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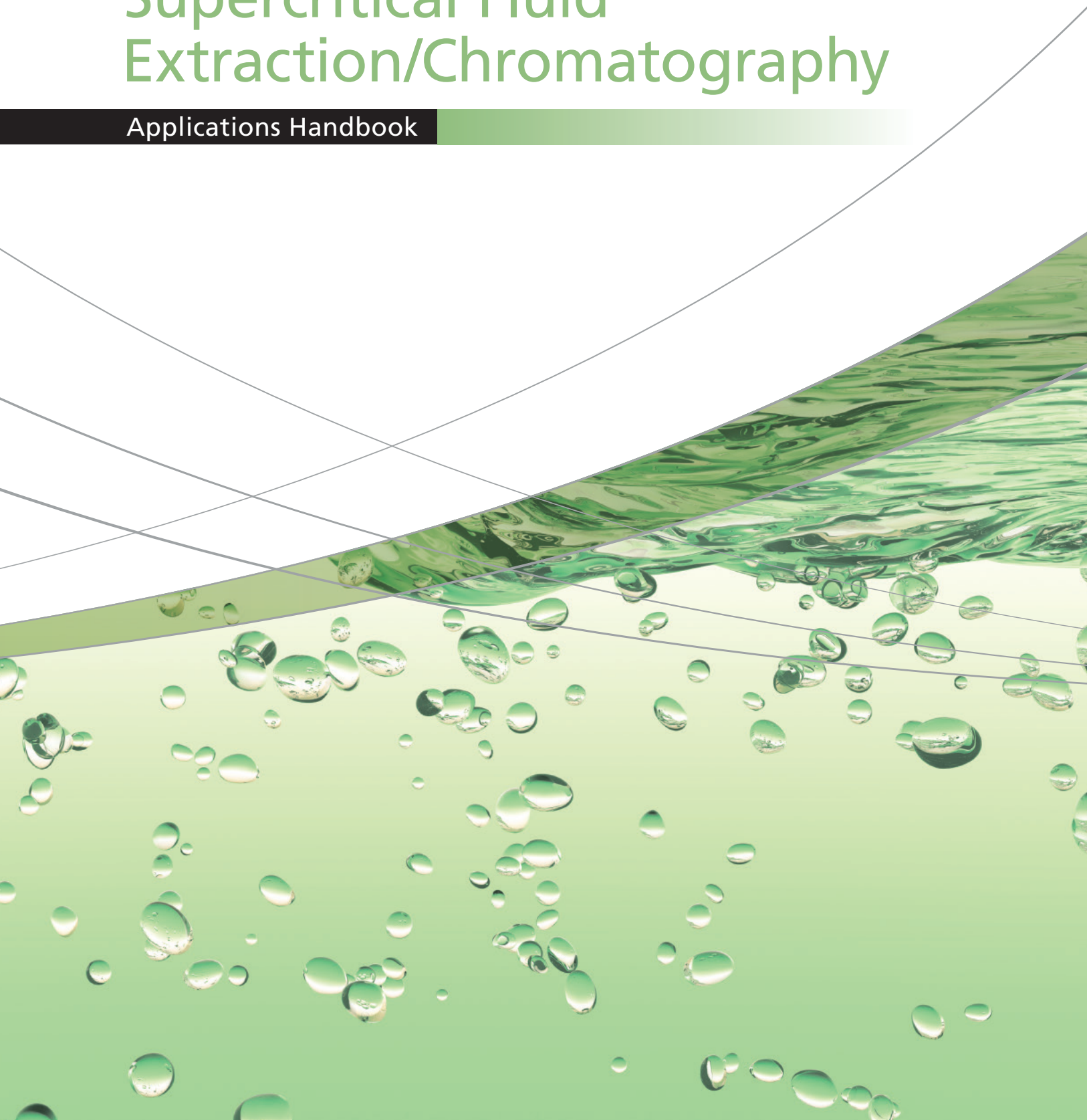
C10G-E059

Supercritical Fluid Extraction/Chromatography

Applications Handbook



Supercritical Fluid Extraction/Chromatography

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Applications Handbook

Pharmaceutical and Nutraceutical

Food Safety and Environmental

Automated Screening of Explosives in Soil Samples by Online SFE-SFC-MS

The recovery from soil sample, comparison between LC/MS/MS and SFC/MS/MS, the linearity results of LC/MS/MS were described.

Technical Report

Supercritical Fluid Chromatography

In this report, starting from the basic principles of supercritical fluid chromatography, we introduce examples of high-speed, high-resolution analysis and chiral separation.

Improved Sample Pretreatment Using Offline Supercritical Fluid Extraction

This article describes the utilization of the Nexera UC SFE pretreatment system, which increases the efficiency of sample pretreatment for analysis.

Online Supercritical Fluid Extraction Supercritical Fluid Chromatography (Online SFE-SFC)

We introduce the basic principle of online SFE-SFC, the characteristics of the "Nexera UC Online SFE-SFC System," and examples of extraction analyses.

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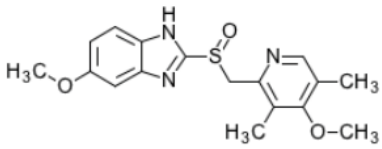
Application News

No.L495

Supercritical Fluid Chromatography

Automated Optimization of Chiral Separation Parameters Using Nexera UC Chiral Screening System

Chiral compounds contain asymmetric carbons in their molecules and are not superimposable on their mirror images. HPLC has been the main method used to separate such chiral compounds, but in recent years, the use of supercritical fluid chromatography (SFC) has been gaining attention. The main mobile phase used for chiral SFC is supercritical carbon dioxide, with low polarity, low viscosity, and high diffusivity, to which polar organic solvents (modifiers) are added to control solubility and polarity. Therefore, chiral compound separation by HPLC, which generally uses normal phase conditions, offers the potential for high speed, low organic solvent consumption, low cost, and low environmental impact. However, chiral SFC requires selecting a variety of separation parameters, such as columns and modifiers, which can involve large amounts of time and effort. This article describes using the Nexera UC chiral screening system to automatically optimize the large number of separation parameters by switching between up to 12 columns and various mixture ratios of four types of modifiers. This can significantly reduce the effort required.



Omeprazole

Fig. 1 Sample Used to Evaluate the Method Scouting Function

Separation Parameters for the Chiral Screening System

Model sample: The structure of omeprazole is shown in Fig. 1. Daicel CHIRALPAK®/CHIRALCEL® series 12 columns for chiral analysis were used for the analysis. These columns offer a line of complementary stationary phase columns that are able to separate a wide variety of chiral compounds. When used in combination with the Nexera UC chiral screening system, which features a method scouting function, optimal chiral separation parameters can be determined easily. In addition, three types of modifiers were used, methanol, ethanol, and a mixture of acetonitrile and ethanol. Details about the separation parameters are indicated in Table 1. The optimal parameters for chiral separation were comprehensively selected from the total of 36 possible combinations of modifiers (3 types) and columns (12 types).

Table 1 Analytical Conditions

Column	: CHIRALPAK®, CHIRALCEL® Series 100 mm L. × 3.0 mm I.D., 3 µm
Mobile Phase	: A; Super critical fluid of CO_2 B; Modifier: Methanol, Ethanol, mixture of Acetonitrile: Ethanol = 3:1 (v:v)
Time Program	: B Conc. 20 % (0 - 8 min) → 40 % (8 - 10 min) → 20 % (10 - 14 min)
Flowrate	: 3 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 µL
BPR Pressure	: 10 Mpa
Detector	: Photodiode Array Detector (Max Plot 210 - 400 nm)

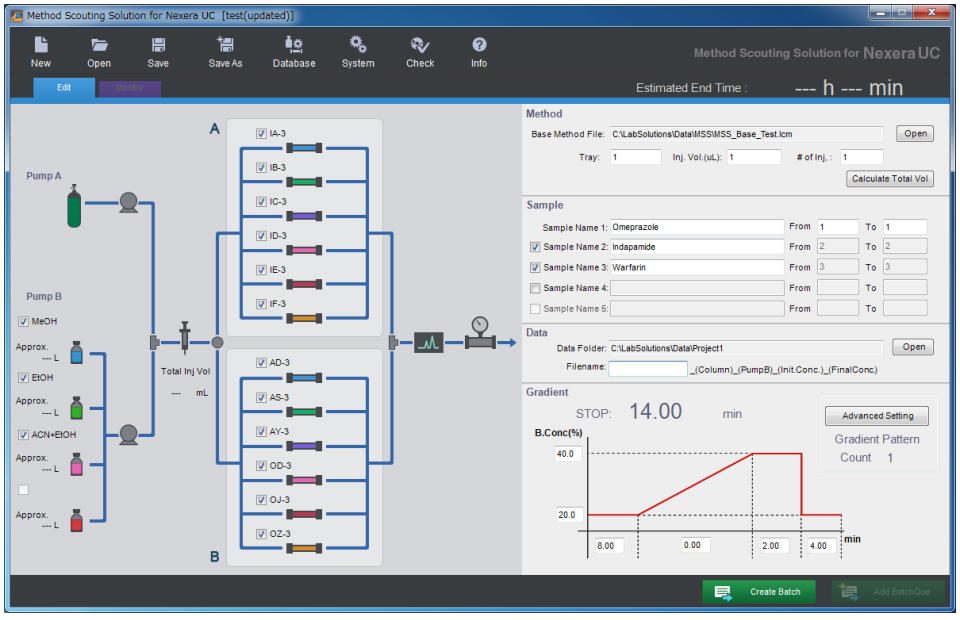
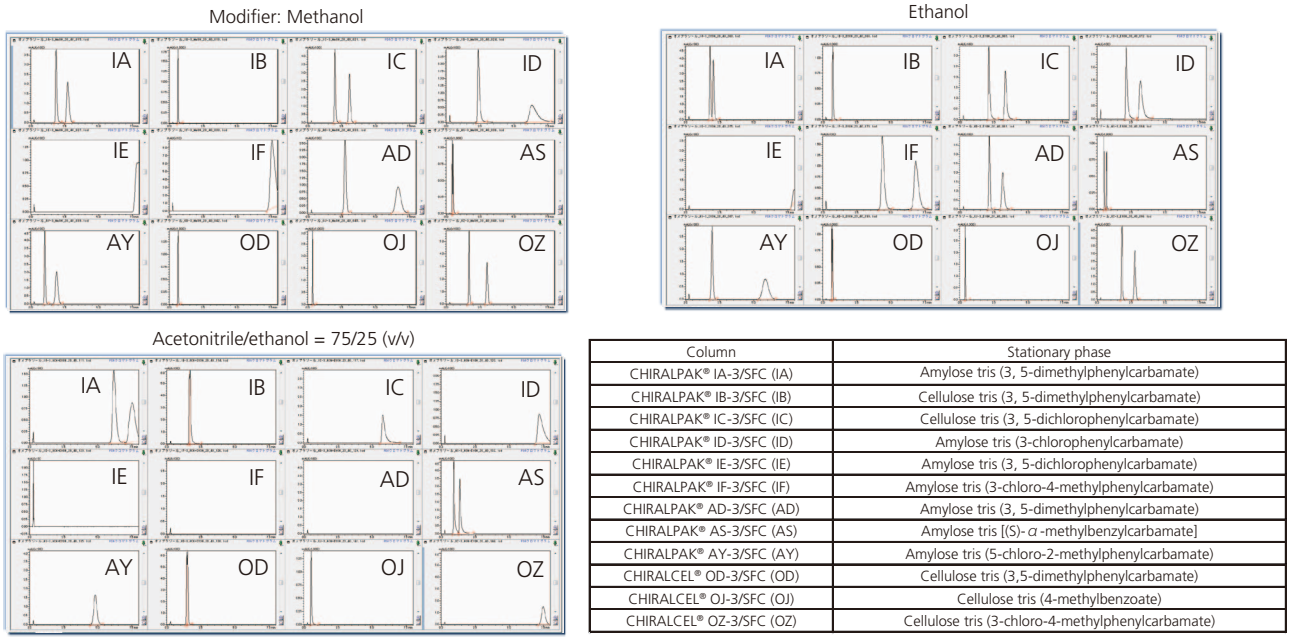


Fig. 2 Method Scouting Solution Operating Screen for Nexera UC

Automated Optimization of Chiral Separation Parameters for Omeprazole

Fig. 3 shows the results from a total of 36 possible combinations of 12 chiral columns and 3 types of modifiers (methanol, ethanol, and acetonitrile/ethanol mixture).

For omeprazole, separation of peaks for two chiral forms were confirmed within 8 minutes of retention. Fig. 4 shows the separation evaluation and optimal parameter ranking results from the optional software. The software automatically ranks all the chromatograms with separation greater than a given criteria (in this case, 1.5). This confirmed the utility of using the Nexera UC chiral screening system to automatically optimize separation parameters for chiral SFC, which otherwise requires a complicated process of selecting analytical conditions.



Column	Stationary phase
CHIRALPAK® IA-3/SFC (IA)	Amylose tris (3, 5-dimethylphenylcarbamate)
CHIRALPAK® IB-3/SFC (IB)	Cellulose tris (3, 5-dimethylphenylcarbamate)
CHIRALPAK® IC-3/SFC (IC)	Cellulose tris (3, 5-dichlorophenylcarbamate)
CHIRALPAK® ID-3/SFC (ID)	Amylose tris (3-chlorophenylcarbamate)
CHIRALPAK® IE-3/SFC (IE)	Amylose tris (3, 5-dichlorophenylcarbamate)
CHIRALPAK® IF-3/SFC (IF)	Amylose tris (3-chloro-4-methylphenylcarbamate)
CHIRALPAK® AD-3/SFC (AD)	Amylose tris (3, 5-dimethylphenylcarbamate)
CHIRALPAK® AS-3/SFC (AS)	Amylose tris [(S)-α-methylbenzylcarbamate]
CHIRALPAK® AY-3/SFC (AY)	Amylose tris (5-chloro-2-methylphenylcarbamate)
CHIRALCEL® OD-3/SFC (OD)	Cellulose tris (3,5-dimethylphenylcarbamate)
CHIRALCEL® OJ-3/SFC (OJ)	Cellulose tris (4-methylbenzoate)
CHIRALCEL® OZ-3/SFC (OZ)	Cellulose tris (3-chloro-4-methylphenylcarbamate)

Fig. 3 Comparison of Separation Using Different Combinations of 12 Chiral Columns and 3 Modifiers

Ranking	Run No.	Analytical Condition	Resolution	Separatoin factor	Symmetry factor		Retention factor		Area%		Peak number
					Peak1	Peak2	Peak1	Peak2	Peak1	Peak2	
1	32	Omeprazole_OZ-3_MeOH_20_40	7.965	1.921	1.16	1.159	6.583	12.644	49.829	50.171	2
2	17	Omeprazole_IC-3_MeOH_20_40	5.587	1.602	1.387	1.274	8.078	12.937	49.971	50.029	2
3	16	Omeprazole_IC-3_EtOH_20_40	5.382	1.639	1.915	1.661	8.617	14.124	49.984	50.016	2
4	31	Omeprazole_OZ-3_EtOH_20_40	5.377	1.599	1.169	1.162	7.229	11.561	49.778	50.222	2
5	1	Omeprazole_AD-3_EtOH_20_40	3.996	1.509	1.257	1.404	8.779	13.25	50.054	49.946	2
6	8	Omeprazole_AY-3_MeOH_20_40	3.55	2.08	1.178	1.145	3.652	7.597	49.974	50.026	2
7	11	Omeprazole_IA-3_MeOH_20_40	3.428	1.523	1.464	1.312	7.435	11.327	49.973	50.027	2
8	4	Omeprazole_AS-3_EtOH_20_40	2.515	1.673	1.657	1.518	1.244	2.081	49.754	50.246	2
9	10	Omeprazole_IA-3_EtOH_20_40	1.586	1.157	1.322	1.279	7.115	8.234	49.347	50.653	2

Separation Parameters for Rank 1
Column: CHIRALCEL® OZ-3/SFC
Modifier: Methanol

Separation Parameters for Rank 2
Column: CHIRALPAK® IC/SFC
Modifier: Methanol

Separation Parameters for Rank 3
Column: CHIRALPAK® IC/SFC
Modifier: Ethanol

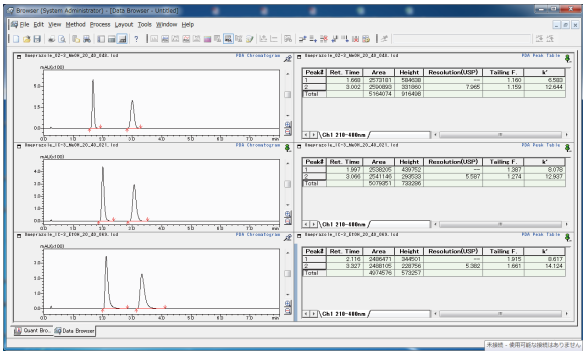


Fig. 4 Evaluation of Separation Parameters and Chiral Separation Chromatogram Using Optimized Parameters

* CHIRALPAK® and CHIRALCEL® are registered trademarks of Daicel Corporation.

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Application News

No.L499A

Supercritical Fluid Extraction / Chromatography

Application of Online SFE-SFC-PDA for Cleaning Validation

Cleaning validation is a process step that is extremely important for ensuring high quality and safety at pharmaceutical manufacturing sites. Cloth used for surface wiping, called a swab, is used to wipe a given part of a piece of manufacturing equipment, and analysis of the wiped area of the swab is performed by using high-performance liquid chromatography (HPLC) or a total organic carbon analysis (TOC). Evaluations using HPLC have been increasingly used in recent years because HPLC enables determination of individual compounds. Prior to analysis, an extraction procedure must be performed on the swab. Using supercritical fluid extraction (SFE) as the pretreatment method allows for simple and quick target component extraction. Using supercritical fluid chromatography (SFC) after SFE also means that analysis results can be obtained simply by preparing the sample for SFE, which unifies the work flow from pretreatment to analysis. Please see Application News L496 for an overview of online SFE-SFC. This article describes the process of column selection using the Nexera-UC Chiral Screening System as the first step in analysis of the target compound alkylbenzenesulfonate.

Analytical Column Selection

For SFC analysis, selection of the optimal column for the sample has a substantial effect on analysis reliability. We performed SFC separation of alkylbenzenesulfonate in four different columns under the conditions shown in Table 1 and Fig. 1, and chose the Shim-pack UCX-SIL analytical column as it had the best peak shape. Based on an investigation of gradient profiles, we also found a relatively steep gradient profile is suitable for quantitative analysis as the properties of alkylbenzenesulfonate, which have different length of carbon chains, mean the significant peak broadening if the gradient slope is not steep. Based on this information, we optimized analytical conditions using the Shim-pack USX-SIL column and performed online SFE-SFC analysis of a sample from a swab.

Table 1 SFC Analytical Conditions for Column Selection

Column	: Shim-pack UCX series columns (250 mm L. × 4.6 mm I.D., 5 µm) (i) UCX-RP (ODS with polar group), (ii) UCX-GIS (ODS), (iii) UCX-SIL, (iv) UCX-DIOL
Mobile Phase	: A: CO_2; B: Methanol
Time Program	: Shown in the figure
Flowrate	: 3.0 mL/min
Column Temp.	: 40 °C
Back Pressure	: 15 MPa
Wavelength	: 220 nm
Injection Vol.	: Shown in figure

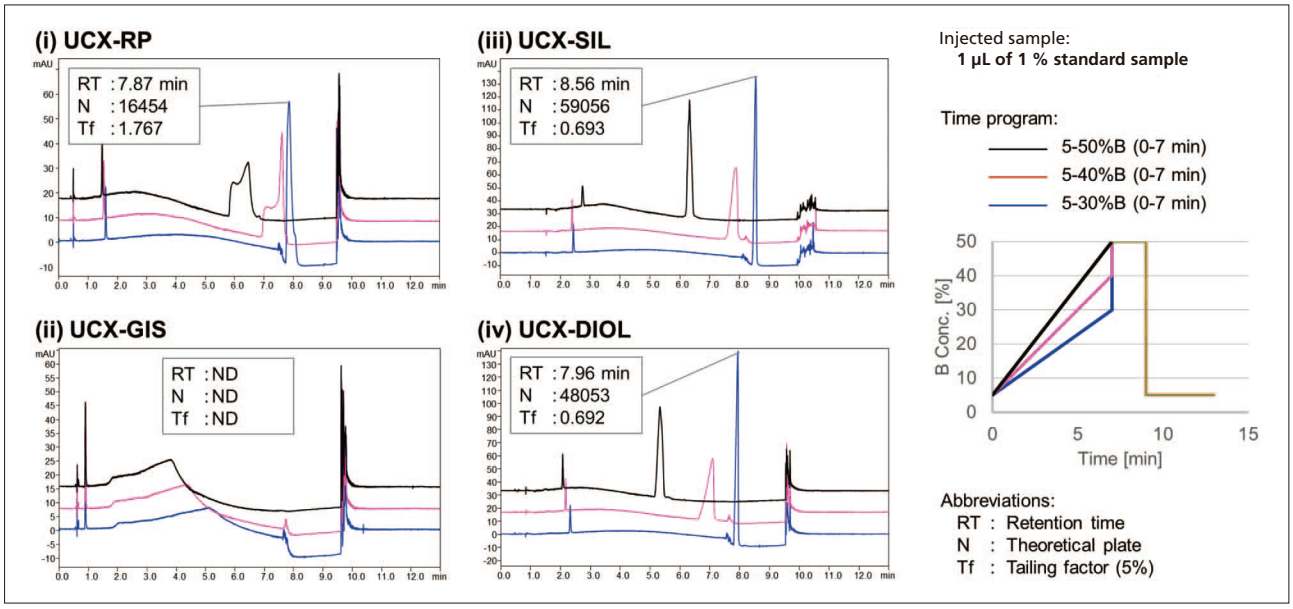

Fig. 1 Comparison of SFC Separation of Standard Alkylbenzenesulfonate in Four Different Columns

Online SFE-SFC Analysis of a Swab Containing Alkylbenzenesulfonate

We investigated column selection by the scouting system, chose the Shim-pack UCX-SIL analytical column, optimized each analytical condition for online SFE-SFC analysis, then performed analysis using the conditions shown in Table 2 below.

Table 2 Analytical Conditions for Online SFE-SFC

[Sample Preparation]	
A total of 10 to 500 µg standard samples in methanol were dropped onto swabs.	
The swabs were enclosed into an extraction vessel and set to the SFE unit.	
[Static Extraction]	
Extraction Time	: 3 min
Mobile Phase	: A: CO_2; B: 0.1 % (w/v) Ammonium Formate in Methanol
B Conc.	: 10 %
Flowrate	: 3.0 mL/min
Back Pressure	: 15 MPa
[Dynamic Extraction]	
Extraction Time	: 3 min
Mobile Phase	: A: CO_2; B: Methanol
B Conc.	: 10 %
Flowrate	: 3.0 mL/min
Back Pressure	: 15 MPa
[SFC]	
Column	: Shim-pack UCX-SIL (250 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: A: CO_2; B: Methanol
Time Program	: 10 %B (0-2 min), 10-60 %B (2-7 min), 60 %B (7-9 min), 10 %B (9-13 min)
Flowrate	: 3.0 mL/min
Column Temp.	: 40 °C
Back Pressure	: 15 MPa
Wavelength	: 220 nm

The peak for the surfactant alkylbenzenesulfonate was well-separated and detected as shown in Fig. 2 below. Fig. 3 shows the results of performing repeated SFE-SFC analyses from the same swab to which had been added an equivalent of 100 ng of alkylbenzenesulfonate. Since there was almost no alkylbenzenesulfonate peak evident from the second and later sample extractions, the extraction procedure was almost entirely complete after the first SFE. Fig. 4 shows the results of adding amounts of alkylbenzenesulfonate to swabs in the range of 10 to 500 µg, and checking linearity. Within this range, the coefficient of determination that represents linearity was 0.996. Fig. 5 shows the result of five consecutive analyses of separate swabs to which were added 100 µg of alkylbenzenesulfonate. Considering the process including extraction, the repeatability of retention times was 0.19 %RSD, and repeatability of peak area was 5.76 %RSD. Based on these results, we confirmed the usefulness of the Nexera-US Online SFE-SFC System in this application.

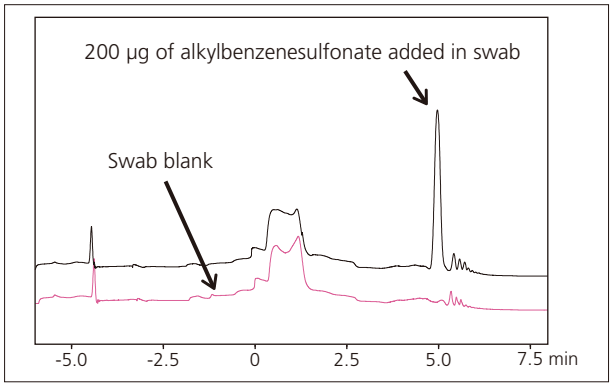

Fig. 2 Online SFE-SFC Analysis of Alkylbenzenesulfonate

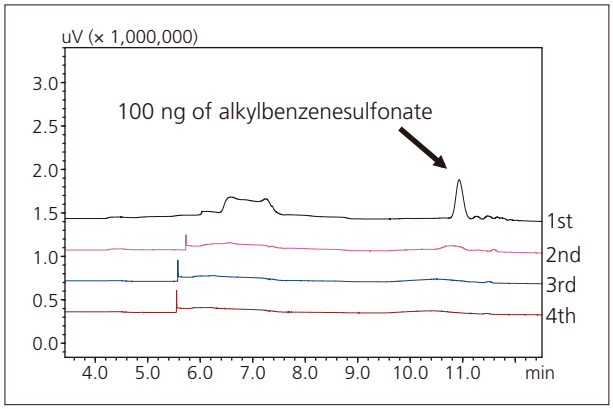

Fig. 3 Confirmation of Online SFE Extraction Efficiency

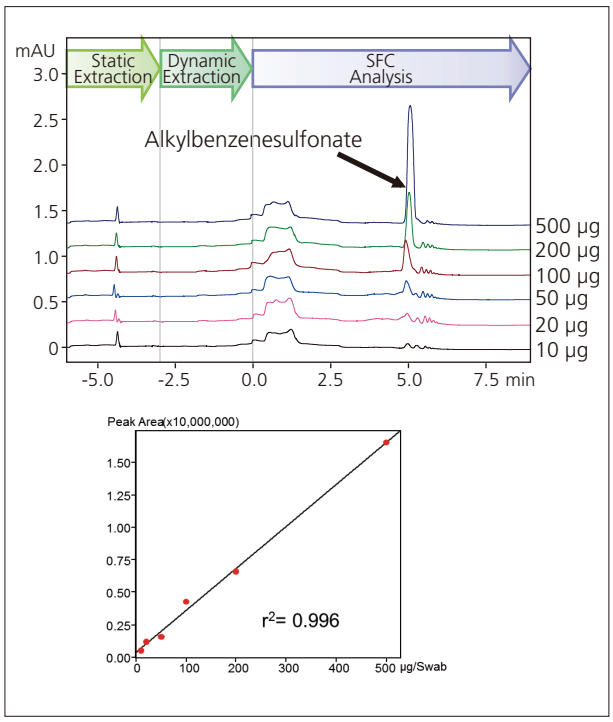

Fig. 4 Linearity of Online SFE-SFC Analysis Using a Swab

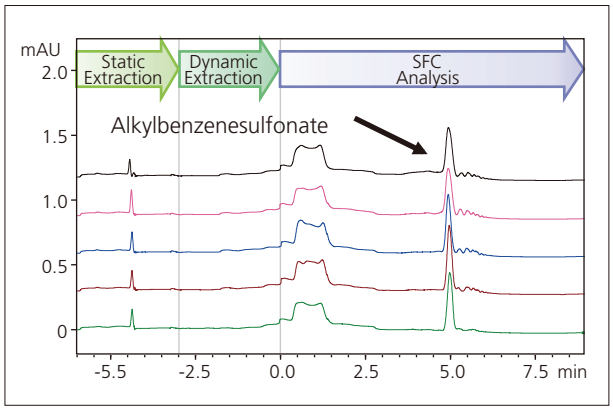

Fig. 5 Repeatability of Online SFE-SFC Analysis Using a Swab

Note: Swab samples were provided by DAIICHI SANKYO COMPANY, LIMITED.

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Application News

No.L501

Supercritical Fluid Extraction / Chromatography

Analysis of Vitamin E in a Commercial Supplement by Offline SFE-SFC-PDA

Vitamin E, also called tocopherol, is a fat-soluble vitamin and an important chemical substance that exhibits an antioxidant effect, particularly in the human body. There are four tocopherols (α, β, γ and δ) that differ based on the number and position of methyl groups. The α-tocopherol exhibits the strongest antioxidant activity, and this is the tocopherol form found in most commercial supplements as vitamin E. Since it is highly fat-soluble, a quick and simple extraction method using supercritical fluid is expected to be applicable. In this article, we introduce a procedure for α-tocopherol pretreatment that uses supercritical fluid extraction (SFE).

Offline SFE System

While the online SFE-SFC system has already been described in several Application News articles, many have expressed the desire to combine SFE with existing analytical methods other than SFC, and SFE has gained attention for its flexibility in terms of sample handling. The advantages of SFE are as follows.

1. Quick and highly efficient extraction using supercritical fluid that is highly permeable and has a high diffusion rate.
2. Extraction of unstable compounds under mild temperature conditions with light-shielding.
3. Low cost compared to solvent extraction.
4. Complete automation of the extraction procedure.
5. Easy handling of the extraction sample.
6. Compatible with various analysis methods.

Fig. 1 shows a flow diagram for an offline SFE system. A supercritical state is present upstream of the BPR back-pressure control unit. Valves inside the SFE unit are controlled to switch between static extraction via enclosure of supercritical fluid in the vessel and dynamic extraction via passage of supercritical fluid through the vessel, which enables quick and highly efficient extraction of the target compounds.

A HPLC pump with a low-pressure GE valve installed is used in the solvent delivery system, and the extraction conditions can be optimized by changing the type of modifier (maximum of four types, including eluent from the trap column) and the concentration relative to carbon dioxide. Extract is retained in the trap column, and the low-pressure GE valve on the solvent delivery pump is switched to the solvent suitable for elution from the trap column. Then the eluent is collected in test tubes with a fraction collector.

SFE Treatment for α-Tocopherol

The commercial supplement used as an actual sample may be present as a paste inside the capsule and may be moisture absorbent. As shown in Fig. 2, we mixed 275 mg of paste supplement with 1 g of Miyazaki Hydro-Protect, which is a dehydrating agent for SFE sold by Shimadzu, and transferred this mixture to the SFE extraction vessel.

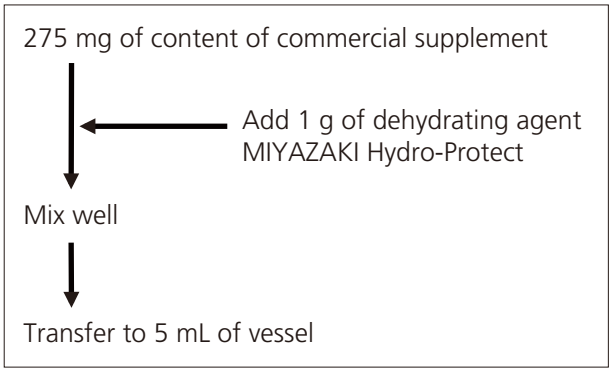

Fig. 2 Preliminary Pretreatment for Supplement Sample Before SFE

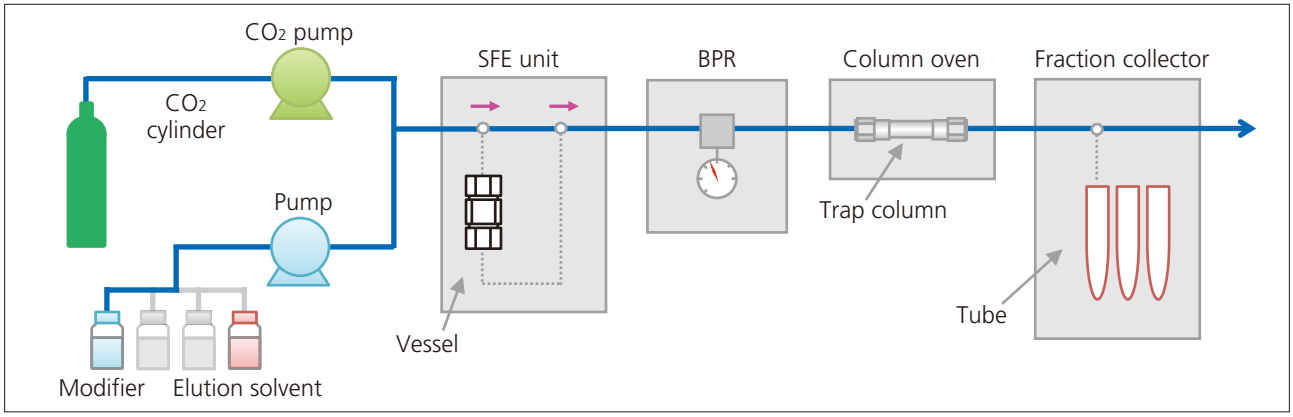

Fig. 1 Flow Diagram of Supercritical Fluid Extraction (SFE) System

The conditions used for SFE are shown in Table 1. We investigated column selection, chose the Shim-pack UCX-SIL analytical column, optimized each analytical condition for online SFE-SFC analysis, then performed analysis using the conditions shown in Table 2.

Table 1 SFE Conditions for α-Tocopherol

Offline SFE:	
Extraction Vessel	: 5 mL
Extraction Solvent	: CO_2
Flowrate	: 5 mL/min
Temperature	: 40 ˚C
Back Pressure	: 15 MPa
Extraction Time	: 15 min (Static 2 min → Dynamic 3 min) × 3 times
Trap & Pressure Down Conditions	
Trap Column	: Shim-pack VP-ODS (50 mm L. × 4.6 mm I.D.)
Temperature	: 60 ˚C
Pressure Down Time	: 10 min (15 - 25 min)
Recovery Conditions	
Elution Solvent	: Hexane
Flowrate	: 2 mL/min
Temperature	: 60 ˚C
Fraction Time	: 3.5 min (25 - 28.5 min)

SFE Evaluation of α-Tocopherol in a Commercial Supplement

For the α -tocopherol extract obtained through offline SFE, we performed SFC under the conditions shown in Table 2 then evaluated the extraction procedure. Extract was mixed with hexane to make up 10 mL before being used for SFC analysis. A representative SFC chromatogram is shown in Fig. 3.

Table 2 SFC Conditions for α-Tocopherol

SFC Conditions:	
Column	: Nacalai COSMOSIL Cholester (250 mm L. × 4.6 mm I.D., 5 µm)
Flowrate	: 3 mL/min
Modifier	: IPA
Gradient	: 2 % (0 min) - 20 % (10 min) - 50 % (10 - 12 min)
Temperature	: 40 ˚C
Back Pressure	: 15 MPa
Injection Volume	: 2 µL

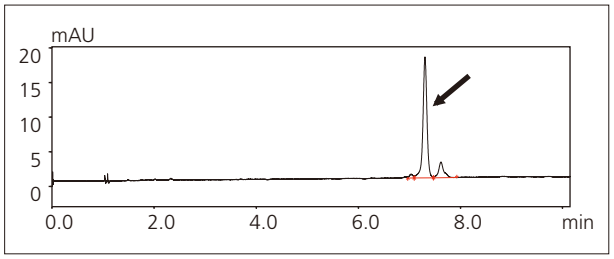

Fig. 3 SFC Analysis of α-Tocopherol Obtained by SFE from a Commercial Supplement

First, we used a standard product to evaluate the suitability of the α-tocopherol SFC conditions used for evaluation of offline SFE. Fig. 4 shows the linearity in the sample concentration range of 0.5 µg/L to 2.0 µg/L, and Table 3 shows the repeatability at a concentration of 1.0 µg/L. Good linearity and sufficient repeatability in terms of retention time, peak area and peak height were obtained.

