Detection of Cocaine in Seized Drug Samples by Capillary Electrophoresis Tandem Mass Spectrometry

Application Note

Forensic Toxicology

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Abstract

A methodology using capillary electrophoresis with tandem mass spectrometry detection (CE-MS/MS) was developed, and applied to determine the cocaine content of seized drugs. The cocaine separation among other concomitants by capillary electrophoresis was achieved in just 4.0 minutes using 30 mM ammonium formate, pH 6.5, as a background electrolyte (BGE). The correlation coefficient of the calibration curve in the range of 0.02 to 1 µg/mL was upper 0.999, and the limits of detection (LOD) and limits of quantification (LOQ) were 0.003 µg/mL and 0.01 µg/mL, respectively. The relative standard deviation repeatability (intraday and interday) was less than 6.5% (peak area). The analysis of seized suspected illegal drugs revealed the presence of cocaine in 93% of samples (n = 11), in amounts ranging from 0.04 to 429 mg/g, showing a high variability of concentrations of cocaine. The user can change the provider and, due to the large variability of concentration, be lead to an accidental overdose.

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Introduction

The development and application of analytical methods to drugs of abuse is essential. Cocaine sold on the street is commonly mixed with a wide variety of adulterants. These include anesthetics, cornstarch, caffeine, or sugar, as well other substances carefully chosen to hide the physical characteristics of the cocaine and impede its detection [1]. The large variability of concentration found in the samples could lead the user to an accidental overdose.

The analysis of seized samples of cocaine by police laboratories is performed using different methodologies [2,3]. In forensic analysis, the results must prove the nature of the substance in criminal processes. In this context, a seized substance is commonly analyzed using different techniques, according to the regulations of each country. The United States Department of Justice [4] classifies chemical analysis techniques into three classes: A, B, and C, according to their specificity and susceptibility to false-positive or negative errors. A conclusive verdict is obtained only when two chemical analyses (a class A test and an A, B, or C test) or three tests (two class B and the third by B or C test) are provided. Table 1 indicates some examples of these techniques.

Table 1. Categories of Analytical Techniques According to the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations (United States Department of Justice)

Category A	Category B	Category C
Infrared spectroscopy	Capillary electrophoresis	Color tests
Mass spectrometry	Gas chromatography	Fluorescence spectroscopy
Nuclear magnetic resonance spectroscopy	Ion mobility spectrometry	Immunoassay
Raman spectroscopy	Liquid chromatography	Melting point
X-ray diffractometry	Microcrystalline tests	Ultraviolet spectroscopy
	Pharmaceutical identifiers Thin layer chromatography	
	Cannabis only: Macroscopic examination Microscopic examination	

Capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) is well suited for the analysis of seized illicit drugs. CE techniques can separate a wide variety of solutes including compounds that are highly polar, cationic, or anionic species, thermally labile or nonvolatile, with high efficiency and selectivity employing a low amount of the seized drug. When coupled with a triple quadrupole mass spectrometer, CE provides an orthogonal approach to analysis in a single analytical run, covering the chemical analysis techniques in classes A and B. CE-MS/MS combines the advantages of both techniques, so that quantitative and migration time information, with molecular masses or fragmentation patterns, can be obtained in one analysis. This would present a high probability to elucidate the chemical compound and its concentration using analytical curve or standard addiction methods.

This application note describes a CE-MS/MS method for the determination of cocaine in seized drug samples. The method is sensitive, fast, and produces a low amount of waste, making it an environmentally friendly technique with high selectivity that assists in the identification of seized samples.

Experimental

Conditions, CE

Instrument	Agilent 7100 CE system
Background electrolyte	30 mM ammonium formate, pH 6.5
Applied voltage	25 kV
Fused-silica capillary	50 µm id × 75 cm total length (p/n 160-2650-5)
Injection	12 seconds at 100 mbar
Temperature	25 °C

Conditions, MS

Instrument	Agilent 6430 Triple Quadrupole LC/MS
Ion mode	ESI, positive ionization
Sheath liquid	5 mM ammonium formate - methanol (50:50, v/v, methanol/H_2O), pH 6.5
Flow rate	6.0 µL/min
Capillary voltage	3,000 V
Drying gas flow (N_2)	8 L/min
Drying gas temperature	180 °C
Nebulizer pressure	10 psi
MRM transitions	*m/z* 304 →182 (16 V) and *m/z* 304 →82 (48 V)
Dwell time	50 ms

