Agilent Hi-Plex Columns for Fingerprinting Organic Acids in Wine

Application Note

Food and Beverage

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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.

The superior separation ability of Hi-Plex H is demonstrated in the quantitative analysis of organic acids in four different samples of wine: red, white, rosé, and dessert wine. This type of analysis is important for wine quality control because the classes and content of organic acids give a characteristic taste to the finished product. Acetic acid, lactic acid, succinic acid, malic acid, citric acid, and tartaric acid are the main organic acids in wine.

The use of a ligand-exchange chromatography column such as Hi-Plex H significantly reduces the need for complicated sample preparation (typically involving elution through an ion-exchange resin bed), as retention is brought about not only by ion exchange, but also by ion exclusion and partitioning on this type of column.

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Materials and Reagents

Sample Preparation

In order to obtain a complete refractive index (RI) profile, each wine was directly injected onto the column without any sample pretreatment. The exceptions to this being the Inniskillin Eiswein, which was diluted by a factor of five with HPLC-grade water, and the Marsala wine, which was diluted by a factor of three with water. Injection volume was 20 µL.

Conditions

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.004 M H_2SO_4
Flow rate	0.4 mL/min
Temperature	75 °C
Detector	RI

Results and Discussion

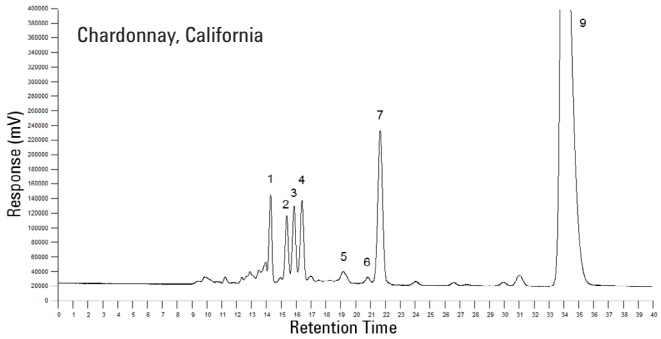
The results demonstrate that it is possible to distinguish between different types of wine (i.e., red, white, rosé, dessert) by HPLC analysis with the Hi-Plex H column. By using an RI detector, levels of organic acids and sugars can be quantified simultaneously.

The rosé and dessert wines all contain very high levels of malic acid and fructose (fruit sugar). In fact, the Inniskillin Eiswein and Masala wines, both dessert wines, contain up to five times as much sugar as ordinary rosé wine and 70 times as much sugar as the red and white wines. Eiswein (commonly called Ice Wine) is produced from grapes that have been frozen, causing some of the water to freeze out, leaving the sugars and other solids dissolved in the remaining juice. The resulting wine is therefore very sweet but has a great deal of balancing acidity, which also explains the high level of malic acid in the wine samples.

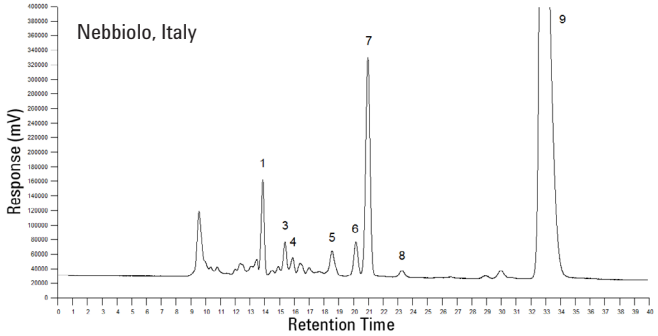
The chromatograms of the red and white wines look very different from those of the other wines, in that they have much lower levels of sugar but much higher levels of lactic acid and glycerol. Red wine is made from the must (pulp) of red or black grapes that undergo fermentation together with the grape skins, while white wine is usually made by fermenting juice pressed from white grapes. During the fermentation process, yeast converts most of the sugars in the grape juice into ethanol and carbon dioxide, which explains the low levels of glucose and fructose in the wine samples. Some wines also undergo malolactic fermentation, where bacteria convert malic acid into the milder lactic acid. All of these factors and the levels of sugars and organic acids produced by the various fermentation processes contribute to the different taste that each wine has and give each one a unique profile when analyzed by HPLC.

Some of the other peaks that appear in the chromatograms are likely to be from the tannins (bitter-tasting plant polyphenols) present in the skins and seeds of the grapes used in the fermentation process.

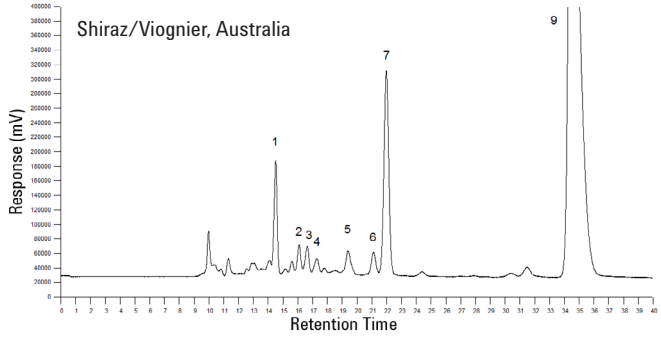
Key
1). Tartaric acid, 2). Malic acid, 3). Glucose, 4). Fructose, 5). Succinic acid, 6). Lactic acid, 7). Glycerol, 8). Acetic acid, 9). Ethanol



