A Review of Methods for Determining Pesticide Residues, Contaminants and Adulterants in Fats and Oils[1]

DAVID FIRESTONE, Division of Food Chemistry, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D. C.

Abstract

Sensitive chromatographic methods are available for determining chlorinated pesticide residues and chick edema factor contaminants in fats and oils. Determination of chlorinated pesticide residues in fats and oils generally involves acetonitrile extraction, Florisil column cleanup, and analysis by electron capture gas-liquid chromatography (ECGLC). However, other procedures are available including dimethyl sulfoxide extraction and sweep codistillation. Sensitive screening tests for chick edema factor involve alumina column fractionation of isolated unsaponifiable matter, sulfuric acid cleanup, and examination by ECGLC. Admixtures of animal and vegetable fats are detected by the gas-liquid chromatography (GLC) of isolated sterols, and individual vegetable oils can be characterized by GLC analysis of the sterols as well as of the other unsaponifiable constituents.

Introduction

ENACTMENT OF FEDERAL LAWS, including the Miller Pesticide and Food Additives amendments to tre Federal Food, Drug and Cosmetic Act of 1938, has stimulated the development of sensitive analytical methods to detect adulteration and contamination of fats and oils, as well as of foods in general. With these methods, pesticides and food additives can be detected at very low levels. Development and improvement of sensitive GLC detection systems with electron capture and phosphorus-sensitive flame detectors now permit the determination of subnanogram amounts of halogen- and phosphorus-containing substances.

Methodology for determining chlorinated pesticide residues and chick edema contaminants, as well as foreign fats in fats and oils, will be discussed in this article. However, discussion of techniques for detecting foreign fats will be limited to methodology involving chromatographic analysis of unsaponifiable constituents, since chemical and physical methods relating to fatty acid composition and many classical identity tests are widely used to identify and characterize fats and oils. The determinative step in the methods discussed is gas-liquid chromatography (GLC), although infrared, nuclear magnetic resonance and mass spectroscopy are often applied as confirmatory tools.

Chlorinated Organic Pesticide Residues

Methods used within the FDA for the isolation of chlorinated pesticide residues from fats and oils generally involve the Mills procedure (1) and various modifications which, in essence, consist of an acetonitrile extraction, Florisil column cleanup, and detection and estimation by paper chromatography, thin-layer chromatography (TLC) or GLC. However, other procedures have been reported. Eidelman (2) used dimethyl sulfoxide (DMSO) to extract chlorinated pesticide residues from acetone and petroleum ether solutions of fats, and McCully and McKinley (3) separated the pesticides by precipitating the fat from a benzene-acetone solution cooled to −70C. Ott and Gunther (4) used a stream of nitrogen to sweep pesticides from butterfat heated to 190C into a cooled trap, and Storherr et al. (5) extended the use of sweep co-distillation, developed for isolation of organophosphates (6), to cleanup of chlorinated pesticide residues in fats and oils.

The *Pesticide Analytical Manual,* Vol. I, prepared by the Food and Drug Administration (7), presents two general cleanup methods for determining multiple residues of chlorinated organic pesticides in fats and oils and fatty foods. The first method (8,9) is based on the Mills procedure. Fat in petroleum ether is extracted with acetonitrile, the acetonitrile extract is diluted with a large volume of 2% NaCl, and the pesticides are partitioned into petroleum ether. The petroleum ether extract is then fractionated on a column of activated Florisil with 6% and 15% ethyl ether in redistilled petroleum ether. The eluates are examined by GLC using electron capture (EC) detectors which are highly responsive to chlorinated organic compounds. Residues are generally confirmed by TLC (10). The 6% Florisil eluate is usually suitable for TLC and ECGLC without further cleanup; the 15% eluate generally requires additional cleanup for TLC by passage of a petroleum ether solution of this fraction through a 1 + 1 MgO- Celite column, saponification, or both.

The second procedure is the DMSO method of Eidelman (2). The fat is shaken with acetone and DMSO, and the lower layer (DMSO) is transferred to a separator holding petroleum ether and acetone. The fat in the upper layer is reextracted with DMSO, and the combined lower layers (DMSO-acetone-petroleum ether mixture) are diluted and mixed with an ice slurry. The aqueous layer is discarded, the petroleum ether extract is reextracted with DMSO by the above technique, and the extract, after transfer back to petroleum ether, is chromatographed on Florisil. A MgO- Celite column and saponification may be required to eliminate oily residues from vegetable and fish oils obtained in the 15% Florisil eluate.

Because of the variability of different production lots of Florisil, the Florisil column must be checked with a standard pesticide mixture before use. Recently, an attempt has been made to produce a Florisil product (designated PR grade) that will function consistently within the scope of the Mills procedure. This Florisil is prepared by the manufacturer by activation at 1250F for 3 hr under controlled conditions (11).

The Pesticide Analytical Manual recommends the use of 6-ft columns (4–6 mm I.D.) packed with 10% DC-200 (12,500 cst) or with combinations of QF-1 and DC-200 on Gas-Chrom Q support for routine ECGLC of isolated pesticide residues after cleanup. Combination columns prepared by mixing equal weights of 15% QF-1 (10,000 cst) on 80–100 mesh Gas-Chrom Q and 10% DC-200 on 80–100 mesh Gas-Chrom Q were effective for the resolution of some chlorinated pesticide mixtures (12,13).

