Application of Supercritical Fluid Extraction/Supercritical Fluid Chromatography to Pesticide Residue Analysis

Yasuhiko NISHIKAWA

Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory,
Takatsukasa, Takarazuka, Hyogo 665, Japan

A new double-stage separation method was developed for residue analysis using direct coupling of supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). While conventional residue analysis requires much time and labor, this new method makes it possible to extract analytes selectively with a small amount of organic solvent and to introduce them into the chromatograph without injection. In this study, the extraction of pesticide residues from soil and various plant samples was investigated. In all cases, the pesticides were successfully extracted with supercritical carbon dioxide, especially with control of their separation from co-extracts in various ways, including adjustment of the modifier. For the soil samples, the extraction behavior of each pesticide from the matrix is discussed in terms of their electronic structures.

Keywords Supercritical fluid extraction, supercritical fluid chromatography, residue analysis, filter paper, soil, crop, electronic distribution

Residue analysis of pesticides requires their selective separation from various samples, such as grain, straw, green tea leaves, vegetables, fruits, and soils. However, the pretreatment processes used, such as homogenization, solvent extraction, partition, and clean-up, are very complicated, laborious, and time-consuming. It would be ideal to be able to perform selective extraction and determination of the pesticides concerned without any pretreatment.

In considering how this might be possible, the use of supercritical fluids has been studied. These fluids were found to have superior extracting power by Hannay *et al.*¹ in the 19th century, but practical application did not begin until the 1950's with the work of Zosel *et al.*² Recently, supercritical fluid extraction (SFE) using supercritical carbon dioxide has been employed by the food industry because of its harmlessness and low cost. Supercritical fluid chromatography (SFC) has also been developed and offers the advantages of both GC and HPLC, due to the density, diffusion coefficient, and viscosity characteristics of the supercritical fluid.

In the field of residue analysis, few applications have been reported for SFE and SFC. Sulfonylurea herbicides were extracted from soil, plant materials, and a cell culture medium;³ chlorinated pesticides were extracted from soil⁴⁻⁶, wheat⁶, fish tissue⁷, and milk samples;⁸ triazine herbicides were extracted from soil⁴, river sediment⁹, and dairy biomass;⁸ organophosphorus pesticides were extracted from fish tissue⁸ and sand.¹⁰ No synthetic pyrethroids were extracted by SFE. In most cases, the residues were analyzed by off-line

GC, HPLC, or GC-MS after SFE. Recently, McNally *et al.*^{3,5,6} reported direct introduction of samples extracted by SFE to SFC.

Direct introduction of SFE extracts into an SFC system is possible with a commercially available JASCO instrument. Using this system, Hondo *et al.*¹¹⁻¹⁵ reported the extraction of caffeine from coffee beans, vitamin E from wheat germ powder, the flavoring material from lemon peel, and vitamin K from human serum.

As described above, the present residue analysis method requires so much complicated pretreatment that we examined the application of SFE/SFC to selective extraction of pesticides, followed by their on-line analysis. In this study, using the JASCO system, an application of SFE/SFC to residue analysis of three representative types of pesticides was investigated: an organophosphorus pesticide, a synthetic pyrethroid and an azole compound.

Experimental

Reagents and materials

Fenitrothion, esfenvalerate and diniconazole were used to fortify each matrix as representative examples of an organophosphorus pesticide, a synthetic pyrethroid, and an azole compound, respectively; fenitrothion [*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate], esfenvalerate [(*S*)- α -cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate], diniconazole [(*E*)-1-(2,4-

Fig. 1 Structure of the pesticides used in the experiments.

Fig. 2 Schematic diagram of SFE/SFC system. a, carbon dioxide; b, modifier solution; c, cylinder-head cooling equipment; d, pump; e, 6-way valve; f, extraction cartridge; g, trap loop; h, loop injector; i, column; j, multi-wavelength UV detector; k, data processor; l, pressure gauge; m, pressure regulator.

dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol]. Figure 1 shows their structures. Fenitrothion was purchased from Wako Pure Chemical Co., and esfenvalerate and diniconazole were prepared by Sumitomo Chemical Co.

Masado soil was used as the soil sample. It is characterized as follows: soil texture, sandy loam; soil separates, 70.0% sand, 19.0% slit, 11.0% clay; 0.3% organic matter content; pH (H₂O), 5.8; cation exchange capacity (CEC), 9.3 meq/100 g dry soil. The pH (H₂O) of the soil was measured by the conventional method.¹⁶

The purity of the carbon dioxide (Neriki Gas Co., Amagasaki, Japan) used as the mobile phase was 99.5%.

Apparatus

The JASCO Super-100 type SFE/SFC system is shown as a schematic diagram in Fig. 2. Its details have been reported by Hondo *et al.*^{11,17} In the present study, the direct coupling mode of SFE-SFC was used. Batch SFE is done in an extraction cartridge and the extracts are transferred to a trap loop for direct introduction *via* valve operation into an analytical column for SFC separation.

The separation column used was a Super SIL C₁₈ (JASCO), except in one case (mentioned later). The

mobile phase used for SFC after extraction, also except for one case (mentioned later), was supercritical CO₂ containing methanol at the flow rates of 4.0 ml/min and 0.1 ml/min, respectively.

Soil samples (Masado) were prepared for extraction using a Riken Co. oil pressure press.

Results and Discussion

Optimization of extraction conditions using filter paper

A piece (7×20 mm) of filter paper (Quantitative No. 6, Toyo Roshi Kaisha, Ltd., Tokyo) spiked with 10 µg of diniconazole was subjected to SFE/SFC, and each extraction parameter of diniconazole on the filter paper was investigated. Figure 3 shows the effect of extraction time, pressure, and temperature on the amount of extracted diniconazole.

The amount extracted reached a plateau in 2 min and over 50°C, respectively. As for the extraction pressure, although the amount extracted increased with the pressure increase, the data variance was large, over 240 kg/cm². The conditions chosen were as follows: extraction time, 5 min; extraction pressure, 200 kg/cm²; extraction and analytical temperature, 50 °C; analytical pressure, 150 kg/cm² (at column outlet).

Reproducibility was examined under the above conditions, except that extraction and analytical temperatures were 40°C, and RSD 3.5% (*n*=5) was obtained.

Examination of extraction conditions of soil samples

Some modifications were needed for this equipment, because it lacks a trap for extracts which will compensate for minor recoveries in one extraction and it has large dead-volumes. These structural issues prevent use of the system for quantitative extraction of total samples in the extraction cartridge in one operation. To examine the extraction conditions of soil samples, the percentage of extract from soil samples to that from filter paper was calculated.

First, Masado soil was fortified with fenitrothion in the usual manner, but the reproducibility of the extracted amount was poor. Further investigation showed that the reproducibility for soil samples depends on the preparation method and sample homogeneity.

Therefore, for the purpose of obtaining homogeneous

Fig. 3 Effect of extraction time, pressure and temperature on extracted amount of diniconazole. (a) effect of extraction time, (b) effect of extraction temperature, (c) effect of extraction pressure. SFE conditions: (a) temperature 40°C, pressure 200 kg/cm²; (b) pressure 200 kg/cm², time 5 min; (c) temperature 40°C, time 5 min. SFC conditions: temperature 40°C, pressure 150 kg/cm² (at outlet), flow rate CO₂ 4.0 ml/min-CH₃OH 0.1 ml/min.

sample surfaces, two methods of the preparation of Masado soil fortified with fenitrothion were studied, and the reproducibilities or the extraction efficiency (evaluated as the percentage of the amount extracted from soil samples to that from filter paper) were compared. Although these methods alter the appearance of the soil samples from their original state, the extent of adsorption/desorption of pesticides does not change because only the surface charge of soils affects adsorption capacity.

(1) Powder method: 10 g of Masado soil (particle size, 0.02 mm; water content, 1.34%) was ground to a powder with an agate mortar and pestle, and fortified with fenitrothion to a concentration of 50 ppm. After drying and stirring, 0.6 g was put in the cartridge for extraction. (2) Tablet method: 300 mg of Masado soil, ground to a powder as described above, was compressed into a tablet using the press. The tablet was fortified with 15 µg of fenitrothion and dried for extraction.

