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Determination of five nitroimidazole residues in artificial porcine muscle tissue samples by capillary electrophoresis

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ABSTRACT

A capillary electrophoresis (CE) method with ultraviolet detection has been developed for simultaneous detection and quantification of five nitroimidazoles including benzoylmetronidazole, dimetridazole, metronidazole, ronidazole, and secnidazole in porcine muscles. Nitroimidazoles in samples were extracted by ethyl acetate with subsequent clean-up by a strong cation exchange solid phase extraction column. The clean extracts were subjected to CE separation with optimal experimental conditions: pH 3.0 running buffer containing 25 mM sodium phosphate and 0.10 mM tetrabutylammonium bromide, 5 s hydrodynamic injection at 0.5 psi and 28 kV separation voltage. The nitroimidazoles could be monitored and detected at 320 nm within 18 min. The limits of detection were below 1.0 μg/kg and limits of quantification were lower than 3.2 μg/kg for all nitroimidazoles in the muscle samples. The recoveries and relative standard deviations were 85.4–96.0, 83.5–92.5, 1.3–3.9, and 1.1–4.2%, respectively for the intra-day and inter-day analyses. The proposed CE method has been successfully applied to determine nitroimidazoles in artificial porcine muscle samples with good accuracy and recovery, demonstrating that it has potential for detection and quantification of multi-nitroimidazole residue in real muscle samples.

1. Introduction

Nitroimidazoles are commonly synthesized from nitrification of iminazole in concentrated sulfuric acid. Among the nitroimidazoles, 5-nitroimidazoles are a well-established group of antiprotozoal and antibacterial agents. So far they have been used in human and veterinary medicine to treat diseases caused by protozoans (e.g., *Giardia lamblia*, *Entamoeba histolicia*) and bacterial infections such as *coccidiosis* and *haemorrhagic enteritis*. In addition, they could accelerate the growth of animals and improve the feed efficiency [1]. However, nitroimidazoles and their metabolites possess some genotoxic, carcinogenic and mutagenic properties in animals [2,3]. They are suspected to be genetic toxic to mammals [4] and could cause harmful effects on humans. Mudry et al. [5] reported that the chromosomes 11 and 17 of *Cebus libidinosus* were more susceptible to lower their replication index when exposed to metronidazole. For this reason, these drugs are currently included in the Annex IV of Council Regulation 2377/90 and 2205/2001 [6,7]; ronidazole in 1993, dimetridazole in 1995 and metronidazole in 1998. They have also been banned from use in food-producing animals in the US [8]. A number of countries which export their food products to the European Union were found to misuse these drugs [9]. Similarly, China has also prohibited the use of dimetridazole and metronidazole in food animals [10]. As such, it is of particular importance to develop a rapid and accurate method to determine nitroimidazoles and their metabolites in animal feeds and tissues.

So far various methods have been developed for analysis of nitroimidazoles and their metabolites in various animal tissues (muscle, liver, kidney, and retina) as well as plasma, serum, egg, faeces and water [11–26]. Most of these methods used electrochemical [27,28], immunoassay [25,29], thin-layer chromatography (TLC) [30], gas chromatography (GC) [11,26], gas chromatography–mass spectrometry (GC–MS) [16,18,31], high-performance liquid chromatography (HPLC) [15,32–34], and liquid chromatography–mass spectrometry (LC–MS) [10,21,35,36]. Among them, chromatography has the unique advantage of higher sensitivity for determination of nitroimidazoles. For instances, Ho et al. [18] utilized GC–MS for the detection of dimetridazole and metronidazole in poultry muscles, porcine liver and kidney, and chicken liver. Sun et al. [33] and Maher et al. [34] proposed HPLC-UV for determination of one or more nitroimidazole residues in animal tissue. Xia et al. [10,35] and Fraselle et al. [36] separated

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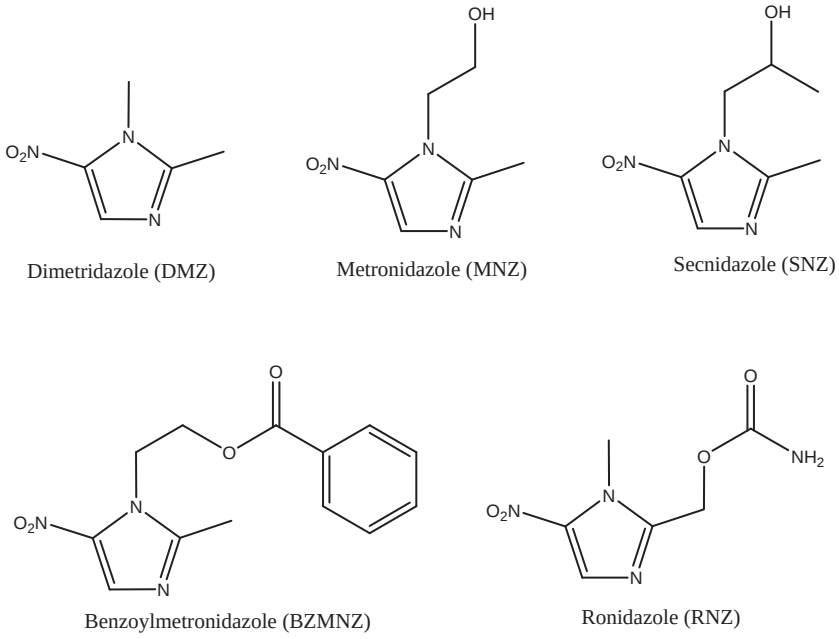

Fig. 1. Chemical structure of the nitroimidazoles.

and detected 5-nitroimidazoles by LC–MS/MS. However, most of these samples require derivatization before they were separated and analyzed by GC or GC–MS. For HPLC analysis of nitroimidazoles, larger sample volume and more solvents are required and the total elution time is longer.

Conversely, capillary electrophoresis (CE) possesses the advantages of high separation efficiency, short run time, small sample volume and less reagent consumption and lower operation cost [37]. These attributes empower CE to be successfully applied to pharmaceutical and biological analyses. For examples, Alnajjar et al. [38] applied CE with ultraviolet light (UV) detection for analysis of tinidazole in pharmaceuticals. Azzam et al. [39] screened the enantiomers of ornidazole in pharmaceutical formulations by CE. Nozal et al. [40] proposed the separation of metronidazole, ronidazole and dimetridazole in pig liver tissue by a supported liquid-CE and UV detection method.

In here, we report the development of a simple validated CE-UV method for simultaneous multi-residue determination of benzoylmetronidazole (BZMNZ), dimetridazole (DMZ), metronidazole (MNZ), ronidazole (RNZ), and secnidazole (SNZ) in porcine muscle tissues. Fig. 1 displays the chemical structures of nitroimidazoles. This work is significant and important as it would provide a simple alternative approach for multi-residue determination of more than one class of nitroimidazoles in muscles simultaneously. Our results confirm that the developed CE method is suitable for use in veterinary drug residue surveillance program, particularly in the perspective of frequent and routine laboratory analyses.

2. Experimental

2.1. Chemicals

Acetic acid, DMZ, MNZ, RNZ, SNZ, and tetrabutylammonium bromide (TBAB) were purchased from Sigma (St. Louis, MO, USA). Benzoylmetronidazole was obtained from AVATAR (Shanghai, China). The individual stock solutions of the nitroimidazoles were prepared in Milli-Q water at a concentration of 1.0 mg/mL and stored at 4 °C prior to use. Various concentrations of standard nitroimidazole solutions were prepared and then spiked into the blank poultry samples to obtain fortified samples containing the five nitroimidazoles at concentrations of 5.00–500 μg/kg. Acetone, acetonitrile, ammonium hydroxide, ethyl acetate, hydrochloric acid, methanol, monosodium dihydrogen phosphate, phosphoric acid, sodium hydroxide, and tris(hydroxymethyl)aminomethane (Tris) were from Fluka (Buchs, Switzerland). All chemicals of analytical reagent grade were used as received without further purification. Milli-Q water (Millipore, Bedford, MA, USA) was used throughout the work. All solutions were filtered through 0.45-μm membrane filters before injecting to the separation capillary.

2.2. Apparatus

The CE analyses were conducted on a Beckman P/ACE MDQ instrument (Fullerton, CA, USA) equipped with an auto-sampler and a diode array detector (PDA). All the CE operations were controlled by the Beckman P/ACE MDQ software. An uncoated fused-silica capillary (Yongnian Ruifeng Optical Fiber Factory, Hebei, China) of 50 cm (effective length 42.8 cm) × 50 μm i.d. was used throughout the experiments.

