

# Comparison among Soil Series and Extraction Methods for the Analysis of Trifluralin

Umadevi I. Garimella,<sup>†,‡</sup> G. Kim Stearman,<sup>†,§</sup> and Martha J. M. Wells<sup>\*,†,#</sup>

Center for the Management, Utilization, and Protection of Water Resources and Departments of Agriculture and Chemistry, Tennessee Technological University, Box 5033, Cookeville, Tennessee 38505

Accurate analytical procedures are needed to improve understanding of the fate and transport of trifluralin, a chemical widely used as a herbicide. Analytical determination of trifluralin is challenging due to its hydrophobic, yet volatile, character and its tendency to degrade into numerous metabolites. In this research, efficient analytical methods for fortified and field-incurred soils were developed for simultaneous quantitation of trifluralin, **I** [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine, CAS Registry No. 1582-09-8; CAS Registry No. have been provided by the author], a trifluralin metabolite, **II** [2,6-dinitro-*N*-propyl-4-(trifluoromethyl)benzenamine, CAS Registry No. 2077-99-8], and a related trifluoromethyl-dinitroaniline isomer of trifluralin, **III** [2,4-dinitro-*N,N*-dipropyl-6-(trifluoromethyl)benzenamine, CAS Registry No. 23106-20-9]. Extractions of trifluralin (0.5 and 2.5  $\mu\text{g/g}$ ) from silt loam, sandy loam, and silty clay soils were compared. A method was developed for the supercritical fluid extraction of trifluralin from soil using modified supercritical carbon dioxide, and the effects of cosolvent, pressure, and flow rate on recovery were evaluated. Supercritical fluid extraction was compared to liquid vortex extraction and automated Soxhlet (soxtec) extraction. Solid-phase extraction was examined for purifying soil extracts. Protocols were developed for analysis of extracts by gas and/or liquid chromatography. Immunoassay was investigated but proven to be impractical for this analysis. Soil properties and extraction methods were observed to affect the level of coextracted background interferences. Trifluralin exhibited concentration-dependent recovery regardless of soil series or extraction method.

**Keywords:** *Herbicide; trifluralin; soil; solid-phase extraction; supercritical fluid extraction; liquid vortex extraction; automated Soxhlet (Soxtec) extraction; biphasic slow desorption; gas chromatography; high-performance liquid chromatography*

## INTRODUCTION

Trifluralin, a dinitroaniline herbicide, is used for preemergent broad-leaf weed control in agronomic and horticultural applications. In the United States, trifluralin is used in the Midwest on soybean and wheat crops and in the South and West on cotton crops (Thurman and Meyer, 1996; Battaglin and Goolsby, 1995). Trifluralin is strongly adsorbed to soil and, therefore, generally thought to have a low potential of leaching (Weber, 1990). However, application by aircraft or large surface runoff may lead to off-site contamination. In regions of high rates of precipitation, trifluralin may reach ground water by macropore movement through shallow soils with low organic content. Trifluralin readily degrades into metabolites that may contaminate nearby ponds, rivers, and ground water.

Existing analytical methods (Foreman et al., 1993; Riley and Keese, 1996; Baez et al., 1997; Miliadis, 1998) for aqueous samples primarily deal with determination of trifluralin as one analyte among multiple types of

herbicide residues and are not necessarily optimized for the recovery of trifluralin. Trifluralin is generally extracted from soil using an organic solvent such as ethyl acetate (Garcia-Valcarcel et al., 1996), acetonitrile (Yordy et al., 1988; An and Qian, 1992; Krause and Niemczyk, 1992), acetone (Balnova and Balinov, 1991), methanol (Lu and Xu, 1991), methylene chloride (Duc, 1992), or diethyl ether (Cabras et al., 1991) either by sonication (Lopez-Avila et al., 1991), mechanical shaking (Garcia-Valcarcel et al., 1996), or Soxhlet extraction (Tutarli et al., 1995). The extract is then cleaned by Florisil (Lu and Xu, 1991; D'Amato et al., 1993; Garcia-Valcarcel et al., 1996) or solid-phase extraction (SPE) (Yordy et al., 1988; Krause and Niemczyk, 1992; Cabras et al., 1991). The extractions consume 50% of the analytical time and involve large quantities of solvent. Using U.S. Environmental Protection Agency (EPA) Method 1618 (U.S. EPA, 1989), the extraction efficiencies from soil samples are low, and acceptance criteria of trifluralin at the 0.2 ng/g level is 3–177%. No EPA method is available for the analysis of trifluralin metabolites.

The principal objective of this research was to develop methodology for the analysis of trifluralin, a trifluralin metabolite, and a trifluralin isomer from soil. The research focused on improving sample preparation by reducing extraction time and by obtaining extracts that are pure enough to be used directly for chromatographic analysis. Analytical methods were developed using

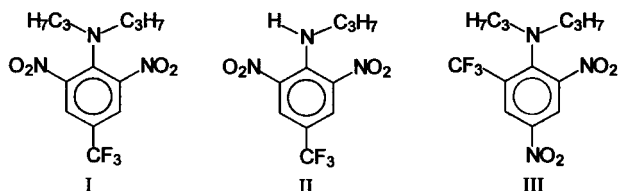
\* Corresponding author [telephone (931) 372-6123; fax (931) 372-6346; e-mail mjmwells@tntech.edu].

<sup>†</sup> Center for the Management, Utilization, and Protection of Water Resources.

<sup>‡</sup> Present address: Volunteer State Community College, 113 Windle Community Rd., P.O. Box 639, Livingston, TN 38570.

<sup>§</sup> Department of Agriculture.

<sup>#</sup> Department of Chemistry.



**Figure 1.** Compounds included in this study: trifluralin, **I** [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine, CAS Registry No. 1582-09-8]; a trifluralin metabolite, **II** [2,6-dinitro-*N*-propyl-4-(trifluoromethyl)benzenamine, CAS Registry No. 2077-99-8]; and a related trifluoromethyldinitroaniline isomer of trifluralin, **III** [2,4-dinitro-*N,N*-dipropyl-6-(trifluoromethyl)benzenamine, CAS Registry No. 23106-20-9].

fortified samples. The parameters determined were then applied to field-incurred residues.

