Application of Supercritical Fluid Technologies to Lipophilic Metabolic Profiling

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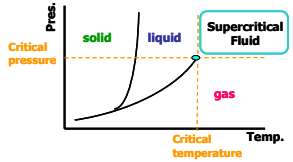
Introduction

Supercritical fluids (SCFs) have several properties (e.g., low viscosity and high diffusivity) that make them suitable for use as mobile phases in chromatography and media in extraction. Supercritical fluid chromatography (SFC) can enable rapid and high-resolution separation of complex metabolites, which are difficult to separate by GC and HPLC. Supercritical fluid extraction (SFE) is a mild and highly efficient process suitable for extracting unstable metabolites. However, SFC and SFE are used to a limited extent. Therefore, to apply them to biometric analysis, an analytical system that takes advantage of the unique properties of SCFs must be developed. In this study, we developed technology for application of SFC-MS to metabolic profiling and a system for analysis of various lipophilic metabolites.

Supercritical fluid chromatography (SFC)

Phase diagram of a single substance



A supercritical fluid is a substance whose temperature and pressure are beyond its critical point; it possesses features such as low viscosity and high diffusivity. Its density is greatly transmutable continuously.

Comparison of physical properties of gas, liquid, and supercritical fluid

Mobile phase	Density (kg/m³)	Viscosity (Pa・s)	Diffusion coefficient (m²/s)
gas	0.6–1	10^{-5}	10^{-5}
Supercritical fluid	200–900	10^{-5}–10^{-4}	10^{-7}–10^{-8}
liquid	1000	10^{-3}	$<10^{-9}$

- Its density and solvating ability are similar to that of a liquid.
- Its viscosity is similar to that of a gas.
- Its diffusion coefficient is intermediate to that of gas and liquid.

It has suitable properties for use as a mobile phase in chromatography

Features of supercritical fluid carbon dioxide

- Low viscosity and high diffusivity
- Noninflammability, low price, nontoxic
- Easy handling (critical pressure: 7.38 MPa, critical temperature: 31.1 ℃)

Features of SFC

- High speed
- High resolution

➡ **High-throughput analysis**

- It is possible to change the polarity of a mobile phase considerably by the addition of a modifier.

➡ **Wide separation modes**

- It is useful for analysis of hydrophobic compounds.

Chromatography variables comparison

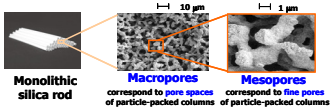
	GC	HPLC	SFC
Temperature	+	-	+
Composition of mobile phase	-	+	+
Solid phase	+	+	+
Pressure	-	-	+

+ shows that the variable can be easily changed to adjust retention and selectivity

Carotenoids: High-throughput analysis of carotenoids using monolithic columns

Monolithic columns

Structures: Monolithic column consists of continuous 3-D structural skeleton and its gaps.



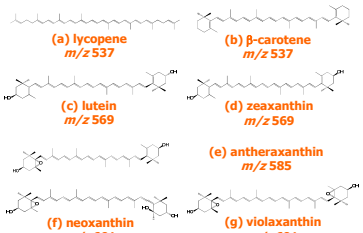
Features:

- Low back pressure

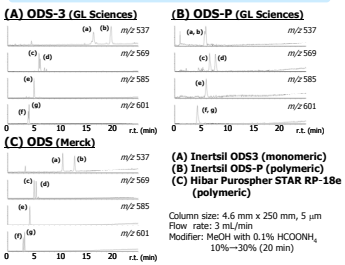
⇨ Shortening analysis time by high-flow rate
High resolution analysis by using longer column

- High efficacy

Structures of target carotenoids



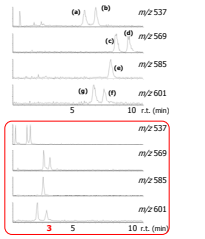
Investigation of columns



(A) Inertsil ODS3 (monomeric)
(B) Inertsil ODS-P (polymeric)
(C) Hibar Purospher STAR RP-18e (polymeric)

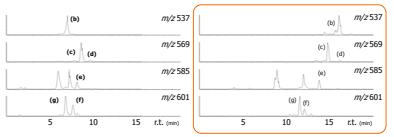
Column size: 4.6 mm x 250 mm, 5 μm
Flow rate: 3 mL/min
Modifier: MeOH with 0.1% $HCOONH_4$
10%→30% (20 min)

