Switching system of SFE/Prep SFC with MS detector

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Introduction

A mass spectrometer provides mass information of analytes, and generally provides high sensitivity combined with selective analysis, compared with conventional optical detectors. In recent years, it has been used as the method of selective detection for target analytes and identification of impurities in the development of pharmaceuticals and in a variety of other industries.

Supercritical fluid extraction (SFE) enables fast and efficient extraction using a supercritical fluid that has the specific characteristics of highly diffusivity, permeability, and solubility. Carbon dioxide (CO2) has been widely used as an extraction solvent in SFE because it easily achieves a supercritical state under moderate conditions (supercritical temperature; 31ºC, supercritical pressure; 7.4 MPa). SFE with supercritical CO_2 has advantages of easy post extraction handling, lower solvent costs, and automation by device control.

We developed a switching system combining SFE with preparative SFC and an MS detector, which provides extraction, preparation and purification with MS trigger in a single system. In this presentation, we will introduce the application of extraction from coffee beans with preparative separation and purification of caffeine using this system.

Experimental

Apparatus

Figure 2 shows the JASCO switching system of SFE/Prep SFC with MS detector used in this experiment. Figure 2 also shows a schematic diagram of this system. The column oven equipped with 10 position-11 port valves enables the switching of several extraction vessels, analytical scale columns, and semi-prep scale columns without re-plumbing. Figure 3 shows the cyclone separator (CS-87) used on the open-bed fraction collector. It significantly increases the fraction recovery. Figure 4 shows the extraction vessel (EV-2) used for SFE.

Fig.1 SFE/PrepSFC-MS Switching System

CO_2 pump:	PU-4387
Entrainer & Modifier pump:	PU-4087
Pump option:	SV unit, DG unit, MX unit
Heater:	HE-01 (Pre), HE-02 (Post)
Heater controller:	HC-4068-01
Autosampler:	AS-4358
Column oven:	CO-4065
Column oven option:	1 in 10 out valve x2
Detector:	MD-4010 (H.P. Prep Cell)
Back pressure regulator:	BP-4340
BP Make up pump	PU-4180
Pump option:	SV unit, DG unit
Splitter:	MRA
MS Make up pump:	PU-4180
Pump option:	DG unit
MS detector:	expression CMS-L
Selector valve:	HV-4088-06
Fraction collector:	Gilson 223 Sample Changer
Fume hood:	FH-4388
System control:	ChromNAV Ver.2
Software option:	Advion expression CMS SMART control driver Fraction collector controller

