

Development of a new method for quantitation and screening analysis of organic acids by means of using IC/MS/MS

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Overview

Purpose: To develop an analytical method for quantitation and screening of organic acids using IC/MS/MS.

Methods: Selected reaction monitoring (SRM) methods were used for the quantitation analysis. Neutral loss, data dependent product ion scans were used for the development of a screening method for organic acids.

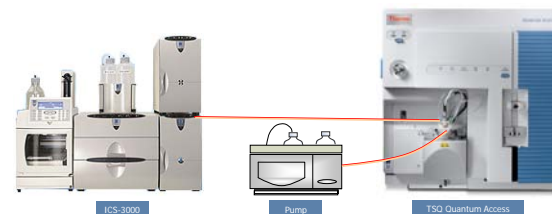
Results: IC coupled with a triple stage quadrupole mass spectrometer can perform both quantitation and screening analyses for multiple organic acids simultaneously.

Introduction

The demand for the quantitative analysis of organic acids, used in plating technology, has been increasing in the Asian chemical industry. As is well known, the quality of products depends on the amount of organic acids; therefore, a quantitative assay of the acids is necessary. The qualitative analysis of organic acids using an ion chromatograph (IC) coupled to a Thermo Scientific Orbitrap mass spectrometer was previously reported by Thermo Fisher Scientific¹. A newly developed quantitation method couples IC with a triple quadrupole mass spectrometer. We have developed a screening method for the detection of these organic acids by utilizing a carboxyl group, common to most organic acids, since they are known to exhibit a neutral loss of CO₂ during the dissociation process.

Methods

Thirty-two organic acid reference standards were used for this experiment. The compounds were dissolved in distilled water and separated using an IC (Dionex ICS-3000) with a suppressor. Acetonitrile was used as a sheath liquid to promote the ionization efficiency of the eluted aqueous solution. The organic acid compounds were ionized using negative electrospray mode and detected with a triple quadrupole mass spectrometer (Thermo Scientific TSQ Quantum Access).



IC conditions (Anion analysis)

Column: IonPac[®] AG20 2x50 mm, IonPac AS20 2x250 mm; Eluent: KOH; Gradient: 1 mM(0 min)–1 mM(5 min)–45 mM(30 min)–45 mM(5 min); Injection Volume: 10μl; Flow: 0.35 ml/min; ASRS[®] 300 2-mm / 50 mA (External Mode)

MS conditions

Ionization: Negative ESI (H-ESI); Spray Voltage: 3500 V; Vaporizer temp.: 300 C; Sheath Gas Pressure: 60 arb; Aux Gas Pressure: 10 arb; Ion transfer capillary temp.: 300 C; Source CID: 5 V; Peak width: Q1 (FWHM) 0.7 u; Collision Gas: Ar; Pressure: 1.5 mTorr; Sheath liquid, Flow: 90% Acetonitrile aq, 0.2 ml/min

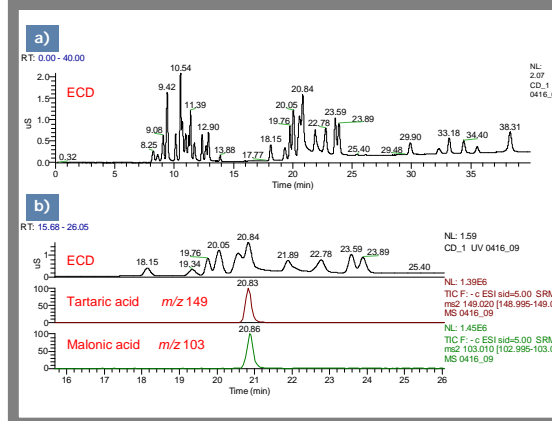
For each organic acid compound, selected reaction monitoring (SRM) methods were used for the quantitative analysis. The collision energy (CE) for each compound was optimized using reference standards and the neutral loss data dependent product ion scan (NLD-MS/MS: neutral loss: 44 Da; CE: 15 eV; data dependent scan threshold: 10⁵e; CE: 25 eV) was used for the development of the screening method.

