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Development of online SFE-LC/MS system for analysis of metabolites in microbial cells

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Overview

Purpose

The aim of this study is to develop an online SFE-LC/MS system that provides completely automated analytical process for metabolites analysis.

Methods

- SFE (supercritical fluid extraction) was used for multi-step extraction.
- Newly developed polymer-based column was used as the trap column for displacing SFE extractant with LC mobile phase.

Results

We successfully developed
Multi-step extraction method that afforded automatic extraction of typical metabolites from microbial cells and low background noise in MS detection due to clean-up step.
An online SFE-LC/MS system using newly developed polymer-based trap column for analysis of metabolites in microbial cells.

Introduction

In bio-based fine chemical production, a short breeding cycle time of microbes is a key issue for improving productivity. Metabolomics has been widely used as a quick and comprehensive analytical procedure due to its excellent features of dynamic monitoring and quick evaluation for bio-production. On the other hand, sample pretreatment procedure is still generally tedious and time consuming while high-throughput analytical methods using LC/MS and GC/MS.
We present the development of online SFE-LC/MS system that provides completely automated analytical process resulting labor-saving.

Methods and Materials

Instrumentation

SFE conditions	
Modifier	: 0.1% ammonium formate-methanol
Flow rate	: 1.0 mL/min
Extraction	: Static extraction : 3 min. Dynamic extraction : 2 min.
BPR	: 15 MPa
Vessel	: 0.2 mL
LC/MS conditions	
Column	: SUPELCO Discovery HS F5-3 (4.6 x150 mm, 3 µm)
Mobile Phase	: 0.1% formic acid-water / 0.1% formic acid acetonitrile
Gradient program	: 0%B (0-2min) => 25%B (5min) => 35% B (11min) => 95%B (15-20min) => 0%B (20.01-25min)
Flow rate	: 0.8 mL/min
Oven temperature	: 40 °C
Ionization	: ESI positive, negative
Mode	: MRM

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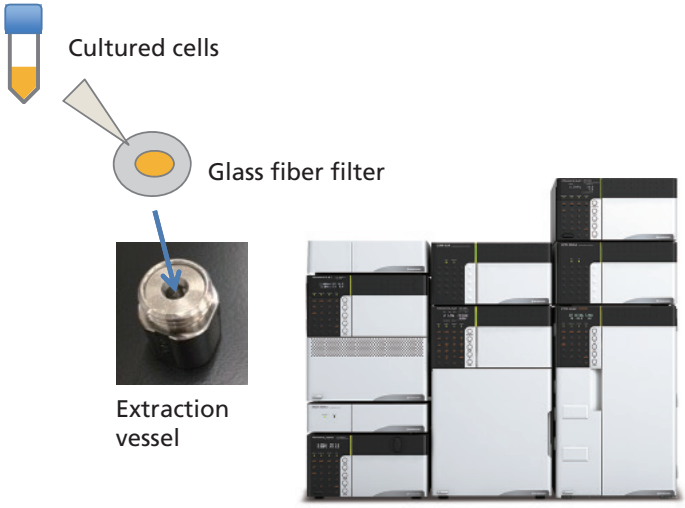

Figure 1 Nexera UC SFE system

Figure 2 LCMS-8060 triple quadrupole mass spectrometer

Samples & target compounds

- E.coli. and yeast cells were used as tested samples.
- Typical metabolic precursors for secondary metabolites on the shikimate, mevalonate, and MEP pathways were selected.

Shikimate	Mevalonate
Dehydroshikimate	Mevalonate-5-phosphate
Shikimate phosphate	Mevalonate-5-diphosphate
DHQ	DXP
Chorismate	MEP
Acetoacetyl-CoA	DOXP
HMG-CoA	Malonyl CoA

