Application Note: 10007

Analysis of Benzoylecgonine in Urine Using Single Quadrupole GC/MS

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Key Words

- DSQ Single Quadrupole GC/MS
- Benzoylecgonine
- Cocaine
- Drugs of Abuse
- Forensics
- Toxicology

Abstract

Cocaine is one of the oldest known drugs. Although it has some medically acceptable uses, the potential for cocaine abuse and addiction raises concern. In the United States, federal guidelines require certain employers to test for cocaine use. Additionally, other public and private entities may test for its use.[1] This testing is commonly performed in urine samples. Typically, initial analysis for cocaine and cocaine metabolites by immunoassay techniques provides tentative evidence of use. Then, confirmation of cocaine use is accomplished through quantitative analysis by gas chromatography/mass spectrometry (GC/MS) of benzoylecgonine (BE), the major urinary metabolite of cocaine. The Thermo Scientific DSQ™ is a quadrupole mass spectrometer that provides the sensitivity and specificity necessary to confirm BE in urine.

To analyze BE by GC/MS, it must be extracted from the urine and then derivatized. The method described here utilizes a 2 mL sample size with all standards and controls prepared in a matrix by spiking with known amounts of BE and a deuterated internal standard. Samples were extracted using a solid phase extraction technique, and the resulting extracts were derivatized with MSTFA with 1% TMCS. Analyses of the derivatized extracts were accomplished on the DSQ in electron ionization (EI) selected ion monitoring mode (SIM).

The calibration curve ranged in concentration from 15 to 1500 ng/mL and had a correlation coefficient of 0.9960. A cutoff concentration of 150 ng/mL and a maximum mandated limit of quantitation (LOQ) of 60 ng/mL are specified for this method.[2] A commercial control, with a nominal value of 112.5 ng/mL, was used to test the calibration.

Introduction

Cocaine is a federally regulated drug, the use of which is tested for a variety of reasons, including employment screening and criminal conviction. It is an extremely addictive drug and stimulant that affects the central nervous system and has short and long term effects on the human body. Cocaine can typically enter the body by being inhaled, injected, or smoked in its various forms. Benzoylecgonine (BE) is a major metabolite of cocaine. The presence of BE in a sample of urine confirms the use of cocaine by the individual undergoing testing. BE can be detected at appreciable levels for up to 48 hours after cocaine has entered the body. The United States Substance Abuse and Mental Health Services Administration (SAMHSA) has set the GC/MS confirmation cutoff for BE in urine at 150 ng/mL. Additionally, SAMHSA requires that laboratories have a LOQ for this assay that is, at most, 40% of the cutoff value. For BE, this mandated LOQ is 60 ng/mL. Since BE contains a carboxyl functional group that impedes successful GC analysis, samples prepared for BE confirmation are typically derivatized to overcome chromatographic issues. While many derivatization methods can be used, this application describes the use of a silylating reagent, which caps the hydroxyl portion of the COOH with a trimethylsilyl group. This enhances response for BE and provides diagnostic high mass ions for SIM analysis. The DSQ (Figure 1), which employs a curved prefilter that minimizes noise created by excited neutrals, was used for this analysis.

Figure 1: DSQ GC/MS

Methods

Two mL aliquots of known blank urine were placed in clean glass tubes. Each tube was then spiked with an appropriate amount of a methanolic standard of benzoylecgonine (Cerilliant Corporation, Round Rock, TX), to generate a calibration curve with points at 15, 30, 60, 150, 600, 1500, and 6000 ng/mL. Two mL of unspiked urine served as a negative control, while two mL of commercial control, MAS(R) DOA-GC/MS Level G2, were used to verify calibration accuracy. One mL of 0.1M phosphate buffer (pH 6.0) and 100 µL of BE-D3 standard (Cerilliant), equivalent to 150 ng/mL as internal standard, were added to each sample. Samples were then mixed, and the pH was checked. Sample pH was adjusted to 6, as necessary, by addition of 0.1M monobasic (lowers pH) or 0.1M dibasic (raises pH) phosphate buffer.

Solid phase extraction was accomplished using Clean Screen® DAU SPE columns from United Chemical Technologies (Bristol, PA, Cat# ZSDAU020) and a 12-place vacuum manifold (Supelco, Bellefonte, PA). The extraction columns were conditioned by sequential

application of 3 mL methanol, 3 mL DI water, and 1 mL 0.1 M phosphate buffer (pH 6.0). The columns were not allowed to dry between applications. The prepared samples were loaded onto the column and extracted under low vacuum at a rate of 1 mL/min. After extraction, the columns were rinsed sequentially with 2 mL DI water, 2 mL 0.1M HCl, and 3 mL methanol. After application of the last methanol rinse, the columns were dried under high vacuum (10 inches Hg) for 5 minutes. The benzoylecgonine was eluted by gravity into clean glass tubes with 3 mL of methylene chloride/isopropanol/ammonium hydroxide elution solvent (78:20:2, prepared fresh daily).[3] The pH of this solution was between 11 and 12. The eluates were evaporated to dryness under nitrogen at 40 °C. A 50 µL aliquot of MSTFA with 1% TMCS (United Chemical Technologies) was added to each sample. The samples were then mixed, capped, and derivatized for 15 minutes at 70 °C. The samples were cooled to room temperature, 50 µL of ethyl acetate were added and vortex-mixed, and then the samples were transferred to GC ALS vials with glass inserts (National Scientific Company, Duluth, GA).

