Method of Analysis for the Determination of Azinphos-Methyl and Its Oxygen Analog in Soil

1 INTRODUCTION

1.1 Scope

This method sets forth the procedure for determining the residues of azinphos-methyl and its oxygen analog in soil.

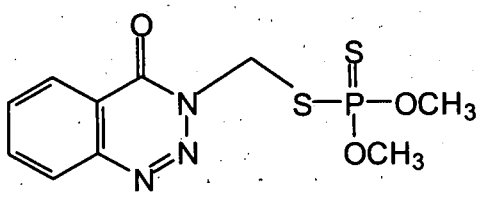
1.2 Principle

Residues of azinphos-methyl and its oxygen analog in soils are extracted with acetonitrile. A dilution in water is made so that the extract is in a 90:10 water: acetonitrile solution and the sample is filtered before injecting onto the LC/MS/MS. Quantification is based on the use of isotopically labeled internal standards and comparison of peak areas with those of known standards.

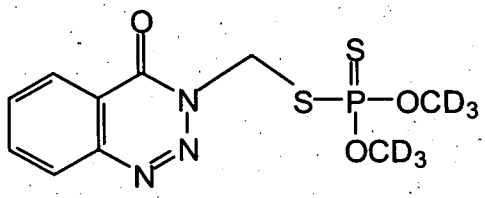
1.3 Method Limits

The target limit of quantitation for this method is 10ppb.

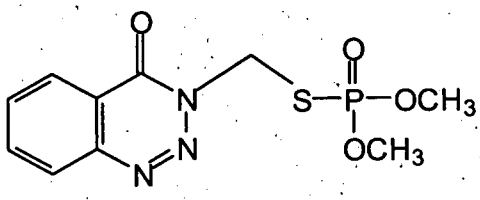
1.4 Structures of the Test Substances



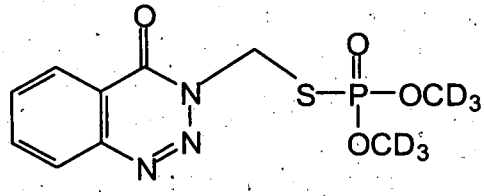
Azinphos-methyl
Molecular weight 317.32
Exact mass 317.00577
M+H=318.013598



Azinphos-methyl, d-6
Molecular weight 323.35
Exact mass 323.04343
M+H=324.05126



Azinphos-methyl Oxygen Analog
Molecular weight 301.26
Exact mass 301.02862
M+H=302.03644



Azinphos-methyl Oxygen Analog, d-6
Molecular weight 307.29
Exact mass 307.06628
M+H=308.07410

Analyte nomenclature:

Azinphos-methyl:

IUPAC name *S*-(3,4-dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate

Chemical Abstracts name *O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate

Azinphos-methyl, oxygen analog or oxon:

IUPAC name *S*-(3,4-dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl) *O,O*-dimethyl phosphorothioate

Chemical Abstracts name *O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorothioate

2 MATERIALS

Unless otherwise noted, equivalent brands and/or suppliers can be used.

2.1 Reagents/Solvents

Acetic acid Guaranteed Reagent (GR) (EM Science Cat. No. AX0073)

Acetonitrile Omni-Solv, (EM Science, Cat. No. AX0142)

Water Omni-Solv, HPLC Grade (EM Science, Cat. No. WX0004)

2.2 Equipment and Supplies

Balance for analytical standards:
Accuracy ± 0.1 mg, Mettler AT 201 or equivalent

Balance for reagents:
Accuracy ± 0.1 g, Mettler PC 4000 or equivalent

Balance for samples: Accuracy ± 0.01 g, Precisa 600 C or equivalent

Mechanical Shaker, Eberbach or equivalent

Centrifuge, Marathon 10K or equivalent

Disposable pipettes

Micropipetter, Eppendorf brand, and pipette tips

Graduated cylinders

Pipette bulb

Volumetric flasks

Volumetric pipettes

250 ml plastic sample bottles, Nalgene P/N 2189-0008 or equivalent

250 ml mixing cylinder and/or 250 ml volumetric flask

20 ml glass or plastic bottle or vial.

1 liter glass containers for HPLC solvent delivery.

Disposable syringe, 3 to 5 ml.

Acrodisk Glass Fiber or PTFE filter disks, 25mm, 0.2 µm

2 ml autosampler vials

2.3 Solutions

Solution of 1.5% acetic acid in HPLC grade water for use as a mobile phase component:

Add about 200ml of HPLC grade water into a 1000ml graduated cylinder or graduated mobile phase reservoir or container.

Transfer 15.0 ml of acetic acid to that cylinder or container, then make up to the 1000ml mark with HPLC grade water.

If necessary, transfer the solution to a clean, dry mobile phase reservoir.

Swirl to mix thoroughly, but do not shake, to prevent dissolving more air into the solution.

Place the container or reservoir in a sonicator bath and apply vacuum while sonicating for about 10 minutes or until air bubble formation or cavitation subsides to a minimum or use an in-line degasser.

Mobile phase is produced by high pressure mixing of the above with pure acetonitrile to produce the mobile phase gradient as outlined in the instrument conditions below. It has not been found necessary to sonicate the acetonitrile.

3 FORTIFICATION AND CALIBRATION SOLUTIONS

3.1 Preparation

All the standard solutions must be stored in amber glass bottles. Standard solutions will be stored in a refrigerator at 4°C ± 5°C when not in use. Solutions should be allowed to warm to room temperature prior to use. The following is an example of a procedure to follow in preparing standard solutions. Alternate or additional standards of appropriate concentration and volume may be prepared as needed. The "~" symbol indicates approximately.

Note: All reusable glassware should be baked in a muffle oven at ~ 400 °C for at least 2 hours to remove possible contamination before use.

3.2 Native, Non- Isotopically Labeled, Stock Standard Solutions:

1. Weigh ~0.0500 g (corrected for purity) each of azinphos-methyl and its oxygen analog into separate 50-ml volumetric flasks and dilute to the marks with acetonitrile. Cap and mix by inversion. The concentration of these stock standards is ~1.0 mg/ml.

2. Transfer 5 ml each of the ~1.0mg/ml of azinphos-methyl and its oxygen analog, via volumetric class "A" pipettes, to one 50 ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~100 µg/ml azinphos-methyl and its oxygen analog.

3. Transfer 5 ml of the 100 µg/ml mixed standard to a 50 ml volumetric flask using a class "A" volumetric pipette. Dilute to the mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~10 µg/ml azinphos-methyl and its oxygen analog.

4. Using a class "A" volumetric pipette, transfer 5 ml of the ~10 µg/ml mixed standard to a 50-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~1.0 µg/ml azinphos-methyl and its oxygen analog.

5. Using a class "A" volumetric pipette, transfer 5 ml of the ~1 µg/ml mixed standard to a 50-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~ 0.10 µg/ml (~100.0 ng/ml) azinphos-methyl and its oxygen analog.

6. Using a class "A" volumetric pipette, transfer 5 ml of the ~ 0.10 µg/ml mixed standard to a 50-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~ 0.01 µg/ml (~10.0 ng/ml) azinphos-methyl and its oxygen analog.

3.3 Fortification Solutions

1. Use the 1.0 µg/ml mixed native solution prepared in Step 3.2.4 to spike soil at the target LOQ. A 25 g sample is fortified to 10 ng/g by the addition of 250 µl of the 1.0 µg/ml solution.

2. Use the 1.0 µg/ml mixed native solution prepared in Step 3.2.4 to spike soil at 5X the target LOQ. A 25 g sample is fortified to 50 ng/g by the addition of 1250 µl of the 1.0 µg/ml solution.

3. Use the 10.0 µg/ml mixed native solution prepared in Step 3.2.3 to spike soil at 10X the target LOQ. A 25 g sample is fortified to 100 ng/g by the addition of 250 µl of the 10.0 µg/ml solution.

3.4 Labeled Internal Standards

1. Weigh ~0.010g each of d-6 labeled azinphos-methyl and its oxygen analog into separate 100-ml volumetric flasks and dilute to the marks with acetonitrile. Cap and mix by inversion. The concentration of these stock labeled standards is ~100 µg/ml.

2. Transfer 5 ml each of the ~1.0 mg/ml solutions, via volumetric class "A" pipettes, to one 50 ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed labeled standard is ~10 µg/ml d-6 labeled azinphos-methyl and its oxygen analog.

3. Using a class "A" volumetric pipette, transfer 5 ml of the ~10 µg/ml d-6 labeled mixed standard to a 50-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed labeled standard is ~1.0 µg/ml d-6 labeled azinphos-methyl and its oxygen analog.

4. Using a class "A" volumetric pipette, transfer 5 ml of the ~1.0 µg/ml d-6 labeled mixed standard to a 50-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed labeled standard is ~0.1 µg/ml (~100.0 ng/ml) d-6 labeled azinphos-methyl and its oxygen analog.

