A Validated Liquid Chromatography–Atmospheric Pressure Chemical Ionization-Tandem Mass Spectrometry Method for Quantitation of Cocaine and Benzoylecgonine in Human Plasma

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Abstract

In order to support studies on various medication protocols for the treatment of cocaine abuse, an accurate, precise, and sensitive (2.5 to 750 ng/mL) liquid chromatography–tandem mass spectrometry assay was developed to determine cocaine and benzoylecgonine in human plasma. Cocaine-d5 and benzoylecgonine-d5 were added as internal standards and samples were subjected to solid-phase extraction. Cocaine recovery was 94.4% and benzoylecgonine was 80.3% at 2.5 ng/mL. The selected reaction monitoring of parent ions at m/z 304 and 290 resulted in strong fragments at m/z 182 and 168 for cocaine and benzoylecgonine, respectively. The method was fully validated. The mean measured concentration at the 2.5 ng/mL, the lower limit of quantitation, was within 10.8% of the target and the precision determined at the low (5 ng/mL), medium (50 ng/mL), and high (650 ng/mL) quality controls ranged from 0.9 to 6.2% CV. Cocaine and benzoylecgonine concentrations in plasma treated with 1% NaF showed changes of less than 10% when maintained at room temperature for up to 7 h and no significant changes when subjected to three freeze-thaw cycles. The concentrations of cocaine and benzoylecgonine remained stable in plasma samples stored at -20°C for up to 11 months. Methanolic stock solutions of both analytes are stable, staying within 2% of the freshly prepared stock solutions, when stored at -20°C for up to 235 days. Both extracted analytes reconstituted in methanolic solutions are stable for up to seven days whether stored at -20°C or at room temperature on the autosampler. The method is rugged, rapid, and robust and has been applied to the batch analysis of more than 700 samples during pharmacokinetic profiling to assess potential interactions between intravenous (i.v.) cocaine challenge and treatment medications. Results from three of these subjects receiving 40 mg (i.v.) cocaine demonstrate the utility of the method.

Introduction

The use of medications to treat drug addiction has grown in recent years (1). From only methadone in 1970, the list of approved medications has grown to include naltrexone and levorotatory acetylmethadol with buprenorphine to soon be added for opiate dependence and replacement nicotine for smoking. Other drugs such as antidepressants are also used to reduce the potential initiation or relapse to drug abuse.

Current efforts are focusing on medications to assist in the treatment of cocaine use (2–4) and will likely expand to methamphetamine use in the near future. During the medication treatment, it is important to accurately and precisely determine the plasma concentrations of not only the medication drugs, but also the targeted drugs of abuse in order to evaluate progress, safety, and effectiveness of the treatment. As part of our research efforts to support studies on the pharmacokinetics, safety, and efficacy of various medication protocols for the treatment of cocaine abuse, we have developed validated methods to quantitate medication drugs such as methylphenidate (5) and selegiline (unpublished data). This report describes our current efforts to develop an analytical method based on mass spectrometry for the determination of cocaine and benzoylecgonine in human plasma. Though developed to support safety, efficacy, and pharmacokinetic studies, the method can also be applied to forensic testing of biological matrices for this drug of abuse and its primary metabolite.

Although a validated positive ion chemical ionization gas chromatography–mass spectrometry (GC–MS) method for plasma cocaine and metabolites had previously been developed in our laboratory (6,7), the procedure is relatively time consuming. Because of the large number of plasma samples that require analysis for safety, efficacy, and pharmacokinetic studies, we undertook development of a liquid chromatographic–tandem mass spectrometric method (LC–MS–MS) that requires less time and offers precision, accuracy, and sensitivity superior to existing...
GC–MS assays (6–10). Although LC–MS and LC–MS–MS assays have been reported for determination of cocaine and benzoylecgonine in biological matrices (11,12), including hair (13), urine (14–18), meconium (19,20), dried blood spots (21), and rat plasma (20,22), to our knowledge, no LC–MS–MS method for quantitation of cocaine and benzoylecgonine in human plasma has been reported. Also, most of the published reports emphasize positive identification of cocaine and metabolites in the biological samples. Quantitative aspects of these methods, especially involving the use of quality-control samples (QCs) for long-term stability studies, have not been fully discussed.

In this study, we report an LC–MS–MS method coupled with solid-phase extraction (SPE) to determine cocaine and benzoylecgonine in human plasma. Validation of the method follows recent guidelines (23). The method has been applied to the analysis of hundreds of plasma samples from patients undergoing treatment for cocaine addiction. Selected data from one clinical study illustrate practical applicability of the assay.

**Experimental**

**Materials**

Cocaine, cocaine-d3, benzoylecgonine, and benzoylecgonine-d3 were purchased from Radian International (now Cerilliant, Austin, TX). All solvents (isopropanol, methylene chloride, and methanol) were high-performance liquid chromatography grade and purchased from Burdick & Jackson (Muskegon, MI). Potassium hydroxide and concentrated hydrochloric acid were obtained from Mallinckrodt Specialty Chemicals, Inc. (Paris, KY). Ammonium hydroxide was purchased from Fisher Co. (Fair Lawn, NJ). Acetic acid was obtained from Sigma (St. Louis, MO). Formic acid was purchased from J.T. Baker (Phipsburg, N J). The Clean Screen Extraction columns (ZSDAU020) were purchased from United Chemical Technologies, Inc. (Torrance, CA).

Calibrator and control preparation

Calibrator samples were freshly prepared in duplicate just prior to extraction by spiking a series of 1-mL blank plasma samples with cocaine and benzoylecgonine to give concentrations of 2.5, 5.0, 10, 30, 90, 350, and 750 ng/mL. QCs were prepared in advance at concentrations of cocaine and benzoylecgonine of 2.5, 5.0, 50, and 650 ng/mL. One-milliliter aliquots were carefully pipetted into 16 × 100-mm silanized borosilicate glass tubes, and the QCs were immediately stored at −20°C until removed for analysis.

Extraction

Extraction was performed using a modification of our previously described procedure (6), that was heretofore only briefly described (7). QCs and appropriate study samples were removed from the freezer and allowed to thaw at room temperature. Calibrator samples were prepared fresh. As soon as the QCs and appropriate study samples had thawed, the samples were spiked with the internal standard solution to give a concentration of 25 ng/mL each of cocaine-d3 and benzoylecgonine-d3. The plasma samples were then vortex mixed and made acidic by adding 4 mL of 0.1M acetate buffer (pH 4.0). After vigorous vortex mixing for 20 s and centrifugation at 2400 rpm for 10 min, the supernatants were subjected to SPE. Each SPE column was conditioned by the following sequence: first with 3 mL methanol, followed by 3 mL deionized water, and then 2 mL acetate buffer (pH 4). No aspiration was necessary. Between additions care was taken to not allow the SPE beds to dry. While the columns were still wet, the supernates of the centrifuged plasma samples were added and allowed to pass through the columns under the force of gravity. The loaded columns were then washed first with 2 mL of 0.1M HCl, followed by 3 mL methanol, and then the columns dried under the house vacuum for at least 15 min. The retained analytes were eluted with 3 mL of a freshly prepared mixture of methylene chloride/isopropanol/NH₄OH (80:20:2, v/v/v), and the eluates were collected in clean 13 × 100-mm labeled glass tubes. The eluates were dried at 40°C under a stream of air. The residues were reconstituted with 100 μL of methanol/0.1% formic acid in Milli-Q water (10:90) and transferred to labeled autosampler vials.

