

Focusing Review

Development of Environmental Analysis Methods Using Supercritical Fluid Extraction and Supercritical Fluid Chromatography

Takashi Yarita

*National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST),**Tsukuba Central 3, Umezono, Tsukuba 305-8563, Japan**Received for review January 11, 2008. Accepted February 4, 2008*

Abstract

Environmental analysis methods have been developed by applying supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). The highly sensitive and selective detection of organochlorine pollutants by SFC was demonstrated using an electron-capture detector. For accurate quantification by SFE, the extraction behavior was investigated in detail using herbicides in soil and polychlorinated biphenyls (PCBs) in sediment as target analytes. For rapid quantification, on-line coupling of SFE and SFC was demonstrated, in which thiolcarbamate herbicides in soil samples could be quantified within 1 h. As an application of the developed analytical method, the production of sediment certified reference materials NMIJ CRM 7304-a and CRM 7305-a, with the use of SFE as one of the extraction techniques for the determination of certified values of PCBs, is also described.

Keywords: Supercritical fluid extraction, Supercritical fluid chromatography, Pesticide, Herbicide, Polychlorinated biphenyls, Certified reference material

1. Introduction

Supercritical fluid chromatography (SFC) was first attempted in 1962 [1]. Although several papers on SFC were published during the subsequent two decades [2], their content did not encourage many researchers to investigate this technique. After the introduction of fused-silica capillary SFC in the early 1980s [3], many papers describing fundamental studies as well as application studies appeared. Today, SFC is recognized as being a separation technique that acts as a bridge between GC and HPLC.

Analytical supercritical fluid extraction (SFE) was demonstrated in the mid-1980s [4]. Since then, the number of papers describing the use of this technique has steadily increased. Because of the preferable properties of supercritical fluids as an extraction medium, SFE is a useful alternative to conventional solvent extraction as well as an "environmentally friendly" technique.

The author and coworkers have made efforts to develop SFE and SFC. In the field of environmental analysis, we developed

electron-capture detection (ECD) in SFC for the highly sensitive and selective detection of polychlorinated biphenyls (PCBs) [5] and organochlorine pesticides [6]. For accurate quantification by SFE, we investigated the effects of operation parameters and the sample matrix on extraction efficiency using organochlorine pesticides in carrots [6], herbicides in soil [7, 8] and PCBs in sediment [9] as target analytes. In addition, we applied an optimized SFE method for PCBs to the production of sediment certified reference materials (CRMs) for which accurate quantification methods were needed to determine certified values [9, 10]. We also demonstrated rapid quantification of thiolcarbamate herbicides in soil using an on-line SFE/SFC system [8].

This paper briefly overviews fundamental and application studies by the author's group on the development of environmental analysis methods using SFE and SFC.

Tel:+81-29-861-9416

Fax:+81-29-861-6865

E-mail:t-yarita@aist.go.jp

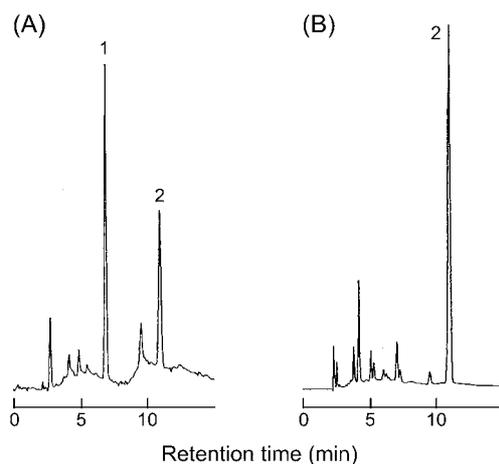


Figure 1. Supercritical fluid chromatograms of a carrot sample. Peak identification: (1), 1 α , 2 α , 3 β , 4 α , 5 α , 6 β -hexachlorocyclohexane (γ -HCH); (2), 1,1-dichloro-2,2-bis-(4-chlorophenyl)ethane (4,4'-DDD). Detection: (A), electron-capture detection; (B), UV detection at 190 nm. Other chromatographic conditions: mobile phase, carbon dioxide; column, L-column ODS (size, 250 mm \times 4.6 mm I.D.; particle size, 5 μ m; pore diameter, 12 nm); column temperature, 55 $^{\circ}$ C; back pressure, 15 MPa. Reproduced by permission of Elsevier [6].

