LC-MS/MS ANALYSIS OF AMINOGLYCOSIDES IN FOODS BY ZWITTERIONIC HYDROPHILIC INTERACTION CHROMATOGRAPHY

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INTRODUCTION

Aminoglycosides (AMGs) are an important class of antibiotics to treat Gram-negative bacterial infections. They can also be used as growth promoters in food-producing animals. AMGs are often analyzed in honey, eggs, milk, muscles and tissues of food-producing animals for control and monitoring purposes. AMGs are highly polar compounds. The analysis of AMGs often employ Hydrophilic Interaction Chromatography (HILIC). Zwitterionic HILIC columns were found better suited for the AMGs than other HILIC columns. However, extremely high mobile phase buffer concentrations were needed for some AMGs analysis.

The goal of this work is to develop a liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS) method for AMGs using a novel zwitterionic HILIC column, the Atlantis Premier BEH Z-HILIC column, which is based on BEH particles and sulfobetaine type stationary phase. The effects of chromatographic conditions on the separation of 15 common AMGs have been investigated. The extraction and clean-up of AMGs in honey, milk, muscles and liver using Oasis HLB SPE cartridge was optimized. The analytical performance of the AMGs analysis will be discussed. The structures of the AMGs included in this study are shown in Fig.1.

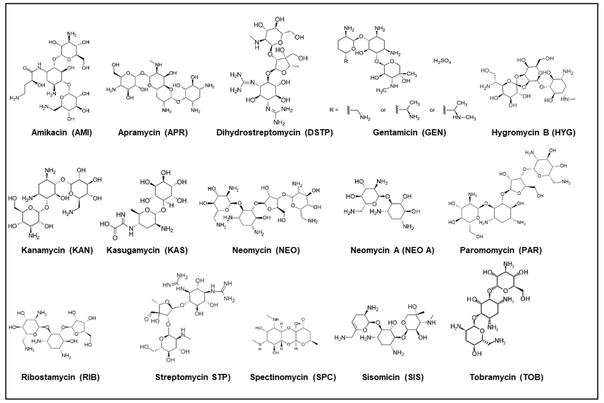

Figure 1. Structures of 15 aminoglycosides. Isomers of gentamicin are also shown (C1A, C2, C1).

METHODS

Samples:

Honey, milk, muscle (bovine and swine), and liver (poultry) samples were purchased in local stores. Honey and milk samples were kept in refrigerator (0-4°C), and the muscle and liver were kept frozen (-20°C) until they were analyzed.

Sample extraction and clean-up

3g of honey, milk or grounded muscle or minced liver tissue was mixed with 20 mL of an extraction solution (10 mM ammonium acetate, 0.4 mM EDTA, 0.5% NaCl, 2% Trichloroacetic acid) in a 50 mL plastic centrifuge tube. These samples were vortexed at high level for 2 min and kept in refrigerator for 30 min. All samples were vortexed again before centrifuged at 3200 g for 5 min at 4oC. The supernatant was transferred by a plastic transfer pipette to another 50 mL plastic centrifuge tube. The pH of the supernatant was adjusted to 6.5 to 7.0 with 50% KOH solutions before loading onto an Oasis HLB SPE cartridge (6cc Vac Cartridge, 500 mg sorbent, 60 µm). The SPE cartridge was previously conditioned with 3 mL of methanol and 3 mL of water prior to the sample loading. After loading, the cartridge was rinsed with 3 mL of water and dried for 15 min under vacuum. The analytes were eluted with 3 mL of elution solution (10 v/v% formic acid, 5 v/v% isopropanol in water) and injected into the LC–MS/MS.

Note: Extraction solution (10 mM ammonium acetate, 0.4 mM EDTA, 0.5% NaCl, 2% TCA): Place 0.375 g of ammonium acetate into a 500 mL volumetric flask. Add approximately 450 mL of MilliQ water and dissolve. Adjust pH to 4.0 with reagent formic acid. Add 0.075 g disodium ethylenediamine tetraacetate, 2.5 g of sodium chloride, and 10 g of trichloroacetic acid (TCA). Mix well to dissolve and bring to the mark with reagent water.

