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Simultaneous Detection Method for Mycotoxins and their Metabolites in Animal Urine by Using Impurity Adsorption Purification followed by Liquid Chromatography-Tandem Mass Detection

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Abstract

A novel method for simultaneous detection of mycotoxins (e.g., aflatoxin B_1) or their metabolic residues in animal urine with impurity adsorption purification followed ultra-performance liquid chromatography coupled tandem mass spectrometry (UPLC-MS/MS) detection has been developed. Extraction of mycotoxins or their metabolites in animal urine sample was performed with 0.1% formic acid-acetonitrile solution after addition of sodium chloride. The extract was then dehydrated and purified with hydrous magnesium sulfate, C_{18}, primary secondary amine, and alumina-A. 3 mL of the supernatant was evaporated and re-dissolved by 0.5 mL of 0.1% formic acid aqueous solution/ acetonitrile (70:30, *V/V*) for UPLC-MS/MS detection. A C_{18} reversed-phase chromatographic column was employed for separation of target analytes. In the chromatographic separation of target analytes, 0.1% formic acid aqueous solution and 0.1% formic acid-methanol solution were used as the mobile phases with the optimum gradient elution procedures. Multiple-reaction monitoring (MRM) mode was applied for qualitative and quantitative analysis, and matrix calibration curves obtained with the external-standard method was used for quantitation of target analytes. Under optimized conditions, the linearity range was 0.05-100 ng/mL, and the limit of quantification of the developed method was 0.05-0.25 ng/mL. The recoveries of mycotoxins and their metabolites spiked in urine samples were from 80.8% to 114.3%, and the relative standard deviation was <15%.

Keywords: Impurity adsorption purification; UPLC-MS/MS; Mycotoxins; Animal urine

Introduction

Mycotoxins are the secondary toxic metabolites generated during the growth process of mold [1]. So far, the number of mycotoxin species with an identified chemical structure have reached over 400 [2], and they are mainly the metabolites of *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, and *Claviceps* [3]. Cereals, oil seeds, and animal feeds made thereof are subject to contamination by various mycotoxins, thereby acutely or chronically poisoning humans and animals [4]. Research indicates that about 25% of agricultural products across the world may be contaminated by mycotoxins, so mycotoxins in the food chain are necessary to monitor and control [5]. World health organization (WHO) has regarded mycotoxins as items that should be monitored under the food-safety system [6], and China has established the maximum limit for mycotoxins in food and animal feeds [7-11]. Generally, mycotoxin exposure is measured by analyzing mycotoxin content in food or animal feeds [12]. In recent years, some researchers have revealed mycotoxin exposure and the *in vivo* absorption and metabolic process of mycotoxins by using mycotoxins in human or animal urine or their metabolites as biomarkers [13-15]. However, most available methods can be used to detect merely a single type of mycotoxin or mycotoxins with similar structure, and simultaneous contamination by different types of mycotoxins is inevitable because animal feeds are made of various raw materials [16]. Thus, a method that can simultaneously detect the various mycotoxins in animal urine is urgent and significant to develop for the purpose of food safety and mycotoxins exposure study.

In this study, a novel and simple clean-up method based on impurity adsorption mechanism has been developed to purify 25 mycotoxins and their metabolites in animal urine. The developed clean-up method with UPLC-MS/MS detection was easy and quick to operate, low cost, can achieve high quantitative accuracy. The proposed simultaneous detection method can also be used to evaluate the coexposure of mycotoxins on animals and to perform toxic kinetic studies of mycotoxins.

Experiment

Reagents and chemicals

The stock standard solutions of mycotoxins and their metabolites were prepared with acetonitrile as solvent and the detailed information of preparation has been listed in Table 1. The stock solution was stored at -20°C. When it was used, an appropriate amount of solution was dried with the vacuum centrifugal concentrator and then dissolved in 0.1% formic acid aqueous solution-acetronitrile (70:30, *v/v*) to prepare standard working solutions of different concentrations. Acetonitrile, methanol and formic acid are chromatographic pure (Fisher, USA), and Milli-Q ultrapure water were used in the experiment. Sodium chloride and anhydrous magnesium sulfate (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. Octadecyl bonded silica (C_{18}), primary secondary amine (PSA), alumina-A (A-AL) and graphitized carbon black (GCB) were purchased from Bonna-Agela Technologies Company, China. Blank pig-urine samples were donated by the National Feed Quality Control Center (Beijing).

