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Determination of benzimidazole residues in animal tissue samples by combination of magnetic solid-phase extraction with capillary zone electrophoresis

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ABSTRACT

Benzimidazole drugs (BZDs) comprise a large number of synthetic anthelmintics, which are widely used in food-producing animals for prophylactic and therapeutic purposes. To protect consumers from the risks related to BZDs residues, a simple, rapid, and efficient method for simultaneous determination of ten BZDs in animal tissues samples was developed. This analytical procedure involved extracting samples with magnetic solid-phase extraction (MSPE) using magnetite/silica/poly (methacrylic acid-*co*-ethylene glycol dimethacrylate) (Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA)) magnetic microspheres, and determination by capillary zone electrophoresis (CZE). To improve the sensitivity of the method, we employed the electrokinetic injection with field-amplified sample stacking technique (FASS). Berbine solution was used as internal standard to minimize the fluctuation of analytical results. Under the optimized extraction conditions, good linearities were obtained for the ten BZDs with the correlation coefficients (R^2) above 0.9920. The limits of detections (LODs) for ten BZDs were 1.05–10.42 ng/g in swine muscle and 1.06–12.61 ng/g in swine liver, respectively. The intra- and inter-day relative standard deviations (RSDs) of the developed method were less than 13.6%. The recoveries of the ten BZDs for the spiked samples ranged from 81.1% to 105.4% with RSDs less than 9.3%.

1. Introduction

Benzimidazole drugs (BZDs) comprise a large number of synthetic anthelmintics, which are widely used in food-producing animals for therapeutic and prophylactic purpose [1–3]. Improper use of BZDs, such as inappropriate withdrawal period and excessive administration, may result in their residues in animal tissues and animal-derived food products. A chronic exposure to BZDs has been associated with several toxic effects such as teratogenicity, congenic malformations, polyploidy, diarrhea, anemia, pulmonary edemas, or necrotic lymphoadenopathy [4]. To protect consumers from the risks related to BZDs residues, maximum residue limits (MRLs) for marker residue (the sum of a parent drugs and/or their metabolites) in animal products have been set by Codex Alimentarius Commission (CAC) and China [5,6], and the values usually range from 10 to 5000 μg/kg according to the different compounds and biological matrix. For example, both of the MRLs for thiabendazole (TBZ)-related drugs (sum of TBZ and 5-OH-TBZ) have been set at 100 μg/kg for muscle and liver. Due to the complexity of samples and the differences of lipophilicity and p*K*a values among BZDs, the multi-residue determination of BZDs in biological matrices is still a challenge. Therefore, the development of sensitive, rapid, and inexpensive analytical method is required [3].

Hereto, the combination of high performance liquid chromatography (HPLC) and mass spectrometry (MS) has become the favored technique for monitoring a wide range of BZDs for highly sensitive and selective determination in edible animal tissues and animal-derived products [1,7–12], while the MS detections are very expensive and not widely applicable for a common laboratory. Gas chromatography (GC) was also applied while extra derivatization step of residues to sufficiently volatilize was required [3]. As a powerful complementary separation technique, capillary electrophoresis (CE) has also been applied in the separation and determination of BZDs due to its unique advantages such as rapid analysis, excellent separation efficiency, versatile separation mode, low cost, and less need for sample and organic solvents [13–15]. However, analytical sensitivity by CE is inferior to HPLC due to both the small injection volumes and the short optical path length

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of the capillary used as detection cell, which severely restricts its direct application in trace analysis. To enhance the instrument sensitivity in CE, various pre-concentration techniques, including electrophoresis-based and chromatography-based, were investigated to improve the sensitivity.

As to electrophoresis-based pre-concentration techniques, several sample stacking modes have been developed, in which field-amplified sample stacking (FASS) is most frequently used and relatively high enrichment efficiency was obtained [16,17]. FASS was achieved based on a discrepancy of the electric conductivity between the sample and the background electrolyte (BGE). Under the applied voltage, the analytes in low conductivity sample solution migrated fast towards the reverse electrode while the migration speed decreased rapidly at the boundary of BGE with high conductivity, where they were concentrated.

Since the animal tissue samples are complicated and the matrix effects can influence the quantification of BZDs, sample preparation is commonly adopted to improve the subsequent analytical performance. Up to date, several pretreatment techniques, such as solid-phase extraction (SPE) and liquid–liquid extraction (LLE) [1,12,18], have been frequently applied for the extraction of BZDs. However, these techniques are usually time-consuming and require large volumes of both samples and toxic organic solvents. Magnetic solid phase extraction (MSPE) has become popular in sample preparation due to the large interfacial area for mass transmission, easy operation, timesaving, high throughput, etc. [19–22]. Recently, our group synthesized a novel magnetic microspheres, i.e. magnetite/silica/poly (methacrylic acid-*co*-ethylene glycol dimethacrylate) (Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA)), and successfully extracted sulfonamides from milk samples [20] and illegal drugs from human urine [21]. Good extraction performance could be achieved quickly and simply. More recently, we also developed a solid-phase microextraction (SPME) method for BZDs based on capillary poly (MAA-*co*-EGDMA) monolith [7]. The results indicated that the poly (MAA-*co*-EGDMA) monolithic material had good extraction efficiency and matrix removal ability. These inspire us to develop a rapid analytical method for the determination of BZDs by combination of Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA)-based MSPE and CZE. To the best of our knowledge, till now there are no reports on the combination of MSPE and CE for the determination of BZDs in food.

