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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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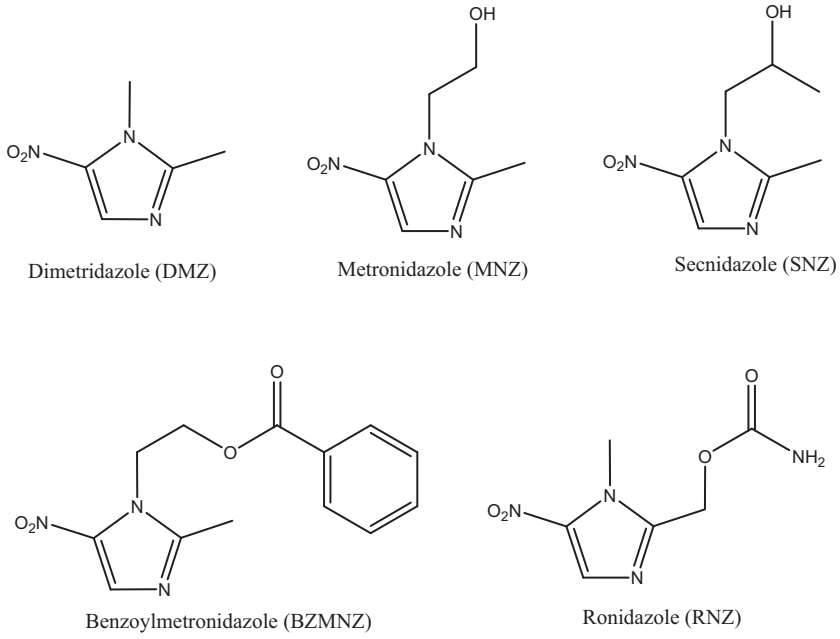

Fig. 1. Chemical structure of the nitroimidazoles.

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,