APPLICATION NOTE

Liquid Chromatography

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Analysis of Organic Acids in Fruit Juices by HPLC and UV Detection

Introduction

In food, organic acids, such as malic, ascorbic and citric acids, originate from natural biochemical processes or are added as preservatives, acidulants and/or stabilizers. Organic acids contribute to the sensory properties of foods and beverages by providing both taste and aroma. In particular, citric acid is widely used in soft drinks to provide the sour citrus taste.

The monitoring of these organic acids is essential for quality control during the processing of juices and related products, as well as for evaluating juice authenticity and purity. In addition, the use of organic acids in foods and beverages is regulated in many countries, though regulations vary widely.[1]

This application note presents a simple and robust HPLC method for the analysis of organic acids typically found in store-bought fruit juices. The method conditions and performance data, including repeatability and linearity, are provided.

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Experimental

Hardware/Software

A PerkinElmer Altus™ HPLC system was used, including the A-10 Solvent/Sample Manager, A-10 column heater and A-10 UV detector (PerkinElmer, Shelton, CT, USA). A PerkinElmer Brownlee™ Validated Aqueous C18 5 µm, 4.6 x 250-mm column was used for all analyses (PerkinElmer, Shelton, CT, USA). All instrument control, analysis, and data processing was performed via Waters® Empower® 3 Chromatography Data Software (CDS).

Method Parameters

The HPLC method parameters are shown in Table 1.

Solvents, Standards and Samples

The water, used for both solvent and diluent, was HPLC-grade. For buffering the mobile phase and adjusting the pH to 2.4, both monobasic potassium phosphate and phosphoric acid were used, obtained from Sigma Aldrich®, Inc (Allentown, PA).

An organic acid standard kit (part # 47264) was obtained from Sigma Aldrich®, Inc. Of the included organic acids, the following were used for this application: oxalic acid, tartaric acid, quinic acid, malic acid, ascorbic acid, shikimic acid, citric acid, succinic acid and fumaric acid. Additionally, acetic acid was added as a standard, which was also obtained from Sigma Aldrich®, Inc.

A stock standard was prepared containing the 10 organic acids indicated above. Individual concentrations varied depending on the individual organic acid's UV absorptivity and expected highest concentration in fruit juices. The concentration, in ppm, of each organic acid in the stock standard solution is presented in Table 2.

Table 1. HPLC Method Parameters.

HPLC Conditions	
Column:	PerkinElmer Brownlee Validated Aqueous C18, 5 µm, 4.6 x 250-mm (Part # N9303549)
Mobile Phase:	Isocratic; 25-mM K-phosphate buffer; pH 2.4
Analysis Time:	8.0 min.; wash/equilibration time = 6.0 min
Flow Rate:	1.5 mL/min. (~3000 psi; 200 bar)
Oven Temp.:	30 °C
UV Detection:	Wavelength: 210 nm
Injection Volume:	20 µL
Sampling (Data) Rate:	5 pts./sec

For calibration purposes, five levels were prepared via serial dilution of the stock standard, using water as diluent.

Commercial fruit juices, including grape juice, orange juice, apple juice and cranberry juice, were obtained from a local store. These were diluted 9:1 with HPLC-grade water, filtered and then injected.

The buffered mobile phase and all standards and samples were first filtered through 0.45 µm filters before injection.

Results and Discussion

Figure 1 shows the chromatogram of a standard mixture of the 10 organic acids, all separated in under eight minutes.

As shown in Figure 2, the chromatographic repeatability of 10 replicates of the standard mix demonstrates exceptional reproducibility. The average retention time (RT) %RSD for all 10 analytes was 0.08 %.

Table 2. Concentrations (ppm) of organic acids in the stock standard solution.

Oxalic	Tartaric	Quinic	Malic	Ascorbic	Shikimic	Acetic	Citric	Succinic	Fumaric
141	543	1002	640	160	21	1005	962	608	81

