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# Separation of Lactose, Lactulose and Epilactose by a new HILIC column

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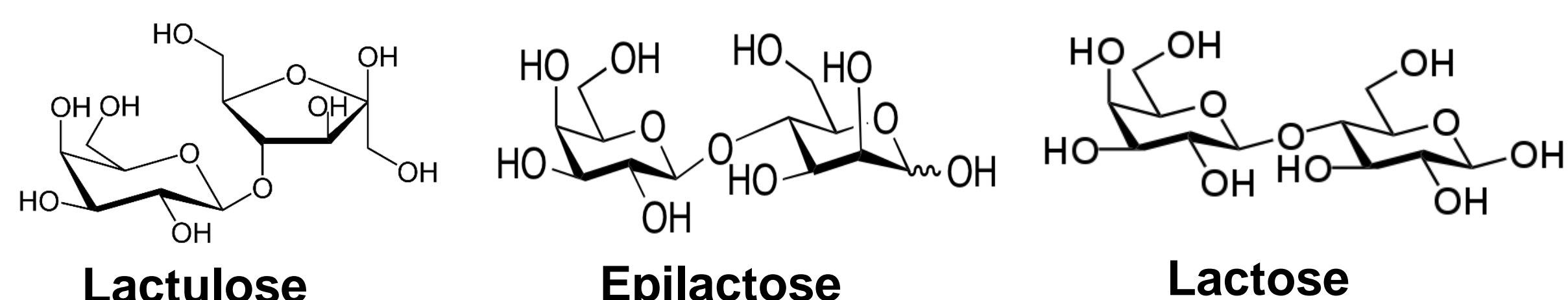
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## Abstract

The HPLC separation of lactose, lactulose and epilactose is outlined in the United States Pharmacopeia Convention (USP). Lactulose, a semi-synthetic disaccharide, is a useful indicator for heat induced modifications in milk. Lactulose and epilactose, isomers of lactose, can be derived from lactose.



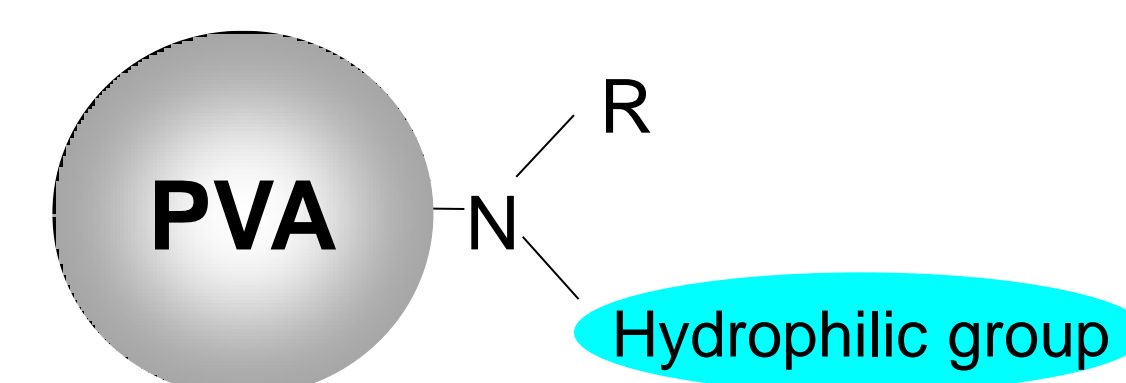
HPLC separation described in the USP method is achieved on an L8 amino propyl functionalized silica support column. Herein, we describe an improved separation for lactulose, epilactose, and lactose on Shodex HILICpak VG-50 4E, a new amino HILIC column.

We have demonstrated the effective, simultaneous separation of lactose, lactulose, and epilactose using the Shodex HILICpak VG-50 4E column (4.6 mm ID x 250 mm), featuring polyvinyl alcohol packing material modified with tertiary amino functional groups. The separation occurs within 15 minutes with resolution >1.6. The calibration curves have demonstrated linearity between 500 µg/L and 10 g/L with RI detection.

