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Application Note

Analysis of Fourteen Organic Acids in Various Beverages Using the ACQUITY UPLC H-Class PLUS and ACQUITY QDa Mass Detector

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Abstract

Organic acids are naturally occurring or frequently used in beverage, food, and feed production. Acidic additives may act as buffers to regulate acidity, antioxidants, preservatives, flavor enhancers, and sequestrants.[1] Organic acid profiles vary between different beverages, and they contribute to the sensory properties of food and beverages, adding to aroma and taste. It is useful to monitor organic acids in beverages for quality control checks, to ensure ingredients are within product specifications, for consistent taste as well as for the evaluation of fruit juice authenticity and purity.

The use of single quadrupole mass detectors such as the ACQUITY QDa, offers several advantages over optical detectors, such as UV-Vis. The advantages include increased selectivity, lower detection limits, and the ability to collect mass spectral information on components of a sample. In this application note, we highlight a method for the separation of fourteen organic acids using the ACQUITY Premier CSH Phenyl-Hexyl Column. The analysis was

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carried out on an ACQUITY UPLC H-Class PLUS coupled with an ACQUITY QDa Mass Detector. The use of the ACQUITY QDa Mass Detector improves the selectivity of the method, resulting in less impact on the analysis from sample matrix co-elutions, allowing for easier integration of the target organic acids and more accurate analysis.

The method was evaluated and studied for retention, peak shape, stability, and linearity. All studied analytes showed acceptable chromatographic retention with this column. The method was tested on various fruit juices, wine, energy, and sports drinks, as well as sparkling water samples. The ACQUITY Premier Column coupled with ACQUITY UPLC-QDa showed sufficient retention time, good resolution, and faster sample analysis time for the studied organic acids when compared to legacy HPLC methods.

Benefits

- The ACQUITY Premier CSH Phenyl-Hexyl Column mitigates analyte interactions with metal surfaces. Excellent method reproducibility with the ACQUITY Premier CSH Phenyl-Hexyl Column

- A single chromatography method for the analysis of fourteen organic acids without the need for sample derivatization, ion-pairing reagents or buffered mobile phase

- Greater selectivity and sensitivity are achieved using the ACQUITY QDa Mass Detector in Single Ion Recording (SIR) acquisition mode, reducing the chromatographic interferences from co-eluting matrix compounds

- Acceptable retention and chromatographic resolution achieved for studied organic acids

- A single method for multiple types of beverage samples such as fruit juices, wines, energy drinks, and sparkling water

- Efficient sample analysis with a run time less than 11 minutes per sample

Introduction

Organic acids may be naturally occurring or are added to beverages as acidulants, for flavoring purposes, or as preservatives. Organic acids play an important role in beverages by adding to the product's aroma, taste, and characteristic flavor profile. Various geographical origins and maturation stages affect the amount and types of organic acids found in different kinds of fruits. The profiling and quantification of organic acids is a useful test for beverages to determine the percentage of fruit juice and for detecting misbranding and/or adulteration in fruit

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juices as well as for quality control purposes. Organic acids are less susceptible to change during processing and storage so they can be a useful index of authenticity for fruit-based products.[2]

Organic acids are challenging to analyze as they are very polar compounds and difficult to retain on traditional silica C_{18} reversed-phase columns. Many published methods suggest ion-chromatography (IC), capillary electrophoreses (CE) or titration methods to determine organic acids in beverages. Ion chromatography methods typically require lengthy analysis times and need specialized instrumentation. Legacy HPLC methods use reversed-phase C_{18} columns for the retention and separation of organic acids with UV-Vis or PDA often used for detection.

Due to the similar structures and characteristics of organic acids, their baseline separations from each other as well as from the matrix components become important, especially when analyzing with a traditional methodology such as UV or PDA detector. One of the challenges with the use of HPLC is that the detection and the peak shape of some organic acids can be compromised by the interaction with surfaces of the analytical flow path, including the column. Achieving acceptable peak shape, retention, and separation between organic acids can be challenging and the presence of co-eluting ingredients from the samples, which can create further complications for routine analysis of organic acids.

This application note describes the analysis of fourteen organic acids in various beverages. The separation of these analytes was performed on an ACQUITY Premier CSH Phenyl-Hexyl Column and analyzed on the ACQUITY UPLC H-Class PLUS System coupled to an ACQUITY QDa Mass Detector. The MaxPeak HPS Technology incorporated in the ACQUITY Premier Column reduces the interaction between analyte and the chromatographic flow path, delivering improved peak shape and sensitivity. Due to use of the ACQUITY QDa Mass Detector, it was possible to simplify the mobile phase composition, removing the need to use a high mM concentration of buffer. The ACQUITY QDa Mass Detector enables the detection of organic acids and provides improved sensitivity compared to UV or PDA detector.

Several performance criteria to assess the performance of the analytical method were evaluated, including retention time, separation, sensitivity, and linearity. The method was used to determine the organic acids in various fruit juices, wine, energy and sports drinks, sparkling water, and energy boosters.

