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Determination of three nitroimidazoles in rabbit plasma by two-step stacking in capillary zone electrophoresis featuring sweeping and micelle to solvent stacking

Xiupei Yang[a,*], Xiumei Cheng[a], Yingyun Lin[a], Zhijing Tan[a], Linxiang Xie[a], Martin M.F. Choi[b,**]

[a] College of Chemistry and Chemical Engineering, Chemical Synthesis and Pollution Control Key Laboratory of Sichuan Province, China West Normal University, Nanchong 637000, China

[b] Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong Special Administrative Region

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ABSTRACT

This study reports a sweeping and micelle to solvent stacking (MSS) method for fast stacking of nitroimidazoles in capillary zone electrophoresis (CZE). The optimal experimental conditions are run buffer of 25 mM sodium phosphate (pH 1.5) and 0.50% (v/v) methanol, micellar solution of 30 mM sodium dodecyl sulfate, applied voltage of 28 kV, and sample and micellar solution injection of 100 s. By applying this new on-line preconcentration technique, the nitroimidazoles content can be determined within 9 min with the limit of detection ($S/N = 3$) ranging 2.3–3.0 ng/mL, which is lower than that of conventional CZE analysis. The proposed MSS technique affords 20-, 12- and 38-fold improvements in sensitivity for the detection of dimetridazole, metronidazole and secnidazole, respectively. The relative standard deviations (RSDs) of intra-day and inter-day are 2.1–3.6% and 2.7–4.6% ($n = 6$), respectively. The recoveries in pretreated rabbit plasma at spiked levels of 5.0–10.0 μg/mL are 92.0–101.1% with RSDs lower than 3.4%. The proposed sweeping and MSS-CZE is a highly sensitive method for the detection of nitroimidazoles in biological and clinical samples and has been successfully applied to analyze nitroimidazoles in pretreated rabbit plasma.

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1. Introduction

Nitroimidazoles are typical antiprotozoal and antibacterial agents that are used primarily to prevent and treat histomoniasis and coccidiosis in poultry and game birds. They are also used for the treatment of genital trichomoniasis in cattle and hemorrhagic enteritis in pigs [1]. However, nitroimidazoles and their metabolites are believed to be carcinogenic and mutagenic to humans. They are banned for use in food producing animals within the European Union under Regulation 2377/90 and 2205/2001 [2], in the USA [3] and in China [1]. Similarly, under the two recently implemented regulations of the Hong Kong Special Administrative Region, Peoples' Republic of China [4,5], the maximum residue limits (MRLs) of dimetridazole (DMZ) and metronidazole (MNZ) in porcine and poultry muscle, kidney, and liver are 5 and 50 μg/kg, respectively.

It is known that the distribution of nitroimidazoles in muscle and liver tissue is not homogeneous and they decline rapidly at storage above 4 °C. Higher concentrations of nitroimidazoles are found in plasma and are more stable under the same storage conditions; thus they could be detected even though the medication has been withdrawn for a longer period of time [6]. As such, plasma is recommended as the target matrices for residue control of nitroimidazoles, especially in poultry.

To date, several analytical methods have been developed for the analysis of nitroimidazoles and their metabolites in plasma, serum, egg, feces, water and various animal tissues (muscle, liver, kidney and retina) [7–15]. Most of them including electrochemical [16,17], thin-layer chromatography (TLC) [18], immunoassay [19], high-performance liquid chromatography (HPLC) [7], liquid chromatography–mass spectrometry (LC–MS) [9–12], gas chromatography (GC) [13] and gas chromatography–mass spectrometry (GC–MS) [15] achieved a limit of detection (LOD) from 0.05 to 10 μg/kg. Among them, chromatography has the unique advantage of higher sensitivity for determination of nitroimidazoles. However, most samples require derivatization before they can be separated and analyzed by GC or GC–MS. HPLC analysis of nitroimidazoles requires larger sample volumes and more solvents and is more time-consuming. Therefore, it is necessary to develop a better, sensitive, efficient and accurate analytical method for rapid determination of nitroimidazoles in biological samples.

* Corresponding author. Tel.: +86 817 2568081; fax: +86 817 2582029.
** Corresponding author. Tel.: +852 34117839; fax: +852 34117348.
E-mail addresses: xiupeiyang@163.com (X. Yang), mfchoi@hkbu.edu.hk (M.M.F. Choi).

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Nowadays, capillary electrophoresis (CE) is recognized as an important separation technique due to its high resolution, less solvent consumption, and rapid separation. Ultra-high-performance liquid chromatography (UHPLC) is a relatively new technique and it provides faster run time, higher separation efficiency and sensitivity than the conventional HPLC [7]. Both CE and UHPLC offer considerable advantages in terms of separation speed and peak efficiency and are especially useful in biological analysis [8]. However, the main drawback of CE is its poor LOD, mainly attributing to small injection volume and short optical path in most UV absorption-based detectors. Thus, several on-line preconcentration strategies have been developed to increase the sensitivity of CE [20–24]. Over the past few decades, a great variety of on-line sample stacking techniques including field amplified sample stacking [25–28], dynamic pH junction [29–31], transient isotachophoresis [32–34] and sweeping [35–37] as well as micelle to solvent stacking (MSS) [38–40] were introduced to CE. The sequential use of two stacking techniques has also become popular for analysis of small molecules in the past decade [41,42]. Quirino [43] reported a new two-step technique for analysis of organic cations in capillary zone electrophoresis (CZE) combining sweeping and MSS. This two-step stacking has been applied to enrich β-blocker and tricyclic antidepressant drugs.