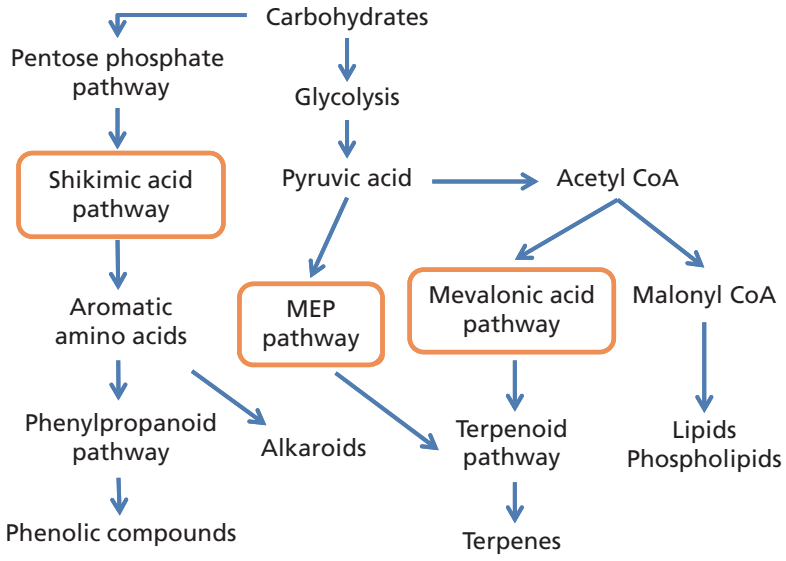

Figure 3 Metabolic pathway

Results

Component technologies for the online SFE-LC/MS

Multi-step extraction with auto-clean up using SFE

- Most of tested metabolites were extracted when the modifier concentration was more than 20% (Fig. 4). Therefore, multi-step extraction method was employed (Fig. 5).
- Some phosphoric compounds were not extracted due to metal ion liganding property.

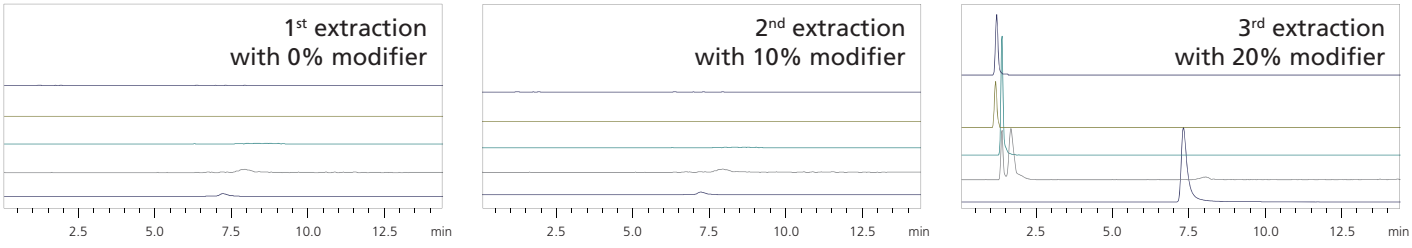

Figure 4 Results of step-wise extraction

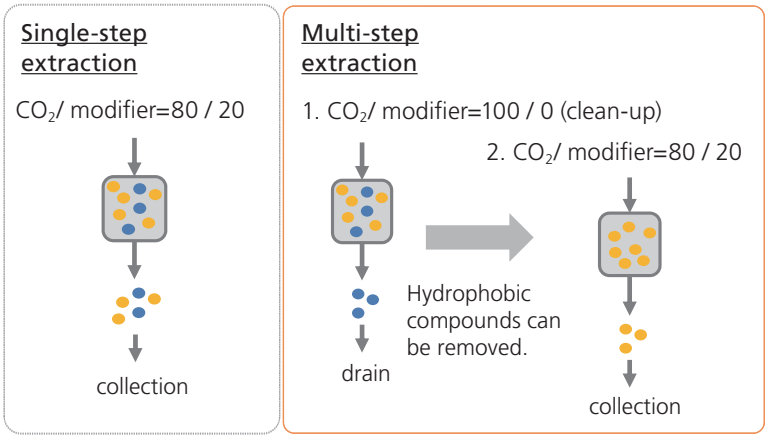

Figure 5 Single / multi-step extraction in SFE

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Multi-step extraction method afforded

- Automatic extraction of typical metabolites from microbial cells without any additional pretreatment.
- Low back ground noise in MS detection due to clean-up step (i.e. 1st extraction with 0% of modifier).

