

ANALYSIS OF ORGANIC ACIDS USING A MIXED-MODE LC COLUMN AND A QDA MASS DETECTOR

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INTRODUCTION

Organic acids (OA) affect the flavor and aroma, the stability, and the microbiological control of juices and beverages. They can be used as markers or fingerprints for authenticity testing.

The analysis of OA is commonly carried out by liquid chromatography (LC) either by anion-exchange, reversed-phase or mixed-mode separation. The main challenge in OA analysis is the separation resolution of OAs and the potential interference from co-eluting compounds.

The objective is to develop an analytical solution that provides adequate resolution of OAs with minimal interference from matrix using Atlantis PREMIER BEH C18 AX column and ACQUITY QDa mass detector.

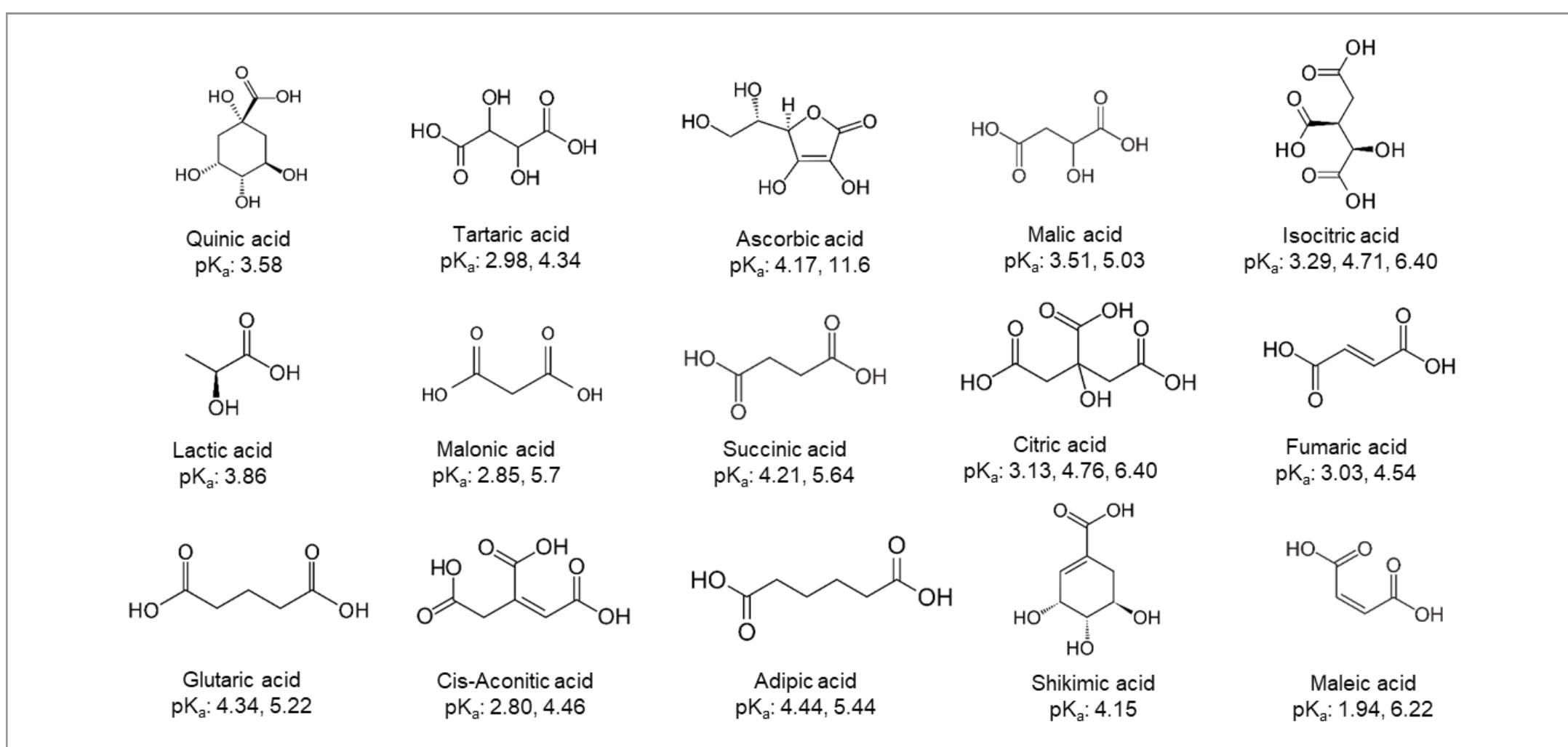


Figure 1. Structures of organic acids and their pK_a values.

METHODS

LC conditions

LC system: ACQUITY H-Class UPLC System
Software: Empower 3 CDS
Column: Atlantis PREMIER BEH C18 AX 1.7 μ m, 2.1 X 100 mm
Column Temp.: 30°C
Flow rate: 0.35 mL/min
Injection vol.: 1.0 μ L
Runtime: 8.0 min
Mobile phases:

A: water with 50 mM ammonium formate and 0.9% formic acid (pH=2.9)
B: acetonitrile with 0.9% FA
C: water with 0.9% FA

Gradient elution program:

Time (min)	%A	%B	%C	Curve
0.0	0	0	100	6
1.4	0	0	100	6
1.5	60	0	40	6
5.0	60	40	0	6
7.0	60	40	0	6
7.1	0	0	100	6
8.0	0	0	100	6

SAMPLE PREPARATION

Samples of fruit juices, including apple, grape, and pomegranate, were purchased from local stores. These juices were diluted with MilliQ water at a 1 to 10 ratio (1 ml juice mixed with 9 ml water) and filtered with a 0.45 μ m glass microfiber membrane filter (GMF). The filtered juice solutions were further diluted with water as needed.

MS conditions

MS system: ACQUITY QDa Detector (Performance)
Ionization mode: ESI-
Capillary voltage: 0.8 V
Cone Voltage: 5.0 V
Probe temp: 600 °C
Acquisition Rate: 1 Hz
SIR masses: [M-H]⁻ (Table 1)

Item	Organic acid	Formula	Monoisotopic mass (Da)	[M-H] ⁻ (m/z)
1	Quinic acid	C ₇ H ₁₂ O ₆	192.06	191
2	Tartaric acid	C ₄ H ₆ O ₆	150.02	149
3	Ascorbic acid	C ₆ H ₈ O ₆	176.03	175
4	Malic acid	C ₄ H ₆ O ₅	134.02	133
5	Isocitric acid	C ₆ H ₈ O ₇	192.03	191
6	Lactic acid	C ₃ H ₆ O ₃	90.03	89
7	Malonic acid	C ₃ H ₄ O ₄	104.01	103
8	Succinic acid	C ₄ H ₆ O ₄	118.03	117
9	Citric acid	C ₆ H ₈ O ₇	192.03	191
10	Fumaric acid	C ₄ H ₄ O ₄	116.01	115
11	Glutaric acid	C ₅ H ₈ O ₄	132.04	131
12	cis-Aconitic acid	C ₆ H ₆ O ₆	174.02	173
13	Adipic acid	C ₆ H ₁₀ O ₄	146.06	145
14	Shikimic acid	C ₇ H ₁₀ O ₅	174.05	173
15	Maleic acid	C ₄ H ₆ O ₄	116.01	115

Table 1. The formula, monoisotopic mass, and m/z of organic acids

RESULTS

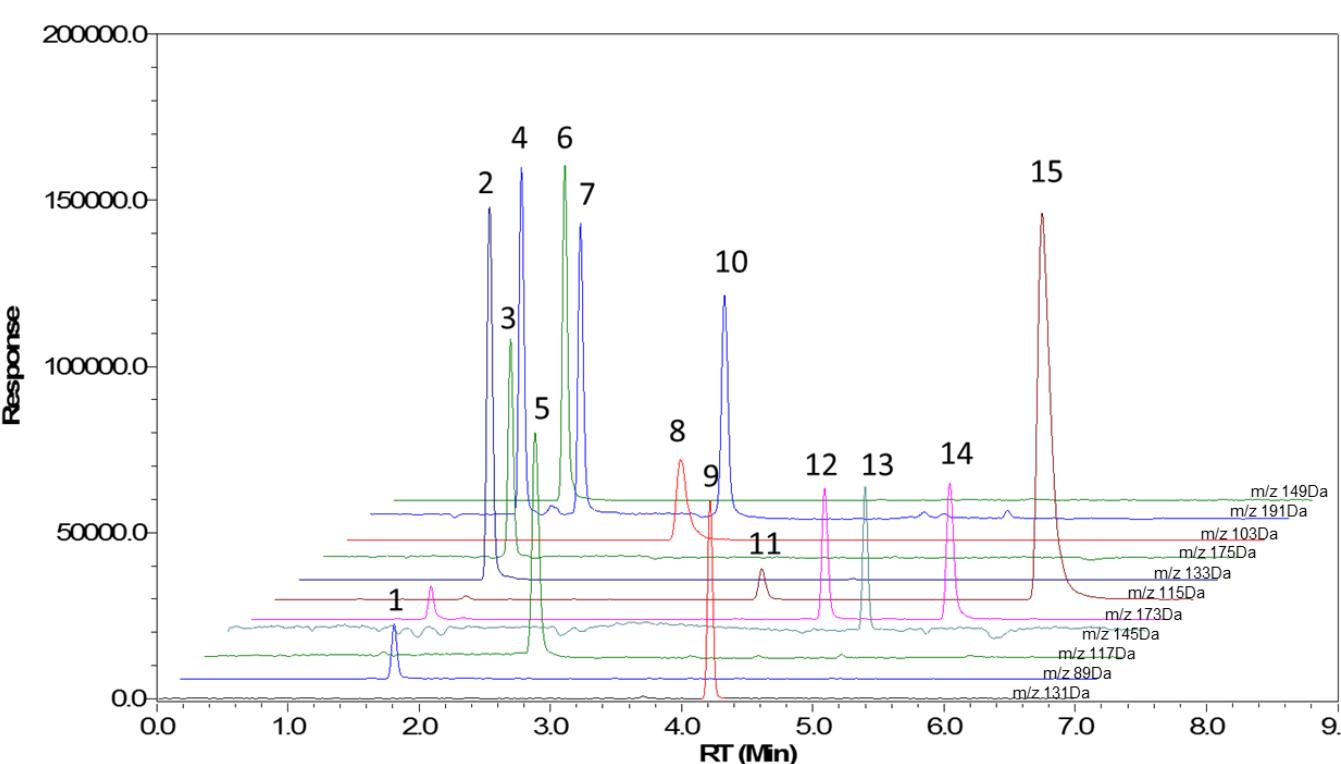


Fig. 2

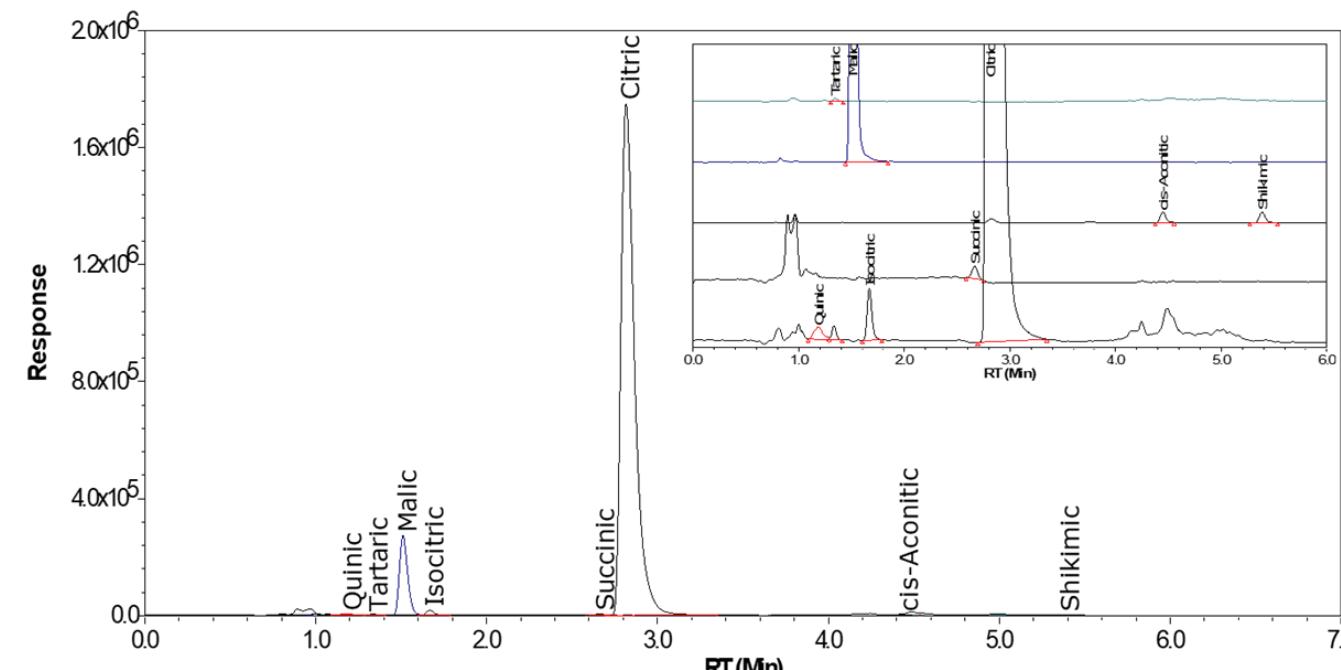


Fig. 3

Figure 2. Chromatograms of 15 organic acid standards in 11 SIR channels. Organic acid concentration: 20 ppm. Column: Atlantis PREMIER BEH C18 AX, 1.7 μ m, 2.1 x 100 mm. Peaks ID: 1. Lactic acid; 2. Malic acid; 3. Ascorbic acid; 4. Quinic acid; 5. Succinic acid; 6. Tartaric acid; 7. Isocitric acid; 8. Malonic acid; 9. Glutaric acid; 10. Citric acid; 11. Fumaric acid; 12. cis-Aconitic acid; 13. Adipic acid; 14. Shikimic acid; 15. Maleic acid.

Figure 3. SIR chromatogram overlay of a pomegranate juice. The juice was diluted 10 times with deionized water before it was analyzed. The insert shows the detected OA chromatograms at enlarged scale with baseline offset.

CONCLUSION

- Greater retention and improved resolution
- Highly selective detection that makes the analysis less prone to interference from co-eluting compounds in the sample matrix
- Highly sensitive detection that is beneficial in certain applications
- Fast analysis with run time less than 8 minutes.

Table 3. The recovery of common organic acids in fruit juices

	Apple Juice		Grape Juice		Pomegranate Juice	
	Original level (mg/ml in juice)	Spiking level (mg/ml in juice)	Original level (mg/ml in juice)	Spiking level (mg/ml in juice)	Original level (mg/ml in juice)	Spiking level (mg/ml in juice)
Quinic Acid	0.24	109%	88%	0	85%	84%
Tartaric Acid	0	135%	89%	0.75	82%	82%
Malic Acid	3.36	N/A*	86%	1.64	N/A*	94%
Citric Acid	0.06	N/A*	119%	0.12	105%	116%
					7.45	N/A*
						163%

*: Recovery is not valid due to low spiking level.

Note: Internal standards were not used in the study. For better results, internal standards are recommended for quantification.