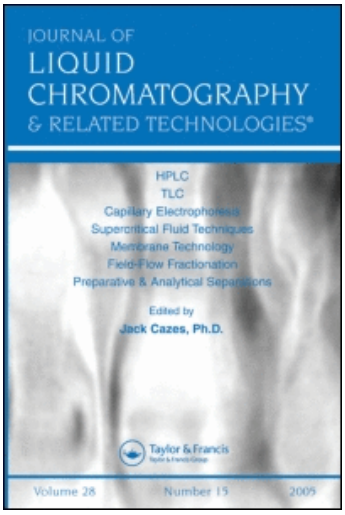

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Determination of Cocaine in Biological Matrices Using Reversed Phase HPLC: Application to Plasma and Brain Tissue

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DETERMINATION OF COCAINE IN BIOLOGICAL MATRICES USING REVERSED PHASE HPLC: APPLICATION TO PLASMA AND BRAIN TISSUE

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ABSTRACT

An HPLC assay using UV detection has been developed for the analysis of cocaine in biological matrices. Samples were precipitated with perchloric acid, extracted in 5% chloroform/pentane at pH 10, and back-extracted into 0.1 N hydrochloric acid. Samples were chromatographed using a Zorbax RX-C18®, 4.6 x 250 mm, 5 μm, column with the absorbance monitored at 235 nm. The method was applied to quantification of cocaine in plasma and brain tissue of Sprague-Dawley rats following intraperitoneal administration of 15 mg/kg cocaine. The method was characterized by its extreme ease of sample preparation and ruggedness over time. The average recovery for the method was marginal; 52 and 38% for cocaine and the internal standard, respectively, in plasma and 42 and 35% of the cocaine and the internal standard, respectively, in brain. However, due to the extreme cleanliness of the sample after preparation, the limit of detection of the method for cocaine was less than 5 ng/ml in plasma and less than 5 ng/g in brain. The limit of quantification was 25 ng/ml in plasma and 25 ng/g in brain.

The method was linear from 50-4000 ng/ml in both plasma and brain. Spiked quality control samples were assayed over a three month period. The inter-day coefficient of variation was 10.2% in plasma at 200 ng/ml (n=22), 11.6% at 200 ng/ml in brain (n=38) and 0.7% at 2500 ng/ml in both plasma and brain (n=23, n=43, respectively). The method would appear to be acceptable for the analysis of cocaine in both saliva and urine.

INTRODUCTION

Cocaine is an infamous drug of abuse. Cocaine acts as a psychostimulant in the central nervous system by inhibiting the reuptake of catecholamines into the presynaptic nerve terminal following release. It also acts as a local anesthetic in the peripheral nervous system by blocking fast opening sodium channels (1). Following repeated intermittent administration of cocaine to rats, progressive augmentation of locomotor activity and stereotyped behavior is observed. Following continuous administration of cocaine, progressive diminution of locomotor activity and stereotyped behavior is observed. The former process is termed sensitization, the latter is termed tolerance (2). One theory regarding the development of sensitization is that it may at least in part be due to increased cocaine brain levels following repeated administration (3). In order to test this hypothesis it is necessary to be able to quantify cocaine and, if possible, its metabolites in biological matrices, particularly plasma and brain tissue.

A variety of methods have been developed using HPLC to assay cocaine concentrations in biological matrices (4-11). However, none of these methods are ideal. Most are developed for a particular matrix, usually plasma (7,9,10), serum (5,6), or urine (4,9). Few are suitable for brain tissue (8, 11). Some require extensive sample preparation prior to actual injection on-column (4,9). Others sacrifice extraction efficiency for sample cleanliness (6,7,11). To

date, no validated method for cocaine analysis in multiple biological matrices, i.e., plasma, brain, urine, and saliva, has been developed.

Previous methods used to detect cocaine and its metabolites by reversed phase HPLC have been problematic both from a technical and theoretical viewpoint. One problem has been the use of lidocaine or other local anesthetics as an internal standard (5,6,8,9). Although lidocaine and bupivicaine (both of which have been used as internal standards in published HPLC methods) are local anesthetics, they are structurally unrelated to cocaine. Ideally, the internal standard would be similar both structurally as well as pharmacologically. Common commercial internal standards meeting the structural similarity requirement are propyl-benzoylecgonine and 3-tropanyl-3,5-dichlorobenzoate (MDL-72222). Unfortunately, these compounds are so lipophilic that long retention times are often seen when using an HPLC system consisting of a moderate percentage of organic in the mobile phase which makes them unsuitable when a rapid turn-around time is needed (Table 1). A seemingly ideal internal standard, structurally similar to cocaine, but less lipophilic than propyl-benzoylecgonine, is m-toluylecgonine perchlorate. This compound has been used in gas chromatography, but has yet to be applied to liquid chromatography (12). Unfortunately, it is not available commercially and must be synthesized by the user. A compromise internal standard is ethyl-benzoylecgonine, i.e., cocaethylene, which is both structurally and pharmacologically similar to cocaine (7). Although cocaethylene has a much shorter retention time than propyl-benzoylecgonine, it cannot be used clinically since cocaethylene is formed metabolically in vivo in subjects taking cocaine and alcohol in combination.

Another problem typically seen in the literature is related to chromatography of amines on octadecyl columns. Since cocaine and its metabolites are tertiary amines, using a reversed phase column, one would expect to see tailing of

TABLE 1
Retention Times and Capacity Factors for Various Drugs of Abuse and Psychotropic Drugs

NAME OF COMPOUND	Retention Time in min	Capacity factor, k'
procainamide	1.4	0.00
flupentixol	1.5	0.02
morphine	1.5	0.02
vancomycin	1.6	0.08
hydromorphone	1.7	0.19
codeine	1.7	0.20
quinine	1.8	0.26
quinidine	1.8	0.27
theophylline	1.9	0.30
hydrocodone	2.1	0.48
3,4-methylenedioxyamphetamine	2.2	0.50
3,4-methylenedioxymethamphetamine	2.4	0.68
caffeine	2.5	0.71
atropine	2.8	0.93
3,4 methylenedioxyethylamphetamine	2.9	1.03
lidocaine	3.0	1.12
chlorpheniramine	3.7	1.55
mepazine	4.2	1.96
methylphenidate	4.6	2.18
gepirone	4.8	2.36
clozapine	5.6	2.92
cocaine	6.5	3.40
procaine	6.9	3.83
meperidine	7.1	3.93
norcocaine	7.4	4.20
pseudococaine	7.9	4.51
chlordiazepoxide	8.3	4.81
phenylpropanolamine	8.5	4.97
salicylic acid	9.2	5.41
RTI-32	9.7	5.80
haloperidol	11.1	6.77
RTI-31	11.4	6.94
acetophenazine	11.4	6.94
pentazocine	11.5	7.01
buspirone	13.1	8.18
trazodone	14.5	9.16
benperidol	18.7	12.05
dextromethorphan	19.6	12.73
amoxapine	21.2	13.83
diphenhydramine	28.8	19.11

(Continued)

TABLE 1 (Continued)

NAME OF COMPOUND	Retention Time in min	Capacity factor, k'
oxazepam	63.5	43.39
methaquaalude	90.5	62.29
diazepam	106.0	73.13
butriptyline	nd	nd
proketazine	nd	nd
meprobamate	nd	nd
nortriptyline	nd	nd
amitryptiline	nd	nd
benztropine methanesulfonate	nd	nd
butaperazine	nd	nd
chlorpromazine	nd	nd
cyclopenzaprine	nd	nd
clomipraime	nd	nd
ethydorvynol	nd	nd
norfluoxetine	nd	nd
fluoxetine	nd	nd
fluphenazine	nd	nd
imipramine	nd	nd
methadone	nd	nd
methamphetamine	nd	nd
nicotine	nd	nd
PCP	nd	nd
phenothiazine	nd	nd
d-propoxyphene	nd	nd
pseudoephedrine	nd	nd
delta-9-tetrahydrocannabinol	nd	nd
ephedrine	nd	nd
propyl benzoylecgonine	28.8	19.14
pentobarbital	30.2	20.10
loxapine	31.1	20.75
flurazepam	33.4	22.36
mesoridazine	36.5	24.49
amphetamine	38.7	26.05
carbamazepine	44.2	29.88
3-tropanyl-3,5-dichlorobenzoate	52.1	35.45
secobarbital	54.6	37.15
acetaminophen	55.9	38.11
WIN 35428	56.2	38.27
hydergine (ergoloid mesylates)	56.3	38.39

Non-extracted samples (~1 μg on-column) were chromatographed for at least 90 min and monitored at 235 nm. Compounds listed as 'nd' were not detected under the conditions described.

peaks that are removed from the solvent front. Indeed, tailing and poor resolution are often seen in chromatograms using published methods (7,9,10). Some papers do not include chromatograms so it is difficult to evaluate these methods critically (8,11).

Our laboratory set out to devise a method for the analysis of cocaine in plasma and brain which was free of previous pitfalls in cocaine analysis. In particular, we wished to develop a method that could be applied to a variety of sample matrices, particularly brain tissue, and was rugged to slight modifications in terms of sample preparation or chromatographic conditions.

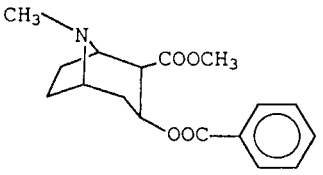
MATERIALS AND METHODS

Reagents and Standards

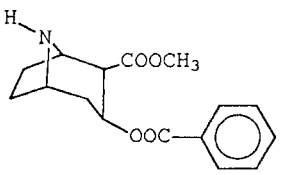
All standards were obtained from the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). The purity of these compounds were greater than 99% as determined by HPLC. All calculations were based on the free base concentrations. Solvents were purchased either from Fisher Scientific (Springfield, NJ) and were at least HPLC grade. All reagents were at least chemical grade. All glassware were silylated prior to use by vapor phase silylation (13). The internal standard, RTI-31 (Figure 1; IS), is available from Research Biochemical International (Natick, MA). Homogenization buffer consisted of 0.5% sodium fluoride in 100 mM sodium phosphate monobasic, pH 4.5. Plasma controls were a gift from UTAK Laboratories, Inc. (Canyon Country, CA).

Instrumentation and Data Analysis

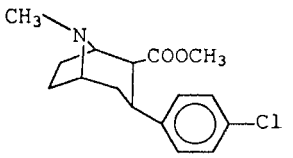
All samples were analyzed using a Waters Assoc. (Milford, MA) 510 pump attached to a Waters Assoc. 710 WISP



Cocaine



Norcocaine



RTI-31
Internal Standard

FIGURE 1: Structure of cocaine, norcocaine, and RTI-31, the internal standard.

autosampler. A Brownlee RP-8 guard column, 15 x 3.2 mm, 7 μm, (Applied Biosystems, Foster City, CA) was attached in line between the injector and column. All samples were chromatographed on a Zorbax® RX-C18 column, 4.6 x 250 mm, 5 μm particle size (Mac-Mod, Chaads Ford, PA). Data were captured using a Waters 780 Data Module. Samples were detected at 235 nm on a Waters 210 variable wavelength ultraviolet detector set at 0.02 AUFS. Samples were centrifuged using a Sorvall RC-5B refrigerated superspeed centrifuge (Dupont Co., Newtown, CT) equipped with a SM24 rotor. All data were collected in peak height mode.

Calibration curves were calculated by linear regression analysis of the ratio of the cocaine peak height to internal standard peak height vs. theoretical cocaine concentration. All data were weighed by 1/concentration and unknown sample concentrations were estimated by inverse prediction. Since

the calibration curves were prepared as ng/ml concentrations, whole brain and striatal levels of cocaine, in ng/g, were determined using the following formula, assuming the density of brain to be 1.0 g/ml:

$$\text{Concentration, ng/g} = \frac{\left(\begin{array}{c}\text{Concentration From}\\ \text{Standard Curve, ng/ml}\end{array}\right)\left(\begin{array}{c}\text{Volume of}\\ \text{Standards, ml}\end{array}\right)\left(1 + \text{Dilution Factor}\right)}{\left(\begin{array}{c}\text{Volume of Tissue}\\ \text{Assayed, g}\end{array}\right)}$$

Sample Preparation

Unknown samples and controls were frozen at -80°C prior to analysis and were allowed to thaw at room temperature. After thawing, a 0.5 ml aliquot was removed for analysis. Plasma standards were prepared as follows: one-half milliliter (0.5 ml) blank plasma was mixed with 15 µl saturated sodium fluoride to inhibit the metabolism of cocaine to ecgonine methyl ester by pseudocholinesterase (14). The standards were then spiked with cocaine prepared in ethanol to give concentrations of 50 to 4000 ng/ml.

Whole brain or striata standards were prepared as follows: homogenized cow brain was diluted 1:8 or 1:4 (w/v), for striata or whole brain, respectively, with homogenization buffer and homogenized using a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY). A 0.5 ml aliquot of homogenate was removed and spiked with cocaine prepared in ethanol to give concentrations of 50 to 4000 ng/ml.

From this point, standards, unknowns, and controls were treated identically. All samples were mixed with 75 µl 150 µg/ml internal standard and precipitated with 50 µl 50% perchloric acid. The samples were mixed vigorously for ~10 s, then allowed to sit at room temperature for 10 min. One (1.0) ml of distilled water was added to each tube. The samples were capped, mixed briefly, and centrifuged at ~2500 for ~30 min at ~10°C using an International Equipment Co.

