NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.289-2023

National food safety standard - Determination of galactooligosaccharides in foods

食品安全国家标准 食品中低聚半乳糖的测定

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Standardization Administration of the PRC

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Foreword

This document was issued on 6 September 2023 by the State Administration for Market Regulation; Standardization Administration of the PRC and takes effect on 6 March 2024.

It is a GB standard without the /T suffix: compliance is mandatory in China.

1 Scope

GB 5009.289-2023 measures galactooligosaccharides in food, principally in infant formula, by high performance liquid chromatography with fluorescence detection. The principle clause sets out the chemistry: the oligosaccharides are extracted into aqueous solution, labelled with 2-aminobenzamide so that they fluoresce, and treated with amyloglucosidase to break down the maltodextrin and starch that would otherwise be counted as product; the derivatives are then separated and quantified against curves built from reference materials, with the lactose and maltose peaks tracked separately. Clauses on reagents and materials, instruments and equipment, the analytical procedure, calculation of the result, precision and other matters complete the method, and the annexes cover identification of other oligomeric reducing substances, the mobile phase elution programme, reference chromatograms of the derivatives, and assignment of retention windows for sugars of different degrees of polymerisation by liquid chromatography with mass spectrometry. A warning is carried for the reducing agents used in derivatisation, which are hazardous. Galactooligosaccharides are added to infant formula as a prebiotic and are declared on the label, so the figure has to hold up. Without the enzyme clean-up and the retention windows,

residual starch and lactose inflate the result and a product can appear to meet a claim it does not meet. Food laboratories, formula manufacturers and market surveillance authorities use it, and it is mandatory.

This Standard specifies the method of liquid chromatography for the determination of

galactooligosaccharides in foods.

This Standard is applicable to the determination of galactooligosaccharides in infant formula

foods (excluding infant formula foods for special medical purposes), infant auxiliary foods,

dairy products, beverages and baked foods.

This Standard is not applicable to the determination of galactooligosaccharides in foods that

contain other oligomeric reducing substances that may cause interference.

2 Principle

The galactooligosaccharides (n is 2 ~ 7) in the specimen is extracted by aqueous solution and

derivatized by 2-aminobenzamide, enzymatically hydrolyzed by amyloglucosidase to remove

the interference of maltodextrin and starch, and then, detected by high performance liquid

chromatograph - fluorescence detector. The collected chromatographic peaks are qualitatively

determined based on the retention time of galactooligosaccharides with the same degree of

polymerization determined by liquid chromatography - tandem mass spectrometry under the

same test conditions. Adopt the external standard method of maltose glycan reference material

corresponding to the degree of polymerization and relative molecular weight for quantitative

determination. After deducting the lactose and maltose content, the galactooligosaccharides

content is obtained.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure,

and

the water is Grade-1 water specified in GB/T 6682.

WARNING---sodium cyanoborohydride and 2-methylpyridine-N-borane are hazardous materials, which will release flammable gases when exposed to water and are dangerous to the environment. Please follow the instructions for use of the reagents. Please wear personal protective equipment and perform all operations under a fume hood.

3.1 Reagents

3.1.1 Dimethyl sulfoxide [(CH3)2SO]. chromatographically pure.

3.1.2 2-aminobenzamide (C7H8N2O).

3.1.3 Sodium cyanoborohydride (NaBH3CN).

3.1.4 2-methylpyridine-N-borane (C6H7N ? BH3).

3.1.5 Acetic acid (CH3COOH).

3.1.6 Ammonium acetate (CH3COONH4).

3.1.7 Ammonium formate (HCOONH4).

3.1.8 Formic acid (HCOOH).

3.1.9 Acetonitrile (CH3CN). chromatographically pure.

3.1.10 Amyloglucosidase (CAS. 9032-08-0). enzyme activity unit ? 60 U/mg.

NOTE. for the determination method of enzyme activity, see A.3 in GB 1866.174-2016.

3.2 Preparation of Reagents

3.2.1 Acetic acid-dimethyl sulfoxide solution (3 + 7, volume ratio). respectively measure-take

3 mL of acetic acid and 7 mL of dimethyl sulfoxide in a beaker, evenly mix it and reserve it for

later use.

3.2.2 2-aminobenzamide derivative solution (0.35 mol/L). accurately weigh-take 476 mg of 2-

amonibenzamide and 630 mg of sodium cyanoborohydride or 1,070 mg of 2-methylpyridine-

N-borane in a beaker, use 10 mL of acetic acid-dimethyl sulfoxide solution (3 + 7) to dissolve

it and evenly mix it.

3.2.3 Ammonium acetate solution (0.2 mol/L, pH 4.5 ? 0.1). weigh-take 1.54 g of ammonium

acetate, use 80 mL of water to dissolve it, then, use acetic acid to adjust pH to 4.5 ? 0.1, and

use water to dilute to 100 mL. Prepare it right before use.

3.2.4 Amyloglucosidase solution (about 180 U/mL). weigh-take amyloglucosidase equivalent

to about 900 U of activity and dissolve it in 5 mL of ammonium acetate solution (0.2 mol/L,

pH 4.5 ? 0.1), stir, until it is completely dissolved. Prepare it right before use.

3.2.5 Ammonium formate solution (50 mmol/L, pH 4.4 ? 0.1). weigh-take 3.15 g of ammonium

formate, use 900 mL of water to dissolve it, then, use formic acid to adjust pH to 4.4 ? 0.1, and

use water to dilute it to 1,000 mL. Prepare it right before use.

3.3 Reference Materials

3.3.1 Maltose reference material (C12H22O11, CAS. 69-79-4), purity ? 98%, or a standard

substance certified by the state and awarded a standard substance certificate.

3.3.2 Maltotriose reference material (C18H32O16, CAS. 1109-28-0), purity ? 98%, or a standard

substance certified by the state and awarded a standard substance certificate.

3.3.3 Maltotetraose reference material (C24H42O21, CAS. 34612-38-9), purity ? 95%, or a

standard substance certified by the state and awarded a standard substance certificate.

3.3.4 Maltopentaose reference material (C30H52O26, CAS. 34620-76-3), purity ? 95%, or a

standard substance certified by the state and awarded a standard substance certificate.

3.3.5 Maltohexaose reference material (C36H62O31, CAS. 34620-77-4), purity ? 95%, or a

standard substance certified by the state and awarded a standard substance certificate.

3.3.6 Maltoheptaose reference material (C42H72O36, CAS. 34620-78-5), purity ? 95%, or a

standard substance certified by the state and awarded a standard substance certificate.

3.3.7 Lactose reference material (C12H22O11, CAS. 63-42-3), purity ? 98%, or a standard