Pesticide Analytical Manual Volume I
10/1999 Revisions

The following pages contain corrections or changes for PAM I. Print these pages and use them to replace the same current pages in the current PAM I 3rd edition (published 1/94, revised 9/96 and 10/99).

Each set of two pages is intended to appear on two sides of the same paper. Different versions of Acrobat Reader vary in their ability to print on both sides of the page. It may be necessary to print one page at a time and turn the paper over to print the second page on the reverse side.

This transmittal does not include updated tables, indices, or appendices. Those files can be found at http://vm.cfsan.fda.gov/~frf/pami2.html#tables.

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1. Title Page	i, ii (not numbered on page)	2, 3
2. Pages 205-1 to 205-2	205-1 to 205-2	4-5
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9. Page 304-1 to 304-2	304-1 to 304-2	84-85

Explanations of changes:

Section 205, page 2, is revised to clarify the directions for weighings of reference standards based on their purity.

Pages 301-1 through 300-5 are revised to include new method modules and to remove references to the DEGS packed GLC column, which is now considered obsolete (it is no longer commercially available).

Pages 302-1through 302-70 now include three additional extraction modules (E5-E7) and one additional cleanup module (C6). Determination modules DG20-DG23 have been removed because the DEGS column is now considered obsolete.

Pages 303-2 and 304-2 have been revised to reflect changes in previous page numbers and to remove DEGS column data.

PESTICIDE ANALYTICAL MANUAL

VOLUME I: Multiresidue Methods

U.S. Department of Health and Human Services • Public Health Service
Food and Drug Administration

PESTICIDE ANALYTICAL MANUAL VOLUME I

3rd Edition, 1994

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205: REFERENCE STANDARDS

The purity of reference standards and use of appropriate preparation and storage techniques for standard solutions significantly affect analytical results. Reliable and accurate data can be obtained only if correct analytical standard solutions are used for identification and quantitation. Each laboratory's quality assurance program plan (Section 206) should include an element on reference standards and standard solutions. Standard operating procedures (SOPs) should include protocols for obtaining, labeling, storing, and handling standards. This section provides rudimentary information that may be incorporated, as appropriate, into such documentation.

205 A: SOURCES

Reference standards are currently available from several commercial sources, including companies that supply only reference standards, suppliers of specialty laboratory chemicals, and suppliers of chromatographic equipment. Each company publishes lists of reference standards for pesticides, related metabolites, and certain industrial chemicals. Eligible laboratories, mainly Federal Government laboratories, may also obtain reference standards for some chemicals from a repository maintained, under contract, by EPA; eligibility is determined by EPA.

Reference standards in "neat" (undiluted) form, preferably certified by EPA, should be used whenever possible. If neat standards are not available, certified solutions of standards may be used.

205 B: EQUIPMENT AND SOLVENTS

Equipment

Equipment used for preparation and storage of reference standards and solutions includes the following essential, but not all-inclusive, items:

1) analytical balance calibrated for accuracy of ±0.05 mg

2) explosion-resistant refrigerator/freezer, used only to store standards

3) standard solution storage containers:

   a) amber colored, screw-cap bottles, 1 and 2 oz

   b) Teflon-lined caps for bottles

   c) vials for working standards

4) desiccators to store reference standards. Larger vials containing desiccant can be used as individual desiccators for vials of standards.

5) appropriate volumetric glassware, pipets, or microliter syringes

Solvents

Pesticide residue quality solvents are essential for preparation of reference standard solutions. Solvents should be checked before use for the presence of interfering substances by injecting the solvent into the determinative system(s) to be used.

Choice of solvent is sometimes restricted by solubility and stability of the particular chemical. The following solvents, in order of preference, should be used to prepare standard solutions, if suitable for the particular chemical: isooctane (2,2,4-trimethylpentane), hexane, acetone, isopropanol, and toluene.

205 C: STORAGE

Reference standards must be stored properly to prevent undesirable reactions, such as oxidation, re-arrangement, or hydrolysis. Improper storage can lead to loss of integrity of previously acceptable standards. Storage conditions must also prevent the possibility of external contamination. Storage requirements are dependent on the chemical and physical properties of the chemical of interest and are much more stringent for volatile, reactive, or unstable compounds. Review the physical and chemical properties of each compound to determine which storage conditions are appropriate. Minimum requirements for long term storage of analytical reference standards follow:

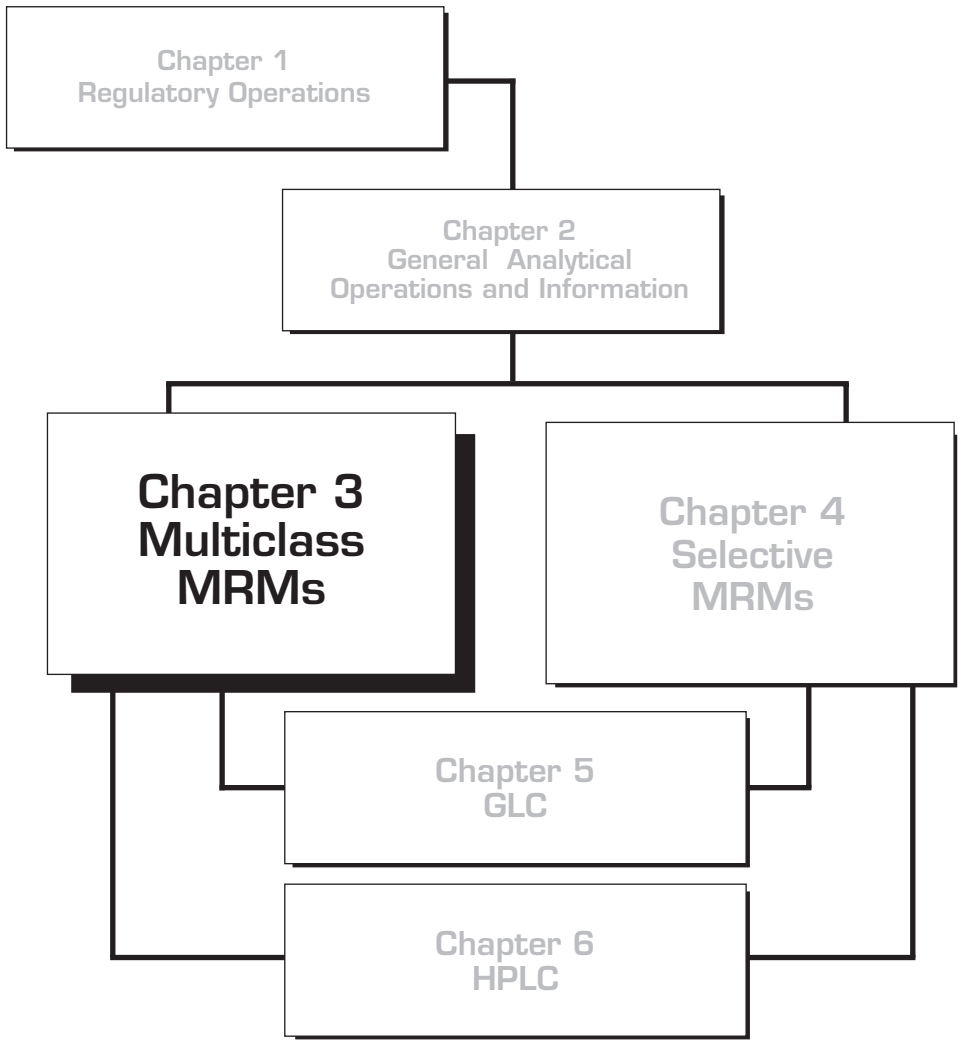
- If at all possible, store reference standards in tightly sealed containers under desiccation in a freezer.
- Store more stable compounds, such as organochlorine pesticides, in a refrigerator if freezer is not available.

Reference standards that have been stored in refrigerators or freezers must be brought to room temperature in a desiccator prior to weighing.

205 D: PURITY

The analyst is responsible for knowing the purity of the reference standard used to obtain reported data. Follow these rules for recording information about reference standard purity:

- Standards with known purity ≥99%: weight may be recorded as measured; it is not necessary to correct for purity.
- Standards with purity <99%: apply appropriate correction factor to measured weights.
- Technical standards with unknown purity (use only if this is the only available reference standard): record weight as measured, do not correct for purity, but include a note on the source and unknown purity of this standard with the results of any analysis whose results rely on this standard.



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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)

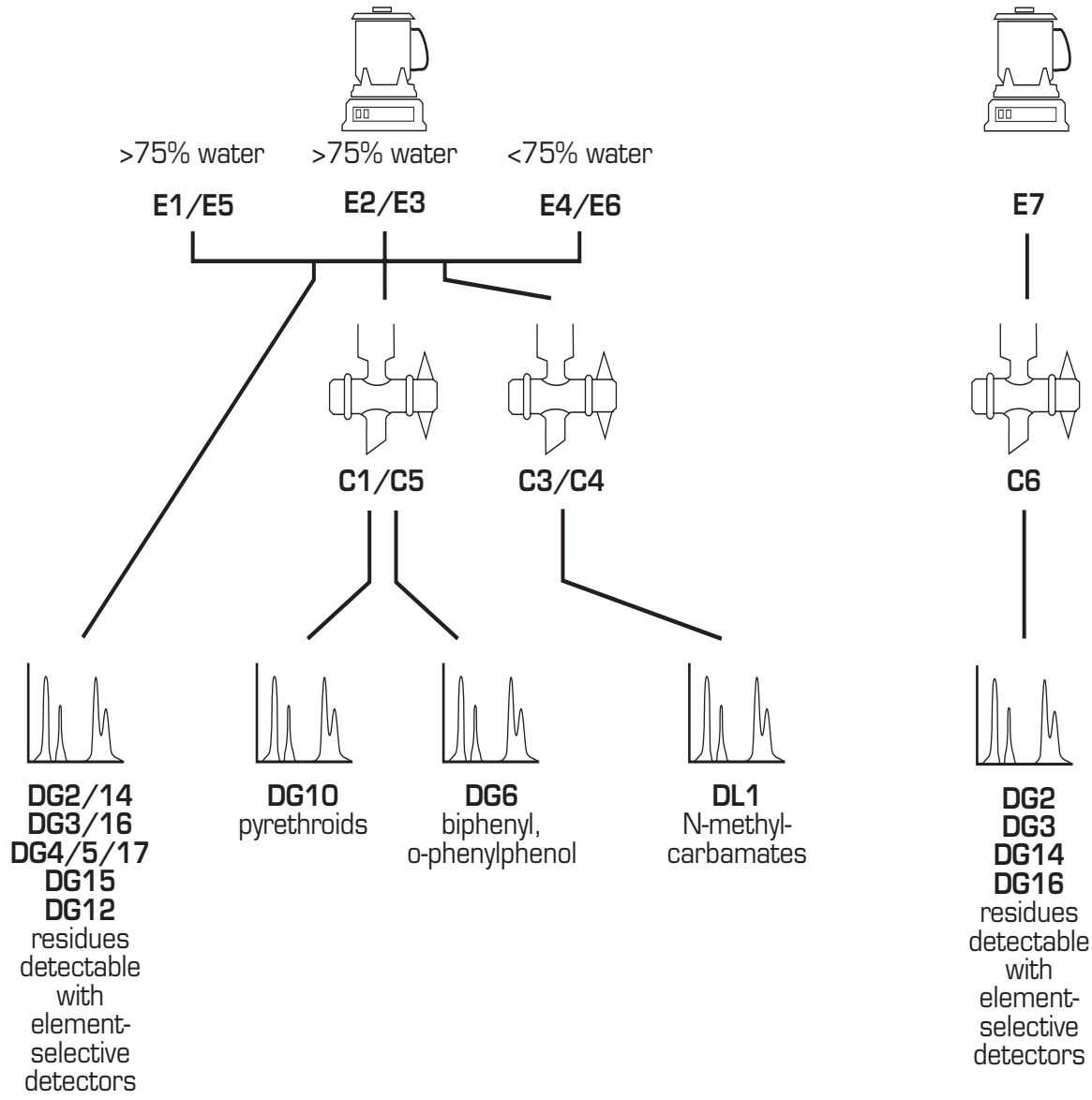
C1	(p. 302-21)	Florisil column (4 g) cleanup, with one methylene chloride eluant	relatively nonpolar residues
C2	(p. 302-23)	Charcoal/Celite/magnesium oxide column cleanup	polar residues
C3	(p. 302-25)	Charcoal/silanized Celite column cleanup	before HPLC determination for N-methylcarbamates
C4	(p. 302-27)	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

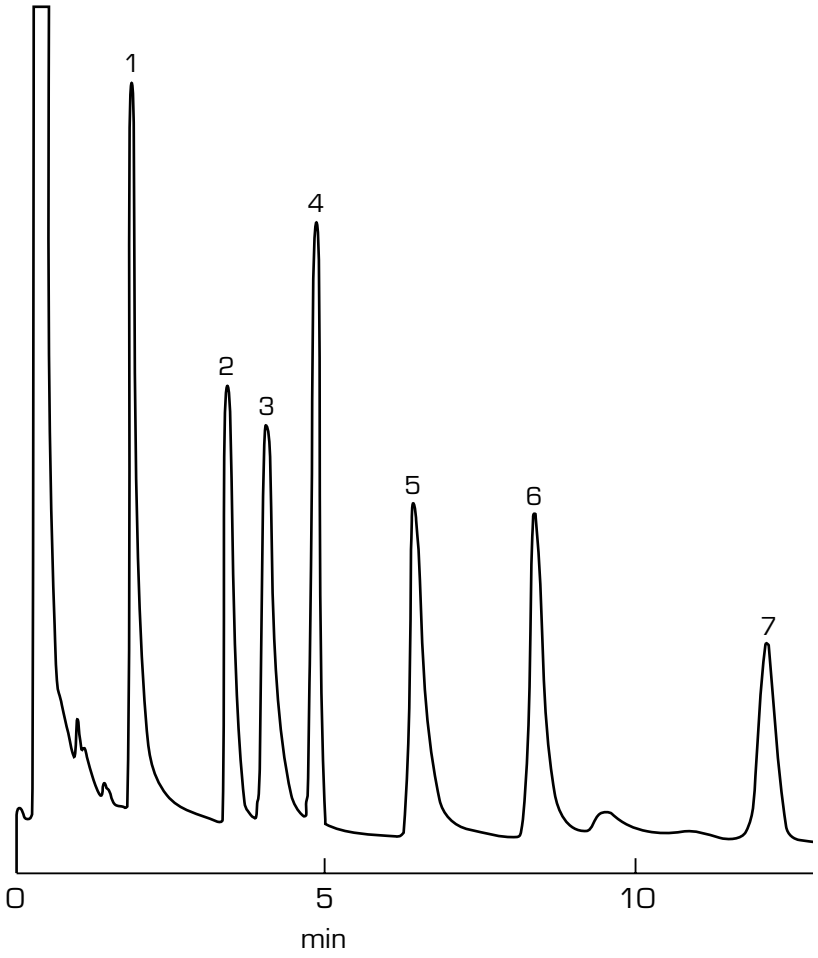
See Section 503 B for other information about EC detector operation.