The gas chromatographic behavior of endrin, a widely used chlorinated pesticide, is used as one of the criteria for satisfactory column performance since its response may be greatly influenced by both the column support and liquid phase (12,13). Thus, Chromosorb P solid support usually produces two peaks (neither peak is endrin) with DC-200 liquid phase, whereas Anakrom ABS column support will produce one peak (endrin) after a long conditioning time (up to 3 to 4 weeks). By use of a Gas-Chrom Q column support the conditioning time may be drastically reduced. Columns are conditioned at 250C–260C with a nitrogen flow of about 100 ml/min for at least 48 hr, or until endrin emerges as a single peak. Preferred operating temperatures for the column, detector, and flash heater, respectively, are 200, 210 (max), and 210C–230C, with a nitrogen (prepurified grade) flow rate of 120 ml/min.

Electron capture detectors are highly sensitive (although not specific) to chlorinated organic compounds at nanogram and subnanogram levels (12,14,15). The working sensitivity

[1] Presented at the AOCS Short Course, "Processing Quality Control of Fats and Oils," East Lansing, Mich., Aug. 29–Sept. 1, 1966.

TABLE I
Recoveries of Pesticides from 2-g Samples of Fortified Refined and Deodorized Corn Oil (19)[a]

Pesticide added (0.25 ppm)	Analysis of unfortified oil (ppm)	% Recoveries[b] (corrected for blank)			
		A	B	C	Av.
Aldrin	0.000	90.7	94.3	90.0	91.7
DDD	0.000	97.4	105.8	101.6	101.6
DDE	0.000	95.6	84.3	95.3	91.7
p,p′-DDT	0.030	103.5	106.8	109.1	106.5
Dieldrin	0.021	95.7	88.3	114.1	99.3
Endrin	0.000	95.5	104.4	98.5	99.5
Heptachlor	0.000	90.3	94.5	90.3	91.7
Heptachlor epoxide	0.000	93.7	99.5	95.7	96.3

[a] Data are reprinted with the permission of the *Journal of the Association of Official Analytical Chemists.*
[b] A, B and C are replicate samples.

of electron capture detectors is generally 1000 times that of microcoulometric and electrolytic conductivity detectors, which also respond to chlorinated compounds (12). Electron capture detection is based on absorption by the pesticide molecule of some of the electrons flowing between the electrodes of the detector cell. The amount of absorption, and thus the response, is affected by electrode spacing, applied voltage, gas flow through the detector, amount and energy of radioactivity available, detector geometry, and electrode contamination. Burke and Giuffrida (14) and Guiffrida et al. (15), as well as other investigators (16,17), have reported that an electron capture detector of concentric geometry has definite advantages over the parallel plate design. With the parallel plate detector, the usable voltage range is limited and is restricted to a voltage range where the detector operation is most troublesome. This voltage range (10–30 volts) is on the steep slope of the volts-amps curve where the standing current is very susceptible to slight changes in the detector (15). The linear range is also greater for the concentric design (17). In practice, the recommended working voltage is beyond that of maximum sensitivity and is chosen so that 1 nanogram of heptachlor epoxide will give a half-scale response at an electrometer setting of 10^{-9} ampere full scale, with aldrin eluting in about 4 to 4.25 min (7,15). Detector contamination because of film deposits on the electrodes from bleeding columns or from inadequate sample cleanup is another important factor. This contamination results in a shift in detector response.

Because cleanup (satisfactory isolation of pesticide from lipid material) is difficult and tedious, constant efforts are being made to improve extraction procedures. Onley and Bertuzzi (18) recently reported a method which combines the use of a mixture of acetone, methyl cellosolve, and formamide to extract chlorinated pesticides from fats or fatty foods at the 0.01 ppm level with the use of calcium stearate to coagulate and hold the lipid constituents. The method of Giuffrida and co-workers (19) uses 10% water-acetonitrile to elute the pesticides from an unactivated Florisil column which retains the fat, followed by cleanup on an activated Florisil column by the method of Mills et al. (20). Detection of several chlorinated pesticides in refined and deodorized corn oil by the latter method (19) is illustrated in Table I. The unfortified oil contained 0.030 ppm of *p,p′*DDT and 0.021 ppm of dieldrin. Recoveries of pesticides from the fortified oil (0.25 ppm added) ranged from 84 to 114%.

Although TLC is commonly used to confirm identity of individual pesticides, other techniques including the formation of derivatives and subsequent GLC analysis have also been employed. Beckman and Berkenkotter (21) used a combination of GLC and sodium/liquid ammonia reduction to examine the reduction products of chlorinated organic pesticides. Klein et al. (22,23) used alkaline hydrolysis (dehydrohalogenation) coupled with ECGLC to analyze DDT in vegetable oils, and mixtures of Perthane, DDD and DDT in leafy vegetables. Beroza and Bowman (24) have described a method of identifying or confirming the identity of pesticides in which the pesticide is distributed between two immiscible liquid phases and the chlorinated organic pesticides in the upper and lower phases are determined by ECGLC. These authors found that the extraction p-value (fraction of a pesticide partitioning into the upper phase of an equivolume two-phase system) remained practically unchanged in the presence of food extractives or large amounts of pesticides. Six binary solvent systems which gave the broadest spread of p-values were recommended for identifying individual pesticides.

