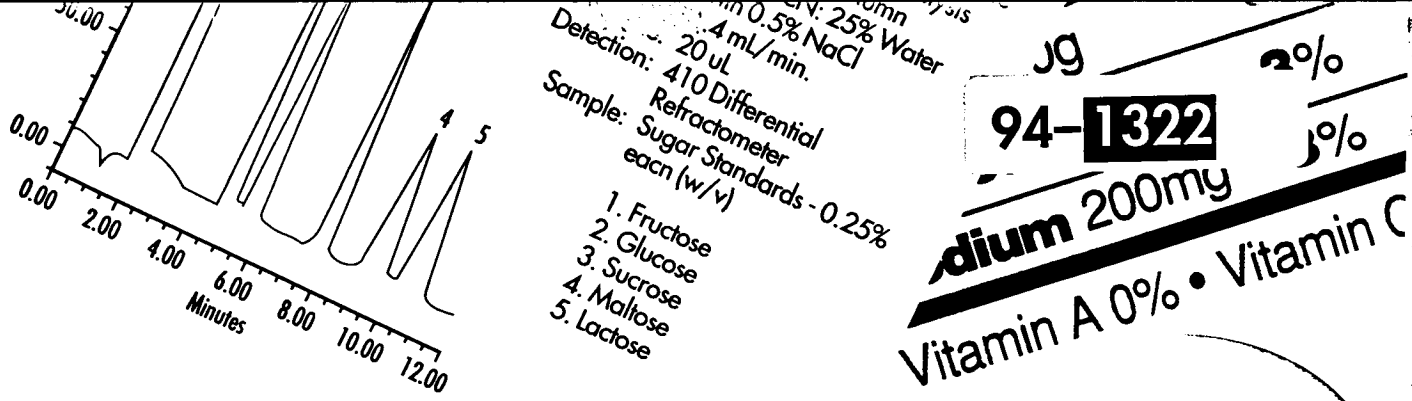

FOOD & BEVERAGE NOTES

VOLUME 6 NUMBER 1

94/322 -01

Challenges of Nutritional Labeling

The deadline for nutritional labeling has arrived and it "will have a far reaching impact on analytical chemists both in the volume of work that will be required and the increased need for more accurate tests of some nutrients."[1] This challenges chemists to incorporate methods that combine valid results with high sample throughput.

In the area of sugar analysis, HPLC is recognized and recommended as the preferred method to meet label claim requirements. Currently, four AOAC methods are cited for sugar analysis by HPLC: 982.14, 980.13, 984.17, and 977.20.

"...high throughput and economical sugar analysis [with an] estimated operating cost for the Waters Sugar Analyzer... less than $4.00 per sample."

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The newest of these AOAC methods is ten years old, and while still valid, improvements in HPLC instrumentation and separation chemistries have produced analytical capabilities never before available. Just as Waters helped pioneer the early revolution of sugar analysis with HPLC, our chemists continue to refine and expand applications of these methods. The need for rugged, reliable techniques which are easily automated to meet nutritional labeling requirements across all matrix classes is achievable with Waters™ Sugar Analyzer and the new High Performance Carbohydrate Analysis Cartridge Column.

The mandatory inclusion of vitamin A on food nutrition information labels has raised several concerns. In addition to determining a methodology to measure vitamin A, what actually constitutes vitamin A needs to be defined.

The AOAC International Task Force on Methods for Nutritional Labeling Analyses identified the need for an official liquid chromatographic method for the determination of vitamin A in all food matrices.

Waters

New Chemistry-Unique Selectivity

As the workload on analytical laboratories increases, so does the desire to shorten analysis time and reduce cost per assay. In response to these needs, Waters has developed a new chemistry and stainless steel cartridge column format for the separation and quantitation of label-required sugars. The new High Performance Carbohydrate Analysis Cartridge Column uses the same propyl amine separation functionality recognized in current AOAC HPLC methods.

The Waters High Performance Carbohydrate Analysis Cartridge Column will perform the desired separation with a high water content in the mobile phase, which offers a number of advantages. Where only 20-25% water concentration is used with most carbohydrate separation chemistries, the high-performance aspect of this new chemistry allows for the use of water concentrations above 25%. Since water is the "strong" eluting solvent in this separation chemistry, higher water concentrations provide shorter run times. Figure 1 demonstrates a fully resolved separation which allows for a 12-minute turn-around-time between injections for accelerated sample throughput. The increased