(Needham Heights, MA) 7R centrifuge. After centrifugation, the supernatant was transferred to a clean 16x125 mm screw-top test tube and 750 µl saturated sodium carbonate added. The samples were briefly mixed and 7.5 ml 5% chloroform in pentane added. The samples were rocked gently for 10 min then centrifuged for ~2 min on a desk-top centrifuge. The samples were then placed in an acetone bath containing dry ice for ~2 min after which the organic layer was decanted into a 16x100 mm conical bottom screw-top test tube containing 250 µl 0.1 M hydrochloric acid. The samples were allowed to sit at room temperature until the aqueous layer was thawed. In some samples, decanting the supernatant onto the dilute acid briefly froze the dilute acid. After the samples had thawed, they were vigorously mixed for ~10 s, then centrifuged on a desk-top centrifuge for 1-2 min. After centrifugation, the samples were placed back in the dry ice bath for 3-5 min. The organic supernatant was discarded and the frozen aqueous layer allowed to thaw. After thawing, the samples were mixed and passed over a nitrogen stream for a few seconds to remove any lingering chloroform/pentane. Allowing the samples to sit at room temperature for ~1 h prior to injection achieved the same result. Seventy-five microliters (75 µl) of the samples was injected on-column. Batch processing of 40 samples could be accomplished within 3 h.

HPLC Conditions

The mobile phase consisted of acetonitrile mixed with 0.5% triethylamine in 100 mM potassium phosphate dibasic, adjusted to pH 2.7 with phosphoric acid (18/82%, v/v). Mobile phase was degassed using helium sparging prior introduction to the column. The flow rate was 2.0 ml/min with a back pressure of ~2700 psi. Under these conditions, cocaine and the internal standard had retention times of 6.5 and 11.4 min, respectively. Chromatography was done at room temperature.

Animal Studies

Female, Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis IN) were dosed intraperitoneally with 15 mg/kg cocaine. At 20 min after administration, the animals were decapitated, trunk blood collected and brain removed. To each ml of blood was added 10 μL saturated sodium fluoride and to the tube of blood was added 50 μl heparin sulfate (1000 U/ml). Striata were dissected out and pooled. Striata and the remaining brain tissue were diluted 1:8 or 1:4 (w/v), respectively, homogenized, and assayed for cocaine. Whole blood was centrifuged, the plasma removed and assayed for cocaine.

RESULTS AND DISCUSSION

Chromatography

Cocaine was adequately resolved from the internal standard (Figures 2 and 3). The only metabolite of cocaine which may interfere with the resolution of cocaine is norcocaine with a retention time of 7.4 min. However, in our studies, norcocaine was always less than 5% of the peak height of cocaine so this does not appear to be a problem. Figures 2 and 3 show chromatograms obtained from blank plasma and whole brain, respectively, spiked with cocaine, norcocaine, and internal standard. Although complete baseline resolution was not achieved between cocaine and norcocaine, the resolution was more than adequate to accurately quantify cocaines peak height. At higher doses, greater amounts of norcocaine may be formed, leading to decreased resolution of cocaine and norcocaine, which may require alterations in the mobile phase composition. Decreasing the percent organic in the mobile phase would achieve the goal of more complete resolution of norcocaine from cocaine, but at the expense of band broadening and

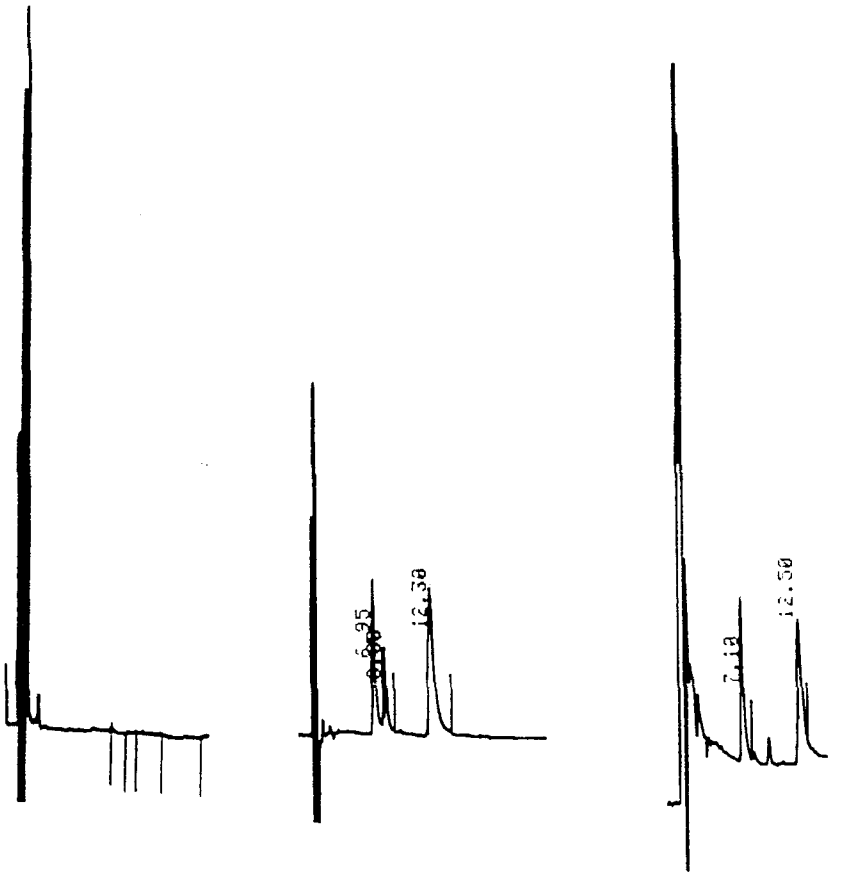


FIGURE 2: Chromatograms from blank plasma (left), plasma spiked with cocaine, norcocaine, and RTI-31 (middle), and plasma obtained from a rat dosed with 15 mg/kg cocaine ip (right).

higher LOD. Alternatively, use of a 3 μm rather than a 5 μm column, should increase resolution sufficiently to provide baseline resolution of cocaine and norcocaine and decrease the LOD, without affecting column pressure to any extent. The average recovery of norcocaine from brain and plasma was 32%.

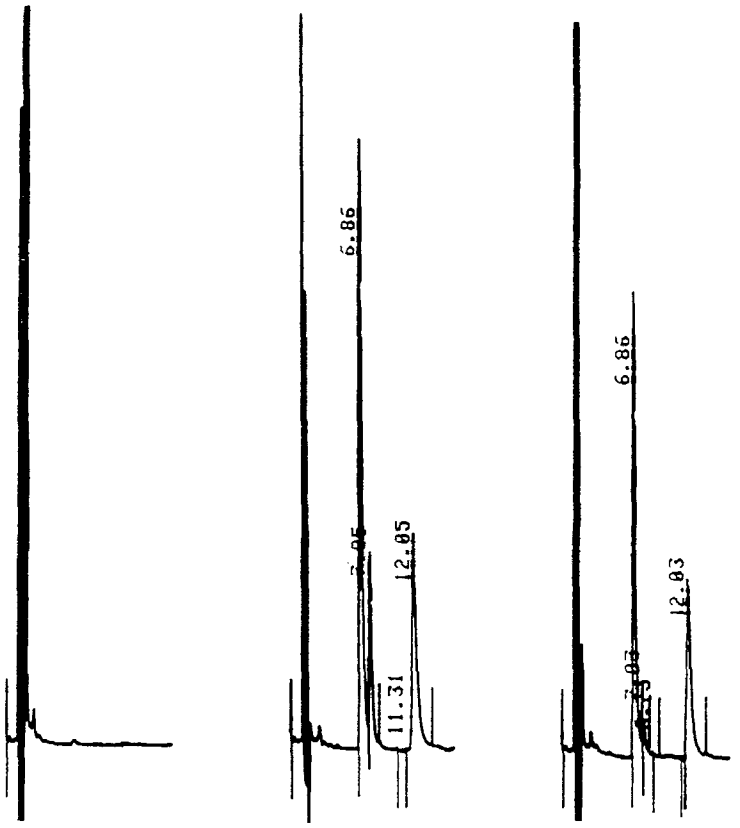


FIGURE 3: Chromatograms from blank whole brain (left), whole brain spiked with cocaine, norcocaine, and RTI-31 (middle), and whole brain obtained from a rat dosed with 15 mg/kg cocaine ip (left).

Figure 4 shows the effect of percent acetonitrile in the mobile phase on retention time of cocaine, norcocaine, and internal standard. As expected, the retention time of the compounds was log-linearly related to percent organic in the mobile phase. The final percent acetonitrile was chosen to maximize resolution of cocaine and norcocaine while minimizing the total run-time. Buffer pH has little effect

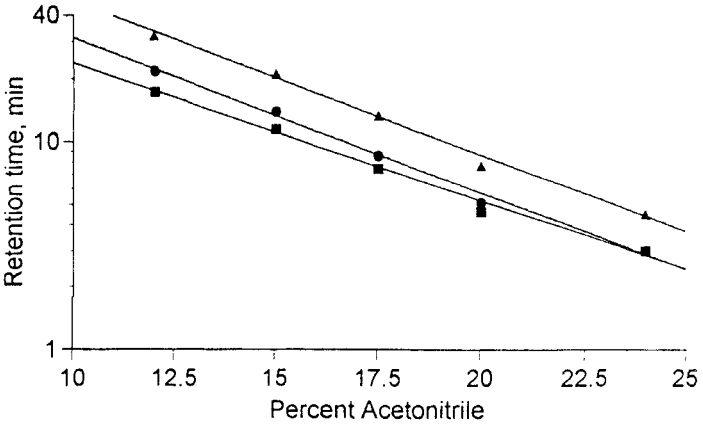


FIGURE 4: Effect of percent acetonitrile on retention time of cocaine (■), norcocaine (▲), and IS (●) when buffer pH was fixed at 2.7.

on retention time, except as the pH begins to exceed 4.0 (Figure 5). Here, pH 2.7 was chosen since it is less than one log unit from the pKa of phosphate ion.

