Determination of 3,4-MDMA in Ecstasy Tablets by CE-MS/MS

Application Note

Forensic Toxicology

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Abstract

A capillary electrophoresis tandem mass spectrometry method (CE-MS/MS) was developed for the determination of 3,4-methylenedioxymethamphetamine (3,4-MDMA) in Ecstasy tablets. In this study, Ecstasy tablets seized in Rio de Janeiro, Brazil, were homogenized and diluted before electrophoretic separation in 0.1 M formic acid electrolyte (pH 2.4) using a polyvinyl alcohol (PVA)-coated capillary. Separation was performed in less than 8 minutes, and the correlation coefficient of the calibration curve in the range of 0.002 to 0.2 µg/mL was greater than 0.998. The average minimal detectable amount for 3,4-MDMA was 0.0005 µg/tablet, which is several orders of magnitude lower than the minimum amount encountered in a tablet. The CE-MS/MS method demonstrated sufficient selectivity to discriminate the presence of 3,4-MDMA from the other drugs present in the seized tablets. The developed method is simple, and does not require an elaborate sample preparation procedure.

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Introduction

The amphetamine derivative 3,4-methylenedioxymethamphetamine (3,4-MDMA), also known as Ecstasy, is a psychoactive compound used mainly as a recreational drug [1]. It is ranked as the second most consumed drug in most European countries, and is only surpassed by cannabis [2]. Figure 1 shows the molecular structure of 3,4-MDMA. The consumption of Ecstasy is considered a public health issue due to different individual responses, arising from harmful to toxic dosages, which depend on tablet purity [3]. Due to the rapidly growing abuse of 3,4-MDMA and related substances, a simple, economic, fast, and consistent method for its determination is necessary for forensic analysis [4].

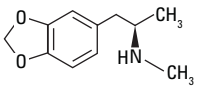

Figure 1. Structure of 3,4-MDMA, Ecstasy.

In forensic analysis, the results generated must prove the nature of the substance, and seized substances are commonly analyzed using multiple uncorrelated techniques [5]. Various analytical methods for the measurement of 3,4-MDMA have been reported, including gas and liquid chromatography coupled to mass spectrometry (GC/MS and LC/MS) and capillary electrophoresis (CE) [6,7]. CE analysis has been gaining recognition in forensics laboratories, especially when coupled with tandem mass spectrometry. CE-MS/MS combines in one analysis the advantages of both quantitative and migration time information with molecular masses or fragmentation patterns. This approach presents a high probability of elucidating the chemical compound and its concentration using an analytical curve or standard addition methods.

This application note describes a CE-MS/MS method for the detection and quantitation of 3,4-MDMA in seized Ecstasy tablets. The method is sensitive, fast, requires low sample volumes (nL), and produces low levels of chemical waste.

Experimental

All separations were performed at 25 °C using 0.1 M formic acid, pH 2.4, as background electrolyte (BGE). The polyvinyl alcohol (PVA) capillary was preconditioned by flushing with Milli-Q water for 3 minutes followed by BGE for 5 minutes. An additional flushing step with BGE for 30 seconds was included between the runs. 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 positive ionization mode, using multiple reaction monitoring (MRM) mode for two specific transitions of 3,4-MDMA: *m/z* 194.1 → *m/z* 163.1 (8 V) and *m/z* 194.1 → *m/z* 105.1 (24 V). The most intense transition was used for quantification, while the second was used as a qualifier ion. The sheath liquid was prepared by diluting the background electrolyte five times with methanol/water 1:1 (v/v).

Sample preparation

Seized Ecstasy tablets were provided by Service of Forensic Chemistry of the Carlos Éboli Institute of Criminalistics, Rio de Janeiro Department of Technical and Scientific Police, Brazil. We had access to 12 samples of tablets with different colors, shapes, and stamped figures. Each tablet was weighed and powdered in a porcelain mortar; 1 mg of the powdered tablet was taken for extraction with 1 mL of methanol. Homogenization was performed by shaking the vial in a vortex mixer for 1 minute. The resulting aqueous supernatant was then removed with a micropipette, and used to prepare 2,000 to 8,000 times diluted solutions. Prior to analysis, all solutions were filtered through a 0.2 µm PVDF and PP membrane (Agilent Captiva filter cartridges p/n A5300002).

