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Short communication

Extraction of cloransulam-methyl from soil with subcritical water and supercritical CO_2

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Abstract

Cloransulam-methyl was extracted from soil samples with supercritical CO_2, subcritical water and conventional organic solvents. Supercritical CO_2 was less efficient than conventional organic solvents; polarity modifiers had no impact on extraction efficiency. Extraction with supercritical CO_2 exhibited a strong temperature dependence. Water was as effective as strong organic solvents for the extraction of cloransulam-methyl; however cloransulam-methyl hydrolyzed when extracted at 150°C. Extraction temperature was the most important variable in increasing the efficiency and rate of extraction, while extraction pressure was not a significant variable.

Keywords: Supercritical fluid extraction; Subcritical water extraction; Soil; Cloransulam-methyl

1. Introduction

Cloransulam-methyl (*N*-(2-methoxycarbonyl-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonanilide) is a triazolopyrimidine sulfonanilide herbicide used for broadleaf weed control in soybeans; its mode of action is through inhibition of acetolactate synthase (ALS). Cloransulam-methyl is a member of the triazolopyrimidine sulfonanilide family of chemistry, which also includes metosulam, flumetsulam, florasulam and diclosulam. It is used both preemergence (soil applied) and postemergence to control weeds such as *Xanthium strumarium* (cocklebur), *Abutilon theophrasti* (velvetleaf), *Ambrosia artemisiifolia* (common ragweed), *Datura stramonium* (jimsonweed). Application rates are low, ranging from 35 to 44 g/ha for preemergence application down to 17.5 g/ha for postemergence application. Cloransulam-methyl is a non-volatile, ionizable compound with a pK_a of 4.81 at 20°C and a vapor pressure of 4×10^{-14} Pa at 25°C. Its water solubility at 25°C is 16, 3 and 184 mg l^{-1} in deionized water (unbuffered), water buffered to pH 5 and water buffered to pH 7, respectively [1–4]. Cloransulam-methyl degrades in soil with half-lives ranging from 13 to 28 days.

Extraction procedures for cloransulam-methyl and other triazolopyrimidine sulfonanilides from soil or crops have typically involved organic solvents plus a strong mineral acid (for example, sonication with 90:10 v/v acetone:1 N HCl)) or a weak organic acid (for example, 79:20:1 acetone/water/glacial acetic acid) [5–7]. Extraction under these conditions typically results in a large amount of coextracted soil organic matter, requiring significant sample cleanup prior to analysis. The objective of this work was to

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determine if supercritical fluids or subcritical water [8–16] could be used to efficiently extract cloransulam-methyl from soil with less coextracted organic matter, simplifying sample cleanup and allowing a more rapid analysis. While supercritical fluid extraction has been used for a number of years for the extraction of pesticides from soil [17–21], the more recently introduced technique of subcritical water extraction offers great promise for overcoming some of the inherent limitations of extraction of polar analytes with a nonpolar supercritical fluid. This work reports the first results on the use of supercritical fluid extraction and subcritical water extraction of triazolopyrimidine sulfonanilide herbicides from soil.

2. Experimental

2.1. Standards and reagents

Cloransulam-methyl radiolabeled in the 7 and 9 positions of the pyrimidine ring was obtained from the Specialty Synthesis group at Dow AgroSciences (Indianapolis, IN). The specific activity of the test material was 9.95×10^{11} Bq/mol (1.39×10^{11} dpm/g). Standard solutions were assayed by liquid scintillation counting (LSC) and high-performance liquid chromatography (HPLC) to check activity and radiochemical purity prior to use in dosing solutions. The radiochemical purity was >99%. Non-radiolabeled cloransulam-methyl (purity >99%) was obtained from Dow AgroSciences. All solvents and chemicals were ACS reagent grade or better and were obtained from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Soil samples

