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Direct determination of ecgonine methyl ester and cocaine in rat plasma, utilizing on-line sample extraction coupled with rapid chromatography/quadrupole orthogonal acceleration time-of-flight detection

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Abstract

Our current experiments assess the applicability of on-line sample extraction with coupled rapid chromatography systems to quadrupole orthogonal acceleration time-of-flight (Q-TOF) detection for the quantitative analysis of cocaine (COC), and ecgonine methyl ester (EME) in rat plasma. Experiments were performed on a Q-TOF instrument, operated in the MS/MS mode. Quantitation was achieved utilizing the most prominent parent–daughter transition and internal standard calibration techniques (COC-d_3: IS). The calibration curves produced for EME and COC ranged from 5.0 to 10 000 and 0.5 to 10 000 ng/ml, respectively. Equations of regression line and correlation coefficients for the pseudo-multiple reaction monitoring (MRM) ion abundance ratio and the corresponding calibration concentrations (r^2) were as follows: $y = 0.0003 + 0.0703x$ ($r^2 = 0.9921$) for EME and $y = 0.0032 + 0.0035x$ ($r^2 = 0.9997$) for COC. The system repeatability, given as percent coefficient of variation (% CV) of mean peak-area ratios, was assessed using 50 injections of a rat plasma sample from the pharmacokinetic study. The analyses were performed over the course of 5 days, rendering % CVs for EME and COC of 0.73 and 0.58, respectively. This method suggests that on-line sample extraction coupled with fast liquid chromatography/quadrupole orthogonal time-of-flight mass spectrometry may be a viable alternative for quantitative analysis of EME and COC in rat plasma.

Keywords: HPLC/(Q-TOF); Cocaine and ecgonine methyl ester analysis; On-line sample extraction

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1. Introduction

Cocaine (COC) is a naturally occurring stimulant derived from the leaves of the *Erythroxylum coca* plant, found mainly in South America. Current estimates report more than 2 million people in the US alone, are frequent COC users [1]. Approaches aimed at reducing cocaine toxicity and abuse through administration of therapeutics that block one or more of the dopamine receptors in the brain, or exhibit a cocaine-like action on these receptors, have not been sufficiently effective in preclinical or clinical investigations [2]. New strategies directed at sequestering cocaine in the periphery, or increasing its rate of metabolic decomposition, have generated considerable interest [3–5]. An essential aspect in assessing the efficacy of these new antibody treatments is the ability to determine ecgonine methyl ester (EME) and COC levels in plasma. Therefore, a sensitive and specific bioanalytical assay would provide insight into the in-vivo disposition of COC and EME.

Currently, quantitative methodology for the determination of COC and EME in biological matrices includes: high-performance liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS), and liquid chromatography/mass spectrometry (LC/MS) [6–8]. Methodologies based on HPLC, GC/MS, and to a lesser degree on LC/MS, necessitate a substantial amount of sample cleanup in order to attain desired levels of detection. Consequentially, sample preparation requirements make HPLC and GC/MS analysis for a range of biologically important compounds a time-consuming process.

LC/MS is an alternative approach that can be implemented to reduce off-line sample preparation, as it allows direct analysis of thermally-labile and non-volatile compounds [9–11]. Furthermore, the utility of atmospheric pressure ionization mass spectrometry (API/MS) has gained widespread popularity as an analytical tool for the quantitative determination and structural characterization of pharmacologically active compounds in biological matrices [12,13]. The high sensitivity and selectivity provided by API when coupled to liquid chromatography/tandem mass spectrometry (LC/MS/MS) have reduced the time required for method development and sample analysis of drugs and their metabolites in biological matrices [10,14].

Time-of-flight (TOF) instruments have been primarily used for large molecule analysis, where the need for higher resolution, mass accuracy, and mass range precludes the use of quadrupole instruments [15,16]. However, the coupling of API interfaces to TOF instruments and the development of rapid chromatographic methods have expanded its role in typical bioanalytical scenarios. Additionally, TOF instruments have two intrinsic advantages over liner quadrupole scanning instruments: innate selectivity and enhanced duty cycles. The ability of TOF instruments to discriminate mass differences well-below 1 μ, affords a level of selectivity comparable to tandem mass analysis, and allows relaxing of separation considerations.

Rapid chromatographic methods can generate extremely narrow peaks, with widths ranging from 2 to 4 s [10,17]. At this speed, quadrupole instruments may begin to alias peaks, due to shorter duty-cycles. As a result, an insufficient number of data points are collected to adequately describe the chromatographic peak — resulting in a loss of accuracy, precision, and sensitivity. The ability of the Q-TOF to collect full spectra every 10–100 μs, allows many spectra to be collected over very narrow peaks. The rapid rate of spectral collection produces a high degree of spectral averaging, increasing the sensitivity of the measurement, while maintaining good definition of the chromatographic peaks for accurate quantitation.