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No	Analyte	Formula	CAS number	Purity (%)	Concentration in stock solution (μg/mL)
1	Aflatoxin B$_1$ (AFB$_1$)	$C_{17}H_{12}O_6$	1162-65-8	99	1
2	Aflatoxin B$_2$ (AFB$_2$)	$C_{17}H_{14}O_6$	7220-81-7	99	1
3	Aflatoxin G$_1$ (AFG$_1$)	$C_{17}H_{12}O_7$	1165-39-5	99	1
4	Aflatoxin G$_2$ (AFG$_2$)	$C_{17}H_{14}O_7$	7241-98-7	99	1
5	Aflatoxin M$_1$ (AFM$_1$)	$C_{17}H_{12}O_7$	6795-23-9	99	1
6	Aflatoxin M$_2$ (AFM$_2$)	$C_{17}H_{14}O_7$	6885-57-0	99	1
7	Sterigmatocysin (STE)	$C_{18}H_{12}O_6$	10048-13-2	99	1
8	T-2 toxin (T-2)	$C_{24}H_{34}O_9$	21259-20-1	98	1
9	Lysergol (LYS)	$C_{16}H_{18}N_2O$	602-85-7	99	1
10	Methylergonovine (MET)	$C_{20}H_{25}N_3O_2$	113-42-8	99	1
11	Roquefortine C (RC)	$C_{22}H_{23}N_5O_2$	58735-64-1	99	1
12	Diacetoxyscirpenol (DIA)	$C_{19}H_{26}O_7$	2270-40-8	99	1
13	Deoxynivalenol (DON)	$C_{15}H_{20}O_6$	51481-10-8	99	5
14	3-AcetylDeoxynivalenol (3-AcDON)	$C_{17}H_{22}O_7$	50722-38-8	99	5
15	15-AcetylDeoxynivalenol (15-AcDON)	$C_{17}H_{22}O_7$	88337-96-6	99	5
16	Neosolaniol (NEO)	$C_{19}H_{26}O_8$	36519-25-2	99	5
17	Wortmannin (WOR)	C23H24O8	19545-26-7	99	5
18	Verruculogen (VER)	$C_{27}H_{33}N_3O_7$	12771-72-1	98	5
19	HT-2 toxin (HT-2)	$C_{22}H_{32}O_8$	26934-87-2	98	5
20	Zearalenone (ZEN)	$C_{18}H_{22}O_5$	17924-92-4	99	5
21	α-Zearalenol (α-ZEL)	$C_{18}H_{24}O_5$	6455-72-8	98	5
22	β-Zearalenol (β-ZEL)	$C_{18}H_{24}O_5$	71030-11-0	98	5
23	Zearalanone (ZAN)	$C_{18}H_{24}O_5$	5975-78-0	99	5
24	α-Zearalanol (α-ZAL)	$C_{18}H_{26}O_5$	26538-44-3	97	5
25	β-Zearalanol (β-ZAL)	$C_{18}H_{26}O_5$	42422-68-4	97	5

Table 1: Information of standards of 25 mycotoxins.

Instrumentation

Ultra performance liquid chromatography (Acquity UPLC) coupled tandem mass spectrometer (XEVO TQ-S, Waters, USA) was employed to detect mycotoxins and their metabolites with MRM mode.

For the chromatographic conditions, the BEH RP18 chromatographic column (Acquity UPLC, 100 mm × 2.1 mm; 1.7 µm; Waters, USA) was used as analytical column to separate target analytes. The column temperature was 40°C and flow rate was 0.3 mL/min. 0.1% formic acid aqueous solution (mobile phase A) and 0.1% formic acid-methanol (mobile phase B) were used as mobile phase with gradient elution. The gradient elution procedures were as followed: 0-2 min, 95% A; 2-4 min, 95%-80% A; 4 min-12 min, 80%-5% A; 12-12.1 min, 5%-1% A; 12.1-14 min, 1% A; 14-14.1 min, 1%-95% A; and 14.1-16 min, 95% A. The injection volume was 5 µL.

The detection of target analytes was carried out on Xevo TQ tandem MS platform fitted with ESI probe operated in the positive ion and negative ion mode and the target analytes was monitored under MRM mode. The optimal parameters as followed, capillary voltage, 2800 V; ion source temperature, 150°C; desolvation gas temperature, 400°C; desolvation gas flow rate, 800 L/h. Argon was used as the collision gas, and the collision cell pressure was 4 m Bar. Other parameters were shown in Table 2. Instrument control, data acquisition and data processing were carried out with Masslynx V 4.1 software (Waters, MA, USA). Positive-ion monitoring mode was used to monitor mycotoxin nos. 1-19, and negative-ion monitoring mode was used to monitor mycotoxin nos. 20-25. Multiple-reaction monitoring (MRM) mode was used to detect and monitor ions, and the parameters including collision energy and cone voltage are shown in Table 2.