Soil samples that were collected as part of a cloransulam-methyl terrestrial field dissipation study [7] were used to determine extraction efficiencies. Details of the application of cloransulam-methyl to each field, including application methods and rate, sample collection, storage, shipment and stability under storage conditions are given in Ref. [7]. Applications of ^{14}C-radiolabeled cloransulam-methyl to bare soil plots in Greenfield, IN (Crosby silt loam soil, pH 7.1, 1.68% organic matter) and Wayside, MS (Commerce silt loam soil, pH 7.8, 0.83% organic matter) were made in June and July of 1993, at a nominal rate of 50 g a.i. ha^{-1}. Soil samples collected at 1 day after treatment (DAT) and 92 DAT (all from 0 to 15 cm depth) were used for this work. The average concentration of cloransulam-methyl (measured at the time of sample collection) was 23 ng g^{-1} in the 1-DAT samples and 0.5 ng g^{-1} in the 92-DAT samples. Unextracted subsamples of these soil samples were stored frozen for up to 3 years after collection prior to the analyses described in this report. Cloransulam-methyl is stable under frozen storage conditions, therefore the concentrations measured by analysis immediately after sampling did not change over the storage time [7]. These stored samples were used because they involved the direct application of cloransulam-methyl to soil and involved the natural sample aging process. Extraction of cloransulam-methyl from these aged samples should be a more accurate predictor of real-world extraction efficiency than using soils spiked in the laboratory. Approximately 5 g (wet weight) of soil was used for each experiment; the soil moisture content was 36% for the Crosby loam soil and 41% for the Commerce silt loam soil. After thawing, soils were extracted as is with no sample preparation or drying.

The total amount of ^{14}C present in each soil sample was determined by combustion of an non-extracted sample followed by liquid scintillation counting (LSC). Extraction results are typically expressed as a percentage of the total ^{14}C in the sample, which may not be equivalent to the total amount of ^{14}C-cloransulam-methyl in the sample (due to biotic or abiotic degradation).

2.3. Organic solvent extraction

Organic solvent extractions were performed by sonicating a 5-g soil sample in 10 ml of 80:20 acetone:1 N HCl for 4 h. At 1-h intervals, the sample was centrifuged, the supernatant was decanted and fresh extraction solvent was added. The extracts were pooled and assayed for ^{14}C by liquid scintillation counting (LSC). Four replicates were typically analyzed from each sample.

2.4. Instrumentation

An ISCO SFX 2200 extraction system (ISCO, Lincoln, NE) was used to perform both the supercritical fluid extractions and the subcritical water extractions. Disposable 10-ml plastic extraction cells with 0.5-μm frits were used. Each cell was reused approximately 50 times before it started to leak and was replaced. Flow-rate was controlled by using fused-silica restrictors, typically 25 cm in length (Polymicro Technologies, Phoenix, AZ). Restrictor inner diameters were 25, 30, 40 and 50 μm; restrictor heaters were not used. An ISCO μLC-500 pump was used to deliver an organic cosolvent for solubility measurements.

2.5. Supercritical CO_2 extraction

Samples were extracted at 50, 100 or 140°C. Samples were extracted with neat CO_2 and CO_2 modified with 10% methanol, 10% toluene, 10% diethylamine or 5% water (v/v). Sample size was approximately 5 g (wet weight). Supercritical fluid extractions were performed at 500 atm of pressure, with a flow-rate of approximately 1 ml/min (measured as liquid CO_2 at the pump), for 60 min. Four samples were analyzed under each set of conditions.

2.6. Subcritical water extraction

Samples were extracted at 50, 100 or 150°C with distilled, deionized water. Extractions were performed at 65, 135 and 500 atm of pressure, at flow-rates ranging from 0.4 to 3.5 ml/min. Sample size was approximately 5 g. Four samples were analyzed under each set of conditions.

2.7. Extract collection

Extracted analytes were collected for the supercritical fluid experiments by bubbling the extraction effluent through approximately 10 ml of methanol. For the subcritical water extractions, the extraction fluid was collected directly in a test tube. Recovery experiments were conducted by applying 660 ng of cloransulam-methyl dissolved in acetone to a filter paper located inside of an extraction cell, allowing 5 min for the acetone to evaporate, followed by extraction and collection. These experiments yielded quantitative recovery of cloransulam-methyl ($96.1 \pm 3.6\%$, $n=5$). Therefore, no loss of cloransulam-methyl due to volatilization was observed, as expected because of its low vapor pressure. No carryover of cloransulam-methyl was observed in extraction blanks following each recovery experiment.

Sample extracts were typically collected as timed fractions. Fraction collection times varied from 1 to 30 min, depending on flow-rate and stage of the extraction. Each fraction was analyzed independently for ^{14}C by liquid scintillation counting (LSC).

