HPLC Analysis of Sugars in Foods Containing Salt

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Utilization of propylamine-bonded silica stationary phase with acetonitrile/water mobile phase provides appropriate selectivity to separate mono- and disaccharides. The usefulness of this separation is limited in measuring sugars in foods that contain salt (sodium chloride) because chloride elutes as an interference with the sugars. The addition of sodium chloride to the mobile phase adjusts the selectivity to sufficiently resolve the chloride peak from the sugars while maintaining resolution of the sugars. This mobile phase modification can be used on a broad range of food matrices including those that do not contain salt.

Keywords: *HPLC; sugars; salt*

INTRODUCTION

The advantages of using HPLC to measure sugars have been recognized for over a decade (AOAC, 1993; Nollet, 1992; Iverson and Bueno, 1981). HPLC has proven to be more selective than conventional wet methods, resulting in less sample pretreatment. Additionally, HPLC allows individual quantitation of several individual sugars in a single chromatographic run.

The Association of Official Analytical Chemists (AOAC) International Task Force on Methods for Nutrient Labeling Analysis has recommended HPLC as a preferred analytical method to meet the FDA nutrition labeling requirement for sugars in food (AOAC, 1993). Sugars have been defined to include mono- and disaccharides. Of the broad offering of carbohydrate separation chemistries available, propylamine-bonded silica possesses the best selectivity to separate mono- and disaccharides from food samples. The separation is facilitated with a mobile phase of acetonitrile and water, usually in a 8:2 ratio, and the sugars are detected with a differential refractometer. A major drawback to this separation chemistry is the interference of sodium chloride with the sugars. Chloride ion elutes with, or near, the sugars being measured, making accurate quantitation difficult. The specific sugar that the chloride ion interferes with differs between the various manufacturer's columns, but chloride is most commonly eluted in the region of the glucose and sucrose peaks. The significance of this interference may contribute to the fact that HPLC on a propylamine-bonded silica stationary phase is an accepted AOAC method for only a limited number of matrices (AOAC, 1990).

In the early 1980s, there were several published reports on removing the salt interference by modifying the mobile phase with an ion-pairing reagent, tetrabutyl ammonium chloride (Wills et al., 1982), or by washing the column with a solution of 0.1% TEPA (tetraethylenepentamine) prior to analysis (DeVries et al., 1983). The former approach suffered from poor reproducibility, while the latter provided only a temporary cure, lasting for approximately 10 injections. A more recent approach is to selectively detect the sugars by pulsed amperometric detection (PAD). PAD detection can detect the sugars with more specificity than differential refractometry, but the sugars must be ionized at high pH in order to be electrochemically active. A basic reagent (sodium hydroxide) can be added postcolumn to ionize the sugars, but this requires an additional pump and mixing coil. Additionally, there is resistance to PAD detection of sugars because it is perceived to be a difficult technique relative to differential refractive index detection. Presently, food analysts are limited in their ability to use the advantages of HPLC to measure sugars for nutrition labeling from a wide matrix of foods.

This paper introduces a simple modification to the acetonitrile/water mobile phase that causes the chloride to elute before the sugars, allowing accurate and confident quantitation of the sugars, free of this interference.

EXPERIMENTAL PROCEDURES

A Waters HPLC system was used, including a 510 pump, a 717 autosampler, a 410 differential refractometer with internal temperature set at 35 °C, and a Millennium 2010 Chromatography Manager. A Waters High Performance Carbohydrate Analysis Column was used at ambient temperature. This column contains a propylamine ligand bonded to 4 μm, 90 Å pore size, spherical silica particles. The column is a 4.6 × 250 mm stainless steel cartridge column.

Sugar standards were purchased from Sigma as solids, dissolved in water 0.25% (w/w), and filtered through 0.45 μm Millex HV filters (Millipore, Bedford, MA). A 20 μL injection volume was used for standards as well as samples.

Food samples were extracted into warm water (80–90 °C) in a blender at high speed for 2 min. The extract was centrifuged or coarse filtered to remove solids. The typical extraction ratio was 1:10 (w/w) food sample to water. Following centrifugation, an aliquot was carefully taken from the center portion of the centrifuge tube, filtered through a 0.45 μm Millex HV filter, and collected in an autosampler vial, ready for injection. To prolong column life, a deproteinization step, such as C18 solid phase extraction cleanup or acetonitrile precipitation, should be included following centrifugation.

