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ARTICLE

Determination of Benzimidazole Residues in Edible Animal Food by Polymer Monolith Microextraction Combined with Liquid Chromatography−Mass Spectrometry

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A sensitive method has been developed for the simultaneous determination of 10 benzimidazole residues and some of their metabolites in egg, milk, chicken, and pork. This method is based on the combination of polymer monolith microextraction (PMME) technique with liquid chromatography and electrospray ionization mass spectrometry (LC-ESI/MS). The extraction was performed with a poly(methacrylic acid-*co*-ethylene glycol dimethacrylate) (MAA-*co*-EGDMA) monolithic capillary column. Under the optimized extraction conditions, good extraction efficiencies for the targets were obtained with no matrix interference in the subsequent detection. The LODs (S/N = 3) for 10 benzimidazoles were found to be 0.56−2.76 ng g^{-1} in egg, 0.50−1.41 ng mL^{-1} in milk, 0.09−0.28 ng g^{-1} in chicken, and 0.08−0.15 ng g^{-1} in pork. The recoveries in egg, milk, chicken, and pork matrices ranged from 75.2 to 116.8% spiked at different levels with analytes, with RSDs of <13.7%. The method was later successfully applied for the determination of primary and metabolite residues in eggs after oral administration of albendazole to hens.

KEYWORDS: Benzimidazole; liquid chromatography−electrospray ionization mass spectrometry; polymer monolith microextraction; animal food

INTRODUCTION

Benzimidazole anthelmintics are broad-spectrum drugs that are widely used for the prevention and treatment of endoparasites in food-producing animals (*1*−*3*). The benzimidazole drugs are the largest chemical family including thiabendazole analogues and benzimidazole carbamates; substitution of various side chain and radicals on the parent benzimidazole nucleus produces the individual members (*3*). Widespread use of benzimidazole anthelmintics increases the possibility of residues appearing in edible animal food, and several toxic effects have been associated with chronic exposure to benzimidazole compounds, such as teratogenicity, congenic malformations, polyploidy, diarrhea, anemia, pulmonary edemas, or necrotic lymphoadenopathy (*4*).

To protect consumers from risks related to benzimidazole residues, maximum residue limits (MRLs) have been set for marker residue (the sum of a parent drugs and/or their metabolites) in animal products. The MRL values range from 10 to 5000 μg kg^{-1} depending on the compound and biological matrix. For example, the MRLs for albendazole (ABZ)-related drugs (sum of ABZ-SO, ABZ-SO_2, and ABZ-NH_2-SO_2) have been set at 100 μg kg^{-1} for milk, muscle, and fat and at 5000 μg kg^{-1} for kidney and liver; the MRL for flubendazole is 400 μg kg^{-1} in egg (*5*).

Because of the differences of lipophilicity and pK_a values among benzimidazoles, the determination of benzimidazole multiresidues in biological matrices is a challenge. The development of a highly sensitive multiresidue methodology that could determine more benzimidazole residues in foods of animal origin is thus highly desirable (*6*). As to the determination of benzimidazole residues, liquid chromatography based on ultraviolet detection (*7*−*9*) and fluorometric detection (*10*, *11*) was frequently used; gas chromatography after derivatization of residues to sufficiently volatilize was also applied (*3*); immunochemical methods (*12*−*14*) and electrochemical potential (*15*) were reported as well. Recently, the combination of HPLC and MS for the determination of a wide range of benzimidazoles and their metabolites has become the favored technique for sensitive and selective detection in complex biological matrices (*16*−*18*).

Due to the complexity of food samples, several pretreatment methods, such as solid-phase extraction (SPE) and liquid−liquid extraction (LLE) (*2*, *7*, *19*), have been reported for the extraction of benzimidazole residues in biological matrices. However, these methods are usually time-consuming, expensive, and require large volumes of both samples and toxic organic solvents. As an alternative, the solid-phase microextraction (SPME) method has become popular in sample preparation due to some advantages, such as high sensitivity, environmentally friendly characteristics, simplicity, and ease of automation(*20*). Recently, a novel microextraction setup named polymer monolith microextraction (PMME) was introduced by our group and has been coupled online or offline with HPLC for the determination of several analytes in different kinds of food samples (*21*−*24*).

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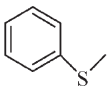
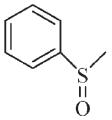
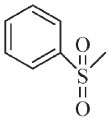
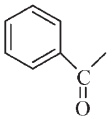
Table 1. Molecular Structures and Properties of Selected Benzimidazole Drugs

Compound	R_1	R_2	Octanol–water partition coefficient	pK_a values		pH range at which substance is in neutral state
				Basic groups	Acidic groups	
ABZ	C_3H_7-S-	$-NH-C(=O)-O-CH_3$	2.2–2.92	N: 5.54	N: 13.11	7.7–11.2
ABZ-SO	$C_3H_7-S(=O)-$	$-NH-C(=O)-O-CH_3$	0.82–0.94	N: 5.69	N: 13.25	7.6–11.2
ABZ-SO_2	$C_3H_7-S(=O)_2-$	$-NH-C(=O)-O-CH_3$	0.9–1.01	N: 3.50	N:11.20	5.5–9.2
ABZ-NH_2-SO_2	$C_3H_7-S(=O)_2-$	$-NH_2$	0.69–0.75	N: 5.98	N: 13.30	7.9–11.3
FBZ		$-NH-C(=O)-O-CH_3$	3.07–4.01	N: 5.12	N: 12.72	7.1-9.8
OFZ		$-NH-C(=O)-O-CH_3$	1.88-2.13	N: 4.13	N: 11.79	6.1–9.8
FBZ-SO_2		$-NH-C(=O)-O-CH_3$	2.13–3.30	N: 3.41	N: 11.12	5.4-9.2
MBZ		$-NH-C(=O)-O-CH_3$	2.44–2.52	N: 4.13	N: 11.79	6.1–9.8
TBZ	H—		1.58–1.76	N: 5.22	N: 12.83	7.2–10.9
5-OH-TBZ	HO—		1.29–1.37	N: 7.65	Phenol: 5.46	Always ionised state

PMME is one kind of SPME technique in which a polymer monolith is used as the sorbent. Different from the traditional SPME fibers or coated capillaries, the format of the extraction material could be regarded as a multichannel separation media, which is combined as a whole and will provide sufficient extraction phase. The extraction efficiency is thus expected to improve greatly (*21*). The good permeability of the monolithic capillary can realize the extraction process very quickly, indicating its wide usage in high-throughput applications. In addition, the polymer monolith showed stability within the entire range of pH and exhibited excellent biocompatibility in dealing with biological samples (*22*, *24*). However, PMME is unsuitable for large volumes of samples such as water; moreover, samples must be filtered to remove large particles of impurities prior to extraction to avoid plugging.