2. Electron-capture detection in SFC

Although many detectors used in GC and HPLC are considered to be applicable to SFC, only flame ionization detection (FID) and UV-VIS detection were widely employed. On the other hand, highly sensitive and selective detection is essential for developing environmental analysis methods. Our first attempt to use SFC for environmental analysis involved developing ECD [5, 6].

We connected a commercially available ECD detector, which had been designed for packed-column GC, to a packed-column SFC system. The mobile phase of SFC was split (split ratio approximately 2%) and introduced into the detector via a capillary tube with a small orifice at one end. Having optimized the position of the capillary tube as well as the detection temperature, we succeeded in detecting small amounts of PCBs and organochlorine pesticides. Figure 1 shows a comparison of ECD and UV detection in SFC of organochlorine pesticides in a carrot sample, in which 1 α , 2 α , 3 β , 4 α , 5 α , 6 β -hexachlorocyclohexane (γ -HCH) could be detected only in ECD [6]. The proposed system is anticipated to be suitable for the microchemical determination of nonvolatile halogenated compounds.

3. Investigation of SFE behavior

In general, the properties of the extraction medium of SFE, such as density, viscosity and diffusion coefficient, are controlled by various operation parameters. When applying SFE to environ-

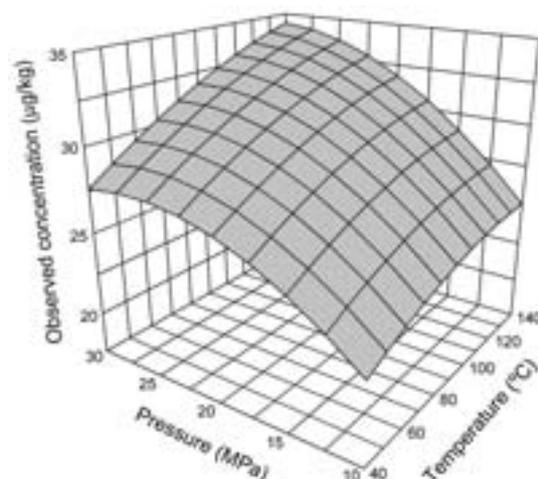


Figure 2. Response surface for SFE of 2,4,4'-trichlorobiphenyl (CB 28) in a sediment sample, showing the effect of the extraction temperature and pressure on SFE efficiency. Other extraction conditions: medium, carbon dioxide; mode and time, static mode for 15 min then dynamic mode for 30 min. Reproduced by permission of the Japan Society for Analytical Chemistry [9].

mental analysis, the effect of these parameters on the SFE yield of the target analyte is not predictable, since the sample matrix has a profound effect on the extraction efficiency. As one example, we have reported the effect of moisture content in soil samples on SFE recovery yields using triazine and thiolcarbamate herbicides as target analytes [7, 8]. The presence of small amounts of water in the matrix was considered to increase the recovery yields of analytes by acting as an internal modifier. In addition, adsorption sites on the matrix might be removed by the presence of moisture. On the other hand, the recovery yields of target analytes from soil containing 30% water were poorer than those from soil containing 10-20% water. We have also found that the concentration level of the triazine herbicides in the sample also influenced the recovery yield [7]. These results indicate that extraction of real samples should be carried out experimentally to permit successful optimization of extraction conditions.

The extraction temperature and pressure are the key parameters that control the physical properties of the carbon dioxide extraction medium. Because the effect of these parameters on extraction efficiency is complex, their optimization sometimes requires a process of trial and error. We have evaluated these parameters by two-way analysis of variance (ANOVA) using experimental SFE results for PCBs in a sediment sample [9]. Each corresponding $^{13}\text{C}_{12}$ -labeled PCB was used as an internal standard. The results indicated that not only extraction temperature and pressure but also their interaction were statistically significant in most target PCBs. We also observed that equilibrium between native PCBs and the

corresponding $^{13}\text{C}_{12}$ -labeled PCBs was not realized. We regressed the experimental data using a second-order polynomial that was used in a fractional factorial design approach [11] and predicted the SFE efficiency, as shown in Fig. 2.

