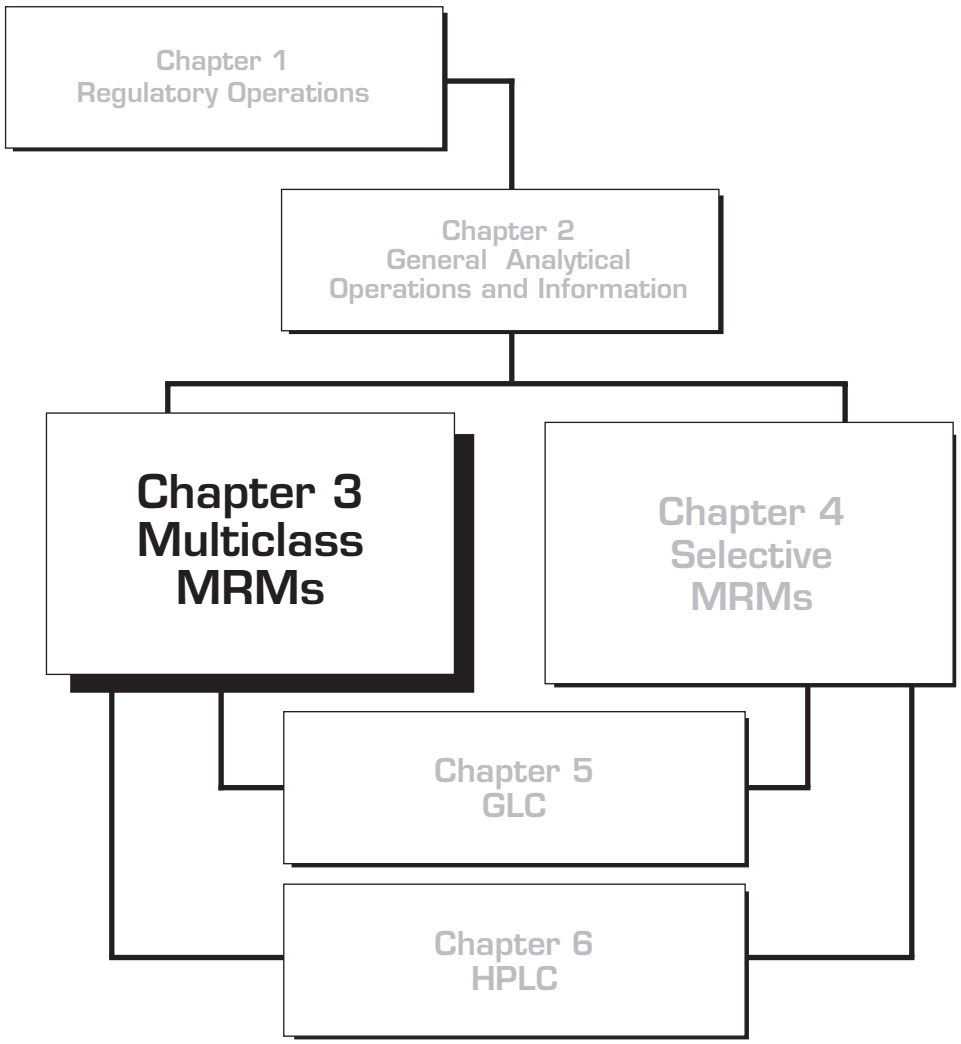

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301: MULTICLASS MRMS: CONCEPT AND APPLICATION

Pesticide multiresidue methods (MRMs) are capable of simultaneously determining more than one residue in a single analysis; this multiresidue capability is provided by a GLC or HPLC determinative step that separates residues from one another before detection. The MRM concept is raised to a higher dimension when a single extract is examined with more than one chromatographic determinative step, each providing coverage of residues in a different class, *e.g.*, chlorinated hydrocarbons, organophosphates, and carbamates. PAM I refers to these broad scope methods as "multiclass MRMs."

A multiclass MRM is potentially capable of determining any residue extracted by its extraction step; PAM I multiclass MRMs extract residues with organic solvents known to remove most nonionic residues from food commodities. Each determinative step in a multiclass MRM provides coverage for a particular group of residues in the extract, and each cleanup step is designed to purify the extract sufficiently to permit accurate determination. A multiclass MRM scheme can be expanded continually as new technologies are developed and adapted.

This introductory section presents a recommended approach to application of multiclass MRMs and background information with which any analyst using such methods should be familiar.

301 A: RECOMMENDED APPLICATION OF MULTICLASS MRMS

Whenever a sample of unknown pesticide treatment history is analyzed, and no residue(s) is targeted, a multiclass MRM should be used to provide the broadest coverage of potential residues; Figure 301-a displays the recommended multiclass MRM for each commodity category. The more detailed scheme provided with each method (Figures 302-a, 303-a, 304-a) directs the user to recommended module(s) for particular commodities. The user may choose as many or as few of these modules as time and resources permit; once residues are extracted, each determinative step extends coverage of the analysis to additional compounds.

Follow these directions to maximize coverage of residues without sacrificing quantitative accuracy:

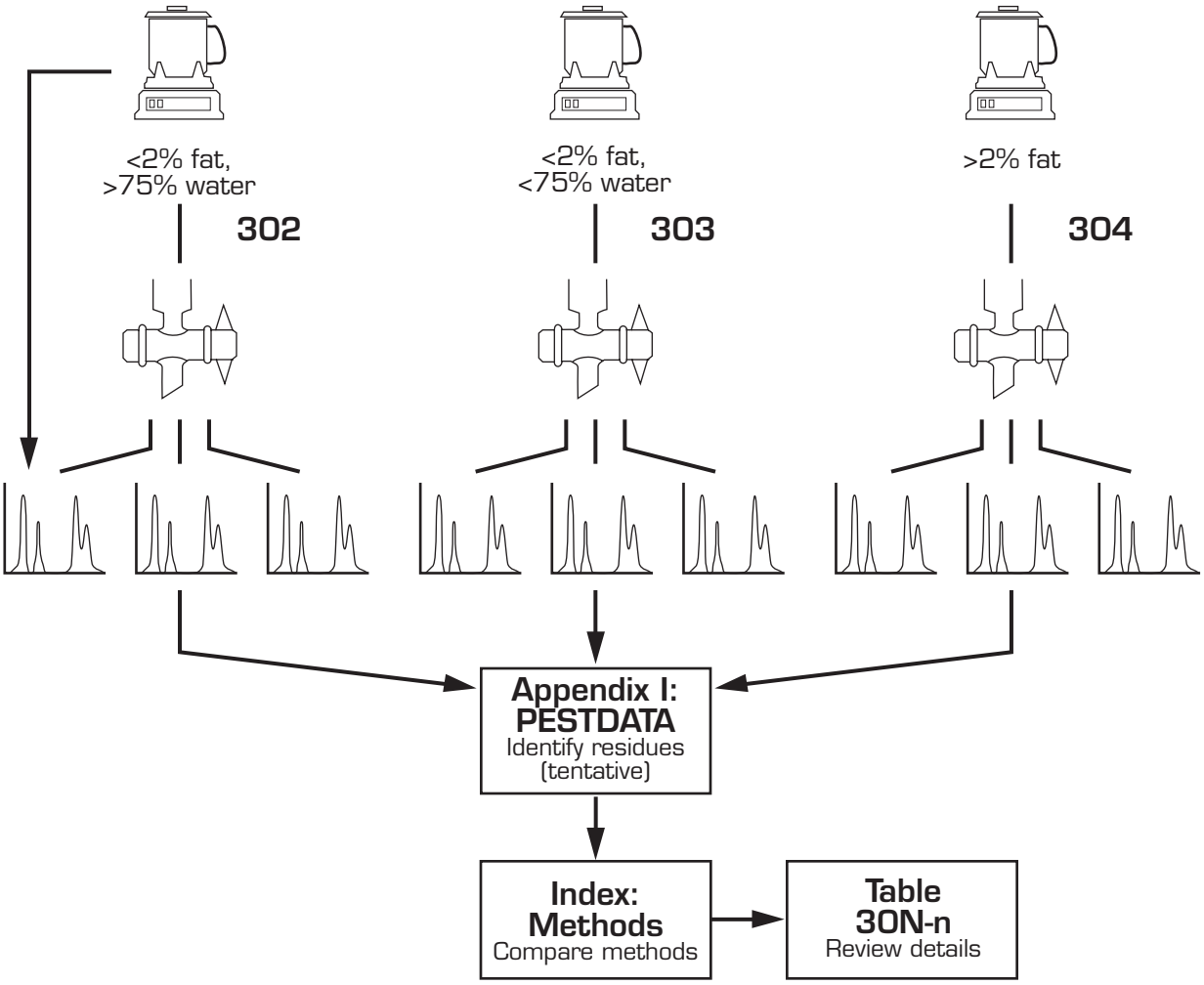
- For broadest coverage of potential residues, examine the uncleaned extract by determinative steps that are sufficiently selective to permit residue identification and quantitation in the presence of co-extractives.

- Following determination by selective determinative steps, clean up the extract as needed to permit additional determinations; these may include determinative steps designed for specific groups of residues (*e.g.*, from Chapter 4 methods).

- When a peak appears in the chromatogram of the extract, use the following PAM I tables to tentatively identify the residue and to choose the additional analyses needed for optimum identification, quantitation, and/or confirmation:

    1) PESTDATA (Appendix I). Compare GLC relative retention time (rrt) of the residue to lists of rrts on several common GLC columns

Figure 301-a
Recommended Approach to Analysis of Foods



for the first clue to residue identity. Review method behavior information for additional clues about which potential candidate(s) behaves in the same way as the residue. Inject solutions of appropriate reference standard(s) for comparison to the residue peak.

If retention times of residue and reference standard match, use PESTDATA information on the chemical's molecular formula and its rrts on other columns as a guide to selecting other determinations that will provide confirmatory evidence. If additional analyses are needed, choose appropriate other methods from PESTDATA listings of recoveries, Index to Methods, and tables related to specific methods, below.

PESTDATA rrts are for GLC systems only. Retention times from the HPLC determinative steps of Sections 401, 403, and 404 are included in the tables that accompany those methods. Use those tables to tentatively identify residues found.

2) Index to Methods. Use this summary as a guide to other method(s) available for a tentatively identified residue. Review method tables, below, for additional details.

3) Tables 302, 303, 304, 401, 402, 403, and 404. When a residue is tentatively identified, review method tables for details about special situations that may diminish recoveries, opportunities to improve recoveries, need for particular determinative step(s), *etc.* Decide what additional analyses are necessary based on this information.

- When tabulated information about behavior of the tentatively identified residue indicates that the method used provided only incomplete recovery, re-analyze the commodity with another method capable of complete recovery of the residue.

  (The analyst should be aware that all data in PAM I tables reflect the best information available but do not guarantee that results will be identical in every situation. Data have been collected for 30 years from many sources, including original method development studies, recovery studies by FDA laboratories, recovery studies by pesticide registrants and/or their contract laboratories, and collaborative and validation studies conducted under the auspices of AOAC International. Particular results may represent many analyses or only one, may have been performed with or without sample present, through complete methods or through individual procedures of a method, and with or without use of lauric acid adsorption value for Florisil column weight adjustment.)

- When the method used has not been previously validated for the residue/commodity combination, develop the necessary validation data.

Inherent in this approach to residue analysis is the acknowledgment that no multiclass MRM is quantitatively valid for all residues it is capable of detecting. Thus, re-analysis by other method(s) is required when a residue(s) is identified by a method known to be incapable of confirmatory identification and/or quantitative accuracy. Demonstration of method validity for any residue/commodity combination that is reported is the responsibility of the analyst using the method.

301 B: CAPABILITIES AND LIMITATIONS OF MRMS

Several aspects of an MRM influence its scope as a multiclass method: (1) thoroughness with which the extraction solvent and physical procedure are capable of extracting residues from the sample, (2) ability of subsequent cleanup techniques to remove sample co-extractives without removing residues, and (3) the number of different determinative steps used to examine the extract. During method development, a researcher evaluates each step of a method and makes choices based on optimum performance. Subsequent interlaboratory validation verifies that the method produces accurate results when performed as written.

This edition of PAM I presents MRMs as a series of modules, in recognition of standard practices in laboratories required to analyze many different commodities for many different potential residues. Modules presented within the same section in this manual were not necessarily developed at the same time or by the same researcher. Module combinations that have undergone interlaboratory validation are listed and recommended, but analysts may find it necessary to combine other modules to meet a particular need. Any such combination must be supported by data that validate its use in the situation. Steps of an MRM must be compatible with one another for the whole method to be applied in a valid manner.

This section provides background information to assist the analyst in making valid choices and avoiding potential pitfalls. Included here are discussions about the overall influence solvents have on method performance and important information about each category of method modules (extraction, cleanup, and determinative steps). Analysts combining method modules must be aware of the following concerns and take precautions to ensure that only appropriate combinations are used.

Influence of Solvents on Methodology

Choice of solvent(s) is among the most important decisions made by a researcher developing an analytical method. Analysts using these methods must also be aware of the following considerations related to solvents used in individual modules:

Availability of Pure Solvent. Solvent purity is essential to avoid potential interferences in the determinative step; impurities are usually concentrated during the evaporation steps included in most residue methods. Higher purity solvents invariably cost more, and it may be possible to use less expensive, lower purity materials if a solvent reagent blank examined by appropriate determinative step(s) (Section 204) supports their acceptability.

Detector Response to Solvent. GLC detectors used in residue determinations are usually selective for an element in the analyte molecule, so the final extract must not be dissolved in a solvent containing element(s) to which the detector(s) respond. For example, no trace of acetonitrile can be present when a nitrogen-selective detector is used, and no methylene chloride when a halogen-selective detector is used. HPLC detectors commonly used in residue determination preclude use of solvents that absorb UV light or fluoresce at the wavelength used during determination.

Solvents can adversely affect detectors in other ways, such as the deleterious but poorly defined effect acetonitrile has on electroconductivity detectors.

Experiences with such effects are usually noted in a method so use of particular solvents can be avoided.

Polarity. Increasing the polarity of an extraction solvent may improve a method's ability to extract particular residues, but it usually also increases the amount of co-extractives. The presence of polar solvents may also affect subsequent cleanup steps, so residues may need to be transferred to a different solvent before the next step of the method is performed.

Boiling Point. Solvents with a low boiling point are preferred, if evaporation to accommodate detector compatibility or appropriate polarity is necessary. In some cases, a solvent with a relatively high boiling point can be evaporated at a lower temperature if an azeotrope is first formed by addition of another solvent. Several types of evaporation apparatus exist (Section 202 C), and choice of which to use is often related to the boiling point of a particular solvent.

Toxicity. Solvents vary in toxicity, and laboratories should choose the least toxic among equivalent choices. Certain solvents (benzene, carbon tetrachloride) should no longer be used in residue analysis. Concentration and evaporation steps must be performed in an adequately ventilated hood, and other standard safety precautions must be followed (Section 207).

Extraction

The necessity of using water-miscible solvents to extract pesticide residues from high moisture products has long been established, as has the necessity of a "blending type" extraction process [1-4]. Acetone (Section 302), acetonitrile (Section 303), and methanol (Sections 401, 403) are used in PAM I multiclass and selective MRMs to extract nonionic residues from fruits and vegetables. Variations in polarity may affect the degree to which each can extract any particular residue [5-8].

Because extraction capabilities of these solvents are similar, other characteristics affect which solvent a developer chooses to use in a method. For example, developers of the method in Section 302 used acetone as extractant instead of acetonitrile (Section 303) because it is less toxic, has a lower boiling point (57° C *vs.* 82° C), does not affect detectors adversely, and does not form a two-phase system with water during analysis of fruit, as acetonitrile does [9].

Liquid-liquid partitioning of residues from initial extractant to nonaqueous solvent is a step common to most MRMs. Nature of the solvent(s) used in this step affects the degree of transfer of both residues and co-extractives. For example, in Section 302 E1, petroleum ether is included in the separator with aqueous acetone and methylene chloride to reduce the amount of polar plant constituents that partition into the organic phase. However, in a method variation targeted at the highly polar methamidophos, petroleum ether is replaced with acetone to improve partitioning of methamidophos from the aqueous to the organic layer [10].

Any MRM is applied with the understanding that certain residues are particularly difficult to extract, *e.g.*, the polar residue methamidophos, above. In such cases, notation of partial recovery is made in the table(s) of data that accompany the method description. Tentative identification of a residue known to be incompletely extracted by the method in use should then lead to re-analysis by another method or variation.

Certain commodities also present greater challenges to the extraction process, and methods may include special steps as an accommodation. Dry products are extracted with combinations of organic solvent and water to make up for the absence of water in the commodity itself. Several studies support the use of water/acetonitrile (Section 303 E3) for this purpose [11-13]. Water/acetone (Section 302 E4) is also used but has been found in some cases to extract less residue than water/acetonitrile [14, 15]; the two methods should be used to check one another when a residue has been identified that can be determined by both methods.

Extraction of residues from fatty products (*e.g.*, Section 304 E1-E5) has traditionally been aimed at nonpolar, lipophilic residues, which are readily extracted from the product when the fat itself is extracted. Currently, no method is available in this manual for quantitative determination of polar residues in fatty products.

Some residues absorbed from soil by plants, *e.g.*, dieldrin in potatoes, have been shown to be incompletely extracted by methods such as Section 303 [13]; other root-absorbed residues (*e.g.*, dieldrin and DDT in carrots) have been extracted completely by the same procedure [16, 17]. Laboratories analyzing root crops must be aware that the method may not be extracting all the residue present. Other, more exhaustive processes, such as use of a Soxhlet extractor [18], may be necessary if the residue or commodity warrants.

Cleanup

Cleanup steps are designed to purify extracts to permit more definitive identification of residues at lower limits of quantitation, and to minimize adverse effects on determinative step instrumentation. However, almost all cleanup steps adsorb, destroy, or otherwise remove at least some residues from the extract. Thus, cleanup may reduce the number of detectable residues in the final extract.

Schemes for multiclass MRMs attempt to determine as many residues as possible by examining uncleaned extracts with selective detectors, *e.g.*, flame photometric and electrolytic conductivity (GLC) and fluorescence (HPLC). Cleanup can subsequently be performed on the extract to permit determination with less selective detectors, *e.g.*, electron capture (GLC) or UV (HPLC). Use of several cleanup steps, each on a separate aliquot of extract, permits examination of each aliquot with a different determinative step. This approach provides coverage for the maximum number of residues, excluding only those not recovered from any cleanup step and also not determined by initial selective detectors.

Residues can often be detected but not reliably quantitated in an uncleaned extract; quantitation may be possible once the extract is cleaned up using a technique known to recover the particular residue. Other residues can be quantitatively measured only by re-analysis with a different extraction step. Tables of recovery data for each method provide the analyst with information to guide the choice of an appropriate cleanup technique or alternative method.

Many cleanup steps involve chromatography of the extract solution on a column or cartridge. Choices of the column/cartridge material and eluting solvent(s) dictate what chemicals can be recovered; *e.g.*, columns of the adsorbent Florisil provide suitable cleanup of relatively nonpolar residues (Sections 302 C1, 303 C1, *etc.*). Increasing the polarity of the eluant permits recovery of more polar residues but decreases the degree of cleanup, because more co-extractives are also eluted.

Very polar residues usually cannot be eluted from Florisil no matter how polar an eluant is used. Instead, charcoal columns are often used for cleaning up extracts containing polar residues, *e.g.*, Sections 302 C2 and C3.

The nature of the solvent in which the extract is dissolved when placed on a cleanup column may affect which residues elute from the column. Recovery data associated with a method are valid only when the extract is in the specified solvent. When combining method modules, the extract added to a cleanup column may be in a solvent different from that originally specified; in such cases, recovery data may not be applicable. To make use of existing tables of data related to chemicals recovered through a method, it may be necessary to change the extract solvent by evaporation or azeotroping.

Determinative Steps

Use of minimal cleanup in an MRM reduces analysis time and reagent costs, but it can jeopardize determinative step reliability by introducing co-extractives that interfere with the determination or cause physical damage to the system. Presence of materials to which the detector responds can cause (1) false reports of residues not actually present, (2) inaccurate quantitation of residues, or (3) complete masking of residues. Risk of chromatographic degradation is increased by repetitive injection of an uncleaned extract.

The analyst using methods from PAM I is responsible for ensuring that extract injected into any determinative step system does not contain potential interferences or materials that adversely affect chromatographic performance. Sections 501 C and 601 E provide recommendations related to determinations with GLC and HPLC systems, respectively. Analytical accuracy and minimal disruption of laboratory operations will result if reasonable use of cleanup steps and regular maintenance of instruments are both employed.

In certain cases, special precautions are needed to detect particular residues. For example, thiometon is known to break down while standing in the extract solution of Section 302 E1; examination of the extract soon after its preparation permits determination of thiometon residues that would not otherwise be detectable. Notes are included in the method tables of data to provide such advice.

References

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[2] Klein, A.K., *et al.* (1959) *J. Assoc. Off. Agric. Chem.* **42**, 539-544

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[4] Porter, M.L., and Burke, J.A. (1968) *J. Assoc. Off. Anal. Chem.* **51**, 63-64

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[8] Sonobe, H., *et al.* (1983) *J. Agric. Food Chem.* **31**, 96-104

[9] Luke, M.A., *et al.* (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020-1026

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[14] Cox, B., and Uribe, M. (Nov. 1984) "Comparison of Acetone and Acetonitrile for Extraction of Endrin from Mung Beans," LIB 2881, FDA, Rockville, MD

[15] Weishaar, J.A. (Nov. 1989) "Comparison of Acetone-Water and Acetonitrile-Water for Extraction of Pirimiphos Methyl from Pasta Products," LIB 3397, FDA, Rockville, MD

[16] Porter, M.L., *et al.* (Dec. 1970) FDA private communication

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302: METHOD I FOR NONFATTY FOODS

BASIC REFERENCES

Luke, M.A., *et al.* (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020-1026

Luke, M.A., *et al.* (1981) *J. Assoc. Off. Anal. Chem.* **64**, 1187-1195

GENERAL PRINCIPLES

Residues are extracted from nonfatty foods by blending with acetone or water/acetone, then transferred from the filtered aqueous extract into organic solvent. The extract is cleaned up if necessary and examined by various determinative steps; the amount of cleanup necessary is dictated by the determinative step(s) to be used and by the type of commodity being analyzed.

APPLICABILITY

Consult Guide to PAM I for additional information pertinent to the appropriate application of multiresidue methodology.

Method is applicable to nonionic residues in nonfatty foods. Cleanup steps may be needed for particularly dirty extracts or for examination by less selective detectors; some residues may be lost during cleanup. Extract is amenable to examination by many determinative steps, and the residues covered by a particular analysis are dependent on the number of different determinative steps used. See Tables 302-a and 302-b, following the method description, for results of recovery tests.

METHOD MODULES

Choose from these method modules, using Figure 302-a for guidance:

Extraction (E)			Recommended Use	
E1	(p. 302-7)	Extraction with acetone, liquid-liquid partitioning with petroleum ether/methylene chloride	nonfatty, high moisture commodities	
E2	(p. 302-9)	Extraction with acetone, removal of water with 40 g Hydromatrix	nonfatty, high moisture commodities	
E3	(p. 302-11)	Extraction with acetone, removal of water with 25 g Hydromatrix	alternative to E2 for reduction in solvent use	
E4	(p. 302-13)	Extraction with water/acetone, liquid-liquid partitioning with petroleum ether/methylene chloride	nonfatty, low moisture commodities	
E5	(p. 302-15)	Extraction with acetone, liquid-liquid partitioning with acetone/methylene chloride	alternative to E1 for relatively polar residues	◀
E6	(p. 302-16)	Extraction with water/acetone, liquid-liquid partitioning with acetone/methylene chloride	alternative to E4 for relatively polar residues	◀
E7	(p. 302-17)	Extraction with acetone and solid phase extraction cartridges, liquid-liquid partitioning	nonfatty, high moisture commodities for relatively polar residues	◀

Cleanup (C)

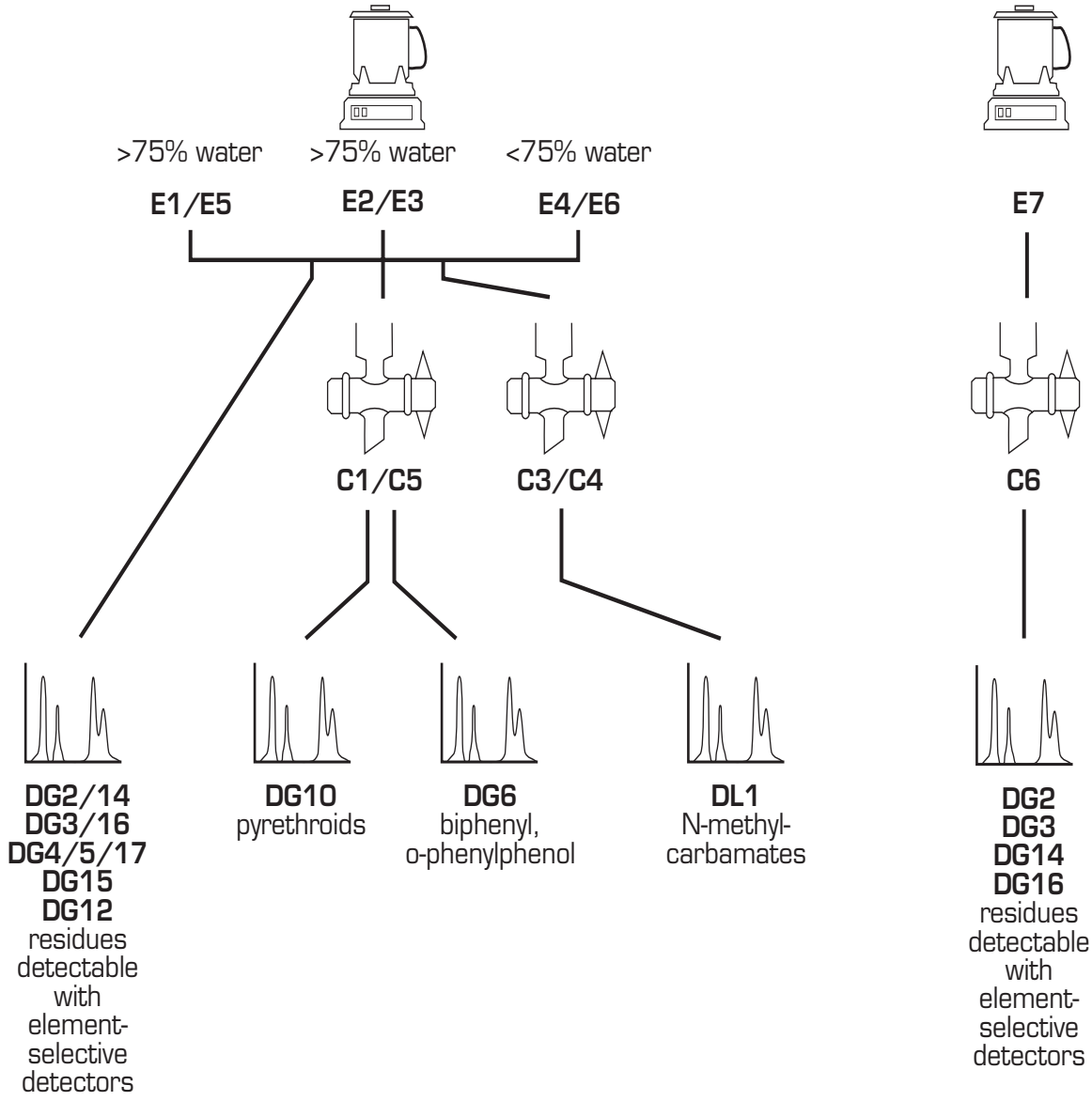
C1	(p. 302-21)	Florisil column (4 g) cleanup, with one methylene chloride eluant	relatively nonpolar residues
C2	(p. 302-23)	Charcoal/Celite/magnesium oxide column cleanup	polar residues
C3	(p. 302-25)	Charcoal/silanized Celite column cleanup	before HPLC determination for N-methylcarbamates
C4	(p. 302-27)	C-18 cartridge cleanup	before HPLC determination for N-methylcarbamates
C5	(p. 302-29)	Florisil column cleanup, with mixed ether eluants	relatively nonpolar residues
▶ **C6**	(p. 302-31)	SAX/PSA cartridge cleanup	polar and nonpolar residues

Determination (D)		**Recommended Use**	
DG 1	(p. 302-33)	GLC, 100% methyl siloxane column, 200°, EC detector	residues with halogen. sulfur, other moities
DG 2	(p. 302-35)	GLC, 100% methyl siloxane column, 200°, FPD-P	residues with phosphorus
DG 3	(p. 302-37)	GLC, 100% methyl siloxane column, 200°, ELCD-X	residues with halogen
DG 4	(p. 302-39)	GLC, 100% methyl siloxane column, 200°, ELCD-N	residues with nitrogen
DG 5	(p. 302-41)	GLC, 100% methyl siloxane column, 200°, N/P detector	residues with nitrogen or phosphorus
DG 6	(p. 302-43)	GLC, 100% methyl siloxane column, 160°, FID	biphenyl, o-phenylphenol
DG 7	(p. 302-45)	GLC, 100% methyl siloxane column, 130°, EC detector	early eluting residues with halogen, sulfur, other moieties
DG 8	(p. 302-47)	GLC, 100% methyl siloxane column, 130°, FPD-P	early eluting residues with phosphorus
DG 9	(p. 302-49)	GLC, 100% methyl siloxane column, 130°, ELCD-X	early eluting residues with halogen
DG10	(p. 302-51)	GLC, 100% methyl siloxane column, 230°, EC detector other moieties	late eluting residues with halogen, sulfur,
DG11	(p. 302-53)	GLC, 100% methyl siloxane column, 230°, FPD-P	late eluting residues with phosphorus
DG12	(p. 302-55)	GLC, 100% methyl siloxane column, 230°, ELCD-X	late eluting residues with halogen
DG13	(p. 302-57)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, EC detector	residues with halogen, sulfur, other moieties

DG14 (p. 302-59)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, FPD-P	residues with phosphorus
DG15 (p. 302-61)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, FPD-S	residues with sulfur
DG16 (p. 302-63)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, ELCD-X	residues with halogen
DG17 (p. 302-65)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, N/P detector	residues with nitrogen or phosphorus
DG18 (p. 302-67)	GLC, 50% cyanopropylphenyl, 50% methyl siloxane column, 200°, EC detector	residues with halogen, sulfur, other moieties
DG19 (p. 302-69)	GLC, 50% cyanopropylphenyl, 50% methyl siloxane column, 200°, FPD-P	residues with phosphorus

►

Figure 302
Recommended Approach: Nonfatty Foods



VALIDATION

Many combinations of method modules are possible. The following combinations have undergone interlaboratory validation and are recommended for use:

E1 + DG2, DG3

Validation report:

> Sawyer, L.D. (1985) *J. Assoc. Off. Anal. Chem.* **68**, 64-71. Collaborative study lead-ing to AOAC official final action status for acephate, a-BHC, chlorpyrifos, dieldrin, monocrotophos, and omethoate in lettuce, strawberries, and tomatoes.
>
> AOAC official method reference: *Official Methods of Analysis of the AOAC* (1990) 15th ed., 985.22.