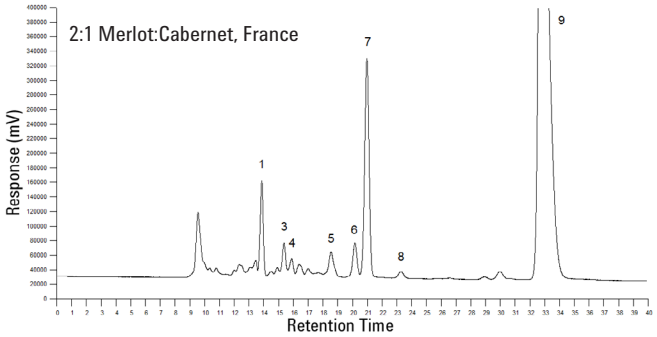
White wine contains a wide variety of organic acids and sugars.



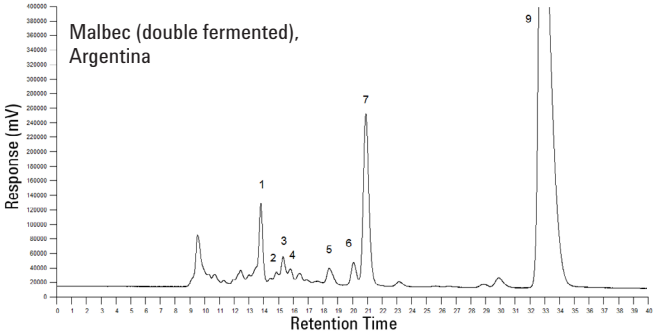
The main constituents of this red wine are tartaric acid, succinic acid, and glycerol.



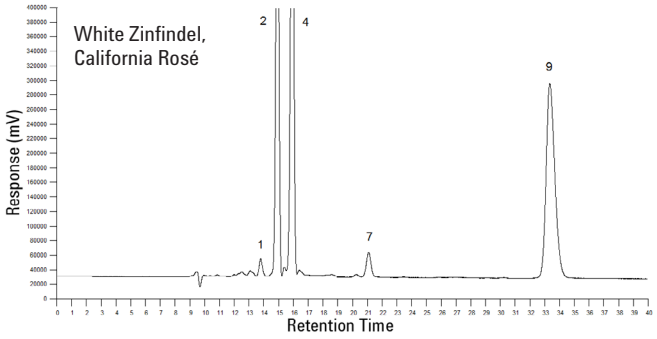
This wine contains slightly higher levels of malic acid and fructose than the Nebbiolo.



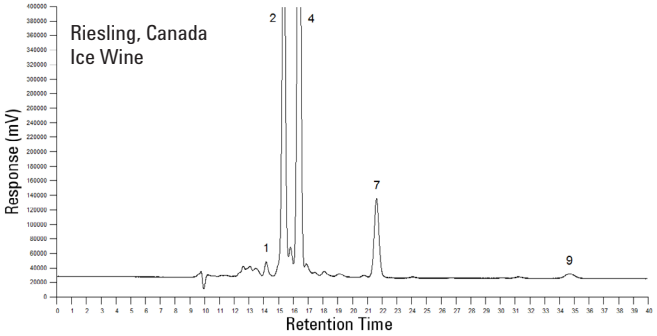
As shown, this red wine contains a slightly higher level of lactic acid than the other wines.



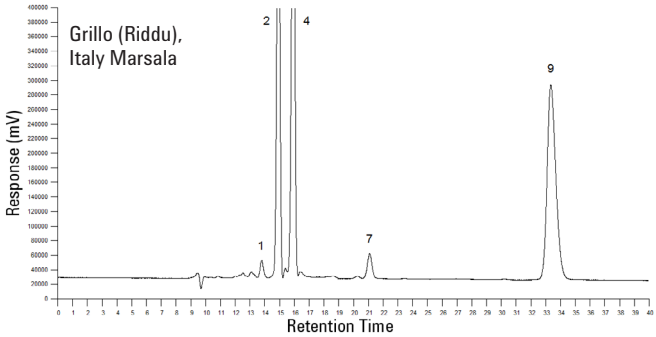
This wine has another unique profile, with differing levels of organic acids and sugar.



Rosé wine contains fewer organic acids and a significantly higher fructose content.



This dessert wine contains very high levels of malic acid, fructose, and glycerol, but little else.



As expected, this dessert wine also contains high amounts of malic acid and fructose.

Conclusion

The analysis of wines demonstrates the use of Agilent Hi-Plex H columns to provide resolution of closely eluting compounds, enabling quantitation of each. These columns are ideal for the analysis of sugar alcohols and sugar molecules, using water as the mobile phase. 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.

References

1. M.Y. Ding, H. Koizumi, and Y. Suzuki. (1995). Comparison of Three Chromatographic Systems for Determination of Organic Acids in Wine. *Analytical Sciences*. 11: 239-243.

2. A. Schneider, V. Gerbi, and M. Redoglia. (1987). A Rapid HPLC Method for Separation and Determination of Major Organic Acids in Grape Musts and Wines. *American Journal of Enology and Viticulture*. 38 (2), 151-155.

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