## Introduction of Shodex™ HILICpak VG-50

### Product specifications and Line up

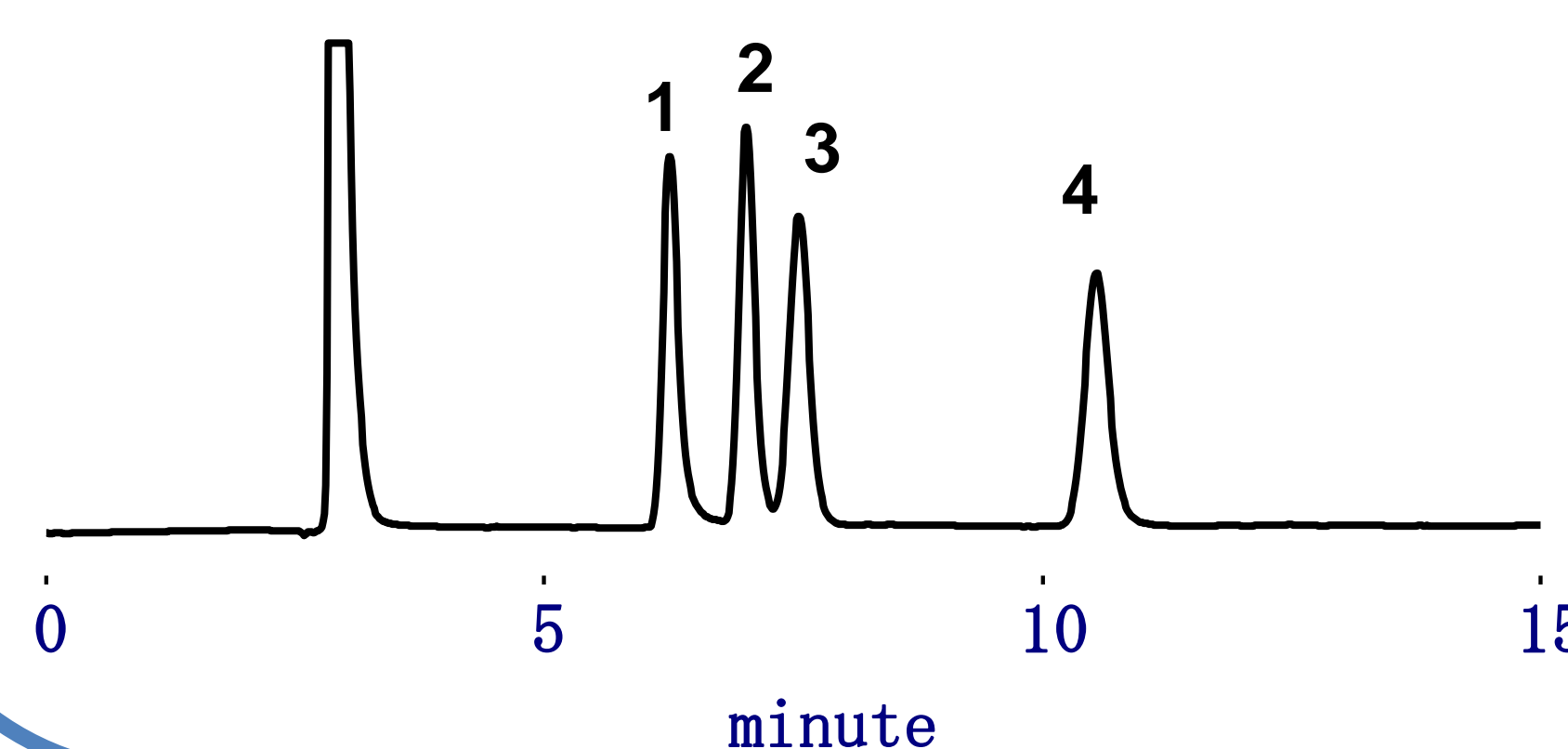
Base Material	Polyvinyl Alcohol
Housing Material	Stainless Steel
Usable Temp.	4–60deg-C
Usable pH Range	2–13
Maximum Flow Rate	1.5 mL/min
Maximum Pressure	15.0 MPa



Base material of VG-50

Product Name	Plate Number (TP/Column)	Functional Group	Particle Size (µm)	Pore Size (Å)	Column Size (ID x length mm)
HILICpak VG-50 4D	5,500	Amino	5	100	4.6 x 150
HILICpak VG-50 4E	7,500	Amino	5	100	4.6 x 250
HILICpak VG-50G 4A	Guard	Amino	5	-	4.6 x 10