TOF instruments have not been widely deployed for quantitation due to limited dynamic range (generally, two orders of magnitude). This limitation is attributable to the detection system. The Q-TOF detection system is a time-to-digital converter (TDC). It registers each ion arrival time in histogram memory as a specific pulse-count event. With high ion currents, the TDC cannot discriminate between a single, and multiple ion arrivals. This leads to lower mass centroids, and inaccurate measurement of peak areas. To overcome this problem, multiple-anode detectors with multiple parallel channels of ion detection are under development, and software controlled

'dead-time' corrections are made to allow accurate registration of ions over a wide ion-current range, improving the dynamic range of the measurement. Recent investigations have examined the utility of API/o-TOF and API/Q-TOF for quantitative bioanalytical applications [18–20].

We report here an assay for the simultaneous quantitative analysis of COC and EME in rat plasma with low ng/ml detection levels. This method is based upon on-line solid phase extraction (SPE)/fast-HPLC/electrospray ionization (ESI)/Q-TOF, and affords simultaneous monitoring of the pharmacokinetic characteristics of COC and EME following intravenous administration of cocaine to rats.

2. Experimental section

2.1. Materials

Cocaine (free base), EME, and cocaine-d_3 (COC-d_3) were obtained from Sigma–Aldrich (Saint Louis, MO). A weighing of each analyte was made from neat material and standard stock solutions (300 000 ng/ml) of these compounds were prepared in HPLC grade dimethyl sulfoxide obtained from J.T. Baker Company (Phillipsburgh, NJ) and stored at 4°C. Primary dilutions of the standard stock solutions were made to provide secondary stock solutions (50 000 ng/ml) in 90% HPLC grade water: 10% HPLC grade acetonitrile, both obtained from the J.T. Baker Company (Phillipsburgh, NJ). Working standards were prepared by appropriate dilution of the secondary stock standards into sterile 'pooled' control rat plasma obtained from Rockland Immunochemicals (Gilbertsville, PA). Aliquots of the EME and COC working standards were combined into a single set, and serially diluted to six different concentrations (0.5, 5.0, 50, 500, 5000 and 10 000 ng/ml). A dilution of the COC-d_3 stock internal standard solution was made into a 90:10 mixture of HPLC grade H_2O and HPLC grade methanol (obtained from the J.T. Baker Company (Phillipsburgh, NJ) to give a working concentration of 300 ng/ml. All specimens were spiked with 200 μl of internal standard before analysis to give a working concentration of ~150 ng/ml. EME and COC quality control specimens were prepared using sterile 'pooled' control rat plasma obtained from Rockland Immunochemicals (Gilbertsville, PA) fortified at 5.0, 1500, and 7500 ng/ml concentrations for EME, and 0.5, 1500, and 7500 ng/ml concentrations for COC.

The extraction mobile phase consisted of 4% 2-propanol (J.T. Baker Company, Phillipsburgh, NJ) in 10 mM ammonium formate (Spectrum Chemical Manufacturing Corporation, Gardena, CA) adjusted to pH 10.4 with ammonium hydroxide (J.T. Baker Company, Phillipsburgh, NJ). The aqueous component of the analytical mobile phase consisted of 50 mM formic acid (Acros, Geel, Belgium) and 100 mM ammonium formate (Spectrum Chemical Manufacturing Corporation, Corporation, Gardena, CA) and the organic component consisted of 60% HPLC grade acetonitrile: 40% acetone (J.T. Baker Company, Phillipsburgh, NJ). All LC/MS/MS mobile phases were filtered through a 0.45-μm TF (PTFE)® membrane filter, Gelman Sciences Inc. (Ann Arbor, MI).

2.2. Mass spectrometry and sample introduction

The analytical instrumentation used consisted of a Q-TOF, hybrid quadrupole: orthogonal acceleration time-of-flight mass spectrometer, Micromass, Inc. (Manchester, UK), CTC PAL Prep and Load System Autosampler, LEAP Technologies, Inc. (Carrboro, NC.), (3) Jasco Inc. HPLC Pumps Model PU-980 and Jasco Inc. HPLC Degasser Model DG-980-50, JASCO Corp. (Tokyo, Japan), Rheodyne LabPro Model PRT750-100-02 6-port column switching valve, Rheodyne L. P. (Rohnert Park, CA), a Compaq Pentium® AP200 Professional Workstation (Houston, TX) data acquisition and processing system operating MassLynx 3.4 Beta build 006 software, Micromass Inc. (Manchester, UK). Extraction chromatography was performed on a 2.0-mm I.D. × 20-mm, Micro BioTrap® 500 MS column, Analytical Sales and Service (Mahwah, NJ). Analytical chromatography was performed on a 2.1-mm I.D. × 30-mm with 5.0 μM packing, Allure Basix® HPLC column, Restek Corporation (Bellefonte, PA).