The DSQ used for this analysis was a 70 L/s turbo pump, EI only system. The TRACE GC Ultra™ was configured with a standard split/splitless injector and an AS 3000 autosampler. The analytical column was a 15 m x 0.25 mm i.d. x 0.25 µm Rtx®-5MS, 95% dimethyl/5% diphenyl polysiloxane column (Restek Corporation, Bellefonte, PA). A 5 mm silanized glass liner was used in the injector. The AS 3000 was programmed to perform a 1 µL hot-needle injection. For a hot needle injection, the sample is drawn up into the syringe, then the plunger draws up air to clear the needle of any sample. The autosampler then inserts the needle into the injector for 3 seconds to allow the needle to reach the desired temperature, and the sample is injected. This allows the sample to volatilize without any discrimination due to condensation on the needle or due to the portion of the sample in the needle volatizing before the portion of the sample in the syringe barrel.

Oven Method	
Initial Temperature (C):	190
Initial Time (min):	1.00
Number of Ramps:	1
Rate #1 (deg/min):	30.0
Final Temperature #1 (C):	270
Hold Time #1 (min):	1.00
Left SSL Method	
Base Temperature (C):	265
Mode:	Splitless
Splitless Time (min):	0.10
Left Carrier Method	
Mode:	Constant Flow
Initial Value (mL/min):	1.00
Vacuum Compensation:	On

Figure 2: TRACE GC Ultra instrument method. This information is saved with every data file and is accessed from Xcalibur's™ Qual Browser.

Acquisition Time:	GC Run Time	
Source Temp:	250 °C	
Start Time:	3.00 minutes	
Detector gain:	3 x 10^5 (Multiplier voltage: 1403 V)	
Scan Event 1		
Scan Mode: SIM		
Mass: 240.0	Width: 1.0	Dwell Time: 80.0
Mass: 346.0	Width: 1.0	Dwell Time: 80.0
Mass: 349.0	Width: 1.0	Dwell Time: 80.0
Mass: 361.0	Width: 1.0	Dwell Time: 80.0
Mass: 364.0	Width: 1.0	Dwell Time: 80.0

Figure 3: DSQ instrument method, also from Qual Browser.

The split/splitless injector was set to 265 °C. A short-duration splitless injection was performed with a splitless time of 6 seconds. The carrier gas (He) was set to a constant flow of 1.0 mL/min. The initial temperature on the TRACE GC Ultra was set to 190 °C. The high temperature at the beginning of the analytical run allowed the BE to elute from the column as quickly as possible. Because of the high boiling point of BE, it was not necessary to use the solvent to recondense the sample at the head of the column. The first temperature was held for one minute. The GC was then ramped at 30 °C/min to a final temperature of 270 °C and held for 1 min (Figure 2). The source temperature was set to 250 °C. The instrument was tuned using the optimal sensitivity option to ensure the best sensitivity across the entire mass range. The detector gain was set to 3 x 10^5, which resulted in a detector voltage of 1402 V. A set of SIM masses was used to detect BE and its deuterated internal standard, BE-D3, and these masses and dwell times are summarized in Figure 3.

