JOURNAL OF APPLIED TOXICOLOGY, VOL. 16(3), 215–219 (1996)

Development and Validation of a High-performance Liquid Chromatography Method for the Determination of Cocaine, its Metabolites and Lidocaine

Stephen A. Barat, Stacey A. Kardos and Mohamed S. Abdel-Rahman†
Department of Pharmacology and Toxicology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ 07103-2714, USA

Key words: cocaine; lidocaine; HPLC; biological fluid.

Street cocaine varies in purity and is often adulterated with various compounds. Some of these additives, such as lidocaine, may increase the toxicity of cocaine. A new precise, accurate and sensitive reversed-phase high-performance liquid chromatography method for the determination of cocaine, its metabolites and lidocaine in plasma samples has been developed and validated. This assay employed a phosphate-buffered aqueous mobile phase (pH 6.0) with an organic component consisting of acetonitrile and methanol and a C-18 column as the stationary phase. Minimum detection limits were 1 ng ml^{-1} for cocaine, 2.5 ng ml^{-1} for ethylcocaine and 5 ng ml^{-1} for benzoylecgonine, norcocaine, norethylcocaine and lidocaine. Linearity was demonstrated over a broad range of concentrations in plasma, with good sensitivity for cocaine and cocaine derivatives.

INTRODUCTION

Cocaine is an alkaloid that is found in the leaves of the South American shrub, *Erythroxylon* coca.[1] Its use has been acknowledged as early as 500 BC in Colombia and Peru in ancient religious rituals.[2] Cocaine abuse is widespread in the USA. In 1990, it was reported that 6.2 million Americans have used cocaine, 336 000 of them on a daily basis.[3] This widespread abuse occurs among all races and sexes. People aged 20–39 years accounted for 83.4% of cocaine-associated emergency room visits in 1989.[4]

Cocaine abusers have been reported to use other substances simultaneously with cocaine. It has been shown in a study that investigated cocaine abusers in an inner city setting that 30% of them have used other drugs with it.[5] Furthermore, other reports indicate that more than 50% of cocaine abusers also use ethanol simultaneously.[6] Under these circumstances, a unique metabolite of cocaine is formed. This metabolite, ethylcocaine, is pharmacologically active and its presence is indicative of simultaneous cocaine and ethanol abuse.[7]

Street cocaine is found in two forms, the hydrochloride salt and the free base which is more commonly referred to as crack. Cocaine free base is prepared from cocaine hydrochloride using common household products, such as baking soda, ammonia and kerosene. Illicit cocaine is often adulterated with various substances.[8] One of the more common adulterants, lidocaine, has been shown to potentiate cocaine's toxicity.[9] A study investigating the purity of street cocaine found that of 634 cocaine samples, 211 had lidocaine in varying amounts.[8] Furthermore, it has been reported that cocaine free base prepared from cocaine hydrochloride containing lidocaine retains 74–90.5% of the lidocaine in the starting material.[2]

Since cocaine is co-abused and adulterated with other drugs, multiple substances may appear in biological fluids. The simultaneous presence of other compounds with cocaine may increase its potential for toxicity. For example, it has been shown that the teratogenic potential of cocaine is increased in the presence of diazepam.[10] In order to investigate the toxicology of these drug interactions, the ability to identify and quantitate each compound is necessary. The aim of this study was to develop a method in order to detect and resolve cocaine, its metabolites and lidocaine by reversed-phase high-performance liquid chromatography.

EXPERIMENTAL

Animals

Male Sprague-Dawley rats (275–300 g) were obtained from Taconic Farms, Germantown, NY. The rats were housed three per cage in a room with constant temperature (25°C) and humidity and access to water and Purina Lab Chow *ad libitum*. All animals were maintained in accordance with the NIH guidelines for the care and use of laboratory animals. Rats were treated with cocaine and/or lidocaine, 5 mg kg^{-1} each, by intravenous injection. The injection (200 μl) was administered into the lateral tail vein under restraint with a commercially available Plexiglas device designed for this purpose. Plasma samples were

† Author to whom correspondence should be addressed.