**LC–MS–MS**

Analyses were performed using a ThermoQuest model TSQ 7000 triple-stage quadrupole MS equipped with an Xcalibur data system (San Jose, CA). The LC system consisted of a Waters 626 pump, a Waters 600 S controller, and an inline degasser (Milford, MA). The LC was interfaced to the MS by means of an atmospheric pressure chemical ionization (APCI) ion source. The autosampler was a CTC 2005 model (used in conjunction with crimp-capped 0.7-ml conical autosampler vials, San Jose, CA). Milli-Q water was placed in wash vial 1, and methanol was placed in wash vial 2. Autosampler set-up consisted of an injection volume of 20 μL and four wash cycles to clean the syringe and the injection loop, one before and three after each injection. Each wash cycle consisted of injection of 20 μL water followed by injection of 20 μL methanol. The LC was operated isocratically with a flow rate of 150 μL/min, and the mobile phase consisted of 50% methanol and 50% water with 0.1% formic acid. The Xcalibur software controlled all instrument parameters. MS conditions for the TSQ 7000 were as follows: corona current, 5 μA; corona voltage, 4.5 kV; vaporizer temperature, 375°C; heated capillary tube temperature, 150°C; and sheath gas (high-purity nitrogen) pressure, 20 psi. The LC–MS–MS acquisition time was 5 min. The following selected reaction monitoring (SRM) transitions were used to quantitate the analytes and internal standards in human plasma: m/z 304 (MH+ to 182, cocaine; m/z 307 (MH+) to 185, cocaine-d3; m/z 290 (MH+) to 168, benzoylecgonine; and m/z 293 (MH+) to 171, benzoylecgonine-d3. The scan time for each transition was 0.2 s. Argon gas at 3–3.5 mtorr was used as the collision agent. The offset energy of the collision chamber was −15 eV. Electron multiplier voltage was 2300 eV. A divert valve was set to direct the LC flow initially to waste for 1 min, then to the APCI source for 4 min, and then back to the waste. Prior to every batch run the MS–MS parameters were adjusted and optimized by tuning the TSQ 7000 using a sample mixture of cocaine and benzoylecgonine (0.1 ng/μL) delivered at 10 μL/min by a syringe pump and mixed via a T-union with 150 μL/min isocratic mobile phase delivered by the LC pump. Finally, before each run, the instrument performance was evaluated by injecting 20 μL of an LC–MS–MS check solution consisting of 10 pg/μL each of cocaine and benzoylecgonine.
cocaine, benzoylecgonine, and the corresponding isotopomers.

Quantitation
The Xcaliber's LCquan software provided a standard method for the quantitative calculations. The peak areas for all of the SRMs were automatically integrated, and the area ratios (cocaine/cocaine-d_3 and benzoylecgonine/benzoylecgonine-d_3) of the calibrators were used to generate linear response curve with 1/Y^2 weighting for cocaine and benzoylecgonine. The response curves were used to recalculate the concentrations of calibrators, QCs, and study samples.

Clinical samples
The clinical data reported here are from a larger study testing for interactions between cocaine and transdermal selegiline (24). Participants were experienced cocaine users who were medically and psychiatrically healthy aside from their drug abuse. The three subjects reported here were healthy aside from their drug abuse. These three subjects were African American, between 35 and 45 years of age with 12-24 years of cocaine use experience and reported using cocaine 12-24 out of the 30 days preceding their study admission. Body weights were 72, 105, and 50 kg for subjects 2, 3, and 6, respectively. The data presented are from single sessions in which each subject received a single dose of 40 mg cocaine HCI administered intravenously (i.v.) in a volume of 1 mL over 1 min. At the time of this session subjects had lived in the residential laboratory 12 days and had received a 20-mg cocaine dose 24 h earlier. Blood was collected and plasma prepared as follows. Sodium fluoride (0.125 mg) and 0.125 mL of 10% acetic acid were added to a 5-mL green-top tube (sodium heparin). Five milliliters of blood was drawn into the tube, and the tube was immediately inverted 3 or 4 times. Samples were spun for 10 min at 3000 rpm at 4°C and aliquoted into a polypropylene tube. Samples were then stored at -70°F until they were shipped to the testing laboratory.

Results and Discussion
APCI versus ESI
Both APCI (21) and electrospray (ESI) (13-15,19,20,22) ion sources have been successfully used for the analysis of cocaine and/or benzoylecgonine in biological samples. Preliminary evaluation using reference solutions indicated that APCI and ESI gave similar responses for cocaine and benzoylecgonine. APCI was selected as the method of ionization because it is less subject to ion suppression due to co-eluting components of biological extracts (25) and tends to give a more reproducible response over the course of an extended run. For example, when we compared the peak areas of a calibrator (350 ng/mL) injected near the beginning to those of a duplicate calibrator injected near the end of a run, we saw very little change in signal intensity (Table I). Although this experiment substantiates the robustness of APCI that we desired for the method, no parallel experiments were performed with ESI that would demonstrate it is any less robust.

Chromatography
Selected ion chromatograms of blank plasma fortified with internal standard demonstrate that no significant peaks were evident at retention times for cocaine and only minor peaks at the retention time for benzoylecgonine (Figure 1, left). When analytes were added at the lower limit of quantitation (LLOQ) of 2.5 ng/mL, sharp peaks with signal-to-noise ratios of 16 were seen for cocaine and benzoylecgonine (Figure 1, right).