The capillary was preconditioned by washing with 0.1 mol/L NaOH solution (2 minutes), deionized water (3 minutes), and BGE (3 minutes) before each injection with BGE for 30 seconds. During the electrophoretic run (at 25 kV), –17 mbar pressure was applied to the inlet vial to compensate for the ESI suction effect [5]. The most intense transition, *m/z* 304 ⟶ 182, was used as a quantifying ion, and *m/z* 304 ⟶ 82 as a qualifying ion for the confirmation of cocaine in the seized drugs samples. Figure 1 shows the molecular structure and pKa values of cocaine as well as two other common contaminates found in cocaine sold on the street. The cocaine (COC), cinnamoylcocaine (CMC), and tropacocaine (TPC) are positively charged amines at pH 6.5; the amino group and pKa values are highlighted.

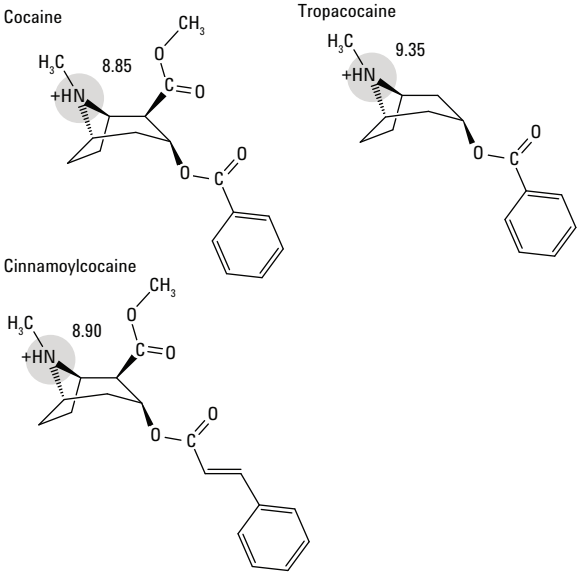

Figure 1. Cocaine, cinnamoylcocaine and tropacocaine structures and pKa values calculated at www.chemicalize.org (accessed in January 2016).

Sample preparation

Samples of seized cocaine were provided by Rio de Janeiro General Department of Technical and Scientific Police. All samples were homogenized with a mortar and pestle, solubilized in 2 mL of methanol:water (50:50 v:v), and diluted a thousand-times or more according to need. The sample was then filtered through a 0.45 µm regenerated cellulose membrane (p/n 5190–5307), and directly injected into the CE-MS/MS equipment.

Results and Discussion

Background electrolyte and sheath liquid composition, applied potential, and hydrodynamic injection were optimized for separation efficiency and sensitivity. Figure 2 shows the MRM electropherogram of a standard solution of cocaine in the background electrolyte (BGE). The time of migration was only 4.0 minutes.

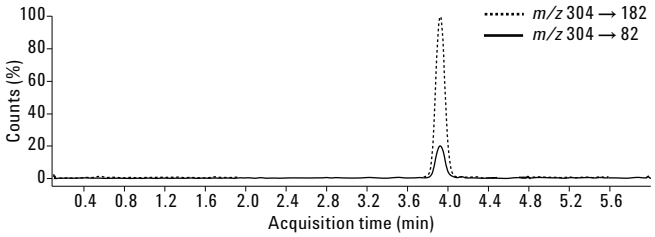

Figure 2. MRM electropherogram at optimum conditions of 0.2 µg/mL cocaine in BGE.

The linearity of the analytical curve was studied in BGE at seven different concentration levels, and analyzed in triplicate, ranging from 0.02 to 1 µg/mL. Using Agilent MassHunter Workstation Quantitative Analysis software, the correlation coefficient (R^2) calculated by linear regression presented a value greater than 0.999, as shown in Figure 3.

The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.003 and 0.01 µg/mL, respectively, defining the LOD as three times the baseline noise, and the LOQ as the concentration that produced a signal 10 times the baseline noise, in a time close to the migration time of cocaine. Interday precision data concerning peak areas expressed as the relative standard deviation percentage (RSD %) was lower than 6.5%. Table 2 presents the results obtained for cocaine in different drugs seizure samples, based on the standard calibration curve.

