Single Quad LC/MS Analysis of Organic Acids Using an Agilent Hi-Plex Column

Application Note

Food Testing

Authors

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Abstract

Organic acids are highly hydrophilic and difficult to retain in reversed-phase mode. Conversely, ion exclusion mode is excellent for separating organic acids. Direct detection by UV or by postcolumn addition of an indicator compound however, does not provide the necessary level of sensitivity for many applications. This application note examines the separation of organic acids by ion exclusion mode, followed by direct detection on a single quadrupole MS.

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Introduction

Organic acid analysis by LC/MS is often performed using a reversed-phase mode. However, since many organic acids are highly hydrophilic, and have little retention in a reversed-phase column, coelution of interfering compounds in samples containing complex matrices is a substantial problem.

For organic acid analysis using an ion exclusion column, both detection by direct UV or by the postcolumn addition of a pH indicator such as BTB have been used. However, it is difficult to detect low concentrations. In this application note, organic acids were separated by ion exclusion mode, and examined by single quadrupole MS detection.

Experimental Conditions

Table 1. LC/MS Analysis Conditions

Parameter	Value
Agilent 1290 Infinity II LC	
Column	Agilent Hi-Plex H, 250 × 4.6 mm, 8 µm (p/n PL1570–6830)
Mobile phase	0.01 % Aqueous formic acid/ACN = 80/20
Flow rate	0.2 mL/min
Postcolumn additive	0.01 % Aqueous NH_4OH/ACN = 80/20
Additive flow rate	0.1 mL/min
Temperature	50 °C
Injection volume	10 µL
Agilent 6120 Single Quadrupole LC/MS	
Ion source	ESI
Drying gas	N_2, 12 L/min
Dry gas temperature	200 °C
Nebulizer pressure	50 psi
Capillary voltage	3,000 V
Mode	Negative
Acquisition parameters	SIM

Table 2. SIM Ion List

	M	M-H	Fragmentor (V)
Acetic acid	60	59	80
Glyoxylic acid	74	73	80
Propionic acid	74	73	80
Glycolic acid	76	75	80
Butyric acid	88	87	80
Pyruvic acid	88	87	80
Lactic acid	90	89	80
Oxalic acid	90	89	80
Valeric acid	102	101	80
Malonic acid	104	103	60
Fumaric acid	116	115	80
Levulinic acid	116	115	80
Maleic acid	116	115	80
Succinic acid	118	117	80
Pyroglutamic acid	129	128	60
Itaconic acid	130	129	60
Malic acid	134	133	60
Adipic acid	146	145	80
Ketoglutaric acid	146	145	80
Tartaric acid	150	149	100
Ascorbic acid	176	175	100
Citric acid	192	191	100
Gluconic acid	196	195	100

Post column ammonium hydroxide addition was made using a Micro T-connector (p/n 5042–8519).

Results and Discussion

Figure 1 shows the SIM chromatogram of a 1 ppm standard solution of each organic acid. A peak was detected for all organic acids.

The S/N value (signal/[peak-to-peak noise] × 2) is shown in Table 3.

Analysis of commercially available yogurt was conducted. Yogurt whey was sampled and filtered through a 0.22 µm filter, and diluted 100 times to prepare a sample. Figure 2 shows the chromatogram of the detected organic acids in this yogurt sample. In Figure 3, a 0.1 ppm malic acid standard was added to this sample, and a recovery test was conducted. The recovery rate was 93 %, with good overall results. In Figure 4, excellent linearity is demonstrated using malic acid over a range of 10 ppb to 0.25 ppm.