2.3. LM/HM and TOF analyzer resolution

The resolving DC voltage on the quadrupole was set to give a constant low mass/high mass resolution of 0.5 m/z at FWHM across the mass range employed. The instrument was mass calibrated utilizing a solution of poly-DL-alanine at a concentration of 1.0 mg/ml in methanol. This calibration rendered mass accuracy's of 1.35, 2.17, and 0.14 ppm measured at m/z's 445.241050, 729.389490, and 1084.575040, respectively. The instrument calibration was verified by co-infusion of a cocaine/leucine enkephalin mixed standard, rendering mass accuracys of 3.6 ppm for both, well within specified instrument tolerance.

2.4. Analytical procedure

Twenty-five microliter injections of rat plasma samples, control blanks, rat plasma quality controls (QCs) and standards were made onto the extraction column. Mobile phase conditions for the extraction column were as follows: 4% 2-propanol in 10 mM ammonium formate pH adjusted to 10.4 with ammonium hydroxide at a flow rate of 0.8 ml/min for 1.2 min. During this initial period, the switching valve was in the divert position, averting presentation of unwanted endogenous material to the ion source. At 1.21 min, the valve was switched in-line with the API ion source and delivered analytical mobile phase consisting of 60% 100 mM ammonium formate: 50 mM formic acid/40% of a 60% HPLC Grade acetonitrile: 40% HPLC Grade acetone mixture at a flowrate of 0.6 ml/min, to the extraction column and subsequently to the analytical column. The output of the HPLC column flowed into the electrospray interface of the mass spectrometer. The valve was held in this elution position for 2.0 min. At 3.2 min, the switching valve was reset and the system was restored to initial conditions. The interface was operated at a source block temperature of 60°C and desolvation temperature of 300°C. The positive ion mode was used for all acquisitions. Conditions for Q1 scans and pseudo-MRM transitions were: ESI electrode voltage of 3500, nebulizer gas pressure at 25 psi, desolvation gas pressure at 250 psi and collision-cell gas pressure at 15 psi. Collision induced dissociation (CID) studies were performed using argon with a collision cell gas pressure of 2.2 mtorr and collision energy of 20 eV. The micro-channel plate (MCP) was operated at 2800 V, function scan duration of 0.2 s and interscan delays of 0.08 s. Post-acquisition, the following pseudo-MRM transitions were extracted for quantitation purposes: EME: 200.2 $m/z \rightarrow 182.2$ m/z, COC: 304.2 $m/z \rightarrow 182.2$ m/z, and COC-d_3: 307.2 $m/z \rightarrow 185.2$ m/z. Three replicate injections of each blank, standard, QC, and sample were made. Quantitative results represent the average of these measurements.

2.5. Sample preparation

Dr. James Woods and Dr. Theodore Baird, University of Michigan Medical School, Behavioral Pharmacology Unit, Ann Arbor, MI, provided rat plasma specimens. The specimens were collected in 1.0-ml Eppendorf® polypropylene microcentrifuge tubes from Brinkman Instruments, Inc. (Westbury, NY). Aliquots (200 μl) of rat plasma samples, rat plasma QC and standard curves were transferred into 96-deep well-plates, Marsh Biomedical Products, Inc. (Rochester, NY) and fortified with internal standard solution. The deep well-plate was placed on a SORVALL® RC *3C* PLUS centrifuge (Sorvall Instruments, Newton, CT) and spun at 4500 rpm for 15 min prior to analysis.

2.6. Data analysis

Calibration curves ranged from 5.0 to 10 000 ng/ml for EME, and 0.5 to 10 000 ng/ml for COC. For each curve, different concentrations distributed throughout the range of the curves were used. Peak-area ratios between EME: COC-d_3 and COC: COC-d_3, were calculated for each concentration using MicroMass MassLynx 3.4 Beta build 006 software. The data were fit to a linear least squares regression curve with a weighting index of $1/x$. Rat plasma blank samples fortified with internal standard were analyzed with each calibration curve.

2.7. Accuracy and precision

QC samples at three different concentrations for each analyte were examined on three separate days to validate the method. The lower limit of quantitation was 5.0 ng/ml for EME, and 0.5 ng/ml for COC. Five replicates for each QC sample concentration were processed and analyzed together with each standard curve sample set. Method accuracy was assessed by comparing the means of COC and EME with the theoretical concentrations in the QC samples, and expressed as percentages. Intra-assay precision was determined by calculating the percent ratio between the relative standard deviation (%RSD) of the five replicates, and their mean at each concentration within the same validation run. Inter-assay precision was expressed as the %RSD of three different validation runs.

