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# Determination of chlorsulfuron and tribenuron-methyl residues in agricultural soils

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**Abstract**

A high-performance liquid chromatography (HPLC) method for the determination of tribenuron-methyl and chlorsulfuron residues on different types of soils is proposed. For the extraction, three procedures using organic solvents, supercritical fluid or solid-phase extraction have been tested. Extraction with water at alkaline pH followed by concentration on octadecylsilane is the most advisable for chlorsulfuron, while for tribenuron-methyl, the use of methanol-modified supercritical $CO_2$ is more adequate. The extracts are analyzed by reversed-phase HPLC with acetonitrile–water (containing 0.1% $H_3PO_4$) (40:60) as mobile phase and UV detection at 220 nm. The type of soil and its herbicide content affect the recoveries obtained. © 1997 Elsevier Science B.V.

*Keywords:* Soil; Environmental analysis; Sample preparation; Pesticides; Tribenuron-methyl; Chorsulfuron; Sulfonylureas

## 1. Introduction

Chlorsulfuron and tribenuron-methyl are two sulfonyl ureas, herbicides with great phytotoxicity even at low concentrations [1].

The extraction of chlorsulfuron from agricultural soils has been carried out by several procedures, such as shaking with organic solvents [2] or mixtures with a high proportion of organic solvent [3], or aqueous solutions at controlled pH (9–10) due to the easy hydrolysis of these compounds [4,5]. The concentration of chlorsulfuron from aqueous solutions by octadecylsilane (ODS) has also been reported [6,7]. On the other hand, extraction with supercritical $CO_2$ of sulfonyl urea herbicides has been considered, the use of high solvent-modified $CO_2$ densities being established as a rule of thumb [8,9].

The determination of tribenuron-methyl has been very poorly studied with regards to other sulfonyl ureas. Recently, an immunoassay method for its determination in vegetals such as sugar-beet and lentils [10], and also, an extraction with supercritical $CO_2$ on inert materials [9] have been developed. The formation of thermally stable derivatives has also been reported [11].

Regarding to the determination in the extracts, high-performance liquid chromatography (HPLC) is mostly used for the analysis of sulfonyl ureas, mainly with UV detection [4,7,12,13], although gas chromatography [5,14], capillary electrophoresis [15] and supercritical fluid chromatography [16] are possible alternatives. A review of extraction and determination methods has been published [17].

The aim of this work was to develop a procedure

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to analyze tribenuron-methyl in soils, making a comparison with the behaviour of a compound of the same chemical family, chlorsulfuron. So, three extraction procedures, (a) mixing with organic solvents, (b) adding a buffer solution and subsequent concentration on ODS cartridges and (c) using supercritical carbon dioxide modified with methanol have been tested on three soils with different textures and different quantities of both herbicides. To analyze the extracts, reversed-phase HPLC with UV detection at 220 nm has been used.

## 2. Experimental

### 2.1. Chemicals

Chromatographically pure chlorsulfuron and tribenuron-methyl standards were obtained from Promochem (Wesel, Germany). Residue analysis-grade methanol, ethyl acetate, ethanol, acetone, acetonitrile, toluene and *n*-hexane were provided by Lab-scan (Dublin, Ireland) and Scharlau (Barcelona, Spain). HPLC-grade acetonitrile was purchased from Lab-scan. Ultrapure water was obtained with a Milli-Q apparatus from Waters (Milford, MA, USA). NaOH, $H_3PO_4$, $HNa_2PO_4$ and $Na_3PO_4$ were supplied by Scharlau. ODS 500-mg Bond Elut cartridges from Analytichem International (Harbor City, CA, USA) were used for solid-phase extraction (SPE). Disposable syringe filter units, 0.50-μm pore size, were obtained from Microfiltration Systems (Dublin, CA, USA).

### 2.2. General instrumentation

A Turbo-vap evaporator system, with thermostated water bath and nitrogen stream, was obtained from Zymark (Hopkinton, MA, USA). A solid–liquid extraction system was purchased from Varian (Harbor City, CA, USA). Finally, centrifuges were supplied by Kokusan (Tokyo, Japan) and mechanic shakers by Selecta (Barcelona, Spain).

