Application solution of galactooligosaccharides in infant formula milk powder by ion chromatography

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Foreword

Galactooligosaccharides(GOS) is a kind of functional oligosaccharide with natural attribute.In nature,there are trace amounts of GOS in animal milk, while human breast milk is abundant. The establishment of bifidobacterial florain infants depends largely on the GOS components in breast milk.

GOS is an excellent nutrient source and effective proliferator of beneficial bacteria such as bifidobacterium and lactobacillus acidophilus in human intestine. It can improve digestive and absorption function of human intestinal tract.The digestive function of the newborn is relatively weak, so infant milk powder has been added with galactooligosaccharides, it can not only improve the digestive function of the baby, but also promote the absorption of calcium in the baby, thereby enhancing the baby's immunity.

Implementation standard

AOAC official method 2001.02 detection method of GOS in food,Ion exchange chromatography

Reagents and standards

All the water used in the detection process of all the preparation solution must be 18MΩ DI water(ultrapure water).

3.1 Phosphate buffer：0.2M，pH6.0。22.0g KH_2PO_4 and 6.0g $K_2HPO_4 \cdot 3H_2O$ are dissolve in the water, diluted to 1L, then sterilized in autoclave at 120℃/30min;

3.2 Hydrochloric acid solution：1M; 8.3mL concentrated HCl is diluted to 1L with water;

3.3 Sodium hydroxide solution：50%，Hydrochloride free;

3.4 Sodium hydroxide solution：1M，54mL sodium hydroxide solution is diluted to 1L with water which is remove CO_2;

3.5 β-galactosidase solution:2000U/mL, 50000U/g β-galactosidase(produced from aspergillus oryzae fermentation) is suspended in the phosphate buffer, and the solution with final activity of 2000U/mL was obtained.One unit hydrolyzes o-Nitrobenzene-β-D-galactose at the condition of pH4.5/25℃, then generate o-

Nitrophenol and D-galactose. Store the suspension in the refrigerator when it is not in use, and fully mix the suspension before use. Use within 8 hours;

3.6 Acetonitrile：LC level；

3.7 Acetonitrile solution：20%(v/v),200mL acetonitrile is diluted to 1L with water;

3.8 Acetonitrile solution：3%(v/v),30mL acetonitrile is diluted to 1L with water;

3.9 Sodium acetate trihydrate：Sodium acetate trihydrate without water，reagent grade；

3.10 Mobile phase A：12.5mM NaOH，without carbonate;

3.11 Mobile phase B：125mM NaOH，without carbonate;

3.12 Mobile phase C：125mM NaOH(without carbonate) and 500mM Sodium acetate trihydrate;

3.13 Galactose：Without water；

3.14 Lactose：一 water(stable at 103℃);

3.15 Sugar standard storage solution: Dry galactose standard sample and lactose at 103℃ for 4 hours to constant weight.Accurately weigh 80mg galactose (S1), put it into a volumetric flask, dilute it to scale with water(0.8mg galactose /mL);Accurately weigh 150mg lactose monohydrate(S_2'),prepare lactose solution (1.425mg anhydrous lactose/mL) in the same way. S_2' multiplying 0.95 obtains the weight of anhydrous lactose(S_2), and compensates the weight of lactose crystal water.

3.16 Working standard solution:Remove S1 solution and S2 solution each 5.00mL (WS_1),and put them into a 1L volumetric flask and dilute them to scale;Repeat the above operation,remove the two kinds of solution for each 10.00mL(WS_2),15.00mL(WS_3) and 20.00mL(WS_4),then dilute them to 1L solution.