In this paper, we developed a simple, rapid, and efficient determination method of BZDs by combination of MSPE with FASS-CZE. The results showed the method possessed good ability to get rid of the interferences from the animal tissue matrix, and FASS technique was applied to further improve the detection sensitivity of BZDs. At last, the MSPE–FASS-CZE method was successfully applied in monitoring the BZDs residues in animal tissue samples.

2. Experimental

2.1. Chemicals and solutions

Acetonitrile (MeCN) and dimethyl sulfoxide (DMSO) were purchased from J&K Chemical (Beijing, China). Sodium hydroxide (NaOH), hydrochloric acid (HCl), disodium hydrogen phosphate (Na_2HPO_4), citric acid (Cit), formic acid (FA), acetic acid (HOAc) and trifluoroacetic acid (TFA) were also purchased from Shanghai general chemical reagent factory and were of analytical reagent grade. Purified water was obtained with an Aike water purification apparatus (Chengdu, China).

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) were all purchased from Sinopharm Chemical Reagent (Shanghai, China). 3-(Methacryloxy) propyl trimethoxysilane (MPS), methacrylic acid (MAA, 98% pure) and ethylene glycol dimethacrylate (EGDMA, 98% pure) were purchased from Acros (New Jersey, USA). AIBN was recrystallized from ethanol, and other reagents were of analytical grade and used directly without further purification.

Albendazole (ABZ), fenbendazole (FBZ), mebendazole (MBZ) and thiabendazole (TBZ) were purchased from Laboratories of Dr. Ehrenstorfer (Augsburg, Germany), and albendazole sulfoxide (ABZ-SO), albendazole sulfone (ABZ-SO_2), oxfendazole (OFZ), fenbendazole sulfone (FBZ-SO_2), 2-amino-albendazole sulfone (ABZ-NH_2-SO_2) and 5-hydroxy thiabendazole (5-OH-TBZ) were purchased from Laboratories of WITEGA (Berlin, Germany). Their chemical structures are shown in Fig. 1. Individual stock solutions were prepared to 500 μg/mL with DMSO–MeCN (2:3, v/v) and diluted with MeCN to obtain a mixture standard solution of 40 μg/mL. Individual stock solution was stable for 6 months and a mixture standard solution was re-prepared every month. Berbine (used as internal standard, I.S.) was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Berbine solution (42 μg/mL) was prepared in MeCN–TFA (100:0.5, v/v). All of the above solutions were stored at 4 °C in the dark.

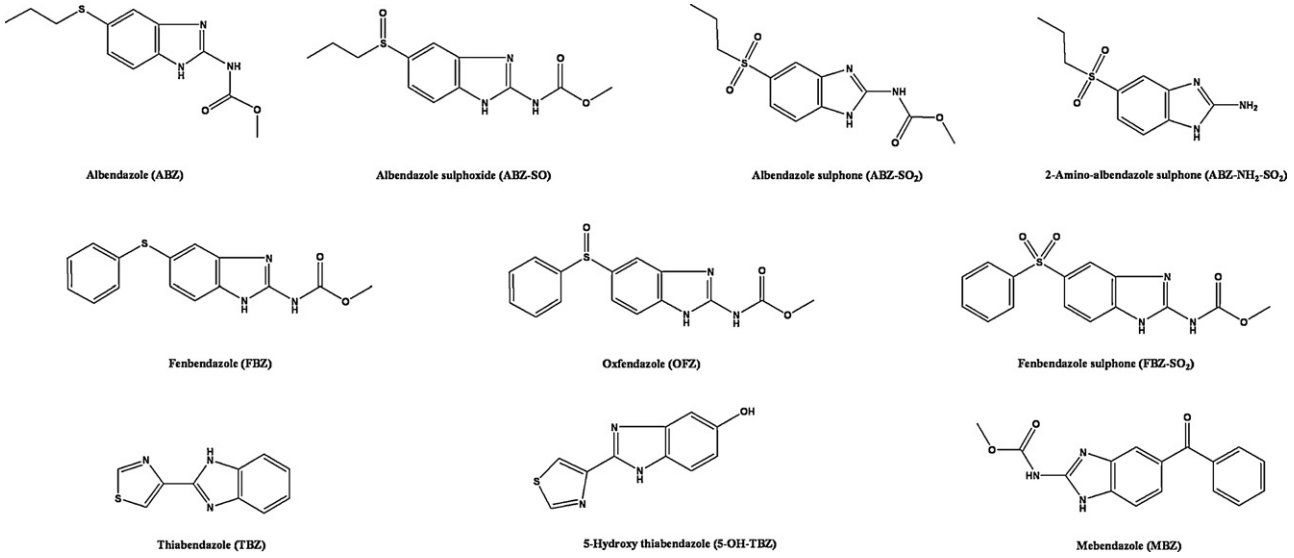

Fig. 1. Chemical structures of the studied benzimidazole drugs.