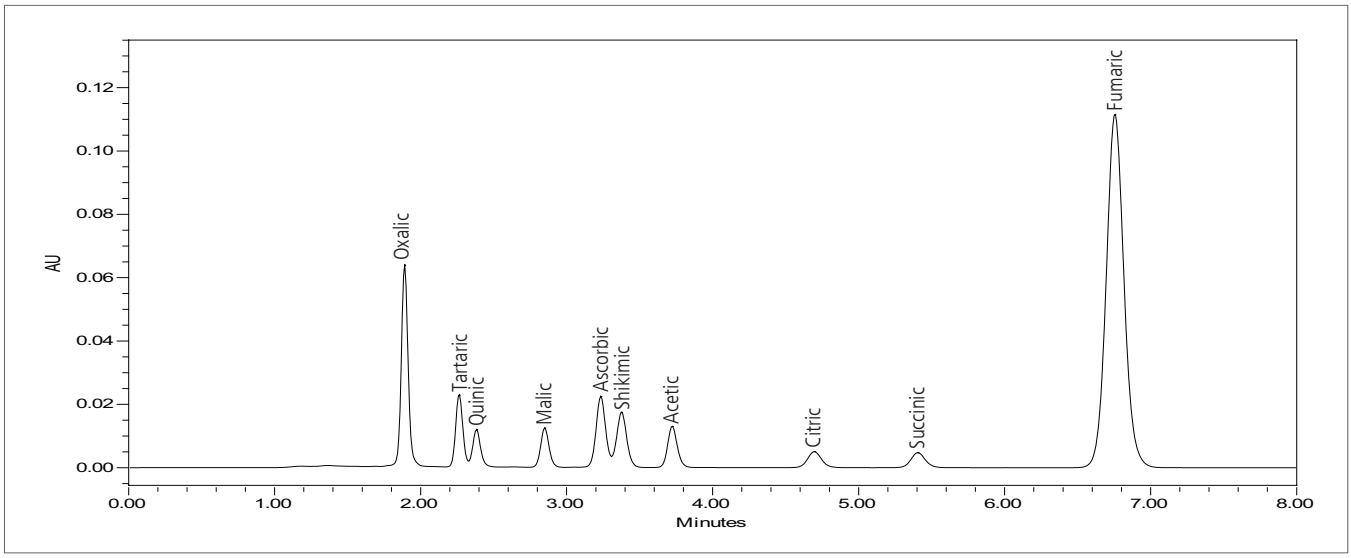

Figure 1. Chromatogram of organic acid standard mix; UV at 210 nm.

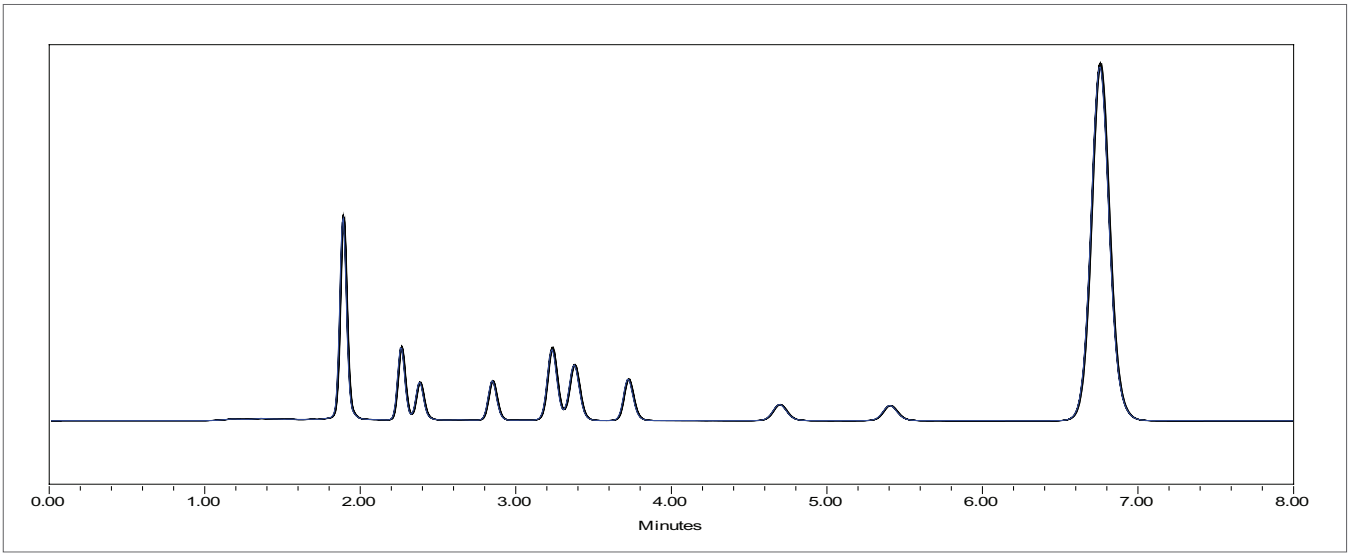

Figure 2. Overlay of 10 replicates of organic acid standard mix.

