CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 43, Issue 2, February 2015
Online English edition of the Chinese language journal

Cite this article as: Chin J Anal Chem, 2015, 43(2), 264–270.

RESEARCH PAPER

Determination of 26 Mycotoxins in Feedstuffs by Multifunctional Clean-up Column and Liquid Chromatography-Tandem Mass Spectrometry

WANG Rui-Guo, SU Xiao-Ou*, CHENG Fang-Fang, WANG Pei-Long, FAN Xia, ZHANG Wei
Institute of Quality Standards and Testing Technology for Agricultural Products, Chinese Academy of Agricultural Science, Key laboratory of Agrifood Safety and Quality, Ministry of Agriculture, Beijing 10081, China

Abstract: A rapid high-throughput method for the determination of 26 mycotoxins involving multifunctional clean-up column coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated for the determination in feedstuffs. The feedstuff samples were extracted by ultrasonic treatment for 1 h, and the mixture of acetonitrile/water/formic acid (84:15.9:0.1, *V/V*) was used as the extraction solvent. After purified by a commercial Mycospin 400 multifunctional clean-up column, 1 mL of the supernatant layer was evaporated and redissolved by 0.25 mL of water/methanol/formic acid (95:4.9:0.1, *V/V*) in a vial for injection into the LC-MS/MS system. Then chromatographic analyses were carried out on a reversed phase C18 column using a gradient elution with 0.1% formic acid aqueous solution and 0.1% formic acid methanol solution. After that，the mass spectrometer was operated in a multiple reaction monitoring (MRM) mode selected one precursor ion and two product ions for each target compound. Validation studies were carried out in maize and soybean meal as representative matrixes. Since most target compounds had different levels of matrix effects, matrix-matched calibration was adopted for quantification. Mean recoveries from spiked samples at three levels ranged from 61.9% to 119.5% with relative standard deviations of 0.8%–18.6%. Limits of quantification ranged from 0. 5 μg kg^{-1} to 50 μg kg^{-1}.

Key Words: Liquid chromatography-tandem mass spectrometry; Feedstuff; Multifunctional clean-up column; Mycotoxins

1 Introduction

Mycotoxins are secondary metabolites produced by different fungal species[1], they can contaminate feedstuffs and cause a toxic response when ingested by vertebrate species[2]. Up to now, approximately more than 400 different mycotoxins have been identified in the world[3]. However, only dozens of mycotoxins are of particular interest for their harmful effects, such as aflatoxins, deoxynivalenol, T-2 toxin and zearalenone, which are associated with acute and long-term chronic effects thus leading to anorexia, lower reproductive performance, higher disease incidence, vomiting, diarrhea and organ necrosis, etc[4]. Now, mycotoxins are adopted to food safety monitoring system by World Health Organization (WHO)[5]. In China, the tolerance limits for aflatoxin B_1, deoxynivalenol, T-2, ochratoxin A and zearelenone in animal feed and feedstuffs were established[6–10]. For analysis, the methods based on thin-layer chromatography (TLC), enzyme-linked immune-sorbent assay (ELISA) and high-performance liquid chromatography (HPLC) were developed. But these methods were commonly adopted as rapid screening, qualitative or quantitative analysis for individual or several kinds of mycotoxins[3]. In fact, feedstuffs are contaminated by various toxins simultaneously[11]. However, due to the advantages such as high efficiency separation, simultaneous qualitative and quantitative analyses, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become a mainstream of mult-mycotoxins detection method in recent years[12].

Received 31 August 2014; accepted 21 November 2014

*Corresponding author. Email: suxiaoou@caas.cn

This work is sponsored by the National Science & Technology Program in Rural Areas during the 12th Five-year Plan Period of China (No. 2011BAD26B0405).

DOI: 10.1016/S1872-2040(15)60807-6

Therefore multi-mycotoxins LC-MS/MS methods were developed for the analysis of Chinese medicines with on-line immune-affinity purifying by Zhao *et al*[13], and for feedstuffs analysis with multi-function cartridges by Ying *et al*[14]. Zheng *et al*[15] detected 13 mycotoxins in grains by LC-TOF-MS. However, the above methods have the some disadvantages, such as the need of special process equipment, high cost of immune affinity column, complex process containing de-fat procedure, or ESI^+ and ESI^- monitoring separately. The objective of this experiment was to develop a rapid high-throughput LC-MS/MS method utilizing a simple and efficient sample extraction to simultaneously detect 26 mycotoxins in feedstuffs.

2 Experimental

2.1 Apparatus and reagents

TQD UPLC-tandem mass spectrometer (Waters, USA), RVC 2-18 desktop vacuum concentrator (CHRIST Inc., Germany), 3K15 high speed refrigerated centrifuge (Sigma, USA), D37520 high-speed centrifuge (Kendro Laboratory Products, Inc., USA), MycoSpin 400 multitoxin clean-up column (Romer Labs, Inc., USA) were used in the experiment.