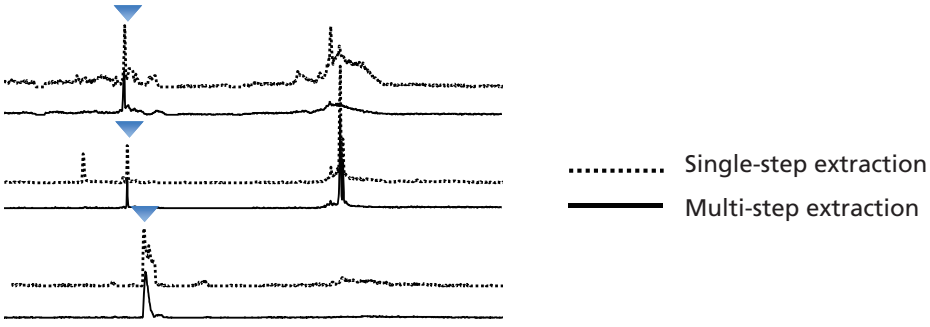
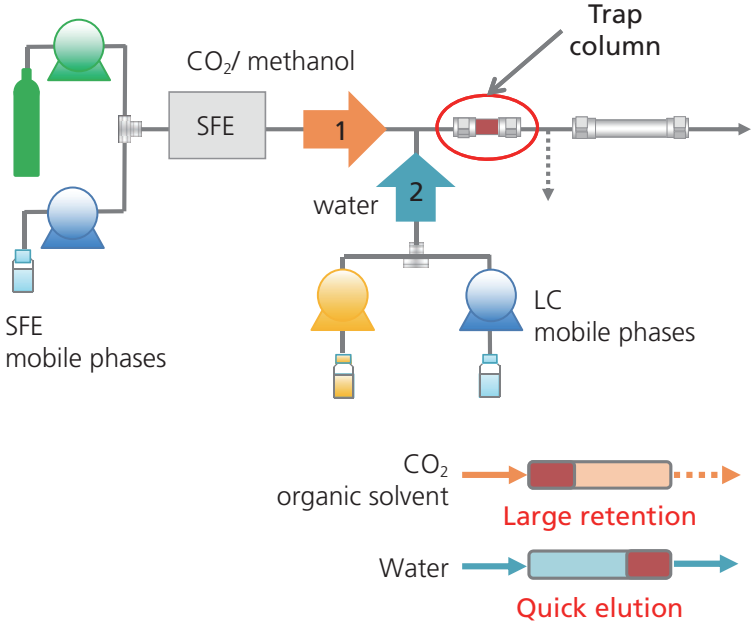


Figure 6 Chromatograms of sample extracts (E.coli.) by single / multi-step extraction

Trapping technique using newly developed polymer-based trap column

- The extract from SFE must be trapped in the trap column before introduction into the LC column for displacing SFE extractant with appropriate solvent due to poor miscibility of SFE and LC mobile phases.
- Newly developed polymer-based column that showed large retention under SFE condition whereas quick elution under LC condition (Fig. 7 and 8) was employed.



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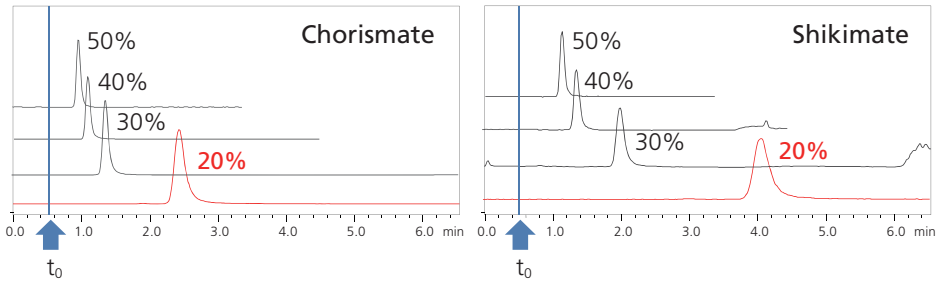
Retention behaviours of the metabolites in new polymer-based column

Under SFE condition

(a) Effect of modifier concentration



(b) Effect of additive in the modifier (modifier conc. 20%)

