Agilent Hi-Plex Columns for Carbohydrates, Alcohols, and Acids

Application Note

Food and Pharmaceutical

Authors

Stephen Ball, Linda Lloyd

Agilent Technologies, Inc.

Introduction

Agilent Hi-Plex columns are ion-exchange ligand-exchange columns used predominantly for the separation of carbohydrates and organic acids. These columns use the preferred separation mechanism for the analysis of simple sugars, alcohols, oligosaccharides, and organic acids in foodstuffs, but can also be used for the separation of other compounds.

The range comprises a 4% crosslinked resin for the analysis of oligosaccharides and an 8% crosslinked resin, with lower exclusion limit, for mono-, di-, and trisaccharide analysis. For carbohydrate and alcohol investigations, Hi-Plex columns use isocratic conditions with water as the eluent and temperature as the main variable for control of resolution. The exception is Agilent Hi-Plex Na (Octo), which is used with sodium hydroxide eluents when pulsed amperometric detection is employed.

In these examples, we use Agilent Hi-Plex H, Agilent Hi-Plex Ca, as well as Agilent Hi-Plex Ca (Duo) for the analysis of organic acids, sugars, and sugar alcohols.

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Organic Acids on Hi-Plex H

Where samples contain organic acids, whether or not in the presence of neutral mono- and disaccharides, Agilent Hi-Plex H is the preferred option. The column is run using water as the eluent for the analysis of sugars and organic acids or, more commonly, dilute acid for separation of organic acids. Refractive index (Figure 1 and Table 1, Figure 2 and Table 2) or UV detection (Figure 3 and Table 3) is used.

Conditions

Sample	Sugars and organic acids
Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	100% 0.0085 M H_2SO_4
Flow rate	0.4 mL/min
Injection volume	20 µL
Temperature	65 °C
Detector	RI

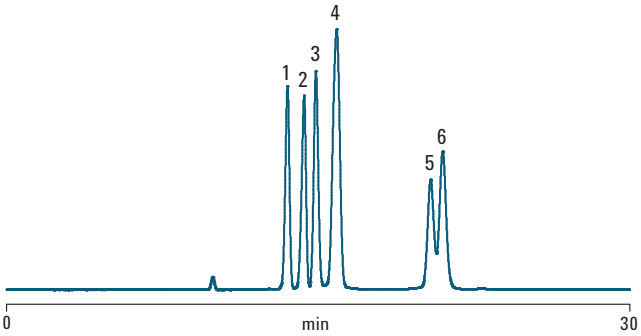

Figure 1. Separation of monosaccharides, organic acids, and glycerol on an Agilent Hi-Plex H column with RI detection.

Conditions

Sample	Organic acids
Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.1 M H_2SO_4
Flow rate	0.6 mL/min
Temperature	50 °C
Detector	RI

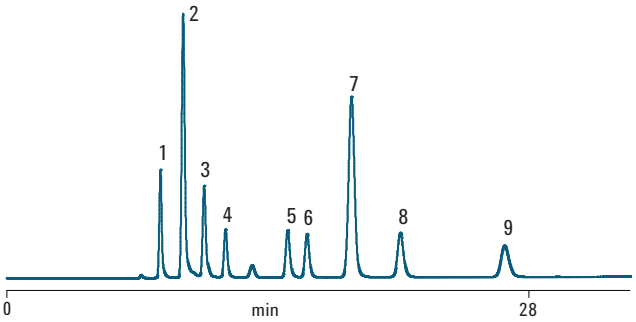

Figure 2. Separation of nine organic acids on an Agilent Hi-Plex H column with RI detection.

Table 1. Peak Data from the Analysis of Monosaccharides, Organic Acids, and Glycerol on an Agilent Hi-Plex H Column Using RI Detection

Peak number	Analyte	As. USP	10% Asymmetry	Plate count	Plates/m
1	Citric acid	0.92	0.92	21207	70691
2	Tartaric acid	0.84	0.85	21475	71583
3	Glucose	1.04	1.04	21805	72684
4	Malic acid, fructose	0.93	0.93	10012	33372
5	Lactic acid	0.89	0.96	19685	65618
6	Glycerol	1.16	1.06	19070	63566

Table 2. Peak Data from the Separation of Organic Acids on an Agilent Hi-Plex H Column Using RI Detection

Peak number	Analyte	As. USP	10% Asymmetry	Plate count	Plates/m
1	Oxalic acid	1.23	1.12	19471	64904
2	*cis*-Aconitic acid	1.29	1.15	16122	53741
3	Tartaric acid	1.30	1.24	19272	64240
4	Malic acid	1.10	1.07	20153	67176
5	Lactic acid	1.16	1.10	21469	71563
6	Formic acid	1.08	1.05	22118	73726
7	Fumaric acid	1.05	1.03	15751	52504
8	Propionic acid	1.12	1.09	20492	68305
9	Butyric acid	1.15	1.13	18181	60603

Conditions

Sample	Organic acids
Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	100% 0.01 M H_2SO_4
Flow rate	0.6 mL/min
Injection volume	20 µL
Temperature	50 °C
Detector	UV, 210 nm

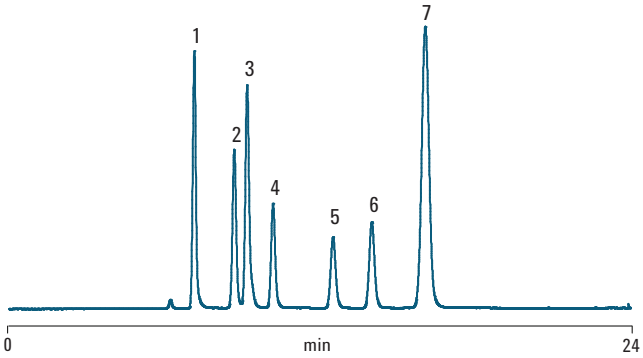

Figure 3. Separation of seven organic acids on an Agilent Hi-Plex H column with UV detection.

