Method of Test for Veterinary Drug Residues in Foods -Test of Avermectins

1. Scope

This method is applicable to the determination of 6 avermectin residues (abamectin etc. listed in Table 1) in poultry, livestock and aquatic foods.

2. Method

After extraction and purification, avermectins 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: XSELECT HSS T3, 2.5 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Centrifuge.

2.1.3. Shaker.

2.1.4. Homogenizer.

2.1.5. Nitrogen evaporator.

2.1.6. Solid phase extraction vacuum manifolds.

2.1.7. Vortex mixer.

2.2. Chemicals

Formic acid, HPLC grade;
Methanol, HPLC grade;
Acetonitrile, HPLC grade;
n-Hexane, reagent grade;
Ammonium solution (30%) reagent grade;
Deionized water, resistivity ≧18 MΩ・cm (at 25℃);
Abamectin, doramectin, emamectin, eprinomectin, ivermectin and moxidectin, reference standards.

2.3. Apparatus and materials

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

2.3.2. Solid phase extraction cartridge: Sep-Pak C8, 6 mL, 500 mg, or an equivalent product.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.4. 5% acetonitrile:

Dilute 5 mL of acetonitrile with deionized water to 100 mL.

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: acetonitrile.

2.6. Standard solution preparation

Transfer about 10 mg of abamectin, doramectin, emamectin, eprinomectin, ivermectin and moxidectin reference standards accurately weighed to each 10-mL volumetric flask, dissolve and dilute with acetonitrile to volume as the standard stock solutions. Store at -20℃. When to use, mix appropriate volume of each standard stock solution, and dilute with acetonitrile to 0.01-5 μg/mL as the standard solutions.

2.7. Sample solution preparation

2.7.1. Extraction

2.7.1.1. Muscle and viscera

Transfer about 5 g of the fine-cut and homogenized muscle sample or 2 g of the fine-cut and homogenized viscera sample accurately weighed into a centrifuge tube, add 25 mL of acetonitrile, vortex-mix for 1 min, shake for 10 min, centrifuge at 3200 ×g for 10 min, and collect the supernatant. Add 20 mL of *n*-hexane, vortex-mix for 1 min, centrifuge at 3200 ×g for 5 min, and collect the lower layer. Add 20 mL of *n*-hexane, repeat this procedure once, and collect the lower layer. Evaporate to dryness with a stream of nitrogen in a water bath at 40℃, add 10 mL of deionized water and 100 μL of ammonia solution, and vortex-mix. Take the solution for purification.

2.7.1.2. Milk

Accurately transfer 10 mL of milk sample into a centrifuge tube, add 25 mL of acetonitrile, vortex-mix for 1 min, shake for 10 min, centrifuge at 3200 ×g for 10 min, and collect the supernatant. Add 10 mL of *n*-hexane, vortex-mix for 1 min, centrifuge at 3200 ×g for 5 min, and collect the lower layer. Evaporate to 8 mL with a stream of nitrogen in a water bath at 40°C, add 10 mL of deionized water and 100 μL of ammonia solution, and vortex-mix. Take the solution for purification.

2.7.2. Purification

Transfer the solution for purification from section 2.7.1. into a solid phase

extraction cartridge prerinsed with 5 mL of acetonitrile and 5 mL of deionized water, and discard the eluent, Wash the cartridge with 10 mL of 5% acetonitrile, and discard the eluent. Add 5 mL of acetonitrile to the cartridge, collect the eluent, and dilute with deionized water to 10 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 eluent. Add 1 mL of the standard solutions, and dilute with deionized water to 10 mL. Filter with membrane filters as the matrix-matched standard solutions. Operate LC-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of each avermectin by the peak areas of each avermectin vs. the added concentrations.

LC-MS/MS operating conditions(note):

Column: XSELECT HSS T3, 2.5 μm, 2.1 mm i.d. × 10 cm.

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

Time (min)	A (%)	B (%)
0.0 → 1.5	100 → 100	0 → 0
1.5 → 3.0	100 → 30	0 → 70
3.0 → 5.0	30 → 5	70 → 95
5.0 → 12.0	5 → 5	95 → 95
12.0 → 12.1	5 → 100	95 → 0
12.1 → 17.0	100 →100	0 → 0

Flow rate: 0.4 mL/min.

Injection volume: 10 μL.

Capillary voltage: 5.5 kV.

Desolvation temperature: 550℃.

Curtain gas: 20 psi.

Ion source gas 1: 50 psi.

Ion source gas 2: 50 psi.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, declustering potential and collision energy are shown in Table 1.

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 avermectin based on the retention time and the relative ion intensities(note). Calculate the amount of each avermectin (ppm) in the sample by the following formula:

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

Where,

C：the concentration of each avermectin 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 sample (g)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions. Maximum permitted tolerances of 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 6 avermectins are listed in Table 2.
2. Further validation should be performed when interfering compounds appear in the samples.

Table 1. MRM parameters of 6 avermectins

Analyte	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)
Abamectin	895 > 751*	51	57
	895 > 449	51	61
Doramectin	921 > 777*	51	59
	921 > 449	51	61
Emamectin	886 > 158*	51	45
	886 > 302	51	39
Eprinomectin	936 > 490*	51	65
	936 > 352	51	69
Ivermectin	897 > 753*	51	57
	897 > 329	51	65
Moxidectin	640 > 528*	51	15
	640 > 478	51	17

* The quantitative ion.

Table 2. Limits of quantification of 6 avermectins

Analyte	LOQ (ppm)		
	Muscle	Viscera	Milk
Abamectin	0.01	0.01	0.005
Doramectin	0.002	0.01	0.005
Emamectin	0.005	0.005	0.005
Eprinomectin	0.01	0.1	0.005
Ivermectin	0.005	0.01	0.005
Moxidectin	0.01	0.03	0.01