High-performance liquid chromatographic (HPLC) and gas chromatographic (GC) conditions for quantification of these compounds were optimized. The method described enables injection of extracts directly into GC or HPLC without further solvent exchange. The HPLC method developed monitors for these compounds in the visible region of the spectrum. A supercritical fluid extraction (SFE) procedure for isolation of the analytes was developed, and the use of SPE for cleaning the extract if necessary was explored. The efficiency of the SFE procedure developed was evaluated by comparison with vortex and Soxtec extraction methods. Immunoassay (EIA) was found to be unsuitable for the analysis of trifluralin because of cross-reaction with the trifluralin metabolite and isomer.

## MATERIALS AND METHODS

**Chemicals.** HPLC or Optima grade methanol, acetone, triethylamine (TEA), and water were purchased from Fisher Scientific. Analytical standards (Figure 1) for trifluralin, **I** [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine, CAS Registry No. 1582-09-8; CAS Registry No. have been provided by the author], a trifluralin metabolite, **II** [2,6-dinitro-*N*-propyl-4-(trifluoromethyl)benzenamine, CAS Registry No. 2077-99-8], and a related trifluoromethyldinitroaniline isomer of trifluralin, **III** [2,4-dinitro-*N,N*-dipropyl-6-(trifluoromethyl)benzenamine, CAS Registry No. 23106-20-9], were obtained from DowElanco, Eli Lilly and Co. Labs, Indianapolis, IN.

**Soil Fortification.** Stock standard solutions were prepared by dissolving analytical standards in acetone and storing in amber bottles at 4 °C. Working standard solutions were obtained by dilution with acetone. Soils (Table 1) were fortified by adding 50 mL of standard in acetone to 50 g of soil, stirring, and air-drying in the dark for 24 h. The fortified soils were then homogenized by grinding with a mortar and pestle. All extractions were conducted in triplicate. Field-weathered soils (Hartsell silt loam) from the Plateau Experimental Station, Crossville, TN, were collected prior to herbicide treatment and during the first month after trifluralin application.

**Analysis.** *HPLC.* HPLC separation was performed with a Hewlett-Packard (Wilmington, DE) series 1050 liquid chromatograph equipped with a Hypersil reversed-phase column (ODS 200 × 2.1 mm, 5 μm particle size). The column was operated at ambient temperature with a flow rate of 1.00 mL/min and an injection volume of 25 μL. The mobile phase consisted of a mixture of acetone/water (80:20, v/v). Absorption maxima were determined using a Perkin-Elmer Lambda 4B UV-vis scanning spectrophotometer, and the HPLC-UV detector was monitored at 386 nm. The samples and standards were diluted to 50% with water prior to HPLC analysis.

*GC.* A Hewlett-Packard 5880 GC with an electron capture detector (70 eV) was used for analysis of trifluralin. The GC was fitted with a wide-bore DB-1701 fused silica column (30 × 0.53 mm i.d., 0.5 μm film thickness). The carrier gas was

helium with a flow rate of 10 mL/min, and the makeup gas was nitrogen. The total flow rate was 60 mL/min. The GC was equipped with a 7673A autosampler and a 5880A terminal integrator level 4. The chromatographic oven temperature was maintained isothermally at 180 °C, the injection temperature at 200 °C, and the detector temperature at 270 °C. The extracts analyzed were dissolved in acetone.

*EIA.* The antibodies of commercially available EIA kits were found to react with all three analytes. Trifluralin metabolite **II** was at least 10 times more sensitive than the trifluralin parent compound, whereas trifluralin isomer **III** demonstrated sensitivity similar to that of trifluralin. Therefore, the EIA method was not appropriate for these analyses.

**Extraction.** *SFE of Soil.* Extraction was conducted with a commercial SFE system (model 703 Dionex, Sunnyvale, CA). Fortified soil (3 g) was packed into the extraction cells having silanized glass wool at both ends. For collection, C<sub>18</sub> traps (Dionex) were used. The collection tubes with sorbent traps (C<sub>18</sub>) were activated by passing methanol (1 mL) followed by acetone (1 mL) through them and fitting into the collection vials. The cells and the vials were placed in the instrument. High-purity CO<sub>2</sub> (SFE grade with 13.8 mPa of helium pressure) was used throughout (Scott Specialty Gases, Plumstead, PA). The extraction was conducted at 20.3 mPa (270 atm) for 3 min followed by a 34.4 mPa (340 atm) extraction for 17 min. The oven temperature was maintained at 60 °C and the restrictor temperature at 125 °C. Acetone (15% v/v) was used as a cosolvent. After extraction, the analytes collected in the traps and vials were eluted into a 5 mL volumetric flask with acetone. This extract was used directly for GC and HPLC analyses.

*Liquid Vortex Extraction of Soil.* The vortex extraction method of Stearman and Adams (1992) was modified to improve the recoveries of trifluralin and make it compatible with GC analysis. Soil (10 g) was extracted with acetone (20 mL). Each sample was vortexed three times for 2 min and equilibrated overnight. The samples were then vortexed four times for 10 s and centrifuged at 870g, and the supernatant was collected.

*Soxtec Extraction of Soil.* Extraction was performed on a Tecator HT 1045 and HT2 1046 Soxtec (automated Soxhlet) system. The cellulose extraction thimbles were presoaked in acetone for 15 min and then air-dried. A soil sample (5 g) was placed in the prepared thimbles. Acetone (75 mL) was added to the extraction cup, and the Soxtec apparatus was assembled. The temperature was set at 130 °C. Boiling for 20 min was followed by 15 min of rinsing. The extract was then concentrated to 2–3 mL, transferred to a 5 mL volumetric flask, and diluted to volume with acetone.