In this study, PMME using a poly(MAA-*co*-EGDMA) monolithic capillary column combined with LC-ESI/MS for the determination of 10 benzimidazole residues has been developed in four different animal products. The effects of various experimental parameters, such as the extraction and desorption conditions and extraction volume, have been investigated. The present method was later successfully applied for the determination of primary and metabolite residues in eggs after oral administration of albendazole to hens.

MATERIALS AND METHODS

Reagents and Solutions. Sodium hydroxide (NaOH), hydrochloric acid (HCl), monosodium phosphate (NaH_2PO_4), phosphoric acid (H_3PO_4), and formic acid (FA) were purchased from Shanghai General Chemical Reagent Factory and were of analytical reagent grade. Acetonitrile (MeCN) and methanol (MeOH) (HPLC grade) were purchased from Fisher Scientific (USA). Purified water was obtained with an Aike water purification apparatus (Chendu, China). Albendazole (ABZ), fenbendazole (FBZ), mebendazole (MBZ), thiabendazole (TBZ), and TBZ NH D6 (internal standard (I.S.)) were purchased from the Laboratories of Dr. Ehrenstorfer (Augsburg, Germany); albendazole sulfoxide (ABZ-SO), albendazole sulfone (ABZ-SO_2), oxfendazole (OFZ), fenbendazole sulfone (FBZ-SO_2), 2-aminoalbendazole sulfone (ABZ-NH_2-SO_2), and 5-hydroxythiabendazole (5-OH-TBZ) were purchased from the Laboratories of WITEGA (Berlin, Germany). The molecular structures of the benzimidazoles studied in this work are shown in **Table 1**.

Individual stock solutions were prepared to 100 μg mL^{-1} with MeOH and diluted with water to obtain a mixture standard solution of 5.0 μg mL^{-1}. A 0.5 μg mL^{-1} solution of the I.S. was prepared in a similar way. With the standard solution, the sample solution was spiked to the desired concentration for experiments. Individual stock solutions were stable for 6 months, and a mixture standard solution was reprepared every month. All of the above solutions were stored at 4 °C in the dark.

LC-ESI/MS Equipment and Conditions. An HPLC-ESI-MS system (Shimadzu LCMS-2010EV, Tokyo, Japan) was used for determination

of the benzimidazole residues. The column was a Shim-pack VP-ODS (Shimadzu, 150 × 2.0 mm, 5 μm). A mixture of 5 mM FA solution and MeCN was used as the mobile phase at a flow rate of 0.2 mL min^{-1}. Solvent A was 5 mM FA solution, and solvent B was MeCN. The gradient profile for the mobile phase was as follows: t_0, 5% B; t_{22}, 60% B; $t_{22.01}$, 80% B; t_{25}, 5% B; t_{30}, 5% B (where t refers to time (min)). The sample injection volume was 10 μL. Selected ion monitoring (SIM) was performed to simultaneously monitor ions at m/z 218, 240, 282, 202, 298, 316, 332, 296, 266, 300, and 208, which corresponded to the protonated molecular ions of 5-OH-TBZ, ABZ-NH_2-SO_2, ABZ-SO, TBZ, ABZ-SO_2, OFZ, FBZ-SO_2, MBZ, ABZ, FBZ, and TBZ NH D6 (I.S.). Capillary voltage was 4.5 kV. Curved desolvation line (CDL) and heat block temperatures for the analysis were set at 250 and 200 °C, respectively. Drying and nebulizer gases of nitrogen were set at 1.5 L min^{-1} with a pressure of 0.04 MPa. The detector voltage was set at 1.4 eV.

Egg Samples of ABZ-Treated Hens. Two laying hens (weight about 1.5 kg) used in this study were bred in cage and acclimated for 15 days prior to dosing. Then ABZ was administered to the test hens orally with 17 mg kg^{-1} dosing daily by manual insertion of the troche into the esophagus. Each treated hen received a single troche on each morning during the 4 consecutive day dosing period. Eggs were collected before dosing (on the day 0) and after dosing on days 2, 3, 5, 8, and 10. Then the egg yolk and the egg albumin were separated and homogenized, respectively. All samples were stored at −20 °C before analysis.

Sample Preparation (*22*, *25*). Pasteurized whole milk, eggs, chicken, and pork were purchased from local markets. These samples were homogenized and stored at −4 °C before use. Preliminary analysis showed they were analyte-free.

For egg and milk samples, I.S. solution (50 μL, 0.5 μg mL^{-1}) was added to 0.5 g of egg or 0.5 mL of milk samples, which were spiked with known variable amounts of benzimidazoles. Fifteen minutes was allowed for equilibration at room temperature, after being mixed with a vortex mixer for 2.0 min. These samples were diluted with 20 mM phosphate solution (pH 5.0) to 5.0 mL. After being mixed with a vortex mixer for 2.0 min again, the samples were centrifuged at 0−4 °C for 10.0 min at 10000 rpm (Anting Scientific Instrument Co., Ltd., Shanghai, China). Then the supernatant was filtered through a 0.45 μm pore filter prior to the PMME step.

For chicken and pork samples, I.S. solution (10 μL, 0.5 μg mL^{-1}) was added to 1.0 g of chicken or pork sample, which was spiked with known variable amounts of benzimidazoles. Fifteen minutes was allowed for equilibration at room temperature. Then 5.0 mL of MeCN was added into the spiked samples. The mixtures were homogenized with an ultrasonic homogenizer model HOM-100 (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) for 1.0 min and then were centrifuged at 0−4 °C for 10.0 min at 10000 rpm. The supertanant was collected and evaporated to dryness under a mild nitrogen stream by a HGC-12A Gas blowing concentrator (Zhongke-Sanhuan Instrument Co., Beijing, China) at 45 °C and reconstituted with 1.0 mL of 20 mM phosphate solution (pH 5.0). The solvent was centrifuged at 0−4 °C for 10.0 min at 10000 rpm. Then the supernatant was filtered through a 0.45 μm pore filter for the following PMME step. All blank samples were prepared in the same way as above but without the compound-spiking step.

