P-W-221

UPLC ANALYSIS OF CARBOHYDRATES;
APPLICATIONS FOR SACCHARIDE ANALYSIS IN FOOD & BEVERAGE PRODUCTS AND PHARMACEUTICAL EXCIPIENTS

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INTRODUCTION

- **Carbohydrates are the most abundant class of organic compounds in nature.**
- **They are found naturally in many raw food stuffs, from fruits and vegetables to grains and dairy products.**
- **They are a common ingredient in most prepared foods, both commercially and in the home. Almost everything we consume has a carbohydrate component.**
- **The pharmaceutical industry frequently uses simple or complex mixtures of sugars or sugar derivatives as pharmaceutical excipients. The analysis of these compounds in drug formulations provides needed information for regulatory compliance.**
- **Many consumer products contain alternative sweeteners, both artificial and natural.**
- **It is this ubiquitous nature of carbohydrates that makes their analyses critical to our understanding of the nutritional consequences of the foods we consume.**

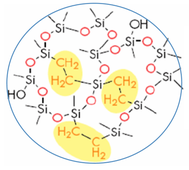
Typical methods of chromatographic analysis frequently utilize ligand/ion exchange or amino based column chemistries. Unique issues encountered with these methods include:

- Complex sample matrices result in chromatographic interferences requiring additional sample preparation steps to clean up the sample prior to analysis
- Silica based columns lack the robustness necessary for high pH applications
- Amino or polyamine based columns are plagued by high levels of column bleed and shortened column lifetime
- Formation of Schiff bases and enamines can lead to the loss of the reducing sugars at higher temperatures or lower flow rates, resulting in inaccurate quantitation and degraded columns
- Mutarotation of the reducing sugars may result in poor peak shape and the undesired separation of the α and β ring forms (anomers)

Here we present our work with the new ACQUITY UPLC® BEH Amide stationary phase which allows fast and efficient separations of simple and complex carbohydrates. The robust nature of the BEH particles enables high pH saccharide separations, which are uniquely suited for detection of these compounds in their native form by negative ion ESI-MS. This eliminates the need for pre- or post-column derivatization for ESI-MS, as well as post-column adduct formation prior to APCI-MS.

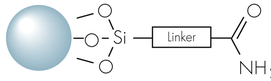
BEH Amide Column Chemistry

The Bridged Ethyl Hybrid technology was selected as the base particle due to its increased thermal and pH stability relative to silica. The amide bonding was selected due to its higher stability relative to the amino phases (reduced column bleed) and the longer lifetime (no bonded phase reaction with the reducing sugars; no formation of Schiff Bases).



BEH Technology™
U.S. Patent 6,686,035 B2

BEH particles are prepared from two high purity monomers: one that forms SiO_2 inorganic-units and another that forms $O_{1.5}Si\text{-}CH_2CH_2\text{-}SiO_{1.5}$ organosiloxane-units. Particles are bonded with a trifunctional amide bonding:



ACQUITY UPLC® BEH Amide

METHODS

Chromatographic Conditions:

All chromatograms were collected on an ACQUITY UPLC® system utilizing the 1.7µm BEH Amide columns in various dimensions. Mobile phases were either acetonitrile/water or acetone/water modified with triethylamine (TEA) or ammonium hydroxide (NH_4OH). Flow rates and temperatures were varied according to the experiment.

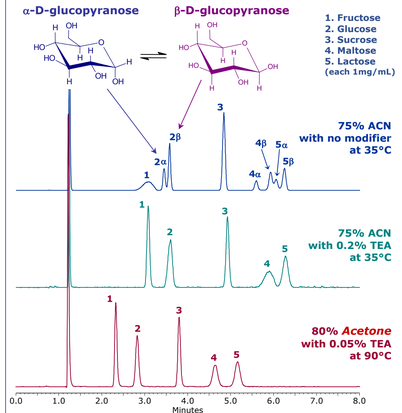
Waters ACQUITY ELS Detection:

Gain:	200	*Nebulizer:*	Cooling
Pressure:	40 psi	*Data Rate:*	10pps
Drift Tube:	40°C	*Time Constant:*	Normal
Data Processing:	Savitsky-Golay Smoothing (Level 17)		

Mass Spec Detection (Waters TQD):

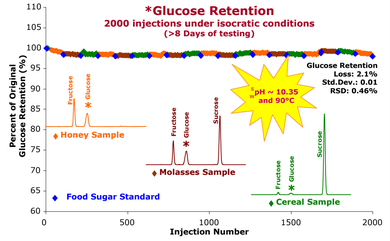
Ionization Mode:	ES-	*Desol Temp:*	350°C
Capillary:	2.8kV	*Desol Gas Flow:*	500L/Hr
Cone Voltage:	25V	*Cone Gas Flow:*	50L/Hr

Demonstration of Anomer Collapse on 1.7µm BEH Amide



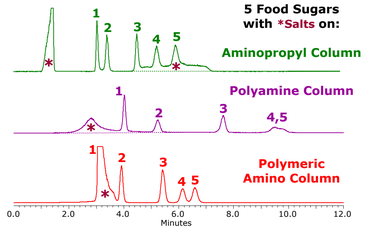
High pH Stability of the 1.7µm ACQUITY UPLC® BEH Amide

