RESTEK HPLC

Applications note

Single-Column Method for HPLC Analysis of Organic Acids in Fruit Juices, Using an Allure™ Organic Acids Column

Organic acids play multiple roles in food and beverage systems: they are important flavor compounds, and they are indicators of product quality. Organic acid profiles are monitored to determine the purity of certain fruit juices. In some food and beverage systems they are added as acidulants, to control the pH of a product. Certain organic acids also can be used as antimicrobial agents; for example, propionic acid can be used to slow mold growth. Malic acid, citric acid, and others are found in fruits. Oxalic acid is present in spinach and rhubarb; grapes contain tartaric acid.

Analysis of polar organic acids can be difficult on conventional reversed phase columns. A highly aqueous mobile phase is needed to increase interaction between the acids and the stationary phase, but stationary phases in conventional C18 columns collapse in 100% aqueous mobile phases. The Allure Organic Acids column was designed to enhance retention and selectivity in challenging applications such as this. Polar embedded groups allow the alkyl groups in Allure Organic Acids columns to remain extended in 100% aqueous mobile phases; retention is stable and reproducible.

The fruit juice industry in the US alone is worth over $12 billion per year[1] and is many times that worldwide. As with other industries in which there is a large potential for profit, unscrupulous juice producers and traders have found ways to replace or extend more valuable juices. This can be done by substituting sugars for juice solids, or by diluting higher cost juices with less expensive ones. For example, white grape juice and pear juice have been used to extend other, more costly juices. To detect these adulterations, a number of laboratories employ fruit juice authenticity testing.

Because juices are chemically quite complex, several complementary tests should be performed to verify authenticity. These can include determining sugar profile and sorbitol content; minerals; anthocyanin pigments; phenolics; oligosaccharides; carbon stable isotope ratio for various components; and organic acid profile. High performance liquid chromatography (HPLC) is a powerful tool in analyses of many of these components. With these complex matrices, the resolving power of HPLC is invaluable for accurately quantifying components.

Organic acids give fruit products their characteristic tartness. Since the organic acids content varies in composition and in concentrations among different fruit juices, organic acid content can be used to identify a product and verify its purity. For example, malic acid is a major component of the organic acid profile of apple juice. If apple juice has been diluted, e.g., with sugar water, the malic acid content will be low. In grape juice, tartaric acid is present at relatively high levels. Cranberry juice, on the other

Figure 1

Excellent separation of tartaric and quinic acids by an Allure™ Organic Acids column.

Peak List:	Conc.
1. tartaric acid	1mg/mL
2. quinic acid	1mg/mL
3. malic acid	1mg/mL
4. citric acid	1mg/mL
5. fumaric acid	0.005mg/mL

Sample:	**Fruit Juice Organic Acid Mixture**
Inj.:	10µL
Conc.:	see peak list
Sample Diluent:	purified water
Column:	**Allure™ Organic Acids**
Catalog #:	9165585
Dimensions:	300 x 4.6mm
Particle Size:	5µm
Pore Size:	60Å
Conditions:	
Mobile Phase:	100mM phosphate buffer, pH 2.5
Flow:	0.5mL/min
Temp.:	ambient
Det.:	UV @ 226nm

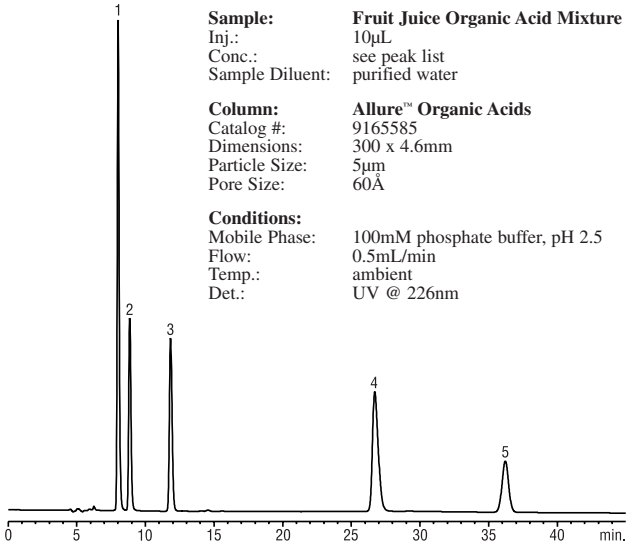

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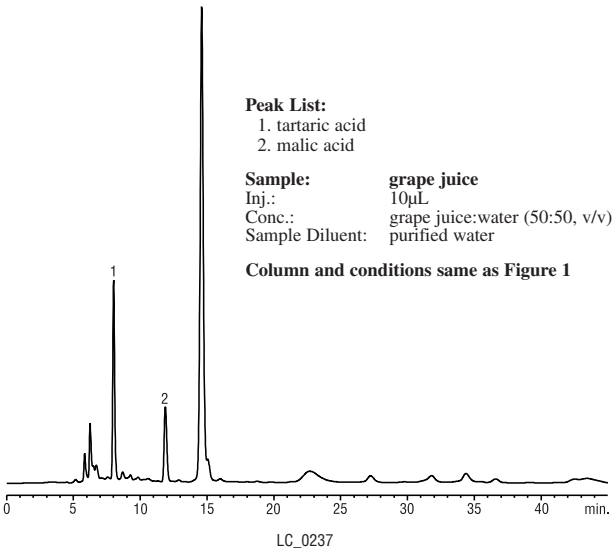
Figure 2

Tartaric and malic acids, the primary organic acids in grape juice, resolved by an Allure™ Organic Acids column.

