Determination of Malic Acid and Lactic Acid in Wine by CE-MS/MS

Application Note

Food

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Abstract

A capillary electrophoresis tandem mass spectrometry method (CE-MS/MS) for the determination of malic acid and lactic acid in wine has been developed. The samples were diluted in Milli-Q water, filtered, and injected for electrophoretic separation in a fused silica capillary using a 25 mM propionic acid, 50 mM NH_4OH, pH 9.3 solution as background electrolyte (BGE). The calibration curve was constructed using eight different concentration levels, from 0.01 to 2 mM. This curve exhibited correlation coefficient values of 0.998 and 0.999 for malic acid and lactic acid, respectively; the limit of detection (LOD) for both analytes was lower than 0.8 µM. The proposed method was successfully applied to determine malic acid and lactic acid concentrations in commercial wine samples.

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Introduction

The type and concentration of organic acids, especially malic and lactic acids, influence the organoleptic characteristics of wine. Malic acid becomes microbiologically unstable when it is used as a substrate for lactic acid bacteria during malolactic fermentation [1]. It is important to be able to quantify the presence of malic and lactic acids in wine to check its quality and fermentation process. Figure 1 depicts the malolactic fermentation reaction.

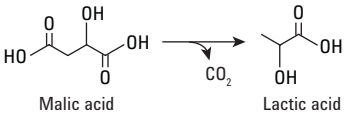

Figure 1. Malolactic fermentation reaction.

Typically, organic acid concentrations can be determined using techniques such as gas chromatography (GC), liquid chromatography (LC), ion chromatography (IC), and capillary electrophoresis (CE) [2,3,4]. These methods are precise and accurate. However, there is a possibility of developing new methods that do not require sample derivatization or treatment, and present the best separation efficiency. This study attempts to develop a sensitive, selective, and fast method for the determination of malic and lactic acid concentrations in wine using CE-MS/MS.

Experimental

CE Conditions

Parameter	Value
Instrument	Agilent 7100 CE system
Background electrolyte	25 mM propionic acid and 50 mM NH_4OH, pH 9.3
Applied voltage	25 kV
Capillary	Fused silica capillary 50 µm id with 60 cm length
Injection	10 seconds at 100 mbar
Temperature	25 °C

MS Conditions

Parameter	Value
Instrument	Agilent 6430 MS
Ion mode	ESI, negative ionization
Sheath liquid	BGE solution diluted 5x with H_2O/methanol (50:50 v/v)
Flow rate	5.0 µL/min
Capillary voltage	4,000 V
Drying gas flow (N_2)	11 L/min
Drying gas temperature	160 °C
Nebulizer pressure	11 psi
MRM transitions	*m/z* 89.0 → 43.0 (40 V), lactic acid as lactate
	m/z 133.0 → 115.0 (76 V), malic acid as malate
	m/z 160.9 → 116.9 (10 V), trichloroacetic acid, TCA, IS*

* Internal standard

All separations were performed at 25 °C using a 25 mM propionic acid and 50 mM NH_4OH, pH 9.3, as background electrolyte (BGE). Fused silica capillaries were preconditioned by flushing with 100 mM NaOH for 1 minute, followed by Milli-Q water for 3 minutes, and finally with BGE for 5 minutes. Samples were introduced hydrodynamically in 10 seconds at 100 mbar, and analyzed with an applied voltage of 25 kV. The mass spectrometer was operated in negative multiple reaction monitoring (MRM) mode using one specified transition for each compound. Trichloroacetic acid (TCA) was used as the internal standard. Samples of wine were diluted 50 times with Milli-Q water, and filtered through a 0.2 µm PVDF and PP membrane (Agilent Captiva filter cartridges p/n A5300002), and analyzed.

Results and Discussion

BGE and sheath liquid composition, applied potential, and hydrodynamic injection were optimized to achieve a good compromise between separation efficiency, sensitivity, and analysis time. Figure 2 shows an MRM electropherogram of a mixture of malic acid and lactic acid at 50 µM each in BGE under optimized instrumental and chemical parameters.

The linearity of the analytical curve was studied in BGE at eight different concentrations ranging from 0.01 to 2 mM. Figure 3 shows that this analysis was performed using Agilent MassHunter Quantitative software. The correlation coefficients (R^2) presented values of 0.998 and 0.999 for malic acid and lactic acid, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were determined considering the corresponding concentration to produce a signal of 3 and 10 times the standard deviation of the blank, respectively. The proposed method allows malic and lactic acids to be determined with LODs lower than 0.8 µM, while the LOQs were lower than 2.6 µM.

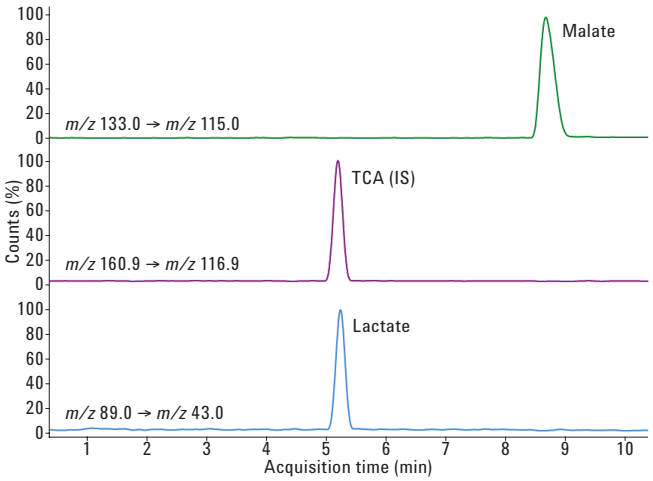

Figure 2. CE-MS/MS electropherogram of a mixture of malic acid, lactic acid, and TCA, 50 µM each in BGE.

