Method of Test for Veterinary Drug Residues in Foods-Multiresidue Analysis of β-Lactam Antibiotics

1. Scope

This method is applicable for the determination of 19 β-lactam antibiotic residues (amoxicillin etc. listed in the attached table) in muscle, viscera, eggs and milk of poultry, livestock and aquatic products.

2. Method

After extraction and purification, analytes are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: Poroshell 120SB-C18, 2.7 μm, 3.0 mm × 15 cm, or an equivalent product.

2.1.2. Homogenizer.

2.1.3. High speed dispersing device: SPEX SamplePrep 2010 GenoGrinder®, ≥ 1000 rpm, or an other mechanical shaker.

2.1.4. Centrifuge: centrifugal force ≥ 3500 ×g.

2.1.5. Nitrogen evaporator.

2.1.6. Vortex mixer.

2.2. Chemicals

Formic acid, HPLC grade;

Acetonitrile, HPLC grade;

Primary and secondary amine (PSA), reagent grade;

Octadecylsilane, end-capped (C18 EC), reagent grade;

Magnesium sulfate anhydrous, reagent grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);

Amoxicillin and other β-lactam antibiotics listed in the attached table, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL.

2.3.2. Centrifuge tube: 15 mL and 50 mL, PP.

2.3.3. Ceramic homogenizer: Bond Elut QuEChERS P/N 5982-9313, or an equivalent product.

2.3.4. Clean-up centrifuge tube(note): containing 150 mg of PSA, 150 mg of C18 EC and 900 mg of magnesium sulfate anhydrous.

2.3.5. Membrane filter: 0.22-μm, PVDF.

Note: Commercial clean-up kits can be used as needed.

2.4. Reagents

2.4.1. 50% Acetonitrile

Mix acetonitrile and deionized water at the ratio of 50:50 (v/v).

2.4.2. 80% Acetonitrile

Mix acetonitrile and deionized water at the ratio of 80:20 (v/v).

2.5. Mobile phase

2.5.1. Solvent A

Dilute 0.05 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Dilute 0.05 mL of formic acid with acetonitrile to 1000 mL, and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 10 mg of β-lactam antibiotic reference standards accurately weighed to each 10-mL volumetric flask, dissolve and dilute to volume with 50% acetonitrile as the standard stock solutions. Store under freezing in the dark. When to use, mix appropriate volume of each standard stock solution, and dilute with deionized water to 0.1-1 μg/mL as the standard solutions.

2.7. Sample solution preparation

Transfer about 5 g of the homogenized muscle or visceral sample accurately weighed; remove eggs' shells, and transfer about 5 g of the mixed egg white and yolk sample accurately weighed; accurately transfer 5 mL of the well-mixed milk sample into a 50-mL centrifuge tube. Add one ceramic homogenizer and 10 mL of 80% acetonitrile, and shake at 1000 rpm for 5 min by the high speed dispersing device. Centrifuge at 3000 ×g for 5 min, and transfer 6 mL of the supernatant to a clean-up centrifuge tube. Shake vigorously several times by hands to prevent coagulation of salts, and then shake at 1000 rpm for 2 min by the high speed dispersing device. Centrifuge at 3500 ×g for 5 min, and collect the supernatant. Take

1 mL of the supernatant, and evaporate to dryness by gently flushing with a stream of nitrogen at 40°C. Dissolve and dilute the residue with deionized water to 1 mL, mix well, and filter with a membrane filter. Take the filtrate as the sample solution.

2.8. Calibration curve

Take a blank sample, add 100 μL of the standard solutions respecitvely, and follow the procedure described in section 2.7 to obtain the calibration standard solutions. Operate LC-MS/MS according to the following conditions. Establish the calibration curve of each β-lactam antibiotics by the peak areas of each β-lactam antibiotics vs. the added concentrations in the range of 0.001-0.01 μg/mL.

LC-MS/MS operating conditions(note)

Column: Poroshell 120SB-C18, 2.7 μm, 3.0 mm × 15 cm.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0 → 2.0	95 → 95	5 → 5
2.0 → 7.0	95 → 50	5 → 50
7.0 → 8.0	50 → 20	50 → 80
8.0 → 8.5	20 → 0	80 → 100
8.5 → 12.0	0 → 0	100 → 100
12.0 → 18.0	0 → 95	100 → 5
18.0 → 20.0	95 → 95	5 → 5

Flow rate: 0.4 mL/min.

Injection volume: 10 μL.