### Example of saccharide analysis

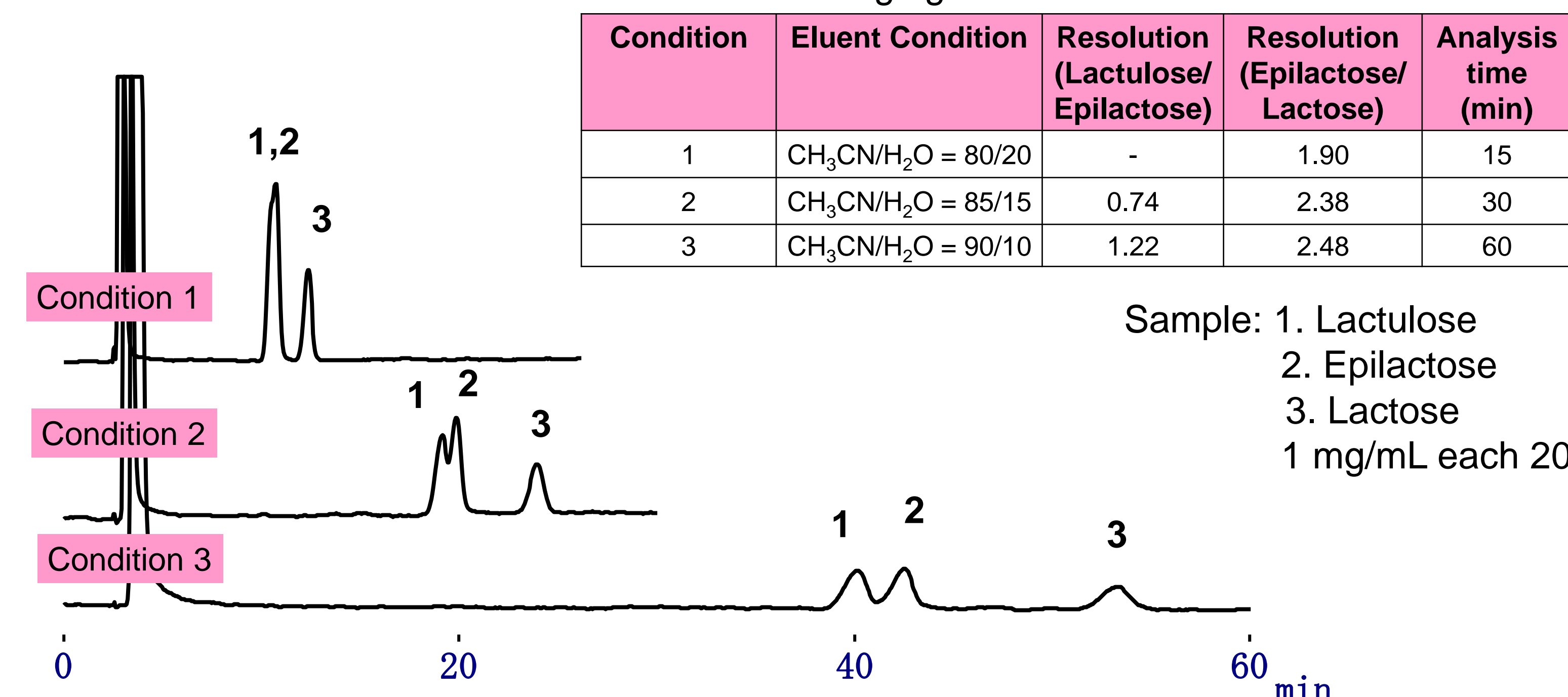


Sample: 1. Fructose  
2. Mannose  
3. Glucose  
4. Sucrose  
1 mg/mL each 20µL

Eluent: CH<sub>3</sub>CN/H<sub>2</sub>O=80/20  
Flow rate: 1.0 mL/min  
Detector: RI  
Column temp.: 40 °C

## Mixture of Acetonitrile and Water as Eluent

Resolution and retention time were studied changing the concentration of acetonitrile.