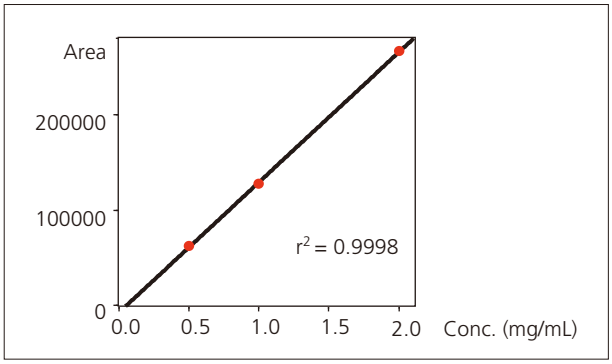

Fig. 4 Linearity for Standard α-Tocopherol Obtained by SFC

Table 3 Repeatability for Standard α-Tocopherol Obtained by SFC (n=6)

No	Retention Time (min)	Area	Height
Average	7.242	127,338	19,682
RSD (%)	0.057	0.573	0.274

Table 4 shows the repeatability of the quantitative α-tocopherol result obtained by repeated SFE treatment, and α -tocopherol recovery relative to the theoretical value (7.4 mg). Fig. 5 shows the overlaid chromatograms for α-tocopherol. Good recovery and repeatability was confirmed after just one extraction, showing that offline SFE is effective for vitamin E compound extraction.

Table 4 Repeatability and Recovery of α-Tocopherol in a Commercial Supplement Using SFE

No	Conc. (mg/mL)	Recovery (%)
1	0.776	104.46
2	0.780	105.00
3	0.772	103.92
4	0.790	106.35
5	0.761	102.44
6	0.758	102.04
Average	0.773	
RSD (%)	1.549	

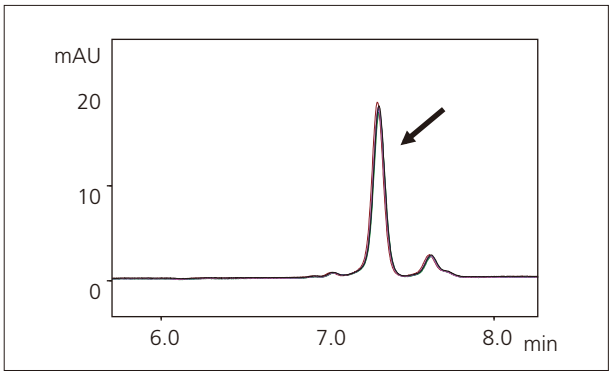

Fig. 5 Overlaid Chromatograms for α-Tocopherol After SFE

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Application News

No. L517

Supercritical Fluid Chromatography

Analysis and Evaluation of Chiral Drugs in Biological Samples Using the Nexera UC-MS/MS System

As introduced in Application News No. L495, the optimization for chiral separation using supercritical fluid chromatography (SFC) starts from employing column scouting to find the column and mobile phase appropriate to separation. This article introduces an example of the selectivity and sensitivity of drug level monitoring in a biological sample and the evaluation results of the analysis method, as an application to the pharmacokinetics research of chiral separation using SFC/MS/MS, after having selected an appropriate column.

Y. Watabe, T. Hattori, T. Iida

Analysis of Omeprazole in a Plasma Sample

The applicability of human plasma matrix to SFC was evaluated taking an example of enantiomeric drug omeprazole, well-known as a proton pump inhibitor. Fig. 1 shows the chemical structure of omeprazole. Fig. 2 shows the pretreatment procedure employed for the blood plasma sample. Table 1 lists the analytical conditions. CHIRALPAK® IC-3 from Daicel Company, which exhibited good separation when utilized in Application News No. L495 was used as the column. Detection was performed using the LCMS-8050 triple quadrupole mass spectrometer.

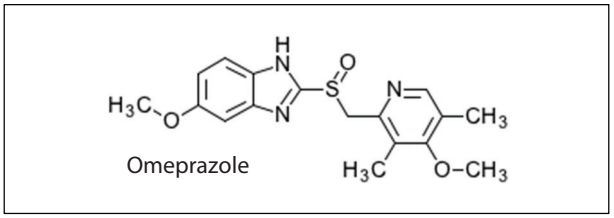

Fig. 1 Omeprazole Structure

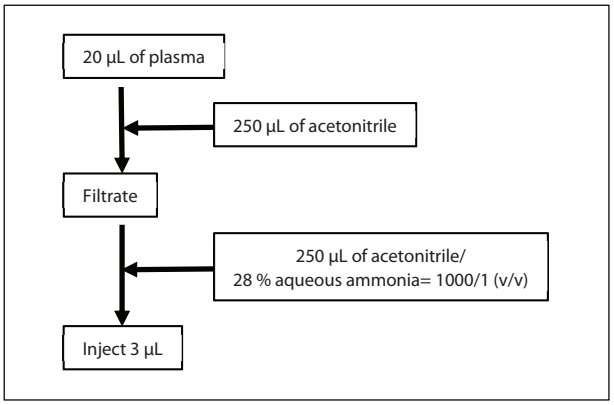

Fig. 2 Plasma Sample Pretreatment Procedure

Table 1 Analytical Conditions

Column	:	CHIRALPAK®, IC-3 (100 mm L. × 3.0 mm I.D., 3 µm)
Mobile phase	:	A) Super critical fluid of CO_2 B) Modifier: Methanol A/B = 5/1 (v/v for omeprazole, isocratic) = 4/1 (v/v for rabeprazole, isocratic)
Flow rate	:	3 mL/min
Column temp.	:	40 °C
Injection volume	:	3 µL
BPR pressure	:	10 MPa
BPR temp.	:	50 °C
Detector	:	LCMS- 8050 (ESI, MRM mode)
Make-up	:	Methanol
Make-up flow rate	:	0.1 mL/min
MRM	:	(+) *m/z* 346.1 > 198.1 (for omeprazole) (+) *m/z* 359.9 > 150.1 (for rabeprazole)

Calibration curve was created based on human plasma samples that contained 1, 2, 10, 2 and 100 µg/L of standard omeplazole to confirm the linearity of loaded amounts.
Fig. 3 and Fig. 4 show the MRM chromatograms for 2 µg/L and 20 µg/L respectively. Among the optically separated peaks, (A) is the fast-eluting isomer and (B) is the slow-eluting isomer. The linearity (r^2) obtained after correcting by 1/(concentration squared) was favorable at 0.99996 for omeprazole (A) and 0.99998 for omeprazole (B).

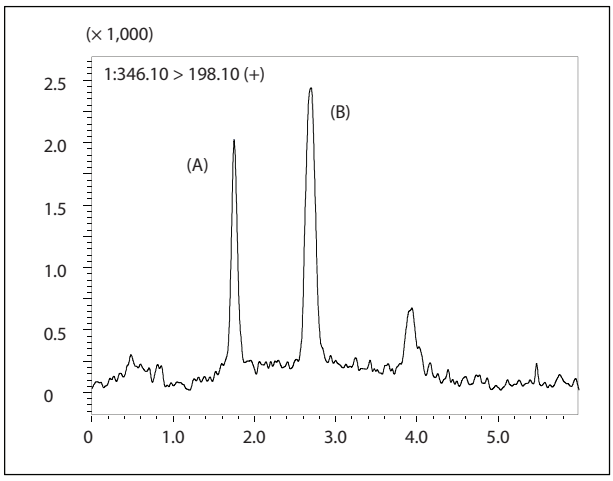

Fig. 3 Omeprazole Added to Human Plasma (2 µg/L)

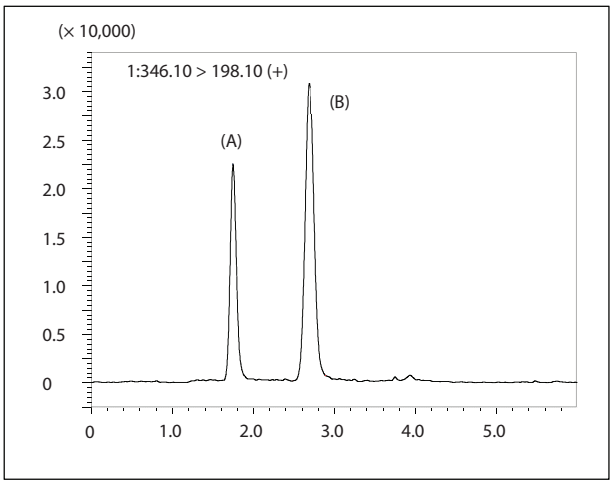

Fig. 4 Omeprazole Added to Human Plasma (20 µg/L)

The repeatability of the area values at 2 µg/L obtained from five repetitions was favorable with RSD values of 4.4 % for both omeprazole (A) and (B). At 10 µg/L, the recovery rates calculated from the results of stock solution analyses were 101.1 % and 100.5 % respectively.

Analysis of Rabeprazole in a Plasma Sample

Rabeprazole, known as a gastric acid secretion inhibitor, has a similar chemical structure to omeprazole, suggesting the possibility of successful chiral separation under similar analytical conditions including the same analytical column. Here we attempted to analyze rabeprazole in a plasma sample based on the analytical conditions used for omeprazole in the previous section. The chemical structure of rabeprazole is shown below. The structural similarity to omeprazole is easily recognized. As shown in Table 1, analysis was successful by merely changing the modifier concentration and the MRM settings.

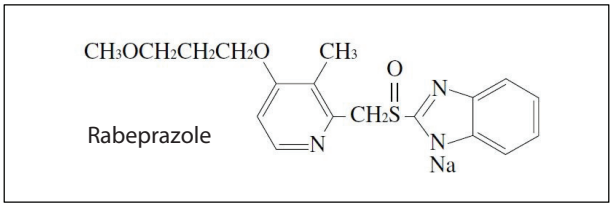

Fig. 5 Rabeprazole Structure

Calibration curve was crated based on human plasma samples that contained 0.3, 1, 3, 10 and 30 µg/L of standard raberlazole to confirm the linearity of loaded amounts. Fig. 6 and Fig. 7 show the MRM chromatograms for 3 µg/L and 30 µg/L respectively. As in Fig. 3 and Fig. 4, (A) is the fast-eluting isomer among the optically separated peaks and (B) is the slow-eluting isomer.
The linearity (r^2) obtained after correcting by 1/(concentration squared) was favorable at 0.99996 for rabeprazole (A) and 0.99999 for rabeprazole (B).

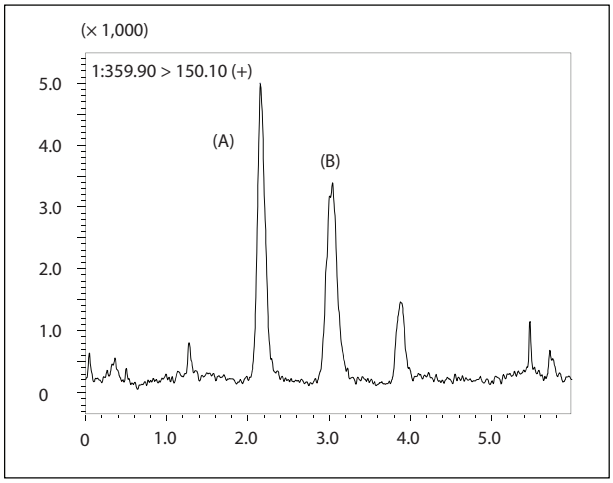

Fig. 6 Rabeprazole Added to Human Plasma (3 µg/L)

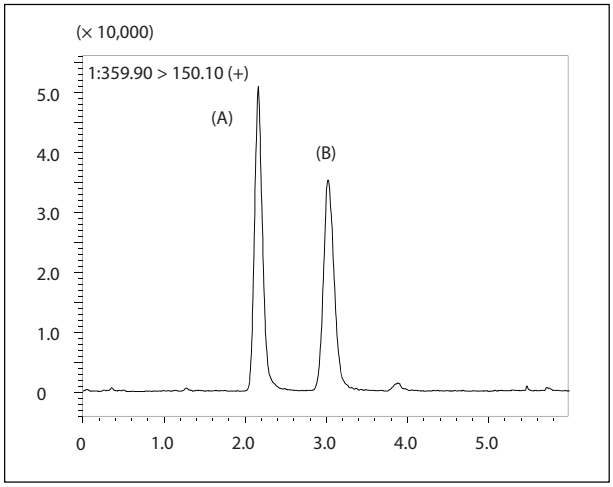

Fig. 7 Rabeprazole Added to Human Plasma (30 µg/L)

The repeatability of the area values at 10 µg/L obtained from five repetitions was favorable with RSD values of 1.8 % and 2.4 % for rabeprazole (A) and (B) respectively. The recovery rates calculated from the results of stock solution analyses were 102.5 % and 100.1 % respectively. Table 2 summarizes the linearity, peak area repeatability, and recovery rate for each compound. These results verify the applicability of this method to the practical analysis of plasma samples.

Table 2 Evaluation Results

	Linearity (r^2)	Area Repeatability (%RSD)	Recovery Rate (%) (4)
Omeprazole (A)	0.99996 (1)	4.4 (3)	101.1
Omeprazole (B)	0.99998 (1)	4.4 (3)	100.5
Rabeprazole (A)	0.99996 (2)	1.8 (4)	102.5
Rabeprazole (B)	0.99999 (2)	2.4 (4)	100.1

(1) 1 to 100 µg/L, (2) 0.3 to 300 µg/L, (3) 2 µg/L, (4) 10 µg/L

Notes: This product has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination and treatment or related procedures.

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Application News

No. L519

Supercritical Fluid Chromatography

Analysis of Choline and Acetylcholine in Rat Cerebrospinal Fluid Samples Using the Nexera UC-MS/MS System

Choline, which is a structural element of cell membranes, and acetylcholine, which is known as a neurotransmitter, are both familiar compounds in the field of bioanalysis. Since acetylcholine is biosynthesized in the body from choline, it is possible to estimate the quality of internal activity by monitoring both of these compounds. This article focuses on the SFC analysis of these compounds in a rat cerebrospinal fluid sample by direct injection of the cerebrospinal fluid to the Nexera UC SFC system. Also introduced is automatic extraction and analysis of a cerebrospinal fluid sample impregnated into filter paper, in consideration of convenience and durability for storage and transport, using the Nexera UC online SFE-SFC-MS/MS system.

Y. Watabe, T. Iida

SFC-MS/MS Analysis

A CN column provided favorable separation of choline and acetylcholine in SFC-MS/MS analysis. Calibration curves were created from the peak area values from six times repeated analyses for each of the three concentrations of 10, 100, and 1000 µg/L. Good linearity was obtained and the quantitation limit (LOQ, ASTM method) was 30 µg/L for choline and 10 µg/L for acetylcholine. Table 1 lists the conditions of SFC-MS/MS analysis. Fig. 1 shows the structural formula of choline and acetylcholine and Fig. 2 shows the obtained calibration curves.

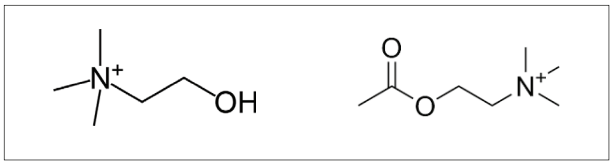

Fig. 1 Structure of Choline (Left) and Acetylcholine (Right)

Table 1 SFC-MS/MS Analytical Conditions

Column	:	Inertsil CN-3 250 mm L. × 4.6 mm I.D., 5 µm
Mobile phase	:	A) Supercritical fluid of CO_2 B) Modifier: Methanol containing 20 mmol/L ammonium formate / water =95/5 (v/v)
Time program	:	B Conc. 10 % (0 min) → 25 % (10 min) → 50 % (10.1-12 min) → 10 % (12.1-15 min)
Flow rate	:	2.5 mL/min
Column temp.	:	40 °C
Injection volume	:	1 µL
BPR pressure	:	10 Mpa
BPR temp.	:	50 °C
Detector	:	LCMS-8050 (ESI, MRM mode)
Make-up	:	Methanol
Make-up flow rate	:	0.2 mL/min
MRM transitions	:	(+) *m/z* 104.1>60.1 (for choline) (+) *m/z* 146. 1>87.1 (for acetylcholine)

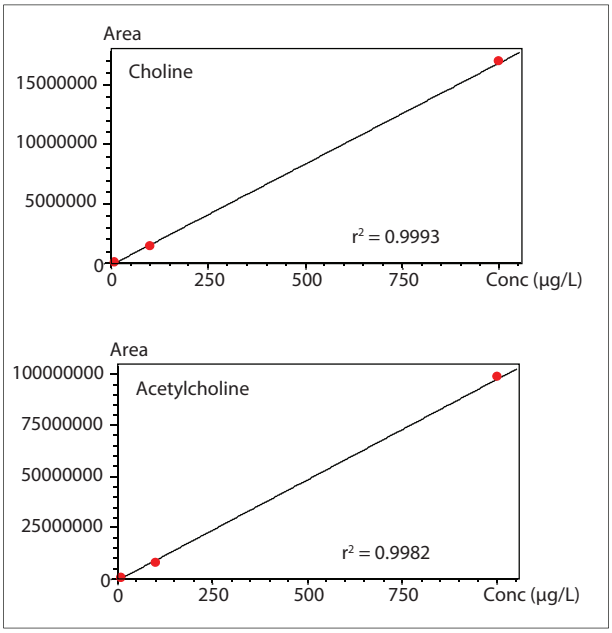

Fig. 2 Calibration Curves of Choline and Acetylcholine

The retention time and peak area repeatabilities after six repetitions at each concentration of 10, 100, and 1000 µg/L was confirmed at calibration curve creation and the results are summarized in Table 2. The linearity (r^2) was 0.9993 for choline and 0.9982 for acetylcholine. Fig. 3 shows the MRM chromatograms for 100 µg/L.

Table 2 Repeatabilities of Choline and Acetylcholine Standards (n = 6)

		Retention time (%RSD)	Peak area (%RSD)
Choline	10 µg/L	0.22	7.5
Choline	100 µg/L	0.05	1.7
Choline	1000 µg/L	0.07	2.2
Acetylcholine	10 µg/L	0.07	5.7
Acetylcholine	100 µg/L	0.06	4.2
Acetylcholine	1000 µg/L	0.07	6.0

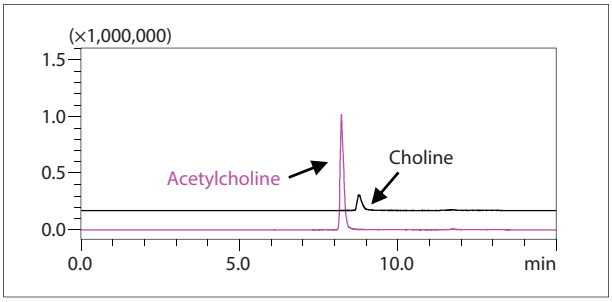

Fig. 3 Choline and Acetylcholine Standards (100 µg/L)

Next, by employing the microdialysis method in which biological compounds are continuously sampled from an awake animal via the semipermeable membrane of a minute dialytic probe connected to a pump, cerebrospinal fluid was sampled from a rat and directly delivered to SFC analysis. The injection volume of cerebrospinal fluid was set to 1 µL due to concerns regarding the miscibility between the aqueous sample and low polar supercritical carbon dioxide, which is the main component of the mobile phase used in SFC. With respect to acetylcholine, the LOQ determined according to the ASTM method was about 10 µg/L. Since the calculated concentration was less than the LOQ, only peak identification was performed. As shown in Table 3, the retention time and peak area repeatabilities were favorable for the six repeated analyses of choline. Fig. 4 shows the chromatograms resulting from SFC analysis of the cerebrospinal fluid sample.

Table 3 Choline Quantitative Value in Rat Cerebrospinal Fluid Sample and Repeatabilities (n = 6)

	Retention time (%RSD)	Peak area (%RSD)
Choline (Concentration 229.6 µg/L)	0.10	3.1

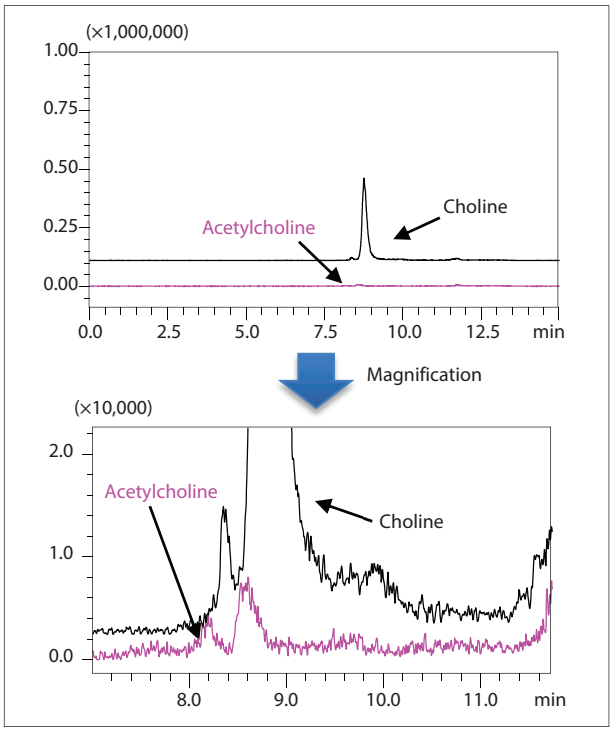

Fig. 4 SFC Analysis of Choline and Acetylcholine in a Cerebrospinal Fluid Sample

Online SFE-SFC-MS/MS Analysis

Next, a sample was prepared by impregnating cerebrospinal fluid sample into filter paper and drying the paper. SFE-SFC-MS/MS analysis was then performed on the sample. The convenience of this method is gaining attention not only because of easy of sample handling but also because of improved miscibility concerns between a mobile phase of low polar supercritical carbon dioxide and an aqueous sample solvent containing a biological sample. Table 4 lists the conditions used in online SFE-SFC-MS/MS analysis.

Table 4 Online SFE-SFC-MS/MS Conditions

Vessel	:	0.2 mL (1 µL of sample was added to filter paper)
Extractant	:	A) Supercritical fluid of CO_2 B) Methanol containing 20 mmol/L ammonium formate / water = 95/5 (v/v) A/B = 9/1 (v/v)
Flow rate	:	2.5 mL/min
Extraction time	:	Static (0-3 min) – Dynamic (3-6 min) – Static (6-8 min) - Dynamic (8-11 min) – Static (11-13 min) – Dynamic (13-16 min)
BPR pressure	:	10 Mpa
Extraction temp.	:	60 °C
Time program	:	B Conc. 10 % (16 min) → 25 % (26 min) → 50 % (26.1-28 min) → 10 % (28.1-31 min)

* SFC-MS/MS conditions are identical to Table 1 except for the time program.

Fig 5. shows the result obtained from online SFE-SFC-MS/MS analysis of a sample created by dropping 1 µL of 100 µg/L standard solution onto filter paper (GA-200 by ADVANTEC). Fig. 6 shows the result obtained by processing the rat cerebrospinal fluid sample in the same manner. The peak obtained for acetylcholine was small like the SFC analysis result, however, since the baseline noise level was improved in comparison, improved LOQ was obtained. Because the S/N value of corresponding peak to acetylcholine was more than 15 based on the baseline noise determined by ASTM method, a simple quantitative calculation was made based on the 100 µg/L standard data in the same way as the more concentrated choline. The obtained choline concentration of 297 µg/L was close to the SFC result and suggested that extraction in online SFE was performed efficiently. For acetylcholine, a calulation result of 1.7 µg/L was obtained from the peak area.

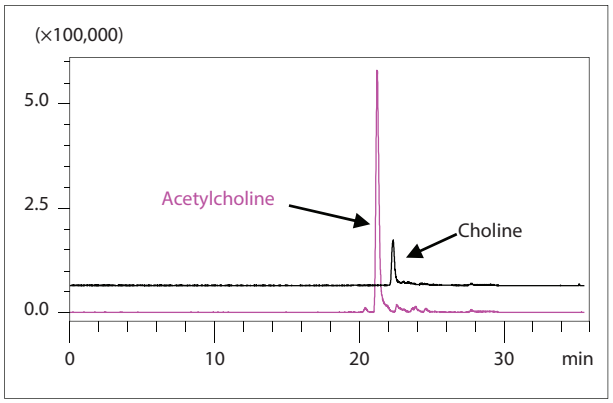

Fig. 5 Online SFE-SFC Analysis of Choline and Acetylcholine Standards

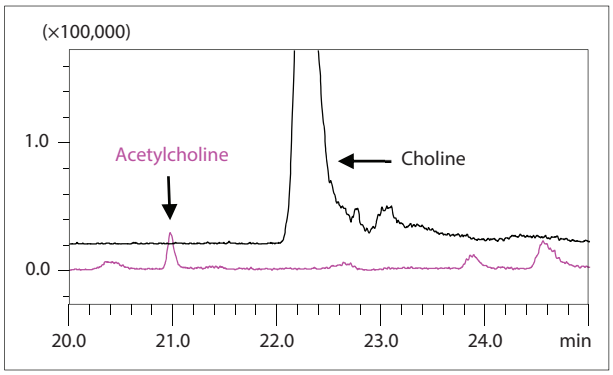

Fig. 6 Online SFE-SFC Analysis of Choline and Acetylcholine in a Cerebrospinal Fluid Sample

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Application News

No.L496

Supercritical Fluid Extraction / Chromatography

Analysis of Unstable Compounds Using Online SFE-SFC

Supercritical fluids have characteristics of both gas and liquid; low viscosity, high diffusivity and solubility. In particular, carbon dioxide becomes a supercritical fluid at a relatively modest critical point (31.1 °C and 7.38 MPa). Due to its low toxicity, inertness, easy availability, and low cost, supercritical carbon dioxide fluid is used in a wide variety of fields. Analytical applications using it include supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC).

Previously SFE and SFC were offline operations for pretreatment or analysis, respectively, and treated as completely separate workflows. However, now SFE and SFC can be connected online using the Nexera UC system, which allows integration of all the processes from pretreatment to data acquisition into a single workflow. This article describes using the Nexera UC system for online SFE-SFC analysis.

Online SFE-SFC

A flow diagram of online SFE-SFC analysis is shown in Fig. 1. Online SFE-SFC involves online introduction of components extracted from an extraction vessel using supercritical fluid to an SFC analytical column, where they are separated and then detected accordingly. The entire process, from extraction to data acquisition, is performed by switching flow lines using a valve inside the SFE unit. Two types of extraction operations are involved. After supercritical fluid is introduced to the extraction vessel, static extraction is performed where components are extracted while fluid flow is stopped. Then dynamic extraction is done to extract components while pumping fluid through the extraction vessel. In the case of online SFE-SFC, the sample is transported through the analytical column during dynamic extraction.
Consequently, the entire online SFE-SFC process, from extraction to separation and detection, can be completed within a single system, which eliminates the need for any complicated pretreatment processes and enables automation. That can significantly reduce the time and effort required for the various operations involved in the analysis.
It also means that the entire process, from extraction to separation and detection, can be performed without exposure to light, without oxidation, and in a moisture-free environment. Therefore, the method is extremely useful for analyzing unstable compounds, such as compounds with components easily decomposed by light, easily oxidized, or easily hydrolyzed. Unlike offline SFE, online SFE-SFC eliminates need for preparing sample solutions, which means it eliminates possibility of dilution of target components by the sample solvent, thus providing an easy way of increasing sensitivity.

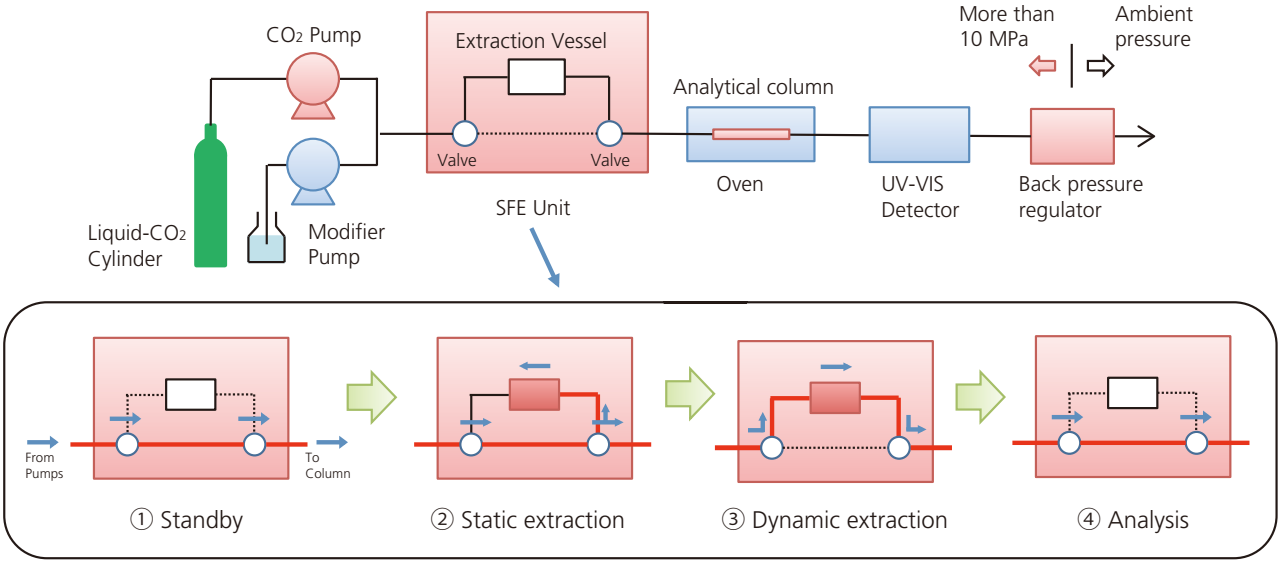

Fig. 1 Process Flow Diagram of Online SFE-SFC System

Online SFE-SFC Analysis of Reduced Coenzyme Q10

Fig. 2 shows the structure of the reduced coenzyme Q10 (ubiquinol). It is easily oxidized to form oxidized coenzyme Q10 (ubiquinone). In this case, both solvent extraction-SFC and online SFE-SFC were used to analyze the reduced coenzyme Q10 contained in a supplement capsule.

Pretreatment operations and analytical conditions for the solvent extraction-SFC analysis are indicated in Fig. 3 and Table 1.

Chromatograms from analyzing the supplement and the oxidized coenzyme Q10 standard sample are shown in Fig. 4.

Table 1 Analytical Conditions for Solvent Extraction-SFC

System	: Nexera UC SFC-UV System
Column	: Shim-pack UC-RP (150 mm L. × 4.6 mm I.D., 3 µm)
Column Temp.	: 40 ˚C
Modifier	: MeOH
Flowrate	: 3 mL/min
Time Program	: 5 % (0 min) → 50 % (5 - 8 min)
BPR	: 10 MPa
Detector	: UV-VIS (220 nm)
Inj. Vol.	: 1 µL

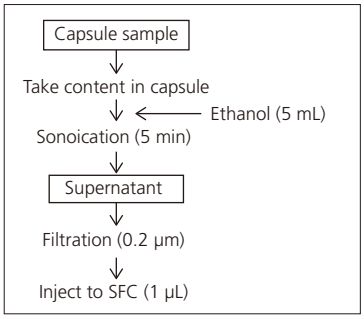

Fig. 3 Pretreatment

Analytical conditions for online SFE-SFC are indicated in Table 2.

About 5 µL each of the liquid sealed inside the supplement capsule and the standard sample of oxidized coenzyme Q10 were dripped onto filter paper. Then a portion of the filter paper was cut with a punch-out device and placed in the extraction vessel for analysis by online SFE-SFC. Chromatograms from analyzing the supplement and the oxidized coenzyme Q10 standard sample are shown in Fig. 5.

Table 2 Analytical Conditions for Online SFE-SFC

System	: Nexera UC Online SFE-SFC-UV System	
SFE		
Extraction Vessel	: 0.2 mL	
Static Extraction	: Time	; 0 - 2 min,
	: B.Conc.	; 5 %
	: BPR	; 10 MPa
	: Flowrate ; 3 mL/min	
Dynamic Extraction	: Time	; 2 - 4 min,
	: B.Conc.	; 5 %
	: BPR	; 10 MPa
	: Flowrate ; 3 mL/min	
SFC		
Column	: Shim-pack UC-RP (150 mm L. × 4.6 mm I.D., 3 µm)	
Column Temp.	: 40 ˚C	
Mobile Phase	: A; CO_2	
	: B; MeOH	
Flowrate	: 3 mL/min	
Time Program	: 5 % (4 min) → 50 % (9 - 13 min)	
BPR	: 10 MPa	
Detector	: UV-VIS (220 nm)	

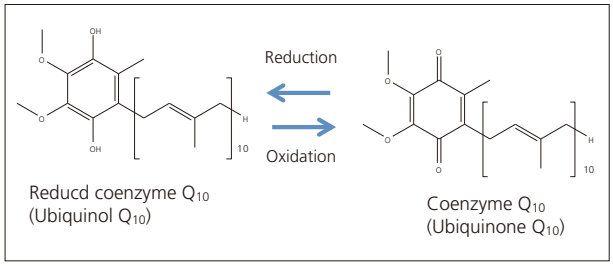

Fig. 2 Structural Formulas

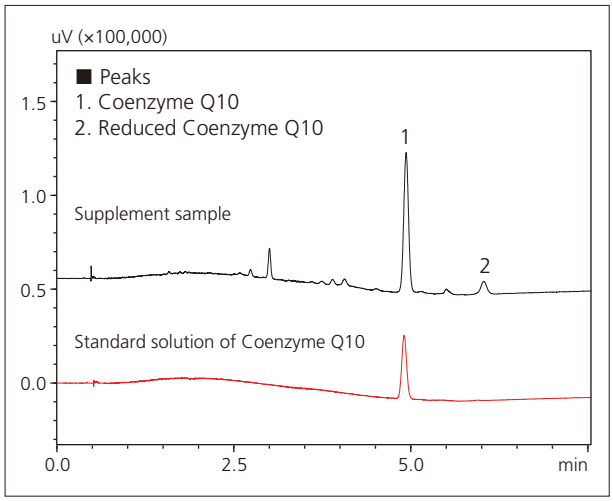

Fig. 4 Chromatograms Obtained by Solvent Extraction-SFC

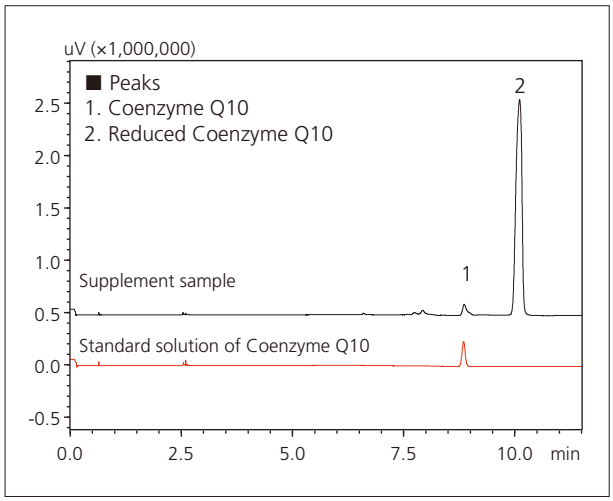

Fig. 5 Chromatograms Obtained by Online SFE-SFC

The results show that the coenzyme Q10 was oxidized during extraction with solvent extraction-SFC, but not oxidized and remained as the reduced coenzyme Q10 form throughout extraction, separation, and detection steps with online SFE-SFC. This shows how online SFE-SFC is an extremely unique analytical technique that can be used to analyze unstable compounds without altering their original form.