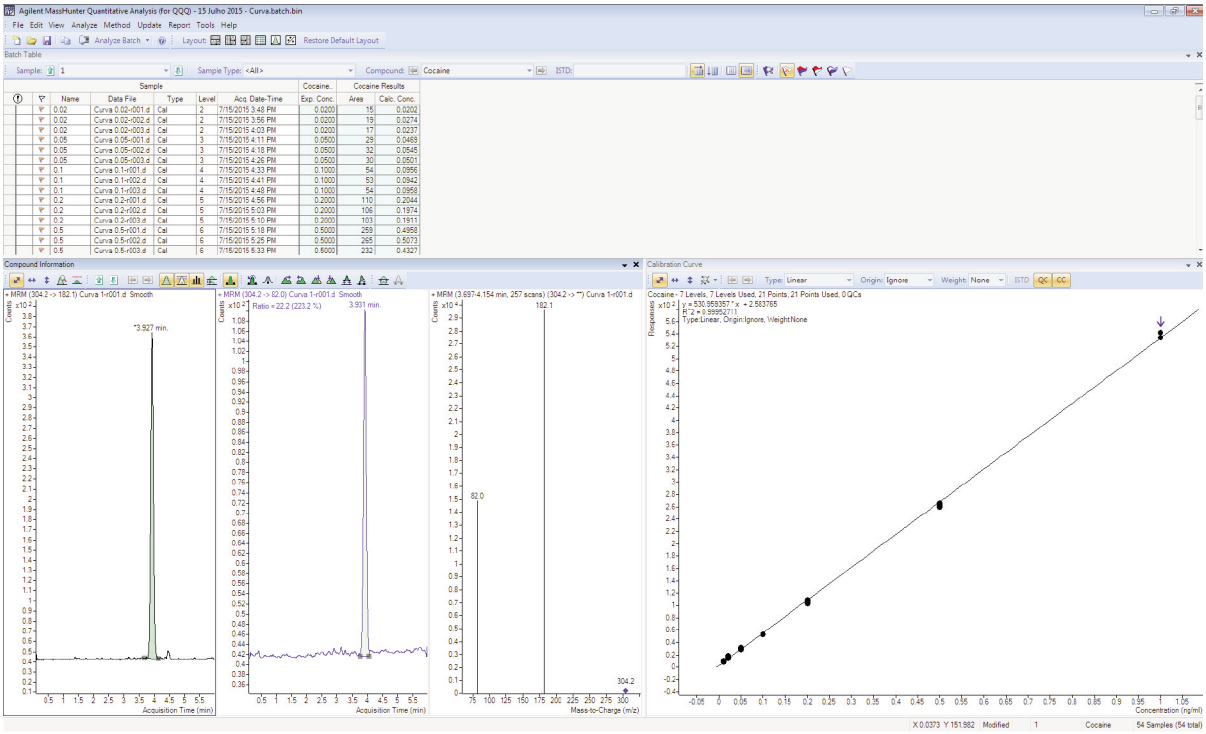

Figure 3. Calibration curve using Agilent MassHunter Quantitative Analysis software.

Table 2. Results for Cocaine in Seizure Samples (n = 3)

Sample	Result	Conc. (mg/g)	RSD (%)
1	Positive	0.040 ± 0.001	2.5
2	Positive	35 ± 2	5.7
3	Positive	242 ± 7	2.9
4	Positive	2.40 ± 0.08	3.3
5	Positive	429 ± 27	6.3
6	Positive	13.9 ± 0.7	5.0
7	Positive	19.7 ± 1.1	5.6
8	Positive	0.098 ± 0.005	5.1
9	Negative	ND	–
10	Positive	75 ± 2	2.7
11	Positive	2.42 ± 0.05	2.0

ND = not detected.

Analysis of seized illegal drugs revealed the presence of cocaine in 93% of samples (n = 11), in amounts ranging from 0.04 to 429 mg/g. The concentrations of cocaine found in seized drug samples were similar to those reported in the literature [6]. Figure 4 shows the MRM electropherogram of siezed drug samples, showing the presence of cocaine, cinnamoylcocaine, and tropacocaine. The presence of these minor cocaine-related alkaloids suggests that they might be useful for comparison purposes [7].