Condition	Eluent Condition	Resolution (Lactulose/Epilactose)	Resolution (Epilactose/Lactose)	Analysis time (min)
1	CH <sub>3</sub> CN/H <sub>2</sub> O = 80/20	-	1.90	15
2	CH <sub>3</sub> CN/H <sub>2</sub> O = 85/15	0.74	2.38	30
3	CH <sub>3</sub> CN/H <sub>2</sub> O = 90/10	1.22	2.48	60

Sample: 1. Lactulose  
2. Epilactose  
3. Lactose  
1 mg/mL each 20µL

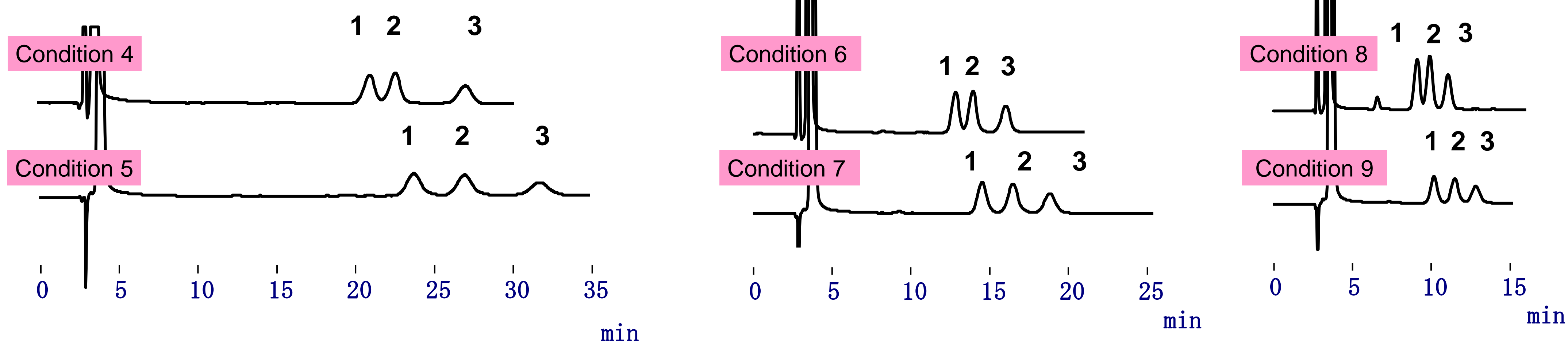
Column: VG-50 4E (4.6 x 250 mm)  
Eluent : See table  
Flow rate: 1.0 mL/min  
Detector: RI  
Column temp.: 40 °C

Saccharides were not separated with 80% and 85% of acetonitrile.  
Saccharides were separated with 90% of acetonitrile; however, the analysis time was 60 minutes.

## Mixture of Acetonitrile, Methanol, and Water as Eluent

Addition of methanol to the eluent decreases analysis time by half. The reduction of analysis time is maximized at 10% methanol.

Further reduction of acetonitrile in the presence of methanol was able to shorten the analysis time by half.



Column: VG-50 4E (4.6 x 250 mm)  
Eluent : See table  
Flow rate: 1.0 mL/min  
Detector: RI  
Column temp.: 40 °C  
Sample: 1. Lactulose  
2. Epilactose  
3. Lactose  
1 mg/mL each 20µL

