DIONEX

Application Note 92

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The Determination of Sugars in Molasses by High-Performance Anion Exchange with Pulsed Amperometric Detection

INTRODUCTION

The accurate measurement of the amount of sugar in final molasses is economically important to sugar mills, because the purchase price of the final molasses is directly related to its sugar content.

Historically, gas chromatography (GC) has been used extensively for sugar analysis. While GC results are accurate, the analytical process involves a time-consuming derivatization procedure. High-performance liquid chromatography (HPLC) was developed in the early 1970s, and sugar mill chemists had hopes of using this new technique for their sugar analysis. However, amino-bonded silica columns gave imprecise results for reducing sugars.[1] In final molasses samples, cation exchange columns gave slightly higher glucose and sucrose response and much higher fructose response when compared with gas chromatography results.[2] The discrepancies were attributed to impurities in the molasses that coeluted with the sugars. The nonspecific refractive index detection used for these analyses could not differentiate between the sugars and other substances present in the molasses.

A high-performance anion exchange method with pulsed amperometric detection (HPAE-PAD) has recently been developed to accurately determine the sugar concentrations of cane and beet final molasses samples.[3] Carbohydrates are weak acids with pK_as above 11. The use of sodium hydroxide as an eluent promotes ionization of the carbohydrates to their anionic form. The pellicular resin of the anion exchange column is stable and durable within the 1–14 pH range. PAD is highly specific when using pulsed potentials optimized for carbohydrates. An eluent pH of greater than 12 facilitates the oxidation reaction at the working electrode and the pulsed potentials keep the electrode clean.

Chemists at the Sugar Milling Research Institute have used HPAE-PAD to measure glucose, fructose, and sucrose in cane molasses samples.[4] J. Thompson refined this method with the use of internal and external standards.[5] C. Day-Lewis compared this improved method with the official GC method and found that the two methods agreed with respect to precision and accuracy.[3] An interlaboratory study using this method has been completed and approved by the International Commission of Uniform Methods for Sugar Analysis (ICUMSA). Submission to the Association of Official Analytical Chemists International (AOAC International) is also planned.

EQUIPMENT

Dionex chromatography system consisting of:

- High-Performance Pump
- Liquid Chromatography Module
- Pulsed Electrochemical Detector
- Eluent Organizer or Eluent Degas Module

Dionex PeakNet Chromatography Workstation or AI-450 Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, 18 MΩ-cm resistance

Sodium hydroxide solution, 50% w/w, low carbonate

Glucose, fructose, lactose, sucrose, and raffinose

CONDITIONS

Columns:	CarboPac™ PA1 Analytical (4 x 250 mm) and guard (4 x 50 mm)
Expected Operating Pressure:	8–10 MPa (1200–1500 psi)
Inj. Volume:	50 µL
Eluent:	150 mM Sodium hydroxide (NaOH)
Flow Rate:	1 mL/min
Detection:	Pulsed amperometry, gold working electrode standard carbohydrate settings

Note: See Dionex Technical Note 21[6] for a discussion of Pulse Potentials.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent: 150 mM Sodium Hydroxide

Dilute 7.8 mL of sodium hydroxide solution (50% w/w, low carbonate) in 1.0 L of helium sparged deionized water. Sodium hydroxide pellets are coated with a layer of carbonate, and will not produce an acceptable eluent.

CALIBRATION STANDARD

Lactose Solution (32g/L)

Lactose is used as the internal standard. In a 250-mL volumetric flask, dissolve 8 g of lactose in deionized water and dilute to the 250-mL mark with deionized water.

Raffinose Solution (7.05g/L)

Weigh 0.14 g of raffinose and 19.86 g of deionized water into a container. Weight/weight measurement is used here for precise concentration.

Molasses Calibration Standards

Dissolve and dilute each of the standard sets in Table 1 (Cane Molasses) and each of the standard sets in Table 2 (Beet Molasses) in individual 100-mL volumetric flasks, with deionized water to the 100-mL mark. Dilute 1 mL of each stock standard to the 100-mL mark with deionized water in another 100-mL volumetric flask.

SAMPLE PREPARATION

Cane

Weigh 1.0 g of cane sugar in a wide-mouth container. Add 5 mL of the lactose solution, dissolve in approximately 50 mL of deionized water, transfer it to a 100 mL volumetric flask, and dilute to the mark with deionized water. Dilute 1 mL of this stock cane sample to the mark of a 100-mL volumetric flask with deionized water. Filter this sample through a 0.45-µm filter. Make a duplicate sample in a separate container.

Beet

Weigh 0.7 g of beet sugar in a wide-mouth container. Add 5 mL of the lactose solution, dissolve in approximately 50 mL of deionized water, transfer it to a 100-mL volumetric flask, and dilute to the mark with deionized water. Dilute 1 mL of this stock beet sample to the mark of a 100-mL volumetric flask with deionized water. Filter this sample through a 0.45-µm filter. Make a duplicate sample in a separate container.

