LC-(DMS)-MS/MS Analysis of Emerging Food Contaminants

Quantitation and Identification of Maleic Acid in Starch-Rich Foods

Fanny Fu[1] and André Schreiber[2]
[1]AB SCIEX Taipei (Taiwan), [2]AB SCIEX Concord, Ontario (Canada)

Introduction

Recent findings (in May 2013) of maleic acid in foods, such as tapioca starch, tapioca balls, rice noodles, and hotpot ingredients, caused the recall of many starch-based food products in Asia.[1-3]

Maleic acid is usually not used in manufacturing of food products, and it is an unapproved food additive.

Occasional consumption of maleic acid at low levels does not pose any significant health risk; however, long term consumption of high levels of the compound can cause kidney damage.

The substance has been traced to a modified starch containing maleic anhydride, a chemical used in the production of food packing materials.

Reliable analytical methods are needed to detect maleic acid in foods to identify potential trace contamination in food production, processing, and packaging and to ensure consumer health.

Maleic acid is *cis*-butenedioic acid (Figure 1) and is closely related to fumaric acid (*trans*-butenedioic acid). The LC-MS/MS-based method presented here can be used to confidently identify and accurately quantify maleic acid even in presence of fumaric acid.

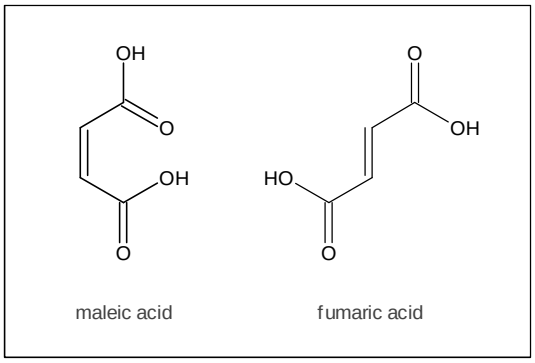


Figure 1. Chemical structures of maleic acid (left) and fumaric acid (right)

Experimental

Sample Preparation

Simple liquid extraction of food samples was performed using the following procedure developed by the Taiwan FDA[4]

- Weigh 1 g of homogenized samples into polypropylene centrifuge tubes (50 mL).
- Add 25 mL of 50% methanol.
- Shake vigorously for 30 min using a shaker.
- Add 20 mL of 0.5 N KOH.
- Vortex and let stand for two hours.
- Add 3 mL of 5 N HCl and bring to a final volume of 50 mL with deionized water.
- Vortex and centrifuge.
- Transfer an aliquot of 100 µL of the extract into an autosampler vial and dilute with 900 µL of water resulting in a total dilution factor of 500.

Further dilution of the extract might be necessary if the sample is heavily contaminated.

LC

Maleic acid and fumaric acid were analyzed using an Agilent 1260 system with a gradient on a Poroshell EC C18 column

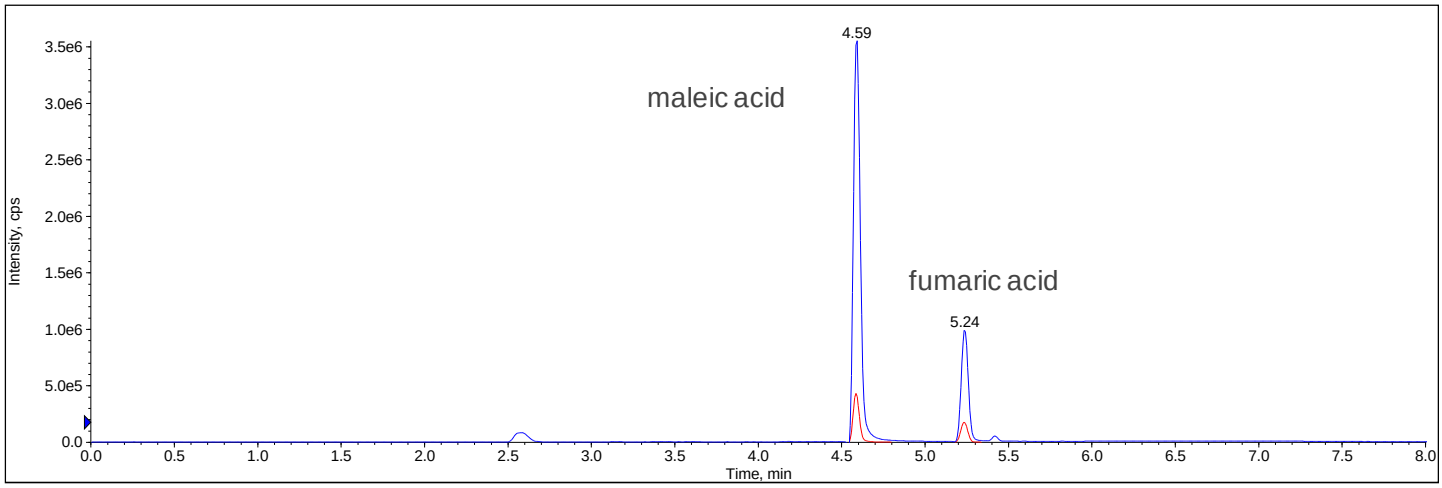


Figure 1. LC-MS/MS analysis of maleic acid and fumaric acid

(150 x 3.0 mm, 2.7 µm) and a mobile phase of water containing 0.1% formic acid (A) and methanol containing 0.1% formic acid (B). The flow rate was set to 0.3 mL/min. Gradient details are listed in Table 1. A sample volume of 10 µL was injected.

Table 1. LC gradient used for the separation of maleic acid and fumaric acid

Time (min)	Flow (mL/min)	A (%)	B (%)
0.0	0.3	98	2
1.0	0.3	98	2
5.0	0.3	5	95
7.0	0.3	5	95
7.5	0.3	98	2
16.0	0.3	98	2

MS/MS

The AB SCIEX QTRAP® 5500 was used with the Turbo V™ source and an Electrospray Ionization (ESI) probe. The mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode using negative polarity. Two selective MRM transitions were monitored using the ratio of quantifier and qualifier ion for identification (Table 2).

In addition, SelexION™ differential mobility separation was investigated to increase selectivity, improve Signal-to-Noise (S/N), and increase confidence in identification.

LC-MS/MS data were processed using the MultiQuant™ software version 2.1.

Table 2. MRM transitions and retention times of maleic acid and fumaric acid

Compound	Q1 (amu)	Q3 (amu)	CE (V)
Maleic acid 1	115	71	-11
Maleic acid 2	115	32	-28
Fumaric acid 1	115	71	-11
Fumaric acid 2	115	32	-28

Results and Discussion

An example chromatogram of the detection of maleic acid and fumaric acid is shown in Figure 1.

First, the limit of quantitation (LOQ), linearity, and repeatability were evaluated using injections of maleic and fumaric acid standards ranging from 0.5 to 200 ng/mL and spiked matrix samples.

Both compounds had LOQ values in the sub ng/mL range, allowing a sample extract dilution to minimize possible matrix effects. Linearity was excellent with a regression coefficient of 0.999 for quantifier and qualifier transitions. The accuracy values ranged from 89.6 to 107.6% across the linear dynamic range (Figure 2).