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.

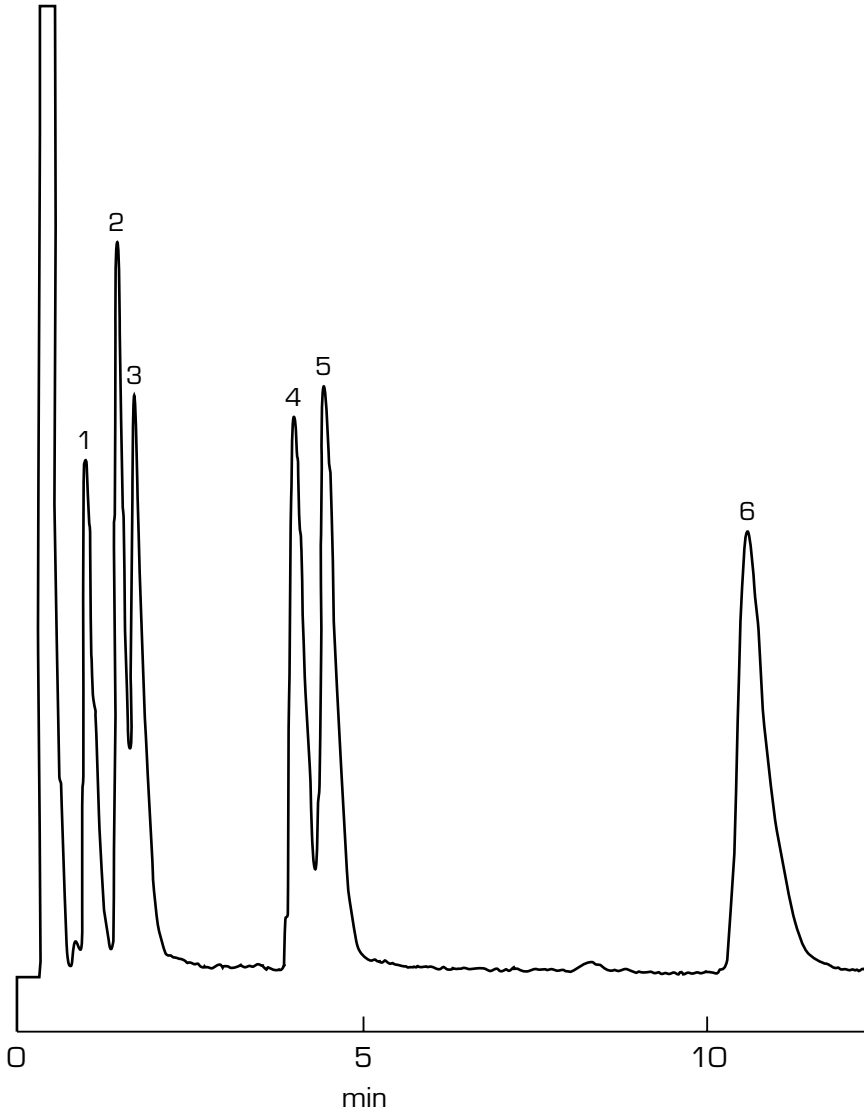
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

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

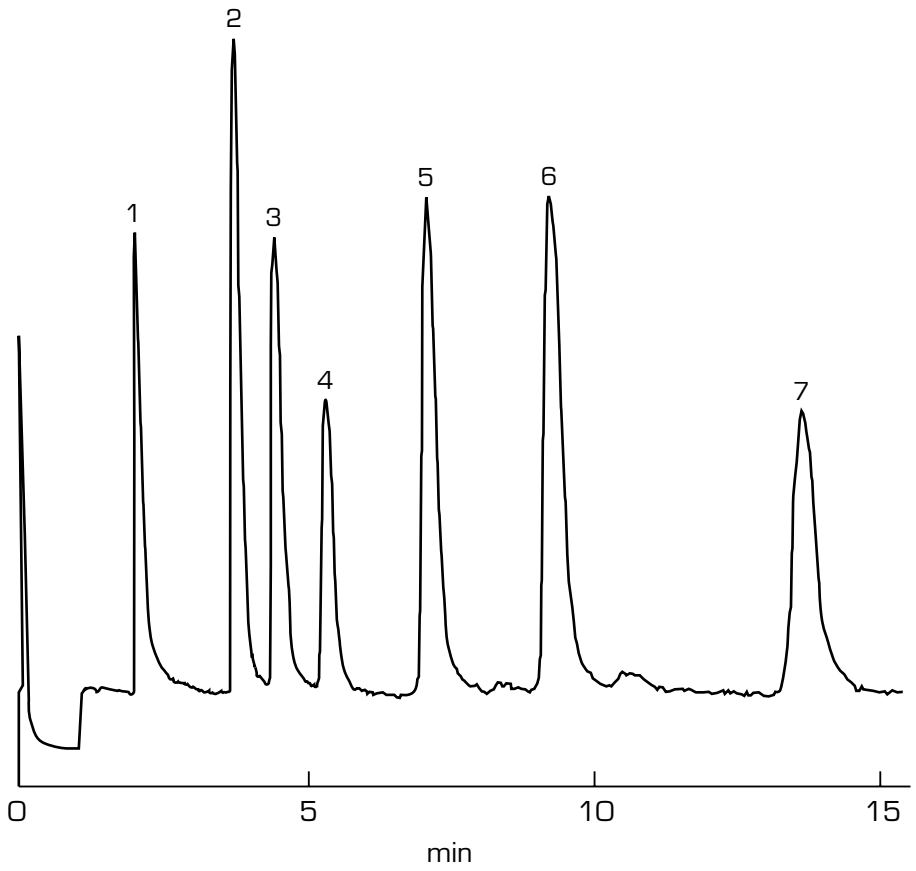
See Section 503 D for other information about ELCD operation.

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.

