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Extraction of Metribuzin from Soil Using Supercritical CO_2 (SFE)

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To validate comprehensive pesticide transport models (e.g., PRZM, LEACHMP, GLEAMS, etc.) on a field or plot scale, analysis of large numbers of soil samples may be necessary. This is especially true if statistically significant results are desired (Smith *et al.* 1987). Supercritical fluid extraction (SFE) has certain advantages over traditional extraction methods: less extraction time required per sample; less solvent usage; less solvent exposure to workers; and less manually intensive.

Recently, supercritical fluid (usually CO_2) has been shown to yield good extraction efficiency for a variety of triazine herbicides (Janda *et al.* 1989*;* Steinheimer *et al.* 1994; Robertson and Lester 1994; van der Velde 1994), These triazine studies did not include metribuzin, the most polar commercially distributed triazine. Metribuzin has a water volubility of 1200 ppm making it moderately polar and supercritical CO_2 is considered non-polar. Using the concept "like dissolves like" supercritical CO_2 would likely be less efficient in extracting metribuzin than in extracting other triazine herbicides. The effect of pH was also beyond the scope of these triazine studies. Metribuzin adsorption to soil is especially sensitive to pH due to its protonation at low soil pH (Ladlie *et al,* 1976). Protonation may increase the difficulty of metribuzin extraction using supercritical CO_2.

The objectives of this study were to examine the effects of several SFE parameters on the extraction efficiency of the moderately polar herbicide metribuzin (solubility=1200 ppm) and to compare SFE results to published metribuzin extraction efficiencies using traditional extraction methods.

MATERIALS AND METHODS

The soil used was a Lowell silt loam (11% sand; 79% silt; 10% clay; pH=6.3; 1.3% organic carbon). The soil was air dried, ground, and passed through a 2 mm sieve. 500 g of soil was placed into a mason jar and spiked with 25 µg of metribuzin methanol) to achieve a soil concentration of 0.05 µ/g. The methanol was allowed to evaporate and the soil was mixed on a rotary tumbler for at least 12 hours at 1 °C. The spiked soil was stored at -18 °C until extracted. Several batches of spiked soil were made. Between batches an unmeasured amount of variability was likely present. Each SFE

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parameter studied (e.g., extraction time, pressure, etc.) used the same spiked soil batch so that soil spike concentration variability would not effect results.

All extractions were performed on a Hewlett Packard Model 7680T supercritical fluid extractor (SFE) module interfaced with a model 1050 HPLC pump (modifier pump) with menu driven control from a Vectra 486/33 computer. This is a commercial system which uses a reciprocating pump, an extraction chamber with a cell loading compartment, a variable restrictor nozzle, and analyte sorbent taps (Cl 8 for this study). This system has the capability to automatically extract up to 8 soil samples of about 9 g each one sample at a time. The maximum soil extracted in this study was 9.2 g and the minimum soil extracted was 8.8 g.

Refer to Table 1 for the base parameters used to extract metribuzin. The trap was rinsed with 1.5 ml of methylene chloride into 2 ml autosample vials. The investigated SFE extraction parameters were 1:) modifier type and amount; 2) nozzle and trap temperature; 3) extraction time 4); CO_2 density; and 5) extraction temperature. Modifiers used in this study were water, methanol, ethanol, and methylene chloride. The modifier pH was increased by addition of triethylamine, After an extraction condition was found to yield the best recovery, it was then used for all subsequent extractions. This approach is similar to Reddy and Locke (1994) and Robertson and Lester (1994).

Table 1. Initial and final SFE conditions*

Parameter	Initial conditions	Final conditions
modifier	2% 1:2 MECL-:MEOH	15% 1:5:10 water:MECL:ethanol (0.05% triethylamine added)
trap temperature	75 °C	105 °C
nozzle temperature	80 °C	105 °C
static extraction time	5 min.	5 min.
dynamic extract. time	20 min.	10 min.
CO2 density	0.85 g/ml	0.75 g/ml
extraction temperature	50 °C	40 °C

* the final conditions were chosen because they either yielded the best recovery or would not damage the equipment (final modifier).

