Aminoglycosides in Bovine Muscle Using Agilent Bond Elut Plexa SPE, an Agilent Poroshell 120 Column, and LC/Tandem MS

Application Note

Food Testing & Agriculture

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Abstract

A method for the simultaneous determination of aminoglycoside residues of spectinomycin, hygromycin B, streptomycin, dihydrostreptomycin, amikacin, kanamycin, apramycin, tobramycin, gentamicin, and neomycin in bovine muscle was developed and validated. The analytes are extracted and cleaned with solid phase extraction and quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry operating in the positive ion multiple-reaction-monitoring mode. The method provides the ng/g level of limit of detection for all aminoglycoside residues in bovine muscle. The dynamic calibration ranges for these compounds were obtained from 10 to 500 ng/g. The overall recoveries ranged from 71 to 98%, with RSD values between 1.4 and 11.2%.

Introduction

Aminoglycosides (AGs) are a class of broad-spectrum antibiotics that have bacterial activity against some aerobic gram-positive and gram-negative organisms. AGs have been extensively employed in animal husbandry for the treatment of bacterial infections or growth promotion. Due to their toxicity and possible antibiotic resistance, considerable attention has been paid to the potential human health risk. The European Union (EU), China, USA, Japan, and other countries have issued strict maximum residue levels (MRLs) for AGs in various animal-origin foods [1, 2].

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The objective of this application note was to develop a multiresidue method that would be simple and fast for routine regulatory analysis of aminoglycoside residues in animal tissue. The method relies on a simple SPE step using a polymer sorbent (Agilent Bond Elut Plexa). Table 1 shows details of the β-agonists.

Experimental

Reagents and chemicals

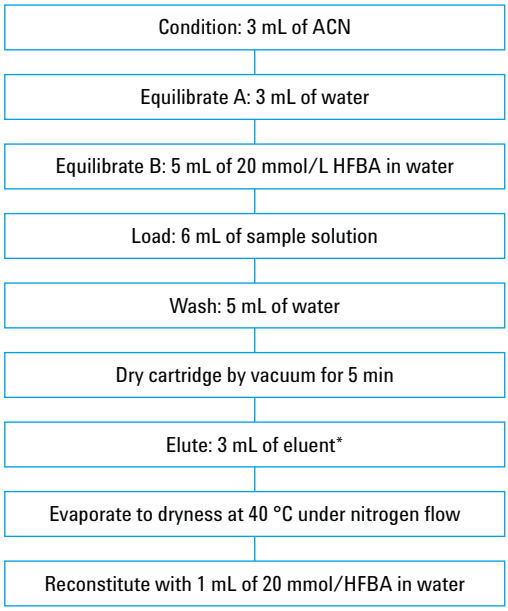
All reagents were MS, HPLC, or analytical grade. Acetonitrile and water were from Honeywell. The standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Bovine muscle was purchased from a local supermarket. Standard solutions (1.0 mg/mL) were made in water individually and stored in a freezer at 4 °C. A combined working solution (10 µg/mL) was made in acetonitrile:water (10:90) and also stored at 4 °C. The spiked solutions were then made weaker by appropriately diluting of the combined working solution in water.

Sample preparation

Bovine muscle (500 g) was minced using a kitchen homogenizer and stored at -20 °C. A 5 g aliquot of homogenized meat was weighed into a 50 mL capped polypropylene tube, and 10 mL of 5% trichloroacetic acid (TCA) in water was added. The mixture was homogenized thoroughly for 1 minute with an Ultra-Turrax T-18 homogenizer (IKA-Labortechnik, Staufen, Germany) and then centrifuged at 4,000 rpm for 5 minutes. The supernatant was transferred to another tube. The same extraction procedure was repeated with 10 mL of 5% TCA in water, and the supernatant was combined into the same tube. A 5 mL volume of 0.2 mol/L heptafluorobutyric acid (HFBA) in water was added to the extracts. After vortex mixing for 1 minute and centrifugation at 4,000 rpm for 5 minutes, the supernatant was adjusted to pH 4.0 with 5% ammonia water. The sample extraction solution was made up to 30 mL with water to be ready for the SPE procedure.

Solid phase extraction

Figure 1 shows the SPE procedure. Bond Elut Plexa cartridges were preconditioned with 3 mL of acetonitrile (ACN) and then equilibrated with 3 mL of water and 5 mL of 20 mmol/L HFBA in water. The 6 mL sample solution was then loaded onto a cartridge and passed through by gravity (approximately 1 mL/min). The cartridges were washed with 5 mL of water. A full vacuum was applied to the cartridge for 5 minutes to completely dry the resin. The compounds were eluted with 3 mL ACN:0.2 mol/L HFBA in water at a rate of 1 mL/min. The eluent was dried under nitrogen at 40 °C. The residue was reconstituted in 1 mL of 20 mmol/L HFBA in water. The sample was then vortex mixed and ultrasonicated to completely dissolve the residue and filtered through a 0.22-µm membrane. Then the sample was finally transferred to a 2 mL chromatography vial for analysis.



* Eluent ACN:0.2 mol/L HFBA in water (8:2).

Figure 1. Bovine muscle clean-up and enrichment – SPE procedure.

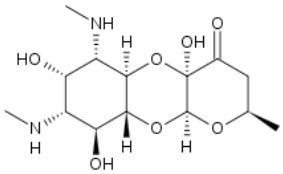
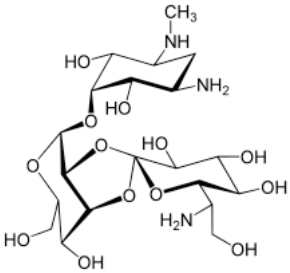
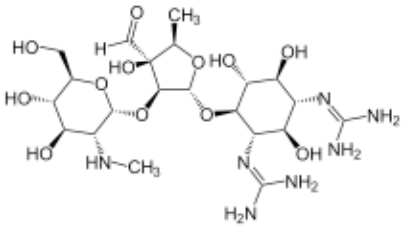
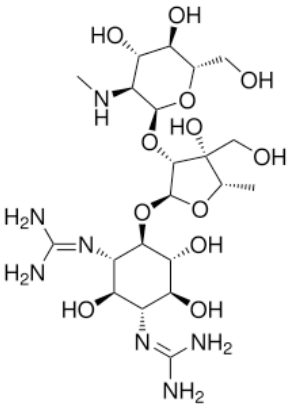
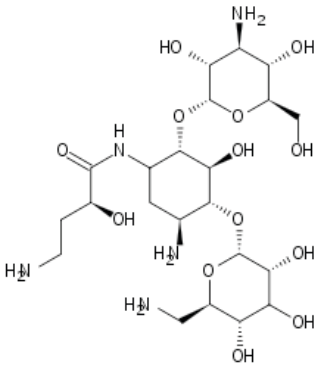
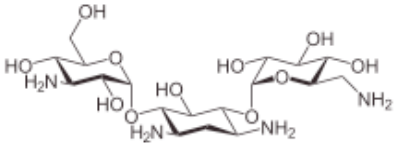
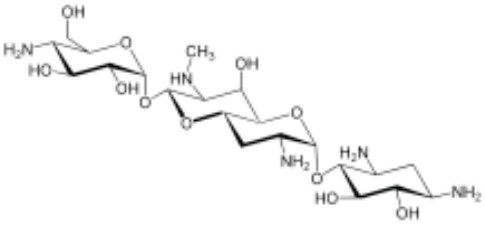
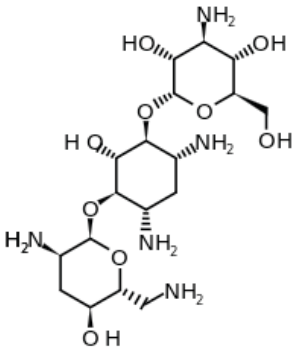
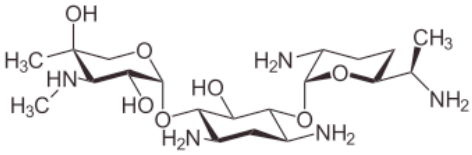
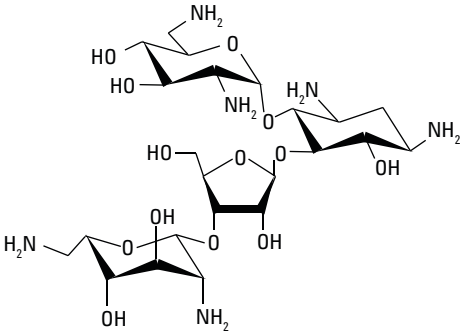
Compound	CAS No.	Log P	Structure
Spectinomycin	1695-77-8	-2.3	
Hygromycin B	31282-04-9	NA	
Streptomycin	57-92-1	-6.4	
Dihydrostreptomycin	128-46-1	NA	
Amikacin	37517-28-5	-7.4	
Kanamycin	59-01-8	-6.3	
Apramycin	37321-09-8	-6.5	
Tobramycin	32986-56-4	-5.8	
Gentamicin	1403-66-3	NA	
Neomycin	1404-04-2	-7.8	

Table 1. Aminoglycoside compounds used in this study.

Conditions

Column:	Agilent Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 µm (p/n 685775-902)		
Sample prep:	Agilent Bond Elut Plexa cartridges, 500 mg, 6 mL (p/n 12259506)		
Mobile phase:	A: Water:acetonitrile (950:50, 20 mmol/L HFBA), B: acetonitrile:water (800:200, 20 mmol/L HFBA)		
Injection volume:	20 µL		
Flow rate:	0.3 mL/min		
Gradient:	Time (min)	%A	%B
	0	85	15
	3	85	15
	9.5	25	75
	9.55	85	15
	10	85	15
Temperature:	Ambient		
Manifold:	Agilent Vac Elut 20 Manifold (p/n 12234101)		
Instrument:	Agilent 1200 Infinity Series LC System Agilent 6460 Triple Quadrupole LC/MS/MS System		

MS conditions

The AGs were monitored in positive mode. Table 2 shows the multiple-reaction-monitoring details.

MS source parameters

Gas temp:	350 °C
Gas flow:	5 L/min
Nebulizer:	45 psi
Sheath gas temp:	400 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	Positive, 0 V
Capillary:	Positive, 3,500 V

Compound	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)	Retention time (min)
Spectinomycin	351.2	333.2	170	15	4.64
		207.1	170	18	
Hygromycin B	528.3	177.1	170	25	6.77
		352	170	20	
Streptomycin	582.4	263.2	180	30	6.98
		245.8	180	35	
Dihydrostreptomycin	584.4	263.3	180	30	7.06
		246.2	180	40	
Amikacin	586.4	163.1	170	30	7.68
		425.2	170	15	
Kanamycin	485.3	163.1	150	20	7.8
		324.2	150	10	
Apramycin	540.3	217.1	140	25	8.32
		378.2	140	12	
Tobramycin	468.3	163.2	125	20	8.42
		324.2	125	8	
Gentamicin	478.3	322.3	125	8	8.64
		157.2	125	15	
Neomycin	615.3	161.1	175	30	8.74
		293.1	175	20	

Table 2. Masses monitored by multiple-reaction monitoring.

Results and Discussion

Linearity and limit of detection

Solutions used to create external calibration curves were prepared by using a combined working solution to spike matrix blank (10, 20, 50, 100, and 500 ng/g). Matrix blanks were created by taking bovine muscle through the entire procedure, including pretreatment and SPE procedures. The limits of detection (LOD) were chosen as the concentration of each compound that gave a signal-to-noise (S/N) ratio greater than 3:1. The results for the calibration curves and LODs are shown in Table 3.

Compound	Regression equation	R^2	LOD in muscle (ng/g)
Spectinomycin	Y=293.4698x-325.2314	0.9998	2
Hygromycin B	Y=270.2367x-424.6557	0.9999	0.5
Streptomycin	Y=28.7892x+10.1849	0.9999	5
Dihydrostreptomycin	Y=458.6225x-1320.7826	0.9999	0.1
Amikacin	Y=572.3138x-923.7852	0.9999	0.2
Kanamycin	Y=508.1929x-905.1314	0.9998	0.5
Apramycin	Y=239.2452x-646.2071	0.9999	0.5
Tobramycin	Y=696.8031x-1922.6636	0.9999	0.1
Gentamicin	Y=1076.2438x-3690.8511	0.9996	0.1
Neomycin	Y=196.7006x-534.5063	0.9997	2

Table 3. Linearity and LODs of aminoglycosides in bovine muscle.

Recovery and reproducibility

The recovery and repeatability for the method were determined at 3 levels; beef spiked to concentrations of 20, 100, and 500 ng/g. The analysis was performed with 6 replicates at each level. Table 4 shows the recovery and reproducibility data. Figure 2 shows the chromatograms of spiked bovine muscle extracts (20 ng/g).

Compound	Spiked level (ng/g)	Recovery (%)	RSD (n=6, %)
Spectinomycin	20	87.7	2.1
	100	79.7	2.4
	500	91.2	3.2
Hydromycin B	20	75.9	3.9
	100	82.1	3.4
	500	85.6	4.0
Streptomycin	20	71.5	11.2
	100	80.0	9.4
	500	74.5	8.0
Dihydrostreptomycin	20	89.1	4.5
	100	91.2	2.3
	500	93.3	3.6
Amikacin	20	85.9	1.8
	100	90.1	2.4
	500	96.5	3.8
Kanamycin	20	86.7	1.4
	100	90.0	2.2
	500	97.6	2.8
Apramycin	20	84.6	4.9
	100	87.6	3.1
	500	95.4	5.4
Tobramycin	20	89.3	4.4
	100	88.1	3.4
	500	97.7	5.8
Gentamicin	20	82.4	3.5
	100	81.2	4.6
	500	95.8	6.8
Neomycin	20	72.1	2.6
	100	82.8	5.6
	500	90.3	5.5

Table 4. Recoveries and reproducibility of aminoglycosides in bovine muscle.

Conclusions

LC/MS/MS was a reliable and powerful technique for the simultaneous quantification and confirmation of aminoglycosides in bovine muscle. Moreover, the result of this study shows that Agilent Bond Elut Plexa can be used as an effective method for purification and enrichment of multiple aminoglycosides in a complex matrix, such as bovine muscle. The recovery and reproducibility results based on matrix spiked standards were acceptable for aminoglycoside residue determination in bovine muscle under international regulations. The impurities and matrix effect were minimal and did not interfere with the quantification of any target compound. The limits of quantitation were significantly lower than the MRLs [3].

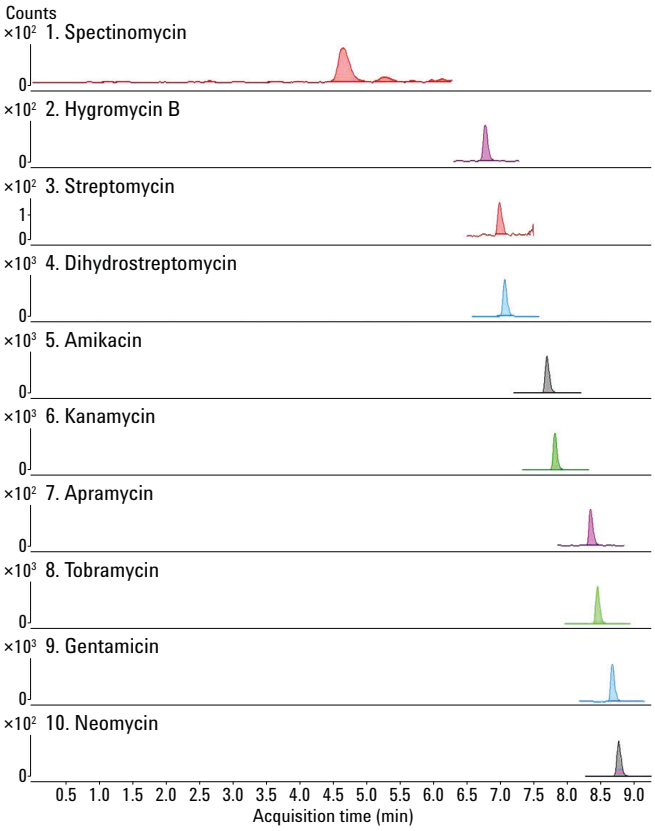


Figure 2. Chromatograms of 20 ng/g spiked bovine muscle sample extract.

References

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2. A. Kaufmann, P. Butcher, K. Maden. *Anal. Chim. Acta* 711, 46 (2012).

3. Anon. GB/T 21323-2007 Determination of aminoglycosides residues in animal tissues - HPLC-MS/MS method. China Standard. General Administration of Quality Supervision, Inspection and Quarantine, Beijing, China.

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Printed in the USA
October 25, 2012
5991-1321EN

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