2.8. Liquid scintillation counting

Radioactive material in solution was quantified by a Packard (Meriden, CT, USA) 2500TR liquid scintillation spectrometer. Ultima Gold scintillation cocktail (Packard, Meriden CT) was added to each sample before counting; samples were generally counted for 3 min. Chemiluminescence was corrected for using the scintillation counter's on-board logic.

2.9. Hydrolysis and aqueous solubility experiments

Hydrolysis experiments were performed by spiking 660 ng of cloransulam-methyl into an extraction cell and then extracting with a buffer solution adjusted to pH 2, 3.5, 5 or 7; the extraction pressure was 135 atm. Sample extracts were analyzed by reversed-phase high-performance liquid chromatography (HPLC) with UV-absorbance detection to determine the amount of cloransulam-methyl present.

Aqueous solubility experiments were performed by loading approximately 2 g of non-radiolabeled cloransulam-methyl into an extraction cell and filling the void volume of the cell with sand. Deionized water at 260 atm was passed through the cell at a flow-rate of 0.3 ml/min (30-μm restrictor) and became saturated with cloransulam-methyl. As the saturated aqueous phase left the extraction unit (and the temperature controlled region), acetone was added at a flow-rate of 0.7 ml/min via a stainless steel tee to prevent precipitation of cloransulam-methyl as the water cooled. Seven fractions (each 3 min in duration) were collected at each of six

different temperatures (35, 50, 75, 100, 125 and 150°C) in a single experiment lasting approximately 3 h. Samples were collected in test tubes and analyzed for cloransulam-methyl by reversed-phase HPLC with UV-absorbance detection.

2.10. High performance liquid chromatography

Samples were analyzed on a modular Hewlett-Packard (Avondale, PA, USA) 1050 liquid chromatograph using a Hewlett-Packard ODS Hypersil column (200×4.6 mm, 5-μm particles). The mobile phase was a binary gradient of acetonitrile (A) and deionized water (B); both mobile phases contained 1% (v:v) acetic acid. Analytes were eluted with a linear gradient from 10% solvent A to 90% solvent A over 30 min. The flow-rate was 1.0 ml/min and detection was by UV-absorbance at 254 nm. Eluent fractions were collected in 1-min increments and analyzed for ^{14}C using off line LSC; radiochromatograms were reconstructed from the scintillation counter results.

3. Results and discussion

3.1. Distribution of radiocarbon in extracted samples

Samples from the 1-DAT and 92-DAT timepoints were analyzed by HPLC to determine the amounts of cloransulam-methyl and it's degradates present. The amount of cloransulam-methyl present in the 1-DAT sample was 90% of the extractable radiocarbon, while it was only 18% in the 92-DAT sample. Extraction results presented are expressed as a percentage of extractable radiocarbon in each subsample analyzed; therefore for the 1-DAT samples the results represent principally (≥90%) cloransulam-methyl while for the 92-DAT samples the results represent principally (≥80%) the degradates of cloransulam-methyl.

3.2. Extraction with supercritical CO_2

Soil samples collected one day after treatment (DAT) with cloransulam-methyl from a field in Wayside, MS were extracted with supercritical CO_2 to determine extraction efficiency for cloransulam-methyl. Results are summarized in Table 1. Extraction efficiencies displayed a strong correlation with extraction temperature, with extractions at 140°C removing 45–60% of the total ^{14}C, compared to 15–30% only at 50°C. Addition of acidic, basic and aromatic modifier fluids had no significant impact on recovery. Note that the conventional organic extraction yielded a recovery of 69% of the total ^{14}C, better than any of the supercritical fluid extractions. Recoveries for all of the extraction techniques are lower than one would expect for a 1-DAT sample. (Extraction efficiencies of >85% would be expected using conventional organic solvent extractions for soil freshly spiked with cloransulam-methyl [22]). This may be due to the frozen storage period (2–3 years) of these samples prior to these extractions increasing the amount of cloransulam-methyl incorporated into the soil organic matter. Comparisons with extractions obtained with fresh soils are not valid in this case and only comparisons with extractions performed at the same time are used in this report.

