Analysis of Functional Sugars in Foods Using HILIC Mode

Introduction

Recently, the effects of functional sugars in foods are gaining scientists' interests, and thus their analytical methods are called for.

Shodex HILICpak VG-50 series used in this application is a set of polymer-based amino columns which effectively separate various saccharides. The packing material consists of a polyvinyl alcohol base with a hydrophilic functional group, a modified tertiary amine (Fig. 1)

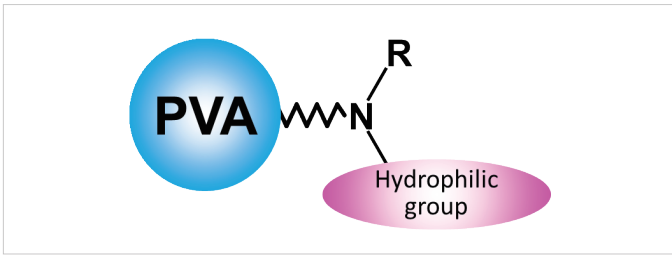


Fig. 1. Schematic diagram of VG-50 packing material

With some columns, reducing sugars form a Schiff base with the packing material and are retained in the column. This does not occur with the HILICpak VG-50 series columns, leading to the series' ability to achieve a high recovery rate. Moreover, column bleeding (elution of column packing material related debris) that is often observed with silica-based amino columns is rarely found with the HILICpak VG-50 series columns, and consequently the related problems of increased background and/or ion suppression in MS are less likely to occur. Another advantage of this column over the silica-based amino column is that the HILICpak VG-50 series columns can be used under alkaline conditions (working pH range, 2 to 13).

In this work, we optimized analytical conditions for the analysis of functional sugars in foods using HILICpak VG-50 4E (4.6 mm I.D. x 250 mm; particle size 5 µm), including the preparation methods for actual food products.

Results and Discussion

1. Analysis of Lactulose

Lactulose is a functional sugar found in dairy products and it regulates intestinal health of humans. Lactulose remains undigested and reaches the intestine unabsorbed, and then Bifidobacterium uses the sugar for its proliferation. The structures of lactulose, lactose, and epilactose are very similar, which makes their chromatographic separation challenging.

A mixture of acetonitrile and water (or buffer) eluent is generally used in HILIC mode separation. We used a mixture of acetonitrile and water as a starting eluent to separate three above mentioned disaccharides. Figure 2 shows the obtained results. We tested different ratios of acetonitrile and water, however, none were able to separate the three saccharides completely. Higher acetonitrile ratio made the retention time longer and increased the baseline noise. This resulted in lowering the signal to noise ratio (S/N).

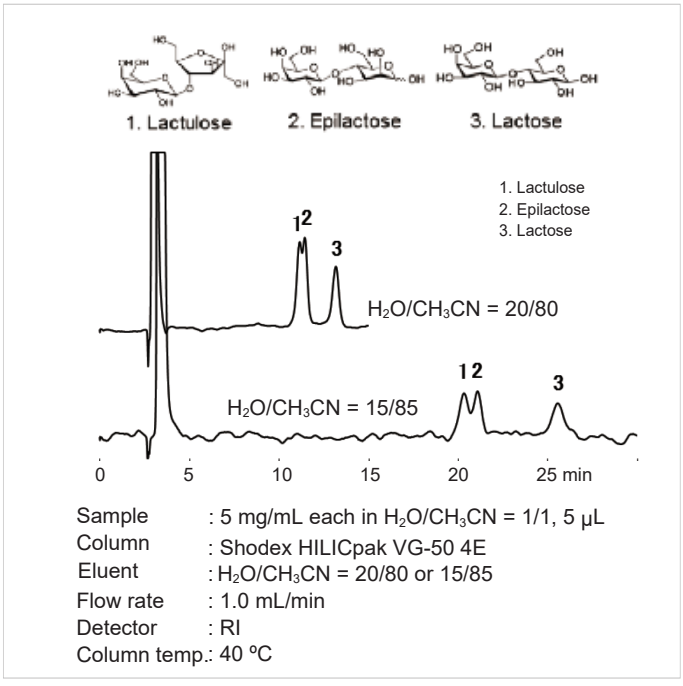
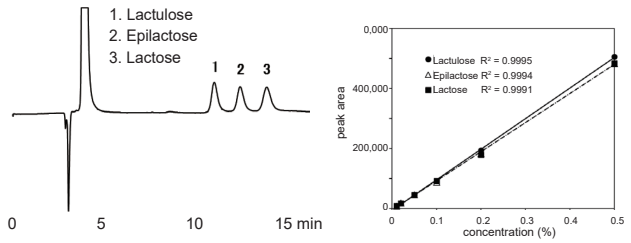


