Carbohydrates

Carbohydrates (saccharides) are a major source of nutrition and a key form of energy for most organisms as well as being structural components of plants. Carbohydrates is one of the major classes of biomolecules and consist of carbon, hydrogen and oxygen atoms with the general formula Cn(H2O)n. Saccharides contain two carbonyl groups that are either of aldehyde or ketone type. Carbohydrates are furthermore divided into four chemical groups:

1) Monosaccharides (examples: Glucose and Fructose)
2) Disaccharides (examples: Lactose, Isomaltose and Trehalose)
3) Oligosaccharides
4) Polysaccharides

In general, the monosaccharides and disaccharides, lower molecular weight carbohydrates, are commonly referred to as sugars. Beside monosaccharides and disaccharides there are also neutral sugars, acidic sugars, amino sugars, sugar alcohols, and their various isomers. Low molecular weight saccharides are common in food, such as fruits, honey and sweets. The separation and identification of saccharides is challenging, especially for compounds having the same chemical formula and only small differences in their molecular structure, i.e. disaccharides maltose and isomaltose. In addition, carbohydrates from simple sugars to oligo- and polysaccharides represent a detection challenge in that they are lacking chromophores. RI and UV (195 - 205nm), are problematic to use due to issues of poor sensitivity, long detector equilibration times and their inability to handle gradient elution. Evaporative light scattering detection (ELSD) is a viable alternative, but just as with RI detectors, ELSD is sensitive to changes in the mobile phase composition making gradient elution difficult.

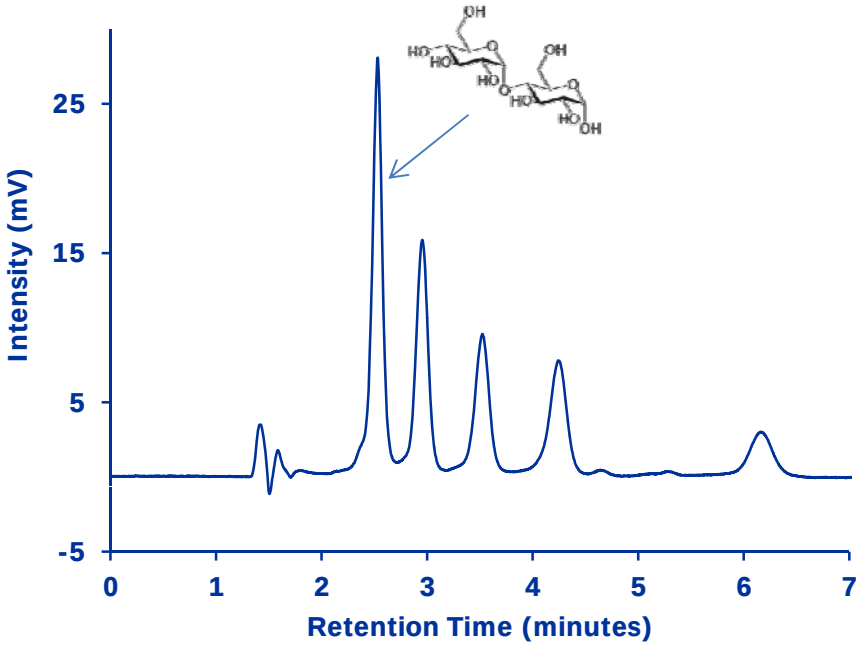
Various separation techniques have been used for carbohydrate analysis; anion chromatography, size exclusion chromatography, reversed phase chromatography, hydrophilic interaction liquid chromatography (HILIC), GC and TLC. HPLC using aminopropyl functionalized columns is one of the more common techniques for analysis of saccharides. In this technique, a mixture of acetonitrile and water is used as the mobile phase and the retention increases with reduction of water content just as in HILIC. In addition, the higher the molecular weight of the sugar, the longer it takes to elute. The aldehyde radicals in sugars can react with the amino radicals in the stationary phase to create Schiff bases, which can sometimes cause significant tailing on peaks for pentasaccharides (such as arabinose and ribose). This can be inhibited by adding a salt to the mobile phase. An advantage with an amino column is that it catalyze the mutarotation of reducing sugars effectively causing the retention time of the sugar to be the average of its two anomers, showing as only one peak in the chromatogram . This application compilation illustrates how Chromolith® NH2 columns can be used for separation of monosaccharides, disaccharides and glycoalkaloids (alkaloids with sugar moieties) .

Linear Oligosaccharides

Chromolith® NH2

Chromatographic Conditions

Column: Chromolith® NH2, 100x4.6 mm (1.52028.0001)
Injection: 2 µl
Detection: UV, 190nm
Flow Rate: 1.0 mL/min
Mobile Phase (v/v): Acetonitrile and Water 65:35 (v/v)
Temperature: 23 °C
Sample: Maltose 21.6 mg/mL. Maltotriose 19.5 mg/mL, Maltotetraose 15.7 mg/mL, Maltopentaose 16.2 mg/mL, Maltoheptaose 10.6 mg/mL in mobile phase
Pressure Drop: 26 Bar (377 psi)



Chromatographic Data

No.	Compound	Retention Time (min)	T_{USP}	Theoretical Plate
1	Void Volume	1.4	-	-
2	Maltose	2.5	0.8	3555
3	Maltotriose	3.0	1.0	3271
4	Maltotetraose	3.5	0.9	3303
5	Maltopentaose	4.2	0.9	3149
6	Maltoheptaose	6.2	1.0	3507