Determination of Amphetamine and Derivatives in Urine

Using a Modified QuEChERS and Capillary Electrophoresis Tandem Mass Spectrometry Analysis

Application Note

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Abstract

A capillary electrophoresis tandem mass spectrometry (CE-MS/MS) method was developed for the simultaneous determination of amphetamine (AM), methamphetamine (MAM), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), and phentermine (PTM) in urine. The urine samples were submitted to a modified QuEChERS extraction procedure followed by electrophoretic separation in 0.1 M formic acid electrolyte (pH 2.4) using a polyvinyl alcohol (PVA)-coated capillary. The correlation coefficients of the calibration curves in the range of 1.0 to 500 ng/mL were up to 0.997. Limits of detection were in the range of 0.01 to 0.02 ng/mL. Precision and accuracy were verified through recovery for spiked urine blank samples at three concentration levels (10, 20, and 50 ng/mL), in triplicate measurements. The recovery values ranged between 90 to 115 %, with a relative standard deviation (RSD) lower than 5.4 %.

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Introduction

Amphetamine (AM) and its derivatives are powerful stimulants of the central nervous system, acting on neurons in the brain to create feelings of pleasure and wellbeing. These compounds are commonly used as performance and cognitive enhancers [1]. In sports requiring intense anaerobic exercise, amphetamines prolong tolerance to anaerobic metabolism [2]. However, the side effects of chronic use of amphetamines can include delusions, hallucinations, psychosis, and depression [3]. Amphetamines and amphetamine-type substances are firmly established on the global illicit drug market. Therefore, there is increased demand for analysis of these illegal drugs in a wide variety of matrices [4].

To successfully analyze large numbers of samples, forensic laboratories require rapid analysis methods. Various analytical methods for the measurement of amphetamine and its derivatives in urine have been reported. These methods include gas and liquid chromatography coupled to mass spectrometry (GC/MS and LC/MS) and capillary electrophoresis (CE) [4,5]. CE analysis has been gaining more recognition in forensics laboratories especially when coupled to mass spectrometry. This recognition is due to unique features of CE such as broad applicability including highly polar compounds, high separation efficiency in short time periods, small sample size requirements, and small amounts of organic solvents and chemicals [6]. CE-MS/MS combines the quantitative and migration time information with molecular masses or fragmentation patterns in one analysis. This presents a high probability of elucidating the chemical compound and its concentration using an analytical curve or standard addiction methods.

Renal excretion is the major elimination route of amphetamine, however, urine matrices are complex. This complexity means that an effective sample pretreatment is necessary to obtain reliable analytical results. Traditional extraction methods, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE), are time-consuming, and need large quantities of organic solvents. QuEChERS is a simple sample preparation technique, and can be a good alternative to traditional extractions methods, reducing material costs and improving sample throughput [7].

The aim of this work was to develop a sensitive, selective, and fast method for the analysis of amphetamine and its derivatives in urine using QuEChERS sample preparation combined with CE-MS/MS.

All separations were performed at 25 °C using a 0.1 M formic acid, pH 2.4, as a background electrolyte (BGE). New polyvinyl alcohol (PVA) capillaries were preconditioned by flushing with Milli-Q water for 3 minutes followed by BGE for 5 minutes. Samples were introduced hydrodynamically in 5 seconds at 100 mBar and analyzed with an applied voltage of 25 kV. The mass spectrometer was operated in positive multiple reaction monitoring (MRM) mode using two transitions per compound. The most intense transition was used for quantification, and the other was used as a qualifying ion. Table 1 lists the monitored ions and other MS/MS acquisition parameters.

