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Study of matrix effects for liquid chromatography–electrospray ionization tandem mass spectrometric analysis of 4 aminoglycosides residues in milk

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ABSTRACT

Matrix effect (ME) is always a major issue for the development of LC–MS/MS method. ME resulting from co-eluting residual matrix components can affect the ionization efficiency of target analytes, leading to quantification errors of the analytes of interest. The present work evaluates MEs of milk samples on simultaneous analysis of four aminoglycosides residues via LC–ESI/MS/MS including streptomycin, dihydrostreptomycin, spectinomycin and kanamycin. Approaches to reduce MEs were examined: optimization of the sample preparation, sample dilution and lower flow rate used. Three commercial sorbents were tested including Oasis MCX, Oasis HLB and Oasis WCX. WCX behaved better for all analytes, but high MEs (80.8–134.9%) were obtained. Therefore, a consecutive SPE of tC18-WCX was found to effectively reduce ME. Milk samples from different manufacturers were analyzed and low MEs (85.6–112.9%) were obtained.

1. Introduction

Aminoglycosides (AGs) are broad-spectrum antibiotics which have bactericidal activity against some aerobic gram-positive and gram-negative organisms [1,2]. AGs have been extensively used against bacteria and parasites in the production of pork, chicken, milk and honey, which would cause side reactions such as ototoxicity, nephrotoxicity, and allergy [1–5]. Some food have been found to contain AG residues originating from animal origins (e.g., milk), which will be a potential hazard for the humans' health due to their toxicity and antibiotic resistance. The European Union (EU), China, JECFA (FAO/WHO, 2005), Japan, and other countries have issued strict maximum residue levels (MRLs) for AGs in many animal-origin foods, which is set to 200 μg/kg for the milk [4,5]. Therefore it is necessary to develop a reliable and sensitive analytical method to determine trace AG residues in milk.

Among many analytical methods, liquid chromatography–electrospray ionization (ESI) tandem mass spectrometer (LC–MS/MS) has well proved to be an efficient tool for identification and quantitation of polar analytes in complex matrix due to its high sensitivity [1,6–8]. One of the limitations, however, is the susceptibility of ESI interfaces to matrix effect (ME), which is defined as the effect of co-eluting residual matrix components on the ionization of the analyte of interest, typically resulting in either signal suppression or enhancement [6,7]. ME can significantly affect the reproducibility and accuracy of the method [8]. Some reviews have in detail discussed this topic [9–13]. Presently the mechanism of ME in LC–MS/MS is still unclear, which may result from a competition between matrix components and target analytes for access to the droplet surface for gas phase emission [6] and Trufelli et al. have reviewed some more relevant mechanisms [14]. Several strategies have been proposed with the aim to minimize or eliminate the MEs, such as improved chromatographic selectivity to avoid co-elution [15], use mobile phase modifiers [6], dilution [7], efficient solid phase extraction (SPE) [1], stable isotope-labelled internal standards [16,17]. Although these approaches were claimed to be effective for model analytes chosen, there are inherent drawbacks for further extension. For example, it is not easy to find suitable internal stan-

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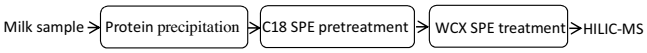

Fig. 1. Schematic diagram of sample treatment procedure.

dard used for lowering ME in Refs. [6,10,11]. The use of improved chromatographic method (two-dimension LC system) [9] can reduce coelution to an extent while the system is rather complex. Also, adding suitable modifiers in mobile phase in Ref. [6] may need much time to optimize the separation condition for different analytes. Dilution of the sample proved to be effective while it also led to poor recovery and sensitivity loss [7]. By comparison, the use of SPE for sample pretreatment is easily-implemented way to reduce MEs [1]. In consideration of highly polar property of AGs, common hydrophobic C18 SPE cartridge should be not effective for capturing these analytes. As a common food, milk is rich in protein and fat, the ME is significant for the analysis of trace AG residues in milk. Although there are many literatures dealing with the analysis of AGs in milk [18–20], to our best knowledge, no report has been given to explore the corresponding MEs. The present work is aimed to evaluate and diminish MEs from milk samples for the analysis of four typical AGs via HILIC–ESI–MS-MS. The main strategies have based on consecutive SPE cartridges and the splitting of flow rate. The optimized method has been successfully applied for analysis of model AG residues in different milk samples. It was found that the matrix effect could be effectively controlled and acceptable recoveries could be obtained as well by choice of a suitable SPE-based sample pretreatment.

2. Experimental

2.1. Chemicals and reagents

Streptomycin, dihydrostreptomycin, spectinomycin and kanamycin were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and their structures are summarized in SI-Fig. 1. 0.5 mg/mL (calculated as dry free base) of individual stock solution was prepared with methanol/water (10/90, v/v) and stored in −20 °C. Working solutions were prepared by diluting stock solution with water/acetonitrile (1:1, v/v), then also stored at −20 °C. Methanol (MeOH), acetonitrile (ACN), isopropanol (IPA) and *n*-hexane, formic acid (FA) were all of HPLC grade (J&K Scientific Corp., Beijing, China). Ammonium formate (NH_4FA) (≥99.95%) was purchased from Sigma–Aldrich. Ammonia aqueous solution (25%, w/w), dipotassium hydrogen phosphate trihydrate (K_2HPO_4), trichloroacetic acid (TCA) and ethylenediamine tetraacetic acid disodium salt (EDTA) were all analytical reagents (Sinopharm Chemical Reagent Corp., Shanghai, China). Unless otherwise stated, ultrapure water obtained by a Milli-Q water-purification system (Bedford, USA) was used for solution preparation.

Commercial solid phase extraction (SPE) cartridges including Oasis HLB (6 mL/500 mg), Oasis MCX (6 mL/500 mg), Oasis WCX (6 mL/500 mg), Sep-pak C18 (6 mL/500 mg) and Sep-pak tC18 (6 mL/500 mg) were from Waters Corp. (Milford, MA, USA).

2.2. LC–MS-MS analysis

2.2.1. LC system

An Agilent series 1290HPLC system (Agilent Technologies, USA) consisted of a binary gradient pump, an autosampler and a column oven. Separation was performed on a Click TE-Cys hydrophilic column (150 mm length × 3 mm i.d., 3 μm dia, Acchrom Corp., Zhejiang, China). The mobile phase of H_2O/FA (99:1, v/v, A) and ACN/H_2O/FA (80:19:1, v/v, B), both containing 30 mM ammonium formate, was used. Gradient elution conditions were as follows: 0–1.0 min, isocratic 10% A; 1.0–10.0 min, linear increase from 10 to 90% A; 10.0–11.0 min, isocratic 90% A; 11.0–12.0 min, linear from 90 to 10% A; and 12.0–20.0 min, isocratic 10% A. The flow rate was 0.4 mL/min. The injection volume of sample was 5 μL.

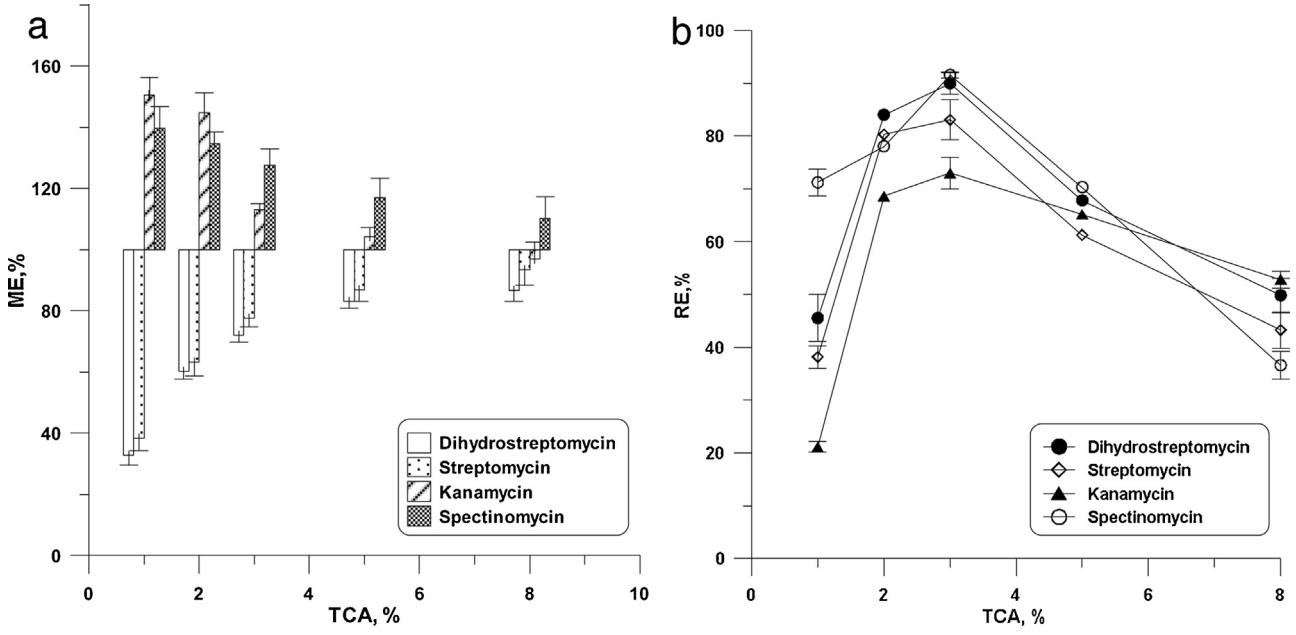

Fig. 2. The effect of TCA amount on matrix effects (a) and (b) of target analytes during protein precipitation.
Conditions: mobile phase, H_2O/FA (99:1, v/v, A) and ACN/H_2O/FA (80:19:1, v/v, B), both containing 30 mM ammonium formate; gradient elution, 10 min, 10–90%A; flow rate: 0.4 mL/ min; sample concentration, 200 μg/kg; injection volume, 5 μL. (n = 3).