### Analysis time and degree of separation in each analysis conditions

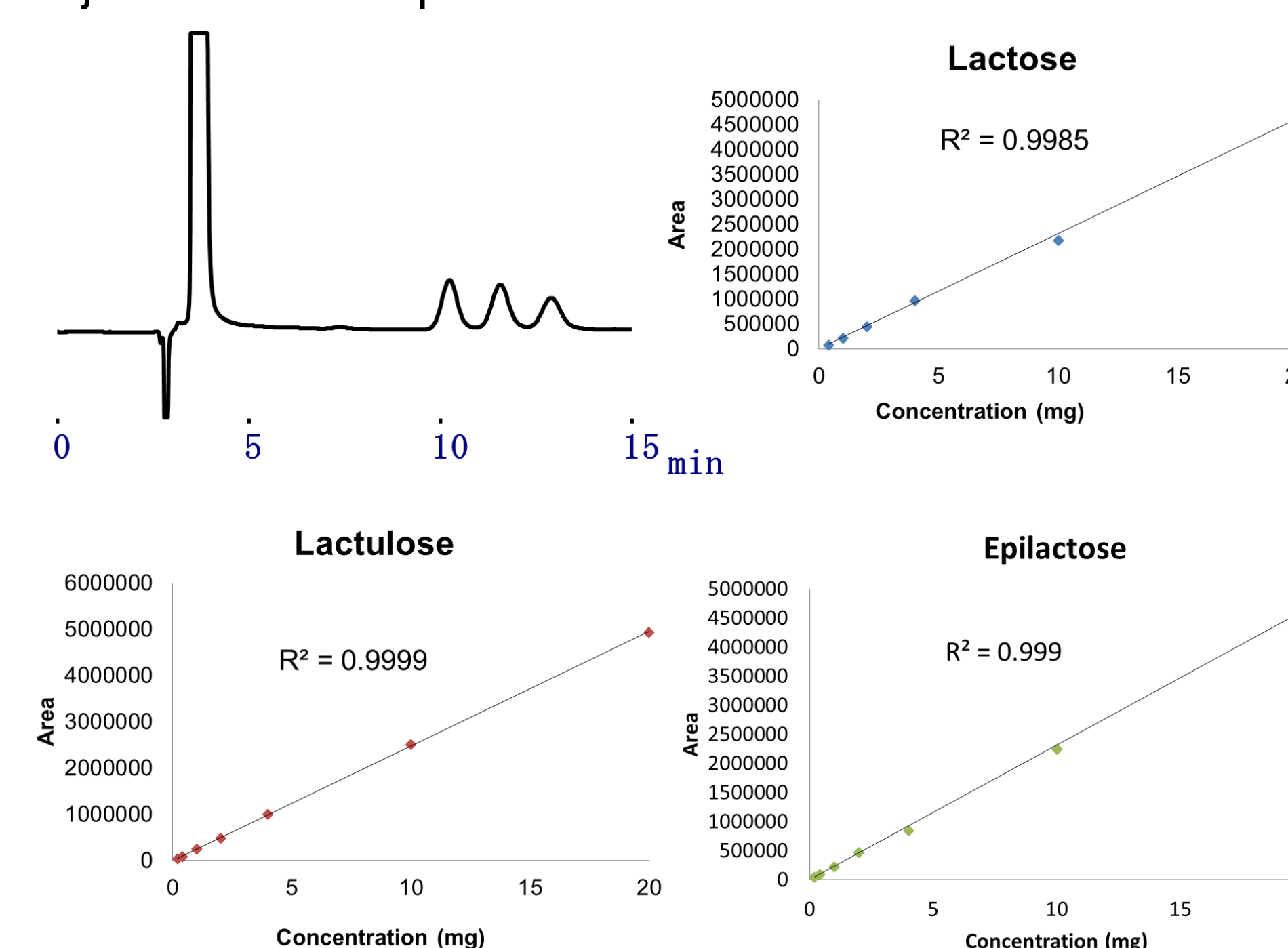
Condition	Eluent Condition	Resolution (Lactulose/Epilactose)	Resolution (Epilactose/Lactose)	Analysis time (min)
4	CH <sub>3</sub> CN/CH <sub>3</sub> OH/H <sub>2</sub> O = 85/ 5/10	1.30	3.21	30
5	CH <sub>3</sub> CN/CH <sub>3</sub> OH/H <sub>2</sub> O = 85/10/ 5	1.93	2.52	35
6	CH <sub>3</sub> CN/CH <sub>3</sub> OH/H <sub>2</sub> O = 80/10/10	1.31	2.32	20
7	CH <sub>3</sub> CN/CH <sub>3</sub> OH/H <sub>2</sub> O = 80/15/ 5	1.89	2.04	25
8	CH <sub>3</sub> CN/CH <sub>3</sub> OH/H <sub>2</sub> O = 75/15/10	1.26	1.72	15
9	CH <sub>3</sub> CN/CH <sub>3</sub> OH/H <sub>2</sub> O = 75/20/ 5	1.76	1.59	15

The optimal resolution and analysis time were achieved using:

**CH<sub>3</sub>CN/CH<sub>3</sub>OH/H<sub>2</sub>O=75/20/5**

## Calibration Curves

Each saccharide was injected in increasing concentration: 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 mg/mL  
Injection size 20 µL

