PO-CON1807E

SHIMADZU
Excellence in Science

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

ASMS 2018 MP 266

Eishi IMOTO[1], Naoki MOCHIZUKI[2], Jun WATANABE[1]
1 Shimadzu Corporation, MS Business Unit, Kyoto, Japan.
2 Yokohama University Pharmacy, Kanagawa, Japan.

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Introduction

There are various substances that can threaten the food safety, such as pesticides, mycotoxins. LC-MS/MS analysis is a prevailing technique for the detection of these substances in food. Mycotoxins are especially frequent contaminants of agricultural products, and brewers are concerned that they can give serious damages to consumers, for example liver cancer, nephritis, pulmonary edema and so on. This is the reason why most countries have adopted regulations to limit exposure to mycotoxins, while the regulated mycotoxins and value differ with countries. The toxicity and potential health hazards induced by mycotoxins demand the need for sensitive, robust analytical methodologies. This research provides a LC-MS/MS system for quantitative screening of mycotoxins and includes a multi-mycotoxin sample preparation column to cover worldwide regulations. Although LC-MS/MS is a highly sensitive analytical technique, the problem of carryover occurs frequently. Metal-free column and multi-rinse mode were performed for reduction of carryover.

Fig 1. LC-MS/MS system (Nexera X2+LCMS-8060, Shimadzu Corporation.)

Methods and Pretreatment

19 mycotoxins (Nivalenol, Patulin, Doxynivalenol-3-Glucoside, Deoxynivalenol, Fusarenon-X, Neosoraniol, 3-Acetyl-Deoxynivalenol, 15-Acetyl-Deoxynivalenol, Aflatoxin B1, B2, G1, G2, Diacetoxyscirpenol, Fumonisin B1, B2, B3, T-2 toxin, Ochratoxin A, Zearalenone) were used for evaluation of matrix effect and recovery rates in wheat. These mycotoxins were diluted with ACN at 5 ng/mL. Ground wheat flour samples were mixed with water/acetonitrile. After filteration, extracts were diluted with aqueous acetic acid solution and mixed with mycotoxins at 5 ng/mL. The solution were loaded to into the spin purification column (MycoSpin™400, Romer Lab) and analyzed using a triple quadrupole mass spectrometer (LCMS-8060, Shimadzu Corp.).

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Table 1. LC and MS conditions

[LC] Nexera™ X2 System

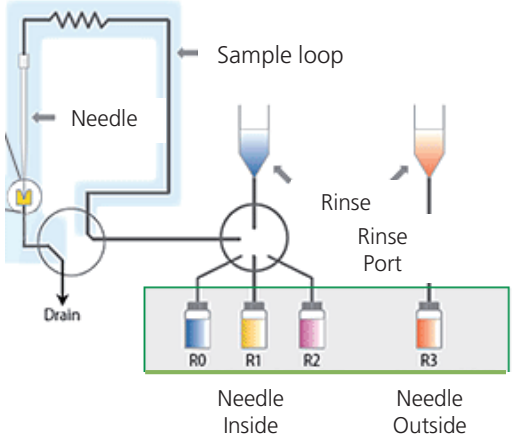
Analytical Column : Mastro™ PFP2 (Shimadzu GLC Ltd) 2.1 mmI.D.×150 mmL., 3 µm
Solvent A : 10 mmol/L ammonium acetate
Solvent B : 2% acetic acid in methanol
Gradient Program :

Time (min)	%B
1.00	40
1.50	40
1.51	50
5.50	50
5.51	65
9.50	70
9.51	95
13.00	95
13.01	20
15.00	STOP

Flow Rate : 0.4 mL/min
Column Temp : 40 °C

[MS] LCMS-8060

Ionization : ESI (Positive/Negative)
Nebulizer Gas : 2 L/min
Interface temperature : 300 °C
Desolvation Line : 250 °C
Heat Block temperature : 500 °C
Heating Gas : 10 L/min
Drying Gas : 10 L/min



< Rinse Program >

R0	10 mmol/L ammonium acetate
R1	10 mmol/L sodium citrate
R2, R3	1% Formic acid + water/MeOH/ACN/IPA= 1/1/1/1 (v/v)

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Table 2. MRM transitions for mycotoxins :Positive :Negative

No.	Mycotoxins	RT (min)	Polarity	transition	CE
1	NIV	1.88	-	371.10>281.10	16
2	PAT	2.22	-	153.00>109.00	11
3	D3G	2.30	-	517.20>427.20	21
4	DON	2.40	-	355.10>265.10	15
5	FUX	2.94	-	413.10>353.10	9
6	NEO	3.05	+	400.20>305.10	-12
7	15-ADON	3.74	+	339.10>261.10	-11
8	3-ADON	3.86	+	339.10>231.10	-14
9	AF G2	4.87	+	331.10>245.10	-31
10	AF G1	5.55	+	329.10>243.10	-30
11	DAS	5.78	+	384.20>307.10	-13
12	AF B2	6.22	+	315.10>259.10	-30
13	AF B1	6.96	+	313.10>241.10	-39
14	FB1	7.37	+	722.40>334.10	-43
15	FB3	8.08	+	706.40>336.10	-38
16	T-2	8.71	+	484.30>185.10	-20
17	FB2	8.97	+	706.40>336.10	-39
18	OTA	9.73	+	404.10>239.10	-24
19	ZEN	10.8	-	317.10>130.10	35

