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Determination of aminoglycosides in honey by capillary electrophoresis tandem mass spectrometry and extraction with molecularly imprinted polymers

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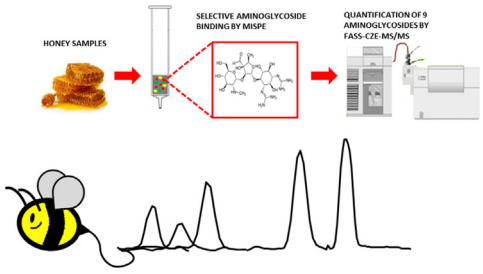
HIGHLIGHTS

- CE-MS/MS proposed the first time for the determination of 9 aminoglycosides in honeys.
- Molecularly imprinted polymer for the selective solid phase extraction of analytes.
- High sensitivity with limits of quantification from 1.4 to 94.8 μg kg^{-1}.
- Recoveries ranged from 88.2 to 99.8%, with RSD lower than 8%.

GRAPHICAL ABSTRACT



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ABSTRACT

A new analytical method based on capillary zone electrophoresis-tandem mass spectrometry is proposed and validated for the identification and simultaneous quantification of nine aminoglycosides in honey samples. Detection using an ion trap mass analyzer operating in the multiple reaction monitoring mode was used. Different parameters were optimized in order to obtain an adequate separation combined with the highest sensitivity. In order to achieve high selectivity in the sample treatment, a commercially-available molecularly imprinted polymer has been used for the solid phase extraction of the analytes. Under optimum conditions, recoveries for fortified samples ranged from 88.2 to 99.8%, with relative standard deviations lower than 8%. The limits of detection ranged from 0.4 to 28.5 μg kg^{-1}. Furthermore, the decision limit and the detection capability were evaluated, ranging from 3.5 to 60.5 μg kg^{-1} and from 6.0 to 103.1 μg kg^{-1}, respectively, demonstrating the sensitivity and applicability of this fast and simple method.

Abbreviations: AG, Aminoglycoside; APM, Apramycin; BGE, Background electrolyte; CZE, Capillary zone electrophoresis; CCα, Decision limit; CCβ, Detection capability; DHS, Dihydrostreptomycin; EU, European Union; FASS, Field-amplified sample stacking; GENT, Gentamicin; HFBA, Heptafluorobutyric acid; HILIC, Hydrophilic interaction chromatography; IT, Ion trap; MRLs, Maximum residue limits; MeOH, Methanol; MIPs, Molecularly imprinted polymers; MRM, Multiple reaction monitoring; NEO, Neomycin; PRM, Paromomycin; IPA, Isopropanol; RASFF, Rapid Alert System for Food and Feed; SPE, Solid phase extraction; MISPE, Solid phase extraction with molecularly imprinted polymers; SPC, Spectinomycin; STP, Streptomycin.

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1. Introduction

Aminoglycoside (AG) antibiotics are widely used in veterinary medicine. They are commonly used to treat foulbrood infection (caused by bacteria), and Nosema disease (caused by protozoa). Veterinary use of antibiotics is regulated by European Union (EU) and maximum residue limits (MRLs) have been established in different edible parts or products derived from animals, such as muscle, kidney, fat, liver, milk, and eggs [1]. EU does not authorize the use of antibiotics, including AGs, in beekeeping and MRLs have not been established for these compounds in honey, applying the so-called "zero tolerance". However, honey and products derived from bees coming from non-EU countries, might be contaminated with AG residues, which is a major concern in the honey trade [2,3]. Some studies have revealed that a substantial part of the currently marketed honey contains residues of antibiotics [4] and since 2003, the EU's Rapid Alert System for Food and Feed (RASFF) has regularly alerted Member States about the presence of antibiotic residues in these products [5].

The EU Community Reference Laboratories for veterinary residues have established "action limits" for the validation of analytical methods in relation to the control of unauthorised analytes in different matrixes [6]. In relation to AGs in honey, a recommended concentration of 40 μg kg^{-1} has been set for streptomycin (SPT), which involves that detection capability (CCβ) for screening methods or decision limit (CCα) for confirmatory methods should be lower than this value.

Traditionally, high performance liquid chromatography (HPLC) methods have been applied for the determination of AGs [7], mainly using as detection system tandem mass spectrometry (MS/MS) with positive electrospray ionization mode (ESI+). However, the high polarity of these antibiotics is a drawback for their analysis by HPLC, as they are not retained in reverse-phase columns. Ion-pair chromatography has been proposed as an alternative to obtain a satisfactory separation of these compounds [8–12]. Nevertheless, ion-pair chromatography requires the addition of an ion-pair reagent (mainly trifluoroacetic acid or heptafluorobutyric acid) in the mobile phase. These ion-pair reagents are rarely volatile acids and can seriously affect the performance of MS, causing ionization suppression of analytes and contamination of the ion source. Hydrophilic interaction chromatography (HILIC) has been recently proposed as an alternative to ion-pair chromatography for the analysis of AGs in honey by LC-MS [11,13–16]. However, in this methodology, a high concentration of salts in the mobile phase is usually needed which can be detrimental for MS detection.

