921077-04

Column Selection and Sample Prep Techniques for the Proposed Requirements for Nutritional Labeling of Carbohydrates

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Recently proposed nutritional labeling requirements for carbohydrates redefine the category of sugars to include mono through tetrasaccharides as well as calling for labeling of the sugar alcohols mannitol, xylitol and sorbitol. Current analytical methods for sugars in foods are not capable of quantifying all these components in one analysis. The method described here is an attempt to meet this labeling requirement across a diversity of matrices using High Performance Liquid Chromatography (HPLC). This article includes a brief review of current HPLC methodologies, the use of differential refractive index detection (RI), sample preparation techniques and a new chromatographic approach.

Current AOAC methods of sugar analysis which cite HPLC are for matrices such as presweetened cereal (982.14), chocolate (980.13), licorice extract (984.17) and honey (977.20). These methods incorporate a propyl amine bonded stationary phase such as the Waters Carbohydrate Analysis Column with an acetonitrile/water mobile phase and dRI detection. This technique is capable of quantifying mono- and disaccharides but falls short for the newly proposed tri/tetrasaccharides and sugar alconols.

Another common HPLC mode for carbohydrate analysis is the use of ion-exchange resin-based columns. These columns have difficulty separating disaccharides from one another, but work well for size separations (mono/di/tri/tetrasaccharides) and alcohols/sugar alcohols.

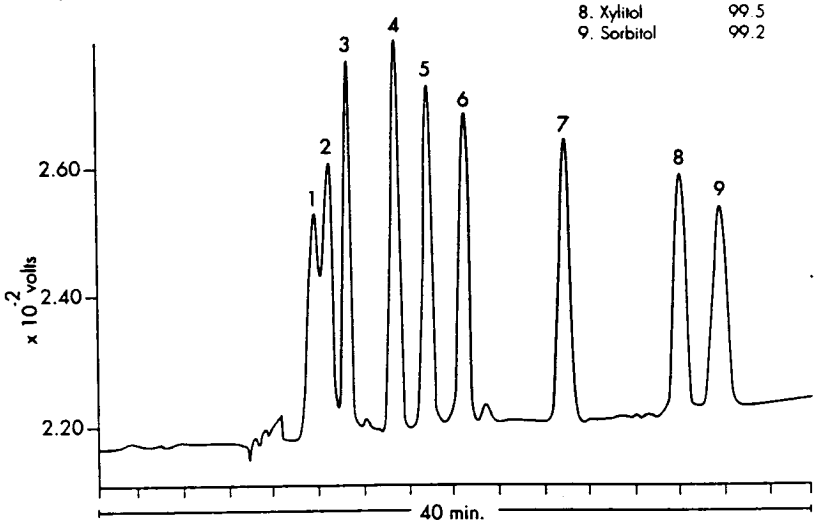
Sensitivity becomes important in the application of HPLC to the carbohydrate nutritional labeling requirements from a sample extraction/preparation point of view. The 410 RI detector provides the level of sensitivity required for these analyses and allows for more dilute extractions which improve the ability to make more thorough extractions (especially tetrasaccharides). Also, since Sep-Pak® cartridges are used in this method, more dilute extractions lower the chances of overloading these solid phase extraction devices with unwanted extractables, resulting in cleaner samples for injection. This article will discuss sample preparation in more detail on an individual sample basis.

The first example (Figure 1) of this method illustrates the uniqueness of the dual separation mode as well as the recoveries obtained with a Sep-Pak C_{18} cartridge.

Figure 1: Carbohydrate Standards - 100 ppm Sep-Pak C_{18} Cartridge Recoveries

Column: KS-801 & SP-0810
Flow Rate: 1.0 mL/min
Mobile Phase: H_2O
Injection Volume: 40 µL
Detection: M410 dRI
Sample: Standards, 100 ppm each with Sep-Pak C_{18} cartridge
Temperature: 80 °C

Name	% Recovery
1. Stachyose	94.6
2. Raffinose	96.2
3. Sucrose	98.2
4. Glucose	98.1
5. Galactose	100.0
6. Fructose	98.3
7. Mannitol	100.0
8. Xylitol	99.5
9. Sorbitol	99.2



Using a KS-801 column in conjunction with a SP-0810 column (with a SP-1010P guard column), the sugars of interest can be resolved with a combined size and ion exchange mechanism using water as the mobile phase. Run times of 40 minutes do not seem all that prohibitive when a total sugar profile, as called for nutritional labeling proposals, can be accomplished. In all the following examples, the same chromatographic and sample clean-up conditions are used.

The samples analyzed were chosen to represent a wide diversity of matrices. The first sample (Figure 2), ready to eat (RTE) breakfast cereal is indicative of a matrix which is high in total carbohydrate content (~85%).

An aqueous extraction with sonication followed by a Sep-Pak C_{18} cartridge clean-up was used to give the results of 8 g/100 g mono and disaccharides in this sample.

The next sample, peanut butter, is shown in Figure 3 and represents a high fat matrix (about 50%). In this case a very large dilution (1/400 w/v) was used to provide an efficient extraction employing a Sep-Pak C_{18} cartridge. At this dilution, 1.3 mg of lipid and 0.7 mg of protein are present per mL of the extract. Typical Sep-Pak C_{18} cartridge bonding capacities are around 5 mg of retained mass, which is adequate to handle 2 – 3 mL of this particular matrix extract. Note that this sample was labeled to contain dextrose (glucose) and sugar (sucrose). Tetrasaccharides were found in this sample also.

Figure 2: Carbohydrates: Ready to Eat Cereal

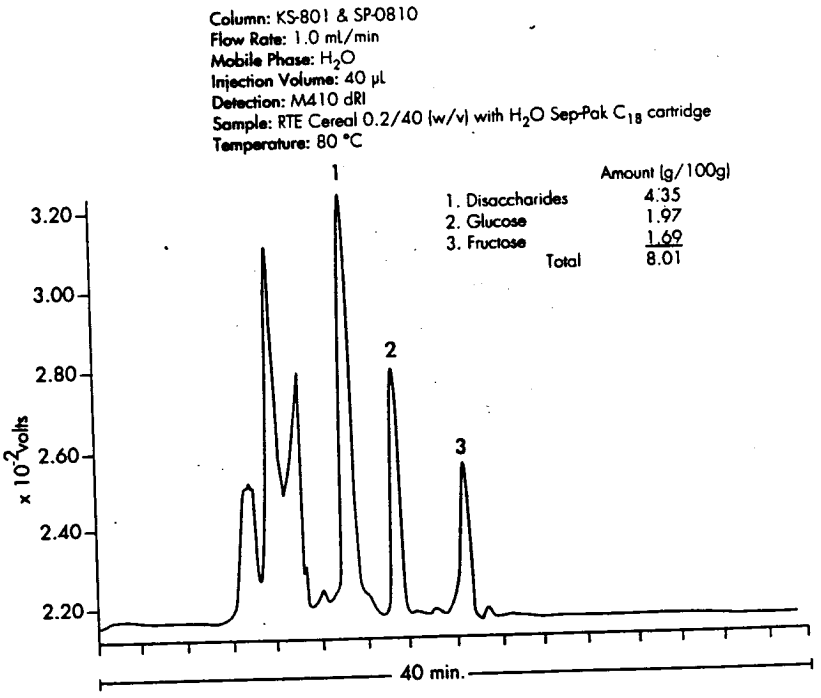
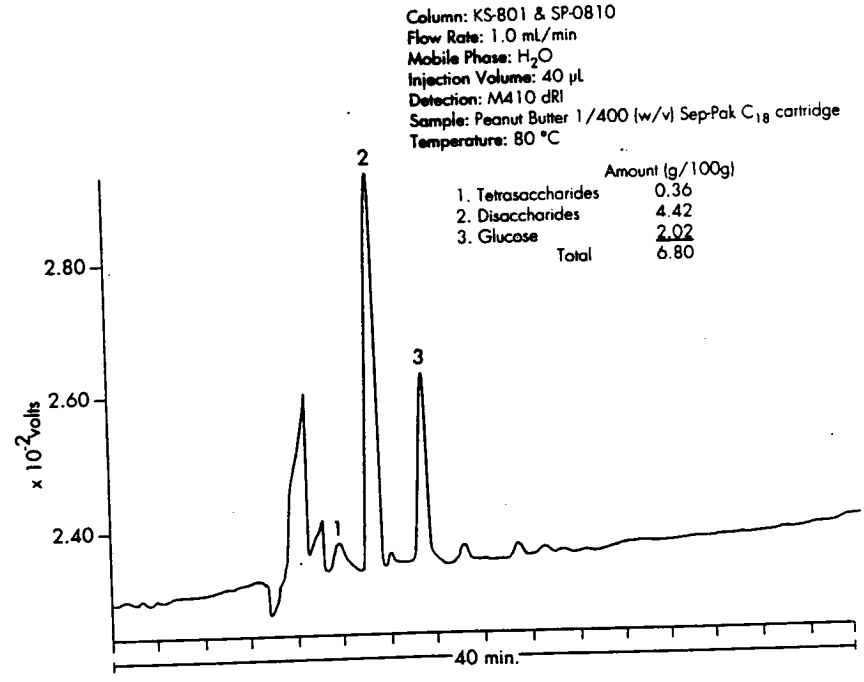


Figure 3: Carbohydrates: Peanut Butter



Flavored potato chips (Figure 4) were analyzed to provide data on how a high salt matrix can be analyzed with this method.

Again, a large dilution (1/200 w/v) allows efficient use of a Sep-Pak C_{18} cartridge to remove lipids and proteins. The SP-1010P Guard-Pak provides additional protection to the analytical columns for high salt samples such as this.

Macaroni was chosen as a sample matrix which provides natural levels of carbohydrates including tetrasaccharides (Figure 5).

The exceptional sensitivity of the Waters 410 refractive index detector allows for a 1/100 (w/v) dilution. This dilution assures complete extraction of these natural levels of sugars from this highly complex carbohydrate matrix.

Figure 4: Carbohydrates: Flavored Potato Chips

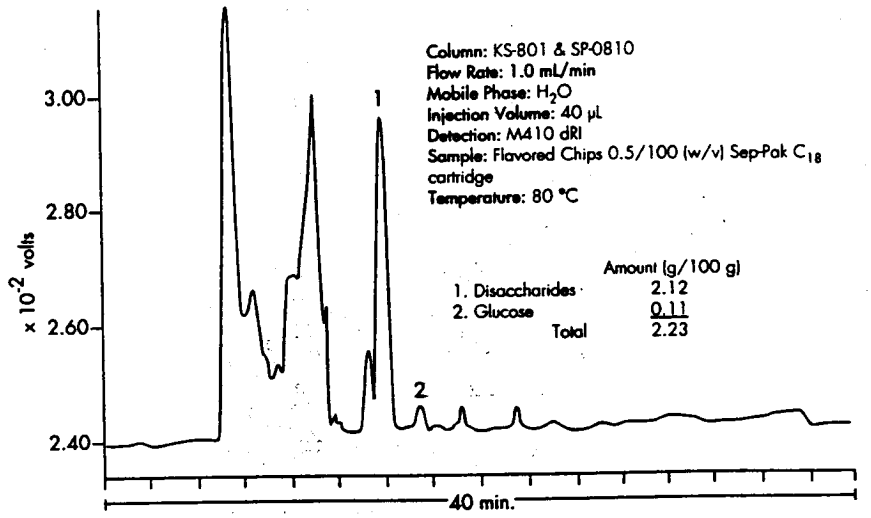


Figure 5: Carbohydrates: Macaroni

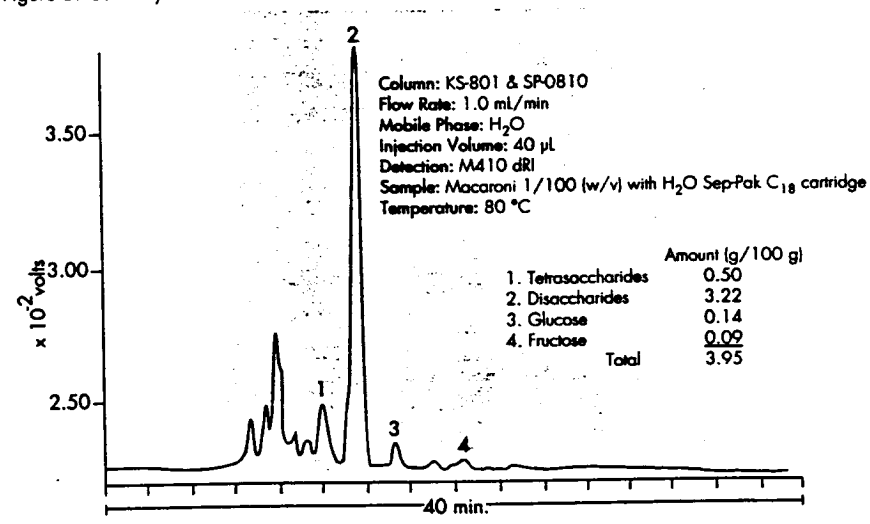
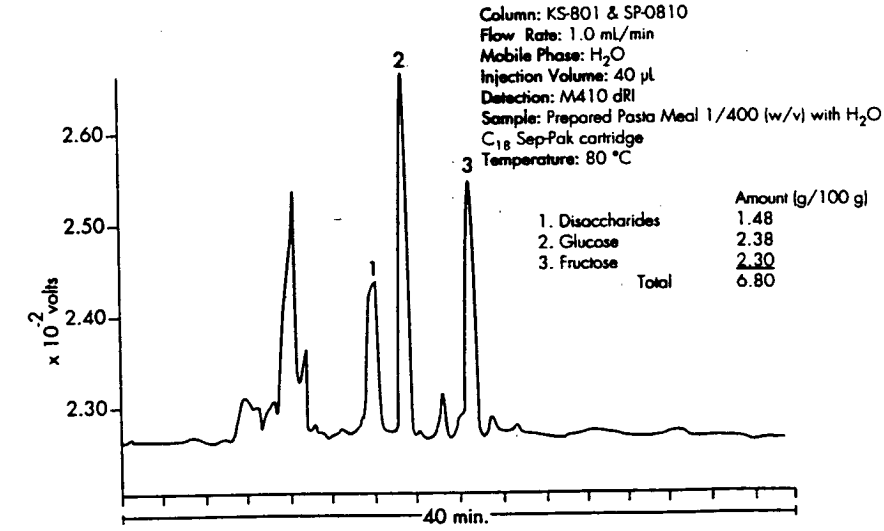


Figure 6: Carbohydrates: Prepared Pasta Meal



Prepared foods contain a more extensive ingredient list which may present a more difficult analytical challenge. This particular product is made with both high fructose corn syrup and sweet dairy whey, so it is an excellent example of the ability of this method to deal with disaccharides (maltose and lactose) as a single entity. The Waters Carbohydrate Analysis column could be used with an acetonitrile/water mobile phase to analyze the maltose and lactose individually if needed.

Although dRI has been used in other methods to provide detection of less than 100 ng of a specific sugar injected onto a column, this method appears to have less sensitive capabilities. The use of two 8 mm x 300 mm columns subtracts from sensitivity (due to sample diffusion on column). However, the 10 ppm standard in Figure 7 (400 ng on column) demonstrates that this method would allow for a 1/100 dilution of a 0.1% sugar-containing sample and still have adequate sensitivity for quantitation.

The retention times of a number of carbohydrates and related compounds using the HPLC method discussed here are shown in Table 1.

The underlined compounds are the ones used in this study to calibrate the system for subsequent sample analyses.

In conclusion, the method presented in this study demonstrates the ability to separate the carbohydrates which are of interest in the proposed labeling regulations from a wide range of sample matrices. Straightforward sample preparation techniques can be incorporated through the use of Sep-Pak cartridges. The 410 dRI detector provides more than adequate sensitivity to meet the method requirements and offers good stability (temperature stabilized) and similar response factors for sugar "classes".

For ordering information on Waters Sep-Pak cartridges and columns for carbohydrate analysis, see page 29. For more information on HPLC and Nutritional Labeling, please check box number 7 on the reply card and return today.

Figure 7: Carbohydrate Standards–400 ng Sensitivity

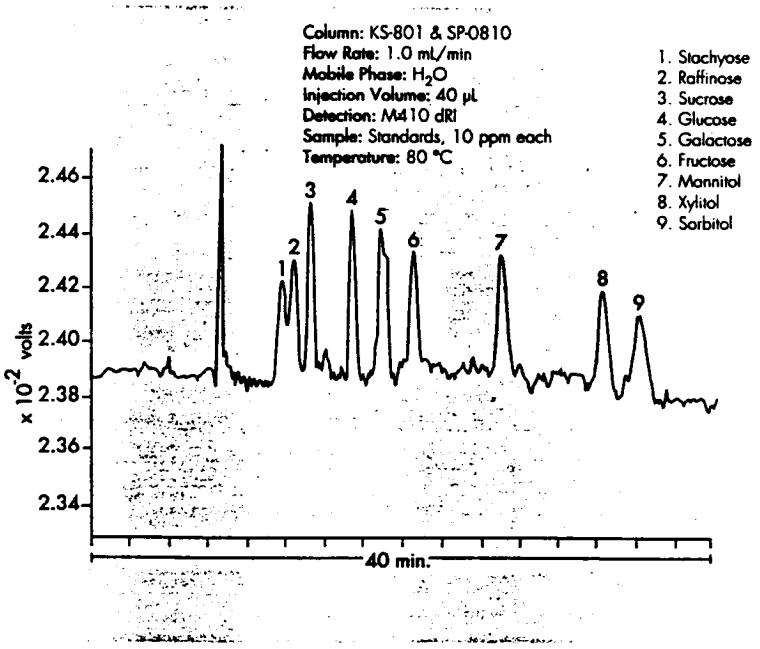


Figure 7 was generated to gain insight into the sensitivity limits of this technique.

Table 1: Carbohydrate Retention Times Using KS-801 and SP-0810 in Tandem

Compound	RT (min)	
Amylose	9.03	Complex Carbohydrate
Stachyose	12.49	Tetrasaccharides
Melezitose	12.45	
Maltotetraose	12.85	
Raffinose	13.26	Trisaccharides
Cellobiose	14.14	Disaccharides
Sucrose	14.31	
Maltose	15.06	
Lactose	15.39	
Glucose	16.91	Monosaccharides
Xylose	18.07	
Galactose	18.89	
Mannose	20.05	
Arabinose	20.30	
Fructose	20.52	
Ribose	31.99	
Maltitol	19.97	Sugar Alcohols & Alcohols
Mannitol	25.38	
Xylitol	30.03	
Sorbitol	32.47	
Ethanol	21.6	
Propylene Glycol	22.40	