### 2.3. Characterization and fortification of soils

Three types of soils with different textures, typical from Castle and Leon (Spain), have been used. Table 1 shows their features: texture, organic matter content, pH and cation-exchange capacity, measured according to official methods [18,19]. All the soils were sieved through a 2-mm mesh, and sterilized by heating at 120°C.

Table 1
Characterization of soils

| Soil | pH | Texture | | Organic matter (%) | Exchange capacity (mequiv./100 g) |
|---|---|---|---|---|---|
| | | Sand (%) | Clay (%) | | |
| A | 7.7 | 71.3 | 9.7 | 0.6 | 1.6 |
| B | 7.8 | 36.7 | 28.4 | 1.1 | 1.8 |
| C | 8.0 | 8.6 | 56.2 | 1.5 | 1.7 |

Soil samples were spiked by adding a volume of 10 ml of an acetonitrile solution containing both herbicides, to ca. 50 g of soil. The soil slurry was then homogenized by shaking for 1 h and the solvent was removed at room temperature (18–22°C) in darkness for 24 h. The dry samples were kept at 4°C until analysis.

### 2.4. Extraction with organic solvents

A sample of soil B (10 g), previously spiked with 0.6 mg/kg of each herbicide, was extracted with 125 ml of different organic solvents, viz. methanol, acetonitrile, ethyl acetate, ethanol, acetone, toluene and *n*-hexane, in glass vessels by mechanical stirring for 1 h. The liquid phase was separated by centrifugation at 3500 *g* for 10 min and collected. The soil sample was subjected to a further extraction with 125 ml of the same solvent and the liquid phase was combined with the previous one. This phase was then evaporated to dryness at 25°C under a nitrogen stream. Finally, the extract was dissolved in 0.5 ml of acetonitrile by sonication and passed through a 0.5-μm pore size PTFE filter.

### 2.5. Solid-phase extraction

Soils were extracted by adding 125 ml of a buffer solution ($PO_4^{3-}$/$HPO_4^{2-}$, 0.1 *M*) at pH 9.5 to 10 g of soil containing 0.06, 0.6 or 1.2 mg/kg of each herbicide. The mixture was shaken in glass vessels for 1 h, then the liquid and solid-phases were separated by centrifugation at 3500 *g*. The extraction

process was repeated and the aqueous phases were combined and acidified with $H_3PO_4$ to pH 3 for chlorsulfuron and pH 7 for tribenuron methyl.

ODS cartridges were rinsed by successive elution of 15 ml of methanol and 10 ml of water at pH 3 or 7 (depending on the herbicide). Then, the aqueous extract was passed through the cartridge at about 5 ml/min using a suction system. Cartridges were dried by passing a nitrogen stream through them for 30 min, and eluted with 4 ml of acetonitrile by gravity. The organic solvent was evaporated and, finally, the residue was dissolved in 0.5 ml of acetonitrile by sonication and filtered for its chromatographic analysis.

The effect of pH on cartridge recovery was also investigated. For this purpose, 250 ml of water containing 0.01 mg/l of each herbicide and pH values of 3, 5, 7 and 9, adjusted with $H_3PO_4$ and NaOH, were tested.

### 2.6. Supercritical fluid extraction (SFE)

A 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; Air Products, Sombreffe, Belgium) was supplied from a cylinder furnished with a direct injection probe.

The following conditions were maintained constant: $CO_2$ flow-rate, 2 ml/min; extraction time, 15 min; nozzle temperature, 85°C; trap temperature during the extraction, 10°C. The extract was eluted from the trap with 1 ml of acetonitrile at a flow-rate of 0.2 ml/min and trap and nozzle temperatures of 30°C. Extractions were performed on 5 g of soil spiked with the herbicides in the 0.06–1.2 mg/kg range. A methanol volume was placed in the bottom of the thimble just before beginning the extraction step. The effect of the equilibrium time, extraction chamber temperature, and methanol volume on the recovery was studied with soil B.

### 2.7. HPLC analysis

The HPLC equipment consisted of a ConstaMetric 4100 pump coupled to an eluent degasser module, an AutoMetric 4100 autosampler and a SpectroMonitor 3200 UV-visible detector, all supplied by LDC Analytical (Riviera Beach, FL, USA). The chromatographic column was a 150×4.6-mm ID Novapak ODS column from Waters, with acetonitrile (ACN)–water (containing 0.1% of $H_3PO_4$) (40:60) as the mobile phase at flow-rate 1 ml/min and room temperature. The retention time for chlorsulfuron and tribenuron-methyl was 3.0 and 7.5 min, respectively. The volume injected was 20 μl and UV detection was performed at 220 nm.

Calibration was made with herbicide standards solved in ACN, on the 0.02–5.00 mg/l range. The regression coefficients ($r$) were, at least, 0.999 for chlorsulfuron and tribenuron-methyl, and the detection limits achieved with standard solutions (three times the signal-to-noise ratio) were about 0.08 and 0.05 mg/l, respectively.

## 3. Results and discussion

### 3.1. Extraction with organic solvents

Fig. 1 shows the recoveries of chlorsulfuron and tribenuron-methyl obtained by solvent extraction on soil B. The recoveries of chlorsulfuron were similar for the different solvents, except $n$-hexane, varying between 63% (ethanol) and 71% (acetonitrile). The recoveries of tribenuron-methyl were lower than those for chlorsulfuron; the highest values were obtained with acetonitrile (53%) and ethyl acetate

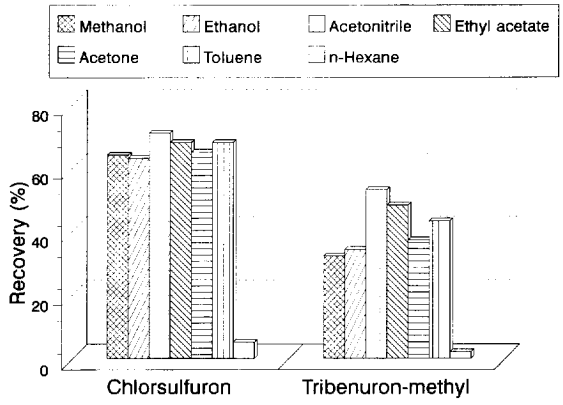


Fig. 1. Recovery of chlorsulfuron and tribenuron-methyl obtained by solvent extraction on soil B (0.6 mg/kg each one), $n=5$.

(48%). The relative standard deviation (R.S.D.) was close to 7–10% in all instances.

In order to improve the performance of the solvent extraction, the soil was extracted with four aliquots of 125 ml instead of two. In this case, the recovery was improved only 3% in the better case (with acetonitrile). On the other hand, the increase in the shaking time up to 2 h did not affect the recovery of the herbicides. The scarce performance of the extraction, mainly for tribenuron-methyl, could be related with the instability of these herbicides in solution [20].

### 3.2. Solid-phase extraction

Fig. 2 shows the recovery of the herbicides obtained by ODS cartridges from water at different pHs. As can be seen, the retention on ODS was favoured by an acidic pH for chlorsulfuron, being higher at pH 3–5. The optimum pH for tribenuron-methyl extraction was 7–9; the recoveries obtained at pH 3 and 5 indicate that tribenuron-methyl is easily hydrolyzed in weakly acid solutions.

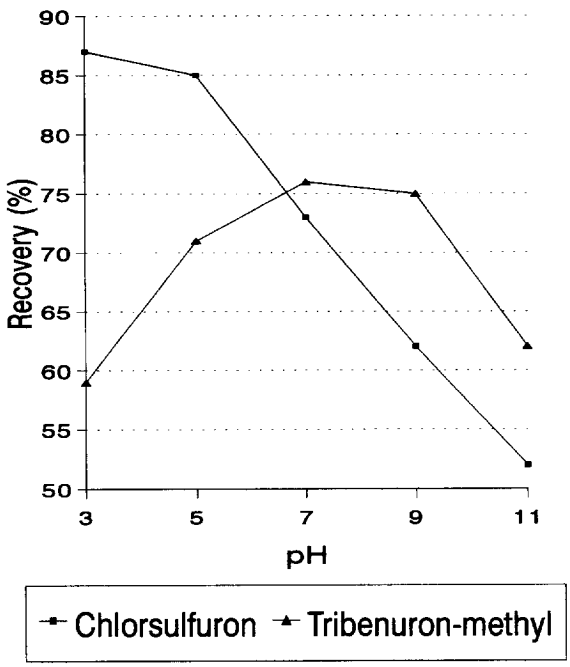


Fig. 2. Recovery of chlorsulfuron and tribenuron-methyl obtained by ODS cartridges from water at different pH values, $n=5$.

Table 2
Recovery of chlorsulfuron and tribenuron-methyl obtained by extraction with a buffer aqueous solution followed of solid-phase concentration from spiked soils, $n=7$

| Fortification level (mg/kg) | Recovery±R.S.D. (%) | | |
|---|---|---|---|
| | Soil A | Soil B | Soil C |
| *Chlorsulfuron* | | | |
| 0.06 | 86.0±3.7 | 86.0±3.8 | 86.3±3.5 |
| 0.6 | 86.5±4.0 | 86.2±4.0 | 85.8±3.9 |
| 1.2 | 85.8±3.3 | 85.9±3.9 | 84.3±3.8 |
| *Tribenuron-methyl* | | | |
| 0.06 | 74.8±4.1 | 75.0±3.9 | 73.9±4.1 |
| 0.6 | 70.6±4.3 | 69.5±4.4 | 68.1±4.0 |
| 1.2 | 65.3±4.0 | 63.7±4.5 | 62.2±4.4 |

R.S.D.: Relative standard deviation.

Table 2 lists the recovery of the two herbicides on the three types of soil and for three fortification levels. The recoveries were about 15% higher in comparison with the acetonitrile extraction, and also, the recovery of tribenuron-methyl (about 70%) was lower than that for chlorsulfuron (about 86%). An analysis of variance (ANOVA) of two ways for the chlorsulfuron results showed that there were not significant differences ($p=0.05$) in the recoveries for the two effects studied: fortification level and texture. However, the ANOVA showed significant differences ($p=0.05$) for the fortification level of tribenuron-methyl on the soils assayed, while the type of soil was not an outstanding factor. In fact, the recoveries decreased notably for high fortification levels while they decreased slowly with the clay content as it is illustrated in Table 2. We have not been able to find a simple explanation for this fact till now.

Extracts of non-treated soils spiked with the herbicides were used to determine the detection limits as three times the signal-to-noise ratio. The detection limits for both herbicides were about 7 μg/kg.

### 3.3. Extraction with $CO_2$

Several extraction parameters were kept constant during the experimentation. Among them, a high density of 0.85 g/ml and a relatively high $CO_2$ flow-rate, 2 ml/min, were used to enhance the

extractant power according to the above mentioned reports [8,9]. Extraction temperature, trap type and its temperature, were settled on the basis of our experiences with polar compounds [21,22]. On the other hand, an equilibrium time of 10 min and a temperature of 50°C were initially used to carry out the experiences.

Fig. 3 shows the variation of the recovery with the volume of methanol added to the sample. As can be seen, the recovery increased gradually with increasing methanol volume, reaching a maximum value. The volumes of 50 and 100 μl were selected as optimum for tribenuron-methyl and chlorsulfuron, respectively.

Fig. 4 shows the influence of the equilibrium time on the recovery, obtained at a temperature of 50°C and methanol volumes of 50 or 100 μl (as the optimum for each herbicide). As in the above case, the recovery increased gradually and then was virtually levelled off. An equilibrium time of 15 min was selected for chlorsulfuron and 10 min for tribenuron-methyl. No further increase in the recovery was observed for higher values.

The effect of the extraction chamber temperature

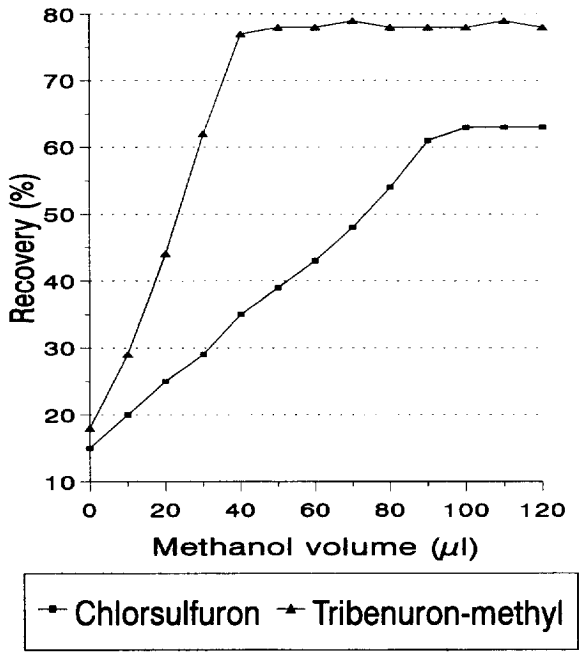


Fig. 3. Influence of the methanol volume on the recovery obtained by supercritical $CO_2$, $n=5$.

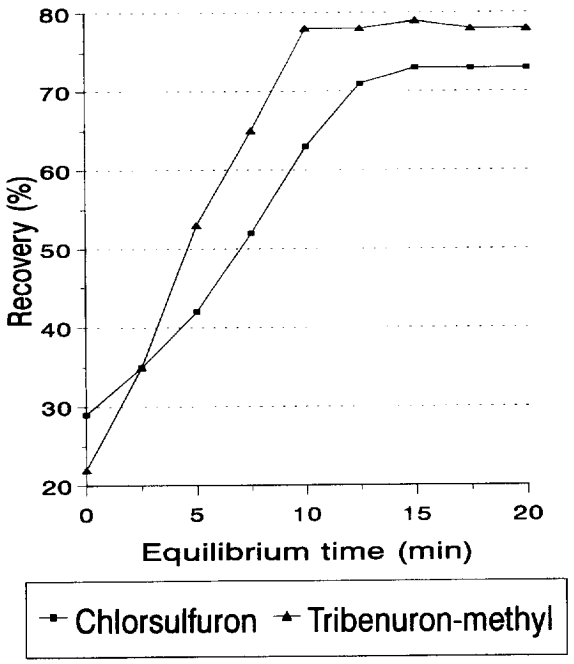


Fig. 4. Influence of the equilibrium time on the recovery obtained by supercritical $CO_2$, $n=5$.

is illustrated in Fig. 5, working with the optimized conditions exposed above. The recovery increased slightly with increase in the temperature up to 50–55°C for chlorsulfuron and 45–50°C for tribenuron-methyl, over which it started to decrease. A temperature of 50°C was considered suitable for the extraction of both herbicides.

Table 3 shows the recoveries obtained by SFE working with the parameters selected: 100 μl of methanol, equilibrium time 15 min and temperature 50°C for chlorsulfuron, and 50 μl of methanol, equilibrium time 10 min and temperature 50°C for tribenuron-methyl. As can be seen, the recoveries decreased slightly on increasing the clay content, and decreased notably for higher fortification levels. An ANOVA revealed that there were significant differences ($p=0.05$) for the fortification level in both herbicides as happened for tribenuron-methyl in the SPE procedure. This last procedure provided recoveries, at least 10% higher for chlorsulfuron and 3–5% lower for tribenuron-methyl in relation to the SFE procedure. The precision of this procedure (R.S.D. about 3%) was similar for both compounds

