ORGANONITROGEN PESTICIDES 5601

FORMULAE: Figure 1	MW: Table 1	CAS: Table 1	RTECS: Table 1

METHOD: 5601, Issue 1　　　　**EVALUATION: FULL**　　　　**Issue 1: 15 January 1998**

OSHA : Table 1　　　　　　　　　　　　　　　　**PROPERTIES:** Table 1
NIOSH: Table 1
ACGIH: Table 1

NAMES and SYNONYMS　(Table 1) Aldicarb　Benomyl　Captan　Carbaryl Carbendazim　Carbofuran
　　　　　　　　　　Chlorprophan　Diuron　Formetanate　Methiocarb　Methomyl　Oxamyl
　　　　　　　　　　Propham　Propoxur　Thiobencarb

SAMPLING

SAMPLER:　FILTER/SOLID SORBENT TUBE
(OVS-2 Tube: 13-mm quartz fiber filter; XAD-2, 270 mg/140 mg)

FLOW RATE:　0.1 to 1 L/min [1]

VOL-MIN:　variable (see Table 2)
-MAX:　480 L

SHIPMENT:　routine

SAMPLE STABILITY:　at least 30 days @ -12 °C [1]
at least 7 days @ 24 °C [1]

BLANKS:　2 to 10 field blanks per set

ACCURACY

RANGE STUDIED:　　　　Table 2

BIAS:　　　　Table 2

OVERALL PRECISION ($\hat{S}_{rT}$): Table 2

ACCURACY:　　　　Table 2

MEASUREMENT

TECHNIQUE:　HPLC, UV detection

ANALYTE:　organonitrogen pesticides (Table 1)

EXTRACTION:　2 mL extraction solution (0.2% V/V 0.1 M aqueous triethylamine phosphate buffer in acetonitrile, pH 6.9 to 7.1)

INJECTION VOLUME:　　5 µL

MOBILE PHASE A: 2% 1-propanol in aqueous 0.02 M triethylamine phosphate (pH 6.9 to 7.1)
MOBILE PHASE B: 2% 1-propanol in acetonitrile

PROGRAM:　mobile phase B, 3 to 95% in 30 min, hold 95% 5 min

COLUMN:　NOVA-PAK® C-18, 30 cm x 3.9-mm ID, or equivalent; ambient temperature (Table 3)

DETECTOR:　UV absorption at 200 and 225 nm

CALIBRATION:　solutions of analytes in extraction fluid

RANGE:　Table 2

ESTIMATED LOD: Table 2

PRECISION ($\bar{S}_r$):　Table 2

APPLICABILITY: The working ranges (Table 2) for aldicarb, carbofuran, and oxamyl range from 0.5 to 10 times the OSHA PEL. Others cover 0.1 to 2 times the OSHA PELs with appropriate dilutions [1].This method may be applicable to the determination of other organonitrogen compounds after evaluation, and to a broad range of pesticides having UV chromophores, e.g., acetanilides, acid herbicides, organophosphates, phenols, pyrethroids, sulfonyl ureas, sulfonamides, triazines, and uracil pesticides.

INTERFERENCES: Because of the broad response of the UV detector at shorter wavelengths, there are many potential interferences. Those tested include solvents (chloroform and toluene), antioxidants (BHT), plasticizers (dialkyl phthalates), nitrogen compounds (nicotine and caffeine), impurities in HPLC reagents (e.g., in triethylamine), other pesticides (2,4-D, atrazine, parathion, etc.), and pesticide hydrolysis products (1-naphthol). Retention times are given in Table 4. Confirmation techniques are recommended when analyte identity is uncertain.

OTHER METHODS: This method may be used to replace previous related pesticide methods: S273 Carbaryl [2]; 5006 Carbaryl [3]; OSHA 63 Carbaryl and 74 Aldicarb [4]; OSHA Stopgap methods for several pesticides [5]; and EPA TO-10 for captan, Folpet, and Mexacarbate [6]. The OVS-2 Tube is similar in concept to the device of Hill and Arnold [7], but offers convenience and lower flow resistance.

REAGENTS:

1. Carbamate, urea, and sulfenimide analytes listed in Table 1; internal standards acetanilide and acetophenone, analytical grade.*
2. Acetonitrile, UV grade.*
3. Methanol, HPLC grade.*
4. Deionized water, ASTM Type II.
5. 1-Propanol, UV grade.*
6. n-Butyl isocyanate.*
7. Triethylamine (TEA), HPLC grade.* Keep refrigerated (0 to 4 °C) and store under nitrogen for longer shelf life [1,8].
8. Ortho-phosphoric acid, >85% by weight, ACS grade or better.*
9. Extraction solution. Prepare separate triethylamine phosphate (TEA-PO₄) preservative and internal standard solutions.
   a. TEA-PO₄ preservative, 0.1 *M*. Dissolve 1.4 mL of TEA in 90 mL of deionized water. Add phosphoric acid to lower pH to 7.0 (± 0.1) as indicated by a calibrated pH meter. Bring volume to 100 mL. Keep tightly capped and refrigerated.
      NOTE: Do not use chloroacetic acid as a preservative [9]. Formetanate, at least, is unstable with chloroacetic acid.
   b. Internal standard stock solution, 5mg/mL. Add 100 mg of each internal standard of choice for each 20 mL of solution required. Dissolve in acetonitrile. Store capped at −12 ± 1 °C.
   c. Final extraction solution. Add 1 mL of the TEA-PO₄ solution and 12 mL of the internal standard stock solution to a 500-mL volumetric flask. Dilute to volume with acetonitrile. Concentration of TEA = 0.2 m*M*, water = 0.2%, and internal standards = 120 µg/mL. Stable up to 30 days at 0 to 4 °C.
10. Individual analyte stock solutions, 5 mg/mL. Add each analyte to acetonitrile in separate volumetric flasks. Use methylene chloride for benomyl and carbendazim. Use 50/50 v/v methanol/acetonitrile for formetanate. Store at −12 ± 1 °C. (Solutions are stable up to 30 days.)
11. Calibration stock solution. Combine stock solutions of analytes of interest in a volumetric flask to produce the highest concentration standard (suggest 120 to 480 µg/mL).
    NOTE: Do not combine benomyl and carbendazim in the same standard

EQUIPMENT:

1. Sampler: OSHA Versatile Sampler (OVS-2 tube), 13-mm OD inlet, 6-mm OD outlet. Front section contains 270 mg 20/60 mesh XAD-2 sorbent held in place by an 11-mm diameter quartz fiber filter and Teflon® ring, separated from the back section of 140 mg XAD-2 sorbent by a short plug of polyurethane foam. The back section is held in place with a plug of polyurethane foam (See Figure 2). The tube is available commercially (SKC #226-58). OVS-2 tubes with glass fiber filters have equivalent desorption efficiencies and are available from SKC (#226-30-16) and Supelco (#ORBO-49P).
2. Personal sampling pump: 0.1 to 1 L/min with flexible and inert connecting tubing.
3. High Performance Liquid Chromatograph capable of mixing two mobile phases in a linear gradient. Must be capable of pumping up to 4000 psi, to accommodate 300 mm long columns.
4. Autosampler, low dead-volume, capable of 5-µL injections. Preservative (TEA-PO₄) in the desorbing solution may be eliminated if a refrigerated autosampler tray is available.**
5. Analytical columns.
   a. Primary column: Base-deactivated octadecylsilyl (C₁₈) column, *e.g.*, NOVA-PAK® C18, 3.9-mm ID X 300 mm, 5-µm particle size or equivalent.
   b. Secondary column: Cyanopropyl silica column, *e.g.*, Supelco LC-CN® 4.6 X 250-mm, 5-µm particle size or equivalent.
6. Guard column, low dead-volume, containing analytical column packing material.**
7. Ultraviolet detector, low dead-volume, with 1-cm path length cell capable of monitoring two wavelengths (200- and 225-nm) simultaneously.
8. Vials: 4-mL with PTFE-lined caps; 2-mL HPLC autosampler vials with PTFE- or polyethylene-lined snap caps.
9. Syringes: 0.01-, 0.05-, 0.1-, 1.0- and 2.5-mL; Luer lock, 1- or 2.5-mL for sample filtering.
10. Volumetric flasks: 2-, 5-, 10-, 25-, 50-, 100-, 500-, and 1000-mL.
11. PTFE syringe filter: 0.45-µm. (Gelman Acrodisc® CR PTFE 0.45-µm filter, Product #4472, Gelman Sciences, Ann Arbor, MI or equivalent.)
12. Forceps.
13. Small vial/tube tumbler capable of 5 to 10 RPM
14. pH meter.
15. Graduated cylinders, 10-mL, 25-mL.

REAGENTS (contd)

solution. [10-12]. See APPENDIX.

12. Quality control spiking solutions: Add analyte stock solutions to acetonitrile at concentrations in the analytical range of the samples. Store in the freezer at −12 ± 1 °C until immediately before spiking.
    NOTE: Spiking solutions must not contain internal standard.

13. Mobile phase A. Combine 20 mL of 1-propanol and 2.8 mL of TEA in a 1-L volumetric flask and bring to volume with deionized water. Adjust pH to 7.0 (± 0.1) with phosphoric acid using a pH meter. Final concentrations: 2% 1-Propanol, 0.02 M TEA-PO$_4$. Degas prior to use.

14. Mobile phase B. Add 20 mL of 1-propanol to acetonitrile in a 1-L volumetric flask and bring to volume. Degas prior to use.

    * See SPECIAL PRECAUTIONS

EQUIPMENT (contd)

16. Pipettes, glass, disposable.

    ** Low dead volumes will give lower dwell volumes [13,14] and better resolution [15].

SPECIAL PRECAUTIONS: Pesticides: Avoid inhaling vapors or dust; avoid skin contact. Wear gloves and suitable clothing when handling pure material. Solvents: Avoid skin contact and open flame. Use in a hood. Phosphoric acid: Avoid skin contact. n-Butyl isocyanate may act as a sensitizer. Avoid skin contact.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Connect the sampler to the personal sampling pump with flexible tubing. Place sampler vertically, with the large end down, in the worker's breathing zone.
3. Sample at an accurately known flow rate between 0.1 and 1 L/min for a total sampling volume up to 480 L. Record volume, and document presence of any known or potential interferences.
4. Cap both ends of the sampler with plastic caps and pack securely for shipment.

SAMPLE PREPARATION:

5. Remove cap from large end. Transfer filter, PTFE retainer ring, and front XAD-2 resin section to a 4-mL vial. Transfer the polyurethane foam divider plug along with the back-up XAD-2 resin bed to a second 4-mL vial.
6. Add 2.0 mL of desorbing solvent with internal standards to each vial using a 2.5- or 5-mL syringe or 2-mL pipette; cap each vial.
7. Mix by rotating the vials end-over-end at 5 to 10 RPM for approximately 45 minutes.
8. Filter an aliquot into a 2-mL autosampler vial through a 0.45-μm PTFE filter.

CALIBRATION AND QUALITY CONTROL:

9. Determine retention times for the analytes of interest using the column and chromatographic conditions of choice for the analysis.
10. Calibrate daily with at least six working standards covering the analytical range for individual analytes.
    a. Prepare working standards by diluting aliquots of the high-level calibration standard with desorbing solution containing internal standard in a volumetric flask. Include an unspiked desorption solution

calibration blank.
    b. Filter aliquots of standards and blanks for analysis (See Step 8).
    c. Analyze together with samples, blanks, and laboratory control samples (Steps 12 through 14).
    d. Prepare a calibration graph (ratio of peak area of analyte over peak area of internal standard vs. µg analyte).
        NOTE: Use of an internal standard is recommended [1,16], but optional if the precision of the injection device and HPLC system are known to be adequate.

11. Prepare desorption efficiency(DE) samples and Laboratory Control Samples (LCS) with each sample set at a rate of 10% of samples.
    a. Remove cap and the PTFE retainer ring from large end of sampler tube (to prevent wicking behind the ring). Apply known volume of calibration solution to face of quartz fiber filter.
        NOTE: Spike no more than 15 to 30 µL at a time. If more needs to be applied, connect the sampler to a vacuum pump with a flow$\leq$1 L/min, then apply spiking solution in 15- to 30-µL aliquots. Allow several minutes for the solvent to evaporate between each aliquot, to prevent wicking along the sides of the tube into the back-up section (5% or more may deposit on the walls of the tube).
    b. Cap and allow to stand a minimum of one hour.
        NOTE: Prepare LCS when samples arrive and store with field samples until analyzed.
    c. Include an unspiked sampler as a media (method) blank.
    d. Analyze with the field samples, blanks, and the liquid standards (Steps 12 through 14).

MEASUREMENT:

12. Set liquid chromatograph according to manufacturer's recommendations and to conditions listed in Table 3. Select two wavelengths for detection with 200 and 225 nm for general-purpose screens. For selected analytes, chose a more specific wavelength {from Table 10 or} from UV spectra where available.
13. Inject sample aliquot with autosampler. See Table 4 for approximate retention times of selected analytes.
    NOTE: If peak area is greater than the area of the highest standard, dilute with desorbing solution containing internal standards and reanalyze. Apply the appropriate dilution factor in calculations.
14. Measure peak area of analyte(s) and internal standard(s). Divide peak area of analyte by peak area of internal standard on same chromatogram.

CALCULATIONS:

15. Determine the mass, µg, (corrected for DE) of analyte found in the sample filter and front sorbent section (W_f), back sorbent section (W_b), and the media blank front (B_f) and back (B_b) sorbent sections from a standard curve.
16. Calculate concentration, C (mg/m³), of each analyte in the air volume sampled, V (L).

$$C = \frac{W_f + W_b - B_f - B_b}{V} \; , \; mg/m^3$$

    NOTE: µg/mL $\equiv$ mg/m³

CONFIRMATION:

Retention Times with Alternate Conditions. Whenever the identity of an analyte is uncertain, confirmation may be achieved by analysis on an alternate column. If primary analysis was performed on a base-deactivated octadecylsilyl (C18) column, identity may confirmed by reanalysis on a cyanopropyl silica column, or by changing to a water/methanol mobile phase (see Table 9 for recommended alternative conditions). See Table 4 for approximate retention times for each column type and condition. Relative retention times (retention indices for a particular set of conditions) are more convenient for the identification of unknown analytes.

UV or Mass Spectra. Confirmation may be achieved through comparison of unknown spectra with reference spectra where available. Relative response ratios (See Table 10 for ratio of absorbances at 225 nm/200 nm for selected analytes) will give a moderate level of confirmation. Some analytes (O-aryl carbamates especially) can be confirmed by GC/MS using highly deactivated injection ports and analytical GC columns, or by HPLC/MS.

EVALUATION OF METHOD:

This method was evaluated over the ranges specified in Table 2 at 25 °C with 240-L air samples. Samplers were tested at 15 and 80% relative humidity and 10 and 30 °C. In these experiments, test atmospheres were not generated; instead, analytes were fortified on the face of the sampler filters. The conditioned air was pulled through the samplers at 1 L/min for four hours. No significant difference in sampler performance was noted at any of these temperature/humidity combinations. Evaluation of sampler precision and stability was conducted at ambient conditions of temperature and relative humidity. Overall sampling and measurement precision, bias, accuracy, and average percent recovery after long-term storage are presented in Table 2. No breakthrough was detected with samplers fortified with 480 µg per analyte per tube after sampling eight hours at 1 L/min. For the estimation of LOD/LOQ, a series of media-spiked standards were prepared in triplicate, analyzed, and responses fitted to a quadratic curve. The Limit of Detection (LOD) and Limit of Quantitation (LOQ), given in Table 2, were estimated according to NIOSH SOP 018 [1, 17]. Criteria established by NIOSH were met [1].