Recently, we have introduced a CZE method to determine five nitroimidazole residues in porcine muscle tissue samples [44]. However, the LOD is above 0.15 μg/mL and is still not good enough for trace analysis. In order to improve detection sensitivity, this work reports the development of a MSS method for fast screening of trace amounts of DMZ, MNZ and secnidazole (RNZ) in biological samples. The sweeping and MSS conditions were systematically optimized to achieve high selectivity and sensitivity for determination of nitroimidazoles in pretreated rabbit plasma sample. To our knowledge, this is the first report on applying an on-line preconcentration method, *i.e.*, sweeping-MSS, with CZE to determine nitroimidazoles in real samples.

2. Experimental

2.1. Chemicals

DMZ, MNZ and RNZ were purchased from Sigma–Aldrich (St. Louis, MO, USA). The individual stock solutions of nitroimidazoles were prepared in water at a concentration of 1.0 mg/mL and kept at 4 °C before use. Working standard solutions were prepared from blank plasma samples spiked with the three nitroimidazoles at requisite concentrations. Sodium dodecyl sulfate (SDS), sodium dodecyl sulfonate, sodium dodecyl benzene sulfonate, and sodium dihydrogen phosphate were obtained from Aladdin Reagent (Shanghai, China). Acetonitrile, hydrochloric acid, methanol, and sodium hydroxide were from Chengdu Kelong Chemical Reagent Factory (Chengdu, China). All chemicals of analytical reagent grade were used as received without further purification. Milli-Q water (Millipore, Bedford, MA, USA) was used throughout this work. The background electrolyte (BGE) was composed of 25 mM phosphate and 0.5% (v/v) methanol, and adjusted to pH 1.5 with 0.10 M HCl. BGE, micellar solutions and sample matrices were prepared each day by dilution of each stock solution with Milli-Q water. All solutions were filtered through 0.45-μm membrane filters before injecting to the separation capillary.

2.2. Apparatus

The experiments were performed on a Beckman P/ACE MDQ instrument (Fullerton, CA, USA) equipped with an auto-sampler and a diode array detector. All CE operations were controlled by the Beckman P/ACE MDQ software. A 50 cm × 50 μm i.d. bare fused-silica capillary (Yongnian Ruifeng Optical Fiber Factory, Hebei, China) with a detector window at 42.8 cm from the inlet end was used for separation.

2.3. Samples preparation

The rabbits from which the blood samples were drawn were obtained from the local markets (Nanchong, Sichuan Province, China). Fresh rabbit plasma was centrifuged at 6000 rpm for 15 min and the supernatant was collected. A de-protein method was applied to obtain the pretreated rabbit plasma. Rabbit plasma, water and acetonitrile (1:2:4.5, v/v) were mixed thoroughly, and stored in a refrigerator at 4 °C for 60 min. Finally, the mixture was centrifuged at 10,000 rpm for 20 min to obtain the supernatant and filtered through a 0.45-μm filter prior to CE analysis.

Rabbit plasma matrix-matched calibration standards were prepared for quantification. Control plasma was prepared as above. One control plasma sample was used for each calibration standard level. Plasma samples (0.5 mL) were pipetted into polypropylene tubes (2 mL). Samples were fortified at 0, 10, 100, 800, 4000 and 20,000 ng/mL by adding mixed working standard solution (1.0 mg/mL).

2.4. General electrophoresis procedure

Prior to use, a new capillary was conditioned by flushing at 20.0 psi sequentially with methanol for 30 min, water for 15 min, 1.0 M HCl for 30 min, water for 15 min, 1.0 M NaOH solution for 30 min, water for 3 min, and finally BGE for 60 min. Between two consecutive analyses, the capillary was rinsed sequentially with 1.0 M NaOH for 1 min, flushed with water for 1 min, and finally with BGE for 5 min. The detector was monitored at 275 nm. Electrophoresis was performed with a constant applied voltage of 28 kV at 25 °C.

2.5. Two-step stacking principle

The two-step stacking of cationic analytes by sweeping and MSS was first reported by Quirino in 2010 [45]. As a stacking strategy in CZE, the improvement in sensitivity is 2–5 times better than that of one-step stacking MSS. In this work, the capillary is initially conditioned with BGE containing an organic solvent. The sample solution is injected and followed by the micellar solution. A voltage is applied with the anode and cathode at the inlet and outlet ends, respectively. The micelle to solvent stacking boundary (MSSB) moves with the electroosmotic flow (EOF). The negatively charged surfactant micelles sweep the cations. The surfactant micelles transport the cations to the MSSB and accumulate at the MSSB. This stacking occurs until all the micelles from the injected micellar solution zone traverse the boundary. The two-stepped stacked cations are then separated by virtue of CZE and move toward the detector.

3. Results and discussion

3.1. Detection wavelength

The pK_a of DMZ and MNZ are 2.81 and 2.58, respectively [46]. It is possible that the pK_a of SNZ is close to that of MNZ, since SNZ contains only one more $-CH_3$ substituent compared to MNZ; so all nitroimidazoles are protonated at pH 1.5 with strong absorption bands in the UV region. The absorption peak maxima for DMZ, MNZ and SNZ are at *ca.* 274–276 nm. The lower the pH, the better is the protonation of these nitroimidazoles. In order to improve the enrichment of nitroimidazoles by the proposed sweeping-MSS

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