Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Sulfometuron Methyl

MRID #: 437648-01

Matrix: Soil

Analysis: HPLC/UV

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Analytical Method for the Determination of Residues of Sulfometuron Methyl in Soil and Water, Using Supercritical Fluid Extraction

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Summary

This analytical method was developed for the determination of sulfometuron methyl (the active ingredient in Oust® Herbicide) residues in soil and water. Residues are quantitated using multi-dimensional HPLC ("heart cut" column-switching) with UV absorbance detection at 235 nm. The Limits of Quantitation were determined to be 0.2 ppb and 0.025 ppb for soil and water, respectively.

This method is highly sensitive, practical, and convenient relative to already existing methods for sulfometuron methyl residue analysis.

Sulfometuron methyl residues are extracted from 5.0- to 10.0-g soil samples using supercritical fluid extraction (SFE); the extraction fluid is CO_2 modified with 80% acetonitrile /20% water (modifier added directly to samples). Extracts are dissolved in bicarbonate buffer (pH 10) and washed with chloroform. After acidifying samples, residues are extracted into toluene. This solvent is evaporated, and residues are transferred to alumina (basic) solid phase extraction cartridges, for clean up. Cartridges are eluted with 2% gl. acetic acid/98% dichloromethane, which is evaporated to dryness.

Water samples (50.0-400.0 g) are acidified, then sulfometuron methyl residues are transferred to "C2" (ethyl-derivatized silica) solid phase extraction cartridges. Cartridges are eluted with ethyl acetate, which is evaporated to dryness.

Samples are reconstituted in 10% acetonitrile/90% water, and analyte concentrations are determined by multi-dimensional HPLC.

The mean recovery (± S.D.) for 42 soil samples (three soil types), fortified over the range 0.2-10.0 ppb, was 82 ± 14%. The mean recovery (±S.D.) for 47 water samples (from two local sources), fortified over the range 0.025-2.00 ppb, was 97 ± 10%.

INTRODUCTION

Sulfometuron methyl (methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoate) is the active ingredient in Oust® Herbicide. Sulfometuron methyl (SM) is used for vegetation management along roadsides, within railroad right-of-ways, and on industrial, noncrop sites. In addition, it can be used for selective weed control in forest site preparation and release of pines. Oust® Herbicide has been shown to control a broad range of herbaceous species (annual grasses, perennial grasses, and broadleaf weeds).

Sulfometuron methyl belongs to the sulfonylurea class of herbicides – the properties of which are well known (1). Their low toxicity combined with low application rates makes these compounds especially attractive from an environmental and human health standpoint.

As part of the U. S. EPA reregistration process for SM (under "FIFRA '88"), The Environmental Fate and Ground Water Branch (U.S. EPA) requested "that the most sensitive analytical methods available to identify sulfometuron methyl residues in soil and water be submitted to the Agency for validation."[1]

At the time of this request, the Agency officially recognized, for regulatory purposes, only the soil and water residue method published by DuPont in 1985 (2). However, based on preliminary experiments in our laboratory, it appeared possible that a method of comparable (if not superior) sensitivity to that described in Reference 2 could be developed using UV absorbance detection (much preferred by most analysts over the photo-conductivity detection scheme in [2]) – but only if a multi-dimensional (i.e., column-switching) HPLC approach was utilized. (See References 3 and 4 for a review of column-switching HPLC.)

This report describes the results of our extended investigation into such an approach.

[1] internal EPA memorandum from Silvia C. Termes to Tom Myers/Doña Canales, dated 04 APR 94 -copy sent to Jack Cain [DuPont] from Doña Canales via FAX, 05 APR 94)

Supercritical Fluid Extraction (SFE)

Early in the method development process, we became very interested in the merits of supercritical fluid extraction (SFE) as a key component of our new soil method. (References 5 and 6 describe supercritical fluids and provide a good overview of SFE.) Our interest stemmed from the following considerations:

- SFE already has a significant but still quickly growing "track record" of being a very practical (if not optimal) technique for extracting environmental samples (7,8).
- SFE is intrinsically an excellent match to the properties of soil (9), especially from the standpoint of high diffusivity (with excellent dissolving power) relative to liquids (see Table I).
- SFE methods are typically very rapid, usually quite quantitative, and the use of organic solvents is minimized (5).
- SFE instrumentation is widely available commercially and already is accepted by the EPA[2] for selected analytical applications (10).
- SFE offers a wide variety of parameters for optimization of extraction conditions, including temperature, extraction fluid polarity (using modifiers), pressure, density, duration of extraction, and ratio of extractant volume to sample volume. SFE methods are often effective using mild conditions.

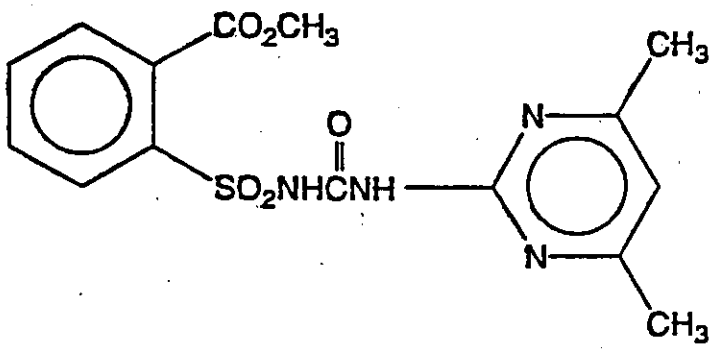
  (The availability of several significant extraction variables/parameters is potentially convenient for dealing with a particularly difficult-to-extract soil, should the need arise. Due to the design of commercially available extraction systems, it is very easy to vary the extraction conditions without a great deal of effort – to be more powerful or to be optimized more closely to the characteristics of a particular soil.)

[2] U. S. EPA Office of Solid Waste, Method 3560 (for extraction of "Total Petroleum Hydrocarbons" from soil and sediment).

MATERIALS

Compound Identity

The *Chemical Abstracts* structure and uninverted, systematic name of SM are as follows:



sulfometuron methyl (SM)

methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]-sulfonyl]benzoate

CAS Registry No. 74222-97-2

Reagents

NOTE: All reagents and solvents were used as received.

Solvents - HPLC grade acetonitrile, chloroform, dichloromethane, ethyl acetate, methanol, 2-propanol, and toluene (EM Science Omnisolv®, VWR Scientific Co., Philadelphia, Pa.). Equivalent solvents may be substituted.

Use water purified by a laboratory Millipore® Milli-Q® reagent-grade water purification system (Millipore Corp., Bedford, Mass.) or equivalent.

Carbon Dioxide - SFC Grade™ CO_2 (Scott Specialty Gases, Inc., Plumsteadville, Pa.) or equivalent.

Pesticide Standards - Sulfometuron methyl (DPX-T5648; 98.5%), E. I. du Pont de Nemours and Company (Analytical Standards Laboratory, Du Pont Agricultural Products, Wilmington, Del.). Store dry analytical standards frozen (≤0°C) until use.

Other reagents - A.C.S. reagent grade (VWR Scientific Co., Philadelphia, Pa.) or equivalent.

Standards (Sulfometuron Methyl)

Note: Store all dissolved standards (stock, fortification, HPLC) refrigerated at 1-5°C when not in use.

Stock Standards - Prepare stock standards (~100 µg/mL) by dissolving approximately 5 mg of the dry standard in 50-mL acetonitrile. Standards are stable in this solvent for six months (1-5°C).

Fortifying Standards - Prepare fortifying standards at the 1-µg/mL level by transferring (with a high-quality syringe) 1.00 mL of stock solution into a clean, dry, 50-mL volumetric flask. Prepare fortifying standards at the 0.1-µg/mL level by transferring (with a high-quality syringe) 1.00 mL of 1-µg/mL standard into a clean, dry, 10.0-mL volumetric flask. Dilute these standards to volume with acetonitrile. Standards are stable in this solvent for six months (1-5°C).

HPLC Standards - Prepare a standard at the 1-µg/mL level by transferring (with a high-quality syringe) 1.00 mL of stock solution into a clean, dry, 100-mL volumetric flask. Dilute to volume with 10% acetonitrile/90% water. Replace this standard weekly (standards are stable for 1 week if stored at 1-5°C). *This standard may also be used for fortifying samples.*

Prepare standards at levels of 0.20, 0.10, 0.05, 0.03, 0.02, and 0.01 µg/mL by transferring (with a high-quality syringe) 2.00, 1.00, 0.500, 0.300, 0.200, and 0.100 mL, respectively, of the 1-µg/mL standard into 10.0-mL volumetric flasks. Dilute to volume with 10% acetonitrile/90% water. Prepare standards at levels of 0.0075, 0.006, 0.005, 0.004, 0.003, 0.002, and 0.001 µg/mL by transferring (with a high-quality syringe) 0.750, 0.600, 0.500, 0.400, 0.300, 0.200, and 0.100 mL, respectively, of the 0.10-µg/mL standard into 10.0-mL volumetric flasks. Dilute to volume with 10% acetonitrile/90% water. Prepare fresh standards weekly (standards are stable for 1 week if stored at 1-5°C).

Equipment

Instrumentation

Note: All the following equipment is readily available from commercial sources.

Liquid Chromatograph [3] - Waters (Milford, Mass.) Model 590 Programmable Solvent Delivery Module; Waters Column Temperature Control System (Cat. No. 38039, includes control module and column heating chamber); Waters Model 486 Selectable Wavelength UV/Vis Absorbance Detector; Valco Model C6W 6-port HPLC valve (Valco Instrument Co., Houston, Tex., used for manual sample injection); SSI Lo-Pulse® pulse damper (Cat. No. 20-0128, Rainin Instrument Co., Woburn, Mass., installed downstream of pump); three-way stream switching valve (Cat. No. 38-082, Rainin Instrument Co., electrically controlled by pump [installed immediately upstream of pump inlet], used for selection of mobile phase supplied to pump); Hitachi Model 655A-40 Autosampler (EM Science, Cherry Hill, N.J.); Valco Model C6W 6-port HPLC valve controlled by Model E60 electric actuator [close mount] (Valco Instrument Co., Houston, Tex., used for column switching); Linseis Model 4000 strip-chart recorder (Linseis, Inc., Princeton Junction, N.J.).

HPLC Columns -

Column I – Zorbax® SB-Phenyl Analytical HPLC Column, 4.6 mm x 150 mm, 5 µm spherical particles (P.N. 883975-912. MAC-MOD Analytical, Chadds Ford, Pa.)

Column II – Zorbax® ODS Analytical HPLC Column, 4.6 mm x 250 mm , 5 µm spherical particles (P.N. 880952-702. MAC-MOD Analytical)

Details of column-switching operation are found in **INSTRUMENTAL ANALYSIS** section.

Use Zorbax® SB-Phenyl (Cat. No. 820674-917) guard cartridges (MAC-MOD Analytical), or equivalent. Install immediately upstream of Column I.

Mobile Phase A – 70% aqueous 0.03 M sodium acetate (pH 4.0)/30% CH_3CN

Mobile Phase B – 60% aqueous 0.03 M sodium acetate (pH 5.0)/40% CH_3CN

Filter mobile phases with a Millipore®, all-glass filter apparatus, Cat. No. XX15 04700, using Durapore® membrane filters (polyvinylidene difluoride), 0.45-µm pores, Cat. No. HVLP 04700 (Millipore Corp., Bedford, Mass.). Filter samples prior to HPLC injection with Millipore Millex-HV® (0.45-µm pores, 4-mm diam.)

[3] Equivalent components may be substituted.

Durapore® membrane filters. Discard filters after use. Equivalent filtration devices may be substituted.

Supercritical Fluid Extractor - ISCO (Lincoln, Neb.) SFE System 2300 (see Figure 1), consisting of one Model 260D pump, one Model 100D pump[4], one system controller (electronic), one Model SFX 2-10 dual-chamber extractor module, and associated valves, fittings, mixing "tee", and connecting tubing; two, 10-mL S.S. sample cartridges; two, extractant collection vials (20 x 150 mm) contained in plastic Erlenmeyer flasks (for safety).

Balances [5] - Weigh analytical standards (dry material) using a Mettler Model AE-163, 4-place analytical balance (Mettler Instrument Corp., Hightstown, N.J.). Weigh samples and reagents on a Mettler Model PE-360, 2-place top-loading balance.

pH Equipment [6] - Fisher Accumet® 15 digital pH meter (Fisher Scientific Company, Pittsburgh, Pa.); Corning X-EL® combination pH electrode (Corning Glass Works, Scientific Products Div., Medfield, Mass.).

Sample-Processing Equipment

NOTE: Equivalent equipment may be substituted for the following, unless otherwise indicated:

Ultrasonic Bath - Branson Model 3200 ultrasonic bath (VWR Scientific Co., Philadelphia, Pa.).

Mixer - Thermolyne Maxi-Mix II™, Model M37615 (Barnstead/Thermolyne, Dubuque, Iowa).

Evaporator - N-EVAP® Model 111 laboratory sample evaporator/nitrogen manifold fitted with Teflon®-coated needles (Organomation Associates, Worcester, Mass.). Attach unit to a clean, dry nitrogen source.

Electronic Pipettor - Biohit Proline® No. 710022, 1000-µL capacity (Cat. No. 53495-205, VWR Scientific Co.)

Syringe - Glass, high-performance, Luer-Lok® tip, 250-µL and 1000-µL capacities (Model Nos. 1725 and 1001, respectively; Hamilton Co., Reno, Nev. [distributed by Alltech Associates, Inc., Deerfield, Ill.]). Use #22 needle with CTFE Luer® hub, Cat. No. 72-15, Alltech Associates, Inc.

[4] Not used, however, for this residue method.
[5] Equivalent components may be substituted.
[6] Equivalent components may be substituted.

Solid Phase Extraction Apparatus - 12-port SPE vacuum manifold, #5-7030M, with Teflon® solvent guide needles, #5-7047 (Supelco, Bellefonte, Pa.)

Solid Phase Extraction Cartridges - **Do not substitute.**

Bond Elut® "ALB", 500-mg basic alumina, 3-mL reservoir (P. N. 1210-2048, Varian Sample Preparation Products, Harbor City, Calif.); Bond Elut® "C2", 500-mg ethyl-derivatized silica, 3-mL reservoir (P. N. 1210-2030, Varian Sample Preparation Products)

Solid Phase Extraction Reservoirs - Empty Bond Elut® polypropylene reservoir (with 20-µm porous polyethylene frits), 75-mL capacity (P. N. 1213-1018, Varian Sample Preparation Products), requires Bond Elut® adapter (P.N. 1213-1001) for attachment to 3-mL cartridge

ANALYTICAL METHOD: SOIL

Principles and Scope

See Figure 2A for a method flow chart.

Method in Brief

Sulfometuron methyl residues are extracted from 5.0- to 10.0-g soil samples using SFE (extraction fluid: CO_2 modified* with 80% acetonitrile/20% water). Extracts are dissolved in bicarbonate buffer (pH 10) and washed with chloroform. After acidifying samples, residues are extracted into toluene. This solvent is evaporated, and residues are transferred to alumina (basic) solid phase extraction cartridges. Cartridges are eluted with 2% gl. acetic acid/98% dichloromethane, which is evaporated to dryness.

Samples are reconstituted in 10% acetonitrile/90% water, and SM concentrations are determined by multi-dimensional HPLC ("heart cut" column-switching) using reversed-phase conditions. UV-absorbance detection at 235 nm is used.

Reliable recoveries were obtained over the range 0.2-10.0 ppb (10.0 ppb was the highest fortification level tested). The Limit of

* Modifier is manually added to samples (contained in extraction cartridges) using the electronic pipettor.

Quantitation was determined to be 0.2-0.5 ppb, depending on soil type (% organic matter).

Sample Preparation and Storage

Prepare representative samples (or subsamples) using a valid, generally-accepted approach to homogenize the soil (mixed with dry ice to prevent degradation of residues) using a Hobart® Food Processor (or similar). Unless the soil is unusually wet, drying the soil should be unnecessary. Store samples frozen (at approximately -20°C) until analysis.

Weigh out representative 5.0-g soil samples (record actual weights); transfer samples to sample cartridges. (If desired, 8.0 or 10.0 g of soil can be analyzed, for low-level work. Or 1 sample can be split between 2 cartridges, to get a greater "concentration factor".) For recovery evaluation, fortify an untreated check sample with the appropriate amount of standard solution as described in the "Standards" section.

Extraction

Notes:

A. Before operating equipment, review extractor rear panel directions and operators manual.

B. If system has not been used recently a "dry run" should be made in order to condition pump seals, remove air from the lines, check the system before committing to run samples, etc. Specifically, carry out Steps 1-19, but with no sample cartridges installed.

Initial Instrument Parameters

Pump A (Model 260D)

Constant Pressure Mode, 5000 psi
Maximum Pressure Limit, 7500 psi
Maximum Flow Limit, 90 mL/min
Minimum Pressure Limit, 10 psi
Minimum Flow Limit, 0.001 mL/min

SFX 2-10 Dual-Chamber Extractor

Temperature, 45°C
Two, 10-mL sample cartridges

Two, extractant collection vials, 20 x 150 mm (30 mL), contained in plastic Erlenmeyer flasks (for safety)

1. Open external valve(s) for liquid CO_2 delivery to system.
2. Turn on controller and extractor.
3. Program the controller to the desired flow rate and pressure conditions (see operators manual for instructions).
4. Fill Pump A with liquid CO_2.
   a. Push "D" ("Select") and "A" keys on the controller, to select Pump A.
   b. Open left (fill) valve on Pump A.
   c. Push "Refill" key, followed by the "A" key.
   d. When pump is full, push "Stop" and "A" keys.
   e. Close left (fill) valve.
5. Set temperature on extractor to desired set point.
   a. Press the "SV" ("Set Value") key on the temperature control panel.
   b. Press and release the "Up Arrow" key to the correct (blinking) numerical value.
   c. Press the "Ent" key.
   d. Press and release the "PV" ("Present Value") key to observe the current temperature.
   e. Allow the temperature to equilibrate to the designated setting before beginning the extraction.
6. Manually add 1.5 (±0.1) mL (per 5 g of soil) of 80% acetonitrile/20% water (v/v) to each sample (contained in sample cartridges at this point), as a modifier.[7]
7. Open the chamber cap assembly and snap in the sample cartridge for extraction (one or two samples). If chamber cap assembly is difficult to open, cautiously open the vent values to release pressure.
8. Screw in the chamber cap assembly.
9. Open the supply valve to the back chamber.

[7]For 8.0- or 10.0-g samples, add 2.4 or 3.0 mL modifier, respectively.

10. Push "D" (select) and "A" keys on controller, to select Pump A.
11. Open the supply valve (right side) on Pump A (be sure to keep the supply inlet valve open for each extraction chamber containing a sample).
12. Push "Run" key (pressure should reach and maintain 5000 psi).
13. At 5 minutes into run push "Stop" and "A" keys.
14. Close supply valve on Pump A (right side).
15. Close supply inlet valves.
16. Avoid abrupt pressure release by slowly opening extract outlet valves and collect sample extract[8] (do not collect, if this is a "dry run"). After cartridges have depressurized, close extract outlet valves. **Slowly open supply inlet and vent outlet valves, to fully depressurize the sample chambers.**
17. Repeat steps 6 through 16 for 2nd extract collection.
18. A final collection (3rd) should be carried out by:
    a. Closing the extract outlet valves.
    b. Opening the sample supply inlet valves.
    c. Open the supply valve (right side) for Pump A.
    d. Push "Run" key on the controller.
    e. At approximately 0.5-1.0 minutes push "Stop" and "A" keys.
    f. Close sample supply inlet valves.
    g. Close Pump A supply valve.
    h. Slowly open extract outlet valves.
    i. Collect final extract.
19. Open (slowly) the vent outlet valves, to release pressure from the system.

[8]Extractant collection vials are empty, initially (analyte is non-volatile).

Clean Up

1. Pour extracts into 70-mL sodium bicarbonate buffer solution (pH 10) (contained in 250-mL Erlenmeyer flasks). Quantitatively transfer each extract by washing tubes into the buffer, using 2 X 0.50-mL acetonitrile followed by 2 X 1.0-mL bicarbonate buffer (briefly sonicate and vortex-mix each time).

2. Transfer samples to 500-mL separatory funnels. Wash these aqueous solutions with 50 mL chloroform by vigorously shaking for 1 min; discard the chloroform (lower) layer (analyte stays in the aqueous layer).

   Repeat this step twice more, for total of 3 times.

   NOTE: Vent the separatory funnel after each mixing period, to release any pressure buildup.

3. Drain each sample into a 400-mL beaker and adjust the pH to 3-4 by adding 5% (v/v) sulfuric acid dropwise, while stirring the sample to prevent localized regions of low pH (which can decompose SM). Perform this step with a calibrated pH meter.

