

Sensitive Simultaneous Analysis of Lactose, Allolactose, Lactulose and Saccharides Contained in Low Lactose Dairy Products Using HPLC with Post-column Fluorescence Derivatization Method

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User Benefits

- ◆ Simultaneous analysis of lactose, allolactose, lactulose and saccharides contained in low lactose dairy products is possible.
- ◆ Highly sensitive analysis of saccharides can be provided using the post-column fluorescence derivatization method.
- ◆ Suitable for the quantitation of trace amounts of lactose, allolactose and lactulose in dairy product.

Introduction

Lactose is a disaccharide composed of galactose and glucose that is found in dairy products. Lactose intolerance refers to the inability to metabolize lactose resulting in diarrhea or other symptoms. Various low lactose and lactose-free products have been developed for people with lactose intolerance. Most of these products reduce the amount of lactose by enzymatic hydrolysis, but the final product may contain small amounts of residual lactose due to incomplete hydrolysis. Some EU countries have set their own threshold levels for the use of the terms "low lactose", and "lactose-free", for foodstuffs other than that intended for infants. These threshold levels are set from 0.01~1 g/100 g of final products. However, currently there are no specific regulations of lactose concentration limits for lactose-free products in the USA, EU, or other regions, except for infant and follow-on formula as less than 10 mg/100 kcal. Allolactose and lactulose may be found in dairy products. It is difficult to separate them from lactose because of their similar chemical structures. Figure 1 shows the chemical structures of lactose, allolactose and lactulose.

This article introduces a sensitive simultaneous analysis of lactose, allolactose, lactulose and other 6 saccharides which were separated by hydrophilic interaction liquid chromatography (HILIC) then detected by fluorescence detector after post-column derivatization with boric acid-arginine. In addition, these 9 saccharides contained in low lactose milk were quantified.

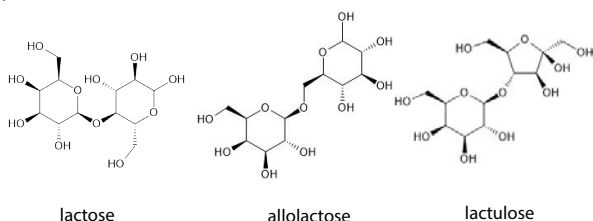


Fig. 1 Chemical Structures of Lactose, Allolactose and Lactulose

Experiment

Table 1 shows the analytical conditions and Figure 2 shows the appearance (a), flow path diagram (b) of the Nexera reducing sugar analysis system used in this article. In this system, saccharides are separated by a HILIC column, and arginine is used as a reaction reagent for post-column derivatization and fluorescence detection. This detection method takes advantage of the fact that saccharides react with arginine in the presence of boric acid to form highly fluorescent derivatives. The derivatized saccharides are detected with high sensitivity by the fluorescence detector. This system enables the analysis of saccharides at low concentrations, which was difficult with differential refractive index detector.

Table 1 Analytical Conditions

System	: Nexera Reducing Sugar Analysis System
<Separation>	
Column	: Asahipak NH2P-50 4E (250 mm × 4.6 mm I.D., 5 μm)
Guard Column	: Asahipak NH2P-50G 4A (10 mm × 4.6 mm I.D., 5 μm)
Mobile Phase A	: Water /85% Phosphoric acid = 1000 : 3
Mobile Phase B	: Acetonitrile /85% Phosphoric acid = 1000 : 3
Flow Rate	: 0.8 mL/min
Time Program	: B Conc. 90% (0 min) - 89% (90 min) -78% (110-120 min) - 90% (120.01-150 min)
Mixer Capacity	: 1.7 mL
Column Temp.	: 45 °C
Injection Vol.	: 10 μL
Vial	: SHIMADZU LabTotal™ Vial for LC 1.5 mL, Glass *1
<Post-column reaction>	
Reaction Reagent	: Mixed aqueous solution of 5 g/L arginine, 0.4 mol/L borate and 0.2 mol/L potassium hydroxide
Flow Rate	: 0.5 mL/min
Reaction Temp.	: 150 °C
Detection	: Ex. 320 nm, Em. 430 nm (RF-20AXS)
Cell Temp.	: 25 °C
Reaction Coil	: SUS tubing, 8 m×0.5 mm I.D.

