GB

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PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Nutrition fortifier - (6S)-5-methyltetrahydrofolate, glucosamine salt

食品安全国家标准 食品营养强化剂 (6S)-5-甲基四氢叶酸，氨基葡萄糖盐

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1 Scope

This standard applies to the food nutrition fortifier (6S)-5-methyltetrahydrofolate, glucosamine salt made from folic acid as raw material through methylation, salification, crystallization, freeze-drying and other processes.

2 Chemical name, molecular formula, structural formula and relative molecular mass

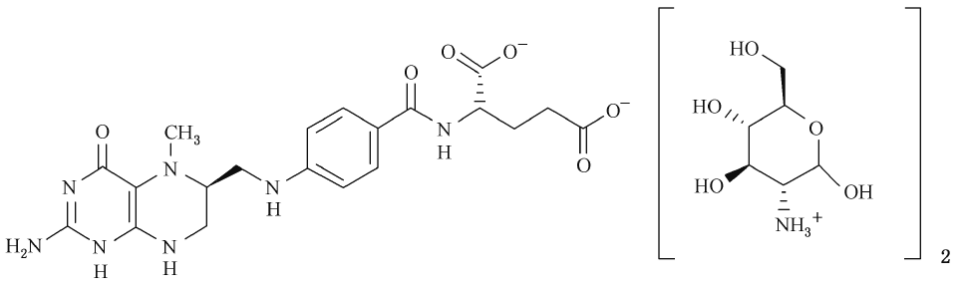
2.1 Chemical name

N-[4-[[[(6S)-2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridine]methyl]amino]benzoyl]-L-glutamic acid, glucosamine salt

2.2 Molecular formula

$C_{32}H_{51}N_9O_{16}$

2.3 structural formula



2.4 Relative molecular mass

817.80 (anhydrous) (according to the 2022 international relative atomic mass)

3 Technical requirements

3.1 Sensory requirements

Appendix A

Test methods

A.1 Safety instructions

Some of the reagents used in this Standard are toxic or corrosive and require caution when handling. If it is splashed on the skin, use water to rinse it immediately. If it is serious, seek medical attention immediately. When using volatile acids, do so in a fume hood.

A.2 General

The reagents and water that are used in this Standard, when no other requirements are specified, refer to analytical reagents and grade-3 water which is specified in GB/T 6682. The standard solutions, standard solutions for impurity determination, preparations and their products used in the tests shall be prepared in accordance with the provisions of GB/T 601, GB/T 602 and GB/T 603 unless otherwise specified. The solutions used in the test shall refer to aqueous solutions unless the solvent used is specified.

A.3 Identification test (IR spectrometry)

Use the potassium bromide tablet method and conduct the test in accordance with GB/T 32199. The infrared spectrum of the sample shall be consistent with that of the reference sample (see Figure B.1 for the spectrum of the reference sample).

A.4 Determination of (6S)-5-methyltetrahydrofolate, glucosamine salt and (6S)-5-methyltetrahydrofolate (dry basis)

A.4.1 Method summary

Separate the sample – which has been dissolved in water – by liquid chromatography; detect by ultraviolet detector or other equivalent detector; quantify by standard single-point method.

A.4.2 Reagents and materials

A.4.2.1 Water: Grade-1 water specified in GB/T 6682.

A.4.2.2 Acetonitrile: chromatographic pure.

A.4.2.3 Potassium dihydrogen phosphate.

A.4.2.4 Potassium hydroxide.

A.4.2.5 (6S)-5-methyltetrahydrofolate calcium standard: purity ≥ 98.0%, CAS number: 151533-22-1, or a national certified standard awarded the standard substance certificate.

A.4.2.6 Potassium hydroxide solution (20 g/100 mL): weigh 20 g of potassium hydroxide; dissolve it in water; fix the volume to 100 mL.

A.4.2.7 Potassium dihydrogen phosphate solution (pH 6.5): weigh about 6.80 g of potassium dihydrogen phosphate to an accuracy of 0.000 1 g; add 900 mL of water to dissolve; use potassium hydroxide solution (A.4.2.6) to adjust the pH to 6.5; add water to fix the volume to 1 000 mL; shake well; filter and ultrasonicate for later use.

