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# High Throughput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS

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## Introduction

Mycotoxins often exist as contaminants in grains. To ensure consumer food safety, manufactures of food and beverages have to strictly manage risks from such contaminants. To maintain the high-quality of food standards it is therefore essential to rapidly determine the concentrations of hazardous mycotoxins in foods or beverages.
UHPLC-MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in complex matrices. In this study, a high throughput method for the quantification of 14 mycotoxins in beers was developed. Highest sensitivity of analysis is crucial to food safety, additionally, autosampler and system carry over need to be monitored to ensure these factors do not become a problem. In these experiments elimination of carry over was investigated through novel rinse condition cycles of the UHPLC autosampler.

## Methods and Materials

14 mycotoxins (patulin(PAT), nivalenol(NIV), deoxynivalenol (DON), aflatoxin(AF) B1, B2, G1, G2, T-2 toxin(T-2), HT-2 toxin(HT-2), zearalenone(ZON), fumonisin(FM) B1, B2, B3 and ochratoxin A(OTA)) were determined by LC-MS/MS using a UFLC HPLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer.
The MRM method of 14 mycotoxins was optimized on each compound-dependent parameter and MRM transition (Q1/Q3). As a result, all compounds were detected with high sensitivity by ESI. AFB1, B2, G1, G2, T-2, HT-2, FMB1, B2, B3 and OTA were detected in positive mode. While PAT, NIV, DON, ZON were detected in negative mode. Ultra Fast Polarity Switching of 15 msec enabled simultaneous determination of the compounds in both modes.

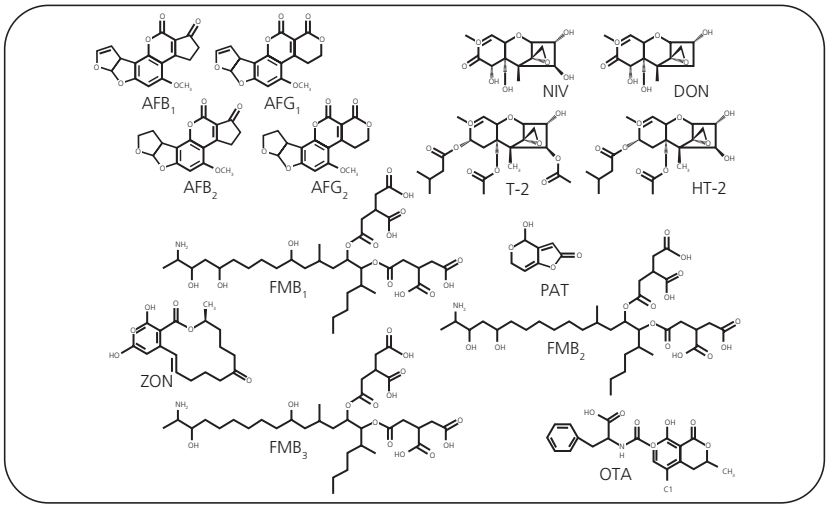


Fig. 1 Structure of mycotoxins

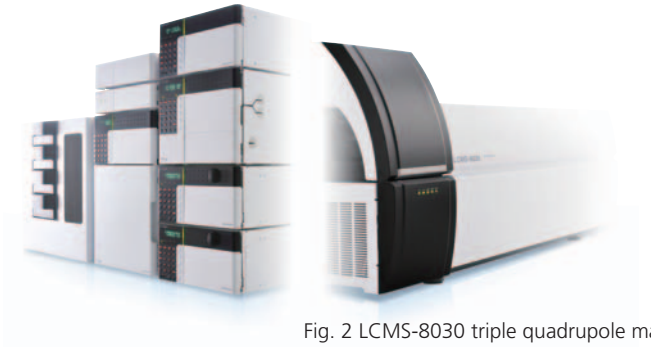

Fig. 2 LCMS-8030 triple quadrupole mass spectrometer

**High Speed Mass Spectrometer**

Polarity Switching
15 msec
Scanning Speed
Max. 15000 u/sec

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# Results

## Method development for 14 mycotoxins

For UHPLC separation, various LC mobile phase conditions were examined. Tailing of fumonisins peaks were observed when only ammonium acetate was added in mobile phase. It was found that pH of a mobile phase effected peak shape of fumonisins. In order to reduce tailing of fumonisins, acetic acid was added in mobile phase B and the gradient program was controlled to maintain high concentration of acetic acid when fumonisins were eluted. By controlling the concentration of acetic acid and ammonium acetate with gradient program, 14 mycotoxins were separated and detected excellently in 11 minutes (Fig. 3).