Fig. 2. Optimization of LC conditions for lactulose analysis (1)

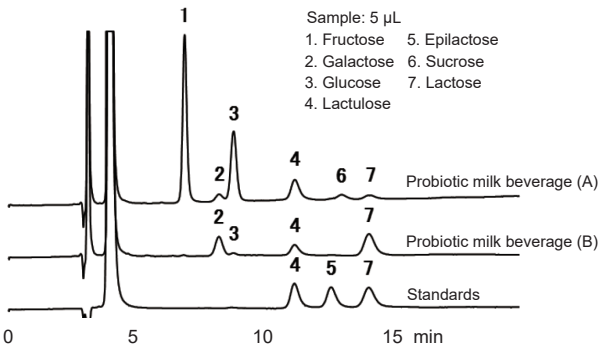
As a next step, we added methanol to the eluent. Figure 3 shows the result of analyzing the three disaccharides using an eluent mixture of water, acetonitrile, and methanol. The addition of methanol to the eluent effectively separated the disaccharides that have very similar structures. Good linearity was obtained for the calibration curves of all sugars, showing the method's applicability for the quantification of the three disaccharides.



Sample : 5 mg/mL each in H_2O/CH_3CN = 1/1, 5 µL
Column : Shodex HILICpak VG-50 4E
Eluent : $H_2O/CH_3CN/CH_3OH$ = 5/75/20
Flow rate : 1.0 mL/min
Detector : RI
Column temp.: 40 ºC

Fig. 3. Optimization of LC conditions for lactulose analysis (2)

To examine its feasibility of analyzing the real samples, we applied the method to analyze a commercial probiotic milk beverage that claims to contain lactulose. As a sample pretreatment, we used the following procedures to remove the protein. Take 1 mL of the probiotic milk beverage and add 1 mL of acetonitrile. Centrifuge the mixture at 890 *g* for 10 minutes, and then filter the supernatant with a 0.45-µm membrane filter. The obtained solution was used for the analysis. Figure 4 shows the result of analyzing lactulose in probiotic milk beverages. Lactulose was detected in the two test samples analyzed, and it was well separated from other saccharides. This demonstrated the effectiveness of the VG-50 for the analysis of lactulose in milk beverages.



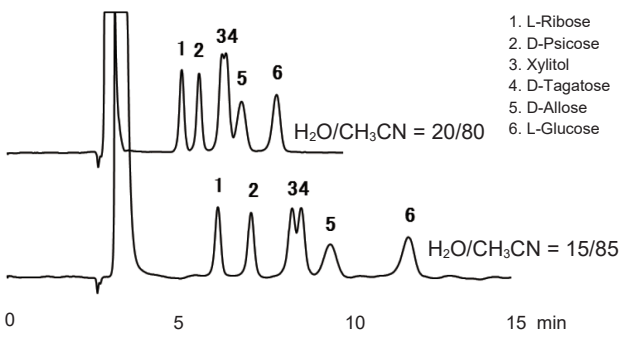
Refer to Fig.3 for analytical conditions used.

Fig. 4. Analysis of lactulose in probiotic milk beverages

2. Analysis of Rare Sugars

Rare sugars slow down the blood sugar level elevation after a meal and inhibit the accumulation of visceral fat. The use of rare sugars in commercial food products have been recently increasing. Moreover, as rare sugars are known to provide various bioactive effects, their potential applications in medicinal products, functional foods, and cosmetics have been studied. In this work, we developed a method for separating six rare sugars, the ones fulfilling the International Institute of Rare Sugar Research and Education's definition of rare sugars (L-ribose, D-psicose, xylitol, D-tagatose, D-allose, and L-glucose).