Analysis of carotenoids mixture using Chromolith™



Analysis of the carotenoids from green algae

Sample: acetone extract of *Chlamydomonas reinhardtii* (green algae)



Monolithic silica column
Chromolith Performance RP-18e
4.6 mm X 100 mm (Merck)
modifier 1-7% (12 min)

Monolithic silica column
Chromolith Performance RP-18e
4.6 mm X 100 mm x 3 (Merck)
modifier 1-11% (20 min)

Methods

Analysis conditions

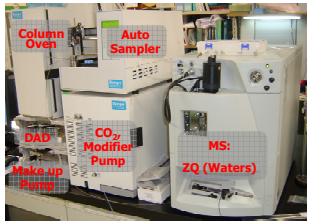
SFC conditions (BERGER SFC™ Analytix, Thar Instruments, Inc.)

Mobile phase : Carbon Dioxide (CO_2, 99.99% grade)
Modifier : Methanol with 0.1% (w/w) ammonium formate
Oven temperature : 35 ℃
Back pressure : 10 MPa

MS conditions (ZQ2000, Waters Co.)

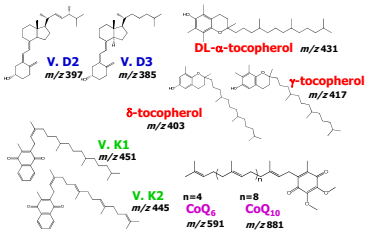
Ionization method : Electrospray ionization (ESI)
Polarity : Positive
Make up : 0.1 mL/min (MeOH with 0.1% $HCOONH_4$)
Capillary voltage : 3.00 kV
Cone voltage : 30 V
Extractor voltage : 2.0 V
RF lenz voltage : 0.2 V
Source temp. : 120 ℃
Desolvation temp. : 350 ℃
Desolvation gas flow : 350 L/hr
Cone gas flow : 50 L/hr

SFC-MS instrument

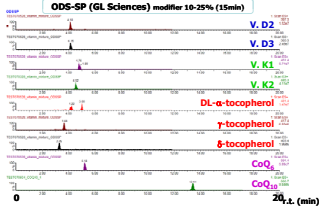


Fat-soluble vitamins: Application of online SFE-SFC-MS analysis to easily oxidized compounds

Structures of target fat-soluble vitamins

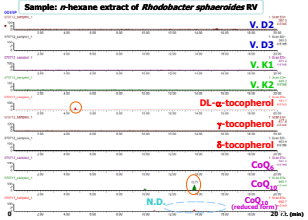


Analysis of vitamins mixture using ODS column



Analysis of the fat-soluble vitamins from photosynthetic bacteria

Sample: *n*-hexane extract of *Rhodobacter sphaeroides* RV



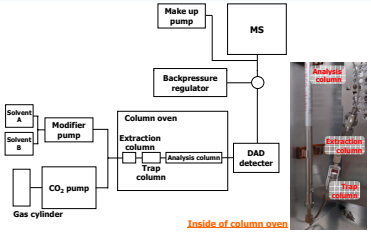
Analysis of the coenzyme Q_{10} from *R. sphaeroides* using online-SFE-SFC/MS system

Extraction:
Supercritical fluid extraction (SFE)
- High efficacy

Separation-detection:
SFC-MS
- High resolution
- High selectivity

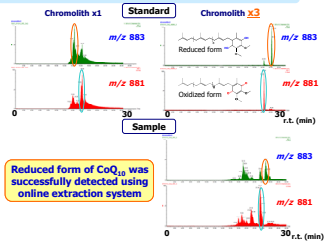
In one operation

Online SFE-SFC-MS System



Advantages of this system

- Shortening total analysis time
- Obtaining accurate profile of easily degraded compounds



Conclusion

<Carotenoids>

The use of a monolithic column resulted in improvement not only in separation but also in throughput, compared with the particle-packed column, as a result of low back pressure that enables the high-flow rate analysis. Furthermore, higher-resolution analysis would also be possible by using a longer monolithic column.

<Fat-soluble vitamins>

Using online-supercritical fluid extracting system, we successfully detected a reduced form of coenzyme Q_{10} in photosynthesis bacteria which was not detected in organic solvent extracts. These results indicated that online extracting system enabled a more accurate analysis by extracting easily-oxidized fat-soluble metabolites stably.

In this study, the utility of SFC in the analysis of hydrophobic metabolites was clearly demonstrated. It is suggested that SFC-MS system can be a powerful tool for metabolic profiling system, especially for hydrophobic metabolite profiling.

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