Table 1. Migration Time (t_M) and MS/MS Acquisition Parameters Used for the Identification and Quantification of Amphetamine and its Derivatives in Urine

Compound	t_M (min)	pKa[a]	Q_1[b] (*m/z*)	Q_3[c] (*m/z*)	CE[d] (V)	FE[e] (V)
Amphetamine (AM)	6.08	10.01	136.1	91.1*	20	70
				119.1	10	
Methamphetamine (MAM)	6.20	10.21	150.1	91.1*	20	75
				65.0	44	
Methylenedioxyamphetamine (MDA)	6.36	10.14	180.1	163.1*	4	80
				105.1	24	
Phentermine (PTM)	6.47	10.25	150.1	91.1*	10	75
				133.1	10	30
Methylenedioxymethamphetamine (MDMA)	6.48	10.14	194.1	163.1*	8	80
				105.1	24	
Methylenedioxyethylamphetamine (MDEA)	6.90	10.22	208.1	163.1*	8	98
				105.1	24	

[a] The pKa values were calculated at www.chemicalize.org (accessed in January, 2016).
[b] Precursor ion (Q1), [c] Fragment ions (Q3), [d] Collision energy, [e] Fragmentor energy.
* Transition used for quantification.

Experimental

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 × 60 cm total length (p/n G1600-67219, 125 cm length, cut to 60 cm)
Injection	5 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

Sample preparation

Samples of blank urine were stored at –20 °C before analysis. Extraction of the amphetamine and derivatives from urine was performed using a modified QuEChERS method. This method involved placing a 10-mL aliquot of the sample into a 50-mL PP tube followed by extraction using 10.0 mL of acetonitrile (containing 10 mg of NaOH, apparent pH 12.4). A partition step was performed by adding 4 g of anhydrous magnesium sulphate ($MgSO_4$) and 1 g of anhydrous sodium chloride (NaCl) using an Agilent Bond Elut QuEChERS AOAC Extraction kit (p/n 5982-5550) followed by shaking for 1 minute, and centrifugation for 5 minutes at 5,000 rpm. Next, a 5-mL aliquot of the supernatant was filtered through a 0.2-µm PVDF and PP membrane (Agilent Captiva filter cartridges, p/n A5300002), and analyzed. The dSPE cleanup step was unnecessary.

The recovery tests were carried out by spiking the samples before the shaking step with a known amount of the analytes. This spiking resulted in three different levels of amphetamine and derivatives (10, 20, and 50 ng/mL) in the blank urine samples. The recovery was determined by comparing the response of amphetamine and derivatives found in the spiked blank sample with the response of the same analytes from postextracted samples at the equivalent concentrations, and was expressed as a percentage.

Results and Discussion

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). This capillary eliminated the interaction between highly polar compounds and the surface of the capillary, avoiding peak tailing. Figure 1 shows an MRM electropherogram of a mixture of AM, MAM, MDA, MDMA, MDEA, and PTM at 0.2 µg/mL each in BGE using a PVA-coated capillary.