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TABLE 1. GENERAL INFORMATION

Name / Synonym	Empirical Formula	MW	Properties	Solubility in H_2O (g/L)	Exposure Limit (mg/m^3)
Aldicarb CAS# 116-06-3 RTECS UE2275000	$C_7H_{14}N_2O_2S$	190.3	MP 99-100 °C; vp 3.9 mPa $(2.9 \times 10^{-5}$ mm Hg) @ 25 °C; LD_{50} 1 mg/kg	6 @ 25 °C	
Benomyl CAS# 17804-35-2 RTECS DD6475000	$C_{14}H_{18}N_4O_3$	290.36	MP decomposes; vp <1.3 mPa $(<1 \times 10^{-5}$ mm Hg) @ 20 °C; LD_{50} >9590 mg/kg	0.002 @ 25 °C	OSHA 5.0 (resp) ACGIH 10
Captan CAS# 133-06-2 RTECS GW5075000	$C_9H_8Cl_3NO_2S$	300.6	MP 178 °C; vp <1.3 mPa $(<1 \times 10^{-5}$ mm Hg) @ 20 °C; LD_{50} 9000 mg/kg	<0.005 @ ~25 °C	NIOSH 5 ACGIH 5
Carbaryl CAS# 63-25-2 RTECS FC5950000	$C_{12}H_{11}NO_2$	201.24	MP 142 °C; vp <5.3 mPa $(<4 \times 10^{-5}$ mm Hg) @ 25 °C; LD_{50} 250 mg/kg	0.12 @ 30 °C	OSHA 5 NIOSH 5 ACGIH 5
Carbendazim CAS# 10605-21-7 RTECS DD6500000	$C_9H_9N_3O_2$	191.21	MP 302-307 °C (decomposes); LD_{50} 6400 mg/kg	0.008/pH 7 @ 24 °C	
Carbofuran CAS# 1563-66-2 RTECS FB9450000	$C_{12}H_{15}NO_3$	221.28	MP 150-153; vp 0.031 mPa $(2.3 \times 10^{-7}$ mm Hg) @ 20 °C; LD_{50} 5.3 mg/kg	0.70 @ 25 °C	NIOSH 0.1 ACGIH 0.1
Chlorpropham CAS# 101-21-3 RTECS FD8050000	$C_{10}H_{12}ClNO_2$	213.68	MP 40.7-41.1 °C; vp 2.7 mPa $(2 \times 10^{-5}$ mm Hg) @ 33 °C; LD_{50} 1200 mg/kg	slightly soluble	
Diuron CAS# 330-54-1 RTECS YS8925000	$C_9H_{10}Cl_2N_2O$	233.11	MP 158-159 °C; vp 0.41 mPa $(3.1 \times 10^{-6}$ mm Hg) @ 50 °C; LD_{50} 437 mg/kg	0.042 @ 25 °C	NIOSH 10 ACGIH 10
Formetanate.HCl CAS# 23422-53-9 RTECS FC2800000	$C_{11}H_{16}ClN_3O_2$	257.75	MP 200-202 (decomposes); LD_{50} 20 mg/kg	>50% as hydrochlor-ide	
Methiocarb CAS# 2032-65-7 RTECS FC5775000	$C_{11}H_{15}NO_2S$	225.34	MP 121.5 °C; vp 0.036 mPa $(2.7 \times 10^{-7}$ mm Hg) @ 25 °C; LD_{50} 60 mg/kg	insoluble	
Methomyl CAS# 16752-77-5 RTECS AK2975000	$C_5H_{10}N_2O_2S$	162.24	MP 78-79 °C; vp 6.7 mPa $(5 \times 10^{-5}$ mm Hg) @ 25 °C; LD_{50} 17 mg/kg	58 @ 25 °C	NIOSH 2.5 ACGIH 2.5
Oxamyl CAS# 23135-22-0 RTECS RP2300000	$C_7H_{13}N_3O_3S$	219.3	MP 100-102 °C; vp 31 mPa $(2.4 \times 10^{-4}$ mm Hg) @ 20 °C; LD_{50} 5 mg/kg	280 @ 25 °C	
Propham CAS# 122-42-9 RTECS FD9100000	$C_{10}H_{13}NO_2$	179.24	MP 90 °C; vp 18 mPa $(1.35 \times 10^{-4}$ mm Hg); LD_{50} 3724 mg/kg	0.25 @ 25 °C	
Propoxur CAS# 114-26-1 RTECS FC3150000	$C_{11}H_{15}NO_3$	209.27	MP 91.5 °C; vp 1.3 mPa (9.75 mm Hg) @ 20 °C; LD_{50} 83 mg/kg	2 @ 20 °C	NIOSH 0.5 ACGIH 0.5
Thiobencarb CAS# 28249-77-6 RTECS EZ7260000	$C_{12}H_{16}ClNOS$	257.81	Not available; LD_{50} 1130 mg/kg	~0.03 @ 20 °C	

Abbreviations: MW=molecular weight (Daltons); RTECS=Registry of Toxic Effects of Chemical Substances[18]; LD_{50}=lethal dose 50% [19,20]; mPa=milliPascals

TABLE 2. METHOD EVALUATION

Compound	Min. Sample Vol (L)	Range Studied (µg/samp)	LOD (µg/samp)	Mean Bias	Overall Precision ($\hat{S}_{rT}$)	Accuracy	Storage Stability 31-Day %Rec. 24 °C	-12 °C
Aldicarb	240	12.0-240	1.2	-0.009	0.066	± 0.131	93.2	95.6
Benomyl	6	12.0-120	0.6	[A]	[A]	[A]	[A]	[A]
Captan	30	48.0-960	4.8	-0.036	0.061	± 0.142	98.7	102.2
Carbaryl	6	12.0-240	0.06	+0.012	0.061	± 0.123	88.2	91.8
Carbendazim	6	[B]	0.6	+0.006	0.061	± 0.121	92.1	89.3
Carbofuran	240	12.0-240	0.6	-0.020	0.060	± 0.126	89.1	92.4
Chlorpropham	6	12.0-240	0.6	-0.017	0.068	± 0.140	84.3	85.9
Diuron	3	12.0-240	0.6	-0.062	0.060	± 0.167	86.0	87.1
Formetanate.	60	12.0-240	0.6	+0.032	0.056	± 0.129	89.8	93.0
Methiocarb	60	12.0-240	0.6	+0.009	0.061	± 0.122	85.1	89.1
Methomyl	12	12.0-120	0.6	-0.002	0.063	± 0.124	90.5	95.2
Oxamyl	240	12.0-240	0.6	+0.037	0.055	± 0.132	94.8	95.9
Propham	3	12.0-240	0.8	-0.053	0.066	± 0.168	88.5	92.5
Propoxur	60	12.0-240	0.6	+0.007	0.079	± 0.156	91.4	95.4
Thiobencarb	6	12.0-240	0.6	-0.068	0.073	± 0.197	75.0	79.8

[A] Results calculated as carbendazim, the primary breakdown product of benomyl.
[B] See range for benomyl, a precursor for carbendazim.

TABLE 3. RECOMMENDED LIQUID CHROMATOGRAPHIC COLUMNS AND CONDITIONS

PARAMETER	HPLC COLUMN AND CONDITIONS			
Column: Solvent:	C_{18} Acetonitrile	C_{18} Methanol	Cyano Acetonitrile	Cyano Methanol
Application:	Primary Analysis	Confirmation	Confirmation	Confirmation
Column parameters:				
Column	NOVA-PAK C18	NOVA-PAK C18	Supelcosil LC-CN	Supelcosil LC-CN
Stationary phase	octadecyl	octadecyl	cyanopropyl	cyanopropyl
Length (mm)	300	150	250	250
ID (mm)	3.9	3.9	4.6	4.6
Particle size (µm)	4	4	5	5
Ligand density[A]	2.7	2.7	5.2	5.2
Mobile Phase A:				
Solvent	water	water	water	water
Organic modifier[B]	2% 1-propanol	none	none	none
Buffer[C]	TEA-PO$_4$	none	TEA-PO$_4$	TEA-PO$_4$
Concentration (Molarity)	0.02 *M*	none	0.02 *M*	0.02 *M*
Mobile Phase B:				
Solvent[D]	acetonitrile	methanol	acetonitrile	methanol
Organic modifier	2% 1-propanol	none	none	none
Mobile Phase Program:				
Initial hold time (min)	0	0	0	0
Program rate	3-95% B	10-80% B	3-60% B	3-95% B
Program time (min)	30	30	30	30
Program type	linear	linear	linear	linear
Final hold time (min)	5	5	5	5
Flow rate (mL/min)	1.00	1.00	1.00	1.00
Column temperature (°C)	ambient(~24)	ambient(~24)	ambient(~24)	ambient(~24)
Dwell volume (mL)	0.6-0.8	3.5-3.8	0.6-0.8	0.6-0.8
Injection volume (µL)	5	30	5	5
Injection solvent	acetonitrile	1:3 acetonitrile:H$_2$O	acetonitrile	acetonitrile

[A] Ligand density (micromole/m^2) is a better description of surface coverage than % carbon loading [22,23].

[B] Choice of alcohol modifier is not critical. Percentages may be varied to adjust retention times and peak shapes for early eluting analytes. Reequilibration time may be shorter with 1-propanol [24-26].

[C] Buffer is very essential for basic analytes such as Formetanate, Carbendazim, and Benomyl [12,27,28]. Formentate is cationic at about pH 7, and its actual elution time is sensitive to small changes in pH and ionic strength of the buffer in mobile phase A.

[D] Acetonitrile is the better choice when monitoring compounds at UV absorptions below 210 nm [20,29].

TABLE 4. APPROXIMATE RETENTION TIMES AND INDICES FOR ORGANONITROGEN PESTICIDES AND POTENTIALLY INTERFERING COMPOUNDS

	HPLC COLUMNS AND CONDITIONS					
Column:	C18			Cyano		
Solvent:		MeCN	MeOH		MeCN	MeOH
COMPOUND[A] (by retention time)	Retention Index[B]	Ret. Time (min)	Ret. Time (min)	Retention Index	Ret. Time (min)	Ret. Time (min)
Solvent void volume	**0.000**	**2.3**	**1.4**	**0.000**	**3.0**	**3.2**
Asulam	0.004	2.3				
Imazapyr	0.372	3.2				
Acetaminophen *//IS*	**1.000**	**4.7**		**1.000**		
Oxamyl	1.269	6.1	9.9	1.865	7.0	6.4
Caffeine	1.397	6.8				
Methomyl	1.445	7.0	10.7	1.915	7.4	6.6
Formetanate	1.573	7.7	C	2.574	12.8	11.3
Sulfometuron methyl	1.868	9.3				
Acetanilide *//IS*	**2.000**	**9.9**		**2.000**	**8.1**	**7.7**
Fenuron	2.053	10.2				
2,4-D acid	2.064	10.2				
Nicotine	2.179	10.8				
Carbendazim	2.192	10.8	C	4.274	13.6	12.9
Chlorimuron ethyl	2.422	11.9				
Aldicarb	2.755	13.5	19.9	3.000	10.5	10.1
Tebuthiuron	2.817	13.8	23.9			
m-Cresol	2.866	14.0				
Bromacil	2.902	14.2				
Hexazinone	2.921	14.3				
Dinoseb	2.928	14.3				
Simazine	2.938	14.3				
Monuron	2.981	14.5				
Acetophenone *//IS*	**3.000**	**14.6**		**3.000**	**10.5**	**9.9**
Cyanazine	3.003	14.6				
Metribuzin	3.115	15.0				
Thiodicarb	3.247	15.5				
Aminocarb	3.301	15.7				
Propoxur	3.317	15.8	22.9	3.675	13.1	11.9
Bendiocarb	3.376	16.0	23.3			
Carbofuran	3.399	16.1	23.2	4.018	14.4	14.1
Fluometuron	3.551	16.6	25.2			
Chloroform	3.601	16.8				
Carbaryl	3.654	17.0	24.5	5.236	19.1	18.2
Atrazine	3.688	17.1				
Metalaxyl	3.837	17.6				
Diuron	3.843	17.6	27.0	5.751	21.1	19.9
DEET	3.851	17.7				
alpha-Naphthol	3.893	17.8				
Propiophenone *//IS*	**4.000**	**18.2**		**4.000**	14.4	
Propachlor	4.241	18.8				
Thiophanate	4.241	18.8	27.6			
Propham	4.267	18.9	25.9	5.092	17.7	17.3
Diethyl phthalate	4.367	19.2				
Clomazone	4.459	19.4				
Siduron	4.615	19.9				
Desmedipham	4.696	20.1				
Phenmedipham	4.700	20.1	>33			

	HPLC COLUMNS AND CONDITIONS					
Column:	C18			Cyano		
Solvent:		MeCN	MeOH		MeCN	MeOH
COMPOUND[A] (by retention time)	Retention Index[B]	Ret. Time (min)	Ret. Time (min)	Retention Index	Ret. Time (min)	Ret. Time (min)
Methiocarb	4.744	20.2	29.3	6.680	22.5	21.8
Linuron	4.848	20.5	28.9			
BDMC /IS	4.904	20.6				
SWEP	4.919	20.7				
Captan	4.926	20.7	27.7	6.172	20.9	21.6
Promecarb	4.981	20.8	20.4			
Butyrophenone /IS	**5.000**	**20.9**		**5.000**	17.4	
Mexacarbate	5.186	21.3				
Toluene	5.269	21.5				
Chlorpropham	5.504	22.1	30.1	6.700	23.4	23.3
Folpet	5.537	22.2	>33			
Barban	5.566	22.3	>33			
Malathion	5.731	22.7				
Fenitrothion	5.802	22.8				
Benomyl	5.822	22.9	[C]	7.391	25.8	23.9
Oryzalin	5.860	23.0				
Metolachlor	5.876	23.0				
Alachlor	5.979	23.3				
Acetochlor	5.983	23.3				
Valerophenone /IS	**6.000**	**23.3**		**6.000**	20.9	
Captafol	6.018	23.4	30.1			
Neburon	6.045	23.4				
Parathion	6.640	24.7				
Hexanophenone /IS	**7.000**	**25.5**		**7.000**	24.0	
Thiobencarb	7.148	25.8	33.4	7.916	26.2	26.8
Heptanophenone /IS	**8.000**	**27.6**		**8.000**	**26.4**	
Di-n-butyl phthalate	8.016	27.6				
Chlorpyrifos	8.701	28.9				
Pendimethalin	8.724	28.9				
2,4-D butoxyethyl ester	8.892	29.2				
Octanophenone /IS	**9.000**	**29.4**		**9.000**	**29.0**	
BHT	9.488	30.2				
Amitraz	9.886	30.9				
Nonanophenone /IS	**10.000**	**31.1**				
2,4-D ethylhexyl ester	10.545	31.9				
Decanophenone /IS	**11.000**	**32.5**				

[A] Organonitrogen pesticides are in bold letters.
[B] Estimated (~) retention times are extrapolated from shorter columns using relative retention times.
[C] Without TEA-PO$_4$ buffer, basic compounds had irregular peak shapes and retention times, or were not detected.
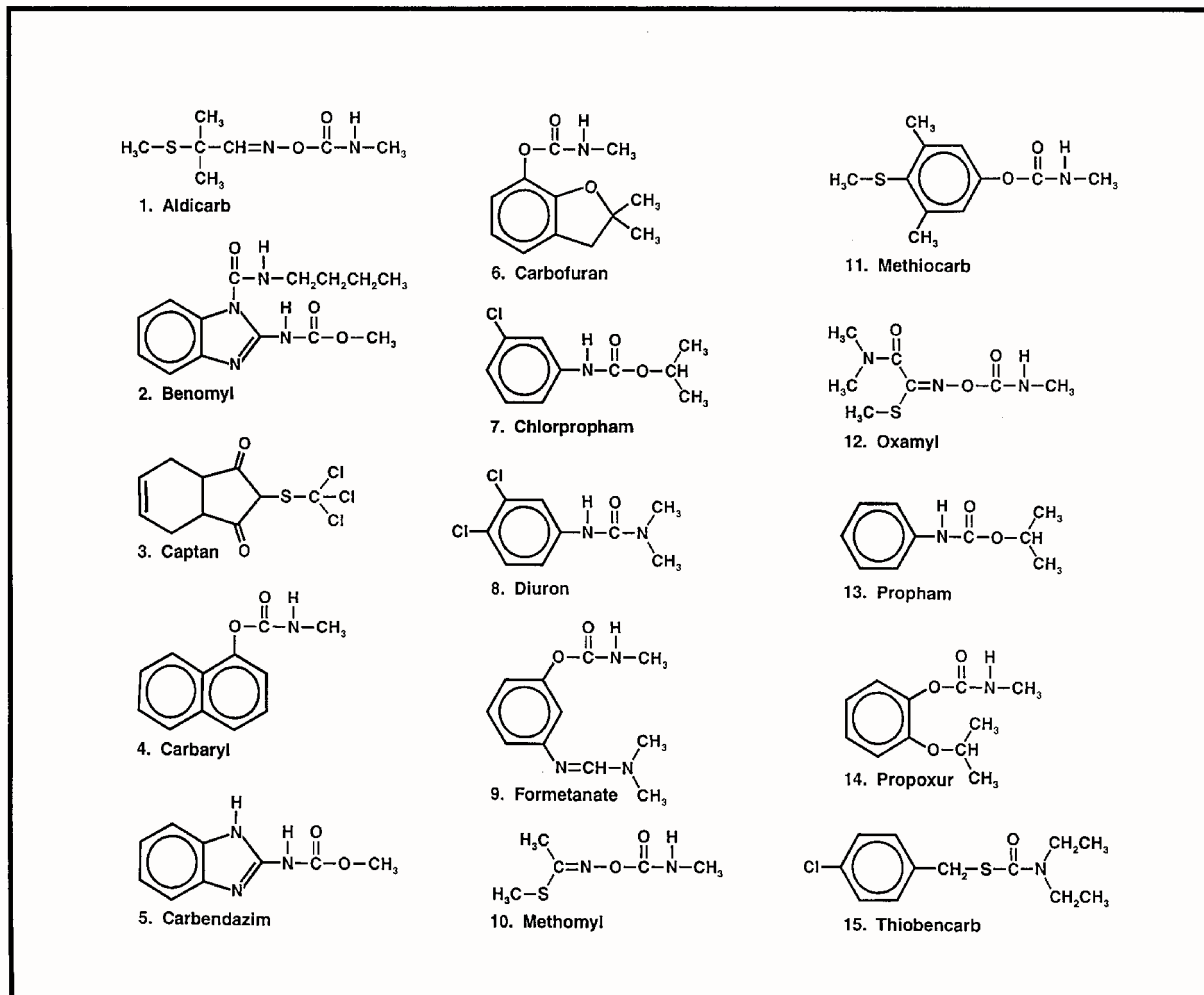Abbreviations: MeCN=acetonitrile; MeOH=methanol; /IS=internal standard.

Table 5. UV Spectra, Orthographic Projection (1)

Approximate nanometers UV absortion maxima (2)

#	Compound (by UV maxima) (3)	max. (3)	Ratio (4) 225/200
1	Dinoseb	373	
2	Metribuzin	293	
3	Benomyl	292	1.074
4	Amitraz	287	
5	Carbendazim	284	0.503
6	Oryzalin	279	
7	Bromacil	277	
8	Parathion	273	
9	Caffeine	270	
10	Fenitrothion	267	
11	Thiophanate	266	
12	Toluene	260	
13	Nicotine	257	
14	Formetanate	254	0.505
15	Asulam	254	
16	Tebuthiuron	251	
17	Neburon	249	
18	Linuron	249	
19	Diuron	248	0.437
20	Chlorimuron ethyl	247	
21	Monuron	246	
22	SWEP	245	
23	Acetaminophen I$	243	
24	Pendimethalin	242	
25	Oxamyl	242	0.867
26	Fluometuron	242	
27	Acetophenone I$	242	
28	Siduron	240	
29	Propiophenone I$	240	
30	Butyrophenone I$	240	
31	Valerophenone I$	240	
32	Hexanophenone I$	239	
33	Heptanophenone I$	239	
34	Octanophenone I$	239	
35	Fenuron	239	
36	Aminocarb	238	
37	Acetanilide I$	238	
38	Chlorpropham	237	0.312
39	Phenmedipham	236	
40	Desmedipham	236	
41	Barban	235	
42	Propham	233	0.393
43	Methomyl	233	1.944
44	Thiodicarb	232	
45	alpha-Naphthol	231	
46	Chlorsulfuron	228	
47	2,4-D acid	228	
48	2,4-D ethylhexyl ester	228	
49	Chlorpyrifos	227	
50	Di-n-butyl phthalate	225	
51	Diethyl phthalate	224	
52	Methiocarb	222	0.269
53	Folpet	221	
54	Atrazine	220	
55	Carbaryl	219	2.595
56	Simazine	219	
57	Thiobencarb	219	0.484
58	Cyanazine	219	
59	BDMC I$		
60	Bendiocarb		
61	BHT		
62	Carbofuran		0.148
63	Propoxur		0.209
64	Mexacarbate		
65	Promecarb		
66	Acetochor		
67	Clomazone	211	
68	Metalaxyl		
69	DEET		
70	Alachlor		
71	Metolachlor		
72	Propachlor		
73	Malathion		
74	Aldicarb		0.169
75	Captan		0.135
76	Captafol		
77	Chloroform		

(1) This table may be used to select wavelengths specific to the compounds of interest.

(2) Approximate UV absorbance: 80-100% | 50-80% | 20-50% | 5-20% | 0-5%

Slight variations in the boundaries should be expected for different scanning UV detectors, compound concentrations, and variations in background subtraction technique. Spectra were obtained at approximately 60ug/mL under analytical conditions on a C18 column. Spectra are scaled independently to 100% and absolute absorbance may vary significantly.

(3) Compounds are sorted by the longest wavelength of a peak or shoulder with an absorbance greater than about 20% of the maximum absorbance between 195 and 400 nm. For peaks at wavelengths less than 210nm, sorting is only approximate due to possible background subtraction errors.

(4) Ratio of UV absorbance, 225 nm/ 200 nm, is a semiqualitative number derived from the average absorbance of 7 replicates analyzed at 60 ug/mL.

FIGURE 1.   STRUCTURE OF CARBAMATE, UREA, AND SULFENIMIDE PESTICIDES

NOTES ON HPLC ANALYSIS OF CARBAMATE, UREA, AND SULFENIMIDE PESTICIDES

It is expected that the analyst will have a well-founded background in the analytical practices and principles that are required for any method to work. The following list of cautions and reminders is provided for convenience; most will impact the success or failure of this method.

A.   ANALYTES

1. Aldicarb
    a. The UV response for aldicarb passes through a minimum at approximately 225 nm. If only two wavelength channels are used (225 nm and 200 nm), the response for aldicarb at 200 nm should be monitored(refer to UV spectrum for aldicarb). At 205 nm the signal will be smaller, but the signal-to- noise ratio may be better on particular instruments. An alternative but lesser maximum is at approximately 245 to 246 nm. This latter maximum, though small, may have less background noise and interferences from coelutants.
    b. Aldicarb is a highly toxic pesticide. Care should be exercised in handling pure stock material.

2. Benomyl (see also Carbendazim)
    a. Benomyl breaks down rapidly by either hydrolysis in protic solvents(*e.g.*, water or methanol) or solvolysis in nonprotic solvents (*e.g.*, methylene chloride or acetonitrile) to carbendazim. The breakdown of benomyl can be so rapid that no benomyl will be detected at all after approximately 4 to 24 hours at room temperature.The rate of solvolysis is slower in the less polar methylene chloride andvery rapid in acetonitrile, even exceeding the rate of hydrolysis [10-12]. Benomyl standards in these nonprotic solvents can be stabilized with the addition of 1% n-butyl isocyanate [30]. The preservative effect is lost as soon as the solution is diluted in solvents that contain or consist of protic solvents, since protic solvents also react with the isocyanates. It is generally unnecessary to add any preservative to the benomyl standard solution (it breaks down anyway). The carbendazim produced can precipitate out, if the solutions are too concentrated. In such an event, the addition of 1% n-butyl isocyanate is essential.
    b. Since benomyl breaks down to carbendazim, do not include both benomyl and carbendazim in the same standard mixture.
    c. When analyzing for benomyl, both benomyl and carbendazim must be determined. The results can be reported as either benomyl or carbendazim by converting the response of one to the equivalent response for the other at any particular wavelength. The relative response of benomyl to carbendazim at 225 nm has been determined to be approximately 1.0738, (adsorption-benomyl/ adsorption-carbendazim). This ratio should be determined for individual instruments with equimolar solutions analyzed separately, and with the benomyl injection solution being preserved with 1% n-butyl isocyanate. To convert carbendazim response to the equivalent benomyl response so that the values can be summed, multiply the carbendazim response by 1.0738 and add this to the benomyl response. Report results as benomyl. Alternately, to report results as carbendazim, divide benomyl response by 1.0738 and then add this to the carbendazim response. Report as total carbendazim.
    d. See notes for carbendazim.

