ATTACHMENT I - Residue Method RM-38-S2

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DETERMINATION OF RESIDUES OF S-1812 and METABOLITES IN SOIL

Method: **RM-38-S2** Date: May 10, 2001

I. INTRODUCTION

This method describes the determination of S-1812 and its soil metabolites S-1812-DPMe, S-1812-DP and HTFP in soils. This modification to RM-35-1S describes a different GC column for the S-1812-DP residue analyses, and a modification to the HPLC gradient profile for the HTFP residue analyses. These modifications were made to achieve better peak shape and/or separation from interfering peaks in the samples.

Briefly, the residues are extracted from the soil using acidified acetonitrile/water. For the determination of S-1812, S-1812-DPMe and S-1812-DP residues, an aliquot of the extract is taken, diluted with a 1% sodium chloride solution, partitioned into hexane, concentrated to dryness using rotary evaporation, redissolved in hexane and passed through a silica cleanup column. Two fractions are collected from the Si column, the first containing S-1812 and S-1812-DPMe and the second fraction containing S-1812-DP. Both fractions are concentrated to dryness using rotary evaporation and the residues are redissolved in toluene for analysis by gas chromatography using a nitrogen/phosphorous detector. For the HTFP residue determination, an aliquot of the initial extract is taken to dryness using rotary evaporation, and passed through a silica cleanup column. The column eluant containing the HTFP residues is concentrated to dryness using rotary evaporation, and is redissolved in acetonitrile/aqueous acetic acid for analysis by liquid chromatography using an ultraviolet detector.

II. EQUIPMENT

Autosampler vials, crimp-capped, and crimping tool
Buchner funnel, 9 cm
Gas Chromatograph (GC): Hewlett Packard 5890 gas chromatograph equipped with a nitrogen-phosphorous detector and a 7673 autosampler, controlled by a Hewlett-Packard Chemstation, or equivalent
GC Column, Rtx 1 (100% dimethyl polysiloxane), 30 m x 0.53 mm x 0.25µm film, Restek
GC Column, DB 5, (5%-phenyl-methylpolysilonaxe), 30 mm x 0.53 mm x 1.5 µm film, Agilent
Graduated cylinders, assorted sizes, including 250, 100, 50, 25, and 10 mL
High Performance Liquid Chromatograph (HPLC): Hewlett Packard Model 1050 HLPC equipped with a quaternary pump, an automatic liquid sampler, a variable wavelength detector, controlled by a Hewlett Packard Chemstation, or equivalent
HPLC Column, Prodigy 5 µ ODS (3), (250 mm x 4.6 mm x 5 µm), Phenomenex
Jars, 1 pt (500 mL) Mason and 250 mL with Teflon lined screw caps
Omni-Mixer with adapter and shafts for use with 1 qt. Mason jars, or similar top-drive blender.
Pipettes, assorted sizes, including 50, 25, 10, 5, 2, and 1 mL

Rotary Evaporator, equipped with temperature controlled water bath
Round-bottom flasks, assorted sizes, including 500, 250, 100, and 50 mL
Separatory funnel, 500 mL and 1 L
Sidearm vacuum flask, 500 mL

III. REAGENTS

Acetic Acid – glacial, reagent grade or equivalent
Acetone – pesticide quality or equivalent
Acetonitrile – pesticide quality or equivalent
Celite® 545 – reagent grade
Ethyl Acetate – pesticide quality or equivalent
Filter paper, Whatman GF/A, 9 cm
Hexane – pesticide quality or equivalent
Hydrochloric Acid - reagent grade or equivalent
Propylene Glycol – 99.5% USP/FCC grade or equivalent
Silica Cartridge, MegaBond Elut®, 2 g, Varian Inc.
Sodium chloride, reagent grade
Toluene – pesticide quality or equivalent
Water, Deionized and HPLC Grade

MIXED SOLUTIONS

Extraction Solution – Acetonitrile / 1% HCl / Water, 180 / 0.5 / 20, v / v / v: Dilute 1 mL concentrated (12 N) HCl in water to make 100 mL of 1% HCL solution. Add 0.5 mL of the 1% HCl solution to 180 mL acetonitrile and 20 mL of water.

