Macrolide Analysis in Pork Using Bond Elut QuEChERS dSPE EMR—Lipid and Poroshell 120

Application Note

Food Testing and Agiculture

Authors

Rong-jie Fu and Chen-Hao (Andy) Zhai
Agilent Technologies Shanghai Co. Ltd.

Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation approach for the extraction and cleanup of seven macrolide residues in pork. We analyzed residues of spiramycin, tilmicosin, oleandomycin, erythromycin, tylosin, roxithromycin, and josamycin. The analytes were extracted with Agilent Bond Elut QuEChERS dSPE Enhanced Matrix Removal—Lipid, and separated on an Agilent Poroshell 120 EC-C18 HPLC column. Quantification was achieved by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC/MS/MS) operated in positive ion multiple-reaction-monitoring mode. The method provided low limits of detection for all macrolides in pork. The dynamic calibration ranges for these compounds were obtained from 5 to 250 µg/kg. The overall recoveries ranged from 63.9 to 98.4%, with RSD values between 3.8 and 10.3%.

Agilent Technologies

Introduction

The use of antibiotics in food animal production has resulted in benefits throughout the food industry. However, their use 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 animals. Some of the more commonly used macrolides are spiramycin, tilmicosin, oleandomycin, erythromycin, tylosin, roxithromycin, and josamycin.

National agencies and international organizations have set regulatory limits on the concentrations of antibiotic residues in foods of animal origin [1,2]. The regulated residue limits vary from zero to 15 mg/kg. An application was developed previously for trace level macrolide residue analysis in honey [3]. The method used Agilent Bond Elut Plexa for sample preparation, and an Agilent Poroshell 120 EC-C18 column for separation. The recovery and reproducibility results based on matrix-spiked standards were acceptable for macrolide residue determination in honey under regulation.

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 pork. A novel sorbent material, Agilent Bond Elut QuEChERS dSPE Enhanced Matrix Removal—Lipid, selectively removes major lipid components from high fat content matrices, such as pork, without unwanted analytes loss. Removal of lipid interferences from complicated matrices has many advantages, including reduced matrix effect to increased mass response, and helping extend the lifetime of LC columns. The superficially porous particle Poroshell 120 HPLC column provides high speed and efficiency with a low backpressure.

Table 1 shows details of the macrolides.

Table 1. Macrolide compounds used in this study (continued next page).

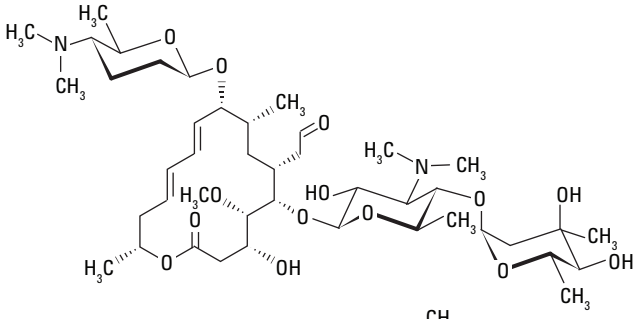
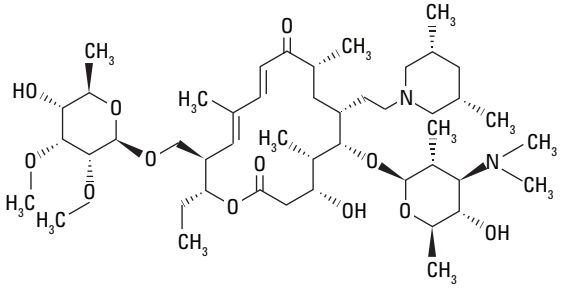
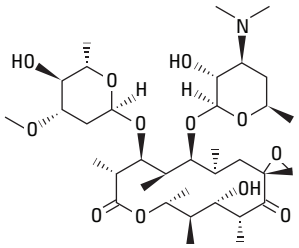
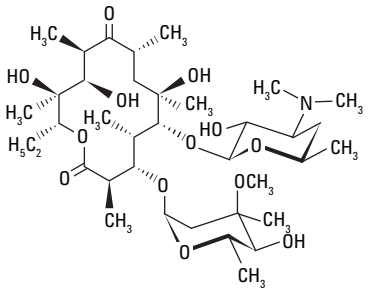
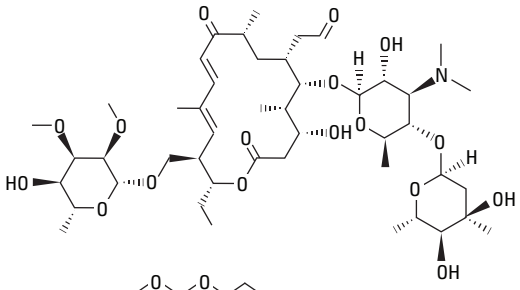
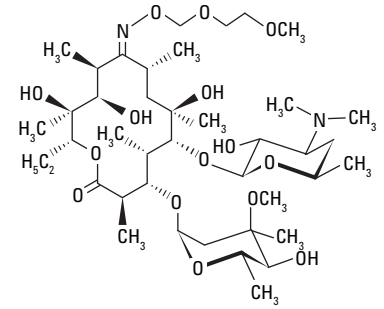
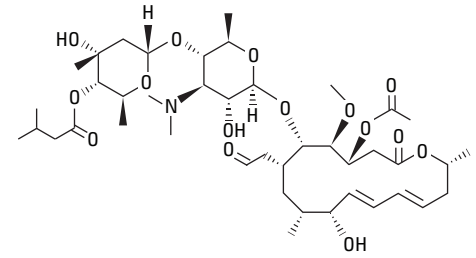
Compound	CAS No.	Structure
Spiramycin	8025-81-8	
Tilmicosin	108050-54-0	

