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On-line coupled supercritical fluid extraction and chromatography for the determination of thiolcarbamate herbicides in soil matrix

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Abstract

Supercritical fluid extraction (SFE) was on-line coupled with supercritical fluid chromatography (SFC) for the determination of thiolcarbamate herbicides in soil matrix. Inert ODS-silica gel packings were used as a trap column for an interface between SFE and SFC and as an analytical column for the satisfactory separation of extracts. Thiolcarbamate herbicides could be extracted satisfactorily from the soil matrix, which had different characteristics. The results indicated that the proposed system was useful for the rapid determination of thiolcarbamate herbicides in soil matrices.

Keywords: Soil; Environmental analysis; Supercritical fluid chromatography; Supercritical fluid extraction; Herbicides; Thiolcarbamates

1. Introduction

Thiolcarbamate herbicides are used for crop protection in the rice field and are widely applied throughout the world. In general, residue analysis of thiolcarbamate herbicides in solid matrix is performed by GC after the solvent extraction which is time consuming and requires large amounts of organic solvents [1].

Supercritical fluid extraction (SFE) is an extraction method in which supercritical fluid is used as an extraction medium. Supercritical fluids have liquid-like solubilities that are conveniently varied by changing the temperature and/or the pressure of the fluids. The mass transfers in supercritical fluids are faster than those in liquids. In addition, carbon dioxide, which is generally used as the extraction medium in SFE, has a mild critical point, a low

toxicity and a low running cost. Therefore, SFE has many potential advantages caused by the properties of supercritical fluid in comparison with classical solvent extraction, such as a soxhlet extraction [2,3]. Up to now, SFE has been recognized as an extraction method that could potentially replace solvent extraction. Various applications of SFE to environmental samples, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and herbicides, have been reported [4,5].

One of the advantages of SFE is its ability to directly couple with the chromatograph system, because carbon dioxide can be evacuated by changing the phase from supercritical fluid into gas under the normal conditions, such as room temperature and atmospheric pressure. The on-line coupling of SFE with the chromatograph system produces the advantages, such as a low sample loss and a short analysis time, which are useful for the trace analysis. There are many examples in the literature that describe GC

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or supercritical fluid chromatography (SFC) connected with SFE [4].

The applications of on-line SFE–SFC to environmental analysis were already reported. McNally and Wheeler [6,7], Nishikawa [8], and Daimon and Hirata [9] have reported the on-line SFE–SFC of pesticide or herbicide residues in complex matrices, such as soil matrices. Ong et al. [10] reported the determination of PAHs in aqueous environmental samples. Fuoco and Griffiths [11] have reported the on-line SFE–SFC of PCBs in a soil matrix using FT-IR spectrometry. Lin et al. [12] have reported the influence of various absorbents on the extraction efficiencies of PAHs, PCBs and organochlorinated pesticides. Nam et al. [13] coupled SFE with a tandem SFC–GC system for the rapid determination of polychlorinated organics in a complex matrix [13].

In this study, we demonstrate the on-line coupling of SFE with SFC using carbon dioxide as both the extraction medium and the mobile phase without a modifier for the determination of thiolcarbamate herbicides in a soil matrix. The recovery test was performed under various conditions of both extraction medium and soil matrix.

2. Experimental

2.1. On-line coupled supercritical fluid extraction–chromatography system

A schematic diagram of the on-line SFE–SFC system employed in this study is shown in Fig. 1.

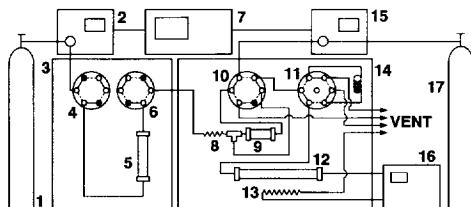


Fig. 1. Schematic diagram of the on-line SFE–SFC system employed in this study. (1) and (17), carbon dioxide cylinders; (2) and (15), pumps; (3) and (14), ovens; (4), (6) and (10), switching valves; (5), extraction chamber; (7), pump controller; (8) and (13), restrictors; (9), trap column; (11), injector; (12), separation column; (16), UV detector.

This system consisted of an SFE part, an SFC part and an interface.