1% NaCl Solution – dissolve 1 gram of NaCl in 100 mL deionized water.

Hexane Saturated with Acetonitrile – Add ca. 50 mL of acetonitrile to ca. 900 mL of hexane in a 1 L separatory funnel and shake to mix thoroughly. Remove excess acetonitrile from the bottom of the separatory funnel.

Keeper Solution – 1% propylene glycol in acetonitrile: Dilute 1 mL of propylene glycol in acetonitrile to make 100 mL of solution.

Eluant 1 – Hexane / Ethyl Acetate, 95 / 5, v / v: Mix 95 mL hexane with 5 mL ethyl acetate.

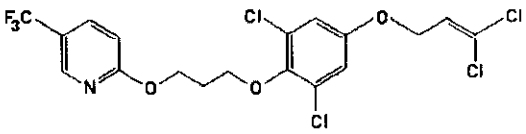
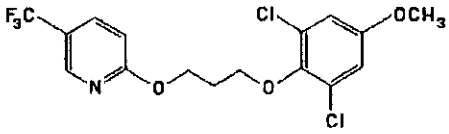
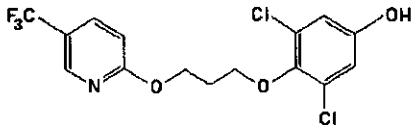
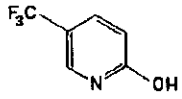
Eluant 2 – Hexane / Ethyl Acetate, 85 / 15, v / v: Mix 85 mL hexane with 15 mL ethyl acetate.

Eluant 3 – Hexane / Acetone, 2 / 3, v / v: Mix 40 mL hexane with 60 mL acetone.

HPLC Mobile Phase – Acetonitrile / 0.01% Acetic acid, 1 / 4, v / v: Dilute 2 mL acetic acid in HPLC grade water to make 200 mL of a 1% acetic acid solution. Dilute 10 mL of the 1% acetic acid solution with HPLC grade water to make 1 L of the 0.01% acetic acid solution. Mix 200 mL of acetonitrile with 800 mL of 0.01% acetic acid solution.

IV. ANALYTICAL STANDARDS

Reference Standards:

Compound	Source / Chemical Name	Structure
S-1812 CAS Number: 179101-81-6	Valent U. S. A. Corporation 2-[3-[2,6-dichloro-4-[(3,3-dichloro-2-propenyl)oxy] phenoxy]propoxy]-5-(trifluoromethyl)pyridine	
S-1812-DPMe	Valent U. S. A. Corporation 2-[3-[2,6-dichloro-4-methoxyphenoxy)propoxy]-5-(trifluoromethyl)pyridine	
S-1812-DP	Valent U. S. A. Corporation 3,5-dichloro-4-[3-(5-trifluoromethyl-2-pyridyloxy)]propoxy phenol	
HTFP	Valent U. S. A. Corporation 2-hydroxy-5-trifluoromethylpyridine	

Stock Standard Solutions: 1 mg/mL Individual Stock Solutions

Weigh 25 or 50 mg of each Reference Standard (correct the amount of standard weighted for the purity of the material) into individual 25 or 50 mL volumetric flasks. Dilute each standard with acetone. Store the Stock Standard Solutions in a refrigerator.

Fortification Standard Solutions: 10 µg/mL Mixed Solution

Pipet 1.0 mL of each of the 1 mg/mL Stock Solutions into a single 100 mL volumetric flask. Dilute to volume with acetone. Store the Fortification Solution in a refrigerator.

Analytical Standard Solutions:

10.0 µg/mL S-1812 + S-1812-DPMe: Pipet 1.0 mL of the 1 mg/mL S-1812 Stock Solution and 1.0 mL of the 1 mg/mL DPMe Stock Solution into a 100 mL volumetric flask. Dilute to volume with toluene.

5.0 ug/mL S-1812 + S-1812-DPMe: Pipet 0.5 mL of the 1 mg/mL S-1812 Stock Solution and 0.5 mL of the 1 mg/mL S-1812-DPMe Stock Solution into a 100 mL volumetric flask. Dilute to volume with toluene.

10.0 µg/mL S-1812-DP: Pipet 1.0 mL of the 1 mg/mL S-1812-DP stock solution into a 100 mL volumetric flask. Dilute to volume with toluene.