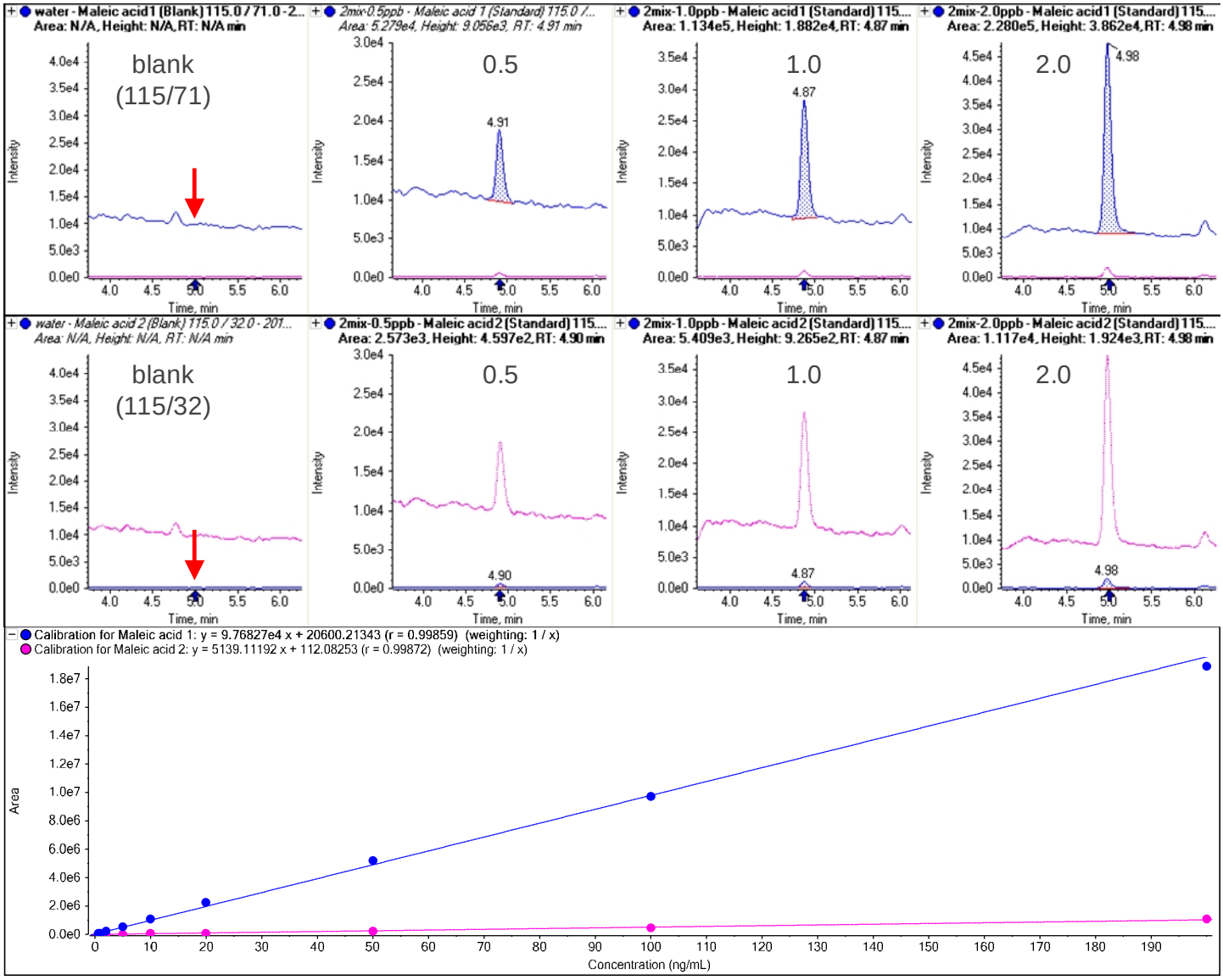


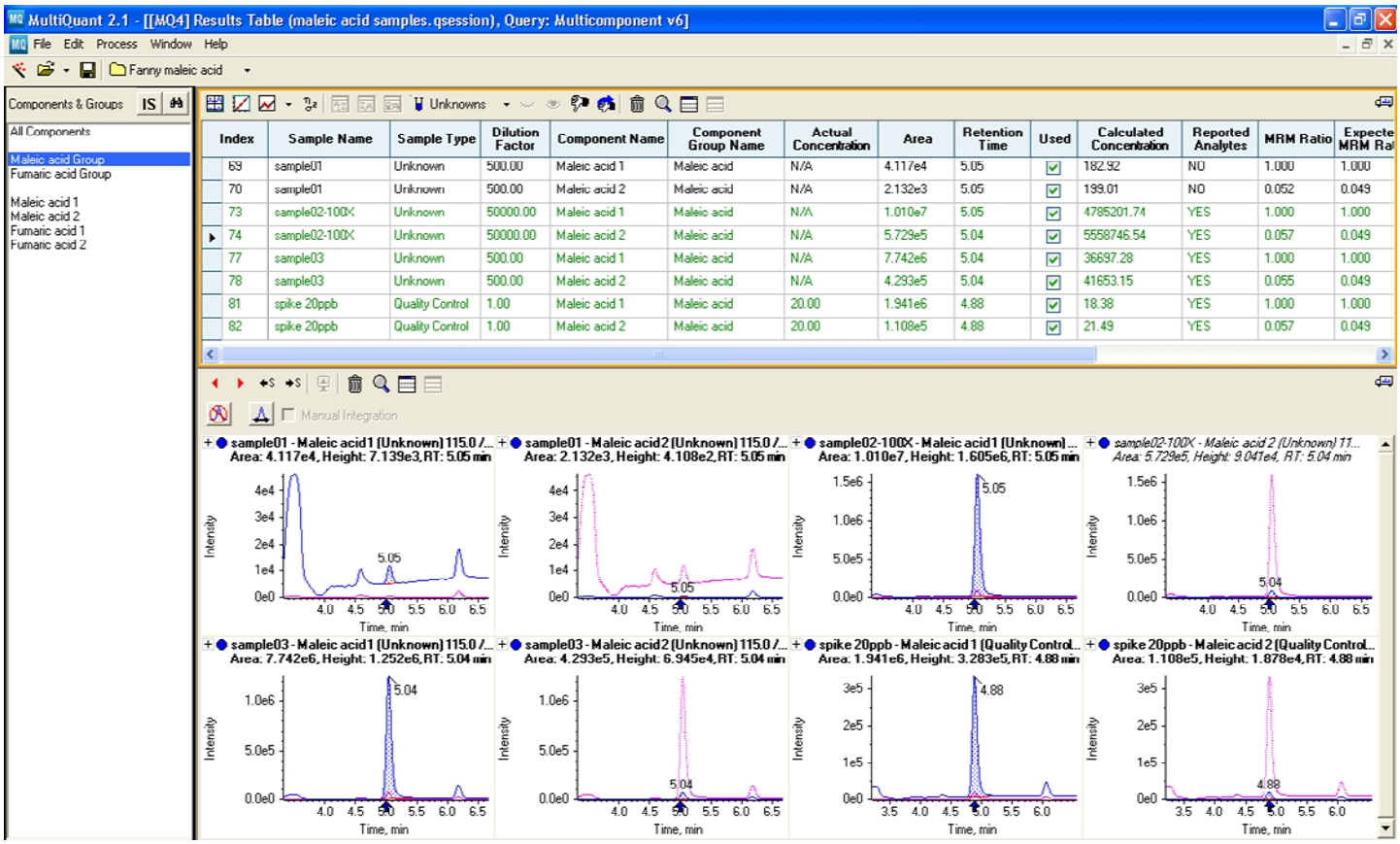
Figure 2. Chromatograms of the quantifier and qualifier transition of maleic acid of the blank sample and at concentration of 0.5, 1.0, and 2.0 ng/mL (top) and calibration lines from 0.5 to 200 ng/mL (bottom)