2.1.1. SFE part

A Shimadzu (Kyoto, Japan) LC-6A pump was used to deliver carbon dioxide as the extraction medium. The pump head was cooled to maintain a stable flow. The pump was controlled by a Shimadzu SLC-6A system controller. An HPLC empty column (stainless steel, 50 mm×4.6 mm I.D.) was used as an extraction vessel and placed inside a DKK (Tokyo, Japan) LSA-EO oven that was equipped with switching valves. The flow-rate of the extraction medium was controlled by a restrictor made of a capillary tube of 100×30 μm I.D.

2.1.2. SFC part

A Shimadzu LC-6A pump, the head of which was cooled, was used to deliver carbon dioxide as the mobile phase. A Shimadzu SLC-6A system controller described in Section 2.1.1 was used for pressure programming. The standard sample solution used to obtain calibration curves was injected into a Rheodyne (Cotati, CA, USA) Model 7125 sample injector with a 20- μl sample loop. A separation column was of 250 mm×4.6 mm I.D. in size and was kept at 55°C in a column oven from a Shimadzu LC-1 system. A Shimadzu SPD-6A UV detector was used for detection at 220nm. The flow-rate of the mobile phase was controlled by a restrictor made of a capillary tube (400 mm×50 μm I.D.).

2.1.3. Interface

The extract obtained by SFE was initially concentrated on a trap column. Then the extract was introduced into the separation column together with the carbon dioxide mobile phase of SFC. An HPLC short column (stainless steel, 35 mm×4.6 mm I.D.) was used as the trap column for the coupling of SFE with SFC. This column was connected to the end of the restrictor of SFE and placed inside the SFC column oven.

2.1.4. Procedure

An approximately 0.6 g portion of soil matrix was packed in the extraction vessel for SFE which was carried out under dynamic mode. Fig. 2 shows the position of the switching valves for the coupling of SFE with SFC. During extraction mode, the pressure

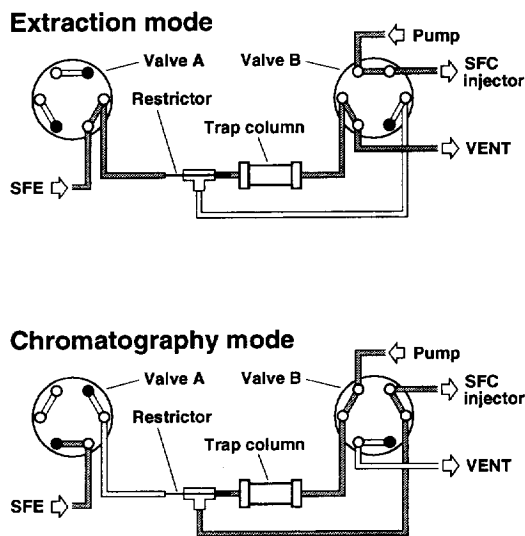


Fig. 2. Flow diagram of the SFE-SFC interface.

of carbon dioxide used as the extraction medium of SFE was reduced to the atmospheric pressure at the outlet of the restrictor and the extract obtained by SFE was concentrated on the trap column. After finishing SFE by changing the position of the switching valve A, the extract was introduced into the separation column of SFC together with the carbon dioxide mobile phase of SFC by changing the position of the switching valve B. During the chromatography mode, the pressure programming was performed as follows: the pressure of carbon dioxide mobile phase was maintained at 10 MPa for 1 min, then raised at 1 MPa/min, and kept at 15 MPa for 25 min.

2.2. Materials and reagents

Two kinds of thiolcarbamate herbicide, thiobencarb and molinate, which were used as the target compounds were obtained from Wako (Osaka, Japan) and GL Sciences (Tokyo, Japan), respectively. The structures of these herbicides are shown in Fig. 3.

A Shiseido (Tokyo, Japan) Capcell Pak C₁₈ UG 120 packed with ODS-silica gel (particle size, 5 μm; pore diameter, 12 nm) was used as the trap column for the coupling of SFE with SFC. Several kinds of separation column packed with ODS-silica gel were examined in SFC. Column I was a Capcell Pak C₁₈

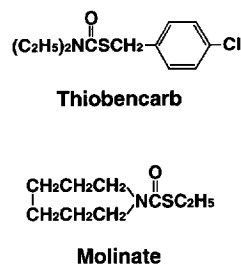


Fig. 3. Structures of the thiolcarbamate herbicides used in this study.

UG 120 (particle size, 5 μm; pore diameter, 12 nm) which was mainly used in this study. Column II (particle size, 5 μm; pore diameter, 12 nm), column III (particle size, 5 μm; pore diameter, 30 nm) and other columns (particle size, 5 μm; pore diameter, 12 nm) were commercially available.

Five kinds of soil matrix were used in this study. The characteristics of these soil matrices are listed in Table 1. Soil A, which was obtained from the Research Institute of the Japan Association for Advancement of Phyto-Regulators (Ushiku, Ibaraki, Japan), was mainly used for the recovery test. Other soil matrices (soils B–E), which were obtained from Japan Plant Protection Association (Tokyo, Japan), were used to investigate the effect of the soil matrix on the recovery of thiolcarbamate herbicides.

2.3. Preparation of soil samples

Most of the soil matrices were air-dried before each was spiked with 1 ppm of thiolcarbamate herbicides, present as a dichloromethane solution. The effect of the moisture level in the soil matrix on the recovery of thiolcarbamate herbicides was investigated using soil samples prepared as follows. The soil matrix was dried at 105–110°C for 15 h and deionized water was added before thiolcarbamate herbicides were added onto this soil matrix.

3. Results and discussion

3.1. Selection of column packing

In packed column SFC, it is well known that the degree of inertness of column packings has consider-

Table 1
Properties of the soil matrices used in this study

| Soil characteristic | A | B | C | D | E |
|----------------------------------|------|------|------|------|-------|
| Organic carbon (%) | 4.92 | 4.67 | 2.29 | 1.75 | 12.91 |
| Cation exchange (me/100 g) | 32.4 | 22.0 | 13.9 | 8.9 | 49.9 |
| Phosphate absorption coefficient | 1980 | 1140 | 530 | 430 | 1850 |
| pH | 5.8 | 5.8 | 6.4 | 6.2 | 7.4 |
| Sand (%) | 38.2 | 44.0 | 60.3 | 71.7 | 30.6 |
| Silt (%) | 32.7 | 30.4 | 21.3 | 13.6 | 49.7 |
| Clay (%) | 29.1 | 25.6 | 18.4 | 14.7 | 19.7 |

able influence on the retention of polar solutes when carbon dioxide is used as the mobile phase without a modifier [14–16]. Thiobencarb and molinate were separated on various kinds of ODS-silica gel columns, using carbon dioxide as the mobile phase alone. Fig. 4 shows SFC chromatograms of thiobencarb and molinate on three kinds of ODS-silica gel columns. Chromatograms A, B and C were obtained by using separation columns I, II and III, respectively. The conditions of SFC were as follows: pressure of carbon dioxide, 18 MPa; detection, UV 210 nm. The peak shape of thiobencarb and molinate obtained by Capcell Pak C₁₈ UG 120 (chromatogram A) was the best among the three chromatograms. Thiobencarb and molinate could not be eluted when ODS-silica gel columns other than the three columns shown in Fig. 4 were used as the separation column.

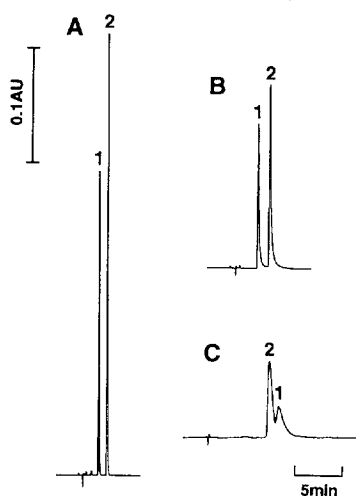


Fig. 4. SFC chromatograms of thiolcarbamate herbicides using different kinds of ODS-silica gel columns. SFC conditions: pressure, 18 MPa; column temperature, 55°C; detection, UV 210 nm. Peaks: 1, molinate; 2, thiobencarb. Separation column: A, Capcell Pak C₁₈ UG 120; B and C, commercially available column.

An ODS-silica gel column was also used as the trap column for the coupling of SFE with SFC. It is considered that the degree of inertness of the trap column influences the peak shape of the extract in the SFC part. Therefore, inert ODS-silica gel columns (Capcell Pak C₁₈ UG 120) were used as the trap column as well as the separation column in this study.