4. Return samples to 500-mL separatory funnels. Extract aqueous solutions with 50-mL toluene by vigorously shaking for 1 min; transfer the organic (upper) layer, which contains the analyte, to 200-mL pear-shaped flasks.

   Repeat this step twice more, for total of 3 times.

   NOTE: Vent the separatory funnel after each mixing period, to release any pressure buildup.

5. Add 1.0-mL glacial acetic acid to each sample (the acetic acid minimizes analyte losses during the next step). Using a rotary evaporator, evaporate the samples to dryness (water bath at about 45°C can be used).

For Step Nos. 6-9, use the Supelco 12-port vacuum manifold for simultaneous preparation and use of a set of SPE cartridges.

6. Condition a set (one/sample) of Bond Elut® "Alumina B" SPE cartridges with 5 mL 75% cyclohexane/12.5% methanol/12.5% 2-propanol. Gravity is sufficient for fluid flow.

7. Dissolve residues (still in pear-shaped flasks) in 1 mL 75% cyclohexane/12.5% methanol/12.5% 2-propanol. Sonicate and vortex-mix, briefly, to remove residues from the flask

walls. Transfer samples (using disposable Pasteur pipettes) to the SPE cartridges. Pass solutions through the SPE cartridges, again using gravity flow. Discard effluents (analyte is sorbed to the SPE sorbent).

8. Repeat step no. 7 four additional times. Wash the entire pear-shaped flask each time with transfer solvent.
9. Elute the SPE cartridges (gravity flow) with 6 mL[9] 2% gl. acetic acid/98% dichloromethane. Collect cartridge effluents in glass test tubes or glass centrifuge tubes (recommend 13-mL capacity).
10. Place the sample tubes in a nitrogen evaporation apparatus (e.g., N-EVAP®) containing a water bath at approximately room temperature. Evaporate to dryness. Store samples refrigerated (0-4°C) until LC analysis.

ANALYTICAL METHOD: WATER

Principles and Scope

See Figure 2B for a method flow chart.

Method in Brief

Water samples (50.0-400.0 g) are acidified, then sulfometuron methyl residues are transferred to "C2" (ethyl-derivatized silica) solid phase extraction cartridges. Cartridges are eluted with ethyl acetate, which is evaporated to dryness.

Samples are reconstituted in 10% acetonitrile/90% water, and SM concentrations are determined by multi-dimensional HPLC ("heart cut" column-switching) using reversed-phase conditions. UV-absorbance detection at 235 nm is used.

Reliable recoveries were obtained over the range 0.025-2.00 ppb (2.00 ppb was the highest fortification level tested). The Limit of Quantitation was determined to be 0.025 ppb.

[9] This elution procedure was worked out during development of this residue method. For best results, the elution profile of SM should be established/calibrated in advance of sample analysis, using SPE cartridges from actual lots to be used for sample analysis.

Sample Preparation

Weigh out representative water samples into 100-mL beakers, as follows:

ppb Level	Sample Weight (g)
≥ 1	50
0.2-1	200
<0.2	400

For recovery evaluation, fortify an untreated check sample with the appropriate amount of standard solution as described in the "Standards" section.

Clean Up

Note: Do not let SPE cartridges dry out during any of the following steps.

1. Adjust the pH of samples to 3.5 (±0.1) by adding 0.1M HCl dropwise, while stirring to prevent localized regions of low pH (which can decompose SM). Perform this step with a calibrated pH meter.
2. Add 5 mL methanol to each sample and stir briefly, to mix.
3. Condition a set (one/sample) of Bond Elut® "C2" SPE cartridges with 10 mL methanol, followed by 25 mL 90% water (pH 3.5[10])/10% methanol. Use vacuum manifold to effect liquid flow rate such that cartridge effluent emerges as discrete drops.

7. Install empty, 75-mL reservoir (with frits, for filtering) on top of conditioned "C2" SPE cartridges. Transfer samples (pour from beakers) to these SPE set-ups. Pass solutions through the SPE cartridges, again using vacuum (same approximate flow rate as for conditioning steps). Discard effluent (analyte is sorbed to the SPE sorbent).
8. Wash the SPE cartridges (vacuum flow) with 10 mL 90% water (pH 3.5[11])/10% methanol. Discard effluent.

[10]Adjusted with 0.1M HCl.

[11] Adjusted with 0.1M HCl.

9. Elute the SPE cartridges (vacuum flow) with 8 mL ethyl acetate. Collect cartridge effluents in glass test tubes or glass centrifuge tubes (recommend 13-mL capacity). If water is present (i.e., 2 layers), transfer the ethyl acetate (upper layer), with a disposable Pasteur pipette, and transfer to a second glass tube (discard water layer).
10. Place the sample tubes in a nitrogen evaporation apparatus (e.g., N-EVAP®) containing a water bath at room temperature. Evaporate to dryness. Store samples refrigerated (0-4°C) until LC analysis.

