHEN ETHANEDISULFONATE



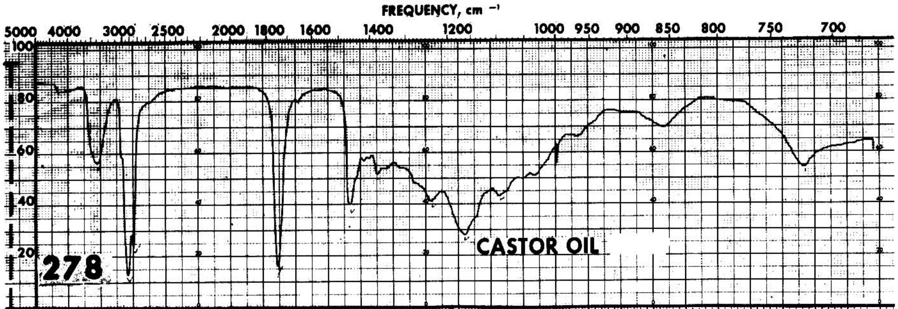
275 CARBIMAZOLE



276 CARBOCAINE HYDROCHLORIDE

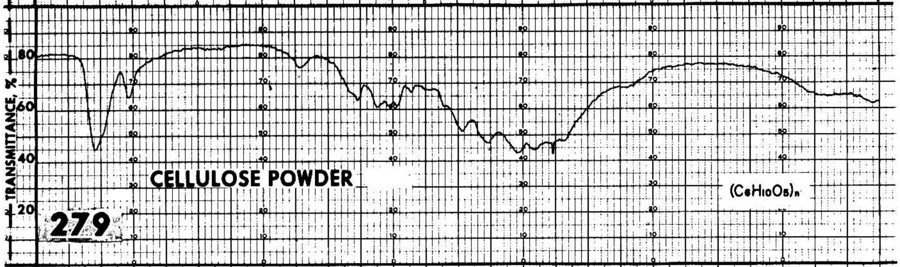


277 CARDRASE



278

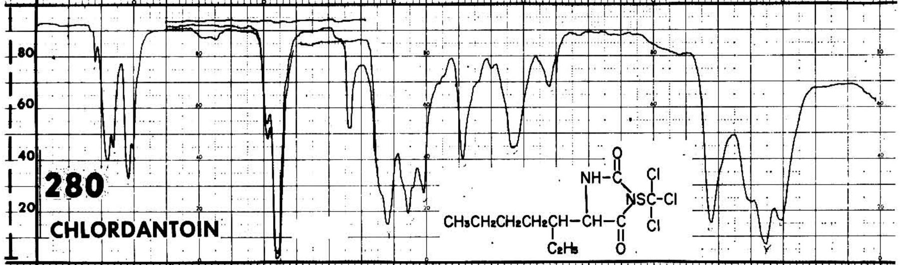
CASTOR OIL



279

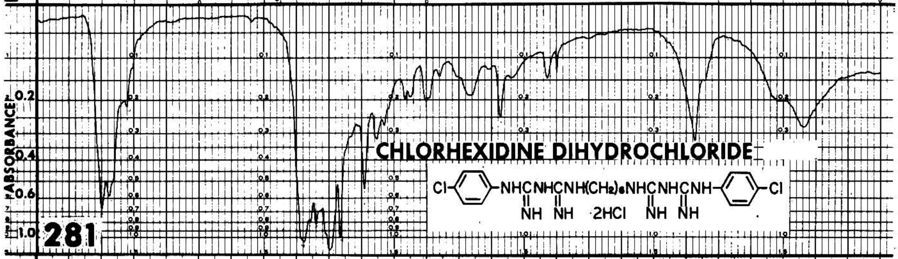
CELLULOSE POWDER

$(C_6H_{10}O_5)_n$



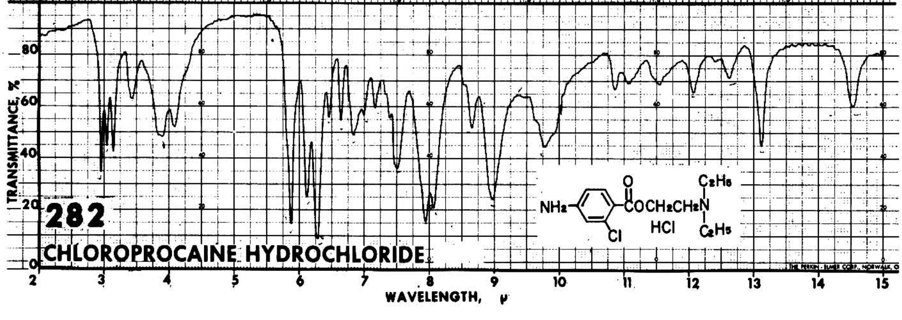
280

CHLORDANTOIN



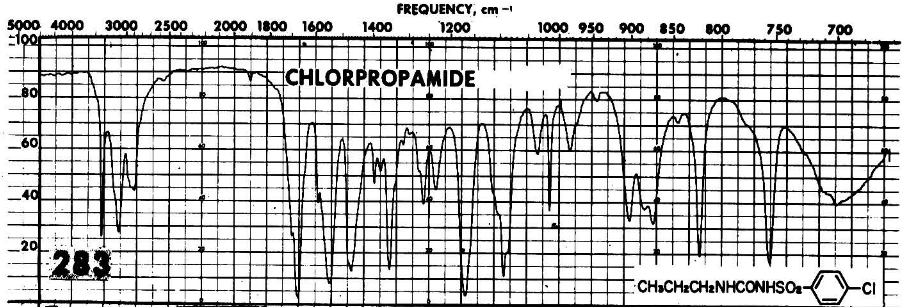
281

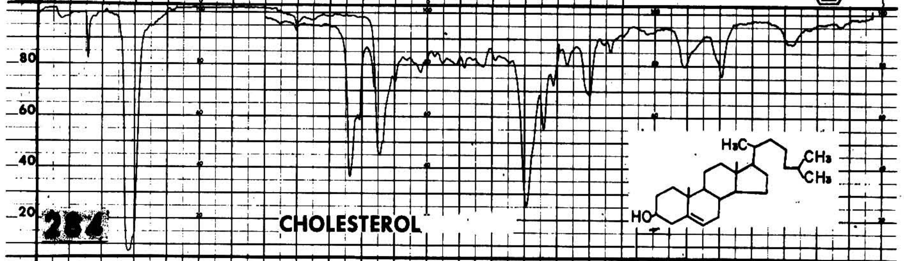
CHLORHEXIDINE DIHYDROCHLORIDE

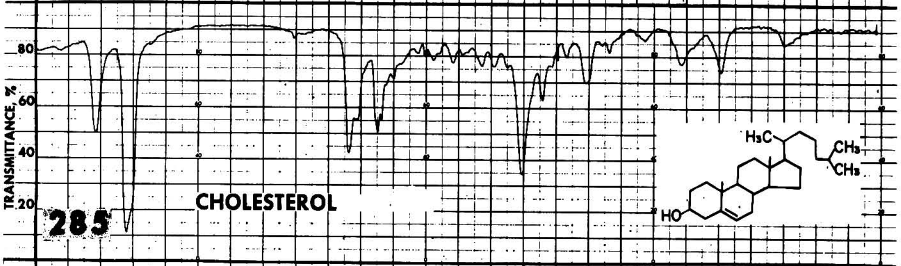


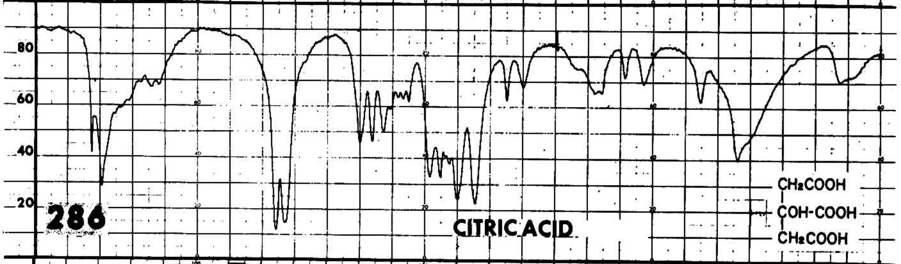
282

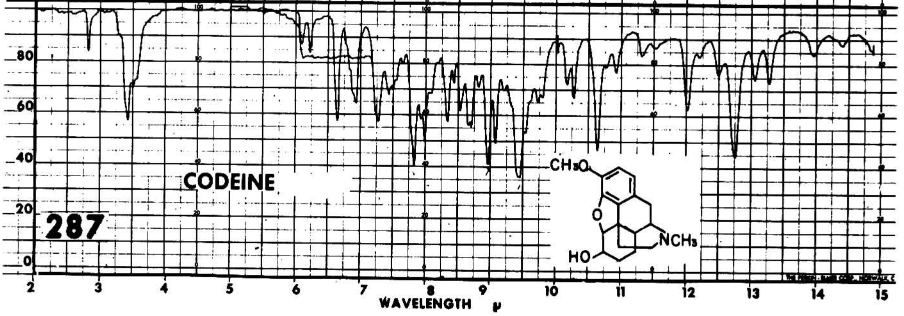
CHLOROPROCAINE HYDROCHLORIDE

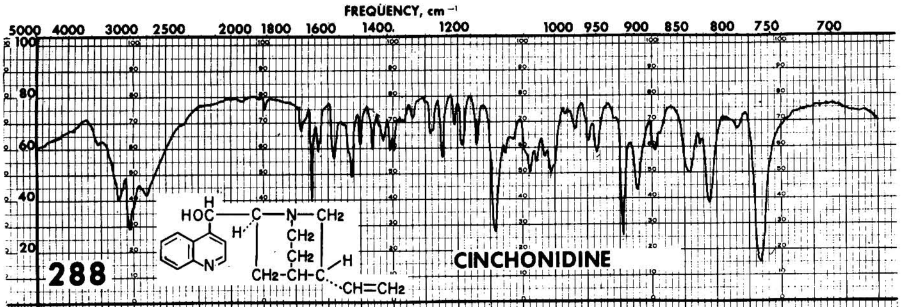

283


284

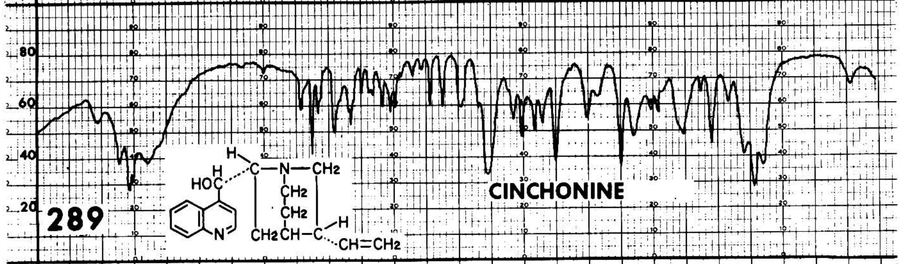

285


286

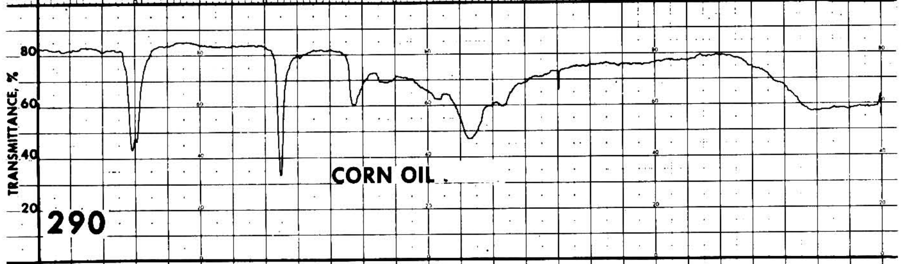

287



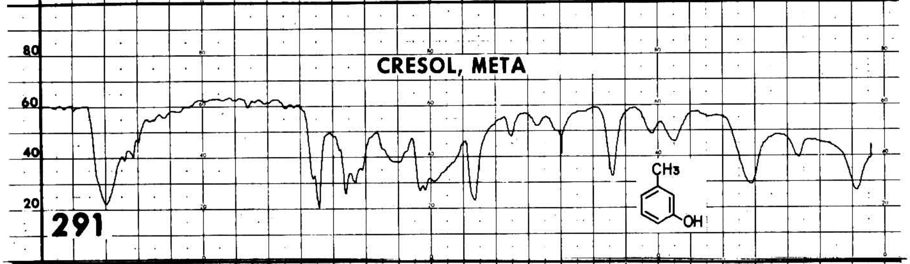
288 CINCHONIDINE



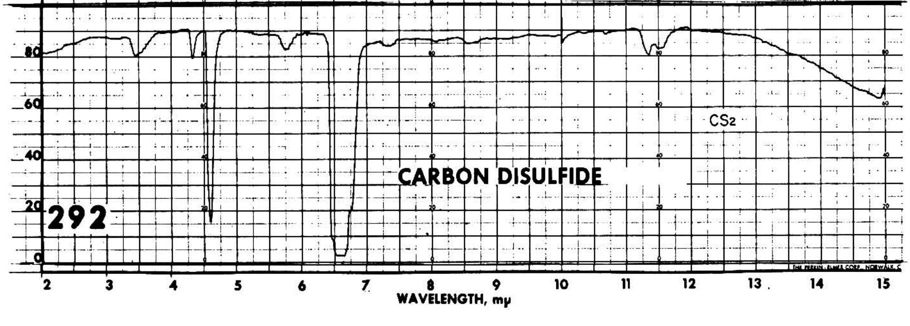
289 CINCHONINE



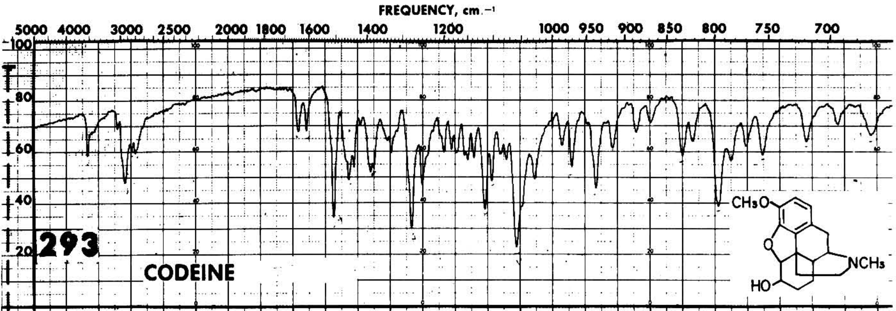
290 CORN OIL



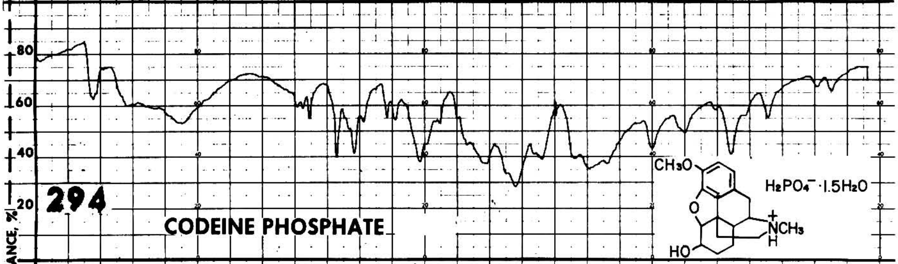
291 CRESOL, META



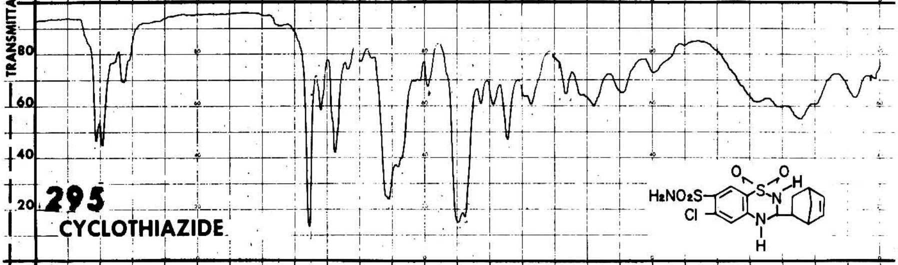
292 CARBON DISULFIDE



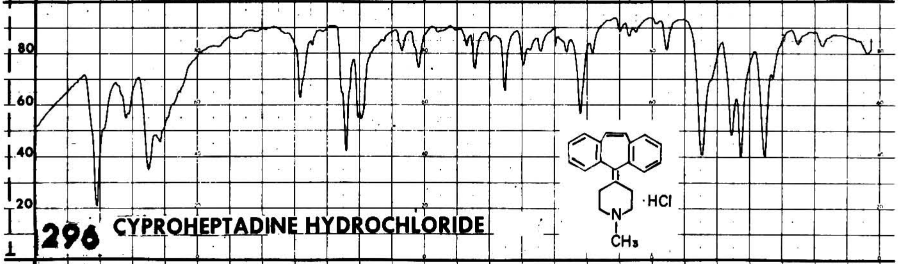
293 CODEINE



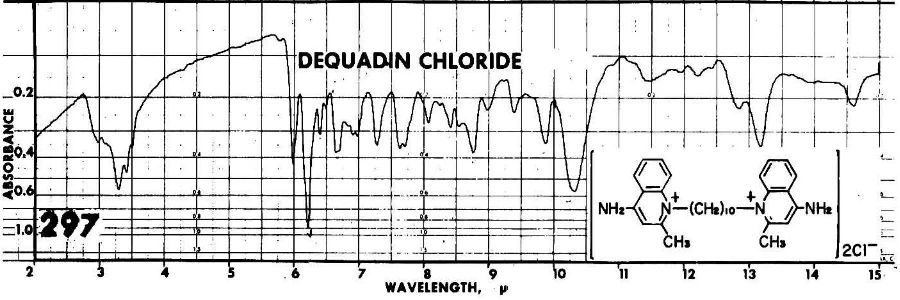
294 CODEINE PHOSPHATE



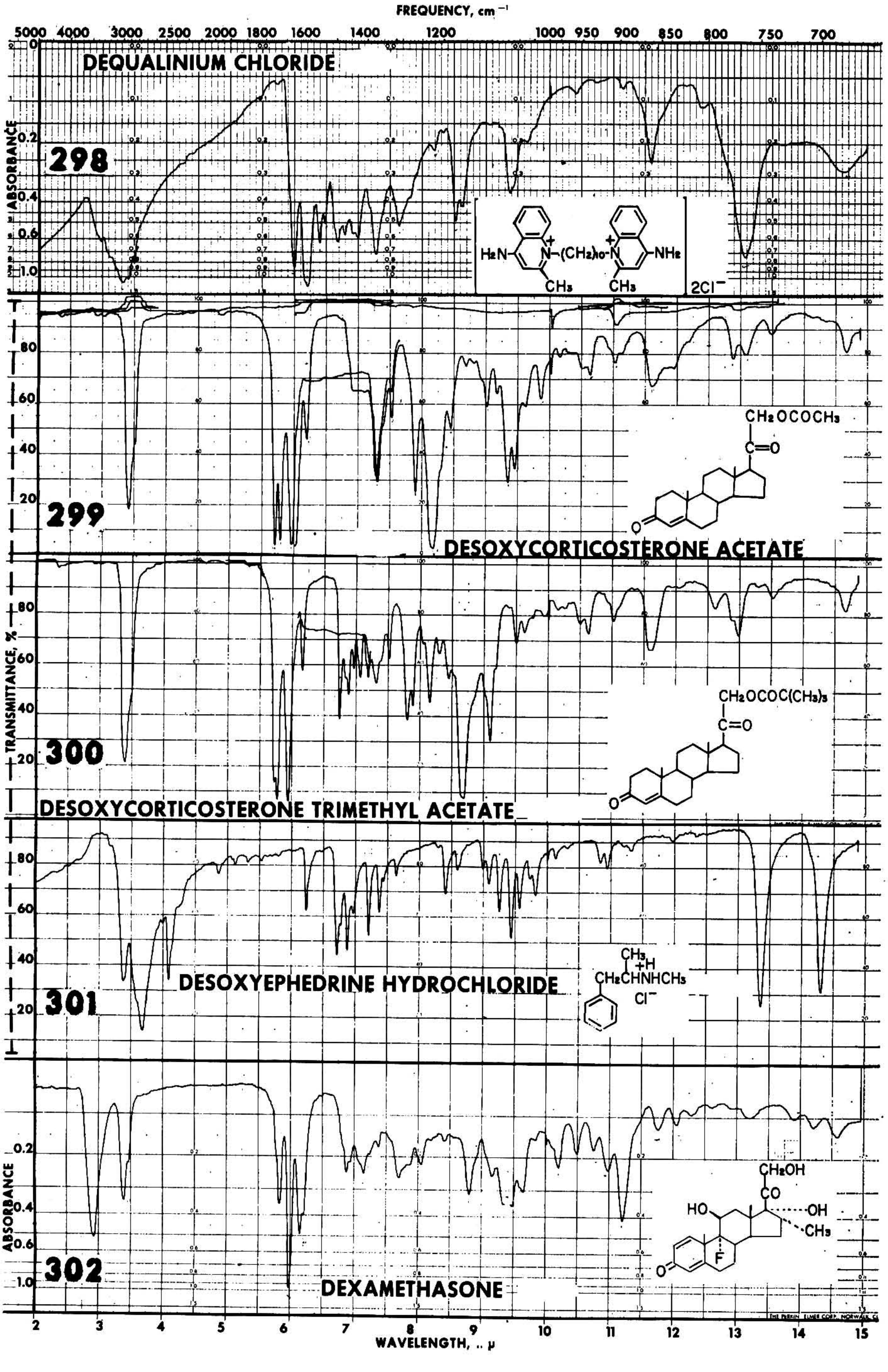
295 CYCLOTHIAZIDE

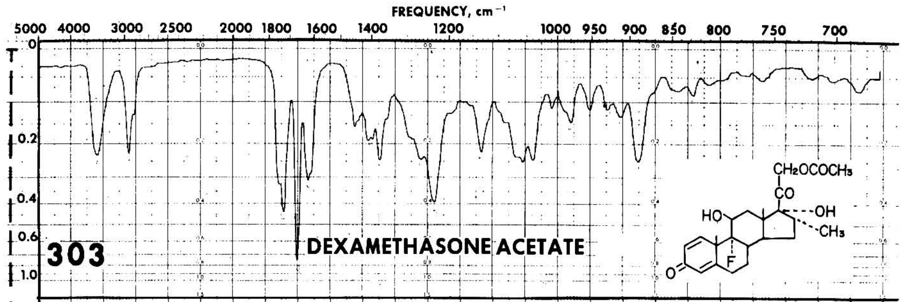


296 CYPROHEPTADINE HYDROCHLORIDE

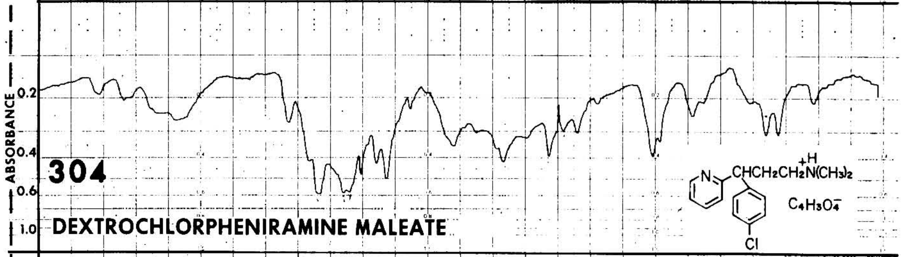


297 DEQUADIN CHLORIDE

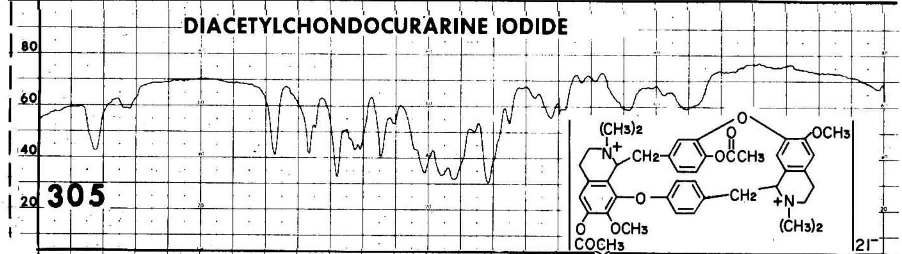

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
298
DEQUALINIUM CHLORIDE
ABSORBANCE
0 0.2 0.4 0.6 1.0
H₂N
(CH₂)₁₀
NH₂
CH₃
CH₃
2Cl⁻
299
DESOXYCORTICOSTERONE ACETATE
CH₂OCOCH₃
C=O
O
80 60 40 20
TRANSMITTANCE, %
300
DESOXYCORTICOSTERONE TRIMETHYL ACETATE
CH₂OCOC(CH₃)₃
C=O
O
301
DESOXYEPHEDRINE HYDROCHLORIDE
CH₃
+H
CH₂CHNHCH₃
Cl⁻
302
DEXAMETHASONE
CH₂OH
CO
HO
OH
CH₃
F
O
0.2 0.4 0.6 1.0
ABSORBANCE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ
THE PERKIN ELMER CORP. NORWALK, CT



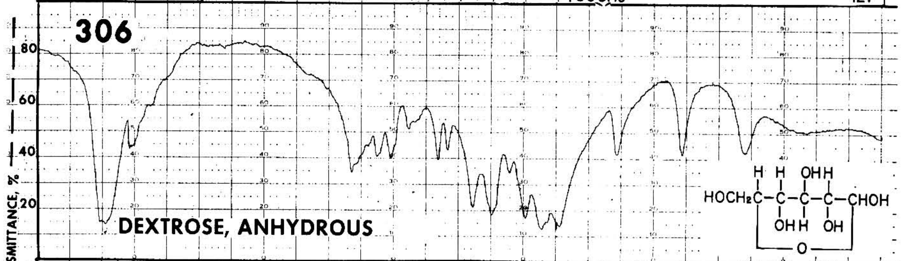
303 DEXAMETHASONE ACETATE



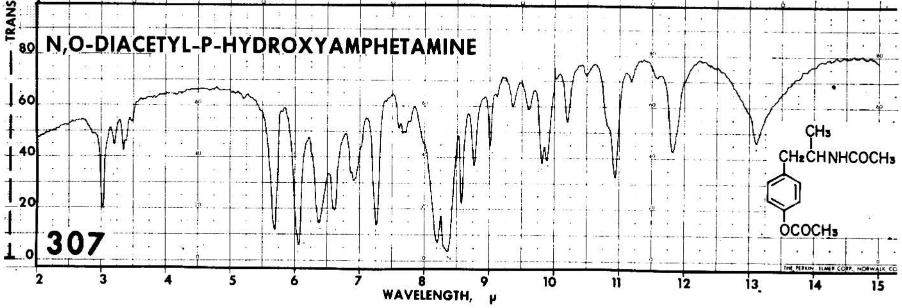
304 DEXTROCHLORPHENIRAMINE MALEATE



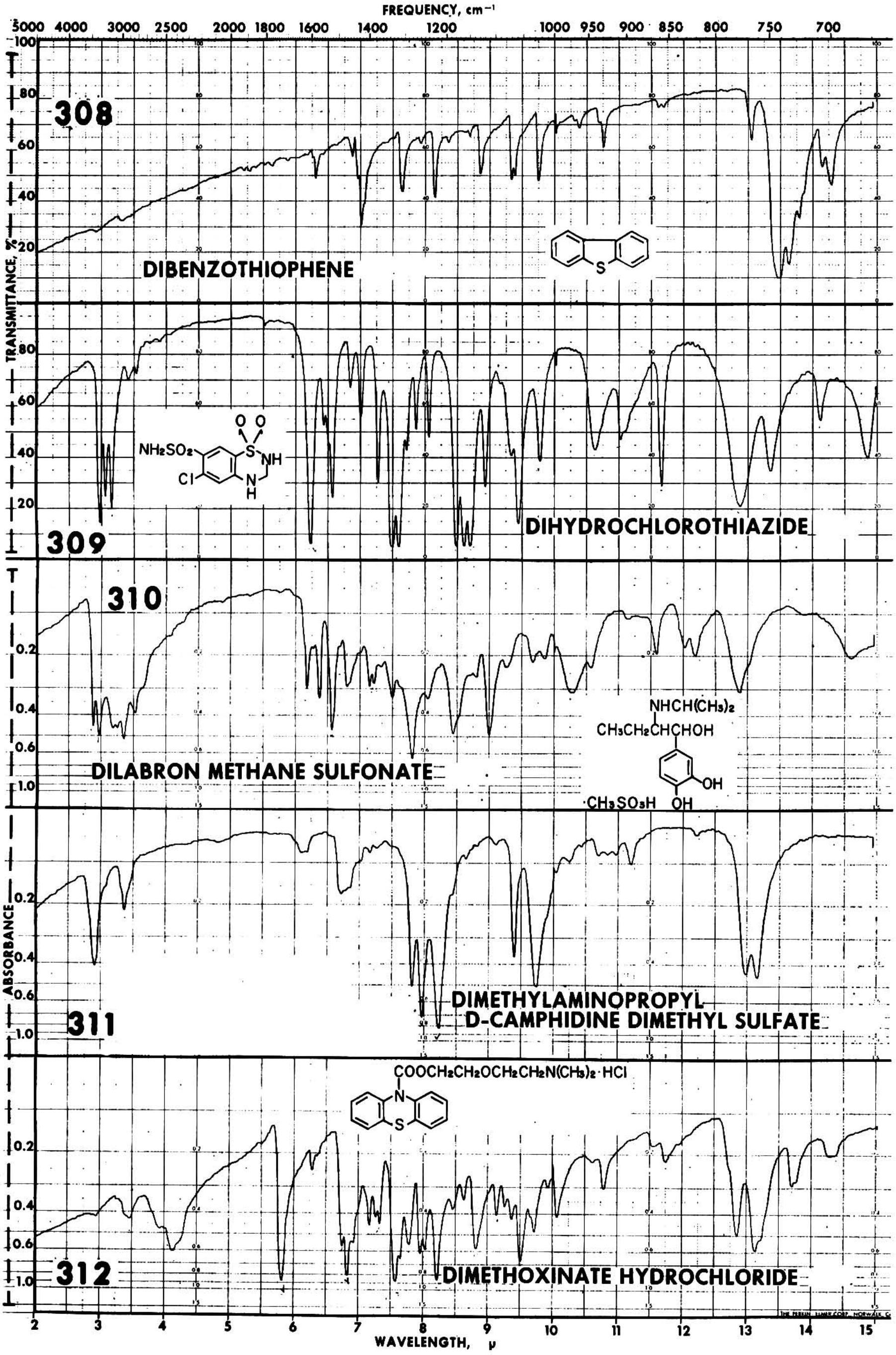
305 DIACETYLCHONDOCURARINE IODIDE

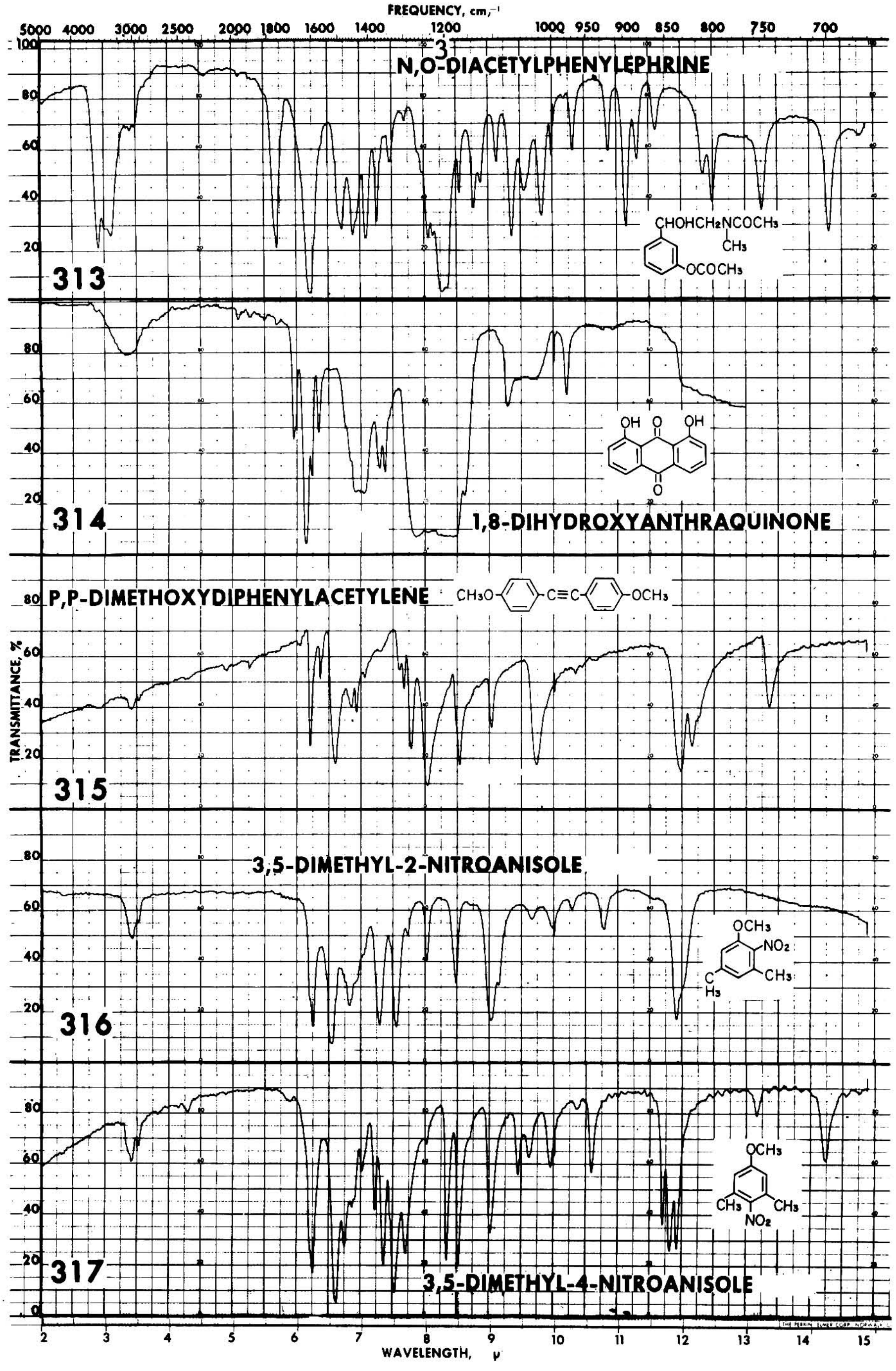


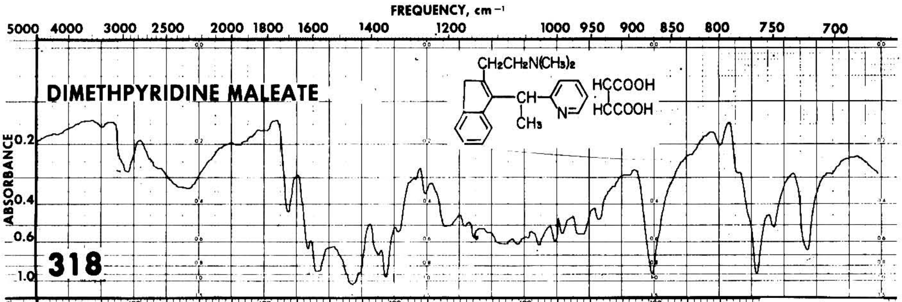
306 DEXTROSE, ANHYDROUS



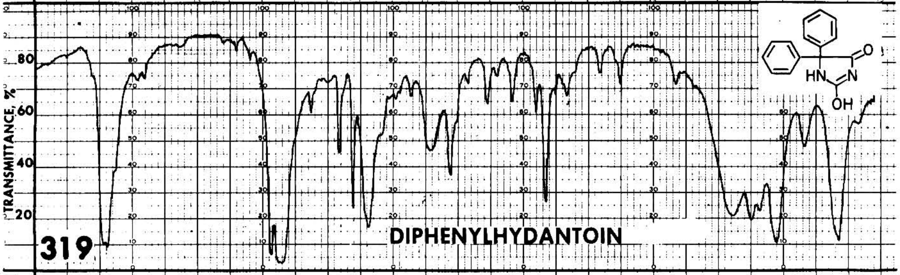
307 N,O-DIACETYL-P-HYDROXYAMPHETAMINE


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100
80
60
40
20
308
DIBENZOTHIOPHENE
S
309
DIHYDROCHLOROTHIAZIDE
NH₂SO₂
Cl
O O
S
NH
N
H
310
DILABRON METHANE SULFONATE
NHCH(CH₃)₂
CH₃CH₂CHCHOH
OH
·CH₃SO₃H OH
ABSORBANCE
0.2
0.4
0.6
1.0
311
DIMETHYLAMINOPROPYL D-CAMPHIDINE DIMETHYL SULFATE
312
COOCH₂CH₂OCH₂CH₂N(CH₃)₂·HCl
N
S
DIMETHOXINATE HYDROCHLORIDE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, µ

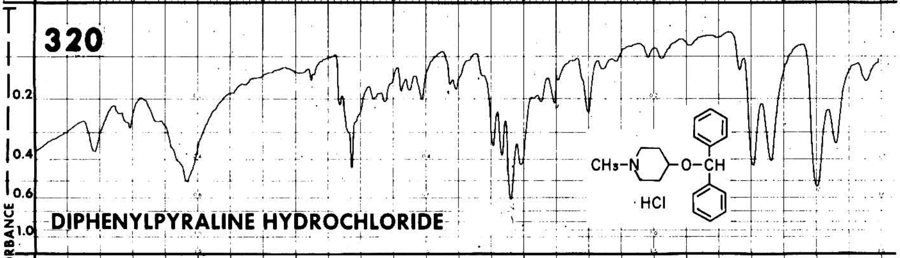

FREQUENCY, cm.⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
N,O-DIACETYLPHENYLEPHRINE
CHOHCH₂NCOCH₃
CH₃
OCOCH₃
313
OH O OH
O
314
1,8-DIHYDROXYANTHRAQUINONE
P,P-DIMETHOXYDIPHENYLACETYLENE
CH₃O C≡C OCH₃
315
3,5-DIMETHYL-2-NITROANISOLE
OCH₃
NO₂
CH₃
CH₃
316
OCH₃
CH₃
CH₃
NO₂
317
3,5-DIMETHYL-4-NITROANISOLE
TRANSMITTANCE, %
0 20 40 60 80 100
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



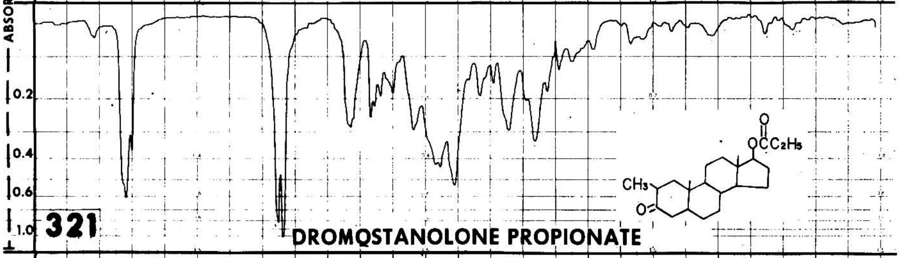
318 DIMETHPYRIDINE MALEATE



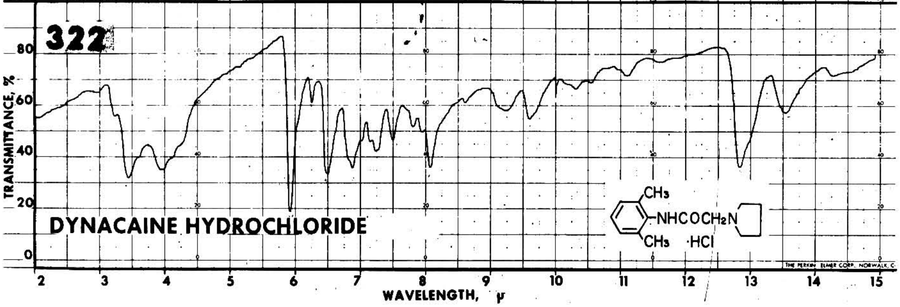
319 DIPHENYLHYDANTOIN



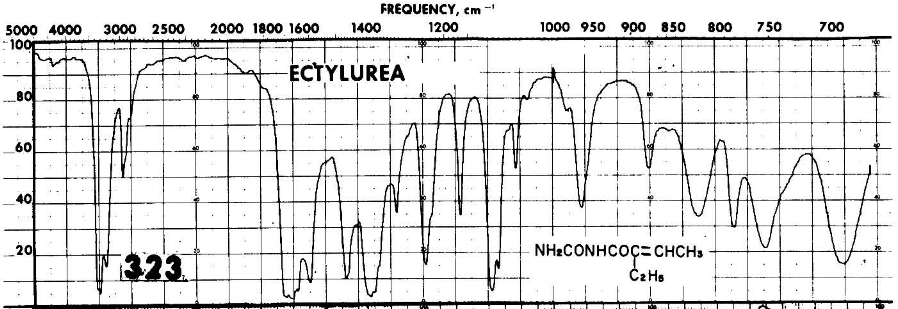
320 DIPHENYLPYRALINE HYDROCHLORIDE



321 DROMOSTANOLONE PROPIONATE



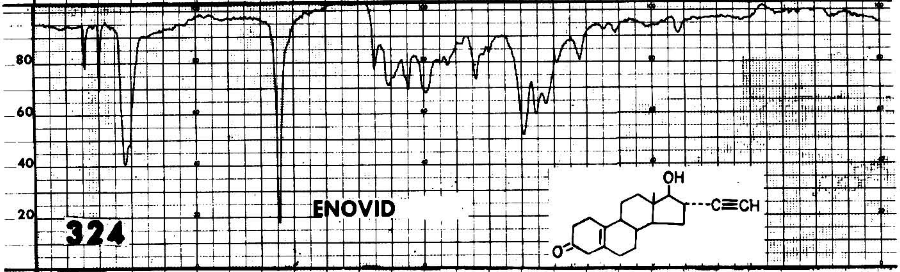
322 DYNACAINE HYDROCHLORIDE



323

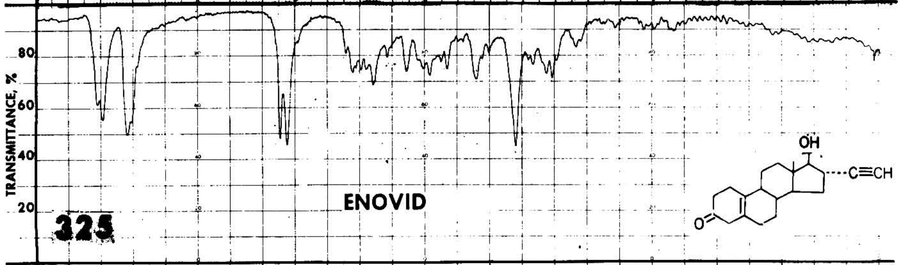
ECTYLUREA

$NH_2CONHCOC(C_2H_5){=}CHCH_3$



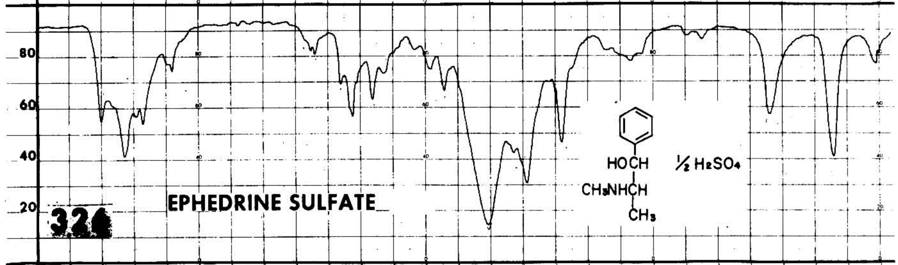
324

ENOVID



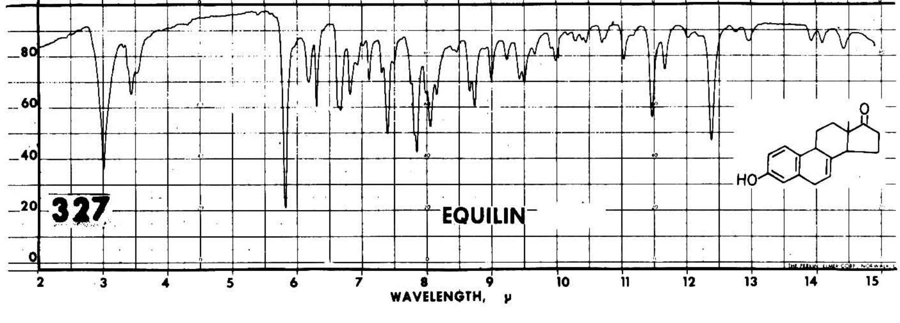
325

ENOVID



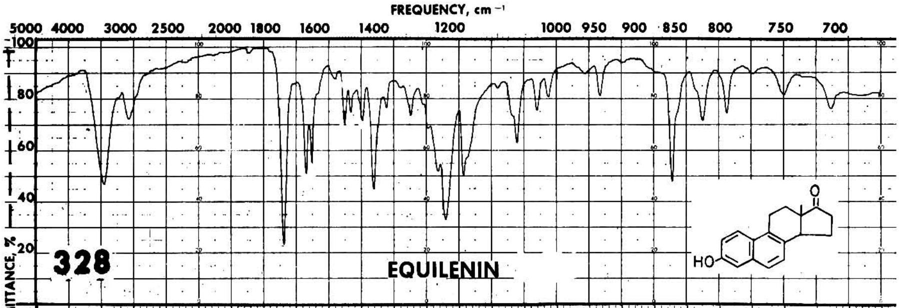
326

EPHEDRINE SULFATE



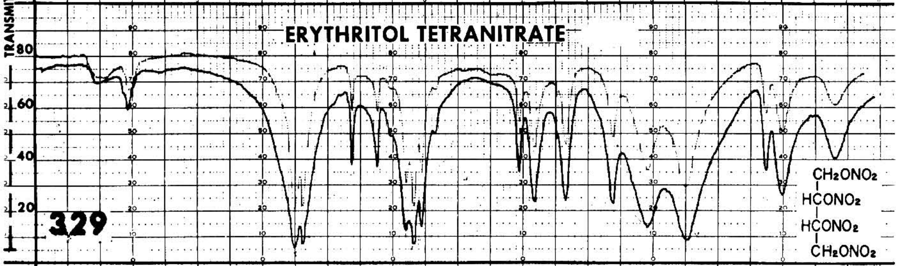
327

EQUILIN



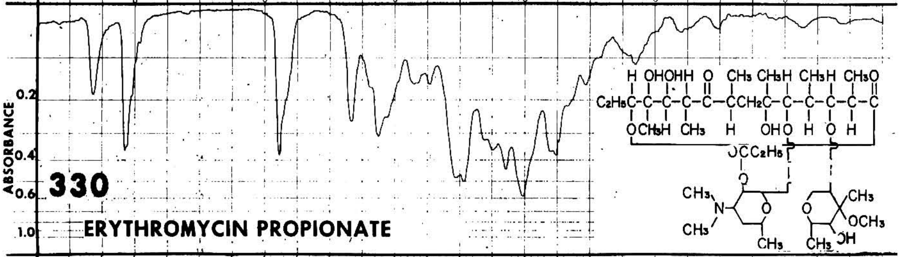
328

EQUILENIN



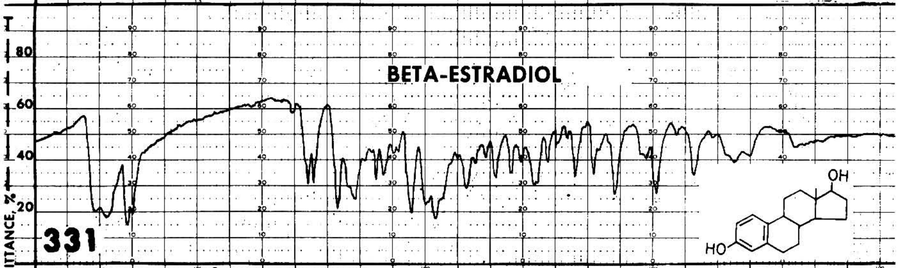
329

ERYTHRITOL TETRANITRATE



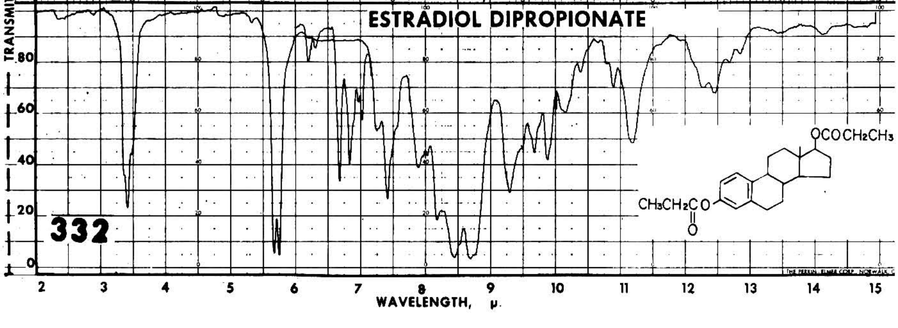
330

ERYTHROMYCIN PROPIONATE



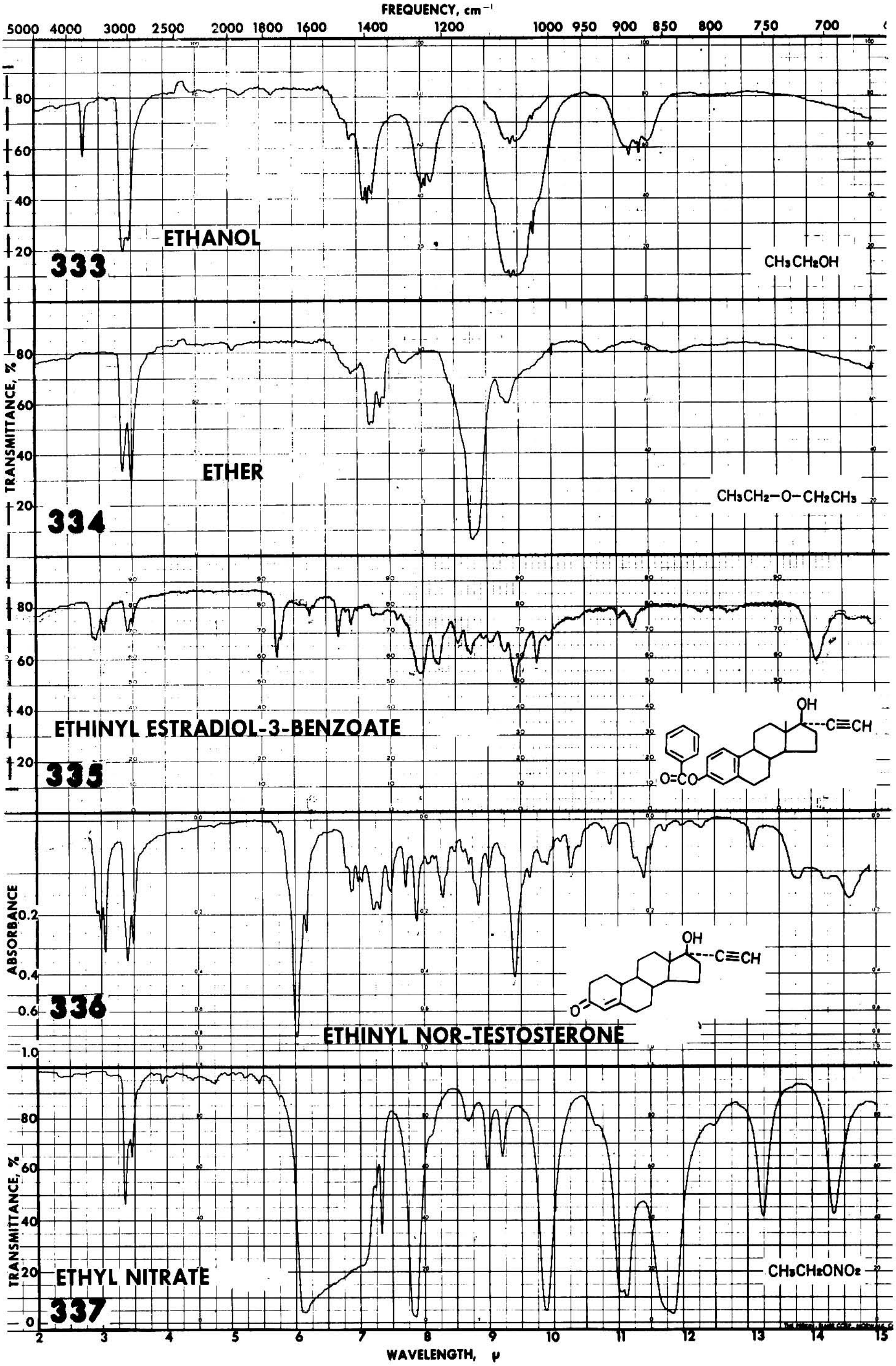
331

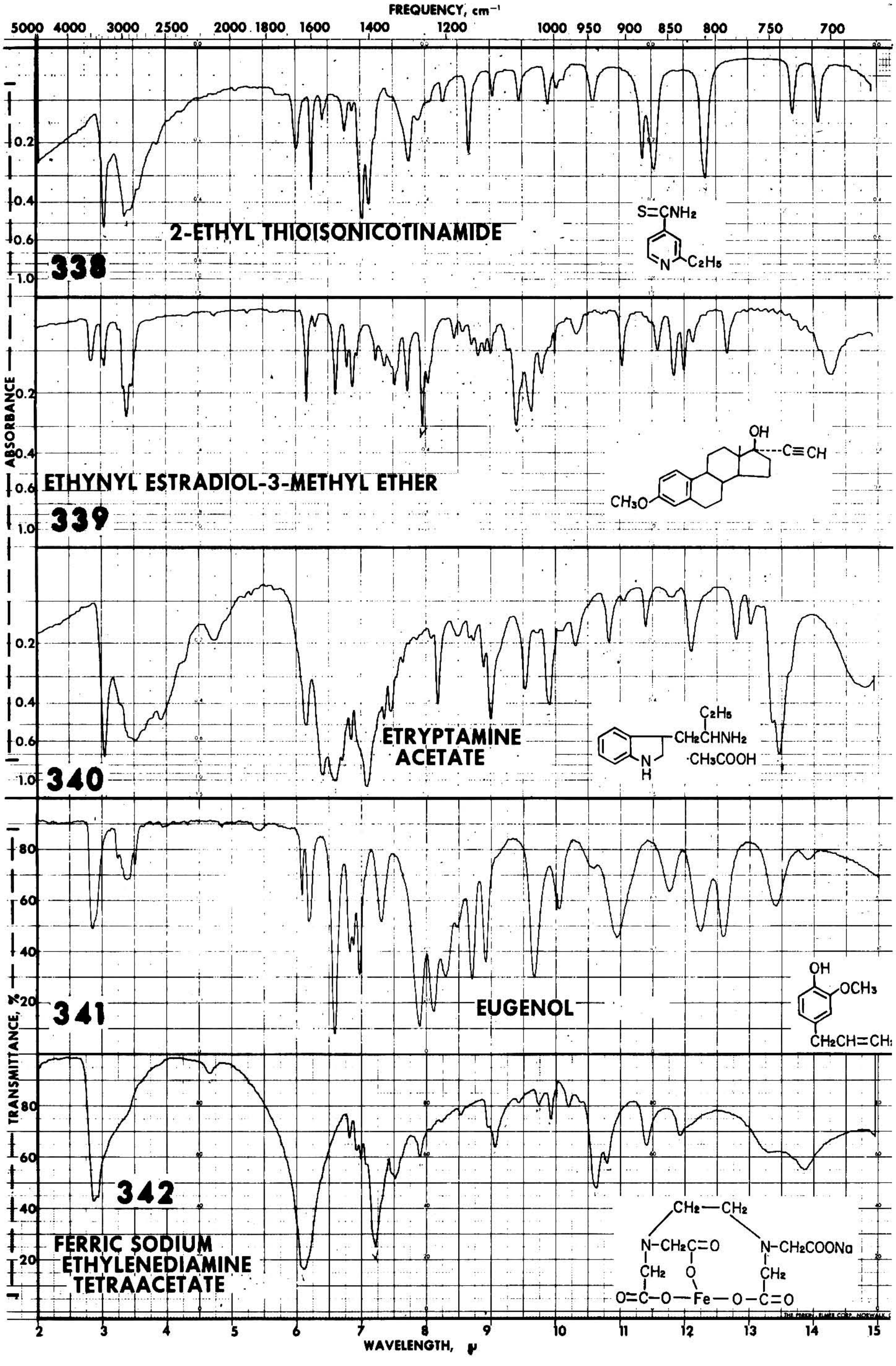
BETA-ESTRADIOL



332

ESTRADIOL DIPROPIONATE


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
80
60
40
20
333
ETHANOL
CH₃CH₂OH
334
ETHER
CH₃CH₂–O–CH₂CH₃
335
ETHINYL ESTRADIOL-3-BENZOATE
OH
C≡CH
O=C
O
ABSORBANCE
0.2
0.4
0.6
1.0
336
ETHINYL NOR-TESTOSTERONE
OH
C≡CH
O
337
ETHYL NITRATE
CH₃CH₂ONO₂
0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