The volume of non-degassed solvent used to rinse the trap varies by about ±10%. Therefore, each autosample vial was spiked with a known mass of a detectable solute to perform quantitative analysis. In this study 0.5 µg of terbutylazine in a 10 µg/ml solution (in methanol) was added to each autosample vial. The volume of rinsate in each vial was determined by the mass of internal standard added to each vial (0.5 µg) divided by the concentration of the internal standard as determined by GC analysis.

Metribuzin analysis was performed using a gas-liquid chromatograph (GC, Hewlett

Packard Company, Model 5890 Series 11, Palo Alto, California), equipped with a nitrogen phosphorus detector GC mas s spectrometry operated in selective ion monitoring mode (m/e=198, 214) was used to confirm metribuzin (HP Model 5971A mass selective detector). GC run conditions included: 225 °C injection temperature; oven program was 190 °C for 14 minutes, 10 °C temperature increase per minute to 220 “C, this temperature was held for 5 minutes; and 240 “C detector temperature. Flows were set at 15, 20, 120, and 5-ml per minute for carrier (He), auxiliary (He), air, and hydrogen, respectively. The column was an RTX-5 (5% diphenyl-95% dimethyl poly siloxane), 30 m, 0.53 mm inside diameter Retention time for metribuzin was 11.1 minutes and for terbutylazine 7.6 minutes.

Analysis of variance was used with the least significant difference (LSD) utilized for means comparison .All extractions were performed in duplicate. More replications were not included because preliminary studies indicated that variability was small. The results presented also indicate this when the average standard deviation between duplicate samples was found to be 3.59 with a high standard deviation of 7.76. When variability was high between duplicates (standard deviation greater than 10) [he problem was always isolated and the extractions rerun, In addition, van der Velde *et a/.* (1992) concluded that good reproducibility was found extracting PCB’s.

RESULTS AND DISCUSSION

Preliminary investigations indicated that a 2:1 aziotropic mixture of methanol and methylene chloride achieved the maximum recovery Addition of water significantly increased efficiency at the 10% and 15% modifier level (Refer to Table 2 columns 2 and 3). Supercritical CO_2 alone (no modifier) has solvent properties similar to hexane, a non-polar solvent. To extract moderately polar herbicides such as metribuzin, the CO_2 needs to be modified. The modifier may not only increa s the volubility of solute but also alter the solute/matrix interaction (Knipe *et al.* 1993). Water may increase extraction efficiency of polar pesticides by competing for adsorption sites on the soil. A higher ratio of water may have yielded better recoveries but preliminary investigations indicated that water accumulation on the trap and finally in the autosample vials would result.

The more basic modifier significantly increased recovery at the 5% modifier level indicating a pH effect on adsorption (Refer to Table 2 columns 3 and 4). This effect was even more dramatic at the 2% modifier addition where the mean recovery increased from 17.01% to 60.63% with the addition of triethylamine (results not reported in Table 2) Metribuzin has been reported to become protonated, thereby increasing adsorption as soil pH decreases (Ladlie *et al.* 1976). The lower the soil pH the higher the degree of protonation. Addition of triethylamine (0.05% by volume) increased the modificr pH from 6 to 10, thereby dcsorbing metribuzin.

Ethanol was substituted for methanol for subsequent extractions because methanol modified supercritical CO_2 is very corrosive . This substitution did not decrease the recovery at the 15% modifier addition (Refer to Table 2 columns 4 and 5).

The results of this study indicate that a temperature of 105 °C gave the best recovery (refer to Table 3). Until the temperature was raised above the boiling point of water no observable differences in recovery were detected.

Table 2. Modifier effect on recovery*

Volumetric modifier added (%)	Modifier type 1:2 MECL: MEOH	 1:5:10 water: MECL:MEOH	 1:5:10 water: MECL:MEOH (0.05% triethylamine)	 1:5:10 water: MECL: ethanol (0.05% triethylamine)
	Recovery (%)			
5	50.07 a† (3.16)‡	53.58 ac (5.85)	78.42 h (2.55)	60.95 bcd (2.69)
10	53.52 ab (6.68)	67.18 defg (7.76)	75.30 gh (1.38)	63.03 bcde (1.90)
15	64.58 def (7.70)	92.52 i (2.81)	69.95 defgh (4.45)	70.04 defgh (2.23)

* Base SFE extraction parameters.