CCC 0260–437X/96/030215–05

Received 18 May 1995
Accepted 13 November 1995

obtained under light ether anestheisa via cardiac puncture with a heparinized syringe. The use of animals in this protocol was approved by the Institutional Animal Care and Use Committee.

Reagents

Cocaine·HCl, benzoylecgonine, norcocaine, ethylcocaine fumarate and norethylcocaine fumarate were provided by NIDA, Bethesda, MD. Lidocaine·HCl was purchased from Astra Pharmaceuticals, Westborough, MA. Propylcocaine·HCl was purchased from Research Biochemicals Incorporated, Natick, MA. Monobasic potassium phosphate was purchased from Mallinckrodt, Inc., Paris, Kentucky. Triethylamine (HPLC grade) was purchased from Aldrich Chemical Co., Milwaukee, WI. Methanol and acetonitrile (HPLC grade) were obtained from JT Baker, Phillipsburg, NJ. Chloroform and isopropanol were purchased from EM Science, Gibbstown, NJ.

Chromatography

A Waters (Waters Associates, Milford, MA) high-performance liquid chromatography system equipped with an automated gradient controller, Model 510 HPLC pump, rheodyne 50-μl loop injector, Model 486 tunable absorption detector and a 746 data module was used. The spectrophotometer was set at a wavelength of 235 nm. Separation was achieved on a Waters Nova-Pak (4.0 μm, 3.9 × 150 mm; Waters Associates, Milford, MA) steel column which was kept at room temperature. The aqueous portion of the mobile phase consisted of 100 mM monobasic potassium phosphate with 30 mM triethylamine, constituting 72% of the total mobile phase. The remaining 28% of the mobile phase consisted of 60% acetonitrile and 40% methanol (v/v). Mobile-phase pH was adjusted to 6.0 with phosphoric acid. Prior to use, the mobile phase was filtered through a 1.2 μm filter (Waters-Millipore, Milford, MA) and degassed under a vacuum. The system flow rate was run at 0.55 ml min^{-1} and increased to 1.0 ml min^{-1} at 11 min into the analysis in order to elute the more hydrophobic compounds.

Standard solutions

A stock solution of each compound was prepared in methanol at a concentration of 1 mg ml^{-1}. Working standard solutions were prepared in the range 1–2000 ng ml^{-1}. Standards were also prepared in plasma from untreated rats. Briefly, 10 μl of a standard spiking solution (0.01–20.0 μg ml^{-1}) and internal standard (10.0 μg ml^{-1}) was added to 100 μl of plasma.

Extraction procedure

In order to extract the compounds from plasma, heparinized blood was obtained from untreated rats via cardiac puncture under light ether anesthesia. Collected blood was centrifuged at 1000 *g* for 15 min to obtain plasma. A 100 μl aliquot of blank plasma was spiked with 10 μl of a 10.0 μg ml^{-1} propylcocaine (internal standard) solution in methanol, yielding a concentration of 1.0 μg ml^{-1}. The extraction procedure employed a modified version of a method described by Lau.[11] A 200 μl aliquot of a 1.0 M borate buffer,[12] pH 9.0, was added to 100 μl of plasma in a glass test-tube. To this, 1.25 ml of a 1:9 mixture of isopropanol:chloroform was added, vortexed for 1 min and centrifuged for 5 min at 1000 *g* at room temperature. The organic layer was retrieved and placed in another glass test-tube and dried down under a gentle stream of air. The dried-down extract was then reconstituted in 100 μl of mobile phase and 50 μl was injected onto the column for analysis.

Precision, reproducibility and accuracy

In order to test the precision, reproducibility and accuracy of this method, the United States Pharmacopoeia XXII was used as a guideline. Assay precision was determined as the mean integrator response of five replicate injections for the peak of interest at 250 ng

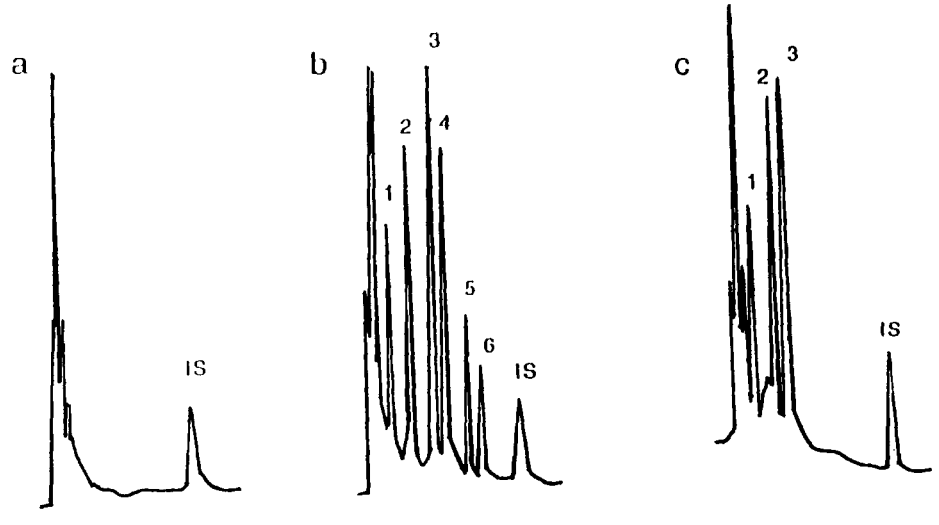

Figure 1. (a) Blank rat plasma with internal standard, 1.0 μg ml^{-1}. (b) Spiked rat plasma with 1.0 μg ml^{-1} of each of the following compounds: 1, benzoylecgonine; 2, lidocaine; 3, cocaine; 4, norcocaine; 5, ethylcocaine; 6, norethylcocaine. (c) Plasma obtained from a rat 5 min after i.v. injection of cocaine and lidocaine, 5 mg kg^{-1} each.