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A novel approach to the analysis of multivitamin by online supercritical fluid extraction/supercritical fluid chromatography

Pittcon 2016 830-12

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A novel approach to the analysis of multivitamin by online supercritical fluid extraction/supercritical fluid chromatography

Introduction

Vitamin is a series of basic trace substances which could maintain normal life forms of the animal body. Due to the chemical structure, fat-soluble vitamins such as vitamin A, vitamin E, etc. have strong hydrophobicity, low solubility in polar organic solvents. The analytical methods of those compounds are various, such as vitamin A by reversed phase liquid chromatography (RP-HPLC), vitamin D by normal phase liquid chromatography (NP-HPLC), and vitamin E normally used gas chromatography. Because of original method diversity, it is difficult to develop a new method of simultaneous analysis for fat-soluble vitamins. Supercritical Fluid Chromatography (SFC) is an unconventional chromatographic separation technology by using supercritical fluid and a small amount of modifier as mobile phase. Supercritical CO_2 ($scCO_2$) with its character of safe, inexpensive, non-toxic, facile, chemical inertness and other factors become the main mobile phase of SFC. Supercritical fluid ($scCO_2$) with low viscosity, high diffusivity and solubility characteristics is used in a wide variety of fields. Nexera UC Online SFE - SFC system is the latest products of supercritical fluid chromatograph in Shimadzu, which realized SFE (supercritical flow extraction) and SFC online combination, and simplify and unify the pretreatment method with high automation, extraction efficiency, and repeatability. In this study, a simultaneous analytical method for fat-solvable vitamins in drug and health care food was developed by using Nexera UC. It provides effective analysis and detection means for a variety of fat-soluble vitamins, and can be the reference for the quantitative study of this kind of material.

Methods and Materials

Sample Preparation

The analytical method for 5 kinds of fat-soluble vitamins was established in this study. Take the five standard include vitamin A acetate (VAA), vitamin A palmitate (VAP), vitamin E acetate (VEA), vitamin D2 (VD2), and vitamin D3 (VD3) and dissolves with n-hexane, diluted to a series of mixture concentration samples with ethanol. Then, dropped them to extraction tank and analyzed for standard curve. For commercially available vitamin A, vitamin E gelatin pearl, capsule and tablets, take out the contents into extraction tank to analyzed.

Experimental condition

Instrument

Nexera UC Online SFE-SFC system

configuration:

SFE-30A (SFE module), LC-30ADSF (CO_2 deliver pump), LC-20ADXR (modifier deliver pump), DGU-20A5 (degasser), CTO-20AC (column oven), SFC-30A×2 (back pressure adjustment module), SPD-20A (UV detector), CBM-20A (system controller), LabSolutions Ver5.8 (workstation).

A novel approach to the analysis of multivitamin by online supercritical fluid extraction/supercritical fluid chromatography

SFE condition	
Extraction agent	: $scCO_2$
modifier	: MeOH(5%)
flow rate	: 5 mL/min
static extraction	: 3 min
dynamic extraction	: 3 min
SFE temperate	: 50 °C
back pressure	: A-14.8 MPa, B-15 MPa
SFC condition	
Column	: GL Science ODS-P 4.6 mm I.D.×250 mm L., 5 µm
Mobile phase A	: $scCO_2$
Mobile phase B	: MeOH
Gradient program	: 0%B (6 min)-2%B (9 min)-10%B (16 min)-50%B(16.1-17 min)
Flow rate	: 3 mL/min
Oven temperature	: 40 °C
back pressure	: 10 MPa
detector wavelength	: 325 nm; 284 nm

Results and Discussion

Supercritical fluid extraction

Samples were loaded to the extraction vessel, and then set in a supercritical fluid extraction module for extraction. Liquid CO_2 and modifier of methanol (98/2, v/v) were delivered through the pumps into the extraction vessel (Figure 1), and changed to supercritical fluid under the setting of temperature and pressure. Methanol, as modifier, is to adjust the polarity, solubility and other properties of supercritical fluid to improve the extraction efficiency. Kept the vessel filled with supercritical fluid in 3 min at a stable temperature and pressure for static extraction.

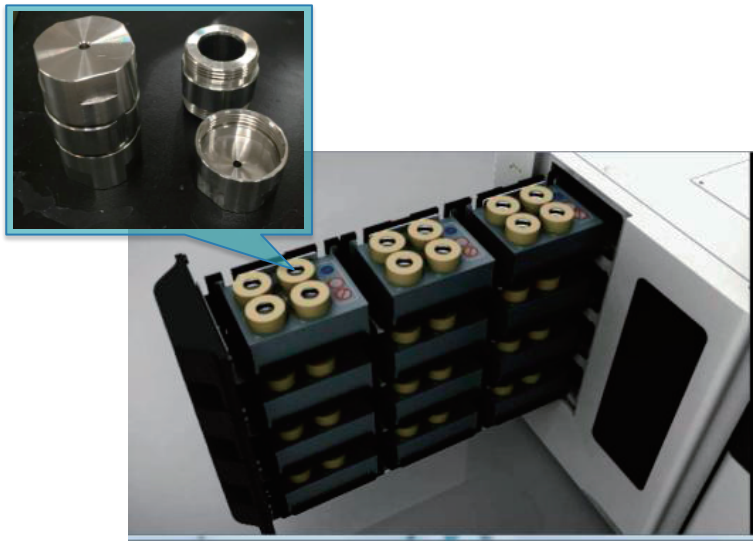

Figure 1 Extraction vessel and SFE

A novel approach to the analysis of multivitamin by online supercritical fluid extraction/supercritical fluid chromatography

Then, through SFE unit flow switch valve, supercritical fluid flow through the extraction vessel and extracted components from sample in 3 min by dynamic extraction. In the process of dynamic extraction, extract was directly introduced into subsequent SFC system. SFC separation and analysis was start after the completion of the extraction. The whole process of online SFE - SFC is shown in figure 2.

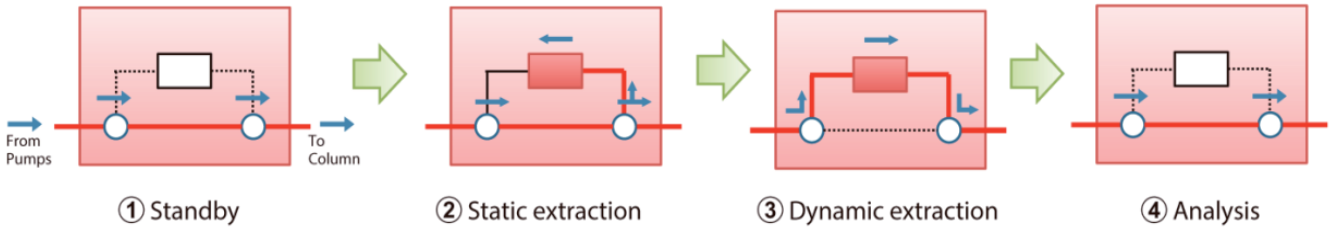

Figure 2 Pretreatment processing of SFE

For estimating the extraction efficiency of 5 compounds under the condition of setting, repeated extraction and analysis for the same vessel was performed. Peak area of every compound was calculated and peak area ratio of first extraction to total three times was recorded in table 2 to show the extraction yield of every compounds. The results showed that the SFE extraction yield of 5 vitamins were above 85% under the condition of settings.

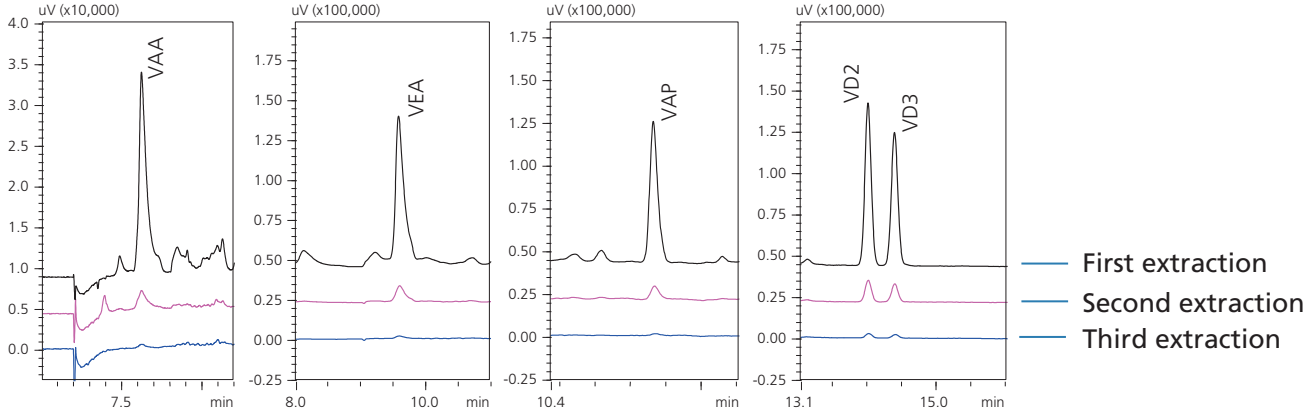

Figure 3 Chromatographs of three extractions for 5 vitamins

Table 1 Yield of three extractions for 5 vitamins

	VAA	VEA	VAP	VD2	VD3
1st extraction	94.2	88.6	89.3	86.2	86.0
2nd extraction	5.8	10.1	9.7	11.6	12.0
3rd extraction	0	1.3	1.0	2.2	2.0

A novel approach to the analysis of multivitamin by online supercritical fluid extraction/supercritical fluid chromatography

Supercritical Fluid Chromatography

The online SFE-SFC analytical results of 5 fat-soluble vitamins were showed in Fig 4. Vitamin A acetate and other four compounds were isolated obviously. The standard curves of absolute amount of compound added in extraction vessel to the detector response shown in Figure 5, it indicates 5 compounds with good linearity in their respective concentration, and regression coefficient of R2 in 0.997-0.999.

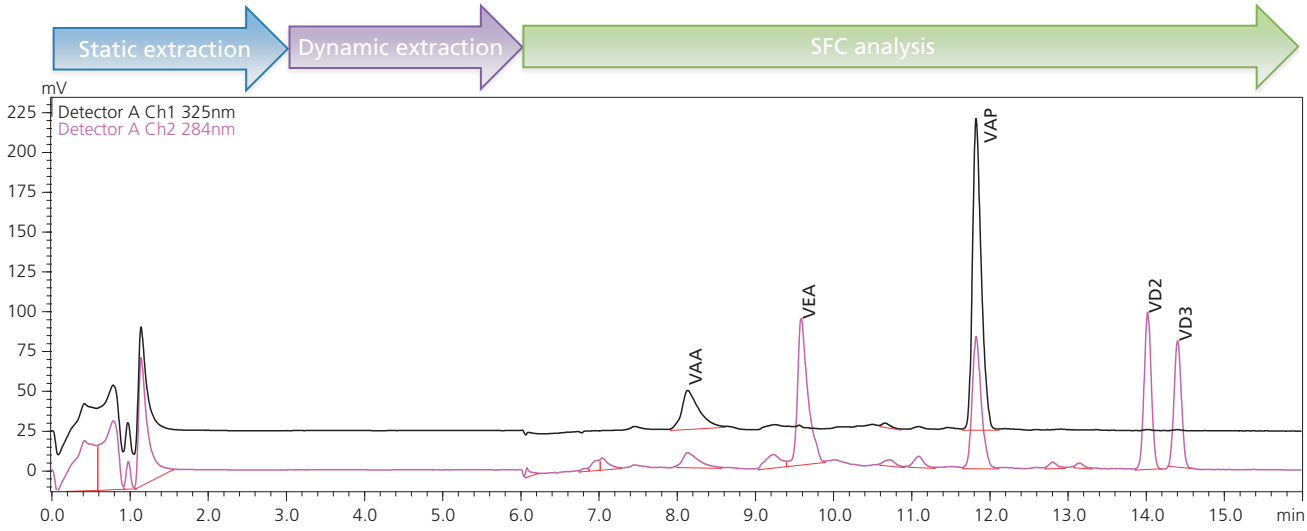

Figure 4 Chromatogram of simultaneous analysis for 5 vitamins

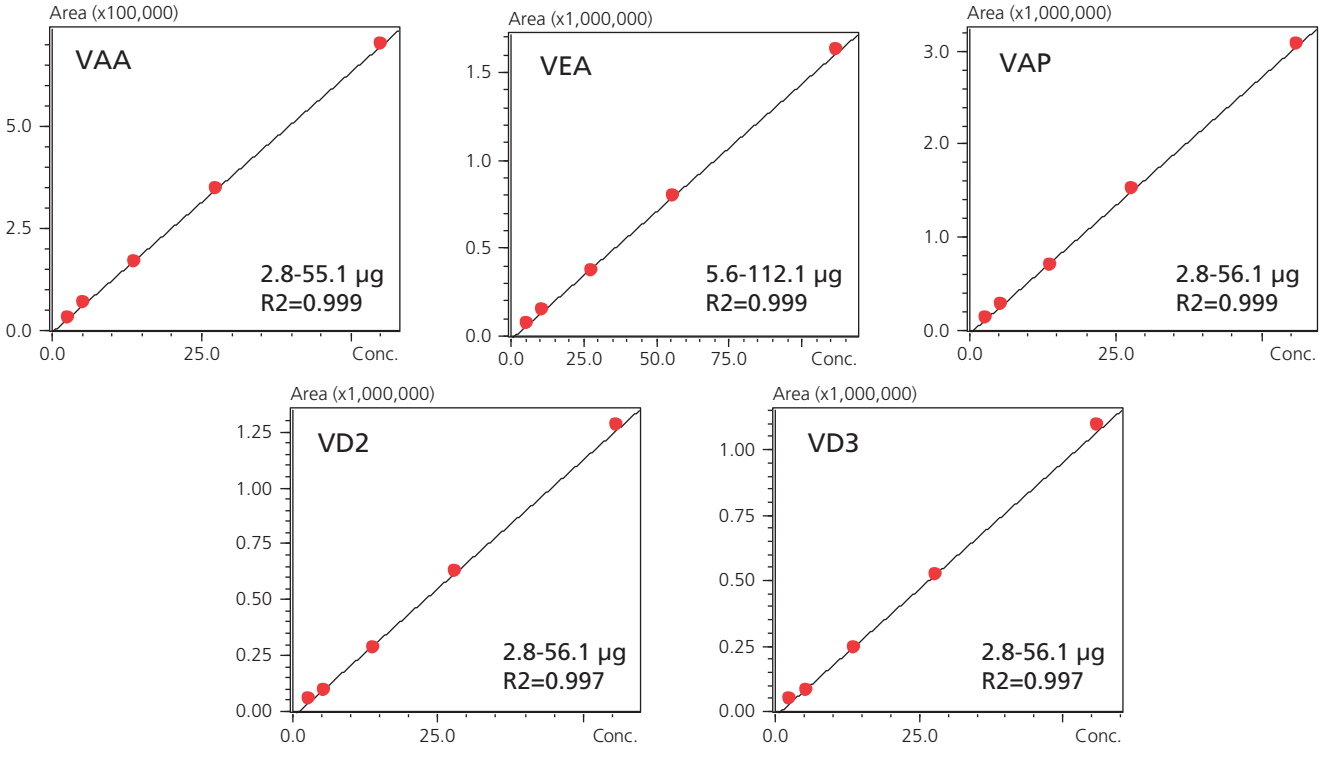

Figure 5 Calibration curves of 5 vitamins

A novel approach to the analysis of multivitamin by online supercritical fluid extraction/supercritical fluid chromatography

Repeatability and recovery

Add 2 times of LLOQ for each compound into extraction vessel to test repeatability and recovery. Results were shown in table 2.

Table 2 Repeatability and recovery of 5 vitamins (n=6)

	VAA	VEA	VAP	VD2	VD3
Rt (RSD%)	0.24	0.15	0.10	0.04	0.03
Area (RSD%)	13.0	5.2	4.1	4.5	5.9
Recovery (%)	94.4	101.1	100.0	90.5	90.5

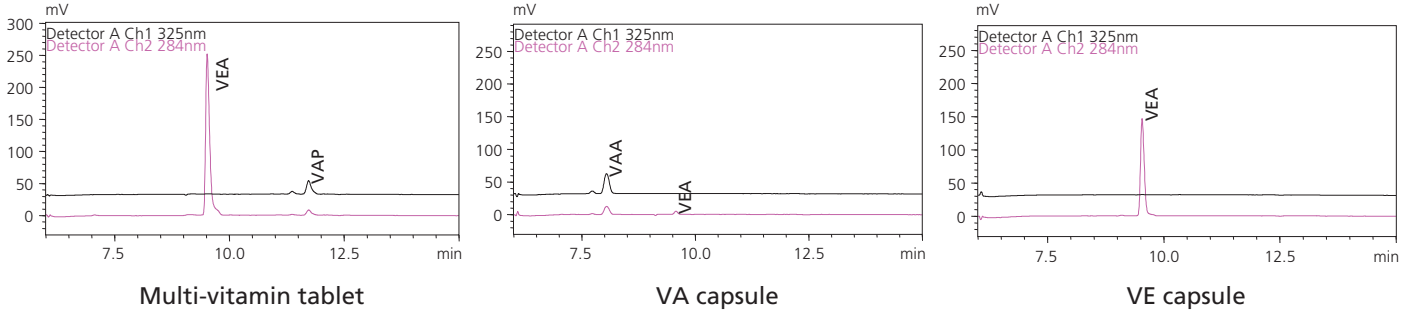

Figure 6 Chromatogram of three real samples which were analysed by using Nexera UC

Conclusions

An Online SFE-SFC method has been developed for quantitative analysis of 5 fat-soluble vitamins in drugs and health care food sample. It provided a new way for simultaneous analysis for 5 vitamins which combined the processing of pretreatment and analysis together. The results showed that this method is rapid and reliable.

First Edition: March, 2016

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Upgrade Your Existing UHPLC to an UHPLC/SFC Switching System

Reduce Instrument Purchase Costs and Enable SFC Analysis Immediately

You can now upgrade to an UHPLC/SFC switching system (Nexera UC/s) by adding the applicable SFC units to your existing UHPLC system.
This enables you to perform both UHPLC and SFC analysis with a single system.

Upgrading Lowers Purchasing Costs and Reduces Installation Space

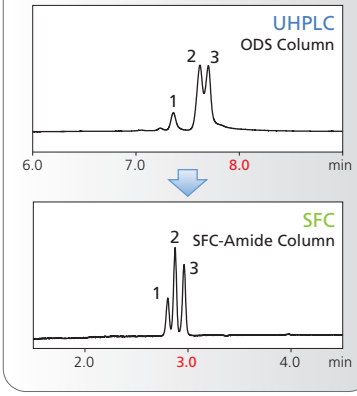
UHPLC System

UHPLC/SFC Switching System (Nexera UC/s)

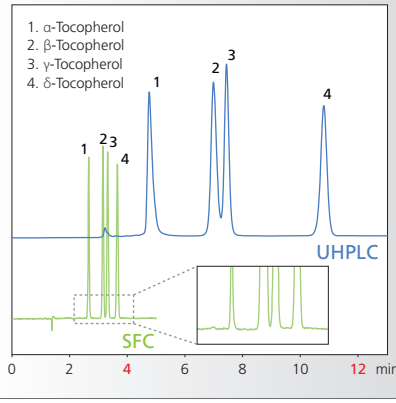
SFC Analysis Improves Separation and Analysis Times, while Reducing Solvent Consumption

In comparison to UHPLC, column efficiency is not impaired in SFC analysis even at a high flow rate. As a result, analysis times are shortened by the increase in speed. At the same time, since the separation characteristics are different, improved separation can be expected for foreign substances and isomers that are not sufficiently separated by UHPLC. Further, the consumed amount of organic solvents can be reduced.

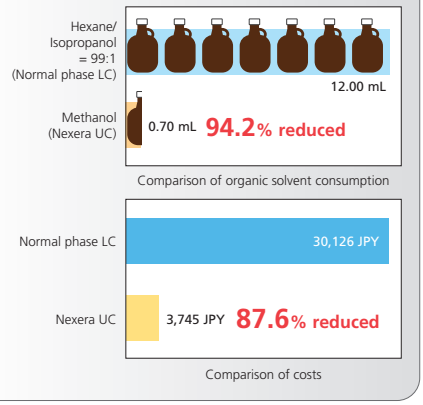
Improved Separation with SFC Analysis



Shortened Analysis Times

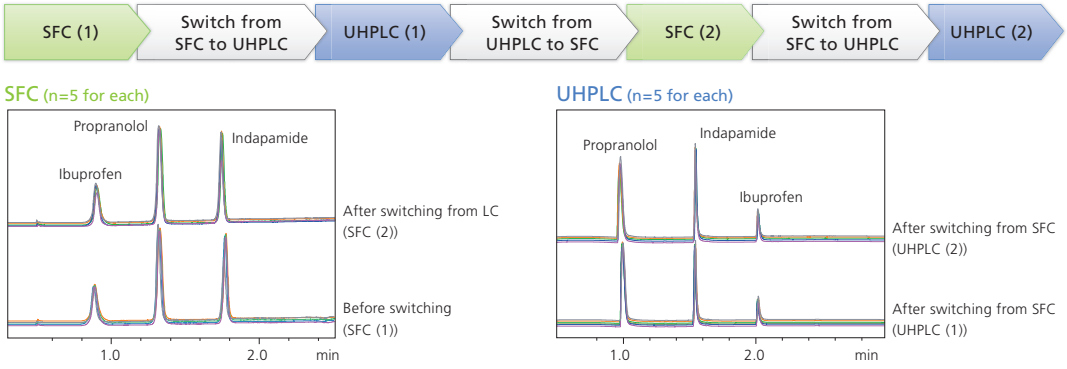


Reduced Solvent Consumption



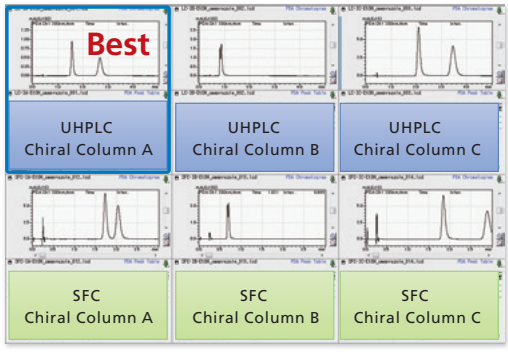
Reliable Analysis Even When Switching Between SFC and UHPLC Modes

SFC and UHPLC utilize significantly different mobile phases and separation characteristics, but analysis can be performed reliably without effects from switching modes by simply purging the flow lines.

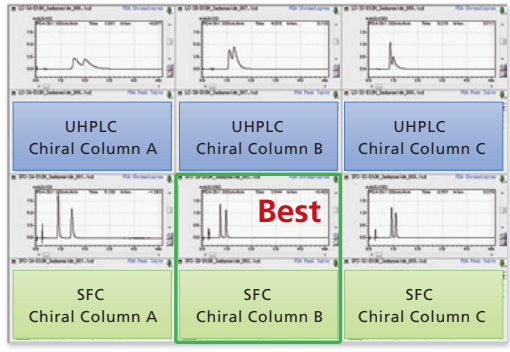


Two Separation Methods Heighten the Efficiency of Examining the Optimal Separation Conditions

Various separation methods, including the separation of chiral compounds and structural isomer, are required in fields such as pharmaceuticals, foods, and the environment. For example, in the case of method scouting with two chiral standard samples, favorable separation is obtained for omeprazole with UHPLC conditions, and imdapamide with SFC conditions. Screening utilizing these two methods makes it possible to construct better analysis conditions in a short time. Switching between SFC and UHPLC analysis methods is easy with the dedicated software.



Omeprazole Method Scouting

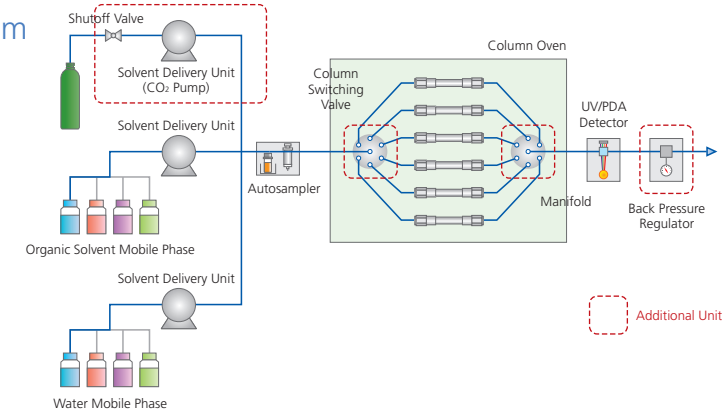


Imdapamide Method Scouting

Kit for Upgrading to an SFC Analysis System

You can upgrade to an UHPLC/SFC switching system (Nexera UC/s) capable of UHPLC and SFC analysis using the existing* solvent delivery unit, autosampler, oven, and detectors.

* The following units can be used in combination when upgrading.
Solvent delivery Unit: LC-30AD
Autosampler: SIL-30AC
Column oven: CTO-20A/20AC
Detector: SPD-20A(V), SPD-M20A
Mass spectrometer: LCMS-2020, LCMS-80X0



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Application News

No.L497

Supercritical Fluid Extraction / Chromatography

Using the Nexera UC Online SFE-SFC-MS System to Analyze Residual Pesticides in Agricultural Products

The Nexera UC online SFE-SFC-MS system combines supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) in one online system, so that the entire process from extraction of target components to acquisition of data can be performed completely automatically. Furthermore, the system can add polar organic solvents (modifiers) to the supercritical carbon dioxide fluid during SFE and SFC, so that the system can be used to extract and analyze components with a wide range of polarities.
Meanwhile, ever since the positive list system was enacted in 2006 in Japan for residual pesticides in foods, which applies to more than 800 types of pesticides, there has been increasing demand for a system able to simultaneously analyze multiple pesticides with a wide range of properties, including pretreating samples.
This article describes an example of using the Nexera UC online SFE-SFC-MS system to analyze residual pesticides in agricultural products.

Online SFE-SFC-MS System

The operating principle of the Nexera UC online SFE-SFC-MS system is shown in Fig. 1. The extraction vessel filled with the sample is placed in the SFE unit and heated to an internal temperature of 40 °C (Fig. 1A). Then supercritical carbon dioxide fluid is pumped into the extraction vessel. After filling the vessel, the flow is stopped to allow static extraction of target components (Fig. 1B). After static extraction, the fluid is pumped through the extraction vessel for dynamic extraction (Fig. 1C). During dynamic extraction, extracted substances flow from the extraction vessel and into the analytical column. However, due to the high level of contaminant components in agricultural products, passing all the extract substances through the analytical column or mass spectrometer could damage the column or contaminate the mass spectrometer. Therefore, the Nexera UC online SFE-SFC-MS system splits the flow to send only a portion of the substances extracted from dynamic extraction through the analytical column. After dynamic extraction, fluid is only sent through the analytical flow line, where the analytical column is used for gradient separation and the mass spectrometer for detecting the target components (Fig. 1D).

Sample Preparation

The QuEChERS is a well-known method that prioritizes simplicity and speed and is commonly used to pretreate agricultural products for residual pesticide analysis. However, the method involves many steps, such as adding reagents, solvent extraction, purification by dispersive solid phase extraction, and centrifugal separation. In contrast, the online SFE-SFC-MS system requires only mixing 1 g of agricultural product crushed with a mixer with 1 g of a dehydrating agent* and placing the mixture in the extraction vessel, as shown in Fig. 2. Consequently, the system improves analytical productivity, reduces the environmental impact, and also avoids human errors involved in the pretreatment steps. Using a dedicated rack changer, the system can continuously extract and analyze up to 48 samples at a time.

* "Miyazaki Hydro-Protect" Patent No. 3645552

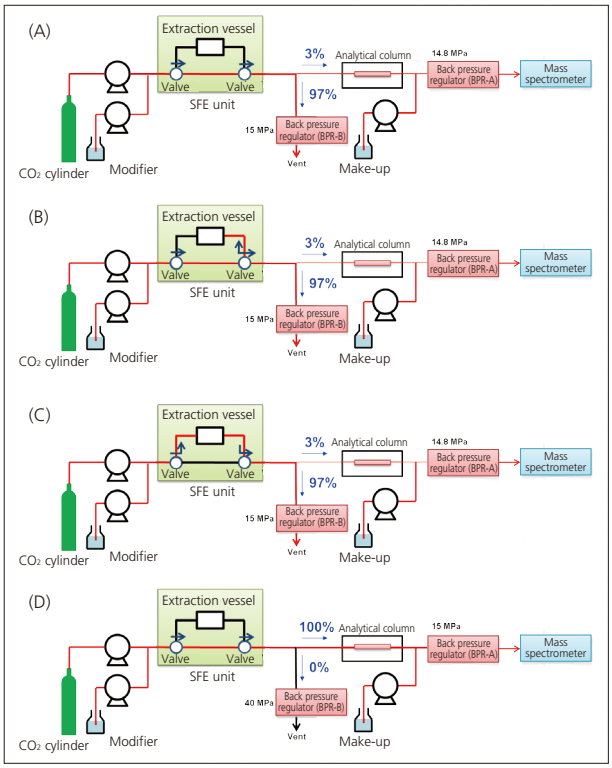

Fig. 1 Analysis Flow by Online SFE-SFC-MS

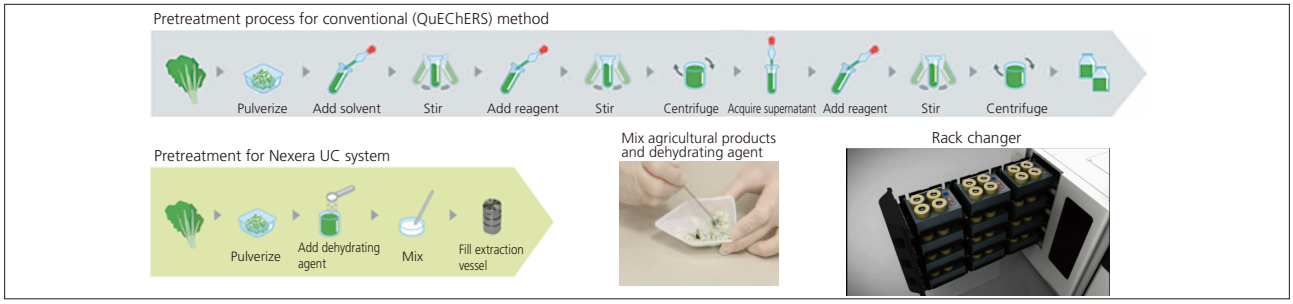

Fig. 2 Sample Preparation

Table 1 Analytical Conditions

[SFE]	
Solvent	: A) Super critical fluid of CO_2 B) 0.1 % Ammmonium formate in methanol
Flowrate	: 5 mL/min
Extraction	: 0-3 min. Static mode (B.Conc. 5 %) 3-6 min. Dynamic mode (B.Conc. 5 %)
Extraction Vessel Temp.	: 40 °C
BPR Pressure	: A) 14.8 MPa, B) 15 MPa (split rate: 3 %)
Make-up	: 0.1 % Ammmonium formate in methanol (0.4 mL/min.)
[SFC]	
Column	: Shim-pack UC-RP (250 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: A) Super critical fluid of CO_2 B) 0.1 % Ammmonium formate in methanol
Time Program	: B.Conc. 0 % (0 min.) → 10 % (11 min.) → 30% (14 min.) → 40 % (14.01-17 min.)
Flowrate	: 3 mL/min
Make-up	: 0.1 % Ammmonium formate in methanol (0.1 mL/min.)
Column Temp.	: 40 °C
BPR Pressure	: A) 15 MPa, B) 40 MPa
Detector	: LCMS-8050 MRM mode

Analysis of Standard Mixture of Pesticides

The standard mixture sample of 510 pesticide components were mixed with a dehydrating agent and analyzed using the analytical conditions indicated in Table 1. Fig. 3 shows the results. Using the system, we were able to accomplish the entire process, from extraction to data acquisition, in about 45 minutes per analysis. For 327 components, we obtained good repeatability for the concentration range from 1 to 100 ng/g (less than 30 %RSD for relative standard deviation for peak area at respective concentrations) and good linearity (contribution ratio of at least R^2 = 0.99). Table 2 also shows how pesticides with a wide range of polarities were analyzed with good repeatability and linearity.

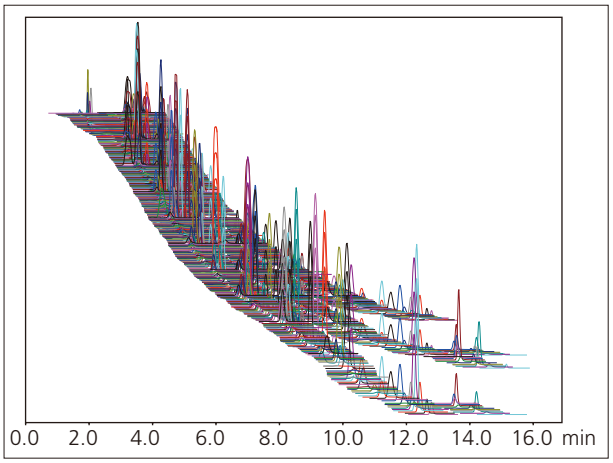

Fig. 3 Mass Chromatogram of Standard Pesticide Mixture Solution

Table 2 Repeatability and Linearity for Representative Pesticides

Compounds	LogPow	Repeatability (%RSD, n=5)	Range (ng/g)	R^2
Ethofenprox	6.9	6.1	1-100	0.9991
Hexaflumuron	5.68	6.8	1-100	0.9992
Benzofenap	4.69	1.4	2-200	0.9990
Mepronil	3.66	4.6	1-100	0.9993
Prometryn	3.34	2.7	1-100	0.9994
Fenamidone	2.8	3.0	2-200	0.9991
Ethylchlozate	2.5	3.0	1-100	0.9996
Imazosulfuron	1.6	6.2	1-100	0.9998
Bensulfuron methyl	0.79	8.1	1-100	0.9996
Primisulfuron methyl	0.2	5.5	1-100	0.9994
Halosulfuron methyl	-0.02	5.5	1-100	0.9996
Azimsulfuron	-1.4	4.2	1-100	0.9998

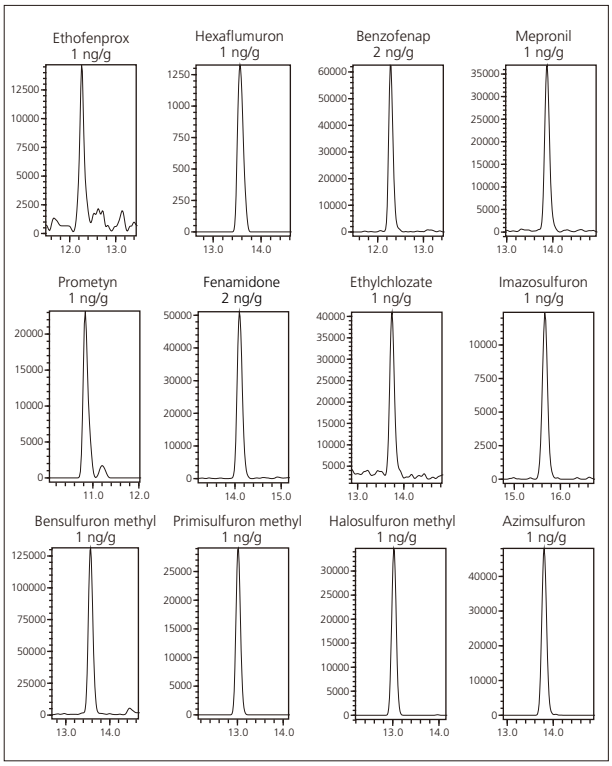

Fig. 4 MRM Chromatograms of Representative Pesticides

Analysis of a Tomato

Analysis of 10 ng/g of 510 pesticide components added to a tomato resulted in good repeatability (less than 20 %RSD for the relative standard deviation of the peak area) and a good recovery rate (70 to 120 %) for 248 components. Plots of LogPow and recovery rate results are shown in Fig. 5. It shows that pesticides with a wide range of polarities were analyzed with good recovery.