Details of sample preparation

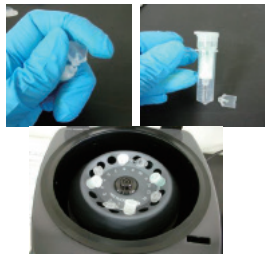
1. Mix a ground wheat flour sample (50.0 g) with 100.0 mL of water/acetonitrile (15/85), and shake for 30 minutes

2. Filter the supernatant using glass-fiber filter paper (pore size < 0.7 um)

3. Add 500.0 µL of acetic acid to the filtrate (10.0 mL): Solution A

4. Load 1.0 mL of Solution A into the spin purification column and mix using vortex mixer for 1 minute while capped

5. Remove the bottom tip of the column and centrifugation for 2 minutes at 10,000 rpm



6. Transfer the supernatant into a vial then serve to the sample

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Results

Typical MS chromatogram for mycotoxins in ACN are shown in Fig. 2. An LC-MS/MS method was developed that achieved good separation and sensitivity for the detection of all mycotoxins without separating method for its polarity. Autosampler rinsing capabilities and metal free column were used to minimize the carryover of the fumonisins. Matrix effect was calculated by the peak area of mycotoxins (5 ng/mL) in ACN and post spiked samples.

Recovery rate was calculated by the peak area of post spiked samples (5 ng/mL) and pre spiked samples (5 ng/mL) which is shown in Table 3. NIV, DON, AF B1, T-2, ZEN were influenced wheat extractions which dramatically decrease the ionization efficiency of the mycotoxins. Recovery rate of the NIV, D3G, DON, T-2, ZEN were also insufficient. Therefore, internal standards are required for achieving accurate quantitative results.

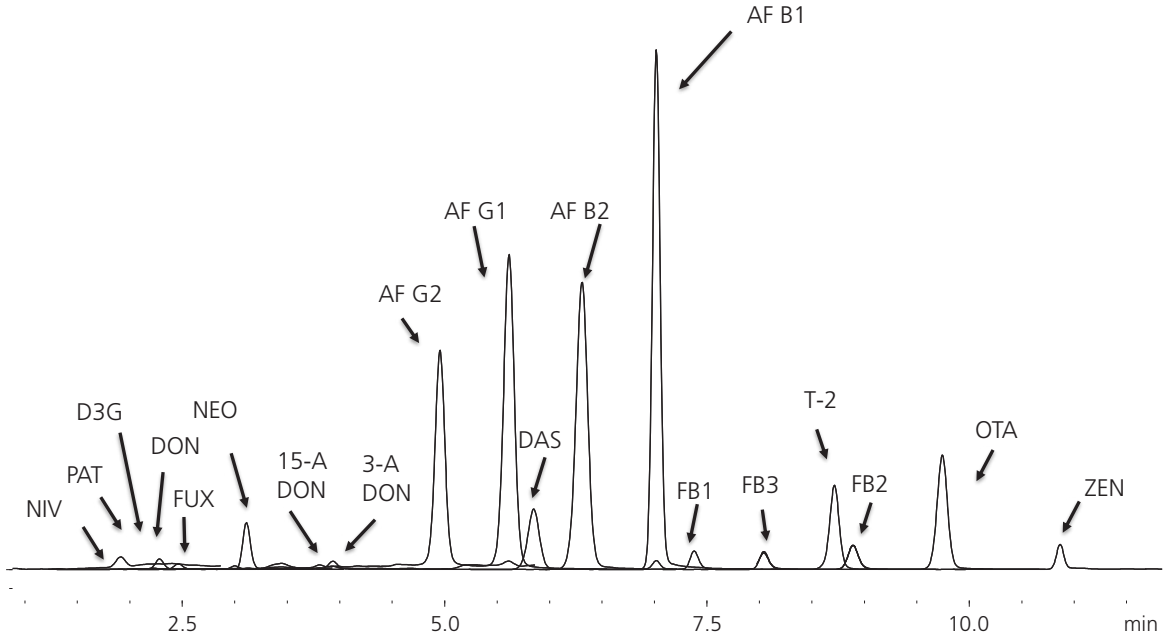


Fig 2. Typical MS chromatogram for mycotoxins mixture (50 ppb)

Table 3. Matrix effect and recovery rate of the mycotoxins in wheat matrix (5 ppb)

No.	Mycotoxins	Matrix Effect (%)	Recovery Rate (%)
1	NIV	35.0	156.6
2	PAT	71.6	115.8
3	D3G	34.4	166.8
4	DON	47.3	143.9
5	FUX	81.8	99.3
6	NEO	74.2	95.1
7	15-ADON	72.6	87.7
8	3-ADON	87.6	78.6
9	AF G2	78.3	70.7
10	AF G1	85.6	65.5