100.0 µg/mL HTFP Solution: Pipet 5.0 mL of the 1 mg/mL HTFP stock solution into a 50 mL round bottom flask. Remove the acetone using a rotary evaporator. Dissolve the residues in ca. 10 mL of acetonitrile/0.01% acetic acid (1/4, v/v) and transfer to a 50 mL volumetric flask. Dilute to volume with acetonitrile/0.01% acetic acid (1/4, v/v), using additional portions of the solvent to rinse the round bottom flask.

10.0 µg/mL HTFP Solution: Pipet 10.0 mL of the 100.0 µg/mL HTFP solution into a 100 mL volumetric flask. Dilute to volume using acetonitrile/0.01% acetic acid (1/4,v/v).

The following dilutions are made for each of the three 10 µg/mL Analytical Standard Solutions.

Dilute the S-1812 + S-1812-DPMe and the S-1812-DP solutions in toluene, dilute the HTFP solutions in 1/4 acetonitirle/0.01% acetic acid as follows:

2.5 ug/mL: Pipet 25.0 mL of the 10.0 µg/mL solution into a 100 mL volumetric flask. Dilute to volume.

1.0 µg/mL: Pipet 10.0 mL of the 10.0 µg/mL solution into a 100 mL volumetric flask. Dilute to volume.

0.5 µg/mL: Pipet 5.0 mL of the 10.0 µg/mL solution into a 100 mL volumetric flask. Dilute to volume.

0.25 µg/mL: Pipet 2.5 mL of the 10.0 µg/mL solution into a 100 mL volumetric flask. Dilute to volume.

0.1 µg/mL: Pipet 1.0 mL of the 10.0 µg/mL solution into a 100 mL volumetric flask. Dilute to volume.

Store the Analytical Standard Solutions in a refrigerator. Similar preparative steps, and serial dilutions, may be used to generate additional analytical standard solutions.

V. INSTRUMENTATION

GAS CHROMATOGRAPH / NITROGEN-PHOSPHOROUS DETECTOR (GC/NPD)

The following operating conditions are suggested for the analyses of S-1812 and S-1812 DPMe in soil. Alternative GC columns and conditions may be used as appropriate.

Column:	Rtx-1, 30 m x 0.53 mm x 0.25 µm	
Injector:	Packed inlet with a Direct Inlet adapter, 2 mm insert with glass wool 250°C	
Injection:	2.0 µL	
Oven:	Initial Temp:	200°C for 2 min
	Rate 1:	15°C/minute
	Temp 1:	275°C for 3.0 min
He Carrier Flow:	13.6 mL/minute (14.6 psi @ 200°C), Constant flow mode	
Detector:	Temp:	280°C
	H_2:	3.6 mL/min
	Air:	100 mL/min
	Aux. Gas (He):	13.8 mL/min
Retention Times:	DPMe:	2.5 minutes
	S-1812:	5.3 minutes

The following operating conditions are suggested for the analyses of S-1812 DP in soil.

Column:	DB 5, 30 m x 0.53 mm x 1.5 µm	
Injector:	Packed inlet with a Direct Inlet adapter, 2 mm insert with glass wool 250°C	
Injection:	1.0 µL	
Oven:	Initial Temp:	250°C for 4 min
	Rate 1:	20°C/minute
	Temp 1:	290°C for 3.0 min
He Carrier Flow:	20.0 mL/minute (21.5 psi @ 250°C), Constant flow mode	
Detector:	Temp:	250°C
	H_2:	4.0 mL/min
	Air:	105 mL/min
	Aux. Gas (He):	10 mL/min
Retention Time:	DP:	3.1 minutes

Alternate GC columns and conditions, such as those described above for S-1812 and S-1812-DPMe may be used for the S-1812 DP analyses, giving a retention time of 3.2 minutes.

HIGH PERFORMANCE LIQUID CHROMATOGRAPH (HPLC)

The following operating conditions are suggested for the analyses of HTFP in soil. Alternative HPLC columns and conditions may be used as appropriate.