Figure 3 presents examples of the linearity plots for four of the organic acids. These four, as well as the other six organic acids, all resulted in R^2 values > 0.999.

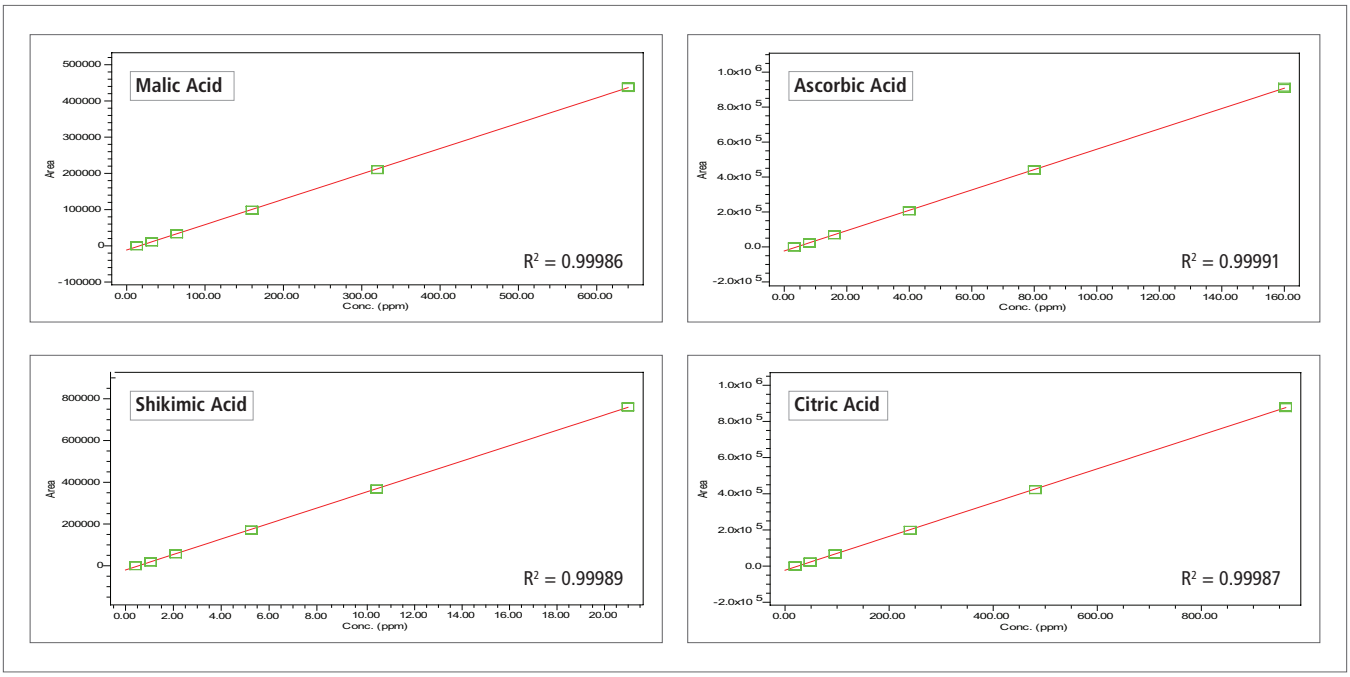

Figure 3. Linearity plots for malic, ascorbic, shikimic and citric acids; UV at 210 nm.

Four fruit juices were subsequently analyzed, with the resulting chromatograms shown in Figures 4 and 5. As an example of the chromatographic overlap of sample and standard, Figure 4b shows the grape juice sample overlaid upon the stock standard mix, both normalized to the highest peak.