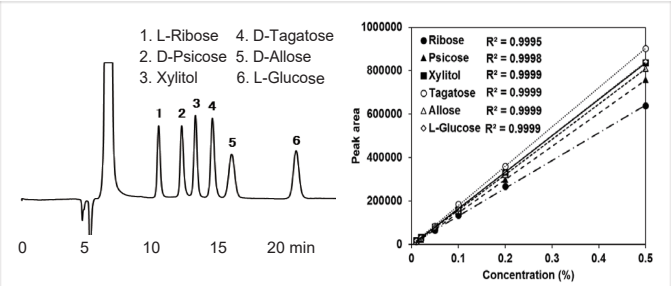
First, similar to the approach we took in "1. Analysis of Lactulose", we used water/acetonitrile eluent for the separation of six rare sugars. Figure 5 shows the resulting chromatograms. Increase of acetonitrile ratio again lowered the S/N, and we concluded that the use of water/acetonitrile eluent cannot provide complete separation of all six rare sugars.



Sample : 5 mg/mL each in H_2O/CH_3CN = 1/1, 5 µL
Column : Shodex HILICpak VG-50 4E
Eluent : H_2O/CH_3CN = 20/80 or 15/85
Flow rate : 1.0 mL/min
Detector : RI
Column temp.: 40 ºC

Fig. 5. Optimization of LC conditions for rare sugar analysis (1)

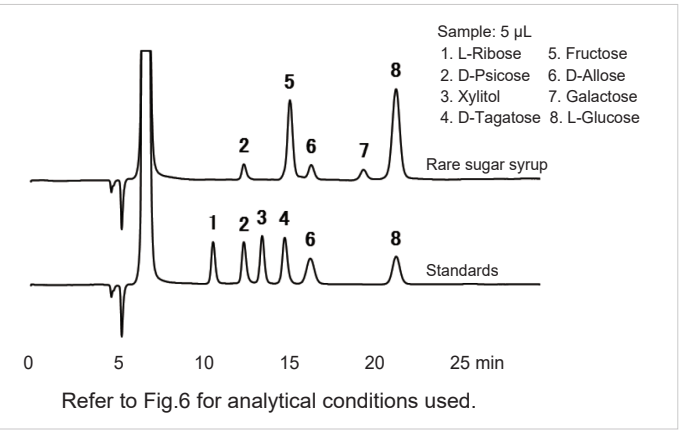
Next, we tested the eluent containing water, acetonitrile, and methanol (Fig.6).The addition of methanol to the eluent effectively separated the six rare sugars. Good lineality was obtained for the calibration curves ofall rare sugars, indicating its feasibility for the quantification of those sugars.



Sample : 5 mg/mL each in H_2O/CH_3CN = 1/1, 5 µL
Column : Shodex HILICpak VG-50 4E
Eluent : $H_2O/CH_3CN/CH_3OH$ = 5/75/20
Flow rate : 1.0 mL/min
Detector : RI
Column temp.: 40 ºC

Fig. 6. Optimization of LC conditions for rare sugar analysis (2)

To examine its feasibility of analyzing the real samples, we applied this method to analyze a commercial syrup containing rare sugars. As a sample pretreatment, we took 0.1 g of the syrup into a test tube and added 2.5 mL of ultra-pure water, capped the test tube and manually shook it to dissolve the syrup. After making sure that the syrup was completely dissolved, 2.5 mL of acetonitrile was added. Then, shook the tube again to mix the solutions and filtered the mixture with a 0.45-µm membrane filter. The obtained solution was used for the analysis. Figure 7 shows the result of analyzing rare sugars in the syrup. By comparing the chromatograms of the syrup and the standards, the syrup most likely contained D-psicose and D-allose. They were well separated from other saccharides. A peak was also detected at L-glucose's position, but as D-glucose also has an identical retention time, its D or L configuration was not identified in this analysis condition.