A.4.2.8 Potassium dihydrogen phosphate-acetonitrile solution (pH 8.0): weigh about 4.08 g of potassium dihydrogen phosphate, accurate to 0.000 1 g; add 600 mL of water to dissolve; mix with 350 mL of acetonitrile; use potassium hydroxide solution (A.4.2.6) to adjust the pH to 8.0; add water to fix the volume to 1 000 mL; shake well; filter and ultrasonicate for later use.

A.4.3 Instruments and apparatuses

A.4.3.1 High performance liquid chromatograph: equipped with ultraviolet absorption detector, or other equivalent detectors.

A.4.3.2 Electronic balance: sensitivity is 0.000 1 g.

A.4.3.3 Acidity meter: accuracy is ±0.02 pH.

A.4.3.4 Ultrasonic cleaner.

A.4.4 Chromatographic reference conditions

A.4.4.1 Chromatographic column: C_{18} column, 250 mm × 4.6 mm, particle size 5 μm; or other equivalent chromatographic columns.

A.4.4.2 Flow rate: 1.0 mL/min.

A.4.4.3 Detection wavelength: 280 nm.

A.4.4.4 Column temperature: 25 ℃.

A.4.4.5 Injection volume: 10 μL.

A.4.4.6 Mobile phase: Mobile phase A is potassium dihydrogen phosphate solution (pH 6.5) (A.4.2.7), and mobile phase B is potassium dihydrogen phosphate-acetonitrile solution (pH 8.0) (A.4.2.8). Use gradient elution, the procedure of which is shown in Table A.1.

w_1 – mass fraction of (6S)-5-methyltetrahydrofolate (dry basis), %;

M_1 – molar mass of (6S)-5-methyltetrahydrofolate, glucosamine salt, in grams per mole (g/mol) ($M_{C_{32}H_{51}N_9O_{16}}$ = 817.80);

M_2 – molar mass of (6S)-5-methyltetrahydrofolate, in grams per mole (g/mol) ($M_{C_{20}H_{25}N_7O_6}$ = 459.45).

A.5 Determination of glucosamine (dry basis)

A.5.1 Method summary

Separate the sample – which has been dissolved in acetonitrile-water solution – by liquid chromatography; detect by ultraviolet detector or other equivalent detector; quantify by standard single-point method.

A.5.2 Reagents and materials

A.5.2.1 Water: Grade-1 water specified in GB/T 6682.

A.5.2.2 Acetonitrile: chromatographic pure.

A.5.2.3 Potassium hydroxide.

A.5.2.4 Potassium dihydrogen phosphate.

A.5.2.5 D-(+)-glucosamine hydrochloride standard: molar mass ($M_{C_6H_{13}NO_5 \cdot HCl}$) is 215.63 g/mol, purity ≥ 98.0%, CAS number: 66-84-2, or a national certified standard awarded the standard substance certificate.

A.5.2.6 50% acetonitrile solution: measure 500 mL of water and 500 mL of acetonitrile and mix well.

A.5.2.7 Potassium hydroxide solution (20 g/100 mL): weigh 20 g of potassium hydroxide; dissolve it in water; fix the volume to 100 mL; shake well and set aside.

A.5.2.8 Phosphate buffered saline solution (20 mmol/L): weigh 2.72 g of potassium dihydrogen phosphate and dissolve it in 900 mL water; use potassium hydroxide solution (A.5.2.7) to adjust the pH to 7.5; add water to fix the volume to 1 000 mL; shake well; filter and sonicate for later use.

A.5.3 Instruments and apparatuses

A.5.3.1 High performance liquid chromatograph: equipped with ultraviolet absorption detector, or other equivalent detectors.

A.5.3.2 Electronic balance: sensitivity is 0.000 1 g.

A.5.4 Chromatographic reference conditions

A.5.4.1 Chromatographic column: NH_2 column, 250 mm × 4.6 mm, particle size 5 μm; or other equivalent chromatographic columns.

