

Determination of Penicillins in Meat by High Performance Liquid Chromatography (HPLC/UV) and HPLC/MS/MS

Application Note

Food

Author

Carol Ball
Agilent Technologies, Inc.
200 Regency Forest Drive, Suite 330
Cary, NC 27511
USA

Abstract

Penicillins are antibiotics widely used to treat diseases in animals. They are occasionally found in animal products destined for human consumption. In this paper, a solid phase extraction method with a high performance liquid chromatograph tandem mass spectrometer (HPLC/MS/MS) is shown for the simultaneous determination of six antibiotic residues: azlocillin, penicillin G, oxacillin, cloxacillin, nafcillin, and dicloxacillin in animal tissues (porcine muscle). In the method, the reversed phase column Agilent ZORBAX Eclipse Plus C18 (3.5 μ m, 100 mm \times 2.1 mm) and an Agilent mixed mode polymer solid phase extraction cartridge (Agilent SampliQ OPT) were combined to give a total solution to the analysis of residual penicillins. The performance of the solid phase extraction procedure on trace residues is quantitatively evaluated by HPLC/MS/MS.



211 Avenue J.F. Kennedy - BP 1140
03103 Montluçon cedex - France
Tél 33 (0)4 70 03 88 55 - Fax 33 (0)4 70 03 82 60
e-mail interchim@interchim.com - www.interchim.com



Agilent Technologies

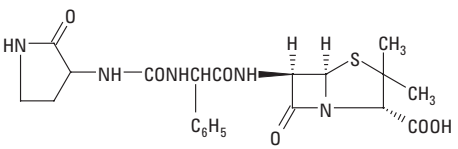
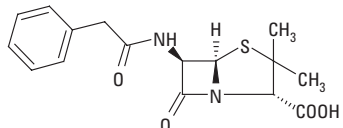
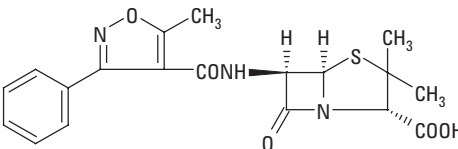
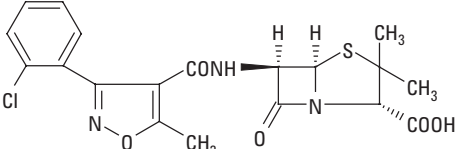
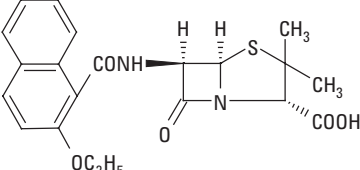
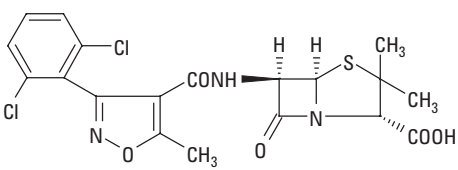
Introduction

β -lactam antibiotics (penicillins and cephalosporins) represent some of the most important antibacterial agents used in animals. However, serious reactions are known to occur in some individuals exposed to penicillins and, as a result, these compounds are carefully monitored in foods. Maximum residue limits (MRLs) for penicillins in a variety of foods are established worldwide and are generally in the range of 1ng/g. These regulations require detection and quantification

by HPLC/MS/MS. This application will show the development of a sample extraction and cleanup method and the quantification by LC/MS/MS

The structures and chemical constants for the compounds used in this study are shown in Table 1. β -lactam antibiotics are readily decomposed in acid or base [1] and experimentally show a 20 percent loss in 70 hours at 4 °C (data not shown). Thus, it is necessary to perform the extractions, cleanup, and analysis within 36 hours.

Table 1. The Compounds in This Study

No.	Name	log P.	pKa	Structure
1	Azlocillin	0.2	2.8	
2	Penicillin G	1.5	2.74	
3	Oxacillin	2.4	2.72	
4	Cloxacillin	2.6	2.78	
5	Nafcillin	3.3	2.65	
6	Dicloxacillin	3.7	2.8	

Experimental

Reagents and Chemicals

Water, acetonitrile, and methanol are all HPLC grade (Honeywell Burdick & Jackson). The standards and other chemicals were purchased from Sigma-Aldrich (Saint Louis, MO).

Phosphate buffer (pH = 8.5), 0.05 mol/L: dissolve 8.7 g of potassium phosphate dibasic in HPLC grade water to make 1 L.

