DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF AMINOGLYCOSIDES IN FOODS USING LC-MS/MS WITH A NOVEL ZWITTERIONIC HILIC STATIONARY PHASE

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INTRODUCTION

Aminoglycosides (AMGs) are broad-spectrum antibiotics that have bactericidal activity against aerobic bacterial infection. They are used as veterinary drugs, feed additives and as growth promoters on food-producing animals. Due to their low cost, there is concern about the overuse of these drugs in commercial animal production. Misuse of veterinary medicines can lead to unacceptance residues in the tissues of the animals. Maximum Residue Limits (MRLs) have been set for specific aminoglycoside in a range of tissues of food producing species and in milk. AMGs are water soluble, highly polar compounds, not amenable to reversed phase chromatography without resort to using ion-paring agents (eg HBFA), which leads to ion suppression and contamination of the LC-MS/MS system. Alternatively, AMGs can be determined using hydrophilic interaction liquid chromatography (HILIC), but limited separation selectivity for these compounds is observed using amide or aminopropyl HILIC stationary phases.

In this study, the Atlantis Premier BEH Z-HILIC column, which has a zwitterionic sulfoalkylbetaine stationary phase attached to BEH particles, was evaluated for the determination of seventeen AMGs in food by LC-MS/MS after extraction with an aqueous solution containing TCA and SPE on Oasis HLB. The performance of the method was evaluated in milk, muscle, liver, and honey.

METHOD

Sample extraction and clean-up

Samples were extracted using a solution that contained 10 mM ammonium acetate, 0.4 mM ethylenediamine tetraacetic acid (EDTA), 0.5% NaCl, and 2% trichloroacetic acid (TCA) and subjected to SPE clean up using Oasis HLB.

Weigh 3 ±0.03 g homogeneous sample into 50 mL plastic Falcon tube

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Add 20 mL extraction solution, vortex (2 min), refrigerate (30 min), centrifuge (3200 g for 10 min at 4°C) and transfer supernatant to a 50 mL plastic Falcon tube

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Adjust pH of the supernatant to 6.75 ± 0.25 with basic solutions (50% and 10% KOH solutions) prior to SPE

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Load of all supernatant onto SPE cartridge (6 cc 500 mg HLB p/n 186000115), wash with 3 mL H_2O, dry (15 min under vacuum) and elute with 3 mL of elution solution (10 % FA, 5 % isopropanol in H_2O)

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The final extract was analyzed by LC–MS/MS without any reconstitution or dilution

Instrumental conditions

The Waters Arc Premier System offers novel MaxPeak High Performance Surfaces (HPS)-based technology within its mid-tier General Purpose LC category, providing a truly inert LC system, and is holistically designed to complement the 2.x µm MaxPeak HPS-enabled columns

LC System:	Arc Premier System with BSM and FLN SM
Column:	Atlantis Premier BEH Z-HILIC (2.5µm 2.1×150 mm)
Mobile Phase A:	20 mM Am formate in water (pH 3.0)
Mobile Phase B:	0.1% FA in acetonitrile
Injection volume:	6 µL
Column temp:	50°C
Purge solvent:	water/acetonitrile (1/9 v/v)

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.70	10	90	Initial
1.00	0.70	75	25	6
5.00	0.70	85	15	6
8.00	0.70	85	15	6
8.10	0.70	10	90	6
10.00	0.70	10	90	6

MS system:	Xevo TQ-S micro
Ionization:	Electrospray
Polarity:	Positive ion mode
Desolvation Temp:	600 °C
Capillary Voltage:	1.5 kV
Cone Gas Flow:	50 /Hr
Source Temp:	150 °C
Desolvation Gas Flow:	1000 L/Hr