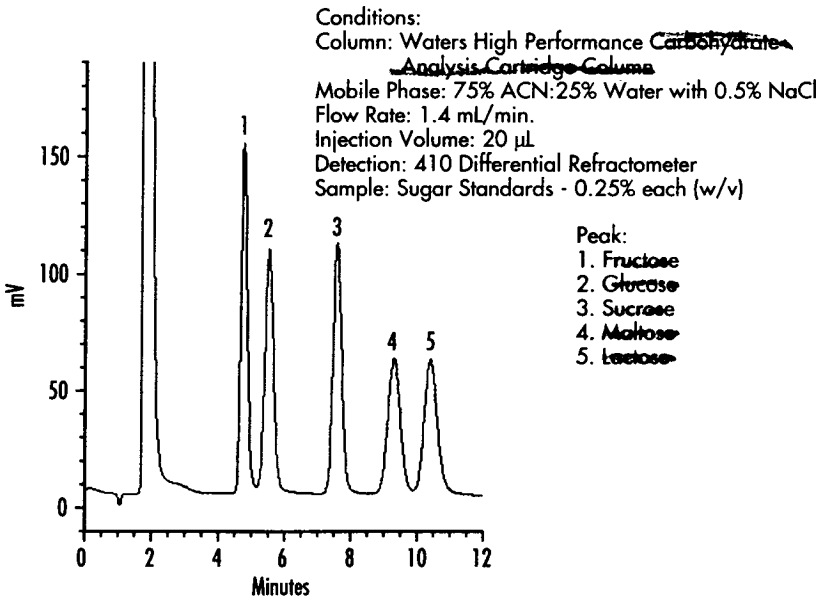


Figure 1. New Chemistry. *The unique selectivity and efficiency of the Waters High Performance Carbohydrate Analysis Cartridge Column delivers fully-resolved sugar separations in about 10 minutes and with low solvent consumption. Only 15mL of acetonitrile are used per assay with a 12 minute turn around time between injections.*

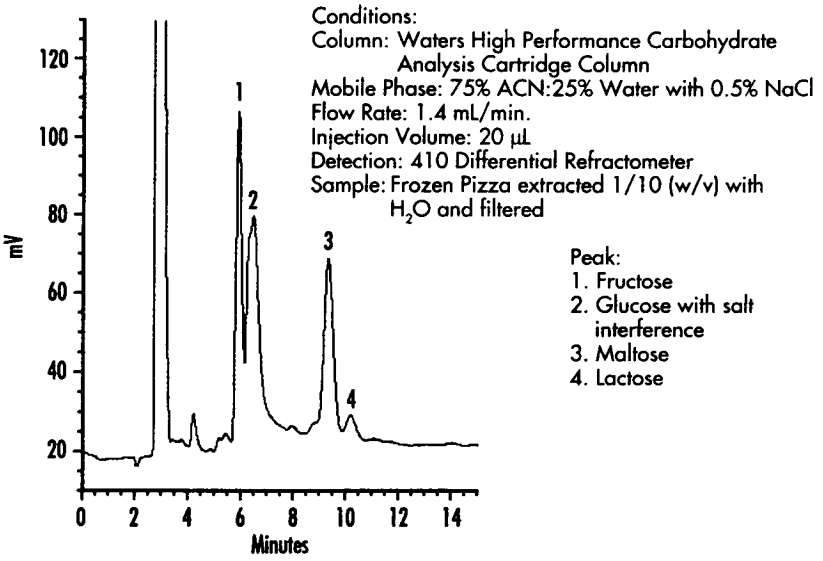


Figure 2. Salt Interferences. *High salt samples have traditionally been problematic with propyl amine column chemistries. In this pizza extract, salt (Cl) is co-eluting with the fructose/glucose portion of the chromatogram and contributing to what appears to be poor resolution of these analytes.*