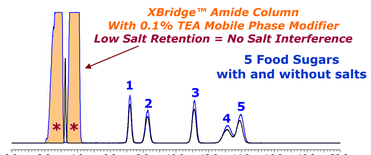
- Unlike silica, BEH particle technology has been shown to have excellent high pH stability
- To confirm the stability of the amide bonding on BEH, repetitive injections of real samples were performed. Intermittent Food Sugar Standard injections were included to monitor column performance during testing
- Testing was performed using a 2.1 x 150mm column under isocratic conditions with 0.05% TEA in 80% Acetone at 90°C ($_n^s$pH~10.35), and a flow rate of 0.15mL/min



Salt Interferences

ELSD using 75% ACN at 1.4mL/min, 35°C
4.6 x 150mm Columns, 10µL injection

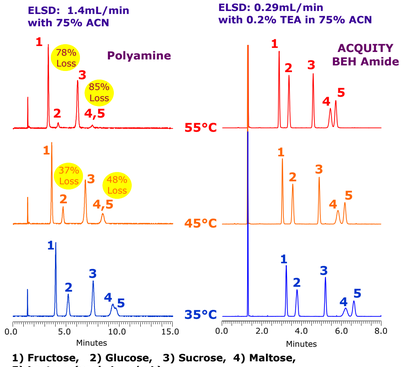




1) Fructose, 2) Glucose, 3) Sucrose, 4) Maltose, 5) Lactose (each 1mg/mL), * Salt Interferences

Loss of Reducing Sugars at Elevated Temperatures

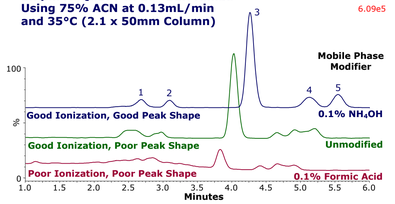
- Substantial loss of the aldohexose reducing sugars is observed at elevated temperatures on amino and polyamine based columns
- No loss is observed on the ACQUITY UPLC® BEH Amide



1) Fructose, 2) Glucose, 3) Sucrose, 4) Maltose, 5) Lactose (each 1mg/mL)

Detection of Saccharides by Mass Spectrometry

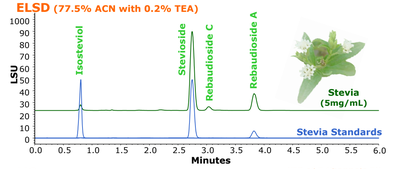
- Saccharide molecules ionize readily under high pH conditions, resulting in increased MS sensitivity without the need for additional pre- or post-column derivatization or adduct formation.

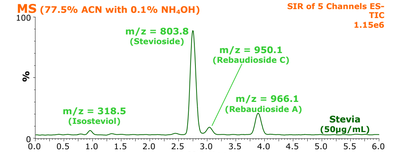


APPLICATIONS

MS and ELS Detection

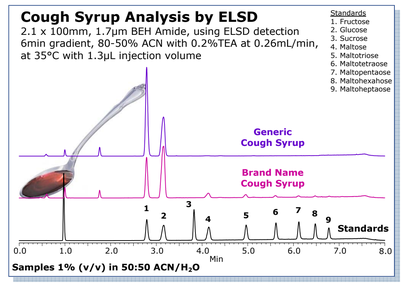
2.1 x 100mm BEH Amide, Isocratic: 0.2mL/min at 35°C





Cough Syrup Analysis by ELSD

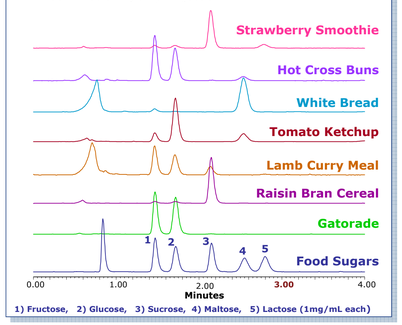
2.1 x 100mm, 1.7µm BEH Amide, using ELSD detection
6min gradient, 80-50% ACN with 0.2%TEA at 0.26mL/min, at 35°C with 1.3µL injection volume



Samples 1% (v/v) in 50:50 ACN/H_2O

Food Sugars on 1.7µm BEH Amide

ELSD on ACQUITY UPLC® System using 0.15mL/min at 85°C
Isocratic: 77% *Acetone* with 0.05% TEA
2.1 x 50mm Column, 0.7µL injection



1) Fructose, 2) Glucose, 3) Sucrose, 4) Maltose, 5) Lactose (1mg/mL each)

MS Analysis of Carbohydrates in Beer

2.1 x 100mm, 1.7µm BEH Amide, 10 minute gradient 75-45% ACN with 0.1% NH_4OH at 35°C, 0.13mL/min, 2µL injection volume
Samples diluted 1:1 in 100% ACN

Standards: 1) Fructose, 2) Glucose, 3) Sucrose, 4) Maltose, 5) Maltotriose
Each 10ppm in 50:50 ACN/Water

CONCLUSIONS

- Anomer peaks arising from reducing sugars can be collapsed into a single peak by using either high temperature or high pH mobile phases
- The BEH particle technology provides a stable and robust substrate for the trifunctional amide bonding, providing extended column lifetime under a wide variety of conditions
- Low salt retention eliminates salt interferences from complex sample matrices
- Both ELS and MS detection allow gradient elution for analysis of more complex polysaccharides
- Saccharides ionize readily under high pH conditions enabling MS detection without pre– or post-column modification

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