2.3. Capillary electrophoretic procedure

Prior to use, the new capillary was conditioned with methanol (30 min), water (15 min), followed with 1.0 M HCl (30 min), water (15 min), 1.0 M NaOH (30 min), and water (15 min), respectively and finally with the running buffer solution for 60 min. Between two consecutive analyses, the capillary was rinsed sequentially with 1.0 M NaOH for 5 min, flushed with water for 3 min, and finally with the running buffer for 8 min. The running buffer was 25 mM sodium phosphate (pH 3.0) containing 0.10 mM TBAB. The sample solution was loaded into the capillary by hydrodynamic injection for 5 s at 0.5 psi. Electrophoresis was performed at a constant voltage of 28 kV with detection at 320 nm. All CE procedures were conducted at 25 °C.

2.4. Samples preparation

Commercial porcine muscle samples were purchased from local supermarkets and retail stores (Nanchong, Sichuan Province,

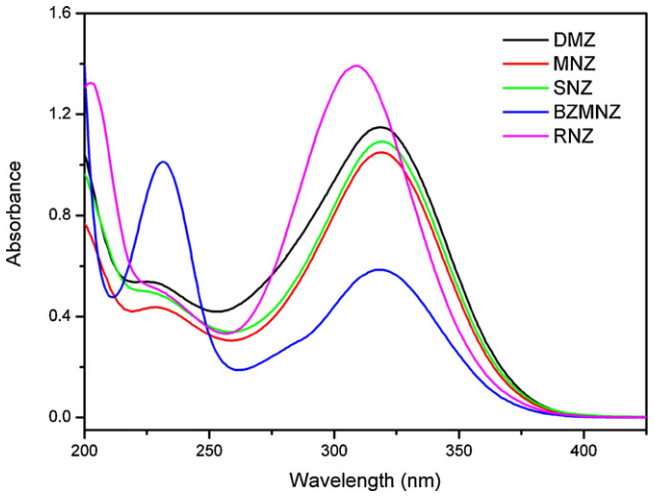

Fig. 2. UV–visible absorbance spectra of 0.13 mM nitroimidazoles in 25 mM phosphate buffer (pH 3.0): benzoylmetronidazole (BZMNZ), dimetridazole (DMZ), metronidazole (MNZ), ronidazole (RNZ), and secnidazole (SNZ).

China). The samples were prepared using procedures as described previously [33] with minor modifications. Briefly, 5.0 g of sample was weighed and homogenized in a high speed blender for 1 min. 1.0 mL of known concentration of standard mixture was spiked into the homogenized sample and mixed thoroughly. Afterwards, 20 mL ethyl acetate was added to the spiked sample, sonicated for 5 min and centrifuged at 3000 rpm for 10 min. The supernatant was collected and the residue was extracted once more with another 20 mL portion of ethyl acetate. The supernatants were combined and evaporated to almost dryness with a rotary vacuum evaporator at 40 °C. The residue was redissolved in 5.0 mL acetic acid–ethyl acetate (1:19, v/v) and subjected to solid phase extraction (SPE) clean-up as follows.

A SCX SPE cartridge (3 mL, 250 mg, Varian, Palo Alto, CA, USA) was initially conditioned by washing sequentially with 5 mL ammonium hydroxide–acetonitrile (1:19, v/v), 10 mL HCl (0.1 M), 20 mL Milli-Q water, 3 mL methanol, and 5 mL acetic acid–ethyl acetate (1:19, v/v). The sample extract obtained above was applied to the cartridge at a flow rate of 2 mL/min. The cartridge was then washed with 2.5 mL acetone, 5 mL methanol, and 5 mL acetonitrile, and finally eluted with 5 mL ammonium hydroxide-acetonitrile (1:19, v/v). The prime 2 mL eluent was discarded and the remaining eluent was collected in a 10-mL vial. It was evaporated to dryness in a nitrogen evaporator at 40 °C. Finally the residue was dissolved in 1.0 mL running buffer and filtered through a 0.45 μm filter prior to CE analysis.

3. Results and discussion

3.1. Detection wavelength

Fig. 2 depicts the UV absorption spectra of these nitroimidazoles in 25 mM phosphate buffer (pH 3.0). All nitroimidazoles possess strong absorption in the UV region. The absorption peak maxima (λ_{max}) for DNZ, MNZ and SNZ are at ca. 320 nm, attributing to the absorption of parent nitroimidazole chromophores. For RNZ, the absorption band is hypsochromatically shifted to 310 nm, possibly due to the presence of amide moiety in RNZ. BZMNZ shows two UV absorption bands with λ_{max} of 235 and 320 nm. The extra absorption band at 235 nm is originated from the phenyl moiety of BZMNZ. In theory, it is possible to use 235 nm but a higher baseline and interference are expected. To compromise, 320 nm was then chosen as the detection wavelength for CE since most nitroimidazoles have strong absorptions at this wavelength.

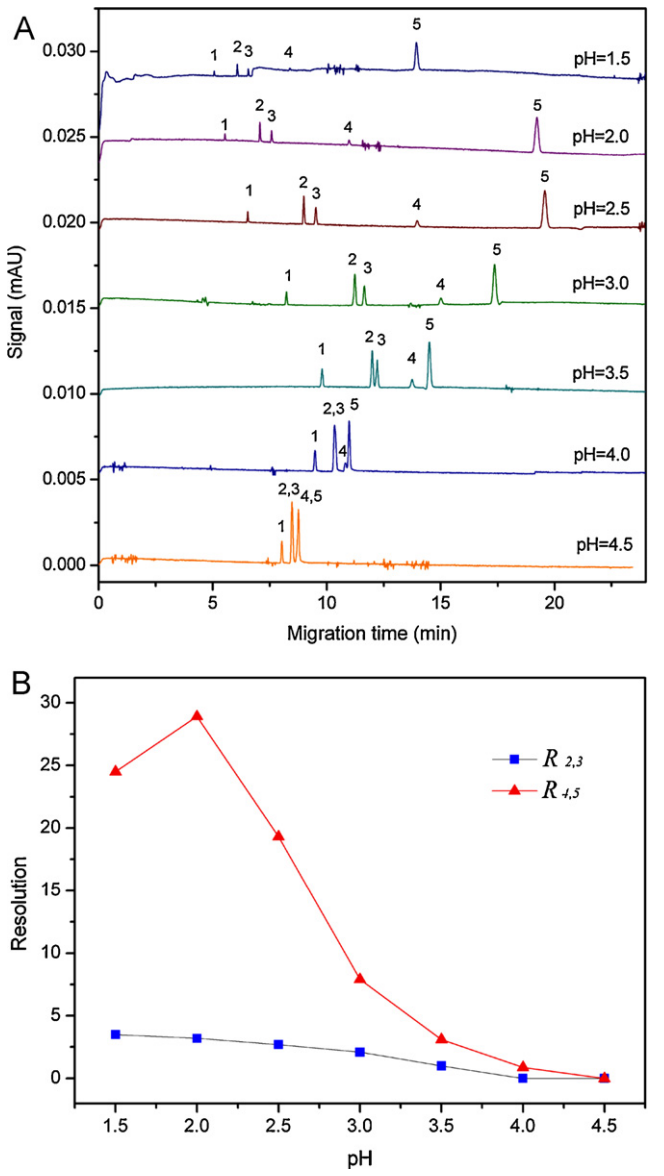

Fig. 3. Effect of running buffer pH on (A) electropherograms of a standard mixture of nitroimidazoles: (1) DMZ, (2) MNZ, (3) SNZ, (4) BZMNZ, and (5) RNZ and (B) resolutions of MNZ-SNZ and BZMNZ-RNZ critical pairs. The running buffer was 25 mM phosphate at pH 1.5–4.5 containing 0.10 mM TBAB. Hydrodynamic sample injection was performed at 0.5 psi and 5 s. The separation voltage and temperature were 28 kV and 25 °C, respectively.

3.2. Optimization of running buffer

Since nitroimidazoles have good solubility in phosphate and Tris buffers, the effect of these buffers on the CE separation of the five nitroimidazoles was investigated. It was found that the phosphate running buffer produces lowest background absorption at 320 nm and better peak shape and separation for nitroimidazoles (not shown). Thus, phosphate buffer was chosen as the running buffer for this work.

Buffer pH is another important factor in controlling and optimizing the CE separation. As such, the effect of pH on the separation of the five nitroimidazoles was studied. Fig. 3a depicts the CE separation of nitroimidazoles at various pH (1.5–4.5) of 25 mM phosphate running buffers containing 0.10 mM TBAB. The addition of TBAB to

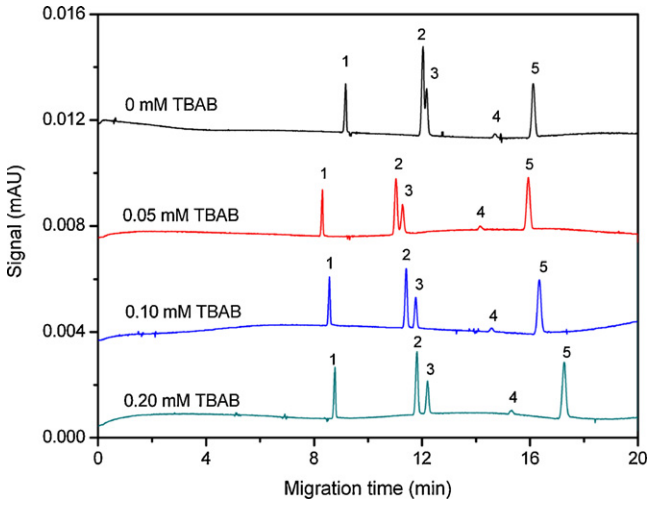

Fig. 4. Effect of TBAB in running buffer on the CE separation of nitroimidazoles: (1) DMZ, (2) MNZ, (3) SNZ, (4) BZMNZ, and (5) RNZ. The running buffers were 25 mM phosphate (pH 3.0) containing various concentrations of TBAB (0.00–0.20 mM). The CE conditions are same as in Fig. 3.

the running buffer will be discussed in the subsequent section. The migration order of nitroimidazoles is: (1) DMZ, (2) MNZ, (3) SNZ, (4) BZMNZ, and (5) RNZ, and is closely related to their charge-to-mass ratios in the running buffer. In general, the larger the charge-to-mass ratio of the solute, the faster it migrates. The pK_a of DMZ, MNZ and RNZ are 2.81, 2.58 and 1.32, respectively [41]. It is possible that the pK_a of SNZ is close to that of MNZ since SNZ contains only one more $-CH_3$ substituent as compared to MNZ. The pK_a of BZMNZ is not available from the literature. However, we guess that its pK_a value (>2.58) would be slightly higher than that of MNZ since it is synthesized from the esterification of MNZ with benzoic acid. When pH is low, nitroimidazoles are protonated and positively charged. The relative molecular masses of nitroimidazoles increase in the order: DMZ < MNZ < SNZ < RNZ < BZMNZ. Apparently, RNZ should migrate before BZMNZ; however, the migration order follows as: DMZ < MNZ < SNZ < BZMNZ < RNZ. The plausible reason is that RNZ exists as the neutral form in the running buffer since it has the lowest pK_a value, resulting in slower migration.

It is found that most of the nitroimidazoles are satisfactorily separated under the investigated pH except the MNZ/SNZ and BZMNZ/RNZ critical pairs. The effect of pH on the resolutions of MNZ/SNZ ($R_{2,3}$) and BZMNZ/RNZ ($R_{4,5}$) are displayed in Fig. 3b. $R_{2,3}$ decreases with the increase in pH. MNZ and SNZ are completely overlapped at pH 4.5. When the pH is 3.5, they are partially separated ($R_{2,3}$ = 1.1). At pH 1.5–3.0, MNZ and SNZ are completely separated. For the BZMNZ/RNZ critical pair, the effect of pH on their resolution is similar. $R_{4,5}$ decreases with the increase in pH (2.0–4.5). When the pH is 4.5, BZMNZ and RNZ are completely overlapped. At pH 4.0, they are partially separated but are completely separated at pH 1.5–3.5. In summary, all these nitroimidazoles are completely separated at pH 1.5–3.0. Although the shortest analysis time is at pH 1.5, the sensitivity of analysis decreases when the pH is below 3.0. As such, 3.0 is chosen as the optimum pH for the running buffer.

Besides pH, the effect of TBAB on the CE separation of nitroimidazoles was investigated. It was found that the addition of TBAB to the running buffer can improve the separation of MNZ and SNZ. Fig. 4 depicts the electropherograms of nitroimidazoles with running buffers (25 mM phosphate at pH 3.0) containing various concentrations of TBAB and their migration times change slightly. Without TBAB, MNZ and SNZ are not completely separated. However, the resolution of MNZ/SNZ critical pair improves with the increase in the concentration of TBAB. Finally, they are completely separated when TBAB is ≥0.10 mM, probably attributing to the fact that SNZ has an extra $-CH_3$ moiety which leads to a better solvophobic interaction with the TBA^+ [42]. As a result, SNZ migrates differently from MNZ.