PMME Procedures. The PMME was the same as that described in previous papers (*22*). The poly(MAA-*co*-EDGMA) monolithic capillary column (2 cm × 530 μm i.d.) from Micromole Separation and Testing Technology (Beijing, China) was selected as the extraction medium for PMME. A laboratory syringe pump (TS2-60, Baoding Longer Precision Pump Co., Ltd., Hebei, China) was employed for the delivery of solutions in the whole extraction process including preconditioning, sorption, washing, and desorption. For preconditioning, 0.5 mL of MeCN and 0.5 mL of phosphate solution (20 mmol L^{-1}, pH 5.0) were ejected through the monolithic capillary at 0.10 mL min^{-1} in turn. For the sorption, 0.5 mL of sample solution was pushed through the capillary at 0.1 mL min^{-1}, and then 0.2 mL of phosphate solution (20 mmol L^{-1}, pH 5.0) and 0.2 mL of water were driven through at the same velocity to eliminate the residual matrix and salts to avoiding interference with separation and detection. Thereafter, the residual water was expelled from the monolithic capillary by air via a clean syringe. For the desorption, 0.10 mL of MeCN was injected into the monolithic capillary at 0.05 mL min^{-1}, and the eluate was collected and evaporated to dryness. The residue was reconstituted with 0.05 mL of MeCN/H_2O (5:95, v/v) for the subsequent analysis by LC-MS.

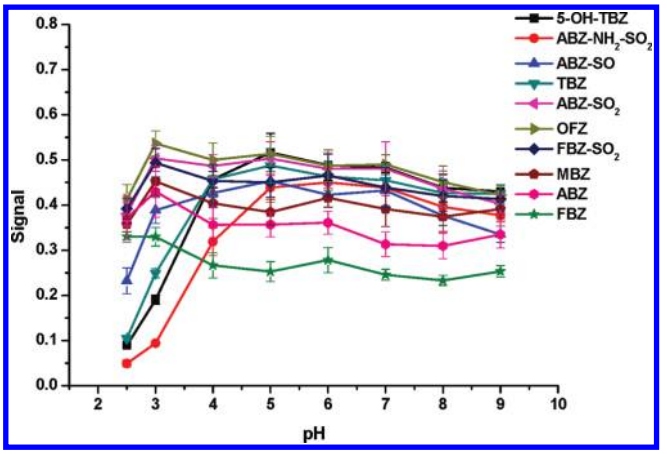


Figure 1. Optimization of the pH in the sample solution for PMME. Sample solutions of benzimidazole drugs spiked at 50 ng mL^{-1} were prepared with 20 mM phosphate solution at pH 2.5−9.0.

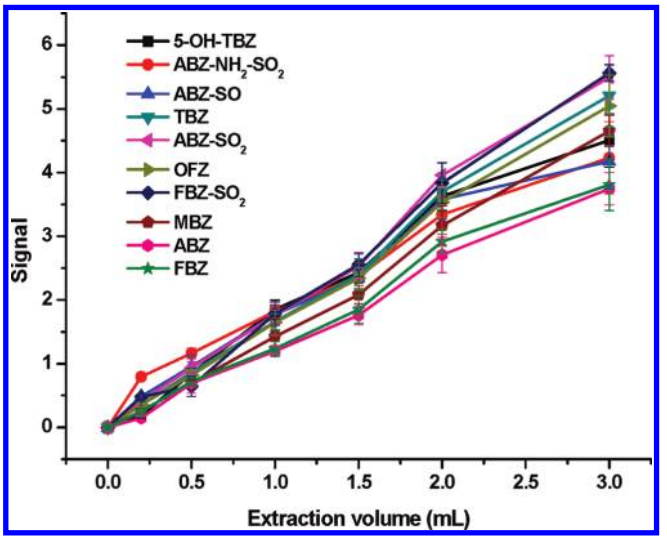


Figure 2. Extraction volume profile in the sample solution for PMME. Benzimidazole drugs were spiked at 50 ng mL^{-1}. Optimal conditions and LC-MS conditions were used.

RESULTS AND DISCUSSION

Optimization of the PMME Conditions. The extraction was optimized with phosphate solution (20 mmol L^{-1}, pH 5.0) spiked with 10 benzimidazoles. Several parameters affecting the extraction efficiency such as pH, extraction equilibrium profiles, washing and desorption step, and flow rate were investigated.

Effect of pH on Extraction Efficiency. It is known that benzimidazoles possess an imidazole ring containing both acidic and basic nitrogen atoms. Under suitable conditions, the molecule may be protonated or deprotonated. The pK_a values and octanol−water partition coefficient of a limited number of benzimidazoles quoted in the literature (*3*) are listed in **Table 1**.

As shown in **Figure 1**, the highest extraction efficiency could be obtained in the pH range of 4.0−7.0. The enhanced extraction efficiency can be explained by the strong ion-exchange interaction between the cationic analytes and the negatively charged poly(MAA-*co*-EGDMA) material. When the pH is increased above 6.0, the most benzimidazole molecules are transformed to neutral state and a few in protonated forms, which results in gradually weakened ion exchange interaction and slightly lower extraction efficiency. This could be attributed to the fact that the hydrophobic interaction and ion-exchange interaction dominate the