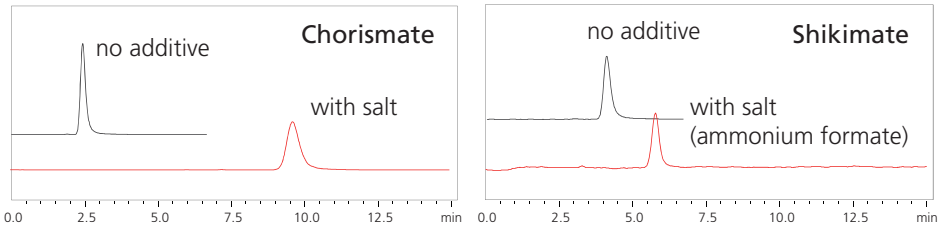


Figure 7 Chromatograms of typical metabolites under SFE condition

Under LC condition

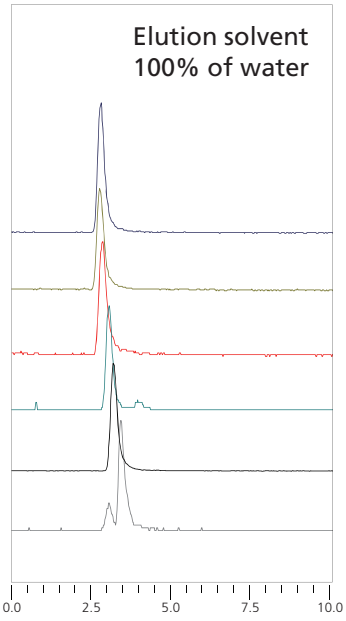


Figure 8 Chromatograms of typical metabolites under LC condition

Component technologies for the online SFE-LC/MS

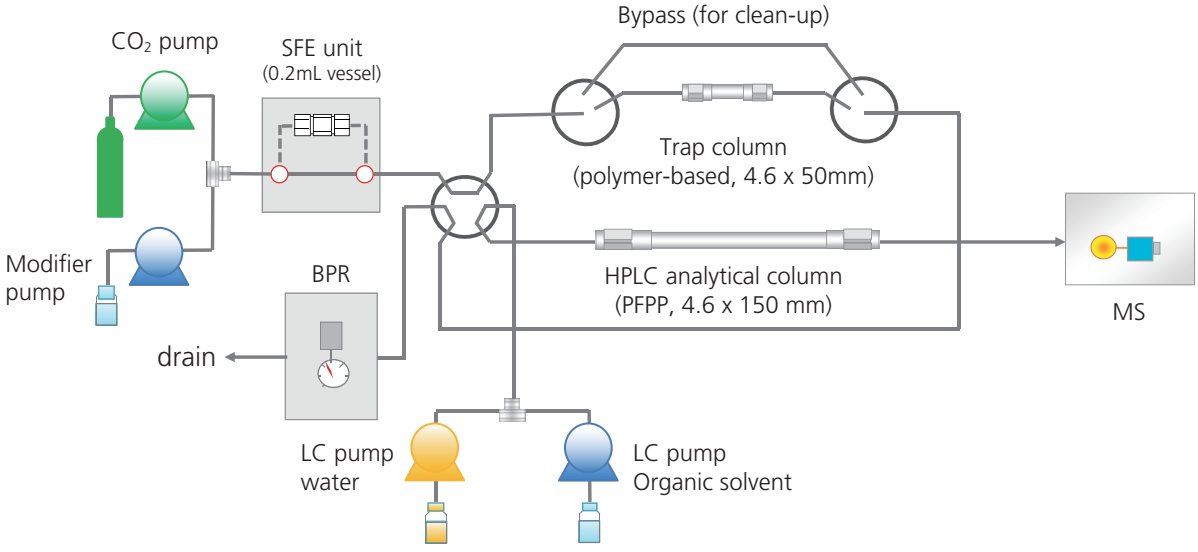


Figure 9 System configuration of newly developed online SFE-LC/MS

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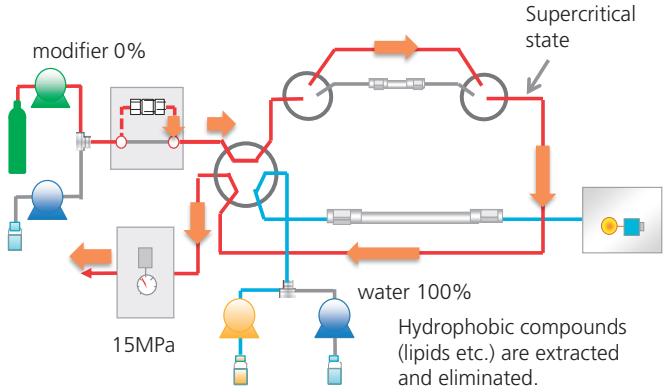
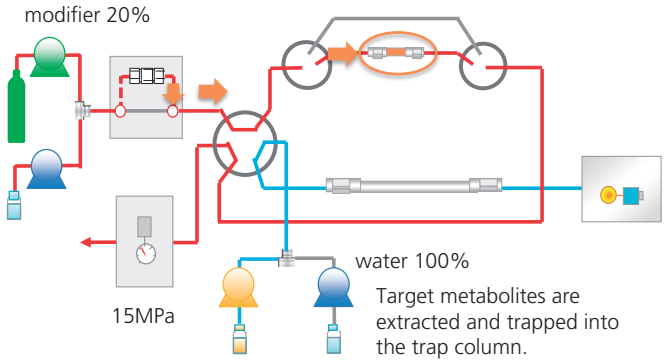
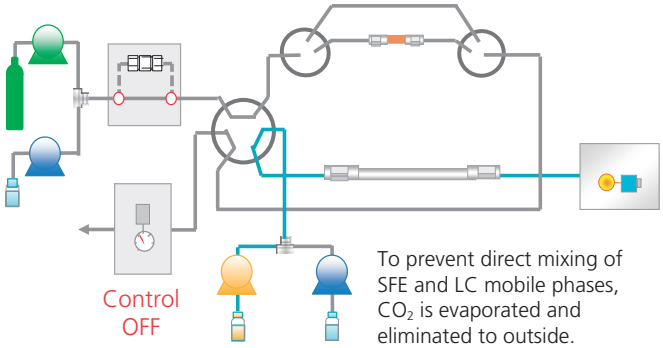
1. 1st step extraction