*SPE of Soil Extracts.* C<sub>18</sub> Mega Bond Elut sorbent (1.0 g columns) and a VacElut extraction manifold (Varian Sample Preparation Products, Harbor City, CA) were used for SPE. The optimized experimental procedure consisted of activating the sorbent with acetone, conditioning the sorbent with aqueous acetone (acetone/water 1:2 v/v), applying a sample across the sorbent in acetone/water 1:2 v/v, washing with 10 mL of aqueous acetone (acetone/water 1:2 v/v), drying the sorbent under vacuum for 15 min, and eluting with acetone (5 mL) into a 5 mL volumetric flask.

**Statistical Analyses.** The Statistical Analysis System (SAS version 8) was used to analyze the data. The SAS General Linear Models (GLM) procedure using least-squares means and Tukey's Studentized range test (HSD) was performed for all main-effects means. Separate statistical analyses were conducted for the effect of extraction solvent composition on SFE recovery of trifluralin, the effect of trifluralin concentration on extraction from soil, the effect of soil series on trifluralin recovery, and the effect of extraction method on trifluralin recovery.

## RESULTS AND DISCUSSION

Trifluralin (Treflan, Treflanocide, Agreflan, Trim, and Elancolan) is a dinitroaniline herbicide (Figure 1).

**Table 1. Physical and Chemical Properties of Soils Examined**

soil series	texture	sand (%)	silt (%)	clay (%)	pH	CEC (cmol kg <sup>-1</sup> )	organic carbon (%)	surface area (m <sup>2</sup> g <sup>-1</sup> )
Maury	silt loam	15.3	60.6	24.0	6.3	17.5	1.3	29.6
Mountview	silt loam	45.1	46.3	8.6	6.3	6.1	0.9	7.5
Baxter	silt loam	9.4	78.2	12.5	5.8	8.0	0.7	21.2
Lindale	sandy loam	55.1	37.5	7.4	4.9	16.1	2.3	9.9
Iberia	silty clay	7.0	43.7	49.3	6.3	40.8	1.5	46.1

Trifluralin is a bright yellow-orange crystalline solid with no appreciable odor. Because of the nitro groups, trifluralin does not exhibit the basicity or the high water solubility of aniline herbicides. The two nitro groups are strongly electron withdrawing and form strong intra- and intermolecular hydrogen bonds (Weber, 1990). Due to its low water solubility and high potential for hydrogen bonding, trifluralin is strongly sorbed to soil, particularly to organic soil constituents, and the degree of sorption increases with increases in the level of organic matter. The experimental sorption coefficient of trifluralin to soil organic matter,  $K_{oc}$ , is 13700 (Kenaga, 1979), a high value compared to that of other pesticides. Trifluralin has a high vapor pressure ( $1.1 \times 10^{-4}$  mmHg) and Henry's constant ( $621 \times 10^{-5}$ ) and is highly hydrophobic ( $\log P = 5.34$ ) (Weber and Monaco, 1972; Probst et al., 1975; Helling, 1976; Brown, 1978; Hansch et al., 1995).

**Stability Study.** Dinitroanilines are known to degrade photochemically when exposed to higher temperatures and light (Golab et al., 1979). Degradation studies of trifluralin in field soil identified >30 transformation products (Golab et al., 1979).

Proper storage conditions that minimized degradation of the standards and extracts in the laboratory were established. Standards were observed to degrade more rapidly in ethyl acetate than in acetone. Standards stored in acetone in amber bottles at 4 °C in the dark were stable for >6 months, whereas those stored in clear bottles at room temperature exposed to ambient light were degraded within 1 week.

**Chromatographic Analyses.** Analytical methods were developed for both HPLC and GC. The compounds (I, II, and III) are characterized by absorbance bands in both the ultraviolet and visible ranges (Wells and Stearman, 1996). Published HPLC methods use the 220 nm band for quantitation of trifluralin with acetonitrile as mobile phase (Cabras et al., 1991; Vitali et al., 1994; Riley and Keese, 1996). In this research, all three compounds were monitored simultaneously in the visible range at 386 nm. This wavelength permitted the use of acetone, which has a UV cutoff at 330 nm, for the mobile phase. As this wavelength is in the visible region where there are fewer absorbing species relative to the UV region, fewer interferences existed. The soil blank showed no interferences with the analyte peak. The detection limit for trifluralin using this method was 0.33 µg/g, based on SFE of a 3 g soil sample.

Trifluralin has a high vapor pressure (U.S. EPA, 1989), so it is volatile enough for direct GC analysis without derivatization. Using an ECD detector, the detection limit for trifluralin was 0.083 µg/g, based on SFE of a 3 g soil sample.

**Extraction Method Development for SFE from Soil.** The liquid vortex extraction procedure (Stearman and Adams, 1992; Stearman et al., 1995) and the Soxhlet extraction procedure (Stearman and Adams, 1992) for trifluralin from soil were adapted from previous research in this laboratory on other soil herbicides.

**Table 2. Effect of Extraction Solvent Composition on SFE Recovery of Trifluralin**

solvent	entrainer	modifier	recovery <sup>a</sup> (%)
supercrit CO <sub>2</sub>			79 ± 9a
supercrit CO <sub>2</sub>	acetone <sup>b</sup>		91 ± 18ab
supercrit CO <sub>2</sub>		acetone (15%)	102 ± 8b
supercrit CO <sub>2</sub>	acetone <sup>b</sup>	acetone (15%)	80 ± 11ab
supercrit CO <sub>2</sub>		acetone/TEA, 90:1.5 v/v (15%)	102 ± 9ab
supercrit CO <sub>2</sub>		acetone/TEA/water, 90:1.5:10 v/v/v (15%)	89 ± 19ab

<sup>a</sup> Mean ± SD. Means followed by the same letter are not significantly different ( $\alpha = 0.05$ ). <sup>b</sup> One milliliter.

However, no SFE procedures existed for trifluralin from soil and were developed in this research.

In the development of the SFE method for trifluralin from soil, the goals were to minimize sample preparation time, to maximize recovery, to obtain an extract free of interfering coextractives, and to produce an extract directly compatible with subsequent chromatographic procedures. Trifluralin and related compounds are thermally labile and volatile. Therefore, moderate SFE oven temperatures (60 °C) and collection vials with C<sub>18</sub> sorbent traps were used throughout this study. Acetone was selected as a preferred cosolvent because (a) the storage stability in acetone was established, (b) solubility of trifluralin in acetone is high, (c) acetone solubility in CO<sub>2</sub> is 20%, which is high compared to that of other solvents, and (d) acetone extracts were directly compatible with both GC and HPLC analytical methods.

**Effect of Cosolvent on SFE Recovery.** The recovery of trifluralin from fortified Mountview soil (2.5 µg/g) using six different solvent compositions was examined (Table 2). Highest recoveries with good reproducibility and cleanest chromatograms were obtained by using 15% acetone as modifier. Extraction with supercritical CO<sub>2</sub> without modifier gave moderately good recovery. However, the addition of acetone, as modifier or entrainer, improved recoveries, suggesting that acetone overcomes solute–matrix interactions and also increases the solubility of analyte in the extraction solvent.

When acetone was added to the soil as an entrainer, greater standard deviations were observed. Possible sources of this error include nonuniform wetting of the soil and restrictor clogging evidenced by reduced flow rates.

The presence of water in the extraction solvent adversely affected the recovery. Trifluralin has low water solubility (3 ppm) and partitions less into a solvent containing water. In addition, water freezes in the SFE restrictor tips due to the Joules–Thompson cooling effect of the expanding gas at the restrictor outlet (Burford et al., 1993). Water also causes swelling in certain soils, which blocks the solvent flow during extraction. Therefore, addition of water was not conducive to SFE of trifluralin from soil matrices.



**Table 3. Effect of Trifluralin Concentration on Extraction from Soil**

concn ( $\mu\text{g/g}$ )	soil series				
	Maury	Mountview	Baxter	Lindale	Iberia
	SFE Recovery <sup>a</sup> (%)				
0.5	68 $\pm$ 11a	69 $\pm$ 7a	61 $\pm$ 5a		71 $\pm$ 23a
2.5	69 $\pm$ 8a	97 $\pm$ 4b	99 $\pm$ 26a	116 $\pm$ 7	108 $\pm$ 15a
	Liquid Vortex Extraction Recovery <sup>a</sup> (%)				
0.5	98 $\pm$ 4a	81 $\pm$ 1a	78 $\pm$ 4a		73 $\pm$ 12a
2.5	92 $\pm$ 2a	92 $\pm$ 3b	93 $\pm$ 4b	94 $\pm$ 4	100 $\pm$ 8b
	Soxtec Extraction Recovery <sup>a</sup> (%)				
0.5	58 $\pm$ 5a	63 $\pm$ 6a	73 $\pm$ 12a		119 $\pm$ 21a
2.5	98 $\pm$ 10b	116 $\pm$ 11b	143 $\pm$ 3b	46 $\pm$ 8	93 $\pm$ 1a

<sup>a</sup> Mean  $\pm$  SD. Means within each column and each extraction method followed by the same letter are not significantly different ( $\alpha = 0.05$ ).

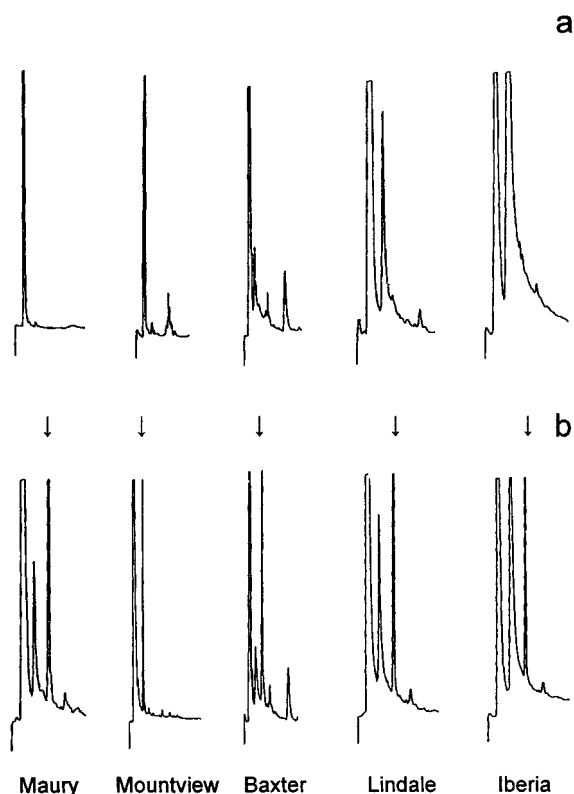
The addition of triethylamine (TEA) to the cosolvent created two problems. Upon analysis by GC, the extracts containing TEA demonstrated higher levels of interfering coextractives, and trifluralin decomposed in refrigerated extracts within 24 h.

**Effect of Pressure on SFE Recovery.** One of the advantages of using supercritical fluids is that varying the pressure at constant temperature allows density and solvating power to be easily and effectively changed (Bartle et al., 1992). Increasing the pressure from 270 to 340 atm resulted in increased recovery of trifluralin from soil (2.5  $\mu\text{g/g}$ ). At 270 atm, only 57  $\pm$  9% of the analyte was extracted, whereas 72  $\pm$  14 and 75  $\pm$  2% recoveries were achieved at 300 and 340 atm, respectively. However, improved extraction (100  $\pm$  6%) was achieved when using a stepwise pressure ramp from an initial low pressure (270 atm for 3 min) followed by higher pressure (340 atm for 17 min). The pressure ramp technique was successfully used previously in this laboratory for other soil herbicides (Stearman et al., 1995). The initial low-pressure facilitates greater diffusion and also lowers the sudden impact of solvent force at high pressure, resulting in improved recovery.

