

VALENT U.S.A. CORPORATION
DUBLIN LABORATORY
DUBLIN, CALIFORNIA

DETERMINATION OF ACEPHATE
AND METHAMIDOPHOS IN CROPS,
EGGS, TISSUES, WATER, AND MILK
METHOD RM-12A-9

DATE: NOVEMBER 3, 1994

INTRODUCTION

Acephate (O, S-dimethyl acetylphosphoramidothioate) is an organophosphate pesticide, which is partially metabolized to methamidophos (O, S-dimethyl phosphoramidothioate). The method of analysis described below determines both acephate and methamidophos. Briefly, the method involves extraction with ethyl acetate or acetonitrile:hexane (oily crops), cleanup using silica gel column chromatography, optional partition cleanup by acetonitrile:hexane and measurement by gas chromatography using a flame photometric detector in the phosphorus mode. This method is the same as described in method RM-12A-6, but includes eggs and animal tissues as a matrix, eliminates the packed column measurement, and describes an alternative wide bore capillary column for the measurement.

REAGENTS

Acetonitrile: Pesticide Quality

Ethyl Acetate: Pesticide Quality

Ethyl Ether: Anhydrous Analytical Reagent; Recommend Mallinckrodt #0848

Hexane: Pesticide Quality

Filter Paper: Whatman 2V

Methanol: Pesticide Quality

Dichloromethane: Pesticide Quality

Phosphoric Acid: Reagent grade, 85%.

Silica Gel: Chromatographic Grade, E. Merck, A. G. Darmstadt (Germany) (70 - 230 mesh) or equivalent

Sodium Sulfate: Anhydrous, granular reagent grade

Acephate Reference Standard

Methamidophos Reference Standard

EQUIPMENT

Omni-Mixer or similar top-drive blender with adaptor and shafts for use with pint (473 mL) or quart (946 mL) glass canning jars.

Osterizer Blender or equivalent

Hobart Food Chopper and meat grinder

Wiley Mill

Separatory funnels - 250 mL, 500 mL and/or 1000 mL capacities with Teflon® stopcocks.

Liquid Chromatography Columns - 25 mm I.D. x 400 mm with Teflon® stopcock plugs

Gas Chromatograph

(Note: Parameters may require adjustments to obtain optimum response. Thus, the following parameters are given only for reference.)

Hewlett-Packard 5890 equipped with FPD in the phosphorus mode, an autosampler, packed column injector, integrator and the following parameters:

(1) Column: 10 m x 0.53 mm I.D. 50% phenyl-methyl silicone (2.0 μ m film thickness) capillary column (Hewlett/Packard, HP-17)

Flow Rates:	Carrier gas (N ₂)	- 20 mL/min
	Make-up gas (N ₂)	- 19 mL/min
	Hydrogen	- 70 mL/min
	Air	- 100 mL/min

Temperatures:	Injector	- 250°C
	Detector	- 250°C
	Column Oven:	
	Initial	- 130°C, hold 1 minute
	Rate	- 10°C per minute
	Final	- 200°C

Retention Time:	Acephate	- 3.96 minutes (Figure 1)
	Methamidophos	- 1.97 minutes

(2) Column: 15 m x 0.53 mm I.D. dimethylpolysiloxane (3 μ m film thickness) capillary column (J & W Scientific, DB-1)

Flow Rates:	Carrier gas (N ₂)	- 10 mL/min
	Make-up gas (N ₂)	- 30 mL/min
	Hydrogen	- 70 mL/min
	Air	- 90 mL/min

Temperatures:	Injector	- 250° C
	Detector	- 250° C
	Column Oven:	
	Initial	- 130° C, hold 1 minute
	Rate	- 15° C per minute
	Final	- 200° C, hold 1 minute
Retention Time:	Acephate	- 3.61 minutes (Figure 2)
	Methamidophos	- 2.15 minutes

EXTRACTION

(Note: Amounts of sample extracted may require adjustment in order to obtain satisfactory recovery efficiencies. Also, quantities stated for sodium sulfate and extracting solvents are approximations only.)

Crops, Eggs and Animal Tissues

Macerate or grind the sample using a food chopper or Wiley Mill. Transfer a 25 g sample to a pint or quart Mason jar. (For recovery purposes, fortify a control sample with an aliquot of an acetone solution of the standards at this point in the procedure.) Add deionized water to wet the sample and mix well. For eggs and muscle tissues only, add 3 mL phosphoric acid and mix well.