E1 + C3 + DL1

Validation report:

> Pardue, J.R. (April 1987) "Recoveries of N-Methyl Carbamates Using a Combination of the Luke (PAM I, 232.4) and Krause (PAM I, 242.24b, 242.25) Procedures," LIB 3138, FDA, Rockville, MD

E2 + C1 + [temperature programmed GLC systems equivalent to] DG1, DG7, DG10, and DG16

Validation report:

> Griffitt, K.R., and Szorik, M.M. (Sept 1989) "The Analysis of 127 Total Diet Items for Chlorinated Residues Using Luke/Solid Phase Extracts," LIB 3366, FDA, Rockville, MD

E1 EXTRACTION WITH ACETONE, LIQUID-LIQUID PARTITIONING WITH PETROLEUM ETHER/METHYLENE CHLORIDE

References

Luke, M.A., *et al.* (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020-1026

Luke, M.A., *et al.* (1981) *J. Assoc. Off. Anal. Chem.* **64**, 1187-1195

Principles

Nonfatty sample is blended with acetone and filtered. Most nonionic residues are extracted into aqueous acetone solution. Residues are transferred from aqueous acetone to methylene chloride/petroleum ether by partitioning, with salt added to aqueous layer after the first partitioning to aid transfer. Concentration step is repeated in the presence of petroleum ether to remove all traces of methylene chloride, then repeated again to produce final extract in acetone solution.

Apparatus

blender, high speed; explosion-proof Waring Blendor, 1 qt jar

Büchner funnel (Büchner), porcelain, 12 cm diameter

filter paper, Shark Skin®, to fit Büchner

long-stemmed funnel, glass, 4" diameter

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated receiving flask

separatory funnel (separator), 1 L

Reagents

acetone, distilled from all-glass apparatus

boiling chips, 20-30 mesh carborundum

glass wool, Pyrex, see Section 204 for handling directions

methylene chloride, distilled from all-glass apparatus

petroleum ether, distilled from all-glass apparatus

sodium chloride, reagent grade

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

Directions

- Prewash filter paper with acetone to remove contaminants.
- Weigh 100 g chopped or blended sample into blender jar, add 200 mL acetone, and blend 2 min at high speed.
- Filter with suction through 12 cm Büchner fitted with Shark Skin® paper; collect extract in 500 mL suction flask. Filtration is normally complete in <1 min. Continuation of vacuum for excessive period can reduce volume of extract and cause error in calculation.
- Place 80 mL sample extract in 1 L separator, and add 100 mL petroleum ether and 100 mL methylene chloride. Shake vigorously 1 min.
- Transfer lower aqueous layer to second 1 L separator.

- Dry upper layer of first separator by passing through about 1.5" sodium sulfate supported on washed glass wool in 4" funnel, collecting in K-D. (If extract will be cleaned up directly with C3, charcoal/Celite column, collect in vacuum rotary evaporator flask.)
- To separator with aqueous phase, add 7 g sodium chloride and shake vigorously 30 sec until most of the sodium chloride is dissolved.
- Add 100 mL methylene chloride, shake 1 min, and dry lower organic phase through same sodium sulfate.
- Extract aqueous phase with additional 100 mL methylene chloride and dry as above. Rinse sodium sulfate with about 50 mL methylene chloride.

  (If extract will be cleaned up directly with C3, proceed to concentration step described there instead of evaporating in K-D as follows.)
- Add boiling chips to K-D and concentrate solvent in K-D; start evaporation slowly by placing only receiver tube into steam. After 100-150 mL has evaporated, concentrator may be exposed to more steam. When liquid level in hot concentrator tube is about 2 mL, add 100 mL petroleum ether through Snyder column and reconcentrate to about 2 mL. Add 50 mL petroleum ether and repeat concentration step. Add 20 mL acetone, and reconcentrate to about 2 mL. Do not allow solution to go to dryness during any of the concentration steps. Adjust volume of extract to suitable definite volume with acetone.
- Calculate equivalent sample weight in final solution:

$$\frac{\text{mg sample equivalent}}{\mu\text{L final extract}} = 100 \times \frac{80}{200 + W - 10} \times \frac{1}{\text{mL final volume}}$$

where:

100 = g sample analyzed

80 = mL filtered extract taken for liquid-liquid partitioning

200 = mL acetone blended with 100 g sample

W = amount (mL) of water present in sample (Section 201; if data are not available for particular raw agricultural commodity, use 85%)

10 = adjustment for water/acetone volume contraction.

Thus, when sample contains 85% water (85 mL/100 g) and final extract volume is 7 mL, each µL contains:

$$100 \times \frac{80}{200 + 85 - 10} \times \frac{1}{7} = \frac{4.15 \text{ mg sample equivalent}}{\mu\text{L final extract}}$$

- Extract may be suitable, as is, for determination by GLC with selective detectors (*e.g.*, DG2, DG3). If co-extractives interfere with determination or adversely affect chromatography, clean up extract with C1, C2, or C5 prior to determination.
- Clean up extract with C1 or C5 prior to determination by electron capture (DG1, DG7, *etc.*) or flame ionization detectors (DG6). Clean up extract with C3 or C4 prior to determination by DL1 for N-methylcarbamates.

E2 EXTRACTION WITH ACETONE, REMOVAL OF WATER WITH 40 G HYDROMATRIX

References

Luke, M.A., *et al.* (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020-1026

Luke, M.A., *et al.* (1981) *J. Assoc. Off. Anal. Chem.* **64**, 1187-1195

Hopper, M.L. (1988) *J. Assoc. Off. Anal. Chem.* **71**, 731-734

Principles

Nonfatty sample is blended with acetone and filtered. Most nonionic residues are extracted from nonfatty foods into aqueous acetone solution. Water is removed from aqueous acetone solution by passing it through a column of specially treated diatomaceous earth (Hydromatrix). Residues are eluted from column with methylene chloride. Up to 13.3 mL water, from 40 mL aqueous acetone extractant, is adsorbed by the column, which is re-usable.

Apparatus

blender, high speed; explosion-proof Waring Blendor, 1 qt jar

Büchner funnel (Büchner), porcelain, 12 cm diameter

filter paper, Shark Skin®, to fit Büchner

chromatographic column, 25 mm id × 500 mm, Teflon stopcock

long-stemmed funnel, glass, 4" diameter

powder funnel, glass, 4" diameter

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated receiving flask

sieve, No. 30

Reagents

acetone, distilled from all-glass apparatus

buffer solution: 0.1 M (13.6 g/L) potassium phosphate monobasic (KH_2PO_4) in water

Hydromatrix material (pelletized diatomaceous earth), Part No. 0019-8003, Analytichem International, Harbor City, CA; also available through Varian

methylene chloride, distilled from all-glass apparatus

potassium phosphate monobasic, certified ACS grade

wire gauze, 40 mesh stainless steel

Directions

- Prepare Hydromatrix column:
  - Cut two pieces stainless steel gauze into circles of diameter slightly larger than chromatographic column id. Place one circle in bottom of column.
  - Place 50 g Hydromatrix material on No. 30 sieve and sieve thoroughly to remove fines.

- Pour 40 g sieved Hydromatrix material into column with aid of powder funnel. Tap end of column lightly on benchtop to settle material. Place second stainless steel gauze circle on top of material in column.
- With stopcock fully open, wash column with 150 mL buffer solution.
- After buffer solution has passed into column and flow has slowed to 3-5 mL/min, wash column with 300 mL acetone. Adjust flow to 50-60 mL/min after first 100 mL acetone has eluted.
- Wash column with 300 mL methylene chloride. Re-adjust flow to 50-60 mL/min after first 100 mL methylene chloride has eluted.

- Prewash filter paper with acetone to remove artifacts.
- Weigh 100 g chopped or blended sample into blender jar, add 200 mL acetone, and blend 2 min at high speed.
- Filter with suction through 12 cm Büchner fitted with Shark Skin® paper; collect extract in 500 mL suction flask. Filtration is normally complete in <1 min. Continuation of vacuum for excessive period can reduce volume of extract and cause error in calculation.
- Prewash Hydromatrix column with 200 mL acetone followed by 200 mL methylene chloride immediately before each use. Discard wash solvents.
- Place K-D under column. (If extract will be cleaned up directly with C3, charcoal/Celite column, collect in vacuum rotary evaporator flask.) Trans fer 40 mL filtered acetone extract to top of column. Let extract pass into column until flow rate has slowed to <1 mL/min. Let column equilibrate 3 min at <1 mL/min.
- Add 50 mL methylene chloride to column. After that has passed into column, add another 50 mL methylene chloride. After that has passed into column, add another 200 mL methylene chloride.
- Collect eluate until flow rate has decreased to slow drip (about 1 mL/min). Total elution time is 6-8 min.

  (If extract will be cleaned up directly with C3, proceed to concentration step described there instead of evaporating in K-D as follows.)
- Add boiling chips to K-D and concentrate solvent in K-D; start evaporation slowly by placing only receiver tube into steam. After 100-150 mL has evaporated, concentrator may be exposed to more steam. When liquid level in hot concentrator tube is about 2 mL, add 100 mL petroleum ether through Snyder column and reconcentrate to about 2 mL. Add 50 mL petroleum ether and repeat concentration step. Add 20 mL acetone, and reconcentrate to about 2 mL. Do not allow solution to go to dryness during any of the concentration steps. Adjust volume of extract to suitable definite volume with acetone.
- If extract will be cleaned up directly with C1, Florisil column, it is not necessary to reconcentrate repeatedly (as above) to remove all traces of methylene chloride. Instead, add boiling chips and concentrate solvent in K-D to <5 mL. Without allowing K-D to cool, add 50 mL acetone through Snyder column, and reconcentrate to suitable definite volume; allow to cool.

- Calculate equivalent sample weight in final solution:

$$\frac{\text{mg sample equivalent}}{\mu\text{L final extract}} = 100 \times \frac{40}{200 + W - 10} \times \frac{1}{\text{mL final volume}}$$

where:

100 = g sample analyzed

40 = mL filtered extract taken for Hydromatrix partitioning

200 = mL acetone blended with 100 g sample

W = amount (mL) of water present in sample (Section 201; if data are not available for particular raw agricultural commodity, use 85%)

10 = adjustment for water/acetone volume contraction.

Thus, when sample contains 85% water (85 mL/100 g) and final extract volume is 5 mL, each µL contains:

$$100 \times \frac{40}{200 + 85 - 10} \times \frac{1}{5} = \frac{2.9 \text{ mg sample equivalent}}{\mu\text{L final extract}}$$

- Extract may be suitable, as is, for determination by GLC with selective detectors (*e.g.*, DG2, DG3). If co-extractives interfere with determination or adversely affect chromatography, clean up extract with C1, C2, or C5 prior to determination.
- Clean up extract with C1 or C5 prior to determination by electron capture (DG1, DG7, *etc.*) or flame ionization detectors (DG6). Clean up extract with C3 or C4 prior to determination by DL1 for N-methyl-carbamates.
- Re-use Hydromatrix column without further rinsing, unless any adsorbed color elutes from column (after about 20 uses). When this occurs, restore column as follows:
  - Do not change stopcock setting. Flow rate will change due to different solvent densities, but this is of no consequence.
  - Wash column with 200 mL acetone, followed by sufficient volume (200-300 mL) buffer solution to remove any color left on column. Once color has been removed, elute with 300 mL acetone followed by 200 mL methylene chloride. Column is now ready for re-use.

ALTERNATIVE:

E3 EXTRACTION WITH ACETONE, REMOVAL OF WATER WITH 25 G HYDROMATRIX

Reference

Palmer, R.E., and Hopper, M.L. (Nov. 1991) "Miniaturized Solid Phase Partition Column for Determination of Organochlorine and Organophosphate Pesticides with PAM I 232.4 (Luke procedure) Acetone Filtrate," LIB 3613, FDA, Rockville, MD

Principles

Smaller size column of Hydromatrix reduces solvent use by 40% over E2, while still removing water from same amount of extract. However, solution eluting from 25 g Hydromatrix column may be cloudy, probably from a small amount of water; this disappears during concentration. The 25 g column may also have a shorter lifetime than the 40 g column. Results using the 25 g column may be somewhat less reliable for certain chemicals; *e.g.*, p,p'-dicofol and dicloran are recovered less reproducibly, and >0.4 ppm methamidophos may be only partially recovered; elution with 300 mL methylene chloride permits complete recovery of the latter.

Directions

- Follow directions of E2, except:
  - Prepare Hydromatrix column from 25 g material instead of 40 g.
  - Prewash Hydromatrix column with 100 mL acetone followed by 100 mL methylene chloride immediately before each use.
  - After transferring 40 mL filtered acetone extract to top of column, elute with 25, 25, and 150 mL methylene chloride, instead of volumes used in E2.
  - Because amount of original sample and amount of filtered acetone extract transferred to Hydromatrix column are the same as in E2, mg sample equivalent is the same as E2.

E4 EXTRACTION WITH WATER/ACETONE, LIQUID-LIQUID PARTITIONING WITH PETROLEUM ETHER/METHYLENE CHLORIDE

Reference

Luke, M.A., and Doose, G.M. (1983) *Bull. Environ. Contam. Toxicol.* **30**, 110-116

Principles

Low moisture nonfatty sample is blended with 35% water/acetone and filtered; the presence of water in the extractant facilitates extraction of residues from the dry product and dilutes co-extractives. Most nonionic residues are extracted into aqueous acetone solution. Residues are transferred from aqueous acetone to organic solvent methylene chloride/petroleum ether by partitioning, with salt added to the aqueous layer after the first partitioning to aid transfer.

Apparatus

blender, high speed; explosion-proof Waring Blendor, 1 qt jar

Büchner funnel (Büchner), porcelain, 12 cm diameter

filter paper, Shark Skin®, to fit Büchner

long-stemmed funnel, glass, 4" diameter

grinder, suitable for reducing dry products to <20 mesh

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated receiving flask

separatory funnel (separator), 1 L

Reagents

acetone, distilled from all-glass apparatus

boiling chips, 20-30 mesh carborundum (optional)

glass wool, Pyrex; see Section 204 for handling directions

methylene chloride, distilled from all-glass apparatus

petroleum ether, distilled from all-glass apparatus

sodium chloride, reagent grade

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

35% (v/v) water/acetone

Directions

- Prewash filter paper with acetone to remove artifacts.
- Grind sample containing <10% fat or oil to <20 mesh.
- Weigh 15 g ground sample into blender jar, add 350 mL 35% water/acetone, and blend 2 min at high speed.
- Filter with suction through 12 cm Büchner fitted with Shark Skin® paper; collect extract in 500 mL suction flask. Filtration is normally complete in <1 min. Continuation of vacuum for excessive period can reduce volume of extract and cause error in calculation.
- Place 80 mL sample extract in 1 L separator containing 100 mL methylene chloride. Add 100 mL petroleum ether and shake vigorously 1 min.

- Transfer lower aqueous layer to second 1 L separator.
- Dry upper organic layer of first separator by passing through about 1.5" sodium sulfate supported on washed glass wool in 4" funnel, collecting in K-D. (If extract will be cleaned up directly with C3, charcoal/Celite column, collect in vacuum rotary evaporator flask.)
- To separator with aqueous phase, add 7 g sodium chloride and shake vigorously 30 sec until most of the sodium chloride is dissolved.
- Add 100 mL methylene chloride, shake 1 min, and dry lower organic phase through same sodium sulfate.
- Extract aqueous phase with additional 100 mL methylene chloride and dry as above. Rinse sodium sulfate with about 50 mL methylene chloride.

  (If extract will be cleaned up directly with C3, proceed to concentration step described there instead of evaporating in K-D as follows.)
- Add boiling chips to K-D and concentrate solvent in K-D; start evaporation slowly by placing only receiver tube into steam. After 100-150 mL has evaporated, concentrator may be exposed to more steam. When liquid level in hot concentrator tube is about 2 mL, add 100 mL petroleum ether through Snyder column and reconcentrate to about 2 mL. Add 50 mL petroleum ether and repeat concentration step. Add 20 mL acetone, and reconcentrate to about 2 mL. Do not allow solution to go to dryness during any of the concentration steps. Adjust volume of extract to suitable definite volume with acetone.
- Calculate equivalent sample weight in final solution:

$$\frac{\text{mg sample equivalent}}{\mu\text{L final extract}} = 15 \times \frac{80}{350} \times \frac{1}{\text{mL final volume}}$$

  where:

  15 = g sample analyzed

  80 = mL filtered extract taken for liquid-liquid partitioning

  Thus, when final extract volume is 2 mL, each µL contains:

$$15 \times \frac{80}{350} \times \frac{1}{2} = \frac{1.7 \text{ mg sample equivalent}}{\mu\text{L final extract}}$$

- Extract may be suitable, as is, for determination by GLC with selective detectors (*e.g.*, DG2, DG3). If co-extractives interfere with determination or adversely affect chromatography, clean up extract with C1, C2, or C5 prior to determination.
- Clean up extract with C1 or C5 prior to determination by electron capture (DG1, DG7, *etc.*) or flame ionization detectors (DG6). Clean up extract with C3 or C4 prior to determination by DL1 for N-methylcarbamates.

ALTERNATIVE: ◀

E5 EXTRACTION WITH ACETONE, LIQUID-LIQUID PARTITIONING WITH ACETONE/METHYLENE CHLORIDE

Reference

Luke, M. A., and Doose, G. M. (1983) *Bull. Environ. Contam. Toxicol.* **30**, 110-116

Principle

Polar pesticides such as methamidophos exhibit variable recoveries when petroleum ether/dichloromethane is used in partitioning. Better recoveries are obtained when acetone is substituted for petroleum ether. Transfer of polar pesticides from the aqueous phase to the organic layer is further facilitated by adding sodium chloride before, rather than after, the first partitioning step.

Directions

- Follow directions of E1 through blending and filtering. Then:
  - Place 80 mL sample extract in 1 L separator, and add 100 mL acetone, 100 mL methylene chloride, and 7 g sodium chloride. Shake vigorously 1 min.
  - Transfer lower aqueous layer to second 1 L separator.
  - Dry upper organic layer of first separator by passing through about 1.5" sodium sulfate supported on washed glass wool in 4" funnel, collecting in K-D. (If extract will be cleaned up directly with C3, charcoal/ silanized Celite column, collect in vacuum rotary evaporator flask.)
  - Add 100 mL methylene chloride, shake 1 min, and dry lower organic phase through same sodium sulfate.
- Continue as in E1, "Extract aqueous phase with additional 100 mL methylene chloride..."

ALTERNATIVE: ◀

E6 EXTRACTION WITH WATER/ACETONE, LIQUID-LIQUID PARTITIONING WITH ACETONE/METHYLENE CHLORIDE

Reference

Luke, M. A., and Doose, G. M. (1983) *Bull. Environ. Contam. Toxicol.* **30**, 110-116

Principle

Polar pesticides such as methamidophos exhibit variable recoveries when petroleum ether/methylene chloride is used in partitioning. Better recoveries are obtained when acetone is substituted for petroleum ether. Transfer of polar pesticides from the aqueous phase to the organic layer is further facilitated by adding sodium chloride before, rather than after, the first partitioning step.

Directions

- Follow directions of E4 through blending and filtering. Then:
  - Place 80 mL sample extract in 1 L separator containing 100 mL methylene chloride. Add 100 mL acetone and 7 g sodium chloride and shake vigorously 1 min.
  - Transfer lower aqueous layer to second 1 L separator.
  - Dry upper organic layer of first separator by passing through about 1.5" sodium sulfate supported on washed glass wool in 4" funnel, collecting in K-D. (If extract will be cleaned up directly with C3, charcoal/silanized Celite column, collect in vacuum rotary evaporator flask.)
  - Add 100 mL methylene chloride, shake 1 min, and dry lower organic phase through same sodium sulfate.
- Continue as in E4, "Extract aqueous phase with additional 100 mL methyl ene chloride..."

E7 EXTRACTION WITH ACETONE AND SOLID PHASE EXTRACTION CARTRIDGES, LIQUID-LIQUID PARTITIONING

Reference

Luke, M. A., *et al.* (Sept. 1994) "An Improved Variation of the Luke Multiresidue Pesticide Procedure for the Analysis of Fruits and Vegetables Using Solid Phase Extraction Cartridges and Element Selective Gas Chromatographic Detectors," LIB 3896, FDA, Rockville, MD

Apparatus

blender, high speed; explosion-proof Waring Blendor, 1 qt jar

Büchner funnel (Büchner), porcelain, 12 cm diameter

filter paper, Shark Skin®, to fit Büchner

500 mL suction flask

long-stemmed funnel, glass, 4" diameter

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated receiving flask

separatory funnel (separator), 1 L

75 mL Bond Elut reservoir or equivalent

25 mm syringe filter, 0.45 µm Nylon 66, with 1 µm prefilter

tC-18 Solid Phase Extraction (SPE) cartridge, 500 mg

Reagents

acetone, distilled from all-glass apparatus

boiling chips, 20-30 mesh carborundum

eluant, water/acetone, 30% (v/v)

glass wool, Pyrex, see Section 204 for handling directions

methylene chloride, distilled from all-glass apparatus

petroleum ether, distilled from all-glass apparatus

sodium chloride, reagent grade

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

Directions

- Prewash filter paper with acetone to remove contaminants.
- Weigh 100 g chopped or blended sample into blender jar, add 200 mL acetone, and blend 2 min at high speed.
- Filter with suction through 12 cm Büchner fitted with Shark Skin® paper; collect extract in 500 mL suction flask. Continuation of vacuum for excessive period can reduce volume of extract and cause error in calculation.
- Attach 0.45 µm Nylon cartridge filter to bottom of 75 mL reservoir; attach tC-18 SPE cartridge to outlet of cartridge filter.
- Wash system with 40 mL acetone, followed by 10 mL eluant. Discard washes.

- Measure 40 mL sample extract and place into reservoir. Elute extract at 3 to 5 mL/min, with air pressure, into 1 L separatory funnel; do not allow level of extract to go below bottom of reservoir.
- Rinse graduated cylinder used for transfer with 10 mL 30% water/acetone; place rinse into reservoir and elute to column dryness.
- Add 50 mL acetone and 100 mL methylene chloride to separatory funnel and shake vigorously 1 min. Let separator stand 5-10 min to allow layers to separate.
- Dry lower organic layer of first separator by passing through about 1.5" sodium sulfate supported on washed glass wool in 4" funnel, collecting in K-D.
- Add 100 mL acetone and 100 mL methylene chloride to separator and repeat shaking. Let separator stand 5-10 min.
- Drain lower organic layer through sodium sulfate into separator. (Sugar content of fruit samples may result in aqueous phase's being the lower layer. In that case, add 5-10 mL methylene chloride and repeat shaking.) Rinse sodium sulfate with about 50 mL methylene chloride.
- Add boiling chips to K-D and concentrate solvent in K-D; start evaporation slowly by placing only receiver tube into steam. After 100-150 mL has evaporated, concentrator may be exposed to more steam. Concentrate solvent to 2-3 mL. After cooling, remove tube from K-D and adjust volume to 5 mL with acetone.
- Calculate equivalent sample weight in final solution:

$$\frac{\text{mg sample equivalent}}{\mu\text{L final extract}} = 100 \times \frac{40}{200 + W - 10} \times \frac{1}{\text{mL final volume}}$$

where:

100 = g sample analyzed

40 = mL filtered extract taken for liquid-liquid partitioning

200 = mL acetone blended with 100 g sample

W = amount (mL) of water present in sample (Section 201; if data are not available for particular raw agricultural commodity, use 85%)

10 = adjustment for water/acetone volume contraction.

