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

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**National Food Safety Standard Determination of 10 Diuretic
Drug Residues in Animal Foods by Liquid
Chromatography-Tandem Mass Spectrometry**

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6.1 Liquid chromatography-tandem mass spectrometer. with electrospray ion source.

6.2 Analytical balance. the sensitivity is 0.00001g and 0.01g respectively.

6.3 Nitrogen blowing concentrator.

6.4 Vortex mixer.

6.5 Ultrasonic generator.

6.6 Centrifuge. the speed is not lower than 8000r/min.

6.7 Solid phase extraction device.

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5.00 µg/kg.

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1. Structure and Drafting Rules for Standardization Documents" drafting.

This document is published for the first time.

National Food Safety Standards

Determination of 10 kinds of diuretics residues in food of animal origin

Liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the method for sample preparation and liquid chromatography-tandem mass spectrometry determination of 10 diuretic drug residues in food of animal origin.

This document is applicable to the muscle of pigs, cattle, sheep, chickens, ducks, acetazolamide, 4-amino-6-chlorobenzene-1,3-disulfonamide, chlorothiazide in eggs and milk.

Determination of 10 kinds of diuretics residues including zine, hydrochlorothiazide, chlorthalidone, furosemide, benfluthiazide, triamterene, spironolactone and canrenone.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents,

Only the version corresponding to the date applies to this document; for undated references, the latest version (including all amendments) applies to this document document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The sample was extracted by acetonitrile-water solution, degreased by n-hexane, purified by reverse-phase mixed hydrophilic-lipophilic solid-phase extraction column, liquid chromatography-tandem mass spectrometer

Determination, quantification by internal standard method.

5 Reagents and materials

Unless otherwise specified, all reagents are chromatographically pure, and water is first-grade water in accordance with GB/T 6682.

5.2 Solution preparation

5.2.1 90% acetonitrile aqueous solution. take 900mL of acetonitrile, add water to dilute to 1000mL.

5.2.2 50% acetonitrile aqueous solution. take 50mL of acetonitrile, add water to dilute to 100mL.

5.2.3 5mmol/L ammonium acetate buffer solution. Take 0.385g of ammonium acetate, dissolve it in water, and dilute to 1000mL.

5.2.4 90% acetonitrile methanol solution. Take 10mL of methanol, dilute to 100mL with acetonitrile, and mix well.

5.2.5 Acetonitrile saturated n-hexane solution. take the same volume of acetonitrile and n-hexane, put them in a separatory funnel, vibrate, let stand to separate layers, and take the upper layer

solution.

5.3 Standards

5.3.1 Standard products of diuretics. acetazolamide, 4-amino-6-chlorobenzene-1,3-disulfonamide, chlorothiazide, hydrochlorothiazide, chlorthalidone, furosemide, Bianfu

Thiazide, triamterene, spironolactone, canrenone, the purity of which is $\geq 98.0\%$. Or the standard product certified by the state and awarded the standard material certificate.

Standard product

See Table A.1 in Appendix A for information.

5.3.2 Isotopic internal standards. acetazolamide-D3, 4-amino-6-chlorobenzene-1,3-disulfonamide-15N2, chlorthalidone-D4,

hydrochlorothiazide-13CD₂,

Chlorthiazide-13C₁₅N₂, furosemide-D₅, benfluthiazide-D₅, triamterene-D₅, canrenone-D₆, all with a purity $\geq 98.0\%$.

Standards with certified reference materials. See Table A.1 in Appendix A for information on isotopic internal standards.

5.4 Preparation of standard solution

5.4.1 Standard stock solution (0.1 mg/mL). Weigh about 10 mg of each standard diuretic drug, weigh it accurately, dissolve in methanol and dilute to volume.

100mL brown volumetric flask, mix well, and prepare diuretic standard stock solution with a concentration of 0.1mg/mL. The standard triamterene should be used first

1mL dimethyl sulfoxide was dissolved, then diluted with methanol and adjusted to the mark. Stored at -20°C, the validity period was 6 months.

5.4.2 Internal standard stock solution (0.1 mg/mL). Weigh about 10 mg of each diuretic internal standard, accurately weighed, dissolve in methanol and make to volume.

into a 100mL brown volumetric flask, mix well, and prepare a diuretic internal standard standard stock solution with a concentration of 0.1mg/mL. Triamterene-D₅ standard

The product needs to be dissolved in 1mL dimethyl sulfoxide first, then diluted with methanol and fixed to the mark. Stored at -20°C, the validity period is 6 months.

5.4.3 Mixed standard working solution. Accurately measure the standard stock solution (0.1 mg/mL) of chlorothiazide, hydrochlorothiazide, fenfluthiazide and canrenone.

1 mL, standard stock solution of chlorthalidone, acetazolamide, furosemide, spironolactone, 4-amino-6-chlorobenzene-1,3-disulfonamide, triamterene

(0.1mg/mL) each 2.5mL, in the same 100mL brown volumetric flask, dilute to the mark with methanol, and prepare the mixed standard working solution

The concentration of chlorothiazide, hydrochlorothiazide, benfluthiazide and canrenone was 1 µg/mL; chlorthalidone, acetazolamide, furosemide, spironolactone and 4-amino-6-

The concentrations of chlorobenzene-1,3-disulfonamide and triamterene were 2.5 µg/mL. Stored in the dark below 4 degrees C, the validity period was 1 month.

5.4.4 Internal standard working solution. Accurately measure 1 mL each of the internal standard stock solution (0.1 mg/mL), put it in a 100 mL brown volumetric flask, and dilute to volume with methanol.

To the mark, prepare the internal standard working solution with a concentration of 1 µg/mL, store it in the dark below 4°C, and have a validity period of 1 month.