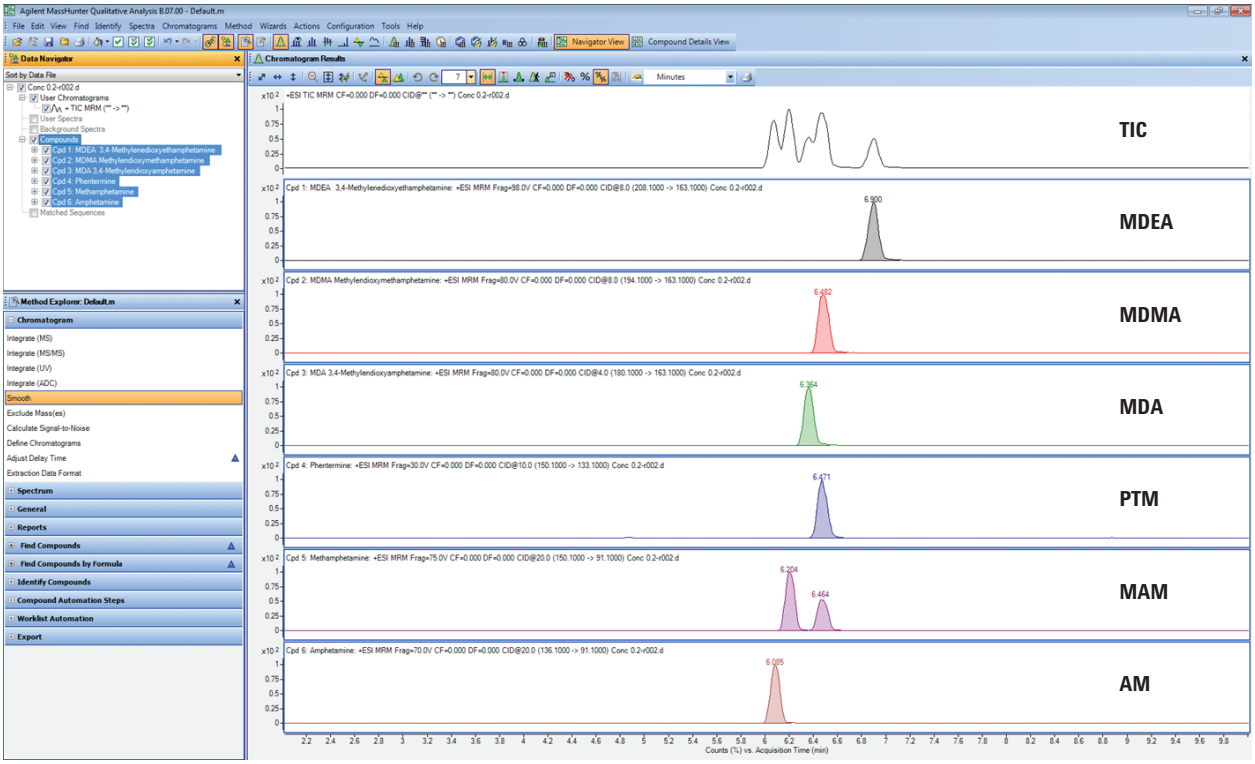

Figure 1. CE-MS/MS electropherogram of a mixture of the amphetamine and its derivatives at 0.2 µg/mL each in BGE using a PVA-coated capillary. Total ion electropherogram (TIC), MDEA, MDMA, MDA, PTM, MAM, and AM.

The linearity of the analytical curve was studied in BGE at 11 different concentration levels ranging from 1.0 to 500 ng/mL using the Agilent MassHunter Quantitative software, as shown in Figure 2. For all calibration curves, the correlation coefficients (R^2) presented values greater than 0.997. The limit of detection (LOD) and limit of quantification (LOQ) were determined using three times the baseline noise and 10 times the baseline noise, respectively, in a time close to the migration time of each target compound. Table 2 summarizes these results.

Table 2. Figures of Merit of the Proposed Method for the Determination of Amphetamine and Derivatives in Urine

Analyte	y = a + bx	R^2	LOD (ng/mL)	LOQ (ng/mL)
AM	a = –35.1 ± 611.8 b = 398019.2 ± 3472.2	0.997	0.02	0.07
MAM	a = 618.0 ± 798.1 b = 779600 ± 4529.5	0.997	0.01	0.03
MDA	a = 326.5 ± 318.1 b = 29228.6 ± 1805.3	0.995	0.02	0.07
PTM	a = 845.5 ± 863.1 b = 499883.6± 3763.6	0.995	0.01	0.04
MDMA	a = 458.2 ± 372.4 b = 252281.5 ± 2113.3	0.997	0.02	0.05
MDEA	a = –12.2 ± 291.8 b = 298436.7 ± 1656.2	0.999	0.02	0.06

