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Comparative study of three extraction procedures for imazamethabenz-methyl in agricultural soil☆

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Abstract

Three procedures for the extraction of imazamethabenz-methyl in soils, using (a) various organic solvents, (b) water at different pH values and (c) supercritical carbon dioxide, were compared. Extraction with methanol, water at pH 11 or methanol-modified supercritical CO_2 were found to be the most suitable methods. These three procedures are suitable for sample amounts of 2 g and fortification levels of 6 mg/kg, giving herbicide recoveries near 90%. Subsequent determination of the herbicide in the extracts is carried out using HPLC with an octadecylsilane column, a mobile phase of acetonitrile–water (40:60), a flow-rate of 1 ml/min and ultraviolet detection at 210 nm. The detection limits achieved in the three procedures were always lower than 0.05 mg/kg in routine analysis.

1. Introduction

Imazamethabenz-methyl, a mixture of methyl 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-*m*-toluate and methyl 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-*p*-toluate, is a relatively recent avenicide of low toxicity and accumulation in animals [1] but long persistence in agricultural soils [2], which may adversely affect the growth of rotation crops of commercial interest such as beetroot.

There are relatively few methods available for the determination of this herbicide. In recent years an immunoassay method has been developed for its determination in cereal grains [3] and a thin-layer chromatographic method for the determination of imazamethabenz-methyl and some of its metabolites in vegetable products [4]. Solvent extraction, prior to GC analysis, has been used for the determination of imazamethabenz-methyl and its acid metabolite in plants and soils [2].

In this work, we carried out a comparative study of several methods for the extraction of imazamethabenz-methyl from agricultural soils, followed by its chromatographic determination, in order to assess residual levels of the avenicide following its application. Three procedures for the extraction of pesticides of medium polarity were tested [5–7], viz., a conventional extraction with an organic solvent, a solid-phase extraction (SPE) and an extraction with supercritical car-

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bon dioxide. For each procedure, the optimum extraction conditions for the determination of imazamethabenz-methyl in soils were investigated. For the determination step, the performances of reversed-phase high performance liquid chromatography (HPLC) and capillary gas chromatography (cGC) were compared, considering two types of detection in the HPLC approach, viz., UV absorption and mass spectrometry (MS).

2. Experimental

2.1. Chemicals

Chromatographically pure imazamethabenz-methyl standards were obtained from Promochem (Wesel, Germany). The commercial formulation Assert, 30% (w/v), from Cyanamid American (Princeton, NJ, USA) was also used.

Residue analysis-grade methanol, ethyl acetate, diethyl ether, dichloromethane, ethanol, acetone and 2-propanol were provided by Scharlau (Barcelona, Spain). HPLC-grade acetonitrile was purchased from Panreac (Barcelona, Spain). Ultra-pure water was obtained with a Milli-Q apparatus from Waters (Milford, MA, USA). Sodium hydroxide and hydrochloric acid were supplied by Scharlau.

Octadecyl, octyl, ethyl, phenyl, cyclohexyl, cyanopropyl, aminopropyl and diol 500 mg Bond Elut cartridges from Analytichem International (Harbor City, CA, USA) were employed for SPE. Disposable syringe filter units, pore size 0.50 μm, were obtained from Microfiltration Systems (Dublin, CA, USA).

2.2. General instrumentation

A Turbo-vap evaporator system was obtained from Zymark (Hopkinton, MA, USA), with a thermostated water-bath and a nitrogen stream. A CL/Samplextract solid–liquid extraction system was purchased from Cromlab (Barcelona, Spain), furnished with a vacuum pump. Centrifuges were supplied by Kokusan (Tokyo, Japan) and mechanical shakers by Selecta (Barcelona, Spain).

2.3. HPLC equipment

The HPLC equipment consisted of a ConstaMetric 4100 pump coupled to an eluent degas module, an AutoMetric 4100 autosampler and a SpectroMonitor 3200 UV–visible detector, all supplied by LDC Analytical (Riviera Beach, FL, USA). Detector signals were acquired and processed by means of a LC Talk workstation from LDC Analytical.

The chromatographic column was a 150 × 4.6 mm I.D. Novapak octadecylsilane column from Waters, with acetonitrile (ACN)–water (40:60) as the mobile phase at flow-rate 1 ml/min and room temperature (retention time 2.1 min). To achieve the optimum mobile phase, a sequential process was carried out: first a gradient from 15% to 100% ACN was run, then a response curve for the capacity factor was drawn from the times obtained for the front, the imazamethabenz-methyl and the overall gradient time. In an attempt to obtain a procedure for rapid routine analysis, it was found that a time of ca. 2 min was adequate, for which a concentration of 40% ACN was chosen. The volume injected was 20 μl and UV detection was performed at 210 nm.

2.4. Mass spectrometric analysis

A model 5989A Engine mass spectrometer from Hewlett-Packard (Avondale, PA, USA) was used. With this instrument, two analytical modes are possible, GC–MS and HPLC–particle beam (PB)-MS. The gas chromatograph was a Model 5989 Series II equipped with a Model 7673 automatic sampler and a 0.25-μm HP-5 capillary column (30 m × 0.25 mm I.D.), all from Hewlett-Packard. The HPLC equipment consisted of a Series 1050 degasser, pump and automatic sampler and the mass spectrometry system was managed by an MS Chemstation; all were supplied by Hewlett-Packard. The room was thermostated at 22°C.

The conditions used in the GC analysis were

as follows: temperature programme, initially 57°C for 1 min, then increased at 10°C/min to 270°C, held for 1 min; transfer line temperature, 280°C; ion source temperature, 200°C; quadrupole temperature, 100°C; injection, splitless mode (purge on at 0.8 min) at 225°C; volume injected, 1 μl; pressure programme, initially 159 kPa for 0.8 min, then decreased at 676 kPa/min to 31 kPa and increased at 2.8 kPa/min to a final pressure of 101 kPa held for 7 min; carrier gas, helium.

For the HPLC analysis, the conditions were as follows: column, octadecylsilane-2 (250 × 2.0 mm I.D.) from Sugelabor (Madrid, Spain); mobile phase, water–methanol (30:70, v/v) prepared by the pump; flow-rate, 0.3 ml/min; volume injected, 4 μl; retention time, 4.3 min. The parameters of the particle beam interface were as follows: desolvation chamber temperature, 60°C; helium pressure 345 kPa; nebulizer position, 9.5; capillary tube, slightly visible. Quadrupole temperature, 100°C; ion source temperature, 250°C. In both GC and HPLC analyses, positive and negative chemical ionization were performed with methane as reagent gas. The electron multiplier voltage was always 350 voltage units above autotune.

2.5. Soil fortification

Soil samples were fortified by adding a volume of 3 ml of an aqueous solution of imazamethabenz-methyl at an appropriate concentration to an amount of soil of ca. 30 g that had previously been sieved through a 2-mm mesh sieve. The soil was then homogenized by shaking for 1 h and the moisture was removed by allowing it to stand at room temperature (18–22°C) in darkness for 24h. The dry samples were then kept at 4°C.

For the experiments, a sandy soil containing 23% clay and 0.8–1% organic matter was used.

2.6. Extraction with organic solvents

An amount of soil (0.25, 0.5, 1, 2 and 5 g), previously fortified with 0.6, 1.2, 3 and 6 mg/kg of the avenicide, was extracted with 50 ml of different organic solvents, viz., methanol, acetonitrile, ethyl acetate, diethyl ether, 2-propanol, ethanol, acetone and dichloromethane, in 100-ml glass vessels by mechanical stirring for 1 h. The liquid phase was separated by centrifugation at 2800 *g* for 10 min and collected. The soil sample was subjected to a further extraction with 50 ml of solvent and the collected liquid phase was combined with the previous one and evaporated to dryness at 35°C under a nitrogen stream. Finally, the extract was dissolved in 1 ml of methanol and passed through a 0.5-μm pore size PTFE filter.

2.7. Solid-phase extraction

Cartridges packed with 500 mg of one of eight different stationary phases (octadecylsilane, octyl, ethyl, phenyl, cyclohexyl, cyanopropyl, aminopropyl and diol) were tested for the solid–liquid extraction of imazamethabenz-methyl dissolved in water. The minicolumns were washed sucessively, with 10 ml of methanol and 10 ml of water, both with elution by gravity. Then, 500 ml of water fortified with 0.1 mg/l of imazamethabenzmethyl was passed through the cartridges at 10 ml/min using a suction system.

The cartridges were then dried by circulating a forced air stream through them for 30 min, eluted with 2 ml of methanol by gravity and then centrifuged in order to recover the remaining methanol. The extract was finally passed through a 0.50-μm pore size PTFE filter and was then ready for chromatographic analysis.

Soils were extracted by adding 100 ml of water to 0.25–5 g of fortified soil containing 0.6–6 mg/kg of the herbicide. The mixture was shaken in 200-ml glass vessels for 2 h, then the liquid and solid phases were separated by centrifugation at 2800 *g*. The extraction process was repeated twice. The collected aqueous phase was filtered through a glass plate and eluted through the selected cartridge. The effect of pH on recovery was also investigated. For this purpose, pH values, of 3.0, 5.0, 7.0, 9.0 and 11.0, adjusted with HCl and NaOH, were tested.

2.8. Supercritical fluid extraction

A Model 7680A supercritical fluid extractor from Hewlett-Packard was used for extraction. The extractor was equipped with a sample thimble of 7 ml and a trap packed with 550–650-μm stainless-steel balls. Liquid CO_2 (99.999% minimum purity; Carburos Metálicos, Madrid, Spain) was supplied from a cylinder furnished with a direct injection probe.

The following conditions were maintained constant: CO_2 flow-rate, 3 ml/min; equilibrium time, 1.5 min; extraction time, 20 min; nozzle temperature, 85°C; trap temperature during the extraction, 10°C. The density (pressure) and extraction chamber temperature were assessed. The effects of the amount of sample (0.25–2.5 g) and the fortification level (0.6–6 mg/kg) were also evaluated. The extract was eluted from the trap with 1 ml of methanol at a flow-rate of 1 ml/min and a trap temperature of 50°C.

Different modifier types were placed in the thimble just before beginning the extraction step, for studying the variation of the CO_2 solvent power and the recovery.

Table 1
Relative abundance of imazamethabenz-methyl fragments in mass spectrometry

m/z	GC	HPLC	
	Methanol	Methanol	Water
Electron impact ionization			
288	6.3	3.2	1.8
256	2.3	58.0	22.1
245	45.6	27.9	16.5
214	22.4	39.4	19.6
187	14.1	91.9	57.2
176	53.4	33.3	23.1
162	5.7	3.6	2.7
144	100.0	100.0	100.0
117	20.5	73.4	42.4
89	23.7	80.4	51.5
Positive chemical ionization			
289	100.0	32.4	18.8
257	85.3	100.0	100.0
229	84.4	82.0	84.6
Negative chemical ionization			
288	100.0	—[a]	—
257	—	100.0	100.0
256	85.2	—	—

[a] Dashes indicate not observed.

3. Results and discussion

3.1. Mass spectrometric characterization

Table 1 shows the ions and relative abundances obtained by injecting imazamethabenz-methyl in water and methanol into the GC–MS and HPLC–PB-MS systems in the electron impact and chemical ionization modes. The base peak in the former mode was that at m/z 144; the abundance of the remaining masses differed as a function not only of the particular chromatographic technique, but also of the solvent in which the analyte was dissolved. The base peak in the positive chemical ionization mode also varied with the technique used, viz. $[M+H]^+$ in GC and $[M-32]^+$ in HPLC, the latter probably due to the loss of CH_3OH. The differences were even greater in the negative chemical ionization mode: the molecular ion, M^+, was the most abundant in GC, but not in HPLC, where the peak at m/z 257 was particularly abundant.

HPLC is more suitable than GC, with hot splitless injection, for the determination of imazamethabenz-methyl on account of the higher repeatability of the chromatographic peaks in the former technique. The relative standard deviation (R.S.D.) ($n=5$) for the area of the most intense chromatographic peak obtained using GC–EI-scan (volume injected 1 μl, concentration 8 mg/l) and that obtained using HPLC–EI-scan (volume injected 2 μl, concentration 45 mg/l) were 40% and 2.8%, respectively. In the HPLC trace only one peak was observed (co-elution of the *meta* and *para* isomers), whereas the GC trace for an imazamethabenz-methyl standard included several badly resolved peaks, the intensity and resolution of which showed poor repeatability; their spectra contained mass fragments similar to those shown in Table 1; the determination of their molecular ion by GC–