Interface voltage: ESI^+, 4 kV; ESI^-, 3 kV.

Interface temperature: 270°C.

Nebulizing gas flow: 3.0 L/min.

Heating gas flow: 15.0 L/min.

Desolvation line temperature: 200°C.

Heat block temperature: 350°C.

Drying gas flow: 5.0 L/min.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, Q1/Q3 cone voltage (Q1/Q3 Pre Bias) and collision

voltage are shown in the attached table.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Identification and quantification

Accurately inject 10 μL of the sample solution and the calibration standard solutions into LC-MS/MS separately. Operate according to the conditions in section 2.8. Identify each β-lactam antibiotics based on the retention time and the relative ion intensities(note). Calculate the amount of each β-lactam antibiotics in the sample by the following formula:

$$\text{The amount of each β-lactam antibiotics in the sample (ppm)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each β-lactam antibiotics in the sample solution calculated by the calibration curve (μg/mL)

V: the volume of 80% acetonitrile for sample extraction (10 mL)

M: the weight of the sample (g or mL)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 100%). Maximum permitted tolerances of relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

Remark

1. Limits of quantification (LOQs) are all 0.002 ppm for 19 β-lactam antibiotics.
2. Further validation should be performed when interfering compounds appear in the samples.
3. The amount of cefapirin is as the sum of cefapirin and its metabolite, desacetyl cefapirin. Cefapirin is rapidly metabolized to desacetyl cefapirin in the kidney matrix.

Reference

1. Li, W., Shen, H., Hong, Y., Zhang, Y., Yuan, F. and Zhang, F. 2016. Simultaneous determination of 22 cephalosporins drug residues in pork

muscle using liquid chromatography–tandem mass spectrometry. J. Chromatogr. B 1022: 298-307.

2. Heller, D. N., Kaplan, D. A., Rummel, N. G. and von Bredow, J. 2000. Identification of cephapirin metabolites and degradants in bovine milk by electrospray ionization-Ion trap tandem mass spectrometry. J. Agric. Food Chem. 48: 6030-6035.
3. Su, W. T., Huang, P. N., Liao, C. D., Kao, Y. M., Chou, H. K. and Chen, H. F. 2014. Method development for multiresidual analysis of β-lactam antibiotics in foods by QuEChERs pretreament. The Research Project of the Food and Drug Administration, Ministry of Health and Welfare, Taiwan.

