

Quantitation of Benzoylecgonine in Urine for SAMHSA-Mandated Workplace Drug Confirmation Testing Using LC-MS/MS

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Key Words

SAMHSA, workplace drug confirmation testing, TSQ Quantum Ultra, TraceFinder, benzoylecgonine, cocaine, SOFT-DFSA

Goal

The goal of this work was to develop a specific and robust dilute-and-shoot quantitative method that meets SAMHSA cutoffs for the confirmation of benzoylecgonine in urine. An additional goal was to have the sample processing method be suitable for all SAMHSA panel compounds to reduce duplicate processing for the different classes of compounds.

Introduction

In the United States, federal employees and public transportation workers are required to pass a pre-employment drug screen known as the NIDA5 or SAMHSA panel. The panel is divided into 5 groups: opiates, amphetamines, cocaine (benzoylecgonine), cannabis (THCA), and phencyclidine (PCP). Per SAMHSA requirements, these five groups are screened with immunoassays and, in the past, confirmed by gas chromatography/mass spectrometry (GC/MS). In October 2010, SAMHSA approved the use of liquid chromatography/mass spectrometry (LC/MS) for confirmation of workplace drug testing samples. This note describes the use of LC/MS with a triple-stage quadrupole mass spectrometer to confirm benzoylecgonine (BE), a metabolite of cocaine.

Experimental

Sample Preparation

Urine was spiked with a deuterated internal standard (BE- d_3) and hydrolyzed with β -glucuronidase. Although BE does not require hydrolysis, other compounds in the SAMHSA panel such as the opiates and THC do. Adding this step enables all SAMHSA panel compounds to be processed with one method. Methanol was added to the hydrolysis mixture and the resulting mixture was centrifuged. Supernatant was further diluted and subjected to LC/MS confirmatory analysis.

Liquid Chromatography

Chromatographic separations were performed with a Thermo Scientific™ Accela™ 600 pump and Accela Open autosampler. The analytical column was a Thermo Scientific Hypersil GOLD aQ™ column (50 x 4.6 mm, 1.9 μ m particle size). The column was maintained at room temperature. The injection volume was 20 μ L. Mobile phases A and B consisted of 5 mM ammonium formate with 0.1% formic acid in water (Fisher Chemical HPLC grade, W5) and methanol (Fisher Chemical HPLC grade, A452), respectively. Mobile phase C was acetonitrile/1-propanol/acetone (45:45:10). The total run time was 4.75 minutes.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra™ triple-stage quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe. Two selected-reaction monitoring (SRM) transitions were monitored for both BE and its deuterated internal standard, BE- d_3 , to provide ion ratio confirmation (IRC). Data was analyzed using Thermo Scientific TraceFinder™ software.

Validation

Standard curves were prepared by fortifying pooled blank human urine with BE. Quality control (QC) samples were prepared in a similar manner at concentrations corresponding to the lower limit of quantitation (LLQC), low (LQC), middle (MQC), and high (HQC) end of the calibration range. Inter-run variability and robustness were determined by processing six replicates of each QC level along with a calibration curve as outlined in the Sample Preparation section on three different days. Matrix effects were investigated by comparing peak areas of analyte and internal standard prepared in twelve different lots of urine to those of a sample prepared in water.

Results and Discussion

The limit of quantitation (LOQ) for BE in this method was 5 ng/mL, which exceeds the SAMHSA confirmation requirement of 100 ng/mL as well as the Society of Forensic Toxicology – Drug Facilitated Sexual Assault (SOFT-DFSA) cut-off of 50 ng/mL. Figure 1 shows an SRM chromatogram at the LOQ. Results were linear from 5 to 2000 ng/mL. Figure 2 shows a representative calibration curve. Quality control results for the validation are shown in Table 1. The quality controls showed good precision and accuracy.

Table 1. Statistics for quality controls run during validation

	LLQC 5 ng/mL	LQC 15 ng/mL	MQC 100 ng/mL	HQC 1500 ng/mL
%CV	5.92%	3.95%	3.68%	9.55%
%Bias	1.52%	-5.36%	-8.87%	1.63%
n	18	18	18	18

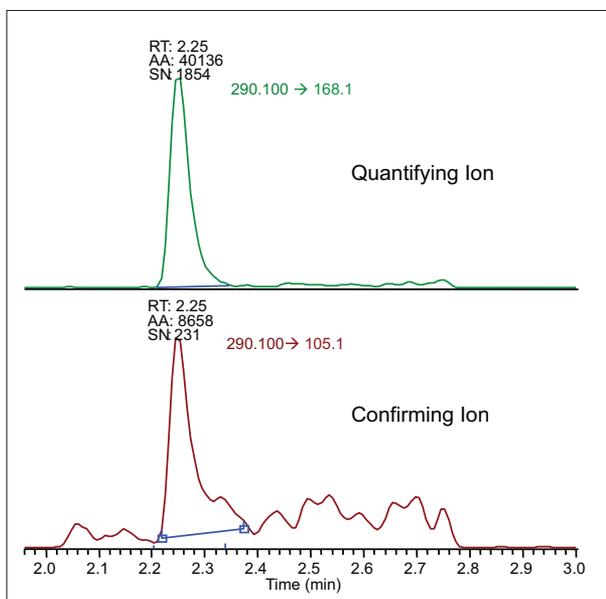


Figure 1. Chromatogram of BE at 5 ng/mL showing quantifying and confirming ions

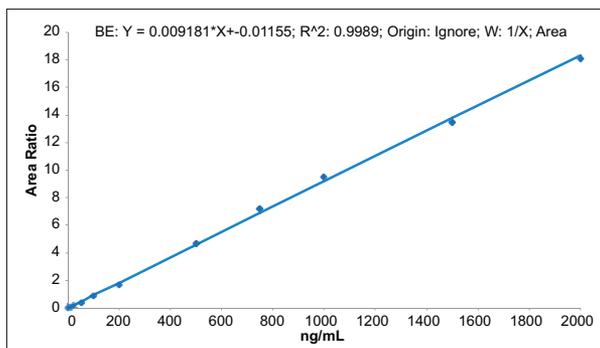


Figure 2. Representative calibration curve for BE in urine

Matrix effects

Peak areas of BE spiked at 10 ng/mL in 12 different lots of urine showed absolute recoveries between 87% and 100% compared to a sample in water, indicating no significant matrix effects. For BE-*d*₃, the absolute recoveries were between 93% and 102%.

Conclusion

- A method with simple dilute-and-shoot sample preparation for the confirmation of BE in urine was developed.
- This method is suitable for SAMHSA-mandated workplace drug testing confirmation, meeting cutoff requirements within a 4.75 minute run.
- The sample processing method enables all SAMHSA panels to be processed at once.
- The method showed good linearity across the required calibration ranges.
- Controls indicated good method precision and robustness.
- No matrix effects were observed.

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