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An integrated electrophoretic mobility control device with split design for signal improvement in liquid chromatography–electrospray ionization mass spectrometry analysis of aminoglycosides using a heptafluorobutyric acid containing mobile phase

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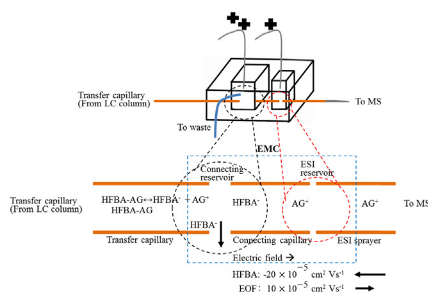
HIGHLIGHTS

- Electrophoretic mobility control was used to alleviate ion suppression due to ion pairing agent.
- An integrated electrophoretic mobility control device with a split design was fabricated.
- The proposed device could be applied to LC-ESI-MS using a conventional HPLC column.
- The device was applied to the analysis of aminoglycosides using the ion pairing agent, HFBA.

GRAPHICAL ABSTRACT



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ABSTRACT

Electrophoretic mobility control (EMC) was used to alleviate the adverse effect of the ion-pairing agent heptafluorobutyric acid (HFBA) in the liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS) analysis of aminoglycosides. Aminoglycosides separated by LC were directed to a connecting column before their detection via ESI. Applying an electric field across the connecting column caused the positively charged aminoglycosides to migrate toward the mass spectrometer whereas the HFBA anions remained in the junction reservoir, thus alleviating the ion suppression caused by HFBA. To accommodate the flow rate of a narrow-bore column, minimize the effect of electrophoretic mobility on separation, and facilitate the operation, an integrated EMC device with a split design was fabricated. With the proposed EMC device, the signals of aminoglycosides were enhanced by a factor of 5–85 without affecting the separation efficiency or elution order. For the analysis of aminoglycosides in bovine milk, the proposed approach demonstrates a sensitivity that is at least 10 times below the maximum residue limits set by most countries.

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1. Introduction

Aminoglycosides are a class of antibiotics and can be used in veterinary medicine and animal husbandry to treat bacterial

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infection. However, their overuse and misuse lead to drug resistance in pathogens [1]. The appropriate use of aminoglycosides is necessary to ensure their effectiveness against bacteria and to protect human health. Many countries, including European Union nations, have established maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin [2].

Analytical methods for determination of aminoglycoside residues in food matrices have been reviewed [3]. High-performance liquid chromatographic (HPLC) methods are reliable and widely used for analyzing aminoglycoside antibiotics. Because of the lack of chromophores or fluorophores in aminoglycosides, labeling tags is necessary before HPLC analysis [4–6]. However, labeling is unnecessary in HPLC coupled with mass spectrometry. In addition, with the use of tandem mass spectrometry, reliable confirmation and higher sensitivity can be achieved simultaneously [7–9].

Aminoglycosides consist of two or more amino sugars linked by glycosidic bonds. Because of their highly hydrophilic nature, aminoglycosides are not retained on reversed phase column. Ion-pair reagents, such as heptafluorobutyric acid (HFBA), are often added to the mobile phase during chromatography to increase hydrophobicity and thus improve chromatographic performance [10–17]. Although perfluorocarboxylic acids have been regarded as volatile ion-pairing reagents, HFBA still exerts adverse effects on ESI ionization efficiency if the concentration is over 5 mM [14]. In addition to the adverse effect on the signal intensity, the ESI source is easily contaminated by HFBA and reduces the time interval of source cleaning. To make the mobile phase more compatible with ESI-MS, separation based on hydrophilic interaction chromatography (HILIC) has been reported [18–21]. However, the performance may not be better than the use of HFBA, especially for late eluting aminoglycosides [12].

A strategy based on post-column electrophoretic mobility control (EMC) was proposed to alleviate the adverse effect of trifluoroacetic acid (TFA) on liquid chromatography mass spectrometry (LC–MS) analysis of peptides [22]. Peptides separated through capillary LC were directed to a connecting capillary before their detection by ESI. By applying an electric field across the connecting capillary, positively charged peptides migrated toward the ESI sprayer, whereas TFA anions remained in the junction reservoir. Because TFA did not enter the ESI source, ion suppression from TFA was alleviated. Signals of peptides were enhanced 9–35-fold without compromising separation efficiency.

