

**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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1. 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 structure. Figure 1 shows the chemical structures of lactulose, lactose, and allolactose. This study describes 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.

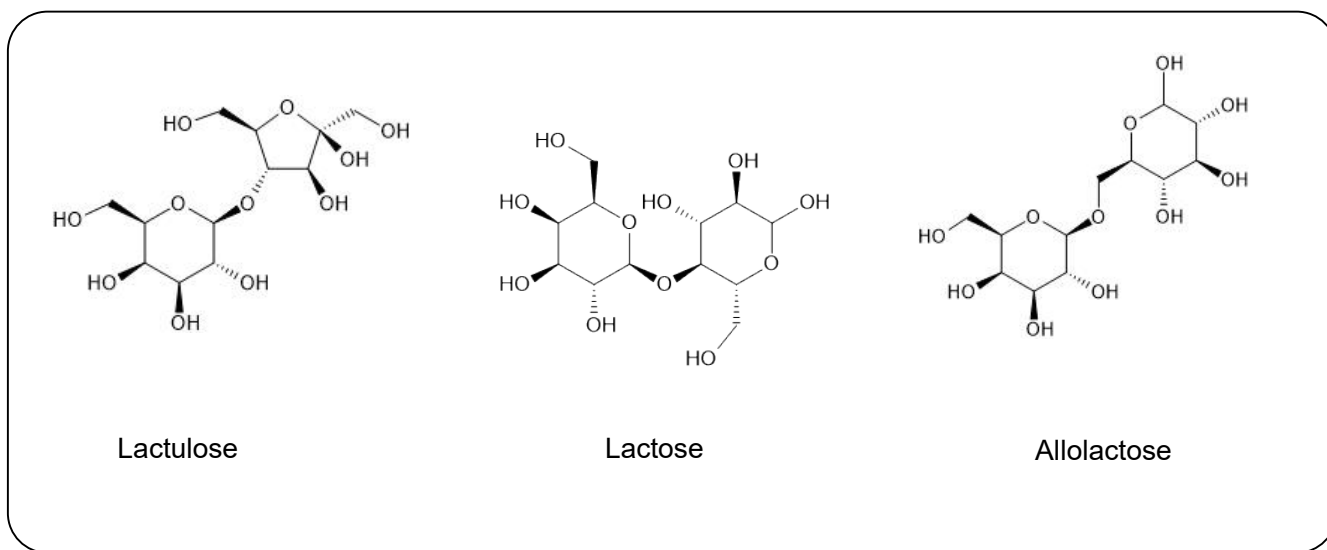


Figure 1 Chemical structures of lactose, allolactose and lactulose

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2. Experiment

Figure 2 shows the appearance(a) and flow diagram(b) of the Nexera™ reducing sugar analysis system used in this study. 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(RF-20Axs). This system enables the analysis of saccharides at low concentrations, which was difficult with differential refractive index detector. Table 1 shows the analytical conditions.

