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Aminoglycoside analysis in food of animal origin with a zwitterionic stationary phase and liquid chromatography–tandem mass spectrometry

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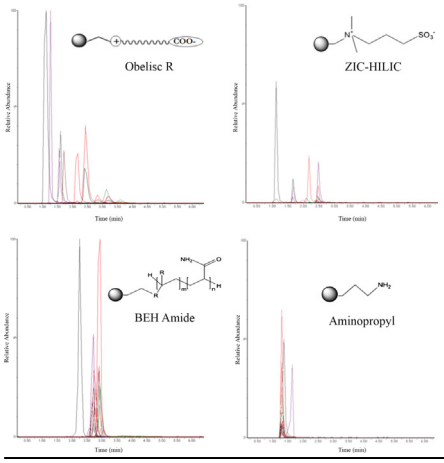
HIGHLIGHTS

- Analysis of 14 aminoglycosides in milk, liver and honey samples was achieved.
- Separation of aminoglycosides was obtained with zwitterionic stationary phases.
- Formic acid mobile phase provided an excellent sensitivity.
- Cation-exchange cartridges provided the best recoveries in all matrices.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, fourteen highly polar aminoglycoside (AGs) antibiotics were selected. Various stationary phases were tested, including Obelisc R, ZIC-HILIC, BEH amide and aminopropyl. The nature of the stationary phase, mobile phase (water or buffer solutions and acetonitrile), pH (percentage of formic acid), gradient conditions and injection solvents were systematically studied as relevant parameters for tuning retention selectivity and detectability of AGs in liquid chromatography electrospray tandem mass spectrometry (LC–(ESI)–MS/MS). Only the two zwitterionic phases (Obelisc R and ZIC-HILIC) achieved a proper chromatographic separation considering interferences due to the crosstalk effect in low resolution mass spectrometers. The water/acetonitrile mobile phase containing 1% formic acid used with Obelisc R provided more sensitivity than the highly concentrated buffered mobile phases required for ZIC-HILIC. A solid phase extraction (SPE) clean-up procedure with polymeric weak cation exchange (WCX) cartridges was optimized for honey, milk and liver samples. Different brands of cartridges and elution solvents were tested, and the Taurus WCX offered the best recovery rate with a buffer elution at pH 3. The final optimized method was validated in these matrices according to Decision 2002/657/EC. A monitoring campaign for sixty honey, milk and liver samples was carried out at the Food Authority

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Control in Geneva. The concentration of dihydrostreptomycin (DSTP) found in one ovine liver exceeded the established maximum residue levels (MRLs) within the European and Swiss legislations but it was compliant taking into account the validation data.

1. Introduction

AGs are an important class of antibiotics used to treat Gram-negative bacterial infections. They tend to be dedicated for serious infections due to their ototoxicity and nephrotoxicity [1]. Many of these drugs are extensively used in human medicine, while others, such as DSTP, are typical veterinary drugs [2]. They can also be used as growth promoters in food-producing animals, which is a banned practice in the EU since 2006 [3]. Due to their high affinity to tissue, AGs result in a high residue level and prolonged withdrawal times [4]. Thus, it is important to monitor residues in food to control AG use and potential abuse that could increase antibiotic resistance and consequently weaken the efficacy of these drugs as human medicines [2]. As a result, AGs are currently analyzed in honey, eggs, milk, tissues and fluids of food-producing animals [2,3,5–8] for control and monitoring purposes. EC Regulation 37/2010 establishes MRLs for some AGs in the kidney, muscle, fat, liver, milk, and eggs of different animal species (Table 1). No MRLs have been set for honey, except in the case of streptomycin, which has a recommended concentration of 40 $\mu g\,kg^{-1}$ set by the Community Reference Laboratory in Fougères (France). Swiss legislation has adopted the same limits.

AGs are relatively large molecules with two or more amino sugars linked with glycosidic bonds to a central hexose/pentose. They are very polar compounds due to the presence of several amino groups that contribute to their weak basic nature and numerous hydroxyl groups that result in high aqueous solubility [9]. AGs exhibit different pK_a values (Table 2) as a result of the steric effects and hydrogen bonds between the amino groups and neighboring hydroxyl groups on the sugar rings [10].