2.2. Instrumentation and analytical conditions

CE analysis was performed on a Beckman Coulter MDQ equipped with a UV–vis detector instrument (Beckman Coulter, Fullerton, CA, USA). Separations were carried out in an 86 cm (effective 75.5 cm, 75 μm i.d.) fused-silica capillary (Yongnian Fiber Plant, Hebei, China). At the start of the daily analysis, the capillary was rinsed at 20 psi sequentially with 1 mol/L HCl (15 min), and water (5 min), 1 mol/L NaOH (15 min), water (5 min), followed by conditioning with BGE for 10 min. Between runs, the capillary was rinsed at 20 psi sequentially with 1 mol/L NaOH, water and BGE at 2 min intervals. The UV absorbance detection was performed at 292 nm. The CZE system was operated using normal polarity (the cathode was located at the outlet). FASS was performed by applying an injection voltage of 10 kV for 12 s to assure the current is less than 30 μA. And all the separations were performed using a buffer of 50 mM Cit-Na_2HPO_4 (pH 2.2) under optimized separation conditions. The applied voltage was 30 kV and the capillary temperature was 20 °C. Before use, the buffer was filtered through a membrane filter (0.45 μm) and degassed in an ultrasonic bath for 10 min.

Berbine solution was added before CE analysis to minimize the variation resulted from the electrokinetic injection and the fluctuation of the electroosmotic flow. Triplicate injections of the sample were performed and relative peak area (analyte area/I.S. area) was used for quantification.

2.3. Preparation of Fe_3O_4/SiO_2/poly (MAA-co-EGDMA)

According to our previous work, Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA) microspheres were synthesized by a three-step procedure [20]. Firstly, the hydrophobic Fe_3O_4 nanoparticles were prepared by chemical co-precipitation of Fe^{3+} and Fe^{2+}. Then, the resultant Fe_3O_4 nanoparticles were coated with SiO_2 by a modified Stöber method, and the surface of Fe_3O_4/SiO_2 was further modified with MPS. Finally, the Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA) microspheres were prepared by distillation–precipitation polymerization with the mixture of MPS-modified Fe_3O_4/SiO_2, MAA, EGDMA, and AIBN.

2.4. Sample preparation

Swine tissue samples were purchased from a local supermarket, these samples were homogenized and stored at −20 °C before use. The thawed sample (1.5 g) was then accurately weighed into a 10-mL centrifuge tube and spiked with different amounts of BZDs. Fifteen minutes was allowed for equilibration at room temperature. After that, 5.0 mL of MeCN was added to the above samples. The mixtures were homogenized with an ultrasonic homogenizer Model HOM-100 (3 mm i.d. chip) (Ningbo Scientz Biotechnology Co., Ningbo, China) for 1.0 min, and then were centrifuged at 0–4 °C for 5.0 min at 10,000 rpm. The supernatant 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 35 °C, then reconstituted with 10 mL phosphate solution (10 mM, pH 5.0) for the following MSPE procedure.

Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA) microspheres (75 mg) were put into a 15-mL vial and firstly activated with MeCN and phosphate solution (10 mM, pH 5.0) in sequence, and then the supernatant was added into the vial. The mixture was mixed with a vortex mixer for 1 min to form a homogeneous dispersion solution, and then magnetic microspheres absorbed BZDs were separated rapidly from the solution under a strong external magnetic field. Subsequently, 1.0 mL phosphate solution (10 mM, pH 5.0) was used as a washing solution. After discarding the supernatant solution, BZDs were eluted from the magnetic microspheres by 1.0 mL of MeCN–TFA (100:0.5, v/v) with 1 min vortex. The solution was separated from the magnetic microspheres by a magnet, and evaporated to dryness under a mild nitrogen stream at 35 °C. The residue was dissolved in 0.1 mL of MeCN–TFA (100:0.5, v/v) for CE analysis.

3. Results and discussion

3.1. Optimization of separation conditions by CZE

Shen [13] reported the optimum buffer was Cit-Na_2HPO_4 when five BZDs were separated by CZE, which produced a good electropherogram baseline and good peak shapes. To achieve the high stacking efficiency and an acceptable current flow, high concentration of buffer to get high conductivity is required. Therefore, 50 mM Cit-Na_2HPO_4 electrolyte was used as BGE. The capillary was kept at 20 °C to maintain reproducibility of analysis.

To get good separation of the ten BZDs in a short analysis time, several parameters such as pH of BGE, separation voltages, and the addition of organic modifier to BGE, were investigated, respectively.

Fig. 2 is the electropherograms of the ten BZDs with the pH range from 2.0 to 3.4. The results showed that the separation became better with the increase of pH value, meanwhile an increase of the migration times was also observed. In CZE mode, the increase in pH of BGE will increase the degree of ionization of silanol group, which therefore increase the velocity of electro-osmotic flow (EOF). On the other hand, the electromigration of analytes is also affected by the electrostatic interaction between analytes and silanol groups on the inner wall of capillary, which plays a dominant role on the separation of the analytes we investigated. Therefore, the migration time of analytes prolonged and the gap of these analytes increased. To gain good separation in a short analysis time, pH 2.2 was chosen for further study. Additionally, the effect of applied voltages on the separation was investigated from 20 to 30 kV. The result (Fig. 3) indicated that the applied voltages contributed little to the peak resolution but the migration time decreased with the increase of applied voltages. To reduce analytical time and keep good separation, the separation voltage was kept at 30 kV.

