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

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1. 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.)

2. Methods and Pretreatment

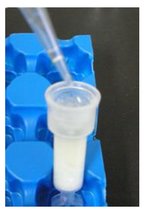
Ground rice flour samples were mixed with water/acetonitrile, and shaked. After filtration on glass fiber paper, extracts were diluted with aqueous acetic acid solution. This solution was then loaded into the spin purification column (MycoSpin™400, Romer Lab) and vortex-mixed before centrifugation. Aflatoxins, Deoxynivalenol, 3-Acetyl-deoxynivalenol, 15-Acetyl-deoxynivalenol, Nivalenol, Fusarenon-X, Fumonisins, Ochratoxin A, T-2 toxin, HT-2 toxin, Zearalenone (Wako, Japan) were spiked to the extraction. Then, samples were analyzed using a triple quad mass spectrometer (LCMS-8060, Shimadzu Corporation).

1. Mix a ground rice 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

Multi-rinse mode

Nexera X2 system has multi-rinse mode which uses 4 types rinses (R0, R1, R2, R3) one by one. R0：10 mM ammonium acetate, R1：10 mM sodium citrate, and R2, R3：H2O：MeOH：ACN：IPA=1：1：1：1 (v/v) with 1% formic acid were used for the solution. Rinsing workflow is R1-R0-R2-R0 for inside and R3-R0 for outside of the needle.

Table 1. LC and MS conditions

[LC] NexeraX2 System			
Analytical Column	: Mastro PFP2 (Shimadzu GLC Ltd., 2.1 mmI.D.×150 mm., 3 µm)		
Mobile Phase A	: 10 mmol/L ammonium acetate		
Mobile Phase B	: 2% acetic acid in methanol		
Gradient program	: 0 min (20%B) -> 1 min (40%B) -> 1.5 min (40%) -> 1.51 min (50%B) -> 5.5 min (50%B) -> 5.51 min (65%B) -> 9.5 min (70%B) -> 9.51 min (95%B) -> 13 min (95%B) -> 13.01 min (20%B) -> 15 min (STOP)		
Flow rate	: 0.4 mL/min		
Column temperature	: 40 ℃		
Injection Volume	: 1 µL		
[MS] LCMS-8060			
Ionization mode	: ESI (positive/negative)	Interface temperature	: 300 ℃
Nebulizer Gas	: 3 L/min	DL temperature	: 250 ℃
Drying Gas	: 10 L/min	Heat Block temperature	: 500 ℃
Heating Gas	: 10 L/min		

Table 2. MRM transitions for mycotoxins

Name	Polarity	Retention Time (min)	Precursor Ion	Product Ion 1	Product Ion 2	Product Ion 3
Aflatoxin B1	+	6.960	313.10	241.10	285.10	269.10
Aflatoxin B2	+	6.224	315.10	259.10	287.10	243.10
Aflatoxin G1	+	5.552	329.10	243.10	311.10	200.10
Aflatoxin G2	+	4.870	331.10	245.10	313.10	189.10
Deoxynivalenol	-	2.404	355.10	295.10	265.10	138.10
3-Acetyl-deoxynivalenol	+	3.861	339.10	231.10	203.10	213.10
15-Acetyl-deoxynivalenol	+	3.737	339.10	261.10	137.10	321.10
Nivalenol	-	1.883	371.10	281.10	311.10	59.00
Fusarenon-X	-	2.943	413.10	353.10	263.10	59.00
Fumonisin B2	+	8.972	706.40	336.10	318.10	354.10
Fumonisin B3	+	8.076	706.40	336.10	318.10	354.10
Ochratoxin A	+	9.730	404.10	239.10	358.10	221.10
T-2 Toxin	+	8.714	484.30	185.10	305.10	215.10
HT-2 Toxin	+	7.449	442.20	263.10	215.10	145.10
Zearalenone	-	10.842	317.10	131.10	175.10	130.10

3. Results

MRM chromatogram for mycotoxins in rice matrix are shown in Fig. 2. The electrospray ionization parameters such as drying gas temperature, drying gas flow rate and nebulizing gas flow rate were optimized by flow injection. An LC-MS/MS method was developed that achieved good separation and sensitivity for the detection of all mycotoxins. Carryover of fumonisins was minimized thanks to the metal-free column and autosampler rinsing capabilities.

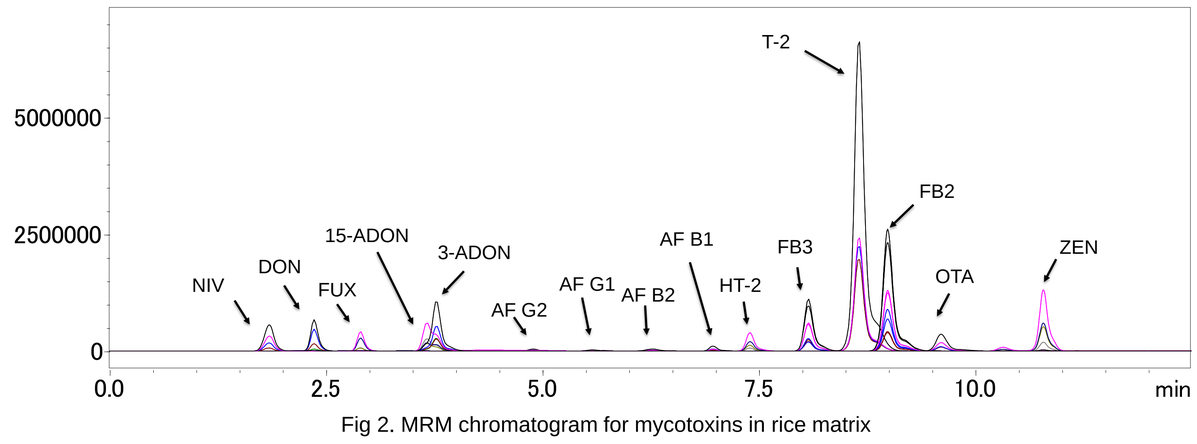


Fig 2. MRM chromatogram for mycotoxins in rice matrix

Table 3. Range, linearity and LOQ of the mycotoxins spiked in rice matrix in comparison with the world wide regulations for mycotoxins in food and feed in 2003

Name	Range (µg/kg)	Linearity	LOQ (µg/kg)	World wide limits (µg/kg) for mycotoxins in food
Aflatoxin B1	0.025-25	0.999	0.05	1-35
Aflatoxin B2	0.025-25	0.999	0.05	1-35
Aflatoxin G1	0.025-25	0.999	0.10	1-35
Aflatoxin G2	0.025-25	0.999	0.10	1-35
Deoxynivalenol	5-5000	0.999	25	300-2000
3-Acetyl-deoxynivalenol	5-5000	0.999	25	
15-Acetyl-deoxynivalenol	5-5000	0.999	25	
Nivalenol	5-5000	0.999	50	
Fusarenon-X	2.5-2500	0.999	25	
Fumonisin B2	2.5-2500	0.999	2.5	1000-3000 (total fumonisins)
Fumonisin B3	2.5-2500	0.998	2.5	
Ochratoxin A	0.0125-12.5	0.998	0.0125	3-50
T-2 Toxin	0.1875-187.5	0.999	0.1875	
HT-2 Toxin	0.1875-187.5	0.999	0.1875	
Zearalenone	0.5-500	0.998	0.50	50-1000

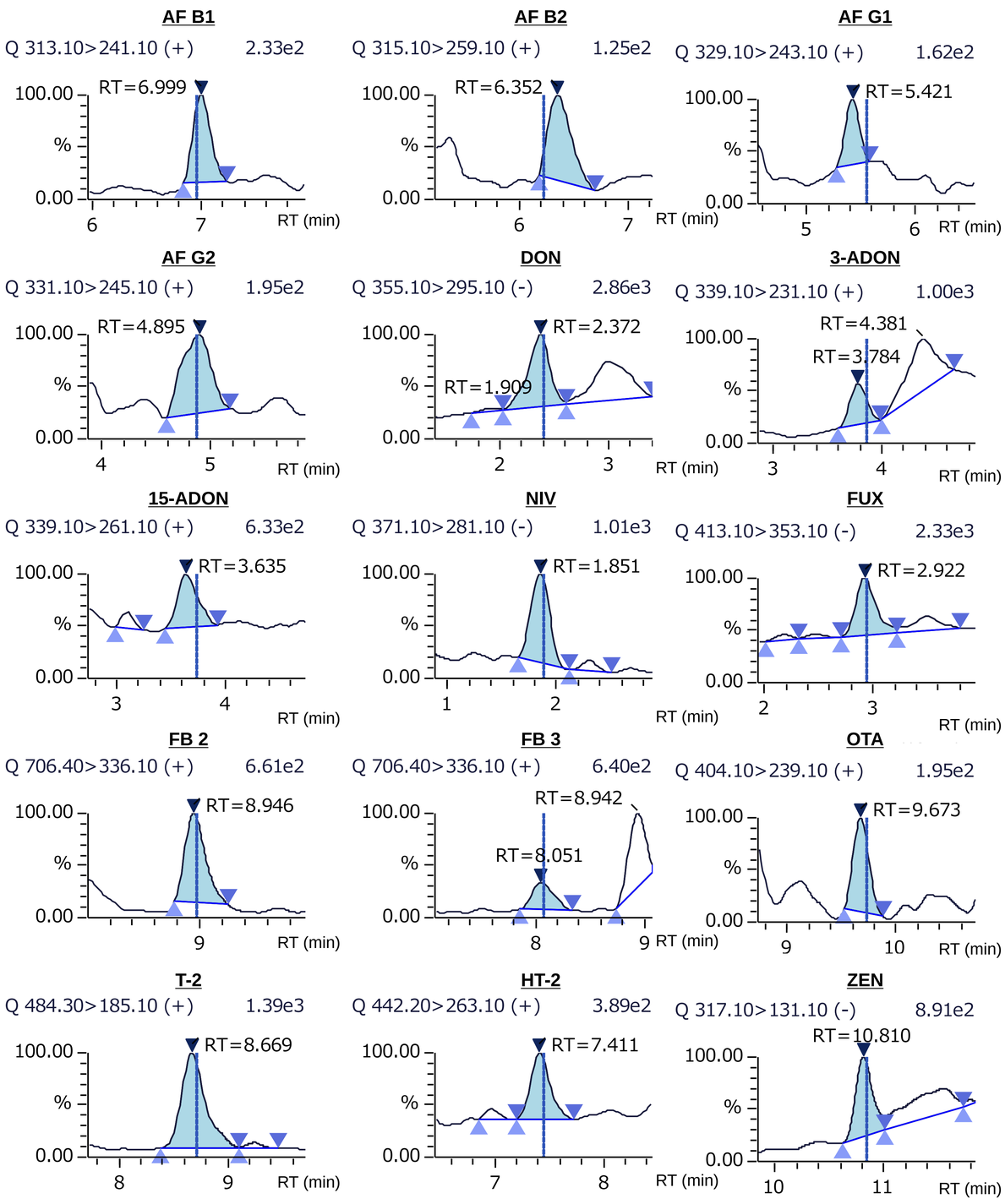


Fig 3. MRM chromatogram for each mycotoxin in rice matrix at LOQ.

5. Conclusion

- This LC-MS/MS method measured various types of mycotoxins at once
- Good linearity and separation were achieved with a single sample preparation

Reference

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