4. On-line coupling of SFE and SFC

Development of high-throughput analytical methods is an important task in environmental analysis. SFE itself is a far more rapid extraction technique than classical solvent extraction, and the obtained extracts can in most cases be subjected to chromatographic systems, since carbon dioxide can be evacuated by changing the phase from supercritical fluid to gas under normal conditions such as room temperature and atmospheric pressure. We demonstrated on-line coupling of SFE and SFC for the rapid determination of thiolcarbamate herbicides in soil [8]. The development of an “organic-solvent free” system is another task in this study.

A schematic diagram of the developed system is shown in Fig. 3. The extract obtained by SFE was initially trapped in a trap column (9), then introduced into an SFC column (12) together with the mobile phase by changing the position of switching valves (6 and 10). Here, ODS columns prepared for HPLC were used as the trap and SFC columns. Because the degree of inertness of the column packings considerably influenced the retention of polar solutes when carbon dioxide was used as the mobile phase without a modifier, the inertness of the column packings was an essential contributor to realizing an organic solvent-free system [12, 13]. We therefore examined various ODS columns, ultimately concluding that a column packed with polymer-coated silica-gel packings showed the best performance. Figure 4 shows a typical chromatogram obtained using the proposed system. We successfully determined two target herbicides in soil samples within 1 h (20 min for extraction and 30 min for chromatographic analysis) without the use of any organic solvents.

5. Application of SFE to the production of certified reference materials

CRMs are playing an increasingly important role in ensuring the reliability of environmental analysis. However, the variety of matrix-type CRMs that are commercially available is limited, and so the development of these CRMs is of great concern. The National Metrology Institute of Japan (NMIJ) has been working on the development of matrix-type CRMs. To establish highly reliable (possibly traceable to the SI units) certified values, we principally apply isotope-dilution mass spectrometry (IDMS), which has the potential to be operated as a primary method of measurement [14], for the determination of certified values. However, incorrectly low

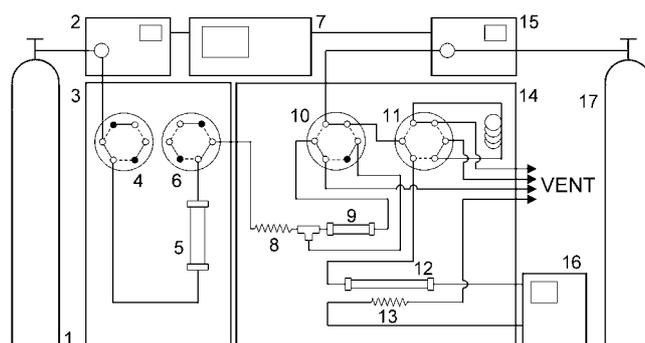


Figure 3. Schematic diagram of the SFE-SFC system. (1) and (17), carbon dioxide cylinders; (2) and (15), pumps; (3) and (14), ovens; (4), (6) and (10), switching valves; (5), extraction chamber; (7), pump controller; (8) and (13), restrictors; (9), trap column; (11), injector; (12), SFC column; (16), UV detector. Reproduced by permission of Elsevier [8].

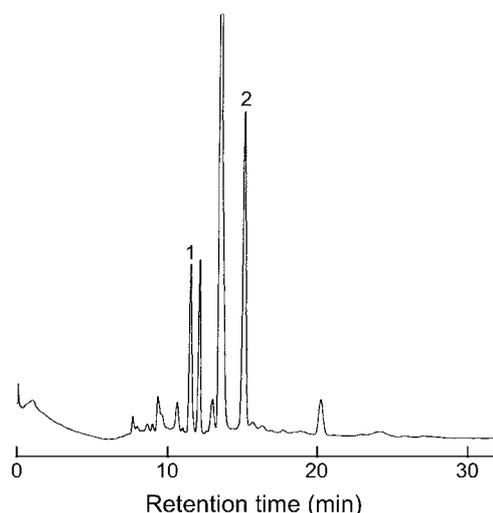


Figure 4. Chromatogram of thiolcarbamate herbicides (each 1 mg/kg spiked) in a soil sample obtained by the SFE-SFC system. Peak identification: (1), molinate; (2), thiobencarb. Pressure programming of carbon dioxide mobile phase: 10 MPa for 1 min, then raised at 1 MPa/min, and kept at 15 MPa for 25 min. Other SFC conditions: column, Capcell Pak C_{18} UG (size, 250 mm \times 4.6 mm I.D.; particle size, 5 μm ; pore diameter, 12 nm); column temperature, 55 $^{\circ}\text{C}$; UV detection, 220 nm. SFE conditions: medium, carbon dioxide; pressure, 20 MPa; temperature, 60 $^{\circ}\text{C}$; mode and time, dynamic for 20 min. Reproduced by permission of Elsevier [8].