Table 1
Summary of recoveries (average±standard deviation) for the supercritical fluid extraction and conventional organic solvent extraction of cloransulam-methyl from Wayside, MS Soil (1 DAT)

Extraction fluid	% Recovery (n=4)[a]	
	50°C	140°C
CO_2	15±3	45±8
CO_2/10% methanol	29±8	43±4
CO_2/10% toluene	20±1	36±6
CO_2/10% diethylamine	15±7	59±13
CO_2/5% water		28±3
80:20 acetone:1 N HCl (4×1-h sonication)		69±7

[a] Recoveries are relative to total ^{14}C determined by combustion.

Since the density (and the solvating power) of supercritical CO_2 decreases with temperature, the increased extraction efficiencies with temperature indicate that solubility is not a limiting factor in the extraction of cloransulam-methyl from soil. It is likely that the limiting step in the extraction is the desorption of cloransulam-methyl from the solid-phase into the mobile phase (the extraction fluid). Given the increase in extraction efficiency obtained by raising the temperature from 50 to 140°C, it is

possible that further increases in extraction temperature would yield increases in the total amount extractable. Unfortunately, the extraction system used in this study was currently limited to 150°C.

A kinetics plot of the amount of ^{14}C extracted as a function of time by supercritical CO_2 at 50 and 140°C is shown in Fig. 1. Kinetics plots for extraction with modified CO_2 were similar. Note that by 30 min, essentially all of the extractable material was removed in the 140°C experiments, while the 50°C extractions had not reached a steady state even after 60 min. These data also suggest that higher temperature extractions (>150°C) could yield complete recoveries of the extractable cloransulam-methyl in 30 min or less.

3.3. Subcritical water extraction

Prior to performing subcritical water extractions, the solubility and hydrolysis rates of cloransulam-methyl in subcritical water were investigated to determine the stability of cloransulam-methyl under experimental conditions.

3.3.1. Hydrolysis

At 25°C, hydrolysis of cloransulam-methyl displays a strong pH dependence with observed half-lives of >365, 230 and 3 days in sterile buffers at pH 5, 7 and 9, respectively [23]. Half-lives in sterilized river water and soil slurries ranged from 10 to 74 days.

Recovery experiments were performed using direct spikes of cloransulam-methyl into an empty extraction cell followed by extraction with buffers at pH 2, 3.5, 5 and 7. No degradation was observed in any of the buffers at 100°C. However, at 150°C, 40±11% (n=4) of the cloransulam-methyl had degraded in each of the buffer systems. Thus, hydrolysis of cloransulam-methyl can be significant over the time scale of an extraction (30 min) at 150°C.

3.3.2. Aqueous solubility

Solubility of cloransulam-methyl increases with temperature, reaching an apparent maximum at 125°C (see Fig. 2). The decrease in concentration of cloransulam-methyl observed at 150°C is likely due to the onset of rapid hydrolysis in the extraction system and not due to a decrease in solubility. These data show that solubility of cloransulam-methyl in deionized water increases from 16 ppm (mg/l) at 25°C to 125 ppm at 125°C, an eight-fold increase

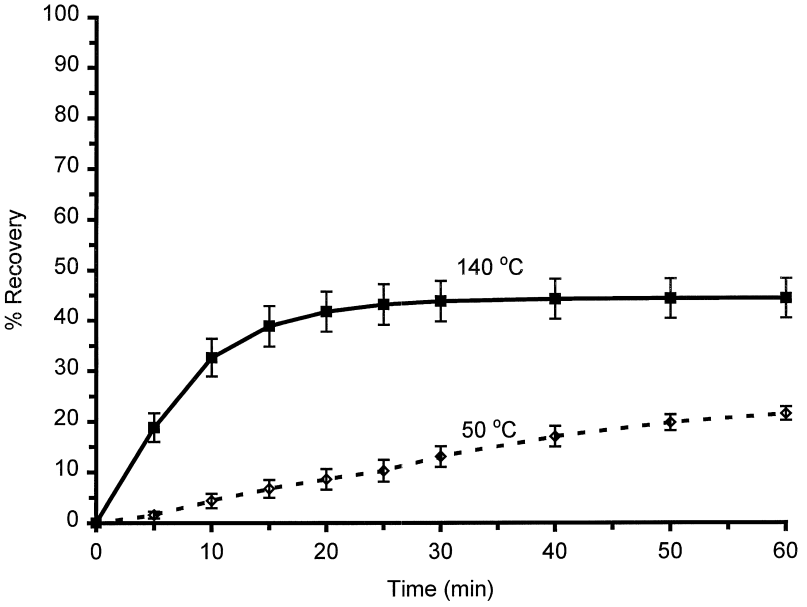

Fig. 1. Cumulative recovery of ^{14}C from Wayside, MS soil with supercritical CO_2 at 50 and 140°C as a function of time.