The information of the standards and mixed standard stock solution are listed in Table 1. Ultrapure water was obtained from a MilliQ Gradient System (Merck Millipore, China). Acetonitrile, methanol, ammonium acetate, and formic acid were of high performance liquid chromatography grade and purchased from Fisher Scientific (USA). The mixed standard stock solution was stored in amber vial and kept in a freezer at –20 °C. A series of mixed work solutions were prepared by accurately pipetting appropriate amount of mixed stock solution, and evaporated with the desktop vacuum concentrator, and then redissolved with water/methanol/formic acid (95:4.9:0.1, *V/V*). The mixed work solutions were prepared immediately before use.

Mycotoxins-free corn and soybean meal samples were kindly provided by China National Feed Quality Control Center (Beijing).

2.2 Sample treatment

The mycotoxins-free corn and soybean meal samples were kept in a dark, cool room at 4 °C until analysis. Then, 5.00 g± 0.01 g of the ground sample was weighted in a 50-mL plastic centrifuge tube and extracted with 20 mL of the organic extraction solvent mixture of acetonitrile/water/formic acid (84:15.9:0.1, *V/V*) by ultrasonic treatment for 1 h and shaking every 20 min. After centrifuged at 10000 rpm for 10 min in a Centrifuge 3K15, 1 mL of the supernatant layer was transferred to Mycospin 400 column. Subsequently, the column was capped and shaken for approximately 2 min by vortex. Then, the bottom tip of the column was broken off and placed into a centrifuge tube with centrifuging for 1 min at 5000 rpm. The total filter solution was evaporated at 60 °C in the desktop vacuum concentrator. After the residue was

Table 1 Mycotoxin information

Serial	Mycotoxin	Abbr.	Molecular formula	CAS number	Purity (%)	Concentration in stock solution ($\mu g\ mL^{-1}$)
1	Deoxynivalenol	DON	$C_{15}H_{20}O_6$	51481-10-8	99	5
2	Nivalenol	NIV	$C_{15}H_{20}O_7$	23282-20-4	99	5
3	Aflatoxin B1	AFB_1	$C_{17}H_{12}O_6$	1162-65-8	99	1
4	Aflatoxin B2	AFB_2	$C_{17}H_{14}O_6$	7220-81-7	99	1
5	Sterigmatocysin	STE	$C_{18}H_{12}O_6$	10048-13-2	99	1
6	Aflatoxin G1	AFG_1	$C_{17}H_{12}O_7$	1165-39-5	99	1
7	Aflatoxin G2	AFG_2	$C_{17}H_{14}O_7$	7241-98-7	99	1
8	15-Acetyldeoxynivalenol	15-AcDON	$C_{17}H_{22}O_7$	88337-96-6	99	5
9	3-Acetyldeoxynivalenol	3-AcDON	$C_{17}H_{22}O_7$	50722-38-8	99	5
10	Methylergonovine	MET	$C_{20}H_{25}N_3O_2$	113-42-8	99	1
11	T2 Triol	T2 Triol	$C_{19}H_{30}O_7$	97373-21-2	99	1
12	Diacetoxyscirpenol	DIA	$C_{19}H_{26}O_7$	2270-40-8	99	1
13	Roquefortine C	RC	$C_{22}H_{23}N_5O_2$	58735-64-1	99	1
14	Neosolaniol	NEO	$C_{19}H_{26}O_8$	36519-25-2	99	5
15	Citreoviridin	CIT	$C_{23}H_{30}O_6$	25425-12-1	99	5
16	HT-2 toxin	HT-2	$C_{22}H_{32}O_8$	26934-87-2	98	5
17	T-2 toxin	T-2	$C_{24}H_{34}O_9$	21259-20-1	98	1
18	Verruculogen	VER	$C_{27}H_{33}N_3O_7$	12771-72-1	98	5
19	Ergomaizeine	ERG	$C_{31}H_{39}N_5O_5$	564-36-3	98	1
20	2-bromo-alpha-ergocryptine	BrERG	$C_{32}H_{40}BrN_5O_5$	22260-51-1	95	1
21	Zearalenone	ZEN	$C_{18}H_{22}O_5$	17924-92-4	99	5
22	α-Zearalenol	α-ZEL	$C_{18}H_{24}O_5$	6455-72-8	98	5
23	β-Zearalenol	β-ZEL	$C_{18}H_{24}O_5$	71030-11-0	98	5
24	Zearalanone	ZAN	$C_{18}H_{24}O_5$	5975-78-0	99	5
25	α-zearalanol	α-ZAL	$C_{18}H_{26}O_5$	26538-44-3	97	5
26	β-zearalanol	β-ZAL	$C_{18}H_{26}O_5$	42422-68-4	97	5