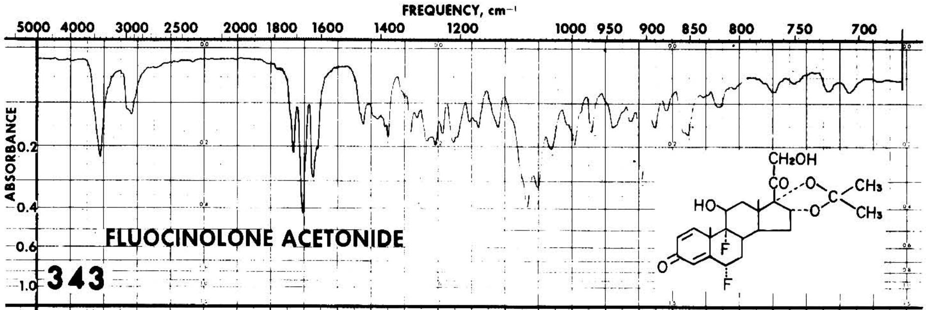
338 2-ETHYL THIOISONICOTINAMIDE

339 ETHYNYL ESTRADIOL-3-METHYL ETHER

340 ETRYPTAMINE ACETATE

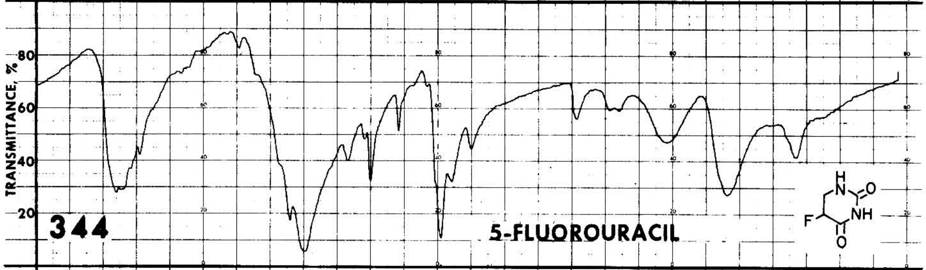
341 EUGENOL

342 FERRIC SODIUM ETHYLENEDIAMINE TETRAACETATE



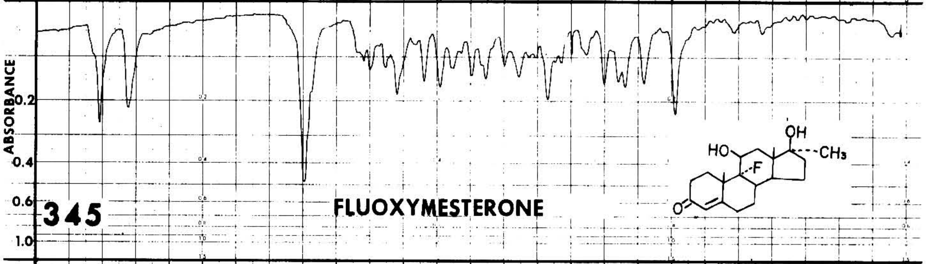
FLUOCINOLONE ACETONIDE

343



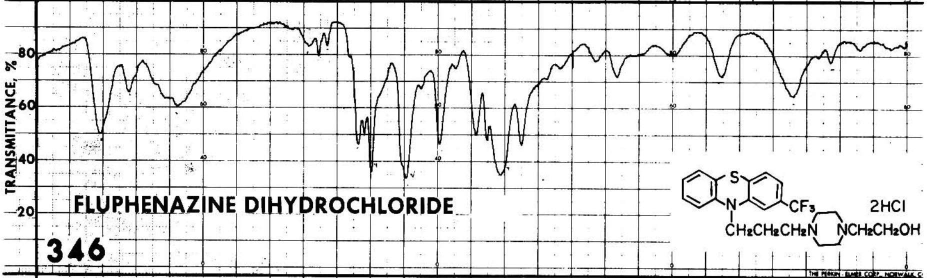
5-FLUOROURACIL

344



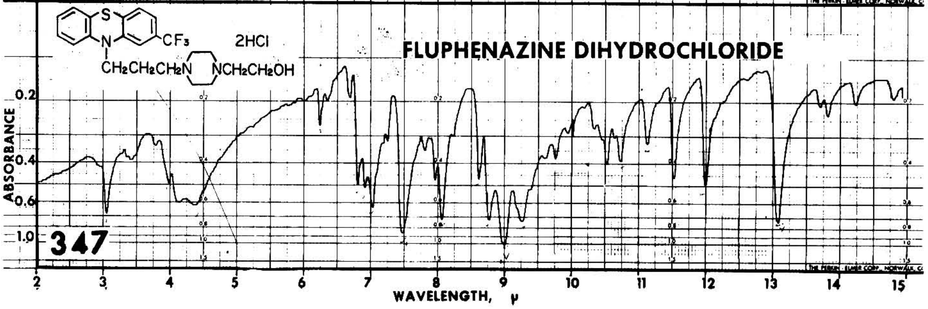
FLUOXYMESTERONE

345



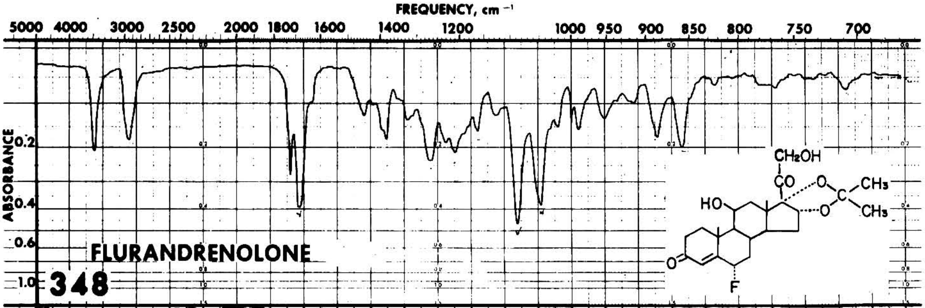
FLUPHENAZINE DIHYDROCHLORIDE

346

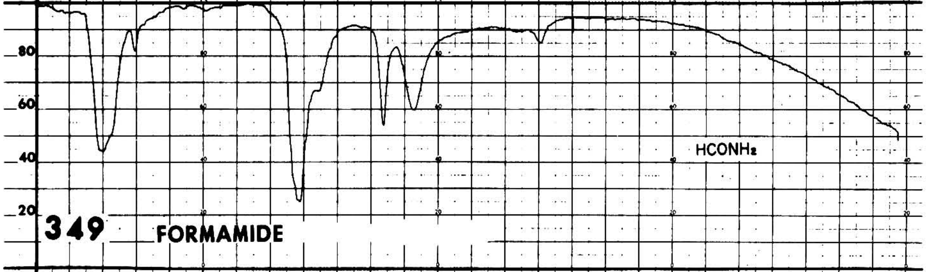


FLUPHENAZINE DIHYDROCHLORIDE

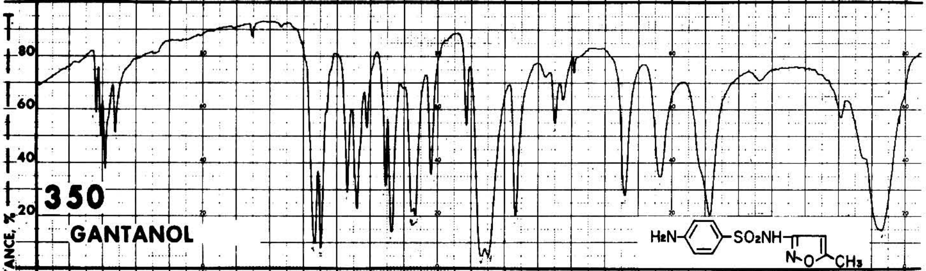
347



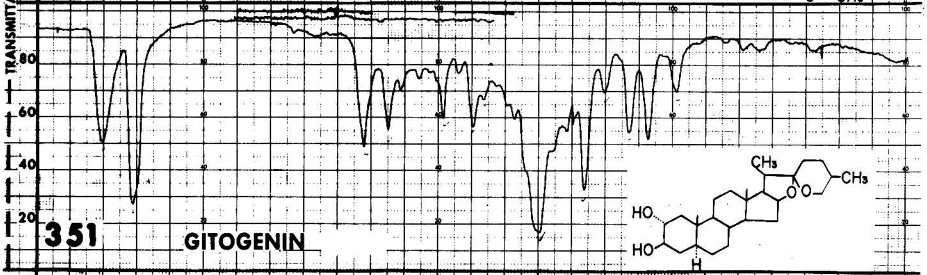
348 **FLURANDRENOLONE**



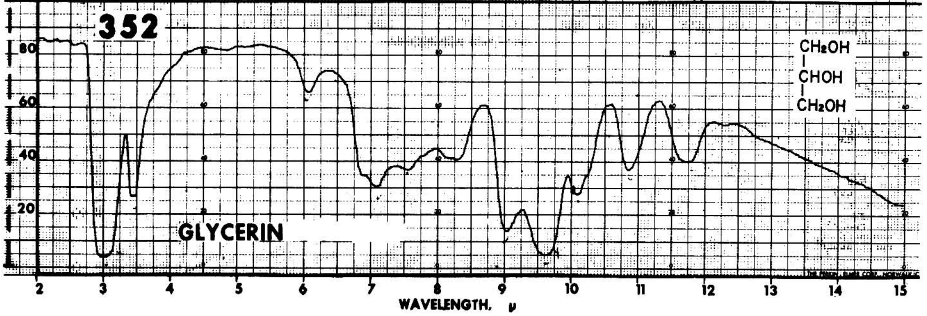
349 **FORMAMIDE**



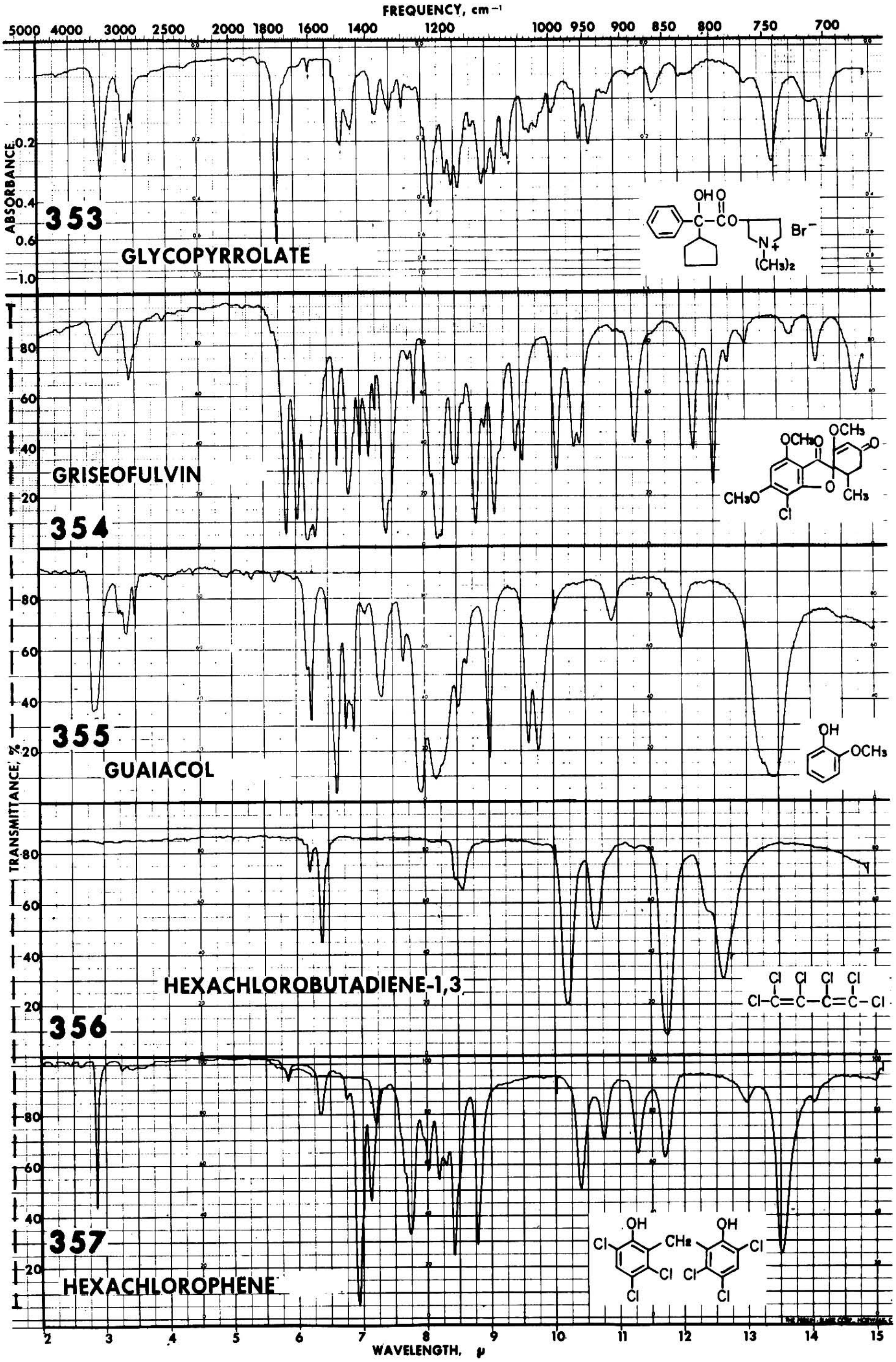
350 **GANTANOL**

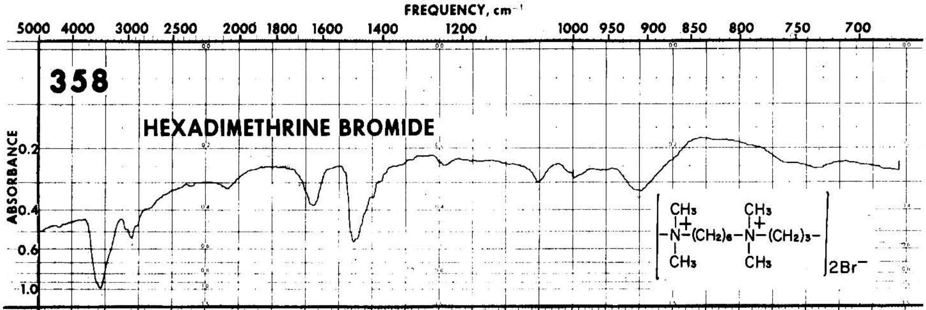


351 **GITOGENIN**



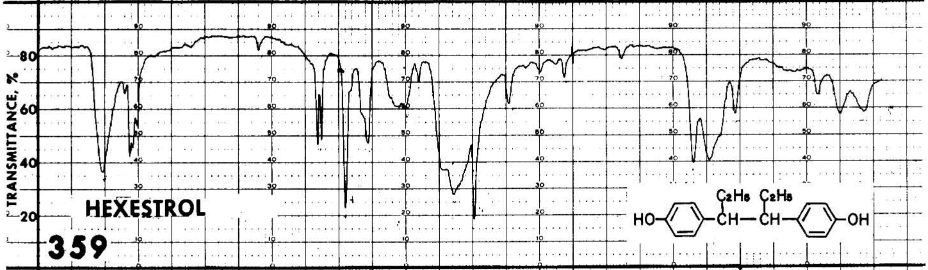
352 **GLYCERIN**


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ABSORBANCE
0.2
0.4
0.6
1.0
353
GLYCOPYRROLATE
OH O
C–CO–O
N+
(CH3)2
Br⁻
80
60
40
20
GRISEOFULVIN
354
OCH3 O
OCH3
O
CH3O
O
CH3
Cl
355
GUAIACOL
OH
OCH3
TRANSMITTANCE, %
HEXACHLOROBUTADIENE-1,3
Cl Cl Cl Cl
Cl–C=C–C=C–Cl
356
357
HEXACHLOROPHENE
OH
CH2
OH
Cl
Cl
Cl
Cl
Cl
Cl
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



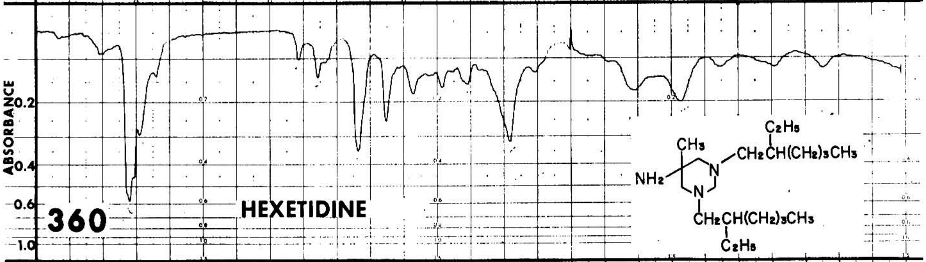
358

HEXADIMETHRINE BROMIDE



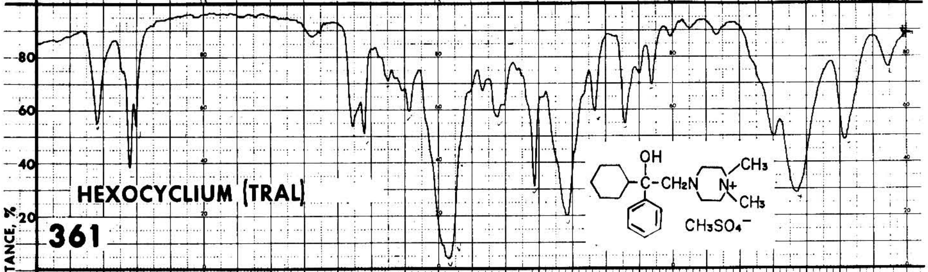
HEXESTROL

359



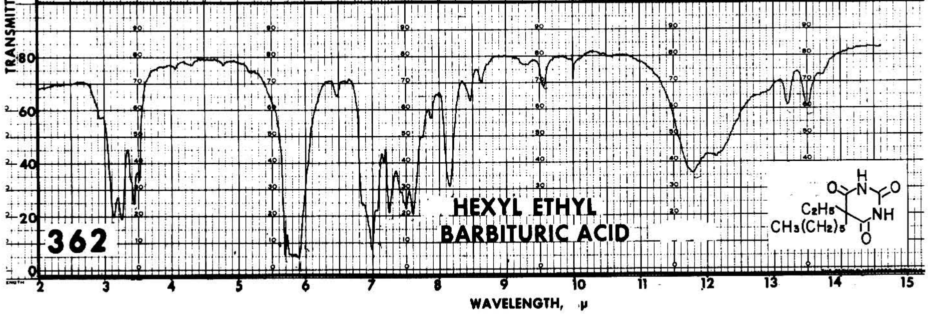
360

HEXETIDINE



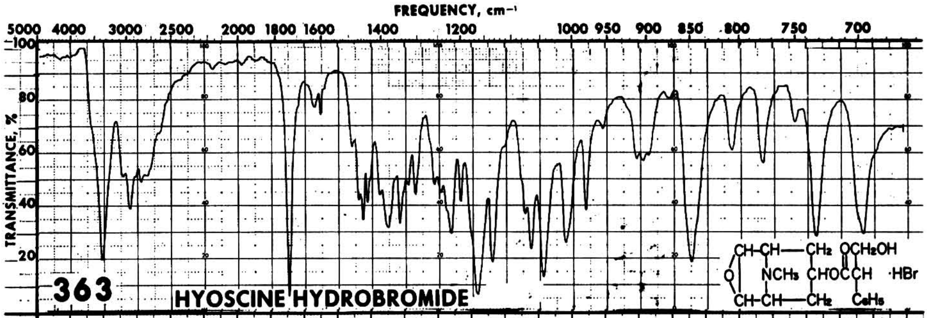
HEXOCYCLIUM (TRAL)

361

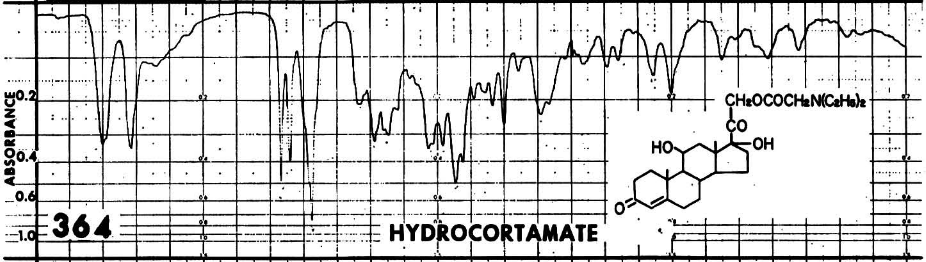


HEXYL ETHYL BARBITURIC ACID

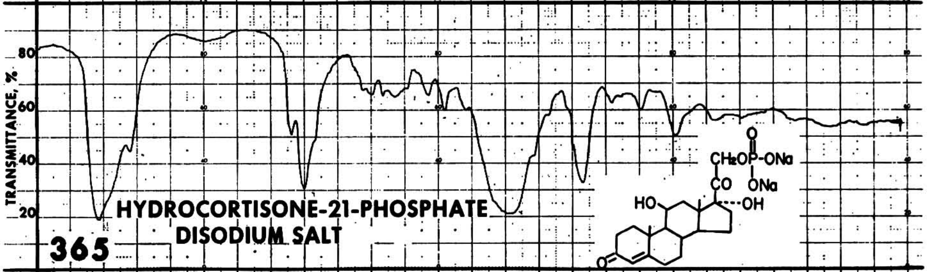
362



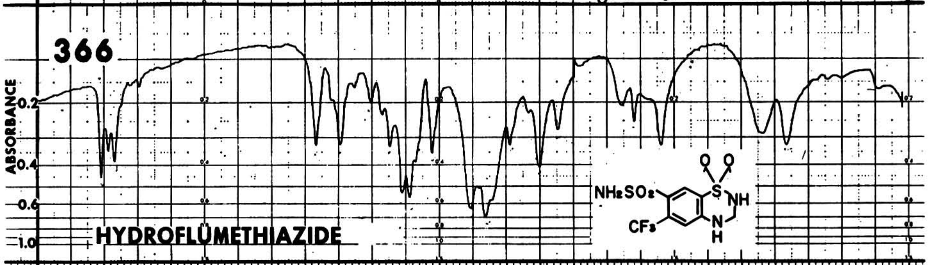
363 HYOSCINE HYDROBROMIDE



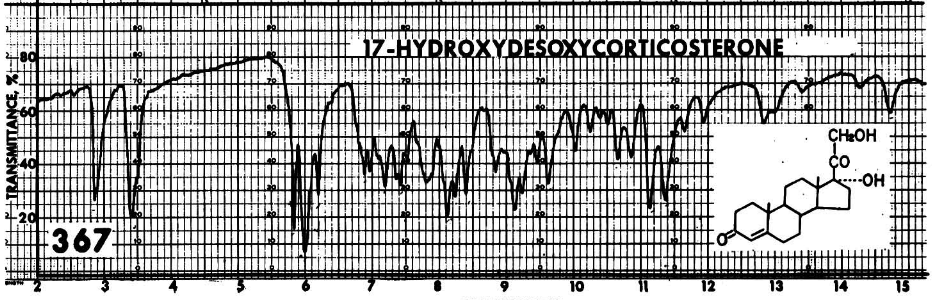
364 HYDROCORTAMATE



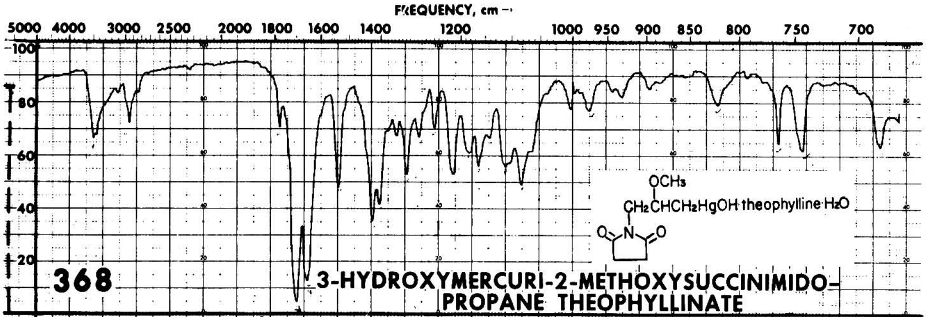
365 HYDROCORTISONE-21-PHOSPHATE DISODIUM SALT



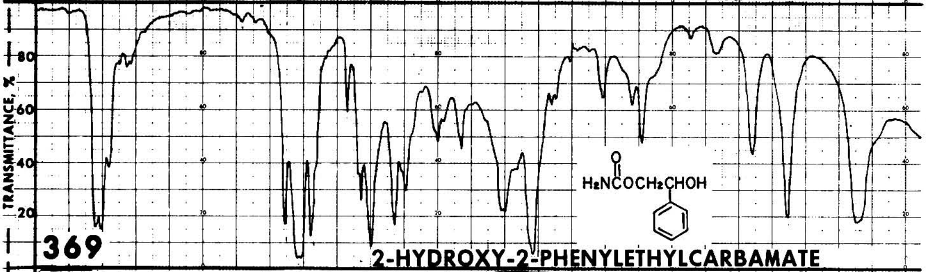
366 HYDROFLUMETHIAZIDE



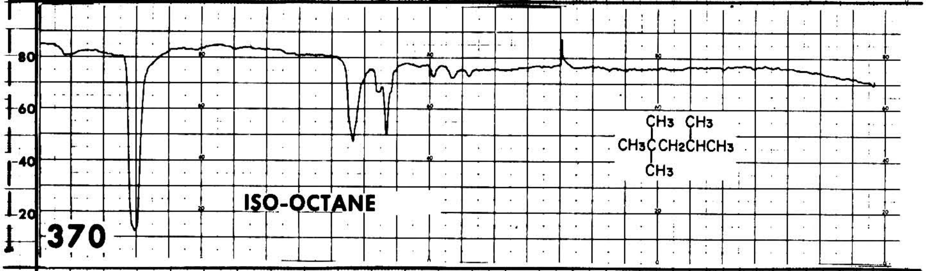
367 17-HYDROXYDESOXYCORTICOSTERONE



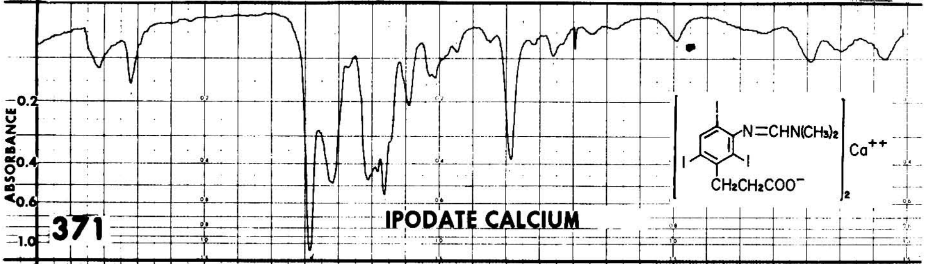
368 3-HYDROXYMERCURI-2-METHOXYSUCCINIMIDOPROPANE THEOPHYLLINATE



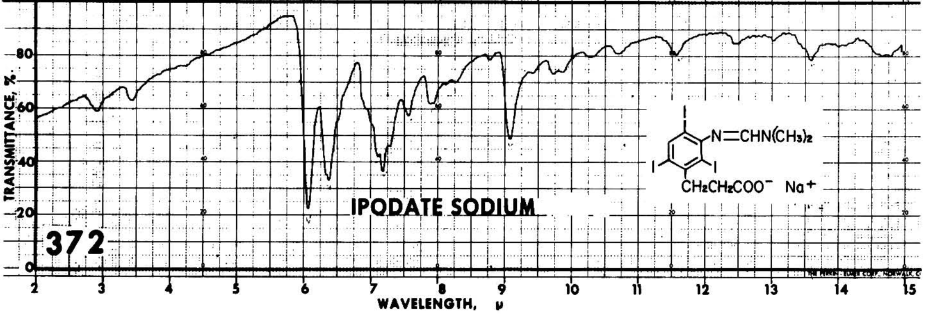
369 2-HYDROXY-2-PHENYLETHYLCARBAMATE



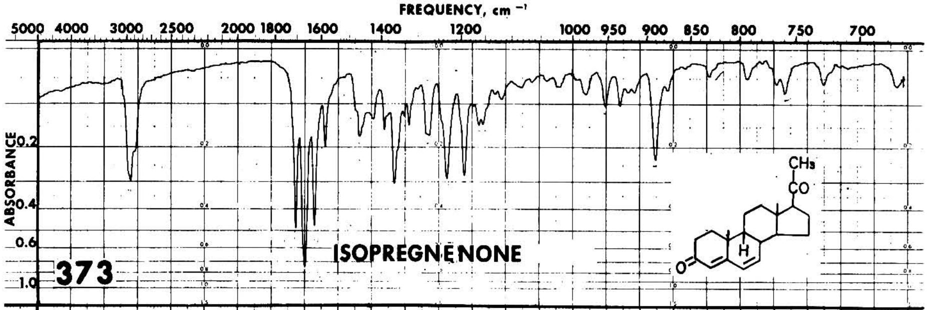
370 ISO-OCTANE



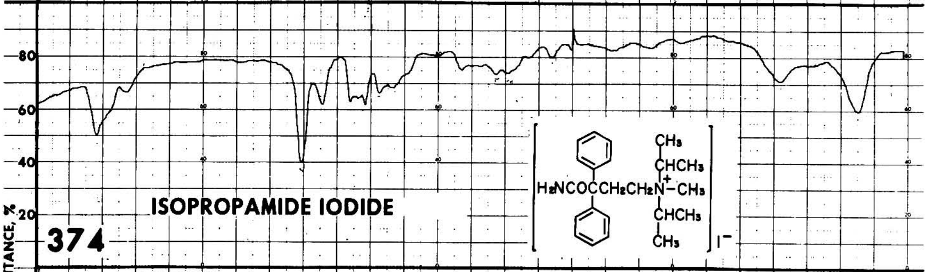
371 IPODATE CALCIUM



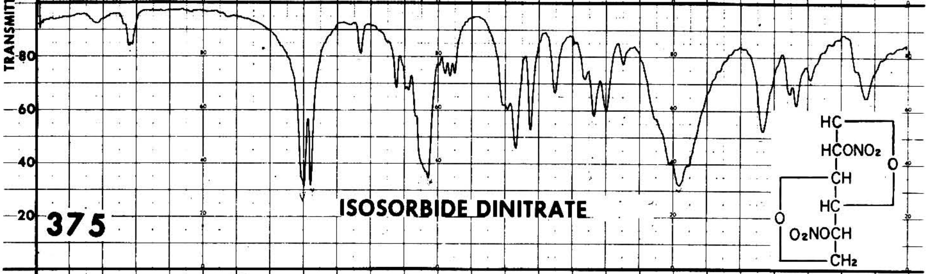
372 IPODATE SODIUM



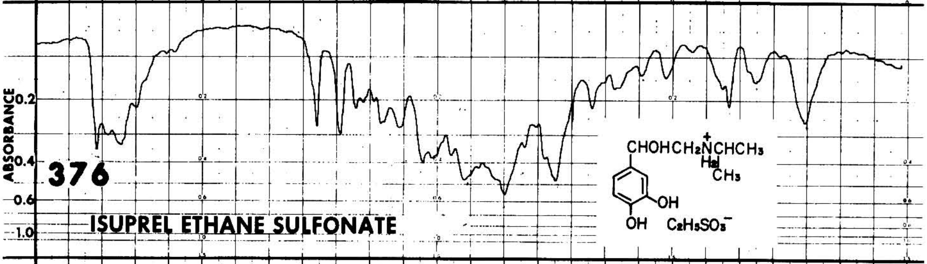
373 ISOPREGNENONE



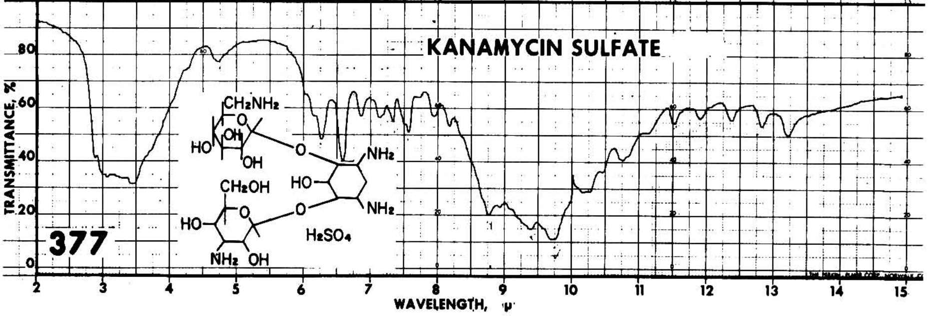
374 ISOPROPAMIDE IODIDE



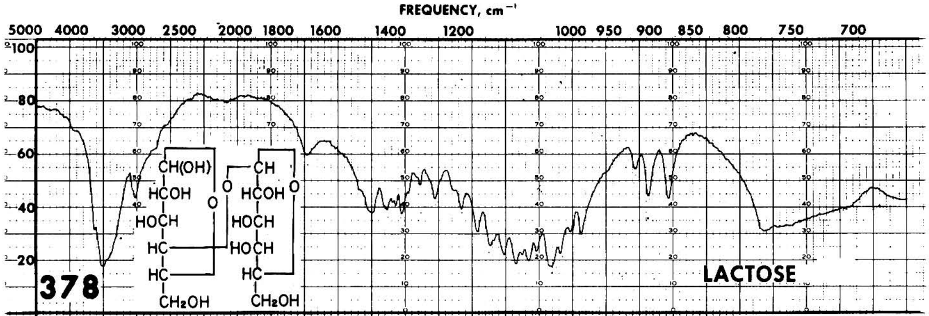
375 ISOSORBIDE DINITRATE



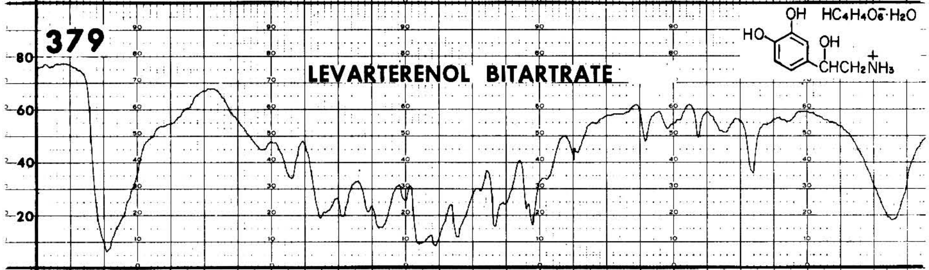
376 ISUPREL ETHANE SULFONATE



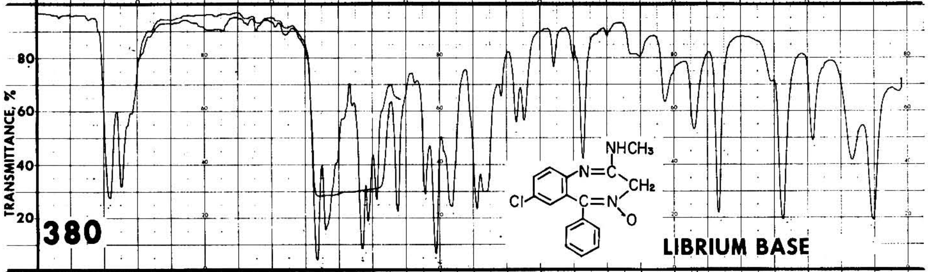
377 KANAMYCIN SULFATE



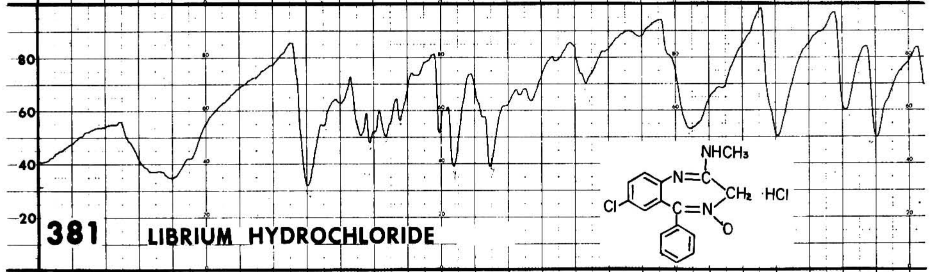
378 **LACTOSE**



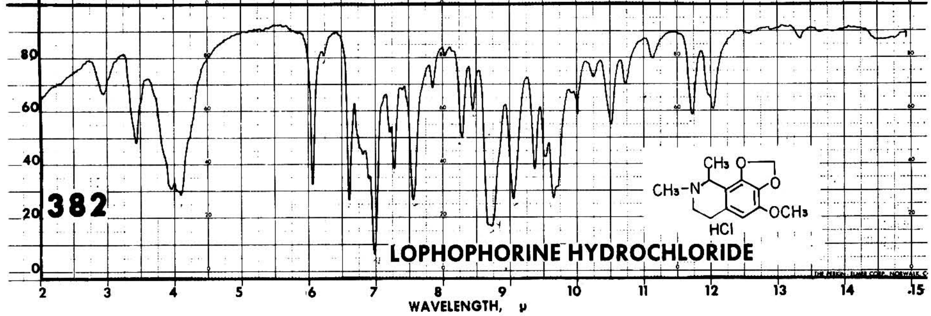
379 **LEVARTERENOL BITARTRATE**



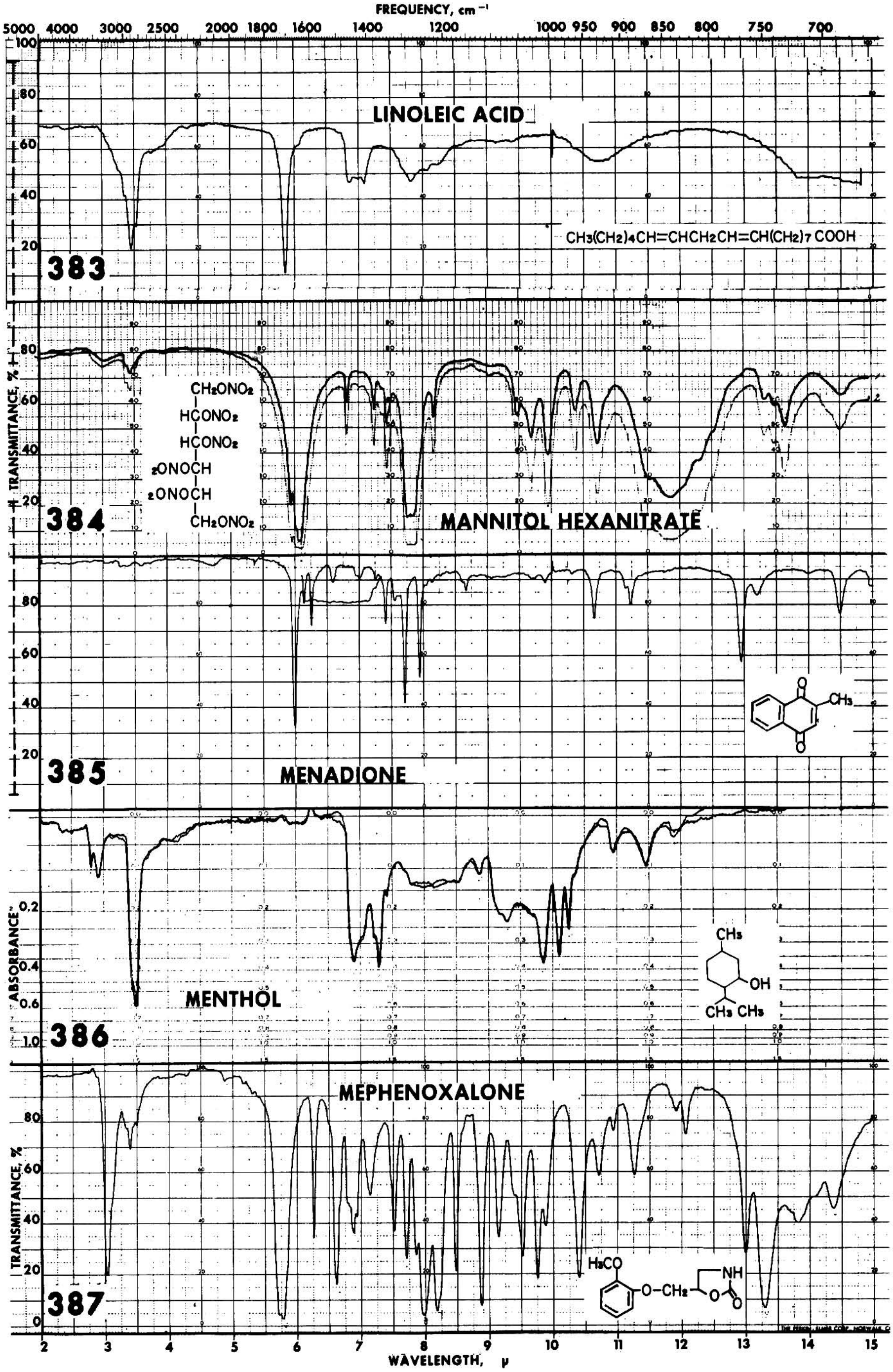
380 **LIBRIUM BASE**

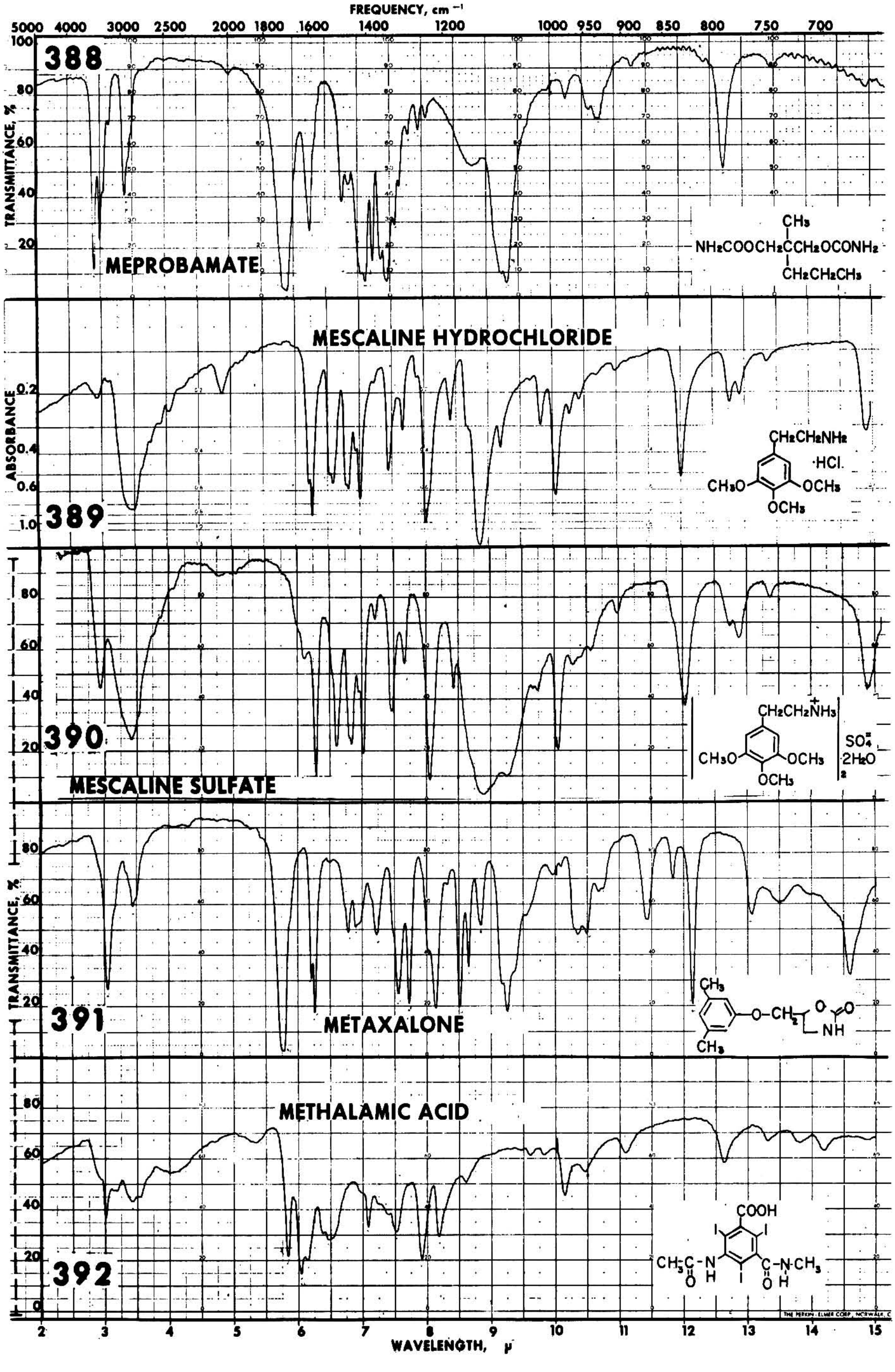


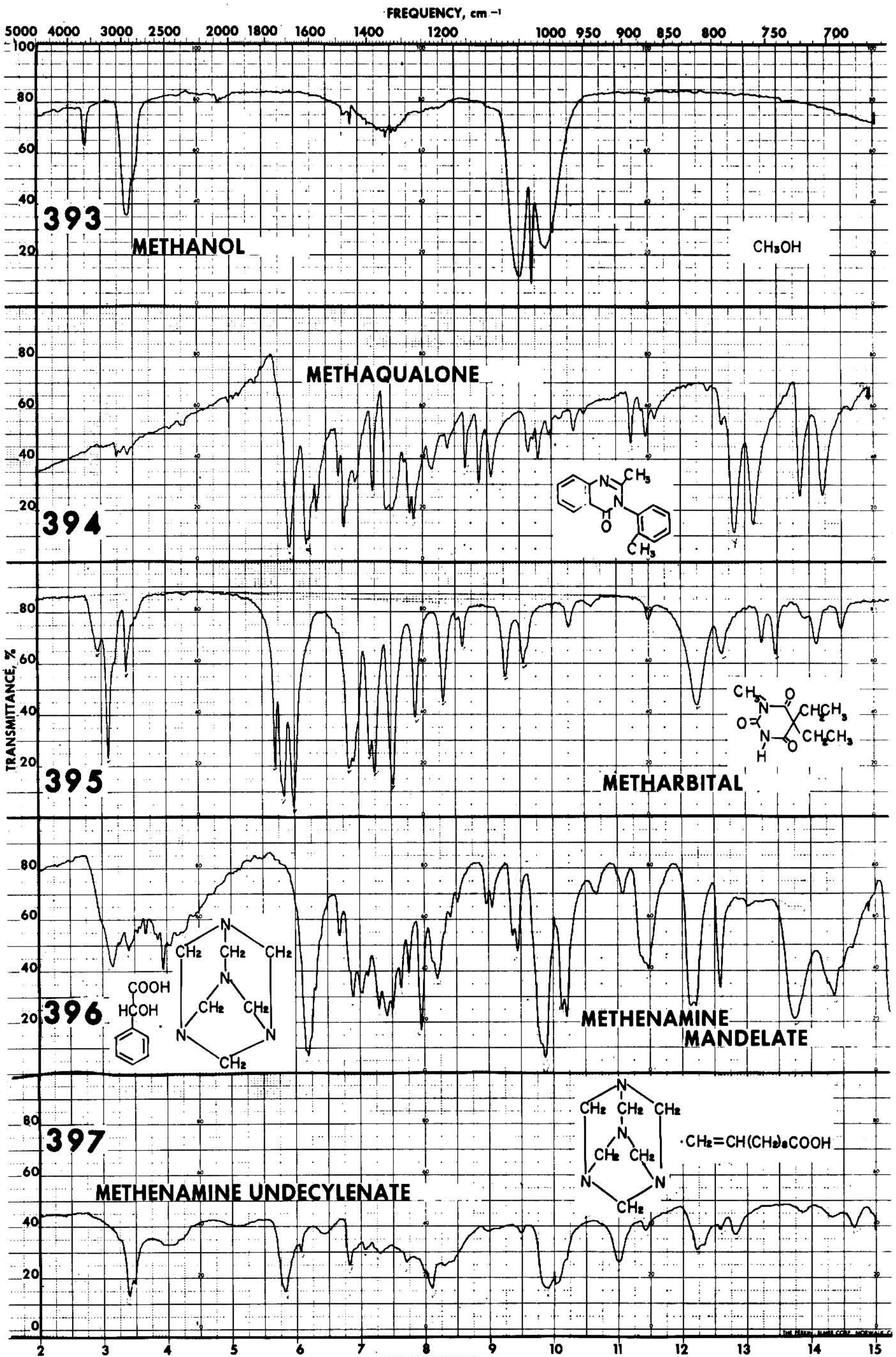
381 **LIBRIUM HYDROCHLORIDE**

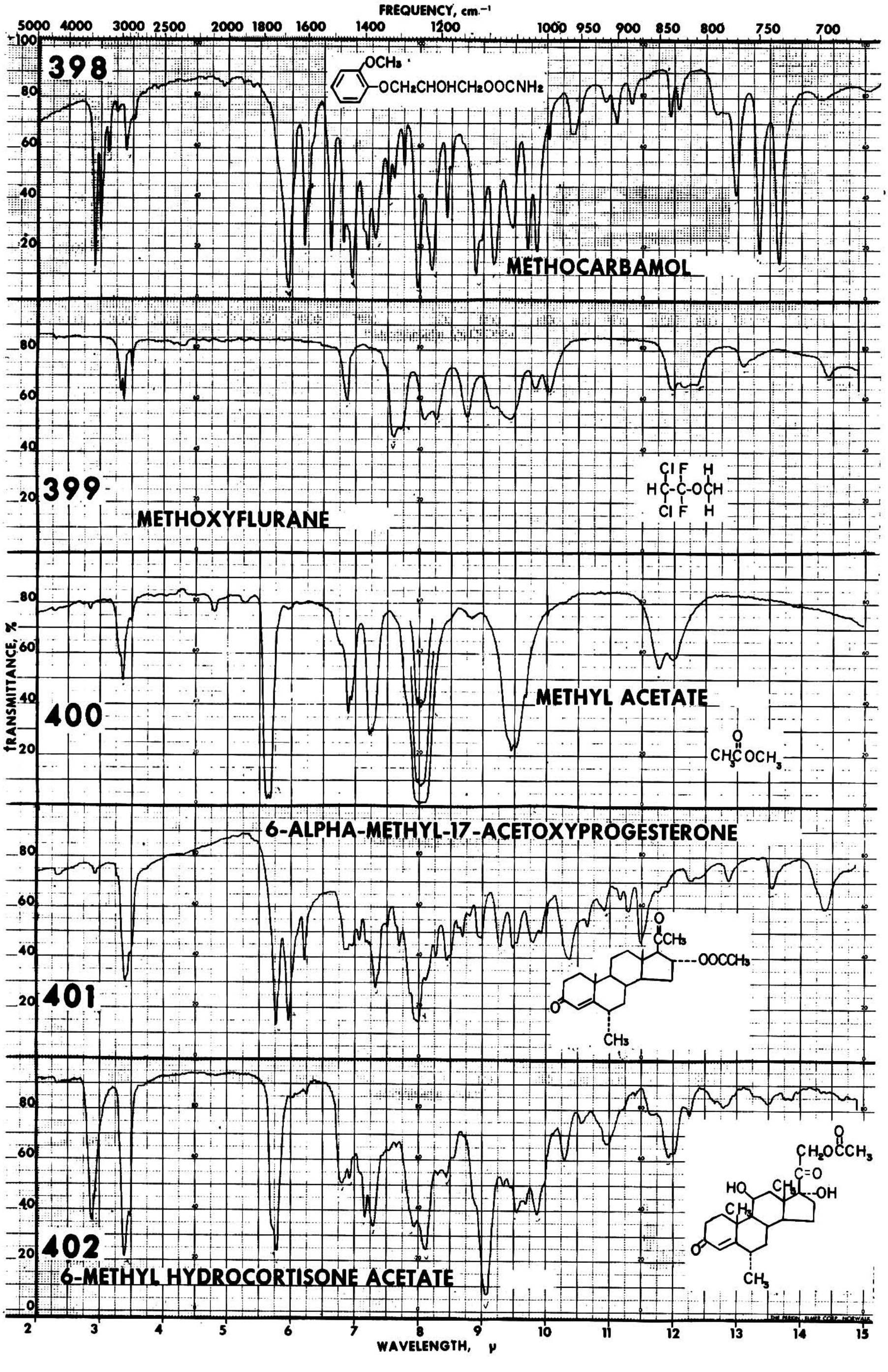


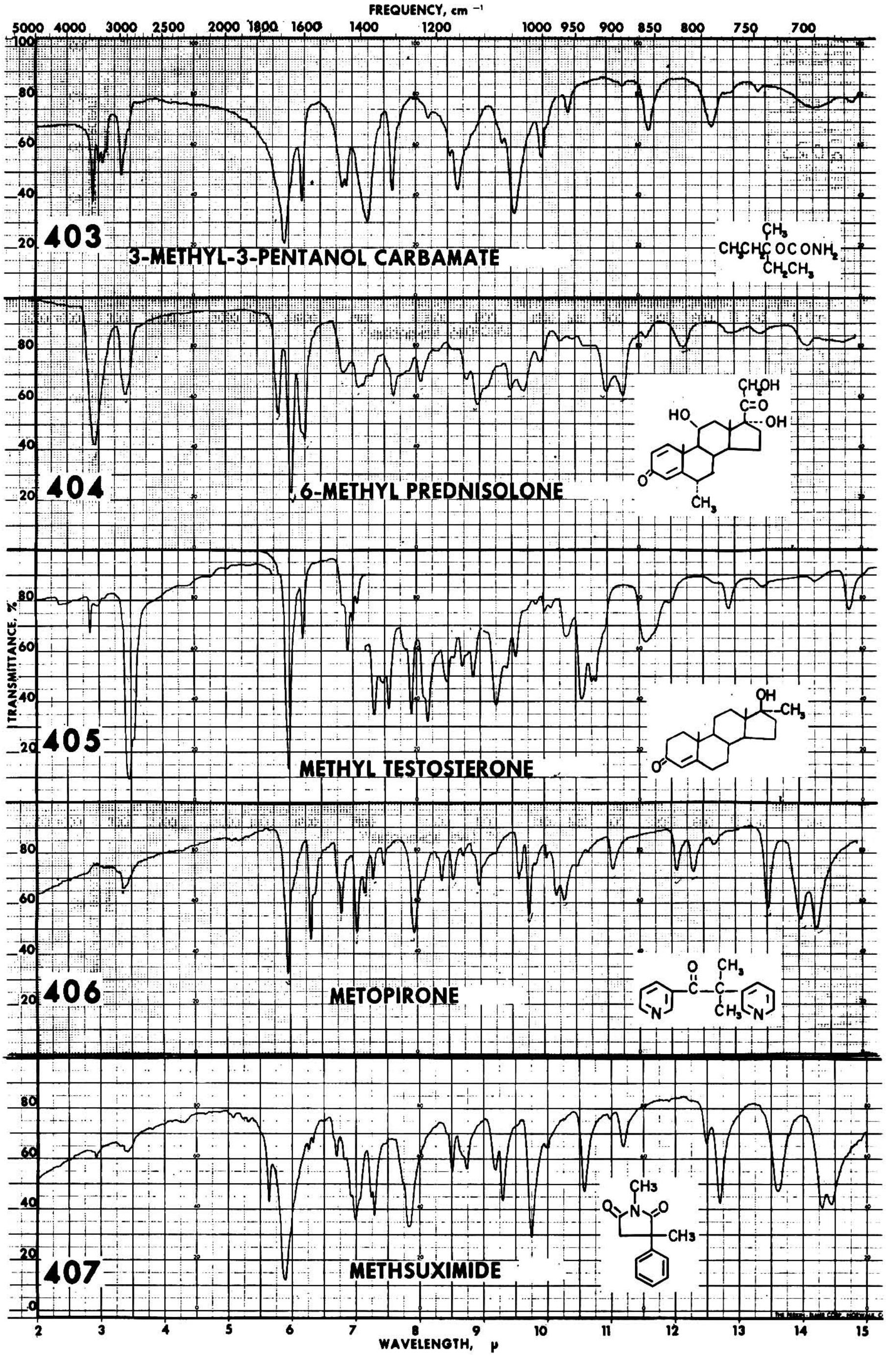
382 **LOPHOPHORINE HYDROCHLORIDE**

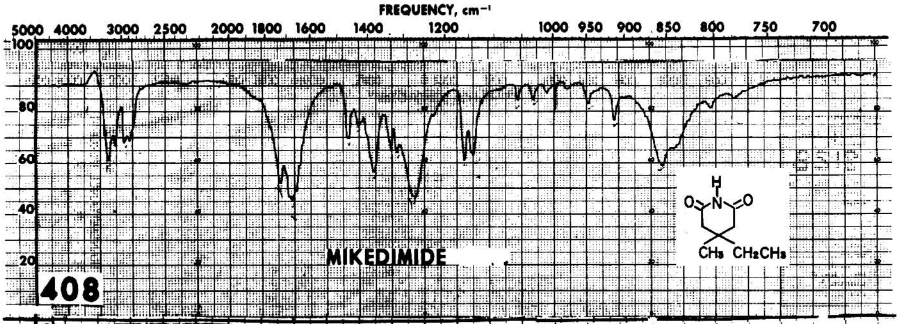

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
LINOLEIC ACID
CH3(CH2)4CH=CHCH2CH=CH(CH2)7 COOH
383
MANNITOL HEXANITRATE
CH2ONO2
HCONO2
HCONO2
2ONOCH
2ONOCH
CH2ONO2
384
MENADIONE
CH3
385
MENTHOL
CH3
OH
CH3 CH3
386
MEPHENOXALONE
H3CO
O–CH2
NH
O
O
387
TRANSMITTANCE, %
ABSORBANCE
100 80 60 40 20 0
0.2 0.4 0.6 1.0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100
80
60
40
20
388
MEPROBAMATE
CH₃
NH₂COOCH₂CCH₂OCONH₂
CH₂CH₂CH₃
ABSORBANCE
0.2
0.4
0.6
1.0
389
MESCALINE HYDROCHLORIDE
CH₂CH₂NH₂
·HCl
CH₃O
OCH₃
OCH₃
T
80
60
40
20
390
MESCALINE SULFATE
CH₂CH₂N̊H₃
SO₄⁼
·2H₂O
2
CH₃O
OCH₃
OCH₃
TRANSMITTANCE, %
80
60
40
20
391
METAXALONE
CH₃
O–CH₂
O
O
NH
CH₃
80
60
40
20
0
392
METHALAMIC ACID
COOH
I
I
I
CH₃C–N
O H
C–N·CH₃
O H
THE PERKIN-ELMER CORP., NORWALK, C
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
393
METHANOL
CH₃OH
394
METHAQUALONE
395
METHARBITAL
396
METHENAMINE MANDELATE
397
METHENAMINE UNDECYLENATE
·CH₂=CH(CH₂)₈COOH
TRANSMITTANCE, %
100 80 60 40 20 0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

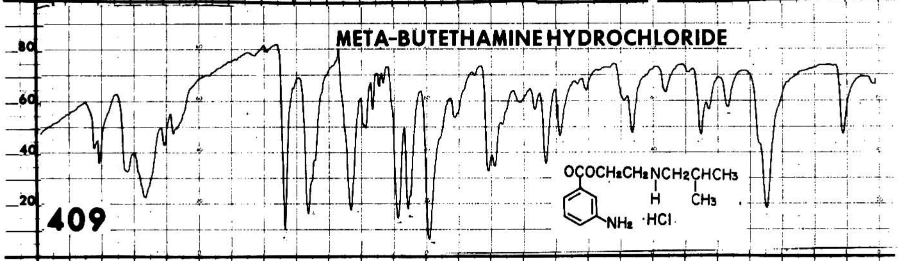

FREQUENCY, cm.⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
398
OCH₃
OCH₂CHOHCH₂OOCNH₂
METHOCARBAMOL
399
METHOXYFLURANE
Cl F H
HC-C-OCH
Cl F H
400
METHYL ACETATE
CH₃COCH₃
O
401
6-ALPHA-METHYL-17-ACETOXYPROGESTERONE
CCH₃
O
OOCCH₃
CH₃
402
6-METHYL HYDROCORTISONE ACETATE
CH₂OCCH₃
O
C=O
HO
CH₃
OH
CH₃
CH₃
TRANSMITTANCE, %
100
80
60
40
20
0
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100
80
60
40
20
0
403
3-METHYL-3-PENTANOL CARBAMATE
404
6-METHYL PREDNISOLONE
405
METHYL TESTOSTERONE
406
METOPIRONE
407
METHSUXIMIDE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



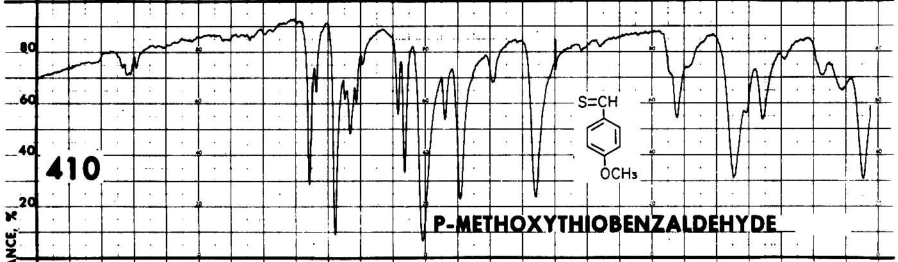
408

MIKEDIMIDE



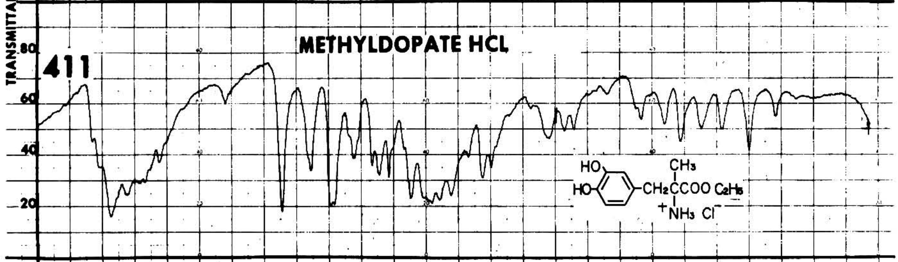
409

META-BUTETHAMINE HYDROCHLORIDE



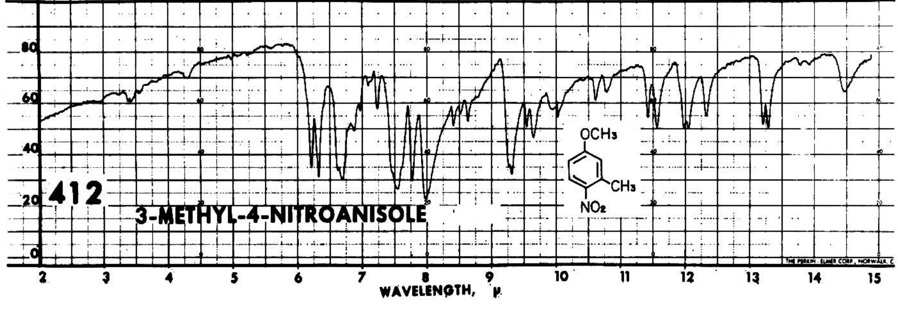
410

P-METHOXYTHIOBENZALDEHYDE



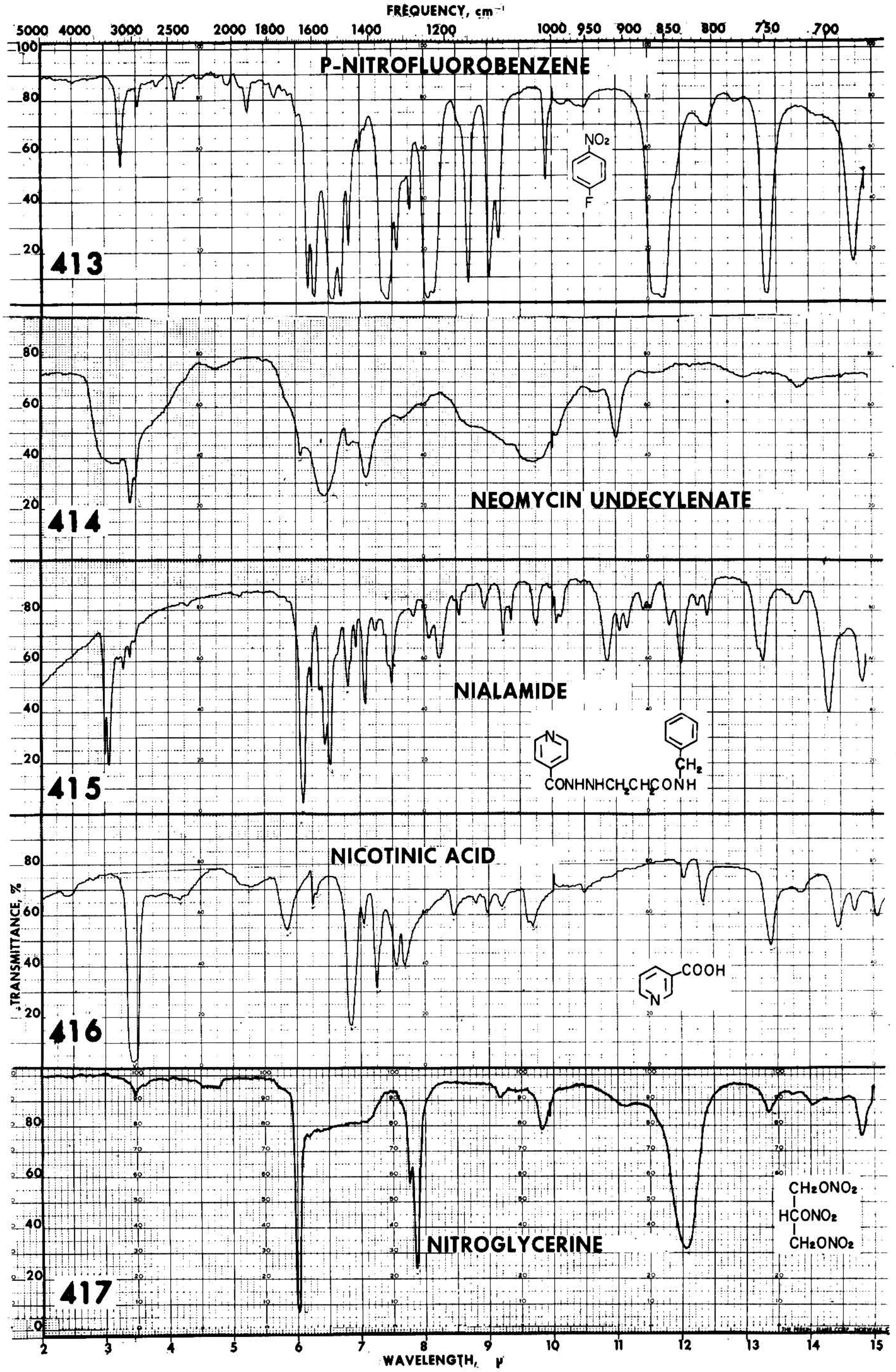
411

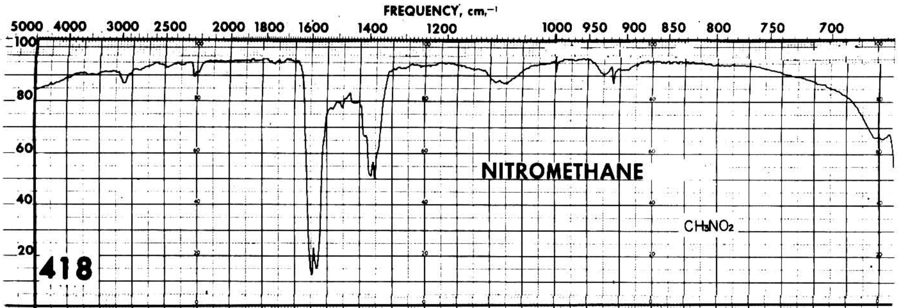
METHYLDOPATE HCL



412

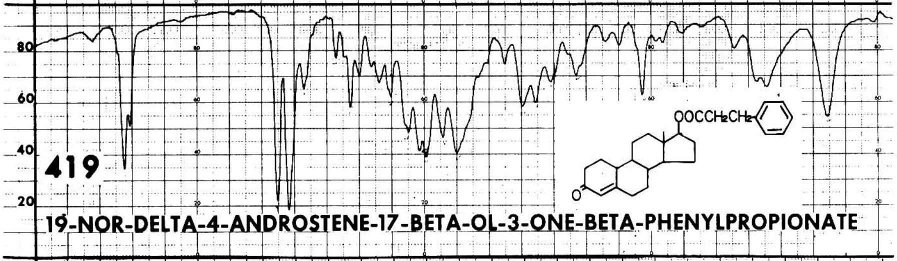
3-METHYL-4-NITROANISOLE


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
100
80
60
40
20
P-NITROFLUOROBENZENE
NO2
F
413
NEOMYCIN UNDECYLENATE
414
NIALAMIDE
CONHNHCH2CH2CONH
CH2
N
415
NICOTINIC ACID
COOH
N
416
TRANSMITTANCE, %
NITROGLYCERINE
CH2ONO2
HCONO2
CH2ONO2
417
0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



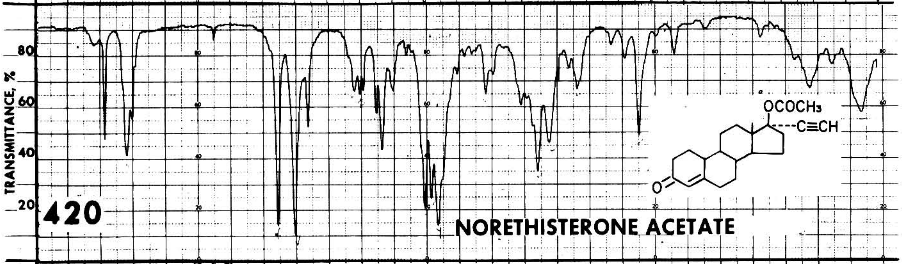
418

NITROMETHANE



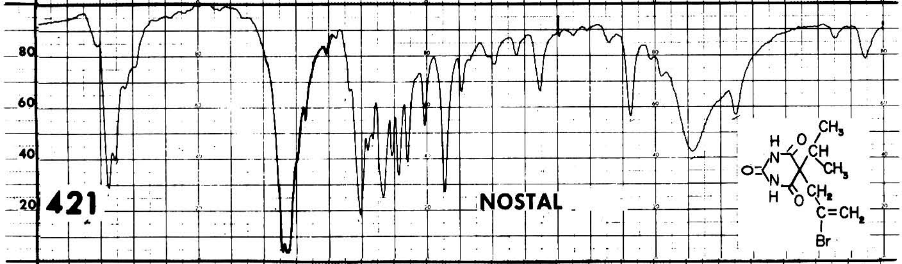
419

19-NOR-DELTA-4-ANDROSTENE-17-BETA-OL-3-ONE-BETA-PHENYLPROPIONATE



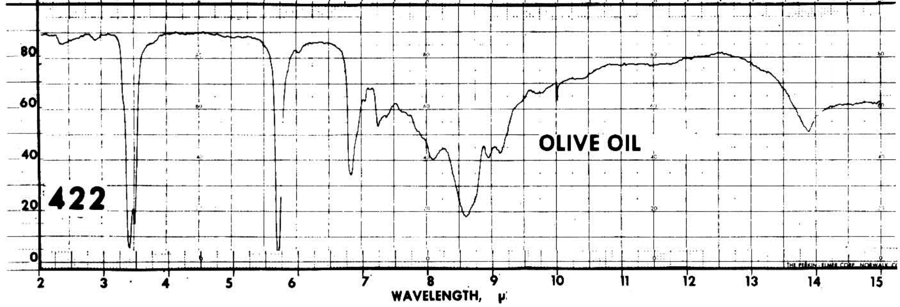
420

NORETHISTERONE ACETATE



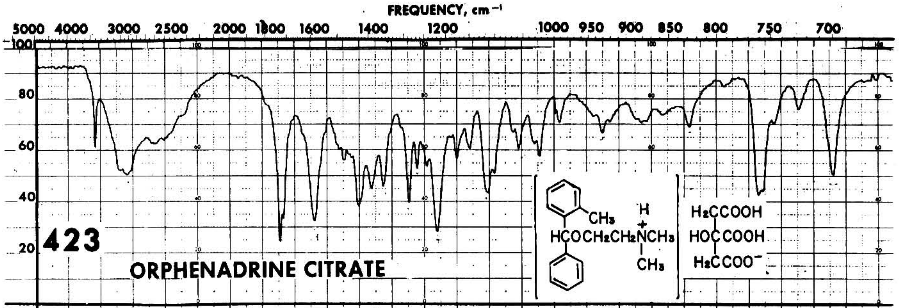
421

NOSTAL



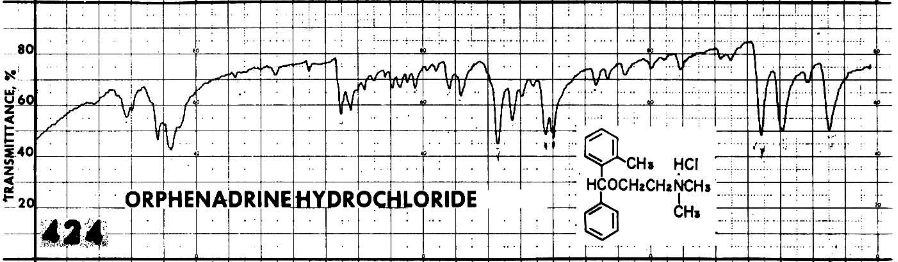
422

OLIVE OIL



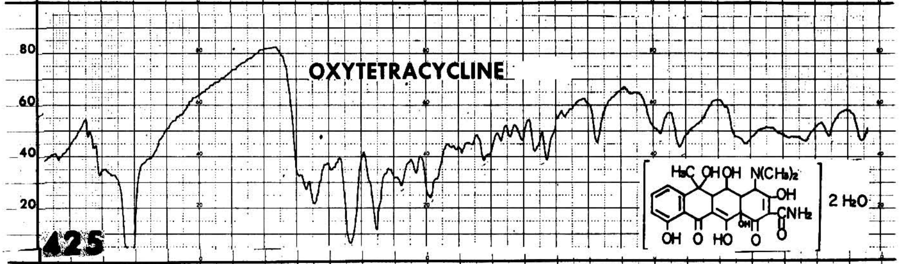
423

ORPHENADRINE CITRATE



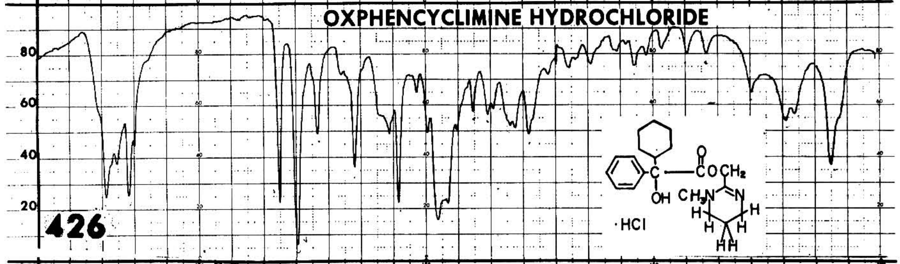
424

ORPHENADRINE HYDROCHLORIDE



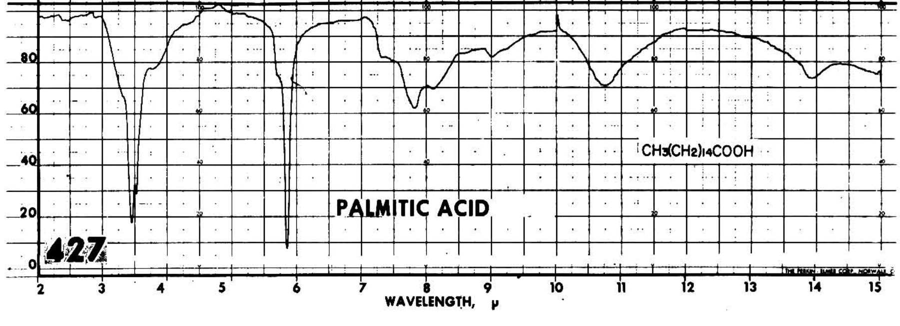
OXYTETRACYCLINE

425



OXPHENCYCLIMINE HYDROCHLORIDE

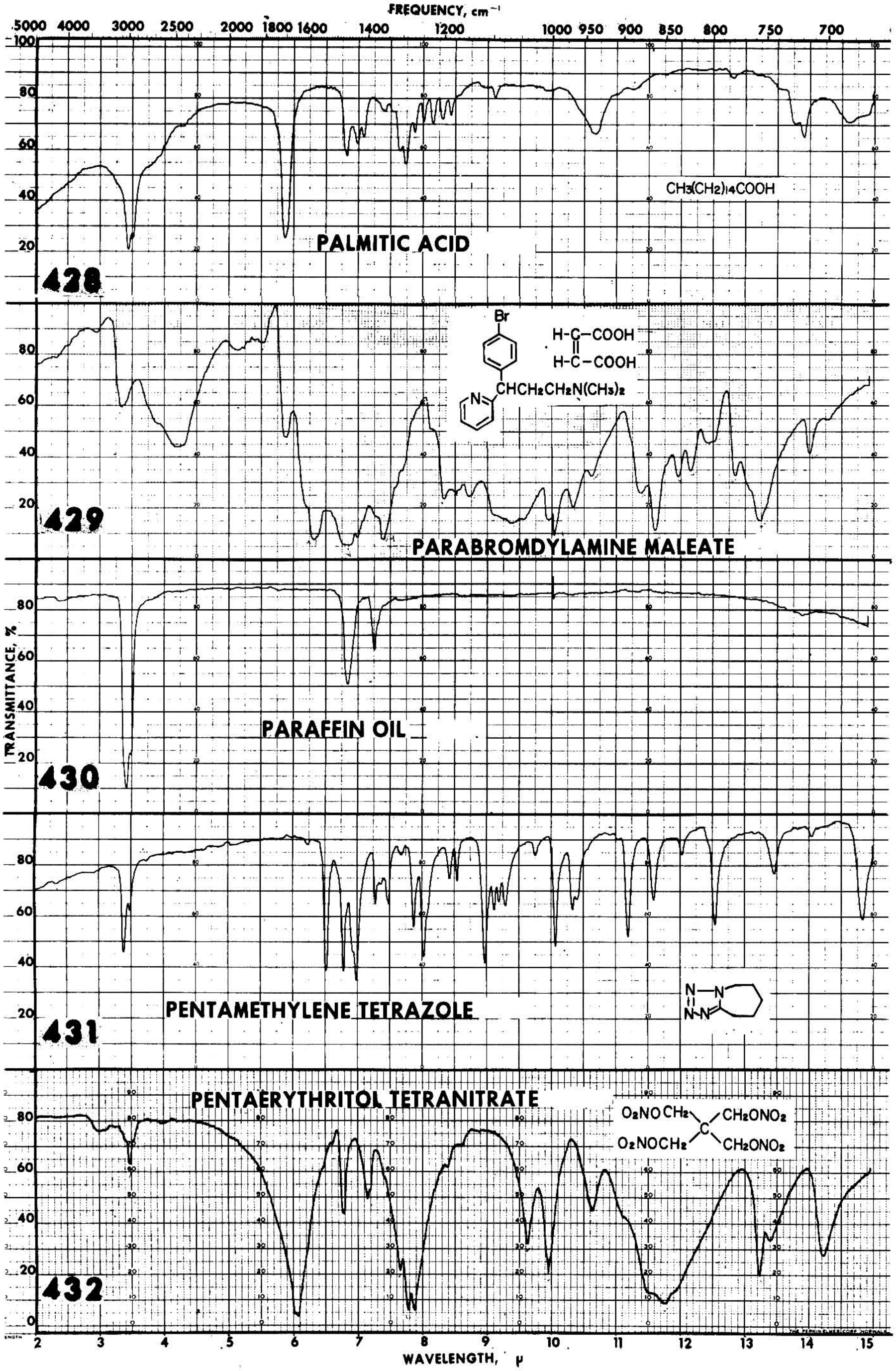
426

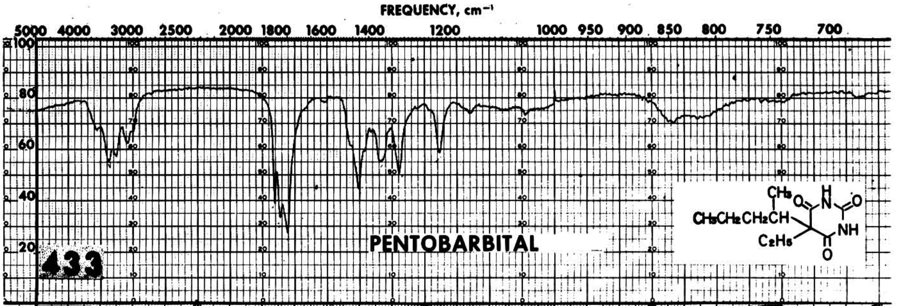


CH₃(CH₂)₁₄COOH

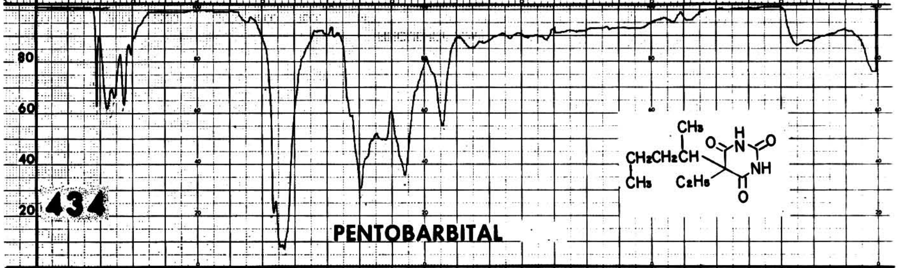
PALMITIC ACID

427

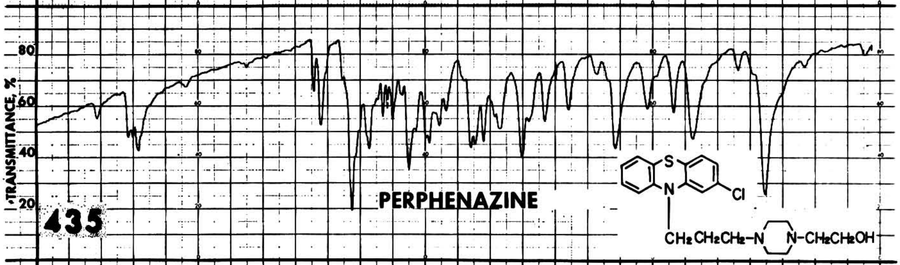

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100 80 60 40 20 0
428
PALMITIC ACID
CH3(CH2)14COOH
429
PARABROMDYLAMINE MALEATE
Br
CHCH2CH2N(CH3)2
N
H-C-COOH
H-C-COOH
430
PARAFFIN OIL
431
PENTAMETHYLENE TETRAZOLE
N—N
N-N
432
PENTAERYTHRITOL TETRANITRATE
O2NOCH2
CH2ONO2
C
O2NOCH2
CH2ONO2
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