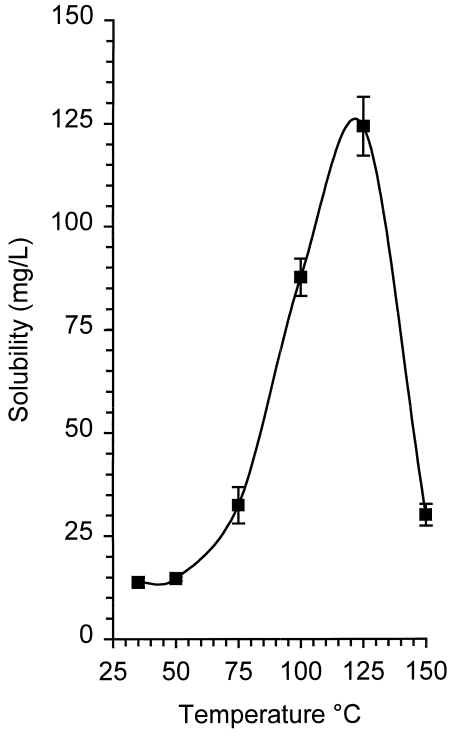

Fig. 2. Solubility of cloransulam-methyl in water as a function of temperature (P=260 atm).

and that hydrolysis of cloransulam-methyl is not observed at 125°C. Thus, the upper temperature limit of extraction of cloransulam-methyl with water is 125°C.

3.3.3. Effect of pressure

Samples from Wayside, MS were extracted with water at three different pressures (65, 135 and 500 atm) and at constant temperature (100°C) and flow-rate (0.4 ml/min). Samples from Greenfield, IN were extracted with water at two different pressures (260 and 400 atm) and at constant temperature (100°C) and flow-rate (0.3 ml/min). Constant flow was maintained by changing the restrictor diameter. Results are shown in Fig. 3. Although the recoveries at 65 atm with the Wayside, MS soil are slightly lower than the other experiments, this effect was not observed in the Greenfield, IN soil. Pressure has a negligible effect on the dielectric constant of water and is not expected to have a significant effect on the aqueous solubility of cloransulam-methyl.

3.3.4. Effect of temperature

Samples from Wayside, MS were extracted with water at three different temperatures (50, 100 and 150°C) at constant pressure (500 atm) and flow-rate (0.4 ml/min). Samples from Greenfield, IN were extracted with water at three different temperatures (50, 100 and 150°C) at constant pressure (400 atm) and flow-rate (1.7 ml/min). Results are shown in Fig. 4.

Increasing the extraction temperature has a significant effect on the total amount extracted and the relative rate of extraction. For example, for the Wayside, MS soil extracts, at t=60 min, approximately 84% of the 50°C extractable material, 88% of the 100°C extractable material and 90% of the 150°C extractable material was removed. Thus, the extraction is approaching completion faster at higher temperatures. The same analysis was not performed for the Greenfield, IN soils because the length of the extraction (120 min) was not sufficient to reach a plateau in the amount extracted at each of the three temperatures.

3.3.5. Comparison with organic solvent extraction

A comparison of extraction of 1-DAT and 92-DAT soils samples by conventional organic solvent extraction and subcritical water extraction is shown in Table 2. Subcritical water extraction at 150°C extracted as much or more total ^{14}C than the strong organic solvents used. Subcritical water extraction at 100°C gave equivalent results for the Wayside, MS soil and slightly lower recoveries from the Greenfield, IN soil.

Recoveries for the 92-DAT Wayside, MS soils were higher using subcritical water than organic solvent extraction. At 92 DAT, cloransulam-methyl has degraded (<20% of the applied ^{14}C remaining as cloransulam-methyl) into a number of compounds that are all more polar than the parent compound [5,7]. Subcritical water may be more effective than organic solvents at extracting these more polar molecules. Since soil metabolites and degradates of pesticides are typically more polar than the parent molecule, water should typically perform better for extracting metabolites than parent. Thus, if a successful extraction method is developed for the parent molecule, the same method (or even milder conditions) should be successful for degradates.

