LT171

GL Sciences Inc.

Analysis of sugars with an InertSphere Sugar-2 Column -Using Ligand-Exchange and Size-Exclusion Modes-

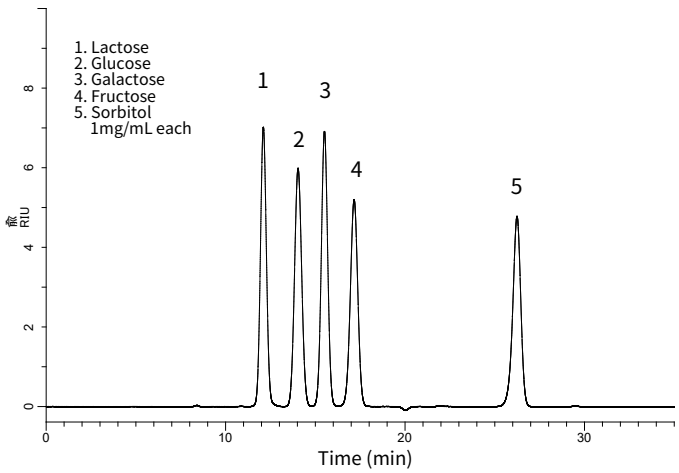
InertSphere Sugar-2 is a sugar analysis column that uses calcium (Ca^{2+} type) as the metal counter ion in the packing material. The separation mainly occurs due to a size-exclusion mechanism and sugars elutes in the order of molecular weight. It also acts in a ligand exchange mode by utilizing the difference in retention between the metal counter ions and hydroxyl groups in the sugars. The bond strength of the complexation depends on the counter ion and the type of sugar.

One of the advantages of this column is that a 100% aqueous eluent is used, eliminating the need for sample preparation.

The InertSphere Sugar-2 column was used to analyze sugars in vegetable juices and yogurts. As a comparison, similar analyses were performed using an amino column (NH2) , separation of difficult components was achieved.. (Y. Yui)

Example: Measurement of standards

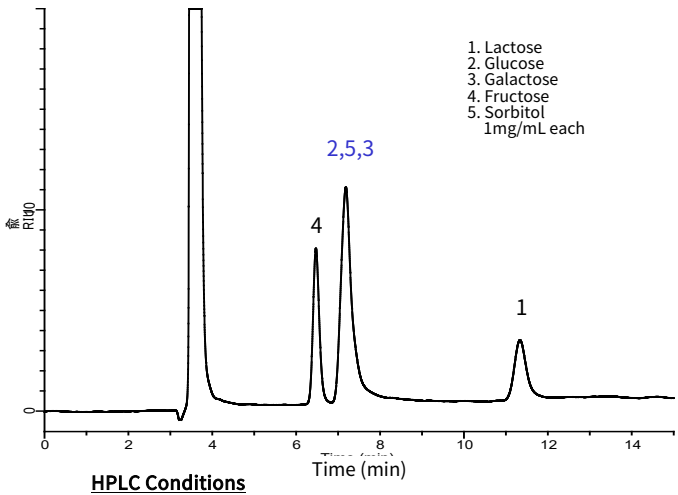
InertSphere Sugar-2



HPLC Conditions

Column size	: 9 µm, 300 x 7.8 mm I.D.
Eluent	: H_2O
Flow Rate	: 0.5 mL/min
Col. Temp.	: 85 ˚C
Detection	: RI
Injection Vol.	: 10 µL
Sample	: Standard

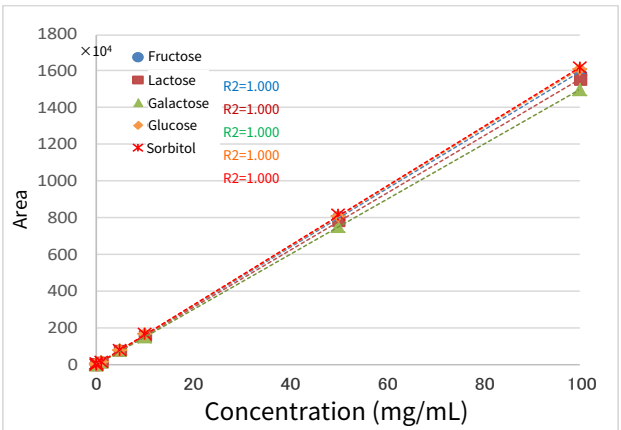
InertSustain NH2



HPLC Conditions

Column size	: 5 µm, 250 x 4.6 mm I.D.
Eluent	: A) CH_3CN B) H_2O A/B = 80/20, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 25 ˚C
Detection	: RI
Injection Vol.	: 10 µL
Sample	: Standard

Typically NH2 columns cannot separate the overlapping peaks of Glucose, Galactose, and Sorbitol,
Using an InertSphere Sugar-2 column, all five components could be separated.



Calibration curve (0 mg/mL - 100 mg/mL)

HPLC-RI Conditions

Column	: InertSphere Sugar-2 (9 µm, 300 x 7.8 mm I.D.)
Eluent	: H_2O
Flow Rate	: 0.5 mL/min
Col. Temp.	: 85 ˚C
Detection	: RI
Injection Vol.	: 10 µL
Sample	: Standard

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Examples of pretreatment and analysis of sugars in vegetable juice.

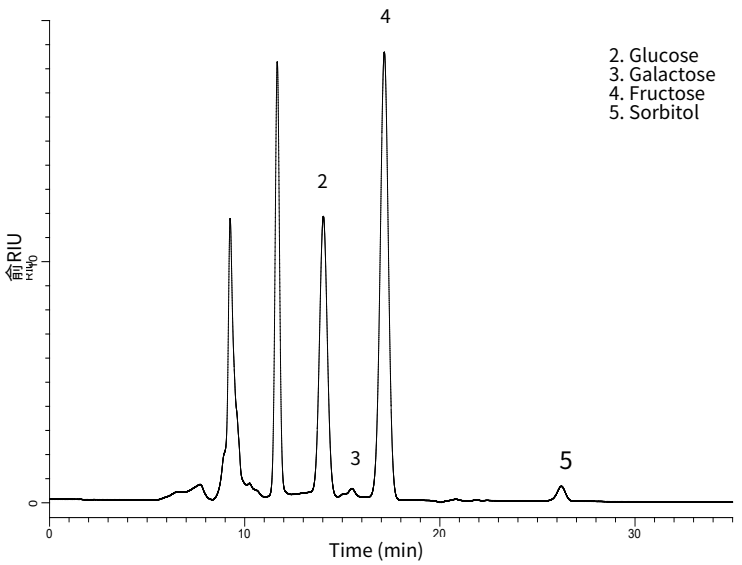
Sample
- Vegetable juice 2.5 g
- Water 15 mL

Neutralization
- 5% (w/v) aqueous sodium hydroxide solution.

Extraction
- Ultrasonic ation for 30 min.)
- Volume made up to 25 mL with water.

Filtration
- GL Chromatodisc (water 25 A 0.45 µm)

HPLC



Examples of pretreatment and analysis of sugars from plain yogurt.

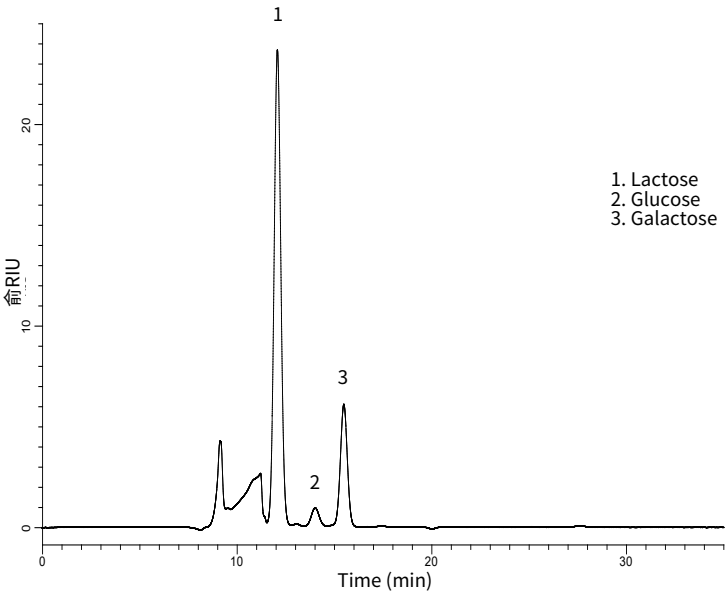
Sample
- Yogurt 2.5 g
- Water 15 mL
- Stir