The urine sample preparation was performed with RVC 20-18 desktop vacuum centrifugal concentrator (CHRIST, Germany), 3K15 high-speed refrigerated centrifuge (Sigma, USA), D37520 high-speed centrifuge (Kendro, USA), and VX-III multitube vortexer (Targin Technology, China).

Sample preparation

Exactly 2.0 mL of pig-urine sample was transferred into a 50 mL plastic centrifuge tube with a 5 mL pipette. 6 mL of 0.1% formic acid-acetronitrile solution was added after addition of 0.8 g of sodium chloride. The mixture was shook with a high-speed vortexer for 1 min and then centrifuged at 8000 r/min for 5 min. 4 mL of upper supernatant was transferred into 10 mL plastic centrifuge tube. 500 mg of anhydrous magnesium sulfate, 50 mg of C_{18}, 50 mg of PSA, and 50 mg of aluminia A were added, successively. The mixture was shook with a high-speed vortexer about 1 min and then centrifuged at 10000 r/min for another 5 min. The supernatant was passed through a 0.22 µm nylon membrane filter. Exactly 3 mL of the filtrate was placed in a 10 mL plastic centrifugal tube, which was then placed in the vacuum centrifugal concentrator to drain the liquid at 60°C and 1500 r/min. Residues were re-dissolved with 0.5 mL of 0.1% formic acid aqueous solution-acetronitrile (70:30, *v/v*) and then centrifuged at 13000 r/min for 5 min. The supernatant was collected in a vial for further detection.

Results and Discussion

Optimization of instrumental parameters

The UPLC-MS/MS is a powerful separation and detection platform in multi residues analysis. The chromatographic separation condition and MS parameters were important factors that needed to optimize in different instrument. In this study, Xevo TQ UPLC-MS/MS was used to determine mycotoxins and their metabolites in throughout study. UPLC separation and MS/MS detection conditions for target analytes were examined.

Methanol-water (50:50, *v/v*) was used as the mobile phase. Combined sample injection was adopted to optimize mass-spectrometry conditions for the 25 mycotoxins and their metabolites. The appropriate molecular ion peaks and ionization mode were selected after a full scan under the positive- and negative-ion modes. $[M+H]^+$, $[M+NH_4]^+$, or $[M+Na]^+$ were obtained under positive-ion mode for parts of mycotoxins (1-19), and $[M-H]^-$ was obtained under negative-ion mode for another parts of mycotoxins (20-25). Based on the scan of the ions in the blank matrix solution and matrix standard solution, the parameters were further optimized, and the characteristic ion pairs collected under MRM mode for each mycotoxin type were identified (Table 2). For isomers featuring identical retention times, i.e., 3-AcDON and 15-AcDON, their respective unique ion fragments were used as product ions for monitoring.

Based on early findings of the experiment [17], 0.1% formic acid aqueous solution and 0.1% formic acid-methanol were used as mobile phases. Gradient elution was carried out, and peak signals of analytes were collected by stages, so the method was applicable to the simultaneous detection of various mycotoxins. Furthermore, isomers with identical ion fragments, i.e., α-ZEL, β-ZEL, and ZAN, as well as α-ZAL and β-ZAL, could be well separated according to their retention times. Figure 1 shows the chromatograms of quantification transitions of the blank pig urine matrix-matched standard.

Optimization of clean-up procedures

Usually, sample preparation is the most crucial step in a simultaneous method due to the different properties of the target analytes that have