Column: Prodigy ODS (3), 250 mm x 4.6 mm x 5 µm
Column Temp.: 35°C
Injection: 50 µL
Gradient:

	% A 1 / 4 Acetonitrile/0.01% acetic acid (v/v)	% B Acetonitrile
0 min	80	20
2	90	10
6	90	10
8	50	50
10	50	50
11	100	0
15	100	0

Detector: Variable Wavelength UV at 235 nm

Retention Time: HTFP: 4.9 minutes

VI. ANALYTICAL PROCEDURE

1. Sample Setup

Weigh 50 g of soil (± 0.5 g) into a 1 pt. (500 mL) jar. If method recovery data is required by the testing facility, fortify control samples with the appropriate amounts of each analyte at this point. (See Note 1).

2. Initial Extraction

Add 100 mL of extraction solution (acetonitrile / 1% HCl / water, 180 / 0.5 / 20, v / v / v), and blend the sample for 10 minutes using the Omni-Mixer. Add ca. 50 mL of Celite, and filter the sample through a 9 mm Whatman GF/A filter paper, pre-wetted with acetonitrile, on a Buchner funnel, using vacuum, into a 500 mL side-arm suction flask. Transfer the sample and filter paper back into the extraction jar, add an additional 100 mL of extraction solution and blend for 10 more minutes. Filter sample as before, combining the filtrates. Rinse the blender jar and filter cake twice with 10 mL of acetonitrile and add to filtrates. Transfer the sample extract to a 250 mL graduated cylinder and adjust the sample volume to 250 mL using acetonitrile. Store the sample extract in a capped 250 mL jar.

3. S-1812, S-1812-DPMe, S-1812-DP Workup

3.1 Hexane / Acetonitrile Partition

Transfer an aliquot equivalent to 10 g of soil (50 mL) from the initial sample extract to a 500 mL separatory funnel, and add 50 mL of a 1% NaCl solution. Partition the sample with 100 mL of hexane saturated with acetonitrile, and allow the phases to

separate. Drain the lower, aqueous phase to a temporary container, and transfer the hexane to a 500 mL round bottom flask. Transfer the retained aqueous phase back to the separatory funnel, and partition twice more with 100 mL portions of hexane saturated with acetonitrile, and combine the hexane fractions. Evaporate the hexane using a rotary evaporator with a water bath temperature of <35°C to approximately 5 mL, and add ca. 20 mL of ethyl acetate. Continue the evaporation just to dryness, add 15 mL of hexane, and rotovap to dryness again. Dissolve the residues in 5 mL of hexane, using sonication to insure the sample is completely dissolved.

3.2 Silica Solid Phase Extraction Cleanup (See Note 2)

Condition a 2 g MegaBond Silica SPE cleanup cartridge by passing 2 x 5 mL of Eluant 1 (95 / 5 hexane / ethyl acetate, v / v) through the cartridge followed by 2 x 5 mL of hexane, using gravity to elute the solvents. The flow should be discontinued when the solvent level reaches the top of the packing. Do not allow the silica packing to get dry during or after conditioning.

Transfer the sample to the column, and rinse the column with 3 x 5 mL of hexane, using the hexane to rinse the round bottom flask. Discard the hexane rinse.

FRACTION A: S-1812 + S-1812-DPMe

Place a suitable collection container under the cartridge (or collect directly in a 50 mL round bottom flask) and elute the S-1812 and S-1812-DPMe with 20 mL of Eluant 1, using the solvent to rinse the 500 mL round bottom flask. As soon as the last of the Eluant 1 reaches the top of the Silica bed, stop the elution from the cartridge and remove collection container. Transfer the sample to a 50 mL round bottom flask using two or three 2 mL hexane rinses.

FRACTION B: S-1812-DP

Place a second collection container under the cartridge (or collect directly in a 50 mL round bottom flask) and elute the S-1812-DP with 15 mL of Eluant 2 (85 / 15 hexane / ethyl acetate, v / v), using the solvent to rinse the 500 mL round bottom flask. Transfer the sample to a 50 mL round bottom flask using two or three 2 mL hexane rinses.

3.3 Concentration

Concentrate each sample fraction just to dryness using a rotary evaporator with a water bath temperature <35°C, and dissolve the residues in 1.0 mL of toluene for analysis, using sonication to insure the sample is completely dissolved. Transfer each sample to crimp capped ALS vial for analysis using GC/NPD.