5. Using a class "A" volumetric pipette, transfer 3 ml of the ~1.0µg/ml d-6 labeled mixed standard to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed labeled standard is ~0.03 µg/ml d-6 labeled azinphos-methyl and its oxygen analog.

3.5 Stock Concentrates in Acetonitrile

Note: Concentrates are prepared in acetonitrile, followed by dilution of each level with water to produce the actual calibration standard solutions. This is done so that fresh calibration standards may be prepared from the concentrates every two to three weeks. The final calibration standard concentrations resulting are ten times less than the stock concentrates.

1. Using a class "A" volumetric pipette, transfer 5 ml of the ~1.0 µg/ml *native* mixed standard solution (from Step 3.2.4) and 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~50 ng/ml azinphos-methyl and its oxygen analog and ~3 ng/ml d-6 labeled azinphos-methyl and its oxygen analog.

2. Using a class "A" volumetric pipette, transfer 3 ml of the ~1.0 µg/ml *native* mixed standard solution (from Step 3.2.4) and 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~30 ng/ml azinphos-methyl and its oxygen analog and ~3 ng/ml d-6 labeled azinphos-methyl and its oxygen analog.

3. Using a class "A" volumetric pipette, transfer 1 ml of the ~1.0 µg/ml *native* mixed standard solution (from Step 3.2.4) and 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~10 ng/ml azinphos-methyl and its oxygen analog and ~3 ng/ml d-6 labeled azinphos-methyl and its oxygen analog.

4. Using a class "A" volumetric pipette, transfer 5 ml of the ~100.0 ng/ml *native* mixed standard solution (from Step 3.2.5) and 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~5 ng/ml azinphos-methyl and its oxygen analog and ~3 ng/ml d-6 labeled azinphos-methyl and its oxygen analog.

5. Using a class "A" volumetric pipette, transfer 3 ml of the ~100.0 ng/ml *native* mixed standard solution (from Step 3.2.5) and 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~3 ng/ml azinphos-methyl and its oxygen analog and ~3 ng/ml d-6 labeled azinphos-methyl and its oxygen analog.

6. Using a class "A" volumetric pipette, transfer 1 ml of the ~100.0 ng/ml *native* mixed standard solution (from Step 3.2.5) and 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~1 ng/ml azinphos-methyl and its oxygen analog and ~3 ng/ml d-6 labeled azinphos-methyl and its oxygen analog.

7. Using a class "A" volumetric pipette, transfer 3 ml of the ~10.0 ng/ml *native* mixed standard solution (from Step 3.2.6) and 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~0.3 ng/ml azinphos-methyl and its oxygen analog and ~3 ng/ml d-6 labeled azinphos-methyl and its oxygen analog.

8. Using a class "A" volumetric pipette, transfer 3 ml of the ~100.0 ng/ml d-6 labeled mixed standard solution (from Step 3.4.4) to a 100-ml volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of the d-6 labeled azinphos-methyl and its oxygen analog is ~3 ng/ml. This is the "zero" stock concentrate and contains only labeled internal standards.

3.6 Calibration Standards in 10% Acetonitrile in Water

Note: Concentrates are prepared in acetonitrile, followed by dilution of each level with water to produce the actual calibration standard solutions.

Using a class "A" volumetric pipette, transfer 5 ml of each concentrate into a separate 50-ml volumetric flask. Dilute each to the mark with water. Cap and mix by inversion. The final calibration standard concentrations resulting are 5, 3, 1, 0.5, 0.3, 0.1 and 0.03 ng/ml (ppb) and 0.0 (internal standard only).

3.7 Stability of the Calibration Standard Solutions

The stock concentrate solutions in acetonitrile when stored under refrigeration at about 4 degrees centigrade should be stable for at least two months, however, the type of glass bottles used for storage may have and adverse effect on the solution stability. It is recommended that the glass be high quality amber borosilicate. The bottles may be rinsed with dilute acetic acid followed by water and then by HPLC grade methanol or acetonitrile. It is also recommended that the water used be vacuum sonicated to remove dissolved air prior to use in making dilutions to reduce oxidation of the analytes. Avoid excessive shaking rather than inverting and swirling as well.