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No	Analyte	Retention time (min)	Precursor ion (*m/z*)	Product ions (*m/z*)	Cone voltage (V)	Collision energy (eV)	Dwell time (ms)
1	AFB_1	8.27	313.14[M+H]$^+$	285.03*/241.15	60/60	36/22	25
2	AFB_2	7.95	315.14[M+H]$^+$	287.15*/259.13	52/52	28/26	25
3	AFG_1	7.75	329.14[M+H]$^+$	199.94*/214.90	40/40	40/30	25
4	AFG_2	7.41	331.14[M+H]$^+$	189.14*/245.18	64/64	38/28	25
5	AFM_1	7.48	329.10[M+H]$^+$	273.15*/311.12	2/2	22/18	25
6	AFM_2	7.05	331.10[M+H]$^+$	273.14*/285.16	2/2	22/22	25
7	STE	10.85	325.14[M+H]$^+$	253.20*/115.11	66/66	60/42	25
8	T-2	9.65	484.28[M+NH_4]$^+$	305.23*/185.17	6/6	18/12	25
9	LYS	4.34	255.23[M+H]$^+$	240.19*/197.15	48/48	22/20	25
10	MET	5.10	340.21[M+H]$^+$	223.22*/208.07	56/56	28/24	25
11	RC	7.82	390.21[M+H]$^+$	193.12*/322.24	52/52	26/20	25
12	DIA	8.10	384.24[M+NH_4]$^+$	247.22*/105.09	6/6	30/14	25
13	DON	4.46	297.20[M+H]$^+$	249.19*/91.05	28/28	42/10	25
14	3-AcDON	6.78	339.19[M+H]$^+$	231.16*/203.21	2/2	14/10	25
15	15-AcDON	6.78	339.24[M+H]$^+$	321.27*/261.19	8/8	12/6	25
16	NEO	6.11	400.24[M+NH_4]$^+$	185.17*/305.24	2/2	20/12	25
17	WOR	9.05	447.33[M+NH_4]$^+$	345.23*/285.23	4/4	20/16	25
18	VER	10.73	534.25[M+Na]$^+$	392.23*/191.10	44/44	20/12	25
19	HT-2	9.05	442.34[M+NH_4]$^+$	263.20*/215.22	10/10	10/16	25
20	ZEN	10.90	317.18[M-H]$^-$	175.04*/131.03	68/68	30/26	25
21	α-ZEL	10.75	319.22[M-H]$^-$	160.09*/130.12	68/68	36/30	25
22	β-ZEL	10.98	319.22[M-H]$^-$	160.09*/275.24	68/68	30/20	25
23	ZAN	10.36	319.22[M-H]$^-$	275.24*/205.15	68/68	22/20	25
24	α-ZAL	10.72	321.24[M-H]$^-$	277.26*/303.26	68/68	22/24	25
25	β-ZAL	10.13	321.24[M-H]$^-$	277.26*/303.26	68/68	28/24	25

Table 2: Optimized MS/MS parameters of 25 mycotoxins.