Chick Edema Factor

Chick edema disease, first encountered in 1957, is caused by a group of chlorinated aromatic compounds of unknown origin which occur as trace contaminants in certain lots of fats and fatty acids. Wootton and Courchene (25) postulated that the chick edema factors are hexachlorohexahydrophenanthrenes; however, recent work based on x-ray crystallographic analysis (26) has shown that one of the factors is a hexachlorodibenzo-*p*-dioxin rather than

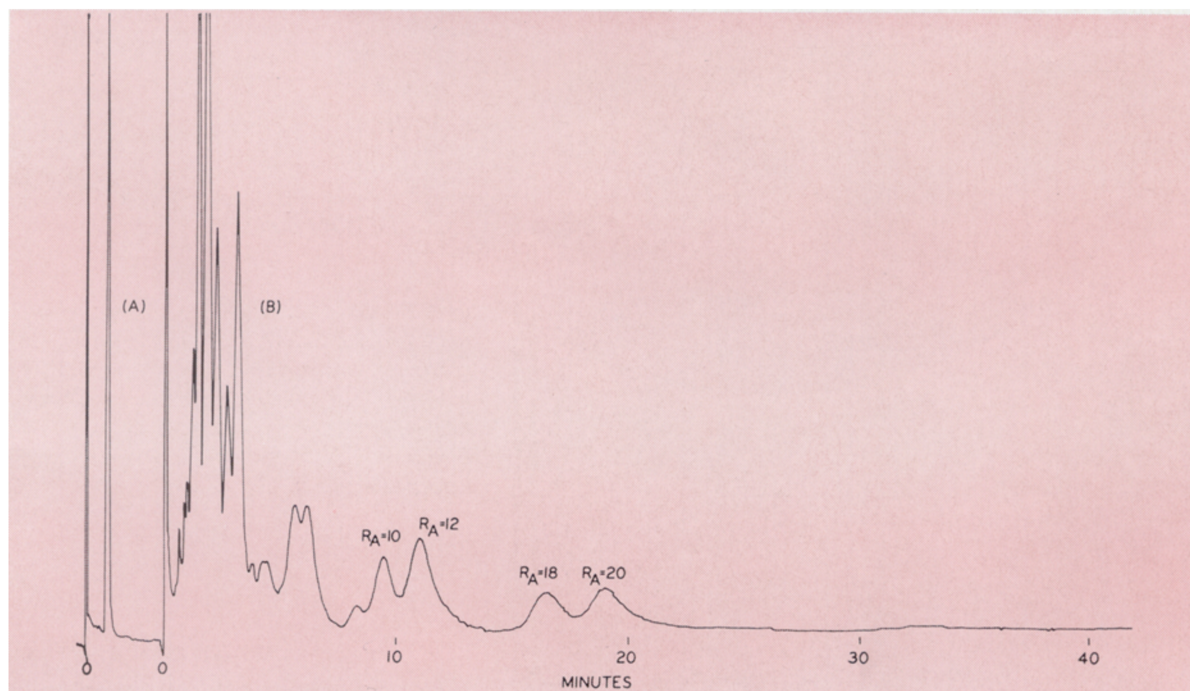

FIG. 1. Electron capture gas chromatograms of (A) aldrin standard (0.1 ng); (B) 5 µl (1/200) of alumina fraction 3 from 10 g of USP (refined and deodorized) cottonseed oil containing 1.5% of toxic fat.

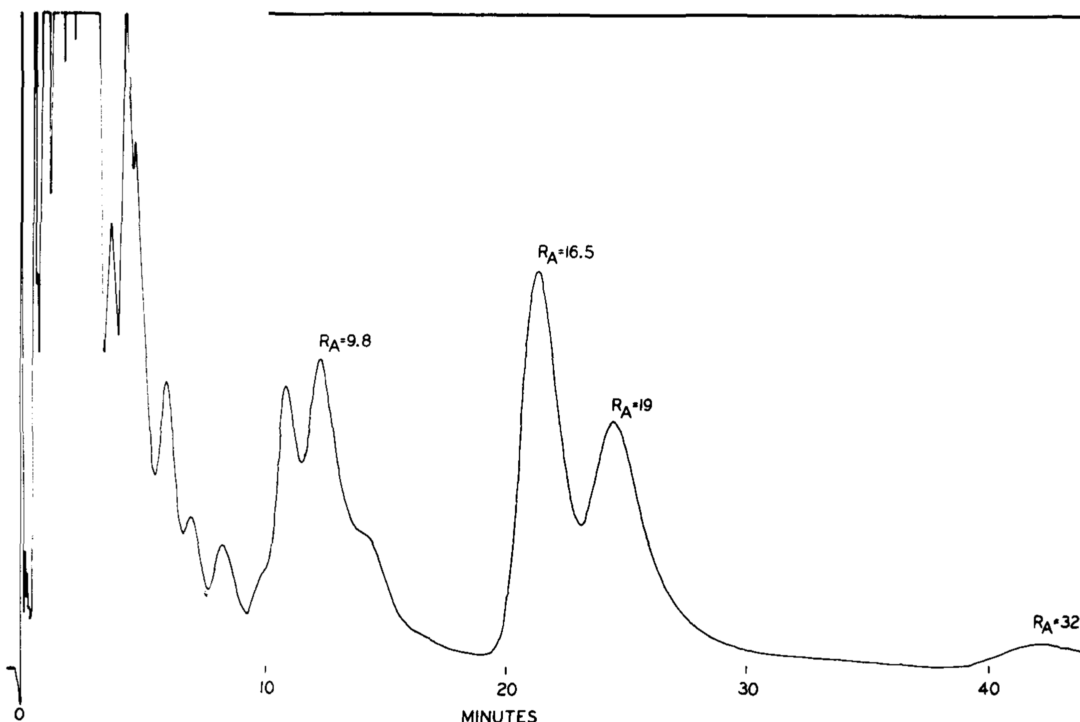

FIG. 2. Electron capture gas chromatogram of 5 µl (1/200) of alumina fraction 3 from 10 g of a toxic commercial glyceryl monooleate.

a hexachloro-hexahydro-phenanthrene. These compounds are extremely toxic to chicks, producing edema, liver and kidney damage, and other symptoms. A screening chromatographic test for chick edema factor (27) developed several years ago consists of adsorption chromatography of extracted unsaponifiables on activated alumina and analysis of a specific fraction by a microcoulometric method. This procedure, requiring 111 g samples, has been tested collaboratively (28) and has been adopted as an official, first action method (29) by the Association of Official Analytical Chemists. The chick bioassay procedure (30) is used for confirmation of chick edema factor contamination.