Continue with the sample analyses as described in Section 5.

4. HTFP Workup

4.1 Sample Concentration

Transfer an aliquot equivalent to 10 g of soil (50 mL) from the initial sample extract to a 250 mL round bottom flask. Add 1 mL of a 1% propylene glycol/acetonitrile keeper solution, and evaporate the sample using a rotary evaporator (water bath temperature <40°C) to approximately 5 mL. Add approximately 50 mL of ethyl acetate, and continue evaporation until just dryness. Immediately add 10 mL of 10/1 hexane/ acetone, v/v, and sonicate the sample to ensure that the residues are completely dissolved.

4.2 Silica Solid Phase Extraction Cleanup (See Note 2)

Condition a 2 g MegaBond Silica SPE cleanup cartridge by passing 2 x 5 mL of 10 / 1 hexane / acetone, v / v through the cartridge followed by 2 x 5 mL of hexane, using gravity to elute the solvents. The flow should be discontinued when the solvent level reaches the top of the packing. Do not allow the silica packing to get dry during or after conditioning.
Transfer the sample to the cartridge, and rinse the cartridge using 3 x 5 mL of hexane, using the hexane to rinse the 250 mL round bottom flask. Discard the hexane rinses. Place a suitable collection container under the cartridge (or collect directly in a 50 mL round bottom flask), and elute the HTFP from the Silica SPE cartridge with 25 mL of Eluant 3 (hexane / acetone, 2 / 3, v / v) using the solvent to rinse the 250 mL round bottom flask. After the solvent has completely eluted from the cartridge, remove the collection container, and transfer the sample to a 50 mL round bottom flask, using two or three 2 mL hexane rinses.

4.3 Concentration

Add 1 mL of the keeper solution to sample, and evaporate just to dryness using rotary evaporator with a water bath temperature <35°C water bath.

Redissolve the HTFP sample in 1.0 mL of HPLC Mobile Phase (acetonitrile/0.01% Acetic acid, 1/4, v/v) for HPLC analysis, using sonication to ensure the sample is completely dissolved. Transfer each sample to an ALS vial for analysis using HPLC/UV.

Continue the analyses as described in Section 5.

5 Sample Analysis

5.1 Sequence

Condition the instruments with at least two sample injections before starting the analyses. Analyze a range of at least four standard concentrations along with each

sample set. Standards should be placed so that there is at least one sample injection immediately prior to the standard injection to maintain instrument conditioning. Each sequence must begin and end with an injection of a standard concentration in the middle of the linear range (reference standard). A typical sequence would be 2 or 3 conditioning samples, reference standard, one to three sample injections, linearity standard, one to three sample injections, linearity or reference standard, . . . , one to three sample injections and a reference standard.

5.2 Residue Calculations

Residues are calculated from a second order quadratic least squares fit curve generated from the standard injections, using the formula:

$$y = ax^2 + bx + c,$$

Where y = µg/mL concentration of the standards,
x = response (area or height), and
a, b and c are constants.

Using a Microsoft Excel spreadsheet, the formula (a, b and c constants) can be derived by plotting the standard data, using the µg/mL concentrations as the y axis and the responses on the x axis, and adding a second order polynomial trendline, with the display formula option chosen. (See Note 3).

Residue amounts (µg/mL) for the samples are calculated from the curve using the formula:

$$\mu g/mL = (a * (response)^2) + (b * response) + c$$

The ppm residues for the samples using the formula:

$$ppm = \frac{\mu g/mL}{(\text{sample weight (g)} / \text{final volume (mL)})}$$

For the fortified samples, the percent recoveries are calculated by:

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

5.3 Example of Residue Calculations

An example of the residue calculations is shown below, using the following data:

Standard	Response (Area) for S-1812
0.50 ug/mL	8783
0.10 ug/mL	1757
0.25 ug/mL	4434
0.50 ug/mL	9063
1.0 ug/mL	18057
2.5 ug/mL	46240
0.50 ug/mL	9142

The formula for the curve (derived from an Excel graph) for the above standards is

$$\mu g/mL = (-4.142e\text{-}11 * x^2) + (5.593e\text{-}5 * x) + 1.445e\text{-}3$$