Calibration and linearity
The calibration curves for both analytes are linear from 2.5 to 750 ng/mL with mean ± S.D. for r^2 from 6 runs equal to 0.997 ± 0.003 (cocaine) and 0.997 ± 0.002 (benzoylecgonine). This quantitation range was chosen based on the statistical results compiled by Paly et al. (26). In that report, chronic cocaine abusers were given free access to cigarettes containing 75 mg of cocaine paste. The subjects developed and maintained cocaine plasma concentration of 253 to 932 ng/mL over a 90-min smoking period, during which they smoked 8 to 10 cigarettes. These cases represent extremes of cocaine abuse. In our analysis of 766 plasma specimens, 5 samples had cocaine concentrations above 750 ng/mL and none had benzoylecgonine concentrations above the upper limit of quantitation.

In the studies where cocaine was given in i.v. doses of 40 mg, we

Table I. Stability of the APCI Ion Source for Cocaine and Benzoylecgonine Analyses

<table>
<thead>
<tr>
<th>Batch</th>
<th>No. of in-between samples</th>
<th>Cocaine</th>
<th>area</th>
<th>diff</th>
<th>area</th>
<th>diff</th>
<th>Cocaine-d_3</th>
<th>area</th>
<th>diff</th>
<th>Benzoylecgonine</th>
<th>area</th>
<th>diff</th>
<th>Benzoylecgonine-d_3</th>
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<td>33209676</td>
<td>14%</td>
<td>1885467</td>
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<td></td>
<td></td>
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<td></td>
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<td>5%</td>
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</tr>
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</table>
could routinely measure cocaine concentrations in samples collected 6 h after dosing and benzoylecgonine in samples collected 24 h after dosing.

Accuracy and precision
Intra-assay accuracy and precision were determined from the analysis of five replicates at each concentration within a single analytical batch (Table II). The mean measured concentration for the QCs at the LLOQ was within 10.8% of the target and the mean measured concentrations for the low, medium, and high QCs were all within 4.2% of their target concentrations. The CVs ranged from 0.9 to 6.2%. The interassay accuracy and precision were determined from five analytical runs performed on separate days (Table II). Interassay measured concentrations for the QCs at the LLOQ were within 8.0% of target and within 4.3% of target for the low, medium, and high QC samples. The interassay CVs ranged from 1.0 to 6.3%.

Extraction efficiencies
The recoveries of cocaine and benzoylecgonine from human plasma were evaluated at three plasma concentrations (2.5, 50, and 650 ng/mL). Two sets of plasma, each containing five replicates at each concentration, were used. One set (A) was spiked with cocaine and benzoylecgonine before extraction, and another set (B) was spiked with cocaine and benzoylecgonine after extraction. For both sets the internal standard was added before extraction. Percent recovery was determined at each concentration by dividing the average peak-area ratio from set A by the average peak-area ratio from set B and multiplying by 100. The recovery

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**Table II. The Precision and Accuracy for LC–MS–MS Determination of Cocaine and Benzoylecgonine in Human Plasma**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Target (ng/mL)</th>
<th>% Target</th>
<th>% CV</th>
<th>% Target</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLOQ</td>
<td>2.5</td>
<td>110.8</td>
<td>4.0</td>
<td>103.6</td>
<td>6.2</td>
</tr>
<tr>
<td>QC1</td>
<td>5</td>
<td>97.0</td>
<td>1.9</td>
<td>97.4</td>
<td>5.1</td>
</tr>
<tr>
<td>QC2</td>
<td>50</td>
<td>98.8</td>
<td>2.0</td>
<td>98.8</td>
<td>1.4</td>
</tr>
<tr>
<td>QC3</td>
<td>650</td>
<td>95.6</td>
<td>0.9</td>
<td>96.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* The intra-assay values are the mean of five QCs analyzed in a single batch. The interassay values are the mean of the average results from five different runs where 3–5 QCs were run in each batch.

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**Figure 1.** Representative ion current profiles from analysis of a blank human plasma sample (left panel) and plasma fortified with 2.5 ng/mL each of cocaine and benzoylecgonine (right panel). Both samples were spiked with 25 ng/mL of each of the internal standard. A smoothing factor of 11 Gaussian was used.
for cocaine ranged from 92.1% at 650 ng/mL to 94.4% at 2.5 ng/mL. Benzoylcegonine recovery ranged from 73.1% at 650 ng/mL to 80.3% at 2.5 ng/mL.

