Macrolides in Honey Using Agilent Bond Elut Plexa SPE, Poroshell 120, and LC/MS/MS

Application Note

Food Testing and Agriculture

Author

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Abstract

A method for the simultaneous determination of macrolide residues of spiramycin, tilmicosin, oleandomycin, erythromycin, tylosin, leucomycin, roxithromycin, and josamycin in honey was developed and validated. The analytes were extracted and cleaned with Agilent Bond Elut Plexa solid phase extraction (SPE), separated on an Agilent Poroshell 120 HPLC column and quantified by liquid chromatography coupled to electrospray-ionization tandem-mass-spectrometry (LC/MS/MS) operating in the positive-ion multiple-reaction-monitoring (MRM) mode. The method provided trace level limit of detection for all macrolides in honey. The dynamic calibration ranges for these compounds were obtained from 0.1 to 100 ng/g. The overall recoveries ranged from 67.8% to 106.6%, with RSD values between 1.9% and 10.7%.

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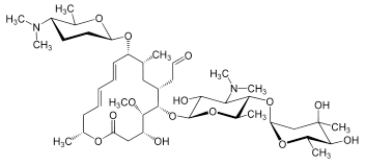
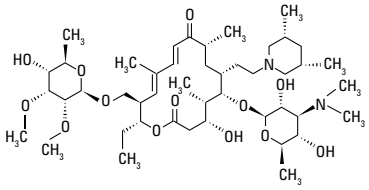
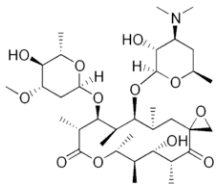
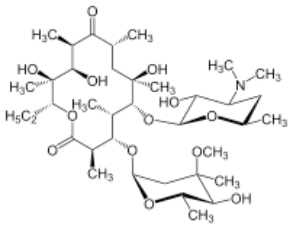
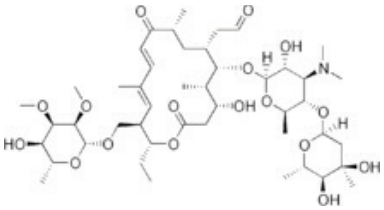
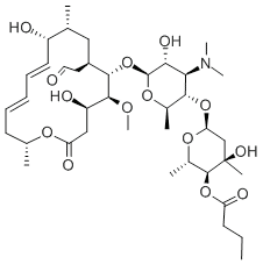
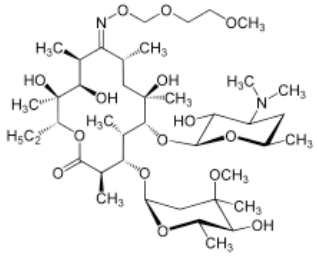
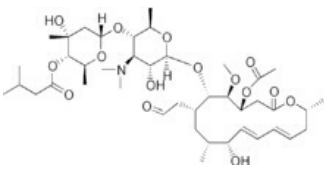
Introduction

The use of antibiotics in food animal production, although beneficial to the food industry, has led to animal and human health safety concerns. Macrolides are a group of antibiotics that have been widely used to treat many respiratory and enteric bacterial infections in humans and animals. Some of the more commonly used macrolides are spiramycin, tilmicosin, oleandomycin, erythromycin, tylosin, leucomycin, roxithromycin, and josamycin.

National agencies and international organizations have set regulatory limits on the concentrations of antibiotic residues in foods of animal origin. Residue limits vary from 0 (no assigned limit) to 15 mg/kg. The objective of this work was to develop a multiresidue method that would be simple and fast for routine regulatory analysis of macrolide residues in milk. The method relies on a simple SPE step using a polymer sorbent (Bond Elut Plexa) and an HPLC column separation using Poroshell 120. Table 1 shows details of the aminoglycosides.

Table 1. Macrolide compounds used in this study.

Compound	CAS No.	Structure
Spiramycin	8025-81-8	
Tilmicosin	108050-54-0	
Oleandomycin	3922-90-5	
Erythromycin	114-07-8	
Tylosin	1401-69-0	
Leucomycin	18361-45-0	
Roxithromycin	80214-83-1	
Josamycin	16846-24-5	

Materials and Methods

Reagents and chemicals

All reagents were MS, HPLC, or analytical grade. Acetonitrile and water were from Honeywell International, Inc. The standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The honey was produced in New Zealand and purchased from a local supermarket. Standard solutions (1.0 mg/mL) were made in methanol individually and stored in a freezer at –20 °C. A combined working solution was made in methanol:water (20:80) and also stored at –20 °C. The spiked solutions were then made daily by appropriately diluting the combined working solution in water.

The extracting buffer was made by dissolving 5.3 g sodium carbonate and 4.2 g sodium biocarbonate in 800 mL water, adjusting to pH 9.3 with sodium hydroxide and diluting to 1,000 mL with water.