*1 P/N : 227-34001-01

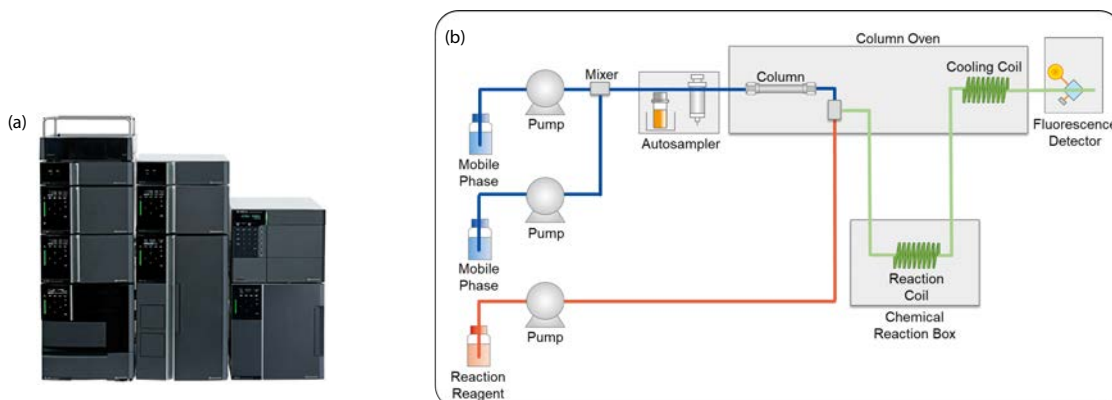


Fig. 2 Nexera™ Reducing Sugar Analysis System
(a) appearance (b) flow path diagram

■ Analysis of Standard Solution

A standard solution of 9 saccharides (xylose, arabinose, fructose, mannose, glucose, galactose, lactulose, lactose, allolactose) was analyzed. The chromatogram is shown in Figure 3. These 9 saccharides are well separated.

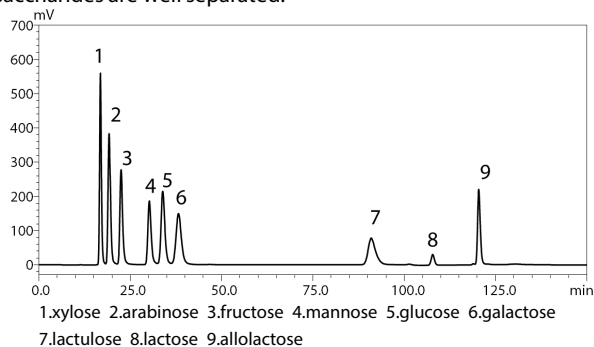


Fig. 3 Chromatogram of Standard Solution

(Concentration of lactose : 400 mg/L; allolactose and lactulose : 200 mg/L;
other 6 saccharides : 100 mg/L)

■ Calibration Curve and Limit of Quantification

Calibration curves were obtained for 9 saccharides, with contribution ratios r^2 are greater than 0.9999. Figure 4 shows the representative calibration curves. The limit of quantification were also calculated from the S/N ratio of the standard solution. Table 2 summarizes the concentration range of the calibration curve, the contribution ratio and the limit of quantification.