The effect of the organic modifier on BGE was evaluated by adding MeCN to the buffer. As shown in Fig. 4, the addition of MeCN could decrease the velocity of EOF, however, MeCN can weaken the electrostatic interaction between analytes and silanol groups. When the MeCN content increased from 0% to 10% (v/v), the influence of MeCN content on the velocity of EOF played a dominant

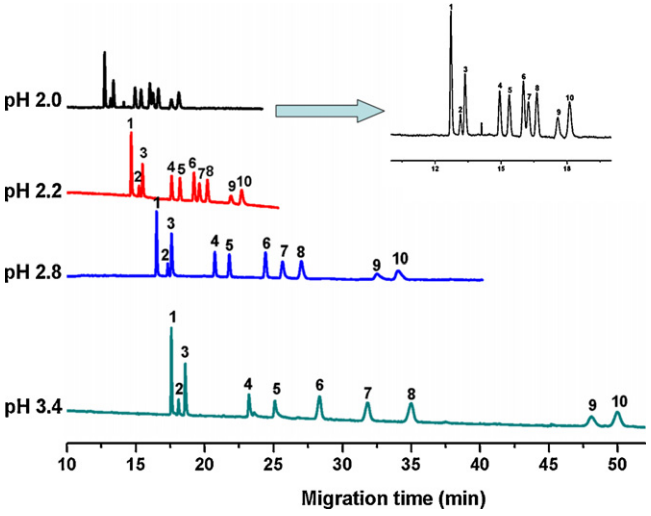

Fig. 2. Electrophoregrams for separation of the ten BZDs at different pH values. CZE conditions: capillary, 86 cm (effective 75.5 cm, 75 μm i.d.); injection: 0.5 psi × 8 s; UV detection at 292 nm; BGE: 50 mM Cit-Na_2HPO_4; applied voltage: 30 kV. Orders of peaks: 1. TBZ, 2. ABZ-NH_2-SO_2, 3. 5-OH-TBZ, 4. ABZ, 5. FBZ, 6. MBZ, 7. ABZ-SO, 8. OFZ, 9. ABZ-SO_2 and 10. FBZ-SO_2.

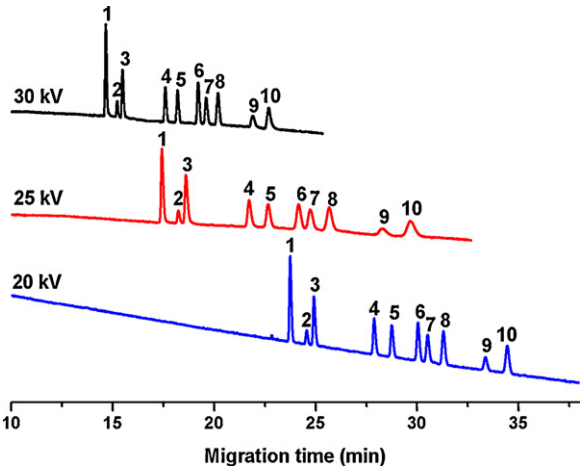

Fig. 3. Electrophoregrams for separation of the ten BZDs at different applied voltages. CZE conditions: capillary, 86 cm (effective 75.5 cm, 75 μm i.d.); injection: 0.5 psi × 8 s; UV detection at 292 nm; BGE: 50 mM Cit-Na_2HPO_4 (pH 2.2). Orders of peaks: 1. TBZ, 2. ABZ-NH_2-SO_2, 3. 5-OH-TBZ, 4. ABZ, 5. FBZ, 6. MBZ, 7. ABZ-SO, 8. OFZ, 9. ABZ-SO_2 and 10. FBZ-SO_2.

role. Further increase of MeCN content from 10% to 30% (v/v) can result in the shorter migration time of analytes, which indicated that influence of decreased electrostatic interaction begun to predominate. Since the organic modifier contributed a little to the peak resolution but increased the migration time, no organic modifier was added for the following experiments.

3.2. Optimization of FASS

To improve the detection sensitivity of CE, FASS was used as on-line preconcentration technique in this study. In this method, sample enrichment was performed by electrokinetically injecting a sample with lower conductivity compared with the BGE. Several parameters affecting FASS were investigated. Firstly, MeCN content in sample solutions was optimized. As shown in Fig. 5A, the signal of each BZDs obtained with various concentrations of MeCN as sample solutions was illustrated. With the increase of the MeCN concentration, the conductivity of the solvent decreased gradually and sample stacking increased rapidly. The highest signal was obtained using the sample solution of MeCN–TFA (100:0.5, v/v), which may

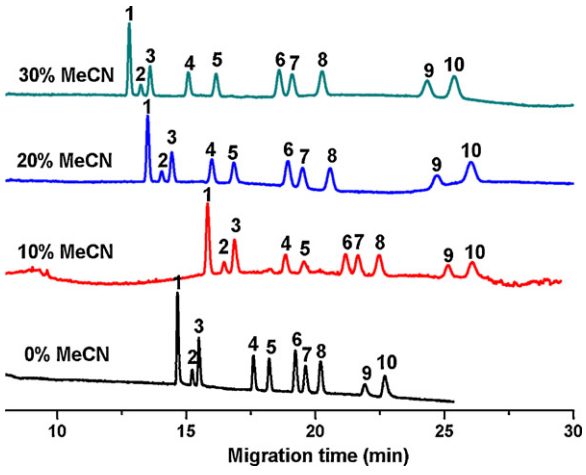

Fig. 4. Electrophoregrams for separation of the ten BZDs at different MeCN content added to the buffer. CZE conditions: capillary, 86 cm (effective 75.5 cm, 75 μm i.d.); injection: 0.5 psi × 8 s; UV detection at 292 nm; BGE: 50 mM Cit-Na_2HPO_4 (pH 2.2); applied voltage: 30 kV. Orders of peaks: 1. TBZ, 2. ABZ-NH_2-SO_2, 3. 5-OH-TBZ, 4. ABZ, 5. FBZ, 6. MBZ, 7. ABZ-SO, 8. OFZ, 9. ABZ-SO_2 and 10. FBZ-SO_2.