The sensitivity of electron capture detection (generally more than 1000 times that of microcoulometric detection) permits the analysis of 10 g or less of fat when combined with a suitable cleanup procedure. In addition, electron capture gas chromatographs are used routinely in many laboratories. An electron capture method for the detection of chick edema factor, developed by Higginbotham and co-workers (31), has recently undergone successful collaborative study. This procedure involves extracting the unsaponifiable matter from 10 g fat, chromatographing the unsaponifiable matter on 50 g of activated alumina, and eluting with 400 ml petroleum ether (fraction 1), 200 ml of 5% ethyl ether in petroleum ether (fraction 2), and 400 ml of 25% ethyl ether in petroleum ether (fraction 3). The third alumina fraction, evaporated to a volume of 2 ml, is shaken for one-half minute with sulfuric acid to eliminate substances which interfere with electron capture detection. The petroleum ether layer is neutralized with sodium carbonate and evaporated to dryness, and the residue is taken up in 1 ml isooctane for ECGLC. Normally, 5 µl or 1/200 of the sample extract is chromatographed.

A Barber-Colman Model 5360 instrument with a tritium foil concentric type detector, operated at 40 volts and 1×10^{-9} amperes full scale, produced satisfactory results with a 7-ft, ¼-in. I.D. glass column packed with 2.5% SE-52 on 60–80 mesh Gas-Chrom Q. The column, detector, and injector temperatures were 200C, 210C and 240C, respectively. Gas chromatographic peaks with retention times vs. aldrin between 10 and 25 indicated the presence of chick edema factor. Fig. 1 shows the typical gas chromatographic pattern obtained from a USP (refined and deodorized) cottonseed oil containing 1.5% of a reference toxic fat. Fig. 2 shows the gas chromatographic pattern obtained from a toxic commercial glyceryl mono-oleate.

A modified ECGLC screening test has been developed (32) which is more rapid, requires less sample, and yields results as satisfactory as those obtained with the original method. The modified procedure involves extraction of unsaponifiable matter from 2.5 g of fat and chromatography on 15 g of alumina; the column is eluted with 100 ml of petroleum ether (fraction 1), 50 ml of 5% ethyl ether in petroleum ether (fraction 2), and 100 ml of 25% ethyl ether in petroleum ether (fraction 3). The third alumina fraction is cleaned up with sulfuric acid. The final extract is dissolved in 250 µl of iso-octane, and a 5 µl sample is examined by ECGLC.

A TLC cleanup procedure has also been developed for the detection of chick edema factor (33). An 8 × 8-in. plate is coated with a 0.25 mm layer of silica gel G-HR which was previously activated by drying at 110C for 2 hr, and a line is drawn 17 cm from the bottom edge. The extracted unsaponifiable material from a 10 g sample is applied in 20 spots across the plate, 3 cm from the bottom edge of the plate, and the plate is developed with benzene/hexane (2/1) until the solvent front reaches the line. A strip 15–17 cm from the bottom edge is removed from the plate and extracted with ethyl ether. The extract is evaporated and the residue taken up in petroleum ether. After treatment with concentrated sulfuric acid, the sample is examined by ECGLC.

Animal and Vegetable Fats in Admixture

A number of methods for detecting animal and vegetable fats in admixture are based on analysis of the sterols by various means. Bömer (34) found that vegetable fat could be detected in animal fat on the basis of differences in melting point of the sterol acetates. A melting point higher than that of cholesterol acetate indicates the presence of vegetable fat. An AOAC procedure for detecting mixtures of animal and vegetable fats (35) involves preparation of purified sterol acetates from the precipitated digitonides for determination of melting point, and was developed for detection of vegetable fat in butter. A GLC method for detecting cholesterol and phytosterols in mixtures of animal and vegetable fats (36) was recently developed. The sterols are precipitated with digitonin; the

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sterol acetates are prepared, then analyzed on a 6-ft column packed with 1% SE-52 on silanized Celite support in an instrument with a radium (argon ionization) detector. Eisner and co-workers (37) found that 1% vegetable oil margarine could easily be detected in butter by GLC analysis of the sterols, isolated by saponification and extraction of the unsaponifiable matter, followed by separation on a Florisil column.

Detection of small amounts of animal fat in vegetable fats generally depends on detection of cholesterol. Animal fats are assumed to contain cholesterol which is absent in vegetable fats. Recourt and Beerthuis (38) found that GLC analysis of the total unsaponifiable matter of vegetable fats and oils sometimes gave an interfering peak at the cholesterol position. These workers concluded, nevertheless, that the vegetable fats and oils contained no cholesterol, and attributed the interfering peak to slight impurities. They reported, however, that palm oil was an exception, and contained sterols with a component whose retention time was equal to that of cholesterol. Investigations in the Food and Drug Administration (39) indicate that vegetable oils contain a substance that behaves chromatographically like cholesterol, but it is present only at low levels (about one-fortieth or less than the levels in animal fats). In addition, cholesterol has been isolated from Red Sea algae (40), fresh water algae (38), *Solanum tuberosum* (potato plant) and *Dioscorea spiculiflora* (41), and has been detected in pine bark sterols (42).

Methods for detection of cholesterol usually involve either direct GLC of whole unsaponifiable matter or GLC after isolation of the sterols by column chromatography or TLC. Ettinger and co-workers (43) reported a method for detection of animal fats in vegetable oils based on the GLC of total unsaponifiable matter. The presence of as little as 2.5% animal fat was detected by comparison with standards containing various levels of lard in vegetable oil; cholesterol was confirmed by GLC of the prepared sterol acetates. The authors used flame ionization detection, 4- or 6-ft columns packed with 3% JXR on 100–120 mesh silanized Gas-Chrom P, and column, detector, and injector temperatures of 230C, 240C, and 200C, respectively.