AG quantification and confirmation at trace residue concentrations has long been a challenge. Due to their extreme polarity, AGs require different sample preparation and chromatographic conditions when compared with other antibiotics [11]. Therefore, they cannot be included in the typical multi-class, multiresidue monitoring methods for veterinary drugs that use reversed phase (RP) LC–MS/MS [12], although some attempts have been made [2,13–16]. AGs show little to no retention in reversed phase columns. Instead, AGs are eluted in the column void and present an asymmetrical peak shape due to strong ionic interactions with the residual silanols groups at the surface of the stationary phase. The only way to achieve suitable retention of these polar compounds on RP columns is to use ion-pairing agents, such as fluorinated acids [1,10], or derivatization agents [17], such as phenyl isocyanate [1]. However, when MS is used as the detection technique, these ion-pairing reagents can contaminate the ion source and cause ion suppression and poor peak shape, thus resulting in the need for extended system rinsing [2].

The introduction of hydrophilic interaction chromatography (HILIC) as a valuable alternative to normal phase LC has provided a more MS-compatible option for the analysis of polar compounds [18]. Unlike normal-phase chromatography in which highly polar analytes may be only sparingly soluble, HILIC uses typical RP mobile phases without the need to employ eluent systems not miscible with water. The HILIC retention mechanism primarily involves the partitioning of polar analytes between a water enriched layer of solvent near the sorbent surface and the slightly more hydrophobic bulk eluent, which is typically acetonitrile. In addition to hydrophilic partitioning, other separation mechanisms, such as ion exchange, hydrogen-bonding, or dipole–dipole interactions, also play a major role in determining retention and selectivity in HILIC [19]. HILIC mobile phases are fully compatible with MS and often provide better sensitivity than the use of ion-pairing reagents. The reason is the enhanced desolvation and ionization efficiencies of the ESI source caused by the acetonitrile-rich mobile phase [10,20–23]. Consequently, the use of HILIC to analyze a wide range of polar contaminants in food and environmental samples is steadily increasing [24]. In addition, HILIC also enables the analysis of a wide range of ionizable analytes [25], being particularly useful for the retention and separation of small polar molecules that are basic or contain nitrogen atoms, such as AGs [1,26].

The primary function of HILIC stationary phases is to bind water at its surface. These phases are made from bare silica or silica bonded with several polar functional groups, such as amino, amide, cyano or diol groups [1]. Other common HILIC phases use ligands capable of undergoing electrostatic interactions, which attract polar molecules using various polar interactions and add an extra dimension to the separation of ionizable compounds. As such, ZIC-HILIC, a sulfoalkylbetaine (zwitterionic) phase, incorporates a positive and negative charge in a relatively long alkyl chain and was recently proposed in several methods for AGs [10,16,27–31]. In addition, a chemical modification of silica gel, commercially termed liquid separation cell technology (LiSC^{TM}), has been recently introduced. In this approach, a liquid separation cell with its own charge characteristics, ionic strength, and hydrophobic properties exists in constant equilibrium with the mobile phase and provides the quick mass transfer of charged analytes. The resulting Obelisc R stationary phase, also a zwitterionic HILIC column similar to ZIC-HILIC, was first applied for the analysis of DSTP and streptomycin (STP) in honey [32] and it has been recently used to analyze 13 AGs in meat [33].

Obelisc R and three HILIC stationary phases were evaluated to optimize and validate a method for the determination of fourteen

Table 1
MRLs of AGs in food of animal origin established by EC Regulation 37/2010.

AGs	MRL ($\mu g\,kg^{-1}$)						
	Muscle	Fat	Liver	Kidney	Milk	Egg	Honey
AMI							
APR	1000[B]	1000[B]	10,000[B]	20,000[B]	NP		
DSTP	500	500	500	1000	200		
GEN (C1 + C1A + C2)	50[B+S]	50[B+S]	200[B+S]	750[B+S]	100[B]		
HYG							
KAN	100	100	600	2500	150		
NEO	500	500	500	5000	1500	500	
PAR	500		1500	1500			
RIB							
SIS							
SPC	300	500	1000/2000[O]	5000	200		
STP	500	500	500	1000	200		40[Foug.]
TOB							

[B]Bovine.
[S]Swine.
[O]Ovine.
[NP]Not permitted in lactating cattle since 2005.
[Foug.]Recommended by EU Reference Laboratory for Antimicrobial and Dye Residues in Food in Fougères (France), CRL Guidance Paper (December, 2007).

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