Thus, when sample contains 85% water (85 mL/100 g) and final extract volume is 5 mL, each uL contains:

$$100 \times \frac{40}{200 + 85 - 10} \times \frac{1}{5} = \frac{2.9 \text{ mg sample equivalent}}{\mu\text{L final extract}}$$

- Clean up extract with C6 prior to determination.

C1 FLORISIL COLUMN (4 G) CLEANUP, WITH ONE METHYLENE CHLORIDE ELUANT

References

Griffitt, K.R., *et al.* (July 1983) "Miniaturized Florisil Column Cleanup of Chlorinated and Organophosphate Eluates in Total Diet Samples," LIB 2722, FDA, Rockville, MD

Griffitt, K.R., and Szorik, M.M. (Sept. 1989) "The Analysis of 127 Total Diet Items for Chlorinated Residues Using Luke/Solid Phase Extracts," LIB 3366, FDA, Rockville, MD

Principle

Residues in solution are separated from sample co-extractives on a small column of Florisil adsorbent, eluting with a single eluant.

Apparatus

chromatographic column, 10 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

Kuderna-Danish concentrator (K-D), 125 or 250 mL, with Snyder column, two-ball micro-Snyder column, graduated or volumetric receiving flask

Reagents

acetonitrile, distilled from all-glass apparatus; see Section 204 for distillation directions

Florisil, PR grade; see Section 204 for handling and testing directions and calculation of lauric acid value

hexane, distilled from all-glass apparatus

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

eluant: 50% methylene chloride/1.5% acetonitrile/48.5% hexane (v/v/v). Pipet 15 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

Directions

- Place activated Florisil (weight = 110/lauric acid value × 4 g) in 10 mm chromatographic column; add about 2 cm sodium sulfate. Completely open stopcock and tap column to settle adsorbent. Prewet column with 15 mL hexane. Do not allow column to go dry. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Dilute extract with hexane to produce solution of 10% acetone/hexane. Volumes depend on concentration of extract, volume taken for cleanup; *e.g.*, dilute 1 mL E1 extract, previously concentrated to 7 mL acetone, to 10 mL with hexane.
- Transfer solution to Florisil column, letting it pass through at about 5 mL/min. Rinse container with two 3 mL portions hexane, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions hexane.
- Elute column at about 5 mL/min with 50 mL eluant.

- Add boiling chip to K-D and concentrate eluate to suitable definite volume. For example, if 1 mL E1 extract (equivalent to 4.15 mg/mL) was cleaned up, concentrate Florisil eluate to 1 mL for same final concentration.

  When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during evaporation.

- Use appropriate determinative steps, such as DG1 or DG13, DG6, DG7, and DG10, to identify and measure residues.

C2 CHARCOAL/CELITE/MAGNESIUM OXIDE COLUMN CLEANUP

References

Luke, M.A., and Doose, G.M. (1983) *Bull. Environ. Contam. Toxicol.* **30**, 110-116

Hardy, R.P. (Fall 1984) "Recoveries of Organophosphorus Compounds Through the Modified Storherr Method Using Charcoal Columns With and Without Magnesium Oxide," LIB 2860, FDA, Rockville, MD

Principles

Polar residues in solution are separated from sample co-extractives on a column of charcoal/Celite/magnesium oxide; cleanup may be necessary for subsequent examination of extract with selective detectors. Aromatic residues are not eluted with this system and must be determined in extract cleaned up by C1, Florisil column. Magnesium oxide may be eliminated to prevent destruction of sensitive residues (*e.g.*, acephate) without diminishing recoveries of other residues normally eluted.

Apparatus

chromatographic column, 22 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated or volumetric receiving flask

Reagents

acetone, distilled from all-glass apparatus

adsorbent mixture, 1:4:2 (w/w/w) charcoal/Celite 545/magnesium oxide or 1:4 (w/w) charcoal/Celite 545

Celite 545. To prepare, slurry about 500 g with distilled water, heat on steam bath about 30 min, and filter with suction. Dry overnight at l05-l30° C and pulverize to pass No. 60 sieve. Store in closed jar.

charcoal, Darco G60 or Norite S.G. Extra

glass wool, Pyrex; see Section 204 for handling directions

magnesium oxide, 200 mesh, adsorptive grade (optional)

methylene chloride, distilled from all-glass apparatus

eluant: 2:1 (v/v) acetone/methylene chloride

Directions

- Place about 1" Celite 545 in column, then add 6 g adsorbent mixture, and top with large plug glass wool.
- Tamp column down firmly and add about 25 mL methylene chloride. Force solvent through column with air pressure until top of solvent reaches top of column. Discard solvent.
- Transfer sample extract quantitatively to column with small portions methylene chloride and force solvent through as before, collecting in K-D.
- Elute with 200 mL 2:1 acetone/methylene chloride; force through as before.

- Mix contents of K-D, add boiling chips, and concentrate solvent; start evaporation slowly by placing only receiver tube into steam. After 100-150 mL has evaporated, concentrator may be exposed to more steam. When liquid level in hot concentrator tube is about 2 mL, add 100 mL petroleum ether through Snyder column and reconcentrate to about 2 mL. Add 50 mL petroleum ether and repeat concentration step. Add 20 mL acetone, and reconcentrate to about 2 mL. Do not allow solution to go to dryness during any of the concentration steps. Adjust volume of extract to suitable definite volume with acetone.
- If magnesium oxide is not used, a white precipitate may form if extract is concentrated to <2 mL; this should not affect GLC.
- Use appropriate determinative steps or confirmatory steps, such as GLC with mass spectrometric detection.

C3 CHARCOAL/SILANIZED CELITE COLUMN CLEANUP

References

Krause, R.T. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 1114-1124

Pardue, J.R. (May 1987) "Recoveries of N-Methyl Carbamates Using a Combination of the Luke (PAM I, 232.4) and Krause (PAM I, 242.24b, 242.25) Procedures," LIB 3138, FDA, Rockville, MD

Principle

Residues in solution are separated from sample co-extractives on a column of charcoal and Celite, cleaning up the extract sufficiently for subsequent determination by HPLC system DL1.

Apparatus

chromatographic column, 22 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

evaporator, vacuum rotary, as described in Section 401 E1

flasks, round-bottom (r-b), 250 and 500 mL, 1 L

magnetic stirrer, star, 10 mm diameter × 8 mm

vacuum adapter, side arm, with Ts bottom joint to fit in 500 mL r-b flask

Reagents

acetonitrile, distilled from all-glass apparatus; see Section 204 for distillation directions

Celite 545, silanized and prepared for use as directed in Section 401 C1

charcoal (Nuchar S-N), produced by Westvaco Corp. and available from Eastman Kodak, Cat. No. 118 0454, purified as directed in Section 401 C1

glass wool, Pyrex; see Section 204 for handling directions

methanol, distilled from all-glass apparatus

methylene chloride, distilled from all-glass apparatus

toluene, distilled from all-glass apparatus

eluant: 25% (v/v) toluene/acetonitrile

Directions

- Test charcoal/silanized Celite column as described in Section 401 C1.
- To the extract in r-b flask, add star magnetic stirrer. Place 250 mL T 24/40 trap on 1 L r-b flask and attach to vacuum rotary evaporator.
- Circulate refrigerated (-15° C) 1+1 water/ethylene glycol through evaporator condensing coils; maintain receiving flask at -15° C by immersion in refrigerated bath.
- Apply vacuum slowly to minimize frothing by regulating with needle valve. After full vacuum is applied, slowly place flask in 35° C water bath.
- Remove r-b flask from evaporator immediately after last traces of solution have evaporated and add 10 mL methylene chloride to r-b flask.

- Fit one-hole No. 5 rubber stopper onto tip of chromatographic column, add side arm vacuum adapter and 500 mL r-b flask, open stopcock, and connect apparatus to vacuum line.
- Place 0.5 g silanized Celite 545 in chromatographic column, tamp, add 5 g charcoal/Celite 545 (1+4) mixture, and tamp again. Add 1-2 cm glass wool plug on top of adsorbent.
- Prewash column with 50 mL 25% toluene/acetonitrile eluant. Close stopcock when prewash solution is about 0.5 cm from top of glass wool.
- Disconnect vacuum, discard solution in r-b flask, and reconnect flask to apparatus.
- Transfer 10 mL methylene chloride extract to column and let pass through column at 5 mL/min.
- Wash 1 L r-b flask with 10 mL methylene chloride and then with 25 mL eluant. Transfer each separately to column and elute each to top of glass wool before adding next solution.
- Add 100 mL eluant and elute column at 5 mL/min. Turn off stopcock when top of eluant reaches top of glass wool.
- Evaporate solution in 500 mL r-b flask just to dryness using vacuum evaporator as above. Remove flask from evaporator immediately after all solution has evaporated.
- Immediately pipet 5 mL methanol into 500 mL r-b flask to dissolve residue. Cleaned up extract contains concentration of sample equivalent (mg/µL) equal to amount of sample in extract taken for cleanup, divided by 5. For example, if entire E1 extract of commodity with 85% water is used, 29 g sample equivalent is cleaned up, *i.e.*, $100\,g \times 80/(200+85-10)$; final concentration of cleaned up extract is 5.8 mg/µL (29 g/5 mL).
- Use determinative step DL1 or DL2 (Section 401) to determine N-methylcarbamates, except use 20 µL injection loop instead of 10 µL loop specified.

C4 C-18 CARTRIDGE CLEANUP

Reference

Sharp, K.B., and Bramlett, C.L. (Dec. 1983) "Analysis for Carbamate Residues in Fresh Produce," LIB 2778, FDA, Rockville, MD

Principle

Residues in solution are separated from sample co-extractives on a C-18 solid phase extraction cartridge, cleaning up the extract sufficiently for subsequent determination by HPLC system DL1.

Apparatus

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated receiving flask

volumetric flask, 5 mL

Reagents

cartridge (solid phase extraction type), C-18, 2.8 mL

methanol, distilled from all-glass apparatus

Directions

- Concentrate extract in K-D to 2 mL. Evaporate almost to dryness (about 0.1 mL) under current of nitrogen.
- Prewet C-18 cartridge with methanol and discard solvent.
- Dissolve residue in receiving flask with 2 mL methanol and transfer quantitatively onto prewet C-18 cartridge. Collect eluate from cartridge in 5 mL volumetric flask.
- Elute cartridge with additional methanol until collected volume is almost 5 mL; add methanol to make volume 5.0 mL. Cleaned up extract contains concentration of sample equivalent (mg/µL) equal to amount of sample in extract taken for cleanup, divided by 5. For example, if entire E1 extract of commodity with 85% water is used, 29 g sample equivalent is cleaned up, *i.e.*, 100 g × 80/(200 + 85 - 10); final concentration of cleaned up extract is 5.8 mg/µL (29 g/5 mL).
- Use determinative step DL1 or DL2 (Section 401) to determine N-methylcarbamates, except use 20 µL injection loop instead of 10 µL loop specified.

C5 FLORISIL COLUMN CLEANUP, WITH MIXED ETHER ELUANTS

Reference

Luke, M.A., *et al.* (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020-1026

Principles

Residues in solution are separated from sample co-extractives on a column of Florisil adsorbent; cleanup is usually necessary for subsequent examination of extract with DG1, electron capture detector.

Apparatus

chromatographic column, 22 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

graduated cylinder (graduate), glass-stoppered (g-s), 100 mL

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, volumetric or graduated receiving flask

Reagents

boiling chips, 20-30 mesh carborundum

eluants: 15% (v/v) ethyl ether/petroleum ether
50% (v/v) ethyl ether/petroleum ether

ethyl ether, distilled from all-glass apparatus, with 2% ethanol as preservative; see Section 204 for peroxide test

Florisil, PR grade; see Section 204 for handling and testing directions and calculation of lauric acid value

petroleum ether, distilled from all-glass apparatus

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

Directions

- Place activated Florisil (4" or weight determined by lauric acid value) in 22 mm id column; add about 0.5" sodium sulfate. Prewet column with 40-50 mL petroleum ether. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Dilute concentrated extract to 10 mL with acetone and transfer to 100 mL g-s graduate, using petroleum ether to rinse. Dilute to 100 mL with petroleum ether; stopper and mix well.
- Transfer diluted extract solution to column letting it pass through at about 5 mL/min.
- Elute column at about 5 mL/min with 200 mL 15% ethyl ether/petroleum ether eluant.
- Change K-Ds and elute at about 5 mL/min with 200 mL 50% ethyl ether/petroleum ether eluant.
- Add boiling chips to K-Ds and concentrate to suitable definite volume. For example, if entire E1 extract of commodity with 85% water is used, and final volume is 5 mL, final concentration of cleaned up extract is 5.8 mg/µL, *i.e.*, 100 g × 80/(200 + 85 - 10) = 29 g; 29 g/5 mL = 5.8 mg/µL.

- When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during final evaporation in receiving flask.
- Use appropriate determinative steps, such as DG1 or DG13, DG6, DG7, and DG10, to identify and measure residues.

C6 SAX/PSA CARTRIDGE CLEANUP

Reference

Luke, M. A., *et al.* (Sept. 1994) "An Improved Variation of the Luke Multiresidue Pesticide Procedure for the Analysis of Fruits and Vegetables Using Solid Phase Extraction Cartridges and Element Selective Gas Chromatographic Detectors," LIB 3896, FDA, Rockville, MD

Principle

SAX and PSA cartridges provide the improved cleanup required for determination with capillary and megabore GC columns; both polar and nonpolar residues can be recovered.

Apparatus

75 mL Bond Elut reservoir or equivalent

25 mm syringe filter, 0.45 µm Nylon 66 with 1 µm prefilter

SAX SPE cartridge or equivalent, 500 mg

PSA SPE cartridge or equivalent, 500 mg

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated or volumetric receiving flask

Reagents

acetone, distilled from all-glass apparatus

petroleum ether, distilled from all-glass apparatus

acetone+petroleum ether, 1+2

Directions

- Attach 0.45 µm filter to bottom of 75 mL reservoir. Attach SAX or equivalent cartridge to filter, and attach PSA or equivalent cartridge to first cartridge.
- Wash cartridges with 40 mL acetone; follow with 10 mL acetone+petroleum ether. Discard washes.
- Dilute the 5.0 mL concentrated acetone extract from E7 with 10 mL petroleum ether and mix. Transfer to reservoir, and elute dropwise with air pressure.
- Rinse tube with five 10 mL portions acetone+petroleum ether. Elute each rinse when the previous solvent has reached top of column.
- Mix contents of K-D, add boiling chips, and concentrate solvent; start evaporation slowly by placing only receiver tube into steam. When liquid level in hot concentrator tube is about 2 mL, add 100 mL petroleum ether through Snyder column and reconcentrate to about 2 mL. Add 50 mL petroleum ether and repeat concentration step. Carefully add 25 mL acetone and reconcentrate to about 2 mL. Do not allow solution to go to dryness during any of the concentration steps. Adjust volume of extract to suitable definite volume with acetone.
- Use appropriate determinative steps, such as DG2, DG3, DG14, or DG16, to identify and measure residues.

DETERMINATION

Inject concentrated extract equivalent to 20 mg (whole high moisture product) into the following GLC systems for determination of residues. (Although AOAC collaborative study for this method involved injection of 12 mg sample equivalent, experience since then has proven that GLC systems can tolerate routine injections equivalent to 20 mg of most nonfatty foods.)

Extract not cleaned up prior to determination:

DG2 or DG14	organophosphorus residues; large amounts of sulfur may interfere
DG3 or DG16	organohalogen residues
DG4 or	organonitrogen residues; selective to nitrogen, but co-extractives may contain nitrogen
DG5 or DG17	organonitrogen and organophosphorus residues
DG15	organosulfur residues; large amounts of phosphorus may interfere
DG12	late eluting organohalogen residues, especially pyrethroids

Additional recommended determinations:

Extract not cleaned up prior to determination:

DG8	early eluting organophosphorus residues
DG11	late eluting organophosphorus residues
DG9	early eluting organohalogen residues

Extract cleaned up on Florisil column, C1 or C5:

DG1 or DG13	residues with halogen, sulfur, or other moieties
DG7	early eluting residues with halogen, sulfur, or other moieties
DG10	late eluting residues, especially synthetic pyrethroids
DG6	o-phenylphenol and biphenyl

Inject concentrated extract equivalent to about 58-116 mg (whole high moisture product) cleaned up by C3 (charcoal/Celite column) or C4 (C-18 cartridge) into following HPLC system:

DL1	N-methylcarbamates (determinative step described in Section 401)

For accurate quantitation, reference standards should be dissolved in same solvent as concentrated extract, only peaks >10% FSD should be measured, and peak sizes of residue and reference standard should match within ±25%.

See Chapter 5 for additional information about operation of GLC systems; Section 504 provides information about quantitation of residues.

See Chapter 6 for additional information about operation of HPLC systems; Section 606 provides information about quantitation of residues.

See Section 205 for additional information about reference standards.

See Section 104 for additional information about reporting residues and determining compliance with regulations.

See Section 105 for additional information about analytical limits of quantitation.

CONFIRMATION

After residues have been tentatively identified and quantitated by comparison to appropriate reference standards, confirm identity according to principles discussed in Section 103. Use appropriate tables of data (PESTDATA, tables accompanying each method, Index to Methods) to choose the most appropriate determinative steps and/or alternative methods for confirmation.

DG1 GLC, 100% METHYL SILOXANE, 200° C, EC

Applicability

Determinative step is applicable to residues containing halogen, sulfur, or other electrophilic moieties. It is a general purpose system, but subject to interferences by nonpesticides.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of p,p'-DDT is 3.1 ± 0.06.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4.0 ± 0.5 min (about 20 mL/min).

Injector temperature: 220-250° C

Detector

Electron Capture (EC)

Detector Operating Conditions:

350° C

Make-up gas: nitrogen or argon/methane (95:5), at 30 mL/min

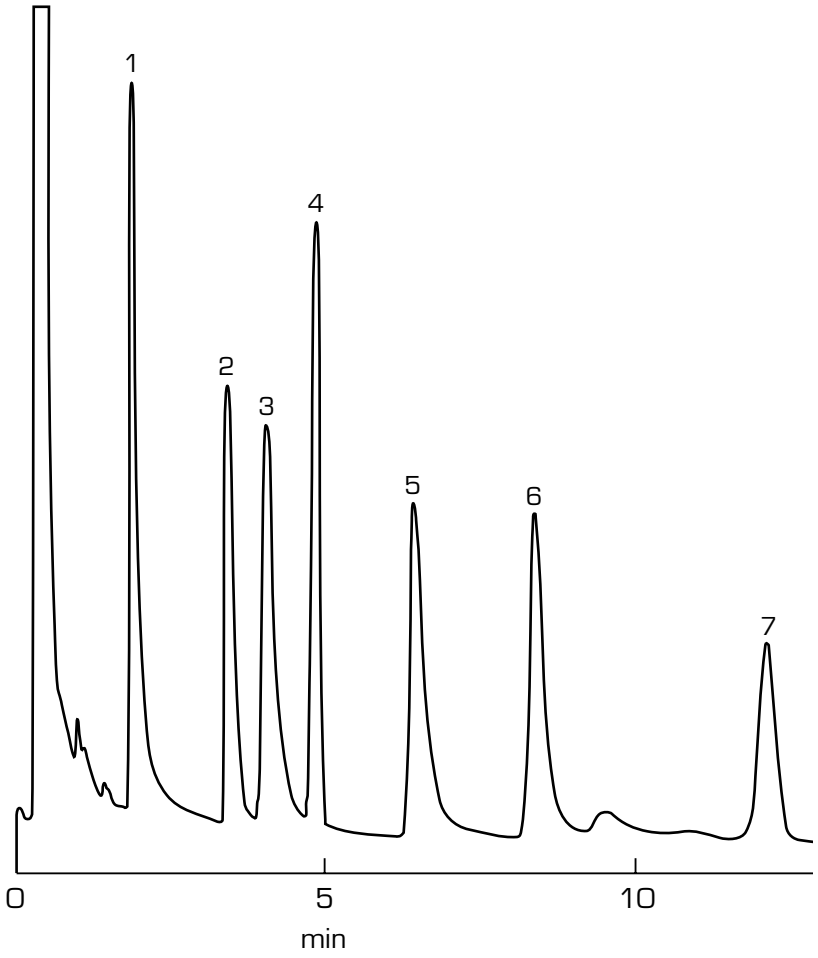
See Section 503 B for other information about EC detector operation.

Set detector electronics (amplification, attenuation) so that response to 0.15 ng chlorpyrifos (or an amount within the detector's linear range) is 50% full scale deflection (FSD).

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column). Response data in Appendix I are based on detector sensitivity of 50% FSD to 1.5 ng chlorpyrifos.

Example chromatogram is on next page. Also see Figures 504-c, d, e, and f.



DG1

Chromatogram of: 1) 0.15 ng dicloran, 2) 0.10 ng heptachlor, 3) 0.19 ng chlorpyrifos, 4) 0.31 ng captan, 5) 0.14 ng endosulfan I, 6) 0.18 ng endrin, and 7) 0.20 ng p,p′-DDT at the conditions described.

DG2 GLC, 100% METHYL SILOXANE, 200° C, FPD-P

Applicability

Determinative step is applicable to residues containing phosphorus. It is particularly useful for residues such as organophosphate pesticides.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of ethion is 2.56 ± 0.05.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4.0 ± 0.5 min (about 20 mL/min).

Injector temperature: 220-250° C

Detector

Flame photometric, phosphorus mode (FPD-P)

Detector Operating Conditions:

225-250° C

See Section 503 C for other information about FPD operation.

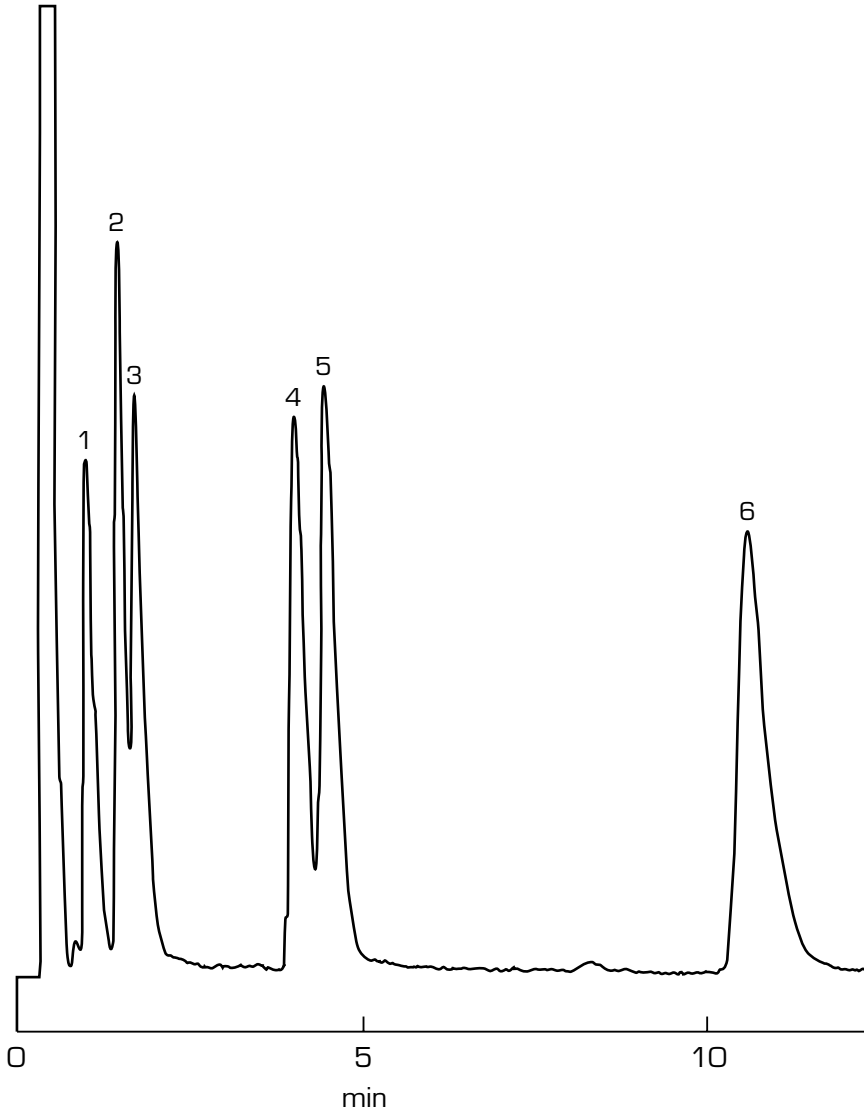
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

A properly functioning system will give a response to 1.5 ng omethoate of ≥50% FSD.

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG2

Chromatogram of: 1) 0.85 ng acephate, 2) 1.73 ng omethoate, 3) 0.68 ng monocrotophos, 4) 1.30 ng malathion, 5) 1.27 ng chlorpyrifos, and 6) 1.26 ng ethion at the conditions described; helium carrier gas flow was 15 mL/min, with 15 mL/min make-up gas being added before the detector. Detector gas flows: 100 mL/min hydrogen, 130 mL/min air.

DG3 GLC, 100% METHYL SILOXANE, 200° C, ELCD-X

Applicability

Determinative step is applicable to residues containing halogen. It is particularly useful for residues such as chlorinated hydrocarbon pesticides and polychlorinated biphenyls.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of p,p'-DDT is 3.1 ± 0.06.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4.0 ± 0.5 min (about 20 mL/min).

Injector temperature: 220-250° C

Detector

Electroconductivity, halogen mode (ELCD-X)

Detector Operating Conditions:

Base temperature 250° C; furnace temperature 900° C (or as specified in operating manual)

Reactor gas: hydrogen, flow as required by specific detector model; see operating manual

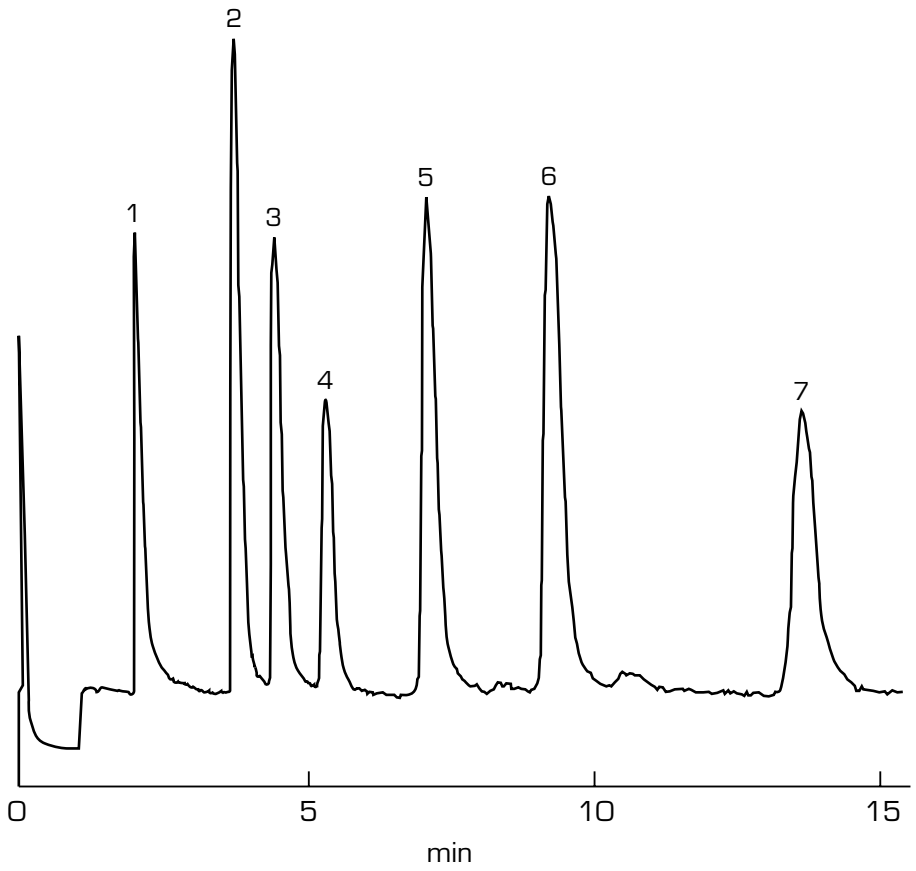
See Section 503 D for other information about ELCD operation.

Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG3

Chromatogram of: 1) 1.44 ng dicloran, 2) 0.98 ng heptachlor, 3) 1.87 ng chlorpyrifos, 4) 2.99 ng captan, 5) 1.37 ng endosulfan I, 6) 1.77 ng endrin, and 7) 1.91 ng p,p'-DDT at the conditions described. Hydrogen reactor gas flow: 40 mL/min, n-propanol electrolyte: 0.3 mL/min.

DG4 GLC, 100% METHYL SILOXANE, 200° C, ELCD-N

Applicability

Determinative step is applicable to residues containing nitrogen. It may be useful for confirmation of residues such as triazines (atrazine, simazine, *etc.*) and triazoles (propiconazole, diclobutrazole, *etc.*).

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C isothermal

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4.0 ± 0.5 min (about 20 mL/min).

Injector temperature: 220-250° C

Detector

Electroconductivity, nitrogen mode (ELCD-N)

Detector Operating Conditions:

Base temperature 250° C; furnace temperature 900° C (or as specified in operating manual)

Reactor gas: hydrogen, flow as required by specific detector model; see operating manual

See Section 503 D for other information about ELCD operation.

Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

No chromatogram currently available.

DG5 GLC, 100% METHYL SILOXANE, 200° C, N/P

Applicability

Determinative step is applicable to residues containing nitrogen. It is particularly useful for residues such as triazines and triazoles.

Column:

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of ethion is 2.56 ± 0.05.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4.0 ± 0.5 min (about 20 mL/min).

Injector temperature: 220-250° C

Detector

Alkali bead detector, nitrogen selective (N/P)

Detector Operating Conditions:

250° C

See Section 503 E for other information about N/P detector operation.

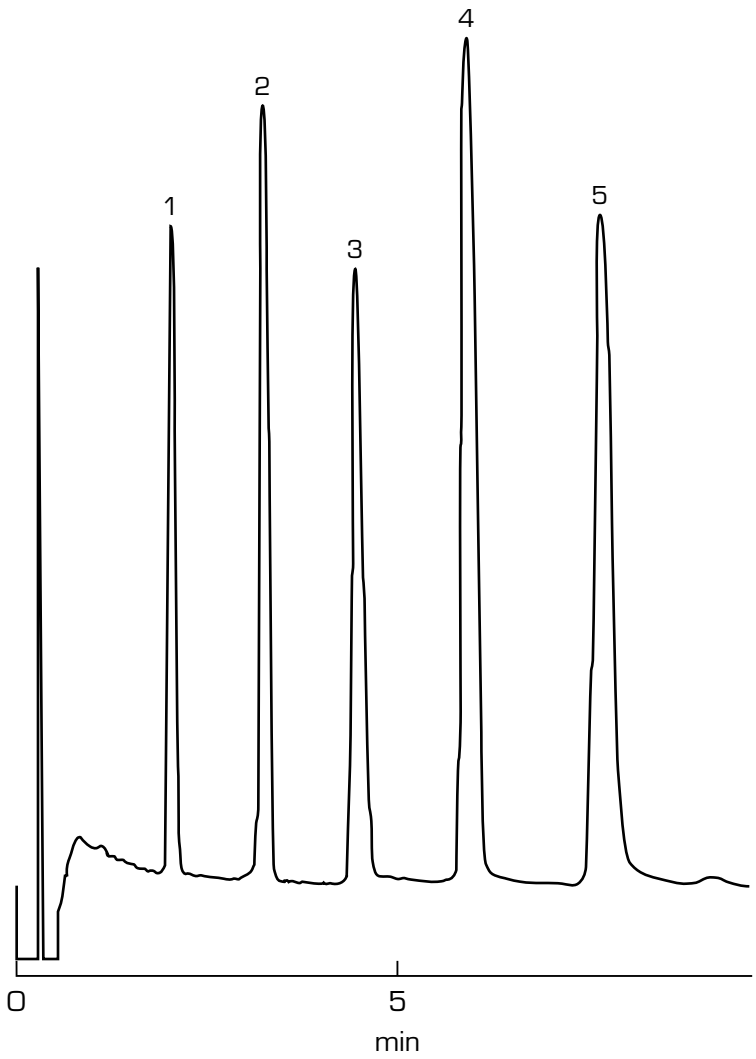
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

A properly functioning system will give a response to 1.5 ng omethoate of ≥50% FSD.

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG5

Chromatogram of: 1) 1.0 ng atrazine, 2) 7.5 ng carbaryl, 3) 1.5 ng chlorpyrifos, 4) 2.5 ng procyazine, and 5) 5.0 ng imazalil at the conditions described.

DG6 GLC, 100% METHYL SILOXANE, 130° C, FID

Applicability

Determinative step is applicable to residues containing no elements to which element-selective detectors respond. It is particularly useful for residues such as biphenyl and o-phenylphenol.

Column

Wide bore capillary, 30 m ¥ 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

130° C isothermal

Carrier gas: helium, about 20 mL/min. At these conditions, chlorpyrifos elutes in about 16 min, and biphenyl and o-phenylphenol elute in <2 min.

Injector temperature: 220-250° C

Detector

Flame ionization detector (FID)

Detector Operating Conditions:

300° C

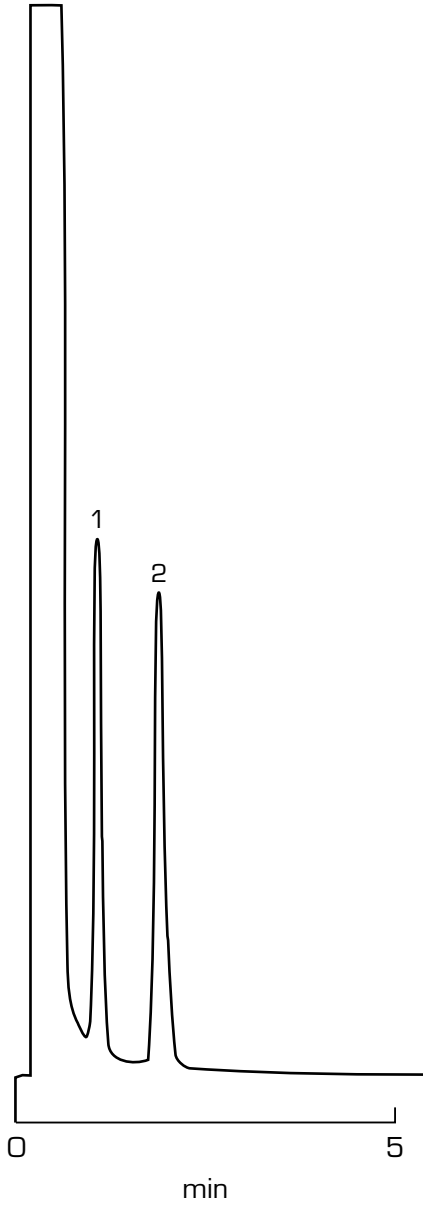
detector gases: hydrogen, 30 mL/min, air, 300 mL/min

set detector electronics (amplification, attenuation) so that response to 50 ng o-phenylphenol is 50% full scale deflection (FSD).

Other Considerations

FID is nonselective and will respond to large quantities of any co-extractive.

Example chromatogram is on next page.



DG6

Chromatogram of: 1) 20 ng biphenyl and 2) 53 ng o-phenylphenol at the conditions described.

DG7 GLC, 100% METHYL SILOXANE, 130° C, EC

Applicability

Determinative step is applicable to residues of high volatility (early elution) and containing halogen, sulfur, or other electrophilic moieties. It is particularly useful for residues such as benfluralin and sulfallate.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

130° C isothermal

Carrier gas: helium; adjust flow rate while column temperature is 200° C so that chlorpyrifos elutes in about 4.0 ± 0.5 min; then change column temperature without changing flow controller.

Injector temperature: 220-250° C

Detector

Electron Capture (EC)

Detector Operating Conditions:

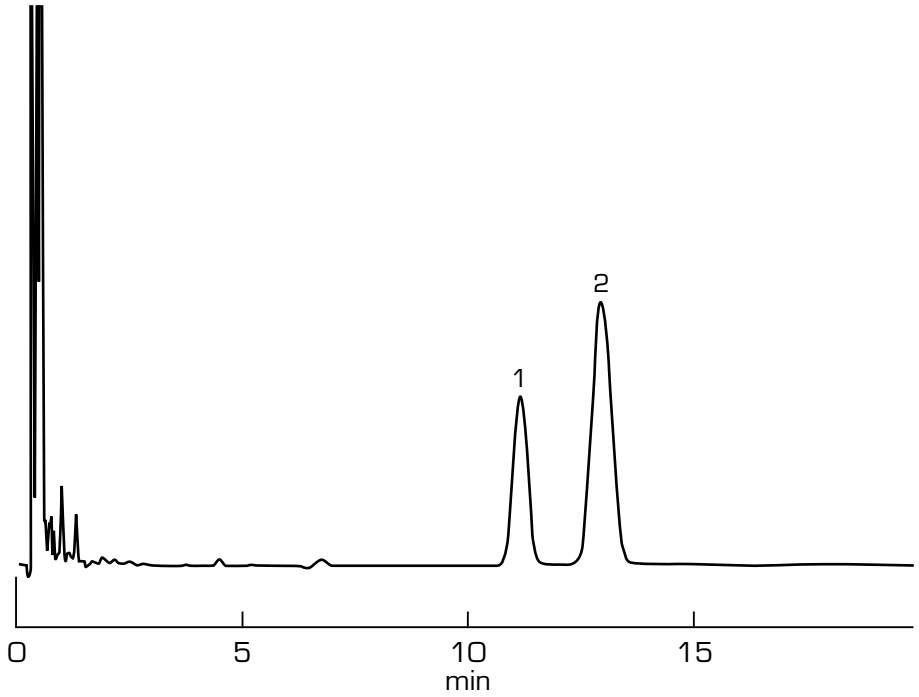
350° C

Make-up gas: nitrogen or argon/methane (95:5), at 30 mL/min

See Section 503 B for other information about EC detector operation.

While column temperature is 200° C, set detector electronics (amplification, attenuation) so that response to 0.15 ng chlorpyrifos (or an amount within the detector's linear range) is 50% full scale deflection; then change column temperature without changing electronics.

Example chromatogram is on next page.



DG7

Chromatogram of: 1) 0.18 ng benfluralin and 2) 0.09 ng sulfallate at the conditions described.

DG8 GLC, 100% METHYL SILOXANE, 130° C, FPD-P

Applicability

Determinative step is applicable to residues of high volatility (early elution) and containing phosphorus. It is particularly useful for residues such as mevinphos, acephate, demeton, and dicrotophos.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

130° C isothermal

Carrier gas: helium; adjust flow rate while column temperature is 200° C so that chlorpyrifos elutes in about 4.0 ± 0.5 min; then change column temperature without changing flow controller.

Injector temperature: 220-250° C

Detector

Flame photometric, phosphorus mode (FPD-P)

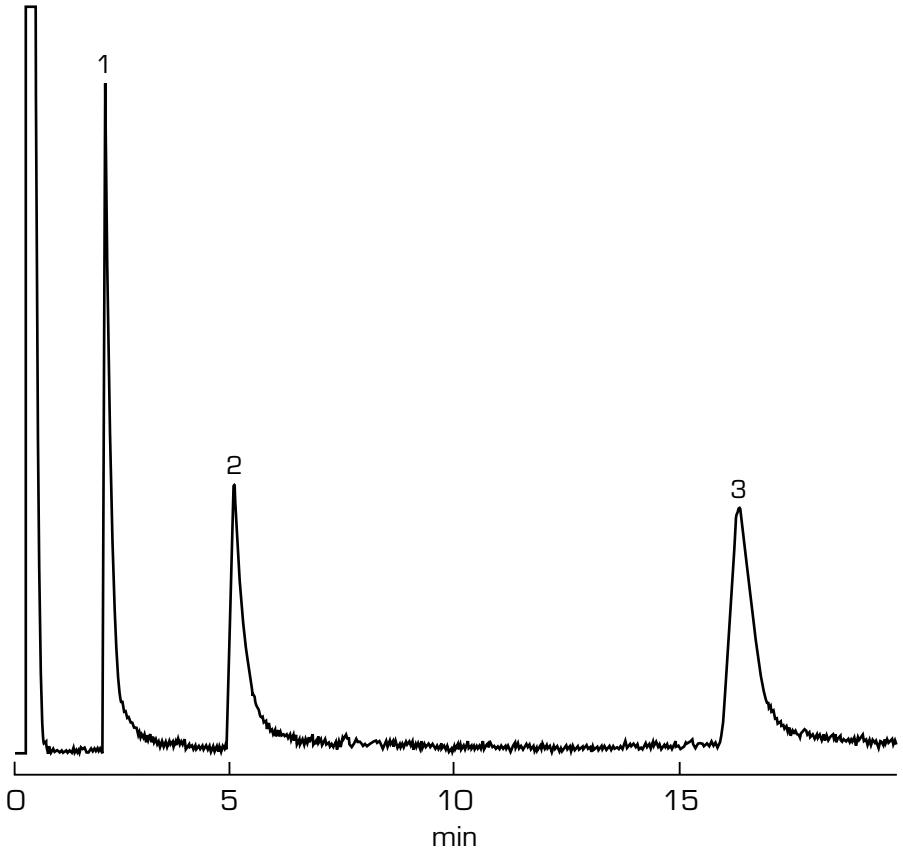
Detector Operating Conditions:

225-250° C

See Section 503 C for other information about FPD operation.

While column temperature is 200° C, set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection; then change column temperature without changing electronics.

Example chromatogram is on next page.



DG8

Chromatogram of: 1) 2.0 ng methamidophos, 2) 2.0 ng acephate, and 3) 4.0 ng dicrotophos at the conditions described.

DG9 GLC, 100% METHYL SILOXANE, 130° C, ELCD-X

Applicability

Determinative step is applicable to residues of high volatility (early elution) and containing halogen. It is particularly useful for residues such as the methyl esters of dicamba, MCPA, mecoprop, dichlorprop, and silvex.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

130° C isothermal

Carrier gas: helium, about 20 mL/min

Injector temperature: 220-250° C

Detector

Electroconductivity, halogen mode (ELCD-X)

Detector Operating Conditions:

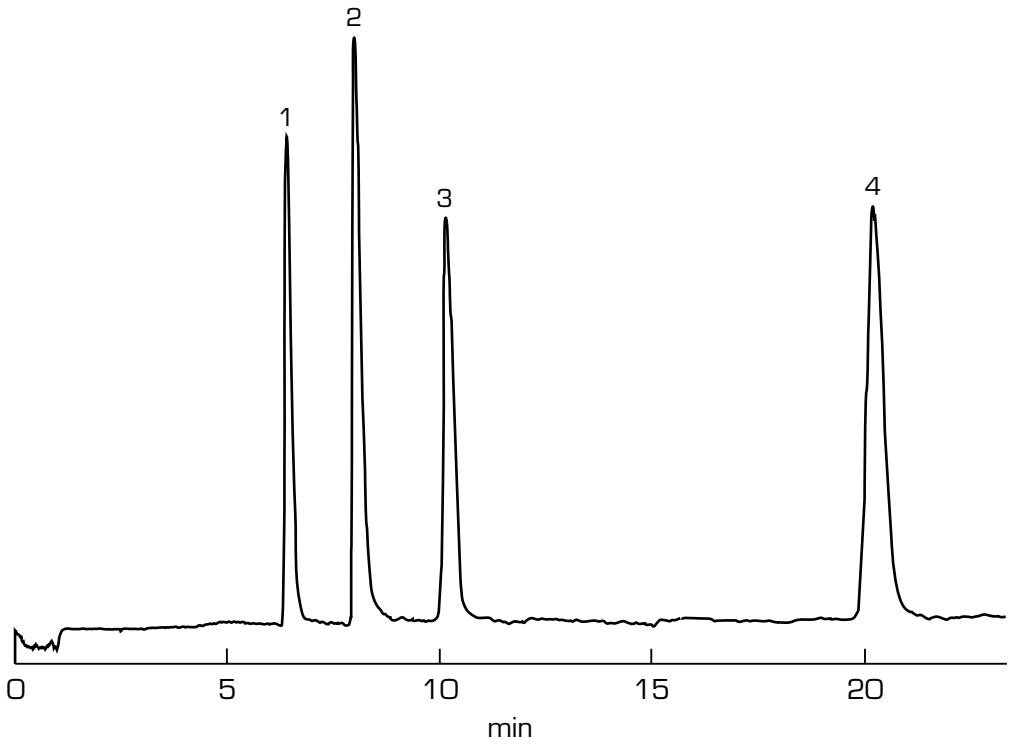
Base temperature 250° C; furnace temperature 900° C (or as specified in operating manual)

Reactor gas: hydrogen, flow as required by specific detector model; see operating manual

See Section 503 D for other information about ELCD operation.

Set detector electronics (amplification, attenuation) so that response to 0.5 ng pentachlorobenzene is 50% full scale deflection.

Example chromatogram is on next page.



DG9

Chromatogram of: 1) 1.0 ng dicamba methyl ester, 2) 3.0 ng MCPA methyl ester, 3) 1.5 ng dichlorprop methyl ester, and 4) 2.0 ng silvex methyl ester at the conditions described, except that carrier gas was hydrogen at 25 mL/min. Hydrogen reactor gas flow: 35 mL/min, n-propanol electrolyte 0.5 mL/min. Pentachlorobenzene eluted in 6.9 min at these conditions, and 0.3 ng pentachlorobenzene caused 40% FSD detector response.

DG10 GLC, 100% METHYL SILOXANE, 230° C, EC

Applicability

Determinative step is applicable to residues of low volatility (late elution) and containing halogen, sulfur, or other electrophilic moieties. It is particularly useful for residues such as pyrethroids, with halogen (permethrin, fenvalerate, deltamethrin) or without halogen (tetramethrin).

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

230° C isothermal; if necessary, adjust temperature so that relative retention time (rrt) to phosalone of cis permethrin is about 1.55.

Carrier gas: helium; adjust flow rate so that phosalone elutes in about 8 min (about 18 mL/min).

Injector temperature: 250° C

Detector

Electron Capture (EC)

Detector Operating Conditions:

350° C

Make-up gas: nitrogen or argon/methane (95:5), at 30 mL/min

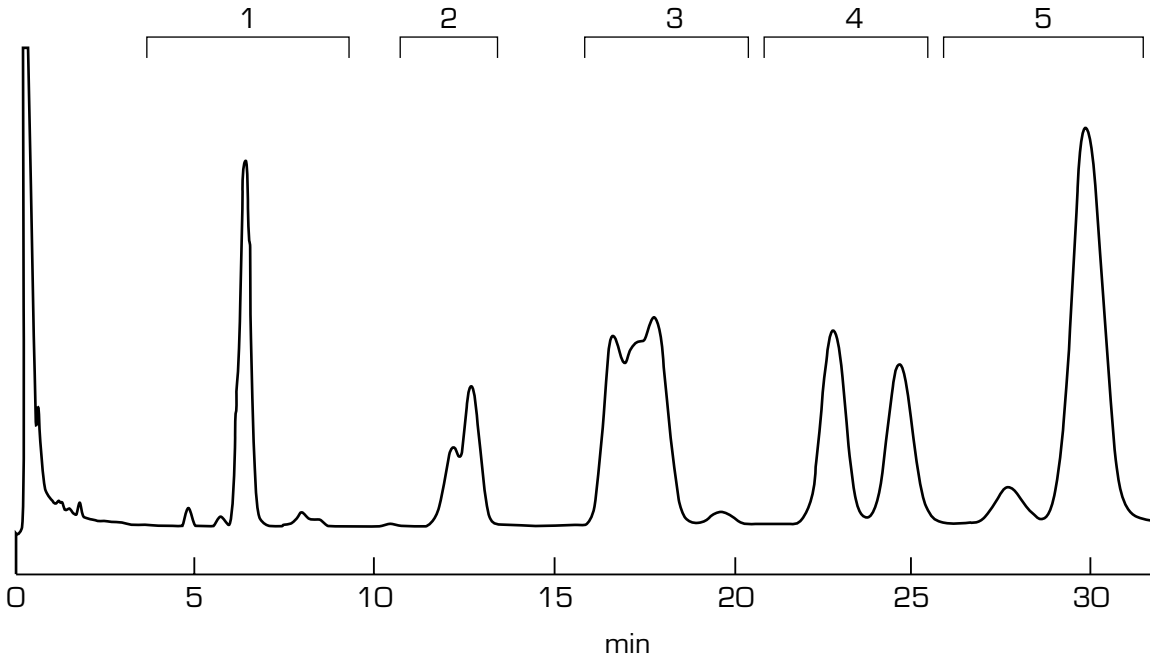
See Section 503 B for other information about EC detector operation.

Set detector electronics (amplification, attenuation) so that response to 0.5 ng phosalone is 50% full scale deflection (FSD).

Other Considerations

Detector sensitivity must be sufficient to measure residues of pyrethroids at ≤0.1 ppm, where some tolerances are set.

Example chromatogram is on next page.



DG10

Chromatogram of: 1) 3.5 ng tetramethrin, 2) 2.3 ng permethrin, 3) 2.1 ng cypermethrin, 4) 1.9 ng fenvalerate, and 5) 2.2 ng deltamethrin at the conditions described.

DG11 GLC, 100% METHYL SILOXANE, 230° C, FPD-P

Applicability

Determinative step is applicable to residues of low volatility (late elution) and containing phosphorus. It is particularly useful for residues such as some organophosphorus pesticides, their oxygen analog sulfones and sulfoxides, and aryl phosphate industrial chemicals.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

230° C isothermal; if necessary, adjust temperature so that relative retention time (rrt) to phosalone of coumaphos is about 1.56.

Carrier gas: helium; adjust flow rate so that phosalone elutes in about 8.5 min (about 18 mL/min).

Injector temperature: 250° C

Detector

Flame photometric, phosphorus mode (FPD-P)

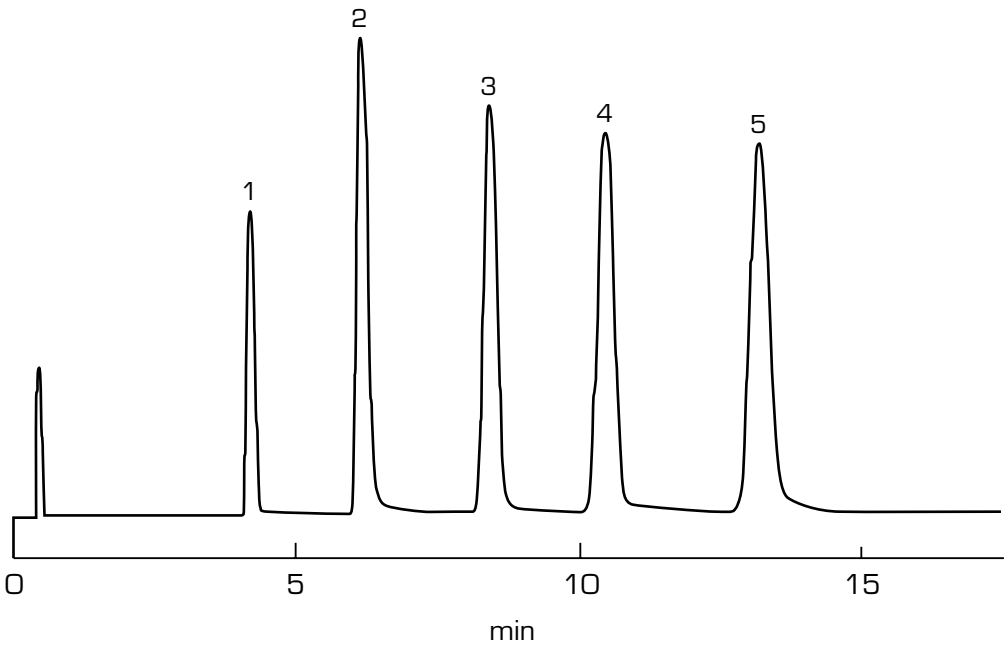
Detector Operating Conditions:

225-250° C

See Section 503 C for other information about FPD operation.

Set detector electronics (amplification, attenuation) so that response to 7.5 ng phosalone is 50% full scale deflection (FSD).

Example chromatogram is on next page.



DG11

Chromatogram of: 1) 1.38 ng ethion, 2) 20.8 ng azinphos-methyl oxygen analog, 3) 7.28 ng phosalone, 4) 7.79 ng pyrazophos, and 5) 10.1 ng coumaphos at the conditions described.

DG12 GLC, 100% METHYL SILOXANE, 230° C, ELCD-X

Applicability

Determinative step is applicable to residues of low volatility (late elution) and containing halogen. It is particularly useful for residues such as halogenated pyrethroids (cyfluthrin, alpha-cypermethrin).

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-1; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

230° C isothermal; if necessary, adjust temperature so that relative retention time (rrt) to phosalone of cis permethrin is about 1.55.

Carrier gas: helium or hydrogen; adjust flow rate so that phosalone elutes in about 8 min.

Injector temperature: 250° C

Detector

Electroconductivity, halogen mode (ELCD-X)

Detector Operating Conditions:

Base temperature 250° C; furnace temperature 900° C (or as specified in operating manual)

Reactor gas: hydrogen, flow as required by specific detector model; see operating manual

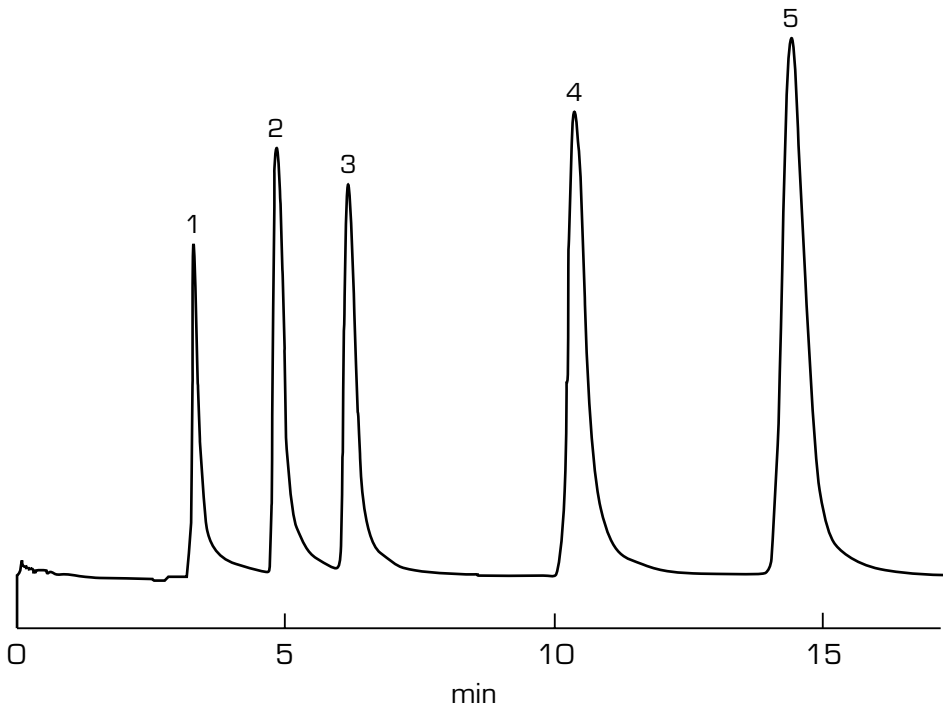
See Section 503 D for other information about ELCD operation.

Set detector electronics (amplification, attenuation) so that response to 18 ng phosalone is 50% full scale defection (FSD).

Other Considerations

Detector sensitivity can probably not be increased to match that of DG10, for the same residues.

Example chromatogram is on next page.



DG12

Chromatogram of: 1) 8.72 ng ofurace, 2) 9.96 ng iprodione, 3) 17.86 ng phosalone, 4) 11.01 ng prochloraz, and 5) 21.06 ng alpha-cypermethrin at the conditions described.

DG13 GLC, 50% PHENYL, 50% METHYL SILOXANE, 200° C, EC

Applicability

Determinative step is applicable to residues containing halogen, sulfur, or other electrophilic moieties. It is a general purpose system, but subject to interferences by nonpesticides.

Column

Wide bore capillary, 30 m m × 0.53 mm id, coated with 50% phenyl, 50% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-17; see Table 502-a for equivalents. See Section 502 C, Recommedned Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C, isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of p,p'-DDT is 3.5 ± 0.07 or rrt_c of ethion is 3.36 ± 0.07.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4 ± 0.5 min (about 20 mL/min).

Injector temperature: 250° C

Detector

Electron Capture (EC)

Detector Operating Conditions:

350° C

Make-up gas: nitrogen or argon/methane (95:5), at 30 mL/min

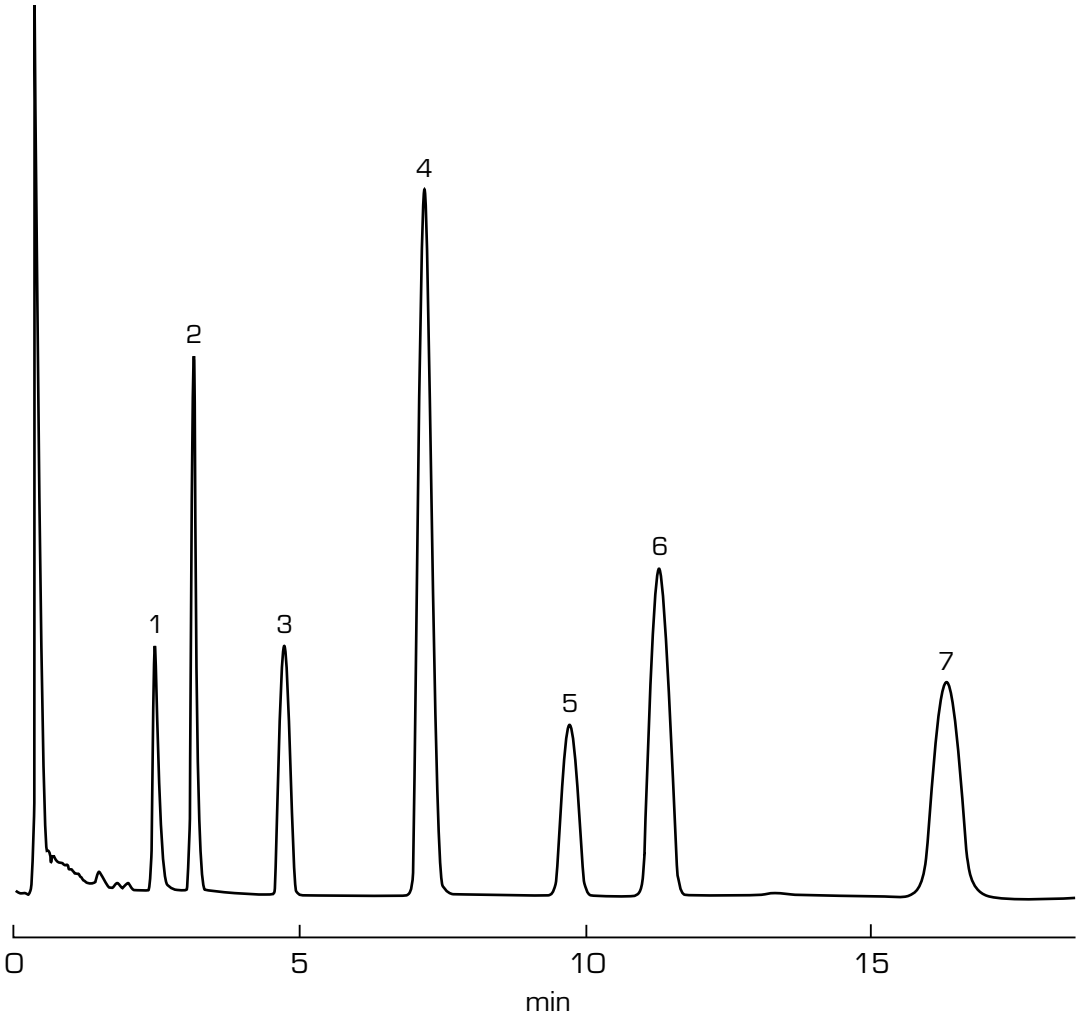
See Section 503 B for other information about EC detector operation.

Set detector electronics (amplification, attenuation) so that response to 0.15 ng chlorpyrifos (on an amount within the detector's linear range) is 50% full scale deflection (FSD).

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column). Response data in Appendix I are based on detector sensitivity of 50% FSD to 1.5 ng chlorpyrifos.

Example chromatogram is on next page.



DG13

Chromatogram of: 1) 0.048 ng dicloran, 2) 0.049 ng heptachlor, 3) 0.15 ng chlorpyrifos, 4) 0.23 ng endosulfan I, 5) 0.22 ng captan, 6) 0.24 ng endrin, and 7) 0.24 ng p,p′-DDT at the conditions described.

DG14 GLC, 50% PHENYL, 50% METHYL SILOXANE, 200° C, FPD-P

Applicability

Determinative step is applicable to residues containing phosphorus. It is particularly useful for residues such as organophosphate pesticides.

Column

Wide bore capillary, 30 mm × 0.53 mm id, coated with 50% phenyl, 50% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-17; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C, isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of ethion is 3.36 ± 0.07.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4 ± 0.5 min (about 20 mL/min).

Injector temperature: 250° C

Detector

Flame photometric, phosphorus mode (FPD-P)

Detector Operating Conditions:

225-250° C

See Section 503 C for other information about FPD operation.

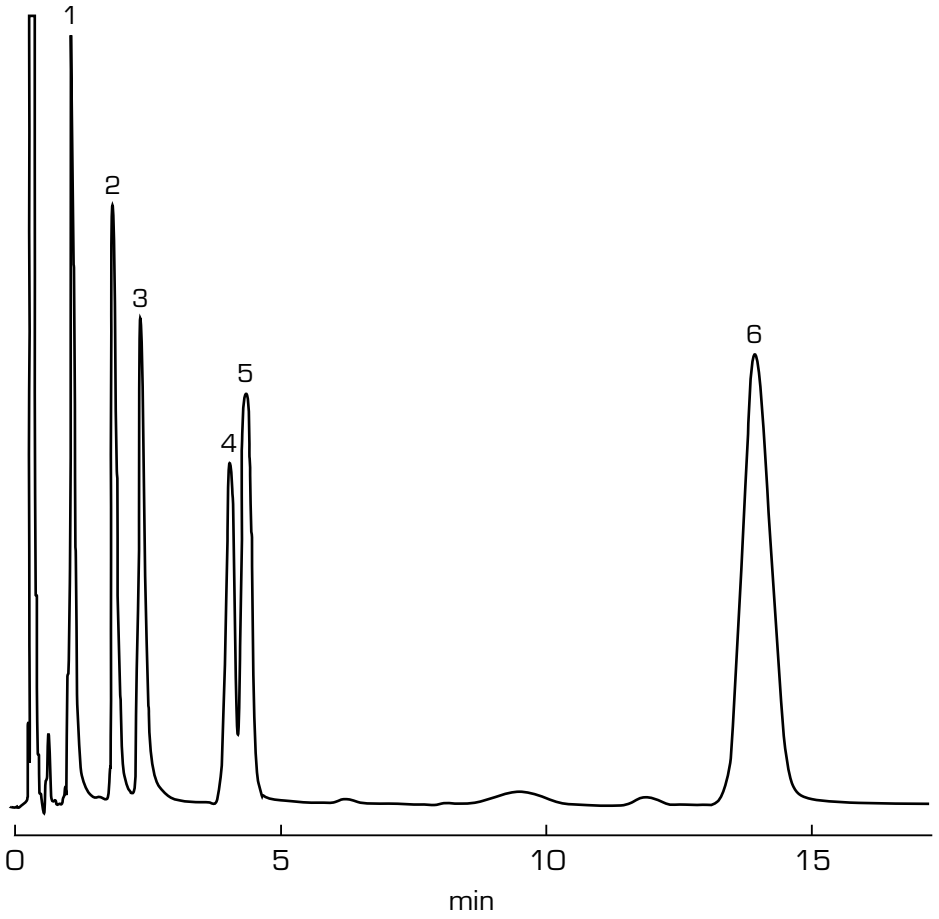
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

A properly functioning system will give a response to 1.5 ng omethoate of ≥50% FSD.

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG14

Chromatogram of: 1) 1.0 ng acephate, 2) 1.5 ng omethoate, 3) 1.0 ng monocrotophos, 4) 1.0 ng pirimiphos-methyl, 5) 1.0 ng chlorpyrifos, and 6) 3.0 ng ethion at the conditions described.

DG15 GLC, 50% PHENYL, 50% METHYL SILOXANE, 230° C, FPD-S

Applicability

Determinative step is applicable to residues containing sulfur. It is particularly useful for residues such as propargite, thiabendazole, and ethofumesate.

Column

Wide bore capillary, 30 m m × 0.53 mm id, coated with 50% phenyl, 50% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-17; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C, isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of ethion is 3.36 ± 0.07.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4 ± 0.5 min (about 20 mL/min).

Injector temperature: 250° C

Detector

Flame photometric, sulfur mode (FPD-S)

Detector Operating Conditions:

225-250° C

See Section 503 C for other information about FPD operation.

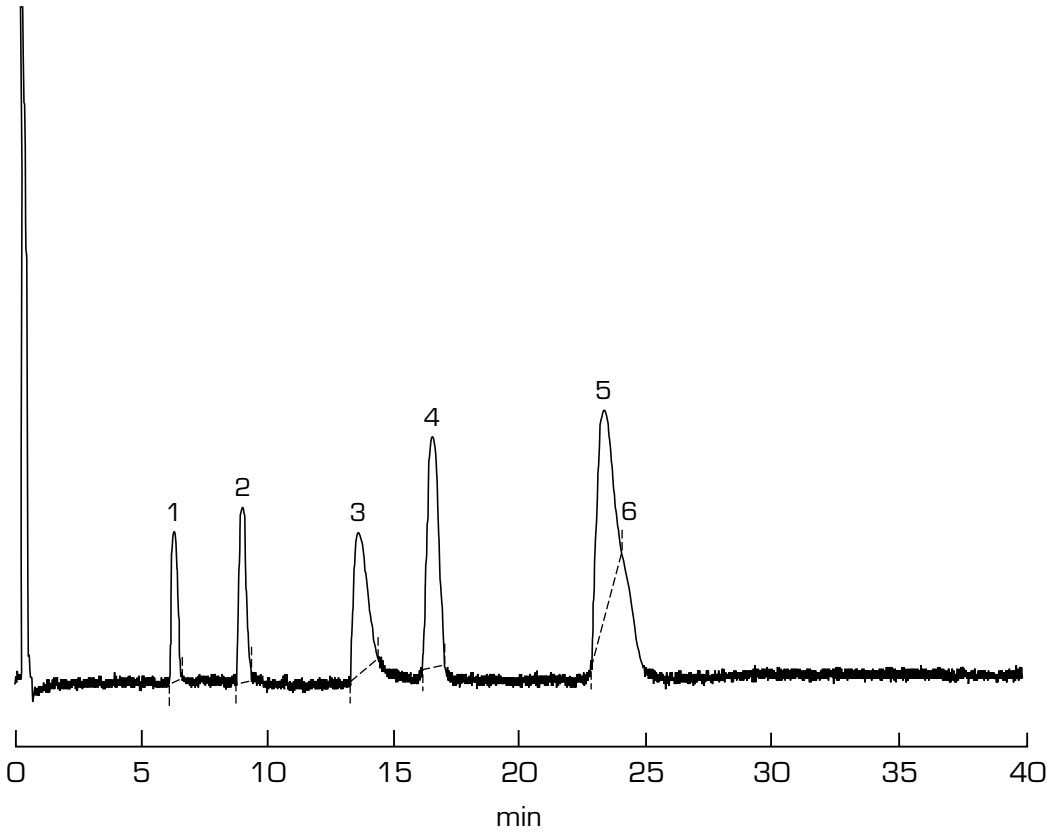
Set detector electronics (amplification, attenuation) to produce greatest possible response (50% full scale deflection [FSD]) to 15 ng chlorpyrifos is reasonable).

Other Considerations

Detector is not linear; quantitation of residues may be calculated from calibration curve (response vs amount injected).

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG15

Chromatogram of: 1) 2.5 ng ethofumesate, 2) 5.0 ng endosulfan I, 3) 12.5 ng thiabendazole, 4) 10.0 ng endosulfan II, 5) 15.0 ng propargite, and 6) 15.0 ng endosulfan sulfate at the conditions described. Using this system, 5.0 ng chlorpyrifos caused about 50% FSD response.

DG16 GLC, 50% PHENYL, 50% METHYL SILOXANE, 200° C, ELCD-X

Applicability

Determinative step is applicable to residues containing halogen. It is particularly useful for residues such as chlorinated hydrocarbon pesticides and polychlorinated biphenyls.

Column

Wide bore capillary, 30 m m × 0.53 mm id, coated with 50% phenyl, 50% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-17; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C, isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of p,p¢-DDT is 3.5 ± 0.07.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4 ± 0.5 min (about 20 mL/min).

Injector temperature: 250° C

Detector

Electroconductivity, halogen mode (ELCD-X)

Detector Operating Conditions:

Base temperature 250° C; furnace temperature 900° C (or as specified in operating manual)

Reactor gas: hydrogen, flow as required by specific detector model; see operating manual

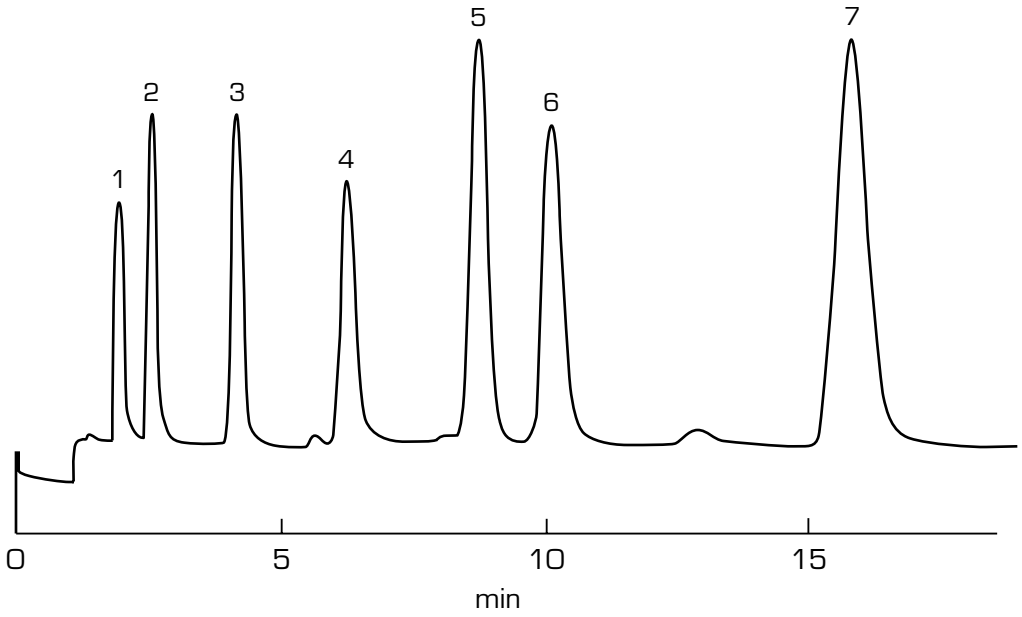
See Section 503 D for other information about ELCD operation.

Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG16

Chromatogram of: 1) 0.85 ng dicloran, 2) 0.58 ng heptachlor, 3) 1.65 ng chlorpyrifos, 4) 1.01 ng endosulfan I, 5) 4.58 ng captan, 6) 1.56 ng endrin, and 7) 3.56 ng p,p'-DDT at the conditions described.

DG17 GLC, 50% PHENYL, 50% METHYL SILOXANE, 200° C, N/P

Applicability

Determinative step is applicable to residues containing nitrogen. It is particularly useful for residues such as triazines, triazoles, and THPI (captan metabolite).

Column

Wide bore capillary, 30 m m × 0.53 mm id, coated with 50% phenyl, 50% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-17; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C, isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of ethion is 3.36 ± 0.07.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 4 ± 0.5 min (about 20 mL/min).

Injector temperature: 250° C

Detector

Alkali bead detector, nitrogen selective (N/P)

Detector Operating Conditions:

250° C

3.7 ± 0.1 mL/min hydrogen and 110 mL/min air

See Section 503 E for other information about N/P detector operation.

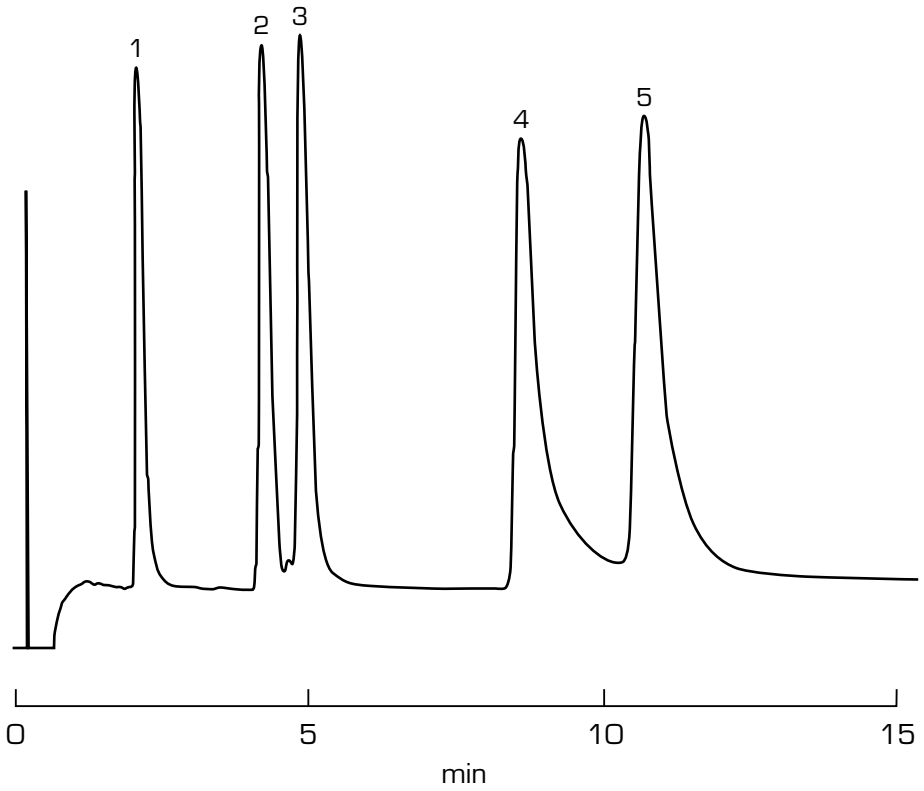
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

A properly functioning system will give a response to 1.5 ng omethoate of ≥50% FSD.

Other Considerations

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG17

Chromatogram of: 1) 1.5 ng atrazine, 2) 1.5 ng chlorpyrifos, 3) 15.0 ng carbaryl, 4) 10.0 ng imazalil, and 5) 5.0 ng procyazine at the conditions described.

DG18 GLC, 50% CYANOPROPYLPHENYL, 50% METHYL SILOXANE, 200° C, EC

Applicability

Determinative step is applicable to residues containing halogen, sulfur, or other electrophilic moieties. It is a general purpose system, subject to interferences from nonpesticides; it is particularly useful for separating BHC isomers and hexachlorobenzene.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 50% cyanopropylphenyl, 50% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-225; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C, isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of lindane is 0.69 ± 0.02 and p,p¢-DDT is 3.6 ± 0.06 or rrt_c of ethion is 3.9 ± 0.1.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 5.5 ± 0.5 min (about 20 mL/min).

Injector temperature: 250° C

Detector

Electron Capture (EC)

Detector Operating Conditions:

350° C

Make-up gas: nitrogen or argon/methane (95:5), a 30 mL/min

See Section 503 B for other information about EC detector operation.

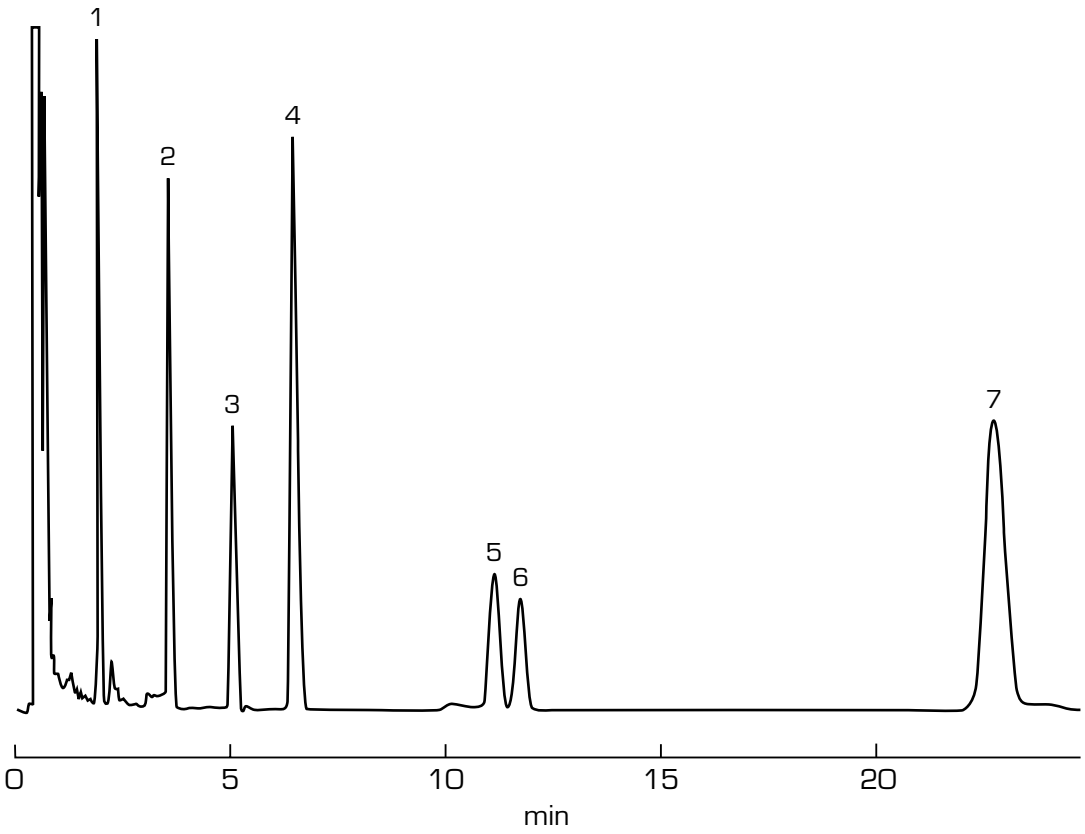
Set detector electronics (amplification, attenuation) so that response to 0.15 ng chlorpyrifos (on an amount within the detector's linear range) is 50% full scale deflection (FSD).

Other Considerations

Columns containing cyano moieties in the phase must not be connected to nitrogen selective or electrolytic conductivity detectors, so this column cannot be used with a different detector to confirm residues tentatively identified using this system.

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column). Response data in Appendix I are based on detector sensitivity of 50% FSD to 1.5 ng chlorpyrifos.

Example chromatogram is on next page.



DG18

Chromatogram of: 1) 0.032 ng hexachlorobenzene, 2) 0.049 ng α-BHC, 3) 0.056 lindane, 4) 0.15 ng chlorpyrifos, 5) 0.054 ng β-BHC, 6) 0.054 ng δ-BHC, and 7) 0.201 ng p,p′-DDT at the conditions described.

DG19 GLC, 50% CYANOPROPYLPHENYL, 50% METHYL SILOXANE, 200° C, FPD-P

Applicability

Determinative step is applicable to residues containing phosphorus. It is particularly useful for residues such as organophosphate pesticides.

Column

Wide bore capillary, 30 m × 0.53 mm id, coated with 50% cyanopropylphenyl, 50% methyl substituted polysiloxane liquid phase, 1-1.5 µm film thickness, bonded and cross-linked. For example, DB-225; see Table 502-a for equivalents. See Section 502 C, Recommended Operating Procedure for Wide Bore Columns.

Column Operating Conditions:

200° C, isothermal; if necessary, adjust temperature so that relative retention time to chlorpyrifos (rrt_c) of ethion is 3.9 ± 0.1.

Carrier gas: helium; adjust flow rate so that chlorpyrifos elutes in about 5.5 ± 0.5 min (about 20 mL/min).

Injector temperature: 250° C

Detector

Flame photometric, phosphorus mode (FPD-P)

Detector Operating Conditions:

225-250° C

See Section 503 C for other information about FPD operation.