Table 1
Figures of merit of the CE-UV analysis of the ten BZDs using FASS as preconcentration procedure.

BZD drugs	FASS[a]		
	Rs (n, $n+1$)	NTP[b]	Fold[c]
TBZ	–	180,789	57.0
ABZ-NH_2-SO_2	3.9	202,297	40.2
5-OH-TBZ	1.2	134,623	53.9
ABZ	11.1	150,483	28.1
FBZ	3.0	137,534	25.7
MBZ	5.5	131,313	21.7
ABZ-SO	2.1	122,238	21.8
OFZ	2.6	116,152	20.3
ABZ-SO_2	8.4	132,542	16.6
FBZ-SO_2	3.0	105,127	15.6

[a] Data given for 0.5 μg/mL.
[b] Number of theoretical plates of column.
[c] Increase in sensitivity compared to the same conditions with pressure injection of sample solution at 0.5 psi for 8 s.

be due to that the lowest conductivity for sample matrix can be obtained with this sample solution. To optimize the condition for solute ionization, three organic acids including FA, HOAc and TFA, were compared. TFA was found to be the best for the FASS process. Subsequently, the effect of TFA concentration on the signal intensities of BZDs was also investigated within the range of 0–2.0% (v/v). As shown in Fig. 5B, the best stacking result was achieved at the concentration of 0.5% TFA. Too low TFA concentration may cause insufficient protonation of the analytes while too high TFA concentration may result in higher conductivity, therefore, samples were dissolved in MeCN–TFA (100:0.5, v/v) and injected electrokinetically at 10 kV for 12 s. Further increase in voltage or injection time may cause current breakdown during the process of separation.

Table 1 lists the resolution, number of theoretical plates (NTP), and enrichment factors of the ten BZDs. The results showed that the detection sensitivity of BZDs increased under the optimized electrophoretic conditions. It can be seen that the resolutions are higher than 1.2 and the NTP are above 105,000 for selected BZDs, which are appropriate to the accurate determination of BZDs. The enrichment folds of the ten BZDs obtained by FASS ranged from 15.6 to 57.0.

3.3. Optimization of conditions for MSPE

The extraction conditions were optimized with phosphate solution (10 mmol/L) spiked with the ten BZDs. Several parameters such as amount of adsorbent, pH, extraction time, desorption time, and desorption solvent were optimized.

To obtain good recovery, the adsorbent amount was firstly investigated from 10 to 100 mg for a certain amount of sample solution. As shown in Fig. 6A, the result indicated the extracted amount of analytes increased rapidly with the increase of adsorbent amount. The signal intensities of analytes obtained by using 75 mg of adsorbent were higher than that of by using 10, 20, and 50 mg sorbent. However, no significant improvement in signal intensities was achieved by using more than 75 mg of adsorbent. Hence, 75 mg of magnetic adsorbent was selected in the further experiment.

The pH of the sample solution not only influenced the degree of ionization of target analytes, but also determined the surface charge of microspheres. It is known that BZDs possess an imidazole ring containing both acidic and basic nitrogen atoms. Under suitable conditions, the molecule may be protonated or deprotonated. The pH optimization was performed within the range from 2.0 to 9.0. As shown in Fig. 6B, the higher extraction efficiency was obtained in the pH range of 4.0–7.0. The enhanced extraction efficiency can be explained by the ion-exchange interaction between the cationic

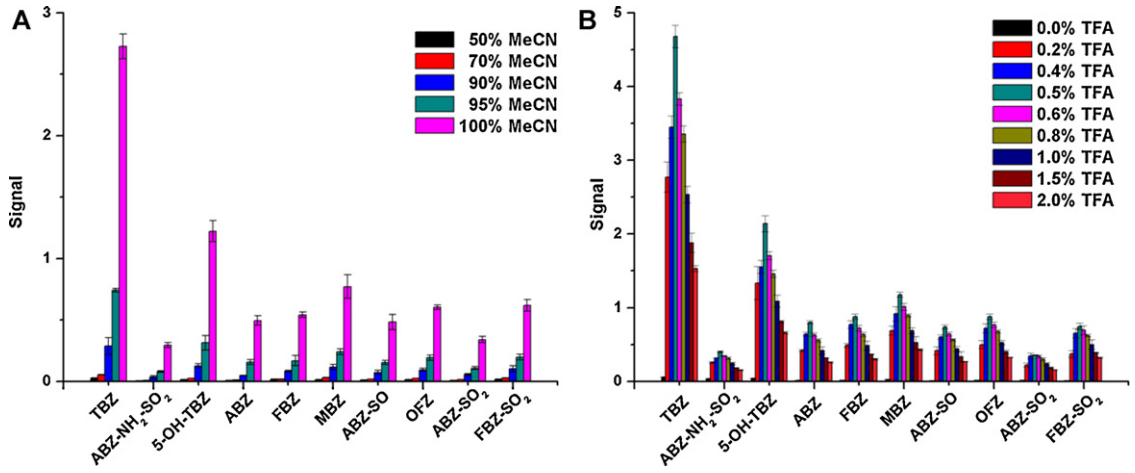

Fig. 5. Optimization of MeCN concentration (A) and TFA concentration (B) added in the sample solutions for FASS.

analytes and the negatively charged adsorbent. When pH value increased above 7.0, the most BZDs molecules were transformed to neutral state and a few were in protonated form, which resulted 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 dominates the retention of BZDs on the adsorbent, which is in accordance with a previous report [7]. To obtain highest extraction efficiency, the sample matrix was adjusted to pH 5.0 for the following experiments.