Table 1. Macrolide compounds used in this study.

Compound	CAS No.	Structure
Oleandomycin	3922-90-5	
Erythromycin	114-07-8	
Tylosin	1401-69-0	
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). Pork was purchased from a local supermarket. Standard solutions (1.0 mg/mL) were made in methanol individually, and stored at –20 °C. A combined working solution was made in acetonitrile:water (20:80) and also stored at –20 °C. The spiked solutions were then made daily by appropriately diluting the combined working solution with water.

Equipment and materials

- Agilent 1290 Infinity LC
- Agilent 6460 Triple Quadrupole LC/MS with electrospray ionization
- Agilent Bond Elut QuEChERS Extraction Kit EN (p/n 5982-5650)
- Agilent Bond Elut QuEChERS dSPE Enhanced Matrix Removal—Lipid (p/n 5982-1010)
- Agilent Bond Elut QuEChERS Final Polish for Enhanced Matrix Removal—Lipid (p/n 5982-0101)
- Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695975-302)
- Eppendorf 5810 R centrifuge (Brinkmann Instruments, Westbury, NY, USA)
- Digital vortex mixer (VWR International, LLC, Radnor, Pennsylvania, USA)

Sample preparation

The final sample preparation procedure was optimized with the following steps.

1. Weigh 2.5 g (±0.1 g) homogenized pork into 50 mL centrifuge tube.
2. Add 8 mL water, vortex for 1 min.
3. Add 10 mL acetonitrile.
4. Add salts in QuEChERS Extraction Kit EN method.
5. Mix sample by shaking for 1 min.
6. Centrifuge at 4,000 rpm for 5 min.
7. Add 5 mL water to a 15 mL EMR—Lipid dSPE tube.
8. Transfer 5 mL of supernatant to EMR—Lipid dSPE tube.
9. Vortex immediately to disperse sample, then vortex for 1 min.
10. Centrifuge at 4,000 rpm for 3 min.
11. Transfer 5 mL of supernatant to a 15 mL EMR—Lipid polish tube containing 2 g salts (1:4 NaCl:$MgSO_4$), and vortex for 1 min.
12. Centrifuge at 4,000 rpm for 3 min.
13. Combine 200 µL of upper ACN layer and 800 µL water in a 2 mL sample vial and vortex.

HPLC conditions

Column:	Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695975-302)	
Mobile phase:	A:10 mM ammonium acetate and 0.1% formic acid in water B: acetonitrile	
Inj vol:	2 µL	
Flow rate:	0.5 mL/min	
Gradient:	Time (min)	%B
	0	20
	5	65
	6	65
	8	20
Temp:	30 °C	

MS conditions

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

MS source parameters

Gas temp:	300 °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, 4,000 V

Table 2. Masses monitored by multiple-reaction monitoring.

Compound	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)	Retention time (min)
Spiramycin	843.4	540.0	270	35	2.178
Spiramycin	843.4	174.1	270	40	2.178
Tilmicosin	869.5	696.4	320	44	2.749
Tilmicosin	869.5	174.1	320	49	2.749
Oleandomycin	688.3	544.3	170	15	2.99
Oleandomycin	688.3	158.2	170	25	2.99
Erythromycin	734.4	576.3	180	14	3.204
Erythromycin	734.4	158.2	180	30	3.204
Tylosin	916.4	772.4	280	30	3.421
Tylosin	916.4	174.2	280	40	3.421
Roxithromycin	837.4	679.3	200	16	4.087
Roxithromycin	837.4	158.1	200	34	4.087
Josamycin	828.4	174.1	250	35	4.461
Josamycin	828.4	109.1	250	46	4.461

Results and Discussion

Linearity and limit of detection

Solutions used to create external calibration curves were prepared from a combined working solution to spike matrix blanks (5, 10, 20, 50, and 250 µg/kg). Matrix blanks were created by taking pork through the entire procedure, including pretreatment and QuEChERS procedures. The data of limits of detection (LODs) were calculated with a signal-to-noise ratio (S/N) of 3 by injecting the postspiked pork matrix at 0.1 µg/kg. All S/N were greater than 3:1. Therefore, all the LODs for these compounds were below 0.1 µg/kg, and conformed to the regulated method. Table 3 shows the results for the calibration curves.