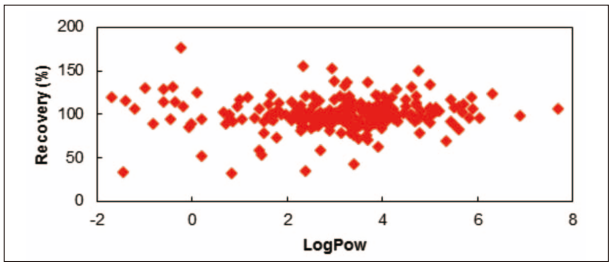

Fig. 5 LogPow vs. Recovery Rate for Tomato Analysis

<Acknowledgments>
This Application News bulletin includes results obtained in cooperation with Osaka University, Kobe University, and the Miyazaki Agricultural Research Institute from the program for the "Development of Systems and Technology for Advanced Measurement and Analysis," sponsored by the Japan Science and Technology Agency (JST). We are deeply grateful to all those involved.

First Edition: Oct. 2015

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Application News

No.L502

Supercritical Fluid Extraction / Chromatography

Analysis of Residual Pesticides in Agricultural Products Using Nexera UC Off-Line SFE-GC/MS System

Since enforcement of the positive list system for residual pesticides in foods in 2006 in Japan, over 800 pesticides have been included in the system. Consequently, there is now a strong demand for effective analytical methods encompassing any sample pretreatment steps that are capable of inspecting large numbers of pesticides.
Conventionally, analysis of residual pesticides in foods has involved pesticide extraction by a solvent extraction method before analysis by LC/MS or GC/MS. The problem with solvent extraction methods is that sample pretreatment requires a substantial amount of time and effort, and large quantities of organic solvents are used.
Supercritical fluid extraction (SFE) that uses supercritical carbon dioxide as the extraction solvent provides good extraction efficiency, where the solvent is similar to gas in terms of low viscosity and high diffusivity, and similar to fluid in terms of high solubility. This allows for extraction within a short period of time. This extraction method is also less damaging to the environment since it uses a smaller amount of organic solvent compared to conventional solvent extraction methods.
We introduce an example GC/MS analysis of pesticides extracted from an agricultural products using the Nexera UC off-line SFE system.

Off-Line SFE System

Fig. 1 shows the principle behind operation of the Nexera UC off-line SFE system. An extraction vessel filled with a sample is placed in the SFE unit, and is heated to 40 °C (Fig. 1 A). The extraction vessel is then filled with supercritical carbon dioxide, and the target components are extracted statically without pumping the liquid (Fig. 1 B). After static extraction, dynamic extraction is performed by pumping supercritical carbon dioxide through the extraction vessel (Fig. 1 C). After trapping the extract material in the trap column, eluate that contains the target components is then collected in the fraction collector (Fig. 1 D).

Sample Preparation

The QuEChERS method that prioritizes simplicity and speed is widely used to pretreat agricultural products for residual pesticide analysis. While there is a special kit available for the QuEChERS method, sample preparation for this kit requires a large number of process steps including reagent addition, solvent extraction, purification by dispersive solid phase extraction, and centrifugal separation.

Meanwhile, as shown in Fig. 2, sample preparation for the Nexera UC off-line SFE system only involves mixing 1 g of agricultural product pulverized in a mixer with 1 g of dehydrating agent*, then filling the extraction vessel with this mixture. This not only results in improved productivity and a reduced environmental burden, but also avoids human errors involved in the sample pretreatment process. Using a specially designed rack changer also allows for extraction of a maximum of 48 samples consecutively.

* "Miyazaki Hydro-Protect" Patent No. 3645552

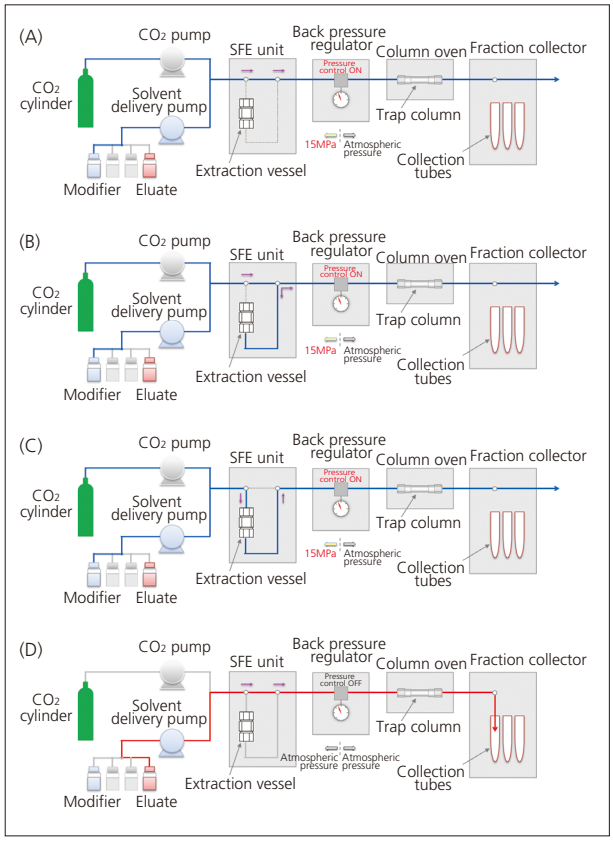

Fig. 1 Flow of Off-Line SFE Extraction

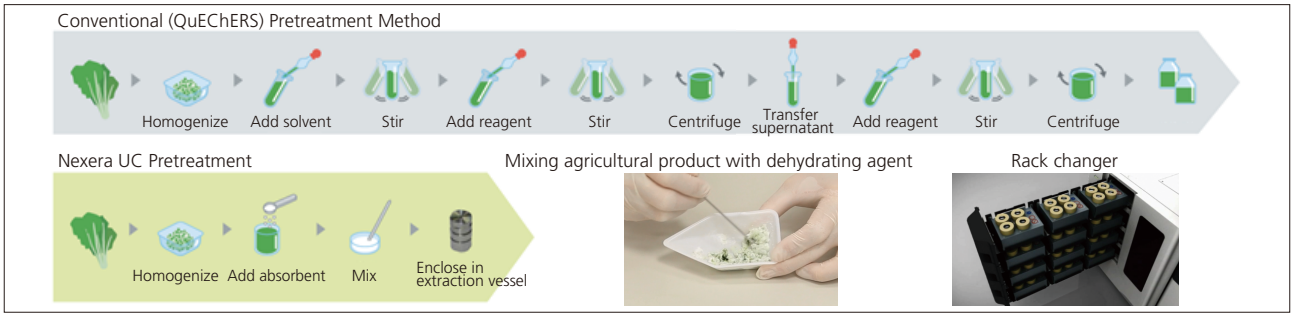

Fig. 2 Sample Preparation

Table 1 Analytical Conditions

[SFE] Nexera UC SFE System	
Extraction Solvent	: A) Supercritical fluid of CO_2 B) Methanol
Flowrate	: 5 mL/min
Extraction	: 8 min (Static mode → Dynamic mode)
Extraction Vessel Temp.	: 40 ˚C
BPR Pressure	: 15 MPa
Trap Column	: Shim-pack VP-ODS (50 mm L. × 4.6 mm I.D., 5 µm)
Elution Solvent	: Acetone/Hexane = 50/50 (2 mL/min, 2 min)
[GC-MS] GCMS-TQ8040	
Column	: Rxi®-5Sil MS 30 m × 0.25 mm I.D., df = 0.25 µm
Column Temp.	: 50 ˚C (1 min) → (25 ˚C/min) → 125 ˚C → (10 ˚C/min) → 300 ˚C (15 min)
Carrier Gas	: He (Constant linear velocity mode)
Linear Velocity	: 47.2 cm/sec
Injection Mode	: Splitless (Sampling time 1.00 min)
High Press Inj.	: 250 kPa (1.5 min)
Injection Volume	: 1 µL
Interface Temp.	: 250 ˚C
Ion Source Temp.	: 200 ˚C
MS Mode	: MRM
Loop Time	: 0.3 sec

Analysis of Brown Rice

A mixed standard solution of pesticides for GC/MS analysis (Hayashi Pure Chemical PL2005 Pesticide GC/MS Mix I-VI, 7) was added to pulverized brown rice to a concentration of 100 ng/g, and SFE was performed using the conditions shown in Table 1. The extraction liquid obtained was made up to 2 mL using eluate, and analyzed by GC/MS. The MRM chromatogram obtained from GC/MS analysis is shown in Fig. 4. Good repeatability (relative standard deviation of quantitation concentration <10 %) and recovery (70-120 %) were obtained for the 301 components. Repeatability and recovery for the 301 pesticides are shown in Table 2. This system uses a very simple pretreatment process, and can perform automated extraction from a single sample in approximately 30 minutes.

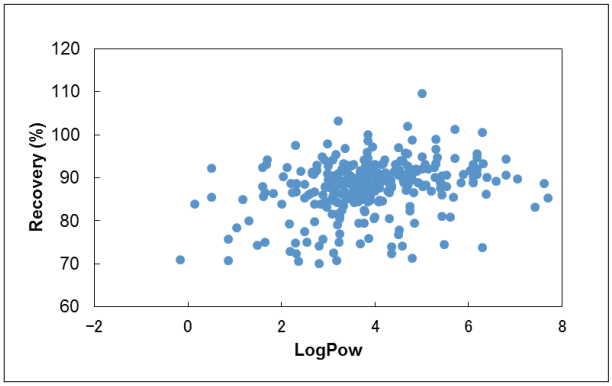

Fig. 3 Recovery in Brown Rice Analysis

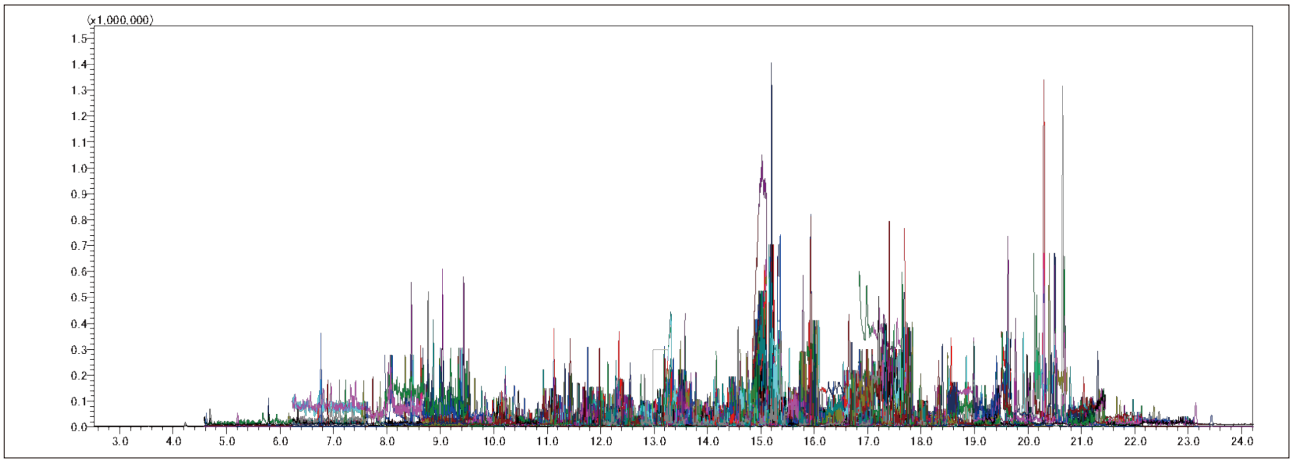

Fig. 4 MRM Chromatogram of Extraction Liquid from Brown Rice

Table 2 Repeatability and Recovery

Compounds	Repeatability (%RSD, n = 6)	Recovery (%)
2-Phenylphenol	3.8	87.0
Acetochlor	5.9	93.1
Acrinathrin-1	6.8	73.8
Acrinathrin-2	3.1	100.6
Alachlor	3.6	88.7
Allethrin-3,4	5.9	102.0
Allidochlor	5.3	86.4
alpha-BHC	4.6	88.9
alpha-Endosulfan	9.5	98.7
Ametryn	4.1	86.3
Anilofos	4.7	86.3
Atrazine	4.8	86.7
Azaconazole	5.5	70.5
Azamethiphos	9.9	78.4
Azinphos-ethyl	5.3	84.3
Azinphos-methyl	2.7	83.1
Benalaxyl	7.0	84.9
Benfluralin	5.2	90.1
Benfuresate	4.1	91.5
Benoxacor	3.2	90.8
beta-BHC	5.3	87.8
beta-Endosulfan	6.5	90.7
Bifenox	4.1	84.5
Bifenthrin	3.3	89.2
Biphenyl	3.5	80.5
Bromobutide	4.6	90.4
Bromophos	5.4	90.1
Bromophos-ethyl	6.0	86.6

Table 2 Repeatability and Recovery (continued)

Compounds	Repeatability (%RSD, n = 6)	Recovery (%)
Bromopropylate	4.1	90.9
Bromuconazole-1	3.7	80.5
Bromuconazole-2	5.3	77.1
Bupirimate	7.9	86.8
Buprofezin	6.6	88.8
Butachlor	6.4	91.6
Butafenacil	4.4	90.4
Butamifos	3.8	90.1
Butylate	4.6	84.7
Cadusafos	4.1	88.1
Cafenstrole	5.1	91.1
Captan	9.1	77.6
Carbofuran	4.7	83.3
Carbophenothion	2.9	91.5
Carfentrazone-ethyl	4.1	96.8
Chinomethionat	4.2	82.1
Chlomethoxyfen	5.8	89.8
Chlorbenside	3.9	81.1
Chlorbufam	4.2	84.7
Chlorethoxyfos	4.6	90.3
Chlorfenapyr	7.5	86.5
Chlorfenson	7.7	91.4
Chlorfenvinphos-(E)	4.4	91.2
Chlorfenvinphos-(Z)	6.5	88.7
Chlormephos	3.1	89.6
Chlorobenzilate	3.6	92.0
Chloroneb	6.0	95.0
Chlorothalonil	5.3	87.7
Chlorpropham	4.9	88.5
Chlorpyrifos	6.2	90.8
Chlorpyrifos-methyl	5.1	90.5
Chlorthiophos-2	9.5	88.4
Chlorthiophos-3	2.8	92.8
Chlozolinate	7.8	82.4
Cinidon-ethyl	4.3	88.8
Cinmethylin	9.9	94.5
Clomazone	4.2	88.6
Clomeprop	3.3	89.8
Crimidine	6.0	80.0
Cyanofenphos	4.7	91.8
Cyanophos	5.0	91.3
Cyflufenamid	8.4	89.6
Cyfluthrin-1	5.1	95.6
Cyfluthrin-2	3.5	94.6
Cyfluthrin-3	4.9	92.0
Cyfluthrin-4	6.0	90.8
Cyhalofop-butyl	4.2	93.4
Cyhalothrin-1	9.1	90.6
Cyhalothrin-2	4.5	94.4
Cypermethrin-1	2.8	99.0
Cypermethrin-2	3.7	96.6
Cypermethrin-3	3.7	93.2
Cypermethrin-4	8.4	93.2
Cyprodinil	4.0	80.9
delta-BHC	2.2	88.2
Deltamethrin-2	3.7	103.2
Dialifos	3.2	91.4
Di-allate-1	2.5	91.5
Di-allate-2	4.7	92.0
Diazinon	7.8	90.0
Dichlobenil	4.0	79.8
Dichlofenthion	5.2	92.1
Dichlofluanid	3.3	87.2
Dichlorvos	3.2	83.9
Diclobutrazol	5.2	87.0
Diclocymet-1	4.3	83.4
Diclocymet-2	5.1	82.2
Diclofop-methyl	4.4	91.0
Diethofencarb	4.8	83.8
Difenoconazole-1	5.5	74.0
Difenoconazole-2	5.2	72.4
Diflufenican	4.4	94.3
Dimepiperate	2.5	87.8
Dimethametryn	6.4	84.8
Dimethenamid	5.4	88.8
Dimethipin	9.9	70.9
Dimethylvinphos-(E)	4.5	86.8
Dimethylvinphos-(Z)	4.9	86.1
Diniconazole	2.3	80.6
Dioxabenzofos	4.4	91.5
Dioxathion	5.4	88.6
Dioxathion deg.	4.4	86.1

Compounds	Repeatability (%RSD, n = 6)	Recovery (%)
Diphenamid	5.7	79.3
Diphenylamine	3.1	91.5
Disulfoton sulfone	5.2	85.0
Ditalimfos	3.2	90.1
Dithiopyr	5.1	90.9
Edifenphos	3.5	95.9
Endosulfan sulfate	6.9	95.4
EPN	3.8	88.0
Epoxiconazole	3.7	83.9
EPTC	4.3	81.6
Esprocarb	2.7	90.6
Ethalfluralin	5.3	93.3
Ethion	3.4	93.1
Ethofumesate	5.7	91.4
Ethoprophos	4.3	91.0
Etobenzanid	3.8	86.6
Etofenprox	3.8	89.7
Etoxazole	8.2	87.9
Etridiazole	3.8	85.3
Etrimfos	2.9	87.9
Famoxadone	5.4	71.2
Fenamidone	5.7	70.1
Fenchlorphos	6.0	92.1
Fenitrothion	6.9	88.7
Fenothiocarb	5.4	88.6
Fenoxanil	6.2	88.2
Fenoxaprop-ethyl	4.1	90.5
Fenoxycarb	6.9	84.4
Fenpropathrin	3.7	91.6
Fenpropimorph	4.7	76.8
Fenthion	3.6	79.5
Fenvalerate-1	5.2	88.4
Fenvalerate-2	4.2	95.0
Fipronil	8.3	86.7
Flamprop-methyl	6.6	85.7
Fluacrypyrim	6.8	97.0
Flucythrinate-1	4.0	92.8
Flucythrinate-2	3.7	95.7
Flufenpyr-ethyl	1.8	98.0
Flumiclorac-pentyl	5.8	91.8
Flumioxazin	9.4	75.0
Fluquinconazole	4.3	81.2
Flusilazole	5.5	86.8
Fluthiacet-methyl	3.8	79.5
Flutolanil	9.6	87.8
Fluvalinate-1	2.6	100.0
Fluvalinate-2	2.6	98.6
Folpet	5.3	87.7
Fonofos	3.8	91.7
Formothion	5.3	74.4
Fosthiazate-2	9.6	93.2
Furilazole	3.3	92.4
gamma-BHC	4.1	88.7
Halfenprox	2.3	85.4
Hexaconazole	8.9	85.6
Indanofan	7.9	86.5
Indoxacarb	3.7	95.7
Iprobenfos	4.4	89.5
Iprodione	2.5	92.7
Iprodione metabolite	3.1	106.2
Isazofos	3.7	94.2
Isocarbophos	6.6	84.0
Isofenphos	3.2	89.0
Isofenphos oxon	5.2	84.5
Isoprocarb	4.5	86.6
Isoprothiolane	7.5	86.1
Isoxadifen-ethyl	5.0	90.5
Isoxathion	6.7	93.2
Kresoxim-methyl	7.0	89.7
Leptophos	3.5	93.3
Malathion	3.2	93.0
MCPB-ethyl	3.5	90.3
Mecarbam	8.4	97.6
Mefenacet	4.5	75.1
Mefenpyr-diethyl	5.0	90.4
Mepronil	4.2	79.5
Metalaxyl	7.0	86.6
Methacrifos	5.9	92.3
Methidathion	4.5	86.0
Methoprene	8.8	109.6
Methoxychlor	3.1	90.6
Metolachlor	2.9	91.1

Table 2 Repeatability and Recovery (continued)

Compounds	Repeatability (%RSD, n = 6)	Recovery (%)
Metominostrobin-(E)	9.6	72.4
Metribuzin	6.5	75.1
Mevinphos-1	9.9	92.3
Mevinphos-2	6.0	85.4
Molinate	3.8	86.0
Myclobutanil	5.9	75.7
Naled	6.1	72.8
Nitralin	4.5	94.2
Nitrofen	8.1	88.9
Nitrothal-isopropyl	2.4	90.2
Oxabetrinil	3.4	91.7
Oxadiazon	3.9	94.7
Oxpoconazole	6.4	74.7
Oxpoconazole-formyl deg.	9.9	88.9
Oxyfluorfen	8.9	88.3
Paclobutrazol	7.5	72.6
Parathion	6.3	90.1
Parathion-methyl	5.1	90.4
Penconazole	4.7	85.0
Pendimethalin	5.1	86.9
Pentoxazone	4.2	95.6
Permethrin-1	4.8	89.0
Permethrin-2	4.0	88.8
Phenothrin-1	7.4	93.1
Phenothrin-2	2.5	90.2
Phenthoate	2.4	91.7
Phorate	4.1	75.9
Phosalone	3.5	88.1
Phosmet	4.2	84.5
Phosphamidon-1	8.6	75.8
Phosphamidon-2	6.6	70.8
Picolinafen	4.0	90.4
Piperonyl butoxide	3.8	89.2
Piperophos	3.5	88.9
Pirimiphos-methyl	5.7	90.8
Pretilachlor	5.6	89.8
Procymidone	7.0	91.6
Profenofos	5.6	94.1
Prohydrojasmon-1	5.5	87.7
Prohydrojasmon-2	8.7	88.6
Prometryn	3.0	86.8
Propachlor	4.4	88.0
Propargite-1	9.3	101.3
Propargite-2	9.5	94.5
Propazine	4.0	97.1
Propiconazole-1	6.7	89.4
Propiconazole-2	3.2	88.3
Propoxur	5.3	83.9
Propyzamide	4.2	81.6
Prothiofos	4.0	85.5
Pyraclofos	5.1	94.1
Pyraclostrobin	4.7	93.1
Pyraflufen-ethyl	4.7	92.7
Pyrazophos	4.2	92.8
Pyrazoxyfen	9.4	91.2
Pyributicarb	3.1	88.1
Pyridaben	3.1	86.1
Pyridaphenthion	5.4	84.2
Pyrifenox-(E)	5.9	85.2
Pyrifenox-(Z)	7.3	92.9
Pyrimethanil	6.0	83.9
Pyrimidifen	4.9	74.2
Pyriminobac-methyl-(E)	3.9	88.6
Pyriminobac-methyl-(Z)	5.2	88.6
Pyriproxyfen	5.7	92.1
Quinalphos	3.3	93.2
Quinoxyfen	3.2	87.1
Quintozene	6.0	90.3
Quizalofop-ethyl	3.0	86.9
Resmethrin-1	6.2	88.5
Resmethrin-2	3.3	86.1
Silafluofen	3.7	88.6
Simazine	5.2	74.9
Simeconazole	6.1	79.1
Simetryn	5.0	74.1
Spirodiclofen	4.6	94.1
Sulfotep	3.9	92.9
Sulprofos	4.8	74.5
Swep	5.3	83.6
Tebufenpyrad	3.6	88.8
Tebupirimfos	4.6	89.4
Tecnazene	3.1	89.5
Tefluthrin	4.5	90.1
Terbucarb	4.0	87.6
Terbufos	3.8	77.9
Terbutryn	4.5	86.0
Tetrachlorvinphos	3.2	93.0
Tetraconazole	7.8	84.3
Tetradifon	5.9	89.5
Tetramethrin-1	6.9	93.8
Tetramethrin-2	4.3	90.9
Thenylchlor	3.5	87.3
Thifluzamide	5.9	84.6
Thiobencarb	3.5	85.6
Tolclofos-methyl	3.9	90.6
Tolfenpyrad	3.6	81.0
Tolylfluanid	5.8	91.1
Triadimefon	3.7	88.3
Triadimenol-1	6.2	70.8
Tri-allate	5.3	91.2
Triazophos	4.7	89.9
Tribufos	6.2	90.6
Trichlamide	5.2	85.3
Trifloxystrobin	5.9	90.7
Trifluralin	3.2	92.5
Vinclozolin	4.2	89.6
XMC	3.9	86.5
Xylylcarb	4.5	85.3
Zoxamide	3.6	82.6

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Application News

No.L503

Supercritical Fluid Extraction / Chromatography

Application of Nexera UC SFE Pretreatment System for Extracting Pesticide Residues from Soil

Evaluating the persistence of pesticides in environmental soil is an important criteria for evaluating the safety of pesticides and analyzing pesticides in soil is extremely important for initial evaluations or registration of pesticides. However, in most cases, analyzing pesticides in soil using liquid-liquid extraction to extract the pesticides is very time-consuming, requires special equipment and reagents, and can cause problems, such as metal ions or other introduced ionic substances contaminating analytical instruments or the target substances being decomposed by oxidation, exothermic reactions, or other consequences of the extraction process.

In contrast, supercritical fluid extraction (SFE) provides excellent extraction efficiency using supercritical carbon dioxide as the extraction solvent, which offers the low viscosity and high diffusivity of a gas and the high solubility of a fluid. Consequently, it extracts target substances quickly using smaller quantities of organic solvent than existing solvent extraction methods, making it a more environmentally-friendly method as well.

This article describes an example of using the Nexera UC SFE pretreatment system to extract residual pesticides from soil.

Off-Line SFE System

The operating principle of the Nexera UC SFE pretreatment system is shown in Fig. 1. An extraction vessel filled with a sample is placed in the SFE unit and heated to 40 °C (Fig. 1 A). The extraction vessel is then filled with supercritical carbon dioxide and the target components are extracted statically without pumping the liquid (Fig. 1 B). After static extraction, the target components are extracted dynamically by pumping supercritical carbon dioxide through the extraction vessel (Fig. 1 C). After trapping the extract material in the trap column, the eluate that contains the target components is then collected in the fraction collector (Fig. 1 D).

Sample Preparation

Liquid-liquid extraction is typically used to pretreat soil samples for residual pesticide analysis. However, due to the extraction time and equipment required, throughput is low, limiting the number of samples that can be processed in a day. It also requires using organic solvent during extraction. Therefore, an alternative extraction method to liquid-liquid extraction is desirable, in terms of both the environment and cost.

In contrast, the Nexera UC SFE pretreatment system requires only mixing 1 g of soil with 1 g of a dehydrating agent* and placing the mixture in the extraction vessel, as shown in Fig. 2. This not only improves productivity and minimizes environmental impact, but also avoids human errors involved in the sample pretreatment process. Furthermore, a specially designed rack changer can be used to perform extraction consecutively for up to 48 samples.

* "Miyazaki Hydro-Protect" Patent No. 3645552

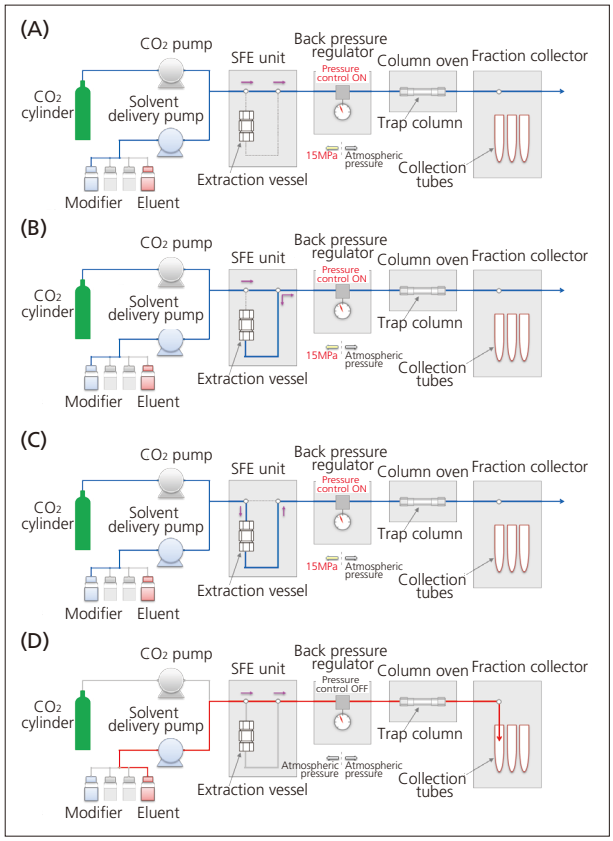

Fig. 1 Process Flow of SFE Extraction

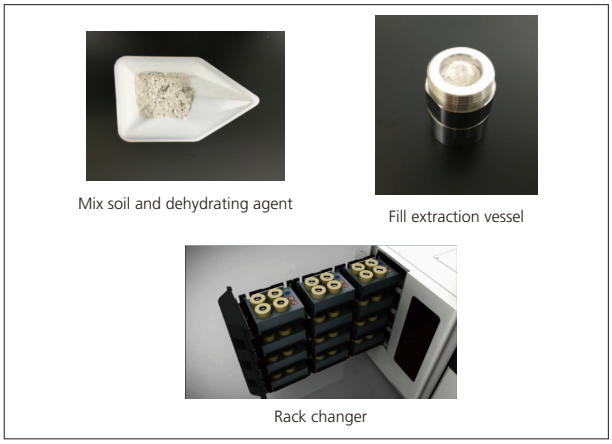

Fig. 2 Sample Preparation

Extraction and Analysis of Residual Pesticides in Soil

Soil was spiked with 200 ng/g each of eight pesticide components, which were then extracted by SFE using the conditions indicated in Table 1. Eluent was added to the extract obtained to make 2 mL, which was then analyzed by LC-MS/MS using the conditions indicated in Table 1. Repeatability and recovery rate values for the eight pesticide components are shown in Table 2. Recovery rates were determined by comparing the area of pesticide peaks measured from the extract obtained from the soil spiked with pesticide and measured from the extract obtained from unspiked soil to which the pesticides were added after extraction. This system uses a simpler and faster pretreatment process than liquid-liquid extraction, which enables it to finish extraction in about 30 minutes per sample. It also uses less organic solvent, so it is superior in terms of the environment and cost as well.

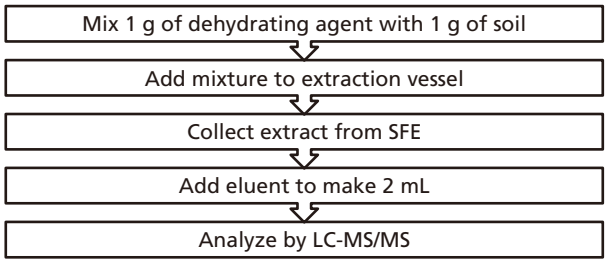

Fig. 3 Process Flow from Pretreatment to Analysis

Table 1 Extraction and Analytical Conditions

[SFE] Nexera UC SFE System
Solvent : A) Supercritical fluid of CO_2
B) Methanol
Flowrate : 5 mL/min
Extraction : 4 min (Static mode → Dynamic mode)
Extraction Vessel Temp. : 40 °C
BPR Pressure : 15 MPa
Trap Column : Shim-pack VP-ODS (50 mm L. × 4.6 mm I.D., 5 µm)
Column Oven Temp. : 40 °C
Elution Solvent : Acetone/Hexane = 50/50 (2 mL/min, 2 min)

[LC] Nexera X2 System
Column : Shim-pack UC-RP (150 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phase : A) 10 mM Ammonium formate
B) 10 mM Ammonium formate in methanol
Time Program : B.Conc. 0 % (0 min) → 100 % (14-17 min) → 0 % (17.1-20 min)
Flowrate : 0.4 mL/min
Column Temp. : 40 °C
Injection Volume : 3 µL

[MS] LCMS-8060 (MRM mode)
Ionization : ESI (positive or negative)
DL Temp. : 200 °C
Block Heater Temp. : 400 °C
Interface Temp. : 300 °C
Nebulizing Gas Flow : 2 L/min
Drying Gas Flow : 10 L/min
Heating Gas Flow : 10 L/min

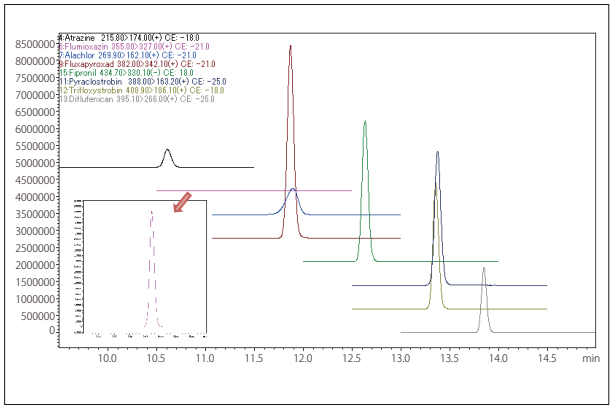

Fig. 4 MRM Chromatogram of Extract from Soil Spiked with Pesticides

Table 2 Repeatability and Recovery

Compounds	Repeatability (%RSD, n=6)	Recovery (%)
Alachlor	1.9	87.0
Atrazine	1.3	75.8
Diflufenican	1.2	86.2
Fipronil	1.5	80.6
Flumioxazin	3.8	70.1
Fluxapyroxad	2.2	72.9
Pyraclostrobin	1.8	73.3
Trifloxystrobin	1.5	87.7

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Application News

No. C162

Liquid Chromatograph Mass Spectrometry

Quantitative Analysis of Highly Polar Pesticides in Food Using SFC/MS

Since achieving sufficient retention and favorable separation in normal batch analysis of highly polar pesticides has proved difficult due to their chemical characteristics, a number of individual analysis methods are employed for LC/MS/MS analysis. To rectify this situation, EURL-SRM (Stuttgart, Germany), an EU Reference Laboratories member in charge of individual analysis method development, is developing a batch analysis method called "QuPPe (Quick Polar Pesticides)" for highly polar pesticides that are difficult to analyze using pretreatment with the QuEChERS method as well as normal batch analysis methods. This method proposes multiple methods to suit each sample and target chemical compound (M. Anastassiades et al; QuPPe of EURL-SRM (Version 9.1; 2016)).

Until now, analysis of highly polar pesticides using LC/MS/MS has used a variety of separation methods including HILIC mode, mixed mode, normal phase, and reversed phase. However, all of these methods have restrictions on the chemical compounds that can be analyzed together and this remains a problem. On the contrary, supercritical fluid chromatography (SFC) has the advantage of being able to separate a wide array of chemical compounds at once due to the characteristics of the mobile phase that is used. In addition, since the separation behavior with SFC differs from that with LC even when using a column of the same separation mode, SFC may be effective for the analyses of chemical compounds for which retention and separation are difficult in LC. This article introduces an example of batch analysis of highly polar pesticides using SFC.

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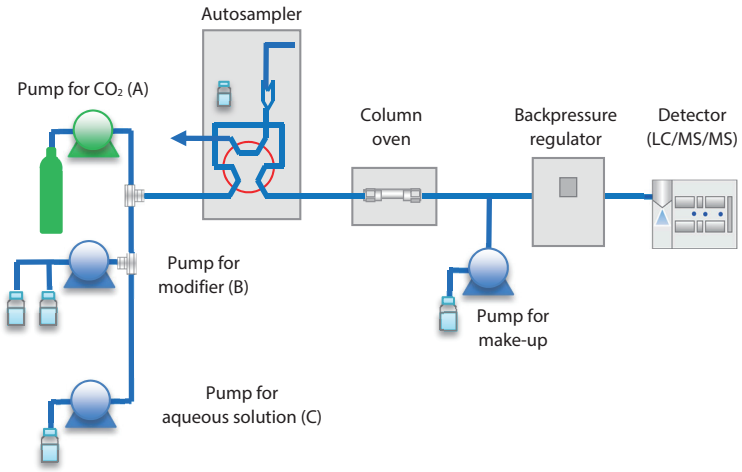


Fig. 1 SFC/MS System Configuration Diagram

In this experiment, an examination of adding a small amount of water to a modifier was performed for the purpose of eluting and separating highly polar pesticides.
In order to simplify this examination, a low-pressure gradient pump (LPGE) was used as pump B and the modifier was automatically prepared by mobile phase blending.

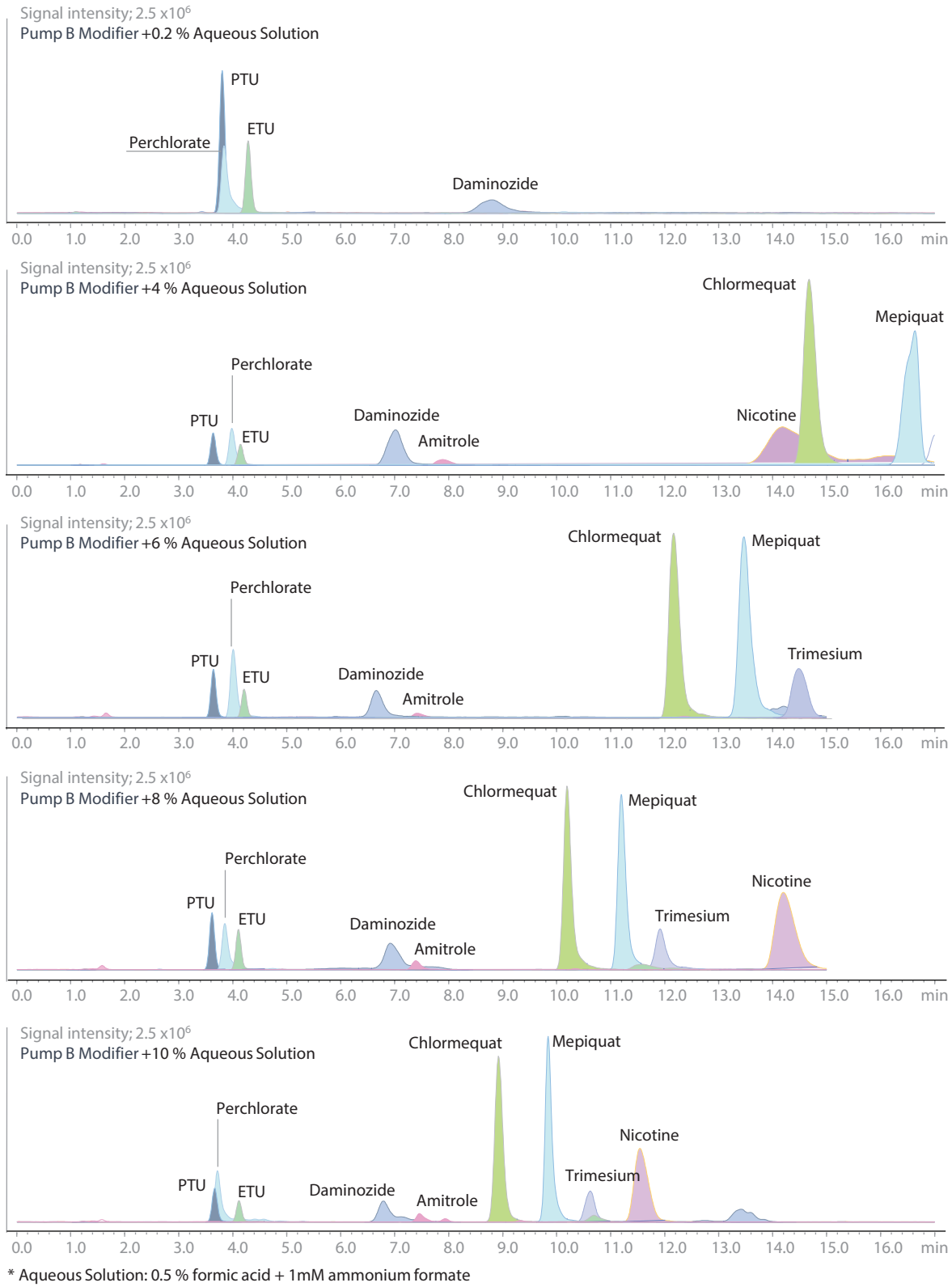
Table 1 SFC/MS Analysis Conditions

Supercritical fluid chromatography		Mass spectrometry	
SFC	Nexera UC system	**LC-MS/MS**	LCMS-8060
Analytical column	Restek Ultra Silica (150 × 2.1 mm 3 µm)	**Ionisation mode**	Heated ESI
Column temperature	50 °C	**Scan speed**	15,000 u/sec
Flow rate	0.8 mL/min (0.6 mL/min 13-22 min)	**MRM Dwell time**	3 msec
Pump A	CO_2	**Pause time**	1 msec
Pump B (modifier solvent)	Acetonitrile + 0.5 % formic acid + 10 mM ammonium formate	**Interface temp.**	300 °C
Pump C (modifier solvent)	Water + 0.5 % formic acid + 10 mM ammonium formate	**Heating block**	350 °C
Pump D (make up solvent)	Methanol	**Desolvation line**	250 °C
Makeup solvent flow rate	0.2 mL/min		

Examination of SFC Separation Conditions

Normally, SFC performs gradient separation using supercritical carbon dioxide and an organic solvent (such as methanol and acetonitrile), which is referred to as a modifier. However, some highly polar chemical compounds exhibit strong retention in columns resulting in cases where separation and elution is insufficient even with 100 % organic solvent. In this experiment, since a number of highly polar pesticides could not be eluted with 100 % organic solvent, separation was examined by adding a small amount of water to the modifier.

Supercritical carbon dioxide has low polarity and low miscibility with water. This means that only a limited amount of water can be added to the modifier (normally about 0.1 to 10 %). We therefore examined separation behavior by adding water by the amount equivalent to 0.2, 4, 6, 8, and 10 % to the modifier. Through examination based on the peak profiles and separation patterns of the eluted components, we adopted a water content of 6 %. However, there were chemical compounds that could not be eluted even with this condition.



* Aqueous Solution: 0.5 % formic acid + 1mM ammonium formate

Fig. 2 Effect of Water on Separation Behavior of Highly Polar Pesticides in SFC/MS

Optimization of SFC Separation Conditions

When we examined addition of water to the modifier, we were able to confirm elution of most chemical compounds with the 6 % aqueous solution. However, nicotine and kasugamycine, which both exhibit strong retention, could not be eluted. Any further addition of aqueous solution in the presence of carbon dioxide adversely affects gradient accuracy and may impair the stability of the analysis method. For this reason, aqueous solution was added using a separate pump (pump C) after the modifier reached 100 % (Fig. 4).

This allowed elution of the remaining highly polar pesticides and enabled batch separation of the highly polar pesticides from logP-3.47 to 1.96.

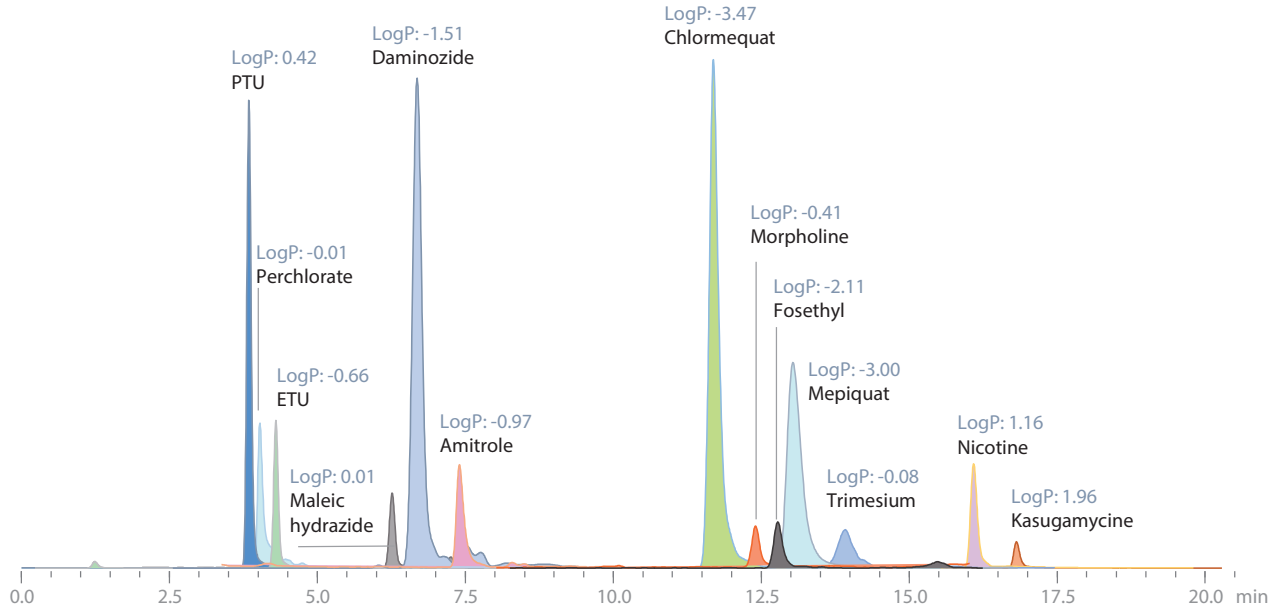
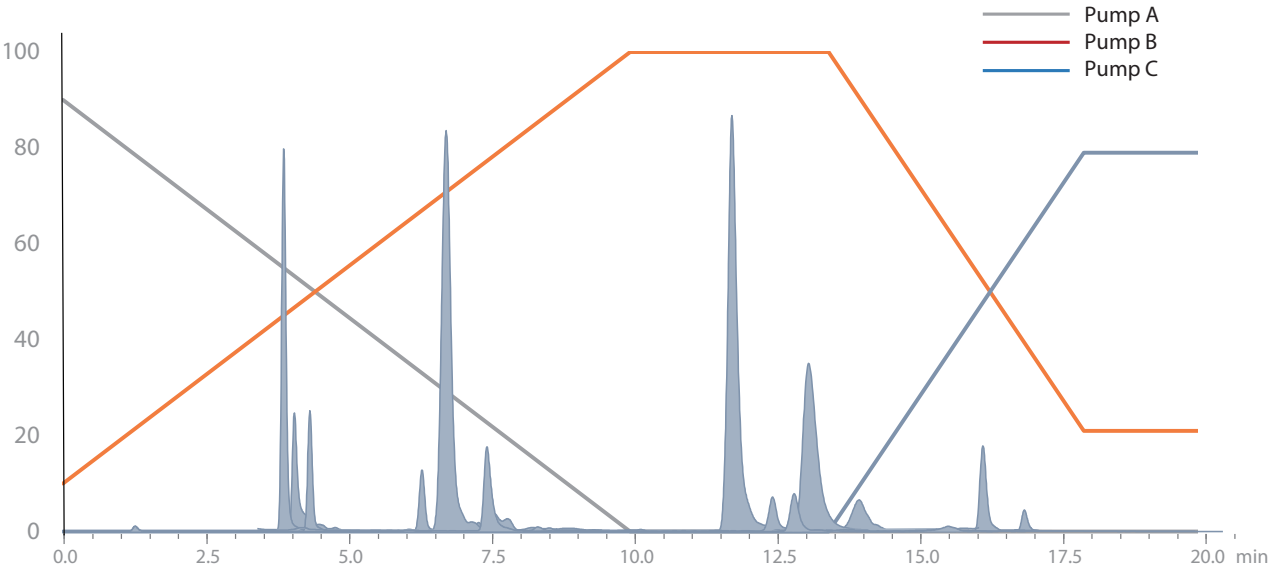


Fig. 3 MRM Chromatogram of Highly Polar Pesticides Using SFC-MS (Addition of 200 ppb Pesticide Standard Solution into Flaxseed Extract Using QuPPe)



The initial SFC/MS conditions;
Pump A 90 % : Carbon Dioxide
Pump B 10 % : 6 % Water in Acetonitrile containing 0.5 % formic acid and 10 mM ammonium formate
Pump C 0 % : Aqueous solution containing 0.5 % formic acid + 10 mM ammonium formate

Fig. 4 Ternary Gradient Program

Sample Preparation and Analysis

Flaxseed and lemon were used as food samples and extraction was performed using a method compliant with QuPPe. (The extracts were provided by Concept Life Sciences, a contract analytical laboratory located in the U.K.) Standard solution of highly polar pesticides was added to these matrix solutions, which were then directly injected into the SFC-MS/MS.

Quantitative Analysis of Highly Polar Pesticides

In order to verify the quantitative performance of the developed SFC/MS analysis method, matrix calibration curves were created using each food extract to which standard solution of the highly polar pesticides was added. The calibration curve range was 10 to 200 ppb and accuracy was verified using the internal standard method regarding components for which an internal standard substance labeled with a stable isotope was obtained.

The calibration curve created for each sample showed favorable linearity for all chemical compounds regardless of the sample matrix.

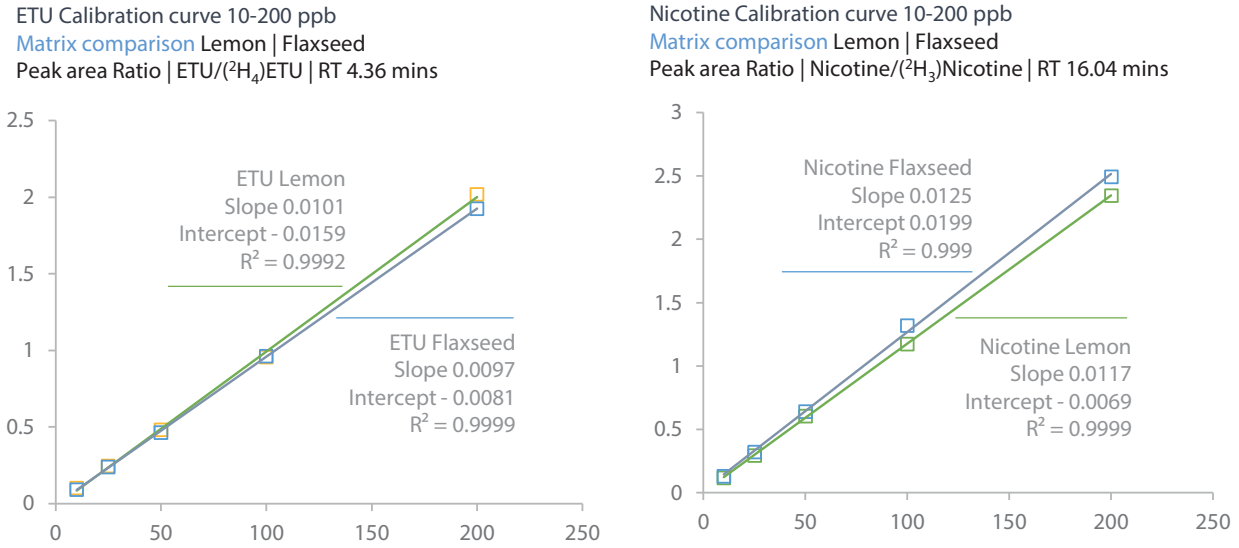


Fig. 5 Matrix Calibration Curves of Representative Highly Polar Pesticides (ETU: fast eluting compound, Nicotine: slow eluting compound, Samples: lemon, flaxseed)

Table 2 Calibration Curve Linearity and Repeatability at 100 ppb of Eight Highly Polar Pesticide Components

Compound	RT (min)	Internal Standard	IS RT (min)	Quan MRM	%RSD 100ppb	R^2
Perchlorate	3.95	$^{18}O_4$ Perchlorate	3.91	99.00 > 82.90	4.98	0.968
ETU	4.36	$^{2}H_4$ ETU	4.26	103.10 > 44.05	4.84	0.999
Maleic hydrazide	6.28	$^{2}H_2$ Maleic hydrazide	6.28	113.00 > 67.10	6.81	0.997
Chlormequat	11.58	$^{2}H_4$ Chlormequat	11.54	121.90 > 58.10	1.75	1.000
Fosethyl	12.50	$^{2}H_{15}$ Fosethyl	12.50	109.00 > 80.95	6.78	0.999
Morpholine	12.19	$^{2}H_8$ Morpholine	12.23	87.90 > 70.05	10.74	0.996
Mepiquat	12.72	$^{2}H_3$ Mepiquat	12.69	114.30 > 98.10	7.66	0.998
Nicotine	16.06	$^{2}H_3$ Nicotine	16.03	163.00 > 130.00	2.31	0.999

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Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perflurocompounds in Textiles

ASMS 2016 MP 283

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Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perflurocompounds in Textiles

Introduction

Supercritical Fluid Chromatography (SFC) with carbon dioxide as eluent has attracted attention recently because of its advantages in low running cost, non-toxicity and wider coverage of analytes in terms of polarity. The combination of SFC with SFE (E=Extraction) has extended the applications to fully-automated sample pre-treatment and analysis as demonstrated by the Nexera UC system introduced by Shimadzu recently. The novel SFE-SFC-MS/MS system has been used successfully for analysis of 510 residual pesticides in agricultural products [1]. One of the main advantages of the Nexera UC platform allows to set up on-line method to analyse directly different types and forms of un-pretreated samples. We describe the development of an approach on the Nexera UC platform, aiming at screening and quantitation of 23 perflurocompounds (PFCs) listed under the Restricted Substance List (RSL) in textile, leather and consumer goods industries [2].

Experimental

A total of 23 PFCs and 2 internal standards (IS), M-PFOS and M-PFOA (refer to Table 2) were obtained from Sigma Aldrich, Wellington Laboratories and Apollo Scientific [3]. Textile samples were cut into smaller pieces and weighed. The sample (60 mg) was loaded into a 0.2 mL stainless steel SFE vessel tightly before proceeding to online-SFE-SFC-MSMS analysis. A schematic diagram of the Nexera UC system employed in this study is shown in Figure 1. The system can be operated for on-line SFE-SFC-MS/MS experiments or only for SFC-MS/MS analysis. The mobile phase is supercritical fluid CO2, with addition of organic modifier like MeOH. Thus, both isocratic and gradient elution modes can be chosen. The details of the analytical conditions are compiled into Table 1.

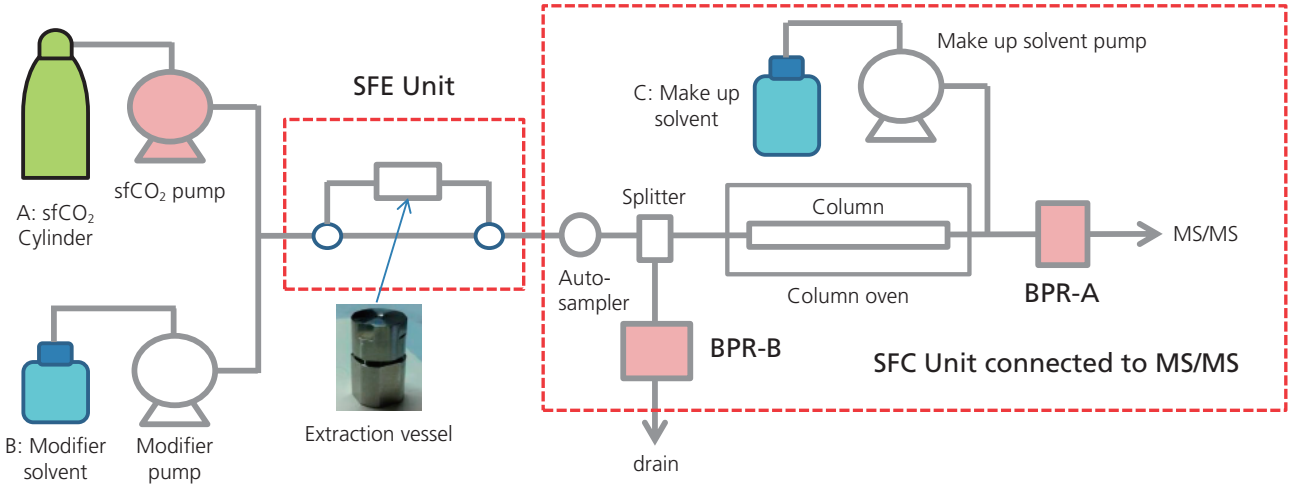

Figure 1: Schematic diagram of Nexera UC system for SFE-SFC-MS/MS analysis of un-pretreated samples

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perflurocompounds in Textiles

Table 1: Analytical conditions of 23 PFCs and 2 internal standard on Nexera UC with LCMS-8050

Column	: Shim-pack UC-X Sil (250 mmL. x 2.1mm I.D., 3µm)
Flow Rate	: 2.0 mL/min
	0.2 mL/min (make up pump of MS)
Mobile Phase	: A : Supercritical Fluid Carbon dioxide (sfCO2)
	B : Methanol with 5 mM ammonium formate
	C : Methanol with 5 mM ammonium formate
Temp.	: Column Oven: 40°C; SFE unit: 40°C or RT
Injection vol.	: SFC: 5 µL; SFE-SFC: 200 µL or 3% split ratio
Elution Mode	: Gradient elution, LC program 7 minute
	0%B (0.00mins to 0.30mins)
	→ 50%B (4.00mins to 4.50mins)
	→ 0%B (4.70mins to 6.00mins)
Interface	: ESI
MS mode	: Negative
Block Temp.	: 400°C
DL Temp.	: 250°C
Interface Temp.	: 300°C
CID Gas	: Ar (270kPa)
Nebulizing Gas Flow	: N_2, 3 L/min
Drying Gas Flow	: N_2, 10 L/min
Heating Gas Flow	: 0 Air, 10 L/min

Results and Discussion

Establishment of SFC-MS/MS method

A SFC-MS/MS method was established for the targeted 23 PFCs with 2 IS first. A Shim-pack UC-X Sil column was used and a gradient elution program was adopted. Two MRM transitions (if available) were used for each PFC, one as quantifier ion and the other for confirmation. The SFC-MS/MS chromatograms and calibration curves (only PFOA and PFOS are displayed) are shown in Figure 2. Calibration curves were built based on the two internal standards with linearity (r^2>0.97) for all 23 PFCs (Table 2). Instead of using the concentration, absolute amount (pg) was used for the calibration curves. The LOQ ranges from 0.03 ~1 ng/mL while the LOD ranges from 0.01 ~ 0.32 ng/mL. The repeatability of the method was evaluated at two concentrations, 5 and 25 pg. The %RSD results of the post-spiked samples are tabulated in Table 2.

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perflurocompounds in Textiles

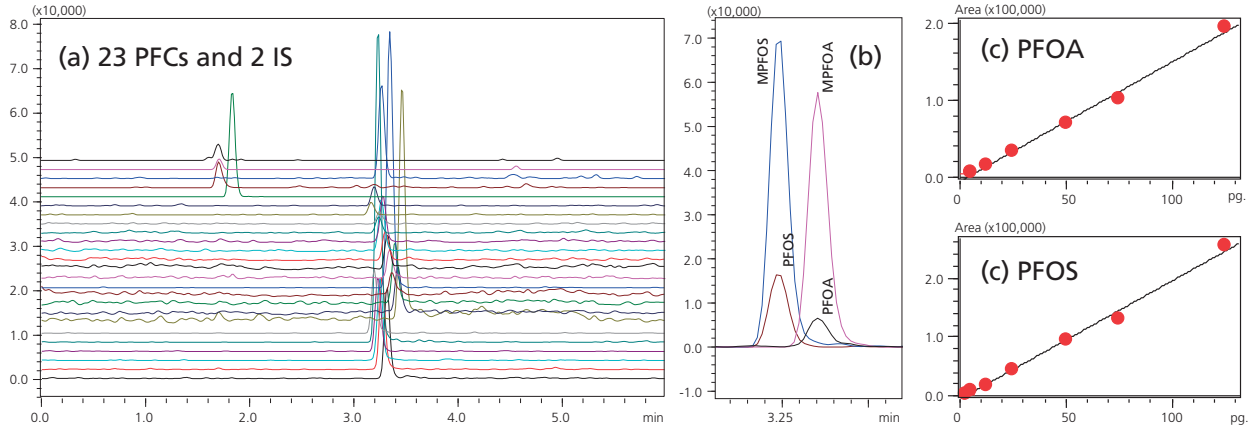

Figure 2: (a) MRM chromatograms of 23 PFC mixed standards with 2 IS, 5 pg for each compound;
(b) Zoomed MRM peaks of PFOA and PFOS with their IS;
(c) Calibration curves of PFOA (5~125 pg) and PFOS (2.5~125 pg) based on quantifier ions as shown in table 3.

Table 2: Calibration curves and performance values of the MRM method for quantitative determination of 23 PFCs on SFC-MS/MS. Absolute amounts (in pg) of analytes are used for convenience (injection volume: 5 µL)

No.	Name	RT (min)	MRM transition	Range (pg)	R^2	LOD (pg)	LOQ (pg)	RSD (%), n=6	
								(5 pg)	(25 pg)
1	N-EtFOSA-M	1.613	526.10>169.00	5 ~ 125	0.9732	< 2.5	5	34.4	32.3
2	N-MeFOSA-M	1.692	512.00>169.15	2.5 ~ 125	0.9966	< 2.5	5	47.1	18.1
3	H4PFUnA	1.695	491.10>367.00	2.5 ~ 125	0.9877	1.2	3.6	59.9	20.7
4	FOSA	1.831	498.00>77.90	2.5 ~ 125	0.9926	< 2.5	5	23.5	7.9
5	PFODA	3.171	913.00>868.90	2.5 ~ 125	0.9916	0.45	1.3	43.9	12.1
6	PFDHxA	3.2	813.00>768.80	2.5 ~ 125	0.9985	0.25	0.8	16.3	8.3
7	PFDS	3.201	599.00>80.00	2.5 ~ 125	0.9942	0.1	0.3	21.5	8.4
8	PFTeDA	3.23	712.90>668.90	5 ~ 125	0.9947	1.55	4.7	14.4	10.4
9	PFOS	3.233	499.00>79.90	2.5 ~ 125	0.9881	0.1	0.35	21	9.5
10	PFHpS	3.253	449.00>79.85	2.5 ~ 125	0.9924	0.15	0.45	15.6	9.6
11	PFTrDA	3.257	663.00>619.00	2.5 ~ 125	0.9986	0.75	2.2	23.7	20.7
12	PFDoA	3.265	612.90>569.00	2.5 ~ 125	0.9943	0.5	1.6	13.5	13
13	PF-3,7-DMOA	3.266	469.05>269.00	2.5 ~ 125	0.9918	0.15	0.4	22.3	9.1
14	PFHxS	3.278	399.00>79.90	2.5 ~ 125	0.9923	0.1	0.35	26.9	5.5
15	PFUdA	3.289	563.00>519.00	2.5 ~ 125	0.9975	0.5	1.55	17.7	8.1
16	PFDA	3.307	512.80>468.90	2.5 ~ 125	0.9946	0.75	2.3	25.4	8.3
17	PFBS	3.322	298.80>79.90	2.5 ~ 125	0.9951	0.15	0.45	18.9	8.8
18	PFNA	3.329	462.90>418.90	5 ~ 125	0.9908	1.5	4.55	23.4	12.3
19	PFOA	3.354	413.10>369.10	5 ~ 125	0.9832	1.6	4.8	15	6.1
20	PFHpA	3.38	313.10>269.05	5 ~ 125	0.996	1.25	3.8	14.4	11.8
21	PFHxA	3.399	263.00>219.00	2.5 ~ 125	0.9951	0.3	0.95	18	13.8
22	PFPeA	3.43	212.90>168.95	2.5 ~ 125	0.996	0.35	1.1	18.7	11.4
23	PFBA	3.466	363.10>319.00	2.5 ~ 125	0.9949	0.15	0.5	11.7	8.7
IS1	M-PFOS	3.235	503.00>79.85	10	Not Available				
IS2	M-PFOA	3.349	416.90>372.00	10	Not Available				

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perflurocompounds in Textiles

Development of on-line SFE-SFC-MS/MS approach

Next, an on-line SFE-SFC-MS/MS approach was developed based on the SFC-MS/MS method established. The mixed standard samples for calibration curve construction could be introduced into the system only by pre-loading them onto filter papers. 50 µL of mixed standard solution was dropped onto half filter paper (recommended by Shimadzu for Nexara UC use) and left it to dryness under N2 flow before loading into the SFE vessel (0.2 mL). The results are shown in Figure 3 and Table 3 (columns 1-7). First, with on-line SFE, the elution peaks of the PFCs become broader and RTs delay slightly (about 0.2 min) in comparison with SFC-MS/MS chromatograms. This peak broadening and delay are due to the larger delay volume by the SFE vessel, needles and the tubing from SFE to column, which caused differences in peak areas and intensity. For direct quantitation of PFCs using on-line SFE-SFC-MS/MS, calibration curves must be established on SFE-SFC-MS/MS too (Figure 3(c) & Table 3 (left).

If we compare the peak areas obtained on SFE-SFC-MS/MS and SFC-MS/MS, system recovery of the SFE-SFC-MS/MS could be estimated (see Table 3, columns 8-11). Although this system recovery may not be highly accurate, it can be used as a reference to understand the performance of the on-line SFE-SFC-MS/MS for quantitation. The average system recovery measured at the absolute loading amounts of 25 pg and 50 pg are 90 % and 74 %, respectively. The average repeatability (RSD%, n=4) of the system with loading amounts of 25 pg and 50 pg are 12 % and 14 %, respectively. It is worth noting that all of the analysis runs shown above are under the condition without splitting of the flow (sfCO2 and MeOH) from SFE to SFC-MS/MS (PBR-B was set to zero flow to drain).

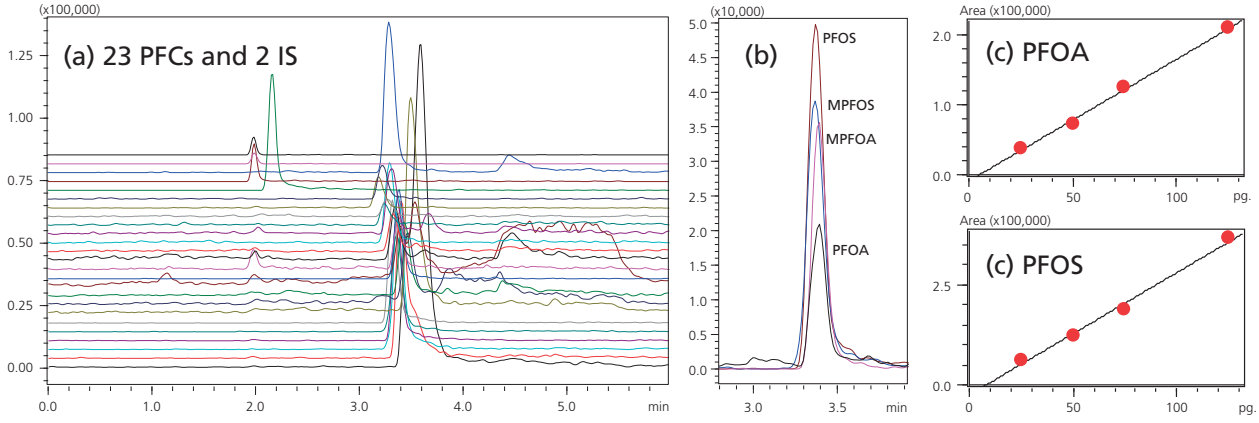

Figure 3: (a) MRM chromatograms of 23 PFC mixed standards with 2 IS on filter paper, 25 pg for each compound.
(b) Separate display of MRM peaks of PFOA, M-PFOA (IS, 10 pg), PFOS and M-PFOS (IS, 10 pg),
(c) Calibration curves of PFOA and PFOS on SFE-SPC-MS/MS.

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perflurocompounds in Textiles

Table 3: Calibration curves of 23 PFCs (on filter paper in 0.2mL SFE vessel) of on-line SFE-SFC-MS/MS method (left table); System recovery estimated by comparison with SFC-MS/MS method (right Table)

ID#	Name	m/z	Ret. Time	Range (pg)	R^2	Accuracy (%)
1	N-EtFOSA	526.10>169.00	1.636	25 - 125	0.964	103
2	N-MeFOSA	512.00>169.15	1.692	25 - 125	0.9668	103.6
3	H4PFUnA	491.10>367.00	1.694	25 - 125	0.9956	101.2
4	FOSA	498.00>77.90	1.828	25 - 125	0.9978	100.7
5	PFODA	913.00>268.95	3.15	25 - 125	0.9946	101.5
6	PFDHxA	813.00>169.10	3.163	25 - 125	0.988	102.3
7	PFDS	599.00>98.80	3.3	25 - 125	0.9998	99.7
8	PFTeDA	712.90>219.20	3.196	25 - 125	0.9968	101.1
9	PFOS	499.00>98.50	3.344	25 - 125	0.993	101.5
10	PFHpS	449.00>99.05	3.344	25 - 125	0.9942	101.4
11	PFTrDA	663.00>169.10	3.198	25 - 125	0.9956	101.3
12	PFDoA	612.90>319.00	3.22	25 - 125	0.9947	99.9
13	PF-3,7-DMOA	469.05>68.80	3.229	25 - 125	0.9949	101.5
14	PFHxS	399.00>79.90	3.401	25 - 125	0.9958	100.7
15	PFUdA	563.00>268.90	3.265	25 - 125	0.995	98.7
16	PFDA	512.80>169.00	3.262	25 - 125	0.9905	101.3
17	PFBS	298.80>98.80	3.357	25 - 125	0.9912	101.6
18	PFNA	462.90>168.90	3.31	25 - 125	0.9986	100.6
19	PFOA	413.10>168.85	3.36	25 - 125	0.9972	100.8
20	PFHpA	363.10>169.05	3.375	25 - 125	0.9904	101.7
21	PFHxA	313.10>119.00	3.397	25 - 125	0.9776	100.3
22	PFPeA	263.00>219.00	3.431	25 - 125	0.9991	100.6
23	PFBA	212.90>168.95	3.446	25 - 125	0.9997	100

25 pg (spiked)		50 pg (spiked)	
Recovery %	RSD% (n=4)	Recovery %	RSD% (n=4)
137.3	19.4	102.0	12.4
89.2	40.1	49.4	33.0
87.8	24.2	51.6	26.0
61.0	18.6	40.2	23.8
91.2	16.9	80.7	20.3
83.9	10.7	74.1	14.5
80.0	11.3	68.9	5.7
104.1	7.7	98.0	13.2
92.6	5.4	75.7	3.5
91.9	1.1	77.5	3.6
72.8	10.0	71.5	19.2
101.3	12.0	87.1	10.2
98.0	8.4	74.4	5.2
87.5	1.1	78.0	4.4
84.4	1.1	76.6	17.7
106.1	4.9	89.6	14.6
89.1	7.9	69.0	8.1
103.6	8.2	78.8	10.4
97.8	7.7	87.3	15.0
95.9	11.0	83.6	4.0
76.6	8.5	74.1	11.4
87.0	7.1	62.4	12.4
53.0	25.7	41.3	32.5

Automated SFE-SFC-MS/MS approach for analysis of PFCs in textiles samples

There are two extraction modes in the on-line SFE stage, static extraction and dynamic extraction with sfCO2 or a mixture of sfCO2 and MeOH (modifier). For more effective extraction, static extraction is performed first for 4 mins at 40 °C, followed by dynamic extraction for 2 mins. Figure 4 shows an example of the analysis of un-pretreated clothing sample and the same sample spiked with mixed PFCs (25 pg each on 60 mg of sample). The results shows that there are no PFC detected in the textile sample, which is in agreement with the offline LC/MS/MS analysis results [4]. On the other hand, all of the 23 PFCs spiked into the sample were detected. The amount of PFCs spiked are equal to 0.42 ng/g (ppb), where detection sensitivity meets the requirement for monitoring PFOA and PFOS in consumer products [2]. This preliminary finding reveals the potential possibility of using on-line SFE-SFC-MS/MS as a new automated screening and quantitation system in analysis of un-pretreated samples directly. However, further studies of this novel approach for PFCs and other targeted analytes in various consumer samples are under investigation for optimizing the SFE conditions and improving the recovery for samples with complex matrix.