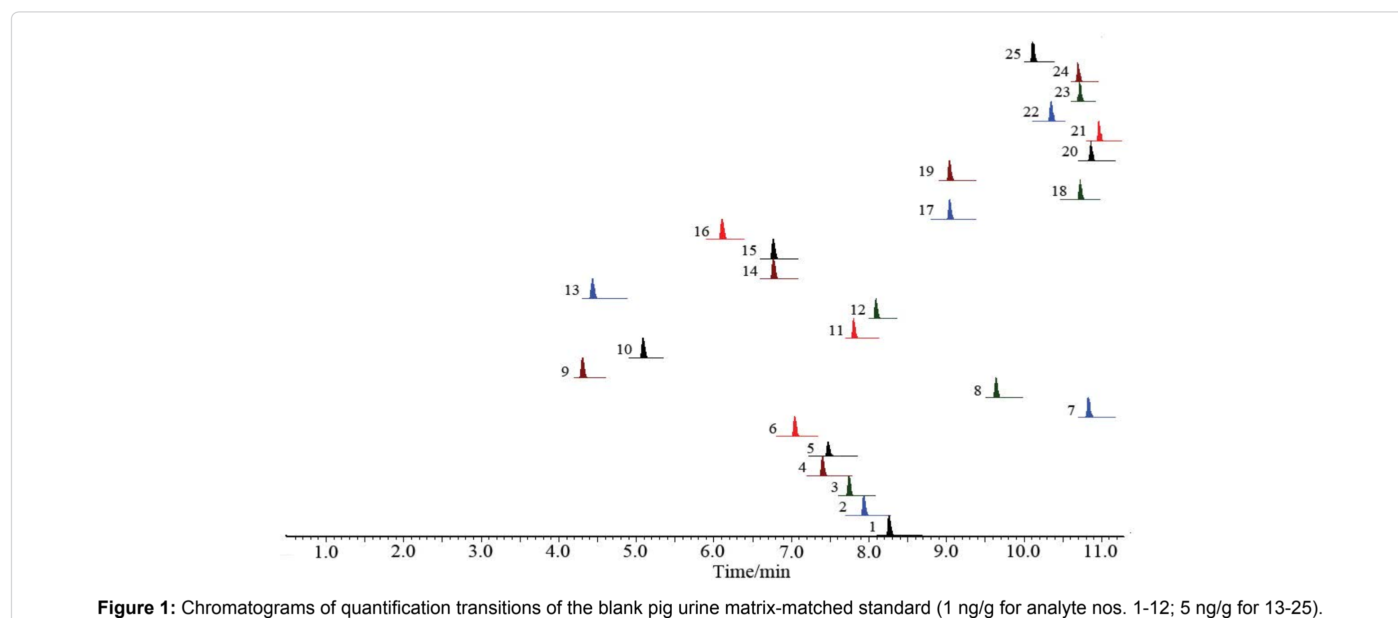

Figure 1: Chromatograms of quantification transitions of the blank pig urine matrix-matched standard (1 ng/g for analyte nos. 1-12; 5 ng/g for 13-25).

to be extracted and cleaned simultaneously. Traditionally, the solid phase extraction and immuno affinity were usually clean-up method for mycotoxins and their metabolite in various samples. In this study, we developed a novel impurity adsorption clean-up method for 25 mycotoxins and their metabolites in urine sample. Firstly, the clean-up effect of C_{18}, PSA, A-AL, and GCB on the target analytes in pig-urine samples was initially evaluated. Results indicated that GCB resulted in poor recovery for most of target analytes due to strong absorbing for mycotoxins and their metabolites. Instead, C_{18}, PSA, and A-AL had no significant absorption effect, with recovery of 80%-120% for all target mycotoxins and their metabolites. C_{18} could significantly reduce the odor of pig-urine samples and can absorb the pigment substance in the samples. PSA could effectively remove the pigment substance from the samples and exerted an enhancing effect on the mass spectrum peak signals of most analytes. A-AL could significantly decrease matrix interference to the analytes including AFB_1, T-2, DON, and ZEA and also improve detection sensibility, although it cannot effectively remove the odor or pigment substance from the samples.

Then the amount of C_{18}, PSA, and A-AL was optimized by examining the recovery of target analytes and baseline of extraction solution. The results indicated that the optimum amount of C_{18}, PSA and A-AL was 25 mg per millitre of urine sample. The developed sample preparation procedures could obtain clean extracts and consistent chromatographic responses, and in addition, the sample treatment time was reduced.

Evaluation of matrix effects

When ESI is used as the ionization technique in mass spectrometry, one of the main problems is the signal suppression or enhancement of the analytes due to the other components present in the matrix (matrix effect). To evaluate matrix effects, 0.1% formic acid aqueous solution-acetonitrile (70:30, *v/v*) was used to prepare mixed solvent standard solutions with different concentrations of target analytes (0.05, 0.1, 0.5, 1.0, 5.0, and 20.0 ng/mL for analyte nos. 1-12; 0.25, 0.5, 2.5, 5.0, 25.0 and 100.0 ng/mL for analyte nos. 13-25). Meanwhile, the pig-urine sample was extracted, purified, and analyzed according to the developed impurity adsorption clean-up procedures. After confirming that no trace analyte remained, the blank sample was used to dilute the matrix-matched standard sample that had the same concentration as the matrix-matched standard sample. The slope and correlation coefficient (R^2) of solvent and matrix-matched calibration curves are shown in Table 3. The slope ratios between pig urine matrix-matched and solvent calibration of the target mycotoxins (Figure 2) reflected the intensity of matrix effect, and the range 80%-120% was regarded as acceptable. A range that exceeded 80%-120% indicated a strong matrix suppression effect or a strong matrix-induced response-enhancement effect. The slopes of target compounds were compared (Table 3), and it can be observed that a matrix effect was noticed for some compounds such as AFM_1, DIA, DON, 15-AcDON and 3-AcDON etc. So it was necessary to avoid the matrix effect, matrix-matched calibration standard curves were selected to quantify target compounds in urine. Therefore, blank pig-urine was selected as representative sample matrix to analysis spiked blank samples of urine at concentration level of 5.0 ng/mL. The recoveries of different class compounds were above 80%. It was demonstrated that the developed extraction and clean-up protocol based on impurity adsorption is suitable for the tested mycotoxins and their metabolites.

