Application Note

Raw Material &
Media Analysis

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Separation of 15 Underivatized Saccharide and Sialic Acid USP Standards

Using an Agilent AdvanceBio MS Spent Media Column with TOF MS Detection

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Abstract

Saccharides and sialic acids are common components of spent media from bioprocessors that are monitored during the process of manufacturing therapeutic proteins such as monoclonal antibodies. This Application Note uses the Agilent AdvanceBio MS Spent Media column with HILIC technology to separate USP standards of these typical spent media components. An Agilent 6230 time-of-flight (TOF) LC/MS was used to analyze these compounds without costly and time-consuming derivatization procedures.

Introduction

Saccharides and sialic acids play a critical role in the production of monoclonal antibody (mAb) products and other therapeutic proteins[1,2,3]. Traditional HPLC carbohydrate separations have consisted of SEC or cation ligand exchange carbohydrate columns. These are heated to high temperatures and used together with refractive index (RI) detectors, as carbohydrates lack a chromophore for UV detection. RI detectors have limitations when separating carbohydrate mixtures, as they are incompatible with gradient methods. Evaporative light scattering detectors (ELSDs) are also used for carbohydrate analysis, but have certain limitations[4]. Electrospray ionization mass spectrometry (ESI-MS) is more sensitive than RI or ELSD detection[5]. Time and labor-intensive derivatization procedures have also been used for LC/MS and GC/MS analysis of carbohydrates.

The AdvanceBio MS Spent Media column uses hydrophilic interaction liquid chromatography (HILIC) technology to separate complex solutions of mono- and disaccharides. This is combined with the sensitivity of an ESI detector to directly, rapidly, and accurately characterize mono- and disaccharides without time and labor-intensive derivatization. The AdvanceBio MS Spent Media column was used with an Agilent 1260 Infinity II LC system and a 6230 TOF LC/MS as a detector. The efficacy of this system was evaluated in the separation of a set of 15 USP standards that were generously donated to us by the USP free of charge. This set included mono- and disaccharides, two sets of five isomers, and three sialic acids.

Experimental

Materials and methods

Samples

USP standard	USP catalog number
Galactose (200 mg)	1287700
KDN (100 mg) (3-deoxy-D-glycero-D-galacto-2-nonulosonic acid)	1354852
Lactose monohydrate (500 mg)	1356701
Lactulose (1 g)	1356803
Maltose monohydrate (500 mg)	1375025
Mannosamine hydrochloride (300 mg)	1375160
Mannose (500 mg)	1375182
N-Acetylneuraminic acid (200 mg)	1612619
N-Glycolylneuraminic acid (200 mg)	1294284
Sucrose (100 mg)	1623637
Tagatose (200 mg)	1642904
Xylose (1 g)	1722005
Fructose (125 mg)	1286504
Epilactose (200 mg)	1236801
Dextrose (500 mg)	1181302

Solvents and consumables

- LC/MS grade ammonium formate, acetonitrile, and ammonium hydroxide from Fisher Scientific
- Agilent vials, screw top, amber, certified, 2 mL, 100/pk. Vial size: 12 × 32 mm (12 mm cap) (p/n 5188–6535)
- Agilent bonded screw cap, PTFE/white silicone septa (p/n 5190–7021)
- USP standards obtained from USP

Column

AdvanceBio MS Spent Media 100 Å, 2.1 × 100 mm (p/n 675775-901)

LC system

- Agilent 1260 Infinity II bio-inert quaternary pump (G5654A)
- Agilent 1260 Infinity II bio-inert multisampler (G5668A)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A)

TOF MS

Agilent 6230 TOF LC/MS

Instrument conditions: Buffers to be specified at 0.4 mL/min, 35 °C column temperature, chiller set at 4 °C

Method development

Individual standard solutions were prepared from the USP standards in 50 % acetonitrile. ESI negative mode was used with the following buffer system: 10 mM ammonium formate, pH 10, and 10 % 100 mM ammonium formate in 90 % acetonitrile. To collapse the sugar anomers into one peak, and provide the sugars with a charge for detection in the TOF MS, pH 10 was selected. At pH 10, only a few of the compounds were present in the chromatograms. Since the pKa of many of the compounds is approximately 12, the pH of ammonium formate was raised to 11.0. At pH 11.0, all the compounds were observed (Tables 1 and 2, Figures 1–4). Adjusting the pH higher than 11.0 may shorten the column lifetime.

