Introduction to sugar analysis application. This work was performed on a Waters Alliance HPLC System consisting of a 2690 Separations Module, 2410 Differential Refractive Index Detector, and Millennium³² chromatography software.
Analysis of mono/disaccharide's; fructose, glucose, and sucrose in an apple. The excellent reproducibility of the 5 overlay chromatograms demonstrates the exceptional flow characteristics of the 2690 Separations Module.
Nutritional Labeling of Food Products requires listing sugar and total carbohydrate content.

Common Sugars defined as:
- Monosaccharides: Fructose and Glucose
- Disaccharides: Sucrose, Maltose, Lactose

AOAC HPLC Methods Recommend the use of Propyl Amine functional columns for analysis of mono and disaccharides in food products.

This slide presents the guidelines for sugar determination according to the Nutritional Labeling & Education Act passed by the U.S. Congress. Note that labeling is not required until the levels are quite high (0.5g total sugar per serving size) and that only the mono-saccharides fructose, glucose and the di-saccharides sucrose, maltose and lactose need to be labeled. These five sugars are the most common in food systems.

In the Official Methods of Analysis of AOAC International (Association of Official Analytical Chemists), the HPLC Methods recommend the use of an analytical column containing a silica stationary phase with a propyl amine functionality.
The Waters Carbohydrate Analysis Column incorporates propyl amine functionality. Baseline resolution of the mono/disaccharides is obtained using the chromatographic conditions described above. Also available, the Waters High Performance Carbohydrate Analysis Column incorporates the familiar chemistry and selectivity of propyl amine functionality bonded onto the highly efficient support of 4 micron Nova Pak silica. This material is packed into a new column format. The Waters stainless steel cartridge column provides the ability to use an integrated guard column to prolong the life of the analytical column. This design also lowers the column replacement cost by incorporating reusable end fittings.
The Alliance HPLC System demonstrates excellent reproducibility for analysis of mono/disaccharides based upon retention time and peak area %RSD (percent relative standard deviation).
Excellent linearity and precision is also obtained. The precision decreases at the 0.005% sugar concentration because we are approaching the method detection limit making it more difficult to integrate the smaller peaks. This is demonstrated in the next slide.
The method detection limit for the mono/disaccharides is 0.0001% or 200 ng of each sugar on column. However, note that labeling is not required until the levels are quite high (0.5g total sugar per serving size).
Analysis of Mono/DiSaccharides
Waters Carbohydrate Analysis Column
Sample Preparation

★ Prepare approximately a 1% Solution of the food matrix
  • Add 80 mL of Warm (80°C) DI Water
  • Sonicate for 15 Minutes
  • Cool and Dilute to 100 mL
  • Pass through a C₁₈ Sep-Pak Cartridge to remove lipid, protein, and suspended solids
  • Use Filtrate for Analysis

Start with a sample concentration of 1 gram to 100 mL.
This is the sample preparation procedure utilized for all of the food products shown in this section.
Analysis of Mono/DiSaccharides
Waters Carbohydrate Analysis Column

1.09g Honey / 100 mL

1  Fructose = 35.30 ± 0.02%
2  Glucose = 31.34 ± 0.02%
3  Sucrose = 0.46 ± 0.01%
4  Maltose = 1.26 ± 0.02%

The following slides demonstrate the different sugar profiles obtained from four food products. Approximately 300 injections were performed on the same column to demonstrate its ruggedness and reproducibility.

Natural honey contains high concentrations of fructose and glucose. Adulteration of honey can be determined by monitoring the fructose/glucose ratio. High fructose corn syrup can be added to provide sweetness at less expense.
Sucrose and lactose (high in calories) are added to milk chocolate to make it sweet.
This breakfast drink is marketed as providing high energy and nutritional value. Note that it also contains high levels of sucrose and maltose. Therefore, it is also high in calories!
Note the contrast in this diet breakfast drink. This product contains significantly lower concentrations of sucrose and lactose so it also has a lot less calories! Less expensive high fructose corn syrup is added to make the product taste good.
Currently there are official AOAC methods for these analytes which incorporates HPLC. It should be noted that they all use propyl amine chemistry bonded to silica stationary phases and that they are very specific to the matrix. Also note that the newest of these methods is from 1984 and are still viable method today (2nd and 3rd digits of the method number represent the year of acceptance). The reasons for the matrix specificity are sample preparation issues and potential matrix interference issues. Both of these issues can be minimized using the column chemistry from Waters.

There are a variety of separation mechanisms and chemistries for the HPLC determination of sugars. Anion exchange, cation exchange, liquid/liquid partition and size exclusion represent a few useful chemistries. However, with the five "food sugars" commonly analyzed by food chemists, the propyl amine chemistry provides the desired separation.