No.	Mycotoxins	Matrix Effect (%)	Recovery Rate (%)
11	DAS	84.6	76.4
12	AF B2	80.6	75.0
13	AF B1	33.8	65.3
14	FB1	73.6	128.6
15	FB3	71.5	120.1
16	T-2	51.8	52.0
17	FB2	68.6	122.2
18	OTA	42.3	111.5
19	ZEN	40.4	28.0

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

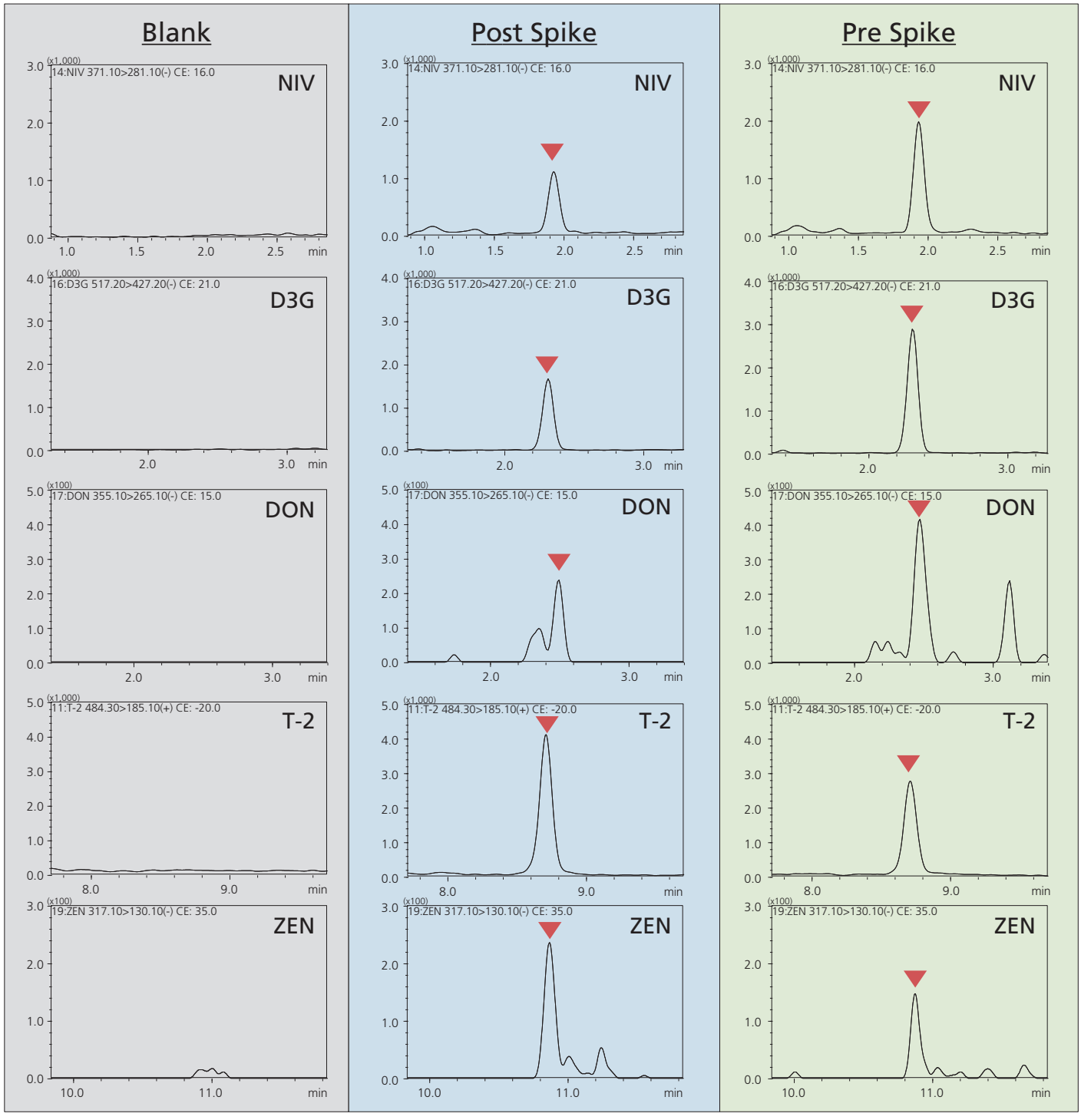


Fig 3. MS chromatograms of NIV, D3G, DON, T-2, ZEN which are pre-spiked in and post-spiked in wheat extraction at 5 ng/mL

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Conclusion

- This LC-MS/MS method and one step sample preparation measured various types of mycotoxins which spiked in wheat matrix.
- Sensitivity of some mycotoxins were decreased because of matrix effect.
- For accurate quantitative measurement, internal standard is necessary

Reference

1) Masayoshi TAMURA, Keiko MATSUMOTO, Jun WATANABE, Naoki MOCHIZUKI, et al., *Journal of separation science*, 2014, **37**, 1552-1560

The product and application are Research Use Only. Not for use in human clinical diagnostics or in vitro diagnostic procedures.

First Edition: June, 2018

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/