

Application of supercritical fluid chromatography to analysis of hydrophobic metabolites

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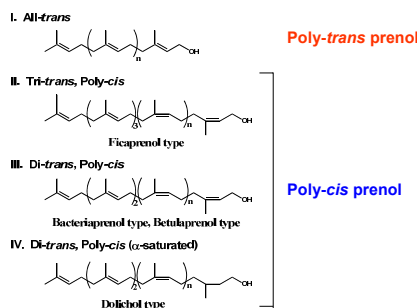
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1. Abstract

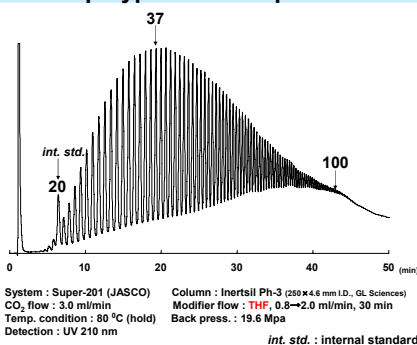
A supercritical fluid is a substance whose temperature and pressure are beyond its critical point; it exhibits properties such as low viscosity and high diffusivity, because of which it is suitable for use as a mobile phase in chromatography. High-speed separation can be carried out by supercritical fluid chromatography (SFC) by utilizing a column with low back pressure; further, SFC can be used to improve the separation resolution by employing a long column. SFC provides a wide range of separation modes, which cannot be achieved by gas chromatography (GC) and high-performance liquid chromatography (HPLC), by adding polar organic solvents and/or by changing temperature and back pressure. Carbon dioxide is most frequently used as the mobile phase in SFC because it is easy to handle, non-flammable, and chemically reactive. Supercritical carbon dioxide has a low polarity that is similar to the polarity of hexane. Therefore, it is suitable as the mobile phase for the separation of hydrophobic compounds. We thus attempted to apply SFC to the analysis of hydrophobic metabolites, as an alternative chromatographic technique to HPLC and GC. Complicated geometric isomers and polymers (having molecular weights greater than 7000), both of which were derived from plants, were successfully separated using SFC, thereby demonstrating the potential applicability of SFC to the analysis of fat-soluble metabolites. Additionally, with the aim of application of SFC to metabolomics, we established an analytical system in which a mass spectrometer used as a detector was coupled to a supercritical fluid chromatograph, and the usefulness of this system was confirmed by a high-throughput analysis of 14 lipid mixtures that included phospholipids, glycolipids, neutral lipids, and sphingolipids.

2. Application of SFC to polyprenol analysis

Chemical structures of natural polyprenols

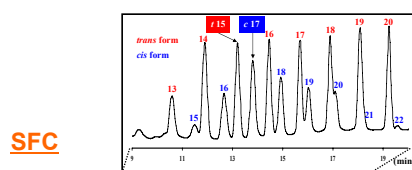
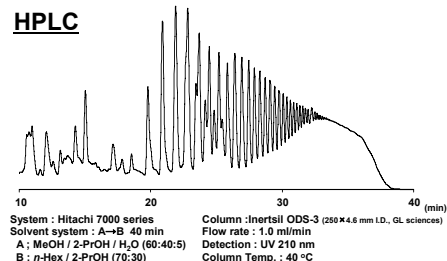


Separation of long-chain polyprenol from plant



Separation of polyprenols from plant

HPLC



3. Application of SFC/MS to lipid metabolomics

General analytical methods for lipid metabolomics

GC/MS

- The lipid target to be analyzed is limited
- The distinction of the lipid class is impossible

LC/ESI-MS

- Low throughput
- Difficult to analyze multicomponents simultaneously

ESI-MS

- Problem of ionization suppression
- The quantitativity is inferior
- It is difficult to detect minor components
- It is difficult to separate the lipid class

It is necessary to establish a new technique for high-throughput analysis of various lipids comprehensively.

Chromatographic separation is important for high resolution and sensitivity analysis.

We attempt to apply supercritical fluid chromatography to lipid metabolomics.

Analysis conditions

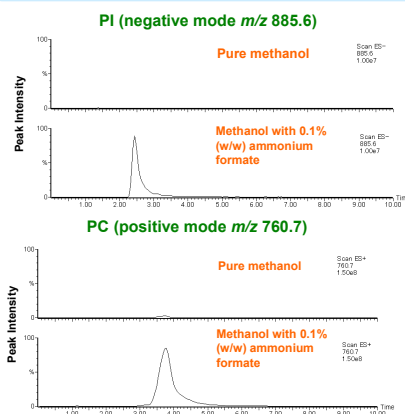
SFC (BERGER SFC™ Analytix)

Mobile phase: Carbon Dioxide (CO₂, 99.99% grade)
Modifier: Methanol with 0.1% (w/w) HCOONH₄
10% → 30%, 20 min
Flow rate: 3 mL/min
Oven temp.: 35 °C
Back pressure: 10 MPa

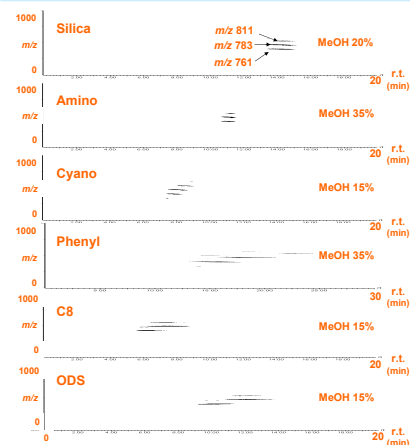
MS (ZQ2000, Waters Co.)

Ionization method: Electron spray ionization (ESI)
Make up: 0.1 mL/min (Methanol with 0.1% HCOONH₄)
Scan range: m/z 250–1200
Capillary voltage: 3.00 kV
Cone voltage: 30 V
Extractor voltage: 2.0 V
RF lens voltage: 0.2 V
Source temp.: 120 °C
Desolvation temp.: 350 °C
Desolvation gas flow: 350 L/h
Cone gas flow: 50 L/h

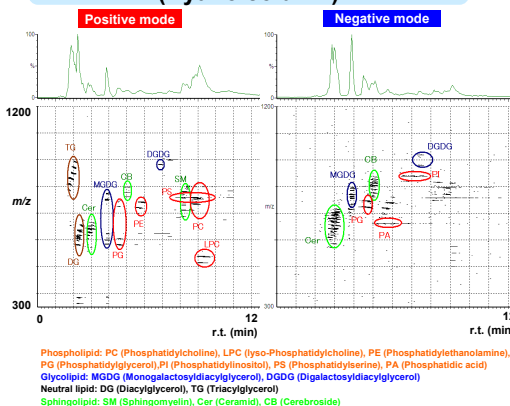
Investigation of modifiers



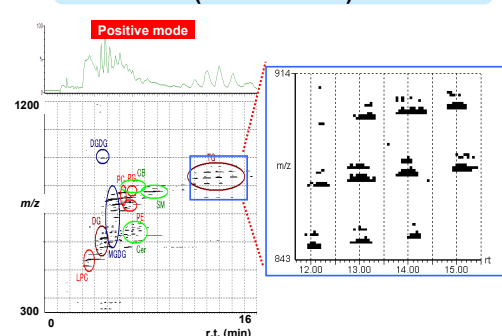
Investigation of columns



Lipid mixture SFC/MS analysis (Cyano column)



Lipid mixture SFC/MS analysis (ODS column)



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