Waters™

응용 자료

Fast, Accurate and Flexible LC-PDA Method for the Determination of Citric Acid In Beverages

Dimple D. Shah

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Citric acid is used as an additive in many beverages to improve taste and flavor as well as an antioxidant. An analytical method for citric acid has been developed using the ACQUITY Premier CSH Phenyl-Hexyl Column, with the ACQUITY UPLC H-Class System coupled with an ACQUITY UPLC PDA Detector. The performance of the method was evaluated by assessing parameters such as peak shape, linearity, and precision. The developed method was applied to energy and sports drinks samples. The quantification of citric acid in the samples was reported using Empower 3 Software. The combination of the ACQUITY Premier CSH Phenyl–Hexyl Column, with the ACQUITY UPLC H-Class, coupled with the ACQUITY UPLC PDA method and Empower 3 Software enables the quantification of citric acid in various beverages.

Benefits

The ACQUITY Premier CSH Phenyl-Hexyl Column with ACQUITY UPLC H-Class System coupled with an ACQUITY UPLC PDA Detector for the analysis of citric acid offers:

- Simple, accurate, and sensitive UPLC-PDA method for citric acid analysis
- Utilization of an ACQUITY Premier CSH Phenyl-Hexyl Column to improve the peak shape and sensitivity of citric acid for the analysis of sports and energy drinks for quality control indicators and to to meet product specifications to simplify this
- Data collection and processing with Empower 3 Quick start Interface

Introduction

Citric acid is a weak organic acid containing three carboxylic acid groups. It occurs naturally in many fruits, especially in citrus fruits like lemons and limes. Although it is naturally occurring, it is also used as an additive in many beverages to improve taste, flavor and as an antioxidant.[1] For quality control checks, to ensure ingredients are within product specifications, for consistent taste, it is useful to monitor citric acid in beverages.

Due to the polar nature of citric acid, it can be challenging to retain on traditional silica C_{18}, reversed-phase columns. Traditional methods of reversed-phase chromatography do not always

yield enough retention or selectivity which may result in co-elution with other ingredients in the beverage, creating peak integration challenges. Many published methods suggest ion-chromatography (IC), capillary electrophoreses (CE), or titration methods for the determination of citric acid.[2,3] These methods can be challenging and time-consuming as well as needing dedicated instrumentation and staff training. To ensure product consistency to get products to market, a reliable, simple, and accurate method is required.

High Performance Liquid Chromatography (HPLC) is an established tool for routine food testing due to its adaptability to run multiple tests on a single platform, along with accuracy and sensitivity, using autosampler technology and an array of detector options. The use of chromatography data software, such as Empower 3 Software, allows for the automatic capture of sample analysis information, data management, and the generation of sample reports, allowing scientists and laboratory managers to easily find information on sample analysis.

One challenge with the use of HPLC is that the detection and the peak shape of the citric acid can be compromised by the interaction with the surface of the analytical flow path including the column. This technology brief describes a UPLC-PDA method for the quantification of citric acid in beverages using the ACQUITY Premier CSH Phenyl-Hexyl Column.

Results and Discussion

Various concentrations of sodium phosphate buffer and formic acid were evaluated for mobile phase composition. Previously, a non-buffer method was developed for various organic acids, including citric acid, using formic acid as an additive in the aqueous and organic mobile phase for LC-MS detector.[4] The PDA detects an entire spectrum with three-dimension, light intensity, time, and wavelength. For HPLC-PDA methods, it is vital to have an absorbance difference between the mobile phase buffer or additives and the analyte of interest for detection purposes. Citric acid has a similar absorbance (210 nm) to formic acid hence 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 LC-PDA detector. A decent peak shape and sensitivity were observed with a lower concentration of sodium phosphate buffer. Hence, the UPLC-PDA method was developed for citric acid using 10 mM sodium phosphate buffer in water and acetonitrile.

Besides, mobile phase additives one of the major factors for peak tailing and reduced peak area for the analyte of interest using the HPLC is the interaction between the analyte and the surface of the

analytical column or LC fluidic path flow. Often analytes show secondary interactions with the surface which is displayed as peak tailing or reduced peak area. There are many procedures available to minimize the interaction between analytes and surfaces by passivating the LC system and column. Some of the passivation methods are incredibly time consuming, require many solvents, and include additives in the mobile phase which can contribute to ion suppression or changes in chromatography. To address metal interactions, 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. Previously, significant improvements in peak shape and sensitivity were observed for citric acid using the ACQUITY Premier Column.[5] The UPLC-PDA method was developed for citric acid using the ACQUITY Premier Column with a gradient of 10 mM sodium phosphate buffer and acetonitrile. The data was collected and processed with Empower 3 Software. The ACQUITY UPLC PDA Detector provides spectral analysis ranged from 190 to 500 nm. The spectral range allows the extraction of the chromatogram from the PDA data where the analyte shows maximum absorbance so 210 nm was selected as the wavelength for this analysis. Therefore, the PDA channel was selected at 210 nm. Figure 1 shows the chromatographic performance and solvent standard calibration curve of citric acid at 200 ppm on the UPLC-PDA. A solvent standard ranging from 12.5 ppm to 600 ppm was prepared in water by serial dilution and injected in triplicate. The entire calibrant range showed linearity above 0.999 with 1/X weighting applied, with residuals less than 5% for citric acid.

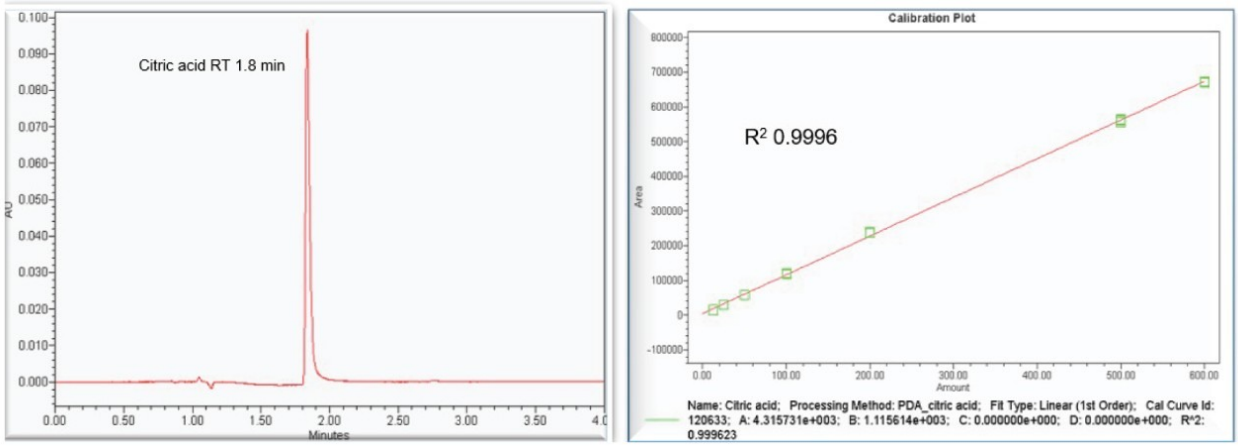
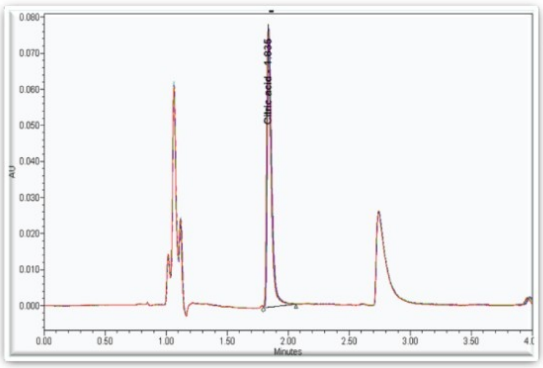


Figure 1. Chromatographic performance and solvent standard calibration curve of citric acid using ACQUITY Premier Column and LC-PDA method. The concentration of the standard is 200 ppm and observed at 210 nm channel on PDA.

The method was applied to analyze energy and sports drinks samples purchased from a local grocery store. Both samples contain citric acid listed on their ingredient label. Both samples were diluted ten times with HPLC grade water and filtered using Acrodisc Syringe Filters 13 mm 0.2 µm PVDF prior to injection. Figures 2 and 3 show chromatograms and a summary of seven-replicate injections from the sports drink and energy drink samples, respectively. As can be seen in Figures 2 and 3, citric acid (RT 1.8 min) peak was sufficiently retained on the column and well resolved from matrix interferences and other components. The retention time, peak area, and calculated amount showed %RSD less than 0.6% for both samples. Both samples showed decent peak shape of citric acid with USP tailing factor less than 2.2. To report the amount of citric acid in samples, the dilution factor needs to be considered. To calculate the amount of citric acid in the samples, the dilution factor was added to Empower Software. The Empower Software quantifies the samples against the solvent standard curve and automatically calculates the amount of citric acid in samples using the dilution factor and generates the report. Providing automatic calculation and report generation reduces the manual work, calculations, and errors.

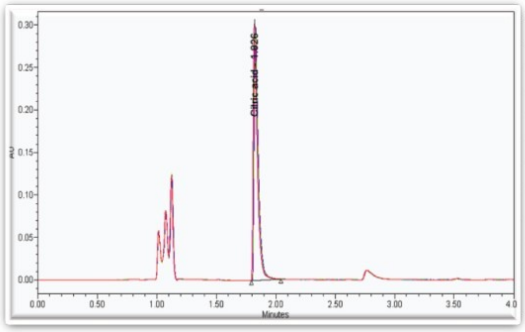
The below report shows the calculated amount of citric acid in the sports drink and energy drink. Citric acid was found at 1706 ppm (1.7 g/L) and 6687 ppm (6.6 g/L) in the sports drink and energy drink, respectively.



Component Summary Table
Name: Citric acid

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	USP Tailing
1	SD 10X	1	PDA Ch1 210nm@4.8nm	Citric acid	1.831	192588	76558	1688	ppm	1.70
2	SD 10X	2	PDA Ch1 210nm@4.8nm	Citric acid	1.835	194575	76687	1705	ppm	1.71
3	SD 10X	3	PDA Ch1 210nm@4.8nm	Citric acid	1.837	194545	77034	1705	ppm	1.71
4	SD 10X	4	PDA Ch1 210nm@4.8nm	Citric acid	1.840	195668	76997	1715	ppm	1.72
5	SD 10X	5	PDA Ch1 210nm@4.8nm	Citric acid	1.839	194262	77106	1703	ppm	1.72
6	SD 10X	6	PDA Ch1 210nm@4.8nm	Citric acid	1.844	195096	77110	1710	ppm	1.74
7	SD 10X	7	PDA Ch1 210nm@4.8nm	Citric acid	1.838	195932	77426	1718	ppm	1.74
Mean					1.8	194666.5		1706.2		
% RSD					0.2	0.6	0.4	0.6		
Std. Dev.					0.0	1102.7		9.9		

Figure 2. An overlay chromatogram of seven injections of sports drink containing citric acid. The Empower summary table reports peak area, retention time, and calculated amount. The mean values, %RSD, and standard deviation are reported here.



Component Summary Table
Name: Citric acid

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	USP Tailing
1	EGRB 10X	1	PDA Ch1 210nm@4.8nm	Citric acid	1.826	746047	300836	6649	ppm	2.18
2	EGRB 10X	2	PDA Ch1 210nm@4.8nm	Citric acid	1.827	753203	299064	6713	ppm	2.19
3	EGRB 10X	3	PDA Ch1 210nm@4.8nm	Citric acid	1.830	753033	298957	6711	ppm	2.20
4	EGRB 10X	4	PDA Ch1 210nm@4.8nm	Citric acid	1.827	746945	299641	6657	ppm	2.19
5	EGRB 10X	5	PDA Ch1 210nm@4.8nm	Citric acid	1.829	748318	299947	6669	ppm	2.20
6	EGRB 10X	6	PDA Ch1 210nm@4.8nm	Citric acid	1.825	753217	301413	6713	ppm	2.19
7	EGRB 10X	7	PDA Ch1 210nm@4.8nm	Citric acid	1.820	751827	301981	6700	ppm	2.18
Mean					1.8	750369.8		6687.4		
% RSD					0.2	0.4	0.4	0.4		
Std. Dev.					0.0	3161.6		28.3		

Figure 3. An overlay chromatogram of seven injections of energy drink containing citric acid. The Empower summary table reports peak area, retention time, and calculated amount. The mean values, %RSD, and standard deviation are reported here.

Conclusion

ACQUITY Premier Column reduces the interaction between analyte and surface and delivered acceptable peak shape and sensitivity. The developed UPLC-PDA method showed good repeatability for retention time, peak shape and peak area for sports and energy drinks. Empower 3 Software provides an automated calculation of the amount present in the sample. The UPLC-PDA analysis method combines with easy data interpretation software yields faster sample throughput and suitable for quality control of citric acid in sports and energy drinks.

References

1. Determination of Citric Acid in Fruit Juices Using HPLC: Katie Weikle, Department of Chemistry, Concordia College, 901 8th St S, Moorhead, MN 56562.
2. Sass-Kiss, A.; Toth-Markus, M.; Sass, M. Chemical Composition of Citrus Fruits (Orange, Lemon, and Grapefruit) with Respect to Quality Control of Juice Products. Nutraceutical Beverages, 2004, 871, 24–34.
3. G.Saccani.; S.Gherardi.; A.Trifirò.; C.Soresi Bordini.; M.Calza.; C.Freddi. Use of Ion Chromatography for the Measurement of Organic Acids in Fruit Juices. <

https://www.sciencedirect.com/science/article/abs/pii/0021967395002063>

4. Dimple Shah, Kerri Smith, Jinchuan Yang, Peter Hancock. Analysis of Fourteen Organic Acids in Various Beverages Using the ACQUITY H-Class Plus UPLC and QDa Mass Detector.

5. Kerri M. Smith, Paul D. Rainville. Utilization of MaxPeak High Performance Surfaces for Improved Separation and Recovery of Analytes Associated with the Tricarboxylic Acid Cycle. Waters Application Note, 720006727 <https://www.waters.com/nextgen/us/en/library/application-notes/2019/tca-cycle-analytes-by-mixed-mode-chromatography-mass-spectrometry.html> , Revised 2020.

Featured Products

ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

Empower Chromatography Data System <https://www.waters.com/10190669>

720007290, June 2021