A.5.4.2 Mobile phase: acetonitrile + 20 mmol/L phosphate buffered saline (75:25, volume ratio).

A.5.4.3 Flow rate: 1.5 mL/min.

A.5.4.4 Detection wavelength: 195 nm.

A.5.4.5 Column temperature: 35 ℃.

A.5.4.6 Injection volume: 10 μL.

A.5.5 Analysis steps

A.5.5.1 Preparation of glucosamine hydrochloride standard solution

Weigh 0.375 g of glucosamine hydrochloride standard to an accuracy of 0.000 1 g; place in a 100 mL volumetric flask; add 50 mL of 50% acetonitrile solution to dissolve; then use 50% acetonitrile solution to dilute to the mark; shake well; filter through a 0.45 μm filter membrane and inject immediately.

A.5.5.2 Preparation of sample solution

Weigh 0.350 g of the sample to an accuracy of 0.000 1 g; place in a 100 mL volumetric flask; add 50 mL of 50% acetonitrile solution to dissolve; then use 50% acetonitrile solution to dilute to the mark; shake well; filter through a 0.45 μm filter membrane and inject immediately.

Note: Solutions must be prepared and used immediately and cannot be stored for long periods of time.

A.5.5.3 System adaptability test

Inject the glucosamine hydrochloride standard solution 6 times to determine the relative standard deviation (RSD) of the peak area, tailing factor and theoretical plate number. Qualification criteria: RSD ≤ 2.0%, tailing factor ≤ 2.0, theoretical plate number ≥ 1 500.

A.5.5.4 Determination

Under the chromatographic conditions of A.5.4, first inject the glucosamine hydrochloride standard solution; perform chromatographic determination according to the above chromatographic conditions; record the chromatogram; take another sample solution and perform determination in the same way. See Figure C.2 for the reference chromatogram of glucosamine standard solution. The retention time of glucosamine is about 12.1 min.

A.6.2.6 Sodium hydroxide solution (10 g/100 mL): weigh 10 g of sodium hydroxide; dissolve it in water; fix the volume to 100 mL.

A.6.2.7 100 mmol/L sodium phosphate buffer solution: accurately weigh 12.0 g of sodium dihydrogen phosphate and dissolve it in 900 mL of water; use sodium hydroxide solution (A.6.2.6) to adjust the pH to 7.0 accurately; add water to fix the volume to 1 000 mL; shake well; filter and ultrasonicate for later use.

A.6.3 Instruments and apparatuses

A.6.3.1 High performance liquid chromatograph: equipped with ultraviolet absorption detector, or other equivalent detectors.

A.6.3.2 Electronic balance: sensitivity is 0.1mg.

A.6.3.3 pH meter.

A.6.3.4 Ultrasonic cleaner.

A.6.4 Chromatographic reference conditions

A.6.4.1 Chromatographic column: HSA chiral column, 100 mm × 4.0 mm, particle size 5 μm; or other equivalent chromatographic columns.

A.6.4.2 Mobile phase: isopropanol + 100 mmol/L sodium phosphate buffer solution (6:94, volume ratio).

A.6.4.3 Flow rate: 0.7 mL/min. Adjust the flow rate so that the retention time of (6S)-5-methyltetrahydrofolate is approximately 4.0 min.

A.6.4.4 Detection wavelength: 225 nm.

A.6.4.5 Column temperature: 30 ℃.

A.6.4.6 Injection volume: 5 μL.

A.6.4.7 Time: 20 minutes.

A.6.5 Analysis steps

A.6.5.1 Preparation of standard solution

Weigh about 0.025 g of (6R, S)-5-methyltetrahydrofolate calcium standard to an accuracy of 0.000 1 g; place in a 100 mL volumetric flask; dissolve in 90 mL of water; ultrasonicate at 20 °C for 1 min; use water to dilute to the mark. Pipette 5 mL of the solution into a 10 mL volumetric flask; use mobile phase to fix the volume; filter through a 0.45 μm filter membrane; inject immediately.

A.6.5.2 Preparation of sample solution

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