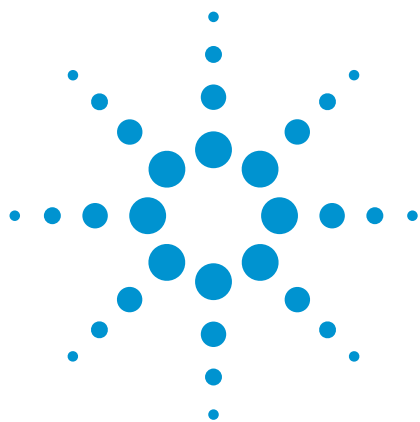




Agilent Hi-Plex Columns for Sugar Separation: Effects of Temperature and Mobile Phase

Application Note

Food

Author

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Introduction

The Agilent Hi-Plex H is a high-performance ligand-exchange chromatography column. The column is based on polystyrene/divinylbenzene with an 8% crosslinking and hydrogen counter ion. Typically used for the analysis of sugars, sugar alcohols, and organic acids, its monodisperse sulfonated packing gives improved column efficiency, lower column pressure, and assured batch-to-batch reproducibility.

This application note investigates the effect of temperature and mobile-phase acid concentration on the separation of sugars and organic acids in wine.



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Materials and Reagents

Sample Preparation

The seven compounds listed in Table 1 were weighed into the same vial in the quantities described and dissolved in 10 mL of 0.01 M H_2SO_4 . Injection volume was 20 μL .

Table 1. Compound Quantities

Constituent	Amount (g)
1. Citric acid	0.1010
2. Tartaric acid	0.1032
3. Glucose	0.1011
4. Malic acid	0.1018
5. Fructose	0.1011
6. Lactic acid	0.1015
7. Glycerol	0.1131

Conditions

Column	Agilent Hi-Plex H, 7.7 $\times$ 300 mm, 8 μm (p/n PL1170-6830)
Mobile phase	0.01 M H_2SO_4
Flow rate	0.4 mL/min
Temperature	> 75 $^{\circ}\text{C}$
Detector	RI

Results and Discussion

This mixture of sugars and organic acids is particularly difficult to analyze as several of the compounds elute very closely together and often simultaneously. However, these results show that as the temperature is increased, the minimum resolution of the separation gradually increases until all seven compounds are nearly separated.

At 35 $^{\circ}\text{C}$, there are two pairs of co-eluted peaks, but by increasing the temperature to 55 $^{\circ}\text{C}$, they have started to split into pairs of peaks. This increase in temperature, however, causes fructose and malic acid to become co-eluted. By increasing the temperature further to 75 $^{\circ}\text{C}$, these two components begin to separate in the reverse order (Figure 1).

To gain complete separation of all seven compounds, this analysis would need to be run above 75 $^{\circ}\text{C}$.

By varying the concentration of the sulfuric acid in the mobile phase, the selectivity of the column can be altered (Figure 2). At high concentrations, fructose and malic acid co-elute. Therefore, by reducing the mobile phase acid concentration, the minimum resolution of the separation can be improved. However, this reduction in mobile-phase acid strength does result in slightly fronted peak shapes for the organic acids. The best separation obtained consisted of a 0.003 M H_2SO_4 , where malic acid elutes almost halfway between glucose and fructose.

By comparing these two sets of results, it can be seen that temperature is a more powerful tool in gaining complete separation of all seven compounds in this particular mixture, as resolution between them can be achieved while maintaining good peak shape.

Peak identification

1. Citric acid
2. Tartaric acid
3. Glucose
4. Malic acid
5. Fructose
6. Lactic acid
7. Glycerol

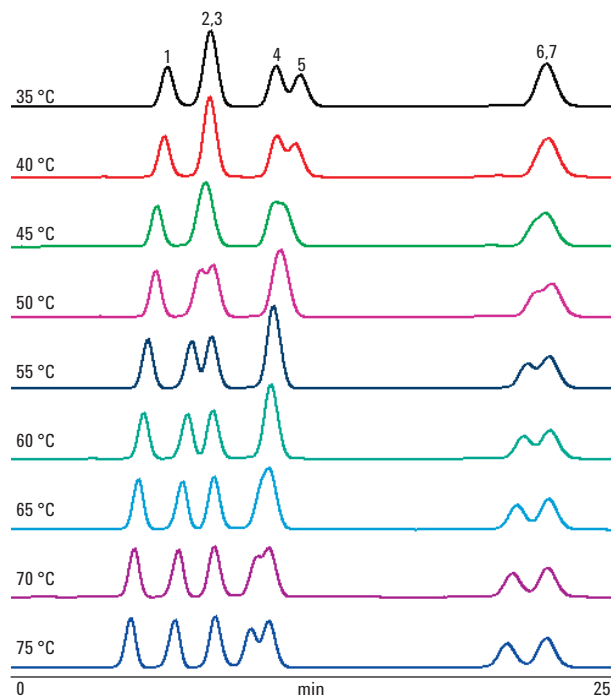


Figure 1. Effect of temperature on the separation of sugars and organic acids on an Agilent Hi-Plex H column.

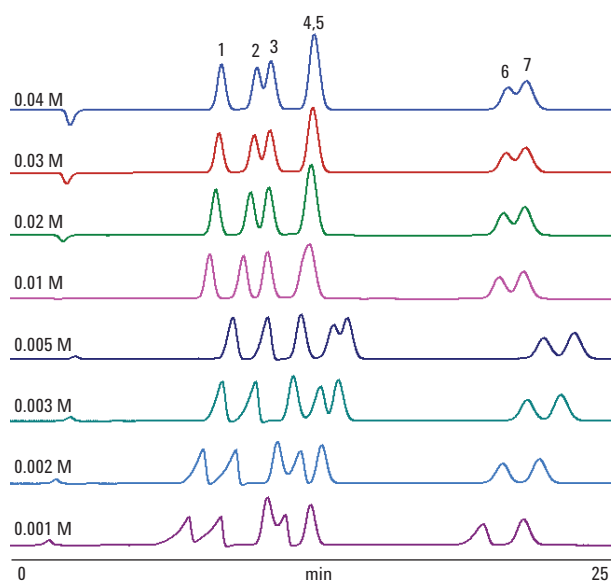


Figure 2. Effect of acid concentration on the separation of sugars and organic acids on an Agilent Hi-Plex H column.

Conclusion

The analysis of wines demonstrates how Agilent Hi-Plex H columns provide optimum resolution of closely eluting compounds, enabling quantitation of each. These columns are ideal for the analysis of sugar alcohols and sugar molecules, using sulfuric acid as the mobile phase. The Hi-Plex H is also the column of choice for the analysis of organic acids, using dilute mineral acid as eluent. By using the columns at higher operating temperatures, closely eluting compounds can be resolved.

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