SHIMADZU

Determination of Aminoglycoside Drug Residue in Honey by LC-MS/MS

Dan Luo[1], Dominika Gruszecka[2]
(1) Shimadzu (China) Co.,LTD; (2) Shimadzu Scientific Instruments, USA.

1. Introduction

Aminoglycosides (AGs) are glycoglycans and aminocyclic alcohols with glycoside bonds. They inhibit bacterial protein synthesis, altering cell wall permeability and exerting antibacterial effects. Streptomycin, as an example, is shown in Figure 1. Recent reports highlight their ototoxicity, nephrotoxicity, and vestibular damage, which can cause shock or death in severe cases. GB 31650-2019 “Maximum Residue Limits of Veterinary Drugs in Food” sets residue limits for gentamicin kanamycin, spectinomycin, streptomycin, dihydrostreptomycin, and neomycin B in various matrices. This work presents a method to detect aminoglycoside residues in honey, involving sample purification with MCX and WCX SPE cartridges. This approach, applicable to antibiotics used in livestock, does not require ion-pairing reagents or high-concentration salt solutions, ensuring accurate detection of residues in honey.

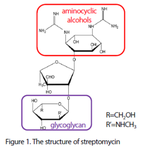

Figure 1. The structure of streptomycin

2. Sample Preparation

Extraction: 5 g of the sample was extracted in 10mL of phosphate buffer. Supernatant was transfered after vortexing, sonication and centrifuging to a new centrifuge tube with 5 mL phosphate buffer and the extraction was repeated, the supernatants were combined, and the volume was adjusted to 20 mL.

Purification: Prepared solution was divided into two aliquots, one passed through the MCX SPE cartridge, rinsed with 7.5 mL water and 7.5 mL methanol, and eluted with 5 mL of ammonia-methanol solution for the analysis of neomycin, kanamycin, apramycin, spectinomycin, hygromycin and tobramycin.

The other solution passed through WCX SPE cartridge after adjusting the pH to 7.5 with sodium hydroxide solution, then washed with 7.5 mL of water, and eluted with 5 mL of methanol-acetate for the analysis of streptomycin, dihydrostreptomycin and gentamicin.

The two parts of the eluate were dried at 40℃ with nitrogen atmosphere, dissolved with 2 mL of 0.3% acetic acid water-HFBA (99:1), filtered through a 0.22 μm membrane, and placed in a plastic vial for LC-MS/MS analysis.

Table 1 Analysis Conditions of Nexera™ and LCMS-8050

System	:	Nexera LC-40 X3
Column	:	Shim-pack Scepter™ C8-120 (100 mm×2.1 mm I.D, 1.9 μm)*1
Temperature	:	35 ˚C
Injection volume	:	5 μL
Mobile phases	:	A-0.5 mM Ammonium acetate+ 0.1 % FA in Water B-Acetonitrile
Flow rate	:	0.3 ml/min
Time program (%B)	:	5 % (0-2 min) → 40 % (6 min) →90 (6.5-7.5 min) →5 % (7.51-12 min)
System	:	LCMS-8050 (ESI Positive)
Nebulizing gas	:	3 L/min
Drying gas	:	10 L/min
Heating gas	:	10 L/min
DL temp	:	150 ˚C
Heat block temp	:	400 ˚C
Interface temp	:	300 ˚C

3. Results

The standard sample was added to the blank matrix sample residue, after dried with nitrogen atmosphere, 2 mL of 0.3% acetic acid water-HFBA (99:1) was added.

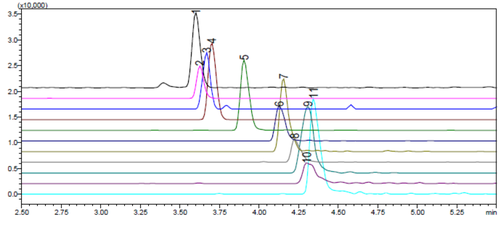

Fig. 2 MRM chromatogram of 5 ng/mL aminoglycosides (1. Spectinomycin; 2. Hygromycin B; 3. Streptomycin; 4. Dihydrostreptomycin; 5. Kanamycin A; 6. Apramycin; 7. Tobramycin; 8. Gentamicin C1a; 9. Gentamicin C2+C2a; 10. Neomycin B; 11. Gentamicin C1)