The calibration standards in 10% acetonitrile water when stored under refrigeration at about 7 degrees centigrade should be stable for 3 to 4 weeks. Thus, fresh calibration standards should be prepared from the concentrates every two to three weeks. Since it has been observed that some standard solutions degrade faster than others, possibly due to variations in the active sites in the glass containers, it is important to examine the calibration area responses for any outliers which could indicate early degradation of some of the solutions.

4. METHOD PROCEDURE

Analysis of Soils by LC/MS/MS

1. Samples are brought to room temperature. Samples should have been homogenized after the core samples were segmented and composited. Avoid weighing out rocks and vegetable matter such as plant roots. Weigh 25 ± 0.05 g of sample into a 250 ml sample bottle

2. Add an appropriate volume of fortification solution to the LOQ and 10X LOQ control samples. For example, add 250 µL of a 1µg/ml fortification solution (from Step 3.2.4) to a 25 g sample for LOQ and 250 µL of a 10 µg/ml fortification solution (from Step 3.2.3) to a 25 g sample for 10X LOQ to give approximately 10 ppb and 100 ppb analyte concentrations respectively.

3. Add approximately 100 ml of acetonitrile to each sample, fortified soil sample or untreated control soil sample. Cap the bottle and shake gently by hand for a few seconds in order to suspend sample in the solvent. To minimize oxidation of the analytes, avoid excessive aeration of the solutions by overly vigorous shaking. Instead, place bottles in a mechanical shaker and shake for 12 minutes at minimum speed.

4. Centrifuge samples so that all the solids are settled at the bottom of the bottle. An rpm set at 2000 for 2 minutes is suggested. The rpm and time does not matter so long as the solids are settled and the supernatant is relatively clear.

5. Decant supernatant into a 250 ml mixing cylinder or a 250 ml volumetric flask.

6. Pour approximately 70 ml of acetonitrile into the original sample bottle. Cap and shake briefly to suspend the soil pellet in the solvent. Place bottles in the mechanical shaker and shake for 12 minutes.

7. Centrifuge samples as in Step # 4.

8. Decant supernatant adding to extract already collected in the 250 ml mixing cylinder or volumetric flask.

9. Repeat Steps # 6 through #8.

10. Bring the Final Volume of the extracts up to 250 ml by adding acetonitrile to the 250 ml mark. To minimize oxidation of the analytes, avoid excessive aeration of the solutions by overly vigorous shaking. Instead, cap the mixing cylinder or volumetric flask and mix by inverting several times.

11. Dilute the extract by pipetting a 1 ml (V_1) aliquot into a 10 ml (V_2) volumetric flask. Add 100 µl of 0.03 µg/ml internal standard solution (ISTD) from Step 3.4.5 and fill to volume with HPLC grade water. Cap the flask and mix by inverting several times. Samples are now in a 90:10 water: acetonitrile solution. ISTD concentration is 0.3 ng/ml.

Note: If the sample extract is too concentrated in one or both analytes for the calibration curves used, the extract will have to be diluted further. For example, to achieve a higher dilution, V_1 can be reduced to 0.4mL while keeping V_2 at 10mL. This results in a V_1/V_2 ratio of 25 and the same amount of internal standard solution is added before filling to the 10mL volume. If V_2 were changed, the internal standard solution volume added would need to be changed accordingly. With a higher dilution, the aqueous portion of the solution is now more that 90% which has no effect on the analysis.

12. Filter extract through an Acrodisk Glass Fiber or PTFE filter disks, 25mm, 0.2 µm into an autosampler vial. Cap vial. Sample is ready to be analyzed by LC/MS/MS.

13. If a reagent blank is run with samples, it should consist of a solution of 10:90 acetonitrile:water, which is the calibration solution solvent.

5 CHROMATOGRAPHIC SYSTEM

Instrumentation: Perkin Elmer Sciex API 3000 LC/MS/MS System
PE Sciex Turbo IonSpray Electrospray Interface.
Shimadzu LC-10AD VP HPLC Pumps (2)
with 250µL High Pressure Mixer
and Shimadzu SCL-10A VP Pump Controller
Perkin Elmer Series 200 Autosampler
or Gilson 215 Autosampler or equivalents

(Note: A low dead volume switching valve may be used to divert mobile phase containing only salts and matrix away from the mass spectrometer before peaks of interest elute.)

HPLC Column: Phenomenex, Luna C8(2), 2.0 x 50 mm, 3µm particle size

Mobile phase solvents: A=100% acetonitrile
B=1.5% acetic acid in HPLC grade water

Gradient program (Mixing dwell volume ~250µL. Flow rates in µL/min.)