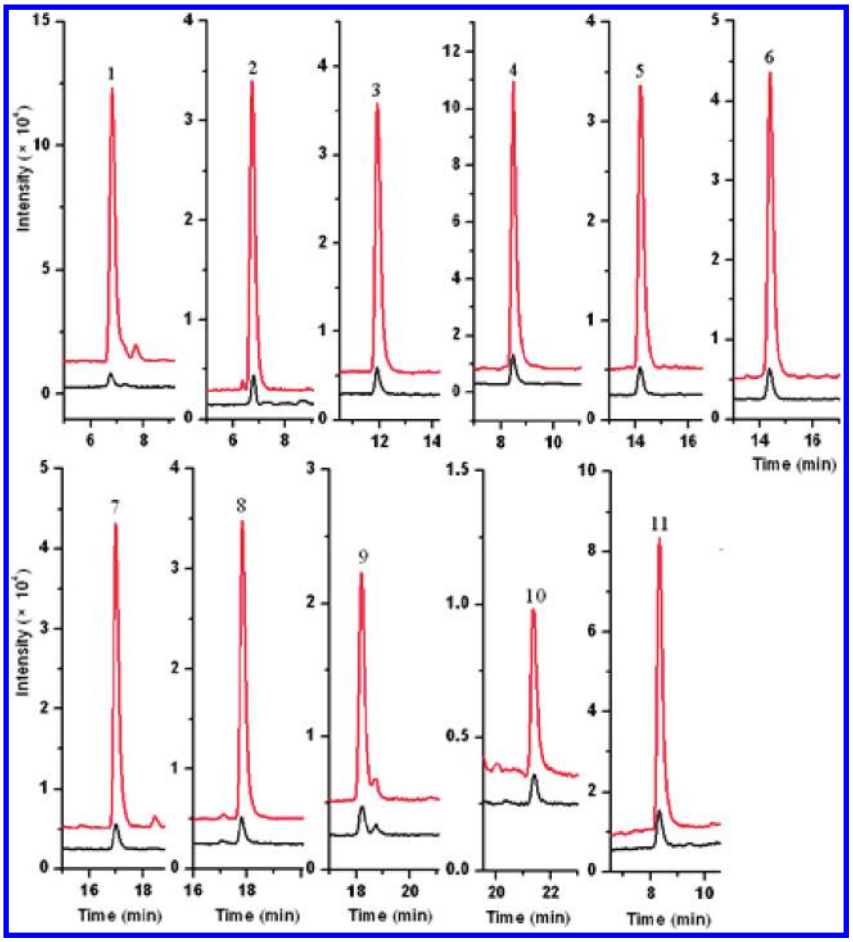


Figure 3. EICs of 10 benzimidazoles (including I.S.) standard samples obtained by PMME (top) and direct LC-MS analysis (bottom). The concentrations of benzimidazoles for PMME and direct LC-MS analysis were 50 ng mL^{-1}, respectively. Direct inject volume was 10 μL. Optimal microextraction and LC-MS conditions were used. Peaks: 1, 5-OH-TBZ; 2, ABZ-NH_2-SO_2; 3, ABZ-SO; 4, TBZ; 5, ABZ-SO_2; 6, OFZ; 7, FBZ-SO_2; 8, MBZ; 9, ABZ; 10, FBZ; 11, TBZ NH D6 (I.S.).

Table 2. Comparison of Sample Preparation Procedures and LODs between Different Methods for Their Application in Animal Products

residues	matrix[a]	extraction	cleanup	determination	LOD (ng g^{-1})[b]	ref
TBZ, 5-OH-TBZ, ABZ 2-NH_2-ABZ, ABZ-SO, ABZ-SO_2, MBZ, FBZ, OFZ, FBZ-SO_2, OXI, TBZ, CAM, FLU	E, Mk, L	ethyl acetate at alkaline	*n*-hexane and SDB-SPE	LC-MS/MS	<6	*2*
FBZ, OFZ, FBZ-SO_2, 4-OH-FBZ	Mk	PBS-Tween + BSA at pH 7.0		ELISA	3−7	*12*
FBZ, OFZ, FBZ-SO_2, ABZ, ABZ-SO, ABZ-SO_2	Mk	MeCN	SPE (C18)	LC-UV	4−26	*7*
FBZ, OFZ, FBZ-SO_2	M	MeCN	SPE (C18, CN)	LC-UV	3.8−4.5	*19*
MBZ, ABZ, CBM, TBZ	M	ethyl acetate	MSPD	LC-UV	10−50	*27*
ABZ, ABZ-SO, FBZ, ABZ-SO_2, FBZ-SO_2, ABZ-NH_2-SO_2, OFZ, MBZ, TBZ, 5-OH-TBZ	E, Mk, M	phosphate solution for milk and egg; MeCN for muscles	PMME (poly(MAA-*co*-EGDMA) monolithic column)	LC-MS	0.08−2.76	this work

[a] Egg, liver, muscle, and milk abbreviated E, L, M, and Mk, respectively. [b] The units for milk sample were ng mL^{-1}.

retention of benzimidazoles on the monolithic column. Considering the extraction efficiency and simplicity of the extraction methods, pH 5.0 was selected as the pH of sample matrix.

Equilibrium Extraction Volume Profiles. To assess the ability of the poly(MAA-*co*-EGDMA) monolithic column to extract the benzimidazoles, the equilibrium extraction time profile was investigated by increasing the extracting volume at constant extraction flow rate (0.10 mL min^{-1}) of sample solution. As shown in **Figure 2**, the extracted amount of these compounds increased rapidly with prolonged extraction volume, indicating the remarkable enrichment ability of the poly(MAA-*co*-EGDMA) monolithic capillary toward these benzimidazoles. The equilibrium of extraction was not obtained even up to 3.0 mL sample volume. To achieve sufficient sensitivity within a short time, 0.5 mL of extraction volume was selected for subsequent analysis.

Optimization of the Washing and Elution Steps. It is important when working with biological samples to apply a washing step immediately after the extraction, which ensures the reduction of the matrix interference in the separation of analytes and prevents the protein from polluting the extraction column and analytical column. In this study, the monolithic column was washed for 2 min by the carrier solution after extraction to eliminate proteins and other weakly adsorbed components.