Sample preparation

A honey sample (5 g) was weighed into a polypropylene centrifuge tube. Extracting buffer (15 mL) was added to the tube. The mixture was vortexed thoroughly for 1 minute; the sample solution was then ready for the SPE procedure.

Solid phase extraction

SPE cartridges:	Agilent Bond Elut Plexa, 200 mg, 6 mL (p/n 12109206)
Manifold:	Agilent Vac Elut 20 (p/n 12234101)
Filter:	Agilent Captiva syringe filter, PTFE, 0.2 µm (p/n 5190-5082)

The SPE procedure is shown in Figure 1. SPE cartridges were preconditioned with 3 mL methanol and then equilibrated with 3 mL water and 5 mL extracting buffer. The sample solution was then loaded onto a cartridge and passed through under gravity (about 1 mL/min). The cartridges were washed with 5 mL water and 5 mL 20% methanol in water. A 15-mm Hg vacuum was applied to the cartridge for 5 minutes to completely dry the resin. The compounds were eluted with 5 mL methanol 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% methanol in water. The sample was then vortex mixed and ultrasonicated to completely dissolve the residue and filtered through a 0.2-µm Captiva syringe filter. The sample was finally transferred to a 2-mL autosampler vial for analysis.

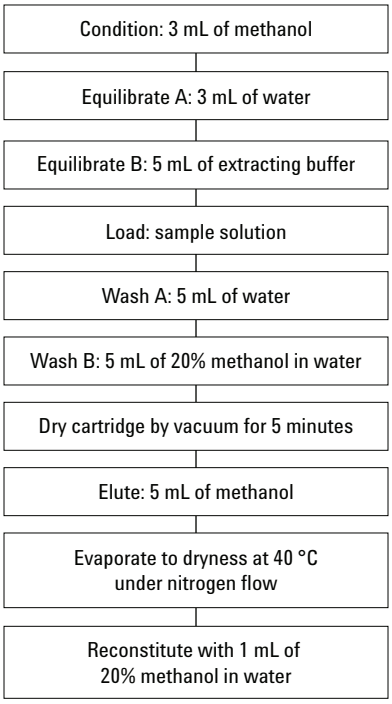


Figure 1. Agilent Bond Elut Plexa SPE procedure for honey cleanup and enrichment.

HPLC conditions

Column:	Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695775-302)
Mobile phase:	A: 0.1% formic acid in water B: acetonitrile
Injection volume:	20 µL
Flow rate:	0.5 mL/min
Gradient:	Time (min) %B 0 20 8 70
Temperature:	Ambient
Instrument:	Agilent 1200 Infinity Series, Agilent 6460 Triple Quadrupole LC/MS system

MS conditions

The macrolides were monitored in positive mode. Table 2 shows the MRM details.

Gas temperature:	300 °C
Gas flow:	5 L/min
Nebulizer:	45 psi
Sheath gas temperature:	400 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	Positive, 0 V
Capillary:	Positive, 4,000 V

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 blanks (0.1, 0.5, 2, 5, and 10 µg/kg). Matrix blanks were created by taking honey through the entire procedure, including pretreatment and SPE procedures. The limits of detection (LODs) were chosen as the concentration of each compound that gave a signal-to-noise ratio greater than 3:1. All of the LODs for the compounds were below 0.1 µg/kg. The results for the calibration curves are shown in Table 3.

Table 2. Masses monitored by MRM.

Compound	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)	Ret Time (min)
Spiramycin	843.4	539.8	270	35	3.70
Spiramycin	843.4	174.1	270	40	3.70
Tilmicosin	869.5	696.4	320	44	4.47
Tilmicosin	869.5	174.1	320	49	4.47
Oleandomycin	688.3	544.3	170	15	4.70
Oleandomycin	688.3	158.2	170	25	4.70
Erythromycin	734.4	576.3	180	14	4.98
Erythromycin	734.4	158.2	180	30	4.98
Tylosin	916.4	772.4	280	30	5.33
Tylosin	916.4	174.2	280	40	5.33
Leucomycin	772.4	174.1	250	30	5.99
Leucomycin	772.4	109.1	250	40	5.99
Roxithromycin	837.4	679.3	200	16	6.19
Roxithromycin	837.4	158.1	200	34	6.19
Josamycin	828.4	174.1	250	35	6.89
Josamycin	828.4	109.1	250	46	6.89

Table 3. Linearity of macrolides in honey.