Analytical performance of the developed method

The blank sample extract was used to prepare the mixed standard solutions with concentrations ranging from 0.05 ng/mL to 20 ng/mL for analytes nos. 1-12 and from 0.25 ng/mL to 100 ng/mL for analytes nos. 13-25. The limit of quantitation (LOQ) of the method was determined according to 10 times of the ratios of signal to noise (S/N). The R^2 that was obtained exceeded 0.99. Results are shown in Table 4.

Recovery studies were performed at three spiked concentration levels (0.5, 5.0 and 50 ng/mL), fortifying six blank pig-urine samples with target analytes at each fortification level. The obtained results are shown in Table 4 and the chromatogram of spiked sample (0.5 ng/mL) was shown in Figure 3. It can be observed that the recoveries of target analytes in pig-urine samples varied from 80.8% to 114.3%. Repeatability (intra-day precision) was evaluated at the three concentration levels of the recovery study, performing six replicates for each level (Table 4), it can be observed that repeatability, expressed as RSD, were lower than 10.3% for all the cases. Whereas reproducibility (inter-day precision) was studied analyzing ten spiked samples at 50 μg/kg during five consecutive days, the RSD were below 12.7%.

Finally the selectivity of the method was evaluated by analyzing control blank samples. The absence of any signal at the same retention time as the selected compounds indicated that there were no matrix interferences that may give a false positive signal (Figure 4).

Application in real urine samples

The developed method was successfully applied to detect target analytes in real samples that were obtained from growing-finishing pigs that were administered DON in different dose by feed (low concentration was 200 μg/kg and high concentration was 2500 μg/kg in feed respectively). The analytical results showed that the low level (133 ng/ml) and high level (1207 ng/ml) of DON were found in low and high concentration diet groups respectively. It was demonstrated that the concentration of DON in pig urine and diet had positive correlation. Meantime, some other mycotoxins (e.g., LYS, ZEA) were found in both groups samples with very low concentration. So, the results indicated that this method can be used for the routine analysis of exposure assessment of the mycotoxins in animal urine samples.

No.	Analyte	Solvent calibration curves		Matrix-matched calibration curves	
		Slope	R^2	Slope	R^2
1	AFB_1	12341.8	0.9993	11264.0	0.9996
2	AFB_2	8711.6	0.9990	8174.8	0.9993
3	AFG_1	11440.5	0.9994	10750.2	0.9996
4	AFG_2	7276.0	0.9992	6264.2	0.9998
5	AFM_1	6212.1	0.9991	4468.1	0.9993
6	AFM_2	20886.7	0.9996	17087.3	0.9999
7	STE	2155.2	0.9998	2333.7	0.9997
8	T-2	1928.4	0.9993	1662.5	0.9996
9	LYS	10975.8	0.9989	11430.2	0.9997
10	MET	9728.3	0.9996	9353.8	0.9998
11	RC	16818.7	0.9997	12530.1	0.9993
12	DIA	2197.7	0.9987	1432.4	0.9996
13	DON	370.2	0.9998	227.8	0.9995
14	3-AcDON	522.7	0.9995	312.3	0.9998
15	15-AcDON	626.6	0.9975	281.8	0.9992
16	NEO	3418.7	0.9988	2238.3	0.9987
17	WOR	283.2	0.9993	203.2	0.9997
18	VER	103.7	0.9990	95.5	0.9993
19	HT-2	262.1	0.9996	172.7	0.9997
20	ZEN	102.5	0.9990	45.7	0.9991
21	α-ZEL	21.7	0.9989	9.6	0.9981
22	β-ZEL	77.4	0.9993	30.3	0.9997
23	ZAN	102.1	0.9991	51.0	0.9995
24	α-ZAL	235.4	0.9993	116.8	0.9997
25	β-ZAL	195.7	0.9989	99.7	0.9996

Table 3: Slope and R^2 of solvent and matrix-matched calibration curves.

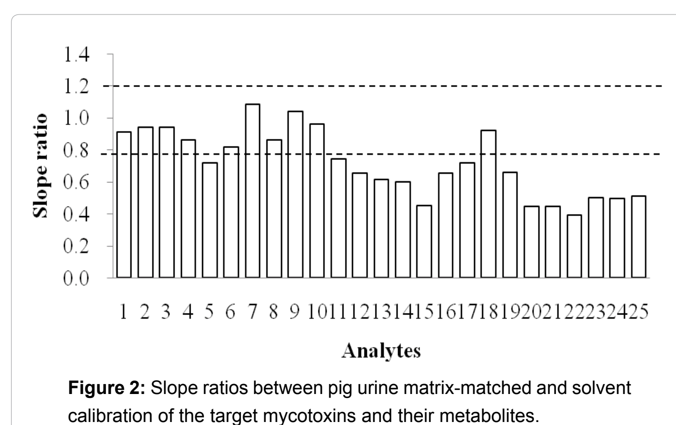

Figure 2: Slope ratios between pig urine matrix-matched and solvent calibration of the target mycotoxins and their metabolites.

