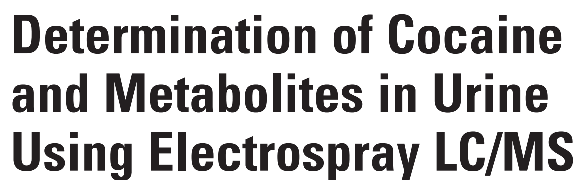

Determination of Cocaine and Metabolites in Urine Using Electrospray LC/MS

Application Note

Drug Testing

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Abstract

A rapid, simple, and sensitive electrospray LC/MS method has been developed for the quantitative analysis of cocaine and benzoylecgonine in urine using electrospray with the Agilent 1100 LC/MSD system. Urine samples were extracted using solid phase extraction cartridges, and the drug and metabolite were analyzed without derivatization using an isocratic separation and selected ion monitoring (SIM).

Introduction

Cocaine has two metabolites, benzoylecgonine (BE) and norcocaine, that are frequently analyzed as markers of cocaine use. The well-established GC/MS analysis of cocaine and BE requires derivatization of the metabolite. Derivatization adds additional variables from the derivatization process and can also introduce aggres-sive derivatizing reagents into the analytical system. These basic molecules show excellent sensitivity

in electrospray mass spectrometry, and the analysis of cocaine and both metabolites can be carried out without a derivatization step. The same solid-phase extraction (SPE) developed for the GC/MS analysis can be used for the LC/MS analysis.

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Materials and Methods

The Agilent 1100 Series system included a binary pump, vacuum degasser, autosampler, thermostatted column compartment, diode-array detector, and an LC/MSD. The LC/MSD was used with the electrospray ionization (ESI) source. The diode-array detector was used during method development only. Complete system control and data evaluation was carried out using the Agilent ChemStation for LC/MS.

Sample Preparation and Extraction

Drug-free urine was fortified with known concentrations of the analytes for preparation of standard curves. Control samples were fortified with known concentrations of the analytes prepared from separate lots of stock solutions. Clean-Screen SPE columns (ZSDAU020, United Chemical Technologies) were conditioned with 3 mL of methanol and 3 mL of Milli-Q water, followed by 1 mL of 100 mM phosphate buffer, pH 6. Urine (1 mL) was mixed with 1 mL of the phosphate buffer, spiked with deuterated internal standards (cocaine-d3 and benzoylecgonine-d3) and loaded on the conditioned column. The column was sequentially washed with 2 mL of Milli-Q water, 2 mL of 100 mM HCl, and 3 mL of methanol. The column bed was dried at full vacuum for five minutes, and the analytes were eluted with 3 mL of dichloromethane/isopropanol/ammonium hydroxide (78/20/2). The eluate was evaporated to dryness with a stream of air at 40°C. The final sample residue was reconstituted in 50 µL of LC mobile phase, and 20 µL was injected for analysis by LC/MS.

Results and Discussion

In the analysis of cocaine metabolites, it is important to be able to distinguish the isobaric BE and norcocaine to allow accurate interpretation of results. The chromatography for this method was therefore optimized to separate BE from norcocaine, and isocratic conditions were found which allow for rapid analysis without column re-equilibration. Figure 1 shows the separation of cocaine, norcocaine and BE using these conditions.

MS parameters which were optimized for this analysis included fragmentor voltage (to give the most intense protonated molecule for each analyte), capillary voltage (for maximum signal), and spray chamber parameters (for maximum signal with minimum noise).

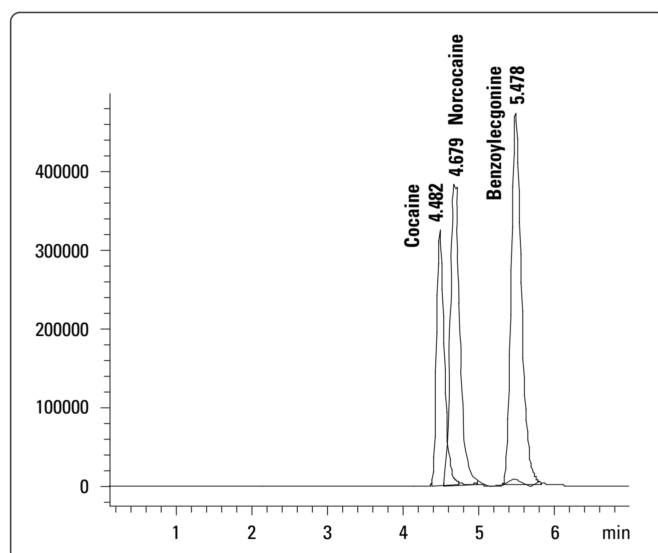

Figure 1. Isocratic separation of cocaine, norcocaine and BE.