3.3. Optimization of other CE parameters

The influence of the separation voltage (22–30 kV) on the CE separation of nitroimidazoles was studied. It is found that the separation voltage does not affect the separation of nitroimidazoles too much. The higher the separation voltage, the faster the nitroimidazoles migrate, resulting in shorter analysis time. However, when the voltage is too high, it will cause excessive Joule heating and will degrade the separation efficiency. As such, 28 kV was chosen as the optimal separation voltage as it produces good separation of nitroimidazoles with short analysis time.

The effect of sample injection time (3–11 s) on the CE separation and sensitivity of detection of nitroimidazoles was investigated. Longer injection time means that larger sample volume is loaded into the capillary; thus, the peaks are larger and the sensitivity of detection is improved. However, if the injection time is too long, the nitroimidazole peaks will be broadened and the resolutions become unsatisfactory. Peak tailing appears and is more pronounced when the injection time is longer than 5 s. Furthermore, the baseline is noisy when the injection time increases. As such, the sample injection time of 5 s at 0.5 psi was selected as it gives better sensitivity and resolution of nitroimidazoles.

It is well known that the separation temperature is crucial to the resolution and reproducibility of a given separation. Various separation temperatures at 19–31 °C were investigated. The migration times of nitroimidazoles increase with the decrease in temperature, attributing to the increase in the running buffer viscosity. On the other hand, higher temperatures cause Joule heating, degrading the separation efficiency. To compromise, 25 °C was chosen as the optimal separation temperature in this work.

In summary, the optimal CE conditions for the separation and determination of nitroimidazoles are pH 3.0, 0.10 mM TBAB and 25 mM phosphate buffer, 5 s of sample injection at 0.5 psi, 28 kV and 25 °C. These conditions were applied for the analysis of nitroimidazoles (*vide infra*).

3.4. Method validation

3.4.1. Stability of standard and sample solutions

The stability of the standard solutions of DMZ, MNZ, SNZ, RNZ and BZMNZ was determined by comparing the response factors (average peak area/concentration) of triplicate solutions stored at 4 °C and in dark. DMZ, MNZ, SNZ, and RNZ did not show evidence of significant degradation for at least 7 days. However, BZMNZ showed a 2% decrease in concentration after 7-day of storage. For this reason, it is advisable to prepare fresh working standard solutions for each analysis.

The stability of spiked porcine muscle tissue samples was evaluated by assessing their peak areas in the electropherograms at different time intervals. It was determined that the fortified samples were stable for at least 12 h. The changes in peak areas are less than 96%. The absorption spectra of the sample solutions did not show any changes within this study period. In essence, the sample solutions do not degrade and can be analyzed by our proposed CE method.

3.4.2. Linearity

Various concentrations of nitroimidazoles were applied to the CE and the peak areas of each nitroimidazole standard were plotted against their concentrations. Linear relationship was found

Table 1
Analytical figures of merit of nitroimidazoles.

Nitroimidazole	Calibration equation[a]	Linear range (μg/kg)	r (n = 5)	LOD[b] (μg/kg)	LOQ[c] (μg/kg)
DNZ	$y = 2.11x + 5.35$	5.00–300	0.9998	0.3	0.9
MNZ	$y = 2.18x - 6.03$	5.00–300	0.9999	0.5	1.3
SNZ	$y = 1.74x - 4.09$	5.00–300	0.9996	0.6	1.7
BZMNZ	$y = 0.51x - 9.16$	10.0–500	0.9994	1.0	3.2
RNZ	$y = 2.57x - 3.42$	5.00–300	0.9999	0.4	1.0

[a] Linear regression by plotting y: peak area (mAU min) *vs.* x: concentration (μg/kg).
[b] Determined with S/N = 3.
[c] Determined with S/N = 10.

between the peak areas and concentrations of nitroimidazoles in the range 5.0–300 μg/kg muscle tissues for DMZ, MNZ, SNZ and RNZ, and 10–500 μg/kg muscle tissues for BZMNZ. Table 1 summarizes the regression analysis of the calibration curves, linear ranges, correlation coefficients (r), limits of detection (LOD at $S/N = 3$), and limits of quantification (LOQ at $S/N = 10$). The calibration curves show good linearity with r ranging 0.9994–0.9999. The LODs were 0.3–1.0 μg/kg and the LOQs were 0.9–3.2 μg/kg.