Experimental

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Standard Preparation

Fourteen organic standards were dissolved in MilliQ water at 10 mg/mL individually to make stock solutions. A mix of all organic acids was prepared at 600 µg/mL (ppm) by mixing individual organic acids stock solution and diluted with MilliQ water. The solvent calibration curve was prepared from 0.2 to 200 µg/mL by serial dilution with MilliQ water from the organic acid mix stock solution.

Sample Preparation

Orange juice, apple juice, red wine, two types of energy drinks, sports drinks, and sparkling water were diluted 10 times with MilliQ water. For Energy booster powder, 9.1 grams were dissolved in 119 mL of MilliQ water and prepared as described on its package. The diluted energy booster was further diluted 100 times, and 500 times with MilliQ water. All samples were filtered after dilution with water.

UPLC-Mass Detector

UPLC system:	ACQUITY UPLC H-Class PLUS with FTN Sample Manager
Mass detector:	ACQUITY QDa
Column:	ACQUITY Premier CSH Phenyl-Hexyl Column 1.7 µm, 2.1 x 100 mm (p/n: 186009475)
Software:	Empower 3 CDS
Mobile phase A:	0.1% formic acid in water (LC-MS grade)
Mobile phase B:	0.1% formic acid in acetonitrile (LC-MS grade)
Injection volume:	5 µL
Column temperature:	50 °C

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Sample temperature: 10 °C

Run time: 11 minutes (including equilibration)

Vials: Waters Total Recovery, deactivated (p/n: 186000385DV)

Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.4	100	0	Initial
0.5	0.4	100	0	6
3.5	0.4	75	25	6
3.6	0.4	5	95	6
4.6	0.4	5	95	6
4.7	0.4	1	99	6
7	0.4	1	99	6
7.1	0.4	100	0	6
11	0.4	100	0	6

MS Conditions

MS instrument: ACQUITY QDa Mass Detector (Performance)

Ionization: Electrospray

Polarity: Negative ion mode

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Capillary voltage: 0.8 V

Cone voltage: 5.0 V

Probe temp.:   600 °C

SIR masses: See Table 2

Organic acid	Monoisotopic mass (Da)	SIR mass [M-H]⁻ (m/z)
Lactic acid	90.03	89
Succinic acid	118.03	117
Glutaric acid	132.04	131
Quinic acid	192.06	191
Isocitric acid	192.03	191
Citric acid	192.03	191
Fumaric acid	116.01	115
Maleic acid	116.01	115
Ascorbic acid	176.03	175
Adipic acid	146.06	145
Shikimic acid	174.05	173
Trans-aconitic acid	174.02	173
Cis-aconitic acid	174.02	173
Tartaric acid	150.02	149
Malic acid	134.02	133

Table 2. Monoisotopic and single ion recording (SIR) mass of studied fourteen organic acids and five internal standards.

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A mixture of organic acids standards outlined in Table 2 was analyzed using the ACQUITY Premier CSH Phenyl-Hexyl Column. A gradient method with a simple mobile phase of water and acetonitrile, each with 0.1% formic acid was used. All studied organic acids were detected using an ACQUITY QDa Mass Detector using negative electrospray ionization (ESI) to produce $[M-H]^-$. The single ion recording masses (SIR) or the molecular ion mass to charge ratio (*m/z*) of all organic acids are listed in Table 2.

Results and Discussion

The chromatography of organic acids can be impacted by interactions between the target analytes and the metal surfaces of the chromatographic flow path. These interactions may result in increased peak tailing and reduced peak area. There are several procedures available to minimize the interaction between analytes and the metal surfaces by passivating the LC system and column. Passivation methods are time consuming and require additional solvents which adds extra time and cost to setup method. Another approach to address metal interactions is the use of additives in the mobile phase. However, these additives can contribute to ion suppression or changes in chromatography, impacting method performance. Waters has developed a new low adsorption ACQUITY Premier System and Columns designed to reduce variability associated with metal-sensitive analytes without time-consuming tasks like system and column passivation. Citric acid is a renowned chelating agent and binds to metal. Figure 1 shows the chromatographic performance of citric acid (RT 1.54 min) on the ACQUITY Premier UPLC System and Column compared to using the standard UPLC system and column. It was observed that peak areas increased 5 times and peak shape was improved using the ACQUITY Premier Column and System.[3] Improvements in peak shape and sensitivity were also still observed when using the ACQUITY Premier Column on a conventional UPLC system.

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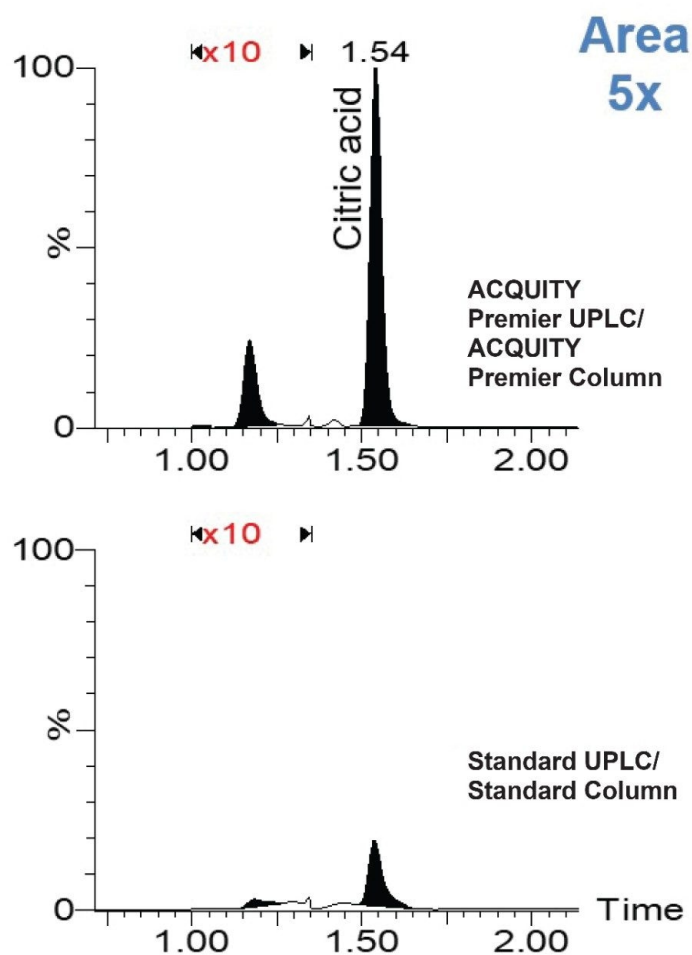
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Figure 1. Improvement in peak area of citric acid using the ACQUITY Premier System and Column compare to standard column and system (LC-MS data).

The baseline separation of all fourteen organic acids and avoiding co-elution with other ingredients of the beverage products can be challenging. The increased selectivity gained with the use of the ACQUITY QDa Mass Detector under the SIR mode, baseline separation may not be required for all analytes. Methods which use optical detectors such as UV-Vis need baseline separation for all analytes as well as can suffer from co-elutions which can create challenges for consistent integration.

Figure 2 shows an example chromatogram generated from the UPLC-PDA method.[4] This method requires buffered mobile phase for detection of organic acids. The UPLC-PDA method requires buffered mobile phase for

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detection purpose. It is vital to have an absorbance difference between the mobile phase buffer or additives and the analyte of interest for the PDA detector. Organic acids with a similar absorbance (210 nm) to formic acid cannot be distinguished from mobile phase background noise resulting in a higher baseline. Therefore, the LC-QDa method developed with formic acid cannot be used for the UPLC-PDA detector. As shown in the UPLC-PDA method (Figure 2), ascorbic acid co-elutes with malic acid and maleic acid co-elutes with adipic acid. While Figure 3 shows separation of fourteen organic acid using ACQUITY QDa Mass Detector as shown in Figure 3.
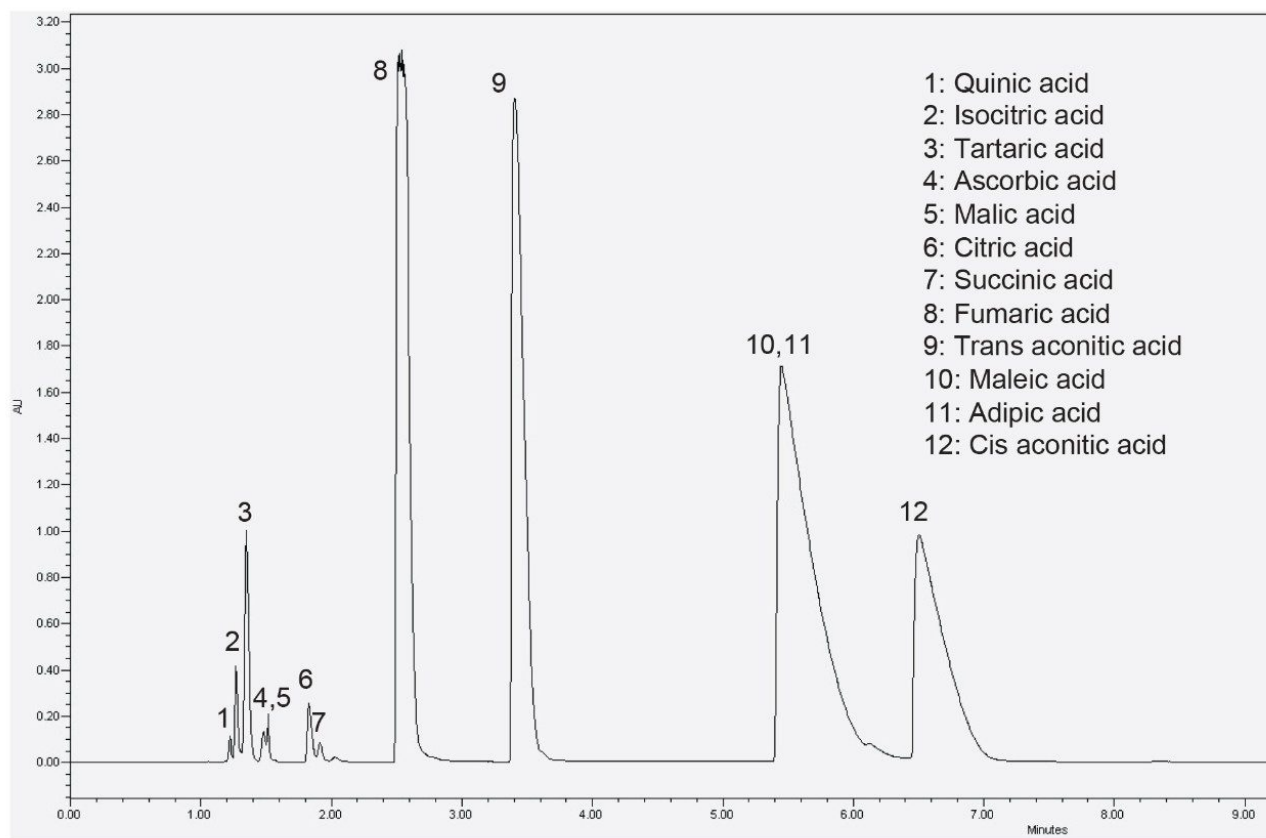
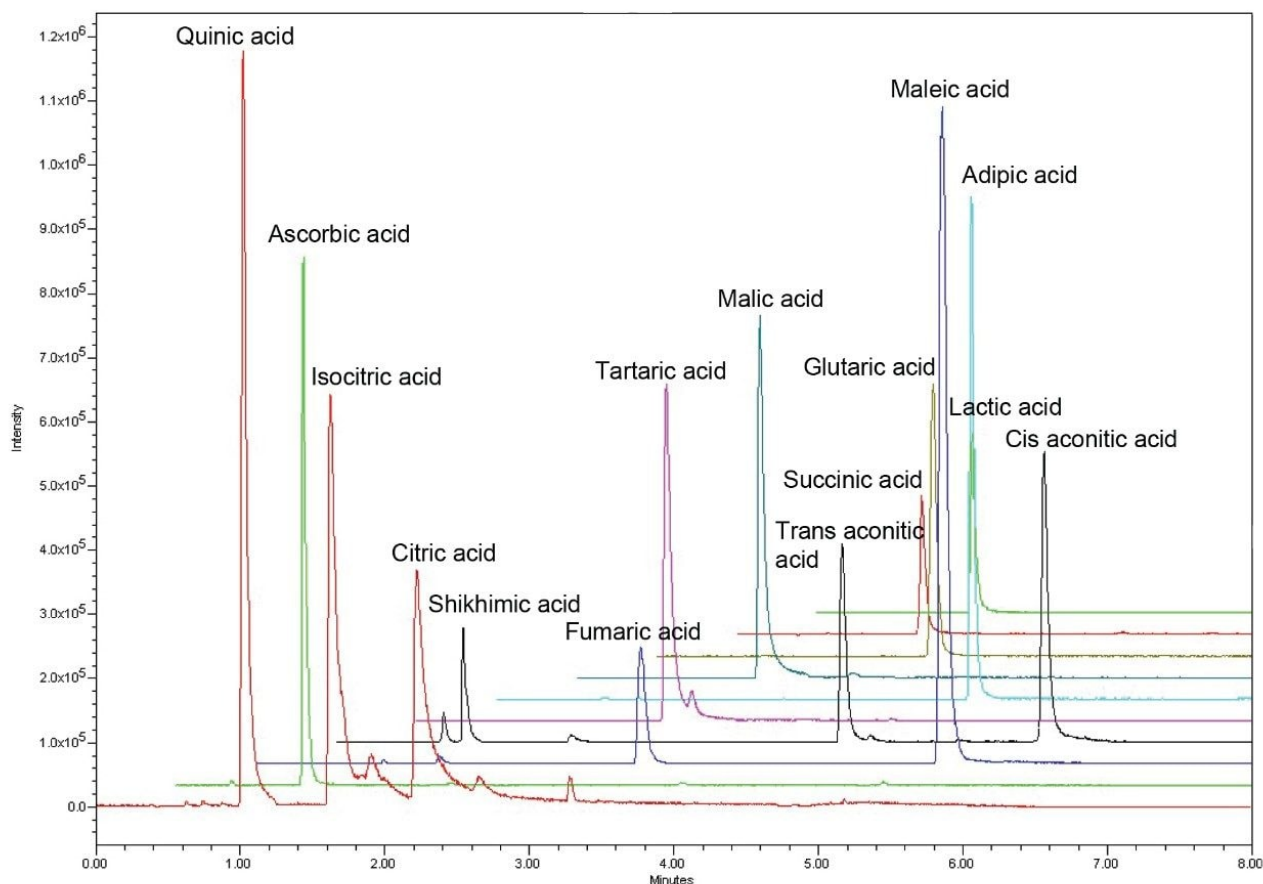*Figure 2. An example chromatogram of various organic acids using UPLC-PDA detector.*

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Figure 3. Chromatographic separation of fourteen organic acids at 6 μg/mL (ppm) using the ACQUITY Premier CSH Phenyl-Hexyl Column. The chromatograms of each compound were overlaid in a single plot with the time axis offset.

While the use of mass detection does provide greater specificity than use of UV-Vis, some key baseline separations are still required. Some of the molecular ions [M-H]⁻ of these organic acids have a similar mass to charge ratios (*m/z*). For example, quinic acid, citric acid, and isocitric acid have *m/z* 191, shikimic acid, and two isomers of aconitic acids (trans and cis) have *m/z* 173 while fumaric acid and maleic acid have *m/z* 115. Isocitric acid is a structural isomer of citric acid, therefore baseline separation between these two organic acids is important.

Organic acids with the same *m/z* were detected in the same SIR channels and ensured they were separated with

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enough resolution between them. Figure 4 shows the baseline separation of these analytes in solvent standards.
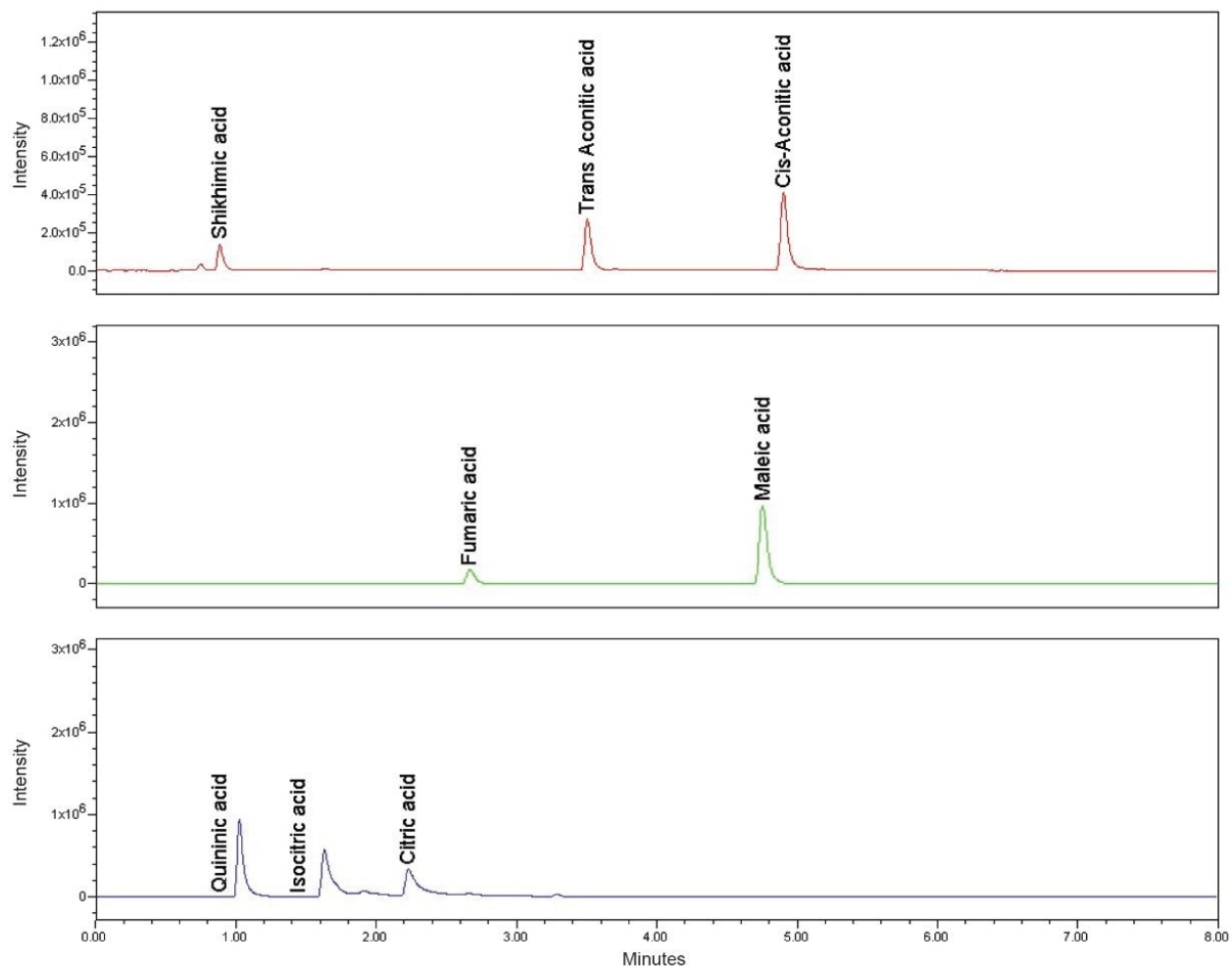

Figure 4. Baseline separation of quinic acid, isocitric acid, and citric acid in the red trace, fumaric acid and maleic acid in the green trace, and shikimic acid, trans aconitic acid, and cis aconitic acid in the blue trace.

To assess the performance of this methodology, several beverage samples were tested to determine their organic acid profiles. As outlined under the sample preparation section, samples were diluted and filtered before analysis.

Energy Drink Samples

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Three energy drink samples were tested, and citric acid was found to be the major organic acid present in the samples. Citric acid is often added to beverages as a flavor enhancer, providing a tart or sour taste. Citric acid was listed under the ingredient list in all three studied energy drinks. Due to the increased selectivity and sensitivity of the ACQUITY QDa Mass Detector, malic acid, and lactic acid were also detected at a lower level in sample A. While sample C showed malic acid, ascorbic acid, and a low level of tartaric acid, malic acid, fumaric acid, and maleic acid. All three samples showed the identified organic acids listed under their ingredient list. Figure 5A, 5B, and 5C shows organic acids found in the energy drink sample A, sample B, and sample C.
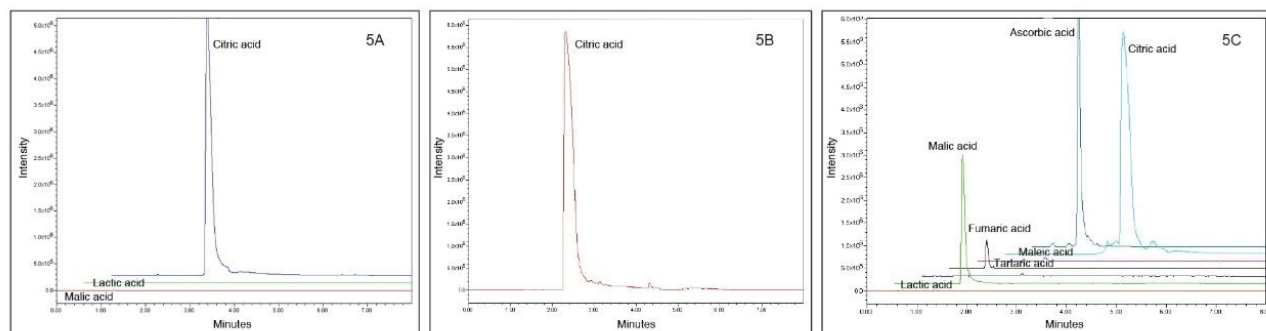

Figure 5. Organic acids detected in energy drink sample A (5A), sample B (5B), and sample C (5C).

Sports Drink and Sparkling Water

The studied sports drink sample contained citric acid as the major organic acid as listed on the label. Along with citric acid, a lower level of malic acid and trans aconitic acid was also detected in the sports drink sample. Lactic acid, succinic acid, fumaric acid, maleic acid, and quinic acid were found at low concentrations in the sparkling water sample. Figure 6A and 6B shows organic acids found in studied sports drink and sparkling water.
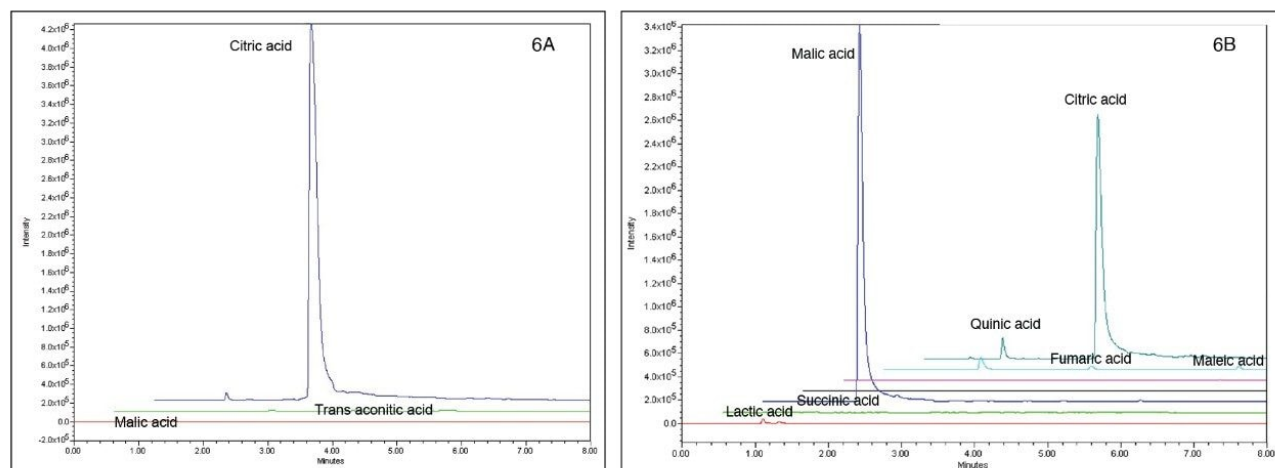
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Figure 6. Organic acids detected in sports drink (6A) and sparkling water (6B).