No.	Analyte	LOQ (ng/mL)	Concentration range (ng/mL)	Spiked levels (ng/mL)	Recovery (%)	RSD(%, n=6)
1	AFB_1	0.05	0.05-20	0.1, 1.0, and 10	96.2-102.4	1.4-3.6
2	AFB_2	0.05	0.05-20	0.1, 1.0, and 10	94.0-100.8	1.9-5.5
3	AFG_1	0.05	0.05-20	0.1, 1.0, and 10	98.3-107.1	2.4~4.6
4	AFG_2	0.05	0.05-20	0.1, 1.0, and 10	91.8-104.2	1.8-3.2
5	AFM_1	0.05	0.05-20	0.1, 1.0, and 10	98.3-110.1	0.8-3.3
6	AFM_2	0.05	0.05-20	0.1, 1.0, and 10	97.7-106.2	1.5-4.5
7	STE	0.05	0.05-20	0.1, 1.0, and 10	91.2-99.4	2.0-6.4
8	T-2	0.05	0.05-20	0.1, 1.0, and 10	88.1-98.6	2.8-5.9
9	LYS	0.05	0.05-20	0.1, 1.0, and 10	86.1-95.7	1.8-4.5
10	MET	0.05	0.05-20	0.1, 1.0, and 10	90.1-101.3	0.8-2.8
11	RC	0.05	0.05-20	0.1, 1.0, and 10	91.2-97.7	1.1-3.7
12	DIA	0.05	0.05-20	0.1, 1.0, and 10	84.3-95.0	1.8-3.5
13	DON	0.25	0.25-100	0.5, 5.0, and 50	89.1-102.4	2.1-5.7
14	3-AcDON	0.5	0.5-100	0.5, 5.0, and 50	90.4-96.5	3.6-8.9
15	15-AcDON	0.5	0.5-100	0.5, 5.0, and 50	89.1-98.5	2.3-7.8
16	NEO	0.25	0.25-100	0.5, 5.0, and 50	88.0-95.5	0.6-2.7
17	WOR	0.25	0.25-100	0.5, 5.0, and 50	84.6-92.4	2.0-5.7
18	VER	0.25	0.25-100	0.5, 5.0, and 50	88.7-96.8	1.8-5.5
19	HT-2	0.25	0.25-100	0.5, 5.0, and 50	92.5-110.2	2.8-4.4
20	ZEN	0.25	0.25-100	0.5, 5.0, and 50	84.2-95.6	2.4-7.4
21	α-ZEL	0.5	0.5-100	0.5, 5.0, and 50	80.8-94.0	3.8-10.3
22	β-ZEL	0.5	0.5-100	0.5, 5.0, and 50	82.6-96.3	2.5-7.7
23	ZAN	0.5	0.5-100	0.5, 5.0, and 50	88.4-96.5	3.2-9.2
24	α-ZAL	0.5	0.5-100	0.5, 5.0, and 50	86.1-105.2	4.2-8.6
25	β-ZAL	0.5	0.5-100	0.5, 5.0, and 50	92.1-114.3	3.6-7.1

Table 4: Performance parameters of the method, including LOQ, concentration range, average recoveries, and relative standard deviation (RSD), of 25 target mycotoxins in pig-urine sample.