Specificity
The assay specificity was evaluated by analysis of plasma collected from six volunteers who had not used cocaine. Three replicate aliquots of each plasma sample were spiked with internal standard at 25 ng/mL. A single sample of each plasma lot was also prepared with no internal standard added. Peak-area ratios for signals at the retention times of cocaine and benzoylcegonine were determined and compared to those of aqueous samples fortified at the LLOQ concentration. For cocaine, these peak-area ratios corresponded to an overall mean of 3.7% of the LLOQ's peak-area ratio, with individual percents of LLOQ peak-area ratios ranging from 0.0 to 11.2%. For benzoylcegonine, these peak-area ratios corresponded to an overall mean of 0.66% of the LLOQ's peak-area ratio, with individual percents of LLOQs ranging from 0.15 to 2.3%.

Short-term room-temperature stability and freeze-thaw stability in plasma
The instability of cocaine and benzoylcegonine in human plasma at room temperature has been well established (27,28). For purposes of this validation, we needed to assure stability during realistic time limits for thawing of plasma prior to aliquoting for extraction. QC at 25 and 650 ng/mL (n = 3) were removed from frozen storage 7 and 4 h prior to 0-h controls. Stability of the cocaine and benzoylcegonine in plasma after three freeze-thaw cycles was also determined. QC controls (n = 3) were taken out of the freezer and allowed to thaw for 0.5 h and returned to frozen storage for at least 12 h. The cycle was repeated two times (three freeze-thaw cycles) prior to analysis with 0-h controls and calibration standards.

The concentration of cocaine in plasma maintained at room temperature for up to 4 h did not change by more than -6.0% from target concentrations, or more than -9.5% from target after 7 h, while benzoylcegonine concentrations increased slightly over the same time period (Table III). These values are still acceptable, but may be indicative of the known instability of cocaine in plasma at room temperature (27,28). The freeze-thaw results show that the cocaine and benzoylcegonine concentrations in plasma do not change significantly when the plasma is subjected to three freeze-thaw cycles (Table III).

Long-term stability
Short-term and freeze-thaw stability are commonly included in publications describing biochemical methods, but long-term stability is rarely presented (29). The long-term stability was evaluated by analyzing low and high QCs (n = 3) when QCs were freshly prepared, after storage at -20°C for three months, and after storage at -20°C for 11 months. Cocaine and benzoylcegonine concentrations showed no significant change in plasma stored at -20°C for up to 11 months (Table IV).

Stock-solution stability
The stability of cocaine and benzoylcegonine in methanolic stock solutions was evaluated. A fresh stock solution containing 1 μg/mL each of cocaine and benzoylcegonine was prepared and placed in plasma at -20°C. An aliquot of this fresh stock was stored at room temperature for 7 h and then returned to storage at -20°C. On the following day, these two sets of stock solutions, and one that had been stored at -20°C for 235 days, were taken for analysis using three aliquots from each stock solution.

Mean (n = 3) peak-area ratios for cocaine and benzoylcegonine stayed within 8% of the 0-h control after 7 h of room temperature storage. Mean peak-area ratios stayed within 2% of the 0-h control after 235 days of -20°C storage. These results indicate that methanolic solutions of both analytes are stable when stored at -20°C for up to 235 days.

Processed-sample stability
The stability of processed samples (extracted and reconstituted in methanol) stored at -20°C for seven days was evaluated as follows: QC's with cocaine and benzoylcegonine at concentrations of 2.5, 5.0, 50, and 650 ng/mL were analyzed on the day that the samples were extracted (Day 0). The autosampler vials containing the processed samples (both QC's and calibration standards) were then stored in a freezer for seven days. After storage for this time, the calibrator and QC's were reanalyzed along with a freshly extracted calibration curve. Concentrations were then determined from the freshly extracted calibration curve and from the calibration standards that had been stored. Cocaine and benzoylcegonine were found to be stable in processed samples stored at -20°C for seven days, retaining accuracy and precision under these storage conditions as shown from use of either calibration curve (Table V).

