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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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National food safety standard--Determination of dinitolmide residues in chicken edible tissues

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1 Scope

This document specifies sample preparation and liquid chromatographic determination for the detection of dinitramide and its metabolites residues in chicken muscle, liver, kidney and fat:

method:

This document is applicable to the detection of dinitramide and its metabolites residues in chicken muscle, liver, kidney and fat:

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

The remaining dinitramide and its metabolites in the sample were extracted with phosphate buffer, purified with HLB solid phase extraction column, separated by liquid chromatography, and then Detection by tube matrix detector 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 Potassium dihydrogen phosphate (KH2PO4):

5:1:2 Dipotassium hydrogen phosphate (K2HPO2):

5:1:3 Sodium hydroxide (NaOH):

5:1:4 Acetonitrile (CH3CN): chromatographically pure:

5:1:5 Formic acid (HCOOH): chromatographically pure:

5:1:6 Hydrochloric acid (HCl):

5:2 Preparation of solution 5:2:1 1:0 mol/L hydrochloric acid solution: Take 21:2 mL of hydrochloric acid, dilute it with water to 250 mL, and shake well:

5:2:21:0 mol/L sodium hydroxide solution: Take 40g of sodium hydroxide, add an appropriate amount of water to dissolve and dilute to 1L, and mix well:

5:2:3 Phosphate buffer (pH 6:0): Take 2:0 g of dipotassium hydrogen phosphate and 8:0 g of potassium dihydrogen phosphate, add an appropriate amount of water to dissolve and dilute to 1000 mL, and mix well:

5:2:4 Phosphate buffer (pH 4:0): Take phosphate buffer (pH 6:0) and adjust the pH to 4:0 with 1:0 mol/L hydrochloric acid solution:

5:2:50:1% formic acid solution: Take 1mL of formic acid, dilute it with water to 1000mL, and mix well:

5:2:60:1% formic acid-acetonitrile: Take 800mL of 0:1% formic acid solution and:200mL of acetonitrile, and mix well:

5:3 Standard products The contents of dinitrotropide and dinitrotropide metabolite (3-ANOT) are >=95%, see Appendix A:

5:4 Preparation of standard solutions 5:4:1 Standard stock solution: Take 10 mg each of dinitramide and 3-ANOT standard, weigh it accurately, dissolve it with an appropriate amount of acetonitrile and dilute it to a 100 mL volumetric flask, shake well: Store below -18°C, valid for 6 months :

5:4:2 Mixing standard stock solutions: Precisely measure 50 mL of each of the two standard stock solutions into a 100 mL volumetric flask, shake well, and get it:

Stored below -18 degrees C, the validity period is 6 months:

5:4:3 Mixed standard working solution: Precisely measure an appropriate amount of the

mixed standard stock solution, and dilute it with 0:1% formic acid-acetonitrile to prepare a solution containing 3-ANOT and dinitramide at a concentration of 25 ug/L, 50 ug/L, and 100 ug/L: L,:200ug/L, 500ug/L, 1000ug/L and:2000ug/L mixed standard working solutions: Prepared for current use:

5:5 Materials 5:5:1 HLB solid phase extraction column: 60mg/3mL, or equivalent:

5:5:2 Filter membrane: pore size 0:22um, organic system:

6 Instruments and equipment

6:1 Liquid chromatograph: equipped with diode matrix detector:

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

6:3 Solid phase extraction device:

6:4 Homogenizer:

6:5 Centrifuge tube: polypropylene plastic centrifuge tube, 50mL:

6:6 Centrifuge: 8000r/min or above:

6:7 pH meter:

7: Preparation and preservation of samples 7:1 Preparation of samples Take an appropriate amount of fresh or thawed blank 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 degrees C and conduct analysis and testing within 3 months:

8 Measurement steps 8:1 Extraction Take 2g of the sample (accurate to +/-0:02g) in a 50mL centrifuge tube, add 10:0mL of phosphate buffer (pH 6:0) [chicken liver, kidney Add phosphate buffer (pH 4:0)], homogenize at 10,000 r/min for 1 min, centrifuge at 8,000 r/min for 5 min, collect the supernatant, and add the corresponding residue to the Add 10:0 mL of phosphate buffer, repeat the process, and combine the supernatants: Accurately pipette 8 mL of the supernatant, and adjust the pH to Centrifuge at 7:0, 8000r/min for 5min, take the supernatant and set aside:

8:2 Purification The solid phase extraction column was activated with 3 mL of methanol and 3 mL of water in sequence: Take the backup solution and pass it through the column: When the liquid level reaches the surface of the column bed, rinse with 3 mL of water:

Wash, drain, and elute with 4 mL of 0:1% formic acid-acetonitrile: Collect the eluate and pass through a 0:22 um filter membrane for liquid chromatography measurement:

8:3 Preparation of standard curve Precisely measure an appropriate amount of the mixed standard working solution, inject it into the liquid chromatograph, and record the chromatogram: Take the peak area of each component as the ordinate, and the corresponding The concentration of the standard solution is the abscissa, draw a standard curve: Find the regression equation and correlation coefficient:

8:4 Determination 8:4:1 Liquid chromatography reference conditions a) Chromatographic column: C18 chromatographic column (100mmx2:1mm, 1:7um), or equivalent;

b) Mobile phase: A is 0:1% formic acid solution, B is acetonitrile, and the gradient elution conditions are shown in Table 1;

c) Flow rate: 0:2mL/min;

d) Wavelength: 246nm (dinitramide); 310nm (3GANOT);

e) Column temperature: 30 degrees C;

f) Injection volume: 10uL:

8:4:2 Determination method Take the sample solution and the standard solution, perform single-point or multi-point calibration, and quantify the chromatographic peak area according to the external standard method: The standard solution and the sample solution The peak area of the target drug in the sample solution should be within the linear range of the instrument detection: The retention time of dinitramide and 3GANOT in the sample solution The deviation from the retention time of dinitramine and 3GANOT in the standard working solution is within +/-2:5%: The liquid chromatogram of the standard solution is shown in Appendix B:

Figure B:1:

8:5 Blank test Take a blank sample and perform parallel operations using the same measurement steps except that no standard solution is added:

9 Calculation and presentation of results The residual amount of dinitramide in the sample is calculated according to the standard curve or formula (1):

10 Sensitivity, accuracy and precision of detection methods 10:1 Sensitivity The detection limit of this method in chicken muscle, liver, kidney and fat is 150 ug/kg, and the quantitation limit is 500 ug/kg:

10:2 Accuracy The recovery rate of this method is 70% to 110% at the added concentration level of 500ug/kg~12000ug/kg:

10:3 Precision The intra-batch relative standard deviation of this method is <=15%, and the inter-batch relative standard deviation is <=20%: