APPLICATION BRIEF

Liquid Chromatography

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The Analysis of a Broad Range of Organic Acids by HPLC with UV Detection

Introduction

Organic acids are prevalently found in all fruit juices and many other beverages. As they are known to impart particular taste and/or aromatic qualities to such drinks, both pleasant and unpleasant, there is considerable interest in monitoring organic acids during production. With this in mind, this application brief provides a routine and reproducible method for the HPLC analysis of 11 organic acids commonly found in juices and other beverages, focusing especially on the relatively higher molecular weight acids.

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Experimental

Hardware/Software

For the chromatographic separations, a PerkinElmer Altus™ HPLC System was used, including the Altus A-10 Solvent/Sample Module, integrated vacuum degasser, A-10 column heater, and Altus A-10 UV detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 Chromatography Data Software (CDS) platform.

Method Parameters

The LC method parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents, reagents, and diluents used were HPLC-grade and filtered via 0.45-µm filters. For all dilutions, HPLC-grade water was used.

The following standards were obtained from Sigma-Aldrich®, Inc (Allentown, PA): lactic acid, acetic acid, butyric acid, isobutyric acid, propionic acid, valeric acid, isovaleric acid, methylvaleric acid, heptanoic acid, hexanoic acid and octanoic acid.

A 1000-ppm working standard solution was prepared by adding 50 µL of each organic acid standard to a 50-mL volumetric cylinder and filling to volume with diluent. Six calibration levels were prepared via serial dilution of the working standard solution. Each standard level was injected in duplicate.

Table 1. LC Method Parameters.

Column:	PerkinElmer Brownlee™ Aqueous C18 5-µm, 250 x 4.6-mm (Part# N9303549)
Mobile Phase:	Mobile Phase A: 10-mM KH_2PO_4, pH 2.4 with phosphoric acid Mobile Phase B: Acetonitrile (ACN) Solvent program:

	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
1	Initial	1.5	80.0	20.0	
2	10.0	1.5	40.0	60.0	6
3	12.5	1.5	40.0	60.0	6
4	12.6	1.5	80.0	20.0	11

Analysis Time:	12.5 min.; 5-min. injection delay time (re-equil.)
Flow Rate:	1.5 mL/min.
Pressure:	2650 psi/177 bar (maximum)
Oven Temp.:	30 °C
Detection:	210 nm
Injection Volume:	20 µL
Sampling (Data) Rate:	5 pts./sec

Results and Discussion

Using the optimized chromatographic conditions described above, Figure 2 shows the HPLC separation of the level-4 (250-ppm) working standard containing the eleven organic acids. All analytes were well resolved, except for butyric and isobutyric acids, which coeluted at 4.5 minutes.

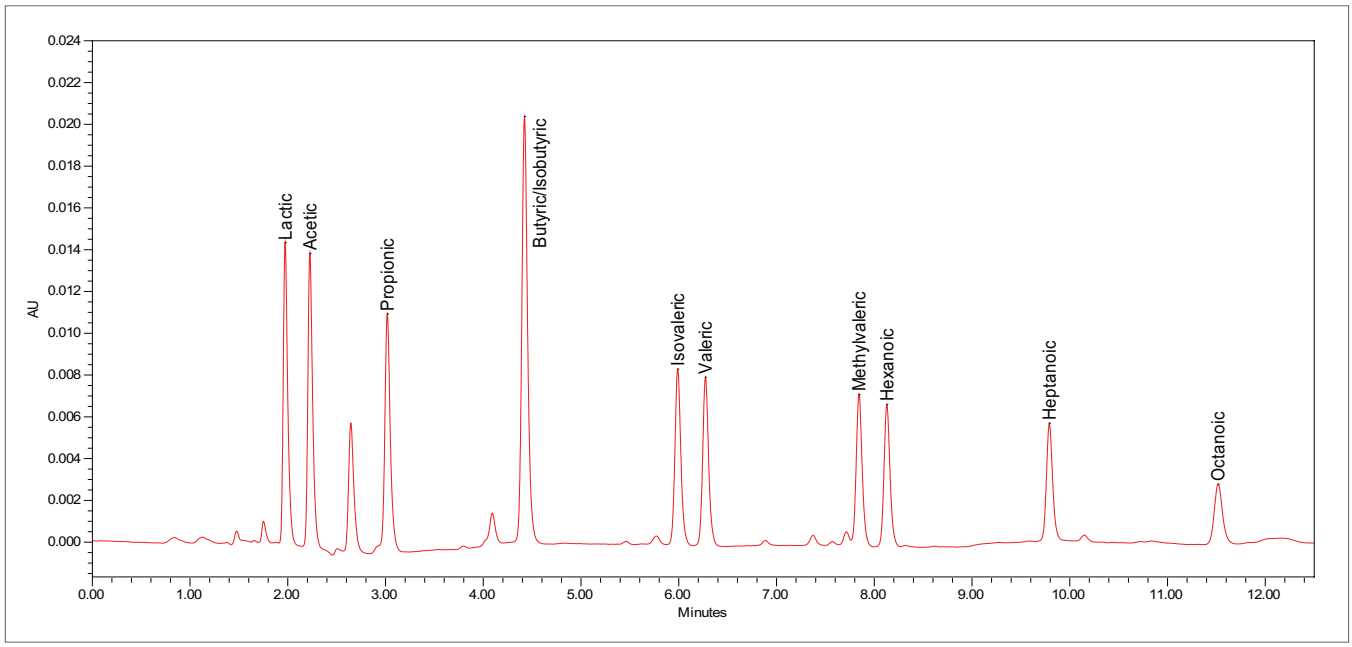

Figure 1. Chromatogram of the level-4 (250-ppm) working standard, containing 11 organic acids; by UV at 210 nm.

Figure 2 shows the overlay of 12 replicate injections of the level-4 (250-ppm) working standard, demonstrating high reproducibility. The retention time precision for all analytes was < 0.075% RSD.

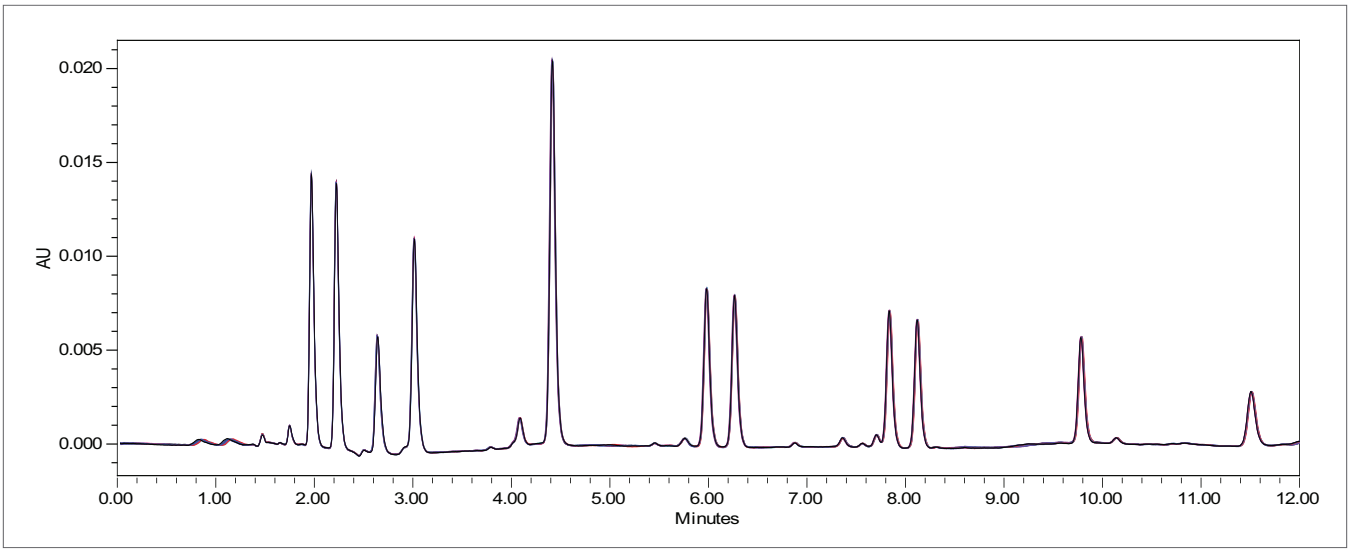

Figure 2. Overlay of 12 replicates of level-4 (250-ppm) working standard, by UV at 210 nm.

Figure 3 shows the calibration results for two representative analytes in the range of 25–1000 ppm. As butyric and isobutyric acids coeluted, the combined concentration range was 50-2000 ppm. All organic acids showed an exceptional linear fit, with R^2 values > 0.9999 (n = 2 at each level) for all analytes.

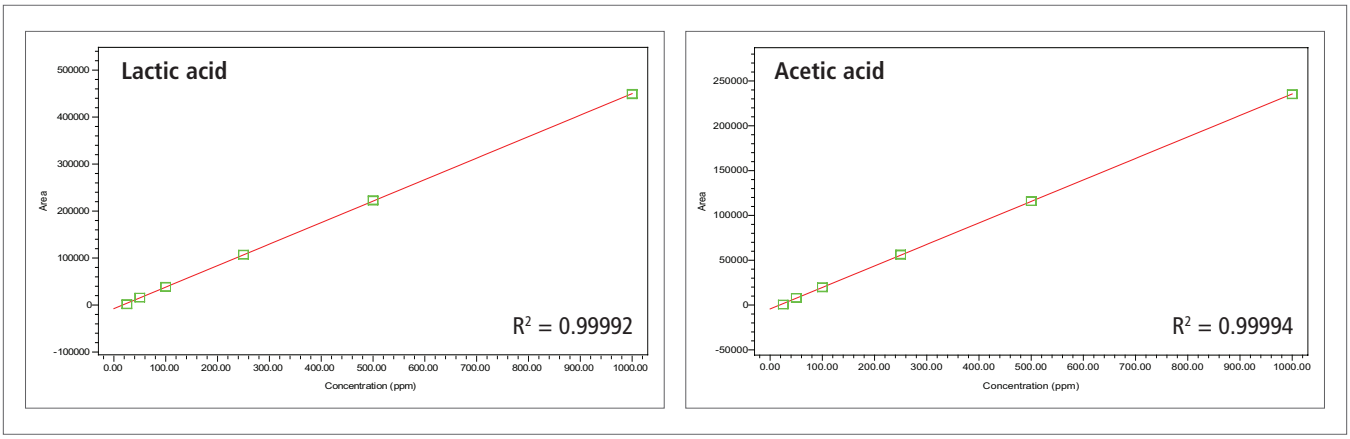

Figure 3. Results of 6-level calibration set for lactic and octanoic acids.

Table 2 presents the calculated limits of detection and quantitation (LOD, LOQ) for the analyzed organic acids. These limits were derived using the signal-to-noise (s/n) results obtained during calibration. The LODs and LOQs ranged from 0.5-1.8 and 1.4-6.0 ppm, respectively.

Table 2. Calculated LOQ and LOD values for the eleven organic acids.

Organic Acids	LOD (ppm) (s/n ≥ 3/1)	LOQ (ppm) (s/n ≥ 10/1)
lactic	0.5	1.4
acetic	0.5	1.4
butyric/isobutyric	0.6	2.1
propionic	0.6	2.1
valeric	0.9	2.8
isovaleric	0.7	2.6
methylvaleric	1.0	3.5
heptanoic	1.1	3.7
hexanoic	1.0	3.5
octanoic	1.8	6.0

Conclusion

The results obtained confirm the applicability of this method for the effective and robust chromatographic analysis of a broad range of organic acids. Apart from the co-elution of butyric and isobutyric acids, the analytes were well separated in under 12 minutes by HPLC using UV detection. The results showed excellent retention time repeatability as well as exceptional linearity over the tested concentration range.

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