Extraction and Clean-up of Aminoglycoside Antibiotics from Chicken Meat Using EVOLUTE® WCX Solid Phase Extraction Columns with Analysis by LC-MS/MS

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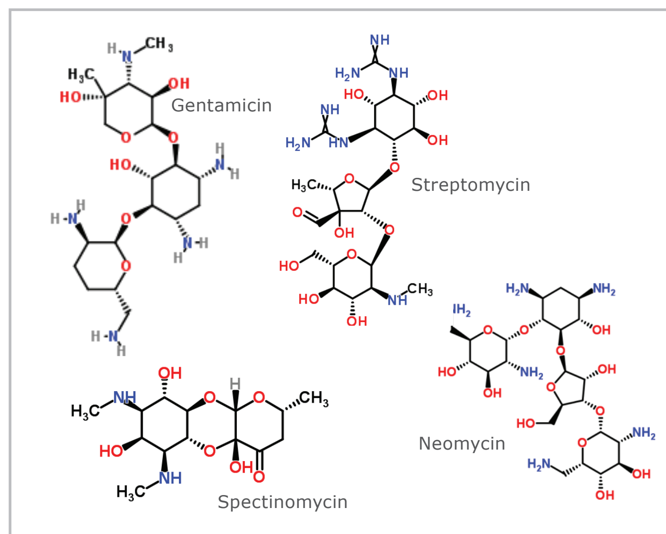

Figure 1. Analyte structures

Introduction

This application note describes a polymer based-based weak exchange mixed-mode SPE protocol for the extraction of the aminoglycoside antibiotics gentamicin, streptomycin, spectinomycin and neomycin from chicken meat prior to LC-MS/MS analysis.

The utility of aminoglycoside antimicrobial additives and foods has recently been challenged due to the effects of bioaccumulation in an animal host. The persistence of these selected residues may result in population resistance to antibiotic treatment and as a consequence, has been considered an issue of public health.

This application note describes an approach to extraction of these analytes from chicken meat, with optimized analyte recovery, minimal ion suppression, and acceptable method precision.

Analytes

Gentamicin, Streptomicin, Spectinomycin, Neomycin

Sample Preparation Procedure

Format: **EVOLUTE® WCX 500 mg/6 mL solid phase extraction columns, p/n 612-0050-C**

Sample Pre-treatment:

Weigh 5 g of chicken meat into a 50 mL conical tube.

Spike with internal standard as required.

Add 20 mL of extraction solution (10 mM NH_4OAc, 0.4 mM EDTA, 0.5% NaCl and 2% trichloroacetic acid in water), vortex for 1 min and shake for 10 mins. After shaking, centrifuge for 10 mins at 4000rpm, and deacant the supernatant into a clean tube taking care not to transfer any of the tissue.

Repeat the extraction procedure and combine the two extracts from each sample. Filter using a glass fibre filter.

Adjust the sample pH to 6.5+/-0.25 using 30% NaOH, 1 N NaOH and 1 N HCl.

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Solid Phase Extraction

Plate Conditioning: Condition each column with methanol (10 mL)

Plate Equilibration: Equilibrate each column with water (10 mL)

Sample loading: Load pre-treated sample (40 mL) using vacuum or positive pressure

Interference Wash : Elute interferences with water adjusted to pH 6.5 (10 mL)

Elution: Elute analytes with 25% formic acid in water (6 mL)

Post Extraction: Add HPLC grade water to the extract to give a total volume of 10 mL, and filter through a 0.2 µm disc

UPLC Conditions

Instrument: Waters Acquity UPLC

Column: Waters Acquity UPLC BEH Amide 1.7 µm 2.1 x 50mm

Mobile Phase: **A:** 20 mM heptafluorobutyric acid (HFBA) in H2O/acetonitrile (95/5, v/v)
B: 20 mM HFBA in acetonitrile

