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NATIONAL STANDARD OF THE
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ICS 07.080

CCS A 40

GB/T 39995-2021

Determination of sterols

甾醇类物质的测定

Issued on: April 30, 2021

Implemented on: November 1, 2021

**Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC.**

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Determination of sterols

1 Scope

This document specifies a method for the determination of sterols by liquid chromatography-mass spectrometry/mass spectrometry.

This document applies to the determination of the content OF β -Cholestanol, brassicasterol, campesterol, stigmasterol, β -Sitosterol, fucosterol, lanosterol, stigmastanol, and ergosterol in the free state IN lard, rapeseed oil, walnuts, hawthorn, lettuce, kudzu root, rice, wheat, and black green beans.

2 Normative references

The contents of the following documents, through normative references in this text, constitute indispensable provisions of this document. Among them, for dated references, only the edition corresponding to that date applies to this document. For undated references, the latest edition (including all amendments) applies to this document.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

GB/T 15687 Animal and vegetable fats and oils - Preparation of test sample

3 Terms and definitions

The following terms and definitions apply to this document.

3.1

Sterols

A class of compounds containing a cyclopentano-perhydrophenanthrene carbon frame structure.

4 Principle

Use reflux of absolute ethanol to extract the sample. After the extract passes through a 0.22 μm nylon filter membrane, use liquid chromatography-mass spectrometry/mass spectrometer for determination; use the standard curve internal standard method for quantification.

5 Reagents or materials

Unless otherwise stated, the reagents used in this method are analytically pure. The water shall be Grade 1 water stipulated in GB/T 6682.

5.1 Methanol: Chromatographically pure.

5.2 Absolute ethanol: Chromatographically pure.

5.3 Sterol standard substances: β -Cholesterol, brassicasterol, campesterol, stigmasterol, β -Sitosterol, fucosterol, lanosterol, stigmastanol, ergosterol, and 6-Ketocholesterol. For compound information, see Table A.1 in Appendix A. The purity is $\geq 93\%$.

5.4 Internal standard stock solution: Accurately weigh an appropriate amount of 6-Ketocholesterol standard substance (accurate to 0.01 mg); use absolute ethanol to dissolve it and prepare an internal standard stock solution with a mass concentration of 500 mg/L. Store in the dark at 0 °C~4 °C for later use. The validity period is 1 month.

5.5 Internal standard working solution: Accurately pipette 2.0 mL of internal standard stock solution into a 1000 mL volumetric flask; add absolute ethanol to dilute and make up to volume and mix well; prepare an internal standard working solution with a mass concentration of 1.0 $\mu\text{g/mL}$. This solution is used right after it is prepared.

5.6 Sterol single standard stock solution: Accurately weigh an appropriate amount of sterol standard substances (accurate to 0.01 mg), respectively. Use absolute ethanol to dissolve and prepare into sterol single standard stock solutions with a mass concentration of 500 mg/L, respectively. Store in the dark at 0 °C~4 °C for later use. The validity period is 1 month.

5.7 Sterol mixed standard solution: Accurately pipette 2.0 mL of each sterol single standard stock solution into a 50 mL stoppered centrifuge tube; use nitrogen to blow dry at 40 °C. Accurately add 10 mL of internal standard working solution to redissolve and mix well; prepare a sterol mixed standard solution with a mass concentration of 100 mg/L. This solution is used right after it is prepared.

5.8 Sterol mixed standard working solution: Accurately pipette an appropriate amount of sterol mixed standard solution. Use the internal standard working solution to dilute AND prepare a series of sterol mixed standard working solutions with mass concentrations of 20 mg/L, 10 mg/L, 5 mg/L, 2.5 mg/L, 1.0 mg/L, 0.5 mg/L, and 0.25 mg/L, respectively. The solutions are used right after they are prepared.

5.9 0.22 μm nylon filter membrane.

of each sterol; refer to Appendix C for the multiple reaction monitoring diagram.

8.3 Parallel tests

According to the provisions of 8.1 and 8.2, carry out two parallel determinations on the same test sample.

9 Test data processing

The calculation of the content of the analyte in the sample is shown in formula (1):

$$X = \frac{\rho \times V}{m} \dots\dots\dots (1)$$

Where:

X - The content of the component tested in the test sample, in milligrams per kilogram (mg/kg);

ρ - The mass concentration of the tested component solution obtained from the internal standard working curve, in micrograms per milliliter (μg/mL);

V - The constant volume of test sample solution, in milliliters (mL);

m - The mass of test sample, in grams (g).

The calculation result shall be expressed as the arithmetic mean of two independent determination results obtained under repeatability conditions. The result is rounded to two decimal places.

10 Precision

The absolute difference between two independent determination results, which are obtained under repeatability conditions, must not exceed 15% of the arithmetic mean.

11 Other

See Table A.1 for the limits of quantification of the 9 sterols determined by this method.

Appendix B

(Informative)

Reference conditions for liquid chromatography-mass spectrometry/mass spectrometry parameters

The reference mass spectrometry conditions are as follows:

- a) Ion source: Atmospheric-pressure chemical ion source (APCI);
- b) Scanning mode: Positive ion mode;
- c) Detection method: Multiple reaction monitoring (MRM);
- d) Drying gas, atomizing gas, and collision gas are all high-purity nitrogen;
- e) Atomizing gas pressure: 0.14 MPa (20 psi);
- f) Ion spray voltage: 3000 V;
- g) Corona needle current: 4 μ A;
- h) Drying gas temperature: 350 °C;
- i) Atomizer temperature: 350 °C;
- j) Drying gas flow rate: 5.0 L/min;
- k) Quantitative ion pairs, qualitative ion pairs, collision gas energy, and in-source fragmentation voltage are shown in Table B.1.

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Contact: Wayne Zheng, Sales@ChineseStandard.net

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