The results obtained with samples prepared by these two methods are shown in Table 1. Both yielded relatively low RSD, and the tablet method increased

Table 1 Comparison of reproducibility and extraction efficiency according to the preparation method

Preparation method	RSD, %	Extraction efficiency ^a , %
(1)	5.6 (n=5)	56.1
(2)	9.6 (n=5)	78.7

a. Extraction efficiency is represented as the percentage of the amount extracted from filter paper adsorbed with the same dosage.

extraction efficiency due to homogeneous fixing of the pesticide. However, the extraction efficiency from the soil samples was inferior to that from filter paper. When only SFC was carried out without extraction, the RSD of the peak area for fenitrothion was 1.46% (n=5, 5 µg fortification, temp. 40°C, column outlet pressure 150 kg/cm²). Therefore, as Hondo *et al.*¹⁴ noted, the reproducibility depends very much on the SFE part.

In addition, recoveries from the filter paper fortified with 15 µg and 30 µg of fenitrothion were investigated; amounts in proportion to the fortified amounts could be recovered.

Also studied was the extraction of the Masado soil samples after fortification of other pesticides.

Diniconazole was so strongly adsorbed onto the soil that it could not be extracted under any of the various extraction conditions. Extraction was finally possible with addition of water to the soils (5% or 10% of the amount of the crude soil). Esfenvalerate could be slightly extracted from crude Masado soils; its extraction efficiency showed a rather low value compared with recovery from filter paper, although it increased with the addition of water. This result was intermediate between those of the two pesticides described previously. These results were greatly influenced by the adsorption behavior of each pesticide onto the soil, which depended upon the pesticide structure.

In general, chemical adsorption of a pesticide onto soil results from the molecular structure of the pesticide compound, especially from the interaction between the electron density (electronic distribution) of the functional group and the surface electronic charge of the soil constituents.^{18,19} Soil analysis showed that the Masado soil used in this study contained quartz as the principal constituent (40–70%) and allophane/kaolinite as clay minerals (20–40%). Acidity pH (H₂O) of the soil was 5.8. The surface charge of the principal constituent quartz is an additional charge derived from the silanol group (=Si-O-H), and that of kaolinite (zero point of charge²⁰ (zpc)=5.2) or allophane (zpc=6.8) has a permanent dipole based upon each constituent.

Therefore, Masado soil is thought to be negatively

Fig. 4 Electronic charge density of the functional groups of the pesticides.

charged on the surface due to its acidity pH value of 5.8. Chemical adsorption occurs corresponding to the electron density of each functional group.^{19,21}

The three pesticides used in this study: fenitrothion, esfenvalerate, and diniconazole, have functional groups with electron densities in the order of: diniconazole < esfenvalerate < fenitrothion, as shown in Fig. 4 (a) – (c).

The azole compound diniconazole is strongly adsorbed by the negative charge on a mineral surface, due to its functional group ($>NH$). However, fenitrothion, whose functional group is the thiono group, is only slightly adsorbed by soils, owing to interaction between the negative charge on the mineral surface and its functional group, $>P^{\ominus}-S^{\ominus}$. Compared with these two pesticides, esfenvalerate is thought to display an intermediate interaction between that of diniconazole and of fenitrothion with minerals, because the electron density of a nitrogen atom in the functional group $-C=N^{\ominus}$ is more effective.

These differences in the adsorption of each pesticide onto the soil constituents lead to their desorption/extraction from the solid phases (soils) in supercritical CO_2 . In other words, the facility of dissolution/partition of a pesticide from the solid phase is the main factor in its extraction into carbon dioxide, a non-polar fluid. Consequently, a pesticide having a weak polar (hydrogen) bond can be extracted into a non-polar supercritical fluid, while an azole compound having a strong polar (hydrogen) bond is difficult to desorb. This hypothesis is well supported by the experimental data.

Although diniconazole is strongly adsorbed by soils, it could be extracted by adding water. This is because water has a large dielectric constant and polarity, which weaken the adsorption of the pesticide and facilitate its partition into supercritical CO_2 .

The higher extraction efficiency of the pesticides from filter paper than from soils suggests that their adsorption on filter paper is mainly due to hydrogen bonds or π -polar bonds between the pesticides and hydroxyl groups of cellulose fiber^{19,22}, which leads to larger partition into supercritical CO_2 .

Examination of extraction conditions of crop samples

The possibility of extracting these three pesticides from crop samples was studied in order to examine qualitative application of SFE/SFC to residue analysis. Fortification of each pesticide per crops in one cartridge was

Fig. 5 Three-dimensional SFC chromatogram of (1) control straw sample (0.1 g) and (2) straw sample fortified with diniconazole (10 μ g added to 0.1 g of straw sample). SFE conditions: temperature, 50°C; pressure, 200 kg/cm²; time, 5 min. SFC conditions: mobile phase, CO_2 (4.0 ml/min)– CH_3OH (0.1 ml/min); temperature, 50°C; pressure, 150 kg/cm² (at outlet); column, Super SIL C₁₈ (4.6 mm×25 cm, 5 μ m).

as follows: 10 μ g diniconazole, 15 μ g fenitrothion, and 60 μ g esfenvalerate.

Straw. Figure 5(1) shows a three-dimensional chromatogram of control extracts of straw sample. (The maximum absorption wavelengths of diniconazole, fenitrothion and esfenvalerate are 250, 260 and 275 nm, respectively.) As is apparent from this figure, most ingredients of straw were not extracted, and every fortified pesticide could be extracted without interference from co-extracts in straw, as shown in Fig. 5 (2).

Green tea. Figure 6 shows a three-dimensional SFC chromatogram of control green tea and a conventional two-dimensional chromatogram. Caffeine was identified by its absorption spectrum which eluted at the

Fig. 6 Chromatograms of control green tea sample (0.3 g). (A) Three-dimensional chromatogram. (B) Chromatogram monitored at 250, 260 and 275 nm. *1, t_R of fenitrothion under these conditions; *2, t_R of diniconazole under these conditions; *3, t_R of esfenvalerate under these conditions. SFE conditions and SFC conditions as in Fig. 5.

retention time of 0.80–0.85 min. Although diniconazole and esfenvalerate could be eluted separately from this co-extract, interference was encountered in the case of fenitrothion. To solve this problem, the following two methods were studied.

The first method involved adjustment using a modifier in the chromatography. The flow of the modifier methanol was stopped when extracts which had been retained in the trap loop were introduced into the analytical column. This caused fenitrothion to be eluted while the interfering co-extracts from green tea remained on the column. Figure 7 shows two chromatograms for which the flow of the modifier was stopped for the first 4 min. Clearly, fenitrothion was eluted at 0.86 min, followed by elution of caffeine and the appearance of methanol absorption spectrum at shorter wavelengths.

The other method examined involved exchanging the ODS column for a silica-gel column. LiChrosorb SI-60 (Merck) gave a different elution pattern of the control extracts, and fenitrothion was eluted at an intermediate retention time between those of the two co-extracts from green tea, as shown in Fig. 8.

Vegetables. Vegetables which contain a lot of water, for example, cucumbers, can cause clogging and therefore are not so suitable for testing with the present SFE equipment. As they usually display lesser adsorption of pesticides and have sufficient water content, their extraction with supercritical fluid will be possible.

Fig. 7 Chromatograms of green tea sample fortified with fenitrothion (15 μ g added to 0.2 g of green tea). (A) Three-dimensional chromatogram. (B) Chromatogram monitored at 260 nm. SFE and SFC conditions as in Fig. 5, except that: mobile phase A (for initial 4 min), CO_2 (4.0 ml/min); mobile phase B (after initial 4 min), CO_2 (4.0 ml/min)– CH_3OH (0.1 ml/min).

Fig. 8 Chromatogram of green tea sample fortified with fenitrothion (15 μ g added to 0.2 g of green tea) using silica-gel column LiChrosorb SI-60 (4.6 mm $\times$ 25 cm, 5 μ m). SFE and SFC conditions as in Fig. 5.

As discussed above, by introducing a technique of micro-scale SFE/SFC, fortified pesticides were successfully extracted from soils and crops, and favorable SFC chromatograms were obtained without the sample pretreatment which is routine in conventional residue analysis. Elimination of the pretreatment shortened the analysis time, and this method offers promise as a routine method for analyzing numerous samples in a unit time. As is obvious from the case of fenitrothion extracted from green tea, it became apparent that a new type of analysis having a double-stage separation process could be developed. Also, although not examined in the present study, another significant advantage of SFC in residue analysis is that it can be used for non-volatile compounds which cannot be studied by GC analysis.