### Analytical Conditions for LC-MS/MS

**HPLC: UFLC system**

Column: YMC-TriartC18 100 mm × 2.0 mm, 1.9 um
Mobile phase A: 10 mM Ammonium acetate - Water
B: 2% Acetic acid - Methanol
Flow rate: 0.4 mL/min
Gradient program: B conc.2% (0 min) - 55% (3 min) - 85% (7.0-8.0 min) - 2% (8.01-11 min)
Column temperature: 40°C

**MS: LCMS-8030 triple quadrupole mass spectrometer**

Ionization: ESI, Positive/Negative MRM mode Ion spray voltage: -3.5 kV

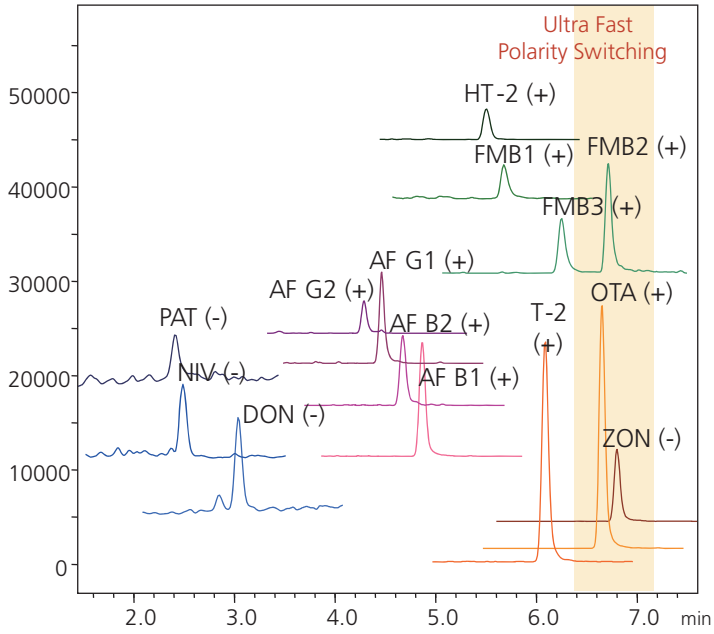


Fig. 3 14 mycotoxins analysis by LC-MS/MS

MRM Transition

| Mycotoxin | MRM transition |
|---|---|
| AF G1 (+) | 329.05 > 243.05 |
| AF G2 (+) | 331.00 > 245.00 |
| AF B1 (+) | 313.00 > 241.05 |
| AF B2 (+) | 315.00 > 259.00 |
| HT-2 (+) | 442.00 > 263.05 ($[M+NH_4]^+$) |
| T-2 (+) | 483.95 > 305.00 ($[M+NH_4^+$) |
| OTA (+) | 404.10 > 238.90 |
| ZON (-) | 317.15 > 273.00 |
| NIV (-) | 371.10 > 281.25 ($[M+CH_3COO]^-$) |
| DON (-) | 355.10 > 295.15 ($[M+CH_3COO]^-$) |
| PAT (-) | 153.10 > 109.20 |
| FM B1 (+) | 722.45 > 334.30 |
| FM B2 (+) | 706.45 > 336.25 |
| FM B3 (+) | 706.45 > 336.25 |

Each mycotoxin standard was analyzed at six concentration levels. Good linearity was observed in the calibration curves, and excellent sensitivity was achieved.

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Table 1 Linearity 14 mycotoxins

| Mycotoxin | Range | Coefficient ($r^2$) | Mycotoxin | Range | Coefficient ($r^2$) |
|---|---|---|---|---|---|
| AF G1 | 0.4-20 ppb | 0.999 | ZON | 2-100 ppb | 0.999 |
| AF G2 | 0.4-20 ppb | 0.999 | NIV | 2-100 ppb | 0.999 |
| AF B1 | 0.4-20 ppb | 0.999 | DON | 2-100 ppb | 0.997 |
| AF B2 | 0.4-20 ppb | 0.999 | PAT | 10-100 ppb | 0.999 |
| HT-2 | 2-100 ppb | 0.998 | FM B1 | 2-100 ppb | 0.995 |
| T-2 | 2-100 ppb | 0.999 | FM B2 | 2-100 ppb | 0.994 |
| OTA | 2-100 ppb | 0.999 | FM B3 | 2-100 ppb | 0.997 |

## Rinse condition for eliminating carry over

Carry over of fumonisins was initially observed using the general rinse condition, because fumonisins formed complexes with trace metal ions in the sample's flow path. Probably, several carboxyl groups of fumonisins coordinated with metal ion (Fig. 4).