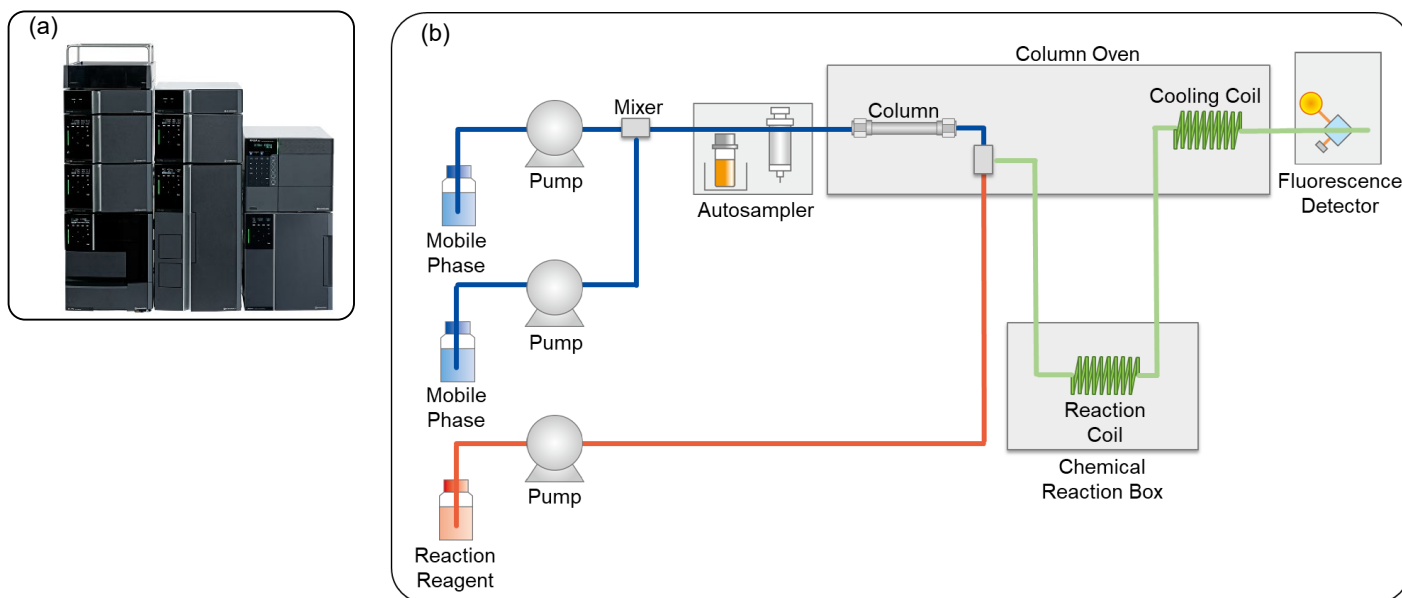


Figure 2 Nexera Reducing Sugar Analysis System (a) appearance (b) flow diagram

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2. Experiment

Table 1 Analytical Conditions

System : Nexera Reducing Sugar Analysis System

<Separation>

Column : Shodex Asahipak NH2P-50 4E (250 mm × 4.6 mm I.D., 5 μm)
Guard Column : Shodex 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™ for LC 1.5 mL, Glass

<Post-column Reaction>

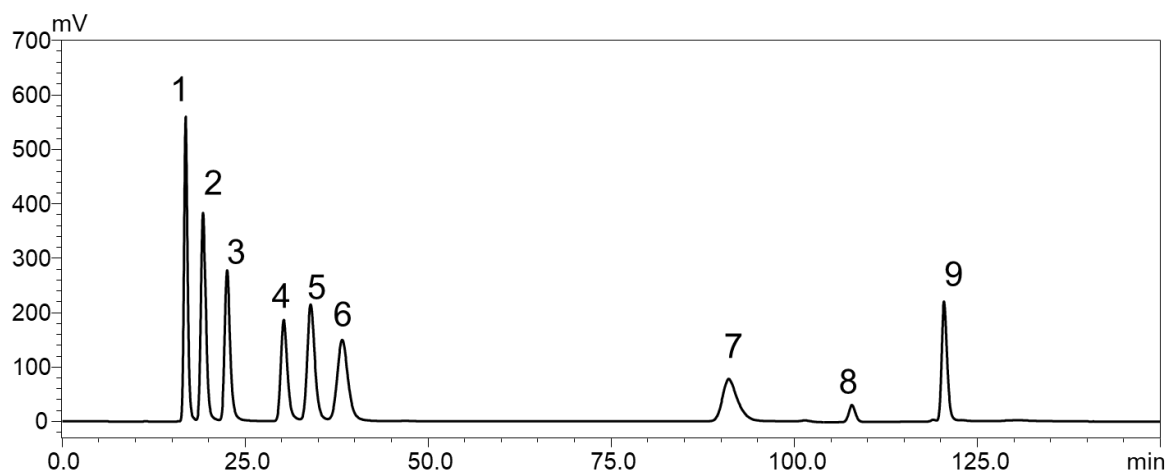
Reaction Reagent : Mixed aqueous solution of 5 g/L arginine,
0.4 mol/L borate and 0.2 mol/L potassium hydroxide
Flow Rate : 0.5 mL/min
Reaction Temp. : 150 °C
Fluorescence Detection : Ex. 320 nm, Em. 430 nm (RF-20Axs)
Cell Temp. : 25 °C
Reaction Coil : SUS tubing, 8 m × 0.5 mm I.D.)