3. Results and discussion

3.1. On-line sample extraction/rapid liquid chromatography/positive ion electrospray Q-TOF mass spectrometry

Inadequate sensitivity and specificity have limited the ability to monitor the plasma pharmacokinetics of COC and EME. The extremely polar nature of EME, and the endogenous interferences found in the plasma matrix are two major factors impacting both the sensitivity and selectivity of methods developed in support of pharmacokinetic studies. We have exploited the utility of on-line extraction coupled to rapid HPLC/Q-TOF mass spectrometry to overcome these problems.

The positive ion ESI daughter-ion mass spectra and proposed fragmentation pathways of the protonated molecules for EME, COC and COC-d_3

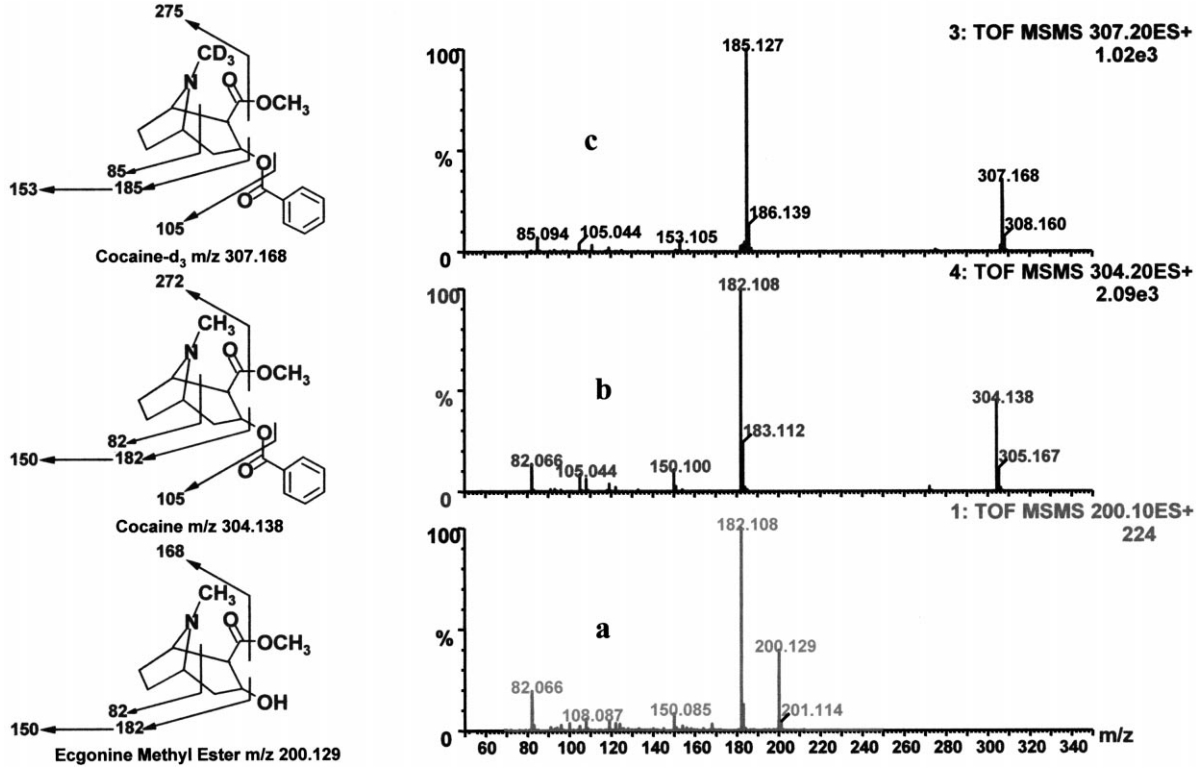

Fig. 1. Electrospray positive ion product mass spectra acquired for ecgonine methyl ester ($[M+H]^+$ m/z of 200.1) (a), cocaine ($[M+H]^+$ m/z of 304.2) (b), and cocaine-d_3 ($[M+H]^+$ m/z of 307.2) (c) at a collision cell gas pressure of 2.2 mtorr and collision energy of 20 eV, and their proposed dissociation pathways.

