NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.309-2025

National food safety standard - Determination of asparagine and glutamine in foods

食品安全国家标准 食品中天冬酰胺和谷氨酰胺的测定

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1 Scope

1 This standard lays down the pre-column derivatization high performance liquid chromatographic method and the liquid chromatography tandem mass spectrometric method for the determination of asparagine and glutamine in foods. In the first method the direct extraction procedure serves for the determination of free asparagine and free glutamine in foods for special dietary uses, and the extraction after enzymatic digestion serves for the determination of total asparagine and total glutamine in foods for special dietary uses. The second method serves for the determination of total asparagine and total glutamine in foods for special dietary uses.

2 First method - principle

2 The sample is either extracted directly or digested with Streptomyces griseus protease; asparagine and glutamine then undergo a derivatization reaction with dansyl chloride, the derivatives are separated on a C18 column and detected with an ultraviolet detector, and quantification is against an external standard.

3 First method - reagents and materials

3 Unless otherwise stated the reagents used in this method are of analytical grade and the water is grade one water as specified in GB/T 6682.

3.1 The reagents listed are methanol and acetonitrile of chromatographic grade; dansyl chloride; anhydrous sodium carbonate; anhydrous disodium hydrogen phosphate; methylamine hydrochloride; tris(hydroxymethyl)aminomethane, that is Tris base; Streptomyces griseus protease of type XIV, from Streptomyces griseus, with an enzyme activity of at least 3.5 U/mg; phosphoric acid of chromatographic grade with a content of at

least 85 %; and concentrated hydrochloric acid.

3.2.1 The dansyl chloride solution of 2.0 g/L is prepared by weighing 0.2 g of dansyl chloride, dissolving it in acetonitrile and making up to 100 mL; it is prepared just before use.

3.2.2 The hydrochloric acid solution of 1.0 mol/L is prepared by taking 8.3 mL of concentrated hydrochloric acid and diluting it with water to 100 mL.

3.2.3 The Tris buffer of 0.05 mol/L is prepared by weighing 6.06 g of tris(hydroxymethyl)aminomethane into a 1000 mL beaker, dissolving it in 800 mL of water, adjusting the pH to 8.0 +/- 0.1 with 1.0 mol/L hydrochloric acid solution and diluting with water to 1000 mL. It is kept at 4 °C and used within 1 month.

3.2.4 The protease solution of 52.5 U/mL is prepared by weighing 0.15 g of Streptomyces griseus protease and dissolving it in Tris buffer to 10 mL; it is prepared just before use.

3.2.5 The sodium carbonate buffer solution of 60 mmol/L is prepared by weighing 0.318 g of anhydrous sodium carbonate, dissolving it in 40 mL of water, adjusting the pH to 9.5 +/- 0.1 with 1.0 mol/L hydrochloric acid solution and making up with water to 50 mL; it is prepared just before use.

3.2.6 The methylamine hydrochloride solution of 20 g/L is prepared by weighing 2.0 g of methylamine hydrochloride and dissolving it in water to 100 mL; it is kept at 4 °C and used within 3 months.

3.2.7 The disodium hydrogen phosphate solution of 10 mmol/L is prepared by weighing 1.42 g of anhydrous disodium hydrogen phosphate, dissolving it in 800 mL of water, adjusting the pH to 6.5 +/- 0.1 with phosphoric acid and diluting with water to 1000 mL.

3.2.8 The 20 % methanol solution is prepared by taking 20 mL of methanol and diluting it with water to 100 mL.

3.3 The standards are asparagine, CAS number 70-47-3, and glutamine, CAS number 56-85-9, each of purity not less than 99 %, or a certified reference material with a national certificate.

3.4.1 and 3.4.2 The asparagine and the glutamine standard stock solutions of 2.00 mg/mL are each prepared by weighing exactly 100 mg of the standard to 0.0001 g, dissolving it in 20 % methanol solution and making up to 50 mL. They are kept at 4 °C in the dark and used within 3 months.

3.4.3 The mixed standard intermediate solution of 20.0 μg/mL is prepared by taking exactly 1.00 mL of each of the asparagine and glutamine stock solutions into a 100 mL volumetric flask, making up to the mark with water and mixing; it is prepared just before use.

3.4.4 The series of mixed working standard solutions is prepared by taking suitable volumes of the mixed standard intermediate solution of 20.0 μg/mL into 10 mL volumetric flasks and making up to the mark with water, which gives mass concentrations of 0 μg/mL,

0.500 μg/mL, 1.00 μg/mL, 2.00 μg/mL, 5.00 μg/mL, 10.0 μg/mL and 20.0 μg/mL; the series is prepared just before use.

3.5 The materials are screw-capped glass bottles of 20 mL and organic microporous filter membranes of 0.22 μm.

5 First method - analytical procedure

5.1 A solid sample that is not a powder is crushed and mixed until uniform; a powdered solid sample or a liquid sample is shaken until uniform.

5.2.1 In the direct extraction procedure, 5.0 g of a solid sample is weighed to 0.001 g into a dry 50 mL beaker, dissolved in a suitable amount of warm water at 40 °C to 45 °C, cooled to room temperature, then transferred and made up to 25 mL. Exactly 1.00 mL of this is transferred to a 150 mL conical flask, about 25 mL of water is added and the flask is mixed on a vortex mixer, the pH is adjusted to 4.5 +/- 0.1 with 1.0 mol/L hydrochloric acid solution, the liquid is transferred to a 250 mL volumetric flask and made up to the mark with water and shaken, and the solution to be derivatized is obtained by centrifuging or filtering. For a liquid sample, 1.0 g to 5.0 g is weighed to 0.001 g into a 150 mL conical flask and the later steps are the same as for a solid sample.

5.2.2 In the enzymatic extraction procedure, 5.0 g of a solid sample is weighed to 0.001 g into a dry 50 mL beaker, dissolved in a suitable amount of warm water at 40 °C to 45 °C, cooled to room temperature, then transferred and made up to 25 mL. Exactly 1.00 mL of this is transferred to a 20 mL screw-capped glass bottle, and 0.5 mL of protease solution, 3.0 mL of Tris buffer and 0.2 mL of methanol are added in that order; the bottle is mixed on a vortex mixer and digested for 16 h in a thermostatic water bath at 37 °C. It is then taken out and cooled to room temperature, the whole digest is transferred to a 250 mL volumetric flask and made up to the mark with water and shaken, and the solution to be derivatized is obtained by centrifuging or filtering. For a liquid sample, 0.5 g to 1.0 g is weighed to 0.001 g into a 20 mL screw-capped glass bottle and the later steps are the same as for a solid sample.

5.3 For the derivatization reaction, exactly 1.00 mL of the solution to be derivatized is taken into a 15 mL centrifuge tube, 1.00 mL of sodium carbonate buffer solution and 1.00 mL of dansyl chloride solution are added, the tube is mixed thoroughly and left to react for 2 h at room temperature in the dark, being taken out and shaken to homogeneity after about 1 h. Then 0.10 mL of methylamine hydrochloride solution is added and mixed on a vortex mixer to stop the reaction, the tube is left to stand in the dark until precipitation is complete, and the liquid is filtered through a 0.22 μm membrane ready for measurement. Exactly 1.00 mL of each of the series of mixed working standard solutions is taken and derivatized at the same time as the sample solutions.

5.4 The reference chromatographic conditions are as follows: a C18 column of 250 mm by