Chromatographic Conditions

Column:	Metasil Basic 3 µm, 3 × 150 mm (Metachem)
Mobile phase:	A = 0.1% formic acid in water
	B = methanol
Isocratic:	51% B
Flow rate:	0.2 mL/min
Column temp:	40°C
Injection vol:	20 µl
Diode-array detector:	signal: 234, 8 nm; reference: 360, 100 nm

MS Conditions

Source:	ESI
Ionization mode:	positive
Vcap:	1500 V
Nebulizer:	20 psig
Drying gas flow:	10 L/min
Drying gas temp:	300°C
SIM ions:	*m/z* 290.1 (BE and norcocaine)
	m/z 293.1 (BE-d3)
	m/z 304.1 (cocaine)
	m/z 307.1 (cocaine –d3)
Peak width:	0.10 min
Time filter:	On
Fragmentor:	70 V

Figure 2 shows the extracted ion chromatograms (EICs) for blank urine fortified with the internal standards. Figure 3 shows the EICs for a urine standard fortified at 25 ng/mL.

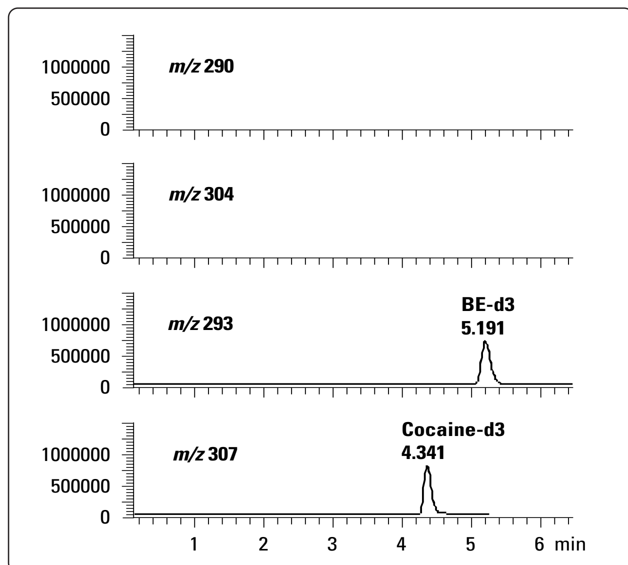

Figure 2. Extracted ion chromatograms of blank urine extract.

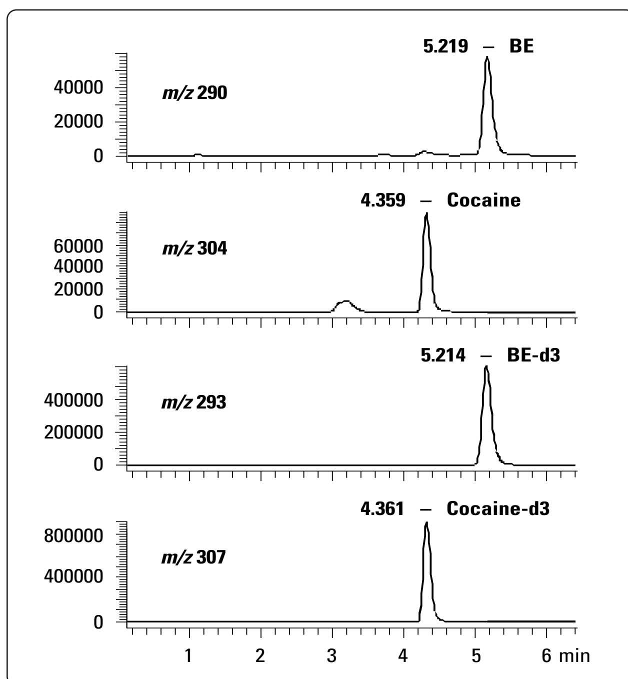

Figure 3. Extracted ion chromatograms of fortified urine extract (25 ng/mL).

The calibration range used for this analysis was 25–1000 ng/mL for both cocaine and BE. The calibration curves were linear across the calibration range without special weighting or curve treatment. Figure 4 shows typical calibration curves for cocaine and BE, with correlation coefficients (r^2) greater than 0.99 (0.99925 for cocaine and 0.99491 for BE).

Figure 5 shows the EICs of a positive urine sample found to contain 640 ng/mL cocaine and approximately 2700 ng/mL BE. The BE quantitation is an estimate, as the concentration is above the calibrated range of the method. Note that norcocaine can be clearly identified because it is chromatographically separated from benzoylecgonine which has the same mass.

Quality control samples fortified with 50 ng/mL and 150 ng/mL of each analyte gave quantitation results within 12% of the target concentration for cocaine and 3% for BE (see Table 1). Coefficients of variation were 7.1% and 5.1% for cocaine and BE respectively as shown in Table 1.

Table 1. Method accuracy and precision. Target concentrations were 50 ng/mL for cocaine and 150 ng/mL for BE.

