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A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

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Overview

Development of a rapid simple screening method by LC/MS/MS for mycotoxins containing fumonisins.

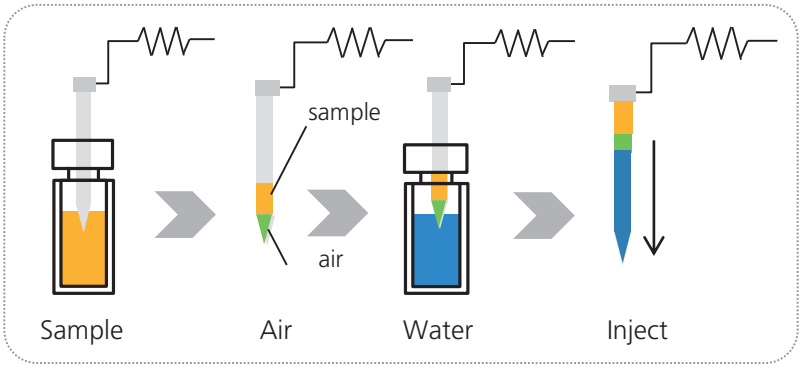
Introduction

Mycotoxin is a chemical substance produced by mold. In terms of harmful substances to health of human and livestock, regulatory limitation is defined in each country. In recent years, the risk management of mycotoxins has been gaining wider acceptance all over the world. In this report, we demonstrate a simultaneous screening analysis for 18 kinds of mycotoxins in grains by LC/MS/MS. For the purification of grain extract, Multitoxin Spin Column (Romer Labs) was utilized in order to perform a simple and rapid clean-up treatment for mycotoxins, which has individual chemical properties, such as aflatoxins, ochratoxin A, trichothecenes, and fumonisins.

Methods and Materials

Analytical conditions

Analysis was performed by a LCMS-8050 which was equipped with a Nexera™ X2 UHPLC. Pentafluorophenyl (PFP) bounded column was used to separate the regioisomeric pair (3-AcDON / 15-AcDON, FB2 / FB3) by gradient elution with a series of mobile phases containing ammonium acetate, acetic acid and methanol. Quantitative limits had been deemed to be less than or equivalent to the minimum values specified in EC/1886/2006.
The developed method achieved the simultaneous determination of mycotoxins such as aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), ochratoxin A (OTA), trichothecenes [(3-acetyldeoxynivalenol(3-AcDON), 15-acetyldeoxynivalenol (15- AcDON), deoxynivalenol (DON), HT-2, nivalenol (NIV), T-2, zearalenone (ZEN)), Fusarenon-X (FUX) , Diacetoxy- scirpenol (DAS)] and patulin (PAT) in 15 minutes analytical cycle. This analytical method was developed by the modified LC/MS/MS method package for mycotoxin (Shimadzu Corporation, Japan).



Inject method

Improving peak shape of NIV solved in more than 50% of acetonitrile aqueous solution, the sample solutions should be injected with additional water. SIL-30AC autosampler has this useful function shown as above.

Nexera is a trademark of Shimadzu Corporation.
MycoSpin is a trademark of Romer Labs Division Holding GmbH.

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High Speed Mass Spectrometer
Ultra Fast Polarity Switching -5 msec
Ultra Fast MRM -Max.555 transition/sec

Figure 1 LCMS-8050 triple quadrupole mass spectrometer

UHPLC conditions (Nexera™ X2 system)	
Column	: Mastro PFP 2 (150 mm×2.1 mm, 3 µm)
Mobile phase A	: 10 mmo/L Ammonium acetate-water
Mobile phase B	: Methanol including 2% acetic acid
Flow rate	: 0.4 mL/min
Time program	: B conc.15%(0 min) -35%(1.51 min) – 45%(5.50 min) - 60%(5.51 min) – 95%(9.50-12.00 min) - 15%(12.01-15.00 min)
Column temp.	: 40 °C
Injection vol.	: 2.5 µL with 50 µL Water
Rince R0	: Mobile phase A
Rince R1	: 10 mmol/L Sodium citrate aqueous solution
Rince R2	: Water/ Methanol / Acetonitrile/ IPA = 1/1/1/1 including 1% formic acid
Needle rinse program	: inside: R1 → R0 → R2 → R0, outside: R3(1 sec) → R0
MS conditions (LCMS-8050)	
Ionization	: ESI, Positive/Negative MRM mode
DL temp.	: 150 °C
Interface temp.	: 200 °C
Heat block temp.	: 400 °C
Nebulizer gas	: 2.5 L /min
Heating gas	: 15 L/min
Drying gas	: 5 L/min

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Table 1 MRM transitions of Mycotoxins

