Determination of Fructan in Milk Powder by Ion Chromatography

Application industry

Food industry, Quality inspection

Key words

Fructan, Infant formula milk powder, Amperometric detector, Ion chromatography

Equipment and Instruments

CIC-D120 Ion Chromatograph, includes：

-Quaternary gradient pump
-PA20 column for sugar analysis
-Pulse amperometric detector, Au Working Electrode, Ag/AgCl Reference Electrode

Analysis condition

Chromatographic column ： Special column for sugar analysis

Eluent：

A：Ultra-pure water；

B：0.50M Sodium hydroxide solution；

C ： 0.15M Sodium hydroxide solution and 0.50M Sodium acetate solution

Flow Speed：0.8mL/min

Injection volume：25μL

Column temperature：30℃

Column pressure：4.9MPa

Reagent

Ultra-pure water: 18.2MΩ·cm;
Fructan reference material ($C_6H_{12}O_6$): purity (>99.0%);
Sodium hydroxide: GR;
Maleic acid ($C_4H_4O_4$): AR;
$NaBH_4$: AR;
CH_3COOH: AR;
$CH_3COONa.\ 3H_2O$: AR;
Sucrase: originated from yeast, enzyme activity (> 300U);
Fructanase: originated from Aspergillus niger, with enzyme activity (> 10 000 U);
50% NaOH: Chromatographic grade;
CH_3COONa: AR.

Pretreatment

Accurately weigh 1.0g (accurate to 0.001 g, containing fructan at least 5mg) sample in 150 mL conical bottle and then added 80 ℃ hot water about 50 mL. Shake 15 minutes at speed 150 r/min in 80 ℃ water bath shaker,

take out of the bottle and cool to room temperature. Transfer constant volume to 100 mL, centrifuge the solution at a high speed and then dilute the supernatant to an appropriate multiple.

Remove 200 μL reserve solution into a 10 mL glass tube with plug and estimate the sucrose content in the sample solution. According to the addition of 300 mL sucrase solution per milligram of sucrose, the solution is mixed by eddy oscillation. It is placed in a constant temperature water bath shaker at 40 ℃. After shaking for 60 minutes at 150 r/min, 300 μL sodium borohydride solution was added. The eddy oscillation is mixed and placed in a constant temperature water bath shaker at 40 ℃ with 150 r/min shaking for 30 minutes. After that, take out and cool to room temperature. The possible fructan content in the sample solution is estimated by adding 750 μL acetic acid solution and standing for 10 minutes. According to the addition of 1.2 mL fructanase solution per milligram of fructan, the whirlpool is mixed and placed in a constant temperature water bath shaker at 40 ℃. After shaking for 30 minutes at 150 r/min, the sample is cooled to room temperature and transferred to 10 mL volumetric bottle, rinsing three times and shook well.

Activate the purification column. The test solution is passed through 0.22 μm water phase filter membrane and purification column in turn. After discarding three times eluent of purification column volume, collect the remaining to be tested, and do reagent blank experiment at the same time.

Results and discussions

Sugars are separated on anion exchange column after ionization in alkaline eluent by ion chromatography. In this paper, a special sugar column for sugar detection is selected, which has good separation effect from fructan to other monosaccharides and disaccharides.

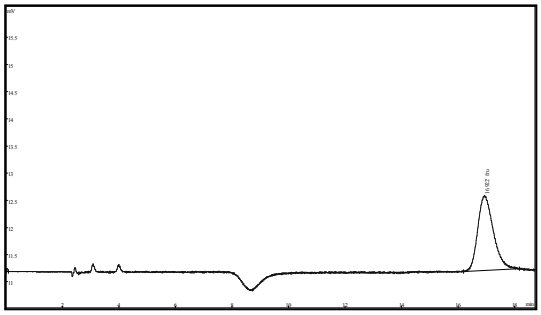

Fig. 1 Ion chromatogram of fructan standard solution

Reproducibility

To investigate the reproducibility of this method, 7 consecutive injections of fructan working solution with the same concentration were carried out to measure the relative standard deviation (RSD) of retention time (t_R) and peak area (A) of the ions measured. The results are shown in Table 1.

Table 1 Relative standard deviations of fructan retention time and chromatographic peak area

	t_R RSD (%)	A RSD (%)
Fructan	1.05	1.92

Linear range and detection limit

The standard working solution of fructan was injected sequentially, the concentration of fructan (c, μg/L) was taken as abscissa coordinate, the peak area of chromatogram (A, mV·min) was taken as ordinate, and the standard working curve was drawn. The linear equation was A=7036c-1596, the linear correlation coefficient was 0.9984, and the linear range was 0.8-20mg/L.

Sample analysis

The content of fructan in milk powder was 4.32 mg/L after sample treatment. The degree of aggregation of fructan and fructooligosaccharide was n=4 in calculation. The detection value of Fructooligosaccharide was 26.52 g/kg, which was close to the label value. It showed that the method was feasible.

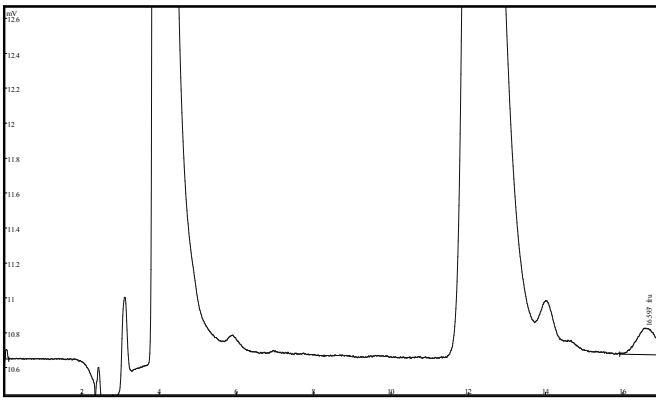

Fig. 2 Ion Chromatography of a Milk Powder Sample

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