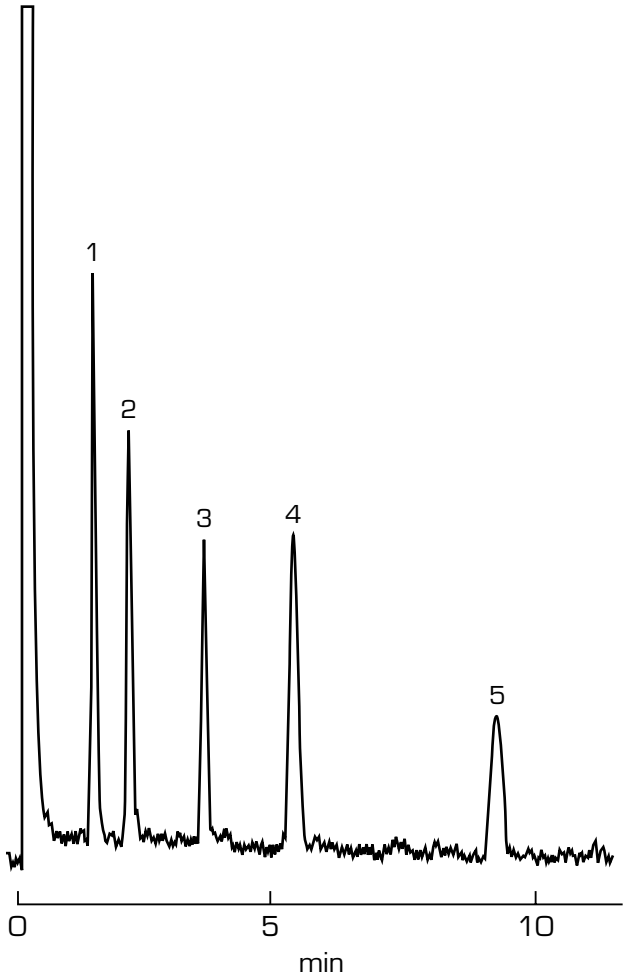
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

Other Considerations

Columns containing cyano moieties in the phase must not be connected to nitrogen selective or electrolytic conductivity detectors, so this column cannot be used with a different detector to confirm residues tentatively identified using this system.

Rrt_cs and ng required to cause 50% FSD response are listed in Appendix I, PESTDATA (many data in PESTDATA were collected using equivalent packed column).

Example chromatogram is on next page.



DG19

Chromatogram of: 1) 0.5 ng methamidophos, 2) 1.0 ng diazinon, 3) 1.0 ng acephate, 4) 1.5 ng chlorpyrifos, and 5) 1.0 ng monocrotophos at the conditions described.

303: METHOD II FOR NONFATTY FOODS

BASIC REFERENCE

Mills, P.A., *et al.* (1963) *J. Assoc. Off. Agric. Chem.* **46**, 186-191

GENERAL PRINCIPLES

Residues are extracted by blending with acetonitrile or water and acetonitrile, then transferred into petroleum ether by liquid-liquid partitioning. Subsequent cleanup of the extract with Florisil column chromatography results in an extract suitable for determination by GLC; two elution systems produce different elution patterns, useful in confirmatory or additional analyses.

The amount of sample represented in the final solution is calculated from the aliquot of acetonitrile extract used and the proportion of petroleum ether retrieved from the partitioning step; this calculation is valid only when the original filtered extract is homogeneous. Variations in the extraction step are used for products of high (>5%) sugar content to ensure homogeneity.

APPLICABILITY

Consult Guide to PAM I for additional information pertinent to the appropriate application of multiresidue methodology.

Method is generally applicable to relatively nonpolar residues in nonfatty commodities, i.e., fruits and vegetables containing ≤2 g fat in 100 g sample. Extraction E1 is applicable to products with high moisture (>75%) content; that extraction is also applicable to eggs if sample size is reduced (Extraction E2). Extraction E3 is applicable to dry products (<75% water), E4 to products with 5-15% sugar, and E5 to products with >15% sugar. See Section 201 for percentages fat, water, and sugar of many commodities. Florisil cleanup step prevents applicability to very polar residues. See Table 303-a, following the method description, for results of recovery tests.

METHOD MODULES

Choose from these method modules, using Figure 303-a for guidance:

Extraction (E)			**Recommended Use**
E1	(p. 303-7)	Extraction with acetonitrile, partition into petroleum ether with high moisture	fruits and vegetables (>75%), and low sugar (<5%), low fat (<2%)
E2	(p. 303-8)	Extraction from eggs with acetonitrile, partition into petroleum ether	whole eggs
E3	(p. 303-9)	Extraction with water/acetonitrile, partition into petroleum ether	dried egg whites, grains, and other foods with low moisture (<75%), low fat (<2%)
E4	(p. 303-9)	Extraction with acetonitrile and water, partition into petroleum ether	fruits and other foods with high sugar (5-15%)
E5	(p. 303-10)	Extraction with heated acetonitrile and water, partition into petroleum ether	fruits and other foods with very high sugar (>15%)

Cleanup (C)

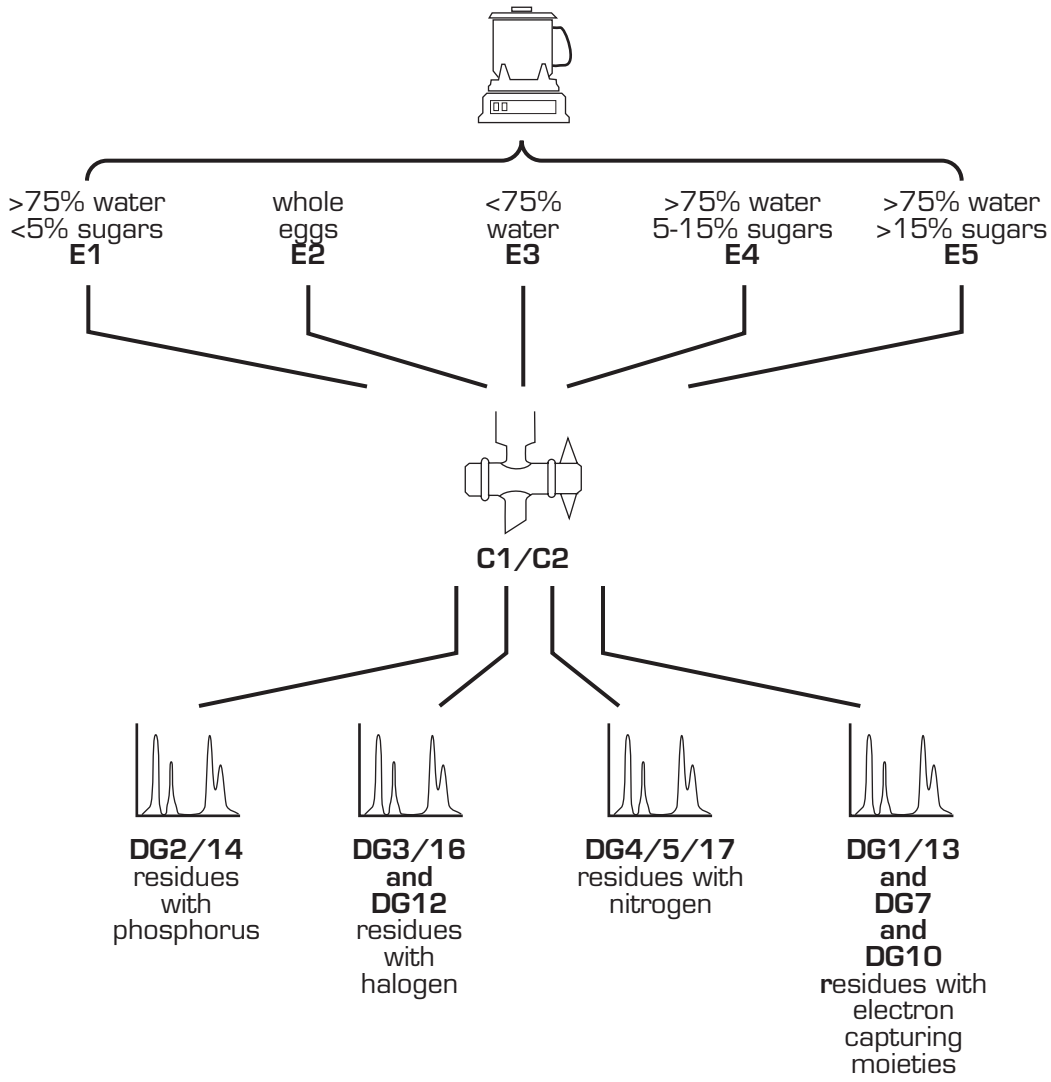
C1	(p. 303-13)	Florisil column cleanup, with three ethyl ether/petroleum ether eluants	for relatively nonpolar residues
C2	(p. 303-14)	Florisil column cleanup, with three methylene chloride eluants	alternative to C1, some additional residues recovered

Determination (D)

(See Section 302 for full details of GLC modules.)

DG 1	(p. 302-33)	GLC, 100% methyl siloxane column, 200°, EC detector	residues with halogen, sulfur, other moieties
DG 2	(p. 302-35)	GLC, 100% methyl siloxane column, 200°, FPD-P	residues with phosphorus
DG 3	(p. 302-37)	GLC, 100% methyl siloxane column, 200°, ELCD-X	residues with halogen
DG 4	(p. 302-39)	GLC, 100% methyl siloxane column, 200°, ELCD-N	residues with nitrogen
DG 5	(p. 302-41)	GLC, 100% methyl siloxane column, 200°, N/P detector	residues with nitrogen or phosphorus
DG 7	(p. 302-45)	GLC, 100% methyl siloxane column, 130°, EC detector	early eluting residues with halogen, sulfur, other moieties
DG10	(p. 302-51)	GLC, 100% methyl siloxane column, 230°, EC detector	late eluting residues with halogen, sulfur, other moieties
DG12	(p. 302-55)	GLC, 100% methyl siloxane column, 230°, ELCD-X	late eluting residues with halogen
DG13	(p. 302-57)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, EC detector	residues with halogen, sulfur, other moieties
DG14	(p. 302-59)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, FPD-P	residues with phosphorus
DG16	(p. 302-63)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, ELCD-X	residues with halogen
DG17	(p. 302-65)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, N/P detector	residues with nitrogen or phosphorus

Figure 303-a
Recommended Approach: Nonfatty Foods



VALIDATION

Many combinations of method modules are possible. The following combinations have undergone interlaboratory validation and are recommended for use:

E1 + C1 + DG1

Validation reports:

Krause, R.T. (1966) *J. Assoc. Off. Anal. Chem.* **49**, 460-463. Collaborative study leading to AOAC official final action status for aldrin, DDE, and methoxychlor in potatoes.

Gaul, J. (1966) *J. Assoc. Off. Anal. Chem.* **49**, 463-467. Collaborative study leading to AOAC official final action status for lindane, heptachlor, and TDE in endive and cauliflower.

Davidson, A.W. (1966) *J. Assoc. Off. Anal. Chem.* **49**, 468-472. Collaborative study leading to AOAC official final action status for BHC, p,p'-DDT, and endrin on apricots and strawberries.

Wells, C. (1967) *J. Assoc. Off. Anal. Chem.* **50**, 1205-1215. Interlaboratory study supporting validity of method for 32 residues in five groups of nonfatty commodities; studies supported extension of official status for previously collaborated resi dues in 13 additional commodities.

Burke, J.A. (1968) *J. Assoc. Off. Anal. Chem.* **51,** 311-314. Interlaboratory study supporting validity of method for nine residues in 21 nonfatty foods; studies supported extension of official status for previously collaborated residues to 15 additional commodities.

Krause, R.T. (1973) *J. Assoc. Off. Anal. Chem.* **56**, 721-727. Collaborative study leading to AOAC official final action status for dieldrin, heptachlor epoxide, mirex, and Perthane in apples and cauliflower.

E1 + C1 + (predecessor to) DG5

Validation reports:

Wessel, J.R. (1967) *J. Assoc. Off. Anal. Chem.* **50**, 430-439. Collaborative study lead ing to AOAC official final action status for diazinon, ethion, malathion, parathion, parathion-methyl, and ronnel in lettuce and apples.

Finsterwalder, C. W. (1976) *J. Assoc. Off. Anal. Chem.* **59**, 169-171. Collaborative study leading to AOAC official final action status for parathion in kale.

Wells, C. (1967) *J. Assoc. Off. Anal. Chem.* **50**, 1205-1215. Interlaboratory study supporting validity of method for 32 residues in five groups of nonfatty commodities; studies were later referenced (Burke, J.A. (1971) *J. Assoc. Off. Anal. Chem.* **54**, 325-327) as support for extension of official status for diazinon, ethion, malathion, parathion, parathion-methyl, and ronnel in barley, broccoli, cabbage, carrots, cauliflower, cucumbers, grapes, green peppers, mustard greens, oats, potatoes, squash, tomatoes, turnips, turnip greens, and wheat.

E2 + C1 + DG1

Validation report:

Finsterwalder, C. W. (1976) *J. Assoc. Off. Anal. Chem.* **59**, 169-171. Collaborative study leading to AOAC official final action status for o,p'-DDT, p,p'-DDT, and p,p'-DDE in kale and p,p'-DDE, dieldrin, lindane, and heptachlor epoxide in eggs.

E3 + C1 + DG1

Validation reports:

Burke, J.A. (1971) *J. Assoc. Off. Anal. Chem.* **54**, 325-327. Referenced the following publications in a recommendation to extend official status to extraction step E3 (for low moisture commodities) for previously collaborated residues on barley, corn meal, hay, oats, popcorn, and wheat:

Bertuzzi, P., *et al.* (1967) *J. Assoc. Off. Anal. Chem.* **50**, 623-627

Wilderman, M., and Shuman, H., (1968) *J. Assoc. Off. Anal. Chem.* **51**, 892-895

Burke, J.A., *et al.* (1971) *J. Assoc. Off. Anal. Chem.* **54**, 142-146. Evaluation of two extraction procedures.

E4 + C1 + DG1 and E5 + C1 + DG1

Validation report:

Burke, J.A. (1970) *J. Assoc. Off. Anal. Chem.* **53**, 355-357. Referenced the following publication in a recommendation to extend official status to extraction steps E4 and E5 (for commodities with high sugar) for previously collaborated residudues:

Porter, M.L., and Burke, J.A. (1969) *J. Assoc. Off. Anal. Chem.* **52**, 1280-1283. Description of way to accommodate commodities with high sugar.

For all combinations above, AOAC official method reference: Official Methods of Analysis of the AOAC (1990) 15th ed., 970.52 A, B, E, H, I, J, K, O, and R.

E1 + C2 + DG1

Validation reports:

Mitchell, L.E. (1976) *J. Assoc. Off. Anal. Chem.* **59**, 209-212. Collaborative study leading to AOAC official final action status for endosulfan, endosulfan sulfate, tetradifon, and tetrasul in apples and cucumbers.

McMahon, B.M. (1988) *J. Assoc. Off. Anal. Chem.* **71**, 94-97. Presentation of additional validation data for previously collaborated residues to 29 additional commodities.

AOAC official method reference: *Official Methods of Analysis of the AOAC* (1990) 15th ed., 976.23.

E1 EXTRACTION WITH ACETONITRILE, PARTITION INTO PETROLEUM ETHER

References

Mills, P.A., *et al.* (1963) *J. Assoc. Off. Agric. Chem.* **46**, 186-191
Porter, M., *et al.* (1967) *J. Assoc. Off. Anal. Chem.* **50**, 644-645

Principles

Residues are extracted from high moisture products by a single blending with acetonitrile, a solvent miscible with the water in high moisture products and also capable of dissolving organic residues. An aliquot of filtered extract is diluted with water and residues are transferred into petroleum ether by liquid-liquid partitioning; transfer to hydrocarbon solvent and removal of all traces of polar acetonitrile permit subsequent cleanup on Florisil. Amount of sample represented in final solution is calculated from aliquot of acetonitrile extract used and proportion of petroleum ether retrieved from partitioning step.

Apparatus

blender, high speed; explosion-proof Waring Blendor, 1 qt jar

Büchner funnel (Büchner), porcelain, 12 cm diameter

filter paper, Shark Skin®, to fit Büchner

graduated cylinders (graduates), glass-stoppered (g-s), 100 mL, and plain, 250 mL

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, graduated or plain receiving flask

separatory funnel (separator), 1 L

vacuum filtration flask, 500 mL

Reagents

acetonitrile, distilled from all-glass apparatus; see Section 204 for distillation directions

boiling chips, 20-30 mesh carborundum

petroleum ether, distilled from all-glass apparatus

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

Directions

- Chop or blend representative sample. Weigh 100 g sample into blender jar and add 200 mL acetonitrile (10 g Celite may be added as filter aid).
- Blend 2 min at high speed and filter with suction through Büchner fitted with filter paper into vacuum filtration flask.
- Transfer filtrate to 250 mL graduate and record volume (F).
- Transfer measured volume of filtrate to 1 L separator.
- Carefully measure 100 mL petroleum ether in same graduate used to measure filtered extract and pour into separator containing extract. Shake vigorously 1-2 min.

- Add 10 mL saturated sodium chloride solution and 600 mL water. Hold separator in horizontal position and mix vigorously 30-45 sec. (Inadequate mixing may lead to low recoveries of some pesticides, e.g., BHC, TDE.)
- Let layers separate, discard aqueous layer, and gently wash solvent layer with two 100 mL portions water. Discard washings, transfer solvent layer to 100 mL g-s graduate, and record volume (P).
- Add about 15 g sodium sulfate to graduate, stopper, and shake vigor ously. Do not let extract remain with sodium sulfate >1 hr or losses of organochlorine pesticides by adsorption may result.
- Transfer solution directly to Florisil column, C1 or C2, or concentrate to 5-10 mL in K-D for transfer.
- Calculate weight sample placed on Florisil column as:

$$g = S \times \frac{F}{T} \times \frac{P}{100}$$

where:

S = g sample extracted

F = volume of filtered acetonitrile extract

T = total volume (mL water in sample + mL acetonitrile added – correction in mL for volume contraction). 5 mL contraction volume is used for 80-95 mL water + 200 mL acetonitrile.

P = mL petroleum ether extract recovered

100 = mL petroleum ether into which residues were partitioned.

See Section 201 for percentage water in commodity; 85% may be assumed for most fruits and vegetables.

ALTERNATIVES:

E2 EXTRACTION FROM EGGS WITH ACETONITRILE, PARTITION INTO PETROLEUM ETHER

Additional Reference

Wessel, J.R. (1969) *J. Assoc. Off. Anal. Chem.* **52**, 172-175

Directions

- Blend combined yolks and whites of whole eggs at low speed at least 5 min or until sample is homogeneous. Low speed blending will minimize foaming or whipping of sample.
- Weigh ≤25g thoroughly mixed yolks and whites into blender jar and add 200 mL acetonitrile.
- Proceed as in E1, "Blend 2 min at high speed... ."
- Calculate g sample to be placed on Florisil column as in E1, except:

  T = 215 (15 mL water in 25 g whole eggs + 200 mL acetonitrile; contraction volume is negligible).

E3 EXTRACTION WITH 35% WATER/ACETONITRILE, PARTITION INTO PETROLEUM ETHER

Additional Reference

Bertuzzi, P.A., *et al.* (1967) *J. Assoc. Off. Anal. Chem.* **50**, 623-627

Principles

Residues are extracted from nonfatty, low moisture products by blending with 35% water/acetonitrile. Water in extractant is needed to obtain adequate extraction of residues from low moisture products. Aliquot (≤250 mL) of filtered extract is diluted with water and residues are transferred into petroleum ether by liquid-liquid partitioning; transfer to hydrocarbon solvent and removal of all traces of polar acetonitrile permit subsequent cleanup on Florisil. Water:acetonitrile dilution ratio of 4:1 is used for transfer of residues to petroleum ether; restricting volume of filtered extract to ≤250 mL allows extract, adequate water for dilution, and 100 mL petroleum ether to fit in 1 L separator.

Directions

- Grind sample to pass 20 mesh sieve.
- Weigh 20-25 g sample into blender jar, and add 350 mL 35% water/acetonitrile (10 g Celite may be added as filter aid). If larger sample is required, add sufficient additional extraction mixture to wet sample and permit thorough blending.
- Blend 5 min at high speed and filter with suction through Büchner fitted with filter paper into vacuum filtration flask.
- Take ≤250 mL filtered extract for analysis. Record volume (F).
- Continue as in E1, "Transfer measured volume of filtrate to 1 L separator…"
- Calculate g sample to be placed on Florisil column as in E1, except:

  T = mL water in sample + mL 35% water/acetonitrile. No correction for volume contraction is needed. If water content of sample is <10%, disregard it and use T = volume extracting mixture.

E4 EXTRACTION WITH ACETONITRILE AND WATER, PARTITION INTO PETROLEUM ETHER

Additional Reference

Porter, M.L., and Burke, J.A. (1969) *J. Assoc. Off. Anal. Chem.* **52**, 1280-1283

Principles

Residues are extracted from nonfatty, high moisture commodities that contain 5-15% sugar by addition of water and subsequent blending with acetonitrile. Water is added to the product to dilute the effect of sugar, which can cause separation of water and acetonitrile phases in the filtered extract and thus disrupt homogeneity of the extract solution. Aliquot (≤250 mL) of filtered extract is diluted with water and residues are transferred into petroleum ether by liquid-liquid partitioning; transfer to hydrocarbon solvent and removal of all traces of polar acetonitrile permit subsequent cleanup on Florisil.

Directions

- Weigh 100 g sample into blender jar and add 200 mL acetonitrile and 50 mL water.
- Blend 2 min at high speed and filter with suction through Büchner fitted with filter paper into vacuum filtration flask.
- Transfer ≤250 mL filtered extract to 250 mL graduate. Record volume (F).
- Continue as in E1, "Transfer measured volume to 1 L separator."
- Calculate g sample to be placed on Florisil column as in E1, except:

  T = mL water in sample + mL acetonitrile added + mL water added – correction in mL for volume contraction. When 50 mL water is added, T is 325 for foods of 85% water content. Contraction volumeof 5 mL is used for 80-95 mL water + 200 mL acetonitrile.

E5 EXTRACTION WITH HEATED ACETONITRILE AND WATER, PARTITION INTO PETROLEUM ETHER

Additional Reference

Porter, M.L., and Burke, J.A. (1969) *J. Assoc. Off. Anal. Chem.* **52**, 1280-1283

Principles

Residues are extracted from commodities with >15% sugar using a heated mixture of water and acetonitrile. Unheated water and acetonitrile (as in E4) is insufficient to prevent separation of water and acetonitrile phases in the filtered extract when sugar is >15%. Aliquot (≤250 mL) of filtered extract is diluted with water and residues are transferred into petroleum ether by liquid-liquid partitioning; transfer to hydrocarbon solvent and removal of all traces of polar acetonitrile permit subsequent cleanup on Florisil.

Directions

- Weigh 100 g sample into blender jar and add heated (75° C) mixture of 200 mL acetonitrile and 50 mL water.
- To analyze raisins, weigh 50 g. Heat 50 mL water and 200 mL acetonitrile separately to 75° C. Add 40-50 mL hot water to container in which raisins were weighed and stir or shake to disperse in water. Transfer to blender jar, using remaining water to rinse container into blender jar. Rinse container with hot acetonitrile and add to blender jar; add remaining hot acetonitrile to blender jar.
- Blend 2 min at high speed and filter with suction through Büchner fitted with filter paper into vacuum filtration flask.
- Before filtered extract cools, transfer ≤250 mL to 250 mL graduate. Record volume (F).
- Continue as in E1, "Transfer measured volume of filtrate to 1 L separator…"
- Calculate g sample to be placed on Florisil column as in E1, except:

  T = mL water in sample + mL acetonitrile added + mL water added – correction in mL for volume contraction. When 50 mL water added, T is 325 for foods of 85% water content. Contraction volume of 5 mL is used for 80-95 mL water + 200 mL acetonitrile.

C1 FLORISIL COLUMN CLEANUP, WITH THREE ETHYL ETHER/ PETROLEUM ETHER ELUANTS

Reference

Mills, P.A., *et al.* (1963) *J. Assoc. Off. Agric. Chem.* **46**, 186-191

Principles

Residues in solution are separated from sample co-extractives on a column of Florisil adsorbent; eluants of increasing polarity sequentially remove residues from the column.

Apparatus

chromatographic column, 22 mm id ¥ 300 mm, Teflon stopcock, coarse porosity fritted disc

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, volumetric or graduated receiving flask

Reagents

boiling chips, 20-30 mesh carborundum

ethyl ether, distilled from all-glass apparatus, with 2% ethanol as preservative; see Section 204 for peroxide test

Florisil, PR Grade; see Section 204 for handling and testing directions and calculation of lauric acid value

petroleum ether, distilled from all-glass apparatus

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

eluants: 6% (v/v) ethyl ether/petroleum ether

15% (v/v) ethyl ether/petroleum ether

50% (v/v) ethyl ether/petroleum ether

Directions

- Place activated Florisil (4" or weight determined by lauric acid value) in 22 mm id column; add about 0.5" sodium sulfate. Prewet column with 40-50 mL petroleum ether. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Transfer sample extract solution to column, letting it pass through at about 5 mL/min. Rinse container (and sodium sulfate if present) with two 5 mL portions petroleum ether, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions petroleum ether.
- Elute column at about 5 mL/min with 200 mL 6% ethyl ether/ petroleum ether eluant.
- Change K-Ds and elute at about 5 mL/min with 200 mL 15% ethyl ether/petroleum ether eluant.
- Change K-Ds and elute at about 5 mL/min with 200 mL 50% ethyl ether/petroleum ether eluant.

- Add boiling chips to K-Ds and concentrate each eluate to suitable definite volume. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during final evaporation in receiving flask.
- Use appropriate determinative steps, such as DG1 or DG13, DG7, and DG10, to identify and measure residues.

ALTERNATIVE:

C2 FLORISIL COLUMN CLEANUP, WITH THREE METHYLENE CHLORIDE ELUANTS

Additional Reference

Mills, P.A., *et al.* (1972) *J. Assoc. Off. Anal. Chem.* **55**, 39-43

Additional Reagents

acetonitrile, distilled from all-glass apparatus; see Section 204 for distillation directions

hexane, distilled from all-glass apparatus

methylene chloride, distilled from all-glass apparatus

eluants:

1—20% methylene chloride/hexane (v/v). Dilute 200 mL methylene chloride with hexane. Allow mixture to reach room temperature, and adjust volume to 1 L with hexane.

2—50% methylene chloride/0.35% acetonitrile/49.65% hexane (v/v/v). Pipet 3.5 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

3—50% methylene chloride/1.5% acetonitrile/48.5% hexane (v/v/v). Pipet 15 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

Directions

- Prepare Florisil column as in C1. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Transfer sample extract solution to column, letting it pass through at about 5 mL/min. Rinse container (and sodium sulfate if present) with two 5 mL portions hexane, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions hexane.
- Elute column at about 5 mL/min with 200 mL eluant 1.
- Change K-Ds and elute with 200 mL eluant 2.
- Change K-Ds and elute with 200 mL eluant 3.
- Add boiling chips to K-Ds and concentrate each eluate to suitable definite volume. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during evaporation.
- Use appropriate determinative steps to identify and measure residues.

DETERMINATION

Inject concentrated extract equivalent to 20 mg (whole product) into the following GLC systems (Section 302) for determination of residues.

Minimum recommended determinations:

DG1 or DG13	residues with halogen, sulfur, or other moieties
or	
DG3 or DG16	organohalogen residues
DG10	late eluting residues, especially pyrethroids
DG12	late eluting organohalogen residues, especially pyrethroids
DG7	early eluting residues with halogen, sulfur, or other moietites
DG2 or DG14	organophosphorus residues; large amounts of sulfur may interfere
DG4	organonitrogen residues; selective to nitrogen, but coextractives may contain nitrogen
DG5 or DG17	organonitrogen and organophosphorus residues

For accurate quantitation, reference standards should be dissolved in same solvent as concentrated extract, only peaks >10% FSD should be measured, and peak sizes of residue and reference standard should match within ±25%.