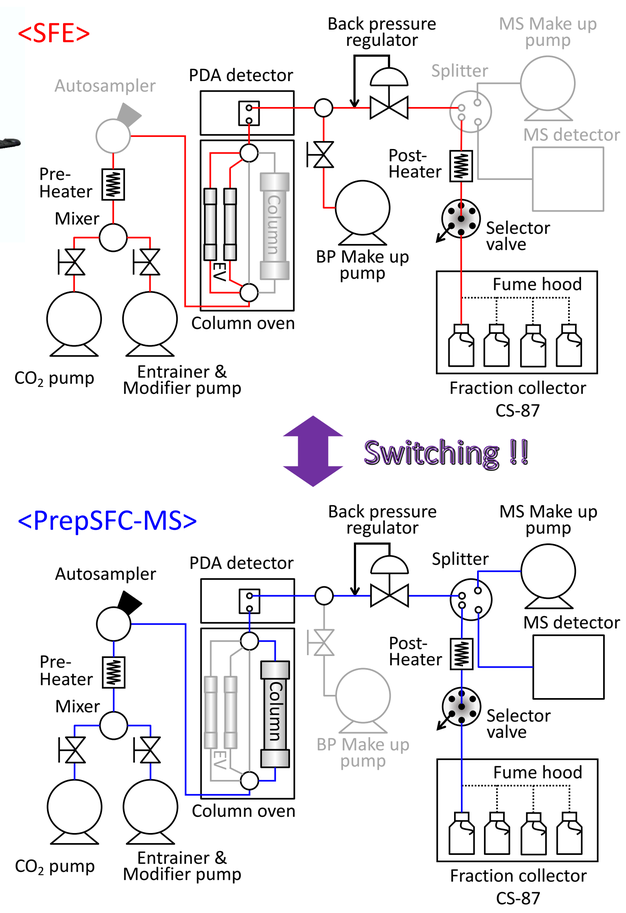


Fig.2 Flow Diagram
Above: SFE, Below: PrepSFC-MS

Fig.3 Cyclone Separator (CS-87)

Fig.4 Extraction vessel (EV-2)

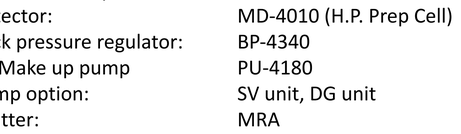
Conditions for SFE and Prep SFC-MS

The SFE procedure was performed by PEEM (Programmed Extraction Elution Method) that enhances extraction efficiency and selectivity with changing the flow rate of an entrainer and back pressure in stages. We used mixtures of methanol/water and ethanol/water as entrainers.

In Prep SFC, we used a silica column (20 mmI.D.), and the separation was performed by gradient elution of CO_2 and methanol at 30 mL/min total flow rate. The MS detector ion source was ESI-positive, and measurement mode was selected ion monitoring (SIM) at 195.2 *m/z*.

<SFE>

CO_2 flow rate:	20 mL/min
Entrainer:	CH_3OH/H_2O (39/1) (Sample No.1) C_2H_5OH/H_2O (39/1) (Sample No.2)
Entrainer flow rate:	0 mL/min (0 ~ 60 min)→1 mL/min (60 ~ 90 min) →5 mL/min (90 ~ 120 min) →0 mL/min (120 ~ 150 min)
Extraction temp.:	40˚C
Extraction vessel:	EV-2 (10 mL)
Pressure:	10 MPa (0 ~ 30 min)→25 MPa (30 ~ 120 min) →10 MPa (120 ~ 150 min)
Wavelength:	270 nm
Sample:	Green coffee beans 4 wet g



<PrepSFC-MS>

<SFC>

Column:	Alcyon SFC SIL (20 mmI.D. x 250 mmL, 5µm)
Eluent:	CO_2/Methanol Gradient 80/20 (0 min)→60/40 (4 min)→ 60/40 (5 min)→80/20 (5.1 min) 1cycle 10 min
Flow rate:	30 mL/min
Column temp.:	40˚C
Wavelength:	270 nm
Pressure:	10 MPa
Injection volume:	500 µL
Sample:	Green coffee beans extraction solution

<MS>

Ion source:	ESI (+)
Mode:	SIM
m/z:	195.2
Make up solvent:	Methanol
Make up solvent flow rate:	1 mL/min

<Splitter>

Flow rate:	30 mL/min
Split ratio:	1000 : 1

Sample Preparation

【Procedure of sample pretreatment of green coffee beans】

Green coffee beans
↓
Soak them in water at room temperature overnight in order to extract caffeine efficiently (Fig. 5).
↓
Take them out of water, and wipe with laboratory tissue paper.
↓
Load 4g of this sample into an extraction vessel.

Fig.5 Coffee beans Left: Dry, Right: Wet

Results and Discussion

<SFE>

Figure 6 shows the extraction result of sample 1. The extracts were collected in 4 vials at each extraction step (extraction 1 to 4). Table 1 shows the loading of green coffee beans into vessels, the composition of entrainers, and the collection volumes.

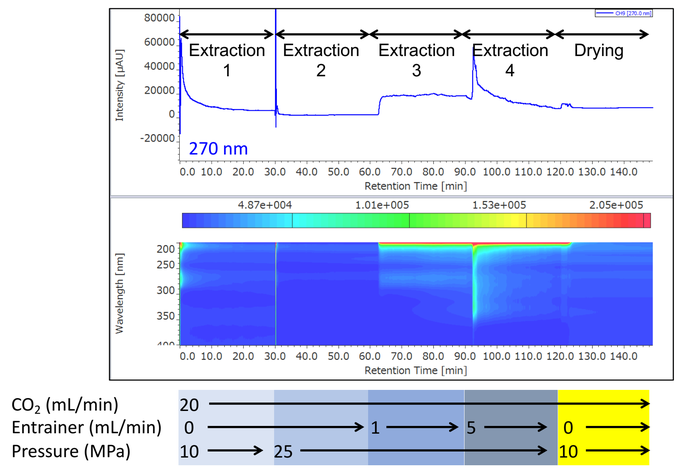


Fig.6 Extraction Result of Sample 1

Table 1 Sample loading, Entrainer composition and Fraction volume

Sample No.	1	2
Sample (wet g)	4.09	4.07
Sample (dry g)	2.18	2.17
Entrainer	Methanol/ Water (39/1)	Ethanol/ Water (39/1)
Collection volume (mL)		
Extraction 1	27	35.5
Extraction 2	39	37.5
Extraction 3	66	67.5
Extraction 4	178	184

<PrepSFC-MS>

Figure 7 shows the chromatograms of each extraction (1 to 4) from sample 1. Table 2 shows the total extraction amount in each extraction, calculated from the peak area of caffeine standard solution (100 ppm, 500 µL). As shown in this table, high extraction efficiency was achieved for sample 1 using a mixture of methanol and water as an entrainer.

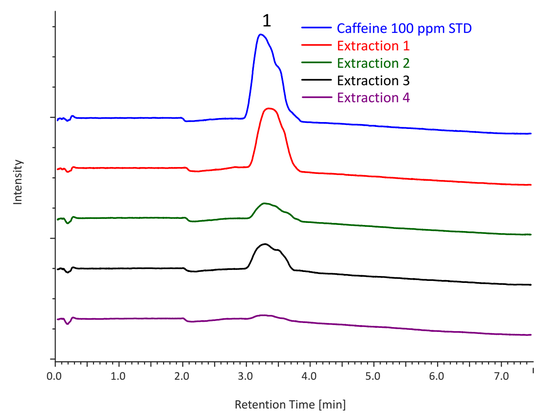


Fig.7 Chromatograms of Extraction 1 to 4 from Sample 1, Peak 1: Caffeine

Table 2 Extraction amount

Caffeine STD	
Concentration (µg/mL)	100
Injection volume (mL)	0.5
Injection amount (µg)	50
Peak area (µV・sec)	406959

	Extraction No.				Extraction amount per Coffee beans 2.18 g (µg)
Sample 1	1	2	3	4	
Peak area (µV・sec)	293049	80299	127776	18218	
Amount per 1 Injection (µg/0.5mL)	36.0	9.9	15.7	2.2	
Collection volume (mL)	27	39	66	178	
Total extraction amount (µg)	1944	770	2072	797	5583

	Extraction No.				Extraction amount per Coffee beans 2.17 g (µg)
Sample 2	1	2	3	4	
Peak area (µV・sec)	131308	34067	34800	13295	
Amount per 1 Injection (µg/0.5mL)	16.3	4.2	4.3	1.7	
Collection volume (mL)	35.5	37.5	67.5	184	
Total extraction amount (µg)	1156	317	583	607	2663

Figure 8 shows the collection results of the caffeine peak in extraction 1 from sample 1, triggered based on the MS signal (CH1: 270 nm, CH2: *m/z* 195.2). MS triggering is effective for the fraction collection of non-chromophoric compounds. ChromNAV with fraction collector management program (chromatography software) supports automatic fractions with advanced fraction algorithms for peak collection, using time, threshold, and slope, and can also be programmed to use signals simultaneously from multiple detectors.

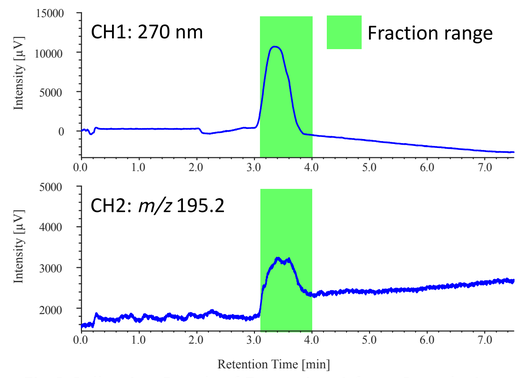


Fig.8 Collection Result of Extraction 1 from Sample 1

<Purity Determination of Extracts by HPLC-PDA>

We determined the purity of extracts collected in SFE by HPLC-PDA. Figure 9 shows the chromatograms of caffeine standard solution and concentrated extracts (mixture of extraction 1 to 4) from sample 1. Figure 10 shows the comparison of caffeine spectra between standard solution and sample solution. A high correlation coefficient was obtained between them (> 0.9995). Figure 11 and Table 3 show the results of peak purity. 98.8% of the peak was determined high-purity caffeine.

【System】

Pump:	PU-4180
Pump option:	LPG unit, DG unit
Autosampler:	AS-4150
Column oven:	CO-4060
Detector:	MD-4010

【Conditions】

Column:	InertSustain C18 (4.6 mmI.D. x 150 mmL, 3 µm)
Eluent:	CH_3CN/H_2O (20/80)
Flow rate:	0.9 mL/min
Column temp.:	40 ˚C
Wavelength:	274 nm
Injection volume.:	10 µL

【Sample Preparation】

Evaporate the extracts (extraction 1 to 4) from sample 1 to dryness.
↓
Dissolve in 3 mL of methanol.
↓
Inject to HPLC.

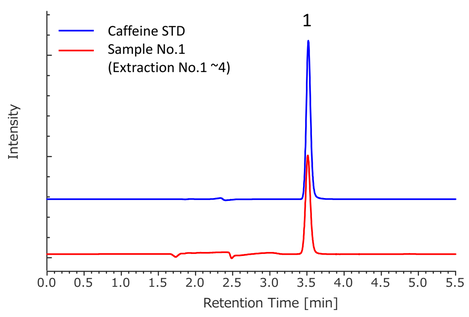


Fig.9 Chromatograms of Caffeine
Peak 1: Caffeine

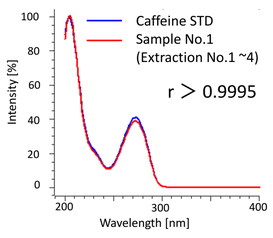


Fig.10 Spectrum Correlation Coefficient

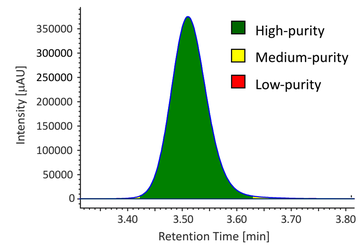


Fig.11 Peak purity

Table 3 Peak purity

Spectrum Correlation	Proportion(%)
＞0.999	98.84
0.90＜ ＜0.999	1.05
＜0.90	0.12

※Correlation coefficients between the peak top spectrum and spectra at all data points within the peak are calculated and presented in different colors according to the degrees of correlation.

Conclusion

- We achieved the extraction and fraction collection of caffeine from green coffee beans using a switching system SFE/Prep SFC-MS.
- The combination of PDA and MS detectors enables the detection and fraction collection of various compounds with or without a chromophore.
- ChromNAV softoware provides automatic and accurate fractions with advanced fraction algorithms, and useful functions (ex. fraction simulation, graphical display of collection monitoring and results).