Add 150 mL of ethyl acetate; add enough (200 to 500 g) sodium sulfate to absorb the amount of water added, and mix thoroughly. Blend on an Omni-Mixer for 5 minutes. Filter the extract through 100-200 grams of sodium sulfate (a glass wool plug or filter paper may be used to contain the sodium sulfate in a 10-12 cm diameter funnel but the use of filter paper results in clearer extracts) into a 1000 mL round bottom flask. Repeat the extraction and filtration step two more times using 100 mL of ethyl acetate each time. (NOTE: If thorough agitation is not obtained during the blending step, additional ethyl acetate may be required. Add more sodium sulfate if emulsions form.) Transfer the contents of the mixing container to the filter cake and rinse the filter cake with 50 mL of ethyl acetate.

Evaporate the combined filtrates to dryness on a vacuum rotary evaporator, azeotroping, when necessary, using a mixture of ethyl acetate and acetone. (Note: some samples, i.e., eggs, begin to foam when near dryness. At that point, remove from evaporator and add ethyl acetate. Return to evaporator and complete evaporation.) If an oily residue remains after evaporation, proceed to Acetonitrile:Hexane Partition cleanup. Otherwise, proceed to Silica Gel Column cleanup.

Milk and Water

Transfer a 50 or 100 g sample to a pint or quart Mason jar. (For recovery purposes, fortify a control sample with an aliquot of an acetone solution of the standards at this point in the procedure.) Add 200 or 400 mL of ethyl acetate and, with stirring to prevent caking, add enough (300-500 g) sodium sulfate to absorb the liquid. Blend on an Omni-Mixer for 5 minutes. Allow the solids to settle and decant the extract through a bed of sodium sulfate (ca 100 g) in a 10-cm diameter filter funnel plugged with glass wool into a 1000 mL round bottom flask. Repeat the extraction and filtration steps two more times using 100 or 200 mL portions of ethyl acetate. Rinse the filter cake with 50 mL of ethyl acetate and evaporate the combined filtrates to dryness on a vacuum rotary evaporator. (NOTE: If caking prevents thorough agitation during the blending step, break the cake with a rod before continuing with the extraction.) Proceed to Silica Gel Column cleanup.

Oily Crops

Macerate or grind the sample using a food chopper or Wiley Mill. Transfer a 10 g sample to a pint or quart Mason jar. (For recovery purposes, fortify a control sample with an aliquot of an acetone solution of the standards at this point in the procedure.) Add deionized water to wet the sample and mix thoroughly.

Add 200 mL acetonitrile. Add 350 g of sodium sulfate with stirring to prevent caking. Add 200 mL of hexane and blend the mixture on an Omni-Mixer for 5 minutes. Filter the extract through a bed of sodium sulfate (ca 100 g) in a 10-cm diameter filter funnel plugged with glass wool into a 1000 mL separatory funnel. Repeat the extraction and filtration steps two more times using 100 mL portions of acetonitrile. Shake the combined filtrates and allow the phases to separate. Discard the hexane layer. Wash the acetonitrile with two successive 100 mL portions of hexane. Evaporate the acetonitrile to dryness on a vacuum rotary evaporator. Proceed to Silica Gel Column cleanup or, if oily residue remains after evaporation, to the Acetonitrile:Hexane Partition cleanup.

Oil

Transfer a 25 g sample to a 500 mL separatory funnel. (For recovery purposes, fortify a control sample with an aliquot of an acetone solution of the standards at this point in the procedure.) Dissolve the sample in 200 mL of hexane. Add 100 mL of acetonitrile and shake the separatory funnel. Allow the phases to separate, and discard the hexane layer. Wash the acetonitrile phase two times with 100 mL portions of hexane. Transfer the acetonitrile to a 250 or 500 mL round bottom flask and evaporate to dryness on a vacuum rotary evaporator. Proceed to Silica Gel Column cleanup or, if oily residue remains after evaporation, to the Acetonitrile:Hexane Partition cleanup. (Direct measurement without cleanup is sometimes possible.)

Cured Tobacco

Grind the sample in a Wiley mill. Transfer a 10 g sample to a Mason jar. (For recovery purposes, fortify a control sample with an aliquot of an acetone solution of the standards at this point in the procedure.) Add 50 mL of deionized water and mix thoroughly. Add 150 mL of ethyl acetate; add 350 g of sodium sulfate with stirring to prevent caking; blend on an Omni-Mixer for 5 minutes. Allow the solids to settle and decant the extract through a filter paper containing a bed of sodium sulfate (ca 100 g) in a 10-cm diameter filter funnel into a 1000 mL separatory funnel. Repeat the extraction and filtration steps two more times using 100 mL portions of ethyl acetate. Rinse the filter cake with 50 mL of ethyl acetate.

Evaporate the combined filtrates to dryness on a vacuum rotary evaporator. Proceed to Silica Gel Column cleanup or, if oily residue remains after evaporation, to the Acetonitrile:Hexane Partition cleanup.

CLEAN UP

(NOTE: For some matrices, it may be necessary to utilize the following cleanup procedures, particularly the silica gel column, more than once.)

Acetonitrile:Hexane Partition

(NOTE: the amounts of the solvents in the following can be adjusted for a particular sample matrix provided the solvent proportions are maintained.)

Quantitatively transfer the residue to a 250 mL separatory funnel with a total of 50 mL of acetonitrile and 100 mL of hexane. (If necessary, add a few grams of sodium sulfate to aid in removal of solid material from the walls of the flask.) Shake the separatory funnel for 1 minute and allow the phases to separate. Transfer the bottom acetonitrile layer into another separatory funnel. (NOTE: If hexane layer is not clear but appears overloaded with sample matrix, i.e., 5 gm citrus soapstock, extract this initial hexane layer with 25 mL of acetonitrile twice, combining all the acetonitrile in one separatory funnel.) Wash the acetonitrile with two 100 mL portions of hexane. Discard the hexane layer. Transfer the acetonitrile to a 250 mL round bottom flask and evaporate to dryness on a vacuum rotary evaporator. Proceed with the Silica Gel Column cleanup step.

Silica Gel Column Chromatography

(NOTE: The activity of silica gel may vary from batch to batch and should be checked by carrying out a recovery from crop extract fortified at 0.25 ppm acephate and 0.1 ppm methamidophos through this cleanup step. In the absence of crop material, the recovery of acephate will be low.)

Prepare a silica gel column as follows: Place a glass wool plug in the bottom of a 25 x 400 mm column equipped with Teflon® stopcock. Add a 15 gram layer of sodium sulfate, 50 mL of ether, and 15 grams of silica gel. Rinse the sides of the column with ether and add a 15 gram layer of sodium sulfate. Again, rinse the sides of the column with ether and allow the ether to drain to the top of the column.

Quantitatively transfer the residue from the extraction step or from the Acetonitrile:Hexane Partition step with four 10 mL portions of ether. (NOTE: Save flask and ensure complete transfer of residue by using to contain all eluants prior to transferring to column.) Wash the column with 100 mL of ether followed by 100 mL of 5% methanol in ether. (NOTE: The following technique is suggested for the 5% wash for samples which adhere to the sides of the flask: add 5 mL of methanol to flask and mix well to dissolve any remaining residue adhering to the sides of the flask. Add 95-100 mL ether to the flask, and mix well before transferring solvent mixture to column.) Elute acephate and methamidophos with 250 mL of 10% methanol in ether (25 mL methanol plus 225 mL ether). Evaporate the eluate to dryness on a vacuum rotary evaporator and proceed with the measurement.

MEASUREMENT

[NOTE: Proper conditioning of the gas chromatographic column is essential for acephate measurements. Therefore, at least two to three conditioning injections, preferably of the samples to be analyzed within the data set, must be made prior to actual measurements to ensure satisfactory reproducibility. (See Note 1.)]

Dissolve the residue in 5.0 mL acetone (25 gram samples) or 10.0 mL acetone (50 gram samples). For other extracted amounts, adjust acetone volume proportionately to obtain the equivalent limit of detection.

Transfer the solutions to be measured to vials for use on the automatic liquid sampler. Load the sampler tray in some order, such as: conditioning, conditioning, standard, fortified, untreated sample, standard, sample, sample, standard, sample, sample, standard. . . . Set the syringe to a specific volume, such as 1 or 3 μL . The standard vials contain 0.5 $\mu\text{g/mL}$ methamidophos and 1.25 $\mu\text{g/mL}$ acephate in acetone.

CALCULATION

$$\text{ppm} = \frac{\text{Peak Units (Sample)} \times C \times \text{DF} \times \text{Final Volume (mL)}}{\text{Average Peak Units of Standard} \times \text{Weight (g) of Sample}}$$

where C is the standard concentration ($\mu\text{g/mL}$) and DF is the dilution factor.

LIMIT OF DETECTION

The limit of detection for acephate is 0.02 ppm and for methamidophos 0.01 ppm, using a 25 g sample and a final volume of 5.0 mL.

LINEARITY

The linearity of the measurement should be verified daily using at least 4 levels which encompass the concentration range of the samples and include the reference standard solution (0.5 µg/mL methamidophos and 1.25 µg/mL acephate in acetone) and a lower limit of 10% of the reference solution. Response factors (response equivalent to 1.0 µg/mL) should have a coefficient of variation of ±10% or less. Exceptions will be allowed only with supervisory approval.

RECOVERY

A fortified control sample should be analyzed concurrently with each set of samples for validation purposes. Method recovery for the fortified sample should be between 70% to 120% to be acceptable. All samples analyzed concurrently with a fortified sample which does not have an acceptable method recovery must be reanalyzed. Exceptions will be allowed only with supervisory approval.

Calculation of percent recovery:

$$\% \text{ Recovery} = \frac{100 \times (\text{ppm in fortified sample} - \text{ppm in control sample})}{\text{ppm fortified}}$$

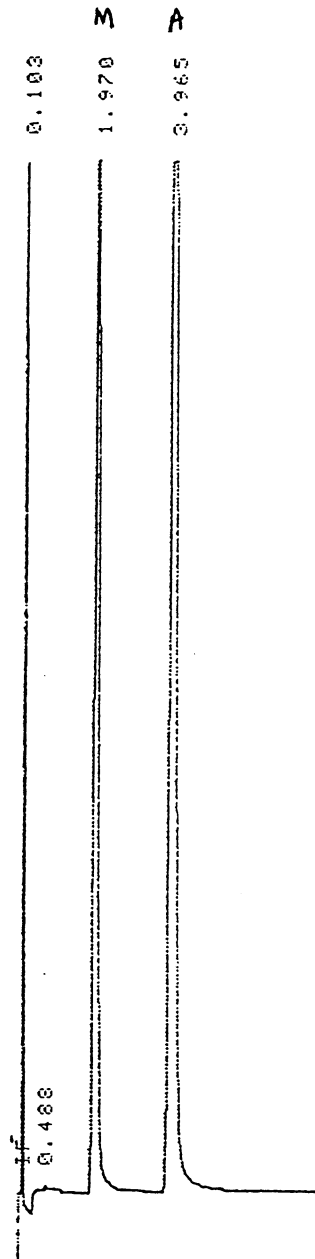
Note 1: For certain matrices, such as cottonseed, it has been necessary to use animal feed diet extracts as conditioning injections just prior to the reference standards to achieve standard reproducibilities of less than ±10% coefficient of variation.

Note 2: When matrices are inadequately cleaned up, resulting recoveries are inordinately high (>150%), due presumably to the non-matrix containing reference standard absorbing onto the residual materials deposited by the matrix on the glass wool insert in the injector liner. Additional column cleanups should be used, i.e., up to 4 silica gel column cleanups for cottonseed samples was sometimes required to achieve acceptable quantitation.

J. C. Lai 12-7-94
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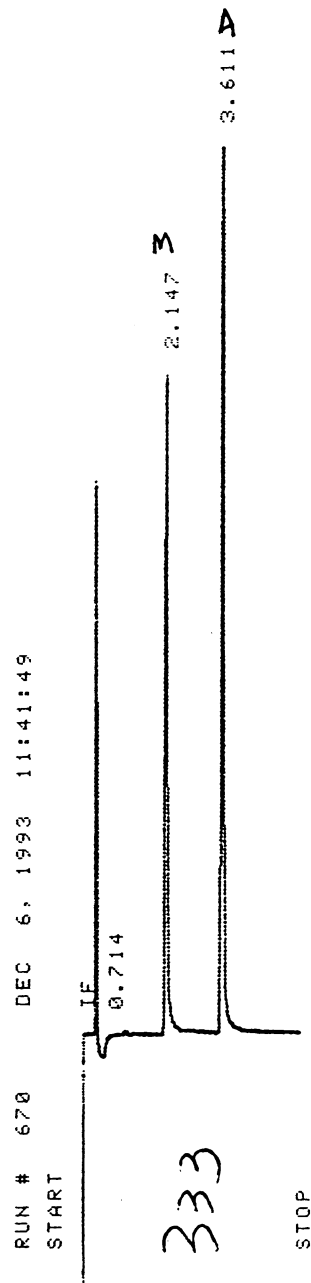
Reviewed by: *J. Whigge 12/11/94*

Figure 1
Reference Standard
Wide-Bore Capillary Column (HP-17)



0.5 $\mu\text{g/mL}$ Methamidophos
1.25 $\mu\text{g/mL}$ Acephate

Figure 2
Reference Standard
Wide-Bore Capillary Column (DB-1)



0.5 $\mu\text{g/mL}$ Methamidophos
1.25 $\mu\text{g/mL}$ Acephate