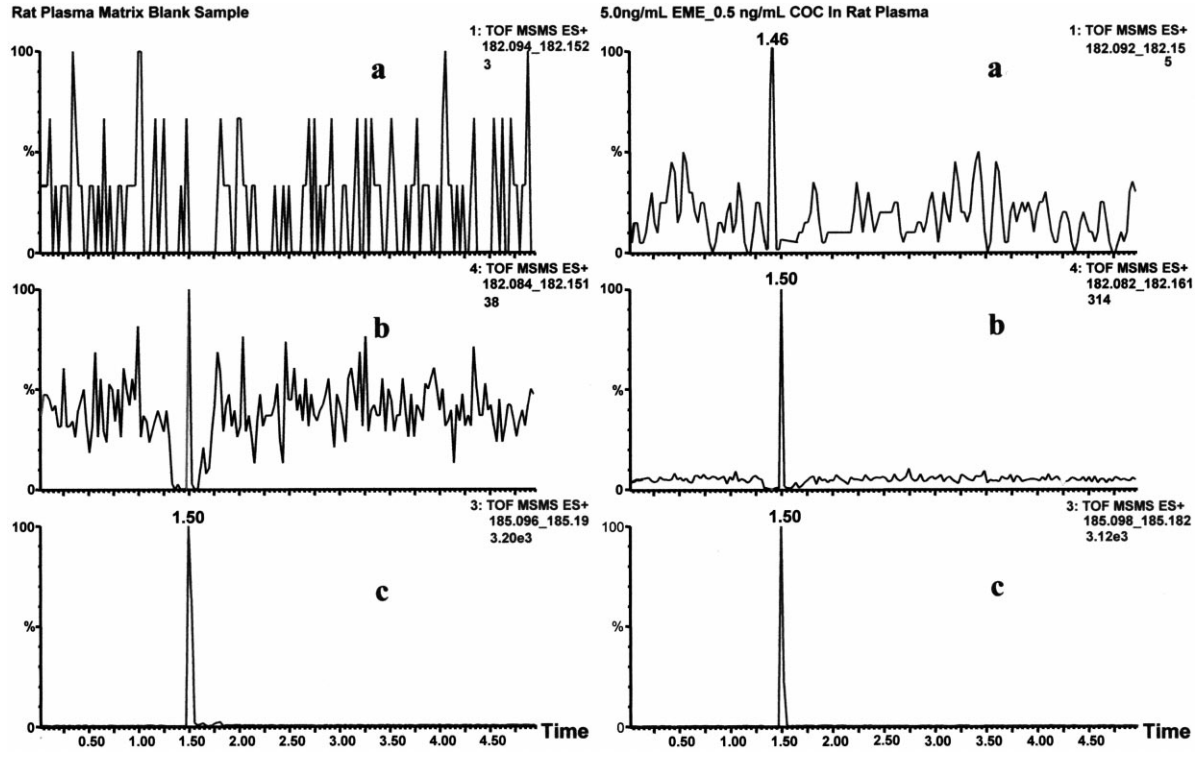

Fig. 2. On-line extraction/HPLC/Q-TOF chromatograms of EME, COC, and corresponding tri-deuterated internal standard of a 'pooled' rat plasma blank specimen and fortified rat plasma standard at the LOQ for EME (5.0 ng/ml) and COC (0.5 ng/ml) (a) pseudo-MRM of ecgonine methyl ester, retention time 1.46 min, (b) pseudo-MRM of cocaine, retention time 1.5 min, and (c) pseudo-MRM of cocaine-d_3, retention time 1.5 min. The analytical separation was performed on an Allure Basix® column (2.1-mm I.D. × 30-mm with 5.0 μm packing).

are shown in Fig. 1. These decompostions have been investigated and reported elsewhere [10,21]. Although $[M + H]^+$ ions were observed for EME, COC, and COC-d_3, the decomposition of EME and COC to ecgonidine methyl ester and COC-d_3 to ecgonidine methyl ester-d_3 represent the base peak of each spectrum. Adducts derived from addition of ammonium salts and ammonium hydroxide, along with cluster ions were not observed. To enhance method sensitivity, the *Z-Spray*® ESI source position and voltages were fine-tuned to allow maximal transmission of these analytes. The retention times for EME and COC were 1.46 and 1.50 min, respectively. The tri-deuterated internal standard, COC-d_3 co-eluted with COC at 1.50 min. A typical on-line extraction/HPLC/Q-TOF of a rat plasma blank sample, and EME/COC rat plasma standard at the LOQ for each are depicted in Fig. 2. Assay selectivity was confirmed by the absence of interfering peaks at the retention time for EME and COC. The specific pseudo-MRM transition and retention time were used to identify the analytes.

Mobile-phase additives are often used to improve chromatographic separations, increase analyte solubility, enhance ESI performance, and heighten ESI response of analytes [22]. The volatile mobile-phase additives employed in this study are commonly used reagents for reversed-phase liquid chromatography (RPLC)/ESI/MS analyses. We recently reported on the affects of mobile-phase additives and eluents on the positive ion responses of ecgonine methyl ester, benzoylecgonine, and cocaine [23]. The results showed that the sensitivity for the test analytes was greatest in a mobile phase consisting of a 1:1

mixture of 60% acetonitrile/40% acetone: 100 mM ammonium acetate. Our current analytical method was based on this information.