Reference chromatogram

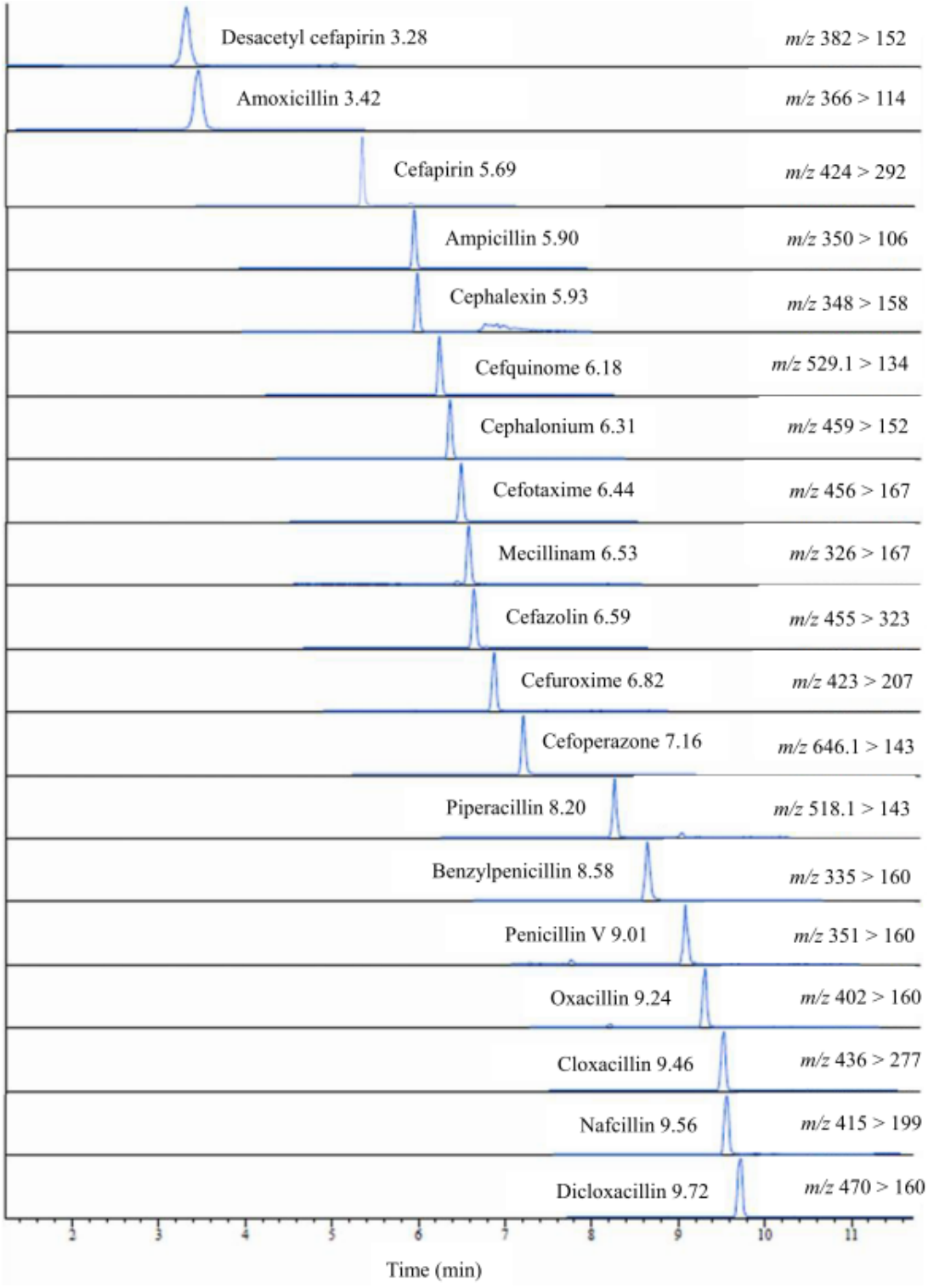

Figure. MRM chromatograms of 19 β-lactam antibiotics analyzed by LC-MS/MS.

Table. MRM parameters of 19 β-lactam antibiotics

No.	Analyte	Ionization mode	Precursor ion (*m/z*) > product ion (*m/z*)	Q1/Q3 Cone voltage (V)	Collision voltage (V)
1	Amoxicillin	ESI^+	366 > 114* 366 > 208 366 > 349	17/20 13/17 10/14	21 13 13
2	Ampicillin	ESI^+	350 > 106* 350 > 114 350 > 192	24/19 17/21 20/22	21 29 16
3	Benzylpenicillin	ESI^+	335 > 160* 335 > 176	16/28 16/18	11 16
4	Cephalexin	ESI^+	348 > 158* 348 > 174	24/28 24/28	10 10
5	Cephalonium	ESI^+	459 > 152* 459 > 337	13/12 17/21	10 15
6	Cefoperazone	ESI^+	646.1 > 143* 646.1 > 530	24/14 22/36	32 13
7	Cefazolin	ESI^+	455 > 323* 455 > 156	21/22 30/28	12 18
8	Cefotaxime	ESI^+	456 > 167* 456 > 396	16/15 22/30	10 15
9	Cefquinome	ESI^+	529.1 > 134* 529.1 > 125	26/13 24/23	18 55
10	Cefuroxime	ESI^-	423 > 207* 423 > 284 423 > 318	12/12 11/18 30/13	15 27 9
11	Cefapirin (Cephapirin)	ESI^+	424 > 292* 424 > 152	20/20 10/15	14 16
12	Cloxacillin	ESI^+	436 > 277* 436 > 160	16/26 16/29	16 14
13	Desacetyl cefapirin (Desacetyl cephapirin)	ESI^+	382 > 152* 382 > 226	18/15 18/15	24 18
14	Dicloxacillin	ESI^+	470 > 160* 470 > 311	13/21 17/28	16 16
15	Mecillinam	ESI^+	326 > 167* 326 > 139	12/17 12/14	23 31

16	Nafcillin	ESI^+	415 > 199* 415 > 171	15/19 15/17	15 33
17	Oxacillin	ESI^+	402 > 160* 402 > 243	14/11 11/24	14 15
18	Penicillin V	ESI^+	351 > 160* 351 > 114	25/11 26/11	13 29
19	Piperacillin	ESI^+	518.1 > 143* 518.1 > 160	26/26 24/30	22 22

*The quantitative ion. The qualitative ion can be selected at least one ion depending on the matrix.