

Study Number: FONO-95-AM-02

Study Title: Fonofos: Determination of Fonofos, Fonofos Oxon and Methylphenylsulfone in Water by Gas Chromatography and Mass-selective Detection

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1 Summary/Introduction

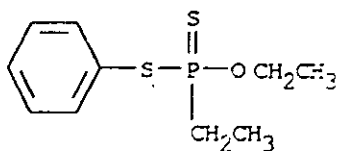
1.1 Scope

A method for the analysis of fonofos, fonofos oxon and methylphenylsulfone in water has been laboratory validated. The concentration range over which this method has been validated extends from 0.1 µg/L to 10 µg/L (parts per billion) in water for each of the compounds listed above. The method detection limit (MDL) for each analyte is listed in Table 2.

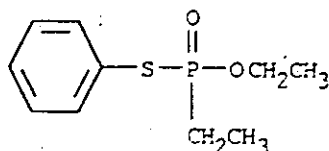
The purpose of this report is to describe an analytical method for the determination of fonofos and its degradates, fonofos oxon and methylphenylsulfone in water. This report also documents a validation study on the method and validation data obtained from the study.

1.2 Principles

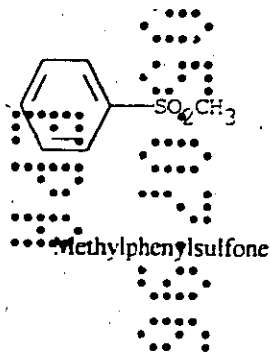
Fonofos is the active insecticidal ingredient in the various formulated products marketed by Zeneca, Inc. under the trademark "DYFONATE®". The Chemical Abstracts Name for fonofos is phosphonodithioic acid, ethyl O-ethyl S-phenyl ester [944-22-9]. The chemical name for fonofos oxon is phosphonothioic acid, ethyl O-ethyl S-phenyl ester [944-21-8]. Methylphenylsulfone is a metabolite that has been identified in a soil-metabolism study. The chemical name for methylphenylsulfone is methylsulfonylbenzene [3112-85-4]. The chemical structures are given below.



Fonofos



Fonofos oxon



Methylphenylsulfone

To determine these compounds, a sample of water is extracted with a solvent mixture of toluene:methylene chloride 5:1. Sodium bisulfite is added to the water to eliminate degradation of the analytes. The toluene-methylene chloride extract is concentrated and analyzed for fonofos, fonofos oxon and methylphenylsulfone by gas chromatography with mass-selective detection.

Method validation was conducted according to Good Laboratory Practice regulations and Zeneca Ag Products standard operating procedures.

2 Materials/Methods

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment capable of providing equivalent sensitivity and selectivity, and reagents of comparable purity can be used.

2.1 Apparatus

2.1.1 Gas Chromatograph

Hewlett-Packard model 5880A designed for use with capillary columns. The gas chromatograph is equipped with a Hewlett-Packard Model 7673 automatic injector, mass-selective detector (HP Model 59970C MS Chemstation), and data acquisition system (HP Chemserver System).

2.1.2 Gas-Purification Traps

Alltech 8121 Inline Gas Purifier and an Alltech OXY-TRAP[®] for removing oxygen from the helium carrier gas.

2.1.3 Gas-Chromatographic Column

DB-17 fused silica capillary, 10 m by 0.18 mm i.d. with a 0.3- μ m film thickness (J&W catalog no. 121-1713).

2.1.4 Syringes

10- μ L capacity (Hamilton 701N) syringe for autosampler, 10-, and 50- μ L capacity Gas-Tight syringes (Hamilton 1700 series) for fortification and standard preparation.

2.1.5 Laboratory Shaker

Eberbach 6010 two speed reciprocating shaker with utility box carrier, VWR Cat. No. 57007-101.

2.1.6 Ultrasonic Cleaner

Branson Model No. B-22-4, stainless-steel tank 2.8 liter capacity, tank size 5" L x 9" W x 4"D.

2.1.7 Evaporator

The Meyer N-EVAP analytical evaporator model 112 from Organomation Associates Inc.

2.1.8 Vortex Mixer

Thermolyne mixer model M-16715.