The study of desorption behavior of pesticides from soils based on the interaction between their structure-based charge and the charge of the constituent minerals can yield very useful information for estimating the effective extraction conditions of various samples.

Further development of equipment having lower detection limits and smaller dead-volumes is needed for an application to quantitative determination. The use of other detectors, improvement of the hardware, or off-line GC/HPLC analysis after SFE using an adsorption column will solve these problems.

The author wishes to thank JASCO Co. for permission to use the Super-100 type SFE/SFC system.

References

1. J. B. Hannay and J. Hogarth, *Proc. R. Soc. [London]*, **29**, 324 (1879).
2. K. Zosel, "Extraction with Supercritical Gases", ed. G. M. Schneider, E. Stahl and G. Wilke, p. 1, VCH, Weinheim, 1980.
3. M. E. McNally and J. R. Wheeler, *J. Chromatogr.*, **435**, 63 (1988).
4. P. Capriel, A. Haisch and S. U. Khan, *J. Agric. Food Chem.*, **34**, 70 (1986).
5. M. E. McNally and J. R. Wheeler, *J. Chromatogr.*, **447**, 53 (1988).
6. J. R. Wheeler and M. E. McNally, *J. Chromatogr. Sci.*, **27**, 534 (1989).
7. K. S. Nam, S. Kapila, D. S. Viswanath, T. E. Clevenger, J. Johansson and A. F. Yanders, *Chemosphere*, **19**, 33 (1989).
8. K. S. Nam, S. Kapila, A. F. Yanders and P. K. Puri, *Chemosphere*, **20**, 873 (1990).
9. V. Janda, G. Steenbeke and P. Sandra, *J. Chromatogr.*, **479**, 200 (1989).
10. V. Lopez-Avila, N. S. Dodhiwala and W. F. Beckert, *J. Chromatogr. Sci.*, **28**, 468 (1990).
11. K. Sugiyama, M. Saito, T. Hondo and M. Senda, *J. Chromatogr.*, **332**, 107 (1985).
12. K. Sugiyama, M. Saito, T. Hondo and M. Senda, Proceedings of 1985 Annual Meeting of The Agricultural Chemical Society of Japan, Sapporo, July 1985, p. 158.
13. M. Saito, T. Hondo, M. Senda and K. Sugiyama, Proceedings of 1985 Annual Meeting of The Agricultural Chemical Society of Japan, Sapporo, July 1985, p. 157.
14. T. Hondo, M. Saito and M. Senda, *Bunseki Kagaku*, **35**, 316 (1986).
15. M. Saito and T. Hondo, *Shokuhin Kikai Souchi*, **22**, 45 (1985).
16. N. Date, "Dojou Hyoujun Bunseki/Sokutei-Hou (Standard Analysis of Soils, in Japanese)", ed. The Japanese Society of Soil Science and Plant Nutrition, p. 70, Hakuyusha, Tokyo, 1986.
17. M. Saito, K. Sugiyama, T. Hondo, M. Senda and S. Tahei, Abstracts of Papers, International HPLC Symposium, Kyoto, 1985, p. 84.
18. I. Yamane, T. Matsui, S. Irisawa, M. Okazaki and M. Hosono, "Zusetsu Nihon no Dojou (a Pictorial Book of the Japanese Soil, in Japanese)", p. 156, Asakurashoten, Tokyo, 1978.
19. S. Kuwatsuka, "Dojou no Kyuuchaku Genshou (Adsorption Behavior in Soils, in Japanese)", ed. The Japanese Society of Soil Science and Plant Nutrition, p. 142, Hakuyusha, Tokyo, 1981.
20. S. Hachisu, "Shin Jikken Kagaku Kouza 18, Kaimen to Koroido (New Experimental Chemistry Series 18, Surface and Colloids, in Japanese)", ed. The Chemical Society of Japan, p. 318, Maruzen, Tokyo, 1977.
21. K. Kawaguchi, "Dojou-Gaku Gairon (An Outline of Soils, in Japanese)", p. 71, Yokendo, Tokyo, 1978.
22. R. T. Parker, R. S. Freedlander and R. B. Dunlap, *Anal. Chim. Acta*, **119**, 189 (1980).

(Received March 18, 1991)

(Accepted May 24, 1991)