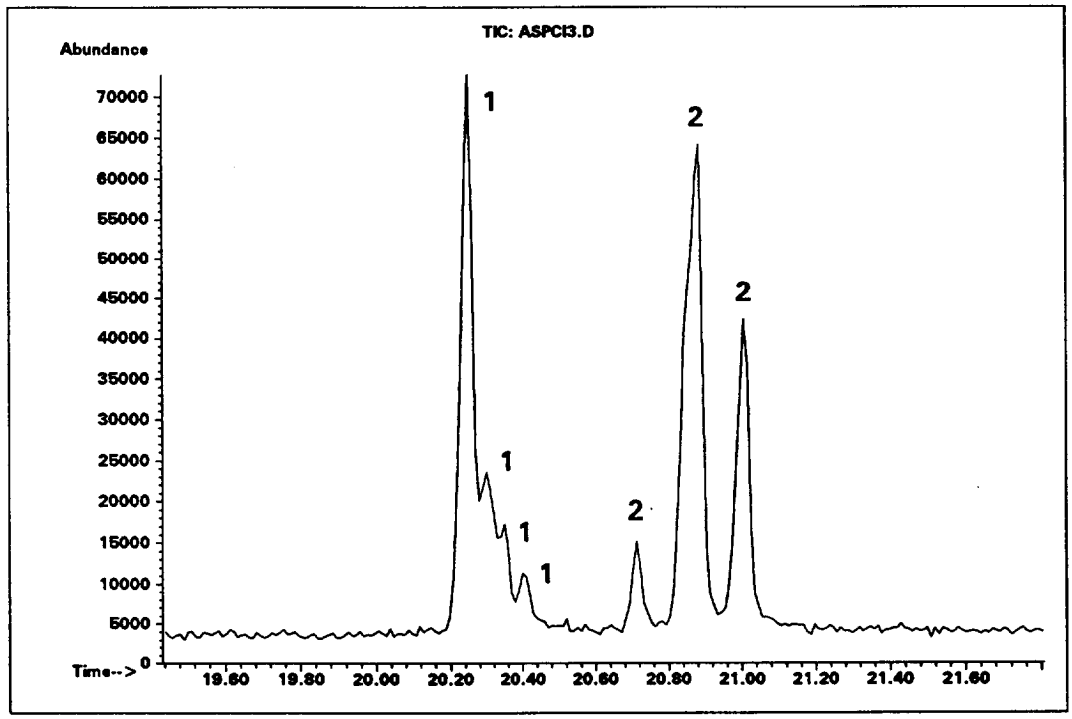

Fig. 1. Chromatogram of an imazamethabenz-methyl standard in methanol obtained by GC–positive CI-MS in scan mode. Peaks: 1 = unknowns, molecular ion 256 u; 2 = similar to imazamethabenz-methyl, molecular ion 288 u.

positive CI-MS revealed the occurrence of up to three compounds of molecular mass 288 u and up to four of molecular mass 256 u (Fig. 1), probably as a result of thermal degradation during the injection or separation process.

3.2. Determination by HPLC

The HPLC determination of imazamethabenz-methyl was addressed by using two different types of detection, viz., UV absorption and MS in the SIM mode. For the determination using HPLC–MS-SIM, the masses 144, 117 and 187 u were monitored; the first was used for measuring the peak area and the other two for corroborating the compound identity.

UV detection proved to be the most sensitive choice; in fact, it provided a detection limit of 0.02 mg/l, compared with 0.10 mg/l for MS detection. The detection limit was always calculated as three times the signal-to-noise ratio. The linear dynamic ranges were at least 0.1–10 mg/l for UV and 1–20 mg/l for MS detection.

3.3. Extraction with methanol

Based on the results given in Table 2, methanol was the most appropriate solvent for extracting the herbicide from soils, followed by acetonitrile, ethyl acetate and acetone. Methanol–water mixtures provided lower recoveries than pure methanol. Extractions were carried out by using an amount of 1 g of soil fortified at the 0.6 mg/kg level.