Table 1 Cane Molasses

Sugars	Std C1	Std C2	Std C3
Glucose (g)	0.02	0.06	0.10
Fructose (g)	0.03	0.07	0.11
Sucrose (g)	0.25	0.31	0.37
Lactose Solution (mL)	5.00	5.00	5.00

Table 2 Beet Molasses

Sugars	Std B1	Std B2	Std B3
Sucrose (g)	0.25	0.30	0.35
Raffinose Solution (mL)	1.00	1.50	2.00
Lactose Solution (mL)	5.00	5.00	5.00

EXPERIMENTAL SETUP

The same protocol applies for both cane and beet samples. The three calibration standards are initially run in sequence and sucrose is checked for linearity. For the sample runs, the middle calibration standard is run first, followed by the two duplicates of a molasses

EXAMPLE 1: Example of calculations for determining sucrose in final cane molasses

Calibration Mass for Sucrose in C2 run before molasses sample ..... 0.3100 g
Calibration Mass for Sucrose in C2 run after molasses sample ..... 0.3100 g
Calibration Height for Sucrose in C2 run before molasses sample 132444
Calibration Height for Sucrose in C2 run after molasses sample 132438

Calibration Mass for Lactose in C2 run before molasses sample ..... 0.160 g
Calibration Mass for Lactose in C2 run after molasses sample ..... 0.160 g
Calibration Height for Lactose in C2 run before molasses sample 161321
Calibration Height for Lactose in C2 run after molasses sample 161830

Mass of molasses sample ..... 1.0725 g
Height for Sucrose in molasses sample 144579
Mass for Lactose in molasses sample ..... 0.160 g
Height for Lactose in molasses sample 165183

RRF of sucrose before sample:

$$\frac{0.3100}{132444} \times \frac{161321}{0.160} = 2.36 \quad \{1\}$$

RRF of sucrose after sample:

$$\frac{0.3100}{132438} \times \frac{161830}{0.160} = 2.37 \quad \{1\}$$

Average of the RRFs:

$$\frac{2.36 + 2.37}{2} = 2.36 \quad \{2\}$$

Percentage of sucrose in the molasses sample:

$$\frac{144579 \times 0.160}{165183} \times 2.36 \times \frac{100}{1.0725} = 30.8\% \quad \{3\}$$

sample, followed by another middle calibration standard, followed by the two duplicates of a different molasses sample, etc. This pattern is repeated until all molasses samples have been run. The middle calibration standards that bracket the molasses sample runs are used to determine the relative response factors (see the "Calculations" section).

CALCULATIONS

Relative Response Factors (RRFs) are calculated for each sugar (i.e., glucose, fructose and sucrose for cane; and sucrose and raffinose for beet) of each calibration standard that is run. For example, the RRF of sucrose in a standard can be calculated by using the following equation:

$$RRF = \frac{M_{suc\text{-}std}}{H_{suc\text{-}std}} \times \frac{H_{lac\text{-}std}}{M_{lac\text{-}std}} \quad \{1\}$$

where: RRF = Relative Response Factor
$M_{suc\text{-}std}$ = Mass of sucrose standard (g)
$H_{suc\text{-}std}$ = Peak height of sucrose standard
$M_{lac\text{-}std}$ = Mass of internal lactose standard (g)
$H_{lac\text{-}std}$ = Peak height of lactose standard

The sugar percentages in each final molasses sample can then be calculated in two steps (see Example 1).

Step 1: Determine the average of the RRFs for sucrose from the standards immediately *before* and *after* the final molasses sample by using the following equation:

$$RRF_{ave} = \frac{RRF_1 + RRF_2}{2} \quad \{2\}$$

where: RRF_{ave} = Average RRF for sucrose
RRF_1 = RRF of sucrose *before* the cane molasses sample
RRF_2 = RRF of sucrose *after* the cane molasses sample

Step 2: Determine the percentage of sucrose in the final cane molasses sample by using the following equation:

$$\%_{suc} = \frac{H_{suc\text{-}smp} \times M_{lac\text{-}smp}}{H_{lac\text{-}smp}} \times RRF_{ave} \times \frac{100}{M_{mol\text{-}smp}} \quad \{3\}$$

where: $\%_{suc}$ = Percentage of sucrose in the sample
$H_{suc\text{-}smp}$ = Peak height for sucrose in the sample
$H_{lac\text{-}smp}$ = Peak height for lactose in the sample
$M_{lac\text{-}smp}$ = Mass of internal lactose standard (g)
RRF_{ave} = Average RRF taken from Step 1
$M_{mol\text{-}smp}$ = Mass of molasses (g)

Relative Standard Deviation (RSD) should not be greater than 1% for duplicates of sucrose, should be less than 2% for duplicates of glucose and fructose, and should be less than 6% for duplicates of raffinose. If the RSD is not within these guidelines, the sample should be reinjected or prepared again.

RESULTS AND DISCUSSION

The CarboPac PA1 column reproducibly separates the sugar components of cane and beet final molasses. PAD, with pulse potentials optimized for carbohydrates, permits detection of the sugars in final molasses samples without interferences from coeluting components, if any.