Table 3. Results for Matrix Effects of the 10 Benzimidazoles for Four Animal Products

	matrix effect[a] (%) ± standard deviation											
	egg			milk			chicken			pork		
analyte	20 ng g^{-1}	100 ng g^{-1}	1000 ng g^{-1}	20 ng g^{-1}	100 ng g^{-1}	1000 ng g^{-1}	20 ng g^{-1}	100 ng g^{-1}	1000 ng g^{-1}	20 ng g^{-1}	100 ng g^{-1}	1000 ng g^{-1}
5-OH-TBZ	8.1 ± 2.0	8.9 ± 3.6	−8.9 ± 3.4	−12.0 ± 2.5	10.5 ± 6.1	−9.2 ± 2.1	2.8 ± 2.4	5.1 ± 2.6	−7.1 ± 0.7	15.3 ± 3.1	−17.4 ± 0.1	−4.4 ± 6.2
ABZ-NH_2-SO_2	−14.3 ± 6.4	−6.1 ± 0.8	−10.7 ± 5.2	−11.5 ± 8.1	−10.4 ± 3.5	−4.4 ± 1.6	−3.3 ± 4.1	−2.3 ± 2.3	−10.0 ± 0.6	−14.5 ± 5.7	−1.2 ± 1.9	−17.1 ± 0.7
ABZ-SO	−3.3 ± 5.2	−2.6 ± 8.8	−5.7 ± 7.6	2.8 ± 3.3	−3.7 ± 0.4	−12.4 ± 0.8	12.4 ± 4.4	−6.6 ± 3.7	−10.7 ± 0.9	−10.0 ± 6.6	−4.6 ± 4.1	−11.2 ± 4.1
TBZ	5.0 ± 9.5	0.6 ± 0.8	−5.3 ± 3.9	2.9 ± 0.6	1.2 ± 2.2	−11.4 ± 4.3	4.7 ± 1.0	1.8 ± 1.6	−9.0 ± 0.1	−8.3 ± 3.3	1.7 ± 0.2	−9.4 ± 1.8
ABZ-SO_2	−3.3 ± 6.2	−1.2 ± 2.9	−2.4 ± 9.2	0.1 ± 0.8	−9.1 ± 2.2	−1.0 ± 5.0	9.0 ± 2.4	−0.9 ± 4.7	−9.7 ± 1.6	−3.9 ± 6.4	2.6 ± 2.4	−7.0 ± 3.6
OFZ	−7.1 ± 5.0	−3.1 ± 3.8	−4.9 ± 8.2	−10.8 ± 1.3	−10.4 ± 3.0	−13.3 ± 3.3	8.2 ± 2.5	−2.7 ± 2.9	−11.2 ± 1.7	−0.7 ± 5.8	−2.5 ± 2.1	−12.9 ± 1.7
FBZ-SO_2	−0.8 ± 1.6	−0.5 ± 2.2	0.5 ± 8.6	4.3 ± 5.6	2.4 ± 4.7	−7.9 ± 2.7	13.7 ± 2.4	2.6 ± 6.0	−8.7 ± 0.6	−2.7 ± 4.1	−0.8 ± 0.0	−9.0 ± 0.9
MBZ	−10.4 ± 4.3	5.8 ± 2.9	−0.8 ± 8.2	13.8 ± 6.0	5.4 ± 2.0	−7.7 ± 3.0	8.7 ± 3.1	7.2 ± 4.4	−9.1 ± 2.2	7.5 ± 3.3	7.4 ± 1.0	−9.8 ± 2.3
ABZ	−18.9 ± 5.8	−16.6 ± 0.7	−11.1 ± 6.3	0.5 ± 1.6	−3.8 ± 2.1	−13.5 ± 2.5	−7.1 ± 1.6	−15.7 ± 2.9	−17.4 ± 1.3	−15.3 ± 3.9	−6.1 ± 7.4	−17.6 ± 6.4
FBZ	−19.2 ± 1.8	−12.1 ± 8.6	−13.1 ± 0.6	−8.2 ± 2.4	2.2 ± 1.1	−13.0 ± 2.8	−16.3 ± 4.6	−16.5 ± 0.4	−12.7 ± 4.6	−14.5 ± 2.6	−15.9 ± 3.2	−14.0 ± 0.7

[a] "+" represents a loss of the analyte signal (ion suppression), 0% represents no matrix effects, and "−" represents an enhancement of the analyte signal (ion enhancement).

Table 4. Linear Range, LOD, and LOQ Data for PMME/LC-ESI/MS of the 10 Benzimidazoles for Four Animal Products

	5-OH-TBZ	ABZ-NH_2-SO_2	ABZ-SO	TBZ	ABZ-SO_2	OFZ	FBZ-SO_2	MBZ	ABZ	FBZ
egg										
LOD (ng g^{-1})	1.47	1.54	1.35	0.89	0.56	0.99	1.96	1.08	1.72	2.76
LOQ (ng g^{-1})	4.89	5.15	4.51	2.97	3.29	1.88	6.54	3.61	5.73	9.20
linear range (ng g^{-1})	10−1000	10−1000	10−1000	5−1000	5−1000	5−1000	10−1000	5−1000	10−1000	10−1000
milk										
LOD (ng g^{-1})	0.92	1.11	1.23	0.64	0.50	0.62	1.05	0.74	1.15	1.41
LOQ (ng g^{-1})	3.05	3.71	4.1	2.13	1.66	2.06	3.49	2.45	3.83	4.69
linear range (ng g^{-1})	10−1000	10−1000	10−1000	5−1000	5−1000	5−1000	10−1000	5−1000	10−1000	10−1000
chicken										
LOD (ng g^{-1})	0.10	0.12	0.09	0.24	0.13	0.17	0.23	0.13	0.24	0.28
LOQ (ng g^{-1})	0.32	0.4	0.28	0.79	0.44	0.56	0.76	0.44	0.79	0.93
linear range (ng g^{-1})	1−200	1−200	1−200	2−200	1−200	1−200	2−200	1−200	2−200	2−200
pork										
LOD (ng g^{-1})	0.08	0.10	0.08	0.12	0.08	0.09	0.10	0.10	0.10	0.15
LOQ (ng g^{-1})	0.27	0.32	0.26	0.40	0.27	0.29	0.32	0.33	0.34	0.49
linear range (ng g^{-1})	1−200	1−200	1−200	2−200	1−200	1−200	2−200	1−200	2−200	2−200