See Chapter 5 for additional information about operation of GLC systems; Section 504 provides information about quantitation of residues.

See Section 205 for additional information about reference standards.

See Section 104 for additional information about reporting residues and determining compliance with regulations.

See Section 105 for additional information about analytical limits of quantitation.

CONFIRMATION

After residues have been tentatively identified and quantitated by comparison to appropriate reference standards, confirm identity according to principles discussed in Section 103. Use appropriate tables of data (PESTDATA, tables accompanying each method, Index to Methods) to choose most appropriate determinative steps and/or alternative methods for confirmation.

304: METHOD FOR FATTY FOODS

BASIC REFERENCE

Mills, P.A. (1959) *J. Assoc. Off. Agric. Chem.* **42,** 734-740

GENERAL PRINCIPLES

Fat and residues are extracted from fatty foods and dissolved in an organic solvent. Residues are separated from the extracted fat to produce a cleaned up extract solution suitable for determination by gas chromatography.

APPLICABILITY

Consult Guide to PAM I for additional information pertinent to the appropriate application of multiresidue methodology.

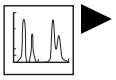
Method is applicable to moderately nonpolar residues in fatty foods. Residue polarity will affect recovery in Cleanups 1 and 2; neither very nonpolar nor very polar residues are recovered completely. See Table 304-a, following the method description, for results of recovery tests.

METHOD MODULES

Choose from these method modules, using Figure 304-a for guidance:

Extraction (E)		Recommended Use	
E1	(p. 304-5)	Extraction of fat with sodium sulfate, petroleum ether	animal tissues, fatty fish
E2	(p. 304-7)	Small scale extraction of fat with sodium sulfate, petroleum ether	animal tissues, fatty fish
E3	(p. 304-9)	Extraction of fat by filtering	butter, oils
E4	(p. 304-11)	Extraction of fat with solvents from denatured product	cheese, milk, egg yolks, dried whole eggs
E5	(p. 304-13)	Extraction of fat with solvents	oilseeds, high fat feeds or feed materials, grains, nuts
Cleanup (C)			
C1	(p. 304-15)	Acetonitrile-petroleum ether partitioning, Florisil column cleanup, three mixed ether eluants	for relatively few samples
C2	(p. 304-17)	Acetonitrile-petroleum ether partitioning, Florisil column cleanup, three methylene chloride eluants	for better cleanup than C1
C3	(p. 304-18)	Acetonitrile-petroleum ether partitioning, Florisil column cleanup, petroleum ether and three mixed ether eluants	to separate PCBs from most pesticides
C4	(p. 304-19)	Acetonitrile-petroleum ether partitioning, Florisil column cleanup, petroleum ether and three methylene chloride eluants	to separate PCBs from most pesticides
C5	(p. 304-21)	Gel permeation chromatography (GPC)	for efficient analysis of many samples (can be automated)

C6	(p. 304-24)	GPC, Florisil column (4 g) cleanup, three methylene chloride eluants	when C5 provides insufficient cleanup
C7	(p. 304-27)	Florisil column (4 g) cleanup, two mixed ether eluants, optional alkaline hydrolysis	to decrease time, solvent use compared to C1
C8	(p. 304-29)	Dispersion on alumina, Florisil column cleanup, three mixed ether eluants	to reduce time compared to C1; screening test only
C9	(p. 304-31)	Dispersion on alumina, Florisil columncleanup, three methylene chloride eluants	to reduce time compared to C3; screening test only

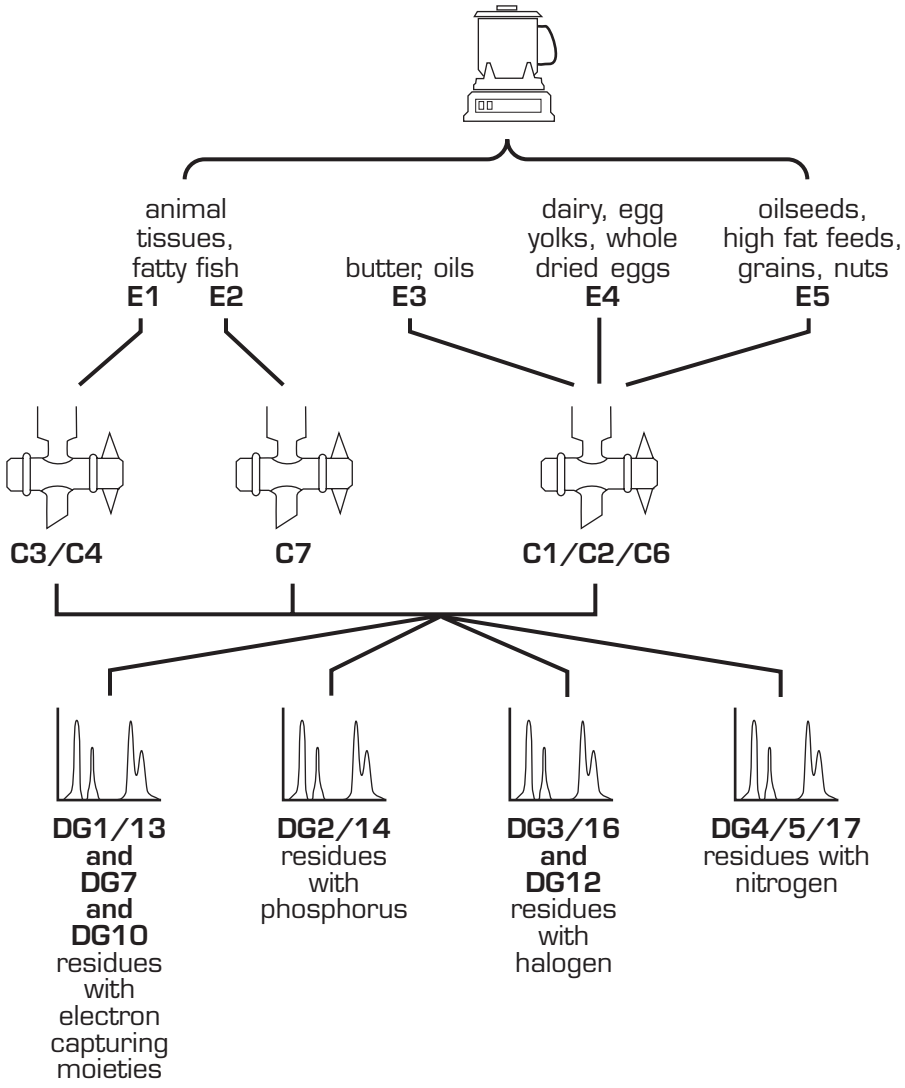


Determinations (D)

(See Section 302 for full details of GLC modules.)

DG 1	(p. 302-33)	GLC, 100% methyl siloxane column, 200°, EC detector	residues with halogen, sulfur, other moieties
DG 2	(p. 302-35)	GLC, 100% methyl siloxane column, 200°, FPD-P	residues with phosphorus
DG 3	(p. 302-37)	GLC, 100% methyl siloxane column, 200°, ELCD-X	residues with halogen
DG 4	(p. 302-39)	GLC, 100% methyl siloxane column, 200°, ELCD-N	residues with nitrogen
DG 5	(p. 302-41)	GLC, 100% methyl siloxane column, 200°, N/P	residues with nitrogen or phosphorus
DG 7	(p. 302-45)	GLC, 100% methyl siloxane column, 130°, EC detector	early eluting residues with halogen, sulfur, other moieties
DG10	(p. 302-51)	GLC, 100% methyl siloxane column, 230°, EC detector	late eluting residues with halogen, sulfur, other moieties
DG12	(p. 302-55)	GLC, 100% methyl siloxane column, 230°, ELCD-X	late eluting residues with halogen
DG13	(p. 302-57)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, EC detector	residues with halogen, sulfur, other moieties
DG14	(p. 302-59)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, FPD-P	residues with phosphorus
DG16	(p. 302-63)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, ELCD-X	residues with halogen
DG17	(p. 302-65)	GLC, 50% phenyl, 50% methyl siloxane column, 200°, N/P detector	residues with nitrogen or phosphorus

Figure 304-a
Recommended Approach: Fatty Foods



VALIDATION

Many combinations of the method modules are possible. The following combinations have undergone interlaboratory validation and are recommended for use:

E1 + C1 + DG1

Validation reports:

Carr, R.L. (1971) *J. Assoc. Off. Anal. Chem.* **54**, 525-527. Collaborative study leading to AOAC official final action status for a-BHC, p,p¢-DDE, p,p¢-TDE, p,p'-DDT, dieldrin, and heptachlor epoxide in fish.

Sawyer, L.D. (1973) *J. Assoc. Off. Anal. Chem.* **56**, 1015-1023. AOAC collaborative study leading to AOAC official final action status for polychlorinated biphenyls in poultry fat and fish.

AOAC official method reference: *Official Methods of Analysis of the AOAC* (1990) 15th ed., 970.52, A, B, E, H, I, J, L, N, O, and R.

E2 + C7 + DG1

Validation report:

Erney, D. R. (1983) *J. Assoc. Off. Anal. Chem.* **66**, 969-973. Collaborative study leading to AOAC official final action status for p,p'-DDE, p,p'-DDT, p,p'-TDE, dieldrin, heptachlor epoxide, and polychlorinated biphenyls in fish.

AOAC official method reference: *Official Methods of Analysis of the AOAC* (1990) 15th ed., 983.21.

E4 + C1 + DG1

Validation reports:

Johnson, L. (1965) *J. Assoc. Off. Agric. Chem.* **48**, 668-675. Collaborative study leading to AOAC official final action status for dieldrin and heptachlor epoxide in dairy products.

Carr, R.L. (1970) *J. Assoc. Off. Anal. Chem.* **53**, 152-154. Collaborative study leading to AOAC official final action status for BHC, p,p'-DDE, p,p'-TDE, o,p'-DDT, p,p'-DDT, lindane, and methoxychlor in dairy products.

Krause, R.T. (1973) *J. Assoc. Off. Anal. Chem.* 5**6**, 721-727. Collaborative study leading to AOAC official final action status for Perthane in dairy products.

Sawyer, L.D. (1978) *J. Assoc. Off. Anal. Chem.* **61**, 282-291. Collaborative study leading to AOAC official final action status for polychlorinated biphenyls in dairy products.

AOAC official method reference: *Official Methods of Analysis of the AOAC* (1990) 15th ed., 970.52, A, B, E, H, I, J, L, N, O, and R.

E3 + C1 + DG1

Validation report:

Wells, C. (1967) *J. Assoc. Off. Anal. Chem.* **50**, 1205-1215. Validation study leading to AOAC official final action status for dieldrin and heptachlor epoxide in vegetable oils.

AOAC official method reference: *Official Methods of Analysis of the AOAC* (1990) 15th ed., 970.52 A, B, E, H, I, J, L, N, O, and R.

E1 + C6 + DG1 and E3 + C6 + DG1

Validation report:

Griffitt, K.R., *et al.* (July 1983) "Miniaturized Florisil Column Cleanup of Chlorinated and Organophosphate Eluates in Total Diet Samples," LIB 2722, FDA, Rockville, MD.

E1 EXTRACTION OF FAT WITH SODIUM SULFATE, PETROLEUM ETHER

Reference

Porter, M.L., *et al.* (1970) *J. Assoc. Off. Anal. Chem.* **53**, 1300-1303

Principles

Fat and residues are removed from fish and animal tissue by dissolving them in petroleum ether. Anhydrous sodium sulfate removes water from the tissue and helps to disintegrate the sample.

Apparatus

blender, high-speed; explosion-proof Waring Blendor, 1 qt jar

Büchner funnel (Büchner), porcelain, 12 cm diameter

chromatographic column, 25 mm id × 50 mm, plain

filter paper, Shark Skin®, to fit Büchner funnel

Kuderna-Danish concentrator (K-D), 500 mL or 1 L, with Snyder column, plain receiving flask

vacuum filtration flask, 500 mL

Reagents

boiling chips, 20-30 mesh carborundum

petroleum ether, distilled from all-glass apparatus

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

Directions

- (Sample size may be adjusted to provide weight of fat appropriate to cleanup step.) Weigh 25-50 g thoroughly ground and mixed fish or animal tissue into blender jar. Add 100 g sodium sulfate.
- Alternately blend and mix with spatula until sample and sodium sulfate are well mixed. Scrape down sides of blender jar and break up caked material with spatula.
- Add 150 mL petroleum ether and blend at high speed 2 min.
- Decant petroleum ether supernate through Büchner fitted with two Shark Skin® papers and filter with suction into vacuum filtration flask.
- Scrape down sides of blender jar and break up caked material with spatula.
- Re-extract residue in blender jar with two 100 mL portions petroleum ether, blending 2 min each time. (After 1 min blending, stop blender, scrape material from sides of blender jar and break up caked material with spatula; continue blending 1 min.) Scrape down sides of blender jar and break up caked material between extractions. Decant petroleum ether supernates through Büchner and combine with first extract.
- After last blending, transfer residue from blender jar to Büchner, rinsing blender jar and material in Büchner with three 25-50 mL portions petroleum ether. Immediately after last rinse, press residue in Büchner with bottom of clean beaker to force out remaining petroleum ether.

- Pour combined extracts and rinses through 25 mm × 50 mm column of sodium sulfate and collect eluate in K-D with plain tube. Wash flask and then column with small portions petroleum ether.
- Add boiling chip to K-D and evaporate most of petroleum ether from combined extracts and rinses.
- Clean up extracted fat using one of the cleanup steps.

E2 SMALL SCALE EXTRACTION OF FAT WITH SODIUM SULFATE, PETROLEUM ETHER

Reference

Erney, D.R. (1974) *J. Assoc. Off. Anal. Chem.* **57**, 576-579

Principles

Fat and residues are removed from fish and animal tissue by dissolving them in petroleum ether. Anhydrous sodium sulfate removes water from the tissue and helps to disintegrate the sample. Sample size and amounts of reagents are somewhat reduced from amounts used in E1, for subsequent small scale cleanup of C7.

Apparatus

funnel, glass

homogenizer, Sorvall/Omni type, with 400 mL cup

volumetric flask (volumetric), 250 mL

Reagents

glass wool, Pyrex, see Section 204 for handling directions

petroleum ether, distilled from all-glass apparatus

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

Directions

- Weigh 20 g thoroughly ground and mixed fish or animal tissue into homogenizer cup. Moisten 40 g sodium sulfate with petroleum ether and add to sample.
- Mix sample, using stirring rod, let stand 20 min, and mix again.
- Add 100 mL petroleum ether to sample and blend 1-2 min.
- Centrifuge balanced homogenizer cup at 2000 rpm 1-2 min to obtain clear petroleum ether extract.
- Place glass wool plug in funnel, overlay with 20 g sodium sulfate, and place funnel in 250 mL volumetric. Decant petroleum ether extract through layer of sodium sulfate into volumetric.
- Mix sample again with stirring rod, add 100 mL petroleum ether, and extract as before.
- Repeat extraction with 70 mL petroleum ether, combining all three extractions in same 250 mL volumetric.
- Dilute to volume with petroleum ether.
- Clean up with C7, Florisil chromatography on 4 g column. No intermediate separation step is required if ≤0.2 g fat is used, as specified in C7.
- Note that this extraction yields a dilute solution of fat, rather than fat with most solvent removed as in other extraction modules. To clean up this extract with a module other than C7, solvent must first be removed.

E3 EXTRACTION OF FAT BY FILTERING

Reference

Mills, P.A. (1959) *J. Assoc. Off. Agric. Chem.* **42**, 734-740

Principle

Fat and residues are removed from butter by melting and filtering to remove solids. No extraction is needed for edible oil.

Apparatus

filter paper

funnel, glass

Directions

- Warm butter at about 50° C until fat separates; decant through dry filter paper placed in glass funnel. Collect oil.
- Clean up extracted fat using one of the cleanup steps.

E4 EXTRACTION OF FAT WITH SOLVENTS FROM DENATURED PRODUCT

Reference

Mills, P.A. (1959) *J. Assoc. Off. Agric. Chem.* **42**, 734-740

Principles

Fat and residues from cheese, milk, egg yolks, or dried whole eggs are dissolved in ethyl ether and petroleum ether after the product has been denatured with oxalate and alcohol. The ether extract is washed with large quantities of water to remove co-extractives.

(Methodology for high fat egg products has not been studied extensively; this method is recommended as the most applicable.)

Apparatus

blender, high-speed; explosion-proof Waring Blendor, 1 qt jar

centrifuge, explosion-proof, to hold 500 mL bottles

centrifuge bottle, glass, 500 mL. Use glass stopper or cover rubber stopper with aluminum foil to avoid contamination.

chromatographic column, 25 mm id × 50 mm, plain

delivery tube apparatus (Figure 304-b), fabricated in laboratory

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, plain receiving flask

separatory funnel (separator), 1 L

Reagents

alcohol, ethyl or methyl

ethyl ether, distilled from all-glass apparatus, with 2% ethanol as preservative; see Section 204 for peroxide test

1+1 (v/v) ethyl ether/petroleum ether

petroleum ether, distilled from all-glass apparatus

sodium chloride, reagent grade

sodium chloride aqueous solution, saturated

sodium (or potassium) oxalate, reagent grade

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

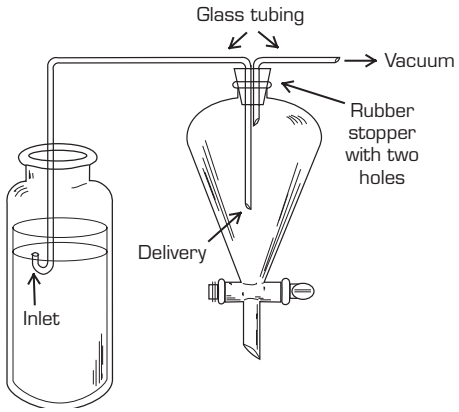
Directions

- *Cheese and Eggs*—If experience with product indicates emulsions will not be broken by centrifuging, add 1 mL water/2 g sample before blending. Place 25-100 g diced cheese or 25-50 g eggs (to provide about 3 g fat), about 2 g sodium or potassium oxalate, and 100 mL ethyl or methyl alcohol in blender jar and blend 2-3 min. Pour blender contents into centrifuge bottle.

  Milk — Dilute evaporated milk with equal amount of water. Place 100 g fluid milk or diluted evaporated milk into centrifuge bottle, add 100 mL ethyl or methyl alcohol and about 1 g sodium or potassium oxalate, and mix.

- To mixture in centrifuge bottle, add 50 mL ethyl ether and shake vigorously 1 min; then add 50 mL petroleum ether and shake vigorously 1 min.
- Centrifuge at about 1500 rpm about 5 min. Never leave stoppers in bottles during centrifuging.
- Transfer solvent layer, with delivery tube apparatus, into separator containing 500-600 mL water and 30 mL saturated sodium chloride solution. Re-extract aqueous residue twice, shaking vigorously with 50 mL portions (1+1) ethyl ether/petroleum ether; centrifuge and transfer solvent layer into separator after each extraction.
- Cautiously mix combined extracts and water. Drain and discard water.
- Gently rewash solvent layer with two 100 mL portions water, discarding water each time. If emulsions form, add about 5 mL saturated sodium chloride solution to solvent layer or include sodium chloride with water wash.
- Pour ether solution through 25 mm × 50 mm column of sodium sulfate and collect eluate in K-D with plain tube. Wash separator and then column with small portions petroleum ether.
- Add boiling chip to K-D and evaporate most of petroleum ether from combined extracts and rinses.
- Clean up extracted fat using one of the cleanup steps.

Figure 304-b
Delivery Tube Apparatus



Glass tube, inserted in one hole of two-hole rubber stopper, is used to draw upper solvent layer from centrifuge bottle into separatory funnel. Siphon tube is straight or bent in U-shape and inlet end placed at interface of two phases in centrifuge bottle. Second hole in stopper is fitted with another glass tube. Vacuum drawn through second tube causes upper phase from centrifuge bottle to transfer into separator.

(Corrigan, E. (Nov. 1963) (FDA) *Bureau By-Lines* **5**, 20; Sawyer, L.D., and Baca, J.R. (May 1978) LIB 2188, FDA, Rockville, MD.)

E5 EXTRACTION OF FAT WITH SOLVENTS

Reference

Sawyer, L.D. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 1122-1128

Principles

Fat and residues are removed from ground oilseeds, high fat feeds or feed materials, grains, or nuts by dissolving in petroleum ether and ethyl ether, followed by ethyl alcohol. The organic extract is washed with large quantities of water to remove co-extractives.

Apparatus

centrifuge, explosion-proof, to hold 500 mL bottles

chromatographic column, 25 mm id, plain

homogenizer, Polytron Model PT 10-35, with PT 35K generator containing knives, head equipped with metal (not Teflon) bushing or Sorvall/Omni type

homogenizer jar, Sorvall/Omni stainless steel cup, 400 mL, or centrifuge bottle, glass, 500 mL

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, plain receiving flask

separatory funnels (separators), 1 L

Reagents

alcohol, ethyl

ethyl ether, distilled from all-glass apparatus, with 2% ethanol as preservative; see Section 204 for peroxide test

1+1 (v/v) ethyl ether/petroleum ether

petroleum ether, distilled from all-glass apparatus

sodium chloride, reagent grade

sodium chloride aqueous solution, saturated

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

Directions

- Weigh 50 g ground sample of oilseeds, high fat feeds or feed materials, grains, or nuts in Sorvall/Omni cup or in centrifuge bottle. Add 200 mL petroleum ether and blend 1.5 min at high speed with Polytron PT 10-35 homogenizer or Omni-Mixer.
- Centrifuge extraction vessel and decant solvent into 500 mL beaker. Never leave stoppers in bottles during centrifuging.
- Add 150 mL (1+1) ethyl ether/petroleum ether to vessel and blend; centrifuge and decant into same beaker as before.
- Set beaker containing combined ethers under gentle air stream to concentrate to about 100 mL.
- Re-extract residue in extraction vessel with 150 mL ethyl alcohol for 1.5 min. Centrifuge and decant solvent into 1 L separator.

- Add 50 mL ethyl alcohol to extraction vessel, wash residue in vessel by gently blending, then centrifuge and decant into same separator as before.
- Add concentrated ethers from first two extractions to separator. Rinse beaker using small (about 5 mL) petroleum ether washes and add washes to separator.
- Mix separator contents well and add 600 mL water and about 40 mL saturated sodium chloride solution.
- Hold separator in horizontal position and mix thoroughly 45 sec. Let layers separate and drain aqueous portion into second 1 L separator containing 100 mL petroleum ether.
- Mix contents of second separator thoroughly about 15 sec and let layers separate. Drain and discard aqueous portion and drain petroleum ether into original separator. Wash combined ethers with two 100 mL portions water.
- Pour ether solution through 25 mm × 50 mm column of sodium sulfate and collect eluate in K-D with plain tube. Wash separator and then column with small portions petroleum ether.
- Add boiling chip to K-D and evaporate most of petroleum ether from combined extracts and rinses in K-D.
- Clean up extracted fat using one of the cleanup steps.

C1 ACETONITRILE-PETROLEUM ETHER PARTITIONING, FLORISIL COLUMN CLEANUP, THREE MIXED ETHER ELUANTS

Reference

Mills, P.A. (1959) *J. Assoc. Off. Agric. Chem.* **42**, 734-740

Principles

Extracted fat is carefully weighed to avoid overloading the capacity of the cleanup step. Pesticide residues are isolated from fat by partition between petroleum ether and acetonitrile. Most of the fat is retained in petroleum ether while residues partition into acetonitrile in proportion to their partitioning coefficient in that system. In the subsequent step, residues in acetonitrile are partitioned back into petroleum ether when added water reduces their solubility in acetonitrile.

Residues in solution are separated from sample co-extractives on a column of Florisil adsorbent; eluants of increasing polarity sequentially remove residues from the column.

Cleanup steps C2, C3, and C4 offer alternative Florisil elution systems. The eluants used in C2 produce different elution patterns than C1, which can sometimes be valuable for confirmation. C2 can also be used as an additional cleanup step.

C3 or C4 are used for analyses directed at the determination of polychlorinated biphenyls. The Florisil column is eluted with petroleum ether prior to elution with the other mixtures, in order to separate the polychlorinated biphenyls from most pesticide residues and to provide a cleaner extract for their determination.

Apparatus

chromatographic column, 25 mm id × 50 mm, plain

chromatographic column, 22 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, volumetric or graduated receiving flask

separatory funnels (separators), 125 mL and 1 L

Reagents

acetonitrile, distilled from all-glass apparatus; see Section 204 for distillation directions

acetonitrile saturated with petroleum ether

boiling chips, 20-30 mesh carborundum

ethyl ether, distilled from all-glass apparatus, with 2% ethanol as preservative; see Section 204 for peroxide test

Florisil, PR grade; see Section 204 for handling and testing directions and calculation of lauric acid value

hexane, distilled from all-glass apparatus

methylene chloride, distilled from all-glass apparatus

petroleum ether, distilled from all-glass apparatus

sodium chloride, reagent grade

sodium chloride aqueous solution, saturated

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

eluants: 6% (v/v) ethyl ether/petroleum ether
15% (v/v) ethyl ether/petroleum ether
50% (v/v) ethyl ether/petroleum ether

Directions

- Cleanup is applicable to ≤3 g fat. Evaporate solvent from solution of extracted fat and determine amount of fat extracted by one of the following operations:
  - When total amount of fat in sample is expected to be >3 g and analysis is not for volatile residues, transfer concentrated solution to tared beaker, using small amounts of petroleum ether, and evaporate to dryness at steam bath temperature under current of dry air. Weigh and record weight of fat extracted. Take ≤3 g fat for cleanup. Calculate weight of sample analyzed as:

$$\frac{\text{weight fat taken for cleanup}}{\text{weight fat extracted}} \times \text{weight original sample}$$

  - When it is known that amount of fat in sample is <3 g, do not evaporate solvent further but clean up total amount of fat solution. Calculate weight of sample analyzed as: weight original sample.
  - When it is known that fat content is >3 g, or that residue level is high, do not evaporate solvent further. Adjust to known, appropriate volume, transfer aliquot to tared beaker, evaporate solvent from aliquot, and weigh to determine fat content. Clean up aliquot of volume containing ≤3 g fat. Calculate weight of sample analyzed as:

$$\frac{\text{volume fat solution taken for cleanup}}{\text{total volume of solution}} \times \text{weight original sample}$$

  - When analysis for volatile chemicals is desirable, do not evaporate petroleum ether at steam bath temperature. Adjust to known, appropriate volume, transfer aliquot to tared beaker, evaporate solvent from aliquot, and weigh to determine fat content. Clean up remaining solution or appropriate aliquot. Calculate weight of sample analyzed as:

$$\frac{\text{weight fat solution taken for cleanup}}{\text{total volume of solution}} \times \text{weight original sample}$$

Acetonitrile/Petroleum Ether Partitioning

- Weigh ≤3 g fat into 125 mL separator, and add petroleum ether so that total volume of fat and petroleum ether in separator is 15 mL. Take smaller weight of fish oil if experience indicates tendency to emulsion formation during partitioning.
- Add 30 mL acetonitrile saturated with petroleum ether, shake vigorously 1 min, let layers separate, and drain acetonitrile into 1 L separator containing 650 mL water, 40 mL saturated sodium chloride solution, and 100 mL petroleum ether.