3.4.3. Specificity

Specificity can be determined by measurement of peak homogeneity. Currently there are different techniques for PDA detection, depending on the impurities or interferences in an electrophoretic peak [43]. In general, it is recommended that several techniques be used for this purpose. In this work, the techniques used for validating the peak purity of the five nitroimidazole in the porcine tissue extraction solutions [44] are: (a) the normalization and comparison of spectra from different peak sections and (b) monitoring the absorbance at 320 nm. Both techniques reveal a high degree of peak purity corresponding to the analytes in the samples.

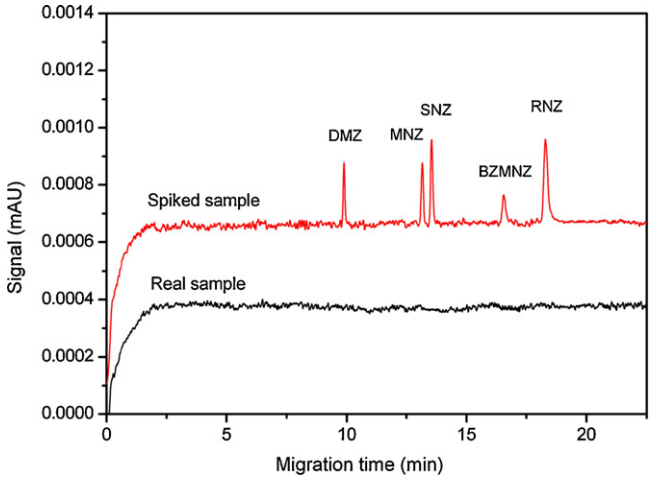

Fig. 5. Electropherograms of the real and spiked samples (25 μg/kg). The running buffer was 25 mM phosphate (pH 3.0) containing 0.10 mM TBAB. The CE conditions are same as in Fig. 3.

3.4.4. Precision and accuracy

The recovery and precision of the proposed CE method were performed to assess its precision and accuracy. The results are summarized in Table 2.

The recovery tests were done by adding known concentrations of nitroimidazoles to the samples at 25 and 150 μg/kg, respectively which were then subjected to sample extraction, clean-up and CE analysis. Fig. 5 displays the typical electropherograms of the porcine and spiked samples (25 μg/kg of nitroimidazoles). It is clear that nitroimidazoles were well separated and detected, indicating that the sample matrix does not affect the determination.

The precision was investigated in terms of intra-day and inter-day precisions. Each sample was repeated for six times. The recovery can reflect the accuracy of an analytical method [45]. The recoveries were good, ranging 85.4–96.0 and 83.5–92.5% for the intra-day and inter-day analyses, respectively. The relative standard deviations (RSDs) for the intra- and inter-day analyses were 1.3–3.9 and 1.1–4.2%, respectively, demonstrating that the proposed CE method is precise within the same day and/or multiple days [46]. The results indicate that our method is accurate, reliable, repeatable and reproducible.

3.4.5. Robustness

Robustness is an important feature of an analytical method since it evaluates the influence of small changes in the operating conditions of an analytical procedure on the measured or calculated responses. Variables including running buffer pH and buffer concentration are proved to be crucial for the development of a CE method. As such, it was decided to explore whether deliberate changes in these parameters could affect the CE separation [47]. The

Table 2
Recovery test and precision of the analysis of nitroimidazoles in porcine muscle samples.

Nitroimidazoles	Spiked level (μg/kg)	Intra-day			Inter-day		
		Found[a] (μg/kg)	Recovery (%)	RSD (%)	Found[a] (μg/kg)	Recovery (%)	RSD (%)
DNZ	25	21.4 ± 0.49	85.6	2.3	22.4 ± 0.29	89.6	1.3
	150	140 ± 3.72	93.3	2.7	131 ± 2.72	87.3	2.1
MNZ	25	22.3 ± 0.29	89.2	1.3	21.3 ± 0.23	85.2	1.1
	150	133 ± 1.72	88.7	1.3	137 ± 2.33	91.3	1.7
SNZ	25	24.0 ± 0.65	96.0	2.7	23.1 ± 0.72	92.4	3.1
	150	133 ± 2.13	88.7	1.6	126 ± 1.89	84.0	1.5
BZMNZ	25	21.9 ± 0.85	87.6	3.9	20.9 ± 0.88	83.5	4.2
	200	182 ± 6.38	91.0	3.5	183 ± 6.21	91.5	3.4
RNZ	25	22.5 ± 0.47	90.0	2.1	22.1 ± 0.35	88.5	1.6
	150	133 ± 2.40	88.7	1.8	134 ± 2.15	89.3	1.6

[a] Six determinations were performed.

