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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.15-2021

National food safety standard - Determination of xylazine and metabolite(2, 6-dimethylaniline)in animal derived food by Liquid Chromatography-tandem Mass Spectrometric method

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Foreword

This document is drafted in accordance with the provisions of GB/T 1:1-2020 "Standardization Work Guidelines Part 1: Structure and Drafting Rules of Standardization Documents":

This document is published for the first time:

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry methods for the detection of xylazine and metabolite 2,6-dimethylaniline residues in animal foods:

This document is applicable to the detection of xylazine and 2,6-dimethylaniline residues in muscle, fat, liver and kidney tissues of pigs, cattle and sheep:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document through normative citations in the text: Among them, the cited documents with dates are:

Only the version corresponding to the date applies to this document; for undated referenced documents, the latest version (including all amendments) applies to this document:

document:

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

3 Terms and definitions

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

4 Principles

Extract xylazine and 2,6-dimethylaniline remaining in the sample with acetonitrile or ammonia-acetonitrile, extract with n-hexane saturated with acetonitrile, and purify:

Determination by liquid chromatography-tandem mass spectrometry in positive ion mode and quantification by 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 n-Hexane (C6H14):

5:1:4 Ammonia (NH3:H2O):

5:1:5 Formic acid (HCOOH):

5:1:6 Anhydrous sodium sulfate (Na2SO4):

5:2 Solution preparation

5:2:1 5% ammonia water and acetonitrile: Take 50 mL of ammonia water and dilute it with acetonitrile to 1000 mL:

5:2:2 20% acetonitrile aqueous solution: Take 20 mL of acetonitrile and dilute to 100 mL with water:

5:2:3 0:1% formic acid solution: Take 1mL of formic acid and dilute it with water to 1000mL:

5:2:4 Acetonitrile-saturated n-hexane: Take 30 mL of n-hexane, add 5 mL of acetonitrile, vortex to mix, shake for 10 min, separate with a separatory funnel, and take the upper liquid:

5:3 Standard products

5:3:2 2,6-dimethylaniline [2,6-dimethylaniline, (CH3)2C6H3NH2, CAS number: 87-62-7], content >=99:0%, see Appendix A:

5:4 Preparation of standard solution

5:4:1 Standard stock solution: Take 10 mg each of xylazine hydrochloride and 2,6-dimethylaniline standard products, weigh them accurately, add an appropriate amount of acetonitrile respectively to dissolve and dilute to a 10 mL volumetric flask, shake well: Prepare standard stock solutions of xylazine and 2,6-dimethylaniline with a concentration of 1 mg/mL: Store below -18°C and have a validity period of 3 months:

5:4:2 Standard working solution: Precisely measure appropriate amounts of xylazine and 2,6-dimethylaniline standard stock solutions into 10 mL volumetric flasks, dilute to volume with acetonitrile, shake well, and prepare respectively: Standard working solution with perazine concentration of 0:4 μg/mL and 2,6-dimethylaniline concentration of 1 μg/mL: Store at 2 degrees C~8 degrees C and prepare for immediate use:

5:5 Materials

Filter membrane: 0:22μm, water phase:

6 Instruments and equipment

6:1 Liquid chromatography-tandem mass spectrometer: equipped with electrospray ion source (ESI):

6:2 Analytical balance: sensitivity 0:00001g and 0:01g:

6:3 Homogenizer:

6:4 Vortex mixer:

6:5 Oscillator:

6:6 Refrigerated centrifuge:

6:7 Nitrogen blower:

6:8 Ultrasound instrument:

7: Preparation and preservation of samples

7:1 Preparation of samples

Take an appropriate amount of fresh or thawed blank or test tissue and homogenize it:

a) Take a homogeneous test sample as a test material;

b) Take a homogeneous blank sample as a blank sample;

c) Take a homogeneous blank sample, add standard working solution of appropriate concentration, and add the sample as a blank:

7:2 Storage of samples

Store below -20 degrees C:

8 Measurement steps

8:1 Extraction

Take 5g of the sample (accurate to +/-0:02g), add 2g of anhydrous sodium sulfate and 10mL of acetonitrile, vortex to mix, shake for 10min, centrifuge at 4°C, 10000r/min for 10min (for the fat sample, add 10mL of 5% ammonia aqueous acetonitrile, Centrifuge at 4°C, 8000r/min for 1 min), take the supernatant, repeat the extraction once, and combine the two supernatants: Blow nitrogen at 40°C until nearly dry, add 1:0 mL of acetonitrile to dissolve, vortex and mix, and set aside:

8:2 Purification

Take the reserve solution, add 0:5 mL of n-hexane saturated with acetonitrile, vortex to mix, centrifuge at 4°C, 10000 r/min for 5 min, and remove the layer solution

200 µL, in a 5 mL centrifuge tube, add 800 µL of water, vortex to mix, centrifuge at 4°C, 10000 r/min for 5 min, take the solution, filter, and use for liquid chromatography-tandem mass spectrometry measurement:

8:3 Preparation of standard curve

Precisely measure appropriate amounts of standard working solutions of xylazine and 2,6-dimethylaniline, dilute with 20% acetonitrile aqueous solution, and prepare xylazine concentrations of 0ng/mL, 0:2ng/mL, 1ng/mL, and 5ng: /mL, 10ng/mL, 50ng/mL, 100ng/mL, 2,6-dimethylaniline concentration series: 0ng/mL, 5ng/mL, 10ng/mL, 20ng/mL, 50ng/mL, 100ng/mL Mix standard solutions for liquid chromatography - string

For mass spectrometry measurement, take the characteristic ion mass chromatographic peak area as the ordinate and the corresponding standard solution concentration as the abscissa, draw a standard curve, and find the regression equation and correlation coefficient:

8:4 Determination

8:4:1 Chromatographic conditions

a) Chromatographic column: C18 (100mmx2:1mm, 3µm), or equivalent;

b) Mobile phase: A is 0:1% formic acid aqueous solution, B is acetonitrile;

c) Flow rate: 0:3mL/min;

d) Injection volume: 5µL;

e) Column temperature: 30 degrees C;

f) The mobile phase gradient elution conditions are shown in Table 1:

8:5 Determination method