Table 3 Calibration Curves of aminoglycosides

No.	Compound	Calibration curve	R^2	Accuracy%
1	Spectinomycin	Y = (11938.8)X + (-6242.93)	0.9979	89.0~111.3
2	Hygromycin B	Y = (2599.09)X + (-2808.64)	0.9978	79.7~118.1
3	Streptomycin	Y = (436.278)X + (-792.476)	0.9973	75.9~114.7
4	Dihydrostreptomycin	Y = (6756.75)X + (-10278.3)	0.9978	86.8~119.1
5	Kanamycin	Y = (12765.4)X + (3397.99)	0.9970	82.9~115.7
6	Apramycin	Y = (7089.01)X + (1225.37)	0.9976	87.5~114.0
7	Tobramycin	Y = (7661.69)X + (-5991.29)	0.9989	83.3~121.3
8	Gentamicin C1a	Y = (6271.04)X + (-13474.4)	0.9980	83.1~107.4
9	Gentamicin C2+C2a	Y = (8137.03)X + (-25856.9)	0.9964	76.3~119.6
10	Neomycin B	Y = (2871.40)X + (-8437.75)	0.9960	77.6~121.4
11	Gentamicin C1	Y = (9089.73)X + (-31827.3)	0.9975	81.6~115.6

Table 4 LOD and LOQ of aminoglycosides

No.	Compound	LOD (ng/mL)	LOQ (ng/mL)
1	Spectinomycin	0.35	1.06
2	Hygromycin B	0.07	0.22
3	Streptomycin	0.90	2.74
4	Dihydrostreptomycin	0.14	0.44
5	Kanamycin A	0.05	0.15
6	Apramycin	0.04	0.12
7	Tobramycin	0.06	0.20
8	Gentamicin C1a	0.20	0.61
9	Gentamicin C2+C2a	0.15	0.46
10	Neomycin B	0.55	1.68
11	Gentamicin C1	0.09	0.28

Table 5 The recoveries of aminoglycosides (n=3)

No.	Compound	25 μg/kg		50 μg/kg	
		Rec.%	RSD%	Rec.%	RSD%
1	Spectinomycin	88.4	3.59	93.4	2.90
2	Hygromycin B	83.7	4.91	86.8	3.74
3	Streptomycin	78.4	7.22	77.9	6.75
4	Dihydrostreptomycin	74.8	6.48	74.1	7.54
5	Kanamycin A	77.8	3.92	83.0	3.54
6	Apramycin	74.4	7.34	79.4	4.12
7	Tobramycin	79.6	7.38	85.7	8.23
8	Gentamicin C1a	69.3	5.66	68.9	3.22
9	Gentamicin C2+C2a	76.2	7.59	80.1	8.13
10	Neomycin B	70.4	9.47	68.9	6.50
11	Gentamicin C1	68.5	8.34	73.6	5.29

Matrix matched external standard calibration was prepared using the standard sample showing good linearity and wide dynamic range from 5 to 500 ng/mL with at R^2>0.996. The accuracy at each calibration point ranged from 75.9~121.4%, shown in Table 3.

Using standard data of 5 ng/mL, the LOD and LOQ of aminoglycosides were calculated with signal-to-noise ratio of 3 and 10, respectively, and the results are shown in Table 4.

The reproducibility of the method was tested by 6 consecutive measurements of matrix standards at 5, 50, and 500 μL/L. The RSD of the retention time were below 0.5% and of peak area below 10% except for Neomycin B at 5 μL/L.

Recovery was tested with spiked 5g honey samples to make the at concentrations of 25 and 50 μg/kg, shown in Table 5.

4. Conclusion

Good linearity in the range of 5 ng/mL to 500 ng/mL, with R^2>0.996 was shown. The recoveries of the samples spiked at 25 and 50 μg/kg ranged from 68.5 to 93.4%. This method only adds ion-pairing reagents to the vials, and analytes were well retained on the C8 column, and with this is sensitive, accurate, and can be used for the determination of aminoglycoside drug residues in honey.