MINOR SACCHARIDES (Liquid chromatography)

PRINCIPLE

A corn sugar solution is passed through a metal ion-modified cation exchange column. The minor saccharides are separated from dextrose by molecular exclusion and ligand exchange, and detected using a differential refractometer. The resulting peaks are quantitated against a maltose standard.

SCOPE

The method is applicable to corn sugars (98+ % dextrose) containing minor amounts of higher saccharides (Note 1).

SPECIAL APPARATUS

1. Integrator

2. Chromatograph Columns: Prepacked cation exchange columns are recommended. These columns are macroreticular polystyrene sulfonate divinylbenzene, 2-8% crosslinked 8-35 μm particle size. The Bio-Rad Aminex HPX-87C for separating DP_1-DP_4 saccharides, is a satisfactory example.

3. Guard Column: Protect the analytical column described above, by inserting a deionizing precolumn available from chromatographic column manufacturers.

4. Chromatographic Column Heater: A thermostatically controlled metal block heater accommodating two columns, capable of operating at temperatures up to 95 °C (± 0.5 °C).

5. Sample Injector: Use a loop injector having a capacity of 10 μL. Rheodyne-type, or equivalent, is recommended. For manual injection, use a precision sampling syringe, 25 μL capacity.

REAGENTS

MINOR SACCHARIDES (Liquid Chromatography) — continued

1. Solvent: Degassed, purified water should be filtered through a 0.22 µm membrane filter prior to use; maintain at approximately 85 °C.

PROCEDURE

Column Conditioning: Install chromatographic and guard columns, begin pumping solvent at 0.1 mL per minute, bring to operating temperature, increase flow rate to 0.5 mL per minute, and allow to equilibrate for 45 minutes.

Approximate Operating Conditions: The parameters listed below may be modified, depending on equipment used.

Liquid Chromatograph

Flow Rate	0.5 mL/minute
Column Temperature	85 °C
Detector Temperature	45 °C
Detector Attenuation	8 X

Inject standard or sample of known composition. Following throughput, examine chromatogram and adjust integrator according to manufacturer's instructions to obtain the desired information.

Standardization: Prepare a standard solution of maltose using characterized (dry substance and saccharide composition) maltose. Weigh 0.3 g of maltose (± 0.1 mg) and transfer to a 50 mL beaker containing 20 mL of purified water (Note 2). Swirl the contents until the sugar is dissolved, and quantitatively transfer to a 25 mL volumetric flask. Dilute to volume and mix. Freeze the solution if future use is contemplated.

Compute the maltose concentration in the standard solution (Note 3).

Standardization is accomplished by injecting 10 µL of the standard maltose solution. The resulting peak is integrated and the integrator response factor (IRF) is calculated (Note 4):

MINOR SACCHARIDES (Liquid Chromatography) — continued

$$\text{IRF for Maltose} = \frac{\text{Maltose Concentration, g/mL}}{\text{Area}}$$

Analysis: Determine the exact dry substance of the sample using refractive index or other appropriate method. Weigh a quantity of the sample and dilute to 100 mL or other convenient volume, with purified water, to obtain a 12% solution for injection. If injecting manually, rinse the syringe with the diluted sample solution at least four times prior to injection. Inject 10 µL of sample solution. Following manual injection, rinse the syringe with warm purified water, allowing the air bubbles to scrub the walls of the syringe.

CALCULATION

Combine the integrals obtained for maltose, DP_3, and DP_{4-}. Follow the equation below, being careful to report the results on an ash-free, dry basis because the sample was ion exchanged.

$$\text{Total Minor Saccharides} = \frac{(DP_2\ \text{Area} + DP_3\ \text{Area} + DP_{4-}\ \text{Area}) \times \text{IRF}}{\text{Sample Wt. (g)} \times \text{Dry Sub., \%}}$$

NOTES AND PRECAUTIONS

1. The following sugars and/or sugar groups may be separated by this technique. Approximate retention times relative to dextrose (Rg = 1.00) are given in parenthesis: excluded peak through DP_4 (0.42 – 0.68); maltotriose DP_3 (0.77); other DP_2 (non-maltulose) (0.85); maltulose (0.91); dextrose (1.00); fructose (1.23).

2. Most purchased maltose is maltose monohydrate and the weight of water in the maltose needs to be taken into consideration.

3. Maltose Concentration, g/mL, Dry Basis =

$$= \frac{\text{Maltose Wt. (g)} \times \left(\frac{\text{Dry Sub., \%}}{100}\right)}{25\ \text{mL}}$$

MINOR SACCHARIDES (Liquid Chromatography) — continued

4. Generally, it is good practice to inject the maltose standard solution several times, and calculate an average integrator response factor (IRF).

METHOD HISTORY

Corn Sugar, Minor Saccharides (Liquid Chromatography) (F51), Date of Acceptance 10-18-1983, Revised 4-01-2009.