High-throughput LC/MS assays tend to relax chromatographic considerations and rely on the resolving ability of the mass spectrometer to provide specificity. However, it is important to ensure that endogenous interferences from the matrix do not impede the analysis [24,25]. For example, there may be endogenous compounds in the biological matrix, which give rise to ions identical to those of the analytes. Additionally, it is possible that interferences present in the clinical samples may not be observed in the rat control plasma. To eliminate these potential problems we developed a high-throughput analytical method that takes advantage of the positive attributes of on-line SPE, rapid chromatography with a new retentive stationary phase, and quantitative quadrupole time-of-flight mass spectrometry.

On-line sample extraction was performed with a 2.0-mm I.D. × 20-mm, Micro BioTrap® 500 MS bio-extraction column. This column was designed to permit continuous direct injections and extraction of plasma, serum, urine, milk, and other biological matrices. The solid support of the column is coated with α-1 acid glycoprotein, and the interior channels are lined with a hydrophilic polymer material. This protein is stable in the pH range of 2–11, and is tolerant of high concentrations of organic modifier in the mobile phase. When an injection from a biological matrix is made onto the column, proteins and other endogenous material from the matrix that are too large to penetrate the pores of the particle channel, are washed off the column to waste. In turn, the analytes become trapped to the hydrophilic polymer until the column is backflushed with analytical mobile phase. When analyzing basic drugs the extraction mobile phase needs to be adjusted to at least a pH of 10, which gives the analyte as low a charge as possible in order to obtain high recoveries. Traces of extraction mobile phase at this elevated pH could have serious affects on a typical silica-based analytical column. Therefore, the analytical mobile phase was adjusted to 50 mM formic acid to eliminate the possibility of adversely affecting the analytical column. The addition of formic acid to the analytical mobile phase also prevents the hydrolysis of EME and COC. More than 500 injections of rat plasma were made onto this column, without observing neither an increase in column backpressure or decrease in extraction performance.

As previously noted, EME is extremely polar and elutes with relatively short retention times on a variety of stationary phases. Needham et al. [26], recently described the use of a pentafluorophenylpropyl (PFPP) stationary phase for the ESI/MS/MS analysis of EME and COC in urine. The PFPP stationary phase was shown to retain EME and COC with 90% acetonitrile in the mobile phase, whereas a C18 stationary phase only required 12% acetonitrile in the mobile phase. We evaluated the use of C1, C6, C8, C18, Supelcosil ABZ + Plus, and Allure Basix columns for the analytical separation. Only the Allure Basix® column met our requirements for asymmetry and retention of COC and EME. The Allure Basix® is a cyanopropyl-based column designed for the analysis of basic drugs, and exhibits retention characteristics similar to the PFPP stationary phase. This column was chosen because it gave excellent peak shapes (asymmetry factor = 0.92) for EME and COC.

Q-TOF instruments have been primarily used for large molecule analysis, where the need for higher resolution, mass accuracy, and mass range precludes the use of quadrupole instruments. With the advent of API, ESI and APCI ionization sources have extended the utility of Q-TOF measurements. Commercial Q-TOF systems capable of performing quantitative analyses have recently become available. Modifications to the detection systems and algorithms have been made to increase the linear dynamic range of the TOF measurement. The calibration curves obtained for EME and COC stretch over 5 orders of magnitude on a Q-TOF instrument with dual-MCPs and the use of 'dead-time' correction. These results were achievable not only due to the detection system, but also to the inherent sensitivity of EME and COC, coupled with the inherent sensitivity of the instrument, that allowed the extension of the lower end of the curve.

The Q-TOF obtains its selectivity via the ability to discriminate between ions below nominal mass resolution. Full product ion spectra were collected for EME, COC, and COC-d_3, post-acquisition pseudo-MRM transitions for each compound were extracted for identification and quantitation purposes. As depicted by Fig. 2, the replots of the ion currents for EME, COC, and COC-d_3 do not indicate the presence of any interferences. Therefore, we determined that the method had adequate specificity for our pharmacokinetic determinations.

3.2. Method validation

3.2.1. Linearity

The standard curves were plots of the ratios of analyte/internal standard responses (peak-area) as a function of analyte concentration. The concentrations of the standards ranged from 5.0 to 10 000 ng/ml for EME, and 0.5 to 10 000 ng/ml for COC. The data were fit to a linear least squares regression curve with a weighting index of $1/x$. Equations of regression line and correlation coefficients for the pseudo-MRM ion abundance ratio and the corresponding calibration concentrations (r^2) were as follows: $y = 0.0003 + 0.0703x$ ($r^2 = 0.9921$) for EME and $y = 0.0032 + 0.0035x$ ($r^2 = 0.9997$) for COC.