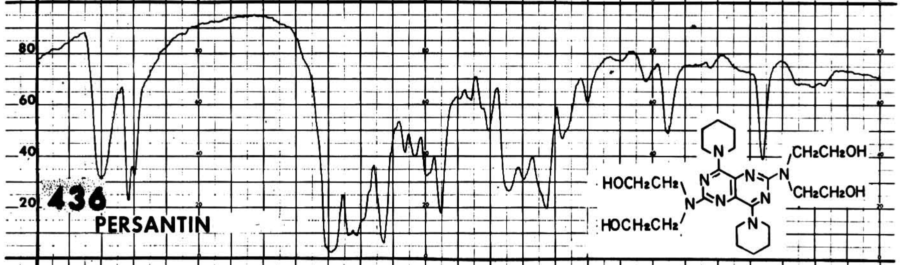
433 PENTOBARBITAL



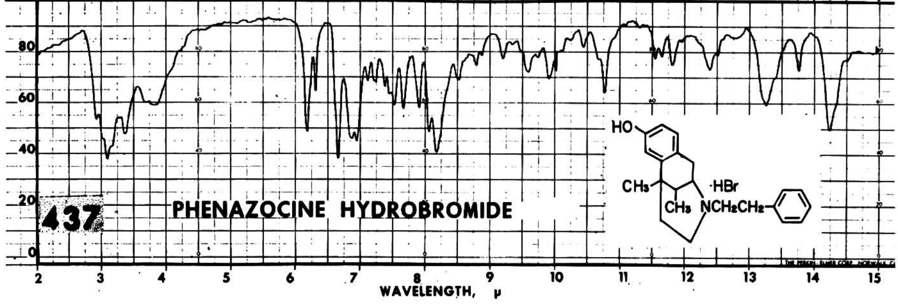
434 PENTOBARBITAL



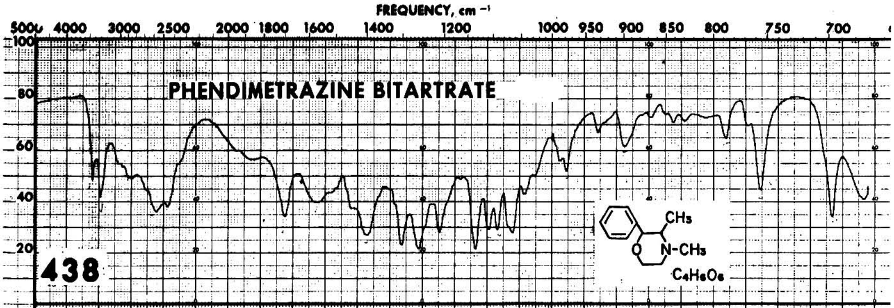
435 PERPHENAZINE



436 PERSANTIN

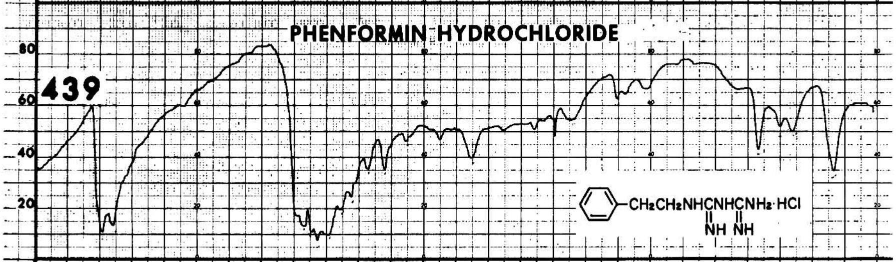


437 PHENAZOCINE HYDROBROMIDE



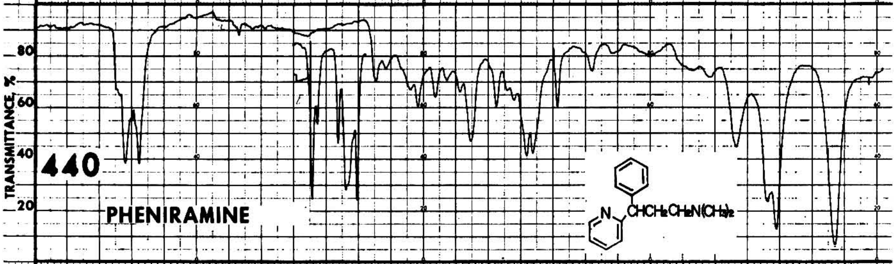
438

PHENDIMETRAZINE BITARTRATE



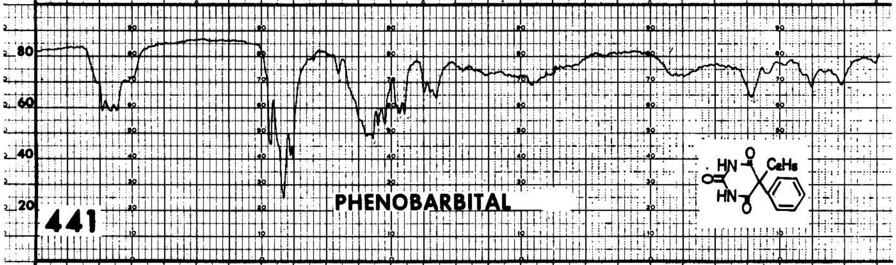
439

PHENFORMIN HYDROCHLORIDE



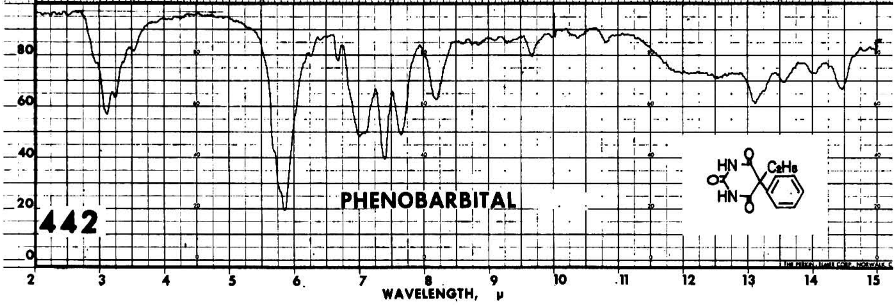
440

PHENIRAMINE



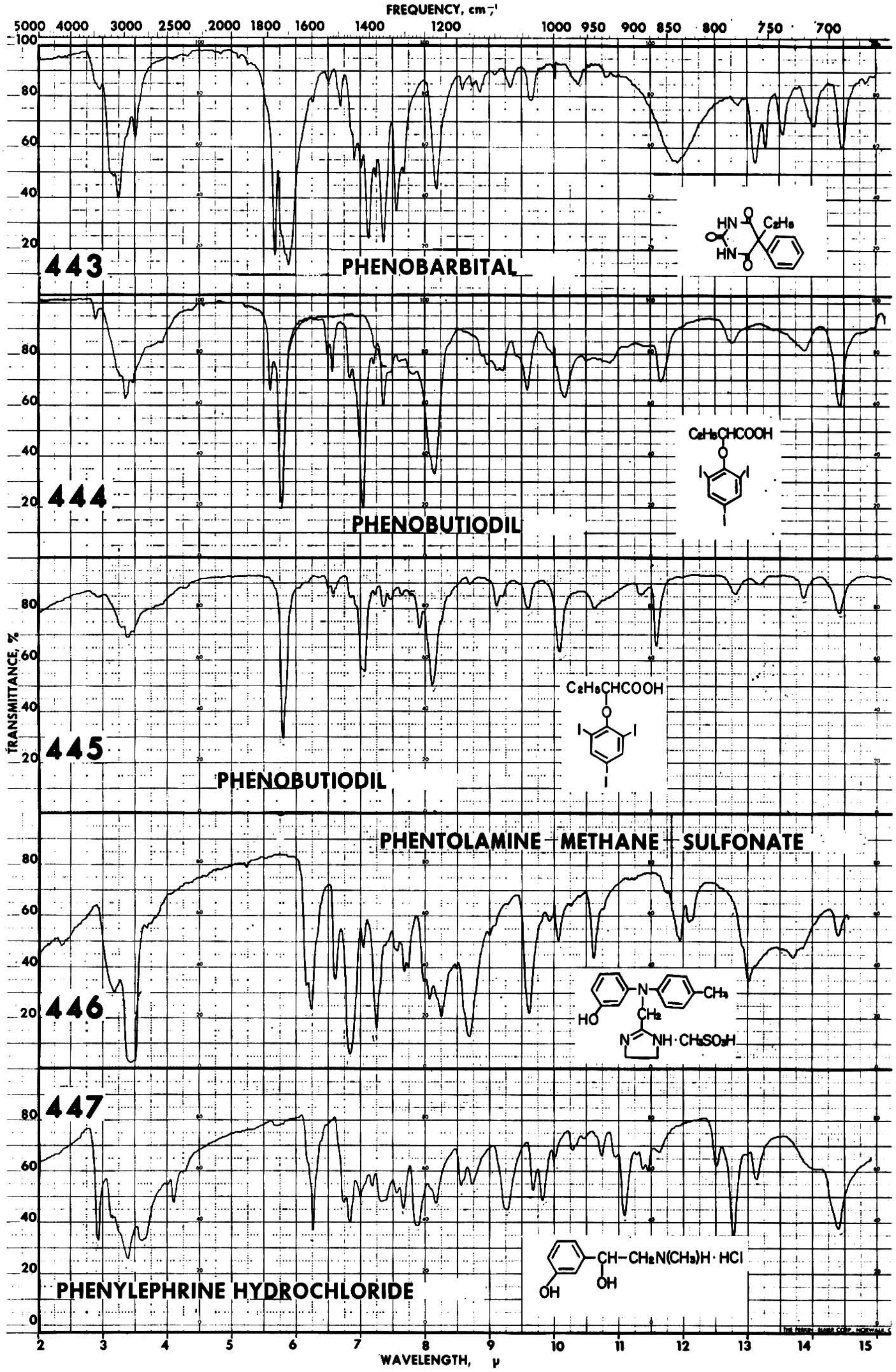
441

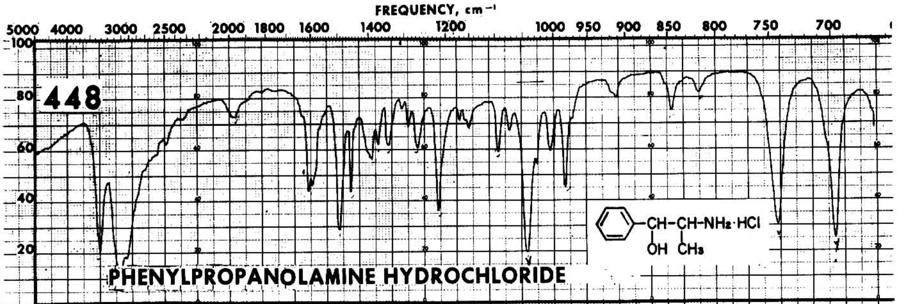
PHENOBARBITAL



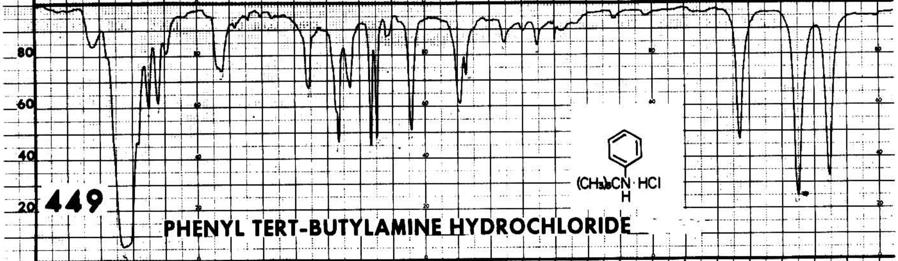
442

PHENOBARBITAL

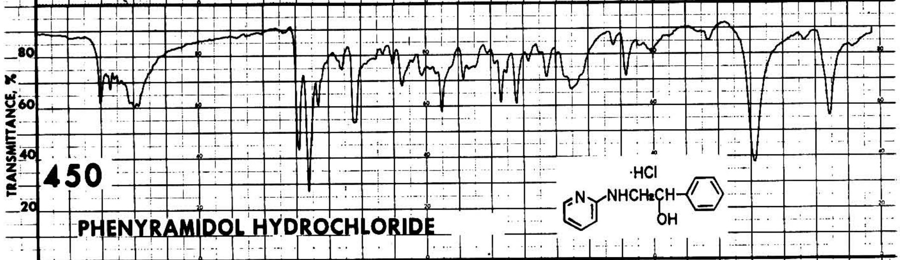

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100 80 60 40 20 0
443 PHENOBARBITAL
HN C2H5 HN
444 PHENOBUTIODIL
C2H5CHCOOH O I I I
445 PHENOBUTIODIL
C2H5CHCOOH O I I I
PHENTOLAMINE METHANE SULFONATE
446
HO N CH3 CH2 N NH·CH3SO3H
447
PHENYLEPHRINE HYDROCHLORIDE
CH–CH2N(CH3)H·HCl OH OH
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15



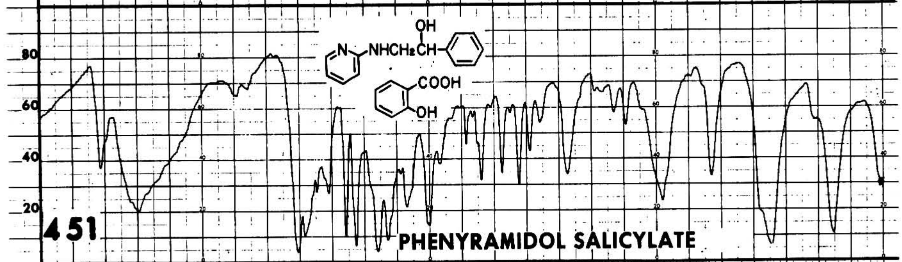
448 PHENYLPROPANOLAMINE HYDROCHLORIDE



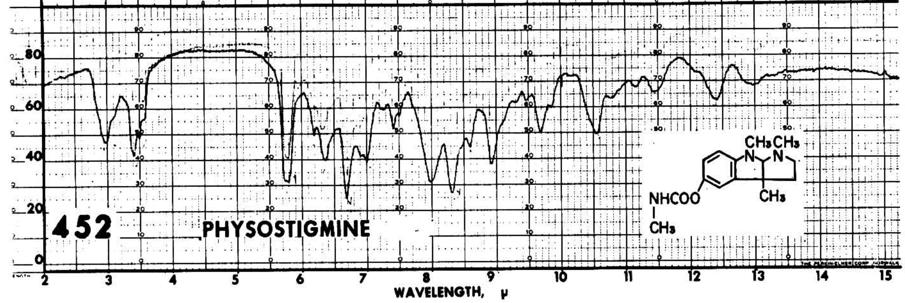
449 PHENYL TERT-BUTYLAMINE HYDROCHLORIDE



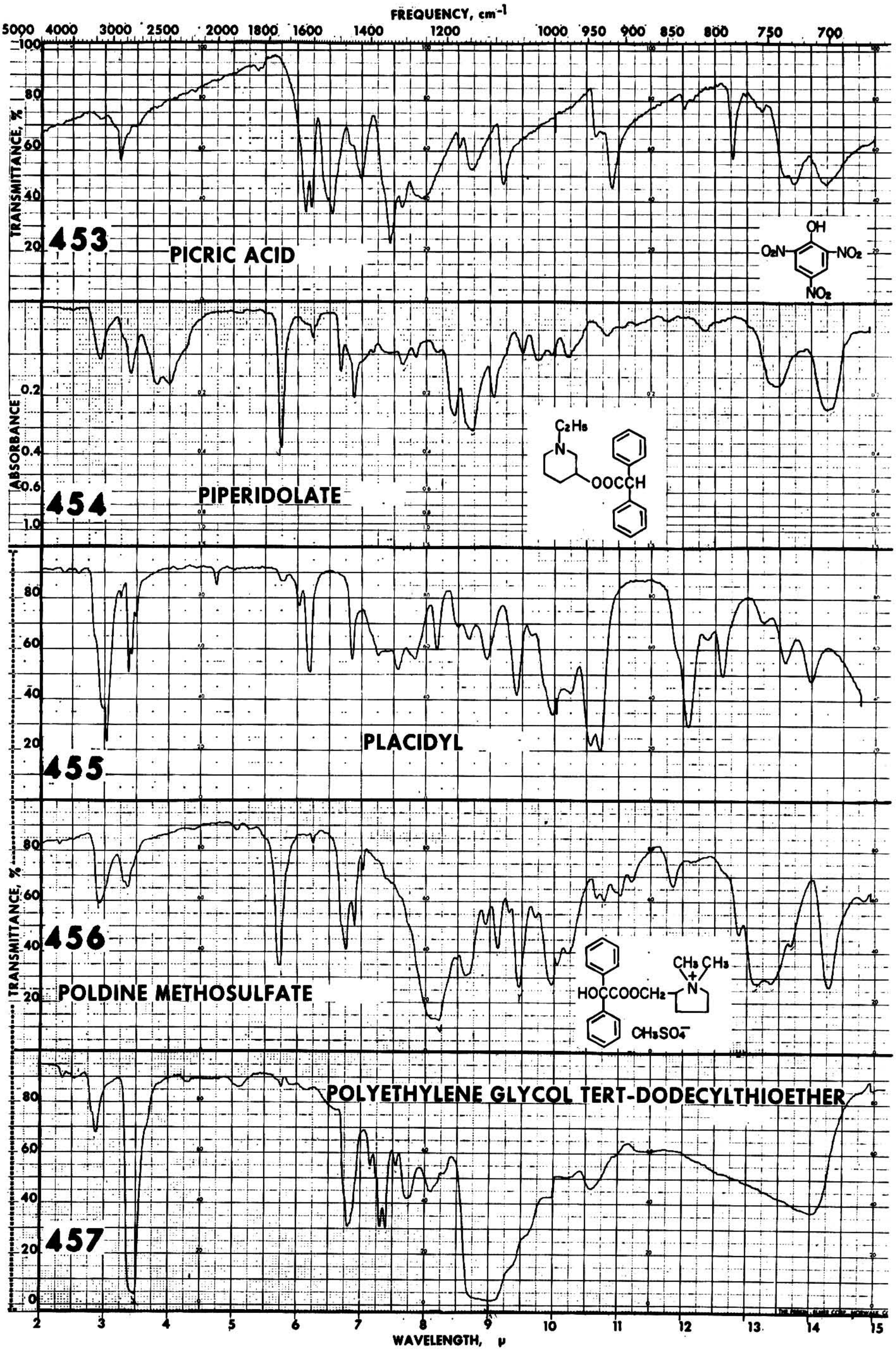
450 PHENYRAMIDOL HYDROCHLORIDE

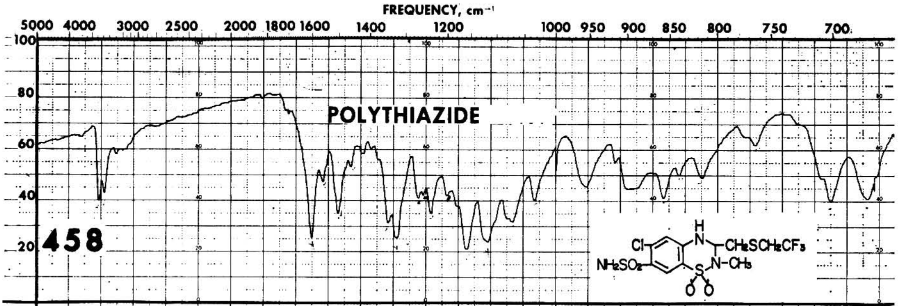


451 PHENYRAMIDOL SALICYLATE

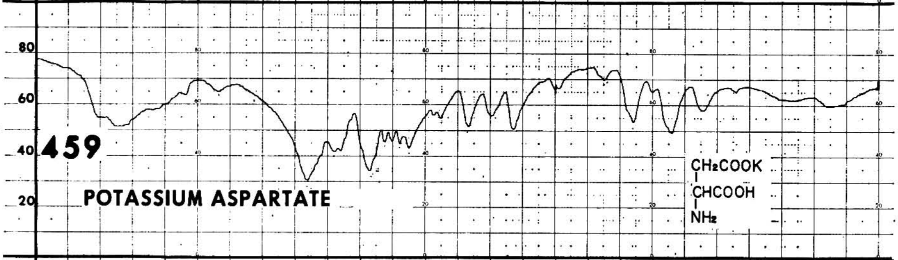


452 PHYSOSTIGMINE

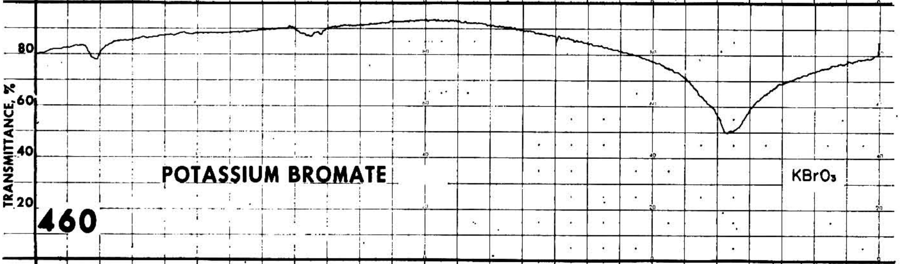

FREQUENCY, cm^{-1}
5000
4000
3000
2500
2000
1800
1600
1400
1200
1000
950
900
850
800
750
700
TRANSMITTANCE, %
100
80
60
40
20
453
PICRIC ACID
OH
O₂N
NO₂
NO₂
ABSORBANCE
0.2
0.4
0.6
1.0
454
PIPERIDOLATE
C₂H₅
N
OOCCH
80
60
40
20
455
PLACIDYL
TRANSMITTANCE, %
80
60
40
20
456
POLDINE METHOSULFATE
CH₃ CH₃
+
N
HOCCOOCH₂
CH₃SO₄⁻
POLYETHYLENE GLYCOL TERT-DODECYLTHIOETHER
80
60
40
20
0
457
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH, μ



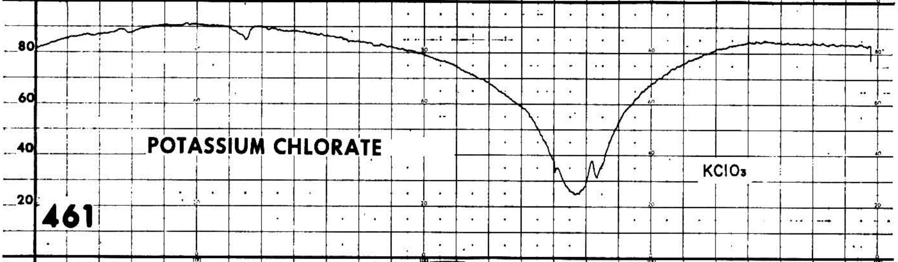
458 POLYTHIAZIDE



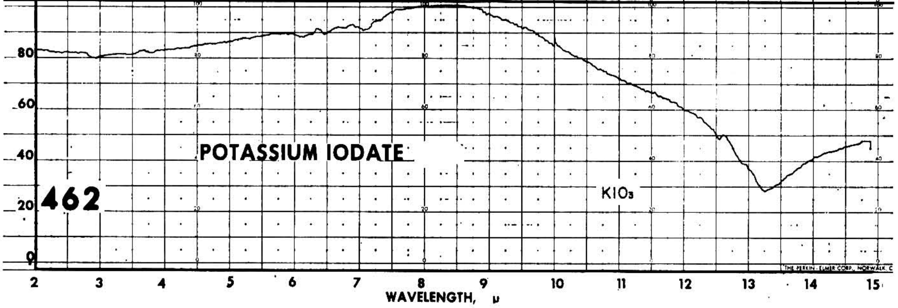
459 POTASSIUM ASPARTATE



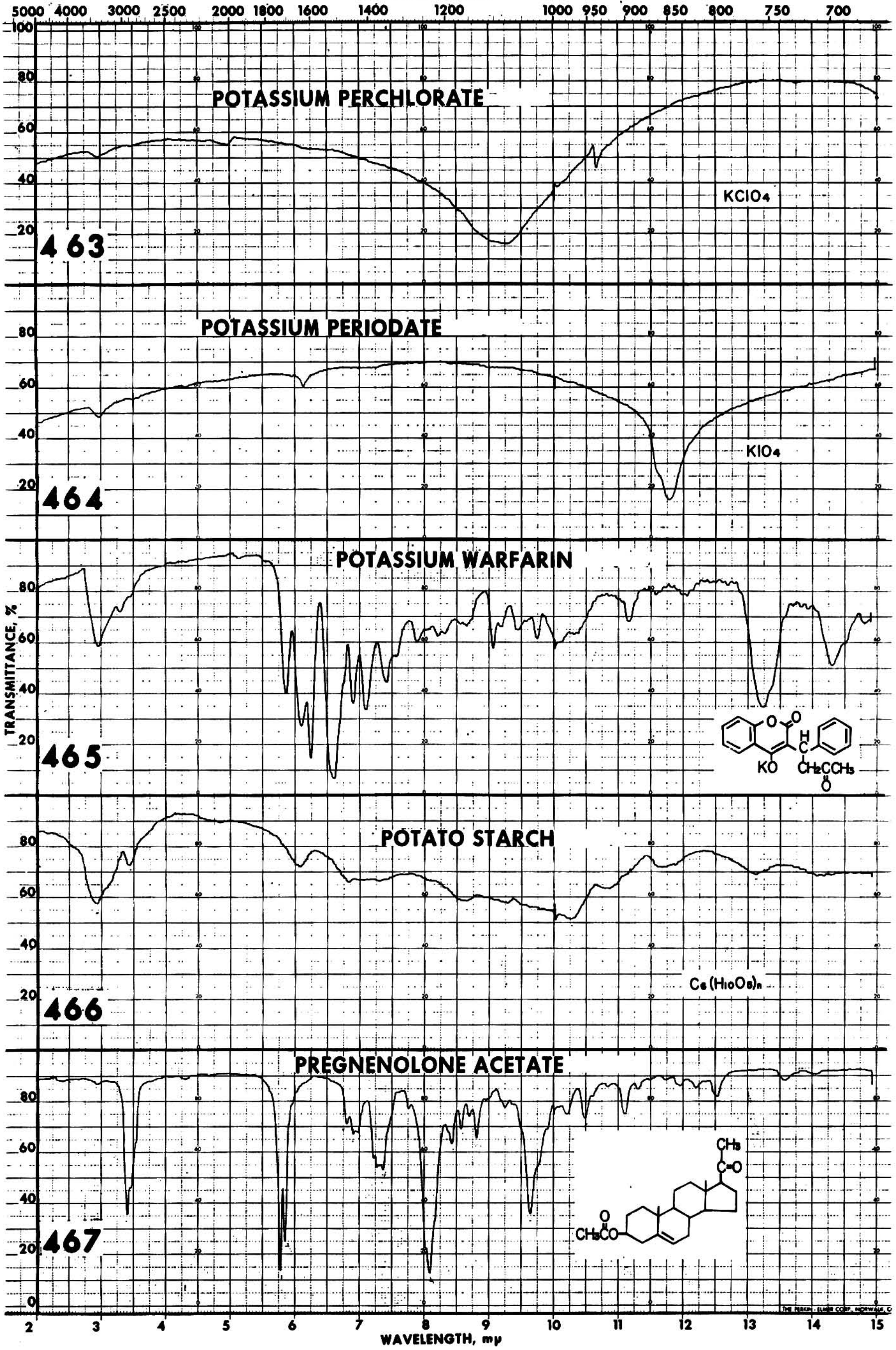
460 POTASSIUM BROMATE

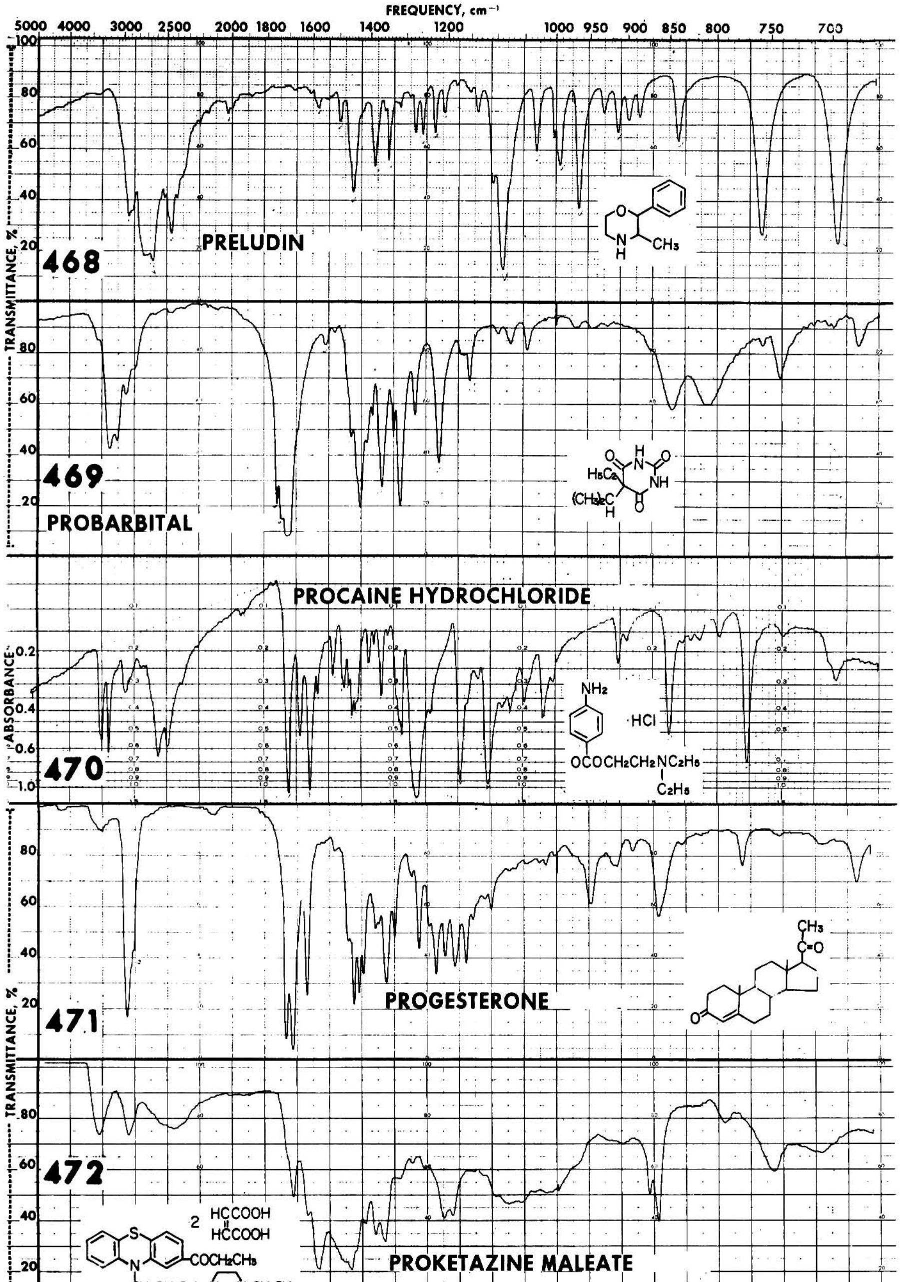


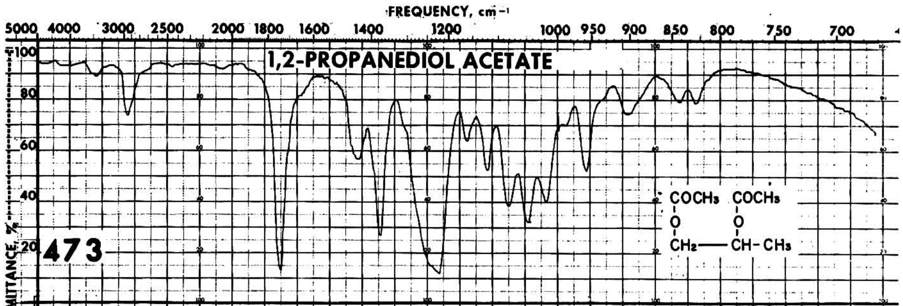
461 POTASSIUM CHLORATE



462 POTASSIUM IODATE

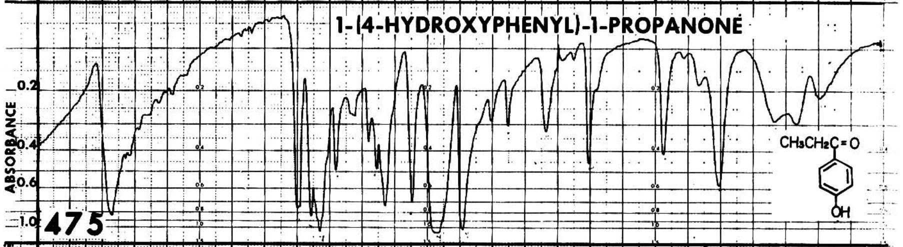

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
POTASSIUM PERCHLORATE
KClO4
463
POTASSIUM PERIODATE
KIO4
464
POTASSIUM WARFARIN
KO
CH2CCH3
O
465
POTATO STARCH
C6(H10O5)n
466
PREGNENOLONE ACETATE
CH3
C=O
O
CH3CO
467
TRANSMITTANCE, %
100 80 60 40 20 0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, mμ
THE PERKIN-ELMER CORP., NORWALK, C


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100 80 60 40 20
468
PRELUDIN
469
PROBARBITAL
470
PROCAINE HYDROCHLORIDE
ABSORBANCE
0.2 0.4 0.6 1.0
NH2
·HCl
OCOCH2CH2NC2H5
C2H5
471
PROGESTERONE
CH3
C=O
O
472
PROKETAZINE MALEATE
2 HCCOOH
HCCOOH
COCH2CH3
CH2CH2CH2-N N-CH2CH2
OH
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15
THE PERKIN-ELMER CORP., NORWALK, C