3. Captan
    a. Captan is not stable in methanol or in aqueous mixtures of methanol or acetonitrile at temperatures greater than –12 °C. Therefore, do not dilute desorbing solutions with water or methanol to weaken the injection solvent. Instead,use small injection volumes of acetonitrile solutions.
    b. Use methylene chloride for making stock standard solutions. See NoteA16 of this Appendix.
    c. Captan has low absorption at 225 nm (see UV spectrum for captan). If only two wavelength channels are used (225 and 200 nm), the response for captan at 200 nm should be monitored. Although a lesser response may be obtained at approximately 205 or 210 nm, this can be accompanied by a proportionately better signal-to-noise response and may be an optional choice of wavelength if it is compatible with other analytical objectives.

4. Carbaryl (see also Diuron): Carbaryl tends to break down to 1-naphthol. Chloroacetic acid in methanol will inhibit this reaction [9], but this reagent is deleterious to other analytes. Carbaryl is

stabilized in acetonitrile desorbing solutions containing the TEA-PO$_4$ preservative for at least 24 hours at room temperature, long enough to permit HPLC analysis. Any 1-naphthol that is detected may represent breakdown of carbaryl prior to desorption or may come from another source. Carbaryl has been stabilized with the TEA-PO$_4$ buffer/preservative for up to three weeks at room temperature.

5. Carbendazim
   a. See notes for benomyl.
   b. Carbendazim can break down to 4-aminobenzimidazole and other compounds. In acetonitrile containing TEA-PO$_4$ preservative, this breakdown appears to be inhibited for up to 24 hours at room temperature, long enough to permit analysis of desorbates.
   c. Carbendazim is itself used as a pesticide. There are also other analytes such as thiophanate methyl that can break down to carbendazim. Therefore, any carbendazim should not automatically be assumed to represent benomyl.

6. Carbofuran
   a. In most instances carbofuran and bendiocarb coeluted under all conditions tested. Their UV spectra are so nearly alike as to be practically indistinguishable. Any positive identification for carbofuran must be tempered by this knowledge and would require an alternate confirmation such as by mass spectrometry or a knowledge of the history of the sample.
   b. Propoxur and carbofuran elute very close together. The separation of the two compounds with nearly baseline resolution is a good test of resolving power of the HPLC system for neutral compounds on a C18 HPLC column. If they cannot be resolved, there may be problems with large dead volumes in the system, analytical column quality, or injection quality.

7. Chlorpropham: No serious problems are expected to be encountered.

8. Diuron: The breakdown product of carbaryl, 1-naphthol, elutes immediately after diuron under the conditions given. On certain columns, it may coelute with diuron, giving false positive responses. The retention time of 1-naphthol relative to diuron must be determined for any particular column if carbaryl is anticipated to have been present in the sample.

9. Formetanate
   a. At analytical concentrations formetanate is not stable either in methanol or in aqueous mixtures of methanol or acetonitrile with or without chloroacetic acid. Therefore, do not dilute desorbing solutions with methanol or water to weaken the injection solvent. Do not use chloroacetic acid as a preservative. Use only small injection volumes of acetonitrile solutions.
   b. The breakdown of formetanate in analytical solutions is inhibited in acetonitrile containing TEA-PO$_4$ preservative at room temperature for up to 24 hours, long enough to permit analysis.
   c. At stock standard concentrations, formetanate dissolves better in 50:50 mixtures of methanol: acetonitrile and is stable if kept in freezer at −12 ± 1 °C.
   d. Formetanate is basic and possesses a single positive charge in solution at neutral pH (6.9 – 7.1). The use of TEA-PO$_4$ buffer and/or base-deactivated HPLC columns is essential for the analysis for this compound.
   e. The exact retention time of formetanate is more affected by exact pH and ionic strength of the buffer (or any other electrolytes) than are the noncharged analytes. Day-to-day inconsistencies in the preparation of mobile phases will cause differences in retention time. If the analyte is eluting too close to methomyl (which just precedes it under the specified conditions), its retention time may be delayed by either raising the pH slightly (by 0.1 to 0.2 units), or by lowering the ionic strength of mobile phase A.

10. Methiocarb: Methiocarb has the same problems as carbaryl, only to a lesser extent. As methiocarb breaks down, there are several other peaks that arise. These have been shown to be alkylthiophenol and/or oxidation products.

11. Methomyl: Attention should be placed on the problem of methomyl as it relates to its early elution time. Refer to Notes B1a and B4 of this Appendix.

12. Oxamyl: Attention should be placed on the problem of oxamyl as it relates to its early elution time. Refer to Notes B1a and B4 of this Appendix.

13. Propham: No problems are expected with propham.

14. Propoxur
    a. With prolonged standing in desorbing solutions at room temperature without preservative, propoxur has been observed to break down to some degree, although much less than carbaryl.
    b. Propoxur elutes very close to carbofuran. See comments under carbofuran.

15. Thiobencarb: Thiobencarb is relatively nonpolar and has lower (but acceptable) recoveries from the XAD-2 resin than the other analytes.

16. Solvent for Analyte Standard Solutions: Most of the analytes (Table 1) are soluble in acetonitrile at stock standard concentrations. The exceptions are benomyl, captan, and formetanate. Appropriate solvents are described under headings for these compounds. In each of the solvents mentioned, analytes are stable at −12 ± 1 °C for at least 30 days.

17. Desorption Solvent and Preservative for Analytes: All analytes (except benomyl) were stable in acetonitrile at −12 °C for at least 48 hours. If refrigerated HPLC autosampler trays are available and all desorbing and tumbling operations are carried out in subambient conditions, no preservative should be needed. At room temperature, several analytes (e.g., carbaryl, methiocarb, and oxamyl especially) degraded unpredictably over 24 to 48 hours. The addition of as little as 0.2% v/v of aqueous 0.1 M triethylamine phosphate (TEA-PO$_4$) buffer, pH 6.9 to 7.1, to the acetonitrile desorption solvent stabilizes all analytes (except benomyl) from hydrolysis or solvolysis for at least 48 hours at room temperature. Methanol, isopropanol, aqueous methanol, and acetonitrile containing any of these alcohols promote degradation of several analytes (especially captan and formetanate) and poor desorption efficiencies. The use of chloroacetic acid, as is required for aqueous samples in EPA Method 531.1 [9], is destructive to at least formetanate. Since the desorption solvent is also the HPLC injection solvent, refer to note on Injection Solvent (B1) for additional comments.

B. HPLC CONDITIONS

    1. Injection Solvent
        a. Normally a solvent producing equal or lower capacity factors than the mobile phase is desirable as an injection solvent in order to produce sharp peaks for early eluting analytes (e.g., oxamyl and methomyl) [31,32]. Since the initial mobile phase mixture of this method is mostly water, the only way to achieve this would be to dilute the samples with water. Water, however, is deleterious to several of the analytes specified in this method (see note A17 above). Therefore, an acceptable alternative is to inject very small volumes, not greater than 5 µL [31], of the desorption solvent (acetonitrile in this method) on a high-resolution HPLC column. If it is known that only analytes that are stable in aqueous solutions are to be determined (e.g., oxamyl and methomyl, but not formetanate or captan), the desorbates may be diluted with water and larger volumes injected. By diluting with water, sharper peaks may be obtained for early eluting compounds with accelerated elution conditions such as the use of shorter HPLC columns, higher percentages of organic modifier, or higher percentages of mobile phase B in the initial conditions.
        b. All sample extracts must be filtered through individual 0.45-micron PTFE filters in order to prolong guard column lifetimes and to protect the injection system valving.

        c. The accidental or intentional inclusion of significant amounts of solvents less polar than acetonitrile in the injection solvent, such as tetrahydrofuran or acetone, may shorten retention times and adversely affect the peak shapes of early eluites (e.g., oxamyl and methomyl) [31].