INSTRUMENTAL ANALYSIS

Liquid Chromatography

Column I – Zorbax® SB-Phenyl Analytical HPLC Column, 4.6 mm x 150 mm (5 µm spherical particles)

Column II – Zorbax® ODS Analytical HPLC Column, 4.6 mm x 250 mm (5 µm spherical particles)

Mobile Phase A – 70% aqueous 0.03 M sodium acetate (pH 4.0)/30% CH_3CN

Mobile Phase B – 60% aqueous 0.00 M sodium acetate (pH 5.0)/40% CH_3CN

Column Temperature – 45°C

Injection Volume – 100-500 µL (250 µL typical) [peaks are sharpened considerably on second column]

Flow Rate – 1.5 mL/min, initially (the flow rates are programmed as described in the attached table)

Detector – UV (wavelength = 235 nm)

Standards/Samples – Prepare in 10% acetonitrile/90% water (due to solubility considerations, when redissolving samples prior to LC analysis, **first add the acetonitrile** [sonicate/vortex-mix briefly] then add the water [sonicate/vortex-mix] to make the final composition 10% acetonitrile).

– For example, for a 2-mL final volume, first add 0.2 mL ACN then add 1.6 mL water.

Isocratic, multi-dimensional HPLC ("heart cut" column switching) is used (see References 3 and 4 for a review of

column-switching methods). A diagram of the columns and switching valve arrangement is shown in Figure 3, where the first column (Column I) is the Zorbax® SB-Phenyl column, and the second column (Column II) is Zorbax® ODS. In valve position 1, the effluent from Column I goes through a 1-µL bypass loop*, back to the valve, and then to the detector. In position 2, the effluent from Column I goes (via the valve) to Column II, back to the valve, and then to the detector.

Table II provides a typical timing sequence for analysis of samples. (In our laboratory, the Waters Model 590 HPLC pump controlled the sequence of operations.)

At the time of injection, put the valve in position 1, so that the HPLC flow bypasses Column II. Pump mobile phase A initially through Column I only (see Table II for flow rate information). When sulfometuron methyl (SM) starts to elute from Column I, switch the valve to position 2 in order to trap the peak on Column II. After the peak has been collected, switch the valve back to position 1. The valve switching times (the "time window") were typically set at -0.30 and +0.30 min around the retention time for SM on Column I as determined each day (with mobile phase A through Column I only, SM has a retention time of approx. 8.5 min). If greater selectivity is desired (due to an interfering peak, perhaps), the time window can be set to be from -0.20 to +0.20 min with respect to the retention time for SM.

After analyte has been trapped on Column II, change the mobile phase from mobile phase A to mobile phase B, and increase the flow rate to 3.0 mL/min, to quickly equilibrate Column I to the new mobile phase and to clean the rest of the sample off the column. After Column I has been cleaned (usually approximately 10 min is required; this step should be carried out until no additional eluting peaks are observed), decrease the flow rate to 1.5 mL/min, and switch the valve to position 2, to elute analyte from Column II. A retention time of 37-38 min is typical, for SM.

After SM has been eluted from Column II, maintain the flow rate (still mobile phase B) at 1.5 mL/min (this cleans Column II). After all peaks have been eluted (usually 10 min is sufficient), return the valve to position 1, and change the mobile phase back to mobile phase A. Set the flow rate to 3.5 mL/min, to hasten the equilibration process. After Column I has re-equilibrated to

* This must be comprised of 0.005" ID tubing, 10-cm length.

mobile phase A (a flat or slightly sloping baseline should be evident), decrease the flow rate to its original value. The system is now ready for the next injection.

Immediately prior to LC analysis, remove residue samples (contained in glass tubes) from the refrigerator and allow them to equilibrate to room temperature.

Dilute samples to an appropriate final volume with 10% acetonitrile/90% water (due to solubility considerations, when redissolving samples prior to LC analysis, first add the acetonitrile [sonicate/vortex-mix briefly] then add the water [sonicate/vortex-mix] to make the final composition 10% acetonitrile).

- For example, for a 2-mL final volume, first add 0.2-mL ACN then add 1.6 mL water.

If stored refrigerated (1-5°C), samples are normally stable for 2 3 days.

Inject samples on the HPLC intermixed with standards (bracket every 2-3 samples with a standard); load vials on the autosampler in a nonsystematic fashion. Select the levels of standards to bracket the expected SM levels (if known) in the samples analyzed. A standard should be the first and last solutions analyzed. (It is often necessary to initially inject one standard [or more, if needed] on the HPLC solely for the purpose of equilibrating the system, prior to starting the "official" HPLC run.)

Calculations

Measure chromatographic peak heights manually from the strip-chart recorder trace (or employ a data system in peak height mode, if applicable).

Generate a peak height (mm) *vs.* known concentration (µg/mL) plot from external SM standards injected. Generate a linear least squares fit calibration curve for the data (use a calculator). The equation for the line is $y = mx + b$, where y is the peak height (mm), x is the concentration of SM (µg/mL), m is the slope of the line (mm/µg/mL), and b is the y-intercept (mm). The solution to the equation for this line gives the concentration of SM found (µg/mL) corresponding to the experimentally observed peak height (mm).

Concentration Sulfometuron Methyl in Soil (ppb)

The parts per billion (ppb) SM found in a soil sample is given by:

$$ppb \text{ sulfometuron methyl detected} = 1000^{12} \times \frac{[\text{concentration found in sample } (\mu g/mL)][\text{final extract vol. } (mL)]}{[\text{original sample weight } (g)]}$$

where

$$\text{concentration SM found in extract } (\mu g/mL) = \frac{\text{SM peak height } (mm) - \text{y-intercept } (mm)}{\text{slope of linear regression line } (mm/\mu g/mL)}$$

% Recovery

$$\text{Recovery } \% = 100\% \times \frac{\text{amount compound found } (\mu g)}{\text{amount compound added } (\mu g)}$$

OTHER CONSIDERATIONS

Timing

Soil Method

Approximately 4-6 samples could be prepared in an 8-hour period by one person. Each analysis on the HPLC required approximately 65 min (using an autosampler, the analysis could be run unattended overnight).

The SFE procedure is much faster than the conventional extraction specified in the original method (from 1985 [2]). A set of 4-6 samples could be extracted in 30-45 min, compared to 2.5-3 hr for the original method. (Also, additional samples can be extracted without increased time if additional extraction modules [Model SFX 2-10] are added to the ISCO SFE system.)

Water Method

Approximately 6 samples could be prepared in a 4-hour period by one person. Each analysis on the HPLC required approximately 65 min (using an autosampler, the analysis could be run unattended overnight).

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12 Converts ppm to ppb.

13 See Table III for soil characterization data.

Special Precautions

A. It is very important that the grade of liquid CO_2 be sufficiently pure such that quantitation limits are not compromised. Since purity and grade characteristics are highly variable among different manufacturers, the suitability of a particular brand of CO_2 should be evaluated (impurities in the extraction fluid are often concentrated in sample extracts), prior to making commitments to a particular vendor (most vendors are quite cooperative, in this regard).

B. Collection tubes for sample extracts should be in a secondary container, to protect against breakage in case of an accidental pressure buildup. We used Teflon® FEP Erlenmeyer flasks, obtained from Nalgene® Corporation (Nalge Co., Rochester, N.Y.).

C. A high-quality supply of HPLC-grade acetonitrile is essential, for preparation of mobile phases. The chromatographic separation is quite demanding, in terms of the solvent purity required, for any low-level work (< 0.05 ppm). If possible, a supply of acetonitrile from a suitable lot should be set aside for this analysis, if significant work requiring this residue method is anticipated.

Cleaning Procedures

All glassware can be cleaned by means of any approach that is consistent with trace organic analysis. Generally, we used the following steps: Rinse the glass items initially with acetone (technical grade). Follow this with a thorough scrubbing with an aqueous soap solution (prepared in tap water). Then rinse the glassware with tap water, followed by another acetone rinse.

Critical items (such as volumetric flasks and centrifuge tubes) were rinsed thoroughly (in addition to the previous steps) with house-purified water (a reverse-osmosis treatment), followed by a rinse with a mixture of 50% methanol/50% 2-propanol (v/v).

RESULTS AND DISCUSSION

HPLC Standards

Detector response was linear for standards within one order of magnitude of each other (e.g., 0.0025-0.0080 µg/mL or 0.0125-0.0300 µg/mL). Typical working curves are presented in Figure 4.

Sample chromatograms are presented in Figure 5. Typical retention times were 7-9 min (Mobile Phase A through the Zorbax® SB-Phenyl column only) and 35-37 min (complete column-switching protocol used).

Method Development: Soil

Initially we pursued a residue method that differed from the already existing method (2) in only two respects:

- substitution of Bond Elut® "ALB" for the Waters Sep-Pak® (specified in original method), and
- employing column-switching (reversed-phase) HPLC with UV detection (instead of the normal phase HPLC conditions, with photo-conductivity detection, specified in original method).

The Bond Elut® format, using standard, medical grade polyethylene syringe barrels, is more general (Waters Sep-Pak® cartridges have a unique design). The second change is actually much more significant. The original method called for use of an obscure, difficult-to-use, HPLC detector – the Tracor® Model 965 Photo-conductivity Detector. Reversed-phase, column-switching HPLC systems are now very readily available and widely used. The column-switching technique adds significant selectivity such that a common UV detector can be used.

Based on fortifications from 0.5 ppb to 5 ppb, a Limit of Quantitation (LOQ) of approximately 1 ppb appears reasonable with this method, based on a Signal-to-Noise ratio (S/N) of 10. Figures 6 and 7 show the results of this approach for the analysis of Donna and Chino soils[13], respectively, fortified at 1.0 ppb with SM.

Because of our interest in achieving lower LOQs, however, we decided to pursue another major method modification – use of supercritical fluid extraction (SFE) (the original method uses a 50% methanol/50% bicarbonate buffer solution and a wrist-action shaker).

As described in the **INTRODUCTION**, SFE is very attractive for environmental analysis from potentially four standpoints:

[13] See Table III for soil characterization data.

- SFE is typically very rapid,
- SFE minimizes the use of organic solvents, and
- SFE offers a wide variety of parameters for optimization of extraction conditions to achieve quantitative, reproducible extractions with significant selectivity (especially when only one analyte is involved). The variety of experimental conditions available with SFE is also potentially quite helpful should a particularly difficult-to-extract soil be encountered.

Based on our previous experience using SFE to extract polar SM degradates (11), we were successful in determining conditions to extract SM (quickly and reproducibly) from a wide variety of soils using supercritical CO_2 modified with 80% acetonitrile/20% water (see **EXPERIMENTAL** section).

Method Validation: Recovery Study, Soil

The soil method was validated using Donna, Chino, and Fargo soils (for soil information, see Table III). Soil samples were generally fortified approximately 15 minutes prior to initiation of the SFE process.