The extraction time (refer to the agitation time on the vortex mixer) was investigated within the range of 0.5–10 min. As shown in Fig. 6C, 1.0 min was enough for effective extraction and the prolonged vortex time did not improve the analytical signals. This phenomenon might be attributed to a large contact surface between the analytes and adsorbent. Hence, 1.0 min was selected as the extraction time.

To ensure compatibility with the sample solution optimized in FASS, MeCN–TFA (100:0.5, v/v) was directly used as desorption solution. The results showed that 1 mL of desorption solution can

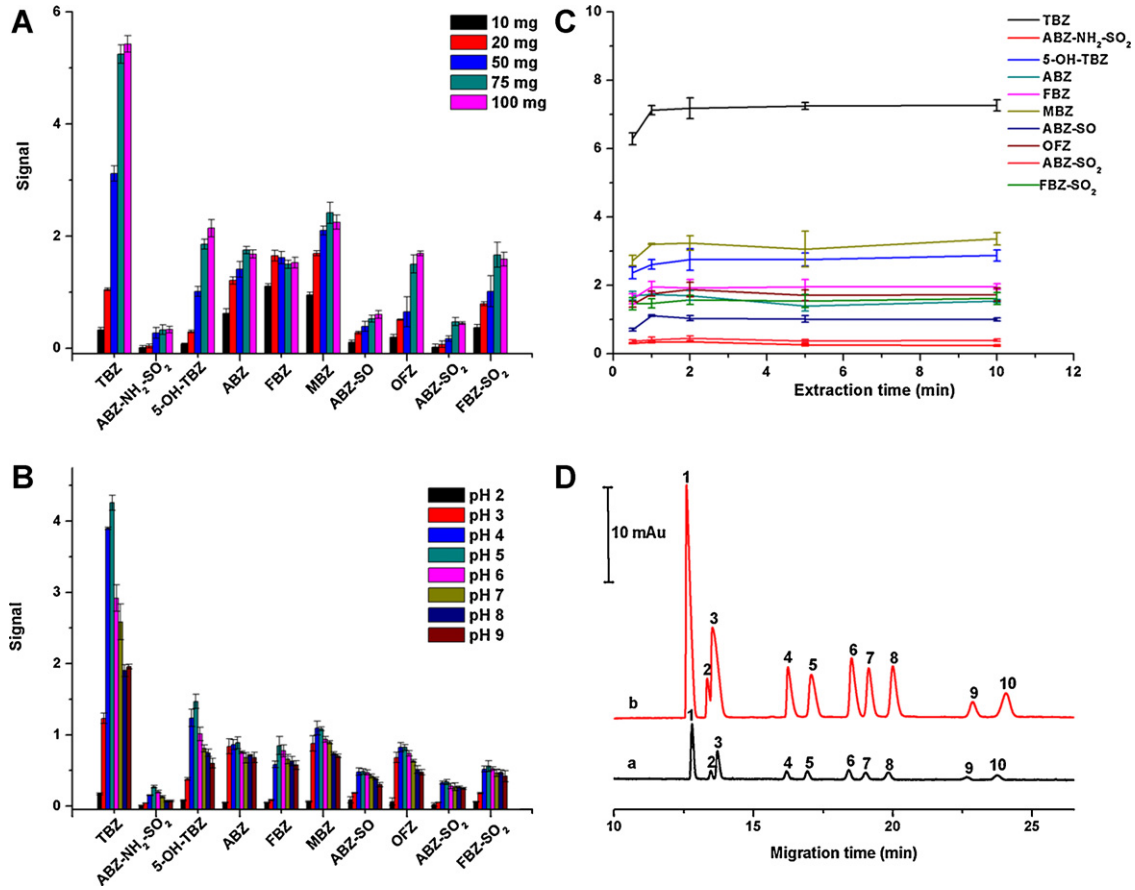

Fig. 6. Effect of adsorbent amounts (A), pH in sample solutions (B), extraction time (C) on extraction efficiency, and (D) electropherograms of the BZDs standard sample obtained by direct CE analysis (a) and MSPE–CE (b). Orders of peaks: 1. TBZ, 2. ABZ-NH_2-SO_2, 3. 5-OH-TBZ, 4. ABZ, 5. FBZ, 6. MBZ, 7. ABZ-SO, 8. OFZ, 9. ABZ-SO_2 and 10. FBZ-SO_2.

completely elute the extracted BZDs from the sorbent. No peak was detected in the following second desorption. However, stacking efficiency was significantly reduced by the residual salt and water in the eluate. Hence, a water-washing procedure (1.0 mL) was performed to remove the residual salt before desorption. Furthermore, the final eluate was dried under a mild nitrogen current at 35 °C to remove the influence of water, and then reconstituted with 0.1 mL MeCN–TFA (100:0.5, v/v) for the following CE analysis.

The electropherograms of the BZDs obtained by MSPE–CE and direct CE analysis under the optimized conditions are shown in Fig. 6D. The results showed that a significant peak height enhancement was observed, indicating the obvious preconcentration ability of the magnetic microspheres.

3.4. Applications to swine muscle and liver samples

Under optimized conditions, the method was applied for the determination of the ten BZDs in swine muscle and liver samples. Fig. 7 shows the electropherograms obtained by MSPE–FASS-CZE of the swine muscle and liver sample spiked with BZDs at the concentration of 100 ng/g. The results showed that no interferences from the matrix were observed for the quantification of the analytes.