To calculate residues and recoveries for a control sample and a fortified sample with the following responses:

Sample	Concentration	Response (Area) for S-1812
Control	10 g / mL	0
Fortified @ 0.1 ppm	10 g / mL	17042

Control µg/mL = $(-4.142\text{-}e11 * 0^2) + (5.593e\text{-}5 * 0) + 1.445e\text{-}3$ = 0.001 µg/mL

Control ppm = 0.001 µg/mL / 10 g/mL = 0.0001 ppm

Fortified ug/mL = $(-4.142\text{-}e11 * 17042^2) + (5.593e\text{-}5 * 17042) + 1.445e\text{-}3$ = 0.943 ug/mL

Fortified ppm = 0.943 ug/mL / 10 g/mL = 0.0943 ppm

% Recovery = ((0.0943 ppm – 0.0001 ppm) / 0.1 ppm) * 100% = 94.2%

5.4 Quality Acceptance Criteria

If the peak response for any sample or fortified sample is greater than 110% of the peak response of the largest linearity standard, the sample must be diluted and the diluted extract analyzed.

Correlation coefficient (r^2) values for the standard data points on the curve need to be 0.99 or greater for the set to be acceptable, and the µg/mL amounts for the standards, when back calculated from the curve need to be within 20% of the standard concentration.

Percent recovery values for the fortified control samples need to be $\geq$ 70% and $\leq$ 120% to be acceptable.

Any deviations to these Acceptance Criteria need to be approved by the chemist responsible for the analyses.

VII. ANALYTICAL LIMITS

The Limit of Detection (LOD) as ppm (µg/g) for this method is determined by dividing the lowest linear standard concentration (µg/mL) by the final sample concentration (g/mL). If the lowest linearity standard is 0.1 µg/mL and the final sample concentration is 10 g/1 mL, the LOD would be 0.01 ppm (0.10 µg/mL standard / 10 g/1 mL sample concentration).

The validated Limit of Quantitation (LOQ) for this method is 0.02 ppm for each compound. This LOQ was validated by spiking control samples at this level, and having successful (≥ 70%) recoveries of each analyte.

VIII. ANALYSIS TIME

A trained analyst, familiar with this method, can complete the initial extraction, and one of the sample workups (S-1812 + S-1812-DPMe + S-1812-DP or HTFP) for a set of twelve samples in approximately eight hours. The second sample workup takes an additional four hours to complete. The analysis time for the S-1812 + S-1812-DPMe is approximately 4.5 hours. The analysis time for the S-1812-DP is approximately 3.5 hours, and the analysis time for the HTFP is approximately 6 hours.

IX. NOTES

1. Valent's Standard Operating Procedures (SOP # VR-002) requires that fortified samples be analyzed with each set of samples. The level of fortifications made during the study need to include fortifications at the LOQ (0.02 ppm, generally a minimum of three) and at levels similar to the detected residues, including the maximum residues found.

2. Each lot of Si SPE Cartridges should be checked for recovery of analytes as follows: Transfer a 0.1 mL aliquot of the fortification standard (10 µg/mL) to a 50 mL round bottom flask, and remove the standard solvent. To check a cartridge for S-1812, S-1812-DPMe and S-1812-DP, redissolve the sample in 5 mL of hexane. For HTFP, redissolve the standard in 10 mL of hexane / acetone (10 / 1, v / v). Transfer the sample to the Si SPE cartridge and elute the analytes as described in Sections 3.2 and 4.2, Sample Cleanup. Evaporate the sample to dryness, and redissolve in 1.0 mL of the appropriate solvent for analysis. Analyze the samples using conditions described in Section V, Instrumentation, a 1.0 µg/mL standard for comparison. If the recoveries of any analyte is <90%, the elution profile should be modified to obtain recoveries >90%.

3. Programs other than Microsoft's Excel, such as CurveExpert 1.3 (Hyams Development, Starkville, MS) may be used to generate the curve and resulting formula.

SIGNATURES

Written by: _____ Date: 5-24-01
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Reviewed by: _____ Date: 5/25/01
G. H. Fujie

Reviewed by: _____ Date: 6/4/01
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