Table IV summarizes recovery data for 19 Donna, 12 Chino, and 11 Fargo soil samples, fortified over the range 0.5-10.0 ppb[14]. Mean recoveries ranged from 80% (Fargo soil, S.D. = 10.%) to 87% (Chino soil, S.D. = 16%). These values indicate good method performance for a variety of soil types.

Chromatograms for unfortified and fortified (0.5 ppb and 5 ppb) soil samples are presented in Figures 8-10, for Donna, Chino, and Fargo soils. The number and intensity of background peaks increases with increasing % organic matter (not unexpected).

A reasonable Limit of Quantitation (LOQ) readily achieved with all three soil types was estimated to be 0.5 ppb. This LOQ represents the fortification level at which the analyte peak was consistently 5-10 times the chromatographic noise at the retention time for SM, using the complete residue analysis method. The chromatographic noise is dominated by peaks from co-extracted materials from the soil matrix, rather than instrument noise.

[14]0.2 -10.0 ppb for Donna soil only.

Donna soil (only 1% organic matter) consistently generated cleaner extracts than did the other 2 soil types. For this soil, the LOQ (defined above) was 0.2 ppb.

Method Validation: Extraction Efficiency, Soil

^{14}C Method Validation

Two, 5-g samples of Chino soil were fortified to 10 ppb with [^{14}C]sulfometuron methyl. Then the residue method was carried out in the usual manner, with one exception:

- Following the SFE procedure, extracts were evaporated to dryness (using N-EVAP®), residues were redissolved in 2.00 mL acetonitrile, and a 200-µL aliquot from each sample was counted using standard liquid scintillation counter (LSC) techniques.

Table V contains the results of this experiment. Based on comparison to the radioactivity present in the original fortification standard, the extraction efficiency (or % recovery up through the extraction procedure, but not going beyond) was determined to be 80.5% (average of 83.4% and 77.7%).

Sample extracts (excluding the aliquot removed for LSC analysis) were processed through the complete residue method, and HPLC/UV analysis was performed in the usual manner. Results are also contained in Table V. The average % recovery was determined to be 84.5% (average of 84% and 85%).

The HPLC result is probably more accurate than the LSC result, based on typical uncertainties and imprecision associated with both techniques. At any rate, these results show SM to be satisfactorily extracted by the SFE technique.

[Some of our method development experiments indicated greater extraction efficiency of residue with a higher extraction temperature. However, we found that higher extraction temperatures led to significant degradation of SM[15], during the extraction. The temperature finally selected – 45°C – prevented SM degradation while ensuring sufficient extraction efficiency.]

15 At higher temperatures (especially at around 60°C) SM hydrolyzed significantly (presumable via the well-known sulfonylurea bridge cleavage reaction) as determined by using an on-line radiochemical HPLC detector.

Analysis of Field-Aged Samples

A two-year field soil dissipation study (12) with Oust® Herbicide was started by DuPont in 1991, to fulfill reregistration requirements of the U.S. EPA. In order to analyze samples with the highest available sensitivity, both a residue method using Liquid Chromatography/Mass Spectrometry (LC/MS) and a method based on an enzyme-linked immunosorbent assay (ELISA) were employed for the determination of SM soil concentrations as low as 0.1 ppb.

This dissipation study provided field-aged samples that served as an additional validation of our new SFE method. Specifically, we analyzed 6 soil samples by our new method and then compared these results with the results already available from these other, already validated techniques. (See Appendix I for relevant soil characterization data.)

Table VI contains the details of this work and the results. Since our primary intent was to evaluate extraction efficiency of the SFE procedure, Figure 11 shows a comparison of residue data, corrected for the recovery variation among these three, very different methodologies. The data obtained using SFE are so similar to that obtained by the other methods that there would seem to be no doubt as to the ability of SFE to satisfactorily extract SM residues. (Note, SM residues have excellent storage stability in soils stored frozen [see Reference 12], so that comparisons such as this are meaningful.)

Method Validation: Recovery Study, Water

The water method was validated using surface water obtained locally (Table VII).

Table VIII summarizes recovery data for 23 Delaware River and 24 Brandywine Creek samples, fortified over the range 0.025-2.00 ppb. Mean recoveries ranged from 95% (Delaware River water, S.D. = 11%) to 99% (Brandywine Creek water, S.D. = 9%). These values indicate good method performance over this fortification range.

Chromatograms for unfortified and fortified water samples are presented in Figures 12-13, for both water types.

A reasonable Limit of Quantitation (LOQ) was estimated to be 0.025 and 0.050 ppb for Brandywine Creek and Delaware River water samples, respectively. This LOQ represents the

fortification level at which the analyte peak was consistently 5-10 times the chromatographic noise at the retention time for SM, using the complete residue analysis method. The chromatographic noise is dominated by background peaks (from matrix and/or reagents), rather than instrument noise.

CONCLUSIONS

A highly sensitive LC/UV method was developed for determining SM residues in soil and water. The minimum Limits of Quantitation were 0.2 ppb for soil and 0.025 ppb for water.

- A recovery study validated this method across three soil and two water types, demonstrating good accuracy and precision (well within the desired range of 70-120% with RSD of <20% for regulatory methods in the U. S.).
- Extraction efficiency for soils was clearly satisfactory, based on analysis of field-aged soils (available from a dissipation study).
- SFE proved very convenient for soils; it was essential for determinations of SM residues below 1 ppb.
- This method is much more convenient and easier-to-perform than the existing regulatory method recognized by the U. S. EPA (2).

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