3.2. Effect of the properties of the extraction medium on recovery

In order to investigate the extraction behavior, the on-line SFE–SFC system was applied to the extraction and analysis of thiolcarbamate herbicides spiked in the soil matrix under various conditions of extraction medium, as shown in Fig. 5. The effect of extraction pressure on recovery was investigated at 10–25 MPa under the following extraction conditions: temperature, 60°C; time, 20 min (Fig. 5A). The recovery of thiobencarb and molinate increased with increasing pressure (density) of the extraction medium and their rates were more than 90%, when the pressure was above 15 MPa. The effect of extraction temperature on recovery was also investigated at 40–90°C under the following extraction conditions: pressure, 20 MPa; time, 20 min. The density of carbon dioxide decreases with increasing temperature, when the pressure of carbon dioxide is maintained at a constant level. Under these conditions, the recovery rates of thiobencarb and molinate were more than 90%. It is indicated that SFE of thiolcarbamate herbicides is not considerably influenced by the extraction temperature under these conditions and can be performed at the moderate extraction temperature. Fig. 5B shows the effect of extraction time on the recovery of thiobencarb and molinate under the following conditions: pressure, 20

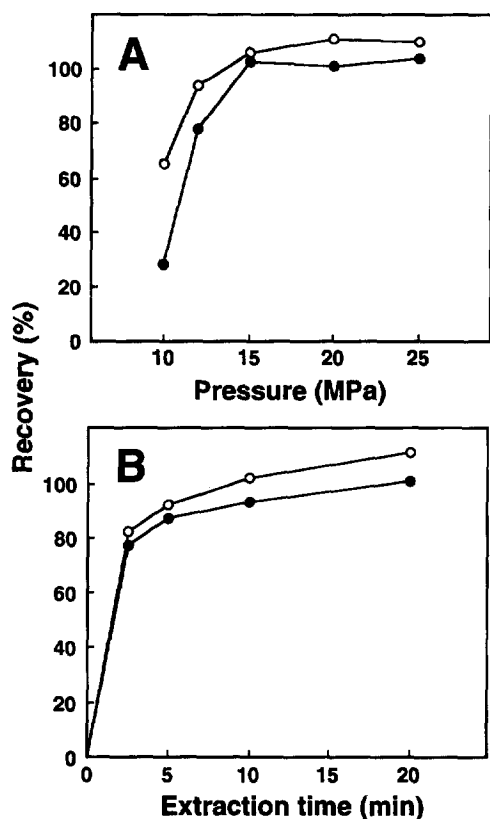


Fig. 5. Effect of extraction pressure (A) and time (B) on the recovery of thiolcarbamate herbicides from a soil matrix. SFE conditions: (A) temperature, 60°C; time, 20 min; (B) pressure, 20 MPa; temperature, 60°C. Samples: ○, molinate; ●, thiobencarb.

MPa; temperature, 60°C. When a capillary tube of 100 mm×30 μm I.D. was used as the restrictor of SFE, the flow-rate of the carbon dioxide extraction medium measured at room temperature and atmospheric pressure was almost 160 ml/min. Under these conditions, 20 min of extraction time was sufficient for the extraction of thiolcarbamate herbicides. In future studies, extraction conditions of pressure, temperature and time were set to 20 MPa, 60°C and 20 min, respectively. The effect of extraction time on the recovery was also investigated under different flow-rate, by using a capillary tube of 100 mm×50 μm I.D. as the restrictor of SFE. When using this restrictor, the flow-rate of the carbon dioxide extraction medium was almost 800 ml/min. Under these conditions, the recovery rates of thiobencarb and molinate were less than 80%.

Fig. 6 shows an SFC chromatogram of thiolcarba-

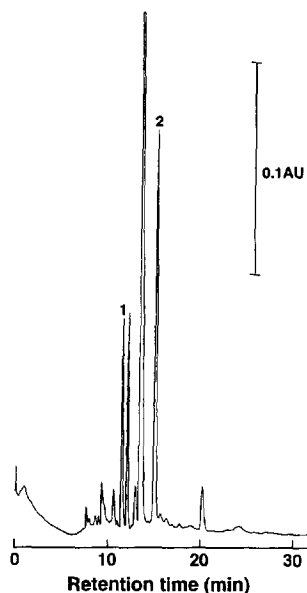


Fig. 6. Chromatogram of thiolcarbamate herbicides in a soil matrix obtained by the on-line SFE–SFC system. SFE conditions: pressure, 20 MPa; temperature, 60°C; time, 20 min. Peaks: 1, molinate; 2, thiobencarb.