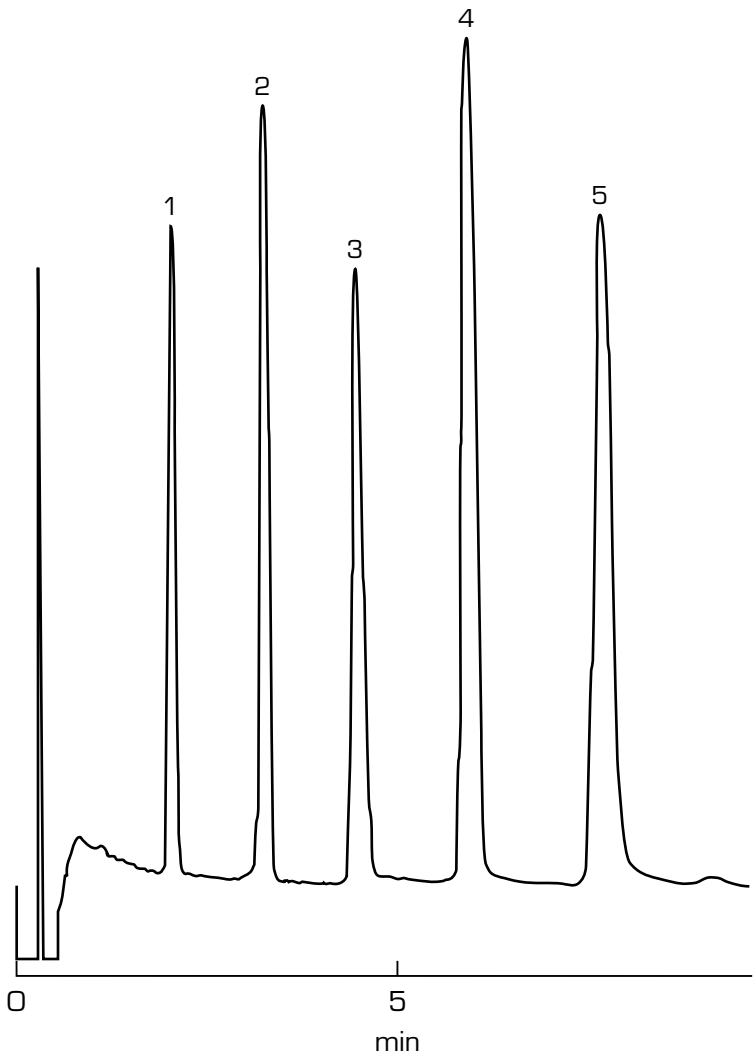
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

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

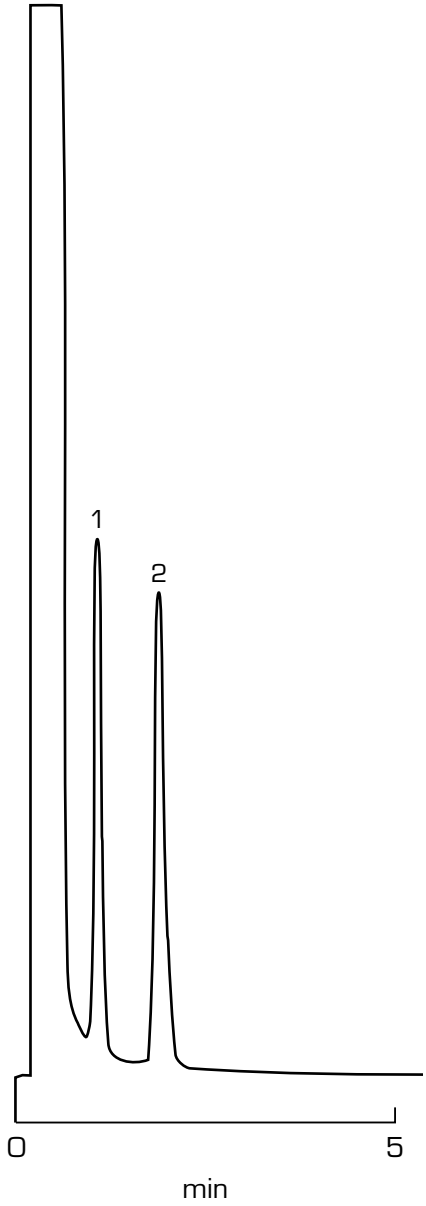
detector gases: hydrogen, 30 mL/min, air, 300 mL/min

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:

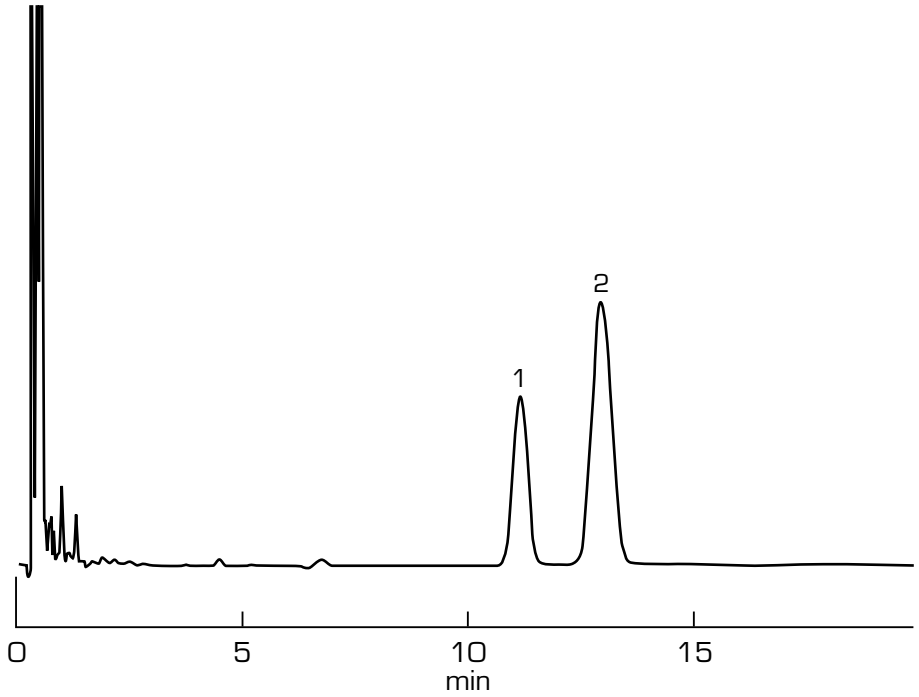
350° C

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)

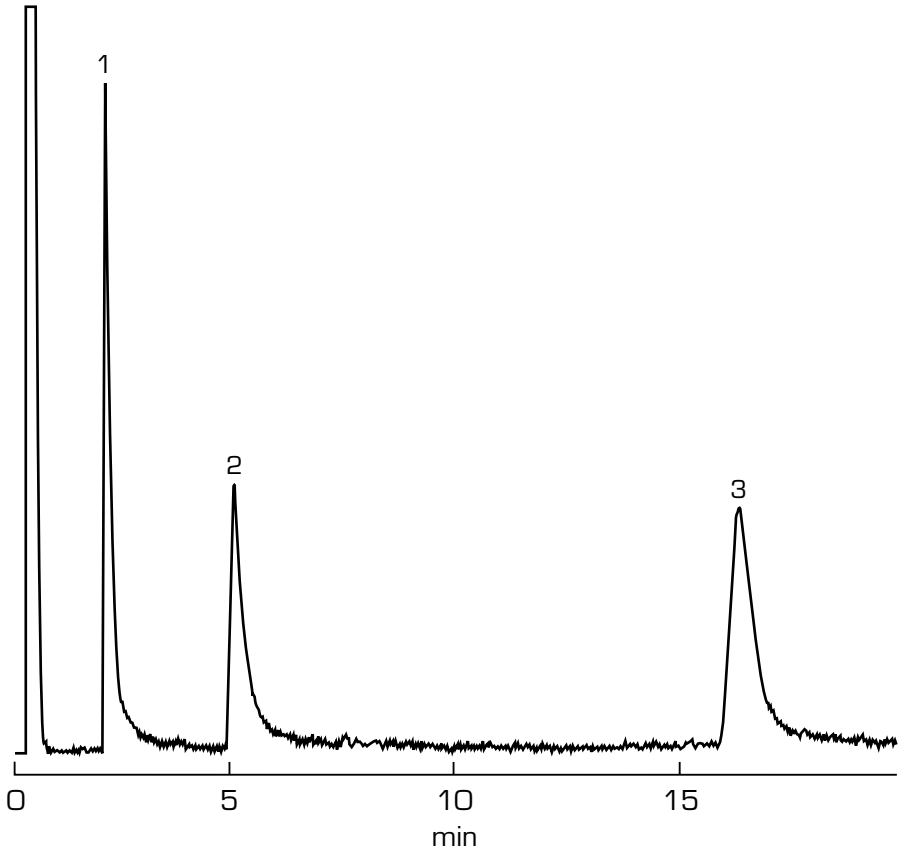
Detector Operating Conditions:

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:

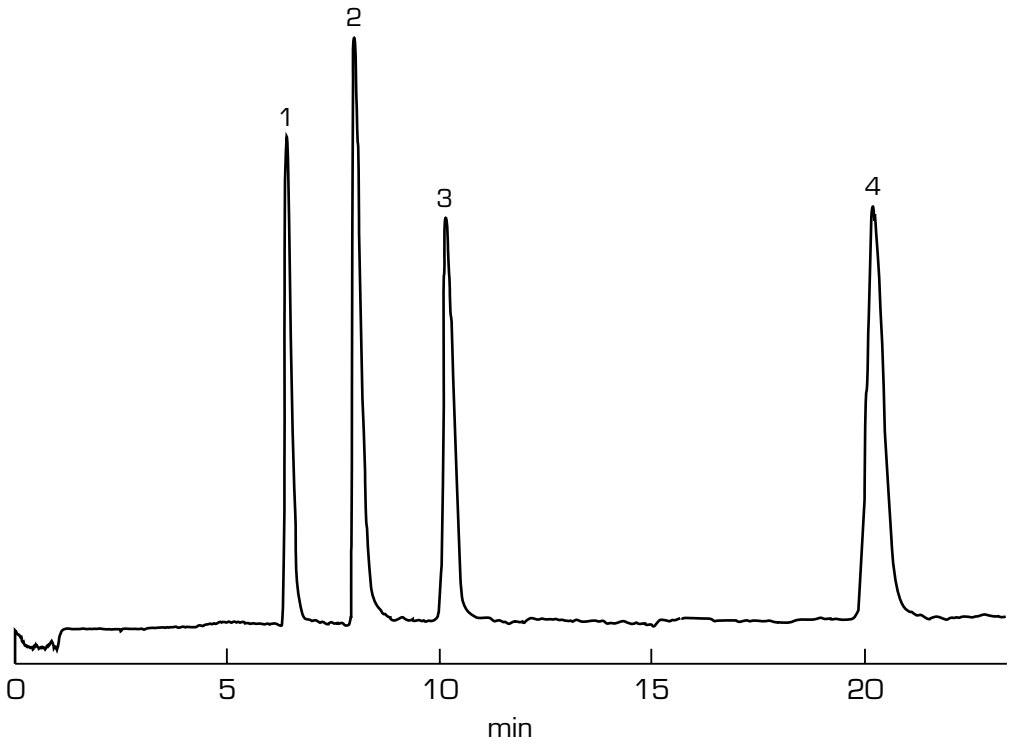
Base temperature 250° C; furnace temperature 900° C (or as specified in operating manual)

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

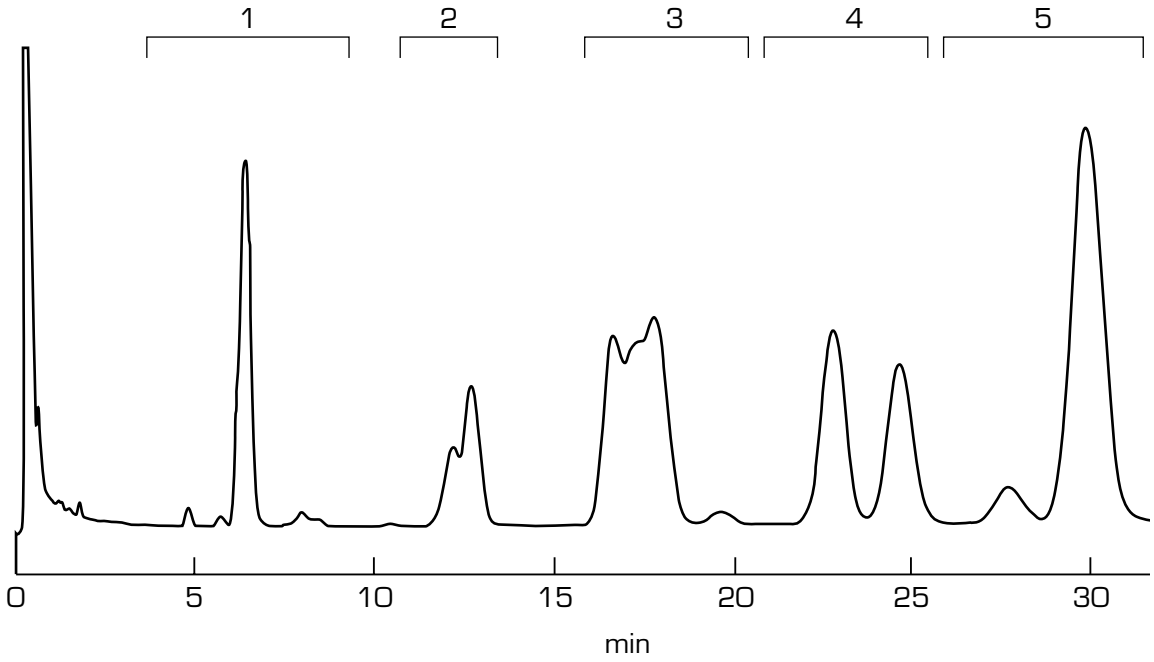
See Section 503 B for other information about EC detector operation.

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)

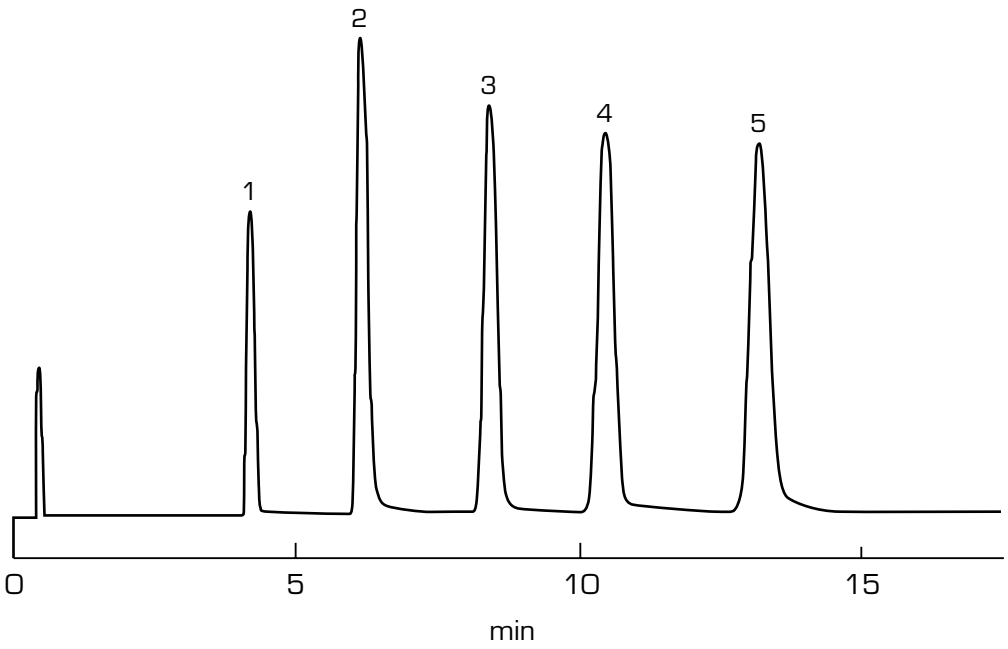
Detector Operating Conditions:

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

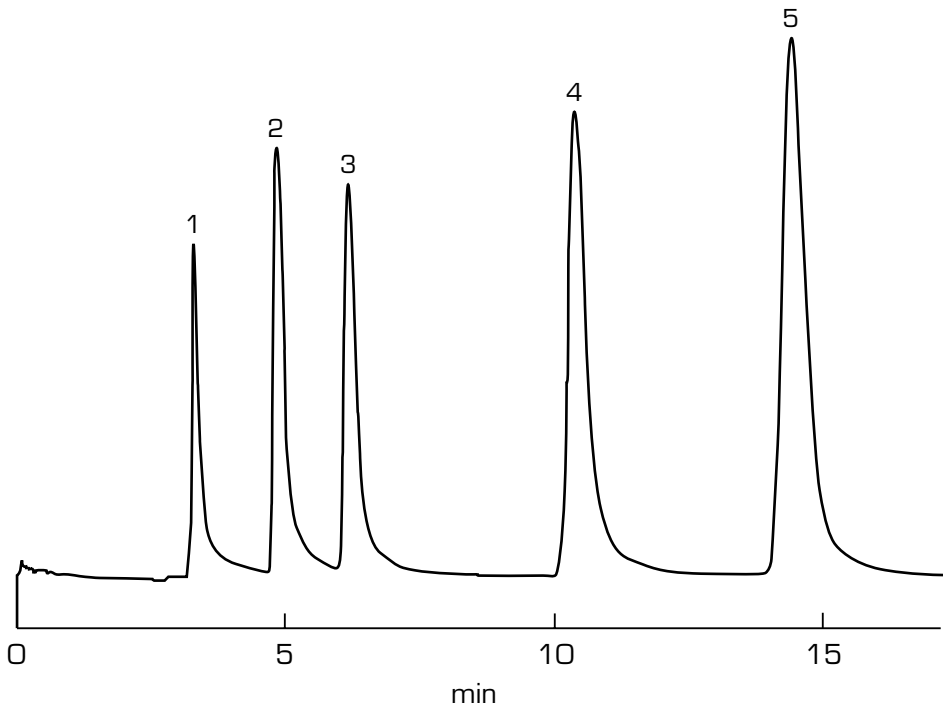
See Section 503 D for other information about ELCD operation.

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

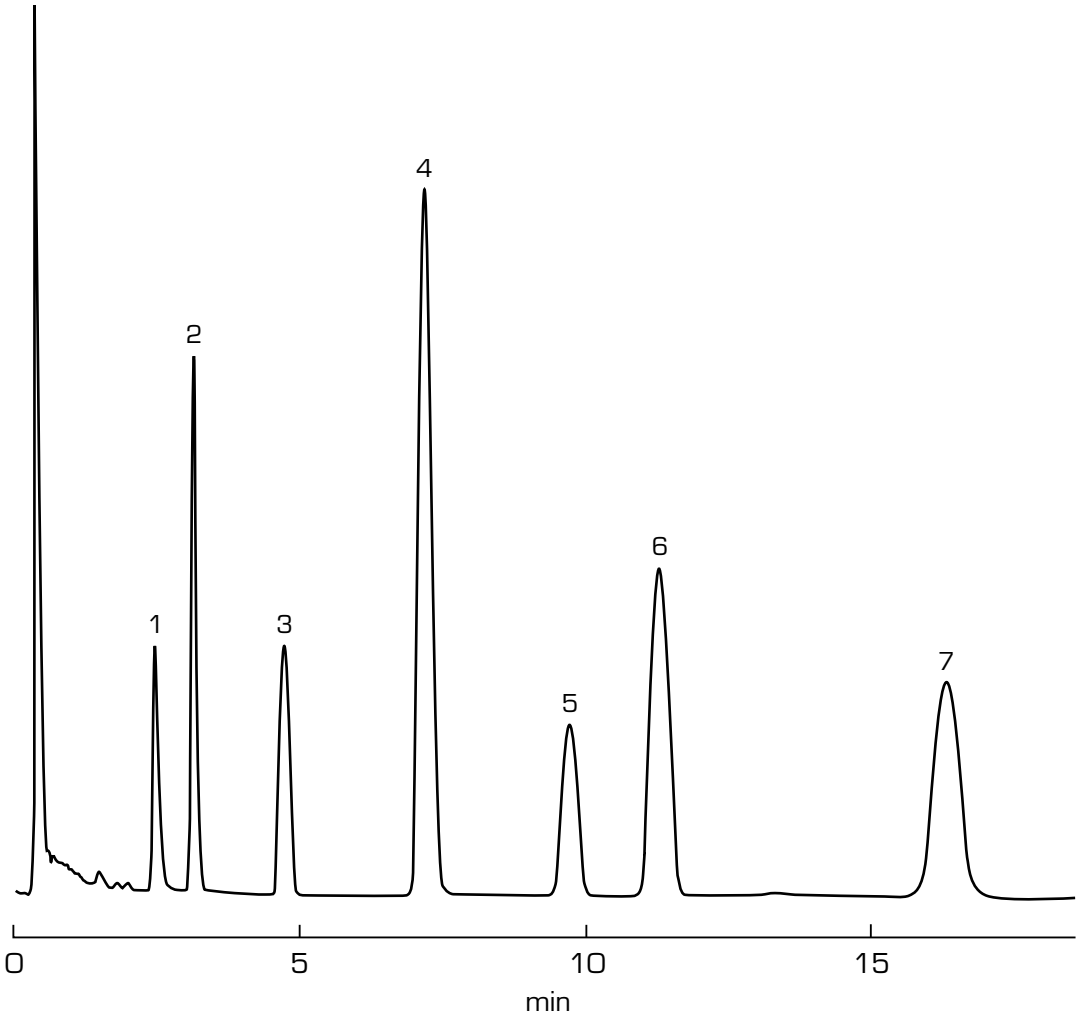
See Section 503 B for other information about EC detector operation.

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.