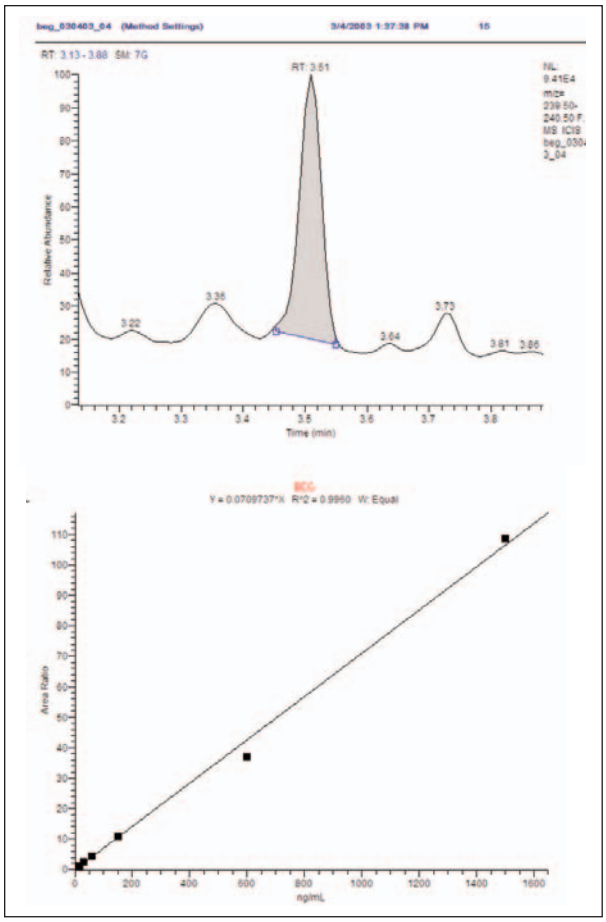

Figure 4: View of the calibration curve from Xcalibur's Quan Browser. The top window shows the integration of BE, mass 240, at 30 ng/mL. The calibration curve on the bottom shows a correlation coefficient of 0.9960.

SAMPLE TYPE	SAMPLE NAME	AREA	LEVEL	AMOUNT	AMOUNT
Std Bracket	15	166568	1	15.000	16.972
Std Bracket	30	293698	2	30.000	33.416
Std Bracket	60	650506	3	60.000	64.448
Std Bracket	150	1501789	4	150.000	151.926
Std Bracket	600	5481448	5	600.000	522.976
Std Bracket	1500	13324114	6	1500.000	1530.351
Unknown	etoac blank			N/A	N/F
Unknown	mb			N/A	N/F
Unknown	neg			N/A	N/F
Unknown	G2	1300508			119.620
Unknown	150	1408386			146.633
Unknown	150	1302817			143.567
Unknown	150	1361196			144.788
Unknown	150	1350047			141.664
Unknown	150	1245122			141.396

Figure 5: Integrated values for the quantitation ion for the sample set. The coefficient of variation of five injections of the cutoff is 1.53% with a % difference of the mean of -4.26%.

SAMPLE NAME	AREA 240	AREA 346	ION RATIO 346/240	AREA 361	ION RATIO 361/240
15	166568	14306	8.589	35669	19.613
30	293698	25912	8.823	57046	19.423
60	650506	46627	7.168	109087	16.769
150	1501789	99486	6.624	260189	17.325
600	5481448	354752	6.472	930622	16.978
1500	13324114	838657	6.294	2251734	16.900
	Average	7.328	**Average**	17.835	
	+20 Percent	8.794	**+20 Percent**	21.402	
	-20 Percent	6.183	**-20 Percent**	14.508	

Figure 6: The ion ratios for qualifier ions were determined using a normal average of these ions from the calibration curve.

Results

BE was eluted and quantitated in less than 5 minutes. The sample set included a solvent blank, negative control, matrix blank, set of calibrators, control sample, and a set of five samples to test reproducibility at the cutoff level. A linear calibration curve was used, and the correlation coefficient was calculated to be 0.9960 (Figure 4). Mass 240 was used as the quantitation mass for BE, and mass 243 was the quantitation mass for BE-D3. The qualifier BE ions used for confirmation were 346 and 361. The determination of ion ratio range was calculated based on a normal average of the calibration curve. The ion ratios were calculated and found to be within a range of +20% (Figure 5). A commercial control, designated as G2, was used to check calibration accuracy. The sample had a stated concentration of 112.5 ng/mL.[4] When analyzed and calculated against the curve, the result was 119 ng/mL. This is a +6.2% difference from the actual concentration, which is well within an acceptable range of ±20% (Figure 6). The reproducibility at the cutoff level gave a coefficient of variation of 1.53%.

Conclusion

This method was found to be both sensitive and reproducible. The LOQ for this method was 15 ng/mL, well below the mandated level of 60 ng/mL. A series of replicate injections at the cutoff level of 150 ng/mL gave a coefficient of variation of 1.53%, and the percent difference of the mean was 4.26%. This method was also linear, yielding a calibration curve from 15 ng/mL to 1500 ng/mL with a correlation coefficient of 0.9960. Since this method was developed in urine matrix, the DSQ has shown its ability to handle matrix contamination and still deliver superior results.

References

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2. Guidance Document for Laboratories and Inspectors, November 2002 Edition. National Laboratory Certification Program, OMB No. 0930-0158.
3. Clean Screen (registered trademark), United Chemical Technologies, Inc. 2731 Bartram Rd. Bristol, PA, 19007-6893.
4. DOA GC/MS Liquid Assayed Drugs of Abuse Control, Medical Analysis Systems, Inc. (MAS), 5300 Adolfo Road, Camarillo, CA 93012.

Acknowledgement

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