The similarity of the pKa values of COC, CMC, and TPC make electrophoretic separation difficult (Figure 4). The separation was mainly based on the size of the molecule; as a result, hydrated ionic radius, and thus the time migration, was 3.29, 3.34, and 3.41 minutes for TPC, COC, and CMC, respectively. The insufficient separation showed in the electropherogram was fully overcome using the MS/MS approach. Since different mass transitions lead to high selectivity, this approach is not viable for other detectors. Thus, CE-MS/MS can be considered a powerful technique to analyze seized samples. The high selectivity, precision, and sensitivity of CE-MS/MS presents adequate analysis according to the regulations of each country.

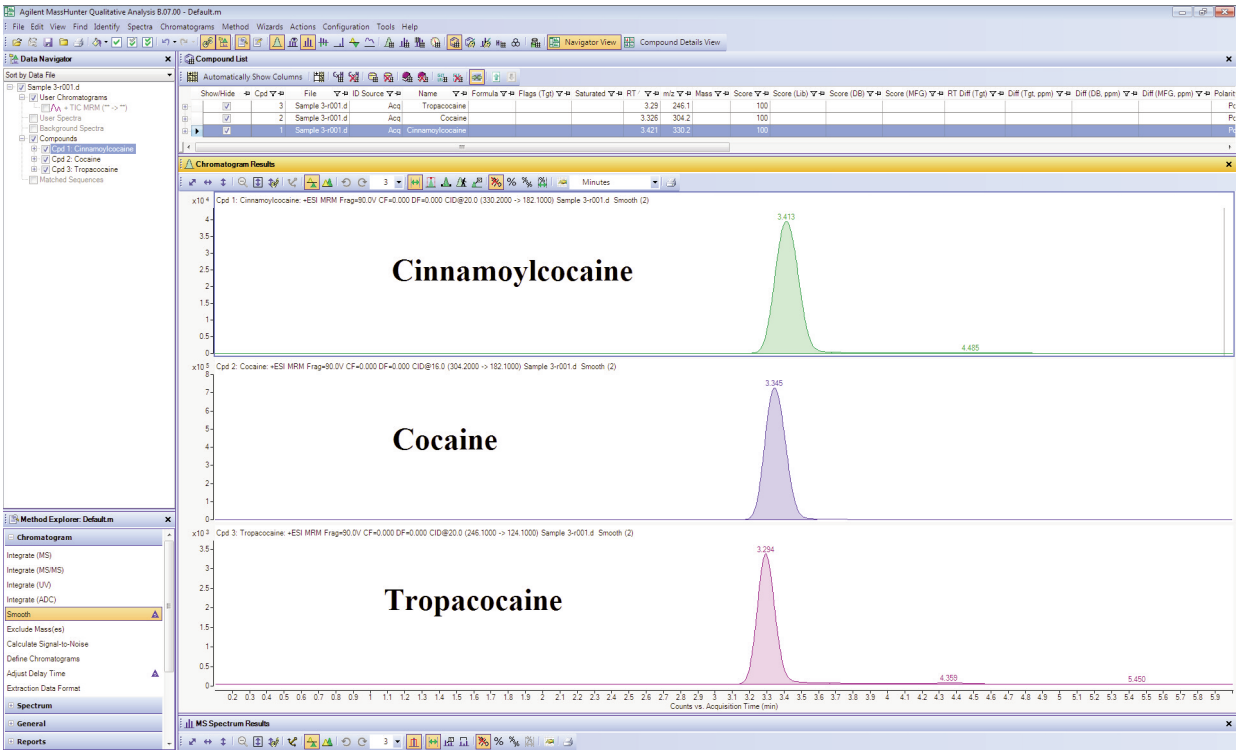

Figure 4. MRM electropherogram of apprehended drug samples, showing the presence of cocaine (COC) as well cinnamoylcocaine (CMC) and tropacocaine (TPC).

Conclusion

The proposed method of CE-MS/MS detection of cocaine in drugs is simple. It uses a small amount of the seized drugs with low chemical consumption, and has easy solubilization and dilution procedures, without need for extra cleanup steps. In addition, the method is fast: less than 5 minutes per sample, and presents linear calibration curves and excellent precision data for replicate injections. The sensitivity and specificity of the analytical method demonstrates its potential for successful application to cocaine analysis. This technique also provides class A and B tests that are required by SWGDRUG, and are met in a single run analysis. In fact, this method is ideal even when cocaine is present in very low concentrations, or is adulterated with several other compounds. If the variability found in cocaine concentrations is too large, a user may suffer an accidental overdose if purchasing a more pure drug from someone other than their usual supplier.

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