One problem that was observed with the chromatography was the shift in retention time during the course of method development. This was probably a result of increased variability in room temperature as the building was shifted from heating to cooling. As such, our chromatography changed and coelution of cocaine and norcocaine became a problem. One way we tried to combat this problem was to add a column heater set to 37°C. To compensate for the expected decrease in retention time, the mobile phase proportions were changed to 15% acetonitrile to 85% buffer with a flow rate of 1.5 ml/min. This change produced similar retention times (cocaine, 7.9 min; norcocaine, 8.8 min; internal standard, 13.7 min), but band broadening significantly decreased peak resolution. As an alternative, and what was finally decided to be the best solution, we fit a cardboard box slightly larger than the column with foam. The column

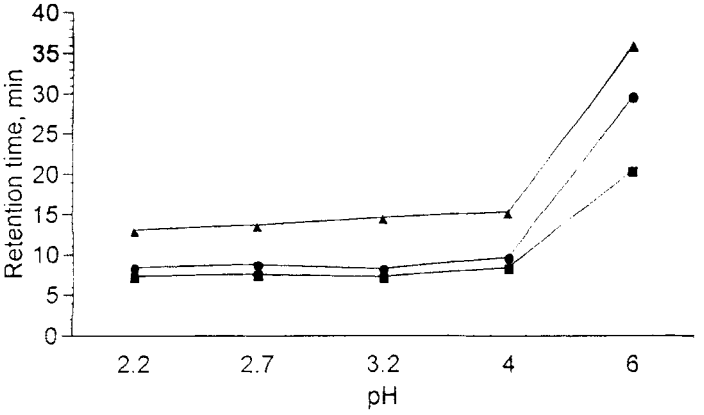


FIGURE 5: Effect of buffer pH on retention time of cocaine (■), norcocaine (▲), and IS (●) when percent acetonitrile was fixed at 18%.

was placed inside the box and sealed. This inexpensive insulation device proved more than satisfactory for any further temperature changes which occurred over time.

No endogenous interferences appear to be present in the assay (Figures 2 and 3). Of more than 70 drugs screened for possible interference, only pseudococaine and pentazocine have the possibility of interfering with the assay (Table 1). All the compounds with retention times in the range of 4.2 to 18.7 min were spiked into plasma to have a concentration of at least 10 μg/ml. In addition to the single drug of interest, each tube was spiked with cocaine, norcocaine, and the internal standard to have a concentration of at least 1 μg/ml. These samples were then processed as described above to determine if any of the drugs recovered to any significant extent and if they coelute with either cocaine, norcocaine, or the internal standard. Of the drugs tested, only methylphenidate, gepirone, clozapine, pseudococaine, chlordiazepoxide, and pentazocine were extracted to any significant extent. Of

these, only pseudococaine and pentazocine coeluted with one of the analytes.

Due to the cleanliness of the final samples, we utilized a "poor-man's" solvent recycling system to decrease the cost of solvent utilization. In effect, we fed our solvent waste line back into our mobile phase reservoir. Our solvent reservoir sat on a magnetic bar stirrer to maintain consistency in the mobile phase. Hundreds of injections were made before a change in resolution was noticed, although retention time began to drift after about 100 samples. Nevertheless, even as the retention time began to change, we were still able to use the method for sample analysis.

Two other biological matrices were examined from spiked samples: saliva and urine. The sample preparation was as used for plasma, described above. Both matrices have very clean chromatography with no endogenous interferences. Although not examined in vivo, saliva offers to be a non-invasive method which can be used to correlate behavioral effects to plasma concentration in humans (15).

