

# Development of on-line SFE-SFC system and its application for food contaminant analysis

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### Introduction

Sample pretreatment is always one of the key issues in a series of analytical procedures. On-line sample pretreatment with an automated chromatographic system is often used for effective and reliable extraction and analysis. On-line solid phase extraction (SPE) has contributed to high-throughput LC-MS/MS analysis. Pretreatment is conducted with a SPE column in a few minutes and the extract is analyzed by UHPLC-MS/MS. Another pretreatment method, supercritical fluid extraction (SFE), has been a commonly used substantial

extraction technique and applied for extraction of bioactive compounds, drugs, lipids, and contaminants from natural products. SFE with supercritical carbon dioxide (SC-CO<sub>2</sub>) has advantages such as low consumption of organic solvents and low toxicity. SC-CO<sub>2</sub> is also readily removed after use. In this study, chloramphenicol (CAP) in honey was determined by on-line SPE-LC-MS/MS and the compound in milk powder was determined by on-line SFE-SFC-MS/MS.

### Methods and Materials

#### Sample Preparation

A honey sample solution was prepared by dissolving honey (1 g) in water (10 mL). Powdered milk samples (0.1 g) were prepared in SFE vessels.

#### Analysis of honey by on-line SPE-LC-MS/MS

##### HPLC

The liquid chromatograph was a Shimadzu LC-30A system (Kyoto, Japan) equipped with LC-30AD pumps, an LC-20AB pump, SIL-30AC autosampler. CTO-30A column oven, and a 6-port flow changeover valve FCV-32AH. A Shim-pack MAYI-ODS (10 mm x 2.0 mm, particle size: 50 μm) was held at room temperature with an eluent composition of water/ACN (95/5) at a flow rate of 1 mL/min. The injection volume was 20 μL. After extraction

(1 min), the valve was switched and mobile phase for analysis was introduced at a flow-rate of 0.4 mL/min. the Shim-pack XR-ODS (75 mm x 2.0 mm, particle size: 2.2 μm) and the column temperature was 40 °C. Mobile phase A was water. Mobile phase B was ACN. The gradient program was: 5%ACN (0-1 min)-95% ACN (2.5-3 min)-5% ACN (3.01-5 min).

##### Mass spectrometry

The triple quadrupole mass spectrometer was LCMS-8080 equipped with an electrospray ionization (ESI) interface. Ionization voltage was -3.5 kV (negative mode). Nebulizing gas flow, heating gas flow and curtain gas flow were 2

L/min, 12 L/min and 3 L/min, respectively. Probe temperature and HSID temperature were 400 °C and 200 °C, respectively. MRM transitions for chloramphenicol was  $m/z$  321.20 > 152.25. Dwell time was 100 ms.

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### Analysis of milk powder by on-line SFE-SFC-MS/MS

#### SFE-SFC

The SFE-SFC instrument was a Shimadzu Nexera UC system (Kyoto, Japan) equipped with SFE-30A extraction unit, SFC-30ADSF pump (for SC-CO<sub>2</sub>), LC-30AD pump (for modifier MeOH), CTO-20A column oven, and SFC-30A backpressure regulators. SFE was conducted under the following conditions: SC-CO<sub>2</sub>/0.1% ammonium formate in MeOH (95/5), temperature; 40 °C, static extraction; 2 min, dynamic extraction; 3 min. The split ratio: (column/waste=3/97) was adjusted by

setting the back pressures (backpressure A: 14.8 MPa, backpressure B: 15.0 MPa). Analysis was conducted at a flow-rate of 2 mL/min. Extracts were separated with Inertsil ODS-EP (150 mm x 4.6 mm, particle size: 5 µm) and the column temperature was 40 °C. Mobile phase A was SC-CO<sub>2</sub>. Mobile phase B was 0.1% ammonium formate in MeOH. Following gradient program was used: 5% MeOH (0-5 min)-35% MeOH (8-8.5 min). The total analysis time was 13 min, including washing step.

#### Mass spectrometry

The triple quadrupole mass spectrometer was LCMS-8050 equipped with an electrospray ionization (ESI) interface. Ionization voltage was -3.5 kV (negative mode). Nebulizing gas flow, heating gas flow and drying gas flow were 3 L/min, 10 L/min and 10 L/min, respectively.

Interface temperature and block heater temperature were 300 °C and 400 °C, respectively. MRM transitions for chloramphenicol was m/z 321.00 > 152.05. Dwell time was 200 ms.

## Results and Discussion

### Analysis of honey by on-line SPE-LC-MS/MS

Retention time of CAP was 2.65 min. A matrix-matched calibration curve of CAP in honey was constructed ranging from 20 pg/mL to 5000 pg/mL. The correlation coefficient was 0.9994. The LLOQ was 20 pg/mL (= 0.2

µg/kg honey). The sensitivity met the requirement of the EU regulation (0.3 µg/kg in honey). Peaks of CAP were found in real samples, however, the amounts were lower than the LLOQ for all samples (Figure 1).

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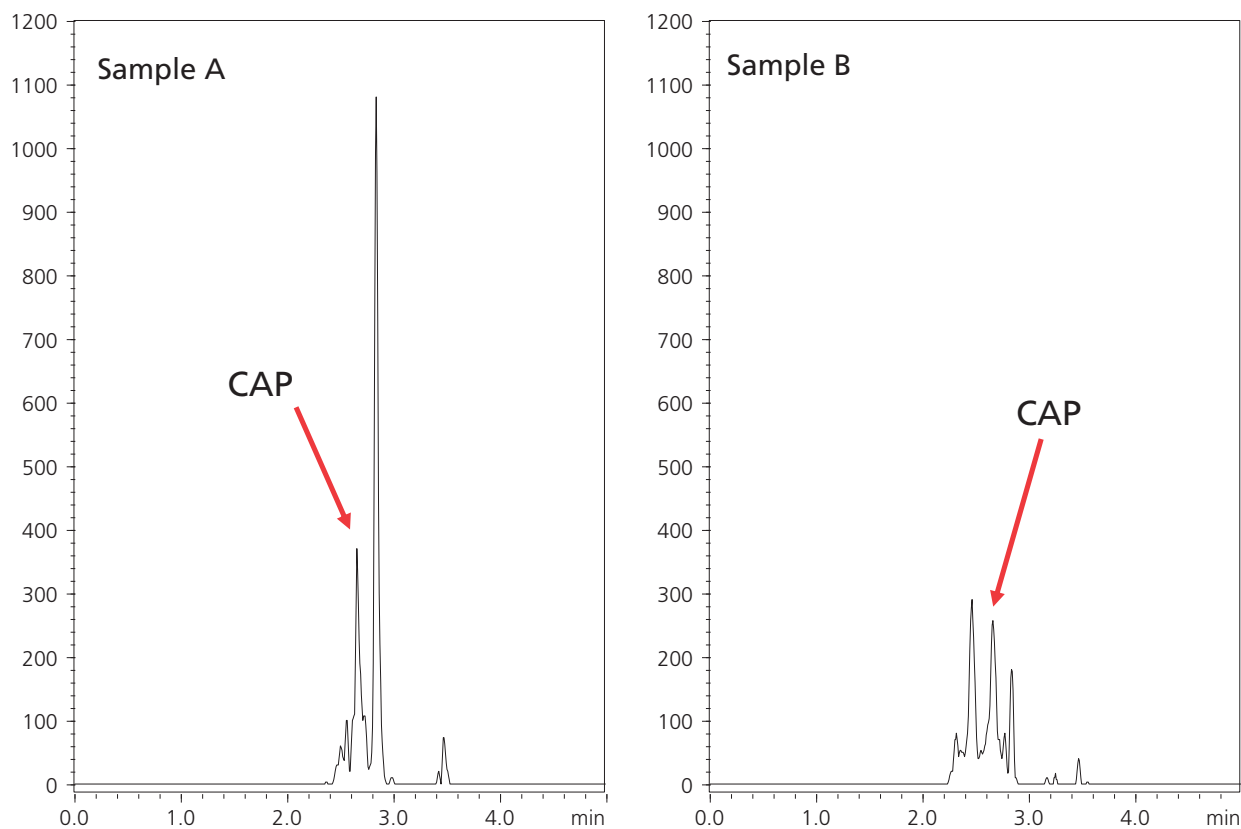


Figure 1 MRM chromatograms of honey samples by on-line SPE-LC-MS/MS

### Analysis of milk powder by on-line SFE-SFC-MS/MS

Retention time of CAP was 7.88 min. A matrix-matched calibration curve of CAP in powdered milk (0.1 g) was constructed ranging from 2 pg to 100 pg. The correlation coefficient was 0.998. The LLOQ was 2 pg (=0.02 µg/kg

milk powder). Peaks of CAP were found in real samples, however, the amounts were lower than the LLOQ for all samples (Figure 2).