Eisner, Firestone, and co-workers (37,42,44) devised a procedure for detecting animal and vegetable fats in admixture. The extracted unsaponifiable matter is chromatographed on a Florisil column to isolate sterols before GLC. The extracted unsaponifiable matter is eluted from the Florisil with 40 ml of hexane (hydrocarbons; fraction 1), 120 ml of 5% anhydrous, alcohol-free ethyl ether (hydrocarbons; fraction 2), 120 ml of 15% ethyl ether (tocopherols, aliphatic alcohols, and triterpenoid alcohols; fraction 3), and 150 ml of 50% ethyl ether (sterols; fraction 4). Fraction 4 is evaporated to dryness and the residue is dissolved in 1 ml of chloroform for GLC.

Florisil is prepared and standardized as follows: PR grade is activated for 2 hr at 260C and cooled to room temperature, and 10.0 ml water is added to 100 g, with swirling to prevent collection of water in one area. The mixture is shaken vigorously for 10- 15 min until the exothermic reaction stops, and then is allowed to equilibrate overnight. Activity is determined by analysis of a mixture of 10 mg each of cholesterol, hexacosanol, and lanosterol. The hexacosanol and lanosterol should elute in the third Florisil fraction as determined by GLC and more than 90% of the cholesterol should elute in the fourth fraction. The water content of the Florisil may be varied in 0.5 ml increments to meet the performance standards described.

A Barber-Colman gas chromatograph or equivalent instrument is used with on-column injection. I have used argon ionization detectors (56μ radium-226 source) but hydrogen flame detectors may also be used under suitable conditions.) A 6-ft glass column, ¼ in. I.D., packed with 3% JXR on Gas-Chrom Q or silanized Gas-Chrom P, 100–120 mesh, is used routinely for argon ionization detection at operating temperatures of 210C–220C, 250C and 275C for the column, flash heater, and detector cell, respectively. A minimum of 1200 theoretical plates is required for the cholesterol peak, and the peak resolution (PR) of cholesterol and campesterol should be two or more. (The PR is calculated by the equation $PR = 2D/(A + P)$, where D is the distance between the peak maxima of the cholesterol and campesterol peaks, and A and P are the triangulated base widths of the cholesterol and campesterol peaks, respectively.) The argon flow rate is adjusted so that cholesterol elutes in about 12–20 min, and samples are analyzed by alternately injecting 2 μl portions of sterol fraction (in 1 ml $CHCl_3$) and cholesterol standards. Identity of cholesterol peaks in the sample is determined by comparison with the retention time of cholesterol, and choles-

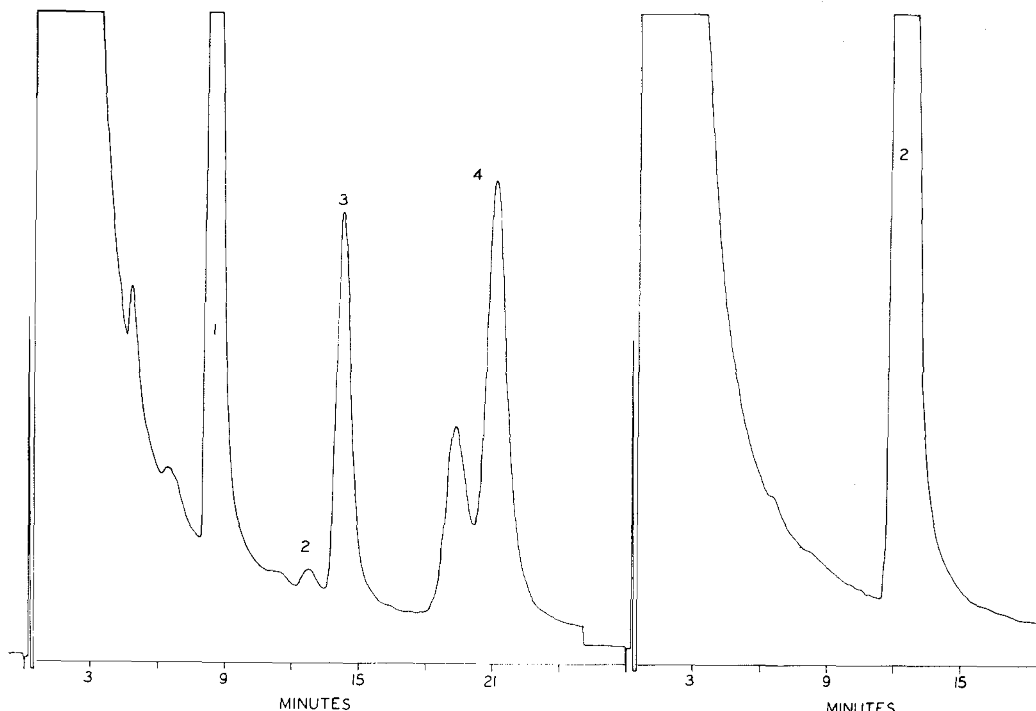

FIG. 3. Gas chromatograms of Florisil fraction 3 (left) and Florisil fraction 4 (right); 1) hexacosanol, 2) cholesterol, 3) α-tocopherol, and 4) lanosterol (mixture of dihydrolanosterol [minor peak] and lanosterol); 6 ft × ¼ in. 3% JXR on 100–120 mesh Gas-Chrom Q at 210C.

