A fast effective sample cleanup method for applications related to food safety surveillance in the screening of commercial meat products for aminoglycoside residues

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Introduction

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. Of particular interest to this investigation are four aminoglycosides: gentamicin, streptomycin, spectinomycin, and neomycin. Strategies to target these compounds have been standardized by FDA method CLG-AMG2.06, "Screening and Confirmation for Aminoglycosides by LC-MS-MS". This report details current sample preparation strategies developed in a collaborative effort with Biotage and Tyson Foods, Inc. Springdale Corporate Laboratory to optimize analyte recovery (>70%), minimize analyte suppression and maintain acceptable method precision. Samples were processed under positive pressure using a manifold (Biotage PPM48), extracted using a polymeric weak cation exchange sorbent chemistry (EVOLUTE WCX)). The diluted extracts were analyzed by liquid chromatography – tandem mass spectrometry. Reduced workflow considerations for method implementation will also be presented.

Analytes of Interest

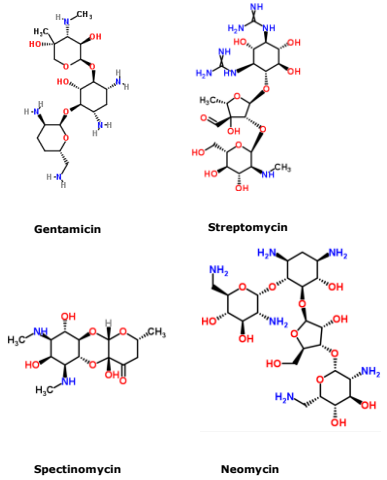

Figure 1: Structure of the analytes of interest

Experimental Procedure

Workflow

5 g of chicken meat samples, as well as 2, 5 g QC samples (aminoglycoside negative chicken meat) were weighed into separate 50 mL conical tubes. One of the QC's was spiked with 10 µg of each of the aminoglycosides tested.[a] To each sample and QC, 20 mL of the extraction solution[b] were added, then vortexed for 1 minute and shaken for 10 minutes. After shaking, the tubes were centrifuged at 4000 rpm for 10 minutes then the supernatant was decanted into a clean tube taking care not to transfer any of the tissue. The extraction procedure was performed one more time and the two extracts from each sample combined. The extractions were filtered through two glass fiber filters. The pH was then adjusted to 6.5 ± 0.25 using 30% NaOH, 1 N NaOH and 1 N HCl. Sample cleanup was performed using Biotage EVOLUTE WCX SPE cartridges. Table 1 shows the optimized SPE parameters. After eluting the analyte from the cartridge, the final volume was brought up to 10 mL using HPLC grade water. Samples were filtered with a 0.20 micron disc filter into a HPLC auto sampler vial. LC-MS/MS standards were made using reagent blank.[c]

a: 100 µl of a 100 ppm aminoglycoside solution
b: 10 mM NH_4OAc, 0.4 mM EDTA, 0.5% NaCl and 2% TCA in water
c: 40 mL of extraction solution run through the produre starting from the filtration step

Reagents

HPLC grade water was obtained via a Millipore Direct-Q water system, HPLC grade methanol, HPLC grade acetonitrile, hydrochloric acid, ammonium acetate, ethylenediamine tetraacetic acid disodium salt dihydrate, sodium hydroxide and sodium chloride were purchased from Fischer Scientific. Gentamicin, streptomycin, spectinomycin, neomycin, heptafluorobutyric acid (HFBA) and trichloracetic acid (TCA) were purchased from Sigma-Aldrich. Formic acid (FA) was purchased from ACROS organics.

Solid Phase Extraction for analyte enrichment and sample cleanup:

Biotage EVOLUTE WCX Solid Phase Extraction Columns (612-0050-C), 500 mg / 6 mL.

Table 1. *Optimized EVOLUTE WCX Procedure*

Step	Details
Sample	5 g chicken tissue
Column Condition/Equilibration	10 mL MeOH followed by 10 mL H2O
Sample Load	40 mL of extraction solution at pH 6.5
Interference Wash 1	Pure H2O at pH 6.5
Analyte Elution	25% FA in H2O

HPLC Conditions – gradient parameters detailed in Table 2

Instrument: Waters Acquity UPLC Xevo TQD Triple Quad Mass Spec

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

Mobile Phase: Mobile phase A (20 mM HFBA in 95/5 H2O/MeCN)
Mobile phase B (20 mM HFBA in MeCN)

Flow Rate: 0.2 mL/min

Injection Volume: 7.5 µL

Autosampler Temperature: 10° C

Column Temp: 40° C

Table 2 **:** *Gradient LC parameters*

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

Mass Spectrometry

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.

Table 3: *CAP MRM transitions for selected analytes*

Analyte	MRM transitions
Gentamicin **C1Gentamicin** **C1aGentamicin C2+C2a**	479>157,160,322 450>112,160,322 464>160,163,322
Streptomycin	582>176,246,263
Spectinomycin	351>98,140,333
Neomycin	615>160,163,293

Results

A summary of the performance for this method is given in Table 4. 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.

A representative chromatogram for the analytes of interest extracted from fortified specimens is detailed in Figure 2. The spiked concentration was 1000 ppb for streptomycin and neomycin, 500 ppb for gentamicin and spectinomycin.

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

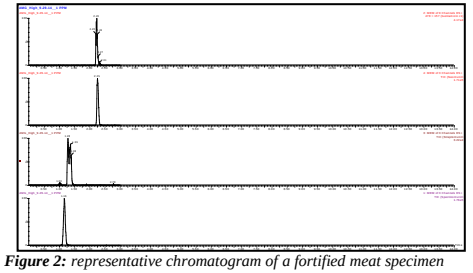

Figure 2: *representative chromatogram of a fortified meat specimen processed using EVOLUTE WCX*

Table 4: *Method performance determined*

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**

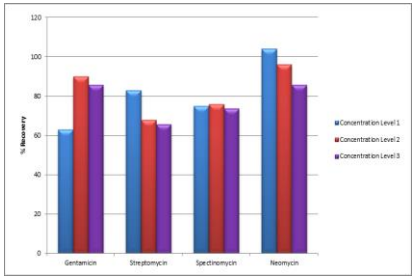

Figure 3: *relative recovery from fortified samples*

Conclusions: The EVOLUTE WCX cartridge format was demonstrated as a viable option for residue measurements over a relevant concentration range in food safety laboratory applications.