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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 31613.1-2021

**National food safety standard--Determination of amprolium residues in
cattle-derived products by liquid chromatographic-tandem mass spectrometry
method and high performance liquid chromatographic method**

**食品安全国家标准 牛可食性组织中氨丙啉残留量的测定 液相色谱-串联质谱法和
高效液相色谱法**

(English Translation)

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1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry methods for the detection of ampropion residues in cattle edible tissues: This document is applicable to the detection of amproline residues in beef muscle, liver, kidney and fat:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document through normative references in the text: Among them, for dated reference documents, only the version corresponding to the date applies to this document; for undated reference documents, the latest version (including all amendments) applies to this document:

GB/T 6682 Specifications and test methods for water used in analytical laboratories

3 Terms and definitions

There are no terms or definitions to be defined in this document:

4 Principles

The remaining amproline in the sample was extracted with phosphate buffer (fat samples were extracted with acetonitrile), purified with HLB solid-phase extraction column, measured by liquid chromatography-tandem mass spectrometry, and quantified by the matrix calibration external standard method:

5 Reagents and materials

Unless otherwise specified, all reagents are of analytical grade and the water is first-grade water that complies with GB/T 6682:

5.1

Reagents

5.1.1

Methanol (CH₃OH)

Chromatographically pure:

5.1.2

Acetonitrile (CH₃CN)

Chromatographically pure:

5.1.3

Formic acid (HCOOH)

Chromatographically pure:

5.1.4

Dipotassium hydrogen phosphate (K₂HPO₄):

5.1.5

Potassium dihydrogen phosphate (KH₂PO₄):

5.2

Standard products Amoprolol Hydrochloride (C₁₆H₁₉ClN₃O₂·HCl, CAS No.: 137-88-2), content ≥98.0%:

5.3

Solution preparation

5.3.1

Phosphate buffer (pH 6.0): Take 2.0g of dipotassium hydrogen phosphate and 8.0g of potassium dihydrogen phosphate, add water to dissolve and dilute to 1000mL, and mix well:

5:3:20.1% formic acid solution: Take 1mL of formic acid, dilute it with water to 1000mL, and mix well:

5:3:30.1% formic acid solution-methanol: Take 900 mL of 0.1% formic acid solution and 100 mL of methanol, and mix well:

5.4

Preparation of standard solution

5.4.1

Standard stock solution (100 mg/L)

Take an appropriate amount of amprolium hydrochloride standard (approximately equivalent to 10 mg of amprolium), weigh it accurately, dissolve it in methanol, dilute it to a 100mL volumetric flask, and shake well: That's it: Stored below -18°C, valid for 6 months:

5.4.2

Standard working solution

Accurately pipet an appropriate amount of standard stock solution, and dilute it step by step with 0.1% formic acid solution-methanol to a concentration of 5 µg/L, 20 µg/L, 50 µg/L, 200 µg/L, 500 µg/L and A series of standard working solutions of: 2000 µg/L: Ready for use:

5.5

Materials

HLB solid phase extraction cartridge: 60 mg/3mL, or equivalent:

6 Instruments and equipment

6.1

Liquid chromatography-tandem mass spectrometer

Equipped with electrospray ion source:

6.2

Analytical balance

Sensitive to 0.00001g and 0.01g:

6.3

Nitrogen blowing instrument:

6.4

Solid phase extraction device:

6.5

Homogenizer:

6.6

Vortex mixer:

6.7

Polypropylene plastic centrifuge tube

50 mL:

6.8

Centrifuge

8000 r/min or above:

7 Preparation and preservation of samples

7.1

Preparation of samples Take an appropriate amount of fresh or thawed air or test tissue, mince it, and homogenize it:

- a) Take the homogenized test sample as the test material;
- b) Take the homogenized blank sample as the blank sample;
- c) Take the homogenized blank sample, add the standard working solution of appropriate concentration, and add the sample as a blank:

7.2

Storage of samples Store below -18°C and conduct analysis and testing within 3 months:

8 Measurement steps

8.1

Extraction

8.1.1

Bovine muscle, liver and kidneys Take 2g of the sample (accurate to ± 0.02 g), add 10.0 mL of phosphate buffer, homogenize at 10000 r/min for 1 min, centrifuge at 8000 r/min for 5 min, collect the supernatant, and add 10.0 mL of phosphate buffer to the residue: , repeat the extraction once, combine the supernatants, and set aside:

8.1.2

Beef fat

Take 2g of the sample (accurate to ± 0.02 g), add 10.0 mL of acetonitrile, homogenize at 10000 r/min for 1 min, centrifuge at 8000 r/min for 5 min, and put the supernatant into a 50 mL plastic centrifuge tube: Add 10 mL of acetonitrile to the residue, repeat the extraction once, combine the two supernatants, blow to dryness with nitrogen in a 50°C water bath, dissolve the residue with 20.0 mL of phosphate buffer, and set aside: