APPLICATION NOTE

Liquid Chromatography

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Analysis of Organic Acids in Food with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

In food, organic acids originate from the natural biochemistry processes or are added as preservatives, acidulants and stabilizers. Organic acids contribute to the sensory properties of foods by providing taste and aroma. Citric acid is widely used in soft drinks to provide them with the sour taste of citrus fruit; fumaric acid is used as an acidulant in baking powder. In the food industry, organic acids are used as antimicrobial agents: formic acid is used as an antibacterial agent in livestock feeds where it can kill salmonella bacteria; proprionic acid is used as preservative in baked goods where it prevents the growth of mold and kills some bacteria. The use of organic acids in food is regulated in most countries, and the type and amount is constantly monitored to insure the food is safe for consumption and complies with regulations.

This application note presents a robust and sensitive reverse phase liquid chromatography method for the analysis of some widely used organic acids (Figure 1). To circumvent the challenge posed by their low retention factors, the mobile phase pH is adjusted to obtain a good separation. Method conditions and performance data including precision and linearity are presented. Red wine vinegar and lemon juice are analyzed and acid type and concentration confirmed.

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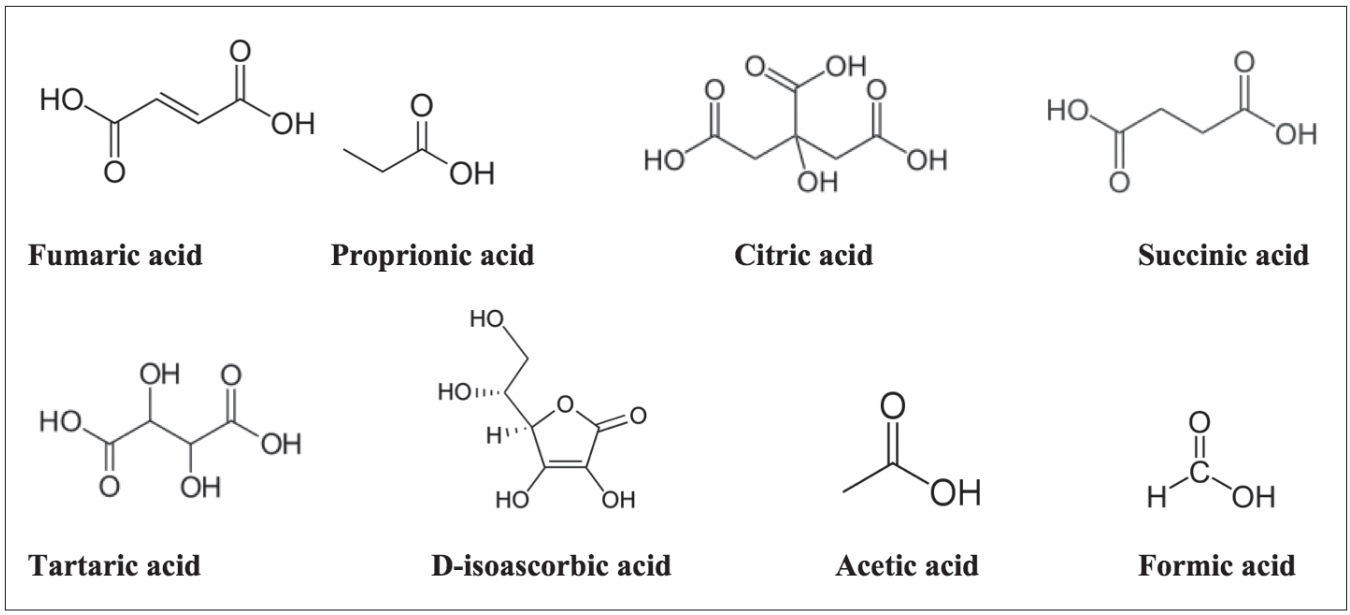

Figure 1. Molecular structure of organic acids studied.

Experimental

The separation was characterized and system calibrated with a mixture of organic acids diluted from neat material. A stock solution of each acid was prepared with water as solvent except for fumaric acid in which ethanol was used. A working standard with 2.7 mg/mL of formic acid, 0.4 mg/mL of D isoascorbic acid, 6.8 mg/mL of acetic acid, 2.6 mg/mL of citric acid, 0.015 mg/mL of fumaric acid, and 9.4 mg/mL of proprionic acid were prepared from the stock standards using water as a solvent. Repeatability was studied with six injections of the working standard. Linearity was determined across a wide range by serial dilution with water from the working standard, from 0.1-0.15 µg/mL for fumaric acid to 120-9400 µg/mL for proprionic acid (see range in Table 2). Samples of 0.1 g/mL vinegar and 0.1 g/mL lemon juice diluted with water were filtered with a 0.2 µm aqueous filter prior to testing.

The PerkinElmer® Flexar™ FX-15 with Flexar FX Photodiode Array Detector (PDA) provided the UHPLC platform for this application. The separation was completed on a PerkinElmer Brownlee™ Analytical C18, 5 µm 100 mm x 4.6 mm column. The run time was approximately 5 min. with a back pressure of 2000 PSI (138 bars). Alternatively, instead of a phosphate buffer a similar separation was obtained with 0.02% formic acid.

***Table 1.* Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC
Setting:	50 µL loop and 15 µL needle volume, partial loop mode injection: 2 µL
PDA Detector:	Recorded at 210 nm, scanned from 190-700 nm
Pump:	FX-15
Column:	PerkinElmer Brownlee Spheri-5 RP-18, 5 µm, 100 X 4.6 mm Cat #0711-0015
Column Temperature:	25 ˚C
Mobile Phase:	B: 70:30 (v:v) Acetonitrile:methanol, A: 5 mM Phosphate monobasic in water adjusted to pH 2.3 with phosphoric acid; alternate A: 0.02% formic acid in water

Time (min)	Flow rate (mL/min)	A%	Curve
4	1.0	100	1
1	1.0	100-85	1

2 min equilibration after each injection (HPLC grade solvent and ACS grade reagent)

Software:	Chromera Version 3.0
Sampling Rate:	5 pts/s

Results And Discussion

Analysis of organic acids in reverse phase LC is challenging due to the low retention factor and subsequent elution close to or within the void volume. In aqueous solutions, organic acids typically dissociate into their ionic form, which tend to be more polar and elute earlier than the non ionic form (In reverse phase LC, the more polar a compound, the faster it elutes). By lowering the mobile phase pH, ionization is prevented causing longer retention and improved resolution between peaks. The study of retention and selectivity based on the mobile phase pH established 2.3 as optimal. The effect of the mobile phase pH on the separation is illustrated in Figure 2.

All acid peaks eluted within 5 min., and the method performance was outstanding: the linearity of the analysis achieved an average r^2 value of 0.9996 and the average precision was less than 2.0% relative standard deviation, ranging from 1.4 % to 1.8%. Details of the method performance are presented in Table 2.

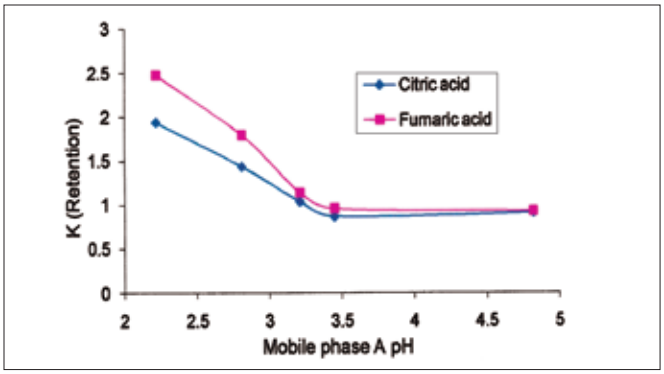

Figure 2. Effect of mobile phase pH on retention.

***Table 2.* Precision, linearity and amount in samples.**

	%RSD (n=6)	Linearity r^2	Linearity Range (µg/mL)	Vinegar	Lemon Juice
Formic acid	1.4	0.9998	30-2650	ND	ND
D isoascorbic acid	1.8	0.9999	2-350	ND	ND
Acetic acid	1.6	0.9998	40-6750	6.4%	ND
Citric acid	1.5	0.9998	15-2600	ND	5.8
Fumaric acid	1.5	0.9998	0.1-15	ND	ND
Proprionic acid	1.6	0.9986	120-9400	ND	ND

With only one injection, a spectrum of each acid was obtained and each wavelength maximum was determined enabling a suitable setting for the analysis. With PerkinElmer's Chromera® software supporting the PDA, a spectral library was created and used to confirm the identity of peaks in samples.

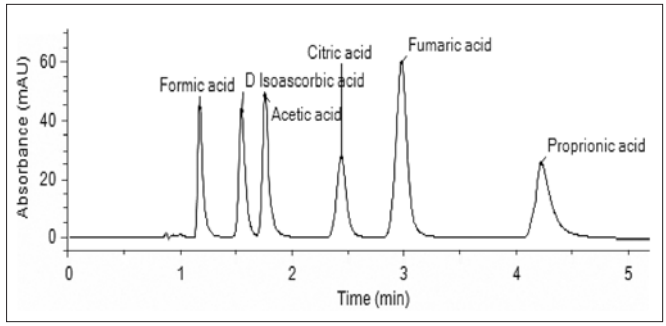

Figure 3. Example chromatogram from the analysis of a standard solution with six acids.

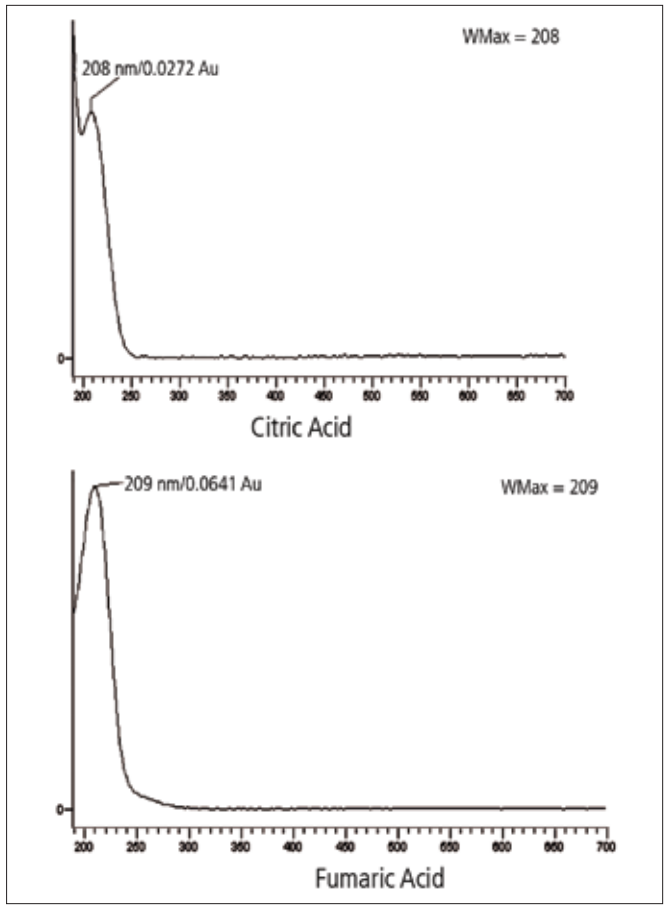

Figure 4. Citric acid and fumaric acid spectra from the analysis of a standard solution.

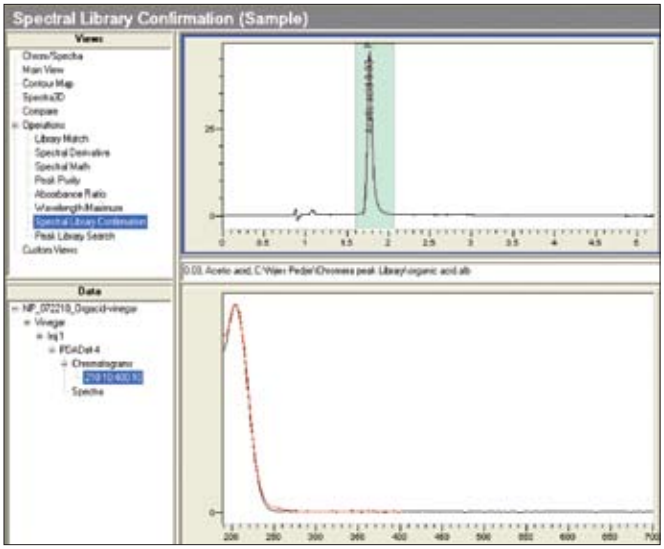

Figure 5. Chromatogram of the analysis of vinegar and the spectra confirmation using a PerkinElmer Chromera PDA spectral library.

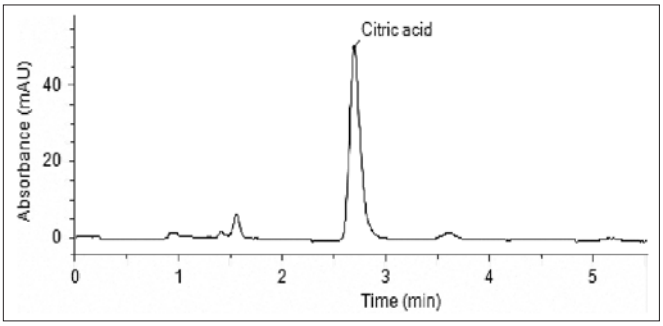

Figure 6. Chromatogram of the analysis of lemon juice.

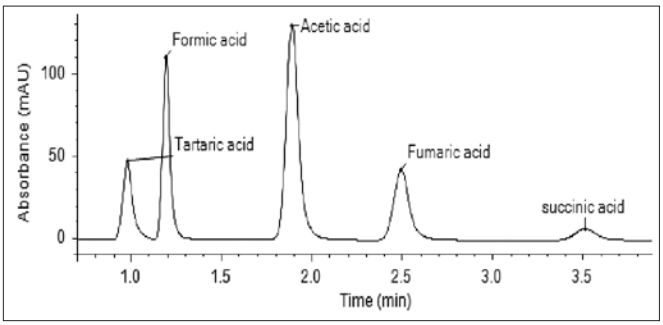

Figure 7. Chromatogram of the analysis of five acids with a 0.02% formic acid mobile phase.

Representative chromatograms of the standard solution and the spectra of two of the six acids tested are presented in Figures 3 and 4. Representative chromatograms of red wine vinegar and lemon juice are presented in Figures 5 and 6. A chromatogram of standard solution using an alternate method with 0.02% formic as mobile phase A is presented in Figure 7.

Conclusion

The method was shown to be linear and peaks were well resolved. The red wine vinegar tested had 6.4% acetic acid, well within the requirement of not less than 4% mandated by the FDA. The lemon juice tested had 5.8% citric acid.

PerkinElemer's PDA provides a rugged and accurate detection over a range of 190 nm to 700 nm encompassing UV and Vis wavelengths. The PerkinElmer Chromera software offers many data acquisition and processing features such as spectral library creation, and peak purity, spectra 3D and contour maps, which are powerful tools for interrogating the information content of a 3D photodiode array chromatogram. The spectral library function allowed the storage of organic acids spectra from the standard solution that were used later for peak identification confirmation in the samples.

References

1. Leo M.L. Nollet, Food Analysis by HPLC, Second Edition, April 2000.

2. FDA, CPG Sec.525.825 Vinegar.

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