Development of Automated Screening and Quantitation Approach on Novel On-Line SFE-SFC-MS/MS Platform – (I) For 23 Restricted Perflurocompounds in Textiles

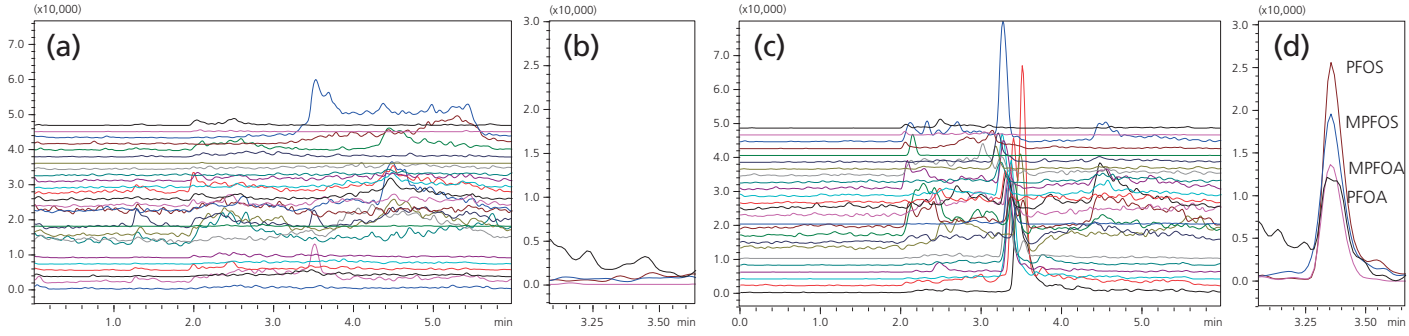

Figure 4: MRM chromatograms for screening of 23 PFCs in a textile sample (a-b) and in the same sample spiked with mixed 23 PFCs standards with 2 IS, 25 pg for each compound (c-d). Only dynamic extraction was used.

Conclusions

In this study, a new analytical approach on the novel SFE-SFC-MS/MS platform was developed for analysis of 23 PFCs including PFOA and PFOS spiked in textiles. The results indicate that the new approach is potentially possible for screening and quantitation of targeted analytes in un-pretreated solid samples directly. The detection sensitivity of the 23 PFCs spiked in clothing sample achieved is at the level of 25 pg or 0.42 µg/kg. However, validation of the SFE-SFC-MS/MS approach for actual samples are not carried out yet.

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1. Shimadzu Application News No LAAN-A-LC-E273, **Using the Nexera UC Online SFE-SFC-MS System to Analyze Residual Pesticides in Agricultural Products (2015).**
2. I. van der Veen, J. M. Weiss, A. C. Hanning, , J. de Boer and P. E. Leonards, *Talanta 147* **(2016)**, 8-15.
3. Wellington Laboratories, "Quick Reference Guide for Perfluoroalkyl Compounds", http://www.well-labs.com
4. J. X. Lee, Z. Sun, J. Xing, J. Y. Tan and Z. Zhan, ASMS 2016, Poster Session TP 485.

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Automated Analysis of Explosives in Soil Samples

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Shimadzu Scientific Instruments, Inc., Columbia, Maryland

Automated Analysis of Explosives in Soil Samples

Introduction

There are a large number of explosives-contaminated sites in the US, Europe, and Asia. High levels of explosives in soil can threaten the health of humans, livestock, and wildlife. A number of remediation efforts are underway, which require the analysis of explosives in soil samples. Recently, a new technique was introduced the allows the automated supercritical extraction and SFC analysis of samples with minimal sample preparation and handling requirements to save analyst time and sample preparation expenses. This technique was applied to the analysis of explosives in soil samples and showed good recoveries of the explosives tested in a number of different soil samples. Automated analysis of up to 48 samples is possible without the need for manual sample preparation to allow quick screening of explosives in numerous soil samples.

Experimental

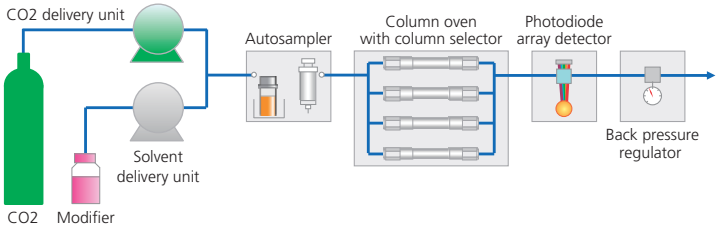
Fig.1 shows a diagram of the SFE-SFC system that was used in this experiment. This system consists of a combination of supercritical fluid chromatography and extraction systems. Method development was initially performed with the SFC method scouting system that automatically allows screening of up to 12 analytical columns with a number of different modifiers.

After determination of the optimal column and modifier combination for an explosives mix (AccuStandard M-8330) was completed, the analysis was moved to the SFE portion of the system for study of the explosives mix from a variety of soil types.

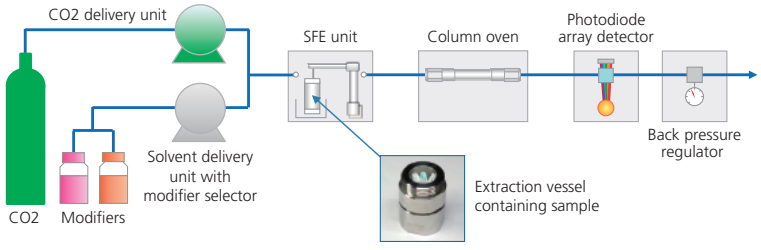
The SFE portion of the system allows the automated analysis of up to 48 soil samples by combining the sample preparation portion with the chromatographic analysis. Samples are extracted from extraction vessels and automatically transferred to an analytical column for analysis.

Instrument Design

1A: Supercritical fluid chromatography (SFC) system for analytical method development



1B: On-line Supercritical fluid extraction/chromatography (SFE/SFC) system



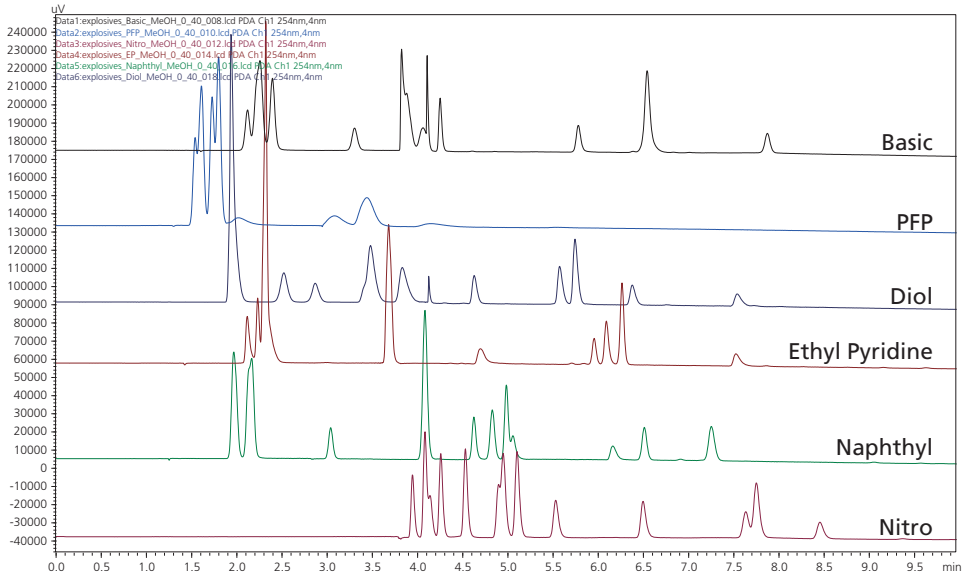
Method Development

Conditions	
Flow rate	: 3 mL/min
Detector	: Photodiode array
Column Temp	: 35°C
Backpressure	: 15 MPa

Mobile Phase	
A	: CO2
B	: MeOH
Gradient	: 1 to 10 min, 0 to 40% MeOH

Columns: 4.6 x 250mm, 5 um
Nexera UC Basic
Nexera UC PFP
Nexera UC Diol
Nexera UC Ethyl Pyridine
Nexera UC Naphthyl
Nexera UC Nitro

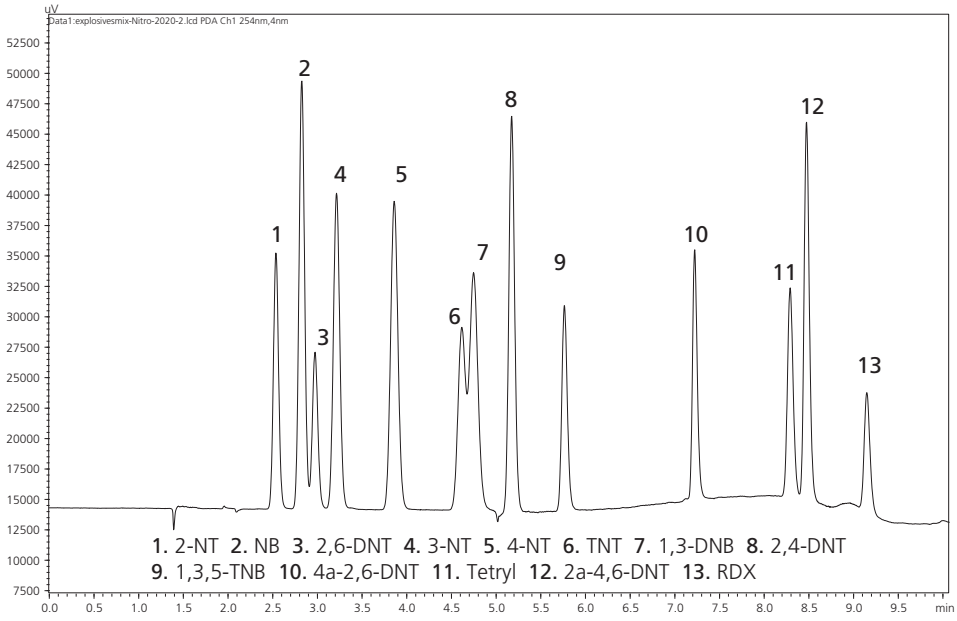
SFC Method Scouting



SFC Column Scouting of Explosives Mix

Optimized SFC Chromatogram

Nexera UC Nitro column



Samples

AccuStandard explosives standards M-8330-05 were used to prepare an explosives mixture. One gram of each soil sample was spiked with 100 uL of a 50 ppm explosives mixture.

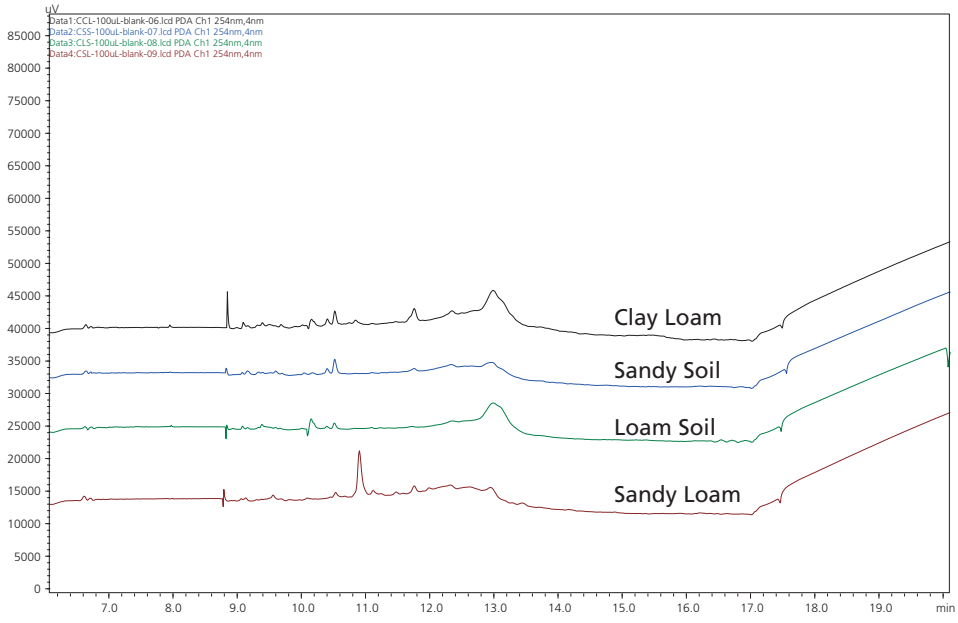
Standards
RDX, TNT, HMX, Tetryl, Nitrobenzene, 1,3-Dinitrobenzene, 2-Nitrotoluene, 3-Nitrotoluene, 2,4-Dinitrotoluene, 4-Amino-2,6-dinitrotoluene, 2,6-Dinitrotoluene, 1,3,5-Trinitrobenzene, 2-Amino-4,6-dinitrotoluene
Soil Samples
1. Clean Sandy Loam 2. Clean Clay Loam 3. Clean Sandy Soil 4. Clean Loam Soil

SFE-SFC Conditions

Extraction Conditions	
Flow rate	: 5 mL/min
95/5 CO2/MeOH	
0-3 min	: Static extraction
3-6 min	: Dynamic extraction

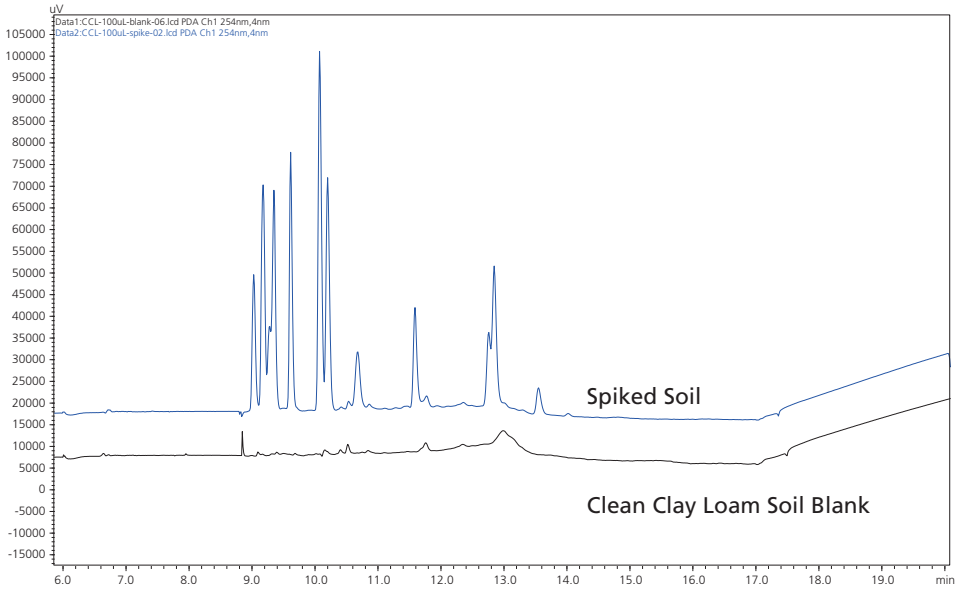
Chromatography Conditions	
Column	: NexeraUC Nitro
Flow rate	: 3 mL/min
6-15 min	: 0 to 40% MeOH
17-25 min	: Wash and equilibration

Blank Soil Sample Extracts

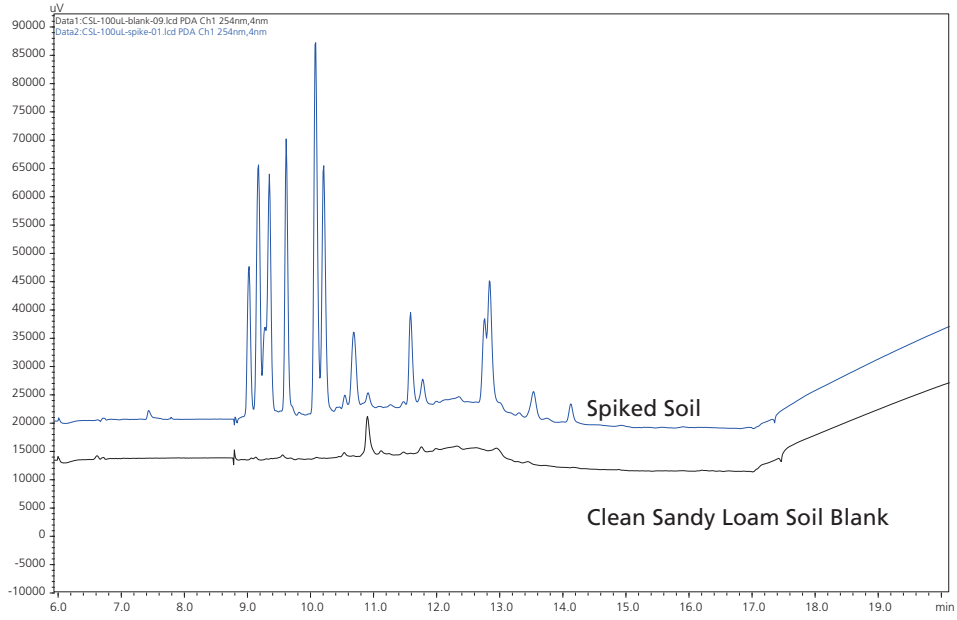


SFE extracts of blank soil types

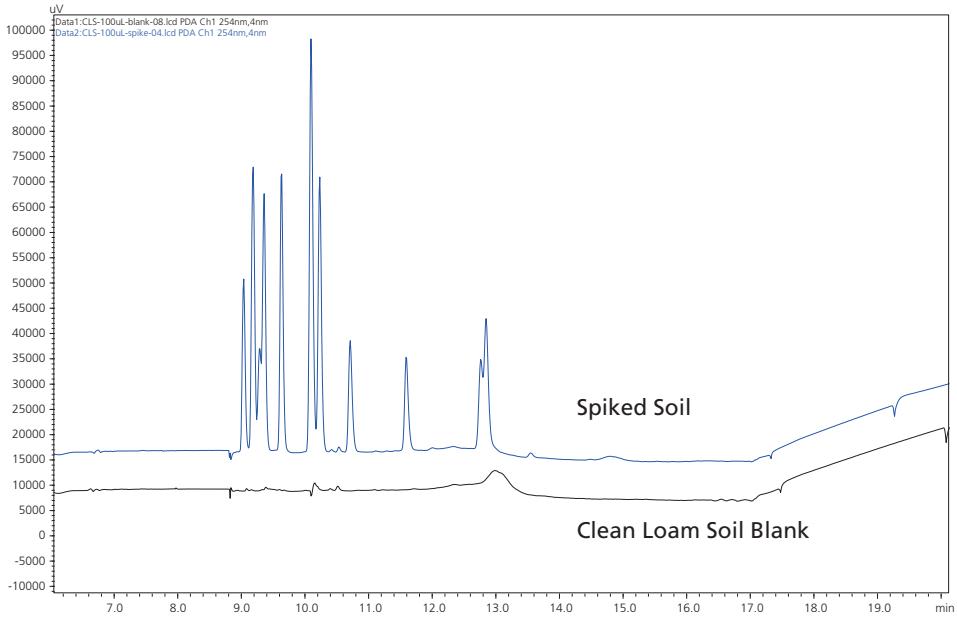
Clean Clay Loam Extract



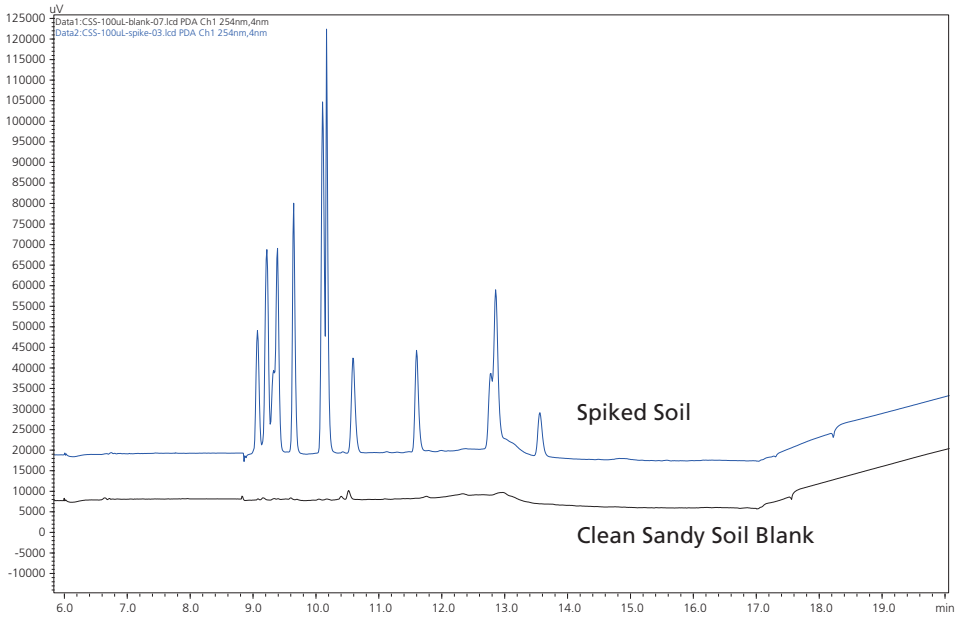
Clean Sandy Loam Extract



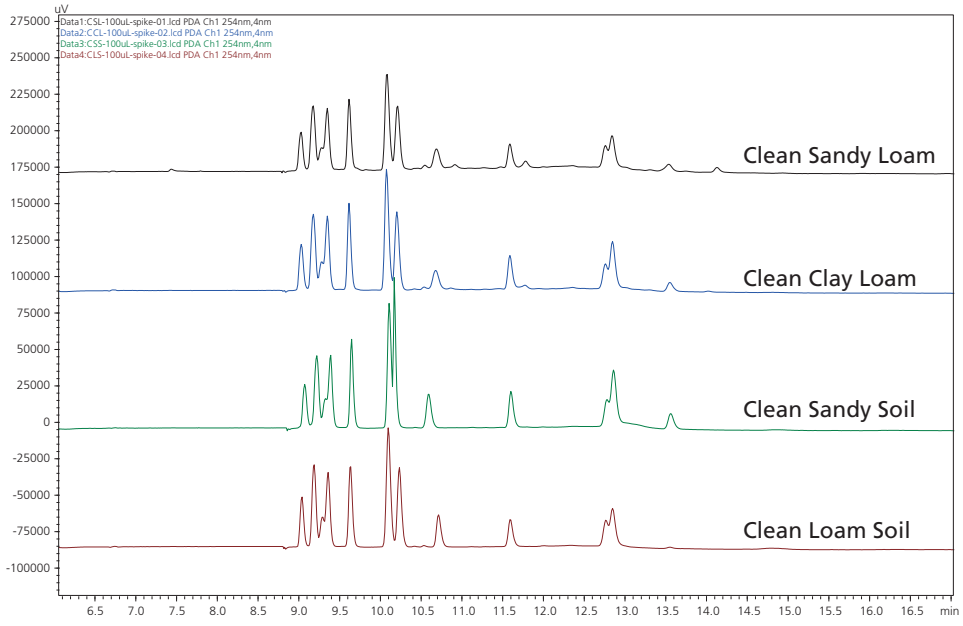
Clean Loam Soil Extract



Clean Sandy Soil Extract

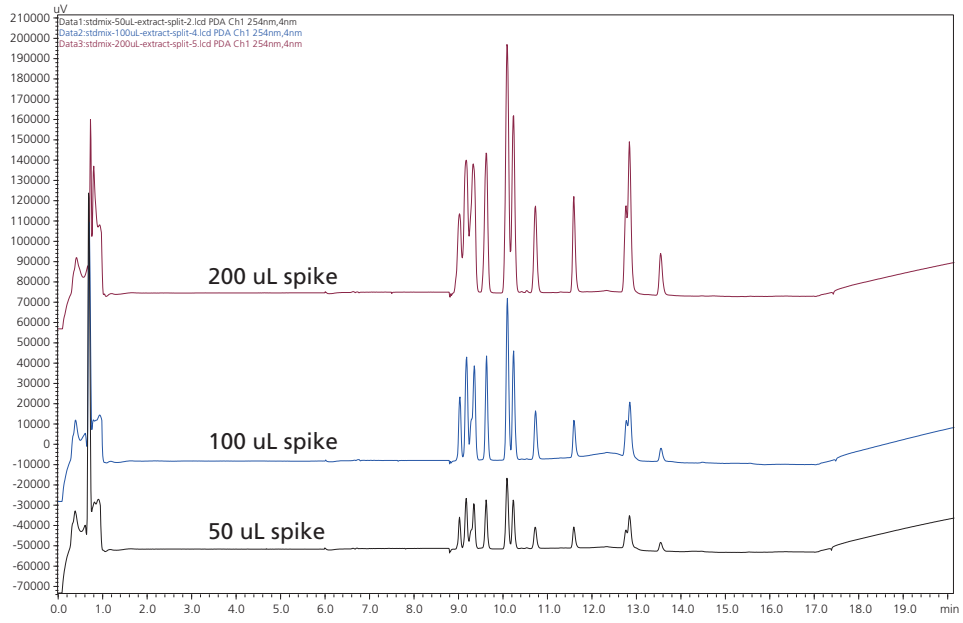


Spiked Soil Extracts Overlay



Spiked explosives standard into four different soil types

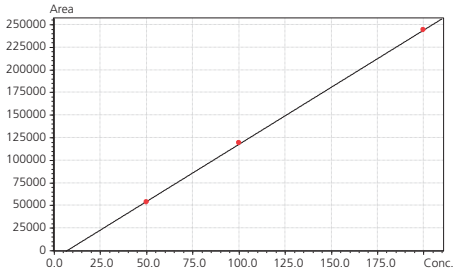
Linearity Study



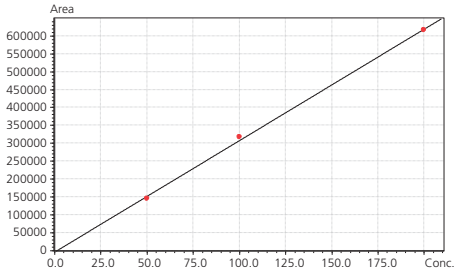
One gram of soil was spiked with 50, 100, and 200 uL of explosives mix

Automated Analysis of Explosives in Soil Samples

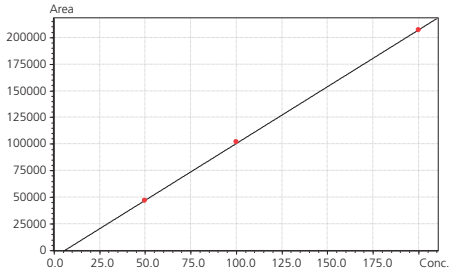
Linearity Results



2-NT
Y = aX + b
a = 1263.65
b = -8615.43
R^2 = 0.9998975
R = 0.9999488



TNT
Y = aX + b
a = 3113.98
b = -3707.87
R^2 = 0.9985081
R = 0.9992538



1,3,5-TNB
Y = aX + b
a = 1066.51
b = -5951.52
R^2 = 0.9998268
R = 0.9999134

Discussion

- A variety of soil samples showed little interference with spiked explosive standards.
- Clean Loam Soil provided poor recovery of the late eluting RDX peak.
- Good reproducibility was observed with the explosive standard extracts from a variety of soil samples.
- Good linearity was observed for the explosive compounds.
- Automated SFE-SFC can be a quick way to screen up to 48 soil samples for explosives in a variety of soil types with minimal sample prep.

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Automated Screening of Explosives in Soil Samples by Online SFE-SFC-MS

ASMS 2016 TP 185

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Jonathan Edwardsen,
Shimadzu Scientific Instruments, Inc.,
Columbia, Maryland

Automated Screening of Explosives in Soil Samples by Online SFE-SFC-MS

Introduction

There are a large number of explosives-contaminated sites in the US, Europe, and Asia. High levels of explosives in soil can threaten the health of humans, livestock, and wildlife. A number of remediation efforts are underway, which require the analysis of explosives in soil samples.

Recently, a new technique was introduced that allows the automated supercritical fluid extraction and SFC analysis of explosives in soil samples with minimal sample preparation and handling requirements to save analyst time and sample preparation expenses.

Experimental Overview

A combined SFC method scouting system and SFE-SFC system was used in this study. Method development was initially performed with the SFC method scouting system that automatically allows screening of up to 12 analytical columns with a number of different modifiers.
After determination of the optimal column and modifier combination for an explosives mix (AccuStandard M-8330) with UV detection was completed, the analysis was moved to the SFE/SFC portion of the system after MS optimization for study of the explosives mix from soil samples.
The SFE portion of the system allows the automated analysis of up to 48 soil samples by combining the sample preparation portion with the chromatographic analysis. Explosive compounds are extracted from the soil samples in the extraction vessels and automatically transferred to an analytical column for analysis after completion of the extraction procedure.

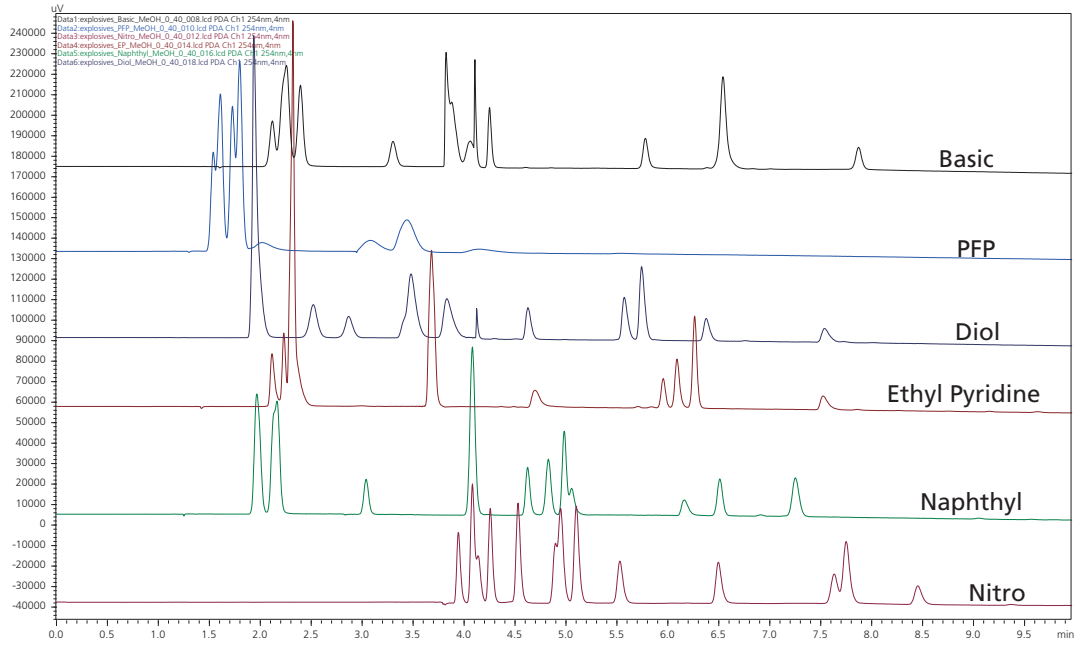
SFC Method Development

Conditions	
Flow rate	: 3 mL/min
Detector	: Photodiode array
Column Temp	: 35°C
Backpressure	: 15 MPa
Mobile Phase	
A	: CO_2
B	: MeOH
Gradient	: 1 to 10 min, 0 to 40% MeOH

Columns: 4.6 x 250mm, 5 µm
Nexera UC Basic
Nexera UC PFP
Nexera UC Diol
Nexera UC Ethyl Pyridine
Nexera UC Naphthyl
Nexera UC Nitro

Automated Screening of Explosives in Soil Samples by Online SFE-SFC-MS

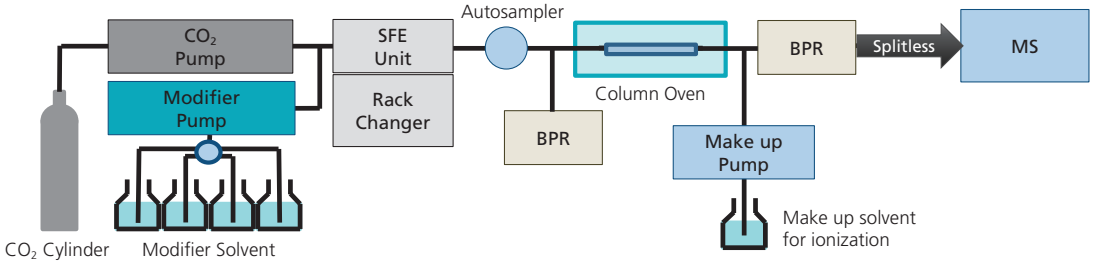
SFC Method Scouting

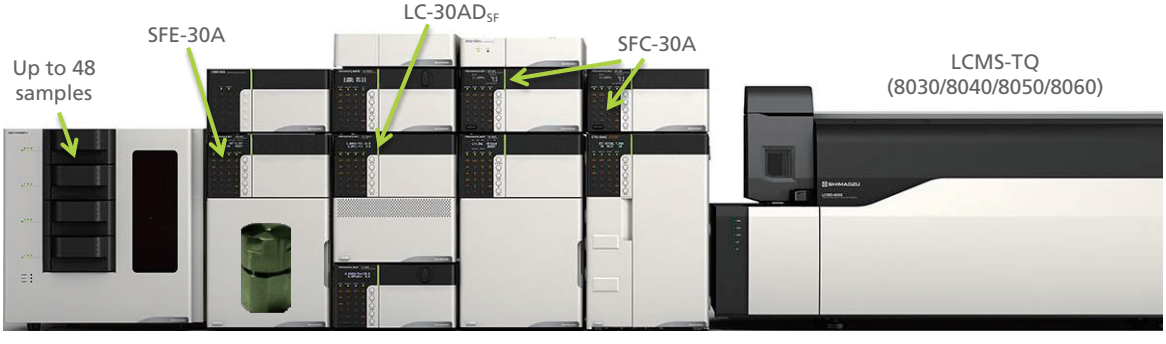


SFC Column Scouting of Explosives Mix

System Configuration – SFE-SFC-MS/MS

Online SFE – SFC – MS System





extraction vessel

SFE/SFC Analytical Conditions

On-line SFE/SFC Analytical conditions

[Sample Preparation]
Various concentrations of explosive standards were pipetted on soil sample and mixed thoroughly. The soil samples were transferred into an extraction vessel and set to the SFE/SFC unit.

[Static extraction]		[Dynamic extraction]	
Extraction time	: 3 min	Extraction time	: 3 min
Mobile phase	: A: CO2; B: Methanol	Mobile phase	: A: CO2; B: Methanol
B conc.	: 10%	B Conc.	: 10%
Flow rate	: 5.0 mL/min	Flow rate	: 5.0 mL/min
Back pressure	: 20 MPa	Back pressure	: 20 MPa

[SFC]	
Column	: Shim-pack UCX-Naphthyl (250 mm L. x 4.6 mm I.D., 5 µm)
Mobile Phase	: A: CO2; B: Methanol
Time program	: 2-40% MeOH over 5 min after extraction
Flow Rate	: 2.5 mL/min
Column Temp.	: 40°C
Back pressure	: 20 Mpa
MS LCMS-8060	: ESI negative mode
Nebulizing Gas Flow	: 3 L/min
Heating Gas Flow	: 10 L/min
Interface Temp	: 300°C
DL Temp	: 250°C
Heat Block Temp:	: 400°C
Drying Gas	: 10 L/min

Samples

AccuStandard explosives standards M-8330-05 were used to prepare an explosives mixture. One gram of each soil sample was spiked with varying amounts of the explosives mixture.

Standards tested

HMX, RDX, Tetryl, TNT, PETN, 1,3,5-Trinitrobenzene,
2-amino-4,6-dinitrotoluene, 2,4-Dinitrotoluene, 4-Amino-2,6-dinitrotoluene

Soil Sample

Fluka Clean Sandy Loam
Lot: CF003 Exp: 12/2016

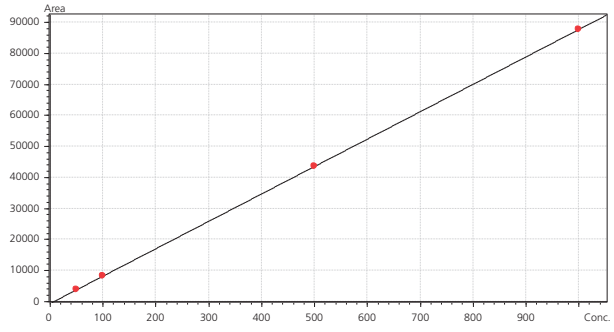
Automated Screening of Explosives in Soil Samples by Online SFE-SFC-MS

SFE-SFC-MS Compound list

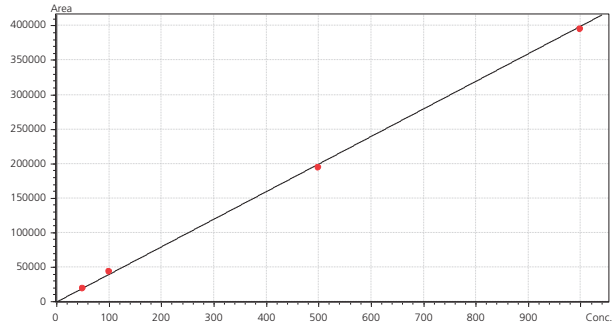
ID#	Name	Ret. Time	m/z
1	HMX	10.403	294.90>146.90
2	RDX	9.665	283.00>45.95
3	Tetryl	10.829	285.90>239.75
4	TNT	9.999	225.90>46.05
5	PETN	8.543	315.10>62.00
6	1,3,5-TNB	11.623	244.00>211.75
7	2-amino-4,6-DNT	9.456	196.00>45.95
8	2,4-DNT	9.428	181.15>46.00
9	4-amino-2,6-DNT	9.688	196.00>45.85

- ESI negative mode was used for this analysis.
- Additional compounds tested but not sufficiently ionized under the SFC conditions tested included: TATP, Nitrobenzene, Nitroglycerin, Nitrotoluene, and Dinitrobenzene

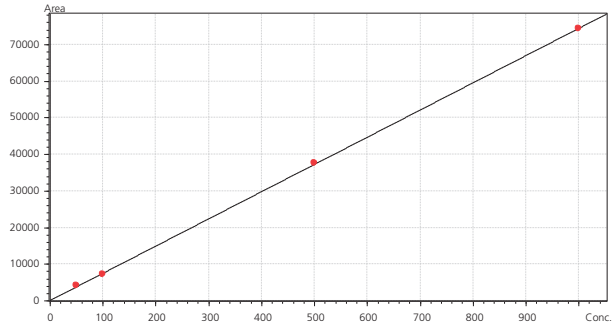
SFC/MS/MS Linearity Results



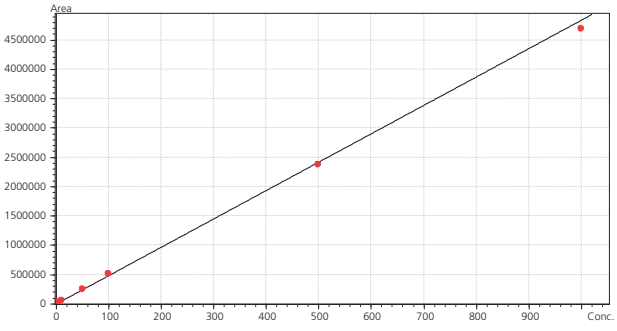
TNT: 50ppb –1ppm r^2: 0.9999



1,3,5-TNB 50ppb-1ppm r^2:0.9957

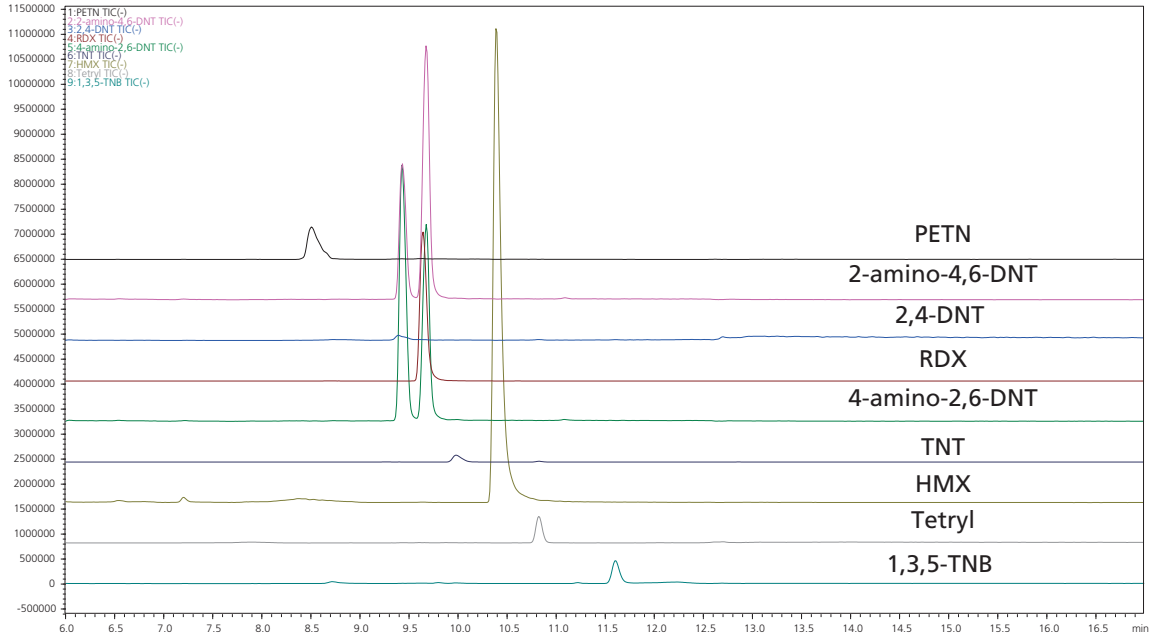


2,4-DNT: 50ppb-1ppm r^2: 0.9999



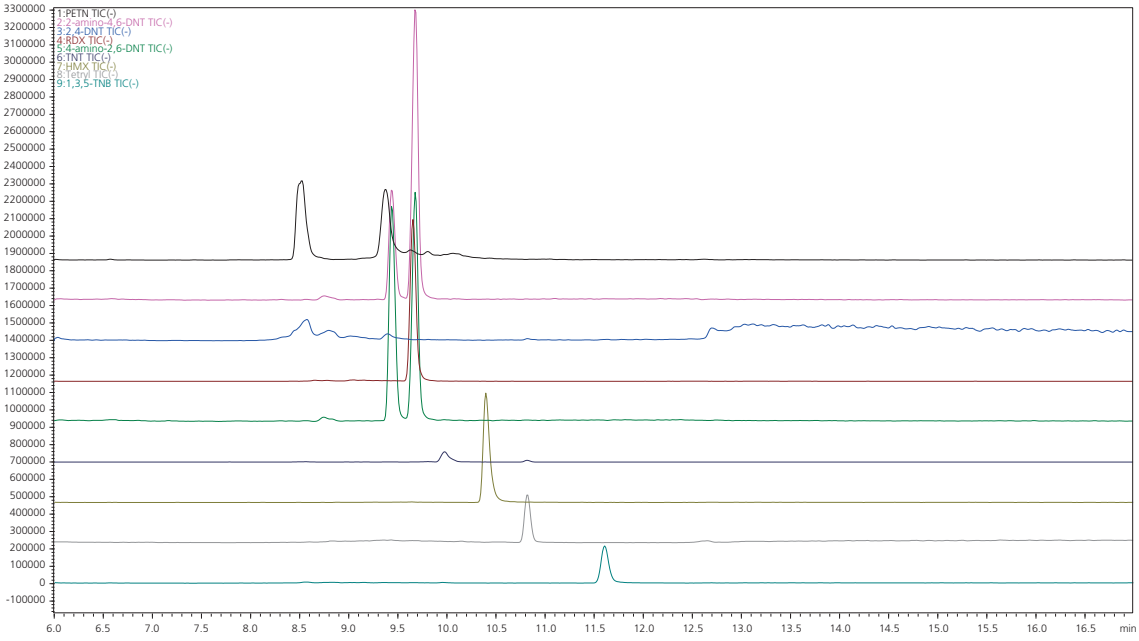
2-amino-4,6-DNT: 5ppb-1ppm r^2: 0.9984

SFE Standard Chromatogram



Standard chromatogram of explosives spiked into extraction vessel without soil

SFE Soil Chromatogram



Standard chromatogram of explosives spiked into extraction vessel with soil

SFE Recoveries from Soil

Recoveries of the explosives from soil was tested by comparing to high and low concentration standards that were added directly to an extraction vessel without any soil present. No heat was applied to the vessels to reduce the possibility of compound degradation.

SFE % recoveries of explosives in soil at different levels

	1 ppm	50 ppb
HMX	12.1	4
RDX	24.8	10.7
Tetryl	36.2	20
TNT	40.4	57.2
PETN	59.6	56.4
1,3,5-TNB	56	49.7
2a-4,6-DNT	28	17.5
2,4-DNT	35.9	32.1
4a-2,6-DNT	26.3	18.2

LC/MS/MS vs SFC/MS/MS

An additional study was made to compare ESI LC/MS/MS results with SFC/MS/MS results for the explosive compounds. Lower quantitation limits were able to be achieved with LC/MS/MS, however SFC/MS/MS allowed the analysis of several more compounds.

Quantitation limits for explosive compounds with LC/MS and SFC/MS

	LC/MS/MS	SFC/MS/MS
HMX	not tested	1 ppb
RDX	0.02 ppb	0.5 ppb
Tetryl	0.02 ppb	10 ppb
TNT	1 ppb	10 ppb
PETN	0.3 ppb	10 ppb
1,3,5-TNB	insufficient signal	50 ppb
2a-4,6-DNT	insufficient signal	5 ppb
2,4-DNT	insufficient signal	50 ppb
4a-2,6-DNT	insufficient signal	5 ppb

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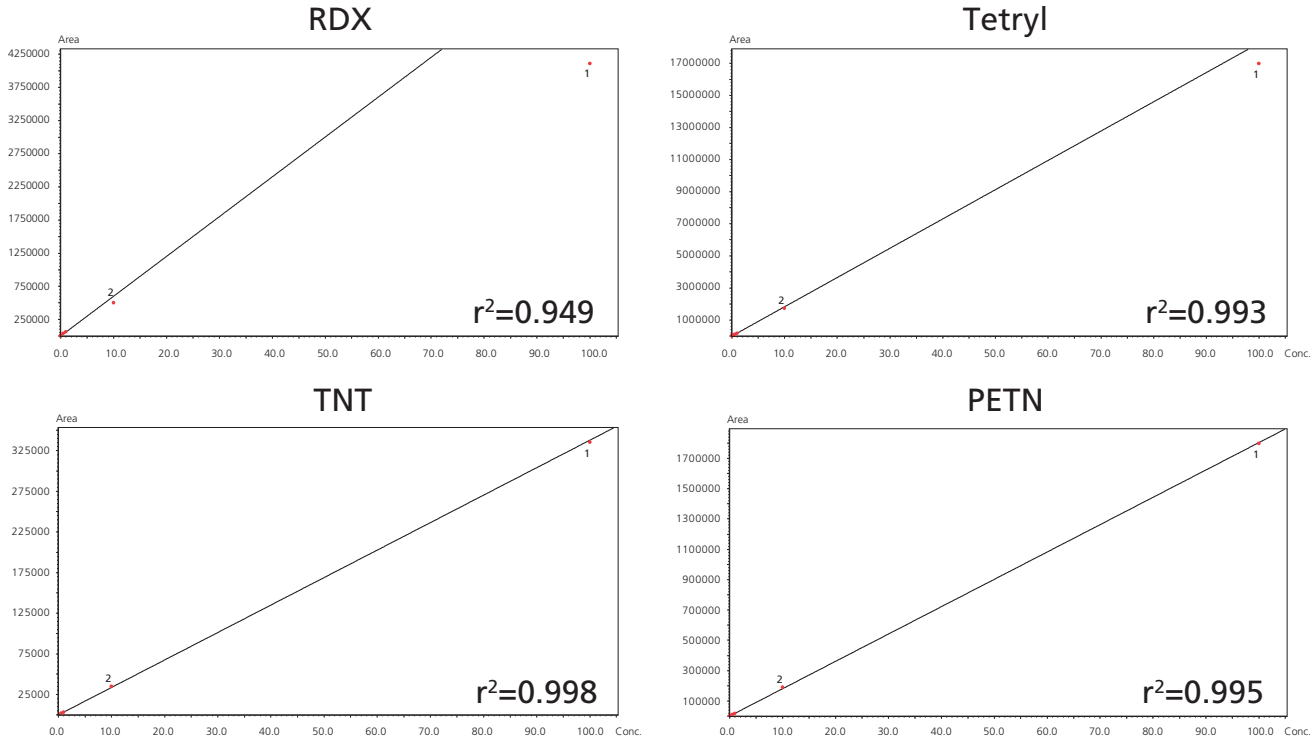
Automated Screening of Explosives in Soil Samples by Online SFE-SFC-MS

HPLC/MS/MS Linearity Results



Discussion

Soil samples showed little interference with explosive standard MS signals suggesting SFE could be a viable technique for high throughput screening of explosives in soil samples.
Lower detection limits for explosives could be achieved by LC/MS/MS, but SFC allowed more compounds to be screened under the conditions tested.
Recoveries from soil typically ranged from 10 to 50% for the extraction conditions tested. It is expected that recoveries could be improved with the addition of vessel heating and adjustment of modifier concentration and CO2 density.

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Technical Report

Supercritical Fluid Chromatography

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Abstract:

Advances in column technology have led to a renewed interest in supercritical fluid chromatography, which uses a supercritical fluid as its mobile phase. Compared to liquid, supercritical fluids have low viscosities and high diffusivities. In this report, starting from the basic principles of supercritical fluid chromatography, we introduce examples of high-speed, high-resolution analysis and chiral separation.

Keywords: supercritical fluid chromatography, SFC

1. Supercritical Fluid

A supercritical fluid is a state of substance wherein the temperature and pressure are both above its critical point (Fig. 1). Supercritical fluids can dissolve substances better than gases and are more diffusive and have lower viscosities than liquids (Table 1). Although various substances have particular critical points, the especially low critical point of carbon dioxide (critical temperature: 31.1°C, critical pressure: 7.38 MPa) makes it easy to handle. As it is non-flammable, inert, low-cost, and non-toxic, it has been widely used in industrial processes, such as for decaffeination of coffee beans and extraction of hops extract and flavor compounds (Fig. 2). Supercritical fluids are also used in analytical fields, including as the main mobile phase in supercritical fluid chromatography (SFC) and the main extracting solvent in supercritical fluid extraction (SFE).

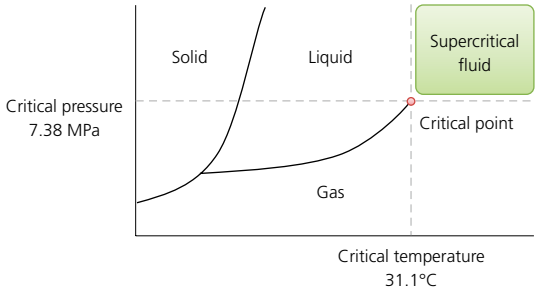


Fig. 1 Phase Diagram for Carbon Dioxide

Table 1 Properties of Supercritical Fluids

	Diffusion coefficient (cm^2/s)	Density (g/cm^3)	Viscosity ($g/cm \cdot s$)
Liquid	10^{-6}	1	10^{-2}
Supercritical fluid	10^{-3}	0.2 to 0.8	10^{-3}
Gas	10^{-1}	10^{-3}	10^{-4}

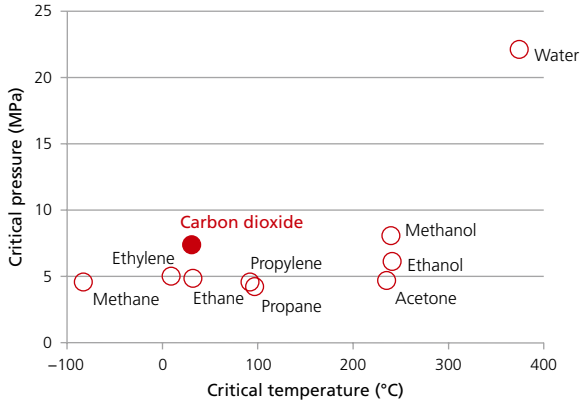


Fig. 2 Critical Points of Various Substances

2. Supercritical Fluid Chromatography

SFC is a separation technique that uses a supercritical fluid as its main mobile phase (often supercritical carbon dioxide). Because of the properties of supercritical fluids, which include low viscosities and high diffusivities, SFC can be performed at a lower column back pressure than conventional high-performance liquid chromatography (HPLC). Additionally, a high-speed analysis can be performed at high flow rates and a high-resolution analysis can be performed by using a longer column. Also, recent advances in SFC systems and in the packed columns made for SFC allow analyses to be performed with HPLC-like operation.

Although supercritical carbon dioxide has a similar hydrophobicity as hexane, this property alone is often insufficient for the elution of target compounds from a column. These target compounds can be eluted by adding an organic solvent, called a modifier, to modify the polarity of the mobile phase. Organic solvents that are compatible with carbon dioxide, such as methanol, ethanol, isopropyl alcohol, and acetonitrile, are used as modifiers. Organic solvents with an added acid (e.g., formic acid or acetic acid), salt (e.g., ammonium formate or ammonium acetate), or base (e.g., diethylamine) are also used as modifiers for the analysis of highly polar compounds.

1 Analytical & Measuring Instruments Division

3. Nexera UC

The Nexera UC platform can accommodate a wide variety of analyses and pretreatments and includes an (1) SFC system, (2) online SFE-SFC system, and (3) offline SFE system. A major difference between a Nexera UC system and a conventional HPLC system is the addition of a back pressure regulator to prevent mobile phase vaporization inside the column and the pump that delivers the carbon dioxide. The Nexera UC platform is based around the Nexera ultra high-performance liquid chromatograph, with each Nexera UC system configured by adding a newly developed carbon dioxide delivery unit (LC-30ADSF), a back pressure regulator unit that allows high-precision pressure control (SFC-30A), and an extraction unit used for SFE (SFE-30A) (Fig. 3). The autosamplers and other units designed for liquid chromatography can be used in the Nexera UC system.

(1) SFC system

SFC systems include an SFC-UV system that uses a UV (or PDA) detector, a UFMS system (SFC-MS) that uses a mass spectrometer (MS) that is suitable for high-speed analyses by SFC, and a chiral screening system that automatically switches between multiple columns and modifiers to examine the analytical conditions.

(2) Online SFE-SFC system

Online SFE-SFC systems combine SFE and SFC online to automatically perform all steps from target compounds extraction from solid samples to analysis.

(3) SFE pretreatment system

Offline SFE pretreatment systems are specifically designed to extract target compounds from solid samples.

The characteristic properties of the supercritical fluid used in the Nexera UC SFC systems, which include high diffusivity and low viscosity, allow for low column pressures even at high flow rates, enabling high-speed analyses while maintaining column efficiency. Because of these factors, the Nexera UC SFC systems can shorten analysis times to between one third and one fifth of the time required for HPLC analysis using the same size column. (Fig. 5).

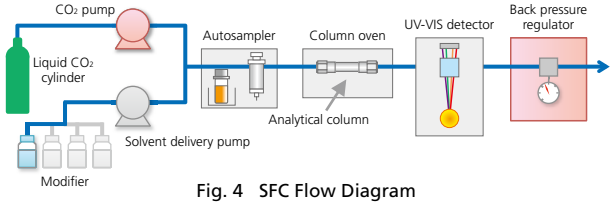
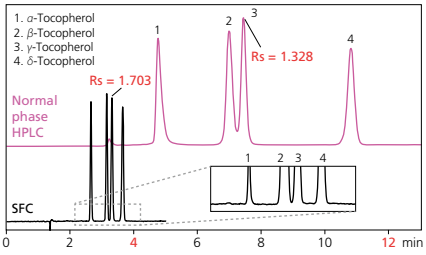


Fig. 4 SFC Flow Diagram



SFC conditions

Column	:	Shim-pack UC-SIL (4.6 mm I.D. × 250 mm L. 5 µm)
Modifier	:	MeOH
Modifier conc.	:	5 %
Flow rate	:	3.5 mL/min
Temperature	:	40°C
Detection	:	UV 290 nm
Back pressure	:	10 MPa

Normal phase HPLC conditions

Column	:	Shim-pack HRC-SIL (4.6 mm I.D. × 250 mm L. 5 µm)
Mobile phase	:	HEX/IPA 99/1 (V/V)
Flow rate	:	1 mL/min
Temperature	:	40°C
Detection	:	UV 290 nm

Fig. 5 Comparison between HPLC and SFC

SFC-UV System

SFC-MS System

Fig. 3 Nexera UC Systems

By using the same column packing material for separation in the Nexera UC systems as that used in normal phase HPLC analysis (e.g., silica gel), normal phase HPLC analyses can be easily transferred to SFC analyses while improving the resolution and increasing the analysis speed, as shown in Fig. 5. Transferring analyses from normal phase HPLC to SFC can also substantially reduce the volume of organic solvents consumed per analysis, as shown in Fig. 6, which also reduces analysis costs. SFC is an environment- and user-friendly technique as it reduces consumption of toxic organic solvents.

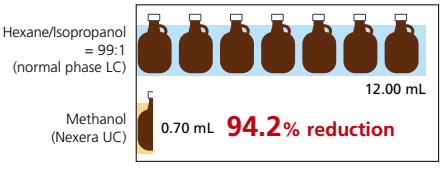


Fig. 6 Comparison between HPLC and SFC of Organic Solvent Consumption

When using a mass spectrometer for SFC, equipment used for LC/MS can be used as is. In SFC, a make-up solution is added after column separation to promote ionization. Conventional SFC systems used pressure regulators that had a large internal volume. This required the flow path of the column eluate to be split before entering the mass spectrometer to suppress the effect of extra-column dispersion (Fig. 7(a)). The Nexera UC systems use a proprietary low-internal volume design for their back pressure regulator (patent pending). This allows the flow path to enter the back pressure regulator and mass spectrometer in series, so all the column eluate enters the mass spectrometer (Fig. 7(b)). Increasing the volume of eluate introduced to the mass spectrometer in this way enables higher sensitivity analysis, and precludes the effects of split ratio variation, etc., resulting in highly reproducible SFC/MS analysis (Fig. 8, Table 2).

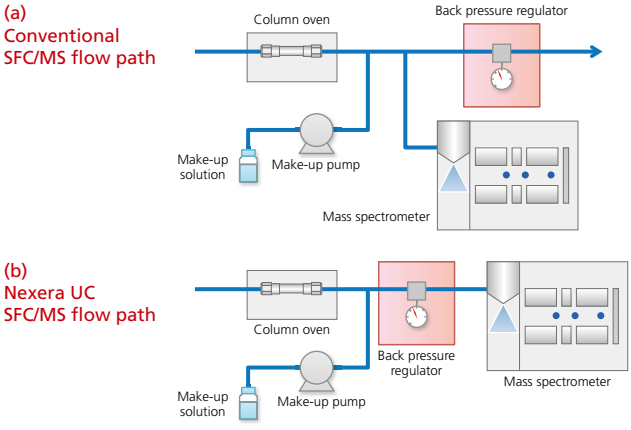
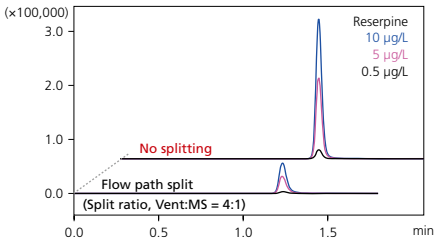


Fig. 7 SFC-MS Flow Path



Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L.
Modifier : MeOH
Modifier conc. : 30 %
Flow rate : 3.0 mL/min
Temperature : 40°C
Back pressure : 10 MPa
Injection volume : 1 µL
Sample : Reserpine (0.5, 5, 10 µg/L)
Detection : TQ mass spectrometer ESI(+) *m/z* 609.3 > 195.0

Fig. 8 Sensitivity With and Without Flow Path Splitting

Table 2 MS Reproducibility With and Without Flow Path Splitting

	Injection volume (µL)	Retention time		Area		Height	
		Ave.	%RSD	Ave.	%RSD	Ave.	%RSD
Flow path split	0.1	0.359	0.64	6,583	18.83	2,361	17.29
	1	0.356	0.25	81,467	4.26	26,656	3.88
	2	0.355	0.32	156,831	2.18	49,721	3.28
No splitting	0.1	0.356	0.09	16,264	6.18	7,673	6.17
	1	0.353	0.05	155,170	2.43	71,971	2.23
	2	0.35	0.07	325,739	1.16	142,350	1.19

Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 30 %
Flow rate : 2.0 mL/min
Temperature : 40°C
Back pressure : 10 MPa
Injection volume : 1 µL
Detection : TQ mass spectrometer ESI(–) *m/z* 351.20 > 271.20 (prostaglandin 100 µg/L)

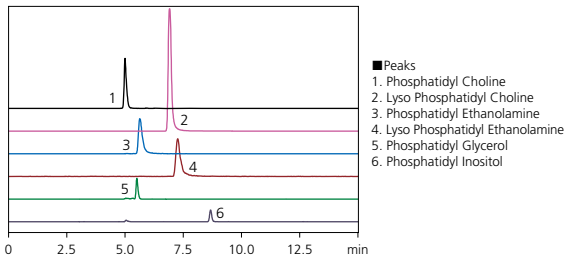
4. Shim-pack UCX Series Columns for SFC

Because of the high diffusivity of the mobile phase used in SFC, the separation behavior substantially changes based on the column stationary phase and modifiers used. The Shim-pack UCX series columns are designed for SFC and encompass eight different stationary phases, as shown in Table 3. This allows the columns to accommodate the separation of a wide variety of compounds.

Table 3 Shim-pack UCX Series Columns

	Functional group	Pore size	Surface area	Carbon content
Shim-pack UC-RP	Octadecyl group + polar functional group	10 nm	—	9%
Shim-pack UC-GIS II	Octadecyl group		450 m^2/g	11%
Shim-pack UC-Diol	Diol group			20%
Shim-pack UC-Sil	—			—
Shim-pack UC-Amide	Carbamoyl group			18%
Shim-pack UC-NH2	Aminopropyl group			8%
Shim-pack UC-Phenyl	Phenethyl group			9.5%
Shim-pack UC-CN	Cyanopropyl group			14%

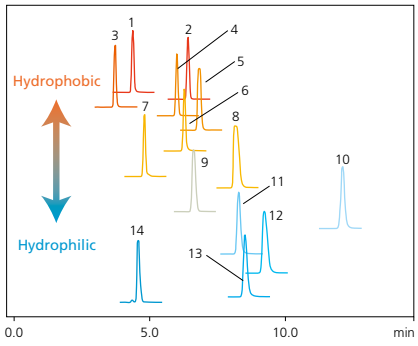
Fig. 9 shows an example analysis of phospholipids using the Shim-pack UCX-Diol column. This column allows separation of phospholipids by class, as with normal phase LC. Phospholipids can also be separated by molecular species using the same modifier conditions paired with a different column, such as the Shim-pack UCX-GIS II, which has an octadecyl group stationary phase. Using different stationary phases but the same mobile phase, SFC can be used to recreate the retention behaviors observed with normal phase and reverse phase HPLC, providing a variety of other separation behaviors. This is of substantial benefit for the analysis of complex samples.



Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 10 % (0 min) → 40 % (10–15 min)
Flow rate : 3.0 mL/min
Temperature : 40°C
Make-up solution : MeOH with 0.1 % w/v ammonium formate
Make-up flow rate : 0.1 mL/min
Back pressure : 10 MPa
Detection : TQ mass spectrometer (ESI)

Fig. 9 Phospholipid Analysis

Fig. 10 shows an example analysis of pesticides of a wide range of polarities—from hydrophobic to hydrophilic—using the Shim-pack UCX-RP column. The Shim-pack UCX-RP column is unique in having a stationary phase that combines octadecyl and polar functional groups. This stationary phase is able to retain a wide range of compounds, including both hydrophobic and hydrophilic compounds. This column allows the simultaneous analysis of pesticides that were previously difficult to analyze without changing the analytical conditions, thereby providing improved analytical efficiency.



No.	Compound	log *P*
1	Carbofuran	7.4
2	Ethofenprox	6.9
3	Fenpropathrin	6.0
4	Pyriproxyfen	5.0
5	Pyraclostrobin	4.0
6	Linuron	3.0
7	Aminocarb	1.9
8	Ethoxysulfuron	1.0
9	Halosulfuron methyl	0.0
10	Bentazone	–0.5
11	Chlorsulfuron	–1.0
12	Rimsulfuron	–1.5
13	Nicosulfuron	–1.8
14	Vamidothion	–4.2

Column : Shim-pack UC-RP 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 0 % (0 min) → 10 % (11 min) → 30 % (14 min) → 40 % (14.01–17 min)
Flow rate : 3.0 mL/min
Temperature : 40°C
Make-up solution : MeOH with 0.1 % w/v ammonium formate
Make-up flow rate : 0.1 mL/min
Back pressure : 15 MPa
Detection : TQ mass spectrometer (ESI)

Fig. 10 Pesticide Analysis

5. Chiral Separation

In the field of pharmaceuticals, research is underway in the area of drug discovery using chiral columns for rapid chiral separation. Finding the appropriate combination of analytical column and mobile phase for a given analyte from the wide variety of chiral columns available requires a substantial amount of time and labor. Therefore, there is a demand to improve the speed of condition scouting for chiral separations.

The speed and labor required for scouting chiral compound separation conditions can be improved by combining Shimadzu's Nexera UC chiral screening system and the wide range of polysaccharide derivative CHIRALPAK and CHIRALCEL series chiral columns (Daicel Corporation).

The Nexera UC chiral screening system includes an SFC system, solvent switching valves, and column switching valves and is able to acquire comprehensive data by automatic and continuous screening of the modifier conditions on a maximum of 12 columns. Its mobile phase blending function can also mix up to four different solvents to user-defined ratios for analysis under a variety of separation conditions, which significantly simplifies the workflow of condition scouting for chiral compounds.

Also, Method Scouting Solution for Nexera UC is software that presents a graphical user interface environment developed to support the process of separation condition scouting for chiral compounds (Fig. 11). This software provides database management for analytical columns, mobile phases, and modifiers, which improves management efficiency and can reduce the number of operating errors that arise with multiple operators. The software provides powerful support for work related to chiral compound analysis, including work such as the calculation of required modifier and sample volumes, column washing, changeover of enclosed liquids at the end of analysis to prevent column degradation, and estimation of analysis completion times.

Here, we present chiral separation screening results for omeprazole obtained from all 36 possible combinations of the 12 chiral columns (Daicel Corporation) and three modifier conditions (Fig. 12, 13).

The Nexera UC chiral screening system utilizes SFC to select the mobile phase and optimize the separation conditions in a short time period, which improves R&D efficiency during the drug discovery stage of pharmaceutical production.

Fig. 11 Configuration Window of Method Scouting Solution Software

Column
(1) CHIRALPAK IA-3/SFC
(2) CHIRALPAK IB-3/SFC
(3) CHIRALPAK IC-3/SFC
(4) CHIRALPAK ID-3/SFC
(5) CHIRALPAK IE-3/SFC
(6) CHIRALPAK IF-3/SFC
(7) CHIRALPAK AD-3/SFC
(8) CHIRALPAK AS-3/SFC
(9) CHIRALPAK AY-3/SFC
(10) CHIRALCEL OD-3/SFC
(11) CHIRALCEL OJ-3/SFC
(12) CHIRALCEL OZ-3/SFC

3.0 mm I.D. × 100 mm L. 3 µm

×

Modifier
(1) MeOH
(2) EtOH
(3) Acetonitrile/EtOH 75/25 (V/V)

Modifier conc	:	20 %
Flow rate	:	3.0 mL/min
Temperature	:	40°C
Back pressure	:	10 MPa
Injection volume	:	1 µL
Detection	:	UV (300 nm)

Fig. 12 Screening Conditions

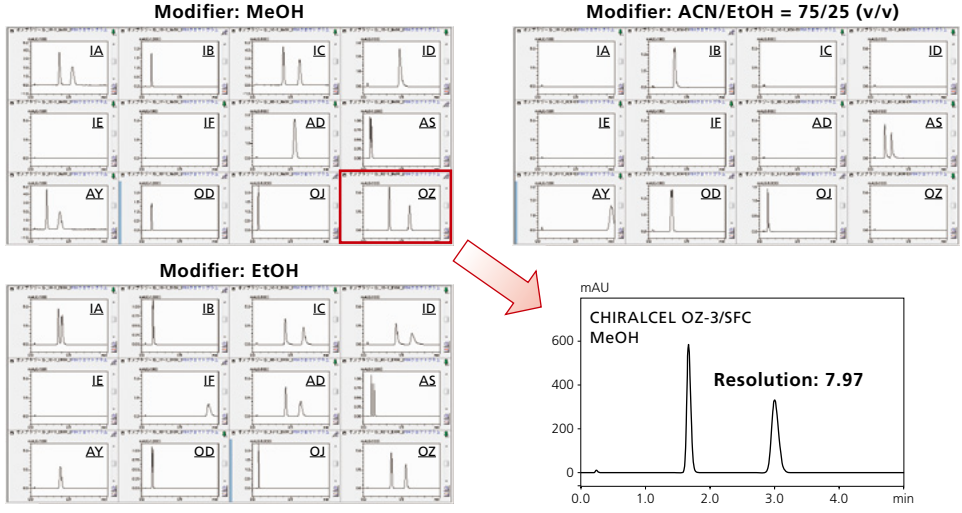


Fig. 13 Screening Results

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Technical Report

Improved Sample Pretreatment Using Offline Supercritical Fluid Extraction

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Abstract:

Separation analysis using HPLC, SFC or GC requires a pretreatment step to efficiently extract a target constituent from the sample in various forms (e.g., solid). Constituents are usually extracted from solid samples using dissolution or solid-liquid extraction methods. Dissolution methods can only be used when the sample is soluble in a given solvent, and they are difficult to optimize depending on the analytical conditions. Solid-liquid extraction methods (e.g., Soxhlet extraction) are not suited to the pretreatment of multiple samples as they require considerable time for extraction and non-extraction (e.g., cleaning, preparation) operations. However, extraction methods involving supercritical fluids can utilize the characteristics of supercritical fluids (e.g., high solubility, permeability) to achieve the elution of a target constituent from a solid sample with high efficiency and also allow automation during the extraction process. This article describes the utilization of the Nexera UC SFE pretreatment system, which increases the efficiency of sample pretreatment for analysis.

Keywords: supercritical fluid extraction, SFE, offline SFE

1. What Is Supercritical Fluid Extraction?

Supercritical fluid refers to the state of any substance at temperature and pressure conditions above its critical point. Supercritical fluids combine the ability of liquids to dissolve materials with high diffusivity and low viscosity properties of gases. Supercritical fluid extraction (SFE) with carbon dioxide is widely employed as a pretreatment method for analysis owing to its low critical points (critical temperature: 31.1 °C, critical pressure: 7.38 MPa), which makes it easy to handle, along with its incombustibility, inertness, and low cost. Some advantages of SFE are shown below:

- Supercritical fluids have high permeability and diffusion coefficients and can therefore be used for highly efficient extraction.
- Supercritical fluids achieve extraction at mild temperatures at which target constituents are unlikely to oxidize.
- Carbon dioxide evaporates, which simplifies sample treatment after extraction.
- Solvent costs are low compared with solvent extraction and other methods.
- The extraction process can be automated.

While supercritical carbon dioxide is as hydrophobic as hexane and is suited for the extraction of fat-soluble compounds, it can also be used for the extraction of compounds with a wide range of polarities by adding modifier substances such as methanol and ethanol.