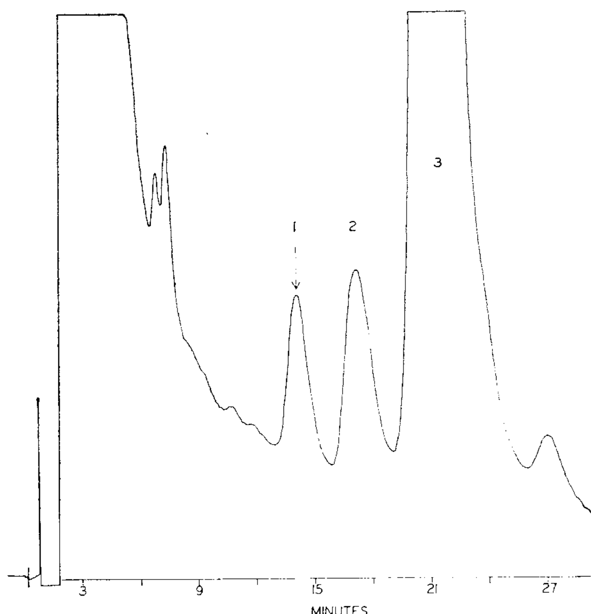

FIG. 4. Gas chromatogram of whole unsaponifiable matter from crude cottonseed oil; 1) retention time of cholesterol, 2) campesterol, 3) β-sitosterol; 6 ft × ¼ in. 3% JXR on 100–120 mesh Gas-Chrom Q at 230C.

terol content is estimated from an area plot (μg cholesterol vs. cholesterol peak area). The sterol acetates are prepared by acetylation with pyridine-acetic anhydride by a procedure recommended by Gregory (45), and the presence of cholesterol is confirmed by GLC of the acetates.

Fig. 3 shows gas chromatograms of several hydroxyl compounds separated on a grade of Florisil which meets the required performance standards. A mixture of 10 mg each of 1-hexacosanol, α-tocopherol, and lanosterol and 20 mg of cholesterol was chromatographed on PR grade Florisil prepared as described earlier, and 4 μl each of fractions 3 and 4 (in 10 ml $CHCl_3$) were gas-chromatographed. The nonsterol hydroxyl compounds eluted in the third Florisil fraction and more than 90% of the cholesterol eluted in the fourth fraction as required. The previously unidentified peak eluting before lanosterol (peak 4) is dihydrolanosterol, an impurity in commercially available lanosterol.

Fig. 4 shows a gas chromatogram of whole unsaponifiable matter from a crude cottonseed oil. At a column temperature of 230C, components with a retention time similar to cholesterol produced a peak indicating considerable contamination with animal fat. Analysis of the same sample after Florisil cleanup (Fig. 5) produced only a small peak with retention time of cholesterol equivalent to less than 1% added animal fat. The cholesterol peak of Florisil fraction 4 increases with the addition of 1.0, 2.5, and 5.0% beef fat to soybean oil, as shown in Fig. 6. With suitable adjustment of gas chromatographic sensitivity, as little as 0.1 mg of cholesterol per 100 g oil can easily be detected; this is roughly equivalent to 0.1% added animal fat.

I have found that a cholesterol-like component is present in small amounts in all common edible vegetable oils including coconut, cottonseed, corn, palm, palm kernel, peanut, safflower, and soybean oils. Table II shows the levels present in various samples. The vegetable oils contained up to 2.5 mg of cholesterol-like component per 100 g oil, whereas the animal fats contained about 90 mg of cholesterol per 100 g or at least 36 times as much cholesterol as the vegetable oils. Results to date suggest that the presence of more than 2.5 mg of cholesterol per 100 g fat indicates the presence of added animal fat.

Small gas chromatographic peaks with retention times close to cholesterol frequently interfere with determination of the cholesterol-like component of vegetable oils. The interfering peaks are usually resolved from the cholesterol-like component when the acetylated sterols are gas chromatographed.

Vegetable Oil Sterols

Vegetable oils contain varying amounts of phytosterols (campesterol, stigmasterol, β-sitosterol, etc.), and the ratios of these components are relatively constant for individual oils (44,46). Table II shows the relative amounts of phytosterols in several vegetable oils, obtained by GLC analysis of the sterols isolated by Florisil column chromatography of the isolated unsaponifiable matter as described earlier. A 6-ft glass column, packed with 1.5% SE-52 on silane-treated Gas-Chrom P, 100–120 mesh, was used, with argon ionization detection and a column temperature of 220C. The amounts of campesterol and stigmasterol differ widely in various oils, and stigmasterol is absent in olive and cottonseed oils. The ratio of β-sitosterol to campesterol plus stigmasterol (R) also indicates the wide difference in sterol composition of different vegetable oils.

Karleskind and co-workers (46) investigated sterol composition and the use of sterol ratios to detect adulteration of one vegetable oil with another. Extracted unsaponifiables were fractionated by TLC on Merck alumina G with

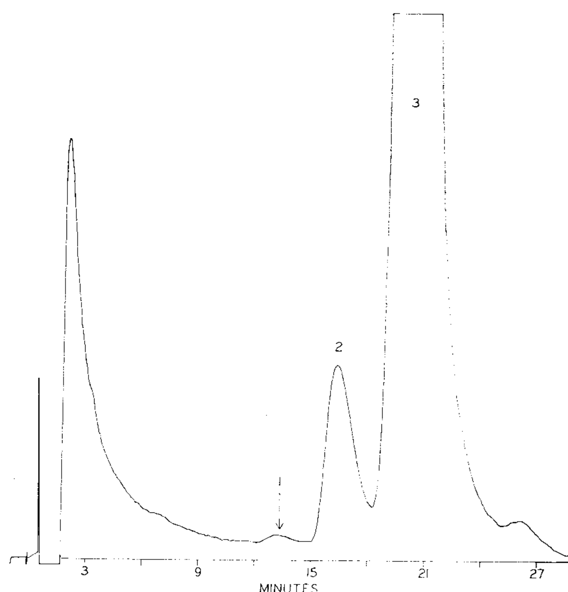

FIG. 5. Gas chromatogram of Florisil fraction 4 (sterols) from crude cottonseed oil; 1) component with retention time of cholesterol, 2) campesterol, 3) β-sitosterol; 6 ft × ¼ in. 3% JXR on 100–120 mesh Gas-Chrom Q at 230C.