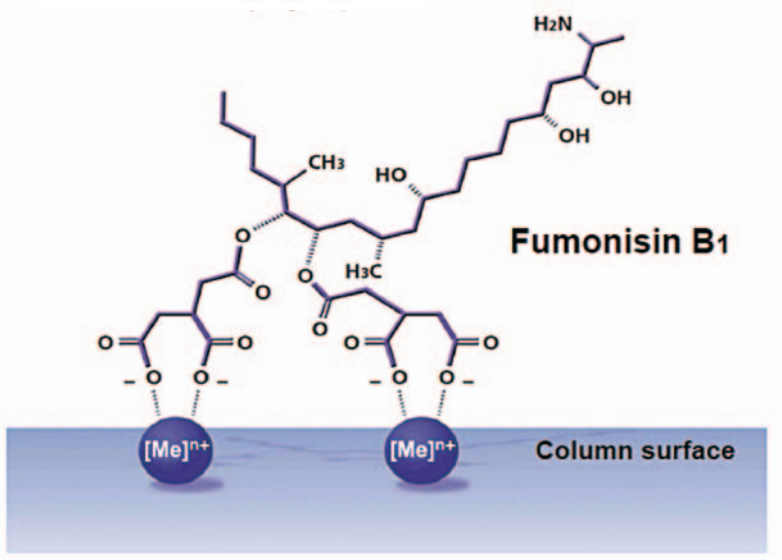


Fig. 4 Possible coordination interaction with metal ion

For eliminating carry over, rinse solvent and rinse method were examined. The performance of Nexera autosampler SIL-30AC, which can wash both inner and outer needle surfaces with 4 different solvents, was used.
It was thought that carboxyl groups of fumonisins may preferentially pair with hydrogen ions in the presence of low pH, therefore formic acid was added to rinse solvent. When investigating rinse methods, it was discovered the inner and outer rinse of needle reduced carry over more than the outer rinse of needle. Finally the modified rinse solvent consisted of:1% formic acid aq./methanol / acetonitrile / isopropanol (1/1/1/1).
To test the modified rinse cycle method, one injection of the 100 ppb fumonisins standard solution was followed by one blank injection to check for carryover. Fig. 5 shows chromatograms of the standards of FMB2 and B3, and the following blank injection. Low carry over was observed in the blank injection. It resulted from washing fumonisins adsorbed inside needle with the needle's inner and outer rinse method and the effective rinse solvent.

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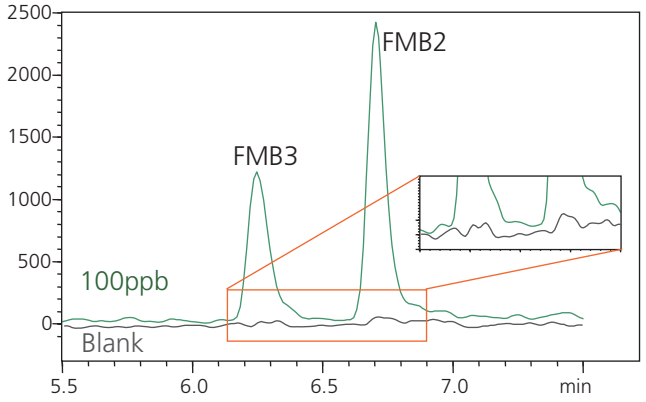


Fig. 5 Carry over evaluation of fumonisins

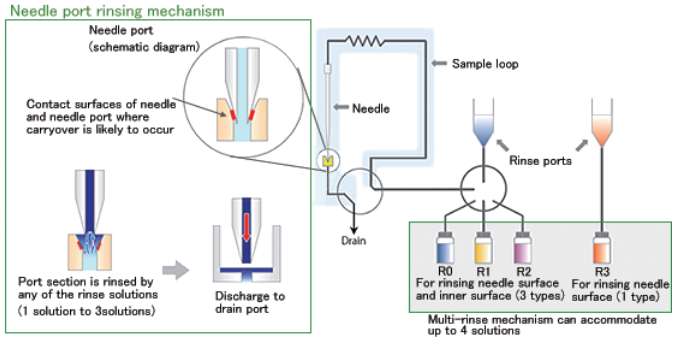


Fig. 6 HPLC path of SIL-30AC

## Quantitative Analysis of 14 mycotoxins in beer-based drinks

Mycotoxins were extracted from samples and were purified with a solid phase extraction (SPE) cartridge.
20 commercial beers were analyzed by using this method. The calibration curves were assessed using beer samples spiked with mycotoxins. PAT, AFB1, B2, G1, G2, NIV, T-2 ,ZON and FMB3 were not detected in any of the beer samples. Some of the tested samples were found to be contaminated with DON, HT-2, OTA, FMB1 and B2 at concentrations of less than their respective LOQs (each 5 ppb).

Table 2 Result of mycotoxin analysis in beer samples (14samples made in 11countries)

| Concentration of mycotoxin/ppb (detected rate) | | | | |
|---|---|---|---|---|
| DON | HT-2 | FMB1 | FMB2 | OTA |
| Trace | Trace | Trace | Trace | Trace |
| 2 | 1 | 2 | 1 | 1 |

Trace is less than 5 ppb

# Conclusions

- High throughput LC-MS/MS method for 14 mycotoxins was developed, and could be applied to the quantification of these compounds in beers.
- Carry over of fumonisins was eliminated by using both the needle's inner and outer rinse method with effective rinse solvent.
- Result of mycotoxin analysis in beer samples indicates that the health risk to consumers posed by intake mycotoxins in commercial beers is relatively low.

# Acknowledgemets

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