

Simple, Reliable HPLC Analyses of Organic Acids

Using Water-Compatible Allure® or Ultra C18 Columns

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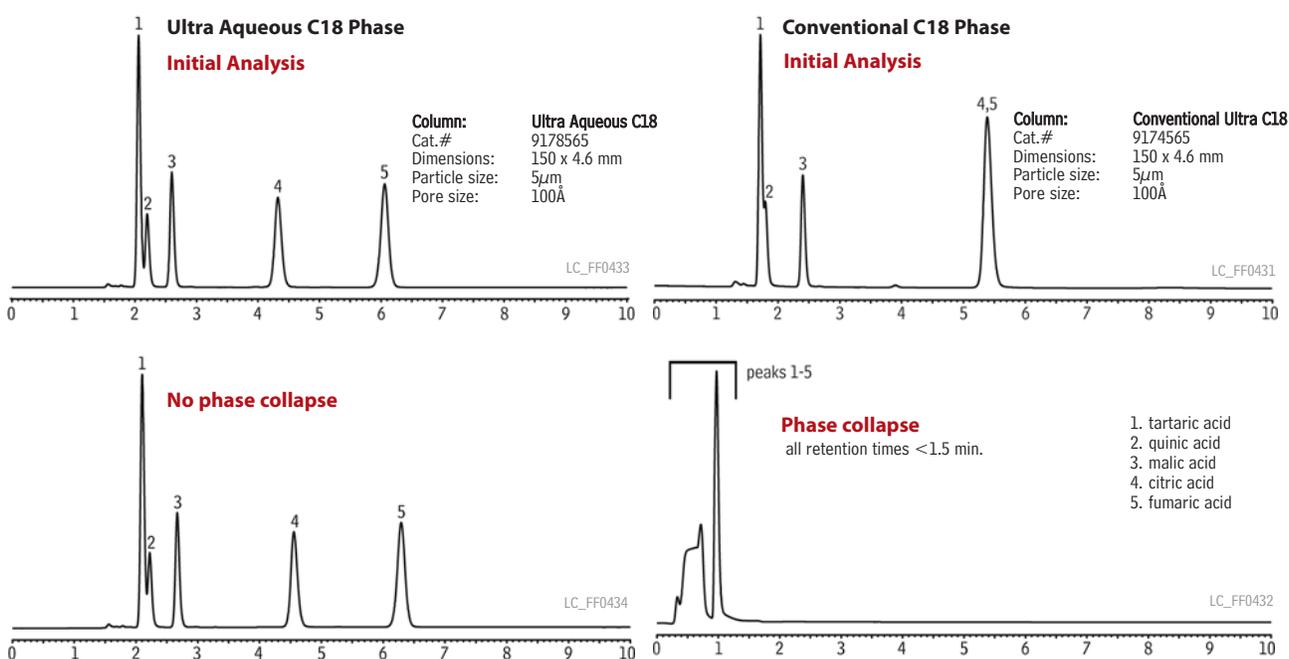
- Use 100% aqueous mobile phases without losing retention.
- Simple, isocratic method.
- Complete resolution of critical fruit juice organic acids, including quinic and tartaric acids.

Organic acids are common components in foods and beverages, and play a critical role in product characteristics like taste and aroma. They can be tested for in many food products including fruits, cheeses, and various beverages such as juices and wines. Organic acids can originate in the foods themselves (e.g. cranberries) or can be produced by food processing (e.g. alcoholic fermentation). A method that allows resolution of organic acids, as well as their quantification, can help determine product quality and authenticity.

Reversed phase HPLC coupled with UV-Vis detection is a popular technique for organic acid analysis. One common method, AOAC method 986.13, stipulates reversed phase HPLC using two C18 stationary phase columns in series. Because organic acids are low in molecular weight, and have polar functionalities, 100% aqueous buffer is needed for adequate retention. A low pH buffer is used to ensure that the organic acids remain protonated or neutral, thus allowing the best interaction between the organic acids and the C18 stationary phase. However, using a 100% aqueous mobile phase can cause the C18 chain in conventional C18 columns to collapse. Phase collapse results in loss of retention, and the column must be flushed with organic mobile phase, a time consuming step, to restore chain structure and column performance.

Three Restek columns – the Ultra Aqueous C18 column, the Allure® Aqueous C18 column, and the Allure® Organic Acids column – use aqueous-compatible C18 phases that do not exhibit phase collapse, even with 100% aqueous mobile phases. The advantage of using these columns is demonstrated in Figure 1 by the fast analysis of organic acids on a Shimadzu Prominence 20A system. Here, we compared the ability of the Ultra Aqueous C18 phase and a conventional C18 phase to withstand phase collapse. Figures 1A and 1B show that the Ultra Aqueous C18 phase resolves organic acids in a 100% aqueous mobile phase without loss of retention. In comparison, the conventional C18 phase shown in Figure 1C and 1D suffers a complete loss of retention following phase collapse when used under the same conditions. Thus, in an analysis that requires, or is improved by, a mobile phase with a high aqueous content, an Ultra Aqueous C18 column is the superior choice.

Figure 1 Restek's water-compatible C18 phase does not collapse in a 100% aqueous mobile phase, compared to a conventional C18 column which shows a complete loss of retention.



Instrument: Shimadzu Prominence 20A

Sample: Inj.: 10µL; Conc.: 2000µg/mL each component except fumaric acid (10µg/mL) (Organic Acids Reference Mixture cat.# 35080); Sample diluent: deionized water

Conditions: Mobile phase: 20mM potassium phosphate (pH 2.5); Flow: 1.0mL/min.; Temp.: 30°C; Det.: UV @ 226nm

Phase collapse caused for experimental purposes by releasing column pressure

Figure 2 Excellent resolution of organic acids, including tartaric and quinic acids, on an Allure® Organic Acids column.

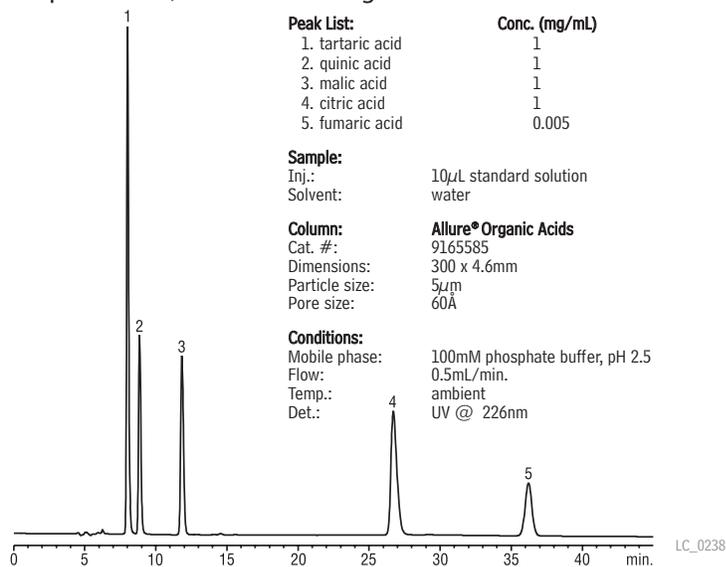
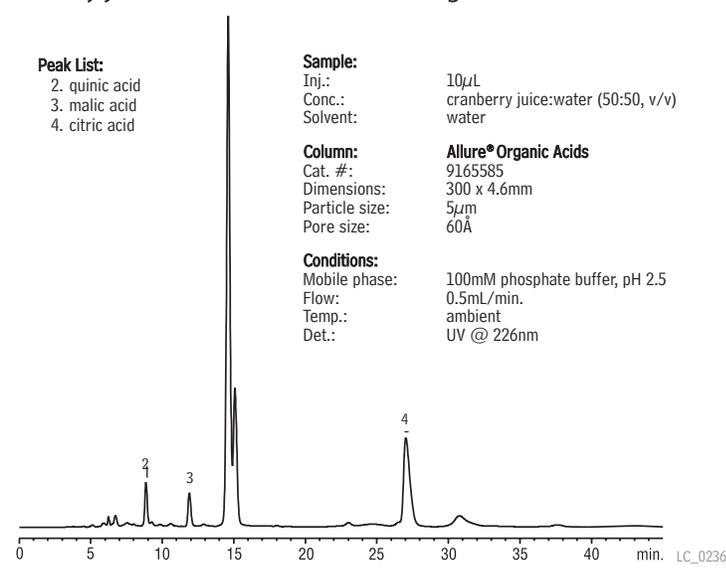


Figure 3 Sharp, easily differentiated organic acid profiles for cranberry juice cocktail on an Allure® Organic Acids column.



In analyses of organic acids, specifically, under high aqueous mobile phase conditions, the Allure® Organic Acids column is the column of choice. We have developed a method using a 300mm Allure® Organic Acids column to separate critical organic acids: tartaric, quinic, malic, citric and fumaric acids. This method calls for 100% aqueous mobile phase as recommended by AOAC method 986.13. The Allure® Organic Acids column is tested specifically for resolving critical organic acids. Figure 2 shows that tartaric and quinic acids are resolved to baseline; Figure 3 shows typical analyses under the conditions we recommend.

References

1. http://www.restek.com/advantage/adv_2003_03_02a.pdf.
2. Official Methods of Analysis (2000). AOAC International, 17th edition, method # 986.13.
3. Manolaki, P. et al., Food Chemistry, 98 (2006), page 658-663.
4. Kafkas, E. et al., Food Chemistry, 97 (2006), page 732-736.

Fruit Juice Organic Acid Standard

citric acid	2000µg/ml	quinic acid	2000
fumaric acid	10*	tartaric acid	2000
malic acid	2000		
In water, 1mL/ampul			
cat. # 35080 (ea.)			
In water, 5mL/ampul			
cat. # 35081 (ea.)			

*Fumaric acid is a trace impurity in malic acid, as well as an added component of the mix. The amount of fumaric acid in malic acid will not affect the stated concentration of malic acid, but can represent a significant and variable deviation from the low concentration of fumaric acid stated to be in the mix. All other components of the mix are at the specified concentration.

Allure® Organic Acids Column

5µm Column, 4.6mm	cat. #
150mm	9165565

Allure® Aqueous C18 Column

5µm Column, 4.6mm	cat. #
150mm	9168565

Ultra Aqueous C18 Column (USP L1)

5µm Column, 4.6mm	cat. #
150mm	9178565

for **more** info

For more information on our Allure® Aqueous C18, Ultra Aqueous C18 and Allure® Organic Acid columns, visit us online at www.restek.com.

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