Results

An IC coupled to the conductivity detector is currently the technique most widely used for ion analyses like organic acids. However, quantitation of multiple compounds is

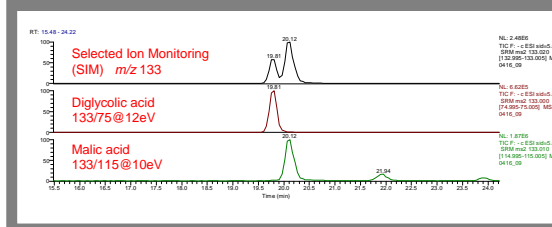
difficult when compounds co-elute while using the conductivity detector (ECD). In this new method, a mass spectrometer is used as the detector and multiple compounds can be successfully identified, even if these compounds elute at the same retention time. For example, tartaric acid (*m/z* 149) and malonic acid (*m/z* 103) have the same retention time but are successfully separated by *m/z*.

FIGURE 1. a) The chromatogram of 32 organic acids using an electrical conductivity detector. b) The ECD chromatogram of an acid mixture and the extracted ion chromatograms of Tartaric acid (*m/z* 149) and Malonic acid (*m/z* 103).



In addition, SRM allowed the separation of ions with the same *m/z*. In the case of diglycolic acid (SRM transition: 133/75) and malic acid (SRM transition: 133/115), it is not sufficient to clearly resolve these compounds using a SIM method on a single quadrupole mass spectrometer. Thus, a highly sensitive quantitation analysis for multiple organic acids became possible by this new method of combining IC with a triple quadrupole mass spectrometer using SRM.

FIGURE 2. The extracted ion chromatogram of *m/z* 133 and the SRM chromatograms of Diglycolic acid (133/75) and Malic acid (133/115).



This highly selective and sensitive analytical method was successful except for the following compounds. No product ions were detected for propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid, while the product ion of glycolic acid was detected at a very low level. To compensate for this, the same *m/z* in Q1 and Q3 was monitored (FIGURE 3).

As a result, the LOQ of the organic acids ranged from 0.5–50 ng/ml, as back calculated using calibration curves (Table 1).

FIGURE 3. The chromatogram of the acid mixture at a concentration level of 1000 ppb.

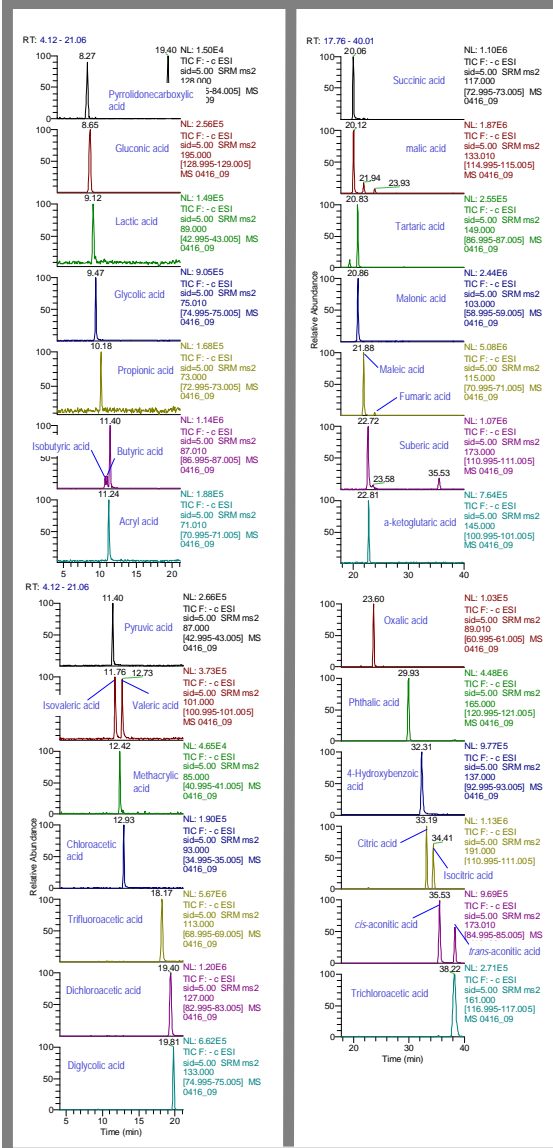


TABLE 1. The SRM transition and calibration curve of organic acids.