Linearity and Limit of Detection

The method was used for routine analysis of brain and plasma samples over a three month period. Fitting daily calibration curves to a simple linear regression model resulted in coefficients of determination ranging from 0.9901 to 1.00 in plasma (n=8) and 0.9900 to 0.9996 in brain (n=14). Fitting each daily calibration curve to a quadratic polynomial resulted in a non-significantly quadratic term. Thus the method was linear from 50 to 4000 ng/ml. No attempt was made to determine the upper-most limit of linearity. The limit of detection (LOD) was calculated to be ~5 ng/ml in plasma and ~5 ng/g in brain. The limit of quantification calculated using a t-value of 10 was ~25 ng/ml in plasma and ~25 ng/g in brain (16). Should a lower

limit of quantification be required, the AUFS on the detector may be decreased to 0.01 with little alteration in baseline noise. The limit of quantification for norcocaine in plasma and brain was ~75 ng/ml and ~75 ng/g, respectively.

Recovery

There was good concordance between recovery from brain, plasma, and saliva. The average recovery from plasma was 52 and 38% for cocaine and the internal standard, respectively. Recovery from brain tissue was 42 and 35% of the cocaine and the internal standard, respectively. The average recovery from saliva was 46 and 40% for cocaine and the internal standard, respectively. Recovery data was not calculated for urine. Both precipitation and extraction from the centrifuged precipitant represent the steps at which the major loss in recovery occurred. Following precipitation, an ~15% loss in recovery was observed which was believed to be due to trapping of analytes in the precipitant. Base extraction from supernatant into 5% chloroform in pentane resulted in ~65% of the analytes being recovered. The final step, back extraction into dilute acid, was complete with ~100% of the analytes being recovered. Obviously, recovery is the major limitation for the method. However, low recovery must be balanced against sample cleanliness. Future studies may wish to examine the effect of other buffers with different pH values on percent recovery.

The solvent used for the extraction, 5% chloroform in pentane, is relatively non-polar in nature and as such should not be expected to extract endogenous, polar substances. However, some sample matrices, possibly forensic samples, may be so "dirty" that some endogenous substances may be extracted into the solvent and interfere with the assay. During the course of method development it was discovered that 35% ethyl acetate in pentane, a relative polar solvent, fails to extract the analytes to any

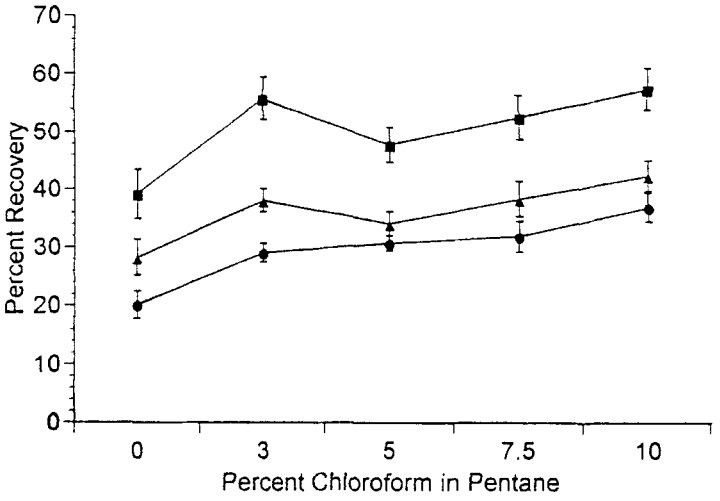


FIGURE 6: Effect of chloroform on percent recovery of cocaine (■), norcocaine (▲), and IS (●) from plasma following a 35% ethyl acetate in pentane wash.

significant extent. This allows polar substances to be extracted but leaves the majority of the analytes behind. Therefore, after precipitation by perchloric acid and transfer to a clean test prior to the addition of saturated sodium carbonate, a wash step using 35% ethyl acetate in pentane may be used as a further clean-up step. For the sample matrices examined herein, plasma, brain, saliva, and urine, this wash step was unnecessary. Figure 6 shows the effect of percent chloroform in pentane on recovery following ethyl acetate washing.

Stability and Precision

Samples were prepared and analyzed. Using a refrigerated autosampler set at 15°C, the samples were reanalyzed 72 h later and the peak heights compared. Using the Wilcoxon signed rank test for differences between days, no significant differences were found ($p > 0.05$).

Therefore, the analytes were stable in 0.1 M hydrochloric acid for a period of 72 h. This should be sufficient time to analyze a 48-position WISP sample carousel.

Blank plasma and cow brain samples were spiked with 200 or 2500 ng/ml (ng/g for brain) cocaine and stored at -80°C until analysis. These samples were treated and stored as if they were unknowns. Over a three-month period, these samples were analyzed in conjunction with experimental samples. The method exhibited adequate within- and between-day precision. The average inter-day coefficient of variation (CV) was 10.2% in plasma at 200 ng/ml (n=22), 11.6% at 200 ng/ml in brain (n=38) and 0.7% at 2500 ng/ml in both plasma and brain (n=23, n=43, respectively). The average intra-day CV ranged from 4.7 to 12.7% in plasma at 200 ng/ml, 0.8 to 9.9% in plasma at 2500 ng/ml, 5.6 to 14.3% in plasma at 200 ng/g, and 2.4 to 14.5% in brain at 2500 ng/g. The mean relative error was 5.0% for both brain and plasma at 200 ng/ml and 3.0% for both brain and plasma at 2500 ng/ml.