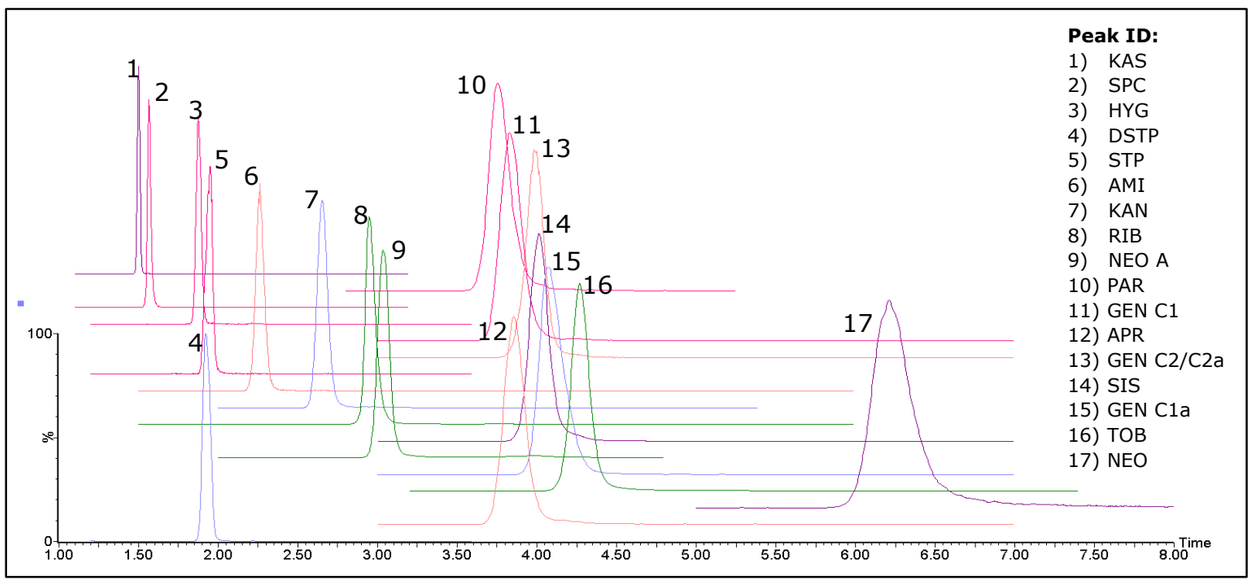
The AMGs included by Waters in this study were amikacin (AMI), apramycin (APR), gentamicin (GEN C1, C1a, C2/C2a), hygromycin B (HYG), kanamycin (KAN), kasugamycin (KAS), neomycin (NEO), neamine (or neomycin A, NEO A), paromomycin (PAR), ribostamycin (RIB), spectinomycin (SPC), streptomycin (STP), dihydrostreptomycin (DSTP), sisomicin (SIS), and tobramycin (TOB).

More details are presented in our application note (see QR code below)

RESULTS AND DISCUSSION

The effects of chromatographic conditions, such as mobile phases, pH, and ionic strength on the separation of seventeen highly polar AMGs on Atlantis Premier BEH Z-HILIC columns were systematically investigated. Gradient elution with a binary mobile phase of aqueous 20 mM ammonium formate at pH 3.0 and acetonitrile with 0.1% formic acid provided a reliable and adequate separation with excellent sensitivity for these AMGs by electrospray with tandem mass spectrometry. Retention times were very stable (RSD 0.01-0.91 %; n=57 in various matrices). More details on the development of the method can be found in the application note.