† Means followed by the same letter are not different at P=0.05 by the LSD test (n=2).

‡ Standard deviation.

Table 3. Trap and nozzle temperature effect on recovery*

Trap temperature (Celsius)	Nozzle temperature (Celsius)	Recovery (%)
75	80	71.10 ab† (2.53)‡
85	85	72.47 ab (6.36)
95	95	65.79 b (4.29)
105	105	80.56 a (2.40)

* Base extraction parameters except 15% modifier (1:5:10 water:MeCl:Ethanol with 0.05% triethylamine).

† Means followed by the same letter are not different at P=0.05 by the LSD test (n=2).

‡ Standard deviation.

The nozzle is the point where the CO_2 enters the trap and moves from the supercritical phase to the gas phase (atmospheric pressure is encountcrcd). The analyte is deposited upon the trap at this .ime, The temperature of the trap and nozzle may effect recovery in two ways: 1) if the temperature is too low, the modifier may condense upon the trap; or 2) if the temperature is too high the trap may thermally degrade the analyte or melt the analyte. Condensation of the modifier may cause two problems: the modifier may move through the trap in liquid form into the waste port thus removing a portion of the analyte with it; or the modifier may be removed during trap rinsing causing erratic rinse volumes or causing damage to GC detectors In .general, the best trap and nozzle temperature is above the boiling point of the modifier being used.

The SFE involves both a static extraction where the sample soaks in supercritical CO_2 and a dynamic extraction where fresh CO_2 is pumped through the sample at a specified flow rate (2 ml/min for this study). The results indicate that a very short static extraction time is necessary to reach equilibrium (about 2.5 min.). The higher static extraction time of 5 min. was chosen for subsequent extractions due to a slightly higher recovery and a lower standard deviation (Refer to Table 4).

Table 4. Static and dynamic extraction time effect on recovery*

Static extraction		Dynamic extraction	
Time (minutes)	Recovery (%)	Time (minutes)	Recovery (%)
2.5	88.22 a† (1.85)‡	5	88.55 a (7.21)
5	92.30 a (0.97)	10	90.20 a (0.58)
7.5	95.42 a (4.94)	20	90.11 a (3.80)
10	94.00 a (2.74)	30	36.64 b (3.22)

* Same extraction conditions listed in Table 2 except 105 “C trap and nozzle temperature.

† Means within a column followed by the same letter are not different at P=0.05 by the LSD test (n=2).

‡ Standard deviation.

No significant recovery differences could be detected between dynamic extraction times of 5 and 20 minutes (Refer to Table 4). At a dynamic extraction time of 30 minutes a significantly lower recovery was found. A dynamic extraction time of 10 minutes was chosen for all subsequent extractions because a 5 minute dynamic extraction had a higher standard deviation and the recovery was slightly lower indicating that equilibrium may not have been reached until 10 minutes The. shortest possible extraction time should be used to reduce CO_2 use (SFE grade CO_2 is expensive) and to maxieizc the number of samples that can be processed in a day.

In the range of densities examined in this study (0.70 to 0.85 g/ml), the C02 density did not significantly effect the recovery (Refer to Table 5), CO_2 density was increased while keeping temperature constant. In general, as CO_2 density increases, the solvent power of the fluid increases (Knipe *et al.* 1993). “Solvent power” in the case of supercritical CO_2 refers to the amount of solute dissolved rather than the polarity of the solvent as this term is traditionally used. For CO_2 as with most fluids, polarity is not a function of pressure. A CO_2 density of 0.75 g/ml was used for all subsequent extractions since it gave the highest recovery and the lowest standard deviation.