A single lyophilized plasma control for cocaine with a target value of 500 ± 50 ng/ml was reconstituted in 5.0 ml water, mixed with 75 ul saturated sodium fluoride, and stored at -80°C until analysis. This control was assayed twice over a period of three weeks. The mean back-calculated concentration was 475 ± 17 ng/ml (n=6, mean ± std dev) after discarding the highest and lowest control with an inter-day CV of 3.6%. Without discarding the highest and lowest control, the mean back-calculated concentration was 488 ± 79 ng/ml with an inter-day CV of 16%.

Whole brain samples obtained from animals dosed with 15 mg/kg cocaine intraperitoneally were homogenized using the phosphate buffer/sodium fluoride buffer were assayed and frozen at -80°C. A random sample was reassayed six-eight months later. Using stochastic regression, the slope of the line relating the reassayed concentration to the original concentration was 0.85, indicating 15% of the cocaine in the

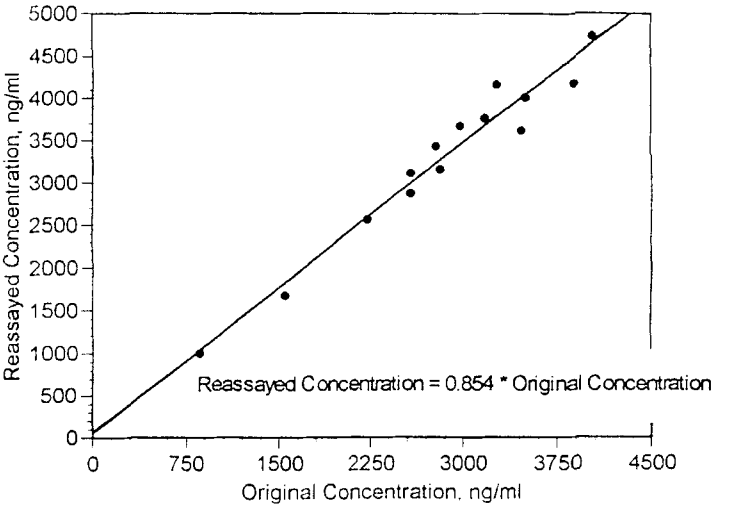


FIGURE 7: Plot of reassayed cocaine concentrations vs. their original concentration after being stored at -80°C for six-eight months. The line is the stochastic regression least-squares fit to the data.

original sample had degraded (Figure 7). This translates to ~2% loss per month.

Plasma and Brain Samples

Table 2 shows the concentrations for the samples and the brain to plasma ratios. Norcocaine was not detected in either plasma or brain. After correction for dose, the reported concentrations are agreement with other studies in animals (17).

CONCLUSIONS

An assay for the quantification of cocaine in both plasma and brain has been developed. The method development

Table 2
Striatum, Whole Brain, and Plasma Cocaine Concentrations in Sprague-Dawley Rats 20 Minutes After Intraperitoneal Administration of 15 mg/kg Cocaine

Animal	Whole Brain Concentration, ng/g	Striatum Concentration, ng/g	Plasma Concentration, ng/ml
1	8.84	9.61	2752
2	8.27	8.79	1582
3	11.54	10.12	1776
4	8.98	11.61	1406
5	11.24	11.66	2080
Mean	9.77	10.36	1919
Std Dev	1.50	1.26	528
CV, %	15.4	12.14	27.5

had three objectives. One was that it would be easy to perform and batch sample processing would be easily accomplished. The ideal method would require no special sample preparation simply because the matrix was not plasma. The second goal was that the chromatography would be similar across sample matrices without any modification of the mobile phase. Finally, the assay would be sensitive and specific for cocaine. The method achieves each goal. The biggest drawback is that the method suffers from marginal recovery, but this is compensated for by a low limit of detection.

We have strived to make this method as rugged as possible. At every point in the assay we have tried to make the method as stable as possible from slight modifications. Hence, during sample preparation no buffers are prepared to a specific pH. We have used saturated buffers and rely on their pKa to maintain the precise pH. Only the mobile phase is prepared to an exact pH and even this was found to be a non-critical factor. These factors all act to increase the stability of the method over time. In summary, a validated HPLC method for the analysis of cocaine has been presented

which is rapid, sensitive, specific, and rugged to slight changes in sample preparation.

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