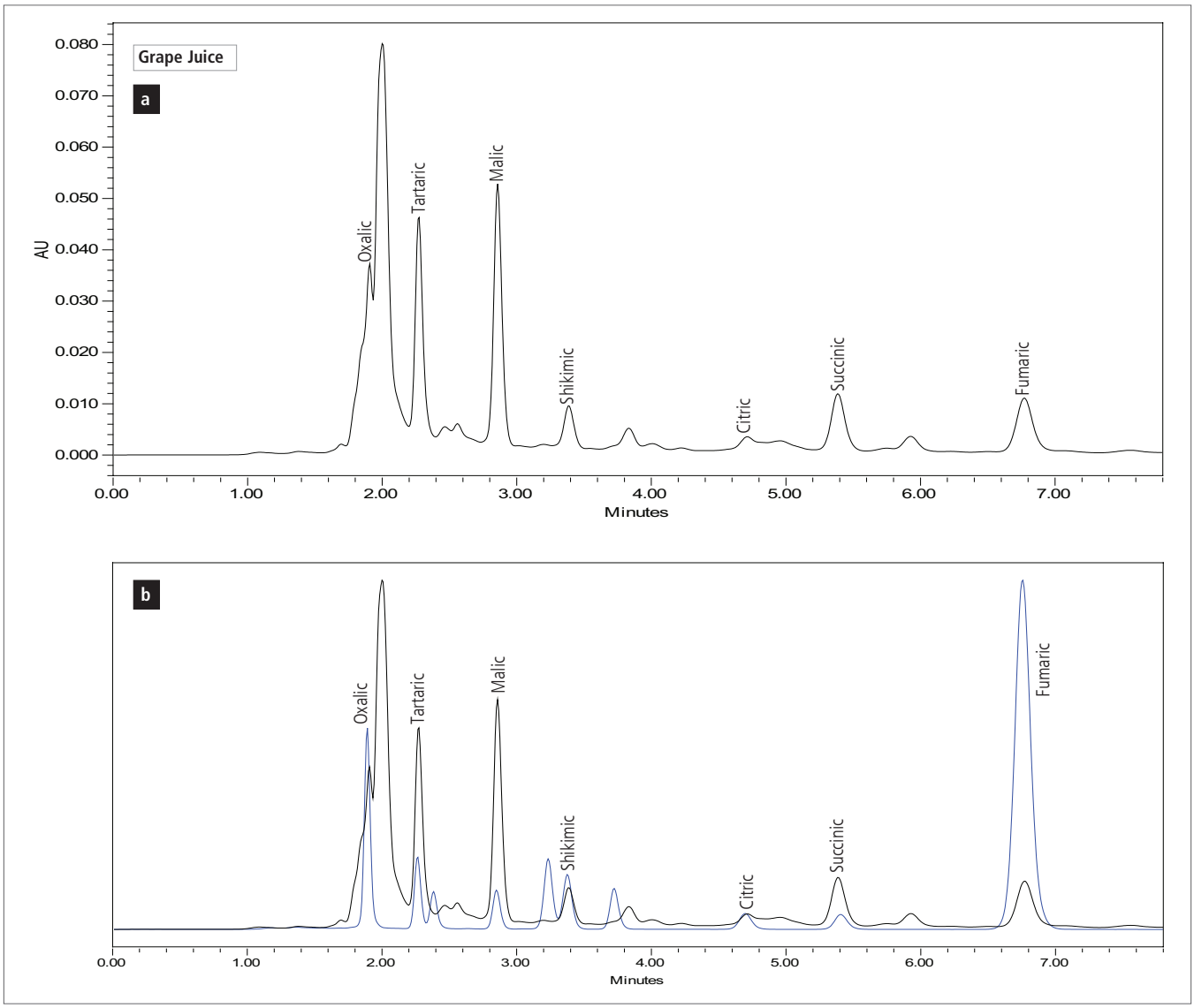

Figure 4. Chromatogram of grape juice sample (a) and same sample overlaid with standard mix (b); UV at 210 nm.

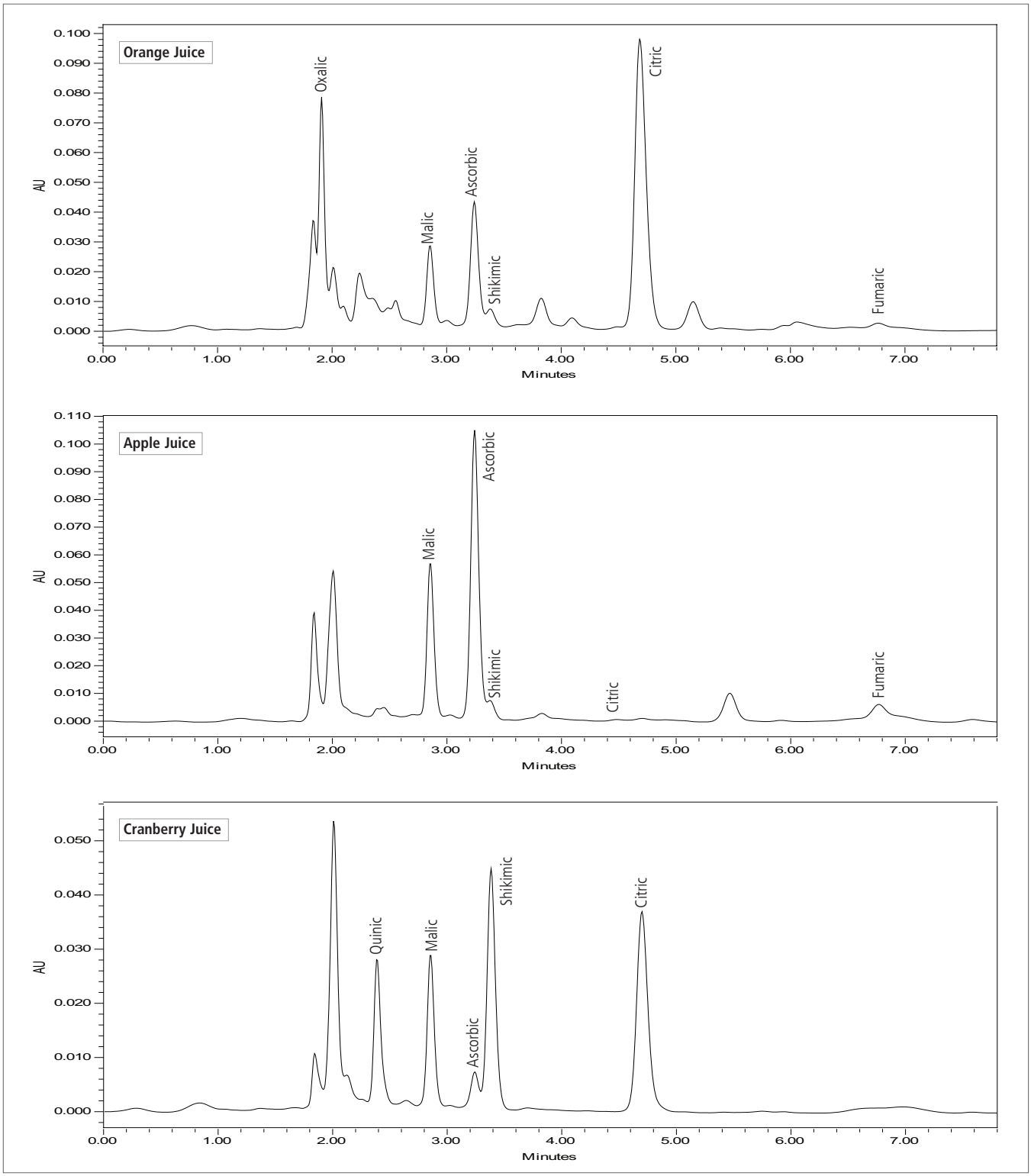

Figure 5. Chromatograms of orange, apple and cranberry juice samples, with identified organic acids; UV at 210 nm.

The individual organic acid content for each of the four juices is presented in Table 3. Comparing the four juices, they varied considerably from one another in the proportion of the organic acids they contained. Overall, both citric and malic acids predominated in all four juices, with small amounts of shikimic acid in each. Interestingly, grape juice was the only juice that showed significant amounts of tartaric and succinic acids, while none of the juices showed any detectable level of acetic acid. Oxalic acid was detected at a significant level in the orange juice, but it was not quantifiable or detected in the other three juices due to significant matrix interference in the early-eluting region.

Table 3. Resulting averaged organic acids content for four analyzed fruit juices. All samples were run in triplicate.

Organic Acid	Grape Juice (ppm)	Orange Juice (ppm)	Apple Juice (ppm)	Cranberry Juice (ppm)
Oxalic	NQ	15.2	ND	ND
Tartaric	135.0	NQ	ND	ND
Quinic	ND	NQ	ND	311.7
Malic	327.4	177.5	365.1	190.2
Ascorbic	ND	39.4	92.1	9.6
Shikimic	1.8	1.6	1.4	6.8
Acetic	ND	ND	ND	ND
Citric	34.9	761.5	27.2	303.8
Succinic	178.2	ND	ND	ND
Fumaric	2.8	2.4	2.6	ND

ND = Not detected NQ = Not quantifiable

Conclusion

This work demonstrated the effective chromatographic separation and quantitation of 10 commonly found organic acids in fruit juices using a PerkinElmer Altus HPLC system with A-10 UV detector. The results exhibited exceptional repeatability and linearity over the tested concentration ranges.

Four different fruit juices were effectively analyzed, with each showing various amounts and combinations of the 10 organic acids. The chromatographic separation was achieved in under eight minutes and, in general, quantitation of the organic acids in all four juices was easily achieved.

References

1. Production and Packaging of Non-Carbonated Fruit Juices and Fruit Beverages (2nd edition), Philip R. Ashurst (editor), Springer Science and Business Media LLC, 1999.

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