    2. Guard Columns: Guard columns are essential to the long life and reproducibility of results on the main analytical column. There are several on the market. Those giving the lowest possible dead volume preserve good peak shapes and resolution of quality analytical columns and are preferred.

3. Analytical Columns
   a. General: The main analytical column specified in this method is a C18 reverse-phase column. Other columns known to perform well also may be used, following manufacturer recommendations. There are many good columns available [28,33].
   b. Base Deactivation: The basic compounds (benomyl, carbendazim, and formetanate) present special challenges that are easily overcome with highly inert or base-deactivated columns [26]. The addition of TEA-PO$_4$ buffer to mobile phase A also greatly improves performance for these compounds [16, 26, 27].
   c. Dimensions: A longer column (300 mm) was used in this method in order to improve resolution for a large number of analytes and interferences, expected and unexpected. Long columns have higher operating pressures; therefore, necessary steps should be taken to provide for them, such as thicker-walled (narrower-bore) transfer lines (if polymer tubing is used), etc. Shorter columns may work well if a limited number of analytes or interferences are expected. The diameter (3.9-mm) is not necessarily critical and should be governed by user preference and equipment, adjusting flow rates and other parameters as necessary. Diameters from 2.0 to 4.6 mm should be expected to perform similarly, as long as the columns are stable and rugged.
   d. Packing Density: Packing (or ligand) density is a better parameter than carbon loading for comparing columns [21,22]. A column with a ligand density near that of the one used in this method should be expected to perform similarly.

4. Mobile Phase Composition
   a. Modifier: Because of the high percentage of water in the initial mobile phase, a condition referred to as hydrophobic collapse of the C18 phase occurs [23,24], which results in poor reequilibration and irreproducible retention times and peak shapes for early eluting analytes such as oxamyl and methomyl. The addition of a constant amount of an alcohol to both mobile phases A and B has been reported and found to improve column performance under these conditions The method specifies 2% 1-propanol to be added to both mobile phases A and B. A concentration of 1-propanol between 3% and 4% has been reported as optimum [24,25]; 2% is a compromise made in order to generate retention (capacity) factors for the earliest eluites, oxamyl and methomyl, of greater than 5, as suggested in the literature [26]. Also, 1-propanol has been reported (and found) to reduce the time required for reequilibration at the end of the run [24]. Other alcohols may be used at higher concentrations (except for reducing reequilibration times) [1]. These are isopropanol (at 3% to 5%) and methanol (at 5% to 10%). If early-eluting analytes are to be determined, or if a column is found that gives sufficiently long retention times for the earliest expected eluite with an initial mobile phase composition as high as 5% to 10% acetonitrile in water, the alcohol could be eliminated (from both phases). Table 4 would not apply if such changes are made.
   b. Mobile Phase B: Pure methanol as mobile phase B results in a steep rise in the baseline UV response, making automatic integrations difficult and UV scanning for confirmation spectra nearly impossible. For this reason acetonitrile has been chosen [28,29]. Methanol is acceptable if these conditions are tolerable and is recommended as an alternate solvent system on a C18 column for confirmation (Section F3); however, precision and LOD values reported in Table 2 would not be applicable.
   c. Solvent Programming: One of the most serious concerns that may be encountered in trying to make adaptations of this method is an attempt to shorten retention times by using higher concentrations of organic modifier in mobile phase A, higher percentages of mobile phase B in the initial solvent program condition, or faster solvent programming. In any case, these changes will seriously affect the peak shape, sensitivity, and retention time reproducibility of early eluting analytes. There is also a greater possibility of false positives from potential interferences because of poor resolution in the early region of the chromatogram. Retention times for the earliest eluting analyte should be kept at 3 to 6 times the retention time of the solvent front (i.e., of an unretained analyte), which is equivalent to a capacity factor of 2 to 5 [5]. The retention or capacity factor for any analyte can be calculated as follows:

   Retention or capacity factor = $(t_r - t_o) / t_o$
   where t_r =   the retention time of the compound
   and t_o =   the retention time of an unretained analyte (the mobile phase

holdup time).

>   The value of keeping analytes from eluting too close to the solvent front cannot be overemphasized.
>
> d. Buffer: For the compounds evaluated, formetanate, carbendazim, and benomyl required a buffer in order to obtain good peak shapes. Of several pHs tested, pH 6.8 to 7.1 gave the best results. Buffer concentration should be 0.01 to 0.05 *M*. The concentration and pH of the buffer had a great effect on exact retention time of formetanate.

5. Dwell Volumes: Dwell volumes can affect the retention times and peak shapes of analytes. This is internal volume of the system from the point of mixture of the mobile phases A and B to the head of the column. It includes the volume of the transfer lines; any in-line filters (which should be between the pump and the sample injector if at all, and definitely not after the sample injector); the sample injector; the guard column; and the head of the analytical column. This volume can range from approximately 0.6 to 3.8 mL. At a flow rate of 1 mL/minute, each mL dwell volume represents a minute delay between the time that the pump produces a given change in a mixture of solvent and the time when the analytical column experiences that change. In effect, it is equivalent to a solvent program delay [13,14]. For this method, lower dwell volumes and no program delays gave better peak shapes for the early eluites.

6. Dead Volumes: Dead volumes can cause the method to fail seriously. Important areas to look for are larger bore and longer-than-necessary transfer lines between the sample injector and the analytical column [15]. Another area of potential large dead volumes are poorly designed or connected guard columns and sample injectors.

7. UV Wavelength Selection
    a. The wavelengths specified in this method are a best compromise for all of the analytes listed in Table 1. There are maxima for each of the pesticides that would give greater sensitivity and/or signal-to-noise ratio. If only a selected few of the pesticides in Table 1 are to be determined, other wavelengths may be considered. For this purpose, Table , an orthographic projection of the UV absorbances, is provided. From this table, a wavelength can be selected in the vertical column that intersects the zones of greater UV absorbance for the compounds of interest. For example, if only ureas are to be determined, the selection of an absorbance band of 240 to 250 nm would give greater sensitivity for these compounds and less interference from others.
    b. Spectra are also provided in the Backup Data Report [1] for many analytes and potential interferences. These were obtained under actual operating conditions. Because of unavoidable errors in background subtractions, the profiles of these spectra are subject to errors at the low wavelength ends of the spectra (190 nm to 210 nm). This could be attributed to the absorbance from the alcohol modifier to the mobile phases, which, in spite of efforts to add an equal amount to both phases, produces a slight baseline rise near the end of the chromatograms.
    c. Many of the O-aryl carbamates and the sulfenimides absorb well only in the low UV range (<215 nm). This is generally an area of great background noise. Many contaminants (plasticizers and solvents) also absorb in this range better than at higher wavelengths. Selecting a longer wavelength that is not at the absorption maximum may give a better signal-to-noise ratio and thus actually increase sensitivity. This should be determined by experimentation for selected analytes.
    d. Bandwidth: A bandwidth of 15 nm was used for evaluation of this method.

C. INTERNAL STANDARDS

1. Calibration Internal Standards: Internal standards are essential for obtaining the precisions listed in Table 2 [1,16]. Acetanilide was found to be unretained by the media during desorption, and therefore, could be added to the extraction fluid. It was also found to be relatively stable and not to interfere with the retention times of other analytes. A second compound, acetophenone, may be added as a back-up internal standard. It is also unretained by all media except XAD-2, on which it has about a 95% recovery. It may be used whenever the acetanilide is interfered with by coeluting analytes or contaminants. It also serves to indicate, by monitoring relative response between the

two internal standards, when the first internal standard has a coeluting interference.

2. Alternate Calibration InternalStandards: It may be wise, especially under isocratic conditions, to select other internal standards having capacity factors closer to particular analytes of interest. There is a wide range of alkyl phenones available for late-eluting analytes, and 4-hydroxy-acetanilide (acetaminophen)for earlier eluting analytes. Their retention times are listed in Table 4. Since these longer alkyl chain phenones have increasingly lower recoveries from the XAD-2 resin, they should not be added to the extraction solutions until after the resin is removed.