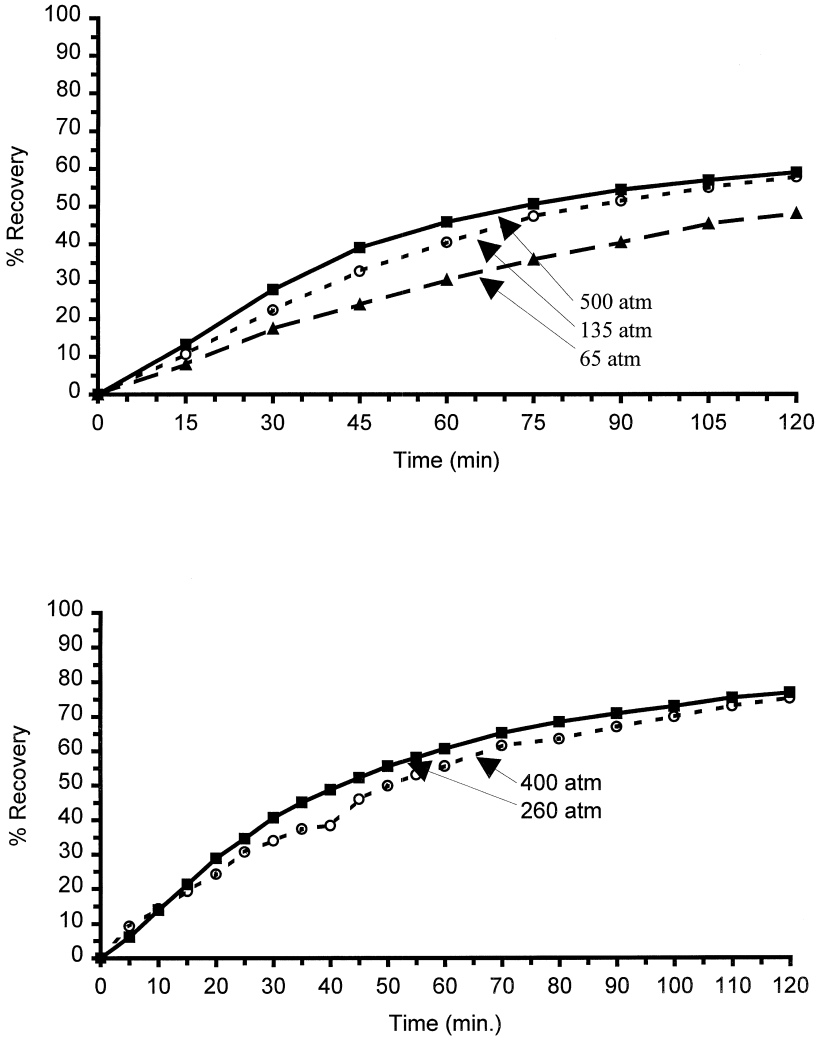

Fig. 3. Effect of pressure on the subcritical water extraction of cloransulam-methyl from soil. Cumulative recovery of ^{14}C versus time for Wayside, MS (top) and Greenfield, IN (bottom) soils.

Subcritical water extraction also allows greater control over the extraction process than using conventional organic solvents. For example, vigorous extraction with organic solvents or hot water does not selectively extract the bioavailable (and environmentally significant) fraction of a molecule in the environment [24–26]. Extraction with subcritical water may allow one to vary the extraction strength of the solvent (by changing the extraction temperature) to selectively extract the bioavailable fraction of a molecule, which then could be followed with a more vigorous extraction to extract the additional, non-bioavailable, fraction of material.

