Development and Validation of a Method for the Determination of Aminoglycosides in Foods using LC-MS/MS with a 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.

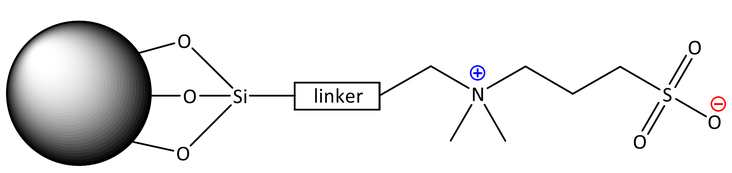
AMGs are water soluble, highly polar compounds, not amenable to reversed phase chromatography without resort to using ion-paring agents (eg HBFA), which leads to ion suppression and contamination of the LC-MS/MS system. Alternatively, AMGs can be determined using hydrophilic interaction liquid chromatography (HILIC), but limited separation selectivity for these compounds is observed using amide or aminopropyl HILIC stationary phases. In this study, a method based upon the Atlantis™ Premier BEH™ Z-HILIC column, developed previously,[1] was implemented using UniSpray™, and the performance further evaluated for the determination of 8 AMGs in milk, eggs and honey, at the laboratory of the State Office for Agriculture, Food Safety and Fisheries Mecklenburg-West Pomerania, Germany.

SAMPLE PREPARATION

Samples were extracted using a solution that contained 10 mM ammonium acetate, 0.4 mM ethylenediaminetetraacetic acid (EDTA), 0.5% NaCl, and 2% trichloroacetic acid (TCA) and subjected to SPE clean up using Oasis™ HLB, prior to LC-MS/MS. Tobramycin was used as an internal standard. Conditions were the same as previously reported[1] but here the platform comprised ACQUITY™ I-Class with BSM and FTN sample manager coupled with Xevo™ TQ-XS using UniSpray.

ATLANTIS PREMIER BEH Z-HILIC COLUMN

This column is packed with a zwitterionic sulfoalkylbetaine stationary phase attached to BEH particles. The zwitterionic sulfobetaine ligand has both positively and negatively charged groups, in a one-to-one ratio, making them net neutral. The sulfobetaine bonding provides a unique selectivity and creates a very hydrophilic surface. A dense/thick water rich layer on the surface further increases polar analyte retention.



CHROMATOGRAPHY

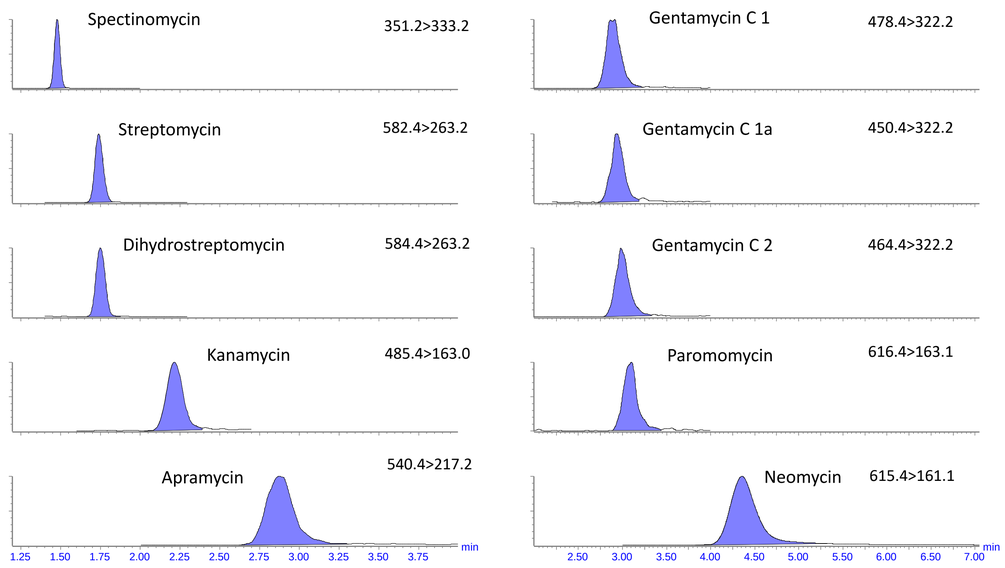


Figure 1. Chromatograms for the AMGs from the analysis of the matrix-matched standard at the various MRLs/level of interest in milk

EVALUATION OF PERFORMANCE

Validation was performed on milk, eggs, and honey based upon requirements of Commission Implementing Regulation (EU) 2021/808. The results from the evaluation of the following parameters are reported here:

- **Identification was assessed by examining retention times, ion ratios, and identification points, and all spikes were found to be within tolerance.**
- **Trueness using measured recovery, repeatability (RSD_r), within-laboratory reproducibility (RSD_{RL}) and decision limit ($CC\alpha$) were derived from data from the analysis of replicate spiked samples, performed on three separate days.**
- **Spikes were prepared at 0.1, 0.5, 1, 1.5 and 2 times the level of interest (typically MRL where available). There are no MRLs for AMG residues in honey, so their presence in honey is not authorised. The level of interest for such unauthorised substances was set at the minimum method performance requirement (MMPR) provided by the EURL and spikes prepared at 0.5, 1, 1.5, 2 and 3 times the MMPR. All calculations, for both MRL and unauthorised substances, were based on InterVAL 3.4.0.4, CD 2002/657 (alternative method).**

The figures below show the results for each spike level in milk. Measured recovery was within the acceptable range (80-120%) at all concentrations, whereas repeatability and within-lab reproducibility were within tolerance (<22%) for the spikes at 0.5x to 2x level of interest, but not at the new 0.1x level. The same trends were observed for eggs and honey (not shown).

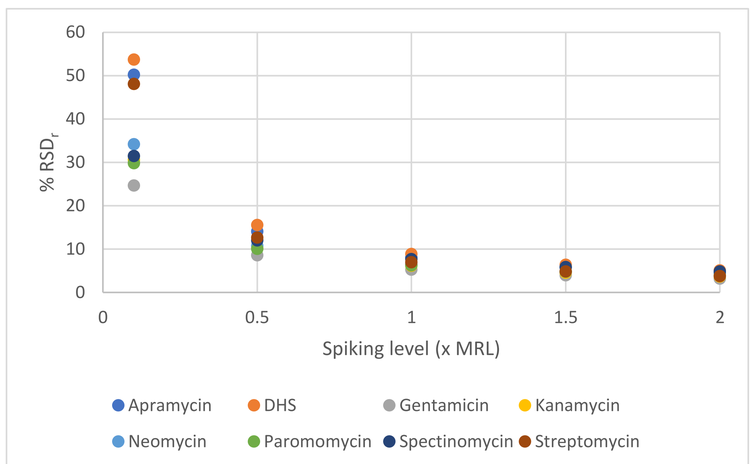


Figure 2. Repeatability in milk

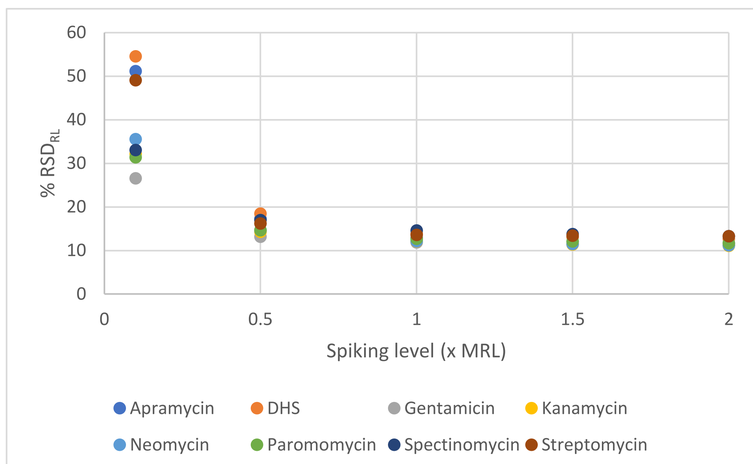


Figure 3. Reproducibility in milk

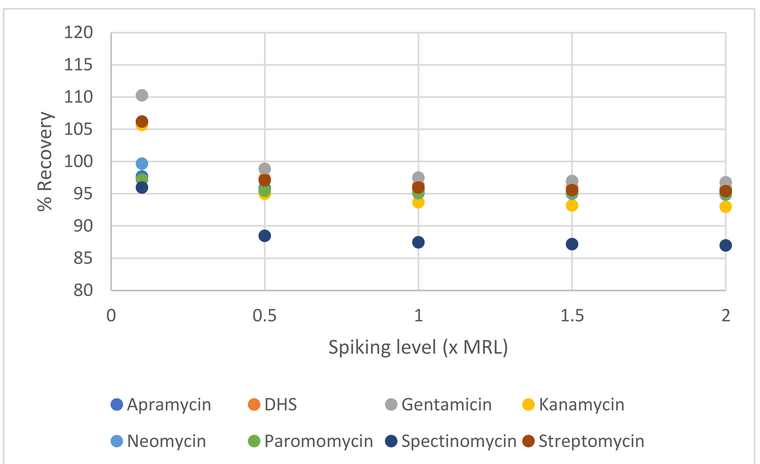


Figure 4. Recovery in milk

Levels of Interest and Decision Limits (µg/kg))

		Apramycin	DHS	Gentamicin	Kanamycin	Neomycin	Paromomycin	Spectinomycin	Streptomycin
Milk	**MRL**	200*	200	100	150	1500	200*	200	200
	CCα	53	254	123	185	1854	40	259	254
Eggs	**MRL**	200*	200*	100*	200*	500	200	200*	100*
	CCα	41	42	18	37	601	242	44	22
Honey	**MMPR**	20	20	20	20	20	20	20	20
	CCα	5.5	8.2	6.0	6.8	6.4	8.5	11	7.8

** There are no MRLs for these analytes in this commodity, so the level of interest has been extrapolated from other MRLs.*

CONCLUSION

- **This poster describes a method for the determination of residues of aminoglycosides using UPLC-MS/MS**
- **Chromatographic retention and separation was provided by the Atlantis Premier BEH Z-HILIC column, using an MS friendly mobile phase**
- **More details on the development of the HILIC method can be found by scanning the QR code to the right**
- **The method is suitable for reliable determination of residues to check compliance with MRLs and in cases where use of the substances is not allowed**
- **The method was successfully validated in three sample types, according to Commission Implementing Regulation (EU) 2021/808**

[1]Analysis of Aminoglycosides in Foods Using a Zwitterionic Stationary Phase and Liquid Chromatography-Tandem Mass Spectrometry. Waters App Note 720007442

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