Peak List:
1. tartaric acid
2. malic acid

Sample:	**grape juice**
Inj.:	10µL
Conc.:	grape juice:water (50:50, v/v)
Sample Diluent:	purified water

Column and conditions same as Figure 1



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hand, contains quinic acid. A cranberry juice that contains measurable amounts of tartaric acid would be suspect. Thus, it is critical to both identify and quantify the organic acids in a fruit juice to determine if the juice is described truthfully.

The organic acid content of fruit juices, such as cranberry juice and apple juice, can be determined using AOAC method 986.13.[2] In this procedure, reversed phase HPLC with a UV detector is used to quantify organic acids. Because several of the organic acids are extremely difficult to resolve, this procedure calls for two reversed phase C18 columns in series. A 100% aqueous mobile phase (phosphate buffer at pH 2.4) is used to maximize the interaction between the acids and the stationary phase.

Now there is a simpler and more reliable approach. A single 30cm Allure Organic Acids column effectively resolves key organic acids, such as tartaric and quinic acids, using the chromatographic conditions specified in AOAC method 986.13!

Figure 1 shows a separation of typical fruit juice organic acids, including tartaric, quinic, malic, citric, and fumaric acids. Note the baseline resolution between tartaric and quinic acids. This superior performance makes interpretation of analytical data more reliable. Similarly, note the distinct organic acid profiles for grape juice and cranberry juice cocktail in Figures 2 and 3.

Analysis of polar organic acids can be difficult on conventional reversed phase columns, even when using highly aqueous mobile phases and two reversed phase columns in series for the separation. In contrast, an Allure Organic Acids column provides enhanced retention and selectivity for these compounds, allowing the separation to be performed on one 30cm column. Retention is stable and reproducible, even with a 100% aqueous mobile phase, as specified in AOAC method 986.13. If you are monitoring fruit juice quality, and want a trouble-free analysis with accurate results, we highly recommend an Allure Organic Acids column.

References

1. *Authenticity of Apple Juice* Technical Bulletin #2 (1996), Analytical Chemical Services of Columbia, Inc.
2. Official Methods of Analysis (2000), AOAC International, 17th edition, method #986.13.

HPLC Catalog

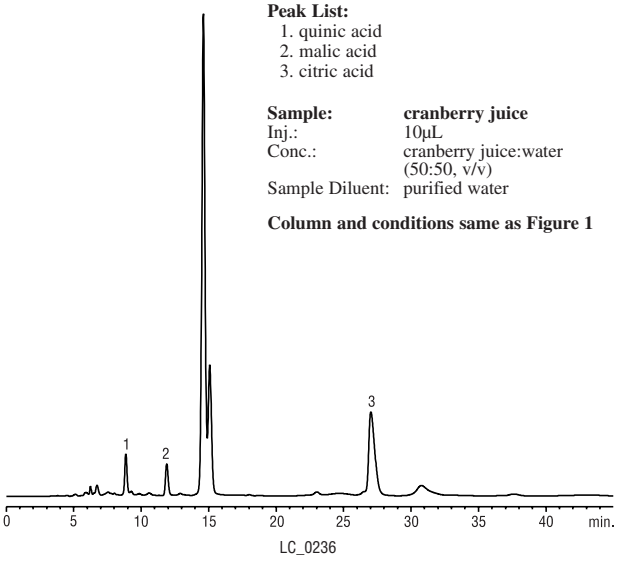
Ability to manufacture silica, synthesize many stationary phases, and perform high-density bonding has made Restek a significant supplier of HPLC columns. This 116-page catalog lists columns in four silica lines, including LC/MS columns and other special-purpose columns, microbore columns, and preparative columns.Many application chromatograms. (lit. cat.# 59241A)

Foods, Flavors, & Fragrances In-Review

Abstracts of 30+ Restek publications on foods - flavors - fragrances applications. Check the publications you want on the pre-paid postcard - or access them on our website. (lit. cat.# 59489)

Figure 3

Quinic, malic, and citric acids in cranberry juice cocktail.



Allure™ Organic Acids Column

Physical Characteristics:

particle size:	5µm, spherical, non-endcapped phase
pore size:	60Å
pH range:	2.5 to 7.5
temperature limit:	80°C

Dimension	qty.	cat.#
300mm x 4.6mm	ea.	9165585

Fruit Juice Organic Acid Mixture

citric acid	2000µg/mL
fumaric acid	10
malic acid	2000
quinic acid	2000
tartaric acid	2000

In water, 1mL/ampul

Each	5-pk.
35080	35080-510

In water, 5mL/ampul

Each	5-pk.
35081	35081-510

Foods, Flavors, & Fragrances

A 48-page overview of the foods, flavors, fragrances applications for which Restek HPLC and GC columns have been used. Applications include amino acids, carbohydrates, chiral separations, essential oils, fats and oils, organic acids, preservatives, vitamins, and more. (lit. cat.# 59260)