**Effect of Flow Rate on SFE Recovery.** The recovery of trifluralin from soil samples was independent of the SFE flow rate. The extraction process appears to have been controlled by desorption, not by the solubility of the analyte (Hawthorne et al., 1995). Therefore, the rate-limiting step in the extraction process must be the initial desorption of the analyte from the bound sites of the matrix.

**Effect of Sample Aging on the SFE Recovery.** Mountview soil was fortified and extracted after 2 days and again following 5 months of being stored at room temperature and exposed to ambient light. Marked reduction in recovery of trifluralin was observed in the aged soils. The recoveries after 5 months were 24 and 39% for the 2.5 and 0.5  $\mu\text{g/g}$  fortified levels, respectively. The reduction can be attributed to losses due to volatility, degradation, and photolysis.

**Comparison of SFE, Vortex, and Soxtec Soil Extraction Methods.** *Effect of Analyte Concentration on Extraction from Soil.* Soils were fortified with 0.5 and 2.5  $\mu\text{g/g}$  trifluralin standard to evaluate extracted recovery and reproducibility (Table 3). Overall, the results were interpreted to demonstrate a tendency toward dependence of recovery on concentration regardless of the extraction method. At the 0.5  $\mu\text{g/g}$  fortified level, 20–40% of the sample, or  $\sim$ 0.150  $\mu\text{g/g}$ , is unrecovered. Translated to the 2.5  $\mu\text{g/g}$  fortified level, a



**Figure 2.** GC of SFE extracts of (a) unfortified and (b) fortified soils. The arrow indicates the elution of the trifluralin peak.

sample loss of 0.150  $\mu\text{g/g}$  would produce a 6% loss in recovery. Therefore, it seems reasonable to conclude that  $\sim$ 0.150  $\mu\text{g/g}$  of trifluralin was irreversibly "bound" to active sites in the soil matrix (Pignatello and Xing, 1996), leading to an apparent dependence of recovery on concentration. Golab et al. (1979) also found that 38% of total trifluralin remained as soil-bound residues. The reduced recovery at lower fortified concentration does not appear to result from the soil type or from the efficiency of the method. The concentration-dependent recovery is therefore concluded to result from inherent characteristics of the analyte.

Concentration-dependent recovery is an analytical chemist's nightmare. To resolve this dilemma and accurately predict actual soil concentrations, a two-phase calibration plot must be prepared: one for higher concentrations and one for lower concentrations. Many chemicals demonstrate slow desorption from natural particles (Pignatello and Xing, 1996). Pignatello and Xing (1996) point out that "the slow fraction is inversely dependent on the initial applied concentration, meaning that it assumes greater importance at lower concentration". Slow desorption manifests itself analytically in concentration-dependent recovery within realistic laboratory time scales.

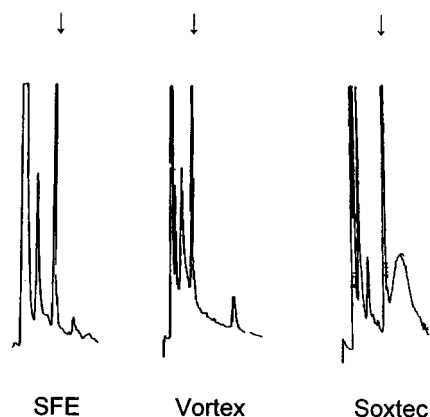
There has existed some question in the scientific community as to whether extractions by SFE would exhibit biphasic, concentration-dependent extraction from soil. These data demonstrate that slow desorption is also evident with SFE.

*Effect of Soil Series on Extraction from Soil.* Soil properties (Table 1) and extraction methods were observed to affect the level of coextracted interfering compounds. The level of interfering coextractives observed in the GC chromatograms of SFE extracts of soils (Figure 2) increased in the following order: Maury  $\approx$

**Table 4. Effect of Soil Series on Trifluralin Recovery**

soil series	method recovery <sup>a</sup> (%)		
	SFE	vortex	Soxtec
2.5 µg/g Fortification Level			
Maury	69 ± 8a	92 ± 2a	98 ± 10ab
Mountview	97 ± 4ab	92 ± 3a	116 ± 11a
Baxter	99 ± 26ab	93 ± 4a	143 ± 3c
Lindale	116 ± 7b	94 ± 4a	46 ± 8d
Iberia	108 ± 15b	100 ± 8a	93 ± 1b
0.5 µg/g Fortification Level			
Maury	68 ± 11a	98 ± 4a	58 ± 5a
Mountview	69 ± 7a	81 ± 1b	63 ± 6a
Baxter	61 ± 5a	78 ± 4b	73 ± 12a
Lindale			
Iberia	71 ± 23a	73 ± 12b	119 ± 21b

<sup>a</sup> Mean ± SD. Means within each column and each fortification level followed by the same letter are not significantly different ( $\alpha = 0.05$ ).



**Figure 3.** GC of SFE, vortex, and Soxtec extracts of Maury soil series. The arrow indicates the elution of the trifluralin peak.

Mountview < Baxter < Lindale < Iberia. Maury, Mountview, and Baxter soils are all classified by soil texture as silt loam soils. Lindale soil is a sandy loam with relatively high organic matter, and Iberia soil is a silty clay with high organic matter and clay content.

Comparing the soil types within extraction method and fortification level revealed that the Soxtec extraction method was most variable at the higher, 2.5 µg/g, fortified level (Table 4). The SFE method was not as good as the vortex or Soxtec extraction methods for the Maury soil series.

The soils in this research ranged from 0.7 to 2.3% in organic carbon. The Lindale and Iberia soils have the highest levels of organic carbon of the soils examined. However, no strong correlation was observed between percent organic carbon and the concentration-dependent recovery of trifluralin from these soils.

