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Liquid Chromatography Mass Spectrometry LCMS-8050

Application News

Determination of Aminoglycoside Drugs Residual in Bee Products by LC-MS/MS

Dan Luo
Shimadzu (China) Co.,LTD

User Benefits

- Heptafluorobutyric acid is added to the injection vial to enhance the retention of aminoglycosides
- There is no need to use ion-pairing reagents and highly concentrated salt solutions in the mobile phase, which might inhibit the mass spectrometry signal

■ Introduction

Aminoglycosides (AGs) are composed of glycoglycans and aminocyclic alcohols combined with glycoside bonds. Figure 1 shows the structure of streptomycin as an example of an aminoglycoside. Their main role is to hinder the protein synthesis of bacteria, so the permeability of bacterial cell walls changes, which exerts antibacterial effects. In recent years, it has been reported that AGs have significant ototoxicity, nephrotoxicity, and vestibular function damage, which can lead to shock and even death in severe cases. GB 31650-2019 "Maximum Residue Limits of Veterinary Drugs in Food" stipulates residue limits of gentamicin, kanamycin, spectinomycin, streptomycin, dihydrostreptomycin, and neomycin B in different matrices.

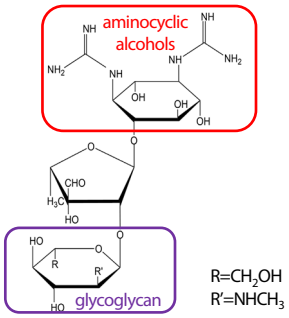

Figure 1. The structure of streptomycin

In this paper, a method for the detection of aminoglycoside residues in honey was established. The extract was divided into two equal parts and purified by MCX and WCX SPE cartridges, respectively. This method covers AGs commonly used in the livestock and poultry industry. Ion-pairing reagents and high-concentration salt solutions are not needed in the mobile phase, and the results are accurate and reliable, and can effectively detect the residues of aminoglycosides in bee products.

■ Sample Preparation

Extraction:

Weigh 5 g of the sample in a 50 mL centrifuge tube, add 10 mL of phosphate buffer (containing 5% trichloroacetic acid and 0.4 mM disodium ethylenediaminetetraacetic acid), vortex for 1 min, sonicate for 5 min and centrifuge at 8000 r/min at 4℃ for 10 min. The supernatant was transferred to another centrifuge tube, 5 mL phosphate buffer was added to the residue, and the extraction was repeated. The supernatants were combined, and the volume was adjusted to 20 mL with phosphate buffer for later use.

Purification:

The MCX SPE column (200 mg/6 mL) and WCX SPE column (150 mg/3 mL) were activated with 5 mL of methanol and 5 mL of water, respectively. The prepared solution was divided into two aliquots, one passed through the MCX SPE cartridge, then rinsed with 7.5 mL of water and 7.5 mL of 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.

■ Analysis Condition

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

*1 P/N：227-31033-05

Table 2 MRM Transition

No.	Compound	Precursor m/z	Product m/z	Q1 Pre Bais(V)	CE (V)	Q3 Pre Bais(V)
1	Gentamicin C1a	450.3	322.4*	-17.0	-14.0	-16.0
			160.2	-17.0	-23.0	-30.0
2	Gentamicin C2+C2a	464.4	160.0	-11.0	-21.0	-10.0
			322.2*	-17.0	-14.0	-23.0
3	Gentamicin C1	478.4	322.1*	-14.0	-15.0	-22.0
			160.1	-11.0	-21.0	-30.0
4	Neomycin B	615.3	161.2*	-22.0	-29.0	-16.0
			293.1	-20.0	-22.0	-20.0
5	Dihydrostreptomycin	584.3	262.9*	-22.0	-31.0	-12.0
			246.2	-22.0	-38.0	-16.0
6	Spectinomycin	351.2	333.2*	-14.0	-20.0	-23.0
			207.1	-13.0	-24.0	-14.0
7	Streptomycin	582.3	263.1*	-22.0	-33.0	-17.0
			246.1	-20.0	-39.0	-11.0

No.	Compound	Precursor m/z	Product m/z	Q1 Pre Bais(V)	CE (V)	Q3 Pre Bais(V)
8	Tobramycin	468.3	324.2*	-17.0	-17.0	-22.0
			163.2	-17.0	-23.0	-17.0
9	Tetracycline B	528.3	177.2*	-20.0	-27.0	-12.0
			352.1	-20.0	-25.0	-25.0
10	Kanamycin	485.3	163.1*	-12.0	-25.0	-16.0
			324.2	-17.0	-17.0	-15.0
11	Apramycin	540.4	217.2*	-20.0	-27.0	-23.0
			378.2	-20.0	-18.0	-18.0

* Quantitative ions

■ MRM Chromatogram

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. The sample was analyzed according to the analysis conditions in Table 1 and 2. As seen from Figure 2, it can be seen that the aminoglycosides were strongly retained on the C8 column, and there was no obvious interference at the target peak.