LC conditions

LC System:	ACQUITY Premier System
MS system:	Xevo™ TQ-S micro system
Run time:	10.0 min
Column:	Waters Atlantis Premier BEH Z-HILIC Column, (2.5 µm, 2.1 × 150 mm)
Temp:	50 °C
Mobile phases:	A: 20 mM ammonium formate in water (pH 3.0). B: 0.1% formic acid in acetonitrile
Injection volume:	6 µL
Gradient program:	

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Ini	0.70	10.0	90.0	Ini
1.00	0.70	75.0	25.0	6
5.00	0.70	85.0	15.0	6
8.00	0.70	85.0	15.0	6
8.10	0.70	10.0	90.0	6
10.00	0.70	10.0	90.0	6

MS system settings:

Polarity:	ES+	Desolvation Temp.:	600 °C
Capillary Voltage:	1.5 kV	Cone Gas Flow:	50 L/Hr
Source Temp.:	150 °C	Desolvation Gas Flow:	1000 L/Hr

RESULTS

1) Chromatography optimization

Various chromatographic conditions have been screened. The pH and the buffer concentration have significant impact on the retention time, the peak shape, and signal intensity for the separation of AMGs.

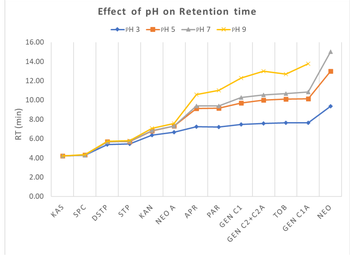

Figure 2. Effect of the pH of the aqueous mobile phase (A) on the retention time of AMGs. Conditions: Atlantis Premier BEH Z-HILIC 1.7µm 2.1 x 100 mm. Flow rate: 0.2 mL/min. Col Temp.: 40°C. Mobile phase A: 20 mM ammonium formate (pH: varies); Mobile phase B: 0.1% formic acid in acetonitrile. Gradient: 20% A to 95% A in 5 min, then keep at 95% A for 10 min.

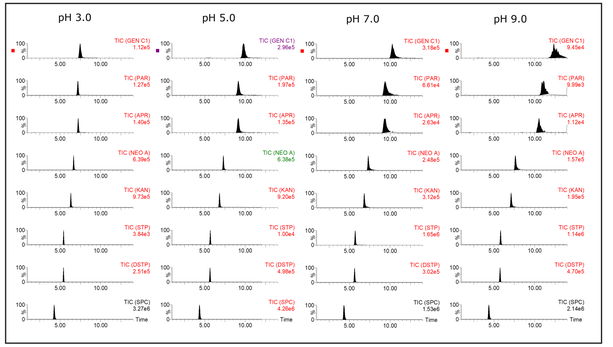

Figure 3. Effects of pH in aqueous mobile phase on the retention, peak shape, peak intensity of AMGs. Conditions: Column: Atlantis Premier BEH Z-HILIC 1.7µm 2.1 x 100 mm; Flow rate: 0.2 mL/min; Col Temp.: 40°C; Mobile phase A: 20 mM ammonium formate (pH: varies); Mobile phase B: 0.1% formic acid in acetonitrile; Gradient: 20% A to 95% A in 5 min, then keep at 95% A for 10 min.

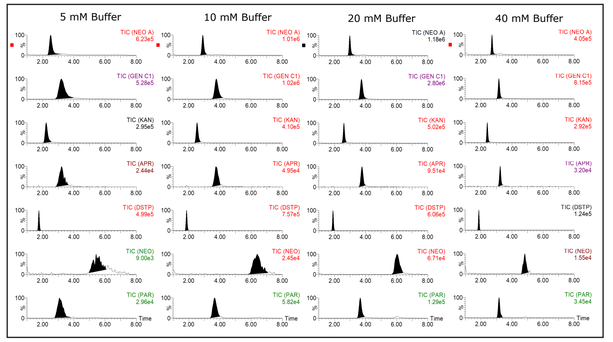

Figure 4. Effect of buffer concentration (Ionic strength) on the chromatography of AMGs. Conditions: Column: Atlantis Premier BEH Z-HILIC 2.5µm 2.1 x 150 mm; Flow rate: 0.7 mL/min; Col Temp.: 50°C; Mobile phase A: ammonium formate in water (pH: 3.0); Mobile phase B: 0.1% formic acid in acetonitrile; Gradient: 10% A to 75% A in 1 min, then to 85% A in 4 min, and stay at 85% A for 3 min.

2) SPE extraction and clean-up

Oasis HLB, Oasis WCX, and Sep-Pak Accell Plus CM SPE cartridges (6mL 500 mg) have been tested. The Oasis HLB SPE cartridges provide good recovery for AMGs in various sample matrices.