No.	Mycotoxin	Retention Time (min)	Polarity	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)
1	Nivalenol (NIV)	2.261	-	371.10	281.10	15
2	Patulin (PAT)	2.569	-	153.00	109.10	13
3	Deoxynivalenol (DON)	2.998	-	355.10	295.10	10
4	Fusarenon-X (FUX)	3.827	-	413.10	353.10	9
5	15-AcetylDeoxynivarenol (15-AcDON)	5.582	+	339.10	261.10	-12
6	3-AcetylDeoxynivarenol (3-AcDON)	5.751	+	339.10	231.10	-14
7	Aflatoxin G2 (AF G2)	7.197	+	331.10	245.10	-30
8	Diacetoxy- scirpenol (DAS)	7.480	+	384.20	307.10	-13
9	Aflatoxin G1 (AF G1)	7.433	+	329.10	243.10	-27
10	Aflatoxin B2 (AF B2)	7.669	+	315.10	259.10	-30
11	Fumonisin B1 (FB1)	7.804	+	722.40	334.10	-42
12	Aflatoxin B1 (AF B1)	7.904	+	313.10	241.10	-39
13	HT-2 toxin (HT-2)	8.060	+	442.20	263.10	-13
14	Fumonisin B3 (FB3)	8.107	+	706.40	336.10	-35
15	Fumonisin B2 (FB2)	8.475	+	706.40	336.10	-39
16	T2-toxin (T-2)	8.705	+	484.30	185.10	-23
17	Ochratoxin A (OTA)	8.987	+	404.10	239.10	-24
18	Zearalenone (ZEN)	9.532	-	317.10	131.10	30

Sample preparation

Analytical samples were prepared through the extraction protocol of MycoSpin™400 (Romer Labs), which is a very convenient method without evaporator nor nitrogen purge procedures. The operation of MycoSpin™400 was completed within 5 minutes.

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2.5 g Wheat / Corn (add 100 ng each standard)

Add 10 mL 50% Acetonitrile Solution
(or 85% Acetonitrile Solution)

Shake for 90 minutes

Centrifuge at 3200 rpm., 10 minutes

1 mL of the supernatant, add 50 µL acetic acid

Transfer 750 µL of the solution
to the MycoSpin™ column

Cap MycoSpin™ column and vortex for 1 minute

Break bottom tip off of MycoSpin™

Centrifuge at 10000 rpm., 30 seconds

Obtain 350 µL of purified extract

Each 45 µL of purified extract with 5 µL standard solution
(0, 100 ,200, 500 ng/mL)

Inject onto the LC/MS/MS system

Figure 2 Protocol of sample preparation

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Result

Analysis of Standard Solution

Figure 3 shows MRM chromatograms of the 18 mycotoxin standards (each 10 ng/mL).
At first, we evaluated the solvent for better recovery of the mycotoxin from MycoSpin™ column.

In comparison, with 85% acetonitrile aqueous solution, better recovery of fumonisins was obtained with 50% acetonitrile aqueous solution (Fig.4). Thus, we decided to use 50% acetonitrile aqueous solution for the extraction solvent.

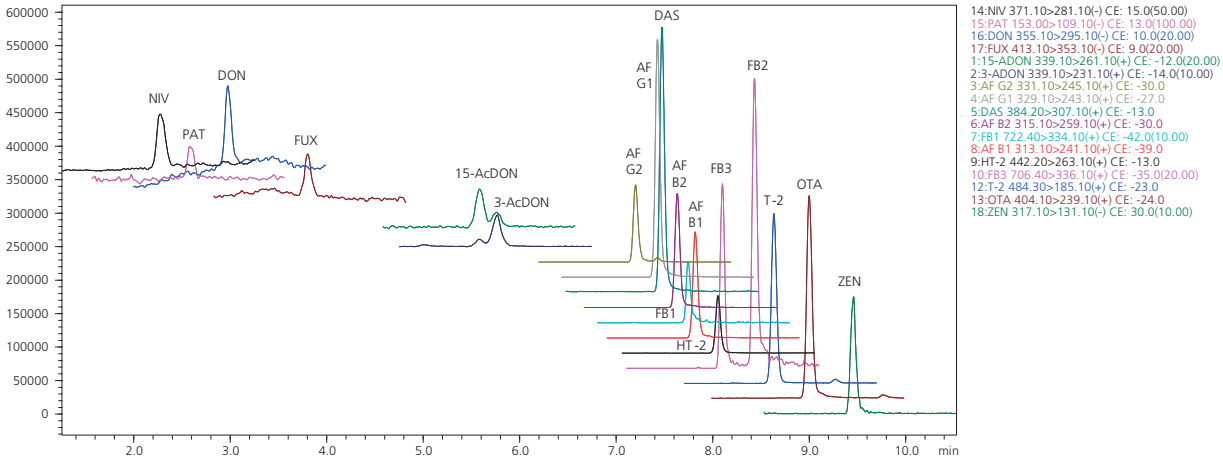

Figure 3 MRM chromatograms of the 18 mycotoxin standards (each 10 ng/mL).