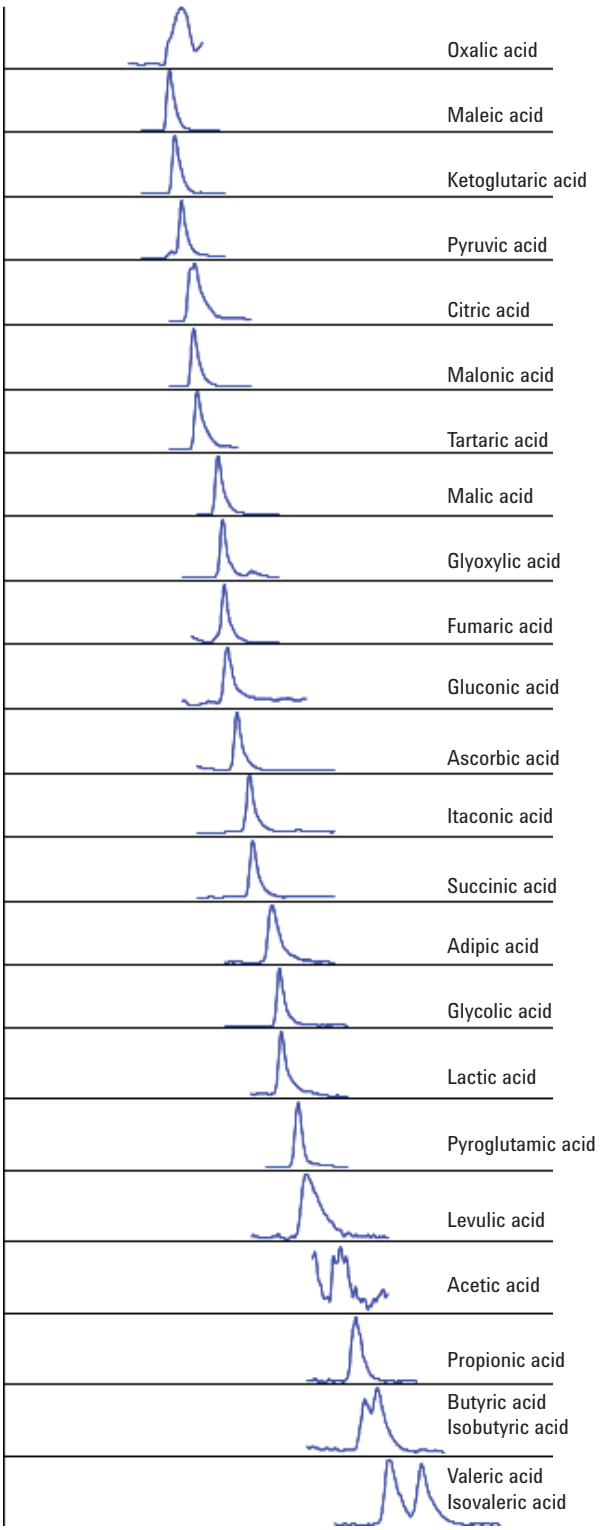

Figure 1. Chromatogram of organic acid standard solution (1 mg/L).

Table 3. Retention Time of Each Organic Acid and S/N at 1 mg/L

	$[M-H]^-$	RT (min)	S/N
Oxalic acid	89	5.897	5.8
Maleic acid	115	6.048	829.3
Ketoglutaric acid	145	6.237	36.3
Pyruvic acid	87	6.49	593.8
Citric acid	191	6.791	527.5
Malonic acid	103	6.964	18.2
Tartaric acid	149	7.051	392.9
Malic acid	133	7.789	84.0
Glyoxylic acid	73	8.005	52.1
Fumaric acid	115	8.088	1,254.1
Gluconic acid	195	8.154	2,740.3
Ascorbic acid	175	8.463	429.4
Itaconic acid	129	8.921	226.8
Succinic acid	117	9.059	555.0
Adipic acid	145	9.745	1,709.1
Glycolic acid	75	10.029	1,339.1
Lactic acid	89	10.123	2,682.3
Pyroglutamic acid	128	10.714	687.1
Levulinic acid	115	11.016	652.6
Acetic acid	59	12.049	1,711.9
Propionic acid	73	12.764	1,288.4
Butyric acid	87	13.117	2,873.6
Valeric acid	101	13.964	58.6

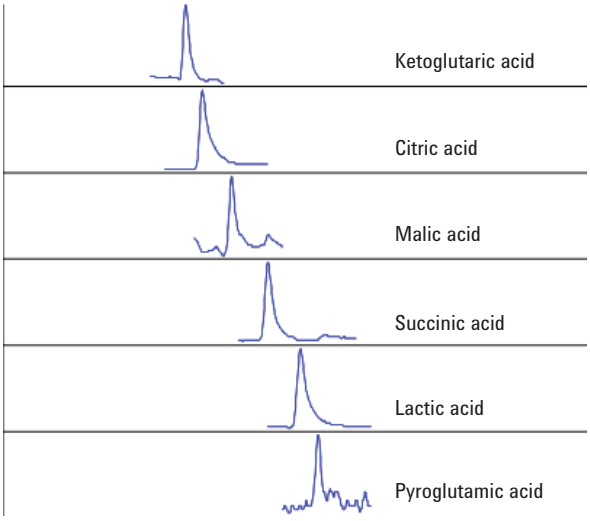

Figure 2. Organic acids detected from commercially available yogurt.

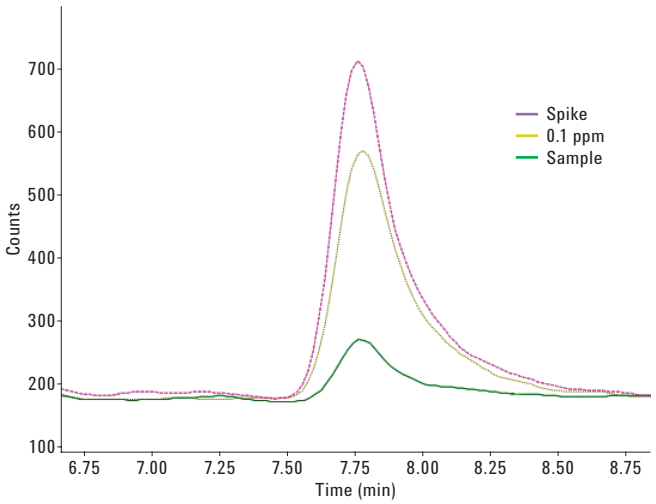

Figure 3. Chromatogram of the malic acid addition recovery test.

Table 4. Area Value in the Malic Acid Addition Recovery Test

Sample	Area
Sample	1,746.8
Sample + 0.1 mg/L spike	10,117.8
0.1 mg/L	7,690.7

Conclusion

A panel of organic acid standards was separated by ion exclusion chromatography, and detected with single quadrupole MS. Good separations were obtained, and peaks were detected at 1 ppm for all organic acids tested.

When applied to a yogurt sample, key organic acids were detected with good sensitivity. Further tests on this real-world sample showed excellent recovery and linear detection down to 10 ppb.

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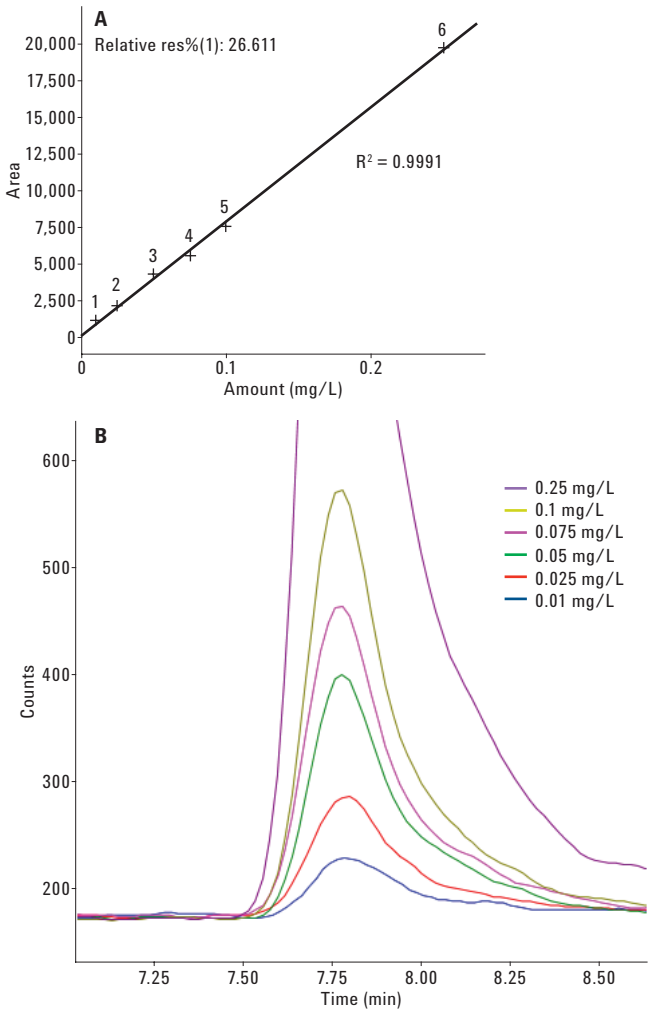

Figure 4. Linearity of malic acid 0.01–0.25 ppm (A) and SIM chromatogram (B).

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