**Effect of Extraction Method on Extraction from Soil.** Comparison of the background interferences of GC chromatograms demonstrated that across all soil types, SFE extracts were cleaner than vortex extracts which, in turn, were cleaner than Soxtec extracts (Figure 3). Extremes in recovery observed using the Soxtec method may be due to interferences with the analyte peak in the GC chromatogram, leading to error in the integration of the area. The cleanliness of the extract affected the accuracy of quantitation and reproducibility of the determination and adversely impacted the lifetime of chromatographic columns used for the analysis. Soxtec extraction was a less suitable procedure as it used more solvent than the other two methods while producing

**Table 5. Effect of Extraction Method on Trifluralin Recovery**

method	soil series recovery <sup>a</sup> (%)				
	Maury	Mountview	Baxter	Lindale	Iberia
2.5 µg/g Fortification Level					
SFE	69 ± 8a	97 ± 4a	99 ± 26a	116 ± 7a	108 ± 15a
vortex	92 ± 2b	92 ± 3a	93 ± 4a	94 ± 4b	100 ± 8a
Soxtec	98 ± 10b	116 ± 11b	143 ± 3b	46 ± 8c	93 ± 1a
0.5 µg/g Fortification Level					
SFE	68 ± 11a	69 ± 7ab	61 ± 5a		71 ± 23a
vortex	98 ± 4b	81 ± 1a	78 ± 4a		73 ± 12ab
Soxtec	58 ± 5a	63 ± 6b	73 ± 12a		119 ± 21b

<sup>a</sup> Mean ± SD. Means within each column and each fortification level followed by the same letter are not significantly different ( $\alpha = 0.05$ ).

extracts that contained more coextracted interfering peaks in the chromatograms.

SFE, vortex, and Soxtec extraction methods were compared for various soil types at two fortification levels (Table 5). The SFE and vortex methods produced equivalent recoveries of trifluralin across soil types except for Maury and Lindale. The sample extraction time was 0.5 h for SFE, 26 h for vortex extraction, and 2 h for Soxtec extraction. The vortex extraction time of 26 h included an overnight equilibration period. The vortex experimental procedure required the least amount of technical expertise to conduct.

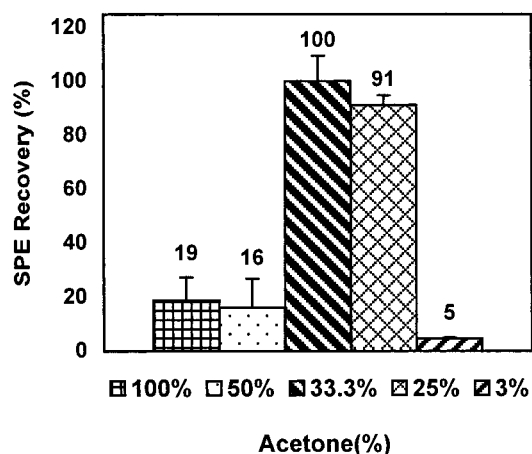
A disadvantage of SFE is the inherent variability of the instrument. The flow rates and the penetration of the solvent in terms of extraction efficiencies directly depend on the packing of the soil, which depends greatly on the analyst. SFE requires sophisticated instrumentation and is the most expensive of the three procedures, whereas the liquid vortex extraction method is the least expensive.

**Application of the Analysis to Related Compounds and Field Samples.** The SFE method developed was extended to the analysis of a trifluralin metabolite, **II**, and a related trifluoromethyl-dinitroaniline isomer of trifluralin, **III**. Mountview soil was fortified with 10 µg/g each of compounds **I**, **II**, and **III**. The compounds were each efficiently extracted by this procedure.

Soil samples (Hartsell silt loam) were collected from the field, including baseline (i.e., pretreated) samples and samples collected 1 month after application of trifluralin. Representative recovery from field soil was compared by SFE (0.31 ± 0.07 µg/g), Soxtec (0.41 ± 0.03 µg/g), and vortex (0.49 ± 0.11 µg/g) methods. The pretreatment (baseline) soil extracts did not contain any significant coextractive peaks. The majority of trifluralin was found in the top 5 cm of soil. The gas chromatogram of the commercial trifluralin spray used in the field indicated the presence of both compounds **I** and **III**.

**SPE of Soil Extracts.** The extracts of all soils studied were analyzed by direct injection with GC and/or HPLC without further sample preparation. However, it was clearly demonstrated that soil properties affect the level of coextracted interferences and that further sample cleanup may be necessary in some cases. Therefore, an SPE purification procedure (without concentration) was developed. The SPE purification procedure developed begins and ends with the extract in 5 mL of acetone. The SPE procedure for soil extracts in acetone is applicable to any of the three soil extraction procedures examined.

**Effect of Sample Solvent Strength on SPE Recovery.** Existing SPE methods for trifluralin use hexane (Krause



**Figure 4.** Effect of sample solvent strength on SPE recovery of trifluralin. Error bars represent standard deviation.

and Niemczyk, 1992), acetonitrile (Vitali et al., 1994), or diethyl ether (Yordy et al., 1988; Cabras et al., 1991) as eluting solvents. In this work it was desirable to use acetone as an eluting solvent because of the established stability of trifluralin in acetone and its compatibility with both GC and HPLC procedures.

The eluotropic solvent strength of the sample drastically affects the retention and desorption of trifluralin by SPE (Figure 4). In reversed-phase chromatography, acetone is considered to be a strong solvent, whereas water is considered to be a weak solvent. In pure acetone (100%), the recovery of trifluralin was 19%. By analyzing the eluate from sample loading in pure acetone, it was determined that the remaining analyte was passing through the column unretained. At the other extreme, low recovery (5%) was also observed from primarily aqueous sample (3% acetone in water). In this case, the analyte was strongly retained on the column, that is, it did not leach from the sorbent during sample loading but was not effectively desorbed. This observation is in agreement with previous research that showed analytes with  $\log P$  values  $>4$  are not eluted completely from alkyl-modified silica due to poor desorption (Nakamura et al., 1996). However, this phenomenon can be overcome by adjustment of the sample solvent strength (Wells et al., 1994; Wells, 2000). As the sample in acetone is diluted with water, the eluotropic strength of the sample matrix decreases in proportion to the concentration of acetone.