The stability of processed samples was also studied in a similar manner during storage on the autosampler for three days and seven days at ambient temperature. The mean concentrations of the low, medium, and high QC samples were well within ± 15% of their target concentrations, and the mean concentration of the
Table V. Cocaine and Benzoylecgonine Stability in Stored Extracts

<table>
<thead>
<tr>
<th>Sample (ng/mL)</th>
<th>Stored Curve (%Target ± %CV)</th>
<th>Fresh Curve (%Target ± %CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stored 7 days at -20°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>116.0 ± 2.4</td>
<td>107.6 ± 3.3</td>
</tr>
<tr>
<td>5</td>
<td>98.8 ± 7.7</td>
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<td>98.0 ± 1.2</td>
<td>91.2 ± 1.3</td>
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<tr>
<td>650</td>
<td>96.0 ± 0.5</td>
<td>89.3 ± 0.5</td>
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<tr>
<td>Benzoylecgonine</td>
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</tr>
<tr>
<td>2.5</td>
<td>105.6 ± 5.3</td>
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<tr>
<td>650</td>
<td>97.5 ± 0.9</td>
<td>90.3 ± 0.9</td>
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<tr>
<td>Stored 7 days on the autosampler</td>
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<td>Cocaine</td>
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<tr>
<td>2.5</td>
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<td>102.0 ± 1.2</td>
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<td>96.5 ± 1.1</td>
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<td>Benzoylecgonine</td>
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<td>103.2 ± 5.8</td>
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<td>50</td>
<td>94.2 ± 1.7</td>
<td>97.4 ± 1.6</td>
</tr>
<tr>
<td>650</td>
<td>94.3 ± 0.8</td>
<td>97.8 ± 0.8</td>
</tr>
</tbody>
</table>

Figure 2. Plasma concentrations of cocaine (A) and benzoylecgonine (B) in three individuals that received a single 40-mg intravenous dose of cocaine.

LLOQ samples were within ± 20% their target concentration using either the stored or fresh calibration curves (Table V).

Application

The current method has been used to measure cocaine and benzoylecgonine in over 700 human plasma samples in support of two National Institute on Drug Abuse sponsored studies on potential cocaine interactions with either selegiline or methylphenidate. The results from three subjects from the former study, who had only received cocaine (40 mg, i.v.), are shown in Figure 2. Cocaine reached maximum plasma concentrations of 308, 131, and 386 ng/mL at 10 min for subjects 2, 3, and 6, respectively. Cocaine concentrations then rapidly decreased in an apparent first-order rate with apparent terminal half-lives of 58 to 73 min. These half-lives are consistent with the majority of those reported for intravenous cocaine (30–33), although Cone and coworkers (32) have described the occasional subject with prolonged terminal half-lives of approximately 9 h. The areas under the curve (ng/mL*h) of 287, 218, and 525 are also consistent with those reported for similar doses of cocaine (32,33).

Benzoylecgonine plasma concentrations reached observed maxima of 263, 162, and 550 ng/mL at 4, 2, and 1.5 h, respectively. The areas under the curves (0 to 6 h, ng/mL*h) were 756, 536, and 1600, respectively. Although these showed some variability, the maximum and areas under the curve were consistent within subject; the ratio of benzoylecgonine to cocaine areas under the curve being 2.46, 2.63, and 3.05, respectively. The variability can be partially explained by the different weight of individuals. Areas under the curve adjusted for weight (μg/mL*h*kg) were 20.7, 22.9, and 26.4 for cocaine and 18.9, 17.0, and 27.5 for benzoylecgonine. These results add to the current literature on the pharmacokinetics of cocaine and benzoylecgonine following intravenous administration of cocaine and demonstrate that the current method provides results consistent with previous studies.

Conclusions

This study demonstrates that the LC–MS–MS method described can accurately quantitate cocaine and benzoylecgonine in human plasma, at concentrations ranging from 2.5 to 750 ng/mL. The current method also demonstrates that cocaine and benzoylecgonine in plasma samples are stable for up to three freeze-thaw cycles as long as the duration of each thaw did not exceed 0.5 h. Because of the known instability of cocaine in plasma at room temperature, extraction of plasma samples should begin as soon as possible after samples are removed from the freezer. The underivatized cocaine and benzoylecgonine were stable in extracts stored at -20°C and on an autosampler maintained at ambient temperature for up to seven days. Methanolic stock solutions are shown to be stable for up to 235 days when stored at -20°C.

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References