Repeatability was evaluated using 7 injections at 5 ng/mL. The coefficient of variation (%CV) was 2.9% for the quantifier transition (115/71) and 1.8% for the qualifier transition (115/32).

A number of food samples were analyzed for maleic and fumaric acids, including noodles, tapioca starch, and processed foods. The analysis of a 20 ppb spiked blank extract gave 91.9% recovery.

Table 3. Maleic acid findings in different food samples

Compound	Concentration (mg/kg)	MRM ratio	Expected MRM ratio
Noodles	0.18	0.052	0.049
Tapioca starch	4790	0.057	0.049
Processed food	36.7	0.055	0.049
20 ppb spike in blank extract	18.4 (91.9% recovery)	0.057	0.049



Index	Sample Name	Sample Type	Dilution Factor	Component Name	Component Group Name	Actual Concentration	Area	Retention Time	Used	Calculated Concentration	Reported Analytes	MRM Ratio	Expecte MRM Ra
69	sample01	Unknown	500.00	Maleic acid 1	Maleic acid	N/A	4.117e4	5.05	☑	182.92	NO	1.000	1.000
70	sample01	Unknown	500.00	Maleic acid 2	Maleic acid	N/A	2.132e3	5.05	☑	193.01	NO	0.052	0.049
73	sample02-100X	Unknown	50000.00	Maleic acid 1	Maleic acid	N/A	1.010e7	5.05	☑	4785201.74	YES	1.000	1.000
74	sample02-100X	Unknown	50000.00	Maleic acid 2	Maleic acid	N/A	5.729e5	5.04	☑	5558746.54	YES	0.057	0.049
77	sample03	Unknown	500.00	Maleic acid 1	Maleic acid	N/A	7.742e6	5.04	☑	36697.28	YES	1.000	1.000
78	sample03	Unknown	500.00	Maleic acid 2	Maleic acid	N/A	4.293e5	5.04	☑	41653.15	YES	0.055	0.049
81	spike 20ppb	Quality Control	1.00	Maleic acid 1	Maleic acid	20.00	1.941e6	4.88	☑	18.38	YES	1.000	1.000
82	spike 20ppb	Quality Control	1.00	Maleic acid 2	Maleic acid	20.00	1.108e5	4.88	☑	21.49	YES	0.057	0.049

Figure 3. Results for maleic acid in different food samples, the 'Multicomponent' query in MultiQuant™ software was used to identify target analytes based on their MRM ratio

Table 3 and Figure 3 show quantitative and qualitative results. MRM ratios were calculated using the 'Multicomponent' query in MultiQuant™ software.

In a last experiment we investigated the use of SelexION™ differential mobility separation (DMS) to increase selectivity and confidence in identification.

SelexION™ uses a planar differential mobility device that attaches between the curtain plate and orifice plate of the QTRAP® 5500 system (Figure 4). An asymmetric waveform, called Separation Voltage (SV), combined with a Compensation Voltage (CoV) is used to separate ions based on difference in their mobility.[5-6]

Chemical modifiers, like isopropanol, methanol, or acetonitrile, can be introduced into the transport gas via the curtain gas to alter the separation characteristics of analytes.