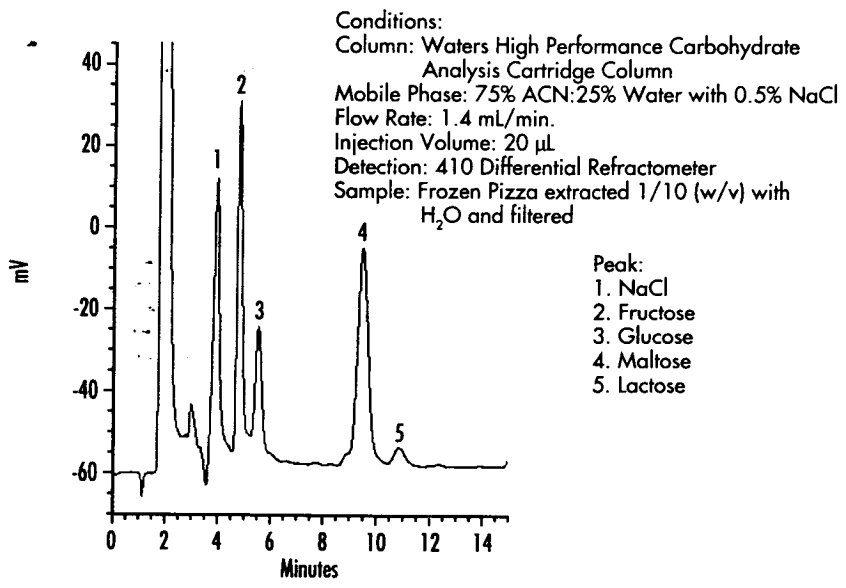


Figure 3. Interference Removal with a Mobile Phase Modifier. *The novel performance characteristics of the Waters High Performance Carbohydrate Analysis Cartridge Column allow the use of NaCl as a mobile phase modifier to shift the interfering salt peak away from the chromatographic region of interest. This example shows the same pizza extract as Figure 2 subjected to separation with the modified mobile phase. Note that the salt peak has migrated away from the sugars resulting in a chromatogram which is easily integrated to give accurate, reliable results.*

water concentration of the mobile phase lowers acetonitrile usage as well and therefore lowers cost per assay. It also provides better solubility for the analytes when higher sugar concentrations are necessary. More water in the mobile phase increases the signal-to-noise ratio and lowers detection levels.

Rare selectivity is the most significant advantage of this new carbohydrate chemistry. Past documentation on the use of propyl amine chemistries for sugar analysis from foods raises a concern that salt (Cl) co-elutes with either the fructose or glucose peak. This co-elution inhibits the ability to accurately quantify the sugars. Figure 2 shows an example of this co-elution problem. The sample, pizza, has a relatively high salt concentration which interferes with the fructose/glucose separation even on the Waters High Performance Carbohydrate Analysis Column. This column is unique, however, in its ability to tolerate the use of a mobile phase modifier (NaCl) which shifts the salt peak in and away from the sugars of interest. Figure 3 demonstrates the removal of the salt peak from the sugars using the Waters High Performance Carbohydrate Analysis Column with a mobile phase modifier, resulting in an easily integrated separation for accurate results.

These selectivity attributes are enhanced by the new cartridge format, featuring reusable endfittings. The novel chemistry is packed in a 4.6 x 250mm stainless steel cartridge column. These dimensions were found to be the optimum for speed, resolution, and longevity. Figure 4 shows a series of up to 350 injections to demonstrate minimal changes in retention time over the extended use of the column. The cartridge column format is compatible with an optional Sentry™ guard column which extends the life of the cartridge for maximum cost effectiveness. Because the cartridge employs reusable endfittings, only the stainless steel cartridge is replaced when performance warrants it, saving you a considerable amount of money long term.

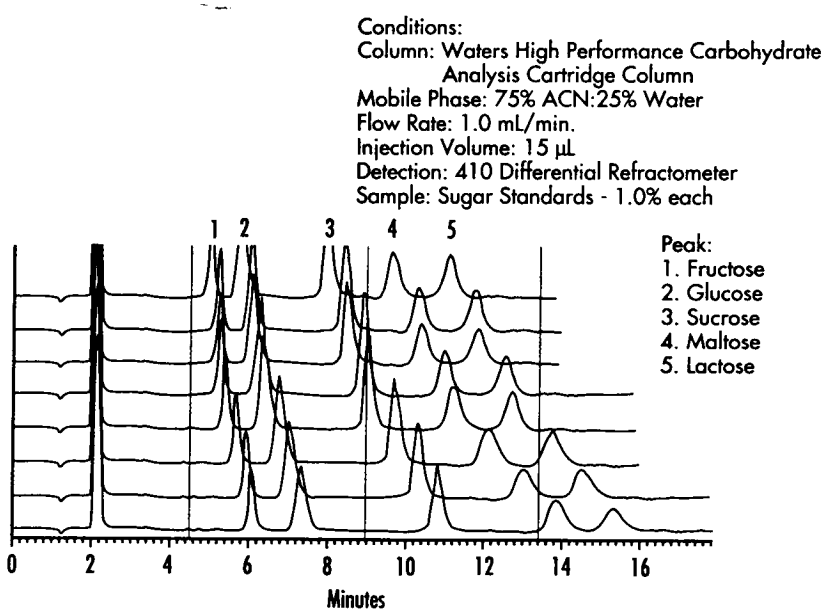


Figure 4. Rugged Chemistry. *Column integrity is maintained even after 350 injections of standards on the new Waters High Performance Carbohydrate Analysis Cartridge Column. Resolution is intact and peak shifts are minimal. In further studies, up to 300 injections of extracted samples have been made with no appreciable change in the column performance. This data is also testimony to the overall system performance during unattended, automated analyses.*

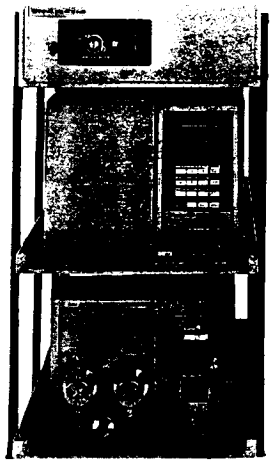
Easy To Use and Reliable Instrumentation

As sample demand increases for any assay, it is often desirable to automate the analysis. This places additional demands on the instrumentation and the analytical methods. Criteria such as ease of use, ease of automation, reliability, ruggedness, cost effectiveness, and confidence in results become desired method performance characteristics in routine assays.

The Waters Sugar Analyzer is a complete HPLC system configured for the analysis of sugars. The system includes all the components necessary to provide optimum HPLC performance for sugar analysis including the unique selectivity of the Waters High Performance Carbohydrate Analysis chemistry. Exceptional flow precision from the Waters 510 solvent delivery system ensures reliable and reproducible retention times for accurate identification of the sugars. Differential refractive index detection with the Waters 410 contributes to the ease of use and day-to-day reliability. Internal detector temperature control and counter-current heat exchangers for system temperature management assure stable performance. The wide dynamic linear range of this detector along with high sensitivity capabilities provides functionality across all sugar levels and matrices required for nutritional labeling. An integrated manual injector provides an economical means for reproducible sample introduction. Figure 4 not only demonstrates column performance but also the excellent reproducibility of the entire system.

Automation Options for High Throughput Situations

Automation of the complete HPLC process can be achieved through the use of a 717plus autosampler and a Millennium® 2010 Chromatography Manager. The 717plus can process up to 96

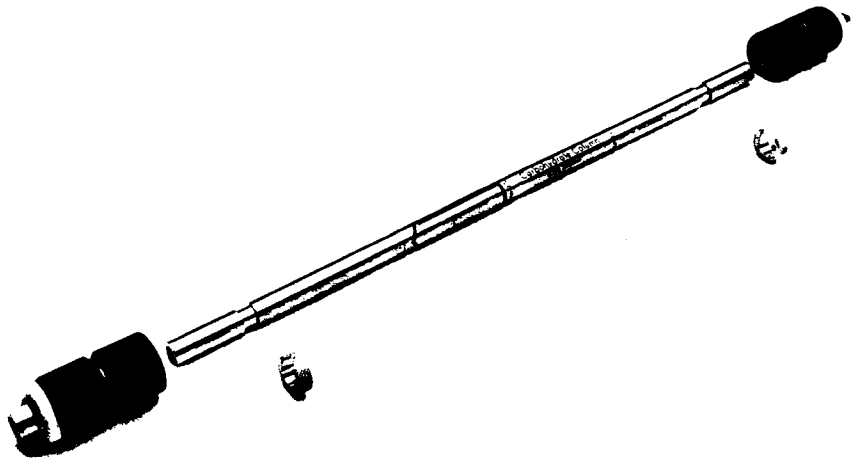


The Waters Sugar Analyzer is a complete HPLC system optimized for the analysis of sugars.

samples automatically with an injection precision of better than 0.5% RSD to generate accurate and reproducible unattended results. Auto Standards routines allow for easy and unattended system recalibration, verification and/or correction. The Priority Sample routines allow priority sampling without stopping the run. The optional heater/chiller for the 717plus ensures sample integrity. Results can be tracked and trends plotted with the Millennium System Suitability software option on a day-to-day or month-to-month basis. Custom report features allow you to format results to meet specific reporting requirements. The Millennium 2010 Chromatography Manager also controls the Sugar Analyzer and 717plus for unattended start up and shut down routines.

Economical turnkey results

HPLC system optimization and high performance chemistry can be employed to successfully meet the high sample demands for nutritional labeling of sugars. Shorter analysis times and reduced acetonitrile usage result in high throughput and economical sugar analysis. The estimated operating cost for the Waters Sugar Analyzer is less than $4.00 per sample. This assay gives results for five analytes, fructose, glucose, sucrose, maltose, and lactose, which equates to about $0.80 per sugar.



Using a new chemistry and stainless steel cartridge column format, the Waters High Performance Carbohydrate Analysis Cartridge Column separates label-required sugars with rare selectivity for more accurate quantitation. When it's time to replace the column, you keep the endfittings and dispose of only the stainless steel cartridge.

Sample extraction and preparation methods (developed by Waters food chemists) exhibit solid analytical performance with almost universal applicability. The Waters Sugar Analyzer delivers rugged, reliable results in an easy-to-use package whether your sugar analysis needs involve analytical method development or food research.

If the challenges of nutritional labeling are placing a burden on your analytical laboratory, consider the dedicated performance and rugged, high-throughput qualifications of the Waters Sugar Analyzer and new High Performance Carbohydrate Analysis Cartridge Column.

References:

1. *The Referee, AOAC International*, Vol. 16, No. 2
2. Devries, J.W. et al, *J. Assoc. Off. Anal. Chem.* (1983) 66,1,197-8