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

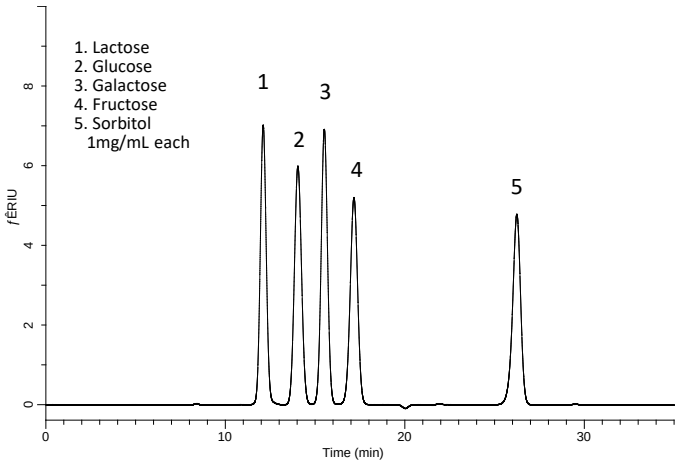
InertSphere Sugar-2 is a sugar analysis column that uses calcium ($Ca^{2+}$ type) as the metal counter ion in the packing material. The separation mainly occurs due to a size-exclusion mechanism and sugars elutes in the order of molecular weight. It also acts in a ligand exchange mode by utilizing the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

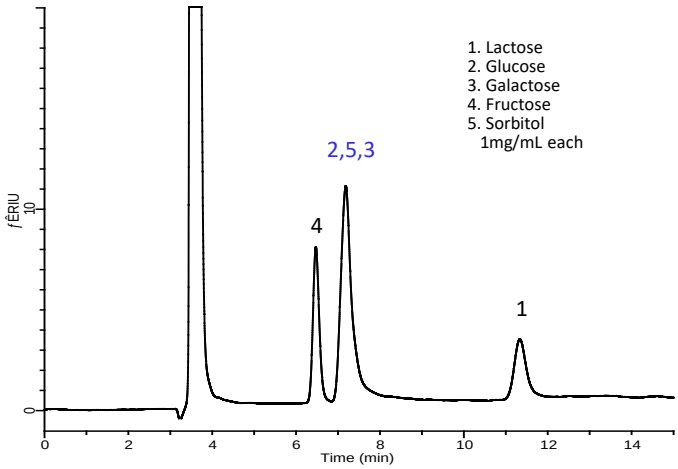
### InertSphere Sugar-2




**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 |

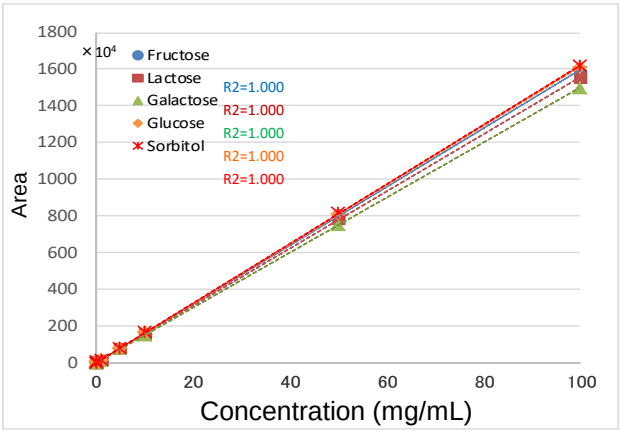
### InertSustain NH2




**HPLC Conditions**

| | |
|---|---|
| **Column size** | : 5 µm, 250 x 4.6 mm I.D. |
| **Eluent** | : A) $CH_3CN$<br>B) $H_2O$<br>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<br>(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.

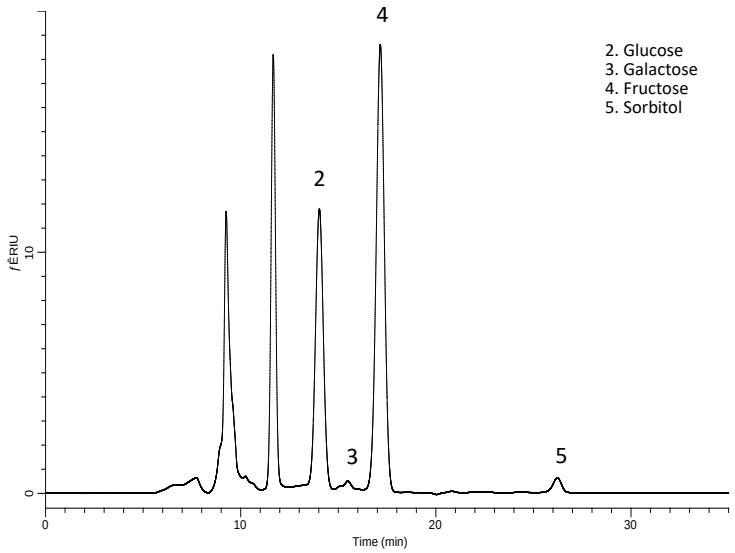
**Sample**
- Vegetable juice 2.5 g
- Water 15 mL

**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.

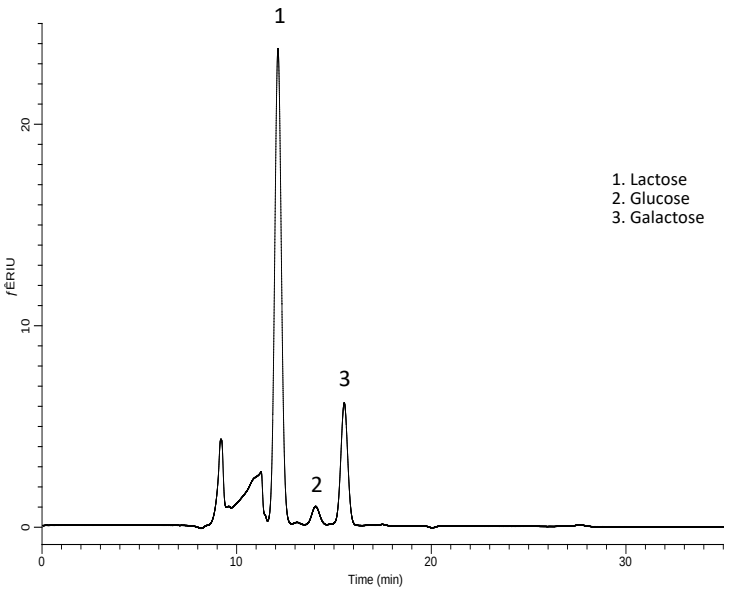
**Sample**
- Yogurt 2.5 g
- Water 15 mL
- Stir

**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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