Quantitative recovery was obtained at an acetone/water ratio of 1:2 v/v (33.3% acetone). The soil extract in acetone was diluted with twice the amount of water to yield the appropriate sample solvent strength. Conversely to adding water to acetone to reduce solvent strength established in this study, it is proposed that to optimize the recovery of trifluralin from water samples, acetone should be added to increase the solvent strength.

Application of the SPE purification procedure to SFE soil extracts resulted in improved chromatograms that further reduced the level of coextracted interferences. During the SPE method development, it was observed that trifluralin strongly adhered to the glass walls of sample containers. Rinsing sample containers with the desorption solvent was absolutely necessary to obtain accurate results. Up to 50% loss due to adsorption was observed when sample containers were not rinsed.

**Conclusion.** Extraction, purification, and chromatographic procedures were developed for the recovery and analysis of trifluralin and related compounds from soil. SFE and liquid vortex extraction of trifluralin from soil were determined to be preferable to Soxtec extraction. SFE produced extracts with slightly fewer interfering coextractives than liquid vortex extraction, but liquid vortex extraction required less technical expertise from the analyst and was inexpensive compared to SFE. The level of interfering coextractives observed increased in the order Maury  $\approx$  Mountview  $<$  Baxter  $<$  Lindale  $<$  Iberia, which correlated somewhat with percent organic carbon and corresponded to soil texture in the order silt loam  $<$  sandy loam  $<$  silty clay. Generally, regardless of extraction method or soil type, a concentration-dependent recovery of trifluralin was observed due to  $\sim 0.150 \mu\text{g/g}$  trifluralin that was not extracted. This reduced extraction recovery at the  $0.5 \mu\text{g/g}$  fortification level more significantly compared to the  $2.5 \mu\text{g/g}$  level. The concentration dependence of trifluralin is attributed to its highly hydrophobic and nonpolar character.

#### ABBREVIATIONS USED

CAS, Chemical Abstracts Service; ECD, electron capture detector; EIA, enzyme immunoassay; EPA, U.S. Environmental Protection Agency;  $g$ , gravity; GC, gas chromatography; HPLC, high-performance liquid chromatography; i.d., inside diameter;  $K_{oc}$ , soil sorption coefficient based on soil organic carbon content;  $P$ , partition coefficient; SD, standard deviation; SFE, supercritical fluid extraction; SPE, solid-phase extraction; TEA, triethylamine; UV, ultraviolet; v/v, volume/volume; vis, visible.

#### LITERATURE CITED

- An, Q.; Qian, W. A Procedure for Determination of Trifluralin Residues in Soils by Capillary Gas Chromatography. *Turang (Nanjing)* **1992**, *24* (1), 48–50.
- Baez, M.; Rodriguez, M.; Lastra, O. Analytical Quality Parameters of the Solid-Phase Extraction Method for Gas-Chromatographic Determination of Pesticide Residues in Water. *Bol. Soc. Chil. Quim.* **1997**, *42* (4), 457–464.
- Balinova, A.; Balinov, I. Determination of Herbicide Residues in Soil in the Presence of Persistent Organochlorine Insecticides. *Fresenius' J. Anal. Chem.* **1991**, *339* (6), 409–412.
- Bartle, K. D.; Boddington, T.; Clifford, A. D.; Hawthorne, S. B. Effect of Solubility on the Kinetics of Dynamic Supercritical-Fluid Extraction. *J. Supercrit. Fluids* **1992**, *5*, 207–212.
- Battaglin, W. A.; Goolsby, D. A. *Spatial Data in Geographic Information System Format on Agricultural Chemical Usage, Land Use, and Cropping Practices in the United States*; U.S. Geological Survey Water Resources Investigation 94-4176; U.S. GPO: Washington, DC, 1995; 87 pp.
- Brown, R. F. *Ecology of Pesticides*; Wiley: New York, 1978; pp 367–368.
- Burford, M. D.; Hawthorne, S. B.; Miller, D. J. Evaluation of Drying Agents for Off-line Supercritical Fluid Extraction. *J. Chromatogr. A* **1993**, *657* (2), 413–427.
- Cabras, P.; Melis, M.; Spanedda, L.; Tuberoso, C. High-performance Liquid Chromatographic Determination of Dinotroaniline Herbicides in Soil and Water. *J. Chromatogr.* **1991**, *585*, 164–167.
- D'Amato, A.; Semeraro, I.; Bicchi, C. Simultaneous Determination of Linuron and Trifluralin Residues in Carrots and their Pulp by LC and GC. *J. AOAC Int.* **1993**, *76* (3), 657–662.
- Duc, P. Determination of Herbicides in Soils. *Analisis* **1992**, *20* (7, Suppl.), S11.