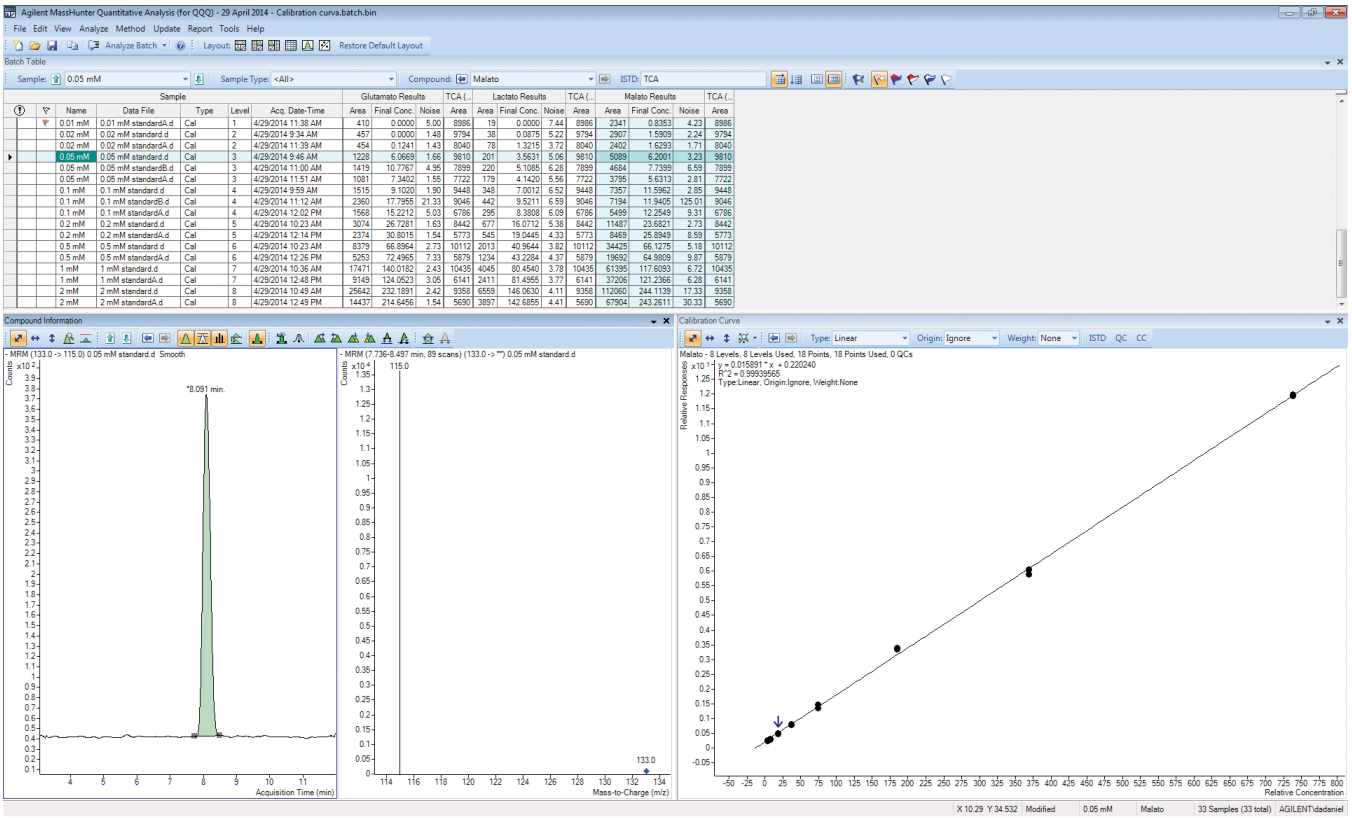

Figure 3. Agilent MassHunter Quantitative window software used for the determination of malic and lactic acids in wine.

An additional standard calibration method was used in the determination of malic and lactic acids in commercial samples of wine to avoid interference from the matrix. Table 1 shows the results obtained for malic and lactic acids in five different samples of wine by CE-MS/MS. Corresponding standard deviations were calculated from three independent measurements of each sample.

Table 1. Results Obtained for the Determination of Malic Acid and Lactic Acid in Wine Samples by CE-MS/MS (n = 3)

Sample	Malic acid (mM)	RSD (%)	Lactic acid (mM)	RSD (%)
Red wine 1	4.4 ± 0.3	2.3	31.1 ± 0.6	2.0
Red wine 2	9.7 ± 0.4	4.4	11.8 ± 0.5	4.1
Red wine 3	27.8 ± 1.3	4.6	3.6 ± 0.5	3.8
White wine 1	1.0 ± 0.0	4.1	12.6 ± 0.6	4.6
White wine 2	0.4 ± 0.3	4.2	11.3 ± 0.4	3.8

Supported by the results shown in Table 1, it is possible to evaluate whether the malolactic fermentation reaction was fully completed. Table 1 shows that samples with high concentrations of lactic acid have low malic acid levels due to the microbiological fermentation. Thus, red wines are frequently richer in lactic acid than white wine. The excess of malic acid is correlated with low microbiological action. Based on these results, efficient quality control can be accomplished using the developed CE-MS/MS method.

Conclusion

A suitable, precise, selective, and accurate method has been presented based on CE-MS/MS for the analysis of malic and lactic acids in wine samples. The proposed method presented a linear response to malic and lactic acids in the concentration range from 0.01 to 2 mM, with the LOD lower than 0.8 µM. It presents linear calibration curves and excellent precision data for replicate injections. This method takes 10 minutes per sample, uses a small amount of sample with low reagent consumption, and only requires dilution for sample treatment. It also has the potential to be successfully applied to other food matrix samples.

References

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