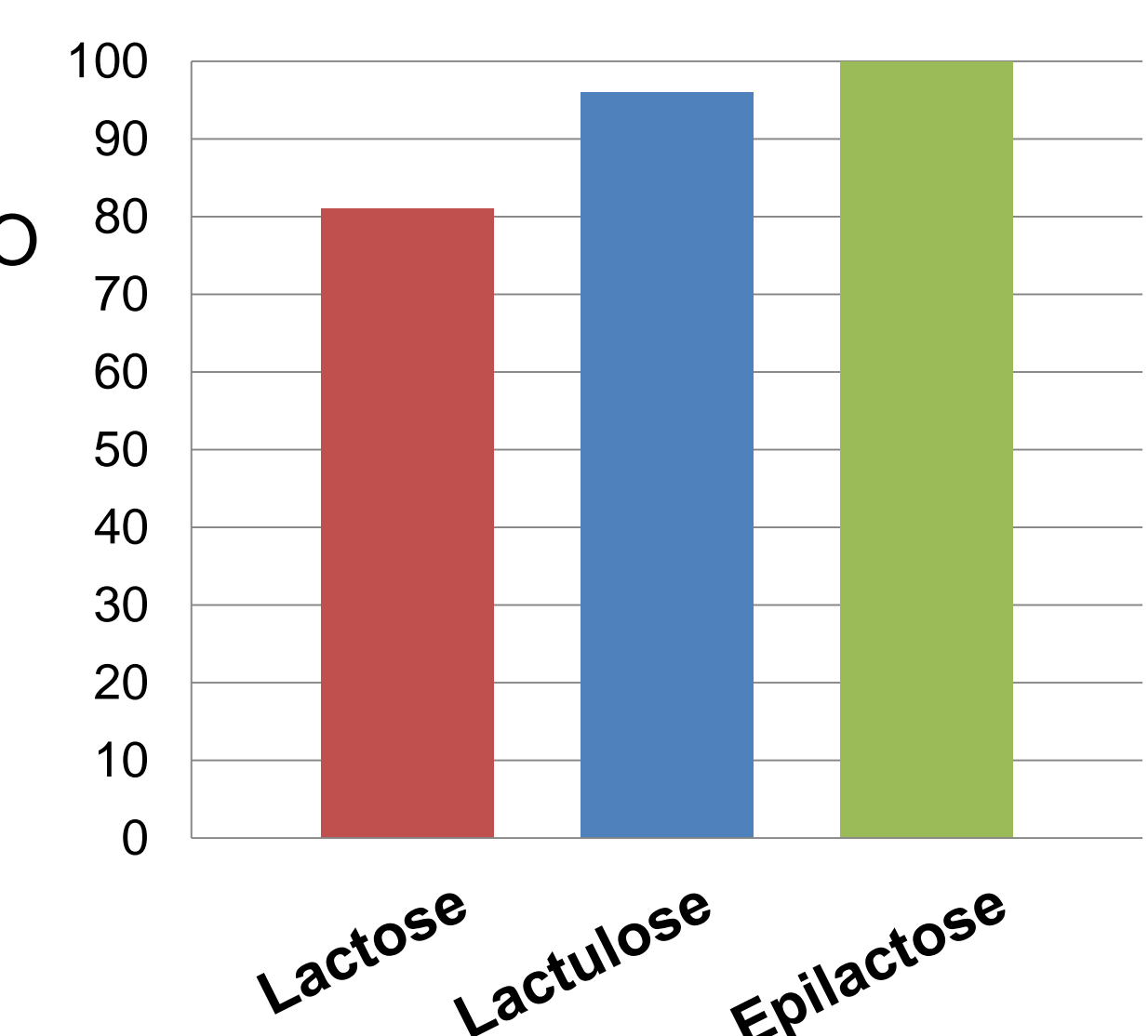


Column: VG-50 4E (4.6 x 250 mm)  
Eluent : CH<sub>3</sub>CN/CH<sub>3</sub>OH/H<sub>2</sub>O=75/20/5  
Flow rate: 1.0 mL/min  
Detector: RI  
Column temp.: 40 °C

The calibration curves showed high linearity between 2µg and 200 µg.  
The correlation coefficient for each saccharide was determined as R<sup>2</sup> = 0.999.

## Recovery ratio

Column: VG-50 4E (4.6 x 250 mm)  
Eluent : CH<sub>3</sub>CN/CH<sub>3</sub>OH/H<sub>2</sub>O = 75/20/5  
Flow rate: 1.0 mL/min  
Detector: RI  
Column temp.: 40 °C



The recovery ratio was calculated by area ratio of Sucrose

The recovery ratio of these three saccharides is high. (80-100%)

## Conclusion

We have demonstrated the separation of lactose, lactulose, and epilactose using our new column, Shodex HILICpak VG-50.

Careful consideration of the eluent conditions allowed maximum resolution in minimal analysis time.

Quantitative data, such as calibration curves and recovery ratios, confirm the value of the separation of lactose, lactulose, and epilactose.