

Determination of 32 Low Molecular Mass Organic Acids in Beverages Using IC/MS

Leo (Jinyuan) Wang, Stacy M. Henday, William C. Schnute; Dionex Corporation, Sunnyvale, CA, USA

INTRODUCTION

Low molecular mass organic acids (LMMOAs) are present in many plants and function in various capacities. In beverages, many LMMOAs are related to organoleptic influences, such as flavor, color, and aroma, as well as stability. They either originated from plants in their natural state or were generated during processing, such as fermentation. For commercial and regulatory purposes, it is necessary to monitor LMMOA levels in raw materials as well as marketed products.

Among the analytical techniques for determination of LMMOAs in beverages, chromatographic methods with various modes of detection provide the most thorough information to profile and monitor LMMOAs. This study describes an ion chromatography method using mass spectrometric detection (IC/MS) for the simultaneous determination of 32 LMMOAs in different beverages. Method performance was evaluated with respect to linearity, range, and detection limits. The LMMOA profiles of several beverages were also determined.

EXPERIMENTAL

Instruments

- Dionex ICS-2000 Reagent-Free™ Ion Chromatography (RFIC™) system with Eluent Generation (RFIC-EG™ system)
- IonPac® AS11-HC analytical column with AG11-HC guard (2 mm)
- ASRS® 300 Anion Self-Regenerating Suppressor (2 mm)
- AS Autosampler with 10 mL vial tray
- MSQ Plus™ Mass Spectrometer
- AXP-MS auxiliary pump x2
- Chromeleon® Chromatography Management Software (6.8, SP3)

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

Conditions

ICS-2000 Conditions

An ICS-2000 ion chromatographic system was operated in external water mode with deionized water as regenerant at a flow rate of 0.5 mL/min delivered by an AXP-MS auxiliary pump. A restriction tubing (0.008 mm ID) was used to generate a backpressure of ~ 1000 psi.

Columns: IonPac AS11-HC Analytical
IonPac AG11-HC Guard
CR-ATC Continuously Regenerated Anion Trap Column

Flow Rate: 0.38 mL/min

Injection Volume: 25 µL

Oven Temperature: 30 °C

Eluent Source: EGC II KOH

Mobile Phase: Hydroxide gradient:

Time /min	Concentration / mM
-10	1.0
0	1.0
8	1.0
18	15
28	30
38	60
40	1.0

Detection: Suppressed Conductivity
MSQ Plus Selected Ion Monitoring (SIM)

MSQ Plus Conditions

A 0.20 mL/min flow of acetonitrile (to assist desolvation) was delivered by the second AXP-MS auxiliary pump and mixed with the eluent by a static mixing tee prior to entering the mass spectrometer. Backpressure tubing was applied to generate a backpressure of ~ 1000 psi.

Ionization Interface: Electrospray Ionization (ESI) source

Nebulizer Gas: Nitrogen at 80 psi

Probe Temperature: 450 °C

Needle Voltage: 3000 V

Scan Mode: negative Selected Ion Monitoring (SIM)
See Table 1 for scan details.