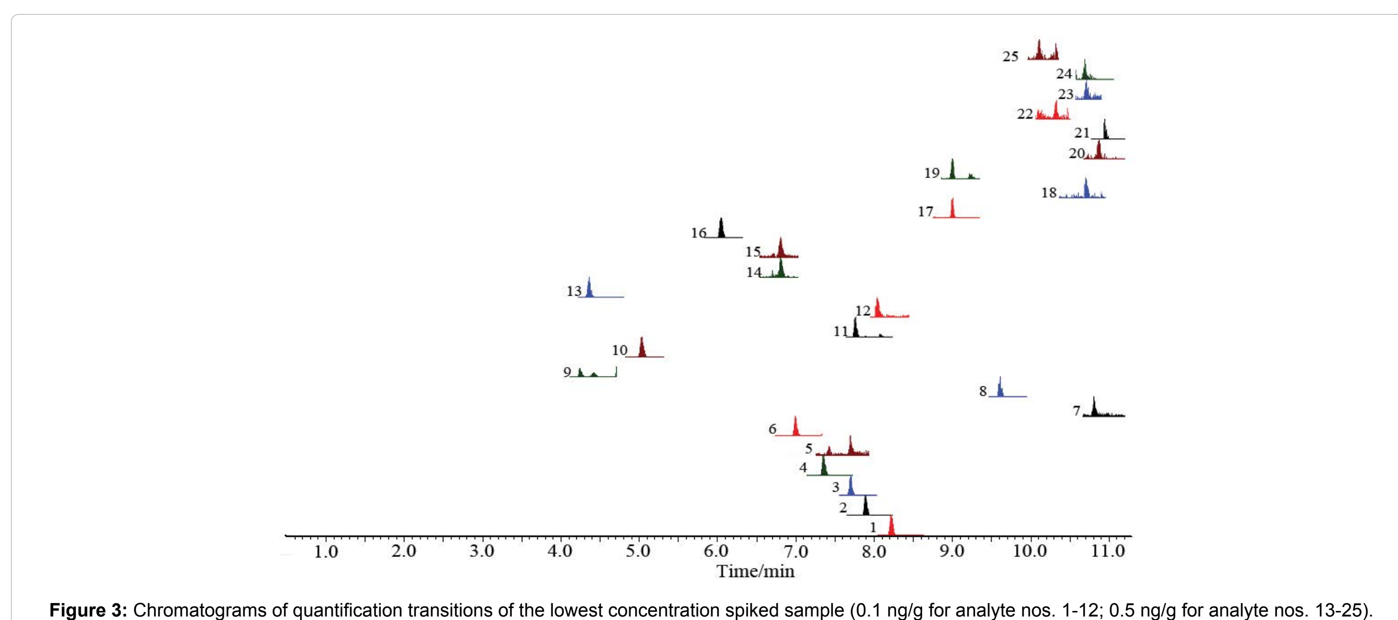

Figure 3: Chromatograms of quantification transitions of the lowest concentration spiked sample (0.1 ng/g for analyte nos. 1-12; 0.5 ng/g for analyte nos. 13-25).

Conclusions

In conclusion, a novel simultaneous detection method for mycotoxins and their metabolites in animal urine by using a simple preparation method based on impurity adsorption mechanism followed UPLC-MS/MS detection was presented. The developed sample preparation procedures could obtain clean extracts and consistent chromatographic responses, and in addition, the sample treatment time was reduced. The compounds examined in this study represent a wide range of physico-chemical properties indicating the potential of impurity adsorption for the extraction of target analytes in animal urine samples.

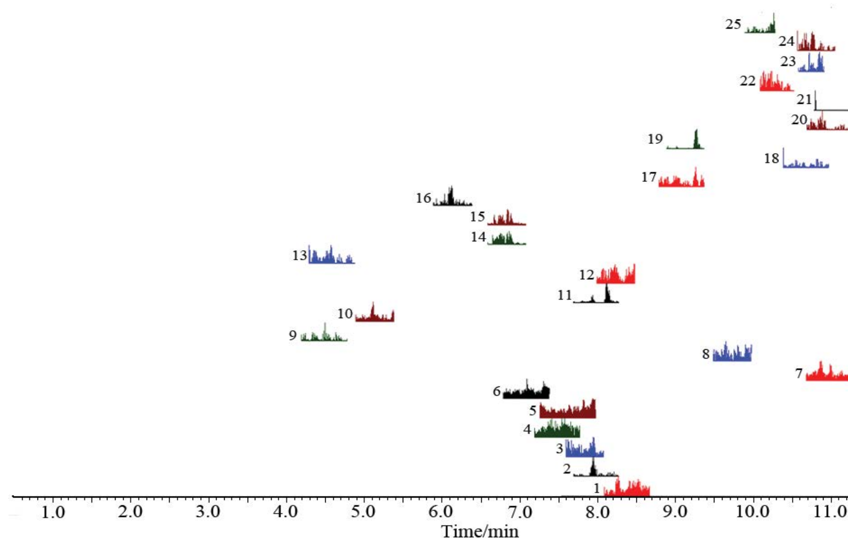

Figure 4: Chromatograms of quantification transitions of the blank pig-urine sample.

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