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Method of Test for Veterinary Drug Residues in Foods - Test of Ionophore Coccidiostats

1. Scope

This method is applicable to the determination of 5 ionophore coccidiostats, lasalocid, maduramicin, monensin, narasin, and salinomycin, in poultry and livestock 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: positive ion electrospray ionization (ESI^+).

2.1.1.2. Column: ACQUITY UPLC BEH C8, 1.7 μm, 2.1 mm × 10 cm, or an equivalent product.

2.1.2. Centrifuge.

2.1.3. Ultrasonicator.

2.1.4. Homogenizer.

2.1.5. Nitrogen evaporator.

2.1.6. Vortex mixer.

2.2. Chemicals

Formic acid, HPLC grade;
Methanol, HPLC grade;
~~A~~cetonitrile, HPLC grade;
n-Hexane, HPLC grade;
Sodium sulfate anhydrous, reagent grade;
Deionized water, resistivity ≥ 18 MΩ•cm (at 25°C);
Lasalocid A sodium salt, maduramicin ammonium, monensin sodium salt, narasin and salinomycin SV sodium salt pentahemihydrate, reference standards.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask: 2 mL, 5 mL and 10 mL.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.4. Reagents

2.4.1. 80% acetonitrile

Dilute 800 mL of acetonitrile with deionized water to 1000 mL.

2.4.2. Acetonitrile containing 5% methanol

Dilute 50 mL of methanol with acetonitrile to 1000 mL.

2.4.3. *n*-Hexane saturated with acetonitrile

Add 50 mL of acetonitrile to 500 mL of *n*-hexane. Shake and then stand until complete layering. Take the *n*-hexane layer.

2.5. Mobile phase

2.5.1. Solvent A

Dilute 1 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Dilute 1 mL of formic acid with acetonitrile to 1000 mL, and filter with a membrane filter.

2.6. Standard solution preparation

Accurately weigh equivalent 10 mg of lasalocid, maduramicin, monensin, narasin and salinomycin reference standards to each 10-mL volumetric flask, dissolve and dilute with methanol to volume as the standard stock solutions. Store at -20°C. When to use, mix appropriate volume of each standard stock solution, and dilute with 80% acetonitrile to 1 μg/mL as the standard solution.

2.7. Sample solution preparation

Transfer about 2 g of the homogenized muscle, visceral or egg sample accurately weighed or 2 mL of the milk sample into a 50-mL centrifuge tube, add 5 g of sodium sulfate anhydrous and 10 mL of acetonitrile containg 5% methanol, vortex-mix for 1 min, ultrasonicate for 10 min, centrifuge at 3200 × g for 10 min, and collect the supernatant. Add 10 mL of acetonitrile containg 5% methanol to the residue, vortex-mix for 1 min, and repeat the extract procedure described above. Combine the supernatants, add 10 mL of *n*-hexane saturated with acetonitrile, and shak for 1 min. Centrifuge at 3200 × g for 1 min, collect the lower layer, and evaporate to dryness with a stream of nitrogen in a water bath at 40°C. Dissolve and dilute the residue with 80% acetonitrile to 2 mL. Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. Matrix-matched calibration curve preparation

Take a blank sample, and follow the procedure described in section 2.7. to

obtain the sample extract after extraction and evaporation to dryness. Add 10-200 μL of the standard solution, dissolve and dilute with 80% acetonitrile to 2 mL. Filter with a membrane filter as the matrix-matched standard solutions. Operate LC-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of each ionophore coccidiostat by the peak areas of each ionophore coccidiostat vs. the added concentrations in the range of 0.005-0.1 μg/mL.

LC-MS/MS operating conditions(Note):

Column: ACQUITY UPLC BEH C8, 1.7 μm, 2.1 mm × 10 cm.

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

Time (min)	A (%)	B (%)
0.0 → 2.0	90 → 30	10 → 70
2.0 → 8.0	30 → 30	70 → 70
8.0 → 10.0	30 → 0	70 → 100
10.0 → 11.0	0 → 0	100 → 100
11.0 → 11.1	0 → 90	100 → 10
11.1 → 15.0	90 → 90	10 → 10

Flow rate: 0.4 mL/min.

Injection volume: 10 μL.

Ionization mode: ESI+.

Capillary voltage: 3.5 kV.

Ion source temperature: 150°C.

Desolvation temperature: 600°C.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are as follows:

Analyte	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	DP (V)	CE (eV)
Lasalocid	613.4 > 377*	44	40
	613.4 > 577	44	34
Maduramicin	939.5 > 877.5*	30	34
	939.5 > 720.4	30	70
Monensin	693.4 > 479*	54	52
	693.4 > 461	54	48
Narasin	787.5 > 279*	62	52

	787.5 > 431	62	48
Salinomycin	773.5 > 431*	58	48
	773.5 > 531	58	40

* Quantitative ion pair.

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 matrix-matched standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.8. Identify each ionophore coccidiostat based on the retention time and the relative ion intensities(Note). Calculate the amount of each ionophore coccidiostat in the sample by the following formula:

$$\text{The amount of each ionophore coccidiostat in the sample (ppm)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each ionophore coccidiostat in the sample solution calculated by the matrix-matched calibration curve (μg/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

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

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

Remark

1. Limits of quantification (LOQs) for ionophore coccidiostats are as follows:

Analyte	LOQ (ppm)			
	Muscle	Viscera	Egg	Milk
Lasalocid	0.02	0.05	0.005	0.005
Maduramicin	0.02	0.05	0.005	0.005
Monensin	0.005	0.005	0.005	0.005

Narasin	0.005	0.005	0.005	0.005
Salinomycin	0.02	0.05	0.005	0.005

2. When the matrix used is not easy to match, the standard addition method can be used for quantification.
3. Further validation should be performed when interfering compounds appear in samples.

References

1. Dubreil-Chéneau, E., Bessiral, M., Roudaut, B., Verdon, E. and Sanders, P. 2009. Validation of a multi-residue liquid chromatography-tandem mass spectrometry confirmatory method for 10 anticoccidials in eggs according to Commission Decision 2002/657/EC. J. Chromatogr. A 1216: 8149-8157.
2. Stubbings, G. and Bigwood, T. 2009. The development and validation of a multiclass liquid chromatography tandem mass spectrometry (LC-MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) approach. Anal. Chim. Acta 637: 68-78.
3. Galarini, R., Fioroni, L., Moretti, S., Pettinacci, L. and Dusi, G. 2011. Development and validation of a multi-residue liquid chromatography-tandem mass spectrometry confirmatory method for eleven coccidiostats in eggs. Anal. Chim. Acta 700: 167-176.