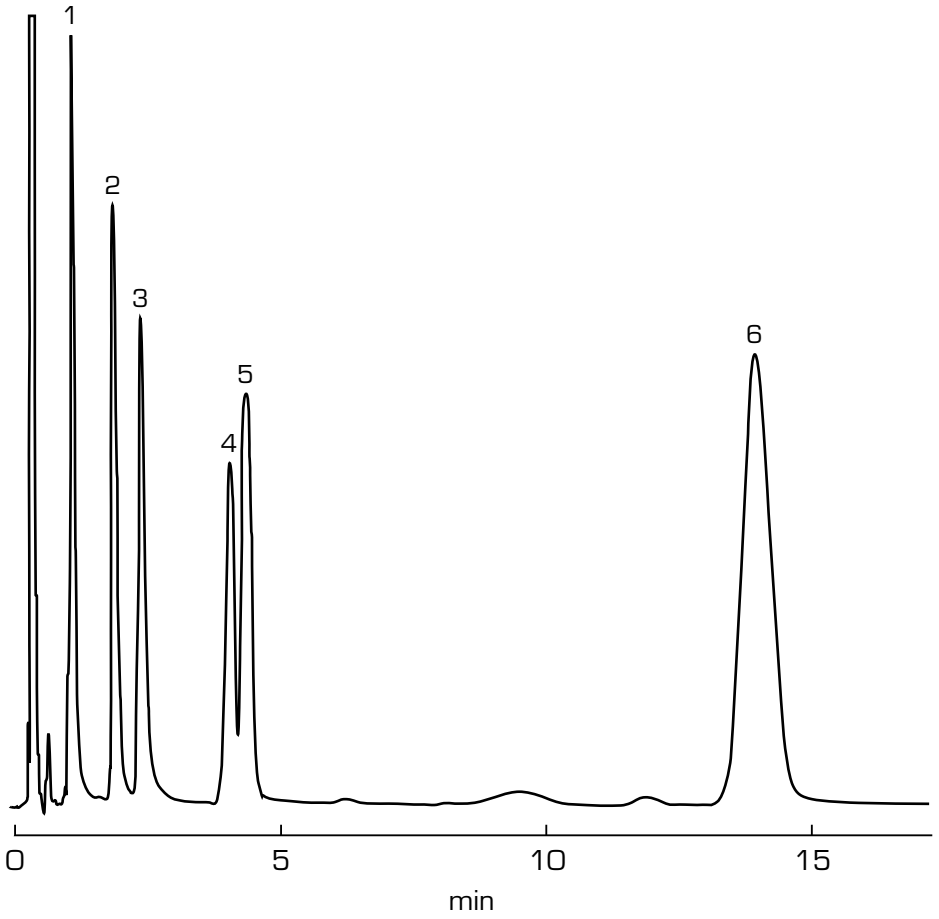
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

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.

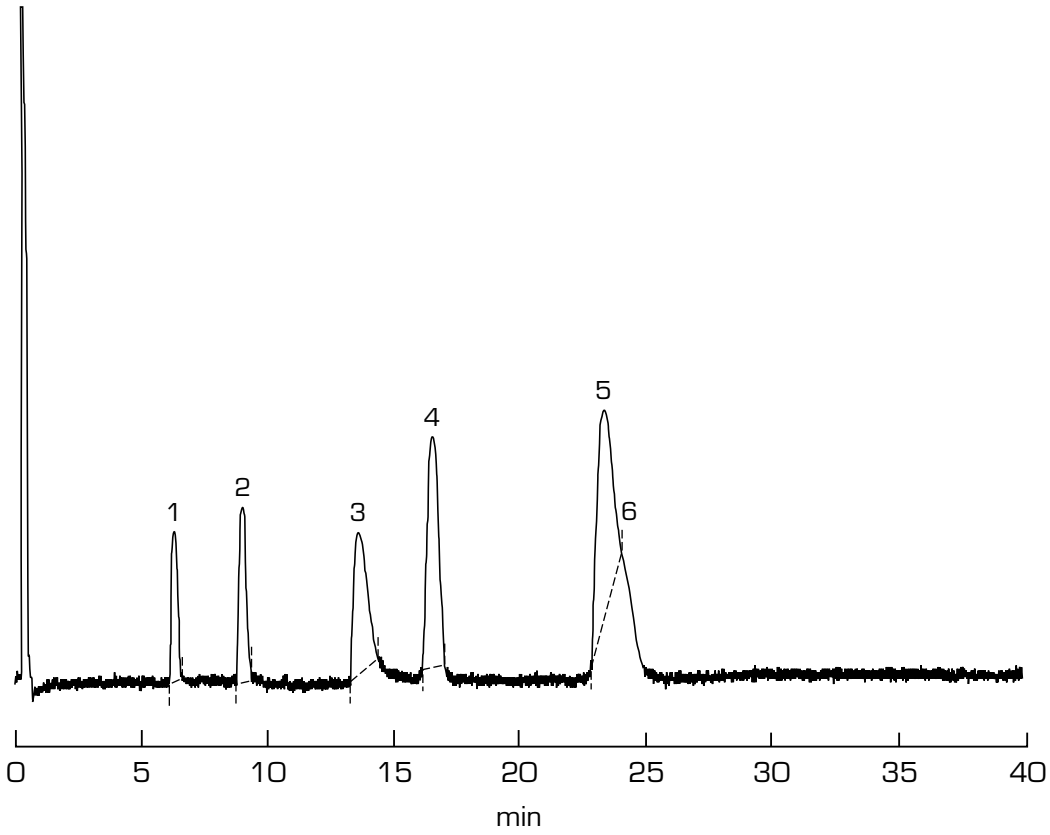
Set detector electronics (amplification, attenuation) to produce greatest possible response (50% full scale deflection [FSD]) to 15 ng chlorpyrifos is reasonable).

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

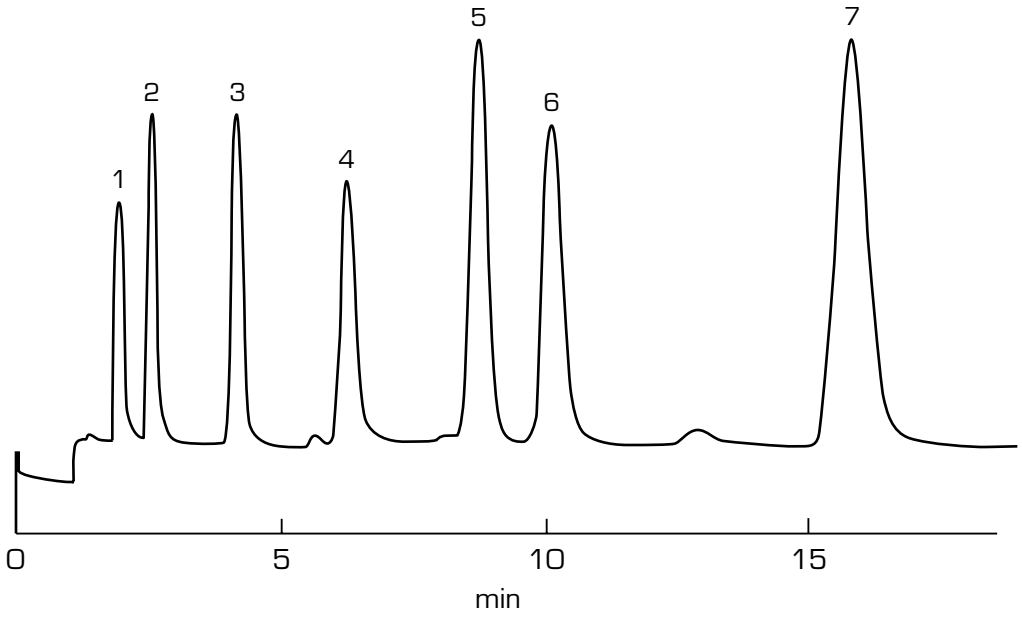
See Section 503 D for other information about ELCD operation.

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.