Flow Rate: 0.2 mL/min

Table 1. Gradient Conditions

Time (min)	Flow rate (mL/min)	% A	% B
Initial	0.2	100	0
0.5	0.2	80	20
1	0.2	80	20
2	0.2	60	40
2.05	0.2	10	90
2.5	0.2	10	90
2.55	0.2	100	0
3	0.2	100	0
3.05	0	100	0

Injection Volume: 7.5 µL

Sample Temperature: 10 °C

Column Temperature 40 °C

Mass Spectrometry Conditions

Instrument: Xevo TQD Triple Quad Mass Spec equipped with an electrospray ionization source operated in positive ion mode. The compound selective MRM transitions are detailed in **Table 3**.

Desolvation Temperature: 350 °C

Ion Source Temperature: 150 °C

Collision Cell Pressure: 3.4 x 10^{-3} mbar

Positive ions acquired in the multiple reaction monitoring (MRM) mode:

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Table 2. MRM transitions for selected analytes

Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
C1 Gentamicin	479>157,160,322	40	25
C1a Gentamicin	450>112,160,322	35	25
C2+C2a Gentamicin	464>160,163,322	35	20
Streptomycin	582>176,246,263	70	32
Spectinomycin	351>98,140,333	40	20
Neomycin	615>160,163,293	52	30

Results

A summary of the performance for this method is given in Table 3. The observed linearity for each analyte over the concentration range of interest was $r^2 > 0.990$. The reference range defined by USDA-FSIS guidelines was 100–500 ppb.

Table 3. Method performance criteria

Analyte	Linearity	LOQ (ppb)	LOQ spec
Gentamicin	0.9915	33	100
Streptomycin	0.9986	382	500
Spectinomycin	0.9959	75	100
Neomycin	0.9993	361	500

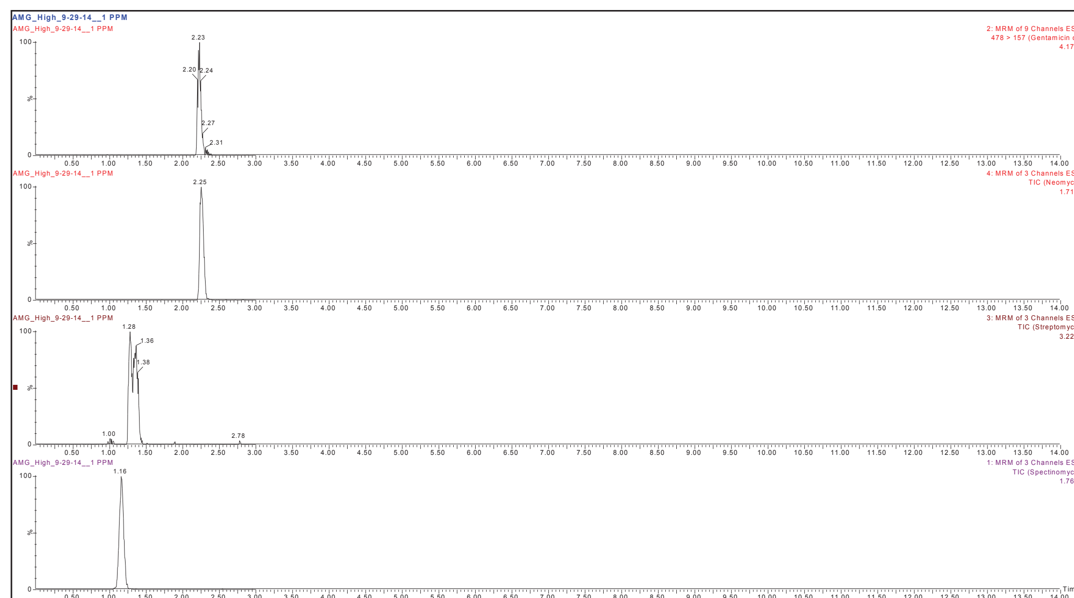

Figure 2. Representative chromatogram of a fortified meat specimen processed using EVOLUTE® WCX

Recovery

Relative recoveries of the selected analyte from fortified specimens were determined at 3 concentration levels. The results are given in **Figure 3**.