Capillary columns are widely used in peptide analysis. However, columns with a much larger inner diameter are often used in other LC–MS applications such as the analysis of aminoglycosides. Unfortunately, the EMC device used in a capillary column [22] could not be applied to a narrow-bore column because of the mismatch in flow rate between a narrow-bore column (approximately 200 μL min^{-1}) and the EMC device (less than 0.2 μL min^{-1}; due to electroosmotic flow (EOF)). To overcome this difficulty, the concept of splitting was adopted for the fabrication of an EMC device. In addition, it was noticed that the order of migration might be affected by the effect of electrophoretic mobility in the connecting column. This effect could be minimized by reducing the length of the connecting column. As a result, to include the concept of splitting, reduce the length of the connecting column, and facilitate the operation, an integrated EMC device with a split design is proposed in this study.

The utility of the integrated EMC device was demonstrated in analyzing aminoglycosides in milk by using LC-ESI-MS. By preventing HFBA from flowing into the ESI source, ion suppression caused by HFBA can be alleviated.

2. Experimental

2.1. Chemicals and materials

The seven aminoglycosides, spectinomycin, streptomycin, dihydrostreptomycin, kanamycin, apramycin, gentamicin (mixture of gentamicin C1, gentamicin C2, gentamicin C1a and gentamicin C2a) and neomycin (Fig. 1) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hydrofluoric acid (48%) and ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) were also purchased from Sigma-Aldrich Chemical Co. Methanol (HPLC grade), acetonitrile (ACN, HPLC grade) were purchased from J. T. Baker (Phillipsburg, NJ, USA). Formic acid (FA) and heptafluorobutyric acid (HFBA) were purchased from Fluka Chemical Co. (Milwaukee, WI, USA). Trichloroacetic acid (TCA) was purchased from Merck (Darmstadt, Germany). Ammonium hydroxide (28–32%) was purchased from ACROS Organics (NJ, USA). Potassium phosphate monobasic was purchased from Tedia Co. (Fairfield, OH, USA). The poly(methyl methacrylate) (PMMA) plates were obtained from Chi Mei Corp. (Tainan, Taiwan). Poly(dimethylsiloxane) (PDMS) monomer and cross-linker (Sylgard® 184) were purchased from Dow Corning (Midland, MI, USA). Deionized water (Milli-Q water system, Millipore Inc., Bedford, MA, USA) was used in the preparation of sample solution, mobile phase and sheath liquid. All fused-silica capillaries (50 μm i.d. × 365 μm o.d. and 100 μm i.d. × 365 μm o.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). poly(ethylene ketone) (PEEK) tubing was purchased from Upchurch Scientific (Oak Harbor, WA, USA). The cartridges used for solid-phase extraction were Oasis HLB cartridges (500 mg, 6 mL) from Waters (Milford, MA, USA). PVDF filter membranes (0.22 μm) were purchased from Merck (Darmstadt, Germany). Milk samples (fat content 3.2%–3.8%) (Kuang-chuan, Taiwan) were purchased at local store.

Stock solutions (10 mg mL^{-1}) were prepared in 0.1% FA (H_2O/FA, 100:0.1, v/v). These solutions were stored in plastic tubes at −4 °C. Standard solutions were prepared daily using a solution of 20 mM HFBA. The milk samples were prepared by spiking with aminoglycosides at different concentration levels.

2.2. Fabrication of the integrated electrophoretic mobility control (EMC) device

The integrated electrophoretic mobility control (EMC) device consisted of a PDMS based microdevice, a connecting capillary, an ESI emitter and a side pipe as shown in Fig. 2. For fabrication of the PDMS based microdevice, the PMMA mold (Fig. 2a) was assembled on a PMMA plate (100 mm × 80 mm × 5 mm) and fixed using the scotch tape (3 M, Taipei, Taiwan). The mold consists of four 25 mm × 20 mm × 3 mm PMMA plates, one PMMA rod, one PMMA square and one PEEK tubing. The hole for inserting a side pipe was drilled on the PMMA back plate using an 800 μm o.d. drill. Two holes (for holding a transfer capillary and an ESI emitter) were drilled on the left and right plates using a 400 μm o.d. drill. A 400 μm diameter channel (for inserting a 375 μm capillary) was drilled near the bottom of the PMMA rod and the PMMA square respectively. After the drilling, a capillary of 375 μm o.d. was inserted from the hole of the left PMMA plate, through the channels of the PMMA square, the PMMA rod and the hole of the right PMMA plate. The distance between the PMMA square and the PMMA rod was approximately 1 cm. The PEEK tubing was inserted through the hole of the back PMMA plate and reached to the PMMA square. PDMS monomer and cross-linker were mixed vigorously and degassed. The mixture was poured on the PMMA mold slowly and cured at 80 °C for 72 h in an oven. After curing, the rod, the square and capillary were carefully withdrawn from the mold. After that,

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