Gradient Table with percentages and flow rates listed as they are at the start of each step:

Time (min)	Duration	Flow	%A	%B	(step description)
0.00	2.00	225	10.00	90.00	(initial isocratic step)
2.00	3.00	225	10.00	90.00	(start linear ramp)
5.00	2.00	225	65.00	35.00	(end ramp)
7.00	0.01	225	65.00	35.00	(end plateau)
7.01	3.00	225	10.00	90.00	(start equilibration)
10.01	0.01	225	10.00	90.00	(end run)

Including the acquisition-file load time and autosampler load time, the total time between injections is about 13 minutes.

Gradient Table as entered into Sciex MassChrom v1.1 (with percentages and flow rates listed as they are at the end of each step):

Step	Time	Duration	Flow	%A	%B	(step description)
0	0.00	-0.00	225.00	10.00	90.00	(equilibration step)
1	0.00	2.00	225.00	10.00	90.00	(initial isocratic step)
2	2.00	3.00	225.00	65.00	35.00	(linear ramp)
3	5.00	2.00	225.00	65.00	35.00	(isocratic plateau)
4	7.00	0.01	225.00	10.00	90.00	(start equilibration)
5	7.01	3.00	225.00	10.00	90.00	(equilibrate to initial)
6	10.01	0.01	225.00	10.00	90.00	(end)

Injection volume: 40 µL

Note: The amount injected may be adjusted if needed, to adjust for variations in sensitivity or concentration detection limits on a particular instrument.

Retention times See chromatograms (Figures 1 and 2)

Suggested MS parameters depending on the instrumentation used:

Ionization and MS Mode:	Electrospray (TurboIonSpray) Positive ion mode
Nebulizer Gas Setting:	15 (Air)
Curtain Gas Setting:	9 (Nitrogen)
Collision Gas Setting:	4 (Nitrogen)
Turbo IonSpray Settings:	Heated air at ~8.5L/min, 500°C
IonSpray Voltage:	In the range of 5500V to 5800V

MassChrom (Mac) Software Specific Instrument Parameters
Q0/IQ1/ST/RO1/IQ2 -10 / -11 / -15 / -11 / -20

Analyst Software Specific Instrument Parameters
EP/IQ1/ST/IQ2 10 / -11 /-15/-20
IQ1 offset = -1
ST offset = -5
IQ2 offset = -10
Note: These Analyst offsets are set via Tune/Tools/Settings/Parameter Settings. It is recommended that the Parameter Settings be printed and saved with the method or with the instrument tune parameters. The required sensitivity may not be obtained without these offsets, even after extensive optimization of the compound dependent parameters.

Resolution settings: Mass peak widths are set to about 1.5amu at half height. Analyst uses "Q1 Low / Q3 Low" with offset drop from unit resolution typically 0.04 to 0.06.
(Note: Use Tune/tools/settings/tuning options/ resolution tab.)

IE1 = Q0 – RO1 (RO1 and IE1 set by resolution tune)
IE3 = R02- R03 (RO3 and IE3 set by resolution tune)
R02 set by collision energy

MRM dwell times: 300ms each per scan for the oxygen analog and the d-6 oxygen analog, 400ms each for azinphos-methyl and azinphos-methyl-d-6, or as needed to obtain sufficient scans across the peaks for good integration. Greater signal to noise ratio is obtained by running azinphos-methyl and the oxygen analog in separate periods.

Compound Dependent and Normally Compound Dependent Parameters:

Compound	Mass Transitions
oxygen analog:	302/160
oxygen analog, d-6:	308/160
azinphos-methyl:	318/160
azinphos-methyl, d-6:	324/160

Compound	CXP = R02-ST3	ST3	R03 (Mac only)
oxygen analog:	-20 – (- 34) = 14V	ST3 = -34V	R03 = -22V
oxygen analog, d-6:	-20 – (- 34) = 14V	ST3 = -34V	R03 = -22V
azinphos-methyl:	-20 – (- 34) = 14V	ST3 = -34V	R03 = -22V
azinphos-methyl, d-6:	-20 – (- 46) = 26V	ST3 = -46V	R03 = -23V

Normally Compound Dependent Parameters: (In this case, same for all four compounds)

OR (DP):	16V
RNG (FP):	104V
Q0- R02 (Collision Energy):	-10 – (- 20) = 10V

Nomenclature for voltages and voltage regions:
OR (DP): Orifice (Declustering Potential); RNG (FP): Ring (Focusing Potential); Q0: Entrance Quadrupole; EP: Entrance Potential; IQ1: Focusing Lens 1; ST: Stubby Prefilter; R01: Quad 1 Potential, IE1: Ion Energy 1; IQ2: Focusing Lens 2; ST3: Stubby Prefilter 3;IE3: Ion Energy 3.