RESULTS

Initial work on this system used a mobile phase consisting of 70% CH_3CN, 30% H_2O pumped at 1.0 mL/min. These conditions base-line-resolved fructose, glucose, sucrose, maltose, and lactose with a run time of about 12 min. Further work indicated that 75% CH_3CN, 25% H_2O at 1.4 mL/min gave optimum resolution of the sugars with a similar run time. Figure 1 shows separation of standards using these conditions. Figures 2 and 3 are food samples run under the same conditions. In these food extracts, coelution of chloride with glucose

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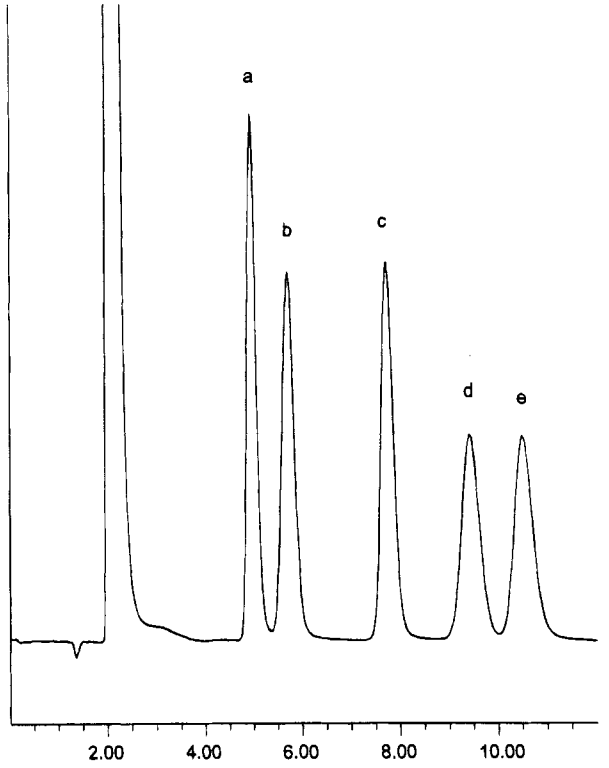

Figure 1. Sugar standards solution containing 0.25% concentration of (a) fructose, (b) glucose, (c) sucrose, (d) maltose, and (e) lactose. Column: Waters High Performance Carbohydrate Analysis Column. Mobile phase: 75% acetonitrile/25% water. Flow rate: 1.4 mL/min. Refractive index detection.

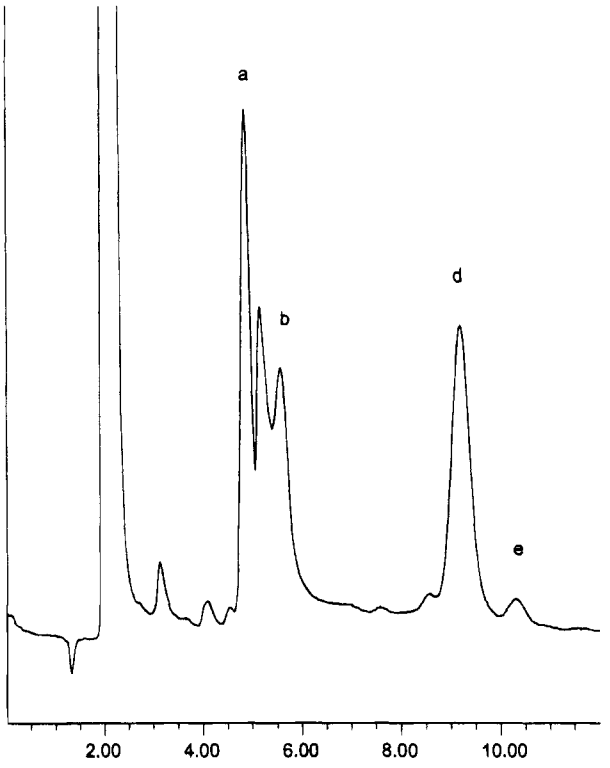

Figure 2. Frozen pizza extract. Conditions are the same as in Figure 1: (a) fructose, (b) glucose plus sodium chloride interference, (d) maltose, and (e) lactose.

is evidenced by poor peak symmetry for glucose as well as erroneously high glucose levels.