mate herbicides in a soil matrix that was obtained by the on-line SFE–SFC system, in which pressure programming of the mobile phase in SFC was performed. The extraction conditions were as follows: pressure, 20 MPa; temperature, 60°C; time, 20 min. In Fig. 6, no interference peaks are observed and all the peak shapes are quite good. SFE and SFC of thiolcarbamate herbicides in a soil matrix could be performed in less than 1 h. It is considered that the on-line SFE–SFC system is useful for the rapid determination of thiolcarbamate herbicides in soil matrices.

3.3. Effects of the properties of soil on recovery

The recovery by SFE is considered to be influenced by the properties of the matrix as well as by those of the extraction medium. The effect of the moisture level in the soil matrix on the recovery of thiolcarbamate herbicides was investigated, as shown in Fig. 7. It was reported that the moisture in solid matrix influenced the recovery in SFE and the mechanism was complicated [3,17–19]. Water is almost immiscible in supercritical carbon dioxide

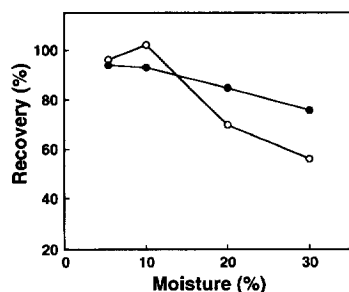


Fig. 7. Effect of moisture level in a soil matrix on the recovery of thiolcarbamate herbicides. SFE conditions: pressure, 20 MPa; temperature, 60°C; time, 20 min. Samples: ○, molinate; ●, thiobencarb.

(less than 0.1%). On the other hand, the existence of water can be considered to increase the recovery of specific samples by acting as an “internal modifier”. The recoveries of thiobencarb and molinate were good at moisture levels of 5–10%, which are almost equal to the moisture level of air-dried soil matrices. In higher moisture levels, the recovery of molinate was poorer than that of thiobencarb; presumably because molinate were more soluble in water than was thiobencarb. The solubilities of molinate and thiobencarb in water are 900 ppm and 30 ppm, respectively [20]. The recovery of thiolcarbamate herbicides decreased with increasing moisture levels in the soil matrix. Snyder et al. [18] described their findings that soils containing high moisture levels require longer extraction times or the use of an alternative extraction fluid. In the case of SFE of thiolcarbamate herbicides, it is thought that the air-drying of soil samples before extraction produces higher recoveries.

It is thought that the characteristics of soil matrices, other than the moisture level, such as organic carbon and particle size, also influence the recovery by SFE. The on-line SFE–SFC system was applied to the extraction of thiolcarbamate herbicides from five kinds of soil matrix that all had different characteristics. Fig. 8 shows the result of the recovery test of thiobencarb and molinate. The R.S.D. values ($n=3$) of SFE shown in Fig. 8 were between 3 and 20%. This poor reproducibility was probably due to the small sample size. Thiobencarb and molinate were satisfactorily extracted from all soil matrices. It is indicated that thiolcarbamate her-

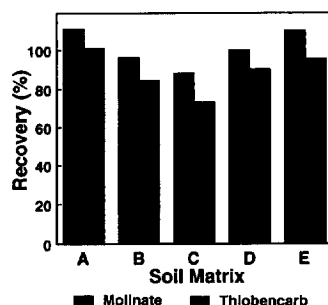


Fig. 8. Recoveries of thiolcarbamate herbicides from five kinds of soil matrix. SFE conditions: pressure, 20 MPa; temperature, 60°C; time, 20 min.

bicides in various types of agricultural soil matrix could be extracted and analyzed rapidly by the proposed system.

4. Conclusions

In this study, we demonstrated the on-line coupling of SFE with SFC and their application to the determination of thiolcarbamate herbicides in a soil matrix. Inert ODS-silica gel packings were needed as the trap column as well as the separation column for the satisfactory separation of thiolcarbamate herbicides in this system in which carbon dioxide was used as the extraction medium and the mobile phase without a modifier. The recovery test of thiobencarb and molinate was performed under various conditions of extraction medium and soil matrix. The results obtained lead to the conclusion that the proposed system was useful for the rapid determination of thiolcarbamate herbicides in soil matrices.

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