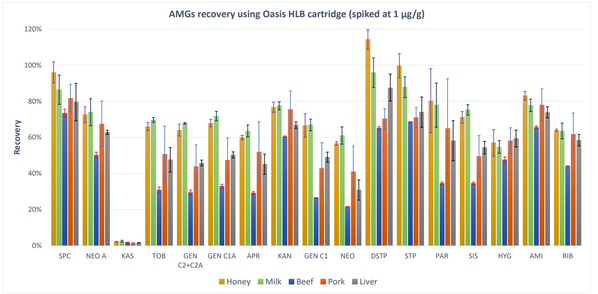

Figure 5. SPE recovery using Oasis HLB SPE cartridge (n=4). This total recovery was obtained by comparing the LC-MS/MS peak areas of food samples spiked at 1 µg/g at the beginning of extraction and after the SPE clean-up.

3) Sensitivity, linearity and linear range

Excellent LOQ and linearity (R^2) were obtained using matrix-matched standard solutions.

Table 1. LOQ, linearity and linear range for AMGs in food matrices

	LOQ (µg/kg)				Linearity (R^2)				Linear range (µg/kg)			
	Milk	Beef	Liver	Honey	Milk	Beef	Liver	Honey	Milk	Beef	Liver	Honey
SPC	10	10	25	100	0.9999	1.0000	0.9999	0.996	10 - 2500	10 - 2500	25 - 2500	100 - 2500
NEO A	10	10	10	10	0.9997	0.9997	0.9992	0.9999	10 - 2500	10 - 2500	10 - 2500	10 - 2500
KAS	10	25	25	100	0.9956	1.0000	1.0000	0.9996	10 - 2500	25 - 2500	25 - 2500	100 - 2500
TOB	10	25	25	10	0.9992	0.9992	0.9995	0.998	10 - 2500	25 - 2500	25 - 2500	10 - 2500
GEN C2+C2A	10	25	25	25	0.997	0.997	0.997	0.994	10 - 2500	25 - 2500	25 - 2500	25 - 2500
GEN C1A	25	25	25	25	0.998	0.998	0.998	0.996	25 - 2500	25 - 2500	25 - 2500	25 - 2500
APR	25	25	25	25	0.996	0.997	0.997	0.999	25 - 2500	25 - 2500	25 - 2500	25 - 2500
KAN	10	10	10	10	0.9997	0.9996	0.9999	0.9994	10 - 2500	10 - 2500	10 - 2500	10 - 2500
GEN C1	10	25	25	25	0.995	0.995	0.996	0.992	10 - 2500	25 - 2500	25 - 2500	25 - 2500
NEO	25	25	25	25	0.9991	0.9985	0.998	0.997	25 - 2500	25 - 2500	25 - 2500	25 - 2500
DSTP	25	25	100	25	0.982	0.9986	0.9998	0.98	25 - 2500	25 - 2500	100 - 2500	25 - 2500
STP	10	25	25	10	0.989	0.9993	1.0000	0.98	10 - 2500	25 - 2500	25 - 2500	10 - 2500
PAR	25	25	25	25	0.9998	0.9998	0.9996	0.9997	25 - 2500	25 - 2500	25 - 2500	25 - 2500
SIS	25	25	25	25	0.998	0.997	0.997	0.995	25 - 2500	25 - 2500	25 - 2500	25 - 2500
HYG	100	100	100	100	0.9993	0.9998	0.9997	0.9999	100 - 2500	100 - 2500	100 - 2500	100 - 2500
AMI	10	10	10	10	0.9987	0.9998	1.0000	0.997	10 - 2500	10 - 2500	10 - 2500	10 - 2500
RIB	10	10	10	10	0.9997	0.9996	0.998	0.9995	10 - 2500	10 - 2500	10 - 2500	10 - 2500

CONCLUSION

LC-MS/MS analysis of AMGs using Atlantis BEH Z-HILIC column offers excellent solution for the screening and quantification of AMGs in foods.

- **Atlantis BEH Z-HILIC column demonstrated adequate separation resolution of 15 common AMGs within a 10 min run.**
- **Oasis HLB SPE cartridge provided satisfactory recoveries for 14 AMGs in milk, muscle, liver, and honey.**
- **Excellent LOQs were obtained for AMGs in food matrices. The low LOQ makes this method suitable for screening of AMGs at much lower concentration than MRLs.**
- **No need to have high buffer concentration in mobile phase**

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