The color of the extracts was markedly different from the different solvent systems. The organic extracts were a dark brown color and would have required extensive cleanup prior to analysis, particularly for a non-radiolabeled analysis. The subcritical water extracts were clear to a faint yellow color and could be analyzed by HPLC without further sample cleanup. If sample cleanup was desired, no solvent evaporation steps would be

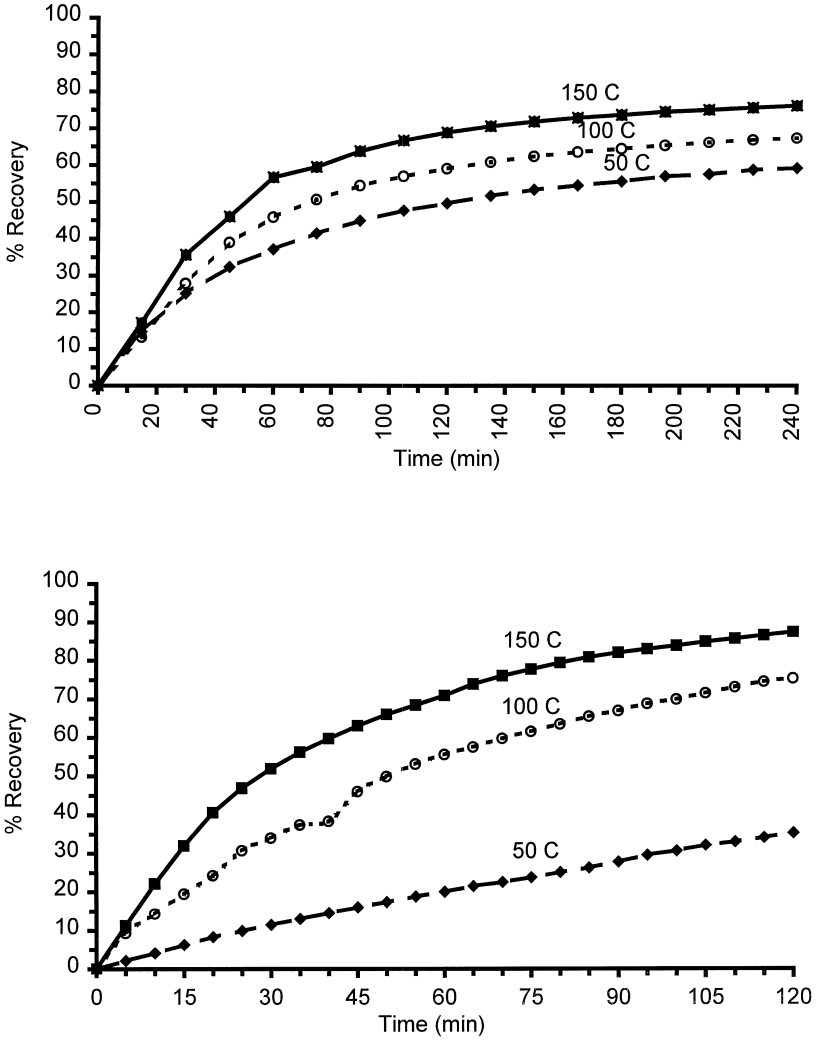

Fig. 4. Effect of temperature on the subcritical water extraction of cloransulam-methyl from soil. Cumulative recovery of ^{14}C versus time for Wayside, MS (top) and Greenfield, IN (bottom) soils. Note that the time ranges are different for the two data sets.

required to remove the organic solvent, so cleanup by solid-phase extraction could proceed quickly.

4. Conclusions

Supercritical CO_2 was less efficient than conventional organic solvents for the extraction of cloransulam-methyl from soil; polarity modifiers had no impact on extraction efficiency. Water was as effective as strong organic solvents for the extraction of cloransulam-methyl. Extraction temperature was the most important variable in increasing the efficiency and rate of extraction, while extraction pressure was not a significant variable. These results indicate that subcritical water is a viable alternative to conventional organic solvents for the extraction of cloransulam-methyl from soil; however, cloransulam-methyl is not hydrolytically stable at 150°C. It is

Table 2
Summary of recoveries (average±standard deviation) for the subcritical water extraction and conventional organic solvent extraction of cloransulam-methyl from soil

Sample	% of ^{14}C Extracted		
	Sonication[a]	Subcritical water extraction	
		100°C	150°C
Wayside, MS (1 DAT)	69±7	68±6	77±4
Wayside, MS (92 DAT)	64±4	77±7	78±6
Greenfield, IN (1 DAT)	88±8	75±9	87±8

[a] Sonication with 80:20 acetone:1 N HCl for 4 h.

clearly important to verify the stability of analytes under subcritical water extraction conditions.

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