Streptomycin and neomycin: Level 1 =400 ppb; Level 2 = 1000 ppb; Level 3 = 10000 ppb

Gentamicin and spectinomycin: Level 1 =50 ppb; Level 2 = 500 ppb; Level 3 = 1000 ppb

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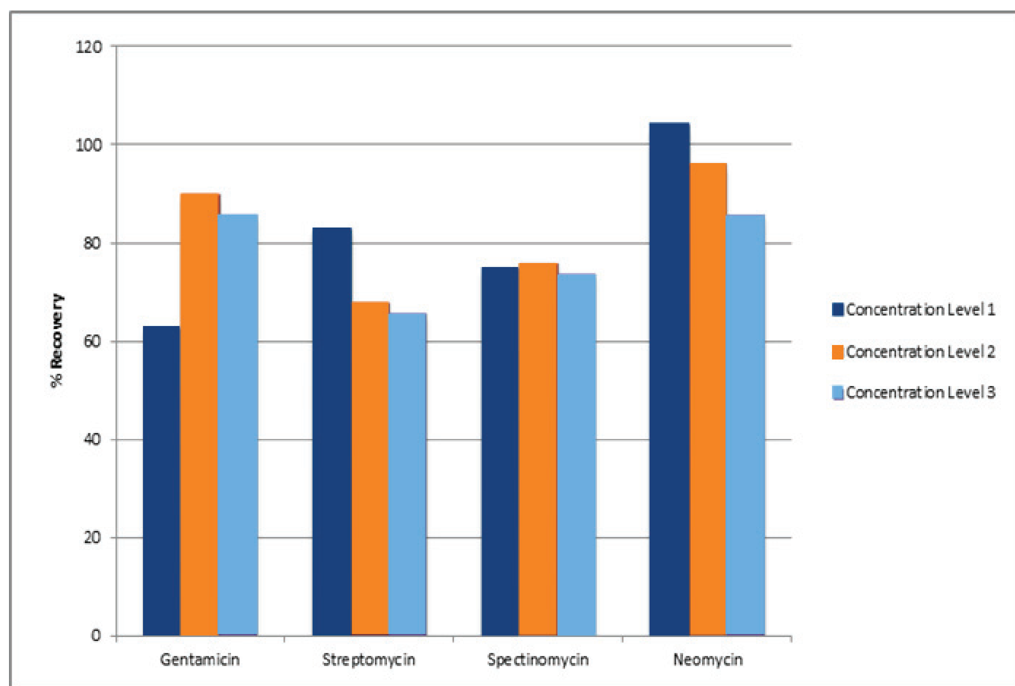

Figure 3. Relative recovery from fortified samples

Conclusions

This method, utilizing EVOLUTE WCX SPE columns was demonstrated as a viable option for residue measurements over a relevant concentration range in food safety laboratory applications.

Additional Notes

1. Buffer Preparation. Aminoglycoside extraction solvent mixture (10mM NH_4OAc, 0.4 mM EDTA, 0.5% NaCl and 2% TCA in water): Add 1.54 g of NH_4OAc to 1.95 L of water. Adjust the pH of the solution to 4.0 with 1N HCl and/or 1 N NaOH. Add 0.3 g $Na_2EDTA.2H_2O$, 10 g of NaCl and 40 g TCA. Mix to ensure the salts dissolve and adjust final volume to 2 L with pure water.
2. Processing Conditions. Samples were loaded on the SPE cartridge at a flow rate of 2 mL/min.

Ordering Information

Part Number	Description	Quantity
612-0050-C	EVOLUTE® WCX 500 mg/6 mL SPE columns	30
PPM-48	Biotage® Positive Pressure Manifold 48 Position	1

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