Table 1. SIM Scan Events					
SIM No.	Analyte	R.T. (min)	SIM (m/z)	Time Range (min)	Cone (V)
SIM 1	formate	11.5	45.0	11.0–14.0	50
SIM 2	acetate	8.8	59.0	7.0–13.0	45
SIM 3	propionate	10.2	73.0	9.5–13.0	50
SIM 4	glycolate	8.8	75.0	8.0–13.0	45
SIM 5	butyrate	12.2	87.0	11.0–15.0	40
SIM 5	pyruvate	12.9	87.0	11.0–15.0	40
SIM 6	lactate	8.2	89.0	7.0–30.0	40
SIM 6	oxalate	25.3	89.0	7.0–30.0	40
SIM 7	isovalerate	13.1	101.1	12.0–18.0	45
SIM 7	valerate	14.3	101.1	12.0–18.0	45
SIM 8	melonate	22.8	103.0	22.0–24.5	40
SIM 9	2-methylactate	8.2	103.1	6.0–11.0	50
SIM 10	IStd valerate-d ₉	14.0	110.0	12.0–18.0	45
SIM 11	maleate	23.7	115.0	23.0–27.0	40
SIM 11	fumarate	25.6	115.0	23.0–27.0	40
SIM 12	succinate	21.9	117.0	21.0–24.0	45
SIM 12	methylmalonate	22.4	117.0	21.0–24.0	45
SIM 13	2-hydroxyvalerate	12.5	117.1	11.5–13.5	45
SIM 14	glutarate	21.6	131.0	21.0–30.0	30
SIM 14	oxalacetate	28.5	131.0	21.0–30.0	30
SIM 15	malate	22.1	133.0	21.0–24.5	45
SIM 16	IStd glutarate-d ₆	21.5	137.0	21.0–23.0	30
SIM 17	adipate	21.7	145.1	20.0–27.0	45
SIM 17	a-ketoglutarate	24.6	145.1	20.0–27.0	45
SIM 18	tartarate	22.8	149.0	22.0–24.5	45
SIM 19	cis-aconitate	35.1	173.0	33.0–38.0	35
SIM 19	trans-aconitate	36.7	173.0	33.0–38.0	35
SIM 20	citrate	33.5	191.0	33.0–36.0	45
SIM 20	isocitrate	34.3	191.0	33.0–36.0	45
SIM 21	quinatate	6.9	191.1	6.0–9.0	80
SIM 22	2-keto-D-gluconate	12.2	193.0	10.0–21.0	50
SIM 22	galaturonate	14.4	193.0	10.0–21.0	50
SIM 22	5-keto-D-gluconate	19.1	193.0	10.0–21.0	50
SIM 23	IStd citrate-d ₄	33.5	195.0	33.0–36.0	45
SIM 24	gluconate	7.7	195.1	6.0–12.0	50
SIM 25	mucate	21.6	209.0	21.0–23.0	50

All SIM scans have the 0.15 second Dwell Time; all SIM scans have a 0.5 m/z scan Span.

RESULTS AND DISCUSSION

Chromatograms of 32 LMMOAs by suppressed conductivity and SIM detections are shown in Figure 1.

Ion Chromatography

The IC method was developed to resolve most analytes, especially analytes with identical or close molecular masses such as butyrate and pyruvate ($m/z = 87.05$ and 87.02 respectively), maleate, and fumarate ($m/z = 115.01$). These analytes cannot be resolved by single quadrupole MS, and some of these analytes cannot be resolved by MS/MS because they either do not fragment or have no uniquely specific fragmentation pattern.

Mass Spectrometry

The mass spectrometer was operated in selected ion monitoring (SIM) mode to provide the most sensitive and selective detection. Organic acids predominantly produce single negatively charged ions $[M-H]^-$ regardless of their valences. $[M-H]^-$ was used to determine and quantify each analyte. Cone voltages controlling the extent of in-source Collision Induced Dissociation (CID) were optimized for each SIM channel. SIM scan events are listed in Table 1.