2.1.9 Evaporation Tubes

15 mL capacity, graduated, Kimax catalog No. 45176.

2.1.10 Glass Bottles

8-oz. narrow-mouth bottles equipped with TEFLON-lined screw-caps, and 1-oz narrow-mouth bottle equipped with Polyseal[®]-lined screw-caps.

2.1.11 Glass Pipettes

1-, 2-, 5- and 10-mL disposable, graduated, glass pipettes for general use.

2.1.12 Calibrated Laboratory Pipettor

Oxford Pipettor Model 10/30, adjustable dispensing range 10 to 30 mL in 1 mL graduations.

2.2 Reagents and Standards

2.2.1 Solvents

Toluene, methylene chloride, methanol and deionized water. Fisher OPTIMA grade toluene, OPTIMA grade methylene chloride, OPTIMA grade methanol, and Millipore Milli-Q[®] filtered water are acceptable. All solvents must be of high purity and suitable for use in trace organic analyses by gas chromatography.

2.2.2 Helium

High-purity gas supplied to gas chromatograph via lines equipped with gas purification traps and appropriate pressure regulators.

2.2.3 Analytical Reference Standards

The following reference standards are available from Zeneca Ag Products, 1200 South 47th Street, Box Number 4023, Richmond, CA 94804-0023:

FONOFOS :	ASW01094-01R; 99.7% w/w purity
FONOFOS OXON:	ASW01703-01F; 95% w/w purity
METHYLPHENYLSULFONE:	ASW01638-01R; 99.4% w/w purity

2.2.4 Sodium Bisulfite

Analytical grade.

2.2.5 Anhydrous Sodium Sulfate

Analytical grade.

2.2.6 Calibration Solutions

Calibration solutions are used to calibrate the instrument. To prepare a stock calibration solution of an analyte, at nominal concentration of 1.0 mg/mL, place a known quantity (± 0.1 mg) of approximately 25 mg of the analyte of a primary standard of known purity into a clean 20-mL beaker. Add toluene to the beaker to dissolve the analyte. Quantitatively transfer the

analyte solution into a clean 25-mL volumetric flask, and dilute to volume with the appropriate solvent. Stopper the volumetric flask, and mix the contents thoroughly. Transfer the contents into a 1-oz bottle, cap the bottle with a Polyseal®-lined cap and keep in a refrigerator when not in use. Calculate the concentration of the stock solution as follows:

$$C = \frac{(W \times P)}{25}$$

where C = concentration of analyte in final solution (mg/mL)

W = weight of primary standard (mg)

P = purity of reference standard (100% = 1.00)

25 = volume of solvent (mL)

To prepare working standard solution for calibration purposes, dilute by volume and combine the calibration stock solution of each analyte, to give a solution mix at concentration of 10 µg/mL each analyte in toluene. This working standard solution is used to prepare daily-use calibration standards in matrix-matched solvent mixture of toluene:methylene chloride 5:1. Matrix-matched calibration standards are recommended to reduce the effects of matrix on the GC/MSD response. Use microliter syringes, clean disposable glassware, and the matrix-matched toluene:methylene chloride 5:1 solvent to make serial dilutions of the working standard solution to give 1.0, 0.5, 0.1, 0.05, 0.01, and 0.005 µg/mL standard solutions in matrix-matched toluene:methylene chloride solvent.

2.2.7 Fortification Solutions

Fortification solutions are used to fortify samples and demonstrate procedural recoveries. To prepare a stock fortification solution of an analyte, at nominal concentrations of 3.0 mg/mL, place a known quantity (± 0.1 mg) of approximately 75 mg of the analyte of a primary standard of known purity into a clean 20-mL beaker. Add methanol to the beaker to dissolve the analyte. Quantitatively transfer the analyte solution into a clean 25-mL volumetric flask, and dilute to volume with the appropriate solvent. Stopper the volumetric flask, and mix the contents thoroughly. Transfer the contents into a 1-oz bottle, cap the bottle with a Polyseal®-

lined cap and keep in a refrigerator when not in use. Calculate the concentration of the stock solution as follows:

$$C = \frac{(W \times P)}{25}$$

25

where C = concentration of analyte in final solution (mg/mL)

W = weight of primary standard (mg)

P = purity of reference standard (100% = 1.00)

25 = volume of solvent (mL)

To prepare working standard solution for fortification purposes, dilute by volume and combine the fortification stock solution of each analyte, to give a solution mix at concentration of 500 $\mu\text{g/mL}$ each analyte in methanol. Further dilute this fortification solution by volume to give a diluted fortification solution at concentration of 5.0 $\mu\text{g/mL}$ each analyte in methanol.