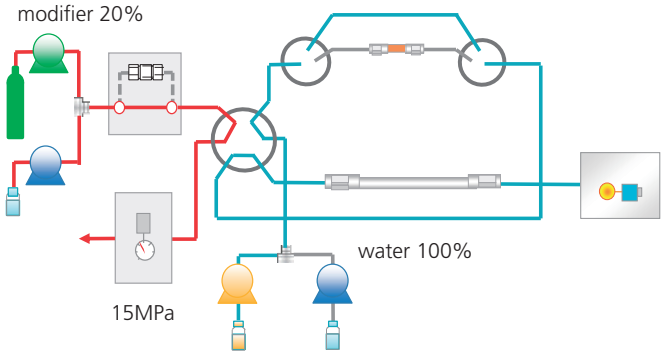
2. 2nd step extraction



3. Releasing back pressure



4. Conditioning



5. LC/MS analysis

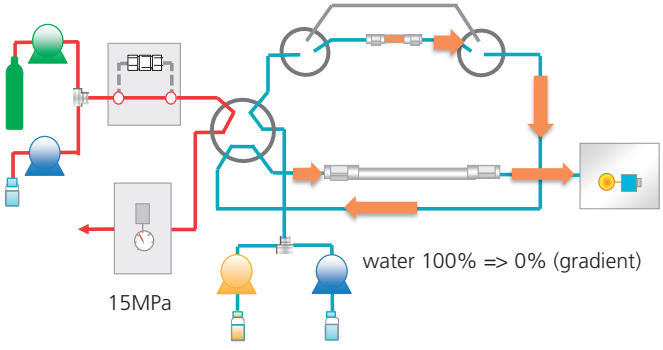


Figure 10 Schematic sequence of the online SFE-LC/MS

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Metabolites analysis in real samples using SFE-LC/MS

- E.coli. and yeast cells collected from their culture mediums by centrifugation were used.
- Some metabolites were successfully extracted from E.coli. and yeast cells without any pretreatment.

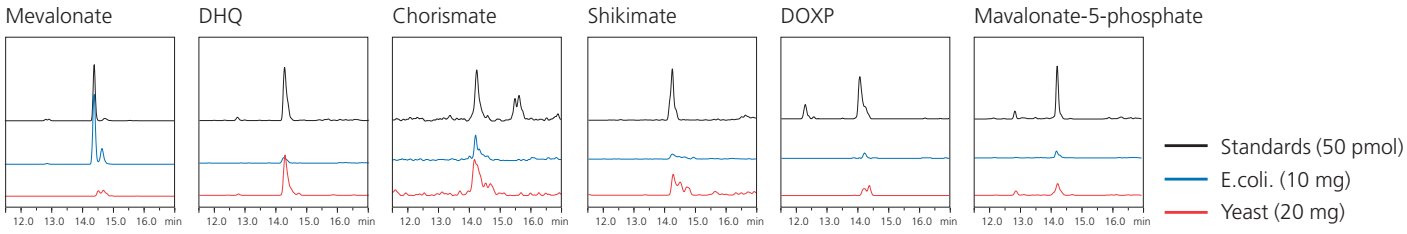

Figure 11 Chromatograms of the metabolites in E.coli. and yeast cells

Conclusions

- An online SFE-LC/MS system using newly developed polymer-based trap column for analysis of metabolites in microbial cells has been successfully developed.
- This system provides completely automated analytical process resulting labor-saving.

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