CE Conditions

Parameter	Value
Instrument	Agilent 7100 CE system
Background electrolyte	0.1 M formic acid, pH 2.4
Applied voltage	25 kV
Capillary	PVA capillary 50 µm id with 58 cm total length (p/n G1600-67219, 125 cm length, cut to 58 cm)
Injection	10 seconds at 100 mbar
Temperature	25 °C

MS Conditions

Parameter	Value
Instrument	Agilent 6430 MS
Ion mode	ESI, positive ionization
Sheath liquid	0.02 M formic acid/methanol (50:50 v/v)
Flow rate	5.0 µL/min
Capillary voltage	4,000 V
Drying gas flow (N_2)	4 L/min
Drying gas temperature	150 °C
Nebulizer pressure	4 psi
MRM transition	*m/z* 194.1 → *m/z* 163.1 (8 V) *m/z* 194.1 → *m/z* 105.1 (24 V)

Results and Discussion

The background electrolyte and sheath liquid composition, as well as applied potential and hydrodynamic injection were optimized for separation efficiency and sensitivity. A PVA-coated capillary (p/n G1600-67219) was used to achieve a good compromise between analysis time and peak resolution by reducing the osmotic flow (EOF). The PVA coating minimizes the interaction between highly polar compounds and the surface of the capillary to avoid excessive peak tailing. Figure 2 shows a representative electropherogram of a standard solution of 3,4-MDMA at 0.02 µg/mL in BGE using a PVA-coated capillary.

The linearity of the analytical curve was studied in BGE at nine different concentration levels, in quintuplicate, ranging from 0.002 to 0.2 µg/mL. The curve was generated using Agilent MassHunter Quantitative software, as shown in Figure 3. The correlation coefficient (R^2) for the calibration curve was greater than 0.998. The limit of quantification (LOQ) was considered as being the lowest level of calibration curve, 0.002 µg/mL. The limit of detection (LOD) was calculated as the LOQ value divided by 3.33, resulting in the concentration of 0.6 µg/L.

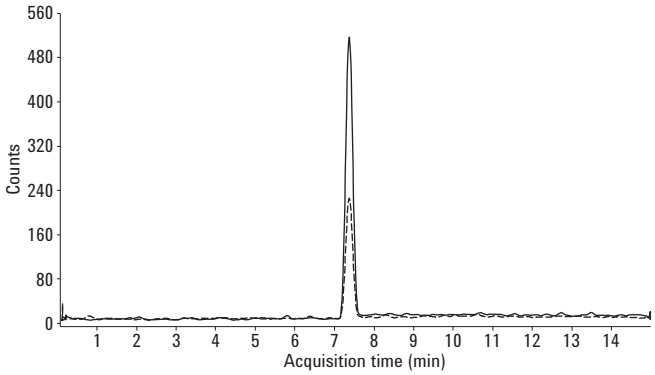

Figure 2. CE/MS/MS electropherogram of a standard solution of 3,4-MDMA at 0.02 µg/mL in BGE using a PVA-coated capillary. Agilent MassHunter Qualitative window software.