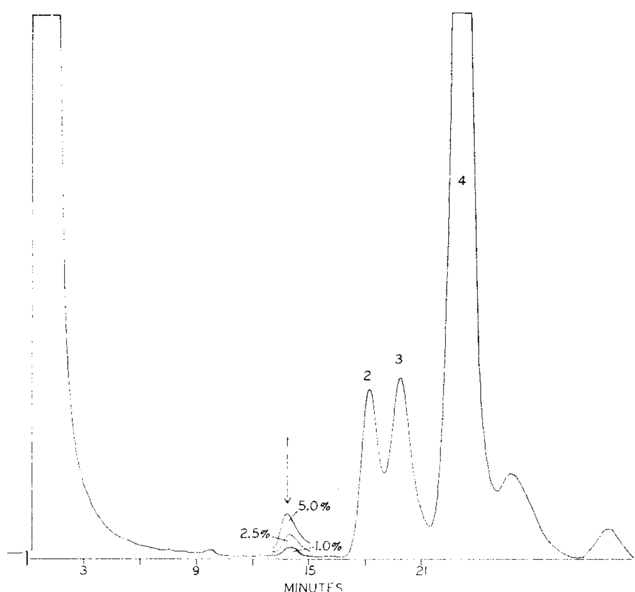

FIG. 6. Gas chromatogram of Florisil fraction 4 (sterols) from 1.0, 2.5, and 5.0 beef fat in refined soybean oil; 1) cholesterol, 2) campesterol, 3) stigmasterol, and 4) β-sitosterol; 6 ft × ¼ in. 3% JXR on 100–120 mesh Gas-Chrom Q at 215C.

TABLE II

"Cholesterol-like" Content of Some Vegetable and Animal Fats

Sample	Cholesterol-like component-expressed as mg cholesterol/100 g
Coconut oil, refined and deodorized	0.4
Corn oil, refined and deodorized	1.4
Cottonseed oil, crude	1.7
Cottonseed oil, refined and deodorized	1.2
Palm oil, refined and hardened	2.5
Palm kernel oil, refined	1.4
Palm kernel oil, refined and hardened	1.2
Peanut oil, refined and deodorized	0.8
Rice bran oil, refined and deodorized	0.8
Safflower oil, refined and deodorized	1.3
Soybean oil, refined and deodorized	0.8
Beef fat	91
Lard	87

hexane/ethyl ether (80/20) as the developing agent. The sterol band, made visible with dichlorofluorescein, was extracted with ethyl ether and gas chromatographed at 265C on a 5-ft column containing 10% SE-52 on 80–100 mesh Chromosorb W, with flame ionization detection. The ratio R (β-sitosterol/campesterol + stigmasterol) of olive oil decreased from 43 to 17 with the addition of 5% corn oil and to 32 with the addition of 5% cottonseed oil. Also, certain sterols are characteristic for certain oils. Thus, brassicasterol, which elutes on silicone columns between cholesterol and campesterol, is the characteristic sterol of rapeseed oil and other oils of the Cruciferae family.

Nonsterol Hydroxyl Compounds

A number of studies (47–51) have shown that vegetable oil unsaponifiable matter contains hydrocarbons, aliphatic alcohols, triterpene alcohols and tocopherols in addition to sterols. Individual compounds vary with different oils, and variations are most pronounced among the triterpene alcohols. The triterpene alcohols and tocopherols, as well as the sterols, are of practical interest because they provide characteristic "fingerprints" for identification of individual oils.

Fedeli and co-workers (49) isolated the triterpene alcohols, as well as the sterols, from the extracted unsaponifiable matter of 18 vegetable oils by TLC on silica gel G plates, developing with hexane/ether (1/1). The triterpene alcohols and sterols were visible under UV light after spraying with the sodium salt of dichlorofluorescein. Strips containing these compounds were extracted with ether for subsequent gas chromatographic analysis on a 6-ft glass column packed with 1% SE-30 on 100–120 mesh silanized Gas-Chrom P, with flame ionization detection. Wide differences were found in the triterpene alcohol composition of the vegetable oils. Eisner and co-workers (51) also found that the nonsterol hydroxyl components of vegetable oils (tocopherols, triterpene alcohols, etc.) isolated by Florisil column chromatography (Florisil fraction 3) displayed characteristic GLC patterns which could be used to identify individual oils.

The triterpene alcohol pattern of gas chromatograms from Florisil fraction 3 has been used to distinguish between pressed and solvent-extracted olive oils (50). The ratio of cycloartenol to butyrospermol was considerably lower in solvent-extracted oils than in pressed oils. Although primarily a test for adulteration of olive oil with teaseed oil, the Fitelson teaseed oil test (52) may also be used to detect solvent-extracted olive oil in pressed olive oil. Capella and co-workers (53) showed that the light red color obtained from solvent-extracted oils was due to the presence of moderate amounts of the triterpene alcohol butyrospermol in these oils.