Table 5. Extraction temperature and CO_2 density effect on recovery

CO_2 density*		Extraction temp.†	
Density (g/ml)	Recovery (%)	Temperature (Celsius)	Recovery (%)
0.70	101.60 a‡ (0.34)§	40	92.12 a (6.87)
0.75	111.62 a (0.30)	50	81.73 a (1.15)
0.80	105.95 a (3.20)	70	67.84 b (4.21)
0.85	102.54 a (7.76)	90	63.65 b (0.89)

Same extraction conditi m Table 3 except a 10 minute dynamic extraction time.

† Same extraction conditions listed in Table 3 except a 10 minute dynamic extraction time and a 0.75 g/ml CO_2 density.

‡ Means within a column followed by the same letter are not different at P=0.05 by the LSD test (n=2).

§ Standard deviation.

Table 6. Solvent extraction of metribuzin

Source	Soil description	Recovery (%)
Allen and Walker (1987)	18 different soils oc=0.6-2.4% pH=5.0-7.4 clay=15-41% sand=11-77%	94.8 (±2.8%)
Bowman (1991)	Plainfield sand	95
Ivany et al. (1983)	fine sandy loam om=2.4% pH=5.4-6.2	92
Sorenson et al. (1991)	silty clay loam	95 (±3%)

In this study an increase in temperature yielded lower recoveries (Refer to Table 5). This was also observed for triazines by Steinheimer *et al.* (1994) where the optimum recoveries were at 40 °C. Extraction temperature was increased while keeping CO_2 density constant. Higher extraction temperatures generally yield higher recoveries. This is due to an increase in the volubility of the solute and an increase in the diffusivity of the fluid at higher temperatures (Knipe *et al.* 1993). The final SFE conditions compared to the initial SFE conditions mey be seen in Table 1.

Conventional metribuzin extraction from most soils will yield greater than 90% recovery (Refer to Table 6). The optimized SFE condition recoveries compare favorably to conventional extraction recoveries. An advantage of conventional extraction techniques compared to SFF. is that more soil may be used thus decreasing the minimum detection limit. This problem may be overcome in SFE by extracting more than onc thimble (putting soil from the same sample in each thimble) without rinsing the trap between thimble extractions.

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REFERENCES

Allan R, Walker A (1987) The influence of soil properties on the rates of degradation of metamitron, metazachlor, and metribuzin. Pestic Sci 18:95-111

Bowman BT (1991) Mobility and dissipation studies of metribuzin, atrazine and their metabotzes in plainfield sand using field lysimeters. Environ Toxicol Chem 10:573-579

Ivany JA, Sadler JM, Kimball ER (1983) Rate of metribuzin breakdown and residue effects on rotation crops. Can J Plant Sci 63:481-487

Janda V, Steenbeke G, Sandra P (1989) Supercritical fluid extraction of s-triazine herbicides from sediment. J Chromatogr A 479:200-205

Knipe CR, Miles WS, Rowland F, Randall LG (1993) Designing a sample preparation method that employs supercritical fluid extraction. Hewlett Packard, Wilmington, DE

Ladlie JS, Meggitt WF, Penner D (1976) Effect of soil pH on microbial degradation, adsorption, and mobility of metribuzin. Weed Sci 24:417-48 I

Reddy KN, Locke MA, (1994) Supercritical CO_2 fluid extraction of imazaquin from soil. Weed Sci 42:249-253

Robertson AM, Lester JN (1994) Supercritical fluid extraction of s-triazines and phenylurea herbicides from sediment. Environ Sci Technol 28:346-351

Smith CN, Parrish RS, Carsel RF (1987) Estimating sample requirements for field evaluation of pesticide leaching. Environ Toxicol Chem 6:347-357

Sorenson BA, Shea PJ, Roeth FW (1991) Effects of tillage, application time and rate on metribuzin dissipation, Weed Res 31:333-345

Steinheimer TR, Pfeiffer RL, Scoggin KD (1994) Extraction of atrazine, cyanazine, desethylatrazine, desisopropylatrazine, and metolachlor from fortified western cornbelt soils by SFE with CO_2. Anal Chem 66:645-650

van der Velde EC, Ramlal MR, van Beuzekom AC, Hoogerbrugge R (1994) Effects of parameters on supercritical fluid extraction of triazines from soil by use of multiple linear regressi.n, J Chromatogr A 683:125-139