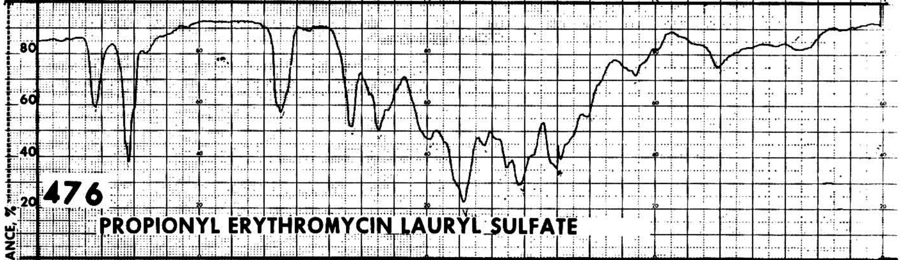


473 1,2-PROPANEDIOL ACETATE

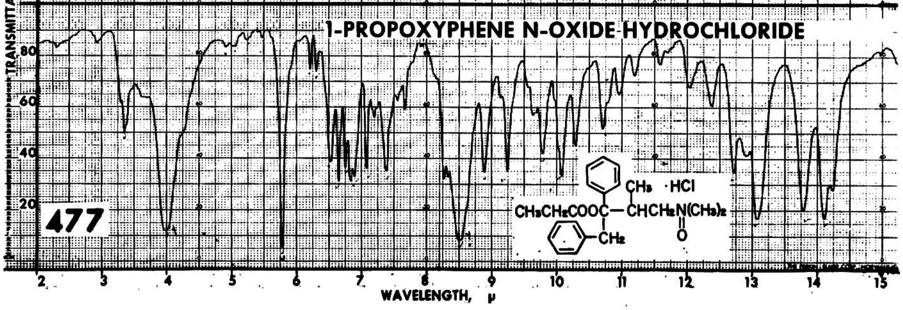
474 PROPANOL



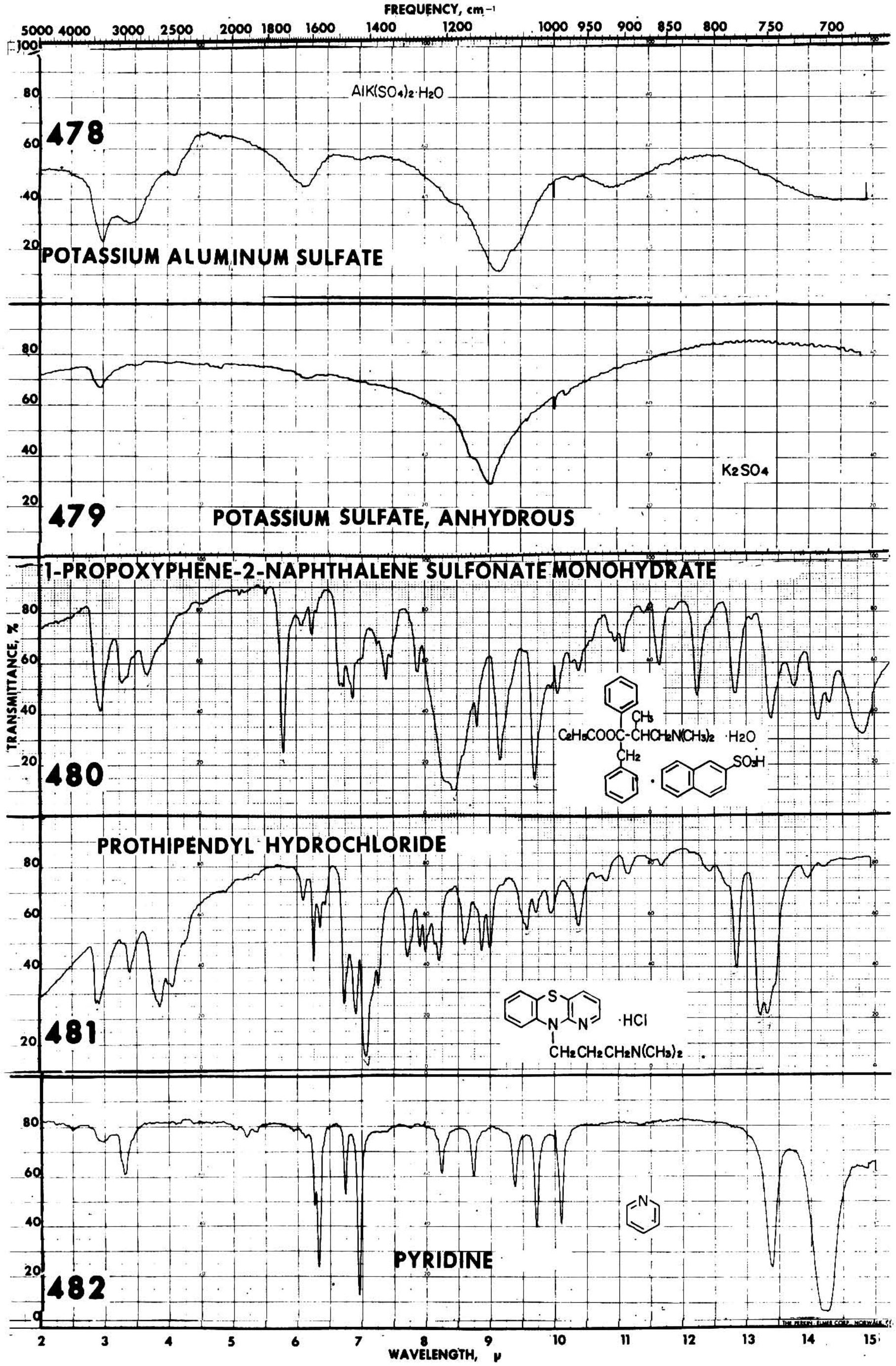
475 1-(4-HYDROXYPHENYL)-1-PROPANONE

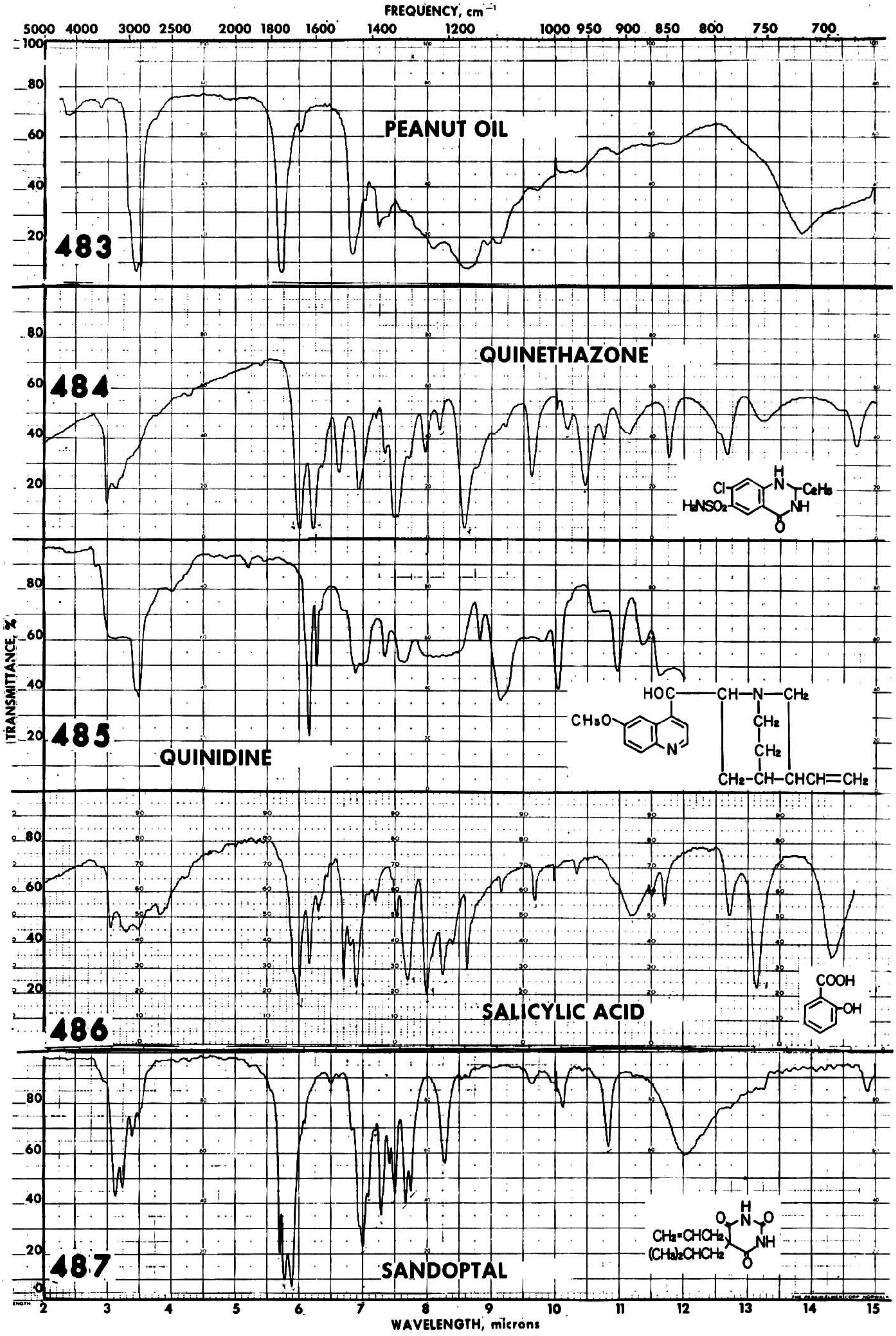


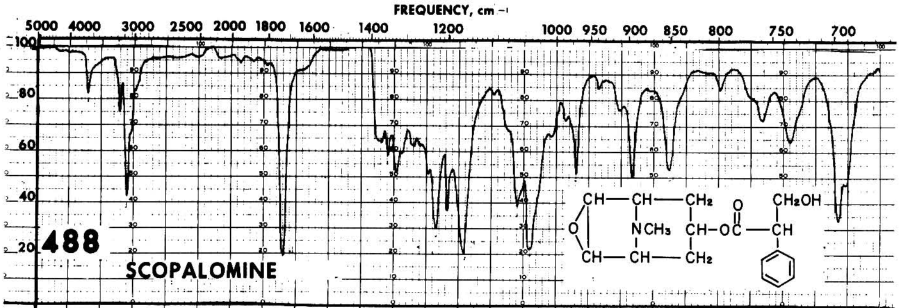
476 PROPIONYL ERYTHROMYCIN LAURYL SULFATE



477 1-PROPOXYPHENE N-OXIDE HYDROCHLORIDE

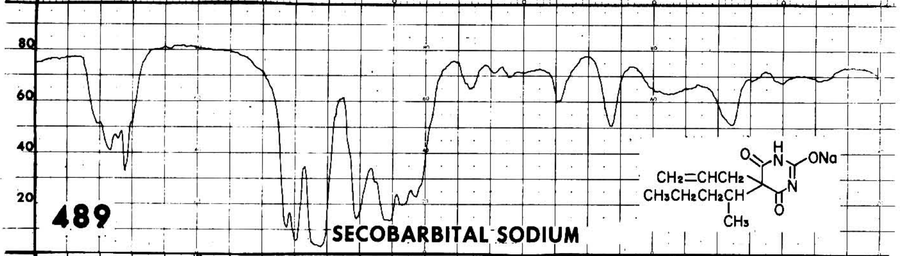

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
AlK(SO₄)₂·H₂O
478
POTASSIUM ALUMINUM SULFATE
K₂SO₄
479
POTASSIUM SULFATE, ANHYDROUS
1-PROPOXYPHENE-2-NAPHTHALENE SULFONATE MONOHYDRATE
C₂H₅COOC-CHCH₂N(CH₃)₂ ·H₂O
CH₃
CH₂
SO₃H
480
PROTHIPENDYL HYDROCHLORIDE
S
N
·HCl
CH₂CH₂CH₂N(CH₃)₂
481
N
PYRIDINE
482
TRANSMITTANCE, %
100
80
60
40
20
0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ
THE PERKIN-ELMER CORP. NORWALK


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100 80 60 40 20 0
483
PEANUT OIL
484
QUINETHAZONE
Cl
H₂NSO₂
H
N
C₂H₅
NH
O
485
QUINIDINE
HOC
CH
N
CH₂
CH₃O
N
CH₂
CH₂
CH₂-CH-CHCH=CH₂
486
SALICYLIC ACID
COOH
OH
487
SANDOPTAL
CH₂=CHCH₂
(CH₃)₂CHCH₂
H
O
N
O
NH
O
WAVELENGTH, microns
2 3 4 5 6 7 8 9 10 11 12 13 14 15



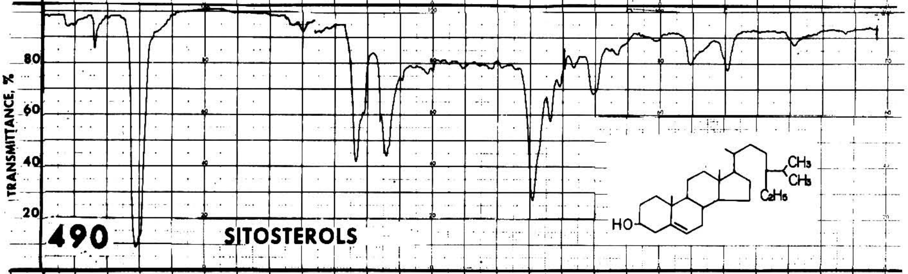
488

SCOPALOMINE



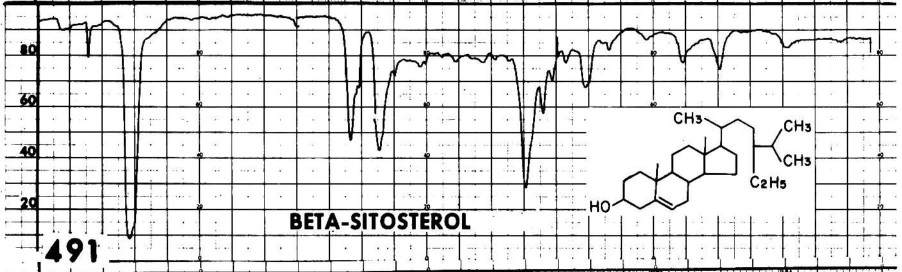
489

SECOBARBITAL SODIUM



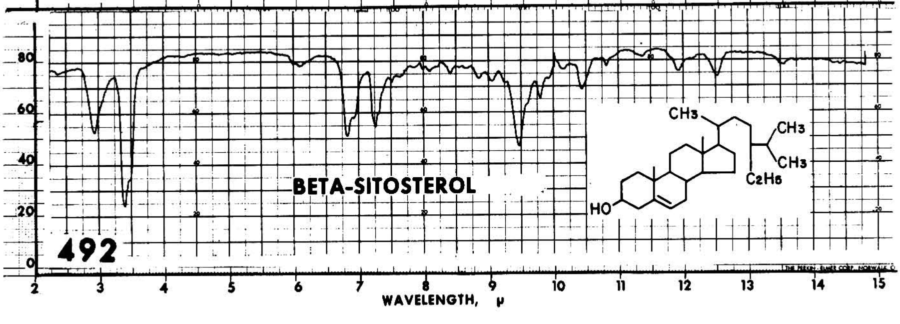
490

SITOSTEROLS



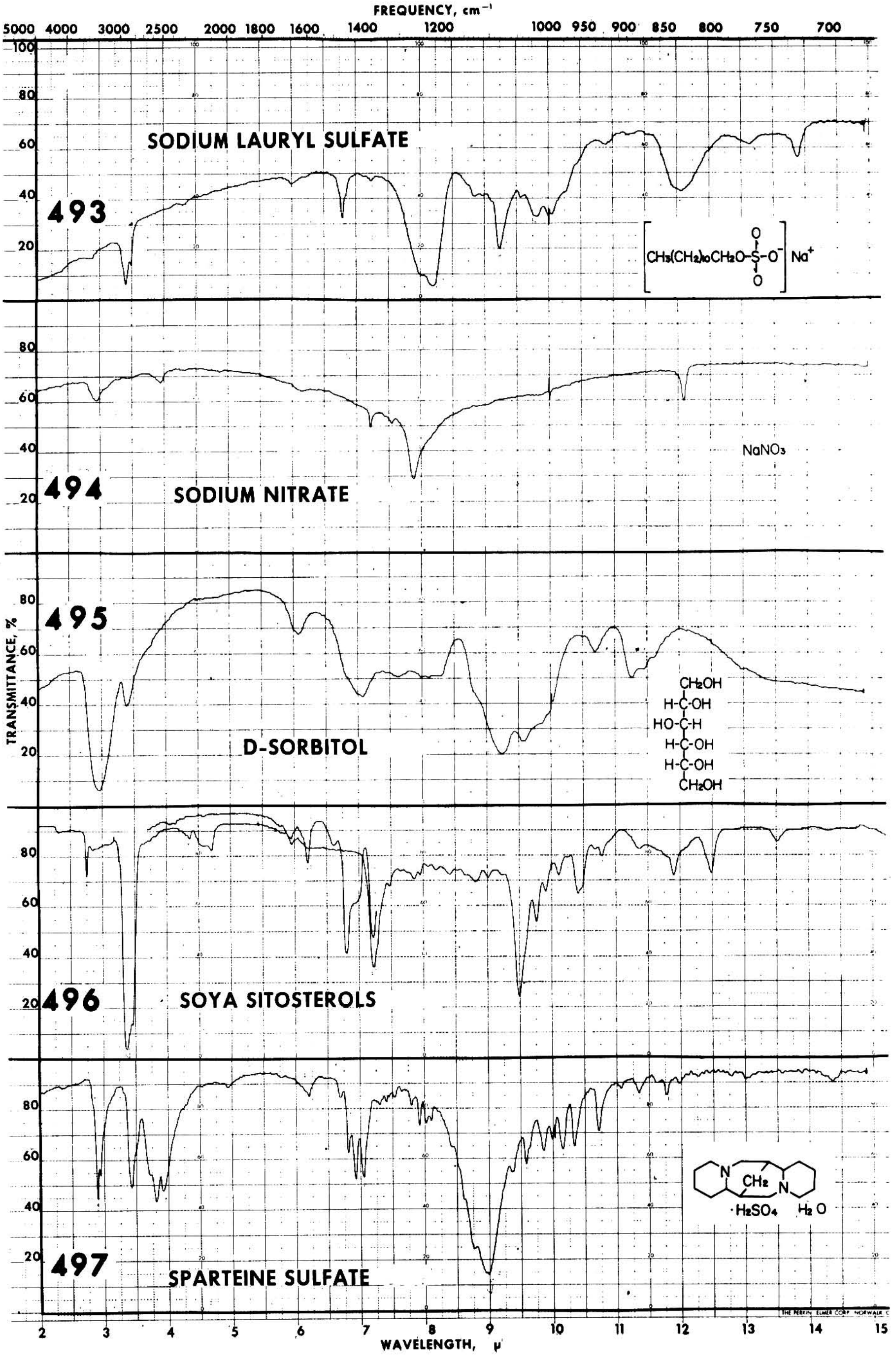
491

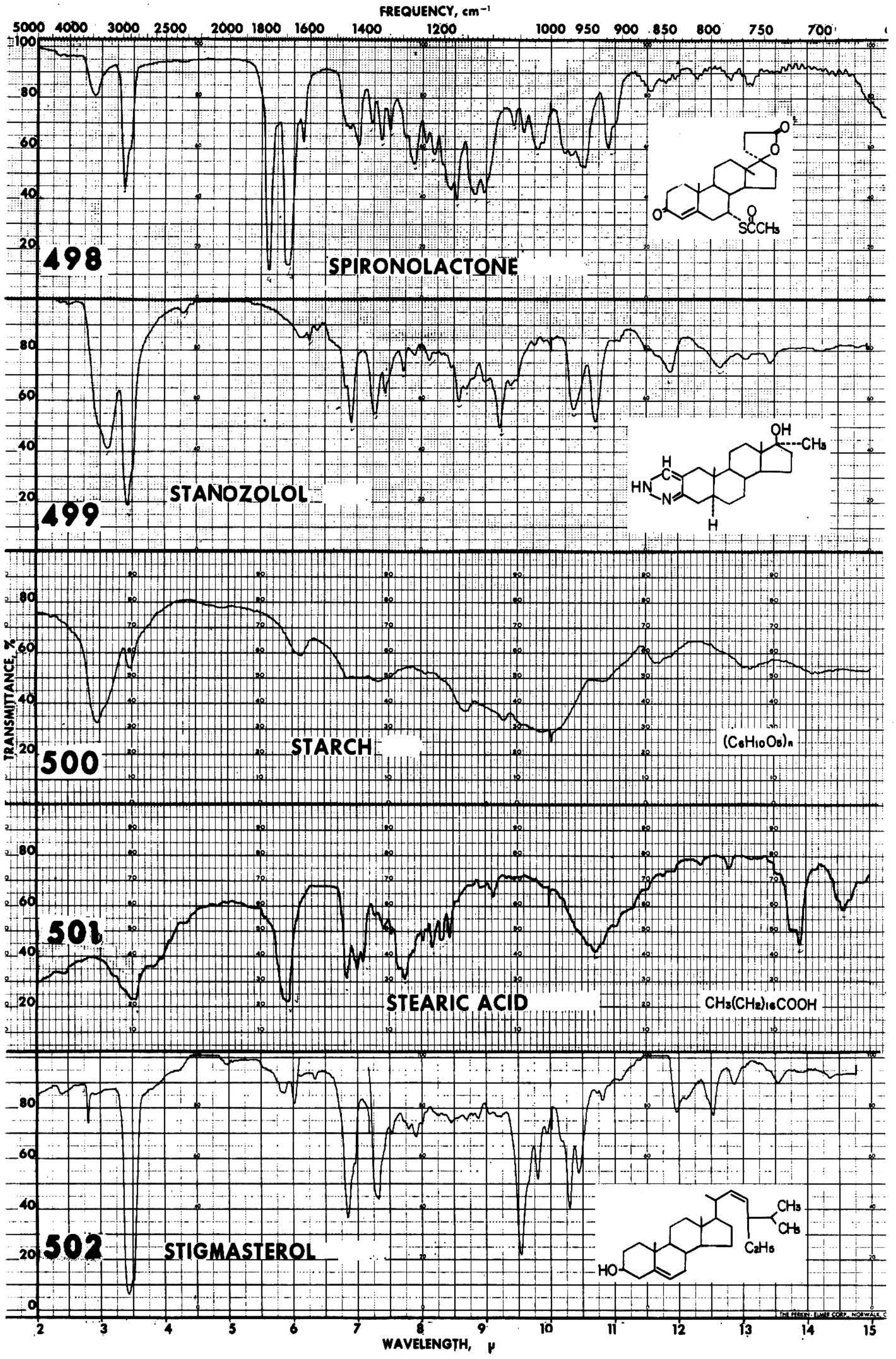
BETA-SITOSTEROL

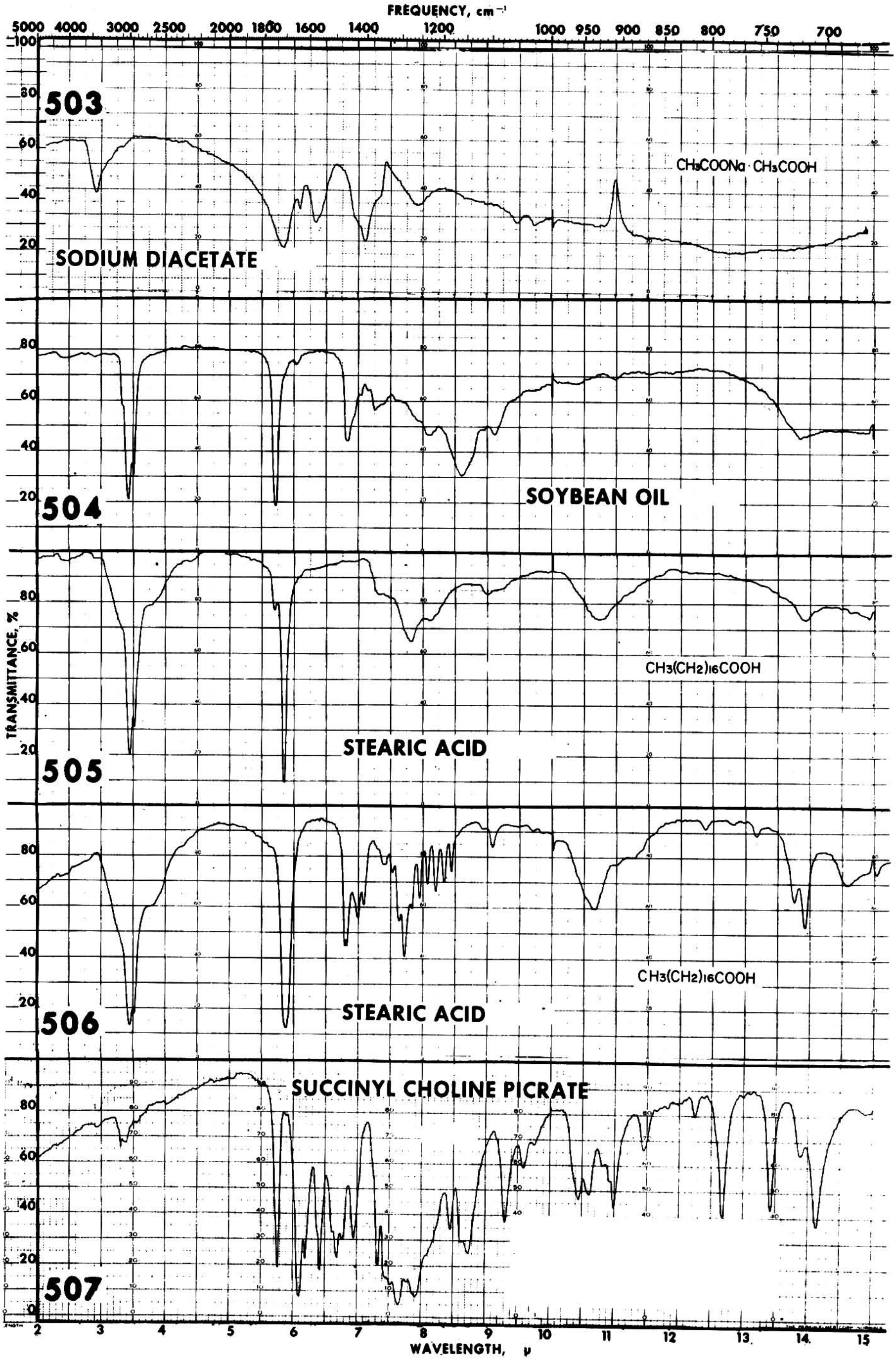


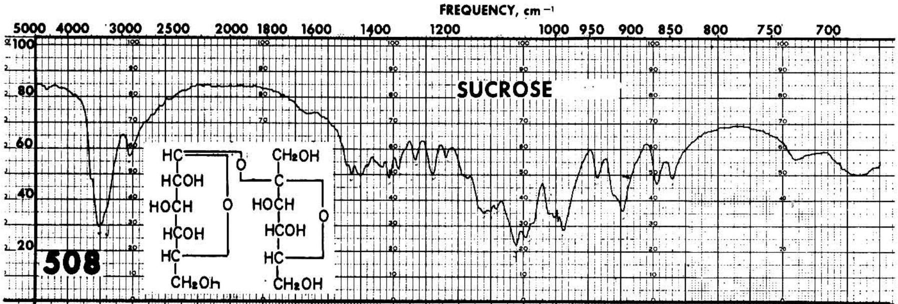
492

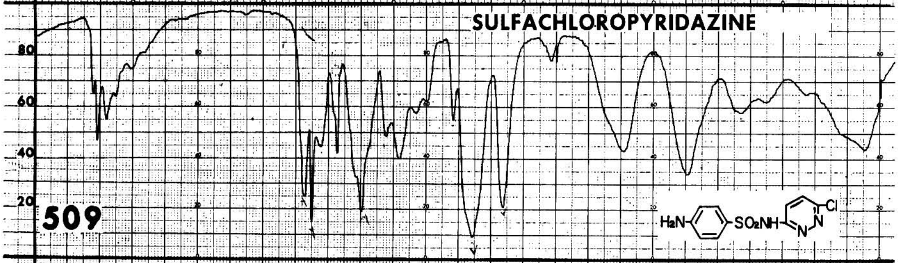
BETA-SITOSTEROL

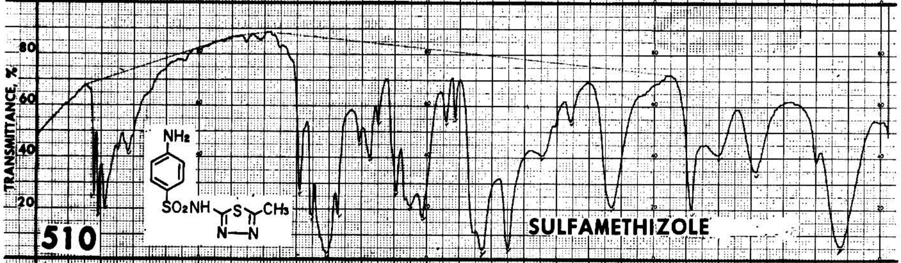

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
493
SODIUM LAURYL SULFATE
[CH₃(CH₂)₁₀CH₂O-S(O)(O)-O⁻] Na⁺
494
SODIUM NITRATE
NaNO₃
495
D-SORBITOL
CH₂OH
H-C-OH
HO-C-H
H-C-OH
H-C-OH
CH₂OH
496
SOYA SITOSTEROLS
497
SPARTEINE SULFATE
N
CH₂
N
·H₂SO₄ H₂O
TRANSMITTANCE, %
100
80
60
40
20
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ
THE PERKIN ELMER CORP. NORWALK C

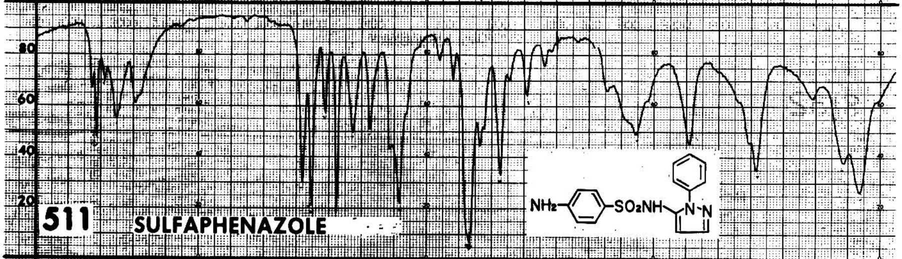

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
100
80
60
40
20
0
TRANSMITTANCE, %
498
SPIRONOLACTONE
O
SCCH₃
499
STANOZOLOL
OH
CH₃
H
HN
N
H
500
STARCH
(C₆H₁₀O₅)ₙ
501
STEARIC ACID
CH₃(CH₂)₁₆COOH
502
STIGMASTEROL
CH₃
CH₃
C₂H₅
HO
THE PERKIN-ELMER CORP., NORWALK, C
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

