

ANALYSIS OF AMINOGLYCOSIDES IN FOODS BY LC-MS/MS USING A ZWITTERIONIC STATIONARY PHASE

Waters™

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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 milk, egg, honey, and muscle 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 analysis of AMGs than other HILIC columns. However, high buffer concentrations in mobile phase were needed for some AMGs analysis.

OBJECTIVE

The goal of this work is to develop a LC-ESI-MS/MS method for AMGs in foods using a MS friendly mobile phase (less buffer or additive).

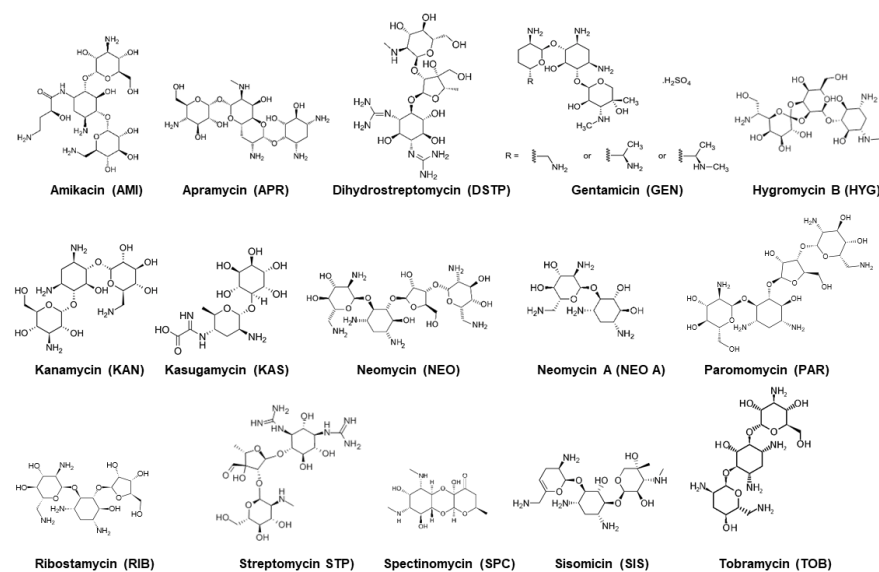


Figure 1. Structures of 15 aminoglycosides. Isomers of gentamicin are also shown (C1A, C2, C1).

EXPERIMENTAL

LC conditions

LC System: Arc™ Premier System (BSM)
MS system: Xevo™ TQ-S micro System
Software: MassLynx™ V4.2 Software
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: water (with 20 mM ammonium formate, pH 3.0).
B: acetonitrile (with 0.1% formic acid)
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

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

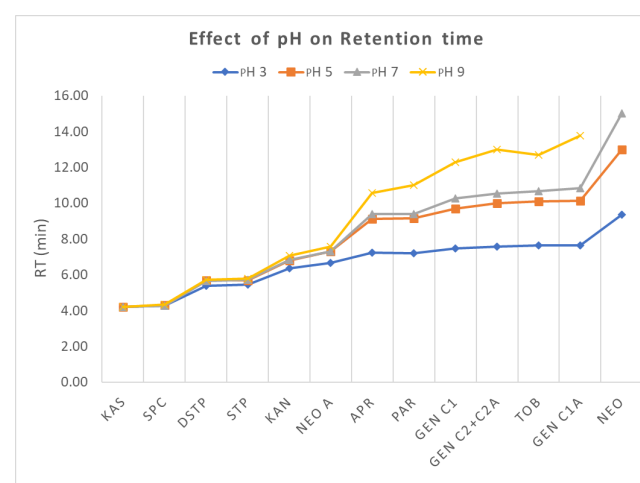


Figure 2. Effect of the pH of the aqueous mobile phase A (water with 20 mM ammonium formate) on the retention time of AMGs. Conditions: Atlantis Premier BEH Z-HILIC Column 1.7μm 2.1 x 100 mm. Flow rate: 0.2 mL/min. Col Temp.: 40°C. Gradient: 20% A to 95% A in 5 min, then keep at 95% A for 10 min.