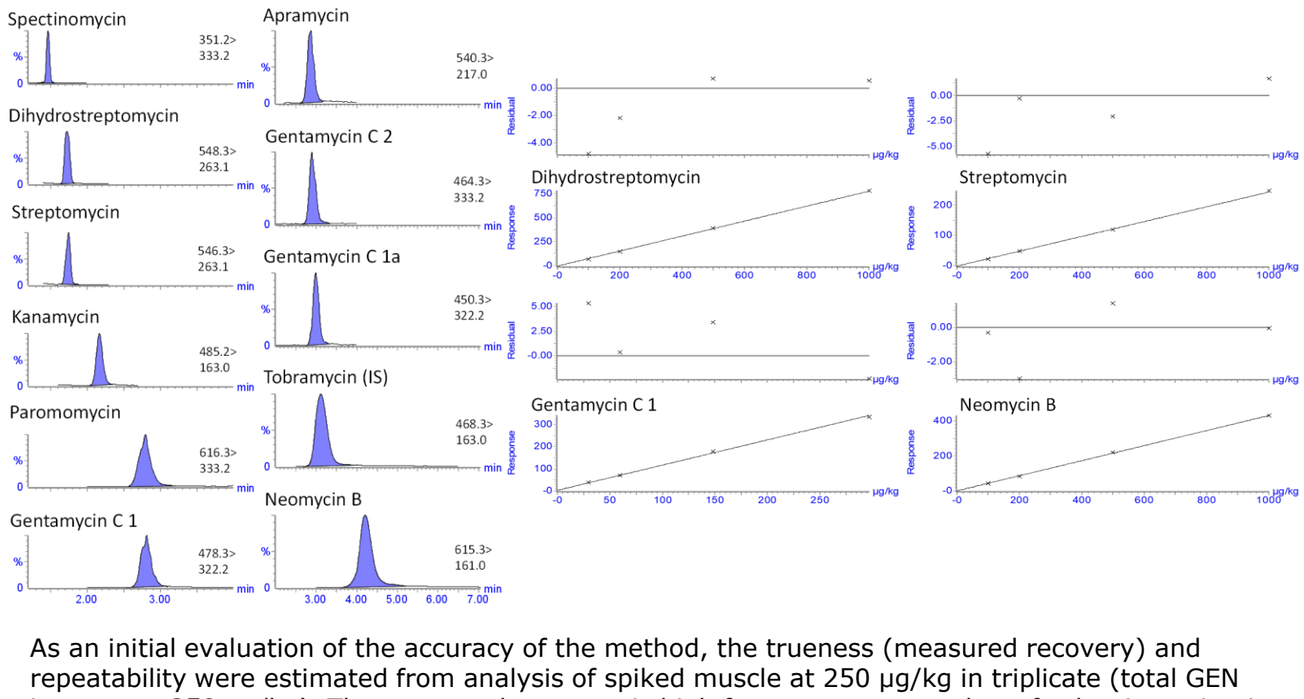
The overlaid chromatograms below show the typical separations obtained from the analysis of spiked milk at 2500 µg/kg.



Matrix effects were observed for these AMGs, from strong ion suppression to strong ion enhancement so matrix-matched calibration is recommended.

The method was then implemented by the laboratory of the State Office for Agriculture, Food Safety and Fisheries Mecklenburg-West Pomerania, Germany. As an initial evaluation of the accuracy of the method, the recovery and repeatability of the key AMGs covered by the method were established from analysis of spiked muscle at 200 µg/kg in triplicate. TOB was used as an internal standard and concentrations in the spikes calculated using a four point matrix-matched calibration.

The method was then implemented on a Xevo TQ-XS by the laboratory of the State Office for Agriculture, Food Safety and Fisheries Mecklenburg-West Pomerania. Matrix effects were observed for these AMGs, from strong ion suppression to strong ion enhancement so matrix-matched calibration was used. TOB was used as an internal standard. Chromatograms from the analysis of the standard at 200 µg/kg (GEN C1=59µg/kg, GEN C1=41µg/kg and GEN C2=100µg/kg) are shown below left and some typical calibration graphs below on the right.



As an initial evaluation of the accuracy of the method, the trueness (measured recovery) and repeatability were estimated from analysis of spiked muscle at 250 µg/kg in triplicate (total GEN isomers at 250 µg/kg). The measured recovery is high for some compounds so further investigation into an appropriate internal standard may be required. Repeatability, albeit with n=3, looks promising.

	Spectinomycin	Streptomycin	Dihydrostreptomycin	Kanamycin	Apramycin
Mean trueness (%)	134	112	111	101	108
Repeatability (% RSD)	2.2	9.1	9.8	3.6	7.1
	Gentamycin C 1	Gentamycin C 1a	Gentamycin C 2	Paromomycin	Neomycin
Mean trueness (%)	123	123	111	109	108
Repeatability (% RSD)	6.6	4.2	7.5	4.9	5.9

CONCLUSIONS

- The effects of chromatographic conditions, including mobile phase composition, buffer concentration, and pH, on the separation of AMGs on the Atlantis Premier BEH Z-HILIC column have been systematically investigated (see application note for more details)
- The final optimized conditions provided reliable separation of 17 AMGs using the Atlantis Premier BEH Z-HILIC column, using MS friendly mobile phases with no ion pair reagents or high concentration of buffer.
- The method appears to be sufficiently sensitive to be able to detect most AMGs of interest at the MRLs.
- An initial evaluation of accuracy, as outlined here and in the Waters application note, indicates that, after further validation, the method could be suitable for the determination of AMGs in milk, muscle, liver, and honey to check compliance with MRLs in Europe.

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