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Method of Test for 2′-Fucosyllactose in Foods

1. Scope

This method is applicable to the determination of 2′-fucosyllactose in infant formula.

2. Method

After extraction, 2′-fucosyllactose is determined by high performance ion chromatograph (HPIC).

2.1. Equipment

2.1.1. High performance ion chromatograph.

2.1.1.1. Detector: pulsed electrochemical detector.

2.1.1.1.1. Gold working electrode.

2.1.1.1.2. Ag/AgCl reference electrode.

2.1.1.2. Column: CarboPac PA20, 3 mm × 15 cm, or an equivalent product.

2.1.1.3. Guard column: CarboPac PA20 Guard, 3 mm × 3 cm, or an equivalent product.

2.1.2. Centrifuge: centrifugal force > 9000 ×g.

2.1.3. Ultrasonicator.

2.1.4. Vortex mixer.

2.1.5. Shaker.

2.2. Chemicals

50% Sodium hydroxide, HPIC grade;

Ethanol, reagent grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);

2′-Fucosyllactose, reference standard.

2.3. Apparatus

2.3.1. Volumetric flask: 1 mL and 20 mL.

2.3.2. Centrifuge tube: 50 mL, PP.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.4. 50% ethanol

Dilute 500 mL of ethanol with deionized water to 1000 mL.

2.5. Mobile phase

2.5.1. Solvent A, deionized water.

2.5.2. Solvent B

Dilute 10.5 mL of 50% sodium hydroxide with deionized water to 1000 mL. Filter with a membrane filter, and take the filtrate as the mobile phase B.

2.6. Standard solution preparation

Transfer about 5 mg of 2′-fucosyllactose reference standard accurately weighed to a 1-mL volumetric flask. Dissolve and dilute to volume with 50% ethanol as the standard stock solution. When to use, dilute appropriate volume of the standard stock solution with deionized water to 1~10 μg/mL as the standard solutions.

2.7. Sample solution preparation

Transfer about 1 g of the homogenized sample accurately weighed into a 10-mL volumetric flask. Add 10 mL of 50% ethanol, vortex-mix, ultrasonicate for 10 min, shake for 10 min, and dilute with 50% ethanol to 20 mL. Centrifuge at 9000 ×g for 30 min. Take 1 mL of the supernatant, and dilute 20 times with deionized water. Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 10 μL of the sample solution and the standard solutions into the HPIC separately, and operate according to the following conditions. Identify 2′-fucosyllactose based on the retention time. Calculate the amount of 2′-fucosyllactose in the sample by the following formula:

The amount of 2′-fucosyllactose in the sample (g/100 g)

$$= \frac{C \times V \times F}{M \times 10000}$$

Where,

C: the concentration of 2′-fucosyllactose in the sample solution calculated by the standard curve (μg/mL)

V: the final make-up volume of the sample (20 mL)

M: the weight of the sample (g)

F: dilution factor (20)

HPIC operating conditions(Note):

Detector: pulsed electrochemical detector.

Gold working electrode.

Ag/AgCl reference electrode.

Column: CarboPac PA20, 3 mm × 15 cm.

Guard column: CarboPac PA20 Guard, 3 mm × 3 cm.

Column temperature: 25℃.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0 → 15	90 → 35	10 → 65
15 → 15.1	35 → 0	65 → 100
15.1 → 25	0 → 0	100 → 100
25 → 25.1	0 → 90	100 → 10
25.1 → 35	90 → 90	10 → 10

Flow rate: 0.5 mL/min.

Injection volume: 10 μL.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. Limit of quantitation (LOQ) for 2′-fucosyllactose is 0.04 g/100 g.
2. Further validation should be performed when interference compounds appear in samples.

References

1. Auer, F., Jarvas, G. and Guttman, A. 2021. Recent advances in the analysis of human milk oligosaccharides by liquid phase separation methods. J. Chromatogr. B 1162: 112497.
2. Taiwan Food and Drug Administration. 2015. Method of test for sugars in foods (TFDAO0022.01). Published on December 12, 2015.

Reference chromatogram

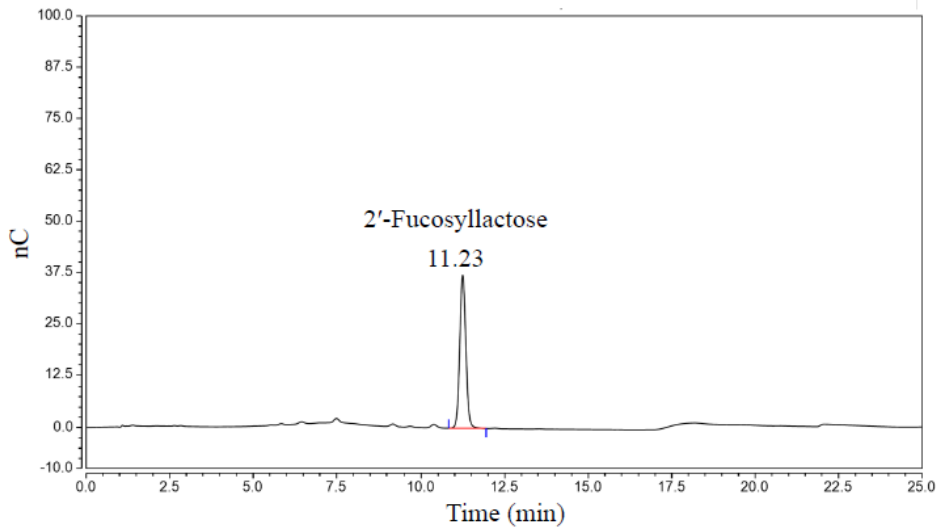

Figure. The HPIC chromatogram of 2′-fucosyllactose standard.