Fig. 2 shows the recoveries obtained by extracting 0.25, 0.5, 1, 2 and 5 g of soil fortified with 0.6, 1.2, 3 and 6 mg/kg of the herbicide. As can be seen, the recovery decreased slightly and progressively with increase in the fortification level (from 90% at 0.6 mg/kg to 87% at 6 mg/kg). On the other hand, there was no clear relationship between the recovery and the amount of sample used. The reproducibility, as

Table 2
Recovery of imazamethabenz-methyl with different organic solvents ($n = 5$)

Extractant	Recovery (%)	R.S.D. (%)	Extractant	Recovery (%)	R.S.D. (%)
Methanol	85.6	3.7	Acetone	65.9	5.0
Ethanol	55.8	4.8	Ethyl acetate	68.6	4.3
2-Propanol	45.0	4.2	Dichloromethane	39.8	3.0
Acetonitrile	78.4	4.0	Diethyl ether	50.4	4.4

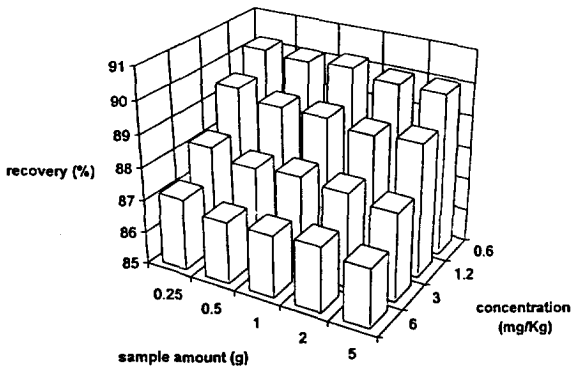

Fig. 2. Recovery of imazamethabenz-methyl obtained by methanol extraction from spiked soils ($n = 5$).

R.S.D. ($n = 5$), was 4%. The detection limit achieved, for 1 g of sample and with UV detection, was 0.02 mg/kg.

3.4. Solid-phase extraction

Table 3 shows the results obtained as regards retention of imazamethabenz-methyl (50 μg) on cartridges packed with various stationary phases (octadecylsilane, octyl, ethyl, cyclohexyl and phenyl, and three of higher polarity, cyanopropyl, aminopropyl and diol). The non-polar phases provided higher recoveries, particularly octadecylsilane (90.5%), which was chosen for subsequent experiments. The retention on octadecylsilane was favoured by a slightly acidic pH, and we chose pH 4 (recovery 94.3%).

The effect of the pH of the water used to extract the soil (1 g fortified with 1.2 mg/kg of herbicide) is illustrated in Table 4. The recovery was favoured by a neutral or alkaline pH in the extracting water. A pH 11 was selected for performing the extractions.

Fig. 3 shows the recoveries obtained by extracting various amounts of soil (0.25, 0.5, 1, 2 and 5 g), containing different amounts of avenicide (0.6, 1.2, 3 and 6 mg/kg), with water at pH 11 and subsequently eluting the acidified

Table 4
Effect of the pH on the recovery of imazamethabenz-methyl in soils extracted with water ($n = 5$)

pH	Recovery (%)
3.0	74.3
5.0	80.1
7.0	89.2
9.0	89.5
11.0	89.6

Table 3
Recovery of imazamethabenz-methyl from water at pH 7 on different 500 mg cartridges ($n = 5$)

Stationary phase	Recovery (%)	R.S.D. (%)	Stationary phase	Recovery (%)	R.S.D. (%)
Octadecyl	90.5	3.6	Phenyl	88.6	4.1
Octyl	82.6	3.5	Cyanopropyl	53.8	4.4
Ethyl	78.4	4.0	Aminopropyl	42.5	4.4
Cyclohexyl	89.4	4.0	Diol	33.7	4.6

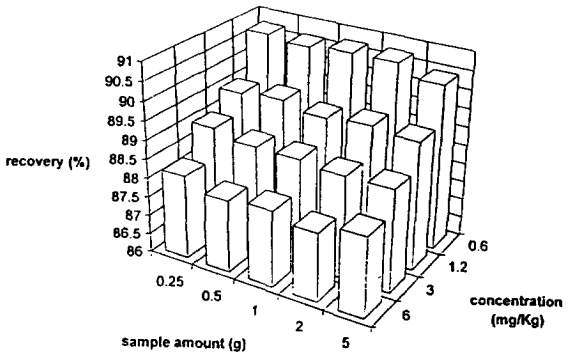

Fig. 3. Recovery of imazamethabenz-methyl obtained by extraction with water and octadecylsilane concentration from spiked soils ($n = 5$).

extracts through octadecylsilane cartridges. As can be seen, the recoveries were similar to those obtained with methanol, viz., 90.5% (0.6 mg/kg) and 88.0% (6 mg/kg). The reproducibility (R.S.D. = 4%, $n = 5$) was also similar. The variation of the sample amount between 0.25 and 5 g was a less important factor than the fortification level in relation to the recovery. The detection limit, however, was higher (0.04 mg/kg), but it is still adequate.

3.5. Extraction with supercritical carbon dioxide

The working conditions for extraction with supercritical CO_2 were optimized by performing several tests using 1 g of soil fortified with 1.2 mg/kg of herbicide. Extractions were initially carried out with no modifier in the fluid; as a result, only 20% of the herbicide was extracted at the highest density tried. Therefore, different compounds as modifiers of the solvent power of CO_2 were tested. As can be seen in Fig. 4a, extraction was favoured by aqueous media, although methanol provided the highest recovery. Fig. 4b shows the variation of the recovery with the volume of methanol added (density 0.8 g/ml, temperature 50°C); as can be seen, the recovery increased with increasing modifier volume up to 100 μl, then virtually levelled off. A methanol volume of 100 μl was selected as optimum, which was then used to obtain the density scan shown in Fig. 5. No further increase in the

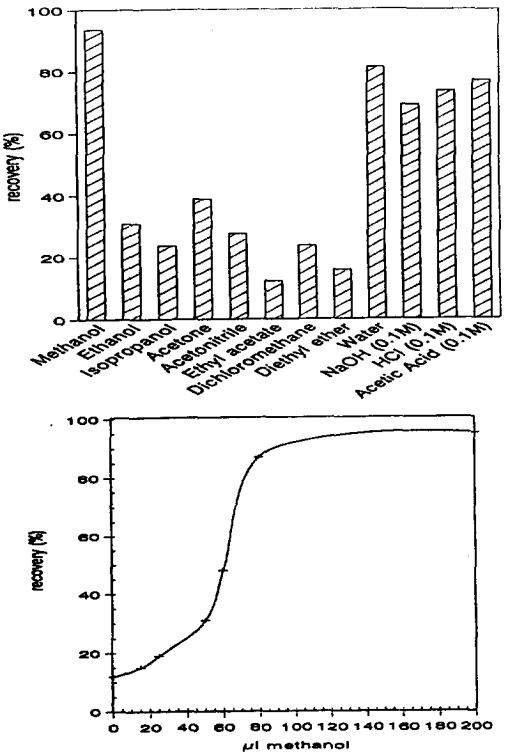

Fig. 4. Influence of the modifier on the SFE recovery. (a) Recovery for different modifiers; (b) recovery for different methanol volumes.

recovery was obtained above a density of 0.85 g/ml. The influence of the temperature in the extraction chamber was then studied in the range 30–80°C at a density of 0.85 g/ml; the recovery

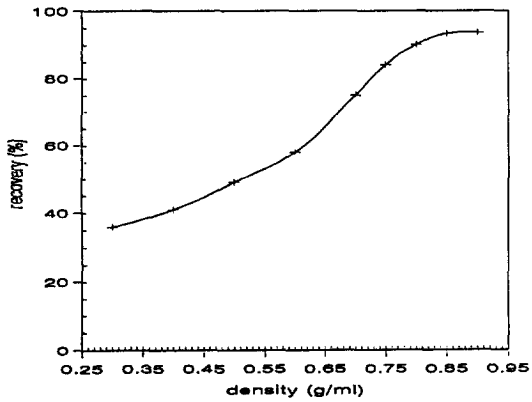

Fig. 5. Influence of the extraction density on the recovery obtained with supercritical carbon dioxide.