Solution	mLS1	mLS1	galactose +lactose
WS1	5.00	5.00	4ug/mL+7.125ug/mL
WS2	10.00	10.00	8ug/mL+14.25ug/mL
WS3	15.00	15.00	12ug/mL+21.375ug/mL
WS4	20.00	20.00	16ug/mL+28.5ug/mL

Configuration and chromatographic conditions

- Type：CIC-D120
- Infusion pump：Four element gradient pump
- IC column：PA20 sugar analysis column
- Eluent:gradient elution
- Flow rate：0.4mL/min
- Sample size：20μL

- Detection: pulsed amperometric detector(Au working electrode，Ag/AgCl reference electrode）

Refer gradient elution procedure:

Mobile phase(%)			
Time(Min)	A	B	C
0.00	95	5	0
20.10	95	5	0
35.00	0	100	0
36.00	0	100	0
36.10	0	0	100
46.00	0	0	100
46.10	95	5	0
61.00	95	5	0

pretreatment

Before analysis,make liquid sample homogeneity.Shatter hard particles, then filter by 1mm^2 sieve(No.18 sieve)

Extract

Weigh accurately to 1mg。Weigh 50mL plastic bottle with spiral lid(M_1). Weigh a certain amount of samples, containing GOS and lactose about 0.1-0.3g, but the sample size should not exceed 10g, then put it in this 50mL plastic bottle(M_2, test sample part).Add about 40mL hot phosphate buffer (about 80 degrees), cover the lid and mix.Let the plastic bottle water bath at 80±2℃,after stirring for 30 minutes,then put it in ice bath and cool it to room temperature.Measure pH value and adjust the pH value to 5.7-6.3 with 1M NaOH or 1M HCl. Dilut with phosphoric acid buffer to about 50mL.Weigh the weight of the bottle, cap and solution, and calculate and test the weight of the extract(M_3).

Enzymolysis

Solution treatment—Weighing plastic bottle with spiral lid(M4), take 20g extract and put it into the bottle, the net weight of sample is M_5. Take 1mL β-galactosidase solution,put it into a bottle,cover tightly with the bottle cap, and mix.

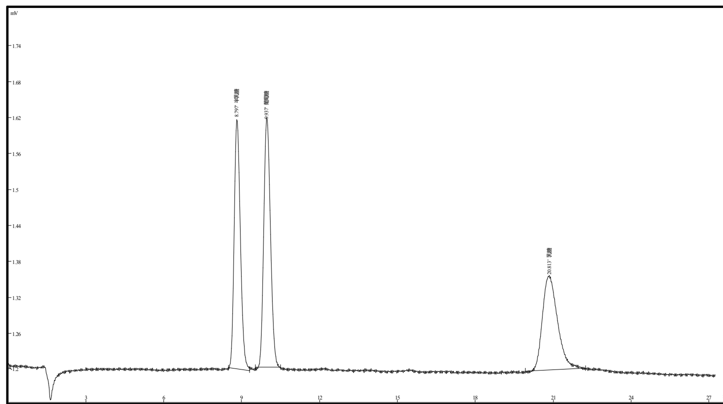
Initial test solution—Weighing plastic bottle with spiral lid(M7),take 1mL β-galactosidase solution and 1mL phosphate buffer and make them water bath for 10minutes at 100℃, then inactivate the enzyme and cool it.Take 20g extract, and put it into the bottle, the net weight of sample is M_8,cover tightly with the bottle cap, and mix.The above active enzyme solution and inactive enzyme solution were cultured at 60±2℃ for 30 minutes,mix it slightly and continuously,and begin to record the heating time when the

solution temperature reaches 60℃. Avoid generating foams or bubbles during mixing.And make it cool with ice bath.

Add 5mL 20% acetonitrile to 6.1 solution, mix, weigh the bottle (M6) with the lid tightly, add 4 mL 20% acetonitrile to 6.2 solution, mix, and weigh the bottle (M9) with the lid tightly.Centrifuge the solution(10000*g, 10 minutes),the supernatant is filtered by 0.2 μm membrane and the filtrate isconcentrated.The enzyme inactivation extract solution is called A1, and the hydrolytic extract solution is called A2.

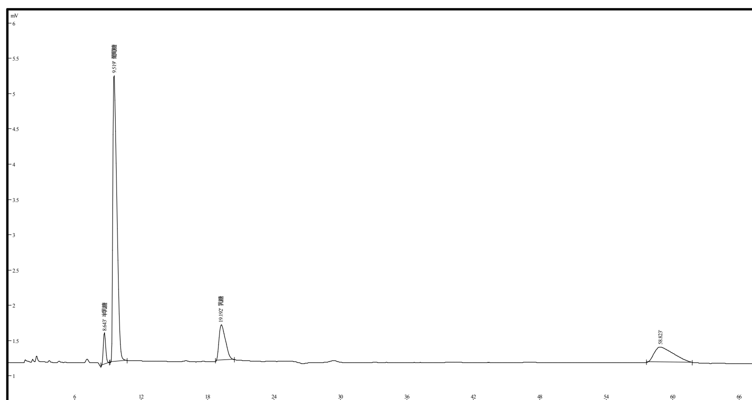
Test spectrum

Standard spectrogram:



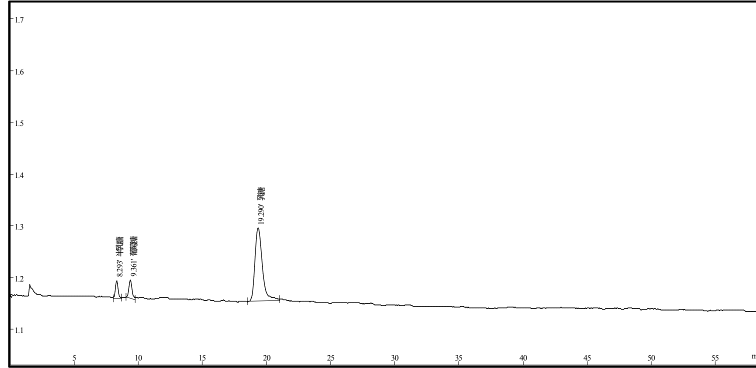
No.	Retention time	Name	Concentration	Peak area	Peak separation degree
1	8.797	Galactose	10	6945	2.48
2	9.937	Glucose	10	7494	14.05
3	20.813	Lactose	20	6452	0.00

Enzymatic hydrolysis samples spectrogram:



No.	Retention time	Name	Concentration	Peak area	Peak separation degree
1	8.643	Galactose	10.4	7223	1.63
2	9.519	Glucose	138.3	103655	11.12
3	19.192	Lactose	63.8	20584	18.45

Enzyme unhydrolyzed sample spectrogram:



No.	Retention time	Name	Concentration	Peak area	Peak separation degree
1	8.293	Galactose	0.8186	569	2.53
2	9.361	Glucose	0.8734	655	13.07
3	19.290	Lactose	18.26	5892	0.00

conclusion

In this paper, Galactooligosaccharides in milk powder is determined by ion chromatography and pulsed amperometric detection. The content of galactooligosaccharides was effectively separated and detected by optimized gradient elution procedure. The method has high sensitivity, wide linear range, good precision and accuracy, and can be used for the determination of galactooligosaccharides in milk powder and other samples.

Product presentation

CIC-D120

CIC-D120 is equipped temperature-control bipolar conductivity detector which can greatly improve the detection performance and stability of the instrument and can be compatible with ampere detector, UV-detector, and UV and post-column derivation device and so on. Combined with Sheng Han's leading chromatography column technology, it can analyze the anions, cations, cyanogen, iodide, sugar, small molecular organic acids etc.and is widely applied in the fields of environment, disease control, food, chemical industry, electronics, mining and metallurgy.

▷**Temperature-control bipolar conductivity detector**（CN 202033335U）

Greater detection range,better precise analysis

▷**Built-in circulating 3D constant temperature technology（CN 204259917U）**

Temperature stability time is less than 30 mins, ensuring the accuracy and reliability of test data

▷**The world's leading full-range series of ion chromatographic columns**

(CN 105126936A、CN 104788603A)

High efficiency, large capacity of the columns for detecting ions of varied compositions

▷**Self-Regenerating Electrolytic Micro-membrane Suppressor**（CN 102735792A）

High pressure resistance, small dead volume, highly responsive to signals

Ion chromatographic separation column

AS the first domestic developer and manufacturer of Ion chromatographic column , now Qingdao Shenghan have the technology of the development and production of three kinds of Ion chromatographic column including ion exchange chromatographic column, ion exclusion chromatographic column and ion pair chromatographic column. In addition, Sheng Han have also successfully developed and produced hydroxyl system of Ion chromatographic column in large scale ranking the second in the world, thus broken the monopoly of imported brands in the high-end ion chromatographic column field more than ten years.Accordingly the application of domestic IC column can reduce the cost of operation and maintenance of users by about 35%.