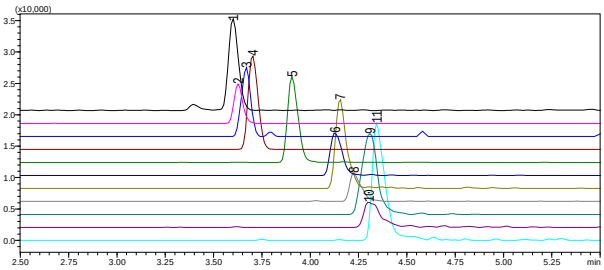

Figure 2. MRM chromatogram of 5 ng/mL aminoglycosides
(1. Spectinomycin; 2. Hygromycin B; 3. Streptomycin; 4. Dihydrostreptomycin; 5. Kanamycin A; 6. Apramycin; 7. Tobramycin; 8. Gentamicin C1a; 9. Gentamicin C2+C2a; 10. Neomycin B; 11. Gentamicin C1)

■ Calibration Curve

As shown from Figure 3, the calibration curve (Matrix matched external standard method) prepared using the standard sample showed good linearity in a wide dynamic range from 5 to 500 ng/mL with a coefficient of determination R^2>0.996. The accuracy at each calibration point ranged from 75.9~121.4%, and specific data are available in Table 3.

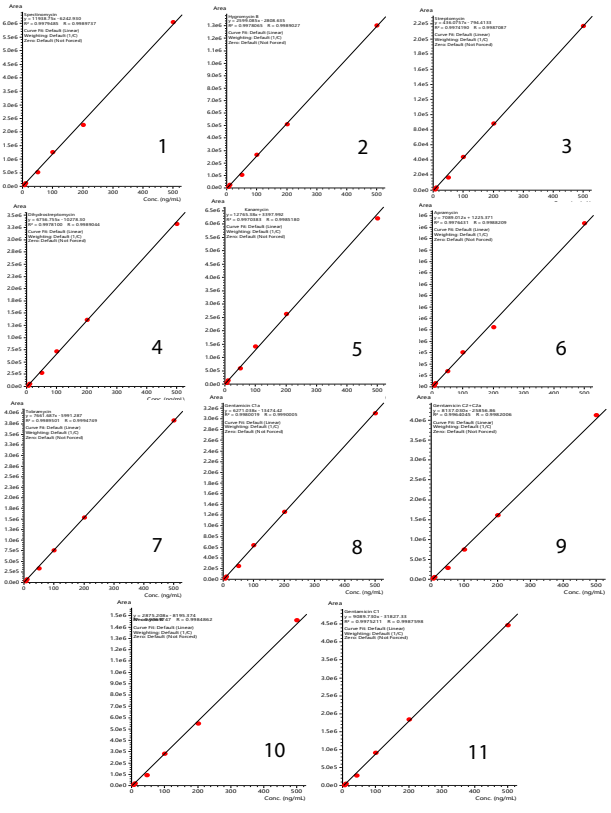

Figure 3. Calibration Curves of aminoglycosides
(1. Spectinomycin; 2. Hygromycin B; 3. Streptomycin; 4. Dihydrostreptomycin; 5. Kanamycin A; 6. Apramycin; 7. Tobramycin; 8. Gentamicin C1a; 9. Gentamicin C2+C2a; 10. Neomycin B; 11. Gentamicin C1)

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

■ Sensitivity

Based on the 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.

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

■ Reproducibility

The reproducibility of the method was tested by 6 consecutive measurements of the matrix standards at concentrations of 5, 50, and 500 µg/L. The relative standard deviations of the retention time and peak area of the analytes are shown in Table 5.

Table 5 RSD% of R.T. and Area

No	Compound	5 ng/mL		50 ng/mL		500 ng/mL	
		R.T.	Area	R.T.	Area	R.T.	Area
1	Spectinomycin	0.37	2.38	0.11	6.24	0.11	2.19
2	Hygromycin B	0.37	5.45	0.11	8.63	0.11	3.07
3	Streptomycin	0.35	9.73	0.13	7.95	0.11	3.10
4	Dihydrostreptomycin	0.40	6.10	0.13	1.66	0.12	2.65
5	Kanamycin A	0.40	3.91	0.12	2.28	0.12	1.55
6	Apramycin	0.39	5.82	0.11	2.21	0.12	1.42
7	Tobramycin	0.39	4.64	0.12	4.36	0.12	3.32
8	Gentamicin C1a	0.34	7.83	0.13	3.39	0.12	1.13
9	Gentamicin C2+C2a	0.46	5.20	0.09	3.22	0.13	1.99
10	Neomycin B	0.40	11.75	0.12	5.17	0.12	1.94
11	Gentamicin C1	0.47	5.43	0.12	6.64	0.12	1.30

■ Recovery

The mixed standard solution was added to 5 g of blank honey samples to make the spiked concentrations of 25 and 50 µg/kg, respectively. The recoveries were determined 3 times in parallel, and the results are showed in Table 6.

Table 6 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

■ Conclusion

A method for the detection of aminoglycoside residues in honey samples was established. This method only adds ion-pairing reagents to the vials, and analytes were well retained on the C8 column. Aminoglycoside drugs have good linearity in the concentration range of 5 ng/mL to 500 ng/mL, with a correlation coefficient R^2>0.996. The recoveries of the samples spiked at 25 and 50 µg/kg ranged from 68.5 to 93.4%. This method is sensitive, accurate, and can be used for the determination of aminoglycoside drug residues in bee products.

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Shimadzu Corporation
www.shimadzu.com/an/

Shimadzu (China) Co., Ltd
www.shimadzu.com.cn