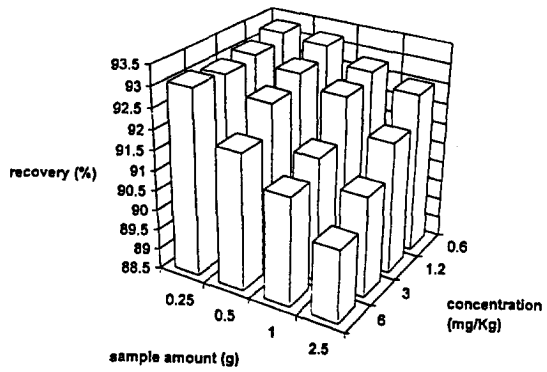

Fig. 6. Recovery of imazamethabenz-methyl obtained by supercritical CO_2 extraction from spiked soils ($n = 5$).

increased slightly with increase in temperature up to 50–55°C, above which it started to drop sharply. The initial equilibration time was found to have little effect on the recovery, so 1.5 min was used.

Fig. 6 shows the variation of the recovery as a function of the amount of sample and fortification level. The recovery was higher than in the previous procedures (nearly 93%) for the extraction of a 0.25-g sample, and was the same for low fortification levels (0.6 mg/kg) as for high levels (6 mg/kg). For sample amounts of $\geq$0.5 g, the recovery decreased with increasing fortification level and, unlike in the other two extraction procedures, also decreased when the amount of sample increased, particularly if the herbicide contamination was high. In any case, the differences in the recoveries in the different experiments were always small. The reproducibility of this procedure was higher (R.S.D. = 3%, $n = 5$). The detection limit for 1 g of sample was similar to those of the other two procedures (0.02 mg/kg). As can be seen in Fig. 7, which shows a chromatogram obtained by this procedure, the chromatographic peak for imazamethabenz-methyl was well resolved and frequently accompanied by a wider and unidentified posterior peak. The chromatograms obtained by extraction with methanol and water were similar, with absence of interferences, except that the second peak was not observed.

4. Conclusions

HPLC is a most suitable technique for determining imazamethabenz-methyl in soils. In fact, it is more reproducible than gas chromatography with hot splitless injection, possibly as a result of the imazamethabenz-methyl being thermally decomposed during the GC process. In conjunction with UV detection, HPLC provides a good detection limit for herbicide analysis. When HPLC was used, no separation between *meta* and *para* isomers was observed, so the determination was effected on a unique compound. It is possible that they could be separated using GC under other conditions, perhaps with cool on-column injection, but in our GC tests it was not possible to confirm this owing to the

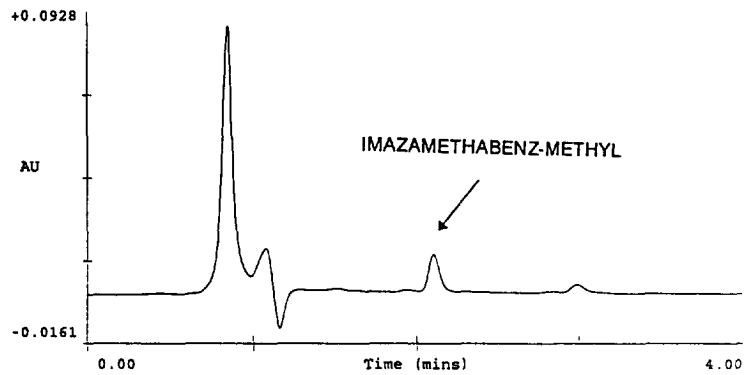

Fig. 7. Chromatogram of a supercritical fluid extract achieved by HPLC with ACN–H_2O (40:60) as eluent and UV detection. Sample amount, 0.5 g; fortification level, 0.6 mg/kg.

non-repeatability of the results and the lack of standards of these isomers.

In the three extraction procedures tested, using methanol, water at pH 11 (following concentration on octadecylsilane) and methanol-modified supercritical carbon dioxide, the recovery depends on the concentration of the avenicide in the soil, whereas the recovery in the SFE procedure is also dependent on the amount of sample, although the differences are slight for the concentration and sample amount levels tested; in fact, they never exceeded 4%, even at an unusually high fortification level for agricultural soils of 6 mg/kg.

All three extraction procedures provided recoveries near 90% by using 1–2 g of sample fortified with no more than 6 mg/kg of herbicide, and with similar reproducibilities. The proposed water and supercritical CO_2 extraction procedures are an advantageous alternative to the conventional procedures that require large amounts of organic solvents.

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