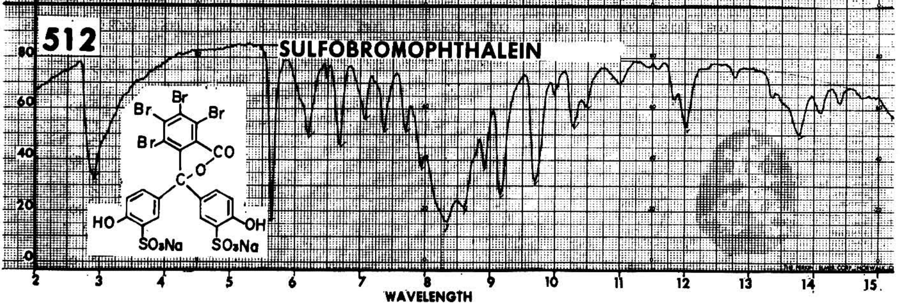

FREQUENCY, cm⁻¹
5000
4000
3000
2500
2000
1800
1600
1400
1200
1000
950
900
850
800
750
700
503
SODIUM DIACETATE
CH₃COONa · CH₃COOH
504
SOYBEAN OIL
505
STEARIC ACID
CH₃(CH₂)₁₆COOH
506
STEARIC ACID
CH₃(CH₂)₁₆COOH
507
SUCCINYL CHOLINE PICRATE
TRANSMITTANCE, %
100
80
60
40
20
0
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH, μ

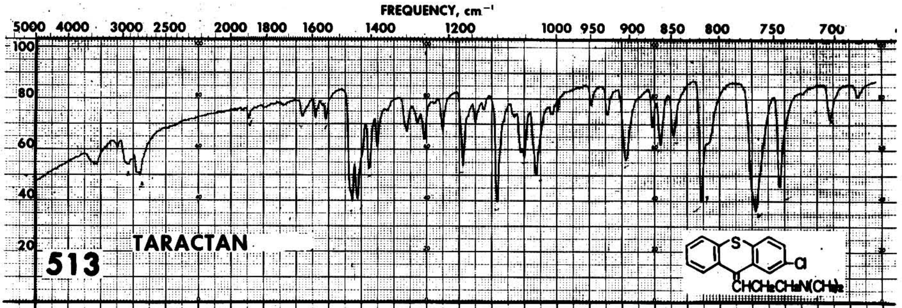

508 SUCROSE


509 SULFACHLOROPYRIDAZINE

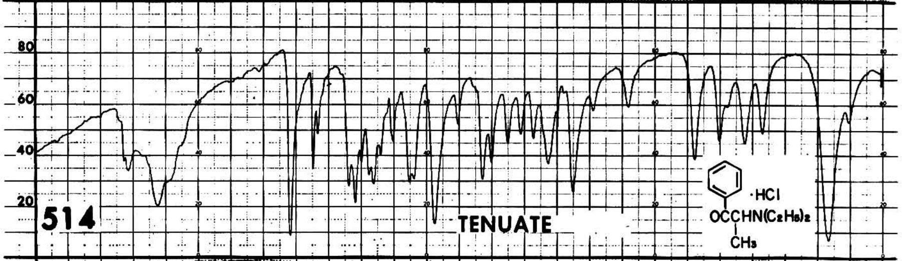

510 SULFAMETHIZOLE


511 SULFAPHENAZOLE

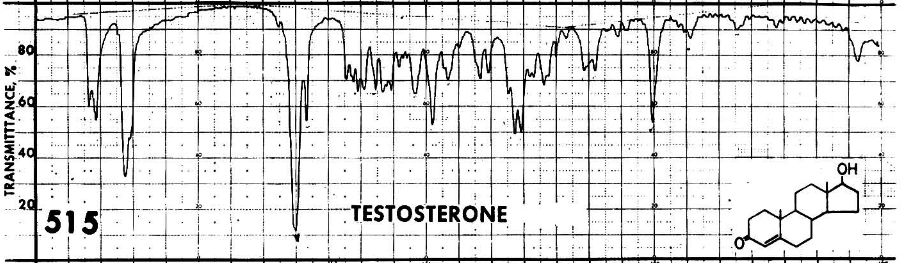

512 SULFOBROMOPHTHALEIN



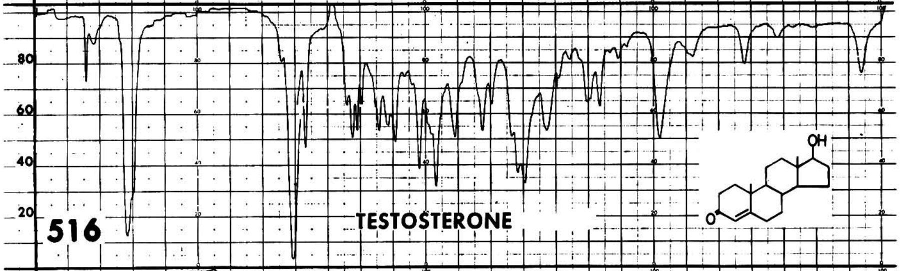
513 TARACTAN



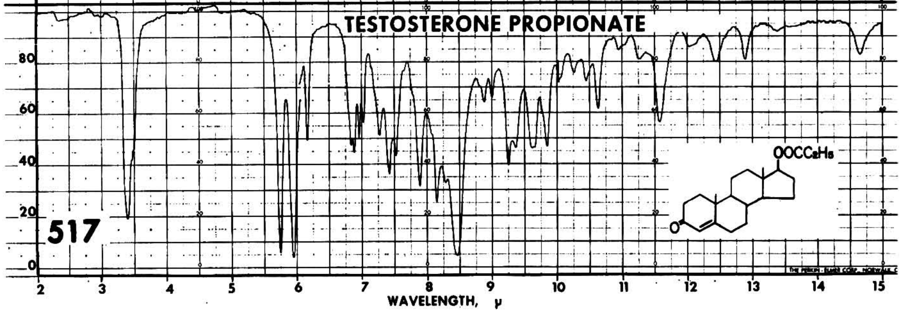
514 TENUATE



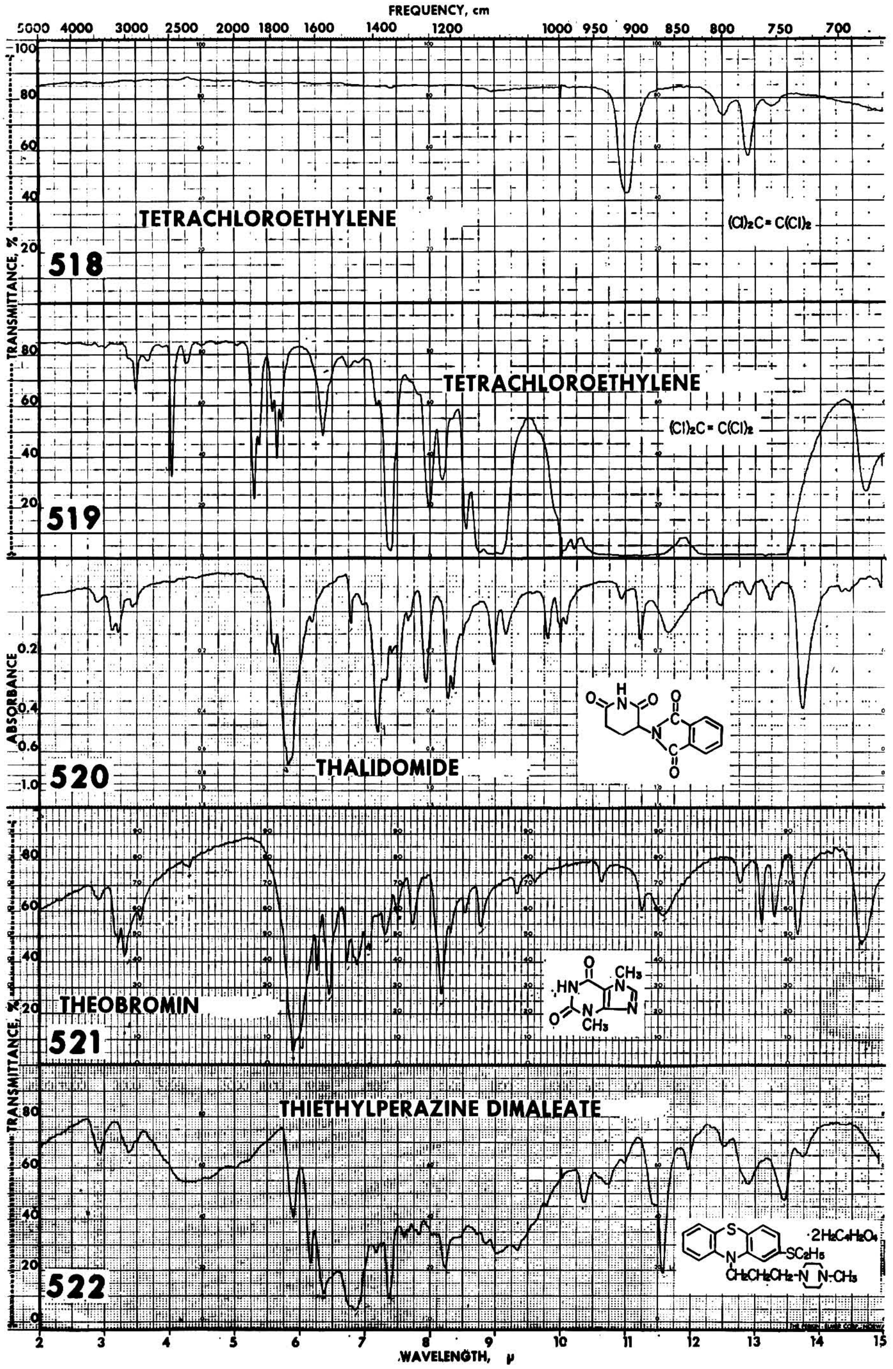
515 TESTOSTERONE

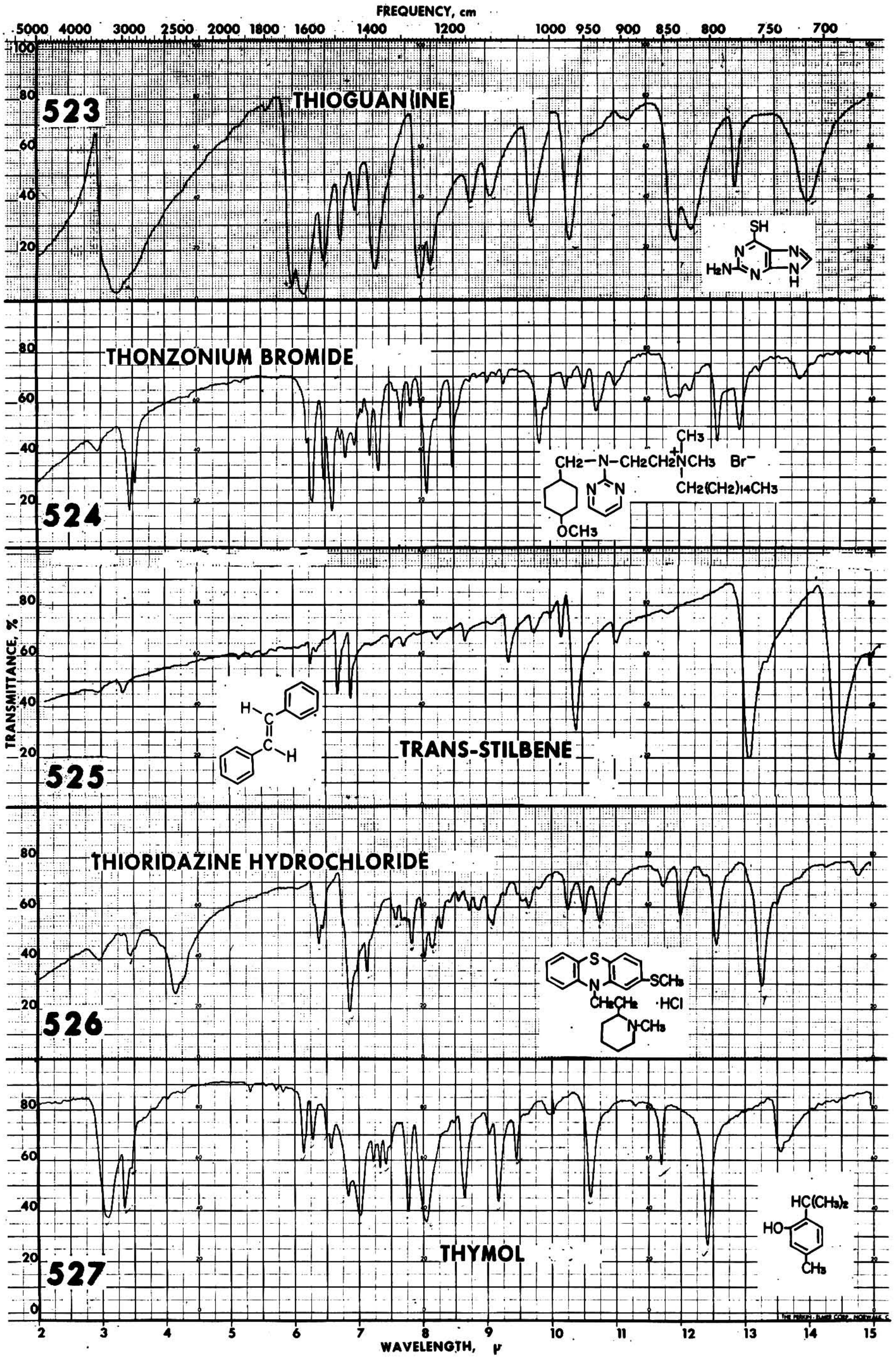


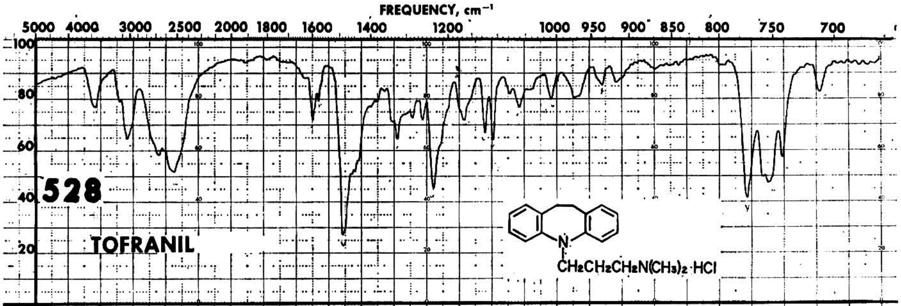
516 TESTOSTERONE



517 TESTOSTERONE PROPIONATE

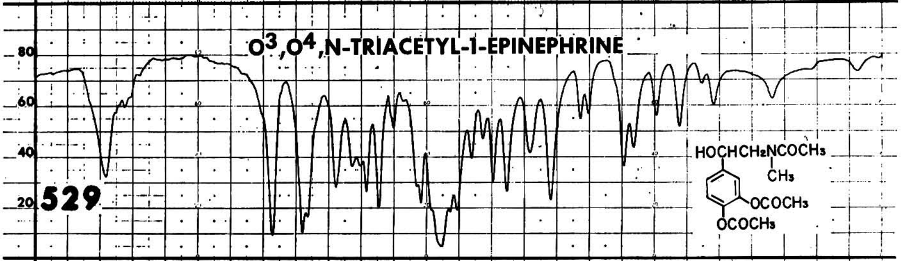

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100
80
60
40
20
TETRACHLOROETHYLENE
(Cl)₂C= C(Cl)₂
518
TETRACHLOROETHYLENE
(Cl)₂C= C(Cl)₂
519
ABSORBANCE
0.2
0.4
0.6
1.0
THALIDOMIDE
520
THEOBROMIN
521
THIETHYLPERAZINE DIMALEATE
·2H₂C₄H₂O₄
SC₂H₅
CH₂CH₂CH₂-N N-CH₃
522
0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
523
THIOGUANINE
SH
H₂N
N
H
524
THONZONIUM BROMIDE
CH₃
CH₂—N—CH₂CH₂NCH₃ Br⁻
CH₂(CH₂)₁₄CH₃
OCH₃
525
TRANS-STILBENE
H
C
H
526
THIORIDAZINE HYDROCHLORIDE
S
SCH₃
N
CH₂CH₂ ·HCl
N-CH₃
527
THYMOL
HC(CH₃)₂
HO
CH₃
TRANSMITTANCE, %
100 80 60 40 20 0
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ



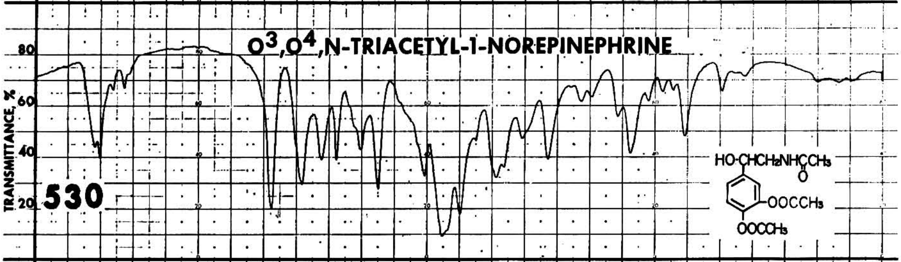
528

TOFRANIL



529

O^3,O^4,N-TRIACETYL-1-EPINEPHRINE

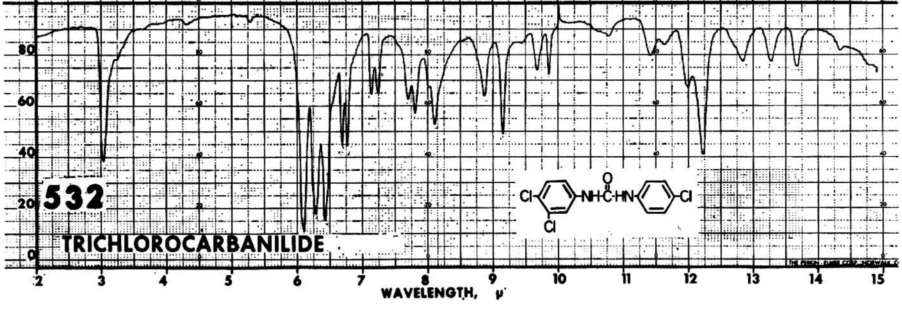


530

O^3,O^4,N-TRIACETYL-1-NOREPINEPHRINE

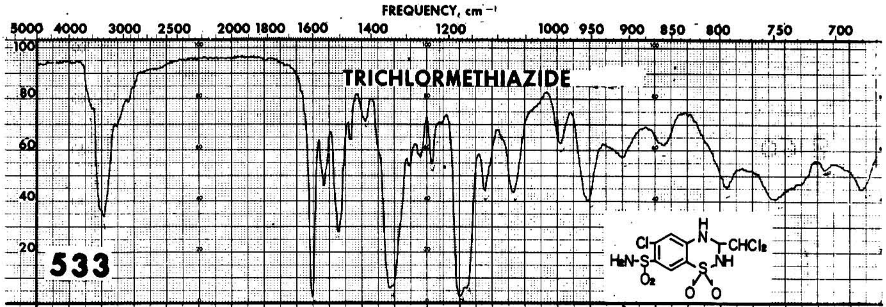
531

TRIBURON CHLORIDE

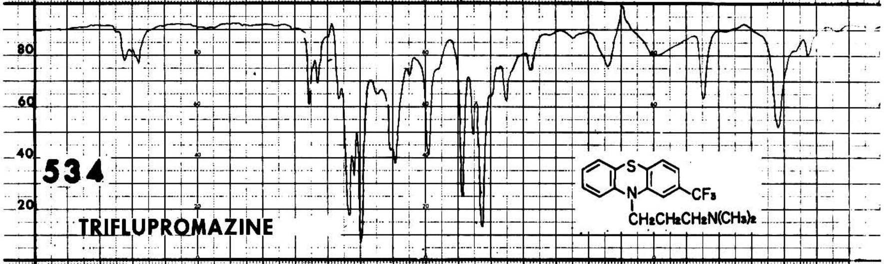


532

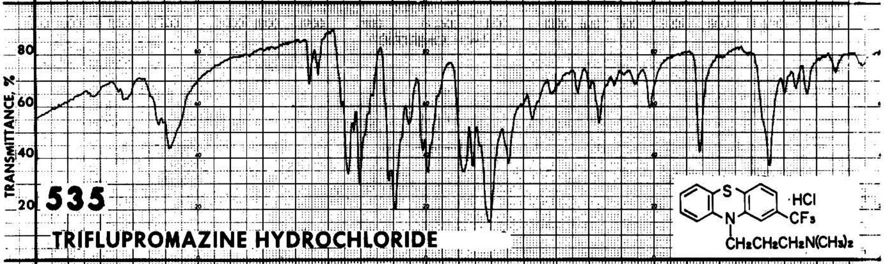
TRICHLOROCARBANILIDE


533

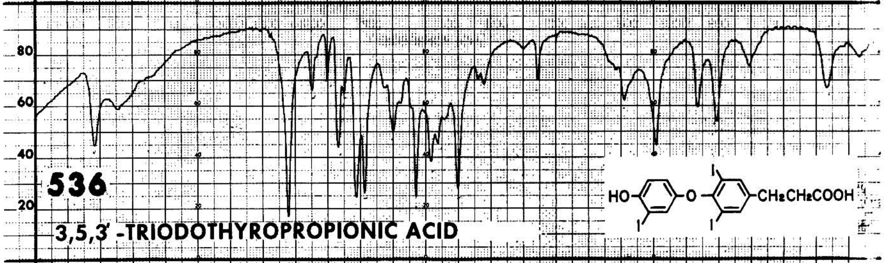
TRICHLORMETHIAZIDE


534

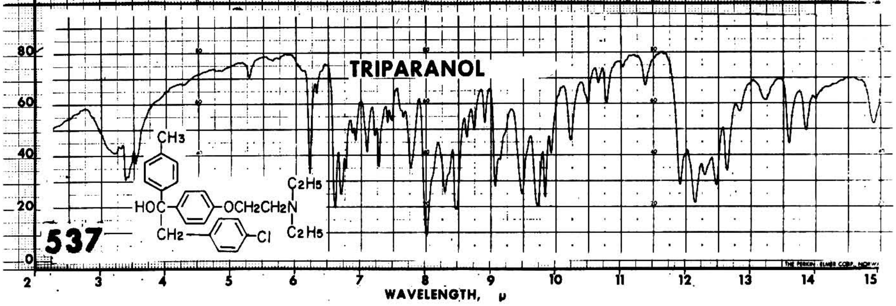
TRIFLUPROMAZINE


535

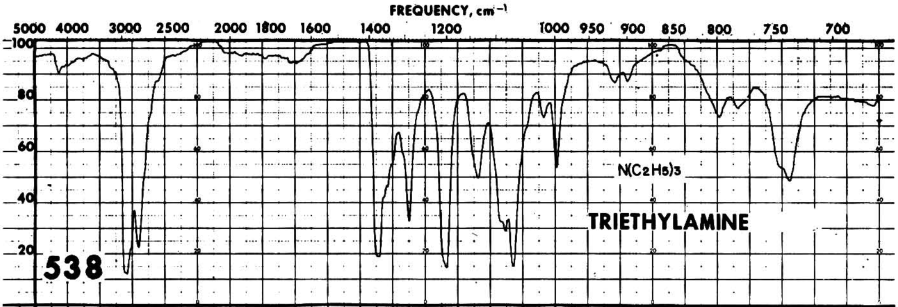
TRIFLUPROMAZINE HYDROCHLORIDE


536

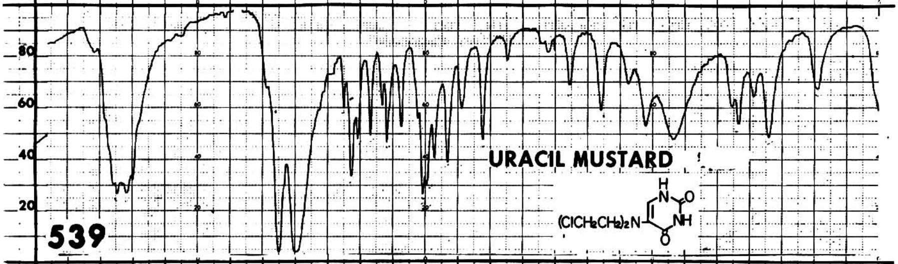
3,5,3′-TRIODOTHYROPROPIONIC ACID


537

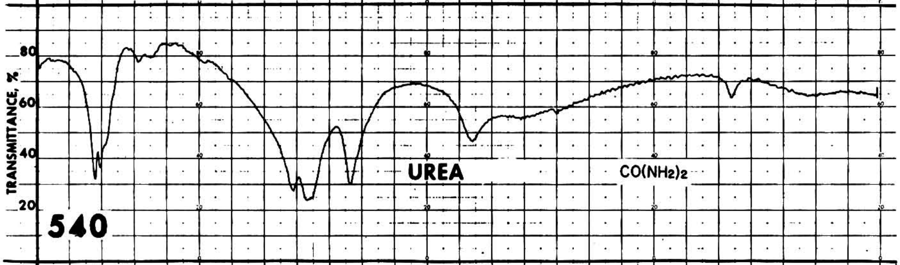
TRIPARANOL



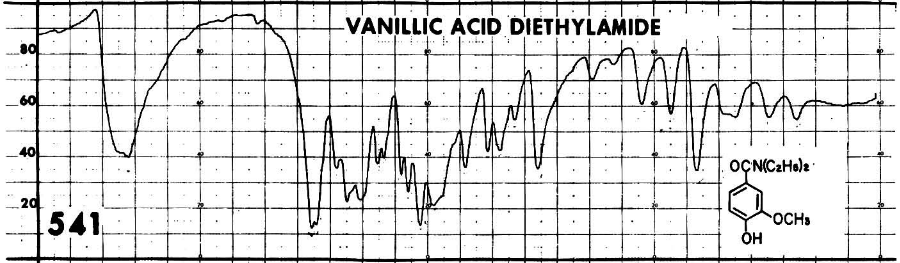
538 TRIETHYLAMINE



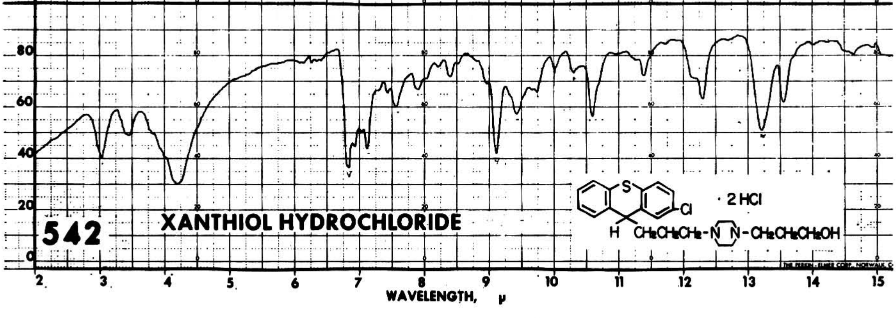
539 URACIL MUSTARD



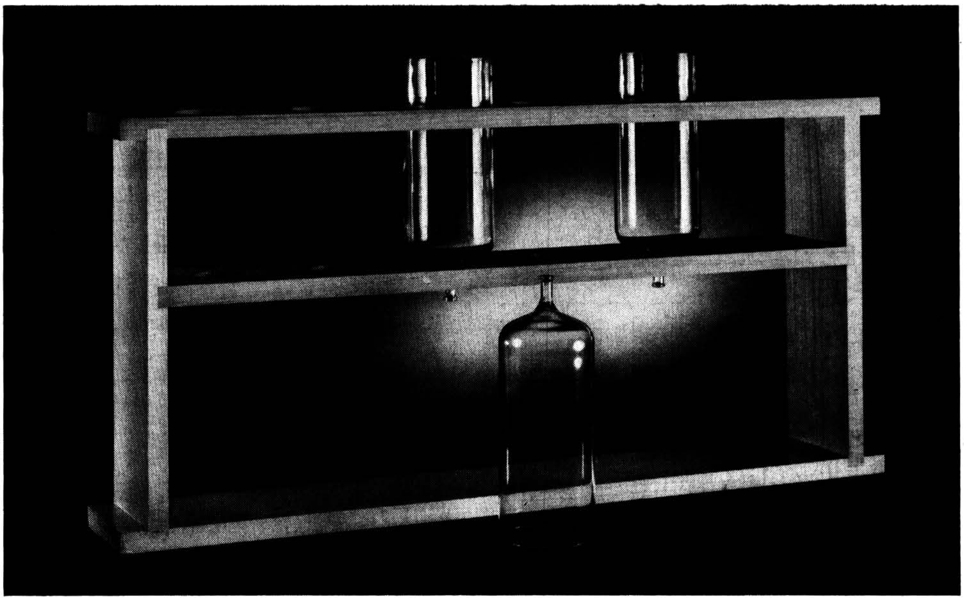
540 UREA



541 VANILLIC ACID DIETHYLAMIDE



542 XANTHIOL HYDROCHLORIDE



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* Described in a paper presented by Robert B. Kilborn, American Association of Cereal Chemists, Minneapolis, Minnesota, April, 1963.

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