Figure 1 shows the separation of the sugars in cane final molasses and the internal standard, lactose. The elution order is glucose, fructose, lactose, then sucrose. After performing the experiment, the percentage of each sugar is calculated (see Example 1). The expected ranges for cane final molasses are 2–10% for glucose, 3–11% for fructose, and 25–37% for sucrose. The percentages determined for this sample, as shown in Figure 1, are 4.39% for glucose, 6.67% for fructose, and 30.8% for sucrose.

Figure 2 shows the separation of the sugars in beet final molasses and the internal standard, lactose. The elution order is lactose, sucrose, then raffinose. The percentage of each sugar is calculated (as in Example 1). The expected ranges for beet final molasses are 35–50% for sucrose and 1–2% for raffinose. The percentages determined for this sample were 47.7% for sucrose and 2.10% for raffinose. Because of the low concentration of raffinose in beet molasses and the high dilution factor required for optimal sucrose analysis, the raffinose percentage is less precise than the sucrose percentage. When necessary, the raffinose can be determined separately using the first 1:100 dilution, rather than the second 1:100 dilution.

HPAE-PAD methodology uses pellicular anion exchange resin technology coupled with selective amperometric detection. The monosaccharides will elute first, followed by disaccharides and then trisaccharides. Pulsed amperometric detection uses a repeating sequence of three potentials, which are applied for specific durations. Using the pulsed conditions in Technical Note 21,[6] detection is optimized for carbohydrates.

For further details concerning pulse sequences used in pulsed amperometric detection, refer to Dionex Technical Note 21.[6] To drive the oxidation reaction at the working electrode of the detector, the eluent pH should be greater than 12. For further details concerning carbohydrate determination, refer to Dionex Technical Note 20.[7]

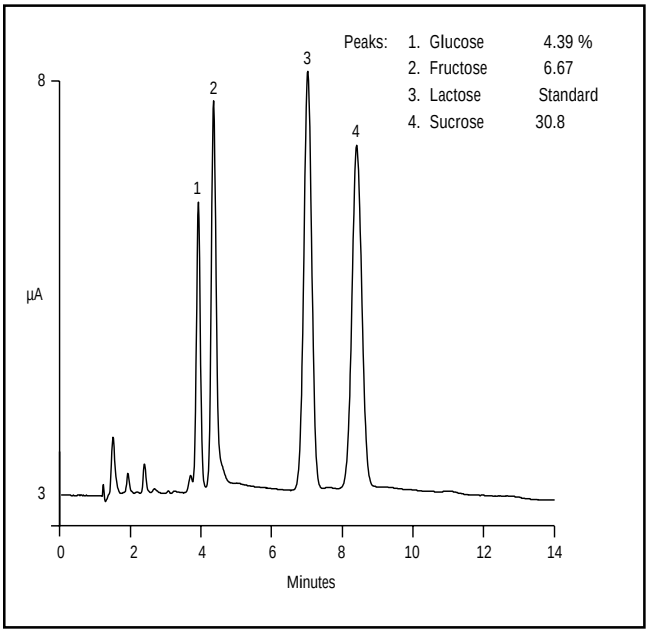

Figure 1 *Sugar cane sample prepared and run by this method.*

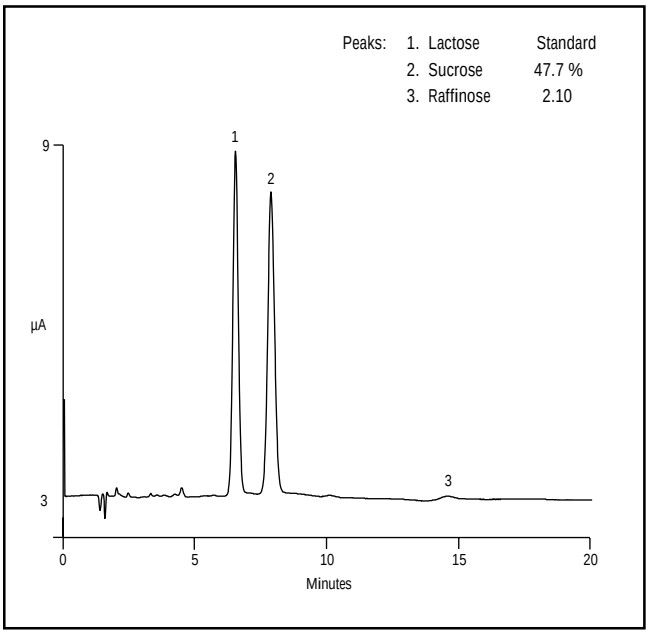

Figure 2 *Sugar beet sample prepared and run by this method.*

PRECAUTIONS

Metal should be eliminated from the eluent flow path, including the injection valve, prior to the column. Metal contamination of the analytical column can result in poor peak efficiency and/or symmetry, which may lead to poor reproducibility.

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6. Dionex Technical Note 21: "Optimal Settings for Pulsed Amperometric Detection of Carbohydrates using the Dionex Pulsed Electrochemical Detector (PED-2) and the Pulsed Amperometric Detector (PAD-2)".
7. Dionex Technical Note 20: "Analysis of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection".

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