The tocopherol composition of vegetable oils may vary considerably among individual oils. This was demonstrated recently by Govind Rao and co-workers (54) who examined cottonseed, neem, soybean and several other vegetable oils. Tocopherols were isolated from extracted unsaponifiable matter by TLC on silica gel G containing sodium fluorescein and developed with petroleum ether/ethyl ether/isopropyl ether/acetone/acetic acid (127/1.5/16/6/1.5). The tocopherols, detected as purple spots under UV light, were extracted and analyzed with the Emmerie-Engel reagent. A trace of β-tocopherol was found in neem oil, but none was detected in any of the other oils examined. Soybean oil contained a moderate amount of δ-tocopherol (26% of total tocopherols) which was not found in cottonseed oil. Sturm and co-workers (55) recently determined the tocopherol composition of New Mexico Valencia peanut oil and other peanut varieties grown in this country. Samples were saponified under a N_2 atmosphere in the presence of pyrogallol. Individual tocopherols were isolated by TLC (silica gel) with chloroform as developing solvent, and determined spectrophotometrically by a modification of the Emmerie-Engle method. Average recovery values for α-, γ-, and δ-tocopherols were 93, 88, and 75%, respectively. Tocopherol composition of a number of peanut oil varieties varied as follows: α-tocopherol, 28–44%, γ-tocopherol, 49–69%, and δ-tocopherol, 2–6%.

In summary, sensitive chromatographic techniques are available for detection of pesticide residues, contaminants, and admixtures of fats and oils. Analysis of the unsaponifiable matter provides a valuable means of detecting animal and vegetable fats in admixture, and for characterizing individual vegetable oils.

TABLE III

Sterol Composition of Some Edible Vegetable Oils by Gas Chromatography (44)[a]

Oil	% of Sterols			R[b]
	Campesterol	Stigmasterol	β-Sitosterol	
Corn	10	1	89	8
Cocoa butter	8	26	66	2
Cottonseed	4	0	96	24
Olive	2	0	98	49
Peanut	13	3	84	5
Rice bran	20	8	72	4
Safflower	13	6	81	4
Soybean	15	13	72	3

[a] Data are reprinted with the permission of the *Journal of the Association of Official Analytical Chemists.*

[b] R indicates ratio of β-sitosterol peak area to stigmasterol plus campesterol peak areas.

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• *Industry Items*

B. L. Thomas

B. L. THOMAS (1953) has formed the Cincinnati-based firm, B. L. Thomas Associates. The firm will serve as consultant in regard to edible fats and oils utilization in the food industry. Mr. Thomas has represented the Procter & Gamble Company in this field for the past 17 years. The associates have had wide experience in in the food industry in consultation with management, engineering, production, and research and development.

The firm's address: B. L. Thomas Associates, P. O. Box 15177, Cincinnati, Ohio 45215; phone, 513/771-7662.

To keep pace with expanding operations and new products, LKB INSTRUMENTS, INC., the U.S. affiliate of LKB PRODUKTER, Stockholm, Swenden, a manufacturer and distributor of instruments for research in biochemistry, has reorganized into three divisions: *Analytical Instruments Division*, which will market calorimetry and gas chromatography-mass spectrometry instruments, with Frederick Schaub as manager; *Ultramicrotomy Instruments Division*, which will include equipment and accessories for electron microscopy, with Robert Evans as Manager; *Laboratory Instruments Division*, which will distribute instruments for Electrophoresis and Chromatography.

Nicholsen New President of Research Administrators

D'Agostino Elected Secretary

Richard Nicholsen

Announcements of the newly chosen president and officers of the Society of Research Administrators were made at the 2nd Annual business meeting held on March 6 at the Shamrock-Hilton Hotel, Houston, Texas.

Richard Nicholsen (1946), research center business manager, Ashland Chemical Company, Minneapolis, Minn., is president for 1968–1969. Born in Austin, Minn., in 1911, Mr. Nicholsen graduated from the University of Minnesota in 1934. He joined ADM Company as a control chemist in 1934 and successively held posts as research chemist, administrative assistant to research director, and research laboratory business manager prior to his current assignment. He has been active in numerous professional societies including the ACS, AOCS, Minnesota Industrial Chemists' Forum, and the Minnesota Federation of Engineering Societies.

Others elected were Ken Hartford, Yale University, President-Elect; Richard D'Agostino (1961), Riker Laboratories, Secretary, and Paul Davis, University of California, Treasurer.

Instrument Society of America Sponsors 1968 Instrumentation Conference

The Instrument Society of America (ISA) will sponsor the 1968 Research Conference on Instrumentation Science, July 29–Aug. 2, 1968, on the campus of Quinnipiac College, Hamden, Conn.

General Chairman for this year's meeting is E. A. Taylor, Rohm & Haas Company. Robert Spooner, IMPAC Instruments, is Vice Chairman in charge of the program. Session topics include: Doppler Ultrasound for Medical Diagnosis; Energy Sensing Transducers; Fluidics, Direct Sensors and System Performance; Computer Challenge to the Accuracy of Sensors; Ultra High Vacuum Measurement; Analytical Instrumentation; Remote Data Read-Out Systems.

Attendance is limited to 100 participants.

For a complete program and an application form, please write: Mariruth Reed, Meetings Coordinator; Instrument Society of America; 530 William Penn Place; Pittsburgh, Pa. 15219.

• *Obituaries*

Word has been received at AOCS headquarters of the death of W. G. REECE (1948). He had been with Central Quality Control, Armour & Co., Foods Division, Chicago, Ill.

ARNOLD ANTONIS (1966), biochemist of the Medical Research Council of Great Britain, Medical Unit, St. George's Hospital, died in December, 1967.

DANIEL PICARD (1910), Emeritus Member of AOCS, died Feb. 6, 1968 in West Palm Beach, Fla.