Table 5. Method Recoveries and Precisions at Three Different Concentrations for PMME/LC-ESI/MS of the 10 Benzimidazoles from Egg (A), Milk (B), Chicken (C), and Pork (D) Samples

	intraday (n = 4) recovery (%) (RSD%)						interday (n = 3) recovery (%) (RSD%)					
	20 ng g^{-1}		100 ng g^{-1}		1000 ng g^{-1}		20 ng g^{-1}		100 ng g^{-1}		1000 ng g^{-1}	
analyte	A	B	A	B	A	B	A	B	A	B	A	B
5-OH-TBZ	78.5 (2.0)	81.3 (3.6)	100.6 (3.6)	109.5 (6.5)	101.2 (6.3)	96.5 (5.7)	98.4 (11.1)	92.1 (3.2)	98.0 (9.1)	101.6 (12.5)	85.5 (4.7)	91.0 (4.0)
ABZ-NH_2-SO_2	110.3 (4.6)	86.4 (6.8)	114.2 (7.1)	90.7 (11.4)	107.5 (7.0)	82.4 (11.8)	86.9 (8.7)	92.8 (5.2)	100.4 (6.8)	93.2 (3.9)	96.7 (12.0)	87.3 (8.6)
ABZ-SO	79.0 (2.0)	84.3 (4.2)	88.2 (2.0)	84.2 (7.6)	108.5 (6.9)	82.1 (6.3)	102.6 (6.4)	105.2 (5.0)	99.0 (9.3)	80.8 (4.0)	90.3 (11.4)	85.2 (3.8)
TBZ	93.4 (0.6)	91.3 (3.3)	101.7 (4.6)	98.9 (2.2)	104.6 (6.9)	94.4 (2.3)	97.8 (9.1)	99.7 (4.0)	99.8 (3.7)	92.8 (2.3)	95.4 (1.5)	97.1 (7.6)
ABZ-SO_2	116.8 (7.2)	92.4 (10.0)	88.6 (4.9)	91.6 (11.0)	109.5 (7.7)	92.9 (3.7)	104.6 (5.7)	88.9 (12.0)	100.3 (3.0)	82.7 (7.8)	91.7 (2.1)	89.7 (6.9)
OFZ	101.5 (3.7)	96.8 (9.1)	81.2 (4.0)	82.3 (7.7)	115.6 (7.4)	88.4 (2.8)	101.5 (1.9)	87.9 (7.1)	99.9 (3.3)	93.9 (9.6)	87.5 (2.2)	84.9 (9.4)
FBZ-SO_2	76.5 (10.4)	108.1 (9.3)	89.8 (2.9)	89.1 (5.2)	106.3 (6.6)	81.3 (12.5)	87.3 (3.8)	82.4 (4.7)	97.7 (4.3)	99.2 (10.3)	88.6 (5.8)	87.6 (5.8)
MBZ	100.6 (4.7)	110.7 (8.5)	77.6 (2.9)	97.2 (6.1)	88.3 (7.1)	85.8 (2.8)	98.7 (8.0)	86.5 (8.1)	99.9 (2.7)	83.1 (4.9)	94.0 (8.4)	83.5 (7.5)
ABZ	75.7 (9.6)	107.9 (5.6)	78.3 (3.6)	88.3 (4.0)	76.3 (7.0)	84.5 (5.4)	104.0 (2.0)	75.3 (9.1)	94.3 (5.4)	98.1 (13.0)	89.7 (11.4)	93.3 (4.3)
FBZ	79.1 (9.0)	87.0 (5.0)	75.7 (9.2)	94.0 (4.0)	80.2 (9.2)	80.7 (7.9)	79.1 (5.7)	115.4 (3.9)	83.1 (10.1)	90.9 (3.8)	86.4 (8.5)	105.9 (7.2)

	intraday (n = 4) recovery (%) (RSD%)						interday (n = 3) recovery (%) (RSD%)					
	2 ng g^{-1}		10 ng g^{-1}		100 ng g^{-1}		2 ng g^{-1}		10 ng g^{-1}		100 ng g^{-1}	
analyte	C	D	C	D	C	D	C	D	C	D	C	D
5-OH-TBZ	86.0 (13.7)	92.3 (10.2)	99.5 (8.3)	107.2 (8.9)	101.7 (10.2)	105.0 (3.3)	83.4 (7.0)	109.2 (8.8)	98.8 (11.6)	89.4 (12.1)	97.5 (9.6)	101.3 (7.3)
ABZ-NH_2-SO_2	85.8 (9.4)	106.9 (8.2)	86.0 (6.8)	101.3 (12.0)	102.6 (3.3)	93.9 (4.0)	95.1 (10.9)	86.0 (10.2)	95.6 (8.0)	90.3 (5.0)	98.1 (12.4)	99.5 (10.5)
ABZ-SO	91.4 (13.1)	100.3 (10.0)	102.0 (5.0)	106.7 (8.4)	105.2 (2.3)	96.6 (2.0)	85.1 (11.3)	100.0 (8.5)	100.8 (4.0)	96.2 (3.6)	96.7 (2.8)	99.7 (5.3)
TBZ	101.3 (8.3)	100.3 (5.2)	75.2 (4.6)	89.9 (2.0)	85.3 (2.8)	106.3 (5.1)	83.7 (3.2)	82.0 (3.8)	86.7 (2.7)	80.9 (2.2)	100.1 (3.1)	94.5 (4.6)
ABZ-SO_2	96.2 (6.6)	96.7 (1.1)	101.3 (5.4)	98.6 (3.6)	81.4 (3.1)	94.9 (9.6)	90.5 (1.8)	100.8 (9.3)	84.1 (2.0)	90.5 (6.1)	95.6 (3.1)	93.0 (6.4)
OFZ	105.2 (4.3)	99.9 (12.0)	95.4 (11.8)	99.1 (5.2)	92.9 (5.5)	93.1 (8.7)	101.1 (7.7)	94.0 (8.4)	90.9 (2.6)	104.4 (5.6)	99.0 (2.5)	88.3 (6.8)
FBZ-SO_2	107.9 (10.6)	99.1 (4.2)	108.5 (13.4)	86.6 (6.1)	85.7 (5.5)	90.9 (7.5)	101.1 (8.3)	86.4 (7.9)	77.0 (2.3)	95.1 (4.4)	95.9 (2.8)	102.2 (9.9)
MBZ	83.8 (5.6)	93.8 (8.1)	85.0 (4.0)	104.1 (7.1)	88.6 (3.0)	100.7 (4.6)	90.0 (6.4)	84.8 (7.3)	80.9 (4.9)	95.5 (8.7)	99.6 (4.6)	96.5 (10.6)
ABZ	100.3 (7.4)	89.2 (10.1)	86.0 (12.1)	92.7 (4.0)	97.9 (5.5)	93.9 (2.6)	80.0 (7.0)	80.5 (7.6)	89.7 (9.5)	98.3 (13.4)	95.7 (8.2)	96.2 (3.7)
FBZ	84.4 (4.5)	101.2 (9.0)	84.8 (3.2)	91.9 (5.9)	112.5 (4.4)	97.4 (5.5)	85.4 (2.6)	85.0 (7.2)	82.9 (7.2)	100.0 (11.7)	95.8 (10.9)	95.9 (1.3)