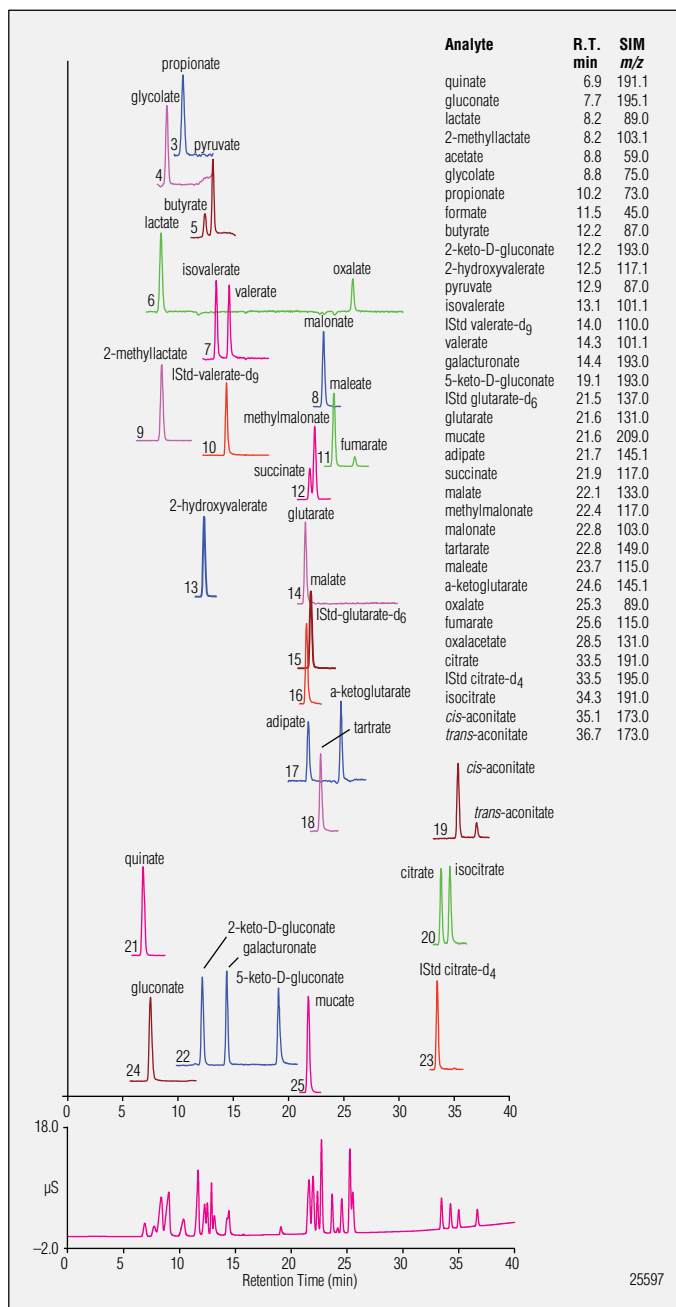
Linearity

Calibration curves were generated from calibrators at 12 levels: 0.000, 1.00, 5.00, 10.0, 20.0, 50.0, 100, 200, 500, 1000, 2000, and 5000 ppb with three isotope-labeled internal standards (valerate-d₉, glutarate-d₆, citrate-d₄) at 100 ppb for each level.

Linearity with correlation coefficients (R^2) greater than 0.99 were achieved for most analytes over three orders of magnitude and details are listed in Table 2.

Precision and Method Detection Limits (MDLs)

Precision was calculated from seven replicated injections (200 ppb) and is shown in Table 2 as %RSD. MDLs were calculated based on the equation: $MDL = t_{99\%} \times s_{(n=7)}$, where t is the Student's t at 99% confidence interval ($t_{(99\%, n=7)} = 3.143$), and s is standard deviation. Seven replicate injections of the level 5 calibrator (20 ppb) were performed and the result was used for MDL calculation using the above equation. MDL of formate and acetate was defined as the lowest injected amount to consistently give a signal to noise ratio (S/N) greater than 3 ($S/N > 3$). The MDLs are summarized in Table 2.



Peak No.	Analyte	R.T. (min)	SIM (m/z)	Fit*			MDL (ng)	%RSD
				From /ppb	To /ppb	R ²		
1	quininate	6.9	191.1	1	5000	0.997	0.062	3.92%
2	gluconate	7.7	195.1	1	5000	0.999	0.091	5.77%
3	lactate	8.2	89.0	20	5000	0.999 ^a	0.129	8.18%
4	2-methyl lactate	8.2	103.1	1	5000	1.000 ^b	0.061	3.86%
5	acetate	8.8	59.0	200	5000	0.999	5.00	N/A
6	glycolate	8.8	75.0	5	5000	0.999 ^a	0.071	4.50%
7	propionate	10.2	73.0	5	5000	0.999	0.065	4.15%
8	formate	11.5	45.0	20	5000	0.999	0.500	N/A
9	butyrate	12.2	87.0	5	5000	0.998 ^a	0.034	2.15%
10	2-keto-D-gluconate	12.2	193.0	1	5000	0.994	0.102	6.48%
11	2-hydroxyvalerate	12.5	117.1	1	5000	0.999 ^b	0.050	3.20%
12	pyruvate	12.9	87.0	1	5000	0.999 ^b	0.056	3.58%
13	isovalerate	13.1	101.1	5	1000	0.997	0.063	7.16%
14	valerate	14.3	101.1	5	5000	0.997	0.059	4.01%
15	galacturonate	14.4	193.0	1	5000	1.000	0.082	5.22%
16	5-keto-D-gluconate	19.1	193.0	1	5000	0.998	0.109	6.92%
17	glutarate	21.6	131.0	1	5000	1.000	0.043	2.72%
18	muconate	21.6	209.0	1	5000	1.000	0.085	5.41%
19	adipate	21.7	145.1	5	5000	0.995	0.047	2.99%
20	succinate	21.9	117.0	1	5000	0.994	0.060	3.79%
21	malate	22.1	133.0	1	5000	1.000 ^b	0.117	7.44%
22	methylmalonate	22.4	117.0	1	5000	0.999 ^b	0.095	6.03%
23	malonate	22.8	103.0	1	5000	0.996 ^c	0.078	4.96%
24	tartarate	22.8	149.0	5	5000	0.997	0.090	5.75%
25	maleate	23.7	115.0	1	5000	0.998 ^b	0.123	3.57%
26	a-ketoglutarate	24.6	145.1	1	5000	0.999	0.135	8.57%
27	oxalate	25.3	89.0	5	5000	0.998	0.112	7.12%
28	fumarate	25.6	115.0	1	5000	0.999	0.074	4.69%
29	oxalacetate	28.5	131.0	N/A	N/A	N/A	N/A	N/A
30	citrate	33.5	191.0	1	5000	0.999	0.076	4.83%
31	isocitrate	34.3	191.0	1	5000	0.996	0.073	4.64%
32	cis-aconitate	35.1	173.0	1	5000	1.000 ^b	0.034	4.31%
33	trans-aconitate	36.7	173.0	5	5000	1.000	0.051	6.45%

Figure 1. CD and MS SIM chromatogram of 32 small organic acids:

1. Each SIM chromatogram is normalized to the greatest peak in that channel
2. 100 ppb for each internal standard
3. MS detection: 50 ppb for each analyte except for cis- and trans-aconitate whose combined total concentration is 50 ppb. Conductivity detection: 5 ppm each for each analyte, aconitantes have a total concentration of 5 ppm
4. SIM channels are sorted by increasing m/z from top to bottom.
5. Formate and acetate are not shown due to weak response.

*For calibration function, linear fit was used unless noted.

a: quadratic fit; b: cubic fit; c: exponential fit.

Beverage Analysis

This method was applied to profile LMMOAs in different beverages. Two bottled tea beverages, two bottled grape juices and two bottles of wine samples (red and white) were purchased from local markets. Beverage samples were homogenized by vigorously shaking and degassed under vacuum. Each sample was then filtrated through a 0.45 μm membrane. The filtered solutions were then diluted 100x in water with internal standards spiked at 200 ppb. One set of calibration standards was run with every batch of unknown samples. Concentrations of LMMOAs are shown in Table 3. Citric acid was observed as the predominant LMMOA in green tea, with a concentration greater than 200 ppm, and a total of

27 LMMOAs were detected in one or both green tea beverages. Gluconic acid, galacturonic acid, malic acid, tartaric acid, citric acid, and isocitric acid were found to be prominent (>200 ppm) in grape juices and 28 LMMOAs were detected. In addition to the prominent acids found in grape juice, lactic acid, acetic acid, and succinic acid were observed in amounts greater than 200 ppm in wine samples, while the citric acid was found to be present in lower concentration. 28 LMMOAs were detected and their concentrations are reported in Table 3. Figure 2 and Figure 3 show the chromatograms obtained from a green tea sample and a red wine sample respectively.

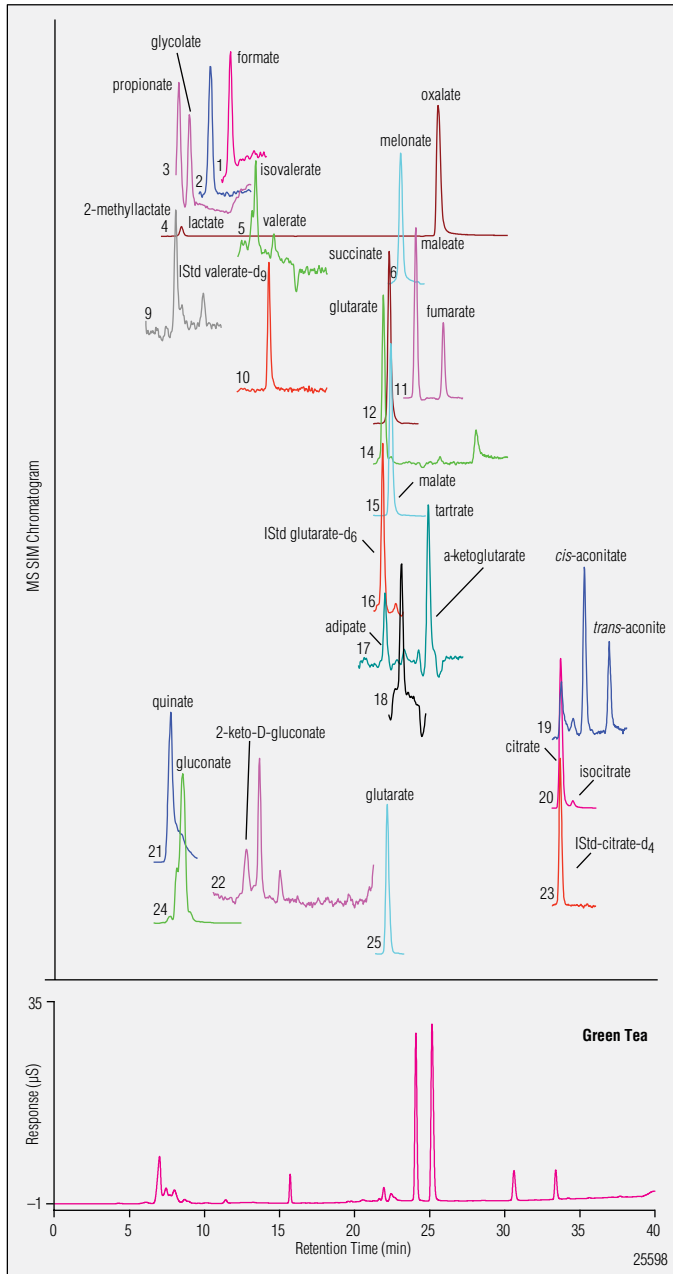


Figure 2. Organic acids in green tea. SIM channels for detected analytes are sorted by increasing m/z from top to bottom.

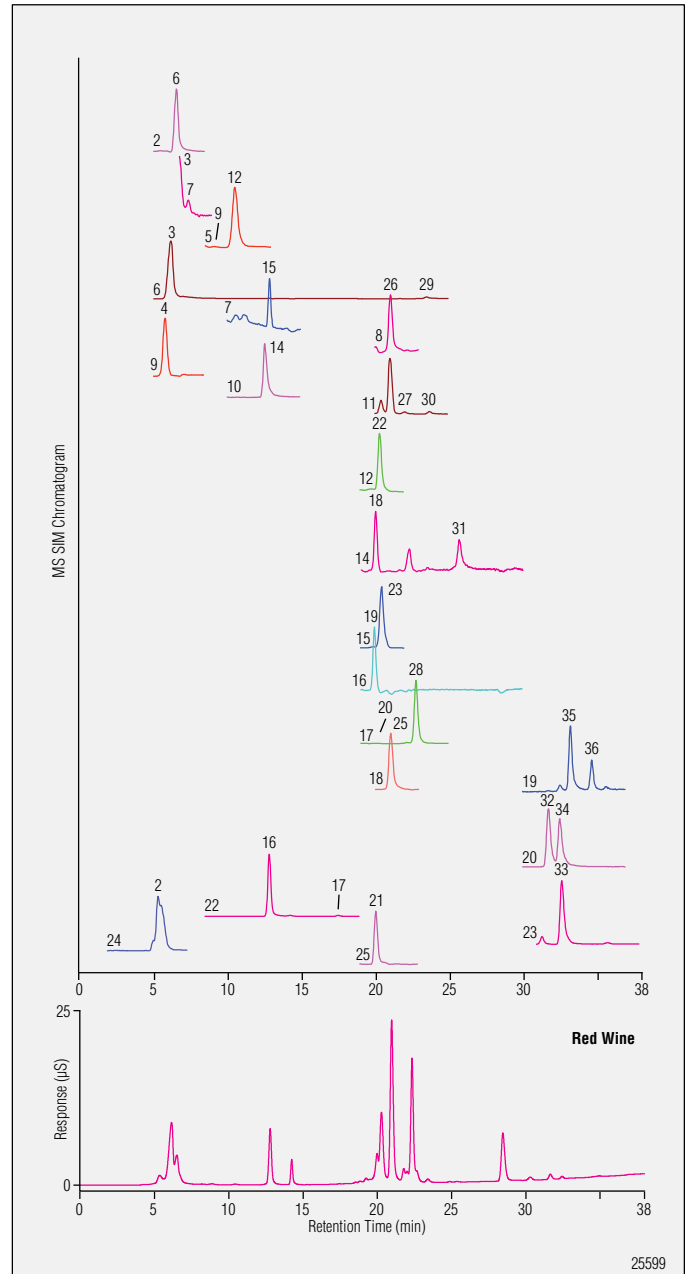


Figure 3. Organic acids in red wine. SIM channels for detected analytes are sorted by increasing m/z from top to bottom.

4 Determination of 32 Low Molecular Mass Organic Acids in Beverages Using IC/MS

Table 3. LMMOAs in Bottled Green Tea, Grape Juice and Wine

No.	Analyte	R.T.	Green Tea				Grape Juice				Wine			
			Brand A		Brand B		Brand A		Brand B		White		Red	
			R.T.	Conc.*	R.T.	Conc.*	R.T.	Conc.*	R.T.	Conc.*	R.T.	Conc.*	R.T.	Conc.*
1	quininate	5.03	5.03	11.5	5.03	78.0	5.03	1.17	5.30	6.20	5.05	4.29	5.42	3.19
2	gluconate	5.55	5.54	5.34	5.61	7.37	5.45	> 200	5.57	> 200	5.43	> 200	5.37	124
3	lactate	5.95	6.03	1.24	5.92	8.39	5.95	12.2	5.97	17.9	6.28	> 200	6.11	> 200
4	2-methylactate	5.96	ND		ND		5.74	0.552	5.77	1.44	5.77	0.280		1.40
5	glycolate	6.31	6.26	6.70	6.27	4.88	6.30	14.4	6.32	12.9	ND		ND	
6	acetate	6.34	8.32	7.80	6.36	2.45	6.33	11.0	6.34	52.5	6.61	> 200	6.48	> 200
7	propionate	7.31	ND		7.33	0.177	ND		ND		7.34	1.57	7.29	1.55
8	formate	8.24	6.41	0.667	8.27	6.45	8.23	26.2	8.20	28.4	ND		ND	
9	butyrate	9.07	ND		9.15	0.024	ND		ND		9.07	1.37	9.06	0.470
10	2-keto-d-gluconate	9.03	8.96	0.778	9.02	0.273	9.02	60.6	9.22	15.2	ND		ND	
11	2-hydroxyvalerate	9.63	ND		ND		ND		ND		ND		9.54	1.77
12	pyruvate	10.47	10.48	0.744	10.47	0.647	10.45	8.02	10.43	7.59	10.47	9.80	10.43	16.9
13	isovalerate	11.11	ND		ND		ND		ND		ND		ND	
15	valerate	12.77	ND		12.80	0.672	12.79	0.901	12.77	1.97	12.80	0.650	12.78	2.02
16	galacturonate	12.78	ND		12.82	1.89	12.79	> 200	12.78	> 200	12.79	> 200	12.79	> 200
17	5-keto-d-gluconate	17.42	17.49	0.734	Trace Amount		17.44	43.6	17.43	42.7	17.44	36.7	17.44	23.1
18	glutarate	19.95	19.95	0.033	19.98	0.090	19.89	1.07	19.88	1.26	19.92	2.20	19.92	3.26
20	adipate	20.04	ND		20.04	0.269	19.83	0.802	20.02	0.773	19.87	0.508	20.01	0.512
21	muicate	20.08	20.10	7.43	20.12	8.37	20.04	77.5	20.00	168	20.04	85.5	20.03	89.6
22	succinate	20.32	20.36	1.80	20.32	6.31	20.25	25.8	20.17	26.7	20.28	> 200	20.26	> 200
23	malate	20.40	20.40	1.80	20.40	46.8	20.33	> 200	20.28	> 200	20.37	> 200	20.37	> 200
24	methylmalonate	20.73	ND		ND		ND		ND		ND		ND	
25	tartrate	21.04	20.92	3.61	Trace Amount		21.01	> 200	20.71	> 200	20.98	> 200	20.97	> 200
26	melonate	21.10	21.09	0.085	21.10	4.85	21.01	4.24	20.99	8.55	21.01	7.71	20.99	12.1
27	maleate	21.95	Trace Amount		Trace Amount		21.85	0.047	Trace Amount		Trace Amount		21.81	0.115
28	a-ketoglutarate	22.69	ND		22.76	3.79	22.72	13.9	22.72	19.1	22.72	53.7	22.69	136
29	oxalate	23.38	23.38	59.1	23.41	80.3	23.39	166	23.38	208	23.40	22.3	23.40	25.5
30	fumarate	23.62	ND		23.69	0.144	23.60	2.17	23.66	1.76	23.60	0.600	23.64	3.93
31	oxalacetate	25.66	Detected, Not Quantified				Detected, Not Quantified				Detected, Not Quantified			
32	citrate	31.67	31.63	> 200	31.62	> 200	31.51	> 200	31.67	> 200	31.67	193	31.66	48.5
34	isocitrate	32.45	ND		ND		32.45	> 200	32.47	92.7	32.48	78.3	32.45	43.7
35	cis-aconitate	33.16	33.28	0.684	33.25	0.351	33.19	4.63	33.17	3.31	33.18	4.00	33.16	3.61
36	trans-aconitate	34.60	34.70	1.05	34.69	0.457	34.62	2.88	34.63	2.19	34.61	2.43	34.60	2.59

Alliquots of each sample were prepared and analyzed in triplicate; retention times and concentrations listed in Table 3 are the mean values. * ppm in original beverage solution

CONCLUSION

An IC/MS method for LMMOA analysis profiling in beverages was developed and evaluated with respect to linearity, range, detection limits, and precision. This method has been successfully applied to green tea, grape juice, and wine analysis. More than 27 LMMOAs were detected and reported at concentrations from 24 ppb to >200 ppm.

MSQ Plus is a trademark of Thermo Fisher Scientific. All other trademarks are the property of Dionex Corporation.

Passion. Power. Productivity.



Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
U.S. (847) 295-7500
Canada (905) 844-9650
South America
Brazil (55) 11 3731 5140

Europe
Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com



LPN 2136-01 09/08
©2008 Dionex Corporation