Table 3. Peak Data from the Separation of Organic Acids on an Agilent Hi-Plex H Column Using UV Detection

Peak number	Analyte	As. USP	10% Asymmetry	Plate count	Plates/m
1	Oxalic acid	1.13	1.02	17164	57212
2	Citric acid	1.11	1.07	17588	58626
3	Tartaric acid	1.30	1.23	19251	64170
4	Malic acid	1.11	1.07	20170	67233
5	Succinic acid	1.08	1.06	19705	65684
6	Formic acid	1.07	1.05	21991	73302
7	Fumaric acid	1.05	1.03	15139	50464

Sugars and Sugar Alcohols on Hi-Plex Ca

Agilent Hi-Plex Ca is recommended for the analysis of samples containing the sweetening sugars (glucose, fructose, and sucrose) and the sugar alcohols (mannitol and sorbitol) (Figure 4). The 4.0 × 250 mm column is referenced in the USP method that specifies L19 media for sugar alcohols analysis.

Conditions

Sample	Sugars and sugar alcohols
Column	Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm (p/n PL1170-6810)
Sample size	10 mg/mL
Mobile phase	100% DI H_2O
Flow rate	0.6 mL/min
Injection volume	10 µL
Temperature	85 °C
Detector	RI

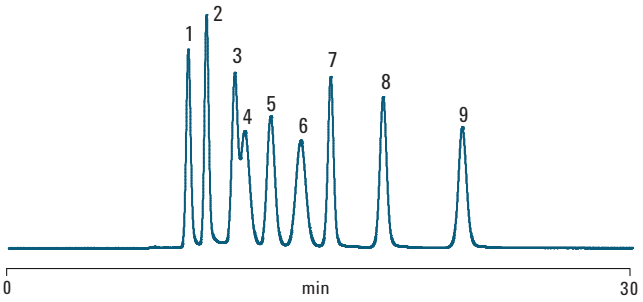

Figure 4. Separation of a mixture of sugars and sugar alcohols on an Agilent Hi-Plex Ca column.

Table 4. Peak Data from the Separation of a Sugars and Sugar Alcohols Mix on an Agilent Hi-Plex Ca Column

Peak number	Analyte	As. USP	10% Asymmetry	Plate count	Plates/m
1	Raffinose	1.12	1.08	7138	23793
2	Sucrose	1.12	1.06	9389	31298
3	Lactulose	0.85	0.92	3858	12861
4	Glucose	1.79	1.59	2986	9955
5	Galactose	1.07	1.07	5008	16694
6	Fructose	1.01	1.01	3727	12423
7	Ribitol	1.00	1.00	14758	49194
8	Mannitol	1.04	1.04	13861	46204
9	Sorbitol	1.04	1.04	14170	47234

Monosaccharide and Oligosaccharide Mixture on Hi-Plex Ca (Duo)

Agilent Hi-Plex Ca (Duo) is an 8% crosslinked material and therefore has a smaller pore size and less resolution for the larger oligomers. However, the Ca counter ion has improved ligand-exchange capability for monosaccharides, and so it is most suited for the analysis of samples containing both mono- and oligosaccharides (Figure 5).

Conditions

Sample	Sugars and sugar alcohols
Column	Agilent Hi-Plex Ca (Duo), 6.5 × 300 mm, 8 µm (p/n PL1F70-6850)
Sample size	10 mg/mL
Mobile phase	100% DI H_2O
Flow rate	0.4 mL/min
Injection volume	10 µL
Temperature	85 °C
Detector	RI

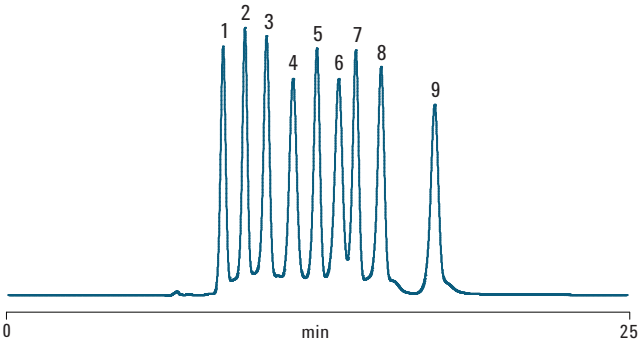

Figure 5. Separation of a mixture of mono- and oligosaccharides on an Agilent Hi-Plex Ca (Duo) column.

Table 5. Peak Data from the Separation of a Sugars and Sugar Alcohols Mix on an Agilent Hi-Plex Ca (Duo) Column

Peak number	Analyte	As. USP	10% Asymmetry	Plate count	Plates/m
1	Raffinose	1.13	1.00	7827	26091
2	Sucrose	0.80	0.96	9363	31211
3	Lactulose	1.03	0.87	7895	26316
4	Glucose	0.99	0.97	6204	20680
5	Galactose	0.85	0.90	10869	36229
6	Fructose	0.82	0.88	7765	25884
7	Ribitol	1.02	0.87	13784	45948
8	Mannitol	0.95	0.87	13431	44771
9	Sorbitol	1.15	0.95	13807	46025

Conclusion

Agilent Hi-Plex columns deliver improved efficiency, lower operating pressures, and longer column lifetimes from monodispersed materials. With a range of ligand counter ions for optimum selectivity and with resolution and materials matched to the USP definitions of media types L17, L19, L34, and L58, the Hi-Plex range is ideal for isocratic separations using water or dilute acid as the eluent. This simplifies system requirements for HPLC and eliminates the use of potentially hazardous organic solvents.

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