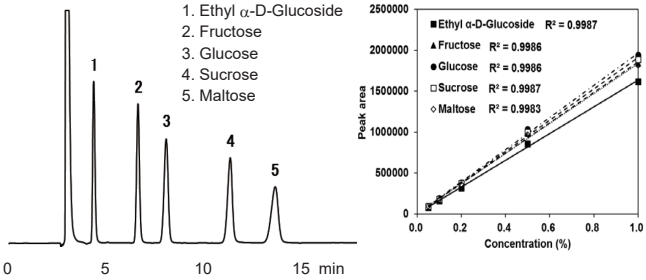


Refer to Fig.6 for analytical conditions used.

Fig. 7. Analysis of rare sugar syrup

3. Analysis of Ethyl α-D-glucoside

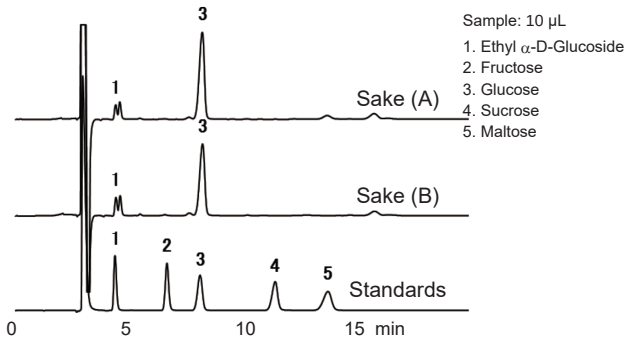
Ethyl α-D-glucoside is an umami component specifically found in sake (Japanese rice wines). Recent studies show that it has a moisturizing effect, and the effects have been focused upon for cosmetic uses. In this study, we developed a method to simultaneously analyze ethyl α-D-glucoside and several saccharides generally present in sake. We used a mixture containing four saccharides and ethyl α-D-glucoside as a standard. Using an eluent containing water/acetonitrile = 20/80 effectively separated the target compounds (Fig. 8). Good linearity was obtained for all analytes, showing the method's feasibility for the quantification of saccharides and ethyl α-D-glucoside.



Sample : 1 mg/mL each in H_2O/CH_3CN = 1/1, 10 µL
Column : Shodex HILICpak VG-50 4E
Eluent : H_2O/CH_3CN = 20/80
Flow rate : 1.0 mL/min
Detector : RI
Column temp.: 40 ºC

Fig. 8. Optimization of LC conditions for ethyl α-D-glucoside analysis

To examine its feasibility of analyzing the real samples, we applied this method to analyze a commercial sake. We prepared the sample as following. Take 1 mL of sake into a test tube and add 1 mL of acetonitrile. Cap the test tube and manually shook it to mix the solutions, and then filter the mixture with a 0.45-µm membrane filter. The obtained solution was used for the analysis. By comparing the chromatograms of the samples and the standards, ethyl α-D-glucoside and glucose were found in the sake. An unidentified peak was found after the ethyl α-D-glucoside peak. The LC/MS result showed that the unidentified compound was consisted of two components having molecular weights of 138 and 252.



Refer to Fig.8 for analytical conditions used.

Fig. 9. Analysis of ethyl α-D-glucoside in sake

Conclusions

This article demonstrated the effectiveness of Shodex HILICpak VG-50 4E with its HILIC mode for the analysis of various functional sugars in foods. The eluent typically used in HILIC mode is a mixture of water and acetonitrile. However, the use of a mixture containing water, acetonitrile, and methanol improved the separation efficiencies of the VG-50 4E for some applications. We also proved the effectiveness of the developed method for analyzing functional sugars in real food products.

Reference

Shodex Technical Article No.3: LC/MS Analysis of Various Hydrophilic Compounds Using HILIC Mode and Alkaline Eluent. The article introduces an LC/MS analysis of nutritional components in a commercial energy drink using the HILICpak VG-50 2D column. The polymer-based packing materials allowed the use of alkaline eluent. The nutritional components included were saccharides, amino acids, caffeine, and water-soluble vitamins. All components were successfully and simultaneously analyzed using the method. Please read technical article No. 3 for details.

https://www.shodex.com/

Resonac Corporation

TA. NO. 010. (2) 20 D. SEP. P