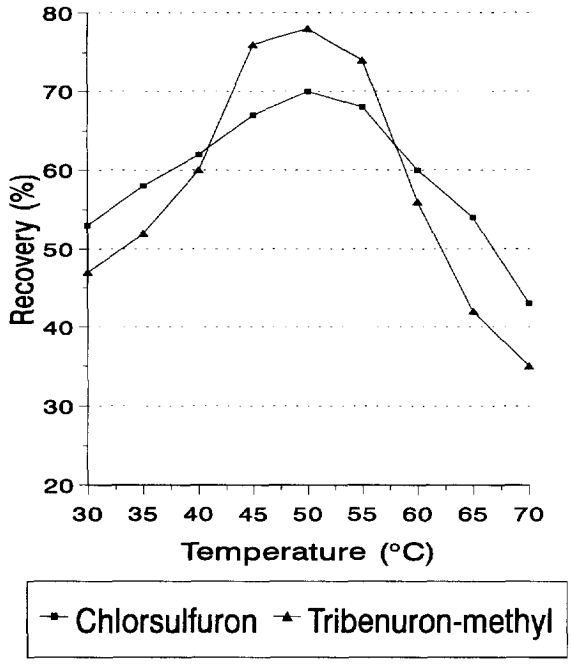


Fig. 5. Influence of the extraction temperature on the recovery obtained by supercritical $CO_2$, $n=5$.

and better than that for SPE (R.S.D. about 4%). The detection limit of this procedure was somewhat worse with regards to the SPE procedure, about 35 μg/kg.

Fig. 6 shows the chromatogram from a SFE extract, which was similar to those obtained by the other two methods. Differences were only found in the front intensity and, to a minor extent, in the presence of small chromatographic peaks not co-eluted with the target-compounds, whose number was higher in the chromatogram of the solvent.

Table 3
Recovery of chlorsulfuron and tribenuron-methyl obtained by extraction with supercritical $CO_2$ from spiked soils, $n=7$

| Fortification level (mg/kg) | Recovery±R.S.D. (%) | | |
|---|---|---|---|
| | Soil A | Soil B | Soil C |
| *Chlorsulfuron* | | | |
| 0.06 | 76.6±2.8 | 76.6±2.6 | 76.6±2.7 |
| 0.6 | 72.1±3.1 | 72.0±2.9 | 71.8±3.0 |
| 1.2 | 69.3±2.5 | 69.2±3.2 | 68.3±2.8 |
| *Tribenuron-methyl* | | | |
| 0.06 | 80.9±3.0 | 78.4±2.8 | 79.4±3.2 |
| 0.6 | 78.4±2.8 | 76.8±2.9 | 76.0±2.9 |
| 1.2 | 72.3±2.7 | 69.8±3.0 | 67.7±2.8 |

R.S.D.: Relative standard deviation.

### 3.4. Comparison of extraction procedures

A comparison of the features of the three extraction procedures for the tribenuron methyl analysis is reflected in Table 4. SPE and SFE are the most adequate in terms of recovering percentage and precision, with acceptable detection limits and being the selectivity of the three procedures acceptable; nevertheless, it must be taken into account that the recovery is affected by the amount of herbicide present in the soil.

As regarding operating time, SFE is the fastest technique. However, SPE could be quicker than SFE because the SPE-vacuum manifold enables the simultaneous extraction of a great number of samples (12–24 samples).

Similar commentaries can be pointed out for chlorsulfuron, except that the recoveries obtained by the three extraction procedures are comparable unlike the tribenuron-methyl results.

## 4. Conclusions

The extraction of chlorsulfuron and tribenuron-methyl by an organic solvent is not advisable in comparison with a solid-phase or supercritical $CO_2$ extraction on the basis of their recoveries. The extraction with water at alkaline pH followed by an acidification and solid-phase concentration provides better recoveries for chlorsulfuron than tribenuron-methyl. SFE is a good alternative for the analysis of tribenuron-methyl as deduced from the assays performed on spiked soils.

The clay content of the soils does not significantly affect the herbicide extraction. However, the herbicide amount in soil is always an important factor for tribenuron-methyl, unlike chlorsulfuron. Both herbicides show a different behaviour.

