

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

Leo (Jinyuan) Wang, Stacy Henday, Björn Ogren, William Schnute, Dionex Corporation, Sunnyvale, CA, USA  
Dave Hazlebeck, Al Roberts, Liqing Zhana, Rodney Corbuz, General Atomics, San Diego, CA, USA

## INTRODUCTION

Low molecular mass organic acids (LMMOAs) are present in many plants and serve various functions. They are also found as degradation products during pretreatment processes for biofuel production. Many of these acids inhibit the microbial fermentation process and thus reduce the conversion efficiency to biofuel. To better understand and optimize the conversion process, it is necessary to monitor the LMMOA profile throughout biofuel production.

Among the analytical techniques for determination of LMMOAs, 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 biomass. Method performance was evaluated with respect to linearity, range, and detection limits. The LMMOA profiles of two biomass samples were also determined.

## EXPERIMENTAL

### Instruments

1. Dionex ICS-2000 Reagent-Free™ Ion Chromatography system with Eluent Generation (RFIC-EG™ System)
2. IonPac® AS11-HC Analytical column/ AG11-HC Guard (2 mm)
3. ASRS® 300 Anion Self-Regenerating Suppressor (2 mm)
4. AS Autosampler with 10 mL vial tray
5. MSQ Plus™ Mass Spectrometer
6. AXP-MS auxiliary pump x 2
7. 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  $\mu$ L

Oven Temperature: 30  $^{\circ}$ 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 used 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 used to generate backpressure of ~1000 psi.

Ionization Interface: Electrospray Ionization  
(ESI) source

Nebulizer Gas: Nitrogen at 80 psi

Probe Temperature: 450  $^{\circ}$ C

Needle Voltage: 3000 V

Scan Mode: Negative Selected Ion  
Monitoring (SIM)  
SIM listed in Table 1.

Dwell Time: 0.15 s

Scan Span: 0.5  $m/z$

Cone Voltage: 30 ~ 50 V

Peak No.	Analyte	R.T. (min)	SIM ( $m/z$ )	Fit*			MDL (ng)	%RSD
				From / ppb	To / ppb	R <sup>2</sup>		
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 <sup>a</sup>	0.129	8.18%
4	2-methylactate	8.2	103.1	1	5000	1.000 <sup>b</sup>	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 <sup>a</sup>	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 <sup>a</sup>	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 <sup>b</sup>	0.050	3.20%
12	pyruvate	12.9	87.0	1	5000	0.999 <sup>b</sup>	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	mucate	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 <sup>b</sup>	0.117	7.44%
22	methylmalonate	22.4	117.0	1	5000	0.999 <sup>b</sup>	0.095	6.03%
23	malonate	22.8	103.0	1	5000	0.996 <sup>c</sup>	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 <sup>b</sup>	0.123	3.57%
26	$\alpha$ -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	<i>cis</i> -aconitate	35.1	173.0	1	5000	1.000 <sup>b</sup>	0.034	4.31%
33	<i>trans</i> -aconitate	36.7	173.0	5	5000	1.000	0.051	6.45%

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

<sup>a</sup>quadratic fit; <sup>b</sup>cubic fit; <sup>c</sup>exponential fit.

## RESULTS AND DISCUSSION

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

### Ion Chromatography

The ion chromatography method was developed to resolve most analytes, especially those with identical or close molecular masses such as butyrate and pyruvate ( $m/z = 87.05$  and  $87.02$  respectively), and maleate and fumarate ( $m/z = 115.01$ ). These analytes cannot be resolved by single quadrupole MS, and some cannot be resolved by MS/MS because 1) they either do not fragment or 2) they 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. And  $[M-H]^-$  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