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3. Result

3.1. Analysis of Standard Solution

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



1.xylose 2.arabinose 3.fructose 4.mannose 5.glucose 6.galactose
7.lactulose 8.lactose 9.allolactose

Figure 3 Chromatogram of Standard Solution of 9 Saccharides
(Concentration of saccharides except lactulose, lactose, allolactose : 100 mg/L ;
lactulose, allolactose : 200 mg/L ; lactose : 400 mg/L)

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3. Result

3.2. Calibration Curve and Limit of Quantification

The linearities of the calibration curves of the 9 saccharides were good. The 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.

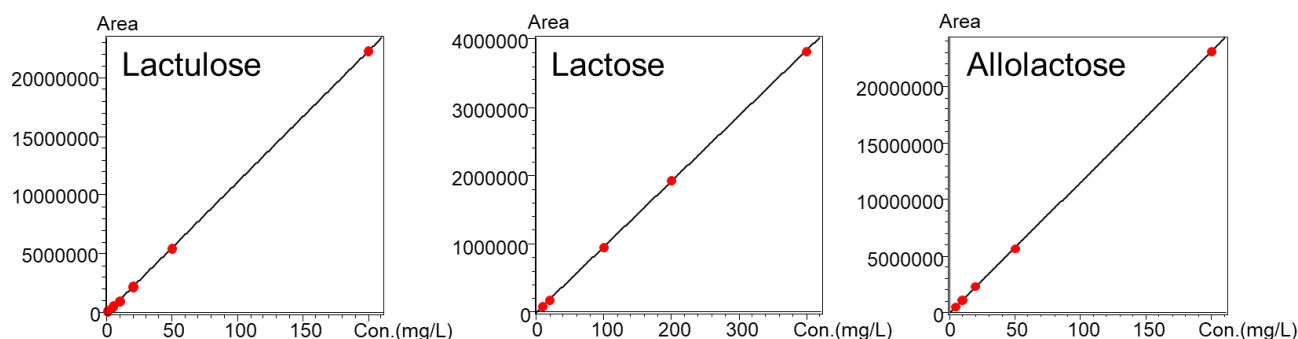


Figure 4 Representative calibration curves

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

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3. Result

3.3. Quantitative Analysis of Low Lactose Milk

Quantitative analysis of low lactose milk was performed. Low lactose milk was extracted with a 70% acetonitrile aqueous solution. Extracts were then prepared by centrifugation and filtration. 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.

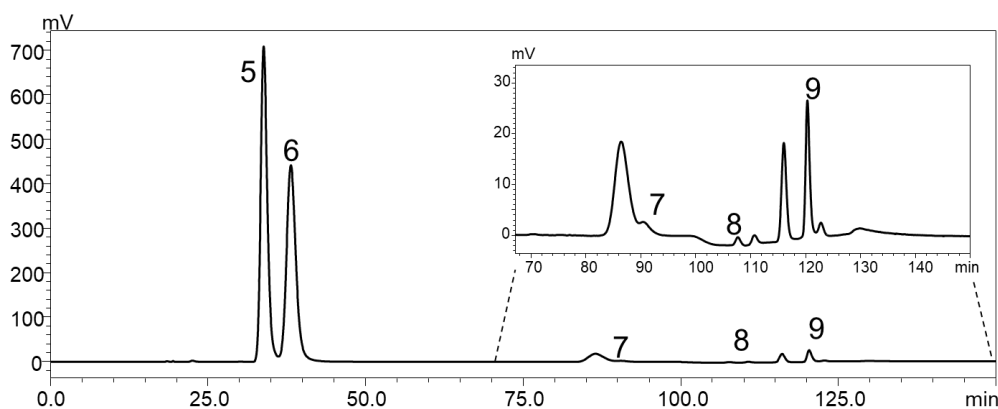


Figure 5 Chromatogram of low lactose milk (100 times dilution)

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

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4. Summary

- 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, lactulose and allolactose in dairy products.

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