3 Procedures

3.1 Extraction of Water Samples

3.1.1 Use a graduated cylinder to add 200 mL of water sample into a 8-ounce bottle. Add 20 mg sodium bisulfite (0.01%) to the water sample. Use a calibrated pipettor to add 25.0 mL of toluene, and use a pipette to add 5.0 mL of methylene chloride into the sample bottle. Seal the sample bottle with the Teflon-lined cap. Shake the sample bottle by hand for a few times to mix the solvents, and sonicate the contents in an ultrasonic cleaner for 20 minutes. Extract the sample by shaking on a reciprocating shaker at high speed for 1 hour. After shaking, place the bottle in its upright position for a minimum of 5 minutes. Allow the organic phase of toluene/methylene chloride to separate from the water phase.

3.1.2 Remove the Teflon-lined cap from the sample bottle. Use a disposable Pasteur pipette to transfer at least 15 mL of the top toluene/methylene chloride extract layer into a 1-oz. glass bottle. Dry the extract with ~4 grams of anhydrous sodium sulfate.

3.1.3 Use a disposable pipette to transfer 10.0 mL of the dried toluene/methylene chloride extract into a clean, graduated evaporation tube. Evaporate to 1.0 mL with a gentle stream of nitrogen using the N-EVAP Model 112 evaporator. Do not evaporate the extract to dryness. If the evaporated volume fall below 1.0 mL, add sufficient solvent mixture of toluene:methylene chloride 5:1 to make up to a final volume of 1.0 mL in the evaporation tube.

3.1.4 Vortex mix the final 1.0 mL extract in the evaporation tube for 1 minute and transfer the extract into an autosampler vial for gas chromatographic analysis.

3.2 Preparation of Fortified Water Samples

Analyze unfortified and fortified control samples with each sample set to demonstrate method recovery. For example, place 200 mL of untreated control water into a 8-ounce bottle. Use a μL -syringe to add 4 μL of fortification solution (5.0 $\mu\text{g}/\text{mL}$) to the control sample to produce a fortification level of 0.1 $\mu\text{g}/\text{L}$. Extract as detailed in section 3.1 above. It is recommended that one unfortified and one fortified sample be analyzed for every set of 10 or less field samples.

3.3 GC Conditions

Follow the manufacturer's instructions for operation of the gas chromatograph and mass-selective detector. The specific conditions listed below were used to generate the data and chromatograms presented in this report.

3.3.1 Instrument

Hewlett-Packard model 5880A designed for use with capillary columns and temperature programming of the column oven. The gas chromatograph is equipped with a Hewlett-Packard Model 7673 automatic injector, mass-selective detector and data acquisition system (Hewlett-Packard Model 59970C MS Chemstation and associated software).

3.3.2 Operating Conditions

Column: DB-17 fused silica capillary, 10 m X 0.18 mm i.d., 0.3 μm film thickness, Cat. 121-1713

Carrier gas: helium

Column head-pressure: ~5 lb/sq. inch

Inlet type: splitless with double restrictor (R&D Separations, Rancho Cordova, CA) with ~ 3 mm silanized borosilicate Pyrex glass wool plug

Inlet temperature: 230°C

Interface temperature: 240°C

Initial oven temperature: 100°C

Initial time: 0.0 min

Oven temperature program rate: 25°C/min

Final oven temperature: 270°C

Final oven temperature time: 5 min.

Volume injected: 3 µL

Splitless valve off: 0.5 min

Total run time: 7.0 min

3.3.3 Mass-selective Detector

Mode: low resolution selective-ion-monitoring (s.i.m.)

Dwell time: 100 msec

Tuning: optics optimized for m/z 69, 100 and 131 with perfluorotributylamine using the HP-supplied manual program

Quantitation: Peak height; external standard

Mass monitored: Methylphenylsulfone m/z 94 & 156,
Fonofos oxon m/z 93 & 230,
Fonofos m/z 109 & 246,

Using the above conditions the elution time of methylphenylsulfone is 3.86 min., fonofos oxon is 5.11 min and fonofos is 5.41 min. See Figures 1 and 3 for typical gas chromatograms.

3.4 Calibration

Calibrate the gas chromatograph by using the 1.0, 0.5, 0.1, 0.05, 0.01 and 0.005 $\mu\text{g/mL}$ calibration standard solutions. Prepare a calibration curve by plotting response versus standard concentration using the HP Chemserver data acquisition system and an appropriate curve fit program. Figures 4 - 6 show examples of typical calibration curves. After every four to six samples, analyze one or more of the calibration solutions to assure that the calibration curve is still valid. After sample analysis has been completed, re-analyze all calibration solutions to ensure the validity of the entire calibration range.

3.5 Analysis of Sample Extracts

Inject the sample extracts on the same day of extraction and calibration, using the same conditions used for the calibration solutions. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of retention times (within 0.05 min) with those of the calibration chromatogram. Reinject the calibration solution after injection of every four to six sample extracts and at the end of the chromatographic run. Calculate the concentration of the analyte in the sample extract by use of a standard curve program or from the average calibration response factor as described in Section 6.

4 Interferences

Analyze extracts from untreated water samples to demonstrate the absence of interferences from samples matrices, solvent and labware.

5 Confirmatory Techniques

Unexpected positive results, as in control samples, should be confirmed by other means. Confirmation can be achieved by quantitation using a different m/z ion or by using a different type of detector, such as a flame photometric detector with a sulfur bandpass filter.

6 Calculations

The concentration of the analyte in the original sample is calculated by using the external standard method, i.e., the response obtained for the analyte in the sample extract is compared to the response obtained from a separate injection of a calibration solution. To use the calculation methods shown below, the injection volumes for all calibration solutions and sample extracts must be fixed at the same volume.

6.1 Linear Detector Response

6.1.1 Calibration Factor

Calculate the response factor, RF, for injection of a calibration solution as follows:

$$RF = \frac{C_{std}}{R_{std}}$$

Where

C_{std} = concentration in $\mu\text{g/mL}$ of the calibration solution

R_{std} = response units (e.g., peak height or peak area) from detector for the calibration solution

6.1.2 Concentration of Analyte in Water Sample

Determine the concentration of each analyte in the original water sample, C_i , from the corresponding average response factor, RF_{avg} , the extraction volume, and the concentration factor as follows:

$$C_i (\mu\text{g/L}) = R_i(\text{sample}) * RF_{avg} * V_1 / V_2 / S$$

Where

$R_i(\text{sample})$ = analyte response unit from detector for the sample extract

RF_{avg} = average response factor over the entire range of calibration for the corresponding analyte

V_1 = volume of extract, mL = 30 mL

V_2 = volume of water extracted, L = 0.2 L

S = concentration factor for the extract = 10

6.2 Nonlinear Detector Response

6.2.1 Calibration Curve

Generate a multiple point calibration curve by plotting the concentration of the calibration solution (C_{std}) as the y-axis and the corresponding response units from the detector (R_{std}) as the x-axis for a range of the analyte concentrations. Examples of calibration curves for fonofos, fonofos oxon and methylphenylsulfone are shown in Figures 4 - 6. Use an appropriate curve fit equation such as the power curve equation to best fit the calibration curve. Take the response units from the detector for the analyte in the sample extract, $R_{(sample)}$, and determine the concentration of the analyte in the sample extract, C_{ex} , by using the corresponding calibration curve.

6.2.2 Concentration of Analyte in Water Sample

Determine the concentration of each analyte in the original water sample, C_i , from the corresponding concentration of the analyte in the samples extract (C_{ex}), the extraction volume, and the concentration factor as follows:

$$C_i (\mu\text{g/L}) = C_{ex} * V_1 / V_2 / S$$

Where

C_{ex} = analyte concentration in the sample extract, $\mu\text{g/mL}$
 V_1 = volume of extract, mL = 30 mL
 V_2 = volume of water extracted, L = 0.2 L
 S = concentration factor for the extract = 10