

Method of Analysis for the Determination of Azinphos-Methyl and Its Oxygen Analog in Surface and Ground Water – Revision 02

1 INTRODUCTION

1.1 Scope

This method sets forth the procedure for determining the residues of azinphos-methyl and its oxygen analog in surface or ground water.

1.2 Principle

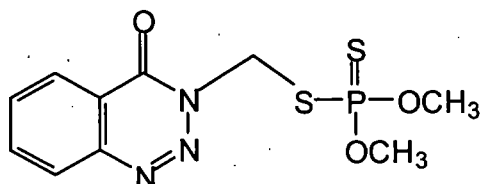
Residues of azinphos-methyl and its oxygen analog in surface or ground water are detected by direct injection onto the LC/MS/MS without any prior sample preparation, clean up or concentration. Quantification is based on the use of isotopically labeled internal standards and comparison of peak areas with those of known standards.

1.3 Method Limits and Performance

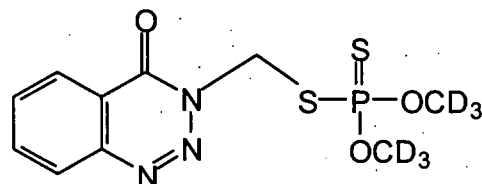
In the validation of this method the method detection limit was demonstrated to fall at or below the target of 10ng/L for both azinphos methyl and the oxon for injection volumes ranging from 20 to 100uL. (See Section 8 below for reference.)

The limit of quantitation was demonstrated to fall at or below the target of 30ng/L for both azinphos methyl and the oxon based on ten times the standard deviation of the 10ng/L spiked samples for injection volumes ranging from 20 to 100uL.

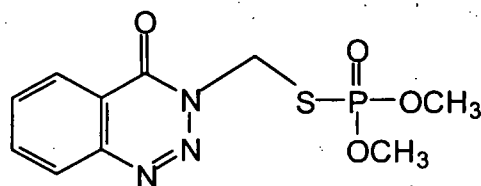
1.4 Structures of the Test Substances



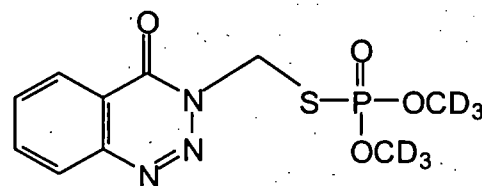
Azinphos-methyl
Molecular weight 317.32
Exact mass 317.00577
M+H=318.013598



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

Disposable pipettes

Micropipetter, Eppendorf brand, and pipette tips

Graduated cylinders

Pipette bulb

Volumetric flasks

Volumetric pipettes

1 liter glass containers for HPLC solvent delivery.

Bottles, Nalgene, amber 4oz. or 8 oz.

Filter disk, Gelman, Acrodisk Glass Fiber, P/N 4523

Filter disk, Gelman, Acrodisk CR, PTFE, 25mm, 0.45um, P/N 4523T

HPLC column: Phenomenex, Luna C8(2), 2.0 x 50 mm, 3 μ m particle size

Pre-column HPLC filter, Upchurch, Part No. A-318 (0.23uL swept volume)
with 0.5micron frit, Part No. A-102.

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 $7^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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 $\sim 400^{\circ}\text{C}$ for at least 2 hours to remove possible contamination before use.

3.2 Native, Non- Isotopically Labeled Standards:

1. Weigh ~0.0500g (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 ~1.0mg/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. Using a class "A" volumetric pipette, transfer 1 mL of the ~100 µg/mL mixed standard to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~1 µg/mL azinphos-methyl and its oxygen analog.
4. 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 azinphos-methyl and its oxygen analog.
5. 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. Using a class "A" volumetric pipette, transfer 3 mL of the ~1.0 µg/mL mixed standard to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~0.03 µg/mL, (~30 ng/ml) azinphos-methyl and its oxygen analog. (Use as target 10X LOQ fortification solution. A 10mL aliquot of sample is fortified to 300ng/L by addition of 100uL of this solution.)
2. Using a class "A" volumetric pipette, transfer 10 mL of the ~0.03 µg/mL mixed standard to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~0.003 µg/mL, (~3 ng/ml) azinphos-methyl and its oxygen

analog. (Use as target LOQ fortification solution. A 10mL aliquot of sample is fortified to 30ng/L by addition of 100uL of this solution.)

3.4 Labeled Internal Standards

1. Weigh ~0.050g (corrected for purity) each of d-6 labeled 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 labeled standards is ~1.0 mg/mL.
2. Transfer 5 mL each of the ~1.0 ng/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 ~100 µg/mL d-6 labeled azinphos-methyl and its oxygen analog.
3. Using a class "A" volumetric pipette, transfer 5 mL of the ~100 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 ~10 µg/mL d-6 labeled azinphos-methyl and its oxygen analog.
4. 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.
5. 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.
6. 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 Calibration Concentrates in Acetonitrile

Note: Concentrates are prepared in acetonitrile, followed by dilution of each level with water to produce the actual standard solutions. This is done so that fresh standards in water may be prepared from the concentrates as needed. The final calibration standard concentrations resulting are 0.5, 0.3, 0.1, 0.05, 0.03, 0.02 and 0.01 ng/mL (ppb).

1. Using a class "A" volumetric pipette, transfer 5 mL of the ~100.0 ng/mL *native* mixed standard solution and 3 mL of the ~100.0 ng/mL d-6 labeled mixed standard solution 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.
2. Using a class "A" volumetric pipette, transfer 3 mL of the ~100.0 ng/mL *native* mixed standard solution and 3 mL of the ~100.0 ng/mL d-6 labeled mixed standard solution 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.
3. Using a class "A" volumetric pipette, transfer 1 mL of the ~100.0 ng/mL *native* mixed standard solution and 3 mL of the ~100.0 ng/mL d-6 labeled mixed standard solution 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.
4. Using a class "A" volumetric pipette, transfer 5 mL of the ~10.0 ng/mL *native* mixed standard solution and 3 mL of the ~100.0 ng/mL d-6 labeled mixed standard solution to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~0.5 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 ~10.0 ng/mL *native* mixed standard solution and 3 mL of the ~100.0 ng/mL d-6 labeled mixed standard solution 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.

6. Using a class "A" volumetric pipette, transfer 2 mL of the ~10.0 ng/mL *native* mixed standard solution and 3 ml of the ~100.0 ng/mL d-6 labeled mixed standard solution to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~0.2 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 1 mL of the ~10.0 ng/mL *native* mixed standard solution and 3 ml of the ~100.0 ng/mL d-6 labeled mixed standard solution to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~0.1 ng/mL azinphos-methyl and its oxygen analog and ~3 ng/mL d-6 labeled azinphos-methyl and its oxygen analog.

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 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 0.5, 0.3, 0.1, 0.05, 0.03, 0.02 and 0.01 ng/mL (ppb).

3.7 Stability of the Calibration Standard Solutions

The calibration solutions when stored under refrigeration at about 4 degrees centigrade should be stable for at least two months.

4 METHOD PROCEDURE

Analysis of Ground and Surface Waters by LC/MS/MS

1. Samples are brought to room temperature. Make sure that there is no portion of the sample still frozen. Mix the sample completely before removing a sub-sample for analysis. Using a large volume auto-pipette and disposable pipette tip, remove a 10mL sub-sample into a suitable container such as a disposable glass or plastic bottle.
2. Add an appropriate volume of internal standard (ISTD) solution for the given sample or sub-sample size. Mix the sample completely. (For example, for a 10mL sub-sample, add 100 μ L of a 0.03 μ g/mL internal standard solution to give approximately 300pg/mL ISTD concentration.)

3. If there are suspended particulates present after mixing, they may be allowed to settle out some before taking an aliquot for analysis.
4. If the sample contains significant suspended particulates, it may be filtered using a disk filtration unit, directly into the HPLC vial for analysis. Glass fiber or PTPE filter disks were tested and gave good recoveries, but nylon gave inadequate recovery. A pre-column filter unit before the analytical column will handle non turbid samples, such as some ground waters, without prior filtration.
5. HPLC grade water or an untreated water sample from the field is used for the untreated control.
6. HPLC water or untreated water from the field is used for the recovery sample and spiked with a known amount of a fortification solution to give the desired level of fortification. (For example, for a 10mL aliquot of sample, add 100 μ L of a 0.03 μ g/mL fortification solution to obtain a level of 300 pg/mL in the sample. Add 100 μ L of a 0.003 μ g/mL fortification solution to obtain a level of 30 pg/mL in the sample.)
7. Analyze samples by LC/MS/MS under the conditions described in Section V.

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 250 μ L 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, 3 μ m particle size

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

Gradient program (Mixing dwell volume ~250 μ L. Flow rates in μ L/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: 30 μ L (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 chromatogram

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 \sim 8 SL/min, 500°C
IonSpray Voltage: In the range of 5500V to 5800V

MassChrom (Mac) Software Specific Instrument Parameters
Q0/IQ1/ST/RO1/IO2 -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

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

Resolution settings: "Q1 Low / Q3 Low"

Mass Spec 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 internal standard 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, a standard at the LOQ or be between the LOQ and LOD should be included.

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,

$$y = A + Bx$$

$$x = \text{Conc. Ratio} = \frac{\text{conc.}}{\text{IS} \cdot \text{conc.}} \quad \text{where IS = labeled internal standard (d-6)}$$

$$y = \text{response ratio} = \frac{\text{response} \cdot \langle \text{area} \rangle}{\text{IS} \cdot \text{response} \cdot \langle \text{area} \rangle}$$

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

$$E = \frac{(y - A)}{B} \times D \times f$$

where: E = concentration of analyte in sample in parts per billion (ppb or ng/g)
 y = ratio of analyte response (area or height) to internal standard response (area or height)
 A = intercept from linear regression analysis (may be forced to zero)
 B = slope from linear regression analysis (area ratio per conc. ratio)
 D = ng/mL, concentration of internal standard in the sample
 f = dilution factor, if applicable

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. This method does not present any specific risks.

8 REFERENCE

Validation of the Method of Analysis for the Determination of Azinphos Methyl and Its Oxygen Analog in Surface and Ground Water, in progress, Document Number GU2004 200791.