Capillary electrophoresis (CE) can be an interesting alternative to HPLC for the analysis of these compounds due to its advantages such as short analysis time, high separation efficiency and low reagent consumption. A drawback is the absence of chromophore groups in AGs, which prevents their determination by UV/Vis, the most common detection method in CE. Several attempts have been made to overcome this problem, i.e. the use of indirect UV detection [17,18] or the application of derivatization methods to form absorbent species in the UV/Vis region [19]. Also post-column derivatization was used to apply laser induced fluorescence detection [20]. Unfortunately, these methods show poor sensitivity because of the short optical path length and the small volume of sample injected or involve tedious and complicated derivatization processes.

MS/MS is an alternative to improve sensitivity and selectivity in CE, allowing the unequivocal identification of antibiotic residues and therefore fulfilling EU regulation requirements [21]. Unfortunately, the small volume of sample injected in CE continues to be a major constraint. In order to mitigate this problem numerous strategies have been developed [22–24]. Among them, field-amplified sample stacking (FASS) is one of the most effective and simplest methods to achieve high sensitivity [25]. In FASS, the sample solvent has conductivity lower than the background electrolyte (BGE). Therefore, when a voltage is applied, the electric field will be higher in the sample zone than in the BGE. As a result, analytes migrate quickly in the sample zone and slow down when they reach the BGE, causing the "stacking" of the analytes around the sample-BGE boundary.

Another issue that needs to be solved in the determination of AGs is the sample preparation. It is not an easy task due to the high polarity of AGs and their tendency to bind strongly to matrix proteins. Most of the methods reported for the analysis of AGs in honey involve the use of solid phase extraction (SPE) cartridges, such as weak cation exchange [14,15], octadecyl [12] or hydrophilic-lipophilic balance [9,16] to ensure a clean extract. Other materials with higher selectivity, such as molecularly imprinted polymers (MIPs) can provide cleaner extracts. MIPs are synthetic materials with artificially generated recognition sites able to specifically capture target molecules [26,27]. Thus, the strong interaction between MIPs and target molecules makes them ideal for the selective extraction of compounds at trace levels, particularly when the sample is complex. Several reviews show their applications in analytical chemistry [28–30]. Specifically, the use of MIPs as SPE sorbents (MISPE) for the selective extraction of antibiotics from food samples has grown significantly in the last few years [31–35]. Recently, Ji et al. have synthesized a MIP sorbent for AGs using SPT as the template molecule, obtaining satisfactory efficiency and selectivity in the analysis of honey samples [13].

In this work, we propose a useful alternative to quantify very low concentrations of nine AGs in honey using a recently commercially available MIPs. The use of CE-MS/MS with FASS preconcentration is also proposed as an approach for improving sensitivity and selectivity. The obtained results demonstrate for the first time the possibilities of MISPE and CE-MS/MS for the quantification of AG residues in honey.

2. Experimental

2.1. Reagents and materials

It has to be highlighted that, because of the high sorption affinity of the AGs to polar surfaces and their high photosensitivity, polypropylene amber vessels (flasks, glass and vials) were used during sample preparation, storage and injection.

Ultrapure water (Milli-Q Plus system, Millipore Bedford, MA, USA) was used throughout the work. Isopropanol (IPA), methanol (MeOH) and acetonitrile (MeCN) (LC-MS HiPerSolv grade) were supplied from VWR (Radnor, PA, USA). Formic acid, acetic acid and heptafluorobutyric acid (HFBA) were obtained from Sigma Aldrich (St. Louis, MO, USA). Potassium hydroxide, ammonium hydroxide (30%), potassium dihydrogen phosphate and dichloromethane were obtained from Panreac-Química (Barcelona, Spain).

Vetranal grade analytical standards of Gentamicin (GENT), which was a mixture of GENT C1, GENT C1a and GENT C2, Neomycin (NEO), Apramycin (APM), Paromomycin (PRM), Dihydrostreptomycin (DHS), Spectinomycin (SPC) and Streptomycin (STP) were supplied by Fluka Analytical (Steinheim, Germany). Individual stock standard solutions of 3 g L^{-1} were prepared by dissolving accurately weighed amounts in water and stored in the dark at 4 °C. They were stable for at least 2 months. Standard solutions containing all the AGs were freshly prepared by proper dilution of the stock standard solutions with water.

MIP extraction cartridges (SupelMIP AGs SPE Column, 50 mg, 3 mL) supplied by Supelco (Bellefonte, PA, USA) were used for