SACCHARIDES (Liquid Chromatography)

PRINCIPLE

A corn syrup solution is passed through a metal ion-modified cation exchange column. The individual sugars are separated by molecular exclusion and ligand exchange. The eluted sugars are detected using a differential refractometer and the resulting peaks are quantified against an appropriate standard with the aid of a modern electronic integrator.

SCOPE

The method is applicable to all corn syrup, including those containing fructose, corn syrups and starch hydrolyzates prepared by acid and/or enzymes conversion (Note 1).

SPECIAL APPARATUS

1. Liquid Chromatograph: A liquid chromatograph capable of accommodating a 22-31 cm temperature-controlled column and equipped with a constant flow pulseless pump and a differential refractometer detector with attenuation capabilities.

2. Integrator

3. Chromatograph Columns: Prepacked cation exchange columns (calcium or silver form) are recommended. These columns are macroreticular polystyrene sulfonate divinylbenzene, 2-8% crosslinked, 8-25 μm particle size. Examples of acceptable columns are: Bio-Rad Aminex HPX-87C for separating DP_1-DP_4 saccharides and Aminex HPX-42C or HPX-42A for separating DP_1-DP_7 saccharides.

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

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

6. Sample Injector: Use a loop injector having a capacity of 10-50 µL. Rheodyne-type, or equivalent, is recommended. For manual injection, use a precision sampling syringe, 100 µL capacity.

REAGENTS

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

2. Carbohydrate Standards: Recommended sugar standards are as follows: fructose; dextrose; maltose hydrate; maltotriose; acid-converted 42 D.E. corn syrup or a maltodextrin of about 10 D.E. (Note 3). When applicable, the purity of a given standard sugar should be determined using liquid chromatographic analysis (Note 4).

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.

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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, so as to obtain the desired information.

Standardization: Prepare a standard solution at 10% solids, using sugars of known purity (e.g., fructose, dextrose, maltose, etc.) that approximates the composition of a sample type, on a dry basis. Dissolve the appropriate dry weight (± 0.1 mg) of each standard sugar in 20 mL of purified water contained in a 50 mL beaker. Heat on a steam bath until all sugars are dissolved; then cool and transfer quantitatively to a 100 mL volumetric flask. Dilute to volume and mix. Freeze the solution if it is to be reused.

If a corn syrup or maltodextrin is used to supply a DP_4 + fraction, care must be taken to include all saccharides in the standard composition calculation.

Compute the dry basis concentration of each individual component in the standard solution according to the equation below (See example of calculation).

$$\text{Sugar Component, \% Dry Basis} = \frac{\text{Component Wt.}}{\text{Dextrose Wt.} + \text{Maltose } (DP_2) \text{ Wt.} + \text{Maltotriose } (DP_3) \text{ Wt.} + \text{Oligosaccharide } (DP_4 +) \text{ Wt.}}$$

Standardize by injecting 10-20 µL (about 1.0-2.0 mg solids) of the standard sugar solution. Integrate the peaks and normalize. Sum the individual DP_4 + responses from the normalized printout to obtain the total DP_4 + normalized response.

$$\text{Area Correction Factor (ACF) (for each component)} = \frac{\text{Known concentration, d.b., \%}}{\text{Measured Concentration, Normalized, \%}}$$

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Compute the integrator response factor for each component using the following equation:

$$\text{Integrator Response Factor (KF) (for each component relative to dextrose)} = \frac{\text{Component ACF}}{\text{Dextrose ACF}}$$

The KF for DP_4 + should be listed as the default value and used to compute higher saccharides.

Analysis: Determine the approximate dry substance of the sample. Dilute the sample, by weight, to about 10% solids with purified water (Note 4). If injecting manually, rinse the syringe with the diluted sample a minimum of 4 times prior to injection. Inject the appropriate volume of sample. Following manual injection, rinse the syringe with warm purified water, allowing air bubbles to scrub the walls of the syringe.

CALCULATIONS

When an electronic integrator with calculation capabilities has been used with the appropriate KF values, the results will be computed automatically (Note 5). List the individual results obtained for fructose, dextrose, maltose and other DP_2. Combine the results obtained for maltotriose and other DP_3 and list the sum as DP_3. Sum the remaining results and list the sum as DP_4 +.

NOTES AND PRECAUTIONS

1. The following sugars and/or sugar groups may be separated by this technique; approximate retention time relative to dextrose as Rg is given in parenthesis: excluded peak through DP (0.42-0.68); maltotriose (0.77); maltose and isomaltose (0.85); maltulose (0.91); dextrose (1.00); fructose (1.23). Sucrose elutes with maltose and isomaltose, and these three disaccharides are rarely separable by this method.

2. The composition of the acid-converted syrup is estimated by determining the level of each saccharide shown for a given dextrose equivalent (D.E.) in the Corn Refiners Association, Inc., Critical Data Tables. The dextrose equivalent

of the syrup should be determined according to the Corn Refiners Association, Inc., Standard Analytical Method E-26. The syrup and maltodextrin are commercially available and approximate compositions are obtainable from the supplier.

3. The best accuracy will be obtained when the dry solids and saccharide compositions of the standard and the sample are about the same.

4. Generally, only one calibration is required per day. When a given standard is analyzed a second time (on a given column having the same resolution), average the two sets of ACF's and use the average.

5. The following is the calculation performed by the integrator.

   List the individual areas for fructose (f), dextrose (d), maltose and other DP_2 (2), as well as Maltotriose and other DP_3 (3). Sum the remaining areas and list the obtained sum as DP_4 +. Use the equations below to compute the concentration of each component. Report as under Calculations.

$$\%\ \text{Component} = \frac{\text{Component Area} \times \text{Component ACF} \times 100}{(\text{Area}_f \times \text{ACF}_f) + (\text{Area}_d \times \text{ACF}_d) + (\text{Area}_2 \times \text{ACF}_2) + \text{- - } (\text{Area}_{4+} \times \text{ACF}_{4+})}$$

METHOD HISTORY

Corn Syrup, Saccharides (Liquid Chromatography) (E-61), Date of Acceptance 9-17-1976, Revised 4-01-2009.