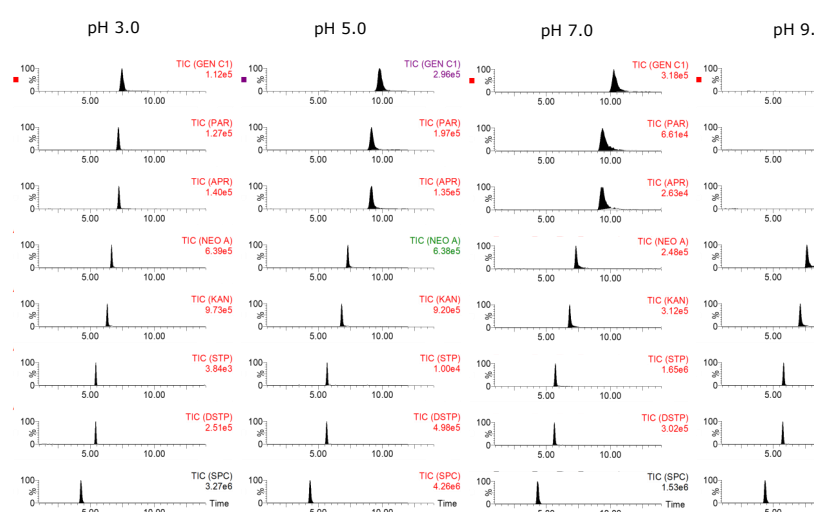


Figure 3. Effects of pH in aqueous mobile phase A on the retention, peak shape, peak intensity of AMGs. Conditions: Column: Atlantis Premier BEH Z-HILIC Column 1.7μm 2.1 x 100 mm; Flow rate: 0.2 mL/min; Col Temp.: 40°C; Gradient: 20% A to 95% A in 5 min, then keep at 95% A for 10 min.

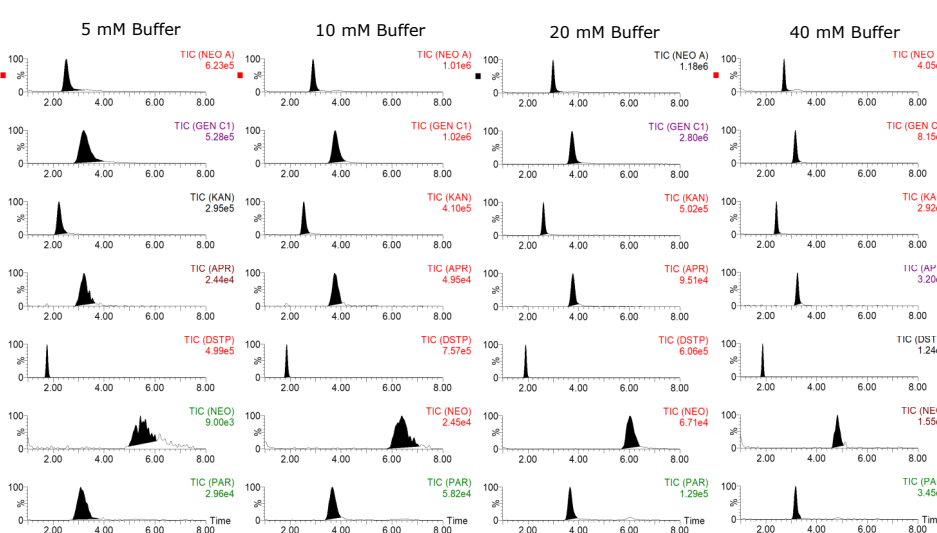


Figure 4. Effect of buffer concentration (ionic strength) on the chromatography of AMGs. Conditions: Column: Atlantis Premier BEH Z-HILIC Column 2.5μm 2.1 x 150 mm; Flow rate: 0.7 mL/min; Col Temp.: 50°C; Mobile phase A: water with ammonium formate (pH: 3.0); Mobile phase B: 0.1% formic acid in acetonitrile;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

RESULTS

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.

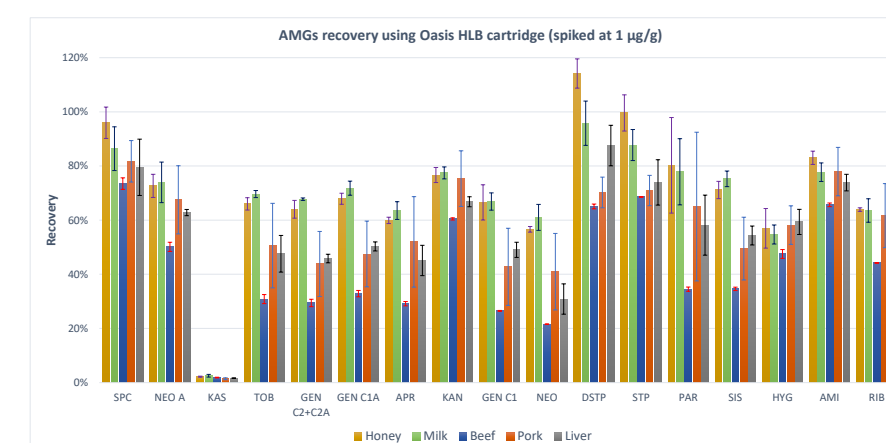


Figure 5. SPE recovery using Oasis HLB SPE Cartridge (n=4). This total recovery was obtained by comparing the LC-MS/MS peak areas of food samples spiked at 1 μg/g at the beginning of extraction and after the SPE clean-up.

3) Sensitivity, linearity and linear range

Excellent LOQ and linearity (R²) 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	0.997	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 using a binary water/acetonitrile mobile phase with 20 mM ammonium formate.
- 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.



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