Standard stock solutions (1 mg/mL) were made fresh daily in methanol. Spiking solutions were made by appropriate dilution of the stock solutions in phosphate buffer.

Equipment

Agilent 1100 HPLC with diode array detector (Agilent Technologies, Inc., Santa Clara, CA, USA)

Agilent 6410 triple quadrupole LC/MS system with electrospray ionization source (Agilent Technologies, Inc., Santa Clara, CA, USA)

Polytron homogenizer (Brinkman Instruments, Inc., PT10-35, USA)

Refrigerated centrifuge (Sorvall Instruments, RC-5B, rotor SA-600)

Rotary evaporator (BÜCHI, Switzerland/USA)

Sample Preparation

Extraction

Weigh 5 g of raw ground pork (accurate to 0.01 g) into a 50 mL capped polypropylene centrifuge tube, add 15 mL of acetonitrile/water (15:2). Homogenize completely using the Brinkman Polytron (1 minute). Centrifuge at 4,000 rpm and 4 °C for 5 minutes. Save the supernatant. Add 10 mL acetonitrile/water (15:2) to pellet, mix with a spatula to resuspend the ground meat. Homogenize for 1 minute. Centrifuge at 4,000 rpm and 4 °C for five minutes. Combine supernatants. Repeat one additional time.

Take the combined supernatants and place into a round bottom flask and evaporate the acetonitrile at 37 °C. There should be approximately 6 mL of water remaining in the flask. Bring the total volume to 20 mL using pH 8.5 phosphate buffer. Filter with a regenerated cellulose, 25 mm, 45 µm syringe filter (Agilent p/n 5185-5831). Load 10 mL of extract onto the Agilent SampliQ OPT 6 mL/150 mg SPE cartridge (Agilent p/n 5982-3067).

Purification

The procedure for SPE extraction is shown in Figure 1. Load 10 mL of the extract onto the conditioned and equilibrated cartridge. The cartridge is washed with 0.1 percent formic acid in water and then pH 8.5 potassium phosphate buffer. Finally, the sample is eluted with 3 mL acetonitrile. The sample is filtered with a 13 mm, 45 µm PTFE syringe filter (Agilent p/n 5185-5836). The eluent is dried under nitrogen at room temperature. The residue is resuspended in mobile phase to 1.0 mL. The sample is vortexed for 2 minutes and then transferred to a 2 mL autosampler vial (Agilent p/n 5182-0864).

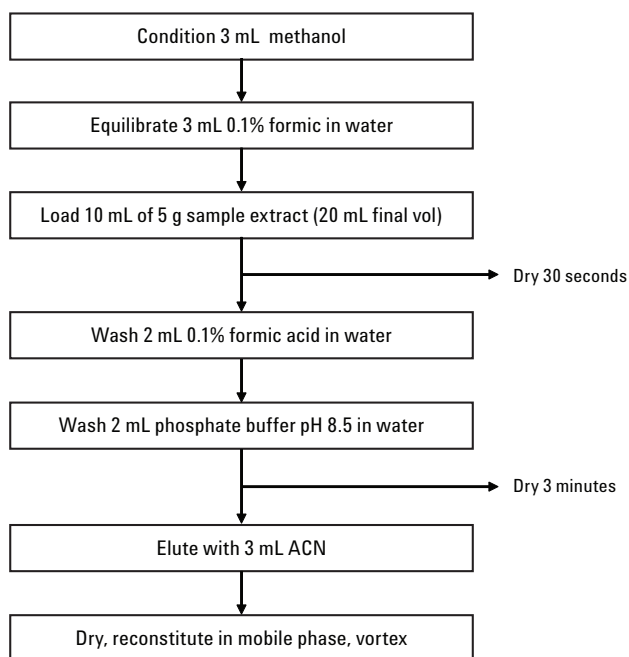


Figure 1. Agilent SampliQ OPT solid phase extraction of penicillins from pork.

Instrument Setting

The HPLC conditions are shown in Tables 2 and 3.

Table 2. HPLC Conditions

HPLC	
Column	Agilent ZORBAX Eclipse Plus, 2.1 mm × 100 mm, 3.5 µm (p/n 959793-902)
Flow rate	0.6 mL/min
Mobile phase	A: water/10 mM ammonium acetate B: acetonitrile
Run time	12 minutes
Post run	3 minutes
Temperature	30 °C
Injection	10 µL

Table 3. HPLC Gradient

Time	%B
0	2
1.2	2
2.0	10
6.0	30
8.0	40
8.5	80
11.9	80
12.0	2

Results and Discussion

Figure 2 shows the chromatograms of the meat spike sample at the limit of quantification (LOQ), 1.0 ng/g (2a), the matrix spiked blank at the LOQ (2b) and the meat extract blank (2c). The cleaned-up pork extract does not show any interferences with the target analytes. HPLC/UV is a significantly less specific detector than HPLC/MS/MS so the impurities remaining after cleanup are more visible using the general UV detector. As shown in Figure 3, the HPLC/UV chromatogram demon-

strates that the sample is extremely clean after the sample extraction and SPE cleanup.

Matrix blank material is prepared by taking the meat through the entire extraction and sample cleanup procedure. External standard calibration curves in spiked matrix blanks are made at concentrations of 0.2, 1.0, 10, and 20 ng/g. Table 4 shows the calculated recoveries of spiked meat taken through the entire sample preparation and SPE procedures. All data were calculated automatically with the Agilent MassHunter Quantitative Data Analysis software. Figure 4 shows these results graphically. All of the compounds show acceptable recovery and low relative standard deviation (%RSD).

Conclusions

The results of this study show that the Agilent SampliQ OPT cartridge provides an effective method for cleaning up complex food samples such as porcine muscle. This is demonstrated using penicillins as target compounds. HPLC/UV is

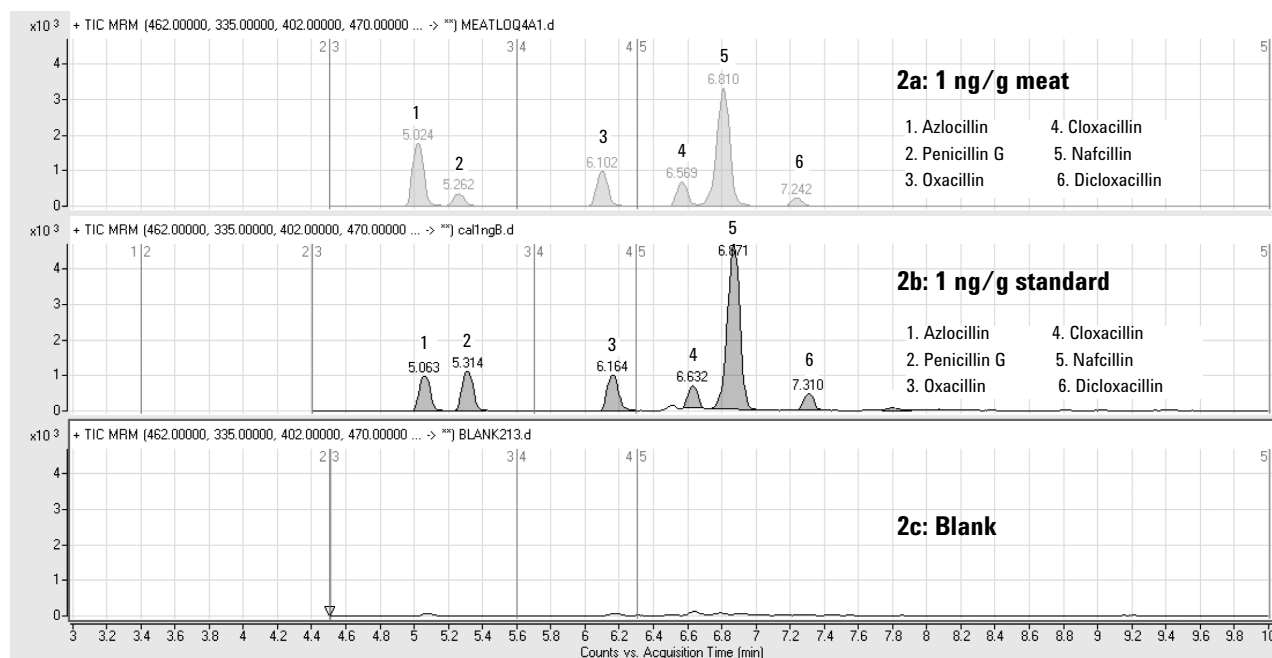


Figure 2. Meat spiked at 1 ng/g taken through extraction and SPE clean-up (2a), meat taken through extraction and clean-up then spiked at 1 ng/g (2b), and unspiked meat taken through extraction and cleanup (2c).