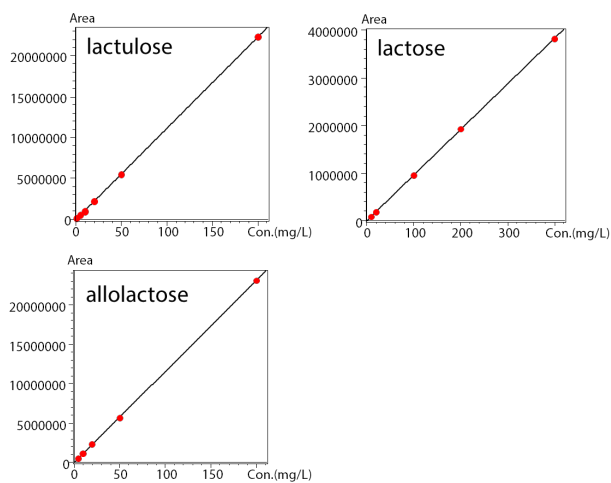


Fig. 4 Representative calibration curves

Table 2 Concentration Range of Calibration Curve, Contribution Ratio and Limit of Quantification

Compound	Conc. range (mg/L)	r^2	Limit of quantification (mg/L)
1 xylose	2.5-100	0.99997	0.020
2 arabinose	2.5-100	0.99991	0.030
3 fructose	2.5-100	0.99997	0.041
4 mannose	2.5-100	0.99994	0.062
5 glucose	2.5-200	0.99997	0.053
6 galactose	2.5-200	0.99998	0.076
7 lactulose	1-200	0.99994	0.294
8 lactose	10-400	0.99997	1.427
9 allolactose	5-200	0.99997	0.103

■ Quantitative Analysis of Low Lactose Milk

Quantitative analysis of low lactose milk was performed. 100 μ L of low lactose milk was extracted with 900 μ L of 70% acetonitrile aqueous solution, and the extracts were then centrifugated (10,000 rpm, 10 min). The supernatant was then filtered through a 0.2 μ m membrane filter and diluted 10 times with 70% acetonitrile aqueous solution. Finally, low lactose milk after sample preparation (100 times dilution) was analyzed and Figure 5 shows the chromatogram. Glucose, galactose, lactulose, lactose and allolactose were detected from the low lactose milk. Table 3 shows the concentrations of the saccharides in the low lactose milk after sample preparation.

Furthermore, three low lactose milk samples were spiked with standards of 9 saccharides and then sample preparation was performed. Table 4 shows the average recovery rates obtained from the results of 3 samples.

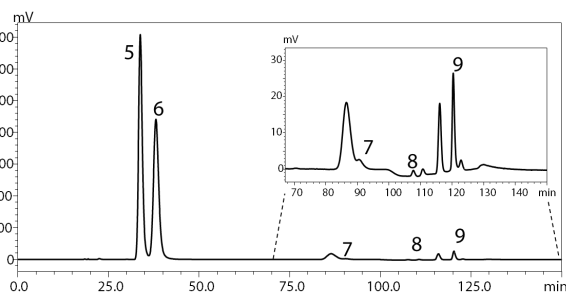


Fig. 5 Chromatogram of low lactose milk (100 times dilution)

Table 3 Concentration of Saccharides in Low Lactose Milk (100 times dilution)

Compound	Concentration (mg/L)
1 xylose	Not detected
2 arabinose	Not detected
3 fructose	Not detected
4 mannose	Not detected
5 glucose	171.6
6 galactose	153.3
7 lactulose	3.8
8 lactose	10.8
9 allolactose	12.8

Table 4 Average Recovery Rate (n=3)

Compound	Spike concentration (mg/L)	Average recovery rate (%) (n=3)
1 xylose	5	103.6
2 arabinose	5	102.7
3 fructose	5	104.1
4 mannose	5	102.0
5 glucose	5	100.7
6 galactose	5	103.8
7 lactulose	10	102.1
8 lactose	20	97.8
9 allolactose	10	94.1

■ Conclusion

Sensitive simultaneous analytical method of lactose, allolactose, lactulose and saccharides contained in low lactose dairy products using HPLC with post-column fluorescence derivatization method was developed. Saccharides contained in low lactose milk were quantified and the recovery rates were good. Since this method is highly sensitive, it can be used to determine trace amounts of lactose, allolactose and lactulose in dairy products.

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