Compound name	RT (min)	SRM channel		Calibration curve		
		Precursor ion (m/z)	Product ion (m/z)	CE (eV)	Range (ng/ml)	Curve Equation
Pyroldonecarboxylic acid	8.24	128	84	12	10-2000	Linear Y = -1640.78+181.658*X R ² = 0.9320
Gluconic acid	8.62	195	129	12	5-2000	Linear Y = -2147.89+3816.65*X R ² = 0.9995
Lactic acid	9.09	89	43	12	50-2000	Linear Y = -28202.3+1621.84*X R ² = 0.9958
Glycolic acid	9.44	75	75	12	5-2000	Linear Y = -6329.98+8903.97*X R ² = 0.9959
Propionic acid	10.15	73	73	10	100-2000	Linear Y = -339615+1303.38*X R ² = 0.9971
Isobutyric acid	10.76	87	87	10	50-2000	Linear Y = -19883-2113.04*X R ² = 0.9968
Butyric acid	11.02	87	87	10	50-2000	Linear Y = -1699.32+2217.92*X R ² = 0.9988
Acrylic acid	11.27	71	27	10	10-2000	Linear Y = -43.7301+132.674*X R ² = 0.9980
Pyruvic acid	11.38	87	43	10	50-2000	Linear Y = -14582-2351.02*X R ² = 0.9997
Isovaleric acid	11.74	101	101	10	50-2000	Linear Y = -13743.4+4232.83*X R ² = 0.9971
Methacrylic acid	12.39	85	41	10	50-2000	Linear Y = -1303.06+487.017*X R ² = 0.9990
Valeric acid	12.71	101	101	10	50-2000	Linear Y = -20551.1+4229.7*X R ² = 0.9984
Chloroacetic acid	12.91	93	35	10	10-2000	Linear Y = -3064.55+1808.63*X R ² = 0.9985
Trifluoroacetic acid	18.15	113	69	12	5-2000	Linear Y = -481605-12665*X R ² = 0.9919
Dichloroacetic acid	19.32	127	83	10	5-2000	Linear Y = -95738.1+19984.3*X R ² = 0.9909
Diglycolic acid	19.78	133	75	12	5-2000	Linear Y = -28712.8+6876.17*X R ² = 0.9919
Malic acid	20.09	133	115	10	5-2000	Linear Y = -3671.8+2474.8*X R ² = 0.9997
Tartaric acid	20.61	149	87	14	10-2000	Linear Y = -2115.04+2930.72*X R ² = 0.9972
Malonic acid	20.84	103	59	10	5-2000	Linear Y = -43831-29531.4*X R ² = 0.9909
Malic acid	21.86	115	71	10	0.5-100	Linear Y = -93075.9+131363*X R ² = 0.9983
Suberic acid	22.70	173	111	14	5-2000	Linear Y = -4730.21+18876.4*X R ² = 0.9988
α-ketoglutaric acid	22.79	145	101	10	10-2000	Linear Y = -4359.36+8988.75*X R ² = 0.9909
Oxalic acid	23.58	89	61	10	10-2000	Quadratic Y = -12794.739+2826*X-0.28273*X ² R ² = 0.9979
Fumaric acid	23.96	115	71	10	50-2000	Linear Y = -28512-2934.83*X R ² = 0.9980
Phthalic acid	29.91	165	121	10	0.5-2000	Linear Y = -156438-77014.7*X R ² = 0.9906
4-Hydroxybenzoic acid	32.29	137	93	12	10-2000	Linear Y = -150691-20382*X R ² = 0.9983
Citric acid	33.17	191	111	12	5-2000	Quadratic Y = -36671.3+9315.41*X+4.12388*X ² R ² = 0.9983
Isocitric acid	34.39	191	111	12	5-2000	Quadratic Y = -25370.1+9458.69*X+2.77746*X ² R ² = 0.9978
cis-Aconitic acid	35.50	173	85	12	1-2000	Linear Y = -35203.9+16760.5*X R ² = 0.9916
Trichloroacetic acid	38.29	161	117	10	10-2000	Linear Y = -140429-9881.1*X R ² = 0.9938
trans-Aconitic acid	38.25	173	85	12	1-2000	Linear Y = -1176.05+9856.43*X R ² = 0.9958