R^2 = Determination coefficient

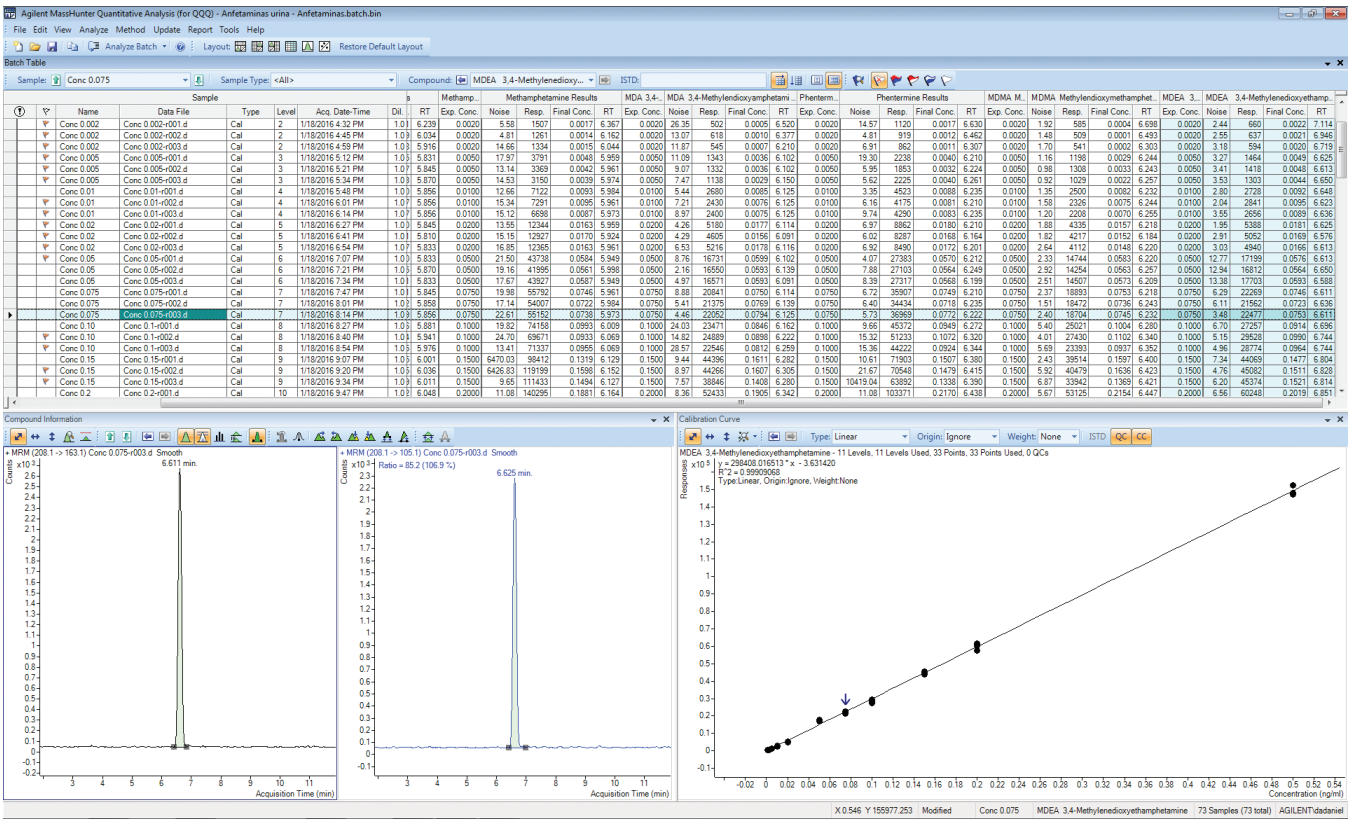

Figure 2. Agilent MassHunter Quantitative window software.

Precision and accuracy, expressed in terms of recovery from urine samples, were studied by analyzing spiked samples at three different concentration levels, in quintuplicate. Table 3 shows these results. Figure 3 shows a blank urine sample spiked with mix of AM, MAM, MDA, PTM, MDMA, and MDEA at 0.01 µg/mL each.