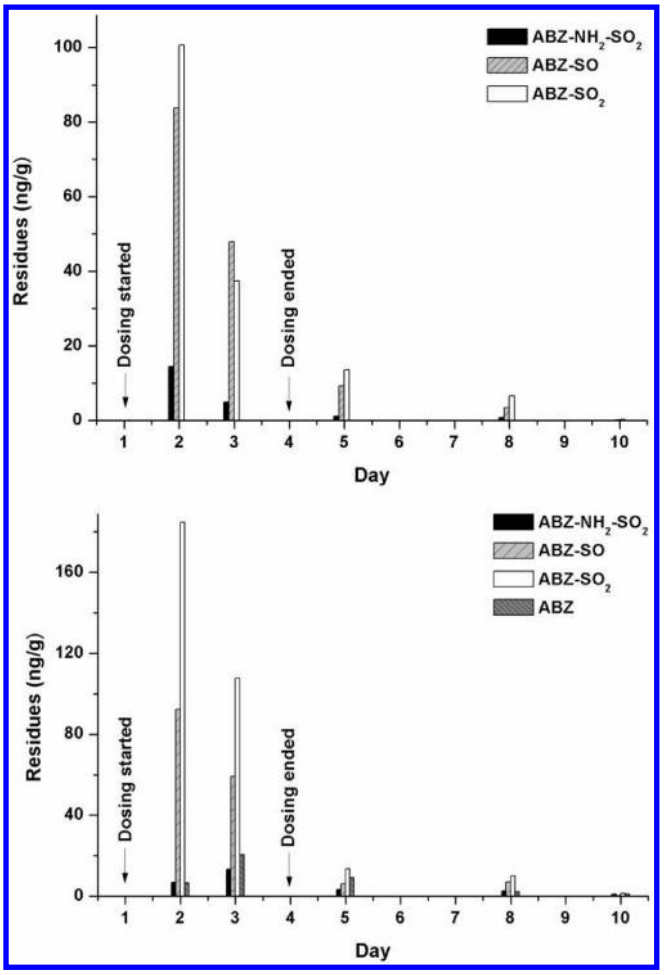


Figure 4. ABZ and its metabolites (ABZ-SO, ABZ-SO_2, ABZ-NH_2−SO_2) residues in egg albumin (top) and yolk (bottom) during and after oral ABZ administration of 17 mg kg^{-1} of feed for 4 consecutive days.

Subsequently, 0.2 mL of water was used to eliminate the phosphate solution, which was unsuitable for MS detection.

The desorption was optimized to achieve accurate quantification of the analytes. After sample extraction, 0.1 mL of MeCN was used to elute all of the analyte. The same procedure was repeated three times. Each of the 0.1 mL eluates was collected and evaporated to dryness. The residue was reconstituted and analyzed by LC-MS. The result indicated that the first 0.1 mL of MeCN could elute nearly all of the extracted benzimidazoles from the monolithic capillary.

Effect of Flow Rate of Adsorption and Desorption on Extraction Efficiency. The flow rate of the sample solution was optimized in the range of 0.05−0.3 mL min^{-1}, with a total loading of 0.5 mL of sample solution. It was found that the flow rate had no obvious influence on the extraction efficiency. Therefore, the flow rate of 0.1 mL min^{-1} was selected considering the extraction time as well as the pressure on the monolithic capillary.

Moreover, the same result was found for the desorption in the range of 0.025−0.1 mL min^{-1}; a flow rate of 0.05 mL min^{-1} was suitable for the desorption step.

Performance of ESI/MS. Above all, the concentration of FA in the mobile phase on ionization efficiency is optimized in the range of 0−25 mM; the result showed the highest ionization efficiency could be obtained at 5 mM of FA.

The EICs of 11 benzimidazoles (including I.S.) obtained after PMME and direct injection under optimal experimental conditions are shown in **Figure 3**. In comparison with the chromatogram of direct injection, an obvious enhancement of the peak height is observed after extraction, indicating the obvious preconcentration ability of the monolithic column.

Application to Edible Animal Products. *Method Comparison.* Comparative study of our developed method with other reported sample preparation procedures was performed, and the results are presented in **Table 2**. It can be seen that the developed method is more sensitive and requires less sample and organic solvent in the pretreatment process. Furthermore, no elimination of fats and protein in samples was required prior to extraction in this study.

Calibration Curves and Detection Limits. Matrix effects were assessed by comparing the ion intensity of sample extracts with the analytes added postextraction to pure solutions prepared in mobile phase containing equivalent amounts of the analytes. The difference in response between the two divided by the pure solution response determines the degree of matrix effect occurring in the analytes in question under chromatographic conditions (*26*). As shown in **Table 3**, the matrix effects of benzimidazoles range from −19.2 to 15.3% in four matrices. The results revealed that the determination of benzimidazoles was affected by the interferences from real samples to some extent. Therefore, to provide reliable results, matrix-matched calibration curves were chosen as reference curves throughout this study.