Results and discussion

A composite sample of each compound was prepared in 62.5 % acetonitrile. All 15 compounds were separated using method 1, which used an 18-minute, shallow gradient in under 13 minutes (Figure 3, Table 2). Isocratic methods did not separate the mixture.

MS parameters	
Ionization mode	ESI negative
Gas temperature	200 °C
Gas flow	10 L/min
Nebulizer	40 psi
Sheath gas temperature	300 °C
Sheath gas flow	12 L/min
Capillary voltage	3,000 V
Nozzle voltage	0 V
Skimmer voltage	65 V
Oct RF Vpp	750 V
Acquisition parameters	Data were acquired at 2 GHz extended dynamic range. MS mass range: *m/z* 50 to 1,000

Table 1. USP standards and retention times.

	Compound	Molecular weight	Retention time
1	Xylose	150.13	2.197
2	Tagatose	180.16	2.606
3	Fructose	180.16	2.99
4	Mannose	180.16	3.618
5	Mannosamine HCl	215.63	3.724
6	Dextrose	180.16	3.947
7	Galactose	180.16	4.242
8	Sucrose	342.3	7.316
9	Epilactose	342.3	8.467
10	Maltose monohydrate	342.3	12.02
11	Lactulose	342.3	12.11
12	N-Acetylneuraminic acid	309.27	12.584
13	Lactose monohydrate	343.3	13.84
14	KDN	268.2	13.91
15	N-Glycolyneuraminic acid	325.27	14.957

Table 2. Method 1 parameters.

Method 1		
Time (min)	%A	%B
0	3	97
15	11	89
15.5	3	97
18	3	37

A = 10 mM ammonium formate, pH 11.0

B = 10 % 100 mM ammonium formate, pH 11.0 in 90 % acetontrile

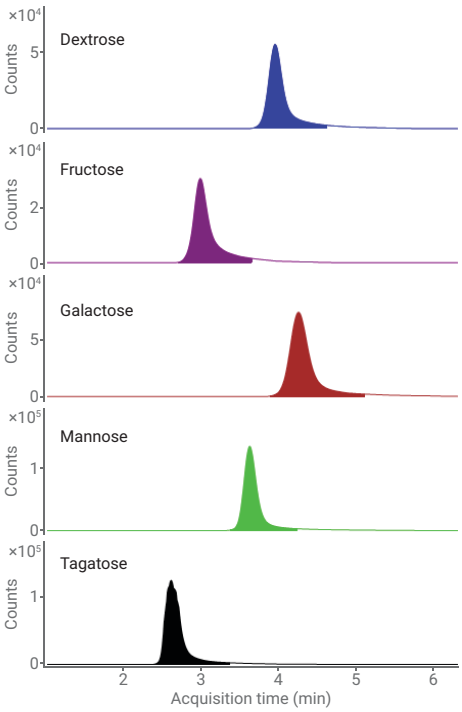

Figure 1. Individually injected USP standards of 6-carbon monosaccharides.

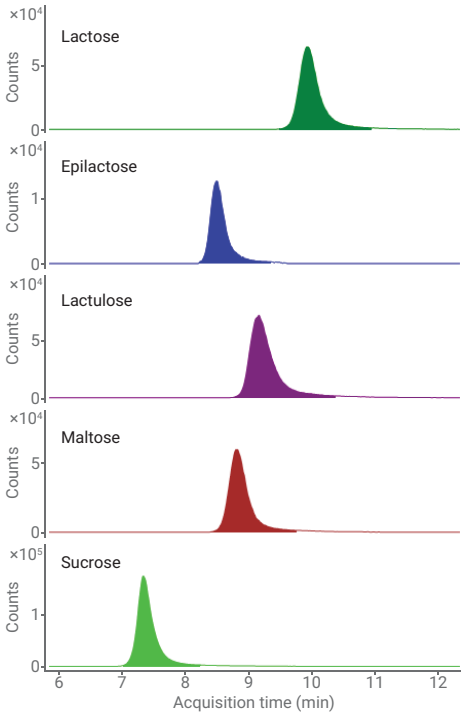

Figure 2. Individually injected USP standards of isomeric disaccharides.