- Foreman, W. T.; Foster, G. D.; Gates, P. M. Isolation of Multiple Classes of Pesticides from Water Samples using Commercial 10-Gram C-18 Solid-Phase Extraction Cartridges. *Natl. Meet.—Am. Chem. Soc., Div. Environ. Chem.* **1993**, *33* (1), 436–439.
- Garcia-Valcarcel, A. I.; Sanchez-Brunete, C.; Martinez, L.; Tadeo, J. L. Determination of Dinitroaniline Herbicides in Environmental Samples by Gas Chromatography. *J. Chromatogr. A* **1996**, *719* (1), 113–119.
- Golab, T.; Althaus, W. A.; Wooten, H. L. Fate of <sup>14</sup>C Trifluralin in Soil. *J. Agric. Food Chem.* **1979**, *27*, 163–179.
- Hansch, C.; Leo, A.; Hoekman, D. *Exploring QSAR [2]. Hydrophobic, Electronic, and Steric Constants*; American Chemical Society: Washington, DC, 1995; 348 pp.
- Hawthorne, S. B.; Galy, A. B.; Schmitt, V.; Miller, D. J. Effect of SFE Flow Rates: Classifying Sample Extraction Behaviour. *Anal. Chem.* **1995**, *67*, 2723–2732.
- Helling, C. S. Dinitroaniline Herbicides in Soils. *Suppl. Proc. Northeast Weed Sci.* **1976**, *30*, 44–51.
- Kenaga, E. E. Predicted Bioconcentration Factors and Soil Sorption Coefficients of Pesticides and Other Chemicals. *Ecotoxicol. Environ. Saf.* **1979**, *4* (1), 26–38.
- Krause, A. A.; Niemczyk, H. D. Simultaneous Gas–liquid Chromatographic Analysis of Trifluralin and Benfluralin Residues in Turfgrass Thatch and Soil by Solid-Phase Extraction Technique. *J. Environ. Sci. Health Part B* **1992**, *B27* (1), 39–51.
- Lopez-Avila, V.; Benedicto, J.; Baldin, E.; Beckert, W. F. Analysis of Classes of Compounds of Environmental Concern: I. Nitroaromatic Compounds. *J. High Resolut. Chromatogr.* **1991**, *14* (9), 601–607.
- Lu, Y.; Xu, J. Determination of Residual Trifluralin in Oil-bearing Crops and Soils. *Shanghai Huanjing Kexue* **1991**, *10* (8), 33–34.
- Miliadis, G. E. Analysis of Pesticide Residues in Water Samples by Gas Capillary Chromatography. *Bull. Environ. Contam. Toxicol.* **1998**, *61* (2), 255–260.
- Nakamura, M.; Nakamura, M.; Yamada, S. Conditions for Solid-phase Extraction of Agricultural Chemicals in Waters by Using *n*-Octanol–Water Partition Coefficients. *Analyst* **1996**, *121*, 469–475.
- Pignatello, J. J.; Xing, B. Mechanisms of Slow Sorption of Organic Chemicals to Natural Particles. *Environ. Sci. Technol.* **1996**, *30* (1), 1–11.
- Probst, G. W.; Golab, T.; Write, W. L. Dinitroanilines. In *Herbicide Chemistry, Degradation and Mode of Action*; Kearney, P. C., Kaufman, D. D., Eds.; Dekker: New York, 1975; Vol. 1, pp 453–500.
- Riley, M. B.; Keese, R. J. Comparison of Solid Phase Extraction Techniques for Herbicides. *Weed Sci.* **1996**, *44*, 689–693.
- Stearman, G. K.; Adams, V. D. Atrazine Soil Extraction Techniques for Enzyme Immunoassay Microtiter Plate Analysis. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 144–151.
- Stearman, G. K.; Wells, M. J. M.; Adkisson, S. M.; Ridgill, T. E. Supercritical Fluid Extraction Coupled with Enzyme Immunoassay Analysis of Soil Herbicides. *Analyst* **1995**, *120*, 2617–2621.
- Thurman, E. M.; Meyer, M. T. Herbicide Metabolites in Surface Water and Groundwater: Introduction and Overview. In *Herbicide Metabolites in Surface Water and Ground Water*; Meyer, M. T., Thurman, E. M., Eds.; American Chemical Society: Washington, DC, 1996; pp 1–15.
- Tutarli, A.; Cici, M.; Celik, S. Determination of Trifluralin and Chloridazon Residues in Agricultural Lands in Elazig Province. *Environ. Technol.* **1995**, *16* (10), 995–1000.
- U.S. Environmental Protection Agency. Office of Water Regulations and Standards Industrial Technology Division, Method 1618. *Organo-halide Pesticides, Organo-phosphorus Pesticides, and Phenoxy-acid Herbicides by Wide Bore Capillary Column Gas Chromatography with Selective Detectors*; U.S. GPO: Washington, DC, 1989.
- Vitali, P.; Venturini, E.; Bonora, C.; Calori, R.; Raffaelli, R. Determination of Triazines and Dinitroanilines by High Performance Liquid Chromatography after Solid-Phase Extraction. *J. Chromatogr.* **1994**, *660* (1–2), 219–222.
- Weber, J. B. Behaviour of Dinitroaniline Herbicides in Soils. *Weed Technol.* **1990**, *4* (2), 394–406.
- Weber, J. B.; Monaco, T. J. Review of the Chemical and Physical Properties of the Substituted Dinitroaniline Herbicides. *Proc. South. Weed Sci.* **1972**, *25*, 31–37.
- Wells, M. J. M. Handling Large Volume Samples: Applications of SPE to Environmental Matrices. In *Solid-Phase Extraction: Principles, Techniques, and Applications*; Simpson, N. J. K., Ed.; Dekker: New York, 2000; pp 97–123.
- Wells, M. J. M.; Stearman, G. K. Coordinating Supercritical Fluid and Solid-Phase Extraction with Chromatographic and Immunoassay Analysis of Herbicides. In *Herbicide Metabolites in Surface Water and Ground Water*; Meyer, M. T., Thurman, E. M., Eds.; American Chemical Society: Washington, DC, 1996; pp 18–33.
- Wells, M. J. M.; Riemer, D. D.; Wells-Knecht, M. C. Development and Optimization of a Solid-Phase Extraction Scheme for Determination of the Pesticides Metribuzin, Atrazine, Metolachlor and Esfenvalerate in Agricultural Runoff Water. *J. Chromatogr.* **1994**, *659* (2), 337–348.
- Yordy, D. W.; Becker, O. D.; Fisher, S. E. Determination of Trifluralin in Soil by Solid-phase Extraction. *Analytical Method, AM-AA-CA-R125-AA-755*; Lilly Research Laboratories: Greenfield, IN, 1988.

Received for review July 7, 2000. Accepted October 5, 2000. This material was based upon work supported by the Cooperative State Research Service, U.S. Department of Agriculture, under Agreement 93-34214-8844, and the Center for the Management, Utilization, and Protection of Water Resources at Tennessee Technological University. Presented in part at the 214th National Meeting of the American Chemical Society, Las Vegas, NV.

JF000839Y