The approach that our laboratory took toward resolving the chloride from the sugars was to add sodium chloride (Sigma) to the mobile phase. It was anticipated

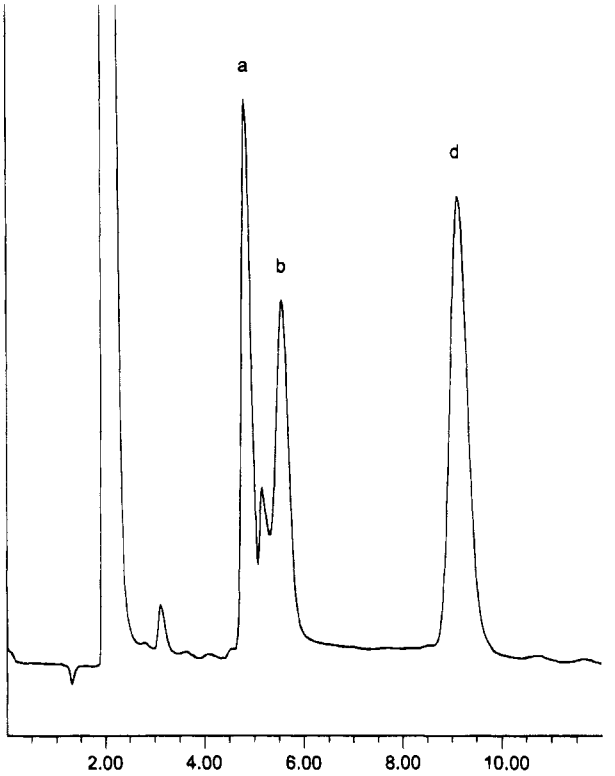

Figure 3. Frozen bagel extract. Conditions are the same as in Figures 1 and 2: (a) fructose, (b) glucose plus sodium chloride interference, and (d) maltose.

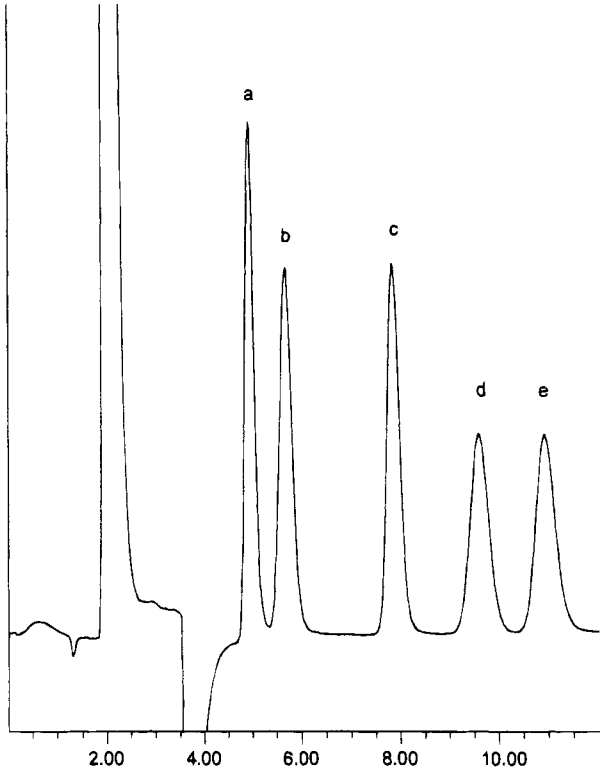

Figure 4. Sugar standards solution containing 0.25% concentration of (a) fructose, (b) glucose, (c) sucrose, (d) maltose, and (e) lactose. Column: Waters High Performance Carbohydrate Analysis Column. Mobile phase: 75% acetonitrile/25% water with 0.5% (w/w) sodium chloride. Flow rate: 1.4 mL/min. Refractive index detection.

that the chloride in the mobile phase would have two effects on the chromatography: first, the chloride ions in the mobile phase would decrease the retention of the