Table 3
Comparison of the proposed CE method with other methods for determination of nitroimidazoles.

Method	Analytes[a]	Sample type	*r*	LOD (μg/kg)	Recovery (%)	RSD (%)	Ref.
Immunoassay	DMZ, DMZOH, IPZ, MNZ, RNZ	Chicken muscle and egg	–	–	18–98	–	[25]
TLC	DMZ, DMZOH, RNZ	Pork and poultry tissue	–	2–10	–	–	[29]
GC	DMZ, MNZ, RNZ	Poultry meat	>0.998	0.2–0.5	80–90	11.2–14.3	[26]
GC–MS	DMZ, HMMNI, IPZ, IPZOH, MNZ, MNZOH, RNZ	Turkey and swine muscles	0.9954–0.9994	0.65–5.2	94.0–118	–	[16]
	DMZ, MNZ	Poultry and porcine tissues	>0.999	0.1–0.6	72–106	7.7–23	[18]
HPLC-UV	DMZ, DMZOH, MNZ, ONZ, RNZ, SNZ, TNZ	Poultry and pork muscles	>0.998	0.2	71.4–99.5	4.0–13.9	[32]
	DMZ, DMOHZ, MNZ, RNZ	Swine tissue	0.9995–0.9998	1.0–2.0	78.9–83.9	2.2–18.9	[15]
LC–MS	DMZ	Poultry tissue and egg	0.999	1.0	93–102	1.2–7.7	[19]
	DMZ, HMMNI, IPZ, IPZOH, RNZ	Pork	>0.997	0.05–0.2	93–106	7–13	[34]
LC–MS/MS	DMZ, MNZ, RNZ	Fresh chicken eggs	0.994–0.999	0.5	76–83	3–9	[22]
CE-UV	BZMNZ, DMZ, MNZ, RNZ, SNZ	Porcine muscle	0.9994–0.9999	0.3–1.0	83.5–96.0	1.3–4.2	This work

[a] Acronyms: DMZOH, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole; HMMNI, 2-hydroxymethyl-1-methyl-5-nitroimidazole; IPZ, ipronidazole; IPZOH, 1-methyl-2-(29-hydroxyisopropyl)-5-nitroimidazole; ONZ, ornidazole; TNZ, tinidazole; DMZOH, HMMNI and IPZOH are the hydroxyl metabolites of DMZ, RNZ and IPZ in animal body, respectively.

results show that our proposed CE method maintains good selectivity and separation of analytes in buffer concentration of 15–35 mM. The buffer pH is restricted between 2.0 and 3.5 in order to achieve a resolution of MNZ and SNZ greater or equal to 1.5. Our proposed CE method is quite robust within this pH range.

3.5. Method comparison

Table 3 summarizes the analytical performance of our CE method as compared to other methods of analyses of nitroimidazoles. The LODs of our proposed CE method are similar to or, in some cases, better than those reported in the literature. In addition, our CE method is superior to some methodologies in terms of the *r* values. Although the recovery of our method is not as good as the LC–MS method, our RSDs are pretty good and even better than other methods of nitroimidazoles determination in biological samples.

The immunoassay method is prone to cross-interference from metabolites of some nitroimidazoles and shows poor recovery. TLC has higher LOD whereas HPLC-UV has relatively poor precision. Among the reported methods, GC–MS and LC–MS/MS seem to perform better than others in terms of LOD, recovery and precision; however, the instrumentation cost is relatively high especially with LC–MS/MS. In fact, the analytical performance of our proposed CE method is close to the GC–MS and LC–MS/MS methods which should provide a good alternative for determination of nitroimidazoles in muscle samples.

4. Conclusion

The separation and detection of five nitroimidazoles has been studied in detail by CE-UV. This method is simple, fast, efficient and reliable. It has been successfully applied to the analysis of nitroimidazoles in complex matrices such as porcine muscles with good recovery, repeatability and reproducibility. It is anticipated that our proposed CE-UV technique could be a cost-effective alternative for the determination of nitroimidazoles in pharmaceutical products and biological samples.

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