Compound	Regression equation	R^2
Spiramycin	Y=1090.2137x+2315.6208	0.993
Tilmicosin	Y=752.7225x-190.6976	0.996
Oleandomycin	Y=2347.6506x-2944.8475	0.998
Erythromycin	Y=640.2091x-632.9097	0.998
Tylosin	Y=756.7928x-742.9858	0.999
Leucomycin	Y=1104.8063x-1270.4446	0.998
Roxithromycin	Y=2988.9663x-2707.5511	0.995
Josamycin	Y=925.6328x-1003.9875	0.996

Recovery and reproducibility

The recovery and repeatability for the method were determined at three levels, in honey, spiked to concentrations of 0.1, 0.5, and 2 µg/kg. The analysis was performed with six replicates at each level. The recovery and reproducibility data are shown in Table 4. The chromatograms of spiked honey extracts (0.1 µg/kg) are shown in Figure 2, and the matrix blank in Figure 3.

Table 4. Recoveries and reproducibility of macrolides in honey.

Compound	Spiked level (µg/kg)	Recovery (%)	RSD (%) n = 6
Spiramycin	0.1	67.8	9.2
	0.5	72.5	10.7
	2	77.8	6.8
Tilmicosin	0.1	73.1	7.8
	0.5	70.6	3.6
	2	82.9	7.1
Oleandomycin	0.1	84.2	7.7
	0.5	88.2	8.3
	2	91.5	4.2
Erythromycin	0.1	94.2	5.4
	0.5	92.0	6.6
	2	79.0	3.1
Tylosin	0.1	77.2	9.3
	0.5	99.3	2.6
	2	96.6	2.5
Leucomycin	0.1	82.0	6.8
	0.5	96.8	9.3
	2	101.9	1.9
Roxithromycin	0.1	86.3	6.7
	0.5	85.3	3.6
	2	105.7	7.7
Josamycin	0.1	106.6	7.6
	0.5	100.8	6.2
	2	88.2	4.9

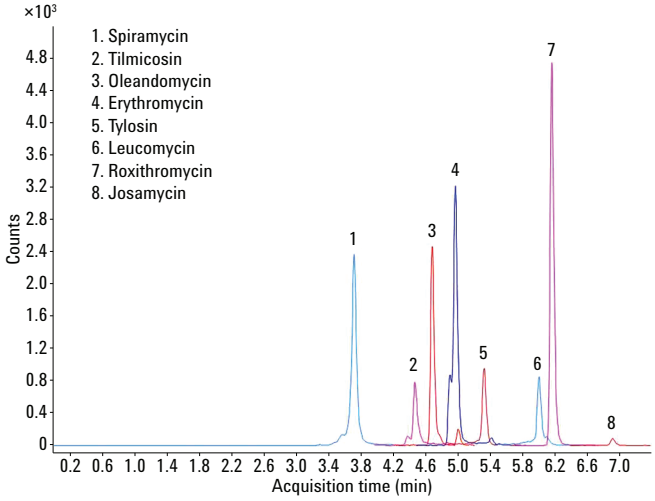


Figure 2. Chromatogram of 0.1 µg/kg spiked honey sample extract.

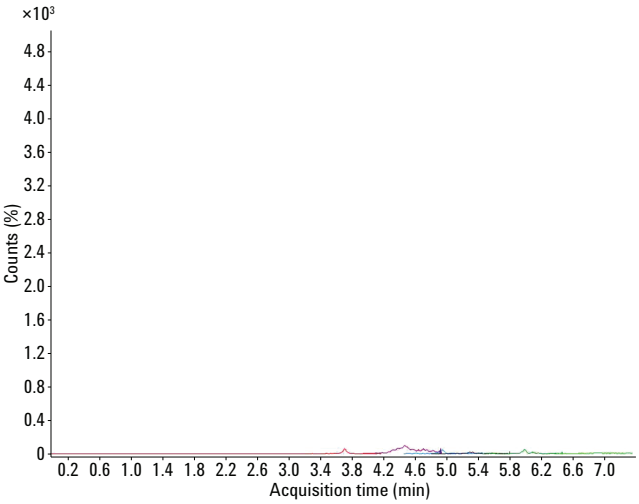


Figure 3. Chromatogram of honey sample blank extract.

Conclusions

LC/MS/MS is a reliable and powerful technique for the simultaneous quantification and confirmation of macrolides in honey. The Poroshell 120 column is an effective tool for the separation for multiple macrolides. Moreover, the results of this study show that Agilent Bond Elut Plexa can be used as an effective method for purification and enrichment of multiple macrolides in a complex matrix such as honey. The recovery and reproducibility results based on matrix spiked standards are acceptable for macrolide residue determination in honey under international regulations. The impurities and matrix effects are minimal and do not interfere with the quantification of any target compound. The limits of quantitation are significantly lower than the MRLs [1,2].

References

1. Anon., "Determination of macrolides residues in honey – LC/MS/MS method," China Standard GB/T 23408-2009. General Administration of Quality Supervision, Inspection and Quarantine, Beijing, China.
2. Anon., "Determination of macrolide antibiotic residues in animal-derived food – part 2: LC-MS/MS method," China Standard SN/T 1777.2-2007. General Administration of Quality Supervision, Inspection and Quarantine, Beijing, China.

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