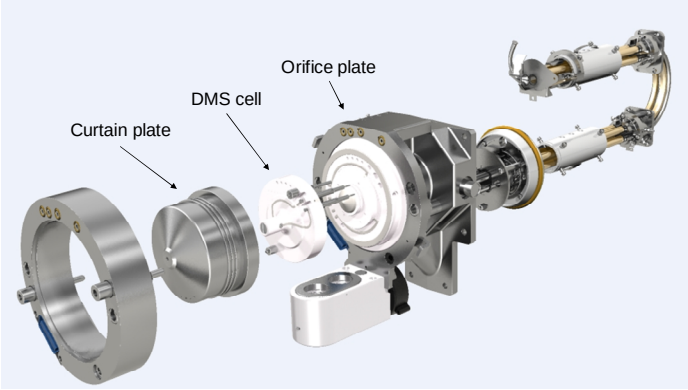


Figure 4. SelexION™ differential mobility separation (DMS)

SV and CoV were optimized for maleic and fumaric acids to separate these two isomers with identical MRM transitions. Best separation and highest selectivity was achieved using an SV of 3600 V and CoV of -8.0 V and -10.5 V, respectively (Figure 5). The added selectivity resulted in reduced background interferences. The presence of an MRM signal in combination with an optimized CoV value can also be utilized as an additional 'identification point' to increase confidence in data quality.

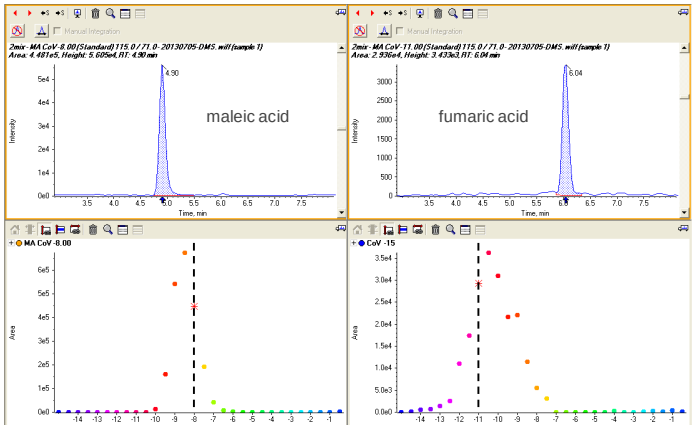


Figure 5. Compensation voltage (CoV) ramps for maleic and fumaric acid, best separation and highest selectivity was achieved using CoV of -8.0 V and -10.5V, respectiviely

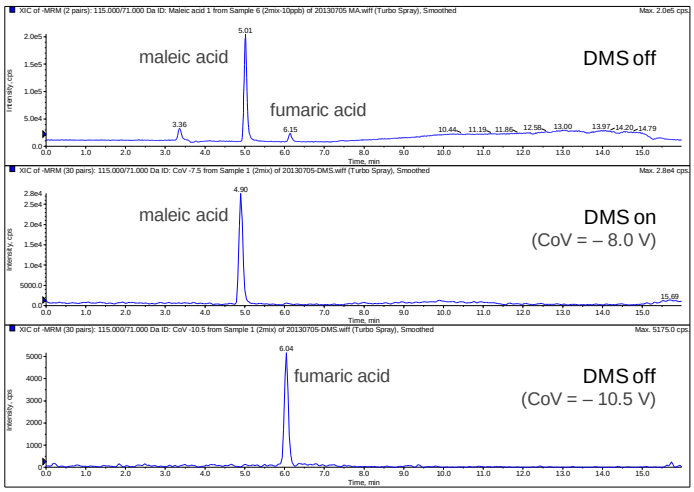


Figure 6. Selective detection of maleic acid and fumaric acid using LC-DMS-MS/MS, the added selectivity resulted in lower background noise and interferences and increased confidence in identification

Summary

The method and data presented here showcase the fast, easy, and accurate solutions for the analysis of maleic acid and fumaric acid in starch-rich foods by LC-MS/MS and LC-DMS-MS/MS. The AB SCIEX QTRAP® 5500 systems provide excellent sensitivity and repeatability for this analysis, with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period.

Maleic acid was quantified in different food samples. MRM ratio calculations in MultiQuant™ software used for compound identification. SelexION™ differential mobility separation was also used successfully to further increase selectivity and to clearly differentiate between isomeric species adding another 'identification point' and increased confidence to the results.

References

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Headquarters
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