Table 1
Accuracy and precision of method for QC samples ($n = 15$)

Ecgonine methyl ester		
5.0 ng/ml	Mean (ng/ml)	4.95
	Accuracy (%)	99.0
	Precision (%)	3.7
1500 ng/ml	Mean (ng/ml)	1530
	Accuracy (%)	102
	Precision (%)	5.9
7500 ng/ml	Mean (ng/ml)	7450
	Accuracy (%)	99.4
	Precision (%)	8.6
Cocaine		
0.5 ng/ml	Mean (ng/ml)	0.52
	Accuracy (%)	104
	Precision (%)	4.8
1500 ng/ml	Mean (ng/ml)	1490
	Accuracy (%)	99.5
	Precision (%)	5.3
7500 ng/ml	Mean (ng/ml)	7620
	Accuracy (%)	102
	Precision (%)	9.3

3.2.2. Accuracy and precision

Plasma blanks showed no interfering signals at the retention times corresponding to EME and COC. Acceptable accuracy and precision were set at $\pm 15\%$ for the rat plasma quality control specimens. The intra-run and inter-run accuracy and precision (% RSD) for the two analytes ranged from 92 to 112%, and 1.1 to 6.2%, respectively. This data is summarized in Table 1.

3.2.3. System repeatability

The system repeatability, given as percent coefficient of variation (% CV) of mean peak-area ratios, was assessed using 50 injections of a rat plasma sample from the pharmacokinetic study. The analyses were performed over the course of 5 days, rendering % CVs for EME and COC of 0.73 and 0.58, respectively.

3.2.4. Limit of quantitation and detection

The lower limit of detection (LOD) was defined as the lowest concentration of the calibration standards fortified in rat plasma yielding a 'signal-to-noise' (S/N) ratio of at least 3.0. The limit of quantitation (LOQ) was defined as the lowest concentration of the calibration standards fortified in rat plasma with both accuracy and precision of $\pm 15\%$. The limit of detection for EME was 0.5 ng/ml, and 0.05 ng/ml for COC. The LOQs for EME and COC were 5.0 and 0.5 ng/ml, respectively, with total coefficients of variation less than 10%. These data suggest that a quantitative assessment of EME and COC in rat plasma can be made with satisfactory assurance.

3.3. Rat plasma pharmacokinetic data

The validated assay was used to examine the ability of Butyrylcholinesterase (BChE) to modify the reinforcing effects of cocaine in rats. BChE in doses of 3.0 and 10 mg/kg, were given as pre-

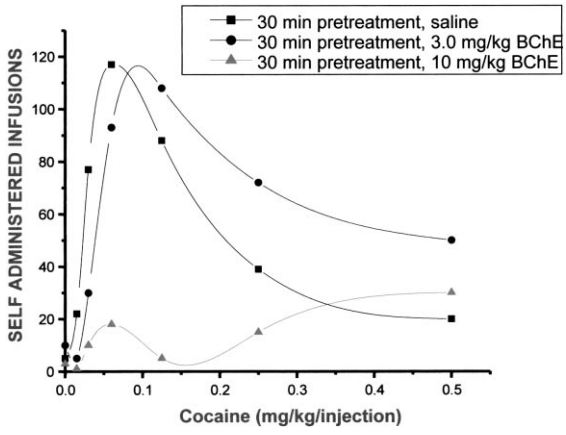

Fig. 3. BChE influence on the reinforcing effect of COC. These data indicate that BChE is producing parallel rightwards shifts in COC's potency as a reinforcer, and that larger doses of COC must be given in order to demonstrate the parallel nature of the interaction.

treatments in rats self-administering cocaine. BChE treatment is thought to alter the metabolic disposition of cocaine by reducing the amount of Benzoylecgonine (BZE) formed, and thereby altering its overall toxicity. Additionally, increasing BChE levels should produce higher levels of EME.

As shown in Fig. 3, the smaller dose produced a slight but non-significant shift parallel rightwards shift in the COC dose-effect curve. The larger dose produced nearly complete suppression of COC-maintained response. These data indicate that BChE is producing parallel rightwards shifts in COC's potency as a reinforcer, and that larger doses of COC must be given in order to demonstrate the parallel nature of the interaction.

As evidenced by Fig. 4A, no hydrolysis of COC was detected in the saline solution throughout the sampling period. A dosage of 3.0 mg/kg of exogenous BChE resulted in minimal metabolism of COC. The addition of 10 mg/kg dose of exogenous BChE led to complete metabolism of COC in 20 min.

