

UPLC/MS/MS Determination of Aminoglycoside Antibiotics in Meat and Milk

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APPLICATION BENEFITS

- Rapid extraction of aminoglycosides from milk or meat
- Rapid LC/MS/MS analysis
- Straightforward SPE cleanup
- Low ppb detection limits

WATERS SOLUTIONS

ACQUITY UPLC® System

ACQUITY® TQD Mass Spectrometer

ACQUITY UPLC HSS PFP Column

Oasis® HLB Cartridge

Polypropylene Vials

KEY WORDS

Aminoglycoside antibiotics, SPE, milk, beef, LC/MS/MS

INTRODUCTION

Aminoglycoside antibiotics, used in veterinary medicine for the treatment of animals bred for meat and milk production, require an effective method for residue analysis in these commodities. This class of antibiotics presents significant challenges for residue analysis. Unlike most other antibiotic classes, these compounds are not amenable to extraction from tissue or milk using acetonitrile or other organic solvents. In this study, the aminoglycosides are extracted from meat tissue or milk using an aqueous buffer with trichloroacetic acid (TCA) added to precipitate protein and inhibit protein binding of the analytes. An effective SPE-based cleanup procedure is employed prior to LC/MS analysis to remove the residual TCA and to minimize co-extracted interferences. For SPE, good recovery and cleanup were obtained for bovine milk and meat using Oasis HLB, a high-performance, water-wettable, reversed-phase sorbent. In the subsequent analysis, excellent UPLC® performance was obtained using the ACQUITY UPLC HSS PFP Column in reversed-phase ion-pairing mode. Heptafluorobutyric acid (HFBA) was used as the ion-pairing reagent. This reagent is volatile and compatible with mass spectrometry.

Worldwide maximum residue levels (MRLs) for the aminoglycoside antibiotics generally range from 100 to 1000 µg/kg milk, as shown in Figure 1. This application note presents methodology suitable for ppb level determination of aminoglycoside antibiotics in bovine milk and tissue and was developed in part from methods presented in references 1 and 2.

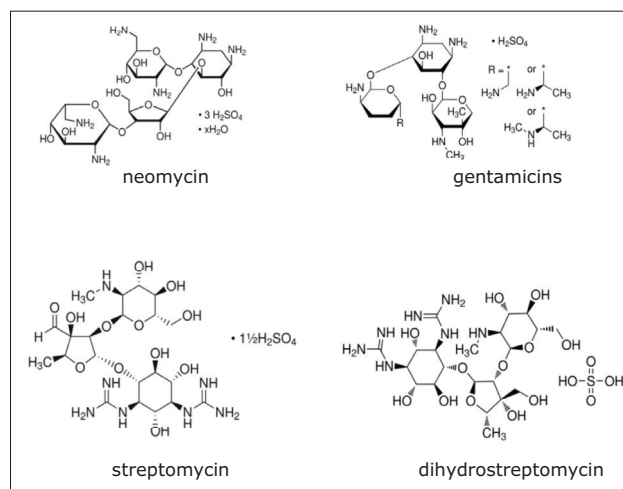


Figure 1. Structures of aminoglycoside antibiotics.

EXPERIMENTAL

UPLC conditions

System:	ACQUITY UPLC
Column:	ACQUITY HSS PFP 2.1 x 100 mm, 1.7 μ m p/n 186005967
Injection volume:	30 μ L
Temp.:	35 °C
Mobile phase A:	20 mM HFBA in water
Mobile phase B:	20 mM HFBA in acetonitrile
Flow rate:	0.50 mL/min
Gradient:	20% B initial, linear gradient to 80% B in 7 min, hold for 8 min, back to 20% B for 8.1 min. Hold and re-equilibrate for 10 min.

MS conditions

Mass spectrometer:	ACQUITY TQD
Mode:	Positive electrospray (ES+)
Capillary:	3.0 kV
Extractor:	3.0 V
Source temp.:	130 °C
Cone gas:	20 L/h
Desolvation temp.:	450 °C
Desolvation gas:	900 L/h
Collision gas:	Argon at 0.20 mL/min

Table 1 summarizes the cone and collision parameters and MRM transitions used in this study.

Aminoglycoside		Cone (V)	Collision (eV)
Streptomycin	582.3 > 246.1	70	28
	582.3 > 263.2	70	28
Dihydrostreptomycin	584.3 > 246.0	60	26
	584.3 > 263.2	60	26
Gentamicin C1a	450.4 > 160.1	35	25
	450.4 > 322.4	35	15
Gentamicin C1	478.5 > 157.2	35	30
	478.5 > 322.1	35	15
Gentamicin C2C2a	464.5 > 160.1	35	20
	464.5 > 322.4	35	15
Neomycin	615.4 > 161.1	55	28
	615.4 > 293.1	55	25

Table 1. Cone and collision parameters and MRM transitions used for this study.

Sample preparation

Extraction Buffer (10 mM NH₄OOCCH₃/0.4 mM Na₂EDTA/1% NaCl/2% TCA):

Place 0.77 g of ammonium acetate (NH₄OOCCH₃) into a 1-L volumetric flask. Add approximately 900 mL of reagent water and dissolve. Adjust pH to 4.0 with 1 N HCl or 1 N NaOH. Add 0.15 g disodium ethylenediamine tetraacetate (Na₂EDTA·2H₂O), 5 g of sodium chloride (NaCl), and 20 g of trichloroacetic acid (TCA). Mix well to dissolve and bring to the mark with reagent water.

Initial extraction:

Place 2 g homogenized bovine tissue or 10 mL milk into a 50 mL centrifuge tube. Add 20 mL extraction buffer, vortex for 10 s, then shake well for 1 min. Centrifuge the sample at 4000 RPM for 5 min, and collect the supernatant. Adjust the pH of the supernatant to 6.5±0.5 using diluted HCl or NaOH as needed.

SPE cleanup:

An [Oasis HLB 96-well Plate \(30 mg\)](#) was used in this study. A 1-cc, 30-mg cartridge can be used if desired. Condition the well or cartridge with 1.5 mL methanol, followed by 1.5 mL water. Set the flow rate at 1 mL/min or less. Load the pH-adjusted supernatant obtained from the initial extraction; a 1-mL aliquot is loaded for tissue samples, a 1.5-mL aliquot for milk samples. Wash with 1 mL water. Elute with 0.5 mL 10:5:85 formic acid/isopropanol/water. Add 1.5 μ L HFBA and analyze using UPLC/MS/MS.

RESULTS AND DISCUSSION

Figure 2 shows a reconstructed UPLC/MS/MS chromatogram obtained from the analysis of a spiked bovine milk sample. The same conditions were used for the bovine muscle samples. The results obtained from the spiking experiments are shown in Table 2 (milk) and Table 3 (muscle tissue). Neomycin (B and C) and gentamycin (C2a and C2) exist as a mixture of isobaric isomers and are partially resolved. The peak area for these pairs was summed for quantification.

Aminoglycoside	% Recovery		% RSD	
	n=6		n=6	
	10 ppb		200 ppb	
Streptomycin	77.7	12.2	81.9	13.1
Dihydrostreptomycin	93.4	3.0	81.9	14.0
Gentamycin C1	79.4	12.0	70.4	10.0
Gentamycin C2C2a	88.0	4.9	79.6	7.1
Gentamycin C1a	78.1	7.4	86.8	9.2
Neomycin	75.5	11.6	78.3	10.1

Table 2. Summary of recovery data for aminoglycosides spiked into bovine milk.

Aminoglycoside	% Recovery		% RSD	
	n=6		n=6	
	50 ppb		1600 ppb	
Streptomycin	102.9	11.7	97.3	4.0
Dihydrostreptomycin	88.4	5.9	89.7	6.1
Gentamycin C1	83.6	9.6	95.3	13.3
Gentamycin C2C2a	93.0	5.5	85.8	9.9
Gentamycin C1a	94.9	10.9	89.1	13.8
Neomycin	86.3	3.0	83.6	11.3

Table 3. Summary of recovery data for aminoglycosides spiked into bovine muscle.

Recovery was determined by comparing the MRM peak areas for samples spiked into the sample matrix prior to sample preparation with the peak areas for samples spiked after all sample preparation steps.

Matrix effects were less than 30% for meat and less than 12% for milk.

Unlike many other antibiotics used in veterinary medicine, the aminoglycosides cannot be extracted from animal tissues and related samples using organic solvents. However, these compounds can be effectively extracted from tissue with an aqueous buffer. This extraction buffer also includes an agent (TCA) to facilitate protein precipitation.

The use of a low organic content eluent for the Oasis HLB SPE protocol gave improved cleanup compared with an alternative methanol-based elution (samples eluted with formic acid/methanol had matrix effects greater than 50%). Apparently, significant matrix interference components remain on the sorbent after elution of the aminoglycosides with the aqueous eluent.

The ion-pairing reversed-phase mode was chosen for the UPLC analysis. The best compromise for peak shape and sensitivity was obtained using 20 mM heptafluorobutyric acid as the ion-pairing agent. UPLC using HILIC columns was also considered; however, peak shape was not as sharp as the chosen method. Also, a disadvantage of the HILIC mode is that the diluent for standards and samples should be acetonitrile. The optimized SPE protocol presented in this application note provides an aqueous-based sample for injection that is better suited for reversed-phase analysis.

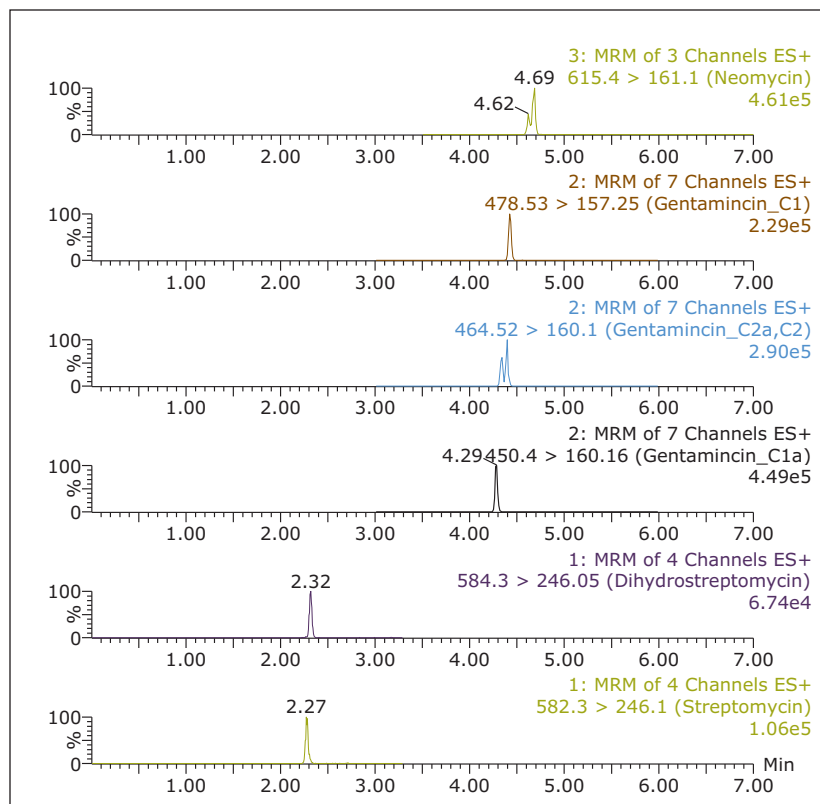


Figure 2. UPLC/MS/MS chromatogram obtained from bovine milk spiked at 10 µg/kg (ppb).

CONCLUSIONS

- Excellent UPLC performance was obtained using the ACQUITY UPLC HSS PFP analytical column for ion-pair reversed-phase chromatography.
- Heptafluorobutyric acid (HFBA) was an effective ion pairing agent for the UPLC analysis.
- A quick and simple procedure is presented for the extraction of aminoglycoside antibiotics from bovine meat and milk; a single operator can easily analyze 20 samples per day.
- An optimized SPE protocol is presented using an Oasis HLB 96-well Plate.
- LOQ of 10 ppb was demonstrated for bovine milk, 50 ppb for bovine muscle; signal to noise ratio was greater than 100:1 for all analytes at those levels.

References

1. Screening and Confirmation for Aminoglycosides by UHPLC-MS-MS (United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science).
2. Plozza T, Trenerry VC, Zeglinski P, Nguyen H, Johnstone P. The Confirmation and Quantification of Selected Aminoglycoside Residues in Animal Tissue and Bovine Milk by Liquid Chromatography Tandem Mass Spectrometry. *International Food and Research Journal*. 2011; 18(3): 1077-1084.

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