Fast Analysis of Fruit Juice Acids with an Agilent Poroshell 120 SB-Aq Column

Application Note

Food & Agriculture

Author

William Long
Agilent Technologies, Inc.

Abstract

Fruit juice producers face the problem of contamination of their product by fermentation-causing bacteria or contamination of juice products with cheaper adulterants. The acid profile can be used to identify a juice or verify its purity. A reversed-phase HPLC method for separation of organic acids (tartaric, quinic, malic, citric, and fumaric acids) in fruit juices is demonstrated using an Agilent Poroshell 120 SB-Aq column. The chromatographic separation was performed with an Agilent 1200 Infinity Series using a potassium dihydrogen orthophosphate buffer (pH 2.5) as mobile phase and diode array detection at λ = 226 nm. Organic acid profiles of several juices are shown.

Introduction

The identification of major organic acids in fruits is considered very important for food and beverage technology and quality evaluation [1]. Organic acids are a useful index of authenticity in fruit products, because they have lower susceptibility to change during processing and storage than other components of fruits [2].

Grapes are known for being acidic, but just how acidic depends on the region in which they are grown. Those grown in cooler climates are more acidic than grapes from warmer areas. Grapes have very low levels of citric acid but have high levels of tartaric acid, which is what makes them sour [3]. Malic acid, from the Latin word malum, or apple, gives green apples their sour quality. It is present in grapes and in most wines. Malic acid confers a tart taste to wine, although the amount decreases with increasing fruit ripeness. Cranberries contain a few different types of acid, including quinic acid, which breaks down the calcium deposits that collect to form kidney stones. Cranberries also contain citric and malic acids. As suggested by the

name, citrus fruits contain a high amount of citric acid, with a general guide that sweeter fruits contain less acid. Limes, lemons, oranges, tangerines, and grapefruits are some acidic citrus fruits. However, berries, such as red currants, raspberries, and blackberries, also have a high amount of citric acid. Figure 1 shows the structures of these common acids.

Acids are generally difficult to separate using reversed-phase liquid chromatography. Bases can be more easily retained at higher pH, where the compounds are not charged. However, in some cases, other compounds in the sample will not be separable at high pH. A possible solution is to use an ion-pair reagent to increase retention. Acidic compounds are also noted as difficult samples to separate or even retain. In general, it is necessary to work below the pKa of the compound, where it will be fully protonated (not charged), and decrease the organic content of the mobile phase [4]. A problem that can occur with many alkyl columns, such as C8 or C18 phases, is poor retention or reproducibility of retention in low organic mobile phase. One of the unique properties of Agilent Poroshell 120 SB-Aq columns is their resistance to dewetting, or what is sometimes referred to as phase collapse [5]. This application note shows separation of some acidic compounds using 100% aqueous conditions.

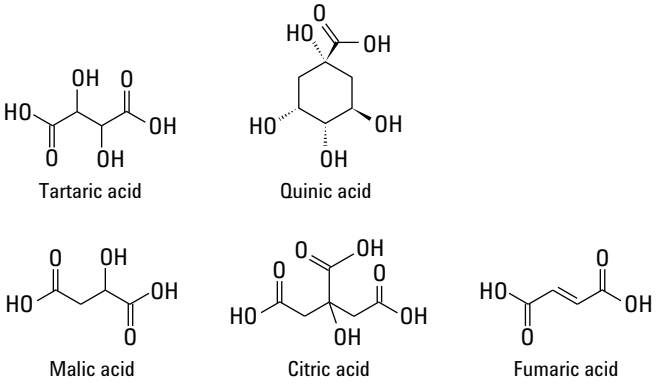


Figure 1. Structures of some fruit acids.

Materials and Methods

HPLC analysis was performed with an Agilent 1200 Infinity Series, comprising:

- Agilent 1200 Series Binary Pump SL (G1312B)
- Agilent 1200 Series Automatic Liquid Sampler (ALS) SL (G1376C)
- Agilent 1200 Series Thermostated Column Compartment (TCC) SL (G1316B)
- Agilent 1200 Series Diode Array Detector (G1315C)

Tartaric, quinic, malic, citric, fumaric acids, potassium phosphate mono basic, and phosphoric acid were purchased from Sigma-Aldrich Corp. Water used in all sample and mobile phase preparation was 18 Mohm.cm Milli-Q (Millipore) produced on site. Juice and cocktail samples were obtained at a local grocery store.

Conditions

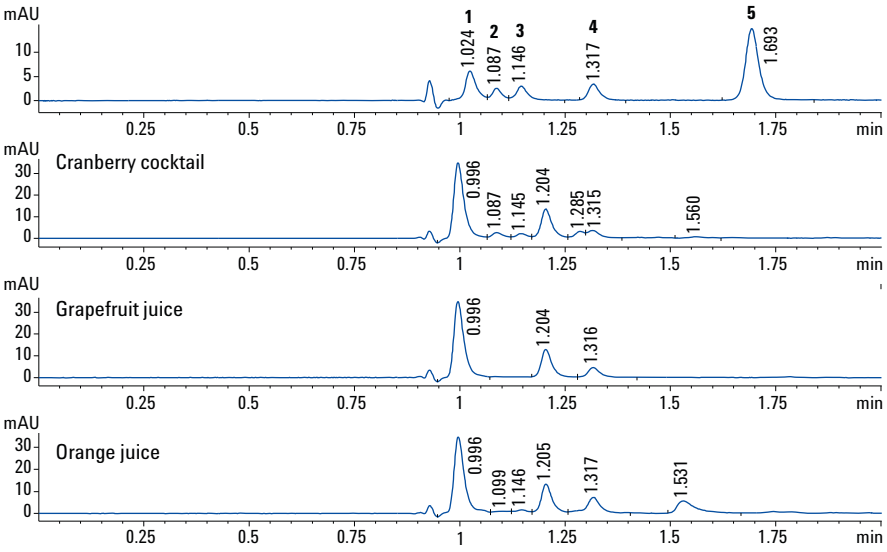
Column:	Agilent Poroshell 120 SB-Aq, 3 × 100 mm, 2.7 µm (p/n 685975-314)
Eluent:	100 mM Potassium phosphate buffer, pH 2.5
Injection volume:	5 µL
Flow rate:	0.5 mL/min
Temperature:	50 °C
Detector:	DAD, at 226 nm

Results and Discussion

All samples were freshly prepared and used within the same day. Some of the acids were found to decay when evaluated over more than 1 day. Storing samples in foil wrapping to reduce light or freezing can be used to extend the life of the standards. However, it is best to analyze samples as quickly as possible to achieve the most accurate results. As shown in this method, the entire analysis including sample preparation was accomplished in under 10 minutes. All samples were diluted to 1/3 of their original concentrations, filtered if pulp was present, and then injected. All samples contained tartaric acid and citric acid. Figure 2 shows a chromatogram of the standards and several sample juices.

A useful feature of the Poroshell 120 SB-Aq column is that it can be used in 100% aqueous mobile phase without phase collapse. Use of the high-water-content mobile phase can lead to a dramatic decrease in retention over time. The loss in retention time observed after moving from an organic-aqueous mobile phase into an aqueous mobile phase can be accelerated by turning off the flow for a period of time. The loss in analyte retention over time (or instantaneously with flow stoppage) using an RP column in a high aqueous mobile phase has commonly, but perhaps incorrectly, been referred to as phase collapse.

Using a similar methodology to a published method [5], a 75% methanol and 25% buffer was run on the column for 20 minutes, followed by a 5 minute equilibration to 100% buffer. A sample was injected after the column equilibrated. After this run, the pump was shut down overnight for 18 hours. In the morning, the pump was started, and a new injection was made. Figure 3 compares the chromatograms with identical retention times for all analytes.



Peak ID

1. Tartaric acid
2. Quinic acid
3. Malic acid
4. Citric acid
5. Fumaric acid

Figure 2. Chromatograms of food acids produced on an Agilent Poroshell 120 SB-Aq column.

Conclusion

This application note is a contribution to the development of a rapid and precise HPLC procedure for quantitative determination of organic acids in fruit juices under reversed-phase conditions. Tartaric, quinic, malic, citric, and fumaric acids were determined simultaneously and eluted from the column within 2 minutes. The method could be used to quantify organic acids in fruit juices. The column was also shown to resist phase dewetting and be effective in a 100% aqueous mobile phase.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

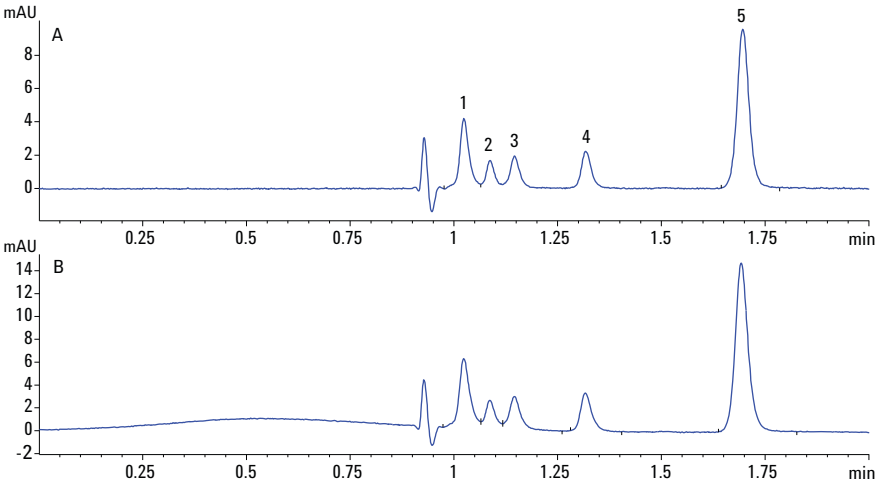


Figure 3. Before (A) and after (B) comparison of two injections of fruit acids after the pump was stopped for 18 hours (injection volume 3 µL, other conditions as before).

References

1. L. Loredana, H. Diehl, C. *Socaciu. Bulletin* USAMV-CN. 62, 288 (2006).
2. K. Weikle. Concordia *Coll. J. Anal. Chem*. 3, 57 (2012).
3. A. J. Pandell. The Acidity of Wine. http://wineperspective.com (1999).
4. L. R. Snyder, J. L. Glajch, J. J. Kirkland. Practical HPLC *Method Development*. Second Ed. John Wiley & Sons, New York (1997).
5. B. A. Bidlingmeyer, A. D. Broske. *J. Chromatogr. Sci*. 42, 100 (2004).

www.agilent.com/chem

Printed in the USA
February 20, 2013
5991-1992EN

Agilent Technologies