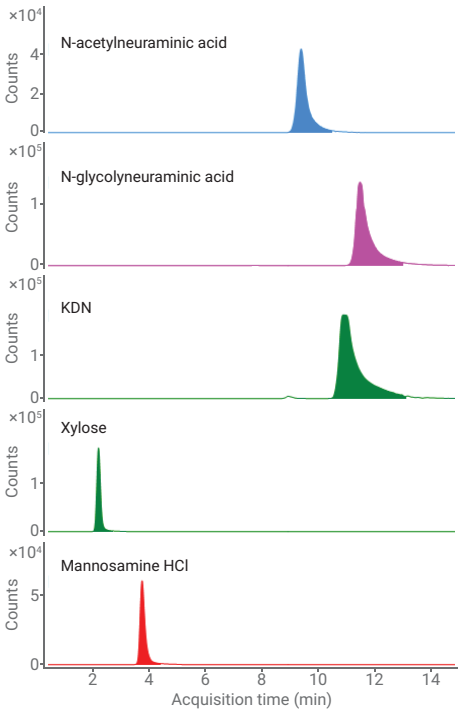

Figure 3. Individually injected USP standards.

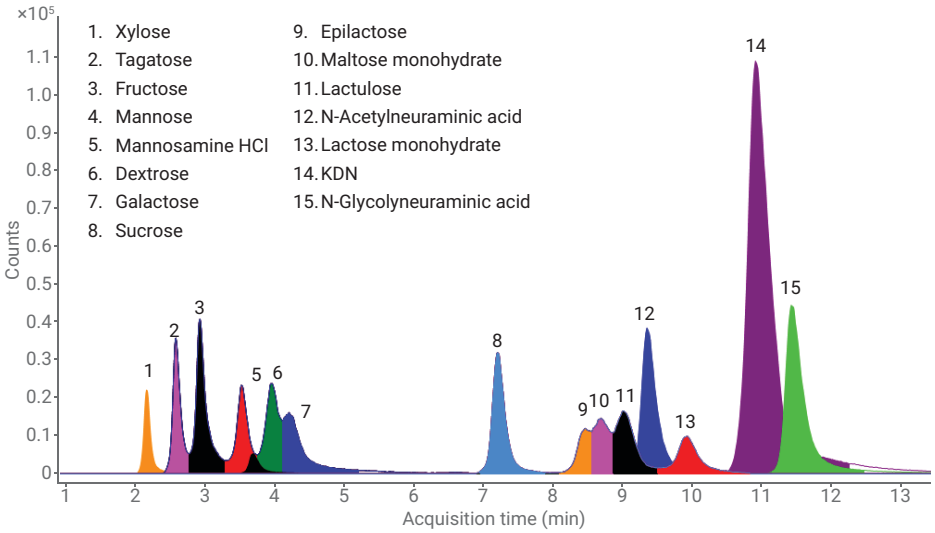

Figure 4. Separation of a composite sample of all 15 USP standards.

Conclusion

A 1260 Infinity II LC system, an AdvanceBio MS Spent Media column, and a 6230 TOF LC/MS in combination provide rapid analysis of complex carbohydrate mixtures. This method has the sensitivity of ESI-MS, without the need for labor and time-intensive derivatization of carbohydrates. Time and money are saved for any labs that currently use derivatization of sugars for LC/MS or GC/MS analysis of simple carbohydrates, increasing lab productivity.

References

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