analytical results are obtained from true values if equilibration is not achieved, so a highly efficient extraction technique is required for accurate determination of certified values. To avoid any possible procedural bias, we apply two or more independent extraction techniques [15]. In the case of the determination of PCB certified

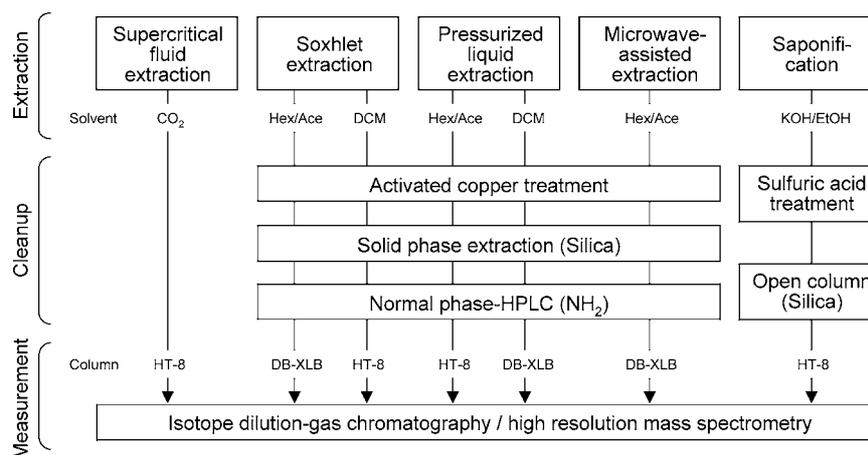


Figure 5. Analytical scheme for the determination of PCB certified values of NMIJ CRM 7304-a. Extraction solvent: Hex, hexane; Ace, acetone; DCM, dichloromethane; EtOH, ethanol.

values in sediment reference materials (NMIJ CRM 7304-a and CRM 7305-a), five independent extraction techniques were applied after the conditions of each had been fully validated or optimized [9, 16-19]. SFE was one of the extraction techniques that operated under optimized conditions, as briefly described in Section 3.

Figure 5 shows an analytical scheme for determining PCB certified values in CRM 7304-a [10]. Here, SFE has a characteristic very different from those of other extraction techniques, so it was regarded as being suitable as one of the extraction techniques in the determination of certified values. That is, supercritical carbon dioxide was used in SFE as an extraction medium without any organic modifier, whereas organic solvents were required in all the other extraction techniques. In addition, SFE did not require a cleanup process of the obtained extracts. Therefore, use of the method based on SFE ensured that no analytical bias was found during the cleanup process of the other analytical methods.

In most cases, no significant differences were found among the analytical results of target PCBs obtained by each extraction technique. In addition, the uncertainties associated with each analytical method were comparable to each other. Using all the results obtained, we provided the certified values and their uncertainties, as listed in Table 1. These CRMs are the first to be obtained by IDMS techniques together with well-validated or optimized extraction processes, and the obtained certified values are traceable to the SI units.

6. Conclusions

The author and coworkers have carried out fundamental and application studies for the development of environmental analysis methods using SFE and SFC. The methods developed by applying SFC demonstrated its advantages over conventional methods in the

Table 1. Certified values^a (mass fraction, $\mu\text{g}/\text{kg}$ dry mass) of PCB congeners^b in NMIJ CRM 7304-a and CRM 7305-a

	NMIJ CRM 7304-a	NMIJ CRM 7305-a
CB 3	0.311±0.085	0.15±0.07
CB 15	2.26±0.24	0.31±0.05
CB 28	34.9±2.3	2.9±0.2
CB 31	27.1±1.8	2.26±0.18
CB 70	60.7±3.8	4.0±0.3
CB 101	31.9±2.6	2.6±0.3
CB 105	18.4±2.0	1.27±0.14
CB 138	13.9±1.1	1.92±0.15
CB 153	15.9±1.0	3.2±0.3
CB 170	3.62±0.22	0.92±0.16
CB 180	9.10±0.69	2.4±0.5
CB 194	1.89±0.11	0.62±0.13
CB 206	0.476±0.050	0.15±0.03
CB 209	1.28±0.20	0.16±0.03

^a Results are expressed as the certified value \pm expanded uncertainty (coverage factor $k=2$)

^b The numbering presented is identical to the IUPAC number

variety of detection techniques as well as the rapidity of analysis by on-line coupling with SFE. Meanwhile, the methods based on SFE provided accurate determination that was applicable to the production of CRMs. The author expects that SFE and SFC have potential for routine application to environmental analyses as a complement to classical analytical techniques.

