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

GB 29704-2013

**Determination of Cyromazine and Melamine residues in animal
derived food by Ultra Performance Liquid
Chromatography-tandem Mass Spectrometric method**

食品安全国家标准 动物性食品中环丙氨嗪及代谢物三聚氰胺多残留的测定 超高效液相色谱-串联质谱法

(English Translation)

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Table of Contents

1 Scope
2 Normative references
4 Reagents and materials
5 Apparatus
6.1 Preparation of the sample
7.1 Preparation of matrix-matched standard curve
7.2 Extract
7.3 Purification
7.4 Determination
7.5 Blank test
9 Detection sensitivity, accuracy and precision
9.1 Sensitivity
9.2 Accuracy
9.3 Precision

1 Scope

This standard specifies the sample preparation and animal foods UPLC cyromazine and melamine metabolite of Residues - string with mass spectrometry.

This standard applies to chicken muscle, kidney and eggs cyromazine residues and metabolites melamine.

2 Normative references

The following documents for the application of this document is essential. For dated references, only applies to the version dated paper Pieces. For undated references, the latest edition (including any amendments) applies to this document.

GB/T 6682 Water for analytical laboratory specifications and test methods Principle 3 the specimens remaining cyromazine and melamine metabolites, extracted with 3% trichloroacetic acid solution, MCX purification column, ultra-high performance liquid chromatography Spectrum - tandem mass spectrometry, external standard.

4 Reagents and materials

The following reagents used, unless otherwise stated who were of analytical reagent; water as a water line with GB/T 6682 provisions.

4.1

cyromazine and melamine reference. content $\geq 98.0\%$.

4.2

TCA.

4.3

Acetonitrile. chromatographically pure.

4.4

Methanol. HPLC grade.

4.5

formic acid.

4.6

ammonia.

4.7

ammonium acetate.

4.8

MCX SPE. 60mg/3mL, or equivalent person.

4.9 3% trichloroacetic acid solution. Take trichloroacetic 3g, dissolve and dilute to 100mL with water.

4.10 2% formic acid aqueous solution. Take acid 2mL, dissolve and dilute to 100mL with water.

4.11 5% ammoniated methanol. aqueous ammonia to take 5mL, dissolved in methanol and diluted to 100mL.

4.12 50% aqueous acetonitrile. take 50mL, dissolve and dilute to 100mL with water.

4.13 0.1mol/L ammonium acetate solution. Take ammonium acetate 7.71g, dissolved and diluted with water to 1000mL.

4.14 1mg/mL cyromazine, melamine standard stock solution. Weigh accurately cyromazine and melamine reference 10mg, respectively, Brown 10mL volumetric flask, dissolved with 50% aqueous acetonitrile and dilute to volume, formulated at a concentration of 1mg/mL of the standard reservoir cyromazine Preparation of standard stock solution and melamine

solution. 2 °C ~ 8 °C storage period of three months.

4.15 10µg/mL cyromazine and melamine working standard solutions. cyromazine precise amount of standard stock solution 1mg/mL and 1.0 mL of each stock standard solution of melamine, to a 100mL volumetric flask, dissolved in acetonitrile and dilute to volume, formulated at a concentration of 10µg/cyromazine and melamine mL of working standard solutions. 2 °C ~ 8 °C storage period of three months.

5 Apparatus

5.1

ultra performance liquid chromatography - tandem mass spectrometer. Distribution ionization source.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

vortex mixer.

5.5

centrifuge.

5.6

Nitrogen blowing instrument.

5.7

centrifuge tube. 50mL.

Microporous filter head 5.8. 0.2µm.

Preparation and Storage of sample 6

6.1.1 Eggs

Fresh or frozen egg white or test, peeled, and homogenized.

--- the test sample taken after homogenization, as the feed try.

--- blank sample taken after homogenization, as a blank sample.

--- blank sample taken after homogenization, adding a suitable concentration of the standard working solution, is added as a blank sample.

6.1.2 Chicken muscle meat and kidney

Fresh or frozen blank or test tissue, minced, and homogenized.

--- the test sample taken after homogenization, as the feed try.

--- blank sample taken after homogenization, as a blank sample.

--- blank sample taken after homogenization, adding a suitable concentration of the standard working solution, is added as a blank sample.

Save 6.2 sample

Or less at -20 °C.

Determination Step 7

7.1 Preparation of matrix-matched standard curve

The precise amount of 10µg/mL cyromazine and melamine working standard solutions amount, diluted with 95% acetonitrile in water to prepare a concentrated it is a series of mixed standard solution 2,5,10,20,50 and 100ng/mL and 1.0 mL from each, are extracted by dissolution, purification and drying 6 parts of the blank sample residue was filtered, for performance liquid chromatography - tandem mass spectrometry. A characteristic ion mass chromatogram peak area for the vertical Coordinate matrix-matched control solution concentration as the abscissa, the standard curve. Seeking regression equation and correlation coefficient.

7.2 Extract

Sample Weigh 2g ± 0.02g, in 50mL centrifuge tube, 3% trichloroacetic acid solution was added 20mL, vortexed 30s, ultrasonic 10min, allowed to stand for 10min, 10000r/min centrifugal 10min, the supernatant was set aside.

7.3 Purification

MCX column washed with methanol and 3mL 3mL water activation, 10mL take stock solution through the column, 3mL plus 2% formic acid, methanol 3 mL rinsed, drained, with 5% ammoniated methanol 3mL elution, drained, eluate dry nitrogen 50 °C, with 95% aqueous acetonitrile the residue was dissolved 1.0mL, membrane filtration, ultra for liquid chromatography - tandem mass spectrometry.

7.4.1 Chromatographic conditions

7.4.1.1

Column. BEHHILIC column (50mm x 2.1mm, particle size 1.7µm), or equivalent person.