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Supercritical Fluid Extraction for Liquid Chromatographic Determination of Pyrazosulfuron-Ethyl in Soils

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Pyrazosulfuron-ethyl (ethyl-5-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-1-methylpyrazole-4-carboxylate) is a class of sulfonylurea herbicides used widely for weed control in rice in Korea. It is active against annual and perennial broad leaf weeds as well as barnyard grass. This herbicide has become more popular due to its high activity at low application rates and low mammalian toxicity. Although pyrazosulfuron-ethyl would appear to be degraded rapidly in soils, as are other sulfonylureas (Kim et al 2003a and 2003b; Mikada et al 1996), the widespread use of this pesticide is a potential concern over soil contamination. Some reports demonstrated that sulfonylureas significantly decreased in soil biota (Bold and Jacobsen 1998; Landstein et al 1995; Wei et al 1998), suggesting that sulfonylureas such as pyrazosulfuron-ethyl pose potentially threat to human. This brings up scientific activities into development of analytical methodologies for monitoring of sulfonylureas in the environment.

A number of methodologies for detection of sulfonylureas have been reported (Marek and Koskinen 1996; Powley and de Bernard 1998; Hickes and Watrous 1999). Sulfonylureas are generally hydrolyzed followed by derivatization prior to gas chromatographic analysis, since they have low volatility and thermal instability. The most common method is high performance liquid chromatograph (HPLC) method. The instrumental methodologies are based on solvent extraction method. They are time consuming and expensive because of high cost of the solvent in sample preparation steps. Supercritical fluid extraction (SFE) has been introduced as an extraction method to avoid the limitations of solvent extraction methods. Many applications of the SFE for extraction of pesticides from soils have been reported (Goli et al 1997; Malone et al 1997; Senseman and Ketchersid 2000). In the present study, we examined a SFE method for the extraction of pyrazosulfuron-ethyl from soils. Two types of soils were treated with pyrazosulfuron-ethyl and incubated for a five-month period. SFE of pyrazosulfuron-ethyl were performed at set intervals over time to determine this pesticide in the soils.

MATERIALS AND METHODS

All chemicals used in this study were of analytical grade, unless otherwise stated.

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Pyrazosulfuron-ethyl standard (98%) was kindly supplied by Nissan Chemical Ind. Ltd. (Japan). All solvents used in this study were obtained from Burdick & Jackson (CA, USA), and of HPLC grade. Two different types, loam and silty loam, of soils were collected from the topsoil of agricultural fields located at Yeongi, Chungnam, and Hanam, Kyunggi, in Korea. The soils had no past history of pyrazosulfuron-ethyl treatment. Some physicochemical properties of the soils are given in Table 1. The soils were passed through a 2 mm-sieve after air-drying, mixed thoroughly, and stored in the dark at 4°C until use.

Table 1. Some physicochemical characteristics of soils used.

Soil	pH	OM	CEC	Soil component (%)			Soil texture
	(1:5H_2O)	(%)	($cmol^+/kg$)	Sand	Silt	Clay	
A	6.2	3.2	3.3	44	44	12	Loam
B	6.4	1.0	5.7	57	23	20	Silty loam

Approximately 2 kg of each soil had been equilibrated in the dark at 27±1°C for a week before pyrazosulfuron-ethyl was added. Fifty-gram portions of each soil were weighed and transferred to a series of triplicate 300 mL flasks. Pyrazosulfuron-ethyl dissolved in methanol was carefully added dropwise to the soils at a concentration of 0.4 ppm, which was the application rate in paddy soil in Korea. Control soils were treated with only methanol. The treated soils were thoroughly mixed after the solvent had evaporated, and distilled water was added to give 1.0 cm of water depth above the soil surface. The flasks were then incubated in the dark at 27±1°C over a five-month period. The water depth was maintained at the initial level by replenishing the distilled water biweekly. Triplicate flasks were taken at an interval time for analysis, and stored at -20°C until analyzed.

For SFE, extraction parameters, such as extraction time, supercritical fluid volume, co-solvent (modifier) type, modifier concentration, pressure, and temperature, were optimized by examining the extraction efficiency of pyrazosulfuron-ethyl from the aged-soil with the pesticide for 24 hr. Soil A was used as a representative sample for optimizing the SFE conditions because it has high organic matter. Thirty gram (wet wt.) portions of the soil were transferred to series of freeze-dryer vessels and dried using a model SFDSM12 Samwon freeze-dryer (Busan, Korea). The freeze-dried soils were thoroughly mixed and 30 g of the soils were transferred to the SFE vessel. The soils were extracted using the SFE, with supercritical CO_2 or supercritical CO_2 containing methanol, as a modifier, where the concentration of methanol was increased with each sample. Extracts were collected every 5 min for 60 min by inserting the outlet of the SFE restrictor into a 50 mL round-bottomed flask. The solvent was evaporated at 40°C using a vacuum evaporator. The residue was then dissolved in 3 mL of methanol and subjected to HPLC analysis.

The soil samples were also extracted by a conventional method using solvent to

compare with the SFE. For this, the soil samples (30 g) were extracted with two volumes of 50% (v/v) aqueous methanol on a shaker at 200 rpm for 1 hr. The extracts were vacuum-filtered on a Büchner funnel layered with a Whatman No. 6 filter paper. The filtrates were transferred into 1L-separatory funnel containing 500 mL of water, 50 mL of saturated NaCl solution, and 2 mL of 6N-HCl solution. The mixtures were then extracted twice with 100 and 50 mL of dichloromethane, respectively. After extraction, the organic phase was dehydrated over anhydrous sodium sulfate and concentrated to a small volume (approximately 3 ml) in an evaporator. The remaining dichloromethane was evaporated to dryness under a gentle stream of nitrogen gas. The dried extract was dissolved in 5 mL of *n*-hexane and subjected to silica gel column chromatography. A chromatographic glass column (15 mm x 50 cm length) was slurry-packed with 10 g of silica gel (70-230 mesh, Sigma, St. Louis, MO, USA) in *n*-hexane. The column was prewashed with 100 ml of *n*-hexane, and the dissolved sample in *n*-hexane was carefully added to the column. The column was washed with 100 ml of a solvent mixture composed of ethyl acetate and *n*-hexane (3/7, v/v). The eluate was then discarded. The column was washed again with 150 ml of a solvent mixture composed of ethyl acetate and *n*-hexane (5/5, v/v). This eluate was collected. The collected eluate was evaporated to about 3 ml by a rotary vacuum evaporator at 40°C. The remaining solvent mixture was evaporated to dryness under a gentle stream of nitrogen gas. The resultant residue was dissolved in 10 ml of acetonitrile for HPLC analysis.

Recovery test was carried out by determining pyrazosulfuron-ethyl spiked in the control soils. For this, the air-dried soils were weighed and placed on an aluminum foil. Pyrazosulfuron-ethyl prepared in methanol was added uniformly to the soils at a concentration of 0.5 and 2.0 ppm. The solvent was allowed to evaporate in a ventilated hood, and the soils were mixed thoroughly. Fifty gram portions of the soils were transferred to a series of triplicate 300 mL flask and then distilled water was added to the soils to give 1.0 cm of water depth above the soil surface. The soils were extracted by the SFE, under the optimized conditions as determined above, and by the solvent extraction method as described above. The concentrations of pyrazosulfuron-ethyl were determined on the standard calibration curve. All experiments were performed three replicates, unless otherwise stated.

A Kontron Model 322 HPLC (Italy) equipped with a Kontron Model 335 UV/Vis detector was used. HPLC column was a Waters Novapak C_{18} stainless column (particle size 4 μm, 4.6 i.d. x 250 mm). Mobile phase was acetonitrile/water (2/1, v/v) containing 0.2% (v/v) acetic acid with a flow rate of 0.6 ml/min. Pyrazosulfuron-ethyl was detected at 254 nm. A Jasco Model PU980 pumps (Japan), with one for pure CO_2 and the other for methanol, were used for the SFE. The pumps were connected to a 10 mL stainless SFE vessel (10 mm in O.D. x 15 cm in length). The vessel was placed in a Jasco Model 965CO column oven chamber to maintain the extraction temperature. The flow rate and pressure of supercritical fluid were maintained by the restrictor (Model 880-81, Jasco). All

the samples were injected onto HPLC in a 10 μl-volume size. Pyrazosulfuron-ethyl concentrations in the samples were calculated from the standard calibration curve. The standard calibration curve was obtained by injecting seven-level concentrations ranged from 0.05 to 4.0 ppm, and measuring the peak areas of their chromatograms. The ratio of signal to noise (S/N) was 3. All experiments were performed three replicates, unless otherwise stated.

RESULTS AND DISCUSSION

Our main contribution in this study was to examine a supercritical fluid extraction method for the extraction of pyrazosulfuron-ethyl from soils. The methodology for extraction of pesticides from complex matrices is one of crucial factors that affect sample preparation step in pesticide residue analysis. SFE conditions for the extraction of pyrazosulfuron-ethyl were established by examining extraction parameters. The extraction parameters were determined by using the aged-soils in order to investigate the extraction efficiency from the actual soil samples. The optimal conditions for the extraction of pyrazosulfuron-ethyl were determined to be 25% (v/v) methanol for the modifier concentration, and 80°C and 300 atm for the SFE apparatus. As a result, these conditions were used for the sample extractions throughout this study. When time-course SFE of pyrazosulfuron-ethyl was performed under the optimized conditions, about 53% of the pyrazosulfuron-ethyl found in the soils was extracted within the first 5 min (Table 2). It took 20 min to achieve more than 94% extraction using the SFE. Approximately 99% extraction was observed in a 30 min-extraction time. Pyrazosulfuron-ethyl was found to be approximately 0.39 ppm in a 30 min-extraction time, close to 0.40 ppm initially added to the soil. An undetectable level of pyrazosulfuron-ethyl was observed in a 50 min-extraction time. The total volume of methanol consumed to reach almost complete extraction was estimated to be 22.5 mL.

Table 2. Time-course percentage values for supercritical fluid extraction of Pyrazosulfuron-ethyl from the aged soils.

Extraction number (Each 5 min)	Extraction percentage (%)[a]
1	53.23 ± 4.22
2	24.60 ± 2.78
3	11.90 ± 1.32
4	4.77 ± 0.55
5	3.23 ± 0.55
6	1.20 ± 0.45
7	0.66 ± 0.25
8	0.26± 0.05
9	0.10 ± 0.10
10	0.05 ± 0.03
11	ND[b]

[a] The data given are means ± SD of triplicate determinations.
[b] Not detectable

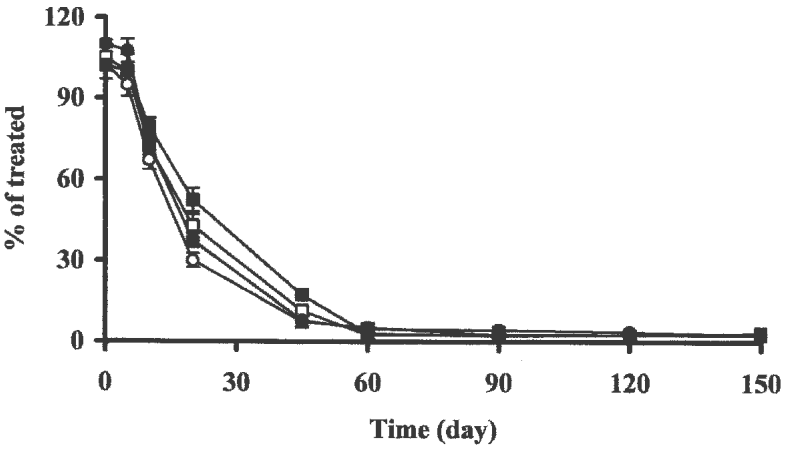

Figure 1. Degradation of pyrazosulfuron-ethyl in the soil A (○, ●) and soil B (□, ■) by SFE (open symbols) and solvent extraction (closed symbols). The data given are the means ± SD of triplicate measurements.

The recovery values of pyrazolsulfuron-ethyl by solvent extraction ranged from 97 to 108%. The mean values of recovery percentage using the SFE ranged from approximately 100 to 107% with a maximum of 6% standard deviation in soil A and 98 to 105% with a maximum of 5% standard deviation in soil B, respectively (Table 3), which was very similar to the values by solvent extraction. These results suggest that the SFE was good enough to extract pyrazolsulfuron-ethyl from the soils as solvent extraction method.

Table 3. Recovery percentage values of pyrazosulfuron-ethyl in soils.

Soil	Fortified level (ppm)	Recovery value (%)[a]	
		SFE method	Solvent extraction method
A	0.5	99.7 ± 4.2	96.4 ± 2.2
	2.0	106.9 ± 6.1	102.6 ± 5.7
B	0.5	97.6 ± 4.8	98.3 ± 3.2
	2.0	104.7 ± 2.5	107.6 ± 2.7

[a] The data given are means ± SD of triplicate determinations.

The data for the concentration of pyrazosulfuron-ethyl extracted from the soils using the SFE are provided in Figure 1. The concentrations of pyrazosulfuron-ethyl at time zero extracted by SFE from the incubated soils were approximately 0.44 ± 0.02 and 0.40 ± 0.01 ppm (n=3) in soil A and soil B, respectively, which was close to the initially added concentration of 0.40 ppm. Approximately 95% of

the treated pyrazosulfuron-ethyl was found by SFE after incubation for 5 days. Pyrazosulfuron-ethyl was determined to be about 67 to 72% of the initially treated concentration by SFE after 10 days-incubation, which was similar to the values by solvent extraction. Pyrazosulfuron-ethyl was found to dissipate continuously with incubation time, giving almost complete dissipation in incubation for 45 days. The half-lives of pyrazosulfuron-ethyl in the soils by SFE were estimated to be 14 and 20 days in the soil A and the soil B, respectively, similar to the half-lives by solvent extraction. Almost the same extraction levels were observed between SFE and solvent extraction after 20 days-incubation. The half-lives by SFE slightly shorter than by solvent extraction was probably due to the fact that pyrazosulfuron-ethyl become solvent non-extractable (bound) residues. Pesticides tend to bind to soils with incubation, which resulted in poor extraction and low degradation of pesticides from the soils (Celi et al 1997; Lee et al 1991; Kim et al 2002). Pyrazosulfuron-ethyl might also bind to soils, as other sulfonylureas (Kim et al 2003a and 2003b). Supercritical CO_2 has solvent properties equivalent to *n*-hexane, a relatively nonpolar solvent. Low extraction efficiency, therefore, would be observed for polar analytes and bound residues.

There have been many studies on the application of the SFE for the extraction of pesticides (Barnabas et al 1994; Papilloud et al1996; Robertson and Lester, 1994; Steinheimer et al 1994). Most of the studies used the SFE for extraction of pesticides from the spiked samples. In our study, we examined a SFE method for the extraction of pyrazosulfuron-ethyl from the actual soil samples incubated with the pesticide. No further sample clean up steps were required using the SFE prior to HPLC injections, suggesting that the SFE is cost effective in terms of saving the material and labor cost. It was suggested that SFE was strong enough to reach the same extraction efficiency as solvent extraction. SFE may be an alternative to solvent extraction in soil extraction of pyrazosulfuron-ethyl, since it minimizes solvent cost, sample preparation steps such as liquid-liquid partitioning and column clean up and waste solvent disposal, and it utilizes a nontoxic solvent such as CO_2.

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