The relative recoveries were calculated by comparing the peak area ratios of the BZDs from the spiked swine muscle and liver samples to those obtained from the standard solutions diluted by phosphate solution. The results showed the relative recoveries of the ten BZDs ranged from 43.7% to 94.7% in two matrices, indicating the determination of the BZDs was affected by the interferences from swine muscle and liver samples to some extent. Therefore, matrix-matched calibration curves were constructed as reference curves for the quantification of BZDs in the following experiments.

Linear regression analyses were performed using relative peak areas against the respective analytes concentration. The results are listed in Table 2. The regression coefficients (R^2) were between 0.9920 and 0.9995, and 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. The LODs of the ten BZDs in swine muscle and liver were in the range of 1.05–10.42 ng/g and 1.06–12.61 ng/g, respectively. The LOQs were in the range of 3.49–34.72 ng/g and 3.55–42.02 ng/g, respectively, which were lower than the regulated MRL values of CAC/MRL (02-2009) and China (No. 235, 2002). Therefore, the MSPE–FASS-CZE method was adequate for the routine residue monitoring of BZDs in swine tissue samples.

The precision of the developed method was assessed by determining the intra- and inter-day relative standard deviations (RSDs). Four extractions of independently prepared samples in a day gave the intra-day precision. The inter-day precision was determined by extracting independently prepared samples for 3 continuous days.

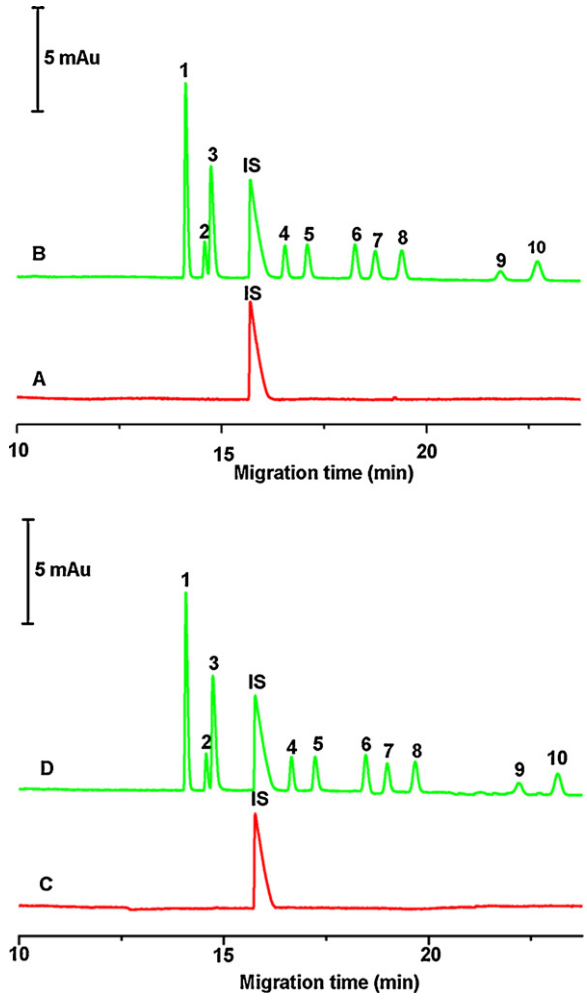

Fig. 7. Electropherograms of the BZDs in blank swine muscle and liver sample (A and C) and spiked swine muscle and liver samples (B and D). Orders of peaks: 1. TBZ, 2. ABZ-NH_2-SO_2, 3. 5-OH-TBZ, 4. ABZ, 5. FBZ, 6. MBZ, 7. ABZ-SO, 8. OFZ, 9. ABZ-SO_2 and 10. FBZ-SO_2. IS was 2 μg/mL.

Three concentrations, involving the low, medium and high level of the calibration curve range, were investigated. As shown in Table 3, the intra- and inter-day RSDs were lower than 13.2% and 13.6% in two matrices, respectively.

The developed method was subsequently applied for analysis of six real samples (3 swine muscle samples and 3 swine liver samples) purchased from three local markets. The results showed that no analytes we investigated were determined in these samples. Satisfied recoveries were obtained in the range of

Table 2
Linear regression and LOD, LOQ data for MSPE–FASS-CZE of the ten BZDs in swine muscle (M) and liver (L) samples.

Analytes	Linear range (ng/g)	Linear regression				R^2		LOD (ng/g)		LOQ (ng/g)	
		Intercept		Slope		M	L	M	L	M	L
		M	L	M	L						
TBZ	50–2000	0.1061	0.4870	0.0012	0.0105	0.9972	0.9920	1.05	1.06	3.49	3.55
ABZ-NH_2-SO_2	50–2000	0.4258	0.0703	0.0068	0.0013	0.9965	0.9934	5.59	6.41	18.62	21.37
5-OH-TBZ	50–2000	−0.2293	0.5259	0.0029	0.0067	0.9930	0.9924	1.84	1.89	6.12	6.31
ABZ	50–2000	−0.2453	−0.1874	0.0031	0.0028	0.9995	0.9935	7.61	8.26	25.38	27.55
FBZ	50–2000	−0.3335	−0.2043	0.0039	0.0030	0.9988	0.9953	7.59	8.02	25.32	26.74
MBZ	50–2000	−0.2349	−0.2635	0.0033	0.0037	0.9952	0.9944	4.59	5.52	15.29	18.42
ABZ-SO	50–2000	−0.2674	−0.2009	0.0036	0.0032	0.9937	0.9959	5.74	6.83	19.12	22.78
OFZ	50–2000	−0.0487	−0.2225	0.0011	0.0035	0.9923	0.9947	5.42	6.56	18.05	21.88
ABZ-SO_2	50–2000	−0.0319	−0.0815	0.0020	0.0011	0.9927	0.9956	10.42	12.61	34.72	42.02
FBZ-SO_2	50–2000	0.1061	−0.0742	0.0012	0.0022	0.9972	0.9941	8.22	9.55	27.40	31.85