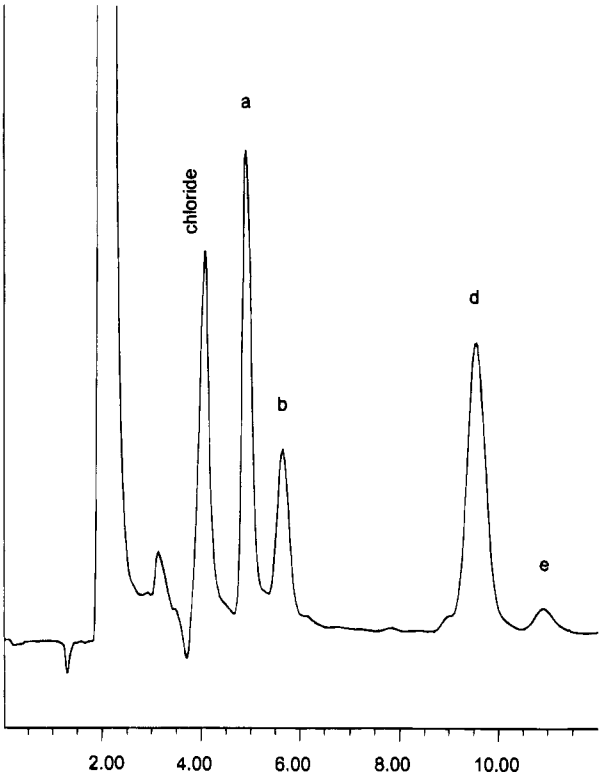

Figure 5. Frozen pizza extract. Conditions are the same as in Figure 4: (a) fructose, (b) glucose, (d) maltose, and (e) lactose. Sodium chloride is fully resolved, eluting earlier than the sugars.

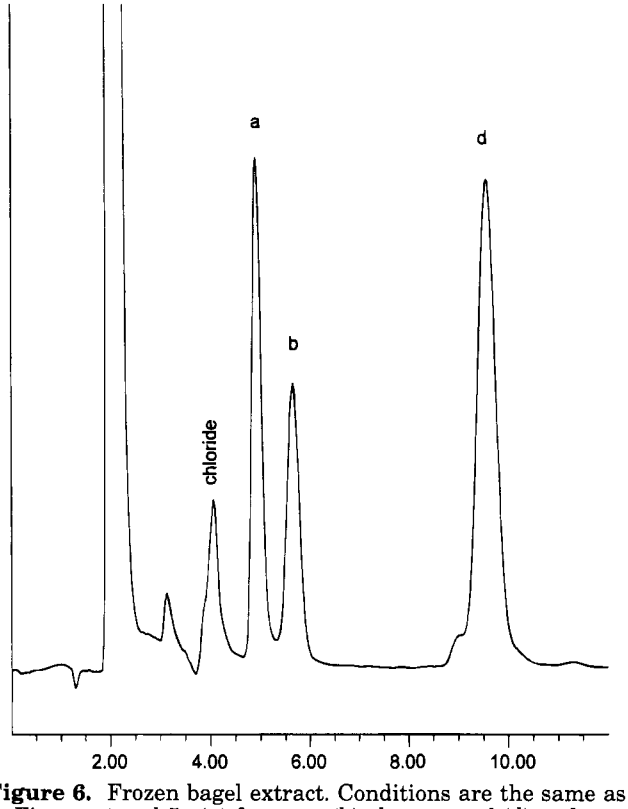

Figure 6. Frozen bagel extract. Conditions are the same as in Figures 4 and 5: (a) fructose, (b) glucose, and (d) maltose.

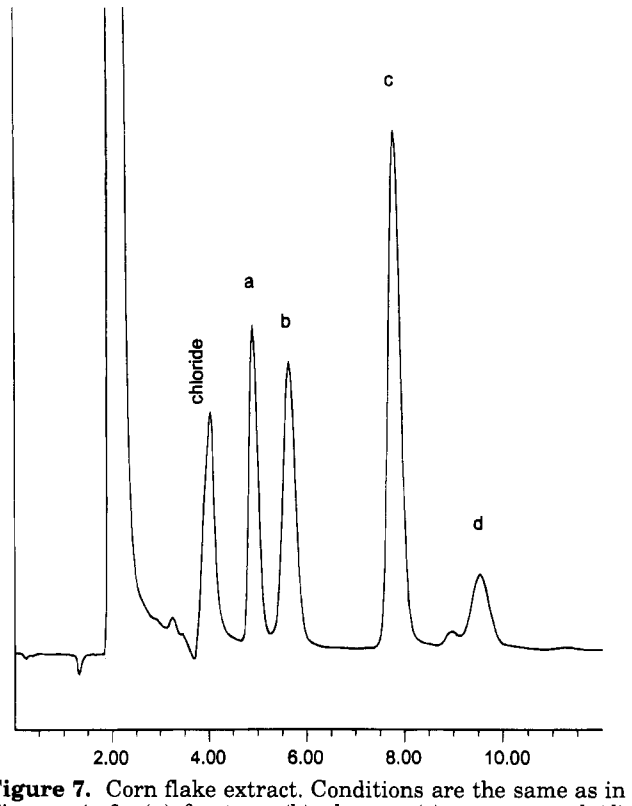

Figure 7. Corn flake extract. Conditions are the same as in Figures 4–6: (a) fructose, (b) glucose, (c) sucrose, and (d) maltose.

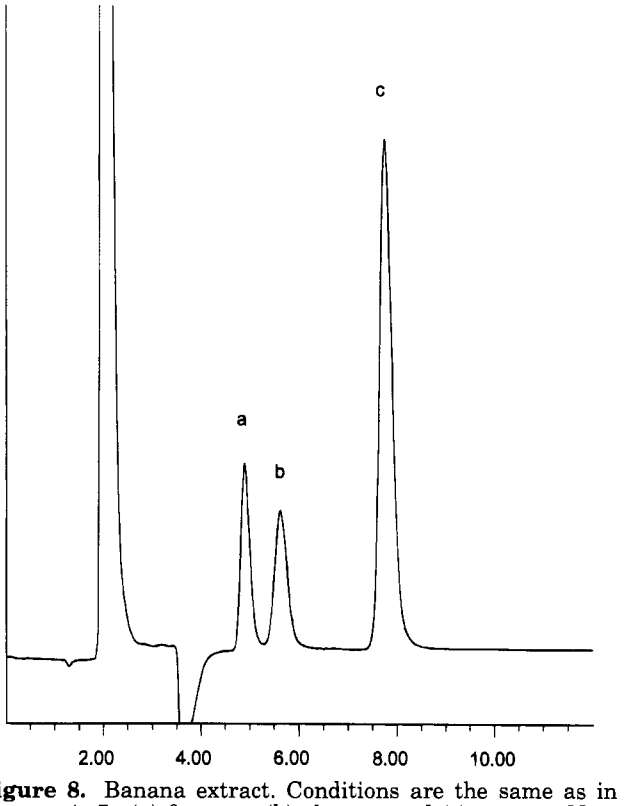

Figure 8. Banana extract. Conditions are the same as in Figures 4–7: (a) fructose, (b) glucose, and (c) sucrose. Note the negative dip in base line from chloride vacancy.

chloride from the food samples retained by an anion exchange mechanism, and second, the sodium chloride in the mobile phase would decrease the differential refractometer response to the chloride injected. Experiments proved that 0.5% (w/w) sodium chloride in the water fraction of the mobile phase successfully resolved the salt peak from the sugars. The resulting concentration of sodium chloride in the total mobile phase is 0.125%. Figures 4–6 are chromatograms of the previously run standard and food extracts that were chromatographed with the addition of sodium chloride to the mobile phase. These results demonstrate the full

resolution of the sugars from the earlier eluting chloride peak, enabling accurate quantitation. Figure 7 demonstrates the resolution of the chloride peak from an RTE cereal sample containing added sucrose as well as added salt. It was of interest to analyze a food sample that contained little or no salt to observe the vacancy peak that results from lack of chloride elution. Figure 8, a chromatogram of a banana extract, provided that data. The vacancy, or negative peak, observed before fructose is caused by the absence of chloride in the sample. The base line stabilizes in sufficient time to accurately quantitate fructose.

Although the addition of 0.125% (w/v) sodium chloride to the mobile phase results in approximately a 10% reduction in differential refractometer response to the sugars, the sensitivity of the method is still sufficient for meeting the nutrition labeling requirements.

CONCLUSION

Experiments show that a mobile phase consisting of 75% acetonitrile/25% water containing 0.5% (w/w) sodium chloride run on this column at 1.4 mL/min has the selectivity to remove a previously interfering sodium chloride peak during sugar analysis. The addition of sodium chloride to the aqueous fraction of the mobile phase has not shown to have any negative effects on column life or retention time reproducibility. This simple modification will be very useful in broadening the range of food matrices that the propylamine-bonded silica stationary phase can be used for, particularly in analyzing sugars for nutrition labeling.

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