The application of the PMME/LC-ESI/MS method for the determination of 10 benzimidazoles was verified using an internal standard for quantification. The internal standard calibration in egg, milk, chicken, and pork samples was performed by plotting peak area ratios (benzimidazoles/I.S.) versus benzimidazole concentrations. Detection and quantification limits were calculated as the concentration corresponding to a signal 3 and 10 times the standard deviation of the baseline noise, respectively. As listed in **Table 4**, the LODs for 10 benzimidazoles were found to be 0.56−2.76 ng g^{-1} in egg, 0.50−1.41 ng mL^{-1} in milk, 0.09−0.28 ng g^{-1} in chicken, and 0.08−0.15 ng g^{-1} in pork. The LOQs were found to be 1.88−9.20 ng g^{-1} in egg, 1.66−4.69 ng mL^{-1} in milk, 0.28−0.93 ng g^{-1} in chicken, and 0.26−0.49 ng g^{-1} in pork. Matrix-matched calibration curves for four matrices were established with $R^2 > 0.9936$.

Trueness and Precision. In this study, the trueness of the method was measured and expressed as recovery. The precision of the method was assessed by determining intra- and interday relative standard deviations (RSDs) of the analysis. Both recoveries and intra- and interday RSDs were calculated with 10 benzimidazoles spiked at three different concentrations in four matrices. The recoveries were determined by comparing the calculated amounts of benzimidazoles in the samples (using matrix-matched calibration curves) with the total spiking amounts. The recovery and RSD data for benzimidazoles spiked in four matrices are summarized in **Table 5**. The intra- and interday recoveries were between 75.7 and 116.8% for egg, between 75.3 and 115.4% for milk, between 75.2 and 112.5% for chicken, and between 80.5 and 109.2% for pork. The intra- and interday precisions for recoveries of the 10 benzimidazoles were less than 11.4% for egg, 13.0% for milk, 13.7% for chicken, and 13.4% for pork. The results demonstrate that the precision and trueness of the present method were acceptable for routine monitoring purposes.

Analysis of Egg Albumin and Yolk Samples of ABZ-Treated Hens. To further validate the feasibility of the method in the practical determination of benzimidazole residues in eggs, we analyzed egg albumin and yolk samples from hens dosed with ABZ over 4 consecutive days.

The distribution of ABZ and its metabolites is shown in **Figures 4** and **5**; both ABZ and its metabolites ABZ-SO,

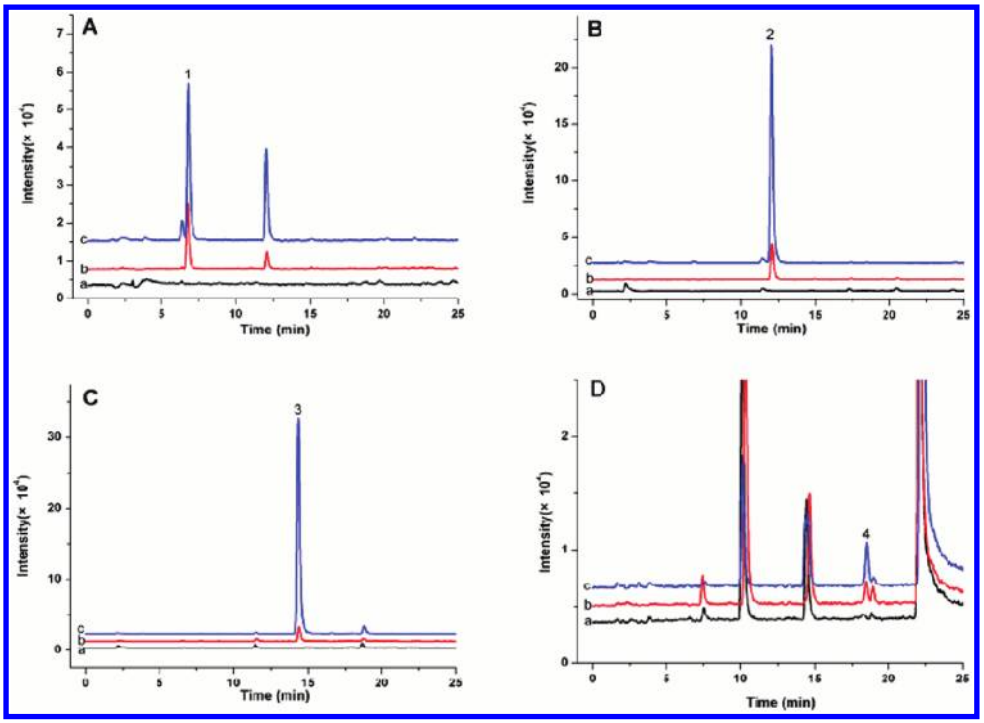


Figure 5. EICs obtained by SPME of ABZ and its metabolites (ABZ-SO, ABZ-SO_2, ABZ-NH_2-SO_2) in control yolk sample (a), spiked yolk sample (b), and yolk sample of ABZ-treated hens (c). Chromatograms A−D represent, respectively, EIC of monitoring *m/z* 240, 282, 298, and 266 for yolk samples. The optimal microextraction and LC-MS conditions were used. Peaks: 1. ABZ-NH_2−SO_2 ; 2. ABZ-SO; 3. ABZ-SO_2; 4. ABZ.

ABZ-SO_2, ABZ-NH_2-SO_2 could be detected simultaneously in yolk, whereas ABZ could not be detected in egg albumin during and after oral administration. Moreover, the concentrations of ABZ and its metabolites were higher in yolk than in egg albumin during and after oral administration, whereas the distribution of ABZ-related compounds between egg albumin and yolk was not quite uniform. The ABZ-related compound concentrations reached peaks in egg albumin and yolk at the second day during oral administration and did not increase further at the third day. Thereafter, the ABZ-related compound concentrations decreased rapidly after administration and reached a low level at the eighth day (the fourth day after dosing) and could hardly be detected at day 10.

Because of the relatively higher lipid solubility for ABZ than its metabolites, ABZ was detected only in yolk and may be contributed by the difference between yolk (predominantly lipoproteins) and egg albumin (water-soluble proteins).

In conclusion, the present PMME/LC-MS method was successfully applied to the determination of 10 benzimidazole residues in egg, milk, chicken, and pork. In comparison to the previously reported pretreatment and analysis methods, the proposed method is environmentally friendly, easily handled, and inexpensive. The high sensitivity makes this method attractive for the trace determination of benzimidazole residues in different biological matrices. Therefore, the proposed method will be useful and practical in residue monitoring and in studying the pharmacokinetics of benzimidazole drugs.

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