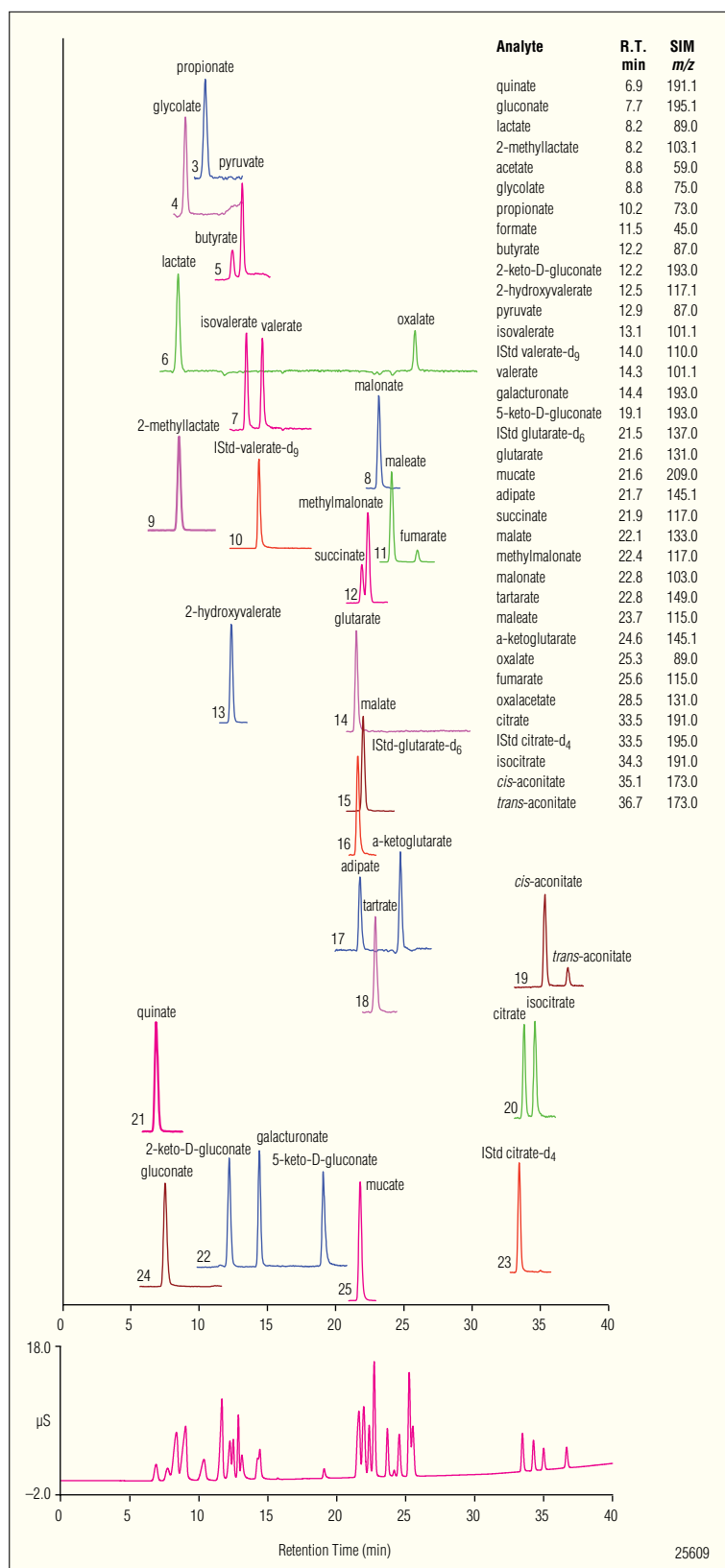
Calibration curves were generated using 12 calibration points (0.000, 1.00, 5.00, 10.0, 20.0, 50.0, 100, 200, 500, 1000, 2000 and 5000 ppb) with three isotope-labeled internal standards (valerate- $d_9$ , glutarate- $d_6$ , citrate- $d_4$ ) at 100 ppb for each level.

Correlation coefficients ( $R^2$ ) greater than 0.99 were achieved for most analytes over three orders of magnitude. Details are listed in Table 1.

**Table 2. LMMOA in Biomass by IC-MS**

Analyte	R.T.	Biomass			
		Sample A		Sample B	
		R.T.'	Conc.	R.T.'	Conc.
5-keto-d-gluconate	17.42	17.39	2.31	17.40	1.62
glutarate	19.95	19.92	0.700	19.92	0.887
adipate	20.04	19.99	0.211	19.99	0.245
mucate	20.08	20.07	7.49	20.08	9.18
succinate	20.32	20.28	> 200 ppm	20.28	> 200 ppm
malate	20.40	20.37	2.78	20.37	5.45
tartrate	21.04	21.03	0.678	21.06	1.84
melonate	21.10	21.06	0.140	21.06	0.356
maleate	21.95	Trace Amount		Trace Amount	
a-ketoglutarate	22.69	22.66	17.2	22.68	16.4
oxalate	23.38	23.41	7.68	23.39	12.1
fumarate	23.62	23.60	3.45	23.60	3.91
oxalacetate*	25.66	Detected, Not Quantified		Detected, Not Quantified	
citrate	31.67	31.69	6.09	31.71	6.29
isocitrate	32.45	32.46	1.26	32.48	0.904
cis-aconitate	33.16	33.18	0.864	33.19	1.01
trans-aconitate	34.60	34.62	0.157	34.62	0.152

\*Oxalacetic acid was not quantified due to its instability.  
Concentration shown as in ppm in original biomass samples.



## Precision and Method Detection Limits (MDLs)

Precision was calculated from seven replicate injections of 200 ppb as %RSD. (Table 1) 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. The 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 ( $S/N > 3$ ). MDLs are summarized in Table 1.

## Biomass Analysis

This method was evaluated by analyzing two biomass samples obtained from microalgae biofuel production processes. Three 1 mL aliquots of each biomass sample were centrifugated at 6000 rpm for 15 minutes. An aliquot of 0.6 mL supernatant was diluted in deionized water with IStd spiked to 200 ppb. The samples were then vortexed and analyzed by IC/MS. Table 2 shows the mean concentration of detected LMMOAs in original biomass samples. The retention times of LMMOAs from the biomass samples agree well with retention times in the standard solution. The presence of seventeen LMMOAs were confirmed in the biomass. Succinic acid was present in biomass as the most prominent LMMOA with concentrations greater than 200 ppm. Mucic acid,  $\alpha$ -ketoglutaric acid, oxalic acid and citric acid were also present in large amounts ( $> 5$  ppm).