Since most organic acids contain a carboxyl group, they are known to undergo a neutral loss of CO₂ during the dissociation process (Figure 4). This presence of a carboxyl group led to the development of a new "neutral loss data dependent product ion scan" method consisting of two events. Event 1 is a neutral loss scan and Event 2 is a product ion scan dependent upon the result of Event 1. The neutral loss mass scan mass spectra of precursor ions show the neutral loss of a selected mass (44Da=CO₂) (Figure 5). NLD-MS/MS, data dependent product ion scan triggered by a neutral loss scan, provides selectivity and structural information of organic acids in a single injection.

FIGURE 4. Product ion scan spectrum of succinic acid

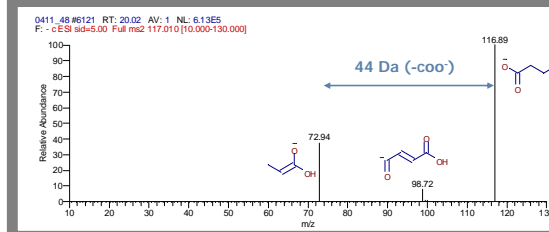


FIGURE 5. Method setting of NLD-MS/MS in Thermo Scientific Xcalibur software.

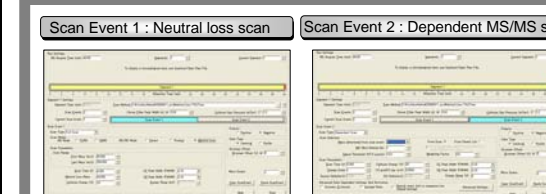


FIGURE 6. Total ion chromatogram and spectra of the acid mixture at a concentration level of 1000 ppb using NLD-MS/MS.

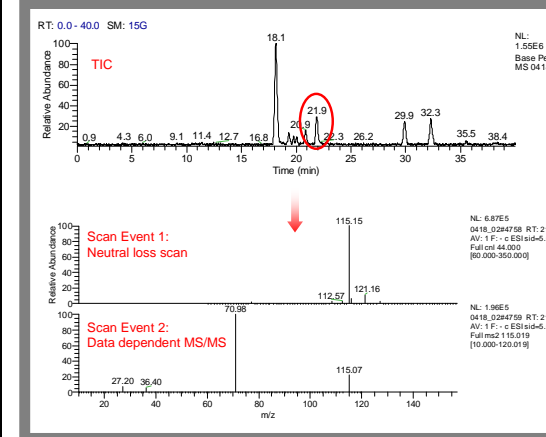


Figure 6 shows the result from a 1000 ppb standard using NLD-MS/MS. For example, RT: 21.9 (maleic acid), not only the *m/z* of the precursor ion but also the product ion spectrum was obtained automatically in a single analytical run. As a result, the precursor ion and the product ion spectrum of 15 compounds were obtained. The other compounds were not detected for the following reasons. Five compounds (propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid) did not dissociate. The product ions of 8 compounds (pyroldonecarboxylic acid, gluconic acid, glycolic acid, chloroacetic acid, malic acid, tartaric acid, citric acid and isocitric acid) were observed; however, these spectrum did not include a neutral loss of 44 Da. Although 44 Da neutral loss product ion spectra of the other 4 compounds (acrylic acid, methacrylic acid, oxalic acid and fumaric acid) could be obtained, they were not detected because the neutral loss spectrum was not above the threshold setting.

Conclusion

IC coupled with a triple stage quadrupole mass spectrometer can simultaneously perform quantitation and screening analyses of multiple organic acids in a single injection.

Reference

- (1) Sakamoto et al., ASMS Conference 2007, Structural Elucidation of Anionic Species by Using Ion Chromatography a Hybrid Linear Ion Trap Fourier Transform Mass Spectrometer.

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