Red Wine

Tartaric acid, succinic acid, and lactic acid were predominantly present in the red wine. The red wine sample was found to contain other low levels of organic acids as well such as shikimic acid, quinic acid, citric acid, cis, and trans aconitic acid, and malic acid. The SIR provides greater selectivity than UV-Vis, this reduces the impact of matrix interferences allowing for the detection and easier integration of low-level organic acids in the red wine samples. The developed method may be applicable to the study and comparison of organic acid profiles in different wines.
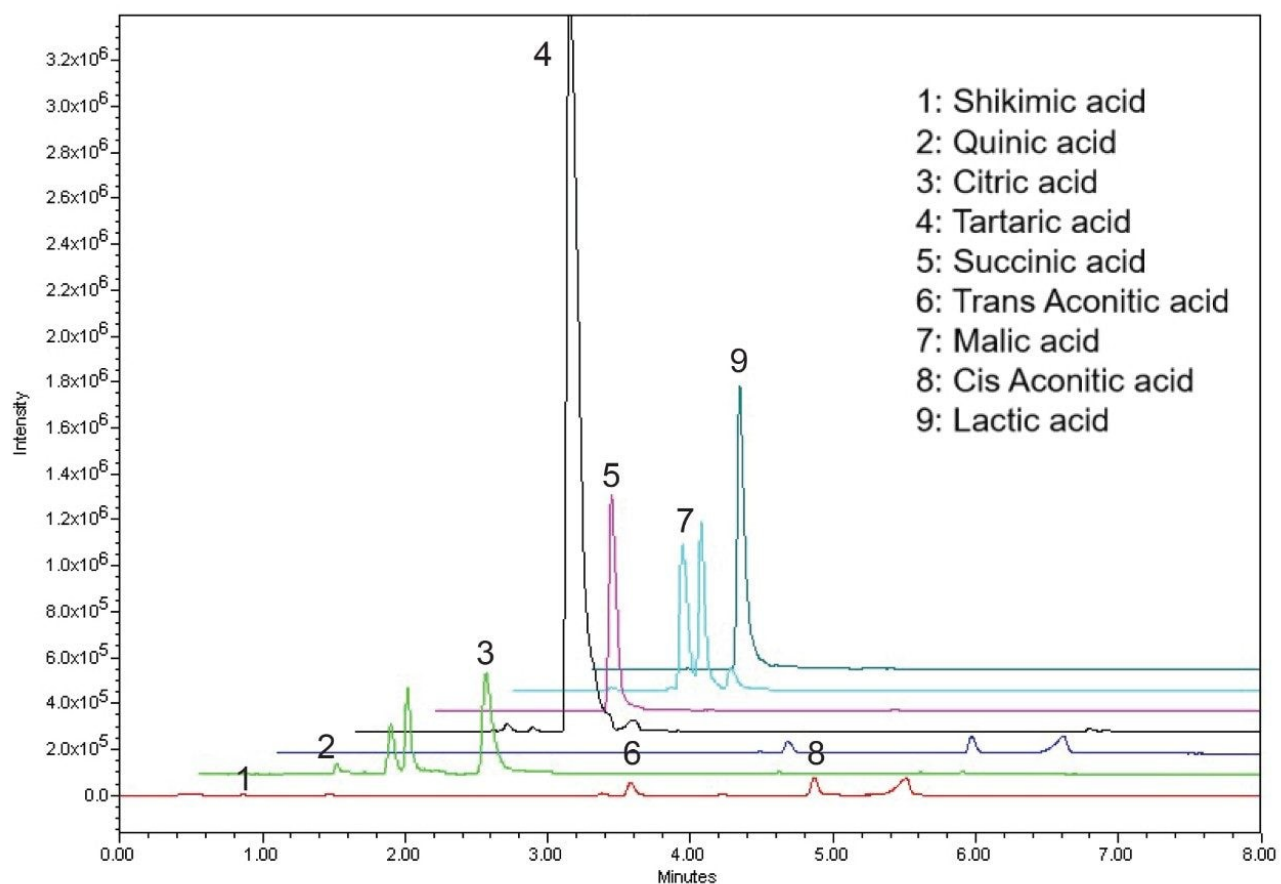
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Figure 7. Organic acids detected in red wine sample.

Fruit Juices: Orange Juice and Apple Juice

Malic acid, citric acid, and quinic acid are the most common organic acids found in apple juice. But, along with these acids, some low-level organic acids were also detected using the ACQUITY QDa Mass Detector. Citric acid was detected at a high level in orange juice. Lactic acid, succinic acid, malic acid, quinic acid, isocitric, tartaric acid, fumaric acid, trans and cis aconitic acid, and ascorbic acid were also detected in orange juice.
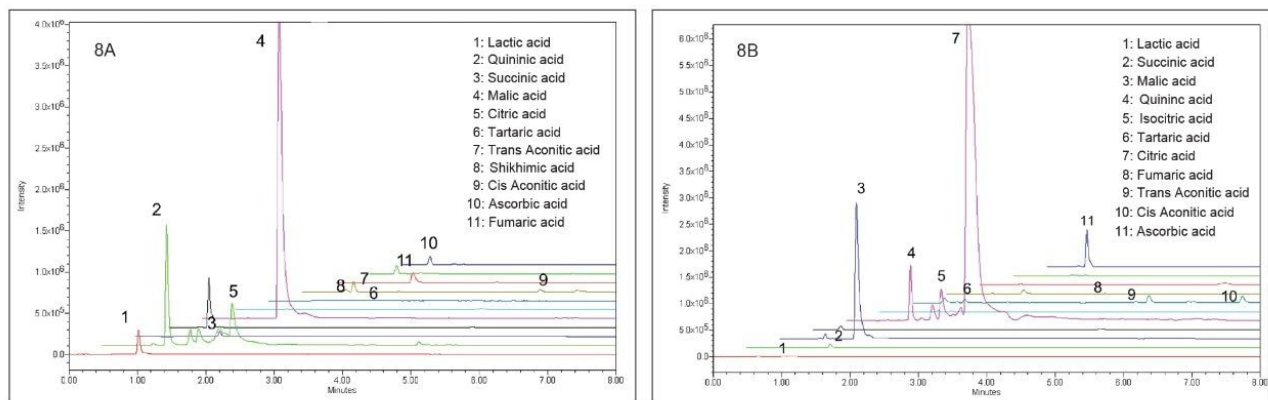
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Figure 8. Organic acids detected in apple juice (8A) and orange juice (8B).

The reproducibility of the method was studied with 50 continuous injections of energy drink, sample G. Figure 9 shows the retention time and peak area of citric acid present in the sample. The %RSD for retention time and peak area was 0.3% and 1.9%, over 50 injections.

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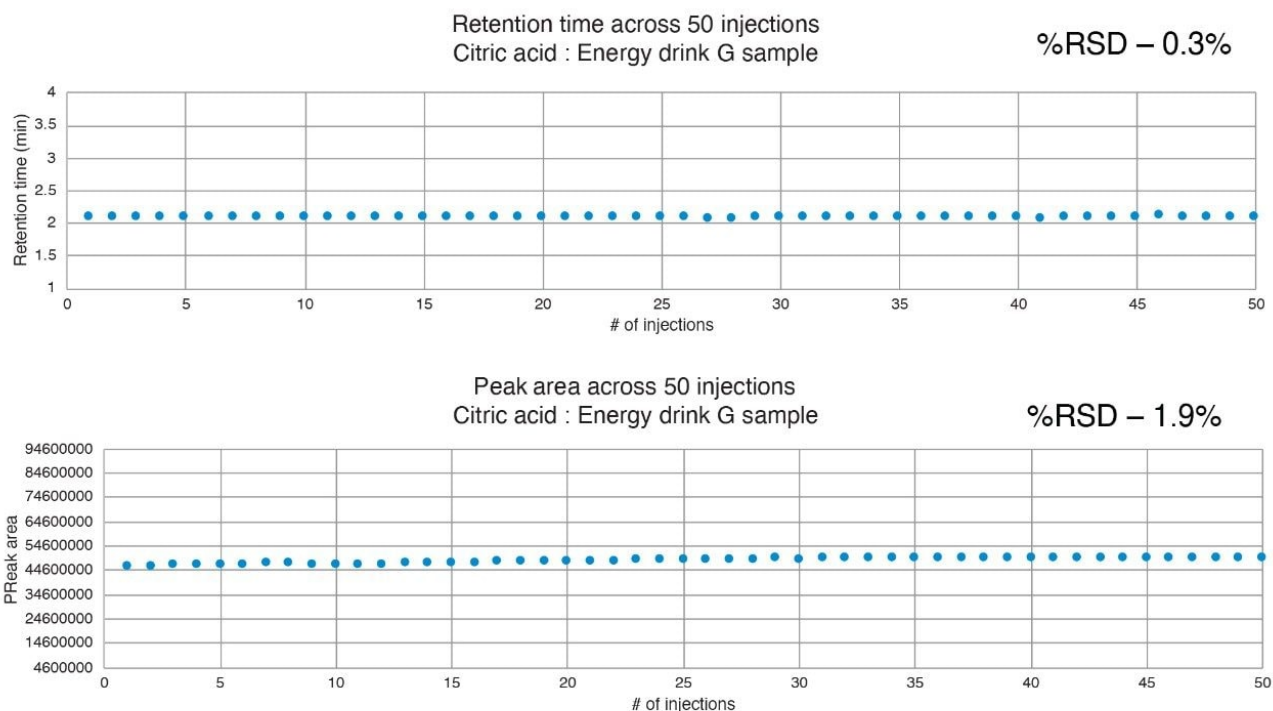
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Figure 9. The ACQUITY Premier CSH Phenyl-Hexyl Column reproducibility over 50 injections of energy drink sample. The retention time (top) and peak area (bottom) of citric acid shown in the plot along with the %RSD.

Conclusion

- This application note highlights a UPLC-MS method for analysis of fourteen organic acids with simple sample preparation and a non-buffered mobile phase.

- The ACQUITY Premier Column showed improved chromatographic peak shapes and sensitivity compared to the conventional column.

- The ACQUITY QDa Mass Detector enables selectivity and sensitivity, compared to UV or PDA detector. This allows for the detection of organic acids that are present at lower levels as well as those at higher levels in the beverage samples.

- The method was able to separate and detect organic acids in various fruit juices, wine, sports drinks, and

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energy drinks.

- The method showed an excellent reproducibility for retention time and peak area of citric acid over 50 injections of an energy drink sample.

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