- Extract petroleum ether solution in 125 mL separator with three additional 30 mL portions acetonitrile saturated with petroleum ether, shaking vigorously 1 min each time, and combine all extracts in the 1 L separator.
- Hold 1 L separator in horizontal position and mix thoroughly 30-45 sec. Let layers separate and drain aqueous layer into second 1 L separator.
- Add 100 mL petroleum ether to second 1 L separator, shake vigorously 15 sec, and let layers separate. Discard aqueous layer, combine petroleum ether with petroleum ether in original 1 L separator, and wash with two 100 mL portions water.
- Discard washings and drain petroleum ether layer through 25 mm × 50 mm column of sodium sulfate into K-D. Rinse separator and then column with three 10 mL portions petroleum ether.
- Add boiling chip to K-D and evaporate combined extract and rinses to 5-10 mL for transfer to Florisil column.

Florisil Column Cleanup

- Place activated Florisil (4" or weight determined by lauric acid value) in 22 mm id column; add about 0.5" sodium sulfate. Prewet column with 40-50 mL petroleum ether. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Transfer sample extract solution to column, letting it pass through at about 5 mL/min. Rinse container (and sodium sulfate if present) with two 5 mL portions petroleum ether, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions petroleum ether.
- Elute column at about 5 mL/min with 200 mL 6% ethyl ether/petroleum ether eluant.
- Change K-Ds and elute at about 5 mL/min with 200 mL 15% ethyl ether/petroleum ether eluant.
- Change K-Ds and elute at about 5 mL/min with 200 mL 50% ethyl ether/petroleum ether eluant.
- Add boiling chips to K-Ds and concentrate each eluate to suitable definite volume. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during final evaporation in receiving flask.
- Use appropriate determinative steps, such as DG1 or DG13, DG7, and DG10, to identify and measure residues. First eluate (6%) is usually suitable for GLC determination without further cleanup.

ALTERNATIVES:

C2 ACETONITRILE-PETROLEUM ETHER PARTITIONING, FLORISIL COLUMN CLEANUP, THREE METHYLENE CHLORIDE ELUANTS

Reference

Mills, P.A., *et al.* (1972) *J. Assoc. Off. Anal. Chem.* **55**, 39-43

Principles

Florisil is eluted with mixtures of methylene chloride, hexane, and acetonitrile. The resulting second eluate is cleaner than the second eluate of C1, although 90% of the fat placed on the column is eluted by the third eluant. The eluants can elute pesticide chemicals of a greater polarity than can be eluted by C1. C2 is preferred for analysis of fats and oils, for residues of endosulfan, and for separation of heptachlor epoxide and octachlor epoxide.

Additional Reagents

eluants: 1—20% methylene chloride/hexane (v/v). Dilute 200 mL methylene chloride with hexane. Allow mixture to reach room temperature, and adjust volume to 1 L with hexane.

2—50% methylene chloride/0.35% acetonitrile/49.65% hexane (v/v/v). Pipet 3.5 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

3—50% methylene chloride/1.5% acetonitrile/48.5% hexane (v/v/v). Pipet 15 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

Directions

- Follow C1 above, except replace directions for Florisil cleanup with the following:
- Place activated Florisil (4" or weight determined by lauric acid value) in 22 mm id column; add about 0.5" sodium sulfate. Prewet column with 40-50 mL hexane. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Transfer sample extract solution to column, letting it pass through at about 5 mL/min. Rinse container (and sodium sulfate if present) with two 5 mL portions hexane, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions hexane.
- Elute column at about 5 mL/min with 200 mL eluant 1.
- Change K-Ds and elute with 200 mL eluant 2.
- Change K-Ds and elute with 200 mL eluant 3.
- Add boiling chips to K-Ds and concentrate each eluate to suitable definite volume. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during evaporation.

C3 ACETONITRILE-PETROLEUM ETHER PARTITIONING, FLORISIL COLUMN CLEANUP, PETROLEUM ETHER AND THREE MIXED ETHER ELUANTS

Reference

Reynolds, L.M. (1969) *Bull. Environ. Contam. Toxicol.* **4**, 128-143

Principles

Polychlorinated biphenyls are separated from most pesticide residues by elution of Florisil column with petroleum ether prior to elution with ethyl ether/petroleum ether mixtures.

Directions

- Follow C1 above, except insert the following before elution of Florisil column with 6% ethyl ether/petroleum ether:
- Elute column at about 5 mL/min with 250 mL petroleum ether. Change K-Ds.

C4 ACETONITRILE-PETROLEUM ETHER PARTITIONING, FLORISIL COLUMN CLEANUP, PETROLEUM ETHER AND THREE METHYLENE CHLORIDE ELUANTS

Reference

Reynolds, L.M. (1969) *Bull. Environ. Contam. Toxicol.* **4**, 128-143

Principles

Polychlorinated biphenyls are separated from most pesticide residues by elution of Florisil column with petroleum ether prior to elution with methylene chloride mixtures.

Directions

- Follow C3 above, except insert the following before elution of Florisil column with eluant 1:
- Elute column at about 5 mL/min with 250 mL petroleum ether. Change K-Ds.

C5 GEL PERMEATION CHROMATOGRAPHY (GPC)

References

Griffitt, K.R., and Craun, J.C. (1974) *J. Assoc. Off. Anal. Chem.* **57**, 168-172

Hopper, M.L (1982) *J. Agric. Food Chem.* **30**, 1038-1041

Principles

Fat is separated from residues by gel permeation (size exclusion) chromatography. The solution of fat extracted from fatty food is placed on a column and eluted with solvents. The fat is eluted first and discarded, leaving residues in the next portion of eluant.

Cleanup C6 offers optional Florisil column cleanup subsequent to GPC.

Apparatus

filtration device for solutions, 10 mL syringe with Luer-Lok tip, fitted with either (a) 13 mm diameter Swinny stainless steel filter holder and 13 mm diameter filters, 5.0 µm LS-type, or (b) disposable membrane filters, 25 mm diameter, 5 µm Teflon membrane, encased in polypropylene. (Pre-assembled devices that do not require a syringe are also available.)

GPC apparatus; automated equipment optional but recommended. GPC apparatus must include:

1) sample introduction valve
2) pump, low pressure, suitable for use with organic solvents, capable of 5 mL/min flow
3) sample loading loop, 1/16" Teflon tubing coiled in cylindrical form, about 13 mL capacity
4) pulse dampener, about 6' of 1/8" copper tubing coiled and closed at one end, installed between pump and sample introduction valve with a connecting tee. Pulse dampener is needed only when pump is not pulseless.

GPC column, glass, 25 mm id × 300 or 500 mm with organic solvent plunger kit

GPC syringe, 10 mL syringe with Luer-Lok tip, with Millipore Swinny stainless steel adapter, Millipore 5.0 µm LS-type filter

graduated cylinder (graduate), glass-stoppered (g-s), calibrated

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated receiving flask

Reagents

acetone, distilled from all-glass apparatus

Bio-Beads SX-3 resin, 200-400 mesh (Bio-Rad Laboratories, Richmond, CA; pretested resin is available from ABC Laboratories)

hexane, distilled from all-glass apparatus

methylene chloride, distilled from all-glass apparatus

eluant: 50% (v/v) methylene chloride/hexane

Preparation of GPC Column

- Weigh 35 g Bio-Beads SX-3 into 400 mL beaker.
- Add 150 mL 50% methylene chloride/hexane.
- Stir beads with glass or steel rod until all beads have swelled and no clumps are present.
- Pour slurry into GPC column with aid of stirring rod.
- Hold column in upright position with plunger tightened about 25 mm from bottom of usable length of column, ignoring threaded ends.
- Continuously add slurry to column so beads never become completely settled until all beads have been added.
- Place other plunger in column after beads have settled and liquid has drained off.
- Compress each plunger equal distance from its respective end until bed length is about 200 mm.
- Connect column to GPC solvent delivery system, and pump solvent from bottom to top of column until all air is expelled.
- Adjust flow rate of system to 5 mL/min and check column pressure. Adjust operating pressure for column to 8-11 psig by moving plunger(s).
- Allow GPC system to equilibrate by pumping solvent through it.
- Re-adjust flow rate to 5 mL/min if it has changed.

Calibration of GPC Column

Elution of Fat

- Melt and filter butter through fluted filter paper into suitable container.
- Weigh 5 g warm filtered butter (do not include water layer) into 25 mL g-s graduate; dilute to 25 mL with 50% methylene chloride/hexane; mix until fat is dissolved (0.2 g fat/mL).
- Filter fat solution through filtration device and load 5 mL fat solution onto GPC column.
- Elute with 50% methylene chloride/hexane.
- Collect column effluent in tared beakers in 10 mL increments from 0 to 100 mL.
- Evaporate solvent, cool and weigh beakers to calculate amount fat eluted in each 10 mL increment. (For manual GPC, collect 10 mL fractions in separate graduates and transfer to tared beakers for evaporation and calculation of fat.)
- Most (98%) fat should elute in first 60 mL. If >5% of fat appears in 60-70 mL fraction or later, reject column and prepare new one by repacking with original batch of beads. Visual evaluation of yellow fat band as it passes through column usually shows tailing or streaking when column is inadequate. Use new batch of beads if second column is still inadequate.

Elution of Pesticides

- Prepare mixed standard solution containing 0.2 µg diazinon/mL, 0.6 µg ethion/mL, 0.1 µg lindane/mL, 0.4 µg parathion/mL, and 0.05 µg pentachloroaniline/mL in 50% methylene chloride/hexane.
- Filter mixed standard solution through filtration device and load 5 mL onto GPC column
- Elute with 50% methylene chloride/hexane.
- Collect 10 mL fractions from 0 through 160 mL.
- Transfer each fraction to K-D fitted with graduated receiving flask and add 50 mL hexane and 2-3 boiling chips; concentrate each to 10 mL.
- Use determinative steps DG2 and DG3 (Section 302) to calculate recoveries; column is normal if diazinon and ethion start to elute in either 50-60 mL or 60-70 mL fraction, and lindane starts to elute in 90-100 mL fraction.
- Determine what volume should be discarded (usually first 60 mL) and what should be collected (usually 60-160 mL fraction) by examining fat and mixed standard elution profiles developed above. Use these calibrated fraction volumes in subsequent calibration steps and in sample cleanup.

Directions

- Use GPC column prepared and calibrated as described above. Column can be used repeatedly.
- Method is applicable to ≤1 g fat sample in 5 mL; better cleanup is provided if weight of fat in 5 mL is restricted to 0.75 g.
- Concentrate solution of extracted fat to small volume. Add 100 mL methylene chloride and reconcentrate.
- Based on estimate of weight of fat in extract, use calibrated graduate large enough to create final solution containing ≤0.2 g/mL. Transfer concentrated solution to graduate. Add enough methylene chloride to provide half the final volume, then fill to final volume with hexane.
- Pipet 1 mL into tared beaker; evaporate solvent and weigh. If weight is >0.2 g, adjust concentration of remaining solution to ≤0.2 g/mL with 50% methylene chloride/hexane.
- Centrifuge cloudy solutions before loading them onto GPC. Filter all solutions through filtration device before GPC.
- Fill GPC sample loading loops with extract using GPC syringe. Load 5 mL fat solution (equivalent to ≤1 g fat) onto GPC column. Load more than one loop with same solution if needed to increase final total sample equivalent.
- Elute column with 160 mL 50% methylene chloride/hexane. Collect and discard volume previously calibrated to contain fat. Separately collect final portion, previously calibrated to contain residues.
- Transfer GPC eluate to K-D with 5 mL graduated receiving flask and concentrate to <3 mL. Add hexane and reconcentrate to <1 mL.

- Use appropriate determinative steps to identify and measure residues. Dilute final concentrated eluate with acetone for determination of organophosphorus residues by DG2 or DG14 (Section 302). Dilute with hexane for determination of organohalogen residues by DG3 or DG16. Clean up on Florisil (C6) before determination of residues with electron capture detector (DG1, 7, 10, and 13).

ALTERNATIVE:

C6 GPC, FLORISIL COLUMN (4 G) CLEANUP, THREE METHYLENE CHLORIDE ELUANTS

Reference

Griffitt, K.R., *et al.* (July 1983) "Miniaturized Florisil Column Cleanup of Chlorinated and Organophosphate Eluates in Total Diet Samples," LIB 2722, FDA, Rockville, MD

Principles

For additional cleanup of samples, residues are further separated from sample coextractives on a small column of Florisil adsorbent; three eluants of increasing polarity sequentially remove residues from the column.

Additional Apparatus

chromatographic column, 10 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

Additional Reagents

acetonitrile, distilled from all-glass apparatus; see Section 204 for distillation directions

Florisil, PR grade; see Section 204 for handling and testing directions and calculation of lauric acid value

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

eluants: 1—20% methylene chloride/hexane (v/v). Dilute 200 mL methylene chloride with hexane. Allow mixture to reach room temperature, and adjust volume to 1 L with hexane.

2—50% methylene chloride/0.35% acetonitrile/49.65% hexane (v/v/v). Pipet 3.5 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

3—50% methylene chloride/1.5% acetonitrile/48.5% hexane (v/v/v). Pipet 15 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

Directions

- Follow directions of C5 up through elution of GPC column. Concentrate GPC eluate to <5 mL.
- Place activated Florisil (weight = 110/lauric acid value × 4 g) in 10 mm chromatographic column; add about 2 cm sodium sulfate. Completely open stopcock and tap column to settle adsorbent. Prewet column with 5 mL hexane. Do not allow column to go dry. Place K-D with volumetric or graduated receiving flask under column to receive eluate.

- Transfer concentrated GPC eluate to column, letting it pass through at about 5 mL/min. Rinse container with two 3 mL portions hexane, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions hexane.
- Elute column at about 5 mL/min with 35 mL eluant 1.
- Change K-Ds and elute with 35 mL eluant 2.
- Change K-Ds and elute with 40 mL eluant 3.
- Concentrate each eluate to suitable definite volume in K-D. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during evaporation.

C7 FLORISIL COLUMN (4 G) CLEANUP, TWO MIXED ETHER ELUANTS, OPTIONAL ALKALINE HYDROLYSIS

References

Erney, D.R. (1974) *J. Assoc. Off. Anal. Chem.* **57**, 576-579

Erney, D.R. (1983) *J. Assoc. Off. Anal. Chem.* **66**, 969-973

Principles

Residues are separated from fat on a small column of Florisil adsorbent. Because no prior isolation step is included to minimize co-extractives, only 0.2 g extracted fat is placed on the Florisil column. Two eluants of increasing polarity sequentially remove residues from the column. The second eluate is cleaned up further with alkaline hydrolysis if needed, a step applicable only to chemicals stable to hot alkali.

Apparatus

chromatographic column, 10 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

Kuderna-Danish concentrator (K-D), 125 mL, with Snyder column, two-ball micro-Snyder column, graduated and 10 mL volumetric receiving flask

Reagents

boiling chips, 20-30 mesh carborundum

ethyl ether, distilled from all-glass apparatus, with 2% ethanol as preservative; see Section 204 for peroxide test

Florisil, PR grade; see Section 204 for handling and testing directions and calculation of lauric acid value

hexane, distilled from all-glass apparatus

petroleum ether, distilled from all-glass apparatus

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

eluants: 6% (v/v) ethyl ether/petroleum ether

15% (v/v) ethyl ether/petroleum ether

2% (w/v) potassium hydroxide in methanol or ethanol

1+1 (v/v) water/alcohol

Directions

Florisil Column Cleanup

- Cleanup is applicable to ≤0.2 g fat.
- Concentration of fat in extract prepared by E2 is about ≤0.2 g in 25 mL. To use this cleanup for extracts prepared by E1, E3, E4, or E5, first dilute all or part of extracted fat to ≤0.2 g in 25 mL.
- Transfer 25 mL aliquot to tared beaker and place on steam bath until solvent is evaporated. Leave on steam bath additional 30 min, remove, and cool. Weigh fat.
- If extract contains ≤0.2 g/25 mL, transfer 25 mL aliquot to 125 mL K-D with graduated receiving flask. If extract contains >0.2 g/25 mL, transfer volume containing ≤0.2 g fat to K-D.

- Add several boiling chips and concentrate solution to about 3 mL on steam bath. Let cool, remove Snyder column, rinse K-D with two 1 mL portions petroleum ether. Use current of air to concentrate sample to 3 mL.
- Place activated Florisil (weight = 110/lauric acid value× 4 g) in 10 mm id glass column; add about 2 cm sodium sulfate. Completely open stopcock, tap tube to settle adsorbent, and mark tube 1 cm above sodium sulfate layer.
- Add about 25 mL petroleum ether to Florisil column. As solvent level reaches mark, place 125 mL K-D with 10 mL volumetric receiving flask under column.
- Using disposable pipet, transfer 3 mL concentrated sample extract to column; wash flask with 1 mL petroleum ether and add wash to column. Solvent level must not go below mark; temporarily close stopcock if necessary.
- Elute column with 35 mL 6% ethyl ether/petroleum ether.
- When solvent level reaches mark, change K-Ds. Elute column with 35 mL 15% ethyl ether/petroleum ether.
- Concentrate each eluate to suitable definite volume in K-D. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during evaporation.
- Use appropriate determinative steps, such as DG1 or DG13, DG7, and DG10, to identify and measure residues. Second eluate may need further cleanup prior to GLC; use optional alkaline hydrolysis if residues are stable to hot alkali.

Optional Alkaline Hydrolysis

- Concentrate 15% ethyl ether/petroleum ether eluate to 2 mL with current of air.
- Add 1 mL 2% potassium hydroxide/alcohol, attach micro-Snyder column to flask, and carefully reduce volume to ≤1 mL on steam bath. Reflux 15 min, then let cool.
- Add 2 mL (1+1) water/alcohol and 5 mL hexane to flask, and shake 1 min. Centrifuge to separate layers.
- Transfer as much hexane layer as possible to second flask, using disposable pipet.
- Add another 5 mL hexane to flask, and repeat extraction.
- Concentrate combined hexane extracts to appropriate volume for determination.

C8 DISPERSION ON ALUMINA, FLORISIL COLUMN CLEANUP, THREE MIXED ETHER ELUANTS

References

Luke, M.A., and Doose, G.M. (Jan. 1978) "A Rapid Analysis for Pesticide Residues in Milk and Other Fatty Foods," LIB 2120A, FDA, Rockville, MD

Gillespie, A.M., and Walters, S.M. (May 1983) "An Alumina Blending Technique for the Separation of Pesticides from Lipids (Based on LIB 2120A)," LIB 2716, FDA, Rockville, MD

Principles

Extracted fat is dispersed on deactivated alumina and pesticide residues are removed with a mixture of water and acetonitrile; most of the fat is retained by the alumina. In the subsequent step, residues in acetonitrile are partitioned back into petroleum ether when added water reduces their solubility in acetonitrile. Residues in solution are separated from remaining sample co-extractives on a column of Florisil adsorbent; three eluants of increasing polarity sequentially remove residues from the column.

Cleanup step C9 offers an alternative Florisil elution system, which produces different elution patterns than C8.

Apparatus

Büchner funnel (Büchner), porcelain, 12 cm diameter

chromatographic column, 25 mm id × 50 mm, plain

chromatographic column, 22 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc

filter paper, Whatman No. 40, 15 cm diameter. Wash paper by soaking in 20% water/acetonitrile to remove substances that interfere in GLC determination. Dry and store in closed container.

homogenizer, Sorvall/Omni type, with 400 mL cup

Kuderna-Danish concentrator (K-D), 500 mL, with Snyder column, two-ball micro-Snyder column, graduated and volumetric receiving flasks

separatory funnel (separator), 1 L

shaker, mechanical, Burrell wrist action

Reagents

acetonitrile, distilled from all-glass apparatus; see Section 204 for distillation directions

alumina, Fisher Adsorption Alumina, No. A-540, 80-200 mesh

boiling chips, 20-30 mesh carborundum

ethyl ether, distilled from all-glass apparatus, with 2% ethanol as preservative; see Section 204 for peroxide test

Florisil, PR grade; see Section 204 for handling and testing directions and calculation of lauric acid value

glass wool, Pyrex; see Section 204 for handling directions

hexane, distilled from all-glass apparatus

methylene chloride, distilled from all-glass apparatus

petroleum ether, distilled from all-glass apparatus

sodium chloride, reagent grade

sodium chloride aqueous solution, saturated

sodium sulfate, anhydrous, granular, reagent grade; see Section 204 for handling directions

20% (v/v) water/acetonitrile

eluants: 6% (v/v) ethyl ether/petroleum ether

15% (v/v) ethyl ether/petroleum ether

50% (v/v) ethyl ether/petroleum ether

Preparation of Alumina

- Wash alumina to remove substances that interfere in GLC determination. Soak in methylene chloride ≥1 hr; decant and discard methylene chloride. Soak in hexane ≥1 hr; decant and discard hexane. Dry alumina on steam bath.
- Place washed and dried alumina in 260° C muffle furnace 4 hr. Transfer to tightly closed container and withdraw convenient amounts (*e.g.*, 500 g) for deactivation. Add water incrementally to final concentration of ≥16% (w/w) but ≤19% (w/w), shaking briefly after each addition. Caution: heat will be produced when water is added. Shake ≥4 hr on mechanical shaker. Store in tightly closed container. Alumina thus prepared has been found to be stable for at least 4 mon.

Directions

Dispersion on Alumina

- Cleanup is applicable to ≤2 g fat.
- Transfer concentrated solution of extracted fat to tared beaker, using small amounts of petroleum ether, and evaporate to dryness at steam bath temperature under current of dry air. Weigh and record weight of fat extracted. Calculate weight of sample analyzed as:

$$\frac{\text{weight fat taken for cleanup}}{\text{weight fat extracted}} \times \text{weight original sample}$$

- Weigh ≤2 g fat into 150 mL beaker containing 50 g deactivated alumina; mix well.
- Transfer mixture to homogenizer cup and add 350 mL 20% water/acetonitrile. Blend 2-4 min at high speed.
- Transfer most of contents, without rinsing, to Buchner fitted with filter paper; filter without suction. Alternatively, centrifuge blended contents 5 min at 1500 rpm and decant supernate through glass funnel with glass wool.
- Record mL solvent recovered; calculate g sample as:

$$\text{g sample} = \text{g fat} \times \frac{\text{mL solvent recovered}}{350}$$

Partitioning

- Transfer filtrate to 1 L separator containing 100 mL petroleum ether and shake vigorously 30 sec.
- Add 10 mL saturated sodium chloride solution and 500 mL water.
- Hold 1 L separator in horizontal position and thoroughly mix 30-45 sec. Let layers separate and drain aqueous layer into second 1 L separator.
- Add 100 mL petroleum ether to second 1 L separator, shake vigorously 15 sec, and let layers separate. Discard aqueous layer, combine petroleum ether with petroleum ether in original separator, and wash with two 100 mL portions water.
- Discard washings and drain petroleum ether layer through 25 mm × 50 mm column sodium sulfate into 500 mL K-D. Rinse separator and then column with three 10 mL portions petroleum ether.
- Add boiling chip to K-D and concentrate combined extract and rinses to 5-10 mL for transfer to Florisil column.

Florisil Column Cleanup

- Place activated Florisil (4" or weight determined by lauric acid value) in 22 mm id glass column; add about 0.5" anhydrous sodium sulfate. Prewet column with 40-50 mL petroleum ether. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Transfer sample extract solution to column, letting it pass through at about 5 mL/min. Rinse container (and sodium sulfate if present) with two 5 mL portions petroleum ether, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions petroleum ether.
- Elute column at about 5 mL/min with 200 mL 6% ethyl ether/petroleum ether eluant.
- Change K-Ds and elute at about 5 mL/min with 200 mL 15% ethyl ether/petroleum ether eluant.
- Change K-Ds and elute at about 5 mL/min with 200 mL 50% ethyl ether/petroleum ether eluant.
- Add boiling chips to K-Ds and concentrate each eluate to suitable definite volume. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during final evaporation in receiving flask.
- Use appropriate determinative steps, such as DG1 or DG13, DG7, and DG10, to identify and measure residues.

ALTERNATIVE:

C9 DISPERSION ON ALUMINA, FLORISIL COLUMN CLEANUP, THREE METHYLENE CHLORIDE ELUANTS

Principles

Florisil is eluted with mixtures of methylene chloride, hexane, and acetonitrile. The resulting second eluate is cleaner than the second eluate of C8, although 90% of the fat placed on the column is eluted by the third eluant. The eluants can elute pesticide chemicals of a greater polarity than can be eluted by C8. C9 is preferred for analysis of fats and oils, for residues of endosulfan, and for separation of heptachlor epoxide and octachlor epoxide.

Additional Reagents

eluants: 1—20% methylene chloride in hexane (v/v). Dilute 200 mL methylene chloride with hexane. Allow mixture to reach room temperature, and adjust volume to 1 L with hexane.

2—50% methylene chloride/0.35% acetonitrile/49.65% hexane (v/v/v). Pipet 3.5 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

3—50% methylene chloride/1.5% acetonitrile/48.5% hexane (v/v/v). Pipet 15 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 L with hexane.

Directions

- Follow C8 above, except replace the directions for Florisil column cleanup with the following:
- Place activated Florisil (4" or weight determined by lauric acid value) in 22 mm id glass column; add about 0.5" anhydrous sodium sulfate. Prewet column with 40-50 mL hexane. Place K-D with volumetric or graduated receiving flask under column to receive eluate.
- Transfer sample extract solution to column letting it pass through at about 5 mL/min. Rinse container (and sodium sulfate if present) with two 5 mL portions hexane, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions hexane.
- Elute column at about 5 mL/min with 200 mL eluant 1.
- Change K-Ds and elute with 200 mL eluant 2.
- Change K-Ds and elute with 200 mL eluant 3.
- Add boiling chips to K-Ds and concentrate each eluate to suitable definite volume. When volume <5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during final evaporation in receiving flask.
- Use appropriate determinative steps, such as DG1 or DG13, DG7, and DG10, to identify and measure residues.

DETERMINATION

Inject concentrated extract equivalent to ≤3 mg fat (or 20 mg whole product) into the following GLC systems (Section 302) for determination of residues.

Minimum recommended determinations:

DG1 or **DG13**	residues with halogen, sulfur, or other moieties
or	
DG3 or **DG16**	organohalogen residues
DG10	late eluting residues, especially pyrethroids
or	
DG12	late eluting organohalogen residues, especially pyrethroids
DG7	early eluting residues with halogen, sulfur, or other moieties
DG2 or **DG14**	organophosphorus residues; large amounts of sulfur may interfere
DG4	organonitrogen residues; selective to nitrogen, but co-extractives may contain nitrogen
DG5 or **DG17**	organonitrogen and organophosphorus residues

For accurate quantitation, reference standards should be dissolved in same solvent as concentrated extract, only peaks >10% FSD should be measured, and peak sizes of residue and reference standard should match within ±25%.

See Chapter 5 for additional information about operation of GLC systems; Section 504 provides information about quantitation of residues.

See Section 205 for additional information about reference standards.

See Section 104 for additional information about reporting residues and determining compliance with regulations.

See Section 105 for additional information about analytical limits of quantitation.

CONFIRMATION

After residues have been tentatively identified and quantitated by comparison to appropriate reference standards, confirm identity according to principles discussed in Section 103. Use appropriate tables of data (PESTDATA, tables accompanying each method, Index to Methods) to choose most appropriate determinative steps.