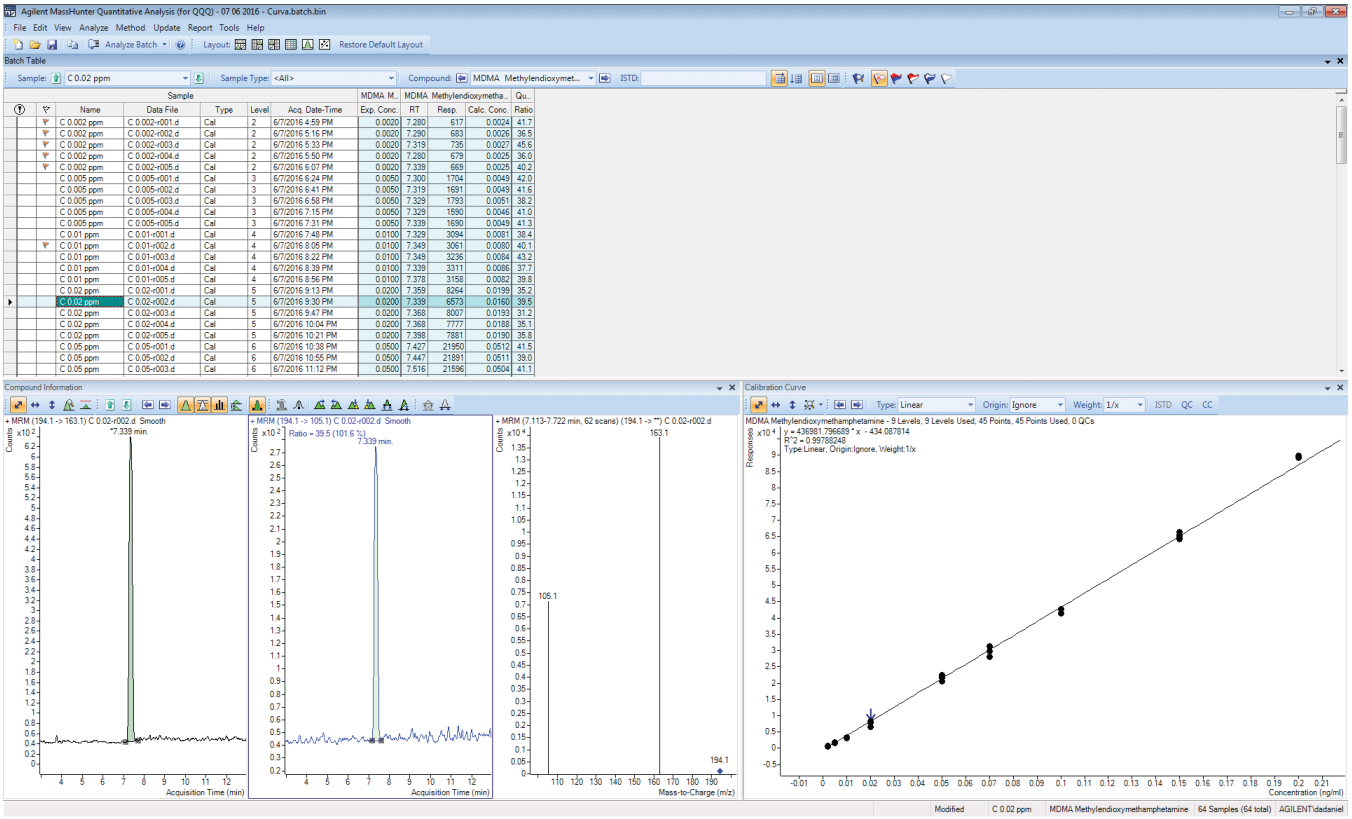

Figure 3. Calibration curve of 3,4-MDMA using Agilent MassHunter Quantitative window software.

Quantification of 3,4-MDMA in seized Ecstasy tablets was performed by external standard calibration, and no interferences from the matrix were observed. Table 1 presents the results obtained for 3,4-MDMA in different Ecstasy tablet samples. The corresponding standard deviations calculated from three independent measurements of each sample are also shown.

Table 1. Results Obtained for the Determination of 3,4-MDMA in Ecstasy Tablets by CE-MS/MS (n = 3)

Sample	Result	Conc. (mg/tablet)	RSD (%)
1	Positive	72.5	2.9
2	Positive	48.6	4.6
3	Positive	90.3	3.5
4	Positive	102.0	1.5
5	Negative	ND*	—
6	Positive	100.3	2.7
7	Negative	ND*	—
8	Negative	ND*	—
9	Positive	87.7	2.0
10	Positive	81.7	4.3
11	Negative	ND*	—
12	Negative	ND*	—

* ND = not detected.

Analysis of the seized Ecstasy tablets revealed the presence of 3,4-MDMA in 58 % of samples (n = 12), in amounts ranging from 48.6 to 102 mg/tablet. The purity of the active ingredient in these tablets was uncertain due to poor methods of production and quality control inherent in the illicit drug market. However, the concentrations of 3,4-MDMA determined in the Ecstasy tablets were similar to those concentrations previously reported in the literature [3]. Negative samples for 3,4-MDMA were also verified for other amphetamines and derivatives. Samples 7 and 8 presented positive results for amphetamine, while samples 11 and 12 presented positive results for methamphetamine. The presence of minor concentrations of other related amphetamines was detected in all samples, with the exception of sample 5, which did not show any positive results.

Conclusion

This study shows that CE-MS/MS is well suited for the analysis of 3,4-MDMA in Ecstasy tablets. The proposed method presented a linear response to 3,4-MDMA in the concentration range from 0.002 to 0.2 µg/mL with excellent precision for replicate injections. It uses a small amount of sample with low reagent consumption, and has easy solubilization and dilution procedures as the only requisite sample treatment steps. In addition, the method developed was fast, taking less than 8 minutes per sample. This method enabled detection of 3,4-MDMA in Ecstasy tablets at concentrations as low as 0.0005 µg/tablet. This concentration is several orders of magnitude lower than the minimum amount encountered in a tablet, therefore the method has excellent potential for use in forensic laboratories.

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