Note: The indicated LC/MS/MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

Note: Phosmet is another organophosphate insecticide having the same nominal parent mass and product ion as azinphos methyl. However, the exact masses are different and the retention time is not the same as azinphos methyl using the above conditions. No interference is expected if the chromatography is not significantly altered.

6 CALCULATIONS

Generate calibration curves for azinphos-methyl and its oxygen analog. A minimum of four standards over a range of concentration levels should be included with a set of samples. To bracket samples with residues near the LOQ, the lowest standard run will be between the LOQ and LOD. After internal standard is added, samples should be diluted only such that any peak areas or heights of the internal standards are still within ±60% of the internal standard peak responses in the calibration standards.

Standards should be interspersed with samples or bracket sample runs to compensate for any minor change in instrument response.

Linear regression coefficients should be calculated for the ratio of analyte to internal standard area or height plotted versus the ratio of analyte to internal standard concentration in the calibration standards. The data from the analytical standards should then be fit to the linear model,

$$y = A + Bx$$

$$x = \text{Concentration Ratio} = \frac{\text{Analyte Concentration}}{\text{Internal Standard Concentration}}$$

$$y = \text{response ratio} = \frac{\text{Analyte area response}}{\text{Internal Standard area response}}$$

A = intercept from linear regression analysis

B = slope from linear regression analysis

A linear through zero regression may be used if it produces a good fit to the data. It is suggested that the linear through zero regression produce a correlation of at least 0.998 and the calculated values for all of the standard levels be within ±20% of their expected values. Use of the linear through zero regression has the advantage of avoiding negative results for detects near the low end of the calibration curve.

The equation to be used to estimate the residues in the samples is:

$$C = \frac{\left[\frac{(y - A)}{B} \times D\right]}{S}$$

where: C = concentration of analyte in sample in parts per billion
D = ng/ml, concentration of internal standard in the extract

The soil/solvent ratio "S" is defined by the concentration of sample in g/ml:

$$S = \frac{W}{V_F} \times \frac{V1}{V_2}$$

Where W = Sample Weight (~25 g)

V_F = Extract Volume (250 ml)

V_1 = Dilution Aliquot Volume (1 ml)

V_2 = Final Dilution Volume (10 ml)

Example Calculation*:

$$S = \frac{W}{V_F} \times \frac{V1}{V_2} \qquad S = \frac{25.03\ g}{250\ ml} \times \frac{1\ ml}{10\ ml} = 0.01001 g/ml$$

$$C = \frac{\left[\frac{(y - A)}{B} \times D\right]}{S}$$

C = Analyte Concentration

$$y = \frac{\text{Analyte area response}}{\text{Internal Standard area response}} = \frac{9580}{76700} = 0.12490$$

A = y-intercept = 0.00368

B = slope = 0.963

D = Internal Standard Concentration = 0.3 ng/ml

$$\text{Analyte Concentration} = \frac{\left[\frac{(0.12490 - 0.00368)}{0.963} \times 0.3 \text{ ng/ml}\right]}{0.01001 \text{ g/ml}} = 3.776 \text{ ng/g (ppb)}$$

* Data from the azinphos-oxon analysis of sample "#2 0.4ng/ml" from Analyst result file "azm 06jan004.rdb".

For a better estimation of any residues between the lowest standard and the limit of detection, the linear through zero regression may be used and high standards may be omitted from the regression, or only the lowest standard and zero may be used.

7 SAFETY

All available appropriate Material Safety Data Sheets should be available to the study personnel during the conduct of the method. General laboratory safety precautions should be taken.

8 REFERENCES

Method of Analysis for the Determination of Azinphos-Methyl and Its Oxygen Analog in Surface and Ground Water - Revision 02, January 7, 2004, Bayer CropScience USA, Method Number GU005_W04_02.

Method of Analysis for the Determination of Azinphos-Methyl and Its Oxygen Analog in Surface and Ground Water - Revision 03, in press, Bayer CropScience USA

Validation of the Method of Analysis for the Determination of Azinphos Methyl and Its Oxygen Analog in Surface and Ground Water, in press, Bayer CropScience Study Number GU112402