	Cocaine	BE
	48.25	146.47
	47.06	155.69
	47.41	158.97
	46.21	148.50
	38.80	147.29
	40.89	146.57
	41.38	167.06
	42.68	159.81
Mean	44.085	153.795
Std Dev	3.570	7.734
C.V.*	7.1%	5.1%

*coefficient of variation = (mean/target)*100

These results compare well with an established GC/MS assay in which intra-assay coefficients of variation were less than 7% for both analytes when tested at 10, 25, 100, and 200 ng/mL.[1] The GC/MS assay gave quantitation results within 4% of the target concentration for cocaine and 5% for BE.

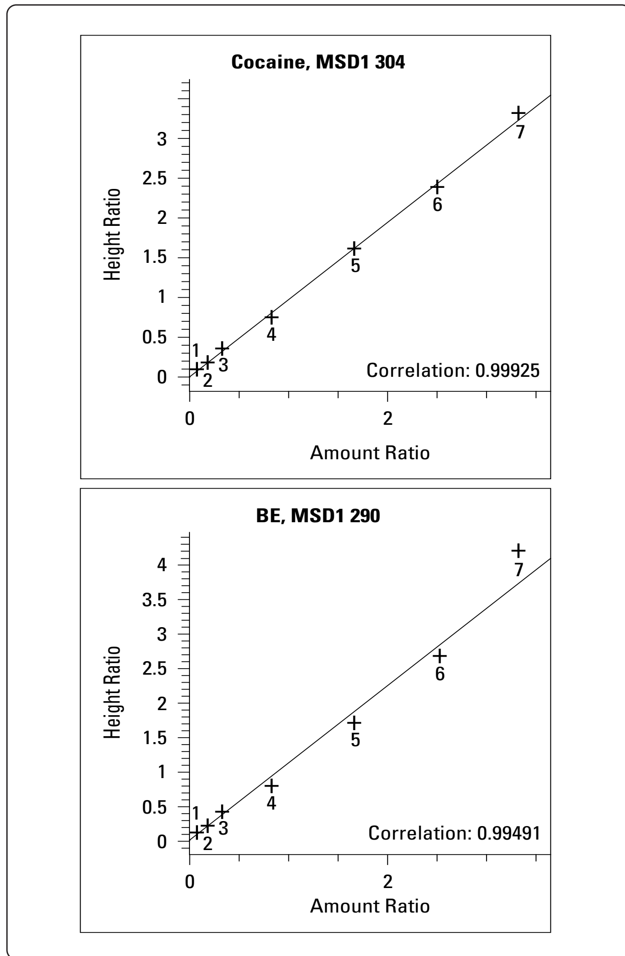

Figure 4. Calibration curves for cocaine and BE.

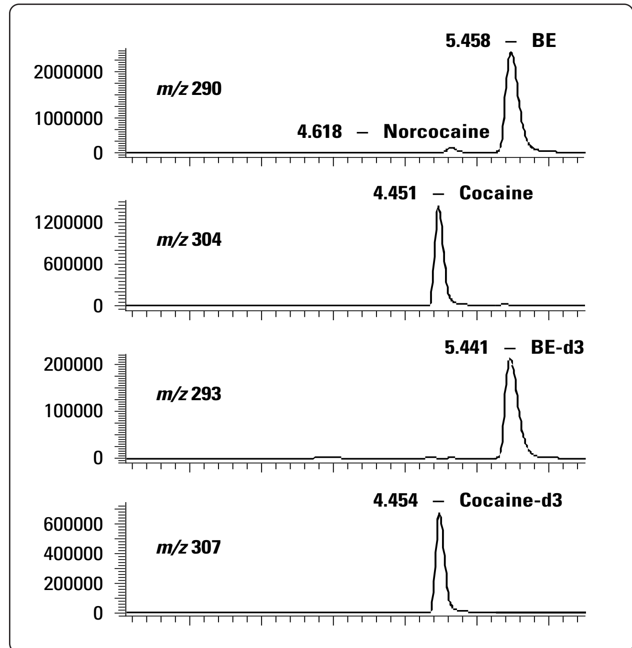

Figure 5. Extracted ion chromatograms from the extract of a positive urine sample.

Conclusions

This note describes an electrospray LC/MS method suitable for routine measurements of cocaine, BE and norcocaine in urine. The assay has a linear range of 25–1000 ng/mL and the precision and accuracy of this analytical method compare favorably to those of the well-established GC/MS method for cocaine and BE. The sample preparation uses previously-described solid phase extraction technology widely used in forensic laboratories and requires no special modifi-cations. In comparison to an existing GC/MS method for these analytes, the LC/MS analytical method is simpler because it does not require derivatization, which involves aggressive reagents, derivatization time, and additional variability. In addition, the overall cycle time for one analysis is shorter for the LC/MS method than for the GC/MS method. This LC/MS method offers several advantages over traditional GC/MS assays with comparable quality of data.

References

1. Crouch, D.J.; Alburges, M.E.; Spanbauer, A.C.; Rollins, D.E.; Moody, D.E.; Chasin, A.A. *Journal of Analytical Toxicology* 1995, 19, 352–358.

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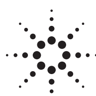

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