Neutralization
- 5% (w/v) aqueous sodium hydroxide solution.

Extraction
- Ultrasonic ation (30 min.)
- Volume made up to 25 mL with water.
- Centrifugal separation、 10,000 rpm (10 min.)

Filtration
- GL Chromatodisc the supernatant Filter (water 25A 0.45 µm)

HPLC



HPLC Conditions

Guard column	: InertSphere Sugar-2 Guard (9 µm, 50 x 6.0 mm I.D.)
Column	: InertSphere Sugar-2 (9 µm, 300 x 7.8 mmI.D.)
Eluent	: H_2O
Flow Rate	: 0.5 mL/min
Col. Temp.	: 85 ˚C
Detection	: RI
Injection Vol.	: 10 µL

Retention time list (reference value)

Natural sugar	
Glucose	12.66
Galactose	13.98
Xylose	13.90
Mannose	14.37
Arabinose	15.69
Fructose	15.45
Ribose	24.10
Disaccharide	
Trehalose	10.50
Sucrose	10.52
Maltose	10.61
Lactose	10.90
Palatinose	10.81
Isomaltose	10.48
Sugar alcohol	
Dulcitol	22.55
Sorbitol	23.55
Myo-inositol	15.75
Xylitol	23.39
Arabitol	20.12
Maltol	65.70
Maltitol	13.99
Lactitol	13.81
Mannitol	19.63
Rare seven monosaccharides	
Glucoheptose	17.24
Rare six monosaccharides	
Sorbose	14.12
Growth	17.58
Tagatose	18.38
Talose	22.09
Fucose	15.66
Rhamnose	14.54
Allulose	18.32
Rare pentasaccharide	
Lyxose	16.39
Rare tetramonosaccharide	
Threose	15.51
Monosaccharide	
N-acetyl-D-glucosamine	13.42
Oligosaccharides	
1-Kestose	9.42
1F-fructofuranosyl nystose	8.63
Nystose	8.90
Maltotetraose	9.18
Maltotriose	9.69
Maltohexaose	8.64
Maltoheptaose	8.50
Maltopentaose	8.59
Meretitose	9.52
Raffinose	9.62
Isomaltotriose	9.46
Amino sugar	
Glucosamine	8.44
Galactosamine	8.45
Artificial sweetener	
Aspartame	14.24
Acesulfam K	8.57
Advantame	20.24
Saccharin	20.16

HPLC Conditions

Column : InertSphere Sugar-2 (9 µm, 300 x 7.8 mm I.D.)
Eluent : H_2O
Flow Rate : 0.5 mL/min
Col. Temp. : 85 ˚C
Detection : RI
Injection Vol. : 10 µL
Sample : Standard

Column

Analytical columns: InertSphere Sugar-2 9 µm, 300 x 7.8 mm I.D.
Cat.No. 5020-11000

Guard columns: InertSphere Sugar-2 Guard 9 µm, 50 x 6.0 mmI. D.
Cat.No. 5020-10999

- Base Material : Styrene-divinylbenzene-based polymer
- Particle size : 9 µm
- Functional Group : Sulfonic acid group
- Counter-ion : Ca^{2+}
- Degree of linking : 8 %
- USP code : L19

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