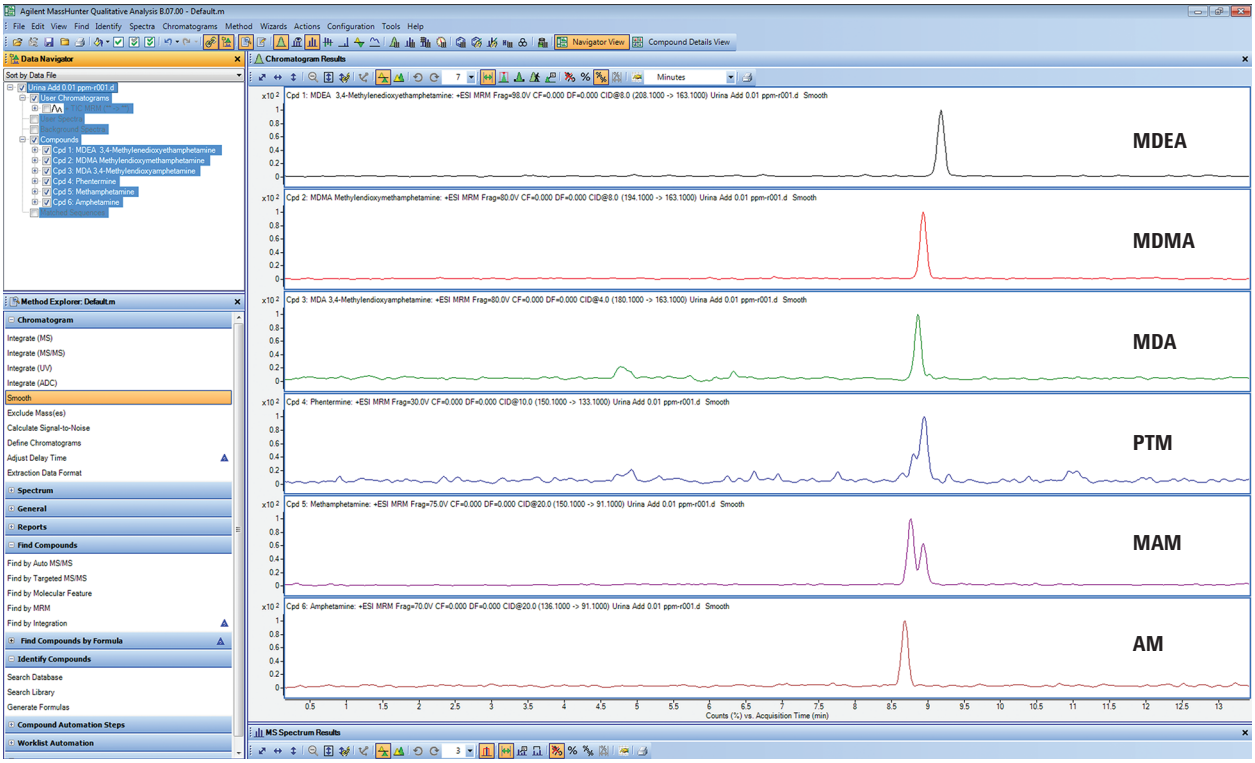

Figure 3. CE-MS/MS electropherogram of a blank urine sample spiked with a mixture of the amphetamine and its derivatives at 0.01 µg/mL each using a PVA-coated capillary. MDEA, MDMA, MDA, PTM, MAM, and AM.

Table 3. Concentration (ng/mL) of AM and Derivatives Spiked into Urine Samples and Recovery Tests Carried Out in These Samples (n = 5)

Analyte	Spiking (ng/mL)	Sample (ng/mL)	Recovery (%)
AM	10	9.9	99
	20	18.4	92
	50	52.0	104
MAM	10	11.5	115
	20	20.9	105
	50	49.3	99
MDA	10	11.4	114
	20	19.3	96
	50	45.9	92
PTM	10	11.1	111
	20	18.9	94
	50	46.9	94
MDMA	10	10.5	105
	20	19.1	96
	50	48.4	97
MDEA	10	10.1	101
	20	20.3	102
	50	45.1	90

Conclusion

We have shown that CE-MS/MS is well suited for the analysis of amphetamine and its derivatives in urine. The analytical method was based on CE-MS/MS to simultaneously determine amphetamine and its derivatives. This method presented efficient separations with high sensitivity. It was able to detect amphetamine with an LOD as low as 20 pg/mL, and with a migration time lower than 7 minutes. The modified QuEChERS extraction was simple and efficient, obtaining certified accurate and precise recoveries ranging from 90 to 115 %. Using a PVA-coated silica capillary permitted EOF suppression, increasing the separation efficiency with no peak tailing effect. The proposed method is simple, and uses a small amount of sample with low reagent consumption and low waste generation. It also has the potential to be successfully applied to other samples in forensic analysis.

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