Table 3. Linearity of macrolides in pork.

Compound	Regression equation	R^2
Spiramycin	Y = 386.144x + 19.317	0.9994
Tilmicosin	Y = 133.272x + 8.018	0.9999
Oleandomycin	Y = 317.284x + 43.963	0.9998
Erythromycin	Y = 848.506x + 119.918	0.9996
Tylosin	Y = 274.158x + 22.703	0.9997
Roxithromycin	Y = 477.739x + 53.019	0.9997
Josamycin	Y = 625.922x + 58.918	0.9998

Recovery and reproducibility

The recovery and repeatability for the method were determined at three levels of pork sample spiked at concentrations of 10, 20, and 100 µg/kg, with six replicates at each level. Table 4 shows the recovery and reproducibility data. Figure 1 shows the chromatograms of 20 µg/kg spiked pork extracts.

Table 4. Recoveries and reproducibility of macrolides in pork (n = 6).

Compound	Spiked level (µg/kg)	Recovery (%)	RSD (%)
Spiramycin	10	89.7	10.3
	20	94.0	8.3
	100	95.2	3.8
Tilmicosin	10	98.4	9.5
	20	90.0	9.7
	100	95.3	7.1
Oleandomycin	10	92.4	5.7
	20	96.4	7.1
	100	97.5	6.2
Erythromycin	10	64.5	8.8
	20	63.9	8.1
	100	68.7	5.1
Tylosin	10	84.1	10.2
	20	93.3	7.4
	100	94.6	5.5
Roxithromycin	10	89.9	9.8
	20	91.6	7.7
	100	92.6	5.1
Josamycin	10	87.9	7.4
	20	92.4	5.6
	100	93.2	4.9

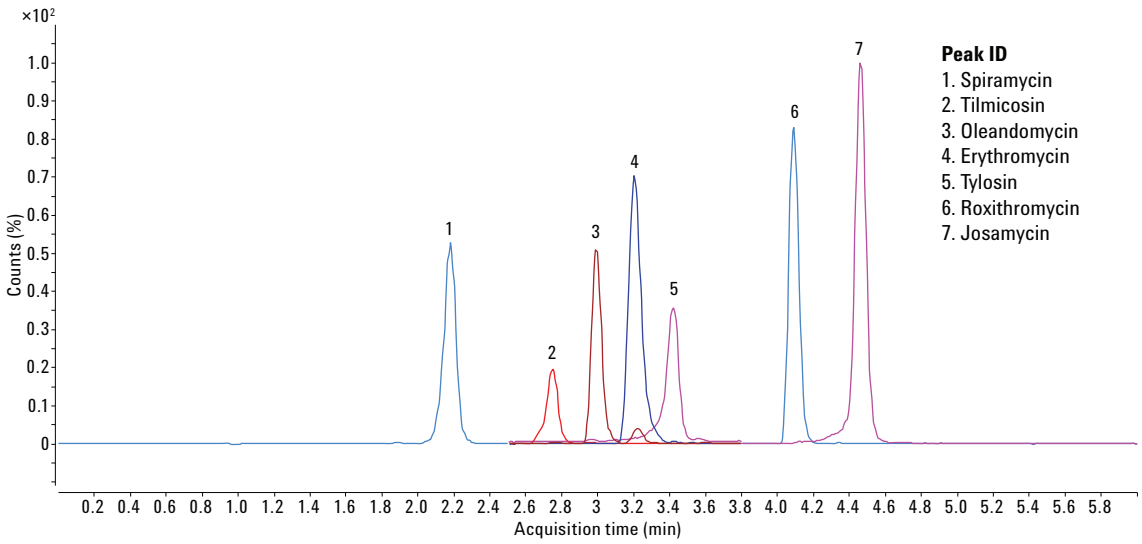


Figure 1. Chromatogram of 20 µg/kg spiked pork sample extract.

Conclusions

The method described in this application note is reliable, quick, and robust for the simultaneous quantification and confirmation of macrolides in pork. EMR—Lipid and Polish provide superior matrix cleanup and remove most matrix, especially lipids, without significantly affecting analyte recovery. The Agilent Poroshell 120 EC-C18 column provides quick separation for multiple macrolides with symmetrical peak shapes and high sensitivity.

References

1. Anon. GB/T 23408-2009. *Determination of macrolides residues in honey – LC-MS/MS method*. China Standard. General Administration of Quality Supervision, Inspection and Quarantine, Beijing, China.
2. SN/T 1777.2-2007. *Determination of macrolide antibiotic residues in animal-derived food – part 2: LC-MS/MS method. China Standard*. General Administration of Quality Supervision, Inspection and Quarantine, Beijing, China.
3. Chen-Hao Zhai, Rong-jie Fu. *Macrolides in Honey Using Agilent Bond Elut Plexa SPE, Poroshell 120, and LC/MS/MS*; Application note, Agilent Technologies, Inc. Publication number 5991-3190EN, **2013**.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
November 12, 2015
5991-6442EN

Agilent Technologies