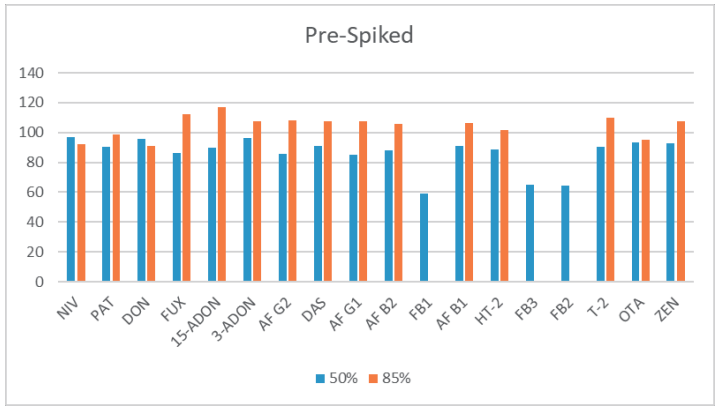

Figure 4 Recovery (%) of the mycotoxin standard from MycoSpin™ column.
(Each 10 ng/mL standard mixture was applied on the column.)

Evaluation of the matrix effect

Figure 5 shows recovery (%) of the mycotoxin standards in the four kinds of extraction as wheat, corn powder, peanut powder, and almond powder. MycoSpin™ protocol was convenient in short timescale. However, even after the clean-up, many matrix compounds remained and was affected.(Fig. 5). Although under this situation, it usually requires each labeled internal standard for target compound, we tried to investigate the quantify by the standard additive method.

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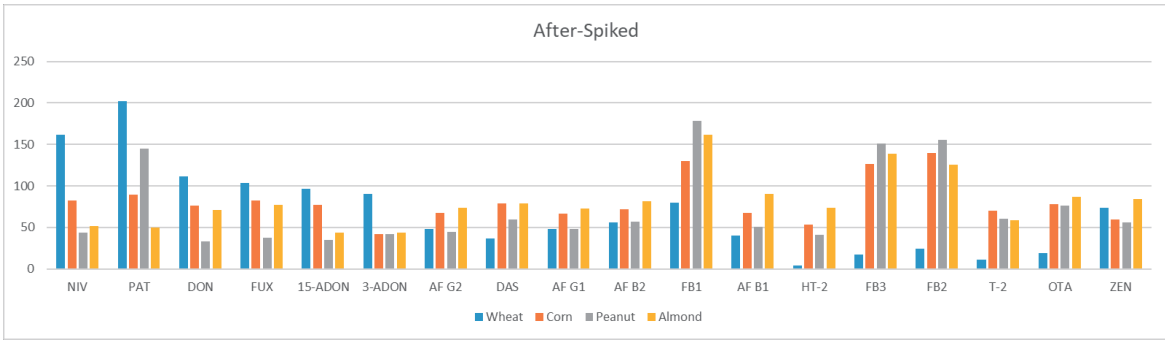

Figure 5 Recovery (%) of the mycotoxins under the four kinds of extractions.
The each standard was spiked after clean-up procedure (final each 10 ng/mL).

Quantitative analysis

The results of quantitative analysis from wheat and corn powder using the sample preparation protocol (2.2) is summarized in table 2 through using the standard additive method instead of the internal standard method. The results indicates that the standard addition calibration method could help correct and improve the recovery rate even under the influence of the matrix effect.
Using this method, only a small amount of mycotoxins were detected in corn powder below the regulation value.

Table 2 The results of wheat and corn powder

	Wheat			Corn		
	Result (mg/Kg)	Recovery (%)	%RSD (n=2)	Result (mg/Kg)	Recovery (%)	%RSD (n=2)
NIV	N.D.	93	0.86	N.D.	134	2.00
PAT	0.0350	94	5.36	N.D.	79	15.60
DON	0.0148	128	6.34	0.1376	114	10.66
FUX	N.D.	101	0.30	N.D.	149	0.02
15-AcDON	N.D.	119	10.73	0.0213	114	14.30
3-AcDON	N.D.	106	7.64	N.D.	117	8.26
AF G2	N.D.	104	4.27	N.D.	115	1.80
DAS	N.D.	118	4.24	N.D.	137	2.25
AF G1	N.D.	101	3.45	N.D.	119	1.11
AF B2	N.D.	110	0.07	N.D.	125	5.95
FB1	N.D.	60	18.33	0.0407	94	15.16
AF B1	N.D.	104	2.06	0.0011	124	2.32
HT-2	N.D.	107	9.21	0.0008	132	3.91
FB3	N.D.	70	19.66	0.0078	83	7.19
FB2	N.D.	66	6.23	0.0094	79	5.80
T-2	N.D.	105	4.79	0.0005	119	1.80
OTA	N.D.	97	12.29	N.D.	109	4.38
ZEN	N.D.	97	0.90	0.0120	103	3.37