Table 3
The method precisions at three different concentrations for MSPE–FASS-CZE of the ten BZDs for swine muscle (M) and liver (L) samples.

Analytes	Intraday (n = 4) RSD (%)						Interday (n = 3) RSD (%)					
	50 ng/g		200 ng/g		2000 ng/g		50 ng/g		200 ng/g		2000 ng/g	
	M	L	M	L	M	L	M	L	M	L	M	L
TBZ	6.8	4.9	5.0	3.1	1.0	7.2	8.8	7.1	9.3	7.8	4.4	7.7
ABZ-NH_2-SO_2	9.6	9.3	6.9	4.9	8.8	5.8	11.3	7.7	6.4	5.0	3.6	5.2
5-OH-TBZ	8.6	10.7	9.6	9.6	3.0	5.8	3.7	7.3	6.4	5.9	5.5	9.2
ABZ	6.6	8.2	3.3	7.4	5.2	6.3	13.2	8.6	5.4	3.6	7.0	8.0
FBZ	9.4	5.8	5.2	3.6	5.6	4.2	9.3	7.2	5.1	5.1	6.7	5.8
MBZ	10.3	9.3	1.6	4.3	5.9	3.9	8.5	7.8	1.5	4.6	4.7	6.4
ABZ-SO	7.6	7.5	7.8	8.0	5.6	8.6	9.6	8.3	4.3	9.1	4.5	7.5
OFZ	5.9	8.4	7.2	6.8	5.8	8.7	8.8	11.6	4.2	6.3	7.5	7.7
ABZ-SO_2	6.0	6.8	8.1	3.3	4.4	5.9	10.5	13.6	11.1	10.2	4.4	6.3
FBZ-SO_2	5.4	8.4	2.0	5.2	7.2	6.2	9.2	8.8	7.8	8.5	6.7	5.0

Table 4
Recoveries of the ten BZDs spiked in swine muscle (M) and liver (L) samples using the MSPE–FASS-CZE method.

Analytes	Swine muscle samples (spiked at 100 ng/g)						Swine liver samples (spiked at 100 ng/g)					
	M-1		M-2		M-3		L-1		L-2		L-3	
	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)	Recoveries (%)	RSD. (%)	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)
TBZ	92.9	7.4	104.9	0.3	96.2	0.8	87.9	3.0	98.2	1.3	99.7	3.1
ABZ-NH_2-SO_2	87.8	8.7	95.7	4.0	81.9	7.3	93.8	6.1	87.5	3.3	100.6	0.5
5-OH-TBZ	102.0	4.6	83.3	6.4	91.1	3.0	103.1	4.4	97.0	0.7	95.7	4.2
ABZ	93.1	8.2	93.3	1.8	94.6	4.8	89.3	7.1	104.6	2.3	98.0	3.3
FBZ	88.6	7.5	93.4	2.4	97.6	5.4	93.0	8.3	98.8	1.1	101.3	3.4
MBZ	91.5	4.1	98.4	1.3	97.5	6.5	89.8	2.8	99.4	0.3	90.8	2.7
ABZ-SO	93.3	8.9	86.0	1.9	93.8	5.5	92.2	7.5	94.2	0.8	97.4	3.7
OFZ	95.8	3.3	90.3	1.0	94.7	5.8	97.5	3.5	105.4	0.4	100.6	2.9
ABZ-SO_2	81.1	9.3	100.7	6.0	102.4	8.6	90.7	5.7	95.4	3.7	95.5	7.8
FBZ-SO_2	91.0	4.2	104.5	2.1	98.5	9.2	94.2	8.5	97.3	4.1	93.4	8.9

81.1–105.4% with RSDs less than 9.3% (as shown in Table 4). These results demonstrated that the proposed method was reliable for the determination of the BZDs residues in swine tissue samples. In addition, the Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA) magnetic microspheres can be recycled and no significant change was observed on extraction efficiency after repeated extraction/desorption cycles.

4. Conclusion

A simple, fast and sensitive method for the determination of ten BZDs in swine muscle and liver samples was established by combining Fe_3O_4/SiO_2/poly (MAA-*co*-EGDMA) magnetic microspheres with FASS-CZE technique. The result showed the magnetic microspheres had obvious preconcentration ability for the ten BZDs. Satisfactory results were obtained with regard to sensitivity, accuracy and precision. Compared with other previously reported methods, the MSPE–FASS-CZE method is inexpensive and applicable for a common laboratory detection of BZDs. Furthermore, this method is effective on removing the potential interferences in edible animal tissue since no interference was observed for the quantification of BZDs. Therefore, the MSPE–FASS-CZE method was adequate for the routine residue monitoring of BZDs in swine tissue samples, and the combination of MSPE and FASS-CE provides a practical tool for veterinary multi-residue determination.

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