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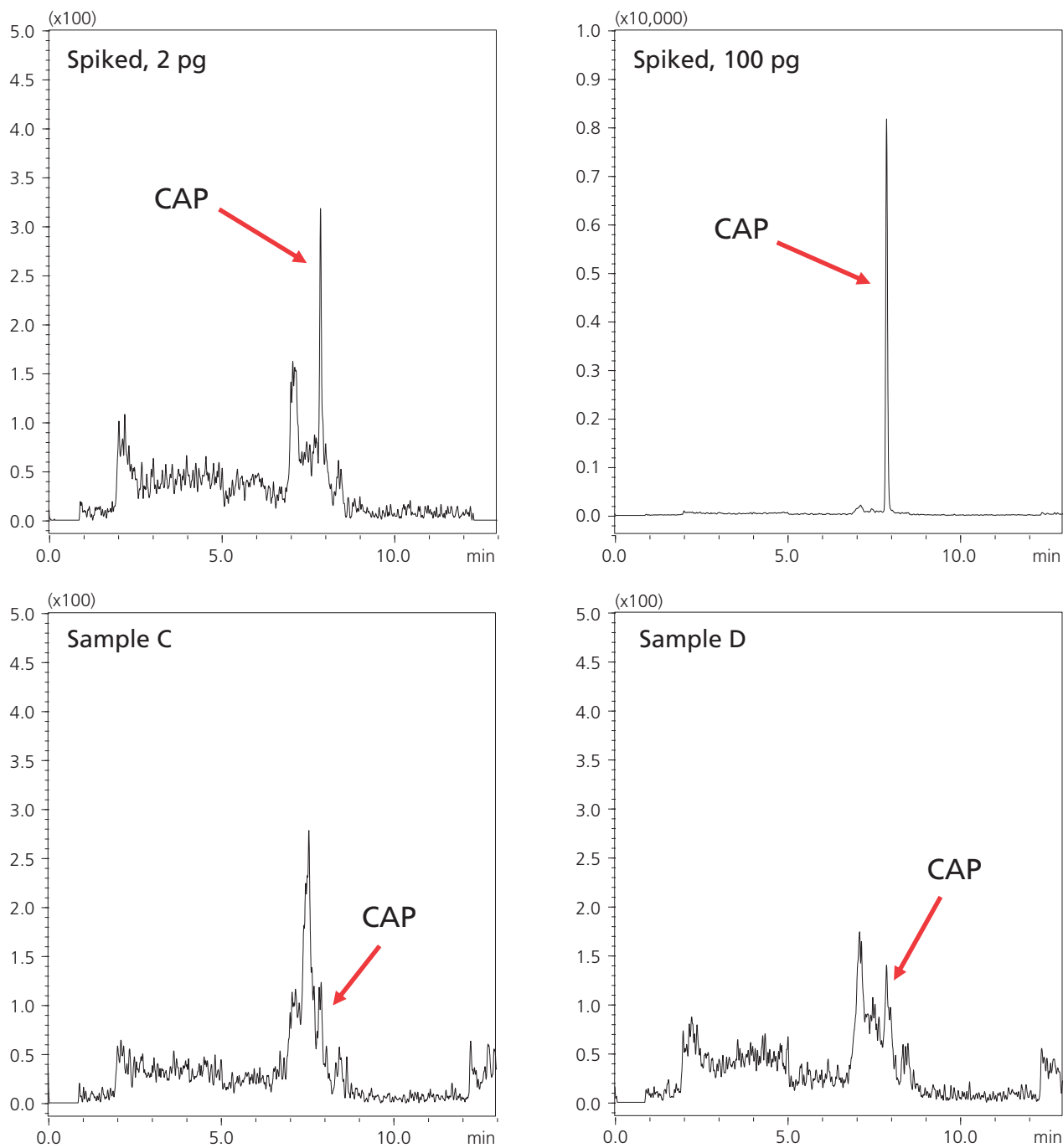


Figure 2 MRM chromatograms of milk powder samples by on-line SFE-SFC-MS/MS

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### Conclusions

Both on-line SPE-LC-MS/MS for liquid samples (diluted honey) and on-line SFE-SFC-MS/MS for solid samples (milk powder) were successfully applied for analysis of CAP. Sensitivity and linearity were excellent and

higher-throughput analysis with on-line pretreatment was achieved. Sample pretreatment can be minimized by using on-line pretreatment systems,

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