## CONCLUSION

This IC/MS method for LMMOA profiling analysis in biomass was developed and evaluated with respect to linearity, range, detection limits, and precision. This method has been successfully applied for profiling LMMOAs in two biomass samples obtained from a biofuel production process. More than 15 LMMOAs were detected and reported with concentrations ranging from 0.140 ppm to  $> 200$  ppm.

Figure 1. CD and MS SIM chromatograms 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 for each analyte, aconitates 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.

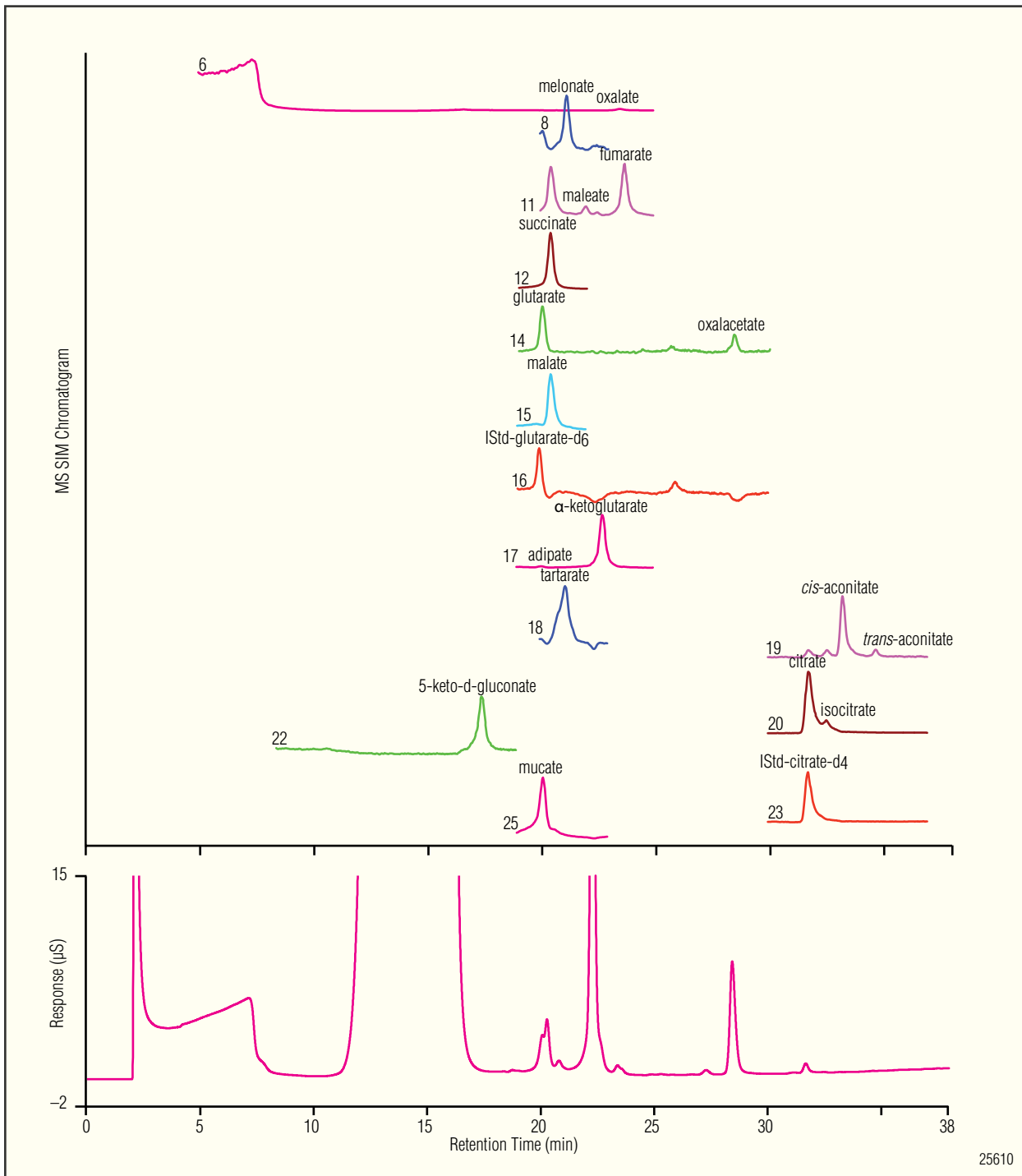


Figure 2. CD and MS SIM chromatograms of detected low molecular weight organic acids in biomass.

IonPac, and ASRS are registered trademarks and Reagent Free is a trademark of Dionex Corporation.  
MSQ Plus is a trademark of Thermo Fisher Scientific.

Passion. Power. Productivity.



**Dionex Corporation**

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

**North America**

U.S./Canada (847) 295-7500

**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](http://www.dionex.com)



LPN 2262-01 05/09  
©2009 Dionex Corporation