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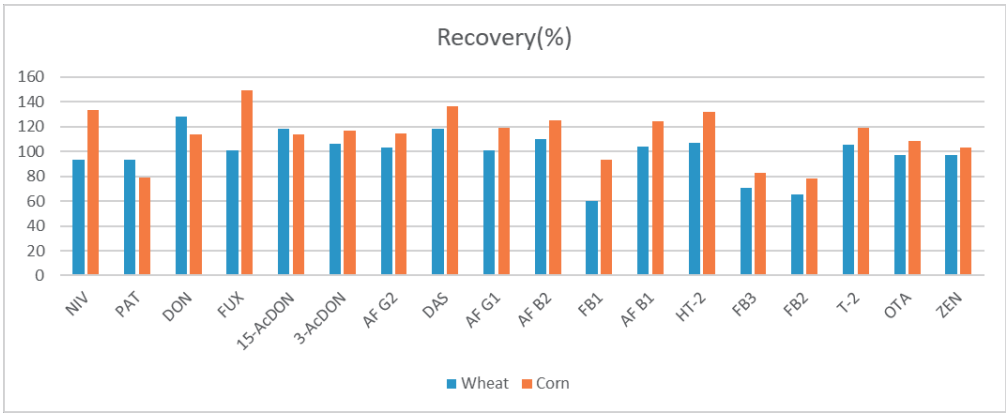

Figure 6 Recovery (%) of the mycotoxin standards from wheat and corn powder (Each 100 ng standard was spiked)

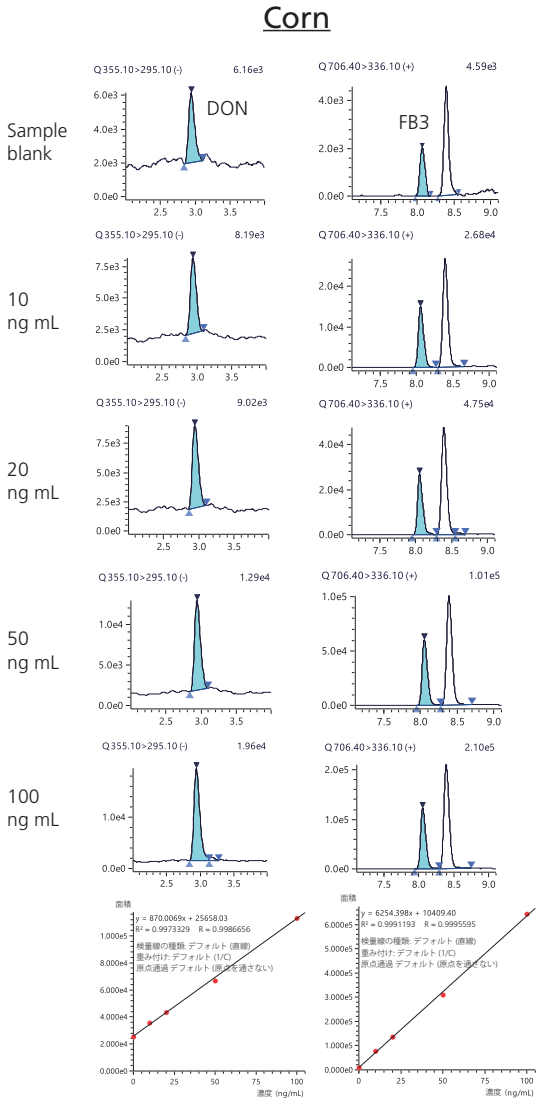

Figure 7 Chromatograms and calibration curves of the corn extraction (DON and FB3).

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Conclusions

- A rapid screening method for mycotoxins had been established.
- The LC/MS/MS method package for mycotoxin (Shimadzu Corporation, Japan) is useful tools for this type of analysis.
- The extraction solvent to improve the recovery rate of fumonisins was optimized.
- We investigated the standard addition method in order to compensate for the effect of the matrix.
- We plan to continue to evaluate multi-function columns or ion exchange columns for further improvement of the clean-up.

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