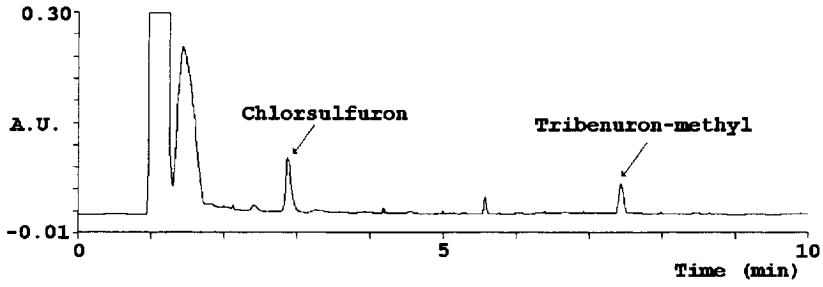


Fig. 6. Chromatogram of an extract obtained by the SFE procedure.

Table 4
Comparison of the extraction procedures for tribenuron-methyl analysis

| Extraction | Efficacy | Precision | Selectivity | Operation time | Affecting factors | Detection limit |
|---|---|---|---|---|---|---|
| Solvent | + | ++ | +++ | ++ | No data | No data |
| Solid-phase | ++ | +++ | +++ | ++ | Concentration | +++ |
| Supercritical fluid | ++ | +++ | +++ | +++ | Concentration | +++ |

+: Bad.
++: Regular.
+++: Good.

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## References

[1] A.M. Blair, T.D. Martin, Pestic. Sci. 2 (1988) 341.
[2] K. Thirunarayanan, R.L. Zimdahl, D.E. Smika, Weed. Sci. 33 (1985) 558.
[3] M.M. Joshi, H.M. Brown, J.A. Romesser, Weed. Sci. 33 (1985) 888.
[4] E.W. Zahnow, J. Agric. Food. Chem. 30 (1982) 854.
[5] I. Ahmad, G. Crawford, J. Agric. Food. Chem. 38 (1990) 138.
[6] P. Klaffenbach, P.T. Holland, J. Agric. Food. Chem. 41 (1993) 396.
[7] G.C. Galletti, A. Bonetti, G. Dinelli, J. Chromatogr. A 692 (1995) 27.
[8] O. Berdeaux, L.F. Dealencastro, D. Grandjean, J. Tarradellas, Intern. J. Environ. Anal. Chem. 56 (1994) 109.
[9] A.L. Howard, L.T. Taylor, J. High Resolut. Chrom. 16 (1993) 39.
[10] E. Kotoula-Sika, I.G. Eleftherohorinos, A.A. Gagianas, A.G. Sficas, Weed. Res. 33 (1993) 355.
[11] P. Klaffenbach, P.T. Holland, Biol. Mass. Spec. 22 (1993) 565.
[12] R.V. Slates, M.W. Watson, Anal. Meth. Pestic. Plant. Growth. Regul. 16 (1988) 53.
[13] G. Nilvé, M. Knutsson, J.A. Jönsson, J. Chromatogr. A 688 (1994) 75.
[14] P. Klaffenbach, P.T. Holland, D.R. Lauren, J. Agric. Food. Chem. 41 (1993) 388.
[15] G. Dinelli, A. Vicari, V. Brandolini, J. Chromatogr. A 700 (1995) 201.
[16] M.E. McNally, J.R. Wheeler, J. Chromatogr. 435 (1988) 63.
[17] A.E. Smith, Intern. J. Environ. Anal. Chem. 59 (1995) 97.
[18] C.A. Blank, D. Evans, J.L. White, L.E. Ensminyer and E.D. Clark (Editors), Methods of Soil Analysis, Part 1, American Society of Agronomy, Madison, WI, 1965.
[19] A.L. Page, R.H. Miller and D.R. Keeney (Editors). Methods of Soil Analysis, Part 2, American Society of Agronomy, Madison, WI, 1965.
[20] C. Tomlin (Editor). The Agrochemicals Handbook, Royal Society of Chemistry, UK, 10nd ed., 1994.
[21] J.J. Jiménez, J. Atienza, J.L. Bernal, L. Toribio, Chromatographia 38 (1994) 395.
[22] J. Atienza, J.J. Jiménez, A. Herguedas, J. Bernal, J. Chromatogr. A 721 (1996) 113.