Fig. 4B gives the time-course for EME production in rats following either pretreatment with

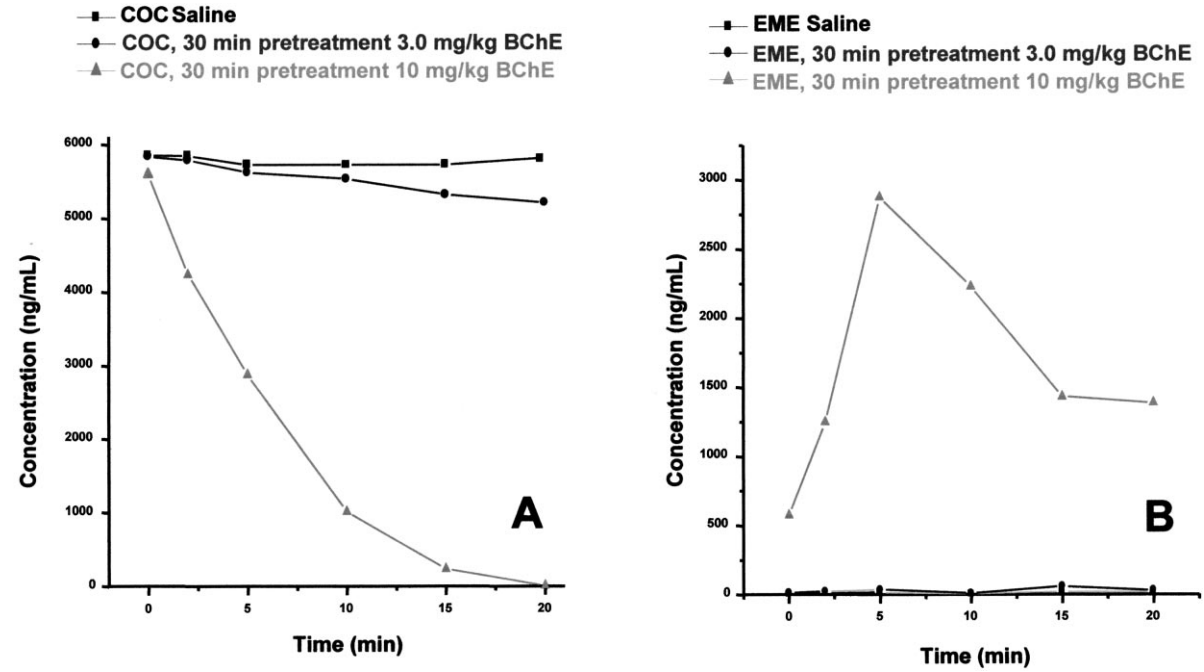

Fig. 4. A: Cocaine metabolism in saline and rat plasma after pretreatment with 3.0 mg/kg BChE, and after pretreatment with 10 mg/kg BChE. B: EME levels are shown for prior treatment with saline, 3.0 mg/kg BChE, and 10 mg/kg BChE.

saline, 3.0 or 10 mg/kg BChE. Saline and 3.0 mg/kg BChE pretreatment did not give rise to appreciable levels of EME, indicating that the normal metabolic pathway for COC had not been altered. However, a 10-mg/kg dose of exogenous BChE is shown to have a marked affect on the conversion of COC to EME. These results confirm those reported by Carmona et al. [5], where endogenous levels of BChE were found not to contribute significantly to COC metabolism. The pharmacokinetic profiles obtained with this methodology will make it possible to examine how protein-based intervention strategies affect cocaine metabolism in-vivo. Future studies will include measurement of the toxic metabolite BZE in order to provide a more detailed time-course study of cocaine disposition following administration of exogenous BChE.

4. Conclusions

Many studies have been conducted to establish the relationships between cocaine metabolism, toxicity, and its rewarding effects. Only recently has sufficient progress been made through in-vitro testing to provide insight into the causative factors involving cocaine metabolism and disposition. Modeling studies have been severely impeded by the lack of appropriate methodology for the quantitation of COC and EME in plasma. We have shown that a combination of on-line sample extraction coupled to rapid chromatography and quadrupole orthogonal acceleration time-of-flight mass detection, provides a sensitive, selective, and rugged system for the analysis of COC and EME in plasma. With this method, reproducible retention times, along with high accuracy and precision were obtained for COC and EME. Total run time was 3.2 min, making it possible to analyze greater than 400 plasma samples in a single daily run. Sample preparation was held to a minimum, and consisted of a sole centrifugation step. This method will provide the foundation for detailed in-vivo studies, aimed at assessing the therapeutic benefits derived from protein-based treatments in preventing COC toxicity and abuse.

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