Acknowledgements

The author acknowledges the fruitful advice of Dr. Akira

Nomura. The author also expresses his gratitude to all collaborators and coworkers involved with these studies, especially Dr. Shigeo Gonda, Prof. Masami Shibukawa, Dr. Yoshiyuki Horimoto, Dr. Akiko Takatsu, Dr. Masahiko Numata, Dr. Kazumi Inagaki and Ms Yoshie Aoyagi. The author is very grateful to the Japan Society for Chromatographic Sciences for selecting him as a recipient of the Encouragement Award in 2007 and for giving him the opportunity to publish this review paper. A part of these studies was financially supported by the Japanese government's Millennium Research Project.

References

- [1] Klesper, E.; Corwin, A. H.; Turner, D. A. *J. Org. Chem.* **1962**, *27*, 700–701.
- [2] Giddings, J. C.; Myers, M.; McLaren, L.; Keller, R. *Science* **1968**, *162*, 67–73.
- [3] Novotny, M.; Springston, S. R.; Peaden, P. A.; Fjeldsted, J. C.; Lee, M. L. *Anal. Chem.* **1981**, *53*, 407A–414A.
- [4] Hawthorne, St. B. *Anal. Chem.* **1990**, *62*, 633A–642A.
- [5] Yarita, T.; Horimoto, Y.; Yamada, J.; Nomura, A. *Anal. Sci.* **1992**, *8*, 811–815.
- [6] Yarita, T.; Nomura, A.; Horimoto, Y.; Yamada, J. *Microchem. J.* **1994**, *49*, 145–149.
- [7] Yarita, T.; Horimoto, Y.; Nomura, A.; Gonda, S. *Chromatographia* **1996**, *42*, 551–554.
- [8] Yarita, T.; Nomura, A.; Horimoto, Y.; Gonda, S. *J. Chromatogr. A* **1996**, *750*, 175–181.
- [9] Yarita, T.; Aoyagi, Y.; Numata, M.; Takatsu, A. *Anal. Sci.* **2006**, *22*, 1449–1454.
- [10] Numata, M.; Yarita, T.; Aoyagi, Y.; Tsuda, Y.; Yamazaki, M.; Takatsu, A.; Ishikawa, K.; Chiba, K.; Okamoto, K. *Anal. Bioanal. Chem.* **2007**, *387*, 2313–2323.
- [11] Fernández, I.; Dachs, J.; Bayona, J. M. *J. Chromatogr. A* **1996**, *719*, 77–85.
- [12] Yarita, T.; Nomura, A.; Horimoto, Y. *J. Chromatogr. A* **1996**, *724*, 373–377.
- [13] Yarita, T.; Ihara, T.; Horimoto, Y.; Nomura, A. *Anal. Sci.* **1999**, *15*, 377–380.
- [14] Milton, M. J. T.; Quinn, T. J. *Metrologia* **2001**, *38*, 289–296.
- [15] Yarita, T.; Takatsu, A.; Inagaki, K.; Numata, M.; Chiba, K.; Okamoto, K. *Accred. Qual. Assur.* **2007**, *12*, 156–160.
- [16] Yarita, T.; Nakama, A.; Numata, M.; Aoyagi, Y.; Yamazaki, M.; Takatsu, A. *Bunseki Kagaku* **2003**, *52*, 1011–1017.
- [17] Yarita, T.; Nakama, A.; Yoshimura, K.; Takatsu, A.; Okamoto, K. *Bunseki Kagaku* **2004**, *53*, 177–182.
- [18] Numata, M.; Yarita, T.; Aoyagi, Y.; Takatsu, A. *Anal. Sci.* **2004**, *20*, 793–798.
- [19] Numata, M.; Yarita, T.; Aoyagi, Y.; Yamazaki, M.; Takatsu, A. *Chemosphere* **2005**, *58*, 865–875.