Fig. 1 The Nexera UC SFE Pretreatment System

2. The Nexera UC SFE Pretreatment System

The Nexera UC SFE pretreatment system (Fig. 1) is a dedicated pretreatment system that performs offline SFE. Solid samples are placed in dedicated extraction vessels (Fig. 2) and introduced into the system, after which the system automatically performs the extraction. The extraction vessels are available in volumes of 0.2 mL and 5 mL such that they can be selected based on the sample to be analyzed. The system allows accommodation of a maximum of 48 extraction vessels, which are mounted into a rack changer (Fig. 3). This setup enables extraction pretreatment of multiple samples via automated sample transfer and cycling. The automation of the multi-sample extraction pretreatment using the Nexera UC SFE pretreatment system substantially reduces the time and labor required for the pretreatment operations while also preventing human error.

Fig. 2 Extraction Vessels

Fig. 3 Rack Changer

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The extraction conditions can be configured such that the pretreatment operations can be run from the same LabSolutions workstation used for the analysis. Thus, the extraction conditions and pretreatment can be intuitively controlled in the same way as sample analysis.

The material extracted by the supercritical fluid is collected in a trap column, subsequently eluted by an organic solvent, and finally recovered using a fraction collector before performing the analysis by LC (LC/MS), GC (GC/MS), or NMR. Comprehensive and complementary sample analysis can be achieved by combining the results from several of these analysis methods.

Compared with Soxhlet extraction, SFE uses a much lower quantity of organic solvent during the pretreatment, thereby reducing costs and allowing a more environmentally friendly pretreatment step.

3. Operating Principles of the Nexera UC SFE Pretreatment System

The schematic diagrams of the Nexera UC SFE pretreatment system showing material flow and principle of operation are shown in Fig. 4. The extraction process can be roughly divided into four operations:

(1) Extraction vessel delivery and temperature control

An extraction vessel is moved from the rack changer to the SFE unit, and the extraction vessel is subsequently heated to the set temperature (40–80 °C).

(2) Static extraction

Once the extraction vessel has reached the set temperature, the supercritical fluid is introduced into the vessel and static extraction occurs. Parameters such as the extraction vessel temperature, pressure and duration of the extraction, and type and quantity of the modifier can be independently controlled during extraction depending on the sample and target constituent to be extracted.

(3) Dynamic extraction

After the static extraction, a dynamic extraction is performed by delivering the fluid through the extraction vessel. This operation allows the extraction of the target material from the extraction vessel and subsequent collection in a trap column located downstream of a back pressure regulator. Downstream of the back pressure regulator is held at close to atmospheric pressure such that carbon dioxide is in a gaseous state while collecting the extraction material in the trap column. ODS and other columns used for HPLC can be used as trap columns. Similar to static extraction, the extraction vessel temperature, pressure and duration of the extraction, and type and quantity of the modifier can be adjusted depending on the sample and target constituent to be extracted.

(4) Elution from the trap column and recovery of the extraction material

Once the dynamic extraction has finished, the fluid delivery is stopped and the back pressure regulator is opened, thereby allowing the system pressure to drop to atmospheric pressure. The delivery pump is then switched from the modifier to the eluent, which is passed through the trap column to elute the extraction material. The eluate is then recovered into collection tubes using a fraction collector. An organic solvent is used as the eluent to simplify concentration and post-treatment steps of the eluate.

(1) Extraction vessel delivery and temperature control

A specified extraction vessel is transferred to the SFE unit and heated to the set temperature.

(2) Static extraction

When the temperature of the extraction vessel has reached the set temperature, the supercritical fluid is introduced and static extraction (i.e., in the absence of fluid flow) is allowed.

(3) Dynamic extraction

The extraction is dynamically performed by passing the supercritical fluid through the extraction vessel. The extraction material is taken from the extraction vessel and collected at atmospheric pressure after evaporation of CO_2 in the trap column downstream the back pressure regulator.

(4) Elution from the trap column and recovery of the extraction material

The delivery pump is used to deliver the eluent through the trap column, thereby eluting the extraction material, which is then recovered using a fraction collector.

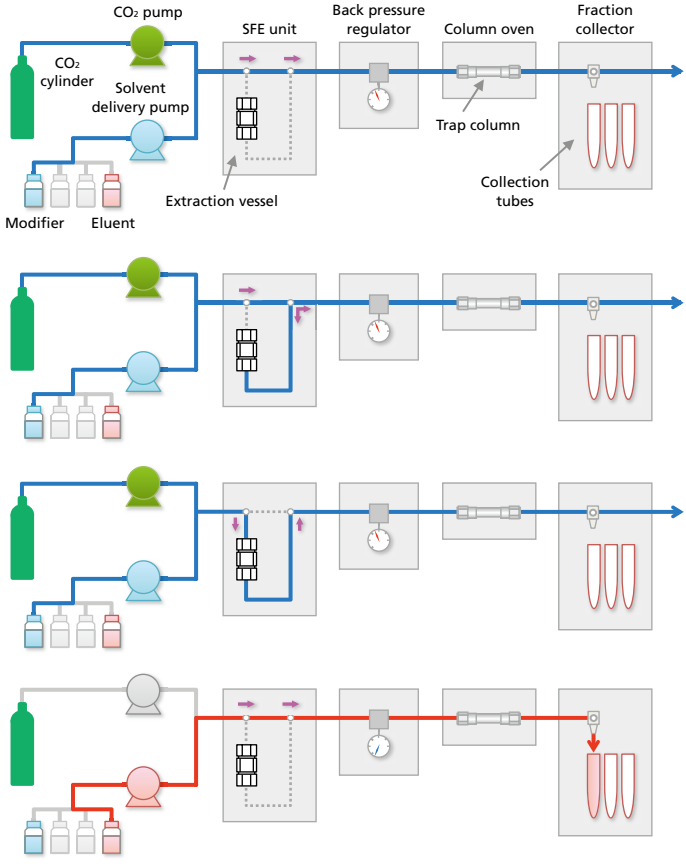

Fig. 4 Material Flow and Principle of Operation

Samples showing high water content complicate the extraction process (i.e., reduce both the extraction efficiency and the repeatability of the pretreatment) as supercritical carbon dioxide does not mix with water. In these cases, the extraction efficiency can be increased by mixing the sample with a dehydrating agent before enclosing it into the extraction vessel. Extraction efficiency can also be low when supercritical carbon dioxide is used for the extraction of highly polar constituents. In this case, the extraction efficiency can be increased by adding modifiers such as methanol during the extraction. In the case of samples with constituents showing ionic polar groups, acid (e.g., formic acid, acetic acid), salt (e.g., ammonium formate, ammonium acetate), and bases (e.g., ammonia, diethylamine) can be added during the extraction. Fine pulverization of the sample normally increases extraction efficiency. For polymer samples, a fine freeze-crushing treatment before the extraction often results in increased extraction efficiencies.

4. Using the Nexera UC SFE Pretreatment System for Extracting Fat-Soluble Vitamins

Vitamin E is a group of fat-soluble compounds widely used as antioxidants and for nutritional support in foods and medicinal products. We present an example of offline SFE using the Nexera UC SFE pretreatment system to extract d-*α*-tocopherol, a vitamin E compound, from a nutritional supplement. The sample used was a commercially available soft capsule supplement containing d-*α*-tocopherol. The soft capsule supplement contained a paste, which was mixed with a dehydrating agent before being enclosed into an extraction vessel. The extraction was exclusively performed with supercritical carbon dioxide, and hexane was used as the eluent after trapping. The detailed extraction conditions are shown in Table 1. The extraction liquid recovered by the fraction collector was diluted to 10 mL with hexane in a measuring flask. The sample extraction vessel contained 7.4 mg of d-*α*-tocopherol, and the theoretical concentration of d-*α*-tocopherol in the final SFE extraction liquid was 0.74 mg/mL.

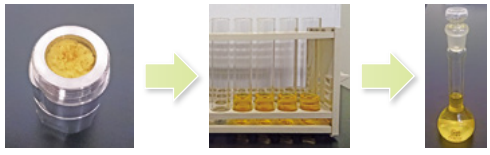

Fig. 5 Sample Before and After the SFE Process

Table 1 SFE Conditions

SFE	
Extraction vessel	: 5 mL
Extraction solvent	: CO_2
Flow rate	: 5 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Extraction time	: 15 min (Static extraction → Dynamic extraction)
Trap & Pressure down conditions	
Trap column	: Shim-pack VP-ODS 4.6 mmI.D. × 50 mmL. 5 µm
Temperature	: 60°C
Pressure down time	: 10 min (15–25 min)
Recovery conditions	
Elution solvent	: Hexane
Flow rate	: 2 mL/min
Fraction time	: 3.5 min (25–28.5 min)

Six extraction vessels were prepared, each containing the same amount of supplement sample. Each extraction vessel was subjected to offline SFE after which the recovered liquid was analyzed by SFC at the conditions shown in Table 2. The recovery and repeatability of the process was confirmed.

The six chromatograms obtained are shown overlapping each other in Fig. 6.

Table 2 Conditions Used for the Analysis of the Pretreated Samples (SFC)

Column	: Nacalai COSMOSIL Cholester 4.6 mmI.D. × 250 mmL. 3 µm
Modifier	: IPA
Gradient	: 2% (0 min) → 20% (10 min) → 50% (10–12 min)
Flow rate	: 3 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Injection volume	: 2 µL
Detector	: UV-VIS (@293 nm)

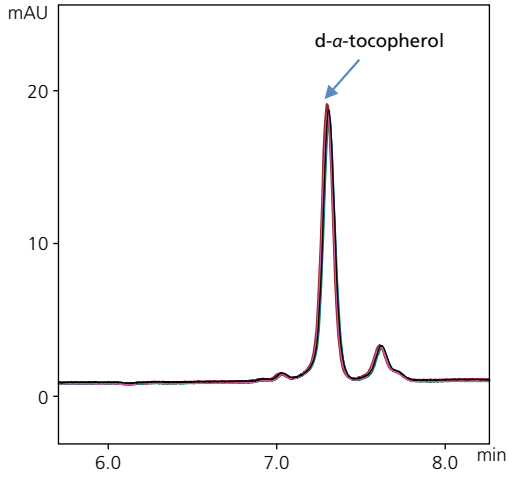

Fig. 6 Results for SFE Extraction Liquid Analyses (The Six Samples Are Shown Overlapping Each Other).

The concentration and recovery of d-*α*-tocopherol in the six SFE extraction liquid chromatograms (Fig. 6) are summarized in Table 3. The extraction pretreatment showed both high repeatability and high recovery, thereby revealing that the Nexera UC SFE pretreatment system can be used for the automated consecutive pretreatment of target constituents in a solid sample with good efficiency.

Table 3 Repeatability and Recovery of Vitamin E Extraction

No.	Conc. (mg/mL)	Recovery (%)
1	0.776	104.46
2	0.780	105.00
3	0.772	103.92
4	0.790	106.35
5	0.761	102.44
6	0.758	102.04
Average	0.773	
RSD (%)	1.549	

5. Using the Nexera UC SFE Pretreatment System for the Extraction of Residual Pesticides from Agriproducts

Over 800 pesticides are subjected to analysis for their residual presence in food products. Analytical methods that enable rapid and simple testing of a large number of pesticides involving any pretreatment operations required for analysis are needed. Conventional analysis for residual pesticides in food normally involves a solvent extraction method to extract the pesticides, followed by LC/MS or GC/MS analyses. The pretreatment operations employed in these analytical methods are highly labor and time consuming, and they use a large volume of organic solvent. We present an example of utilizing the Nexera UC SFE pretreatment system to extract residual pesticides before analyzing them using a GC/MS/MS system. 1 g of dehydrating agent was added to 1 g of pulverized brown rice*. This mixture was then enclosed into an extraction vessel, and an extraction pretreatment was performed using the conditions shown in Table 4. The extraction liquid recovered by the fraction collector was diluted to 2 mL with an acetone/hexane (1/1, V/V) mixture in a measuring flask and then analyzed using GC/MS/MS under the conditions shown in Table 5. The components included in pesticide standard mixture solutions for GC/MS (PL2005 Pesticide GC/MS Mix I to VI and Mix 7, Hayashi Pure Chemical Ind., Ltd.) were analyzed.

* "Miyazaki Hydro-Protect" Patent No. 3645552

Table 4 SFE Conditions

Offline SFE	
Extraction vessel	: 5 mL
Extraction solvent	: CO_2 + Methanol
Flow rate	: 5 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Extraction time	: 8 min (Static extraction → Dynamic extraction)
Trap & Pressure down conditions	
Trap column	: Shim-pack VP-ODS 4.6 mmI.D. × 50 mmL. 5 µm
Temperature	: 60°C
Pressure down time	: 4 min (8–12 min)
Recovery conditions	
Elution solvent	: Acetone/Hexane = 1/1 (V/V)
Flow rate	: 2 mL/min
Fraction time	: 2 min (12–14 min)

The pesticide standard solutions were added to an brown rice sample (pesticide concentrations of 100 ng/g). An MRM chromatogram of the extraction liquid obtained from this sample is shown in Fig. 7. The theoretical concentration of each pesticide in the extraction liquid used for GC/MS/MS analysis was 50 ng/mL.

The extraction was performed on six samples to which the abovementioned pesticide standard solutions were added; each pesticide was quantified using a matrix calibration curve created using the SFE extraction liquid obtained from a blank brown rice sample, after which repeatability and recovery were confirmed. Good repeatability (relative standard deviation of quantified concentration: <10 %) and good recovery (70 %–120 %) were obtained for the 301 pesticides studied. An excerpt from these results showing the repeatability and recovery for some representative pesticides is shown in Table 6. The Nexera UC SFE pretreatment system can be used for automated consecutive pretreatment of up to 48 samples while consuming low amounts of solvent.

Table 5 Conditions Used for the Analysis of the Pretreated Samples (GC/MS/MS)

Column	: Rxi-5Sil MS 30 m × 0.25 mmI.D., df = 0.25 µm
Column temp.	: 50°C (1 min) → (25°C/min) → 125°C → (10°C/min) → 300°C (15 min)
Carrier gas	: He (Constant linear velocity mode)
Linear velocity	: 47.2 cm/sec
Injection mode	: Splitless (Sampling time 1.00 min)
High press inj.	: 250 kPa (1.5 min)
Injection volume	: 1 µL
Interface temp.	: 250°C
Ion source temp.	: 200°C
MS mode	: MRM
Loop time	: 0.3 sec

Table 6 Repeatability and Recovery of Representative Pesticide Extraction

Compounds	Repeatability (%RSD, n=6)	Recovery (%)
Cyhalofop-butyl	4.2	93
Etofenprox	3.8	90
Iprodione	2.5	93
Malathion	3.2	93
Piperonyl butoxide	3.8	89

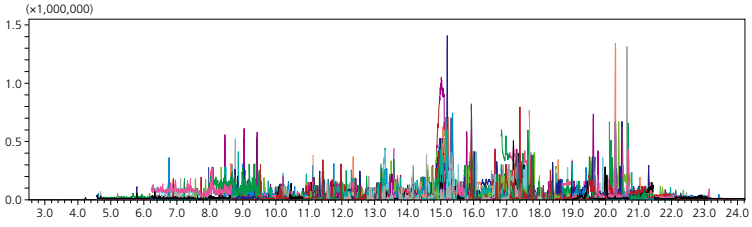

Fig. 7 MRM Chromatogram of the Brown Rice Extraction Liquid

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Technical Report

Online Supercritical Fluid Extraction-Supercritical Fluid Chromatography (Online SFE-SFC)

Takato Uchikata[1], Hidetoshi Terada[1], Keiko Matsumoto[1], Takanari Hattori[1], Yoshiyuki Watabe[1], Tadayuki Yamaguchi[1], Yasuhiro Funada[1]

Abstract:

Online supercritical fluid extraction-supercritical fluid chromatography (online SFE-SFC) system is that directly connects the supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). By using the online SFE-SFC, pretreatment to analysis can be automated, creating advantages such as simplification of pretreatment, analysis of unstable compounds, and high-sensitivity analysis. Here, we introduce the basic principle of online SFE-SFC, the characteristics of the "Nexera UC Online SFE-SFC System," and examples of extraction analyses.

Keywords: supercritical fluid chromatography, SFC, supercritical fluid extraction, SFE, online SFE-SFC

1. What is Supercritical Fluid?

Supercritical fluid is a material for which the temperature and pressure are at or over the critical point, has high diffusivity and low viscosity like gas, and solubility like liquid. Carbon dioxide has a critical temperature of 31.1°C and a critical pressure of 7.38 MPa. Since its critical point is low, its handling is easy, and as it is non-flammable, inert, and low cost, it is widely used in industrial settings like in the decaffeination of coffee. In analytical fields, supercritical fluid extraction (SFE) that uses supercritical carbon dioxide as the extraction medium and supercritical fluid chromatography (SFC) that mainly uses supercritical carbon dioxide as the mobile phase are currently being used.

SFE can extract at high efficiency using the high permeability and diffusivity of the supercritical fluid. Since the critical temperature of carbon dioxide is low, allowing for extraction under mild conditions, the target component can be extracted in a condition in which it is unlikely to be decomposed.

SFC uses supercritical fluid characterized by high diffusivity and low viscosity as the mobile phase. Hence, compared to the traditional HPLC analysis, the pressure on the column decreases, and even under faster flow rate conditions, separation functioning does not decrease in turn. Therefore, high-speed analysis becomes possible. In addition, the polarity of supercritical carbon dioxide is generally said to be near the level seen with hexane, but by mixing with a polar solvent such as methanol, it can respond to a wide range of polarities. Therefore, it can be applied to the analysis of a wide range of compounds, including hydrophobic and hydrophilic ones.

2. What is Online SFE-SFC?

Online SFE-SFC is an extraction separation technique that introduces the component extracted via SFE directly into the column and separates with SFC. It can be actualized in the Nexera UC Online SFE-SFC-MS System shown in Fig. 1.

Online SFE-SFC only requires the user to fill the sample in the extraction vessel, and automatic operation from extraction to analysis is possible. Since analysis can be performed without the extract being exposed to light or air, it is useful for compounds that are unstable with light or that tend to oxidize. Since water is not used as the extraction medium, it is can also be employed for compounds that are susceptible to hydrolysis. Since all the extracted compounds are introduced to the detector, the load of the target component can be reduced. As it allows for highly sensitive analysis, it is useful for components for which sensitivity is insufficient and a higher concentration is required, or for a small amount of sample.

The Nexera UC system can load a maximum of 48 samples using the rackchanger, allowing for the continuous processing of multiple samples. There are two types of extraction vessels: a 0.2 mL vessel for a small amount of sample, such as dried blood spot (DBS), and a 5 mL vessel for a large amount of sample, such as agricultural products. It can handle a wide range of samples.

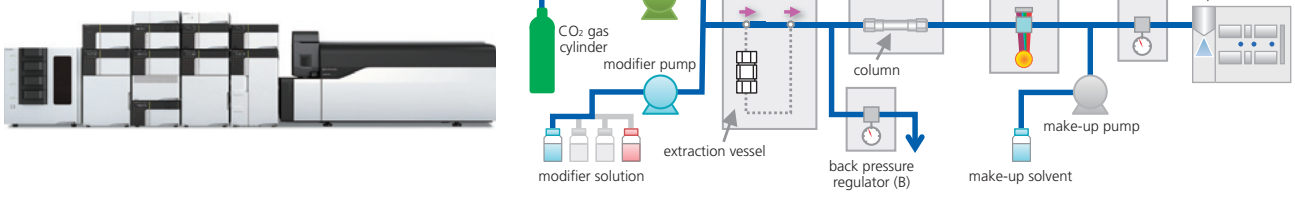

Fig. 1 Structure and Flow Path of Nexera UC Online SFE-SFC-MS System

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3. Operating Principle of Online SFE-SFC

The operating principle of the Nexera UC Online SFE-SFC extraction separation analysis is shown in Fig. 2. These three processes follow:

(1) Extraction

(1)-1 Transportation and Temperature Control of the Extraction Vessel

Designated extraction vessel is transported to the SFE unit and is temperature controlled until it reaches the set temperature. The extraction temperature can be set between 40 and 80°C.

(1)-2 Static Extraction

When the temperature of the extraction vessel reaches the designated temperature, the supercritical fluid is introduced into the extraction vessel. After the introduction, the fluid is not passed into the extraction vessel, and extraction is performed in a static state.

(1)-3 Dynamic Extraction

After the static extraction, extraction is performed while passing the supercritical fluid through the extraction vessel. The extract is removed from the vessel and introduced to the analytical column*.

* With the Nexera UC Online SFE-SFC System, when sample concentration is extremely high or when a sample with a high amount of matrix is extracted, part of the extract can be introduced into the column performing a split.
The ratio of the split can be adjusted by changing the pressure using two back pressure control valves.

(2) Separation

After completing the extraction, the extraction vessel is separated from the flow path, and by increasing the concentration of the modifier in the mobile phase, the extract is separated.
Regardless of the type of column and modifier, as the concentration of the modifier increases, the elution strength increases.
The modifier solvent can be changed so that it switches between separation and extraction.

(3) Detection

The eluted compound is detected by a photodiode array detector or mass spectrometer. When using a mass spectrometer and the main mobile phase is carbon dioxide, the ions to be used for ionization are insufficient and sensitivity is decreased; therefore, an organic solvent (make-up solvent) to support ionization is added with a make-up pump.
For the make-up solvent, a different solvent from the modifier can be used; therefore, one can select a solvent that allows for a highly sensitive analysis of the compound of choice.

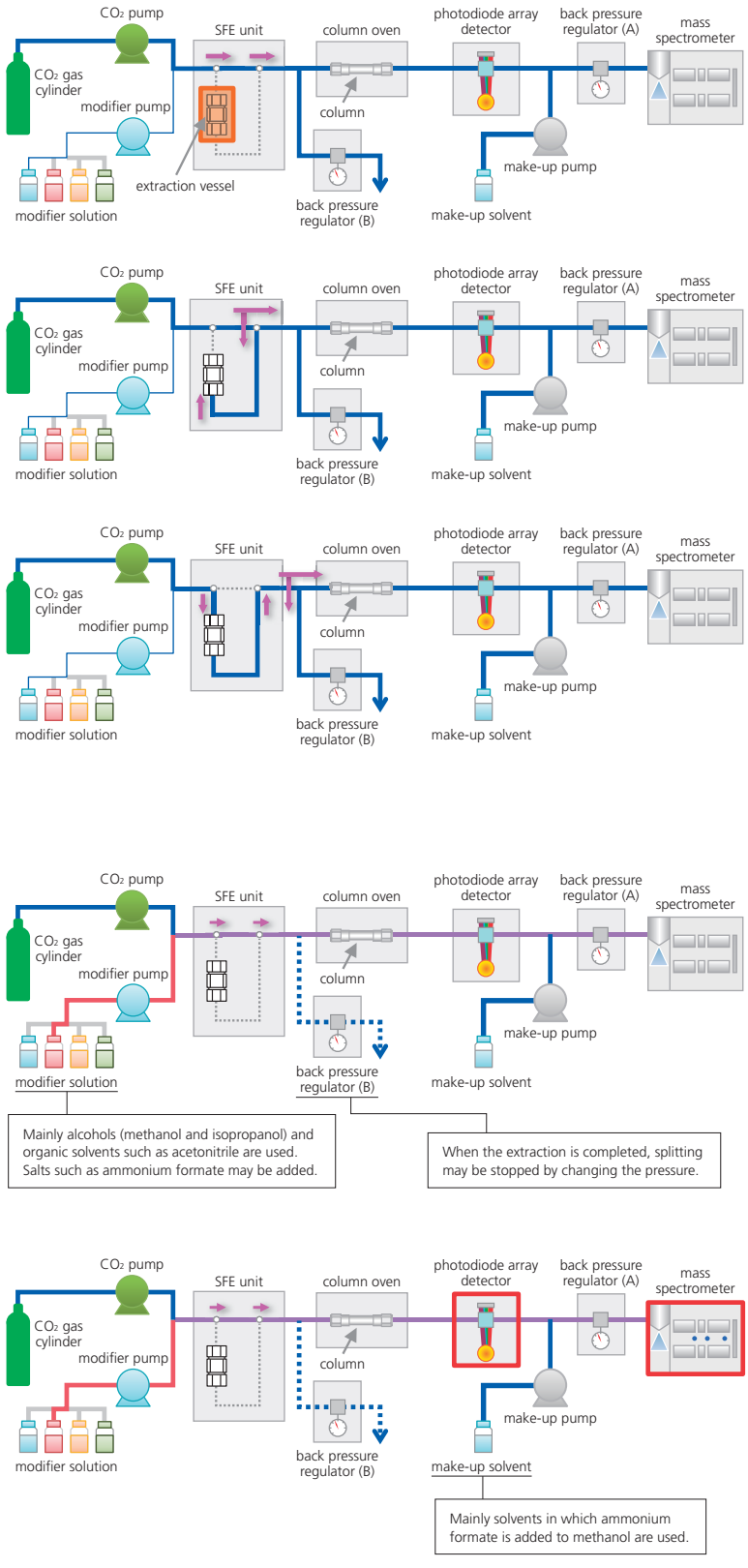


Fig. 2 Operating Principle of Nexera UC Online SFE-SFC System

4. Analytical Conditions

Using the Nexera UC Online SFE-SFC System, the following parameters can be employed to examine extraction conditions and separation conditions. By adjusting each parameter, the extraction efficiency and separation can be optimized.

Extraction conditions (static extraction and dynamic extraction)	Separation conditions
• Time • Temperature • Pressure • Type of modifier solvent • Adding a modifier	• Type of column • Type of modifier solvent • Adding a modifier • Column oven temperature • BPR pressure

5. Extraction Vessels

With the Nexera UC, there are two types of extraction vessels, and the following characteristics are observed depending on the vessel:

Capacity	Shape	Characteristics
0.2 mL		Since the capacity is small, peaks tend to be sharp in online SFE-SFC analysis.
5 mL		About 1 g of sample can be loaded. But since the capacity is large, this takes more time to remove the extracts from vessel.

6. Advantage of Introducing Splitting

In the Nexera UC Online SFE-SFC System, if the capacity of the extraction vessel is 5 mL, and the total volume of the extract is introduced into the column, the load on the column becomes too large, which causes peak broadening. Therefore, by introducing only a portion of the extract to the column using the split, the load can be reduced. Since the flow of the mobile phase in the column can be reduced, this creates a condition in which the target compound tends to remain at the top of the column (Fig. 3).

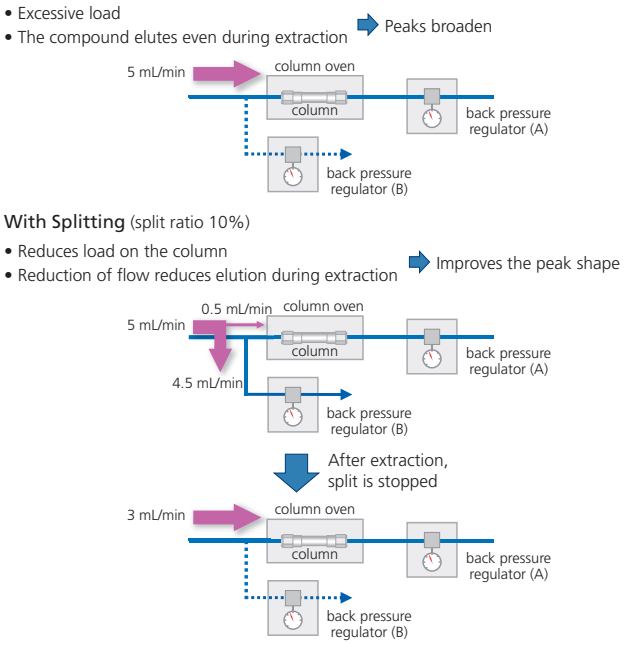
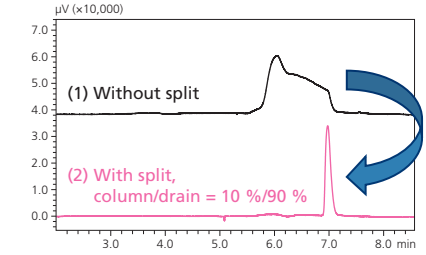


Fig. 3 Introducing the Split

By splitting, the elution of the extract can be suppressed; therefore, the shape of the peak can be improved. Fig. 4 shows an analytical example of the split using caffeine. (1) Without the split, the peak is quite broad, but (2) by introducing the split, the shape of the peak is improved.



Extraction vessel	: 5 mL
Sample	: caffeine
Modifier	: MeOH
Extraction time	: 4 min (static 2 min, dynamic 2 min)
Modifier ratio	: 10% (0–4 min) → 50% (4–6 min)
Flow rate	: 5 mL/min (at extraction), 3.0 mL/min (at analysis)
Back pressure	: 15 MPa
Column	: Shim-pack UC-SIL 4.6 mmI.D. × 250 mmL., 5 μm
Detection	: photodiode array detector (at 272 nm)

Fig. 4 An Example of Caffeine Analysis with Split

7. High-sensitivity Extraction Analysis

In the pretreatment with the traditional solvent extraction, a solvent was added to the sample for extraction. Therefore, the extract was diluted with the extraction solvent, decreasing the compound concentration in the extraction fluid. Furthermore, under analytical-scale conditions, since the inner diameter of the column is very narrow, only several microliters of the extraction fluid could be introduced. Therefore, sensitivity is insufficient when a sample with a small amount of extract is analyzed, and multiple steps of extraction and concentration become necessary. In contrast, since the extract can be directly introduced onto the column in the Nexera UC Online SFE-SFC System, the amount of sample introduced to the column can be significantly higher than that with the solvent extraction. An example of the comparison* is shown in Fig. 5. When the sample is directly introduced with the Nexera UC, compared to the 1 μL injection of 10 mL solvent in the solvent extraction, the amount introduced into the column is 10,000 times higher, allowing for a highly sensitive analysis.

* Assuming that the extraction efficiency is equivalent, and the target compound in the sample is 1%.

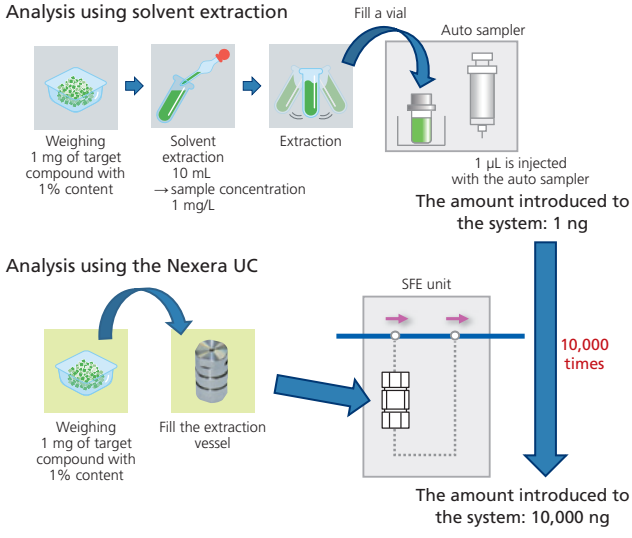


Fig. 5 High-sensitivity Analysis

8. Unstable Compound Extraction Analysis

With the Nexera UC Online SFE-SFC System, it is possible to use chemically stable carbon dioxide for extraction, and since the sample is not exposed to light or oxygen after being filled in the extraction vessel until detection, its extraction analysis of the unstable compound is effective. The reduced form of coenzyme Q_{10} (CoQ_{10}) is easily oxidized, but by using online SFE-SFC, the reduced form can be accurately extracted and analyzed (Fig. 6).

Please refer to Application News No. L496 for details such as analytical conditions.

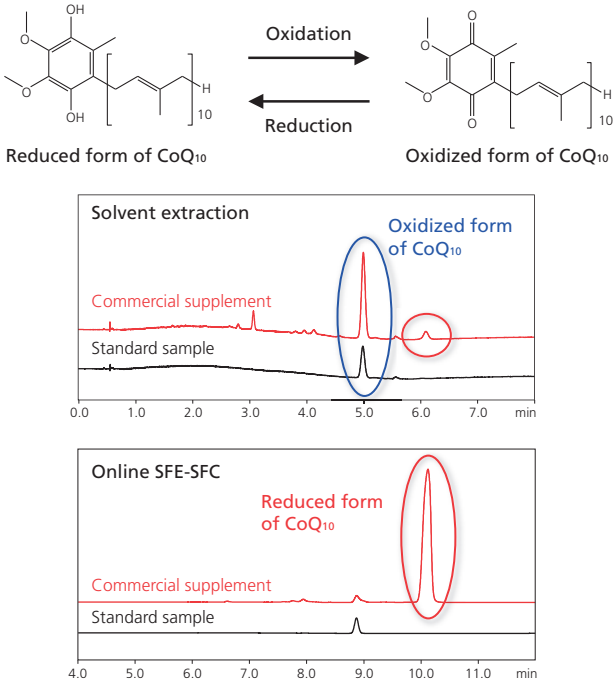


Fig. 6 Application of CoQ_{10} to Online SFE-SFC

9. Simplifying the Pretreatment and Multi-sample Processing: Application of Residual Pesticide to the Nexera UC Extraction Analysis

Faster and simpler methods of analyzing pesticide residues in food are strongly desired due both to the large number of pesticides and to reduce the time required for testing. One of the existing methods is a pretreatment using organic solvent, but it involves many processes, which require much time and effort. For the Nexera UC pretreatment, a dehydrating agent is mixed in to remove moisture, and the pretreatment is completed by filling the extraction vessel (Fig. 7). This is because supercritical carbon dioxide does not mix with water, and if there is moisture, the extraction efficiency might decrease. The actual results of an online SFE-SFC-MS analysis of pesticides are shown in Table1. These can be applied to pesticides with a wide range of polarity, from hydrophilic Log P_{ow} –1.4 compounds to hydrophobic Log P_{ow} 6.9 compounds.

In addition, by using a rack changer, 12 racks—each able to hold four extraction vessels—can be used. Therefore, a maximum of 48 samples can be analyzed consecutively.

With the use of online SFE-SFC, the complex pretreatment process can be simplified, improving the operation efficiency.

Please refer to Application News No. L497 for details such as analytical conditions for pesticide residue.

Table 1 Repeatability and Linearity for Representative Pesticides

Compounds	Log P_{ow}	Repeatability (%RSD, n=5)	Range (ng/g)	R^2
Ethofenprox	6.9	6.1	1–100	0.9991
Hexaflumuron	5.68	6.8	1–100	0.9992
Benzofenap	4.69	1.4	2–200	0.9990
Mepronil	3.66	4.6	1–100	0.9993
Prometryn	3.34	2.7	1–100	0.9994
Fenamidone	2.8	3.0	2–200	0.9991
Ethylchlozate	2.5	3.0	1–100	0.9996
Imazosulfuron	1.6	6.2	1–100	0.9998
Bensulfuron methyl	0.79	8.1	1–100	0.9996
Primisulfuron methyl	0.2	5.5	1–100	0.9994
Halosulfuron methyl	–0.02	5.5	1–100	0.9996
Azimsulfuron	–1.4	4.2	1–100	0.9998

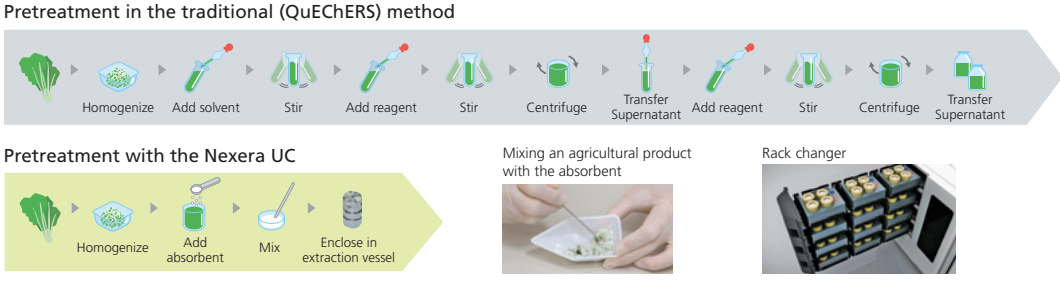


Fig. 7 Comparison of Pretreatments for Pesticides

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