3. Retention Index Reference Standards:These are optional standards and are used to establish relative retention times for qualitative purposes. These are highlighted in Table 4 and include a homologous series of alkyl phenones and acetanilides. Establishing retention times between two nearest-eluting reference standards gives a more consistent retention value than retention time alone or relative retention time using a single internal standard alone. This value, the Retention Index, varies according to column and analytical conditions, but should be relatively consistent for any one set of conditions and more reliable as a qualitative tool over a long period of time. This value is calculated as follows:

$RI_{(A)}$ = Retention Index of Analyte "A"

$$\frac{Tr_{(A)} - Tr_{(RS-P)}}{Tr_{(RS-F)} - Tr_{(RS-P)}} + N_{(RS-P)}$$

where
- $Tr_{(A)}$ = retention time of analyte A
- $Tr_{(RS-P)}$ = retention time of preceding reference standard
- $Tr_{(RS-F)}$ = retention time of following reference standard
- $N_{(RS-P)}$ = a number assigned to preceding reference standard, with zero assigned to the first peak in the series.

(Numerical assignments for the acetanilide and phenone series are suggested in Table 4.)

In order to avoid confusion in the chromatograms, the retention index reference standards are analyzed periodically as external standards and not added to the analytical samples themselves. The use of these reference standards is optional but is suggested where application of this method is expected to encounter consistently a broad range of unknown analytes and to augment other confirmatory techniques.

D. INTERFERENCES:

The UV detector responds to many compounds. Retention times of some common potential interferences are provided in Table 4.   Interfering compounds that may be encountered are discussed below.

1. Impurities in Mobile Phases and Additives: Only HPLC-grade solvents should be used. Triethylamine (TEA) was found to develop unidentified impurities over time which contributed to significant irregularities in the baseline. This degradation was reduced or eliminated by storing the TEA under nitrogen in a small desiccator at 0 to 4 °C [8].

2. Organic Solvents or Fuels
   a. Chloroform showed a response that nearly coelutes with carbaryl (Table 4). Benomyl and captan, therefore, are made up in methylene chloride, which is more UV transparent than chloroform.
   b. Toluene (Table 4).
   c. Blends of solvents having ketones, ester, or any of the above compounds, such as lacquer solvents, gasoline, paint stripper, and cleaning solvents, are to be avoided or documented during sample collection and handling.

3. Industrial Chemicals (Plastic and Rubber Additives)
   a. Several plasticizers might elute in the window of interest depending upon the selection of column and conditions. These include diethyl and dibutyl phthalates. Dibutyl phthalate is typically found in polyvinyl gloves, flexible tubing, and in coatings on bottles and tool handles. Contact with these materials should be avoided or documented. Other plasticizers, such as dioctyl phthalate and bis-ethylhexyl adipate, have late elution times and will elute after about 30 minutes under the conditions specified. However, if the run times are shortened, these may be carried over on-column to subsequent analyses, causing interferences.
   b. Common antioxidants such as BHT (2,6-di-tert-butyl-4-methyl phenol) also elute late and may be carried over on-column, creating interferences in subsequent analyses.

4. Other Pesticides: Spray mixtures very often contain a mixture of pesticides. It is not uncommon to find chlorpyrifos, an organophosphate, or a pyrethroid pesticide in combination with propoxur, for example. Both of these noncarbamate pesticides can be detected under the conditions of the method. The retention time and spectra of chlorpyrifos is included for qualitative purposes. Most pyrethroids elute later than most of the carbamates; if present, they may elute in a subsequent run if run times are too short. It is also not uncommon to mix herbicides of different classes such as diuron with bromacil, atrazine, or 2,4-D. Because of this possibility, retention times and spectra of other common herbicides are also provided in Table 4 for qualitative purposes.

5. Miscellaneous Chemicals: There are a number of chemicals which elute in the retention time window of interest for carbamates and urea pesticides and may, with particular columns or conditions, interfere with analytes of interest. The presence or the known use of these compounds or their sources should be documented as part of the sample history. Table 4 lists a few of the compounds, which include the following.
   a. The common insect repellent, DEET (N,N-diethyl-meta-toluamide). Since DEET may be heavily used by outdoor workers, its presence on exposed areas of the skin or clothing may contribute to sampler contamination either through direct contact with the face of the sampler or through collection of vapors if the sampler is in close proximity to areas of application or is exposed to overspray during application from a spray can or bottle.
   b. Inadvertent collection of tobacco side-stream smoke may introduce potentially interfering compounds, one of which is nicotine.
   c. Compounds in beverages used during work periods, which include at least caffeine.

E. SAMPLER

1. The OVS-2 Sampler. The OVS-2 (OSHA Versatile Sampler with XAD-2) combines both filter and XAD-2 sorbent in one unit. The filter is necessary to trap submicron aerosols that would pass through the XAD-2 bed. Substitutions should not be made.

2. Quartz Fiber and Glass Fiber Filters (GFF). The OVS-2 tubes are available with either glass or quartz fiber filters. OSHA Methods specify GFF. This method specifies a quartz fiber filter. For analytes being desorbed with acetonitrile, no difference in desorption efficiency was observed between glass fiber and quartz fiber filters. Therefore, the tubes may be interchanged for the analytes specified in this method.

3. Flow Rates. The OVS-2 sampler is designed for a flow rate of 1 L/min. At slower flow rates, 0.1 to 0.2 L/min, there may not be enough capture velocity for aerosols.

4. Applying Liquid Spikes. The Teflon® retainer ring should be removed when spiking the face of the OVS-2 tubes with more than approximately 10 µL, in order to prevent wicking of the carrier solvent behind the ring and consequent loss of standard. Volumes of spiking fluid greater than 15 to 30 µL will flood the XAD-2 section and possibly wick into the back-up section. Whenever more than 15 to 30 µL is to be applied to the tubes, air must be drawn through the tubes at approximately 1 L/min during the spiking procedure and the solvent added in 15- to 30-µL increments with a few minutes between each addition allowed for drying of the solvent.

F. CONFIRMATION

1. By Relative Retention Times (Retention Index).The Retention Index (RI) may vary considerably from column to column and from one set of conditions to another. But it will be reasonably consistent once a set of conditions has been chosen and will be much more reliable for day-to-day comparisons than will absolute retention times. Actual RIs need to be established for each set of conditions. Compounds that are ionic under elution conditions or that interact strongly with polar sites on the column will have the most variable retention times and retention indices.

2. By Second Column. The cyanopropyl stationary phase strongly induces some exchanges in elution orders and alters relative spacings between adjacent analytes in the chromatogram that may be useful in confirmations.

3. By Alternate Solvent. As mentioned earlier (Section B1), methanol as the mobile phase B solvent on a C18 column can be used just as effectively to establish confirmations because methanol interacts differently with the stationary phase than acetonitrile, and so different molecular forces come into play. Significant alterations in retention order are thus obtained; for some analytes this is more dramatic than with a cyanopropyl column.

4. By Ratio of Two UV Absorption Bands. As long as the UV absorption channels are not saturated, there should be a consistent ratio between the background-corrected absorption bands that is characteristic of each analyte and should reflect the ratio of relative heights of the absorption spectra at their respective UV bands in the UV spectra. This ratio is dependent, however, on the bandwidth of the adsorption bands employed, and consistent bandwidths must be used. The consistency of the ratio of absorption across the HPLC peak is also indicative of the purity of the peak. A constantly changing ratio indicates that the peak may have multiple components.

5. By Matching to Reference UV Spectra. Unknown spectra should not be oversaturated in any portion. They need to be background-corrected properly. If the baseline is rising, a background selected from the backside of the peak may induce losses of absorption in the region below 210 nm. Conversely, one selected from in front of the unknown HPLC peak may add to this region of the spectra. It is better to get an averaged background first. Spectra maxima should match within a few nanometers. Relative absorbance at each maxima may vary, even after background subtraction, depending upon the concentration of the analytes and the characteristics of different scanning UV detectors.