Table 4. Calibration Results for Spiked Meat Blanks

Name	Linear regression	R ²	1.0 ng/g		20 ng/g	
			% recovery n = 6	%RSD n = 6	% recovery n = 6	%RSD n = 6
Azlocillin	y = 6089x – 1283	0.9590	77.8	24.2	75.5	6.7
Penicillin G	y = 2690x – 513	0.9924	43.6	6.3	48.8	6.8
Oxacillin	y = 2319x + 1487	0.9794	96.5	6.0	102.8	8.9
Cloxacillin	y = 2229x + 128	0.9848	86.3	7.3	95.3	8.1
Nafcillin	y = 16654x – 1264	0.9891	98.5	6.6	101.9	3.1
Dicloxacillin	y = 1899x – 113	0.9870	105.7	13.5	103.9	3.6

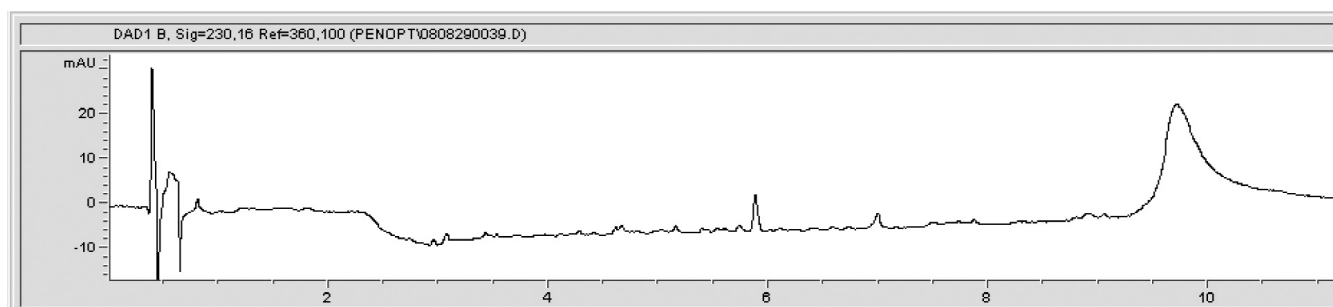


Figure 3. HPLC/UV chromatogram of an unspiked meat sample taken through extraction and SPE cleanup. Wavelength 230 nm.

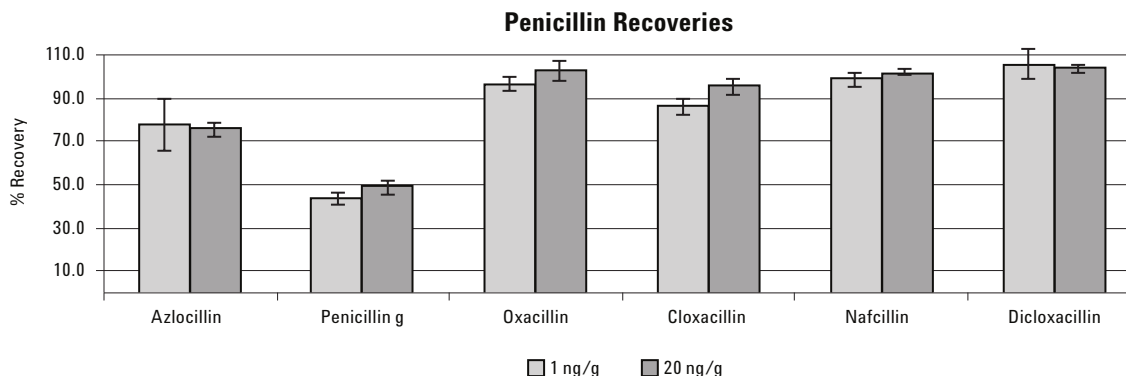


Figure 4. Recovery data for meat extracts at 1.0 and 20 ng/g.

used as a detector to demonstrate the extent of cleanup, which is found to be excellent. The LC/MS/MS is used to demonstrate the recovery of the penicillins at trace level. Even with extensive extraction and SPE cleanup, recoveries are acceptable and reproducibilities are excellent.

Reference

1. Xinbo Lu, Huabin Xing, Baogen Su, and Qilong Ren, Effect of Buffer Solution and Temperature on the Stability of Penicillin G, *J. Chem Eng.* 53 (2), 543–547, 2008

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.