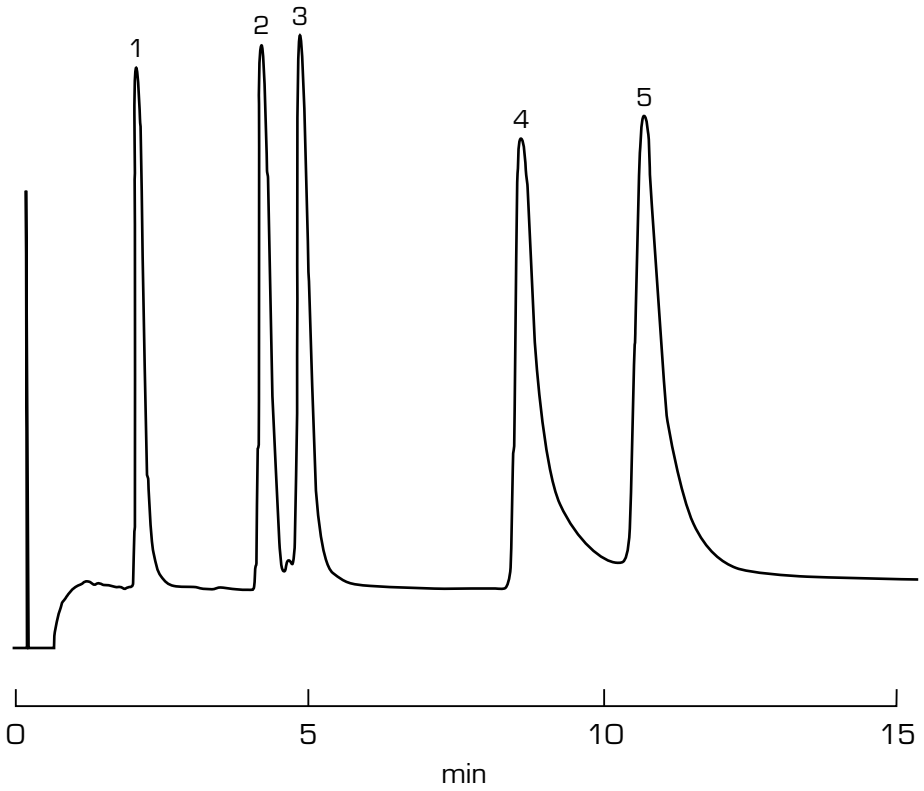
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

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.

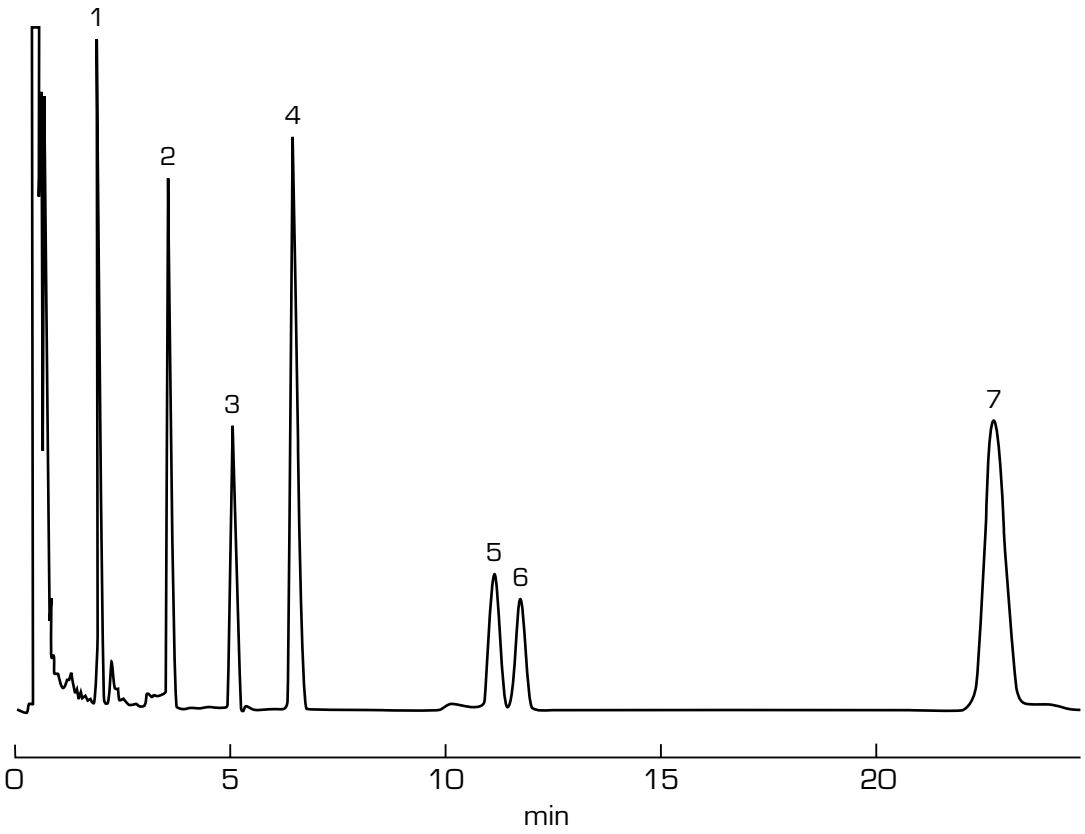
Set detector electronics (amplification, attenuation) so that response to 0.15 ng chlorpyrifos (on an amount within the detector's linear range) is 50% full scale deflection (FSD).

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.

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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.

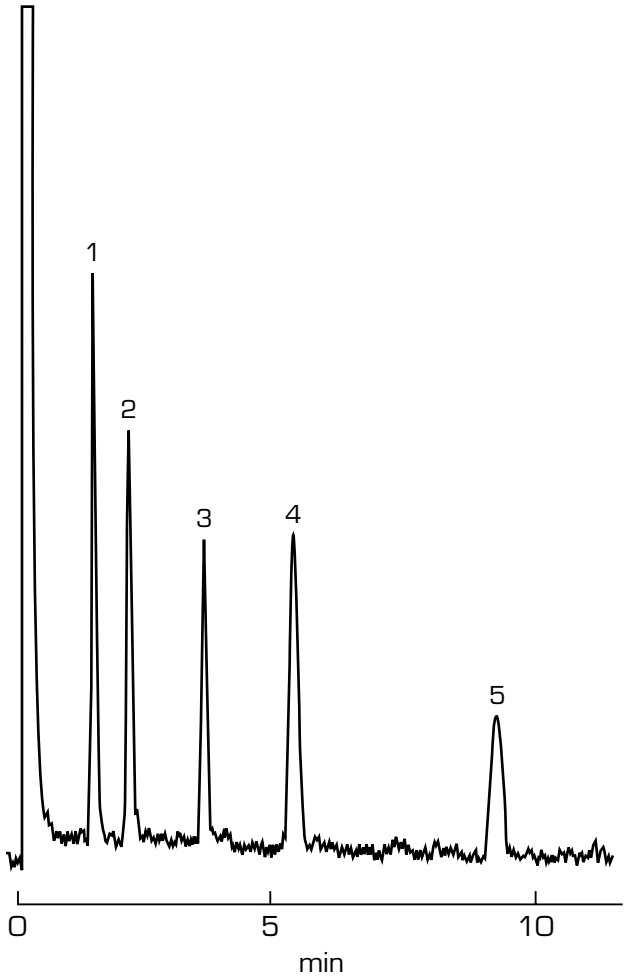
Set detector electronics (amplification, attenuation) so that response to 1.5 ng chlorpyrifos is 50% full scale deflection (FSD).

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

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.

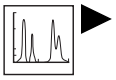
Method is applicable to moderately nonpolar residues in fatty foods. Residue polarity will affect recovery in Cleanups 1 and 2; neither very nonpolar nor very polar residues are recovered completely. See Table 304-a, following the method description, for results of recovery tests.

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