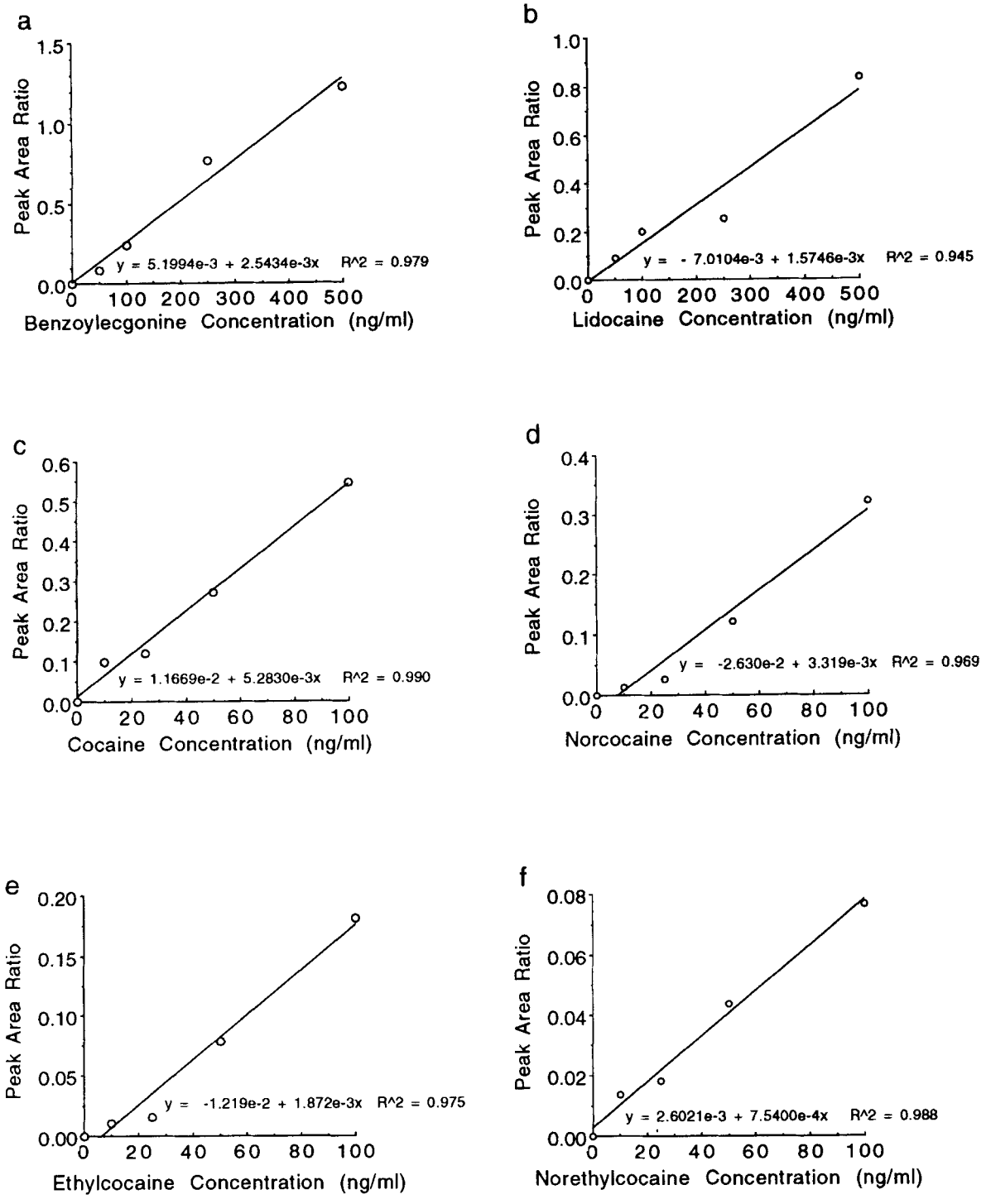

Figure 2. Plasma linearity studies for: a, benzoylecgonine; b, lidocaine; c, cocaine; d, norcocaine; e, ethylcocaine; f, norethylcocaine.

ml^{-1}. Theoretical plate count was determined by the formula $N = 16(T/W)^2$, where T-retention time of the peak and W = width of the peak. The capacity factor was calculated as $(t_1 - t_0)/(t_0)$, where t_0 = retention time of the solvent peak and t_1 the retention time of the peak of interest. Resolution was calculated as $2(t_2 - t_1)/(W_1 + W_2)$ where t_1 = retention time of the peak before the peak of interest, t_2 = retention time of the peak of interest, W_1 = width of the peak before the peak of interest and W_2 = width of the peak of interest.[13]

RESULTS AND DISCUSSION

Figure 1 shows chromatograms of: blank rat plasma with internal standard showing no interfering peaks with those desired for analysis; rat plasma spiked with cocaine, its metabolites, lidocaine and internal standard; and plasma obtained 5 min after injection in a male Sprague-Dawley rat. Figure 2 depicts the linearity studies of cocaine, its metabolites and lidocaine in plasma. The graphs are expressed as the peak area ratio of the

Table 1. HPLC assay precision

Compound[a]	Mean peak area[b]	Standard deviation	Relative standard deviation[c]
Benzoylecgonine	42124.4	2741.3	6.51
Lidocaine	23961.6	1638.8	6.84
Cocaine	99883.6	945.2	0.95
Norcocaine	47945.0	1357.5	2.83
Ethylcocaine	46456.0	1319.5	2.84
Norethylcocaine	25209.8	1308.9	5.19

[a]Listed in order of elution.
[b]Values represent mean integrator response from five replicate injections of 250 ng ml^{-1}.
[c]Calculated as 100 × (standard deviation/mean).

Table 2. HPLC assay resolution

Compound[a]	Mean[b]	Standard deviation	Relative standard deviation[c]
Benzoylecgonine	1.591	0.003	0.189
Lidocaine	1.536	0.009	0.586
Cocaine	1.383	0.014	1.012
Norcocaine	0.646	0.007	1.084
Ethylcocaine	1.394	0.004	0.287
Norethylcocaine	0.693	0.006	0.866

[a]Listed in order of elution.
[b]Values represent mean of five replicate injections of 250 ng ml^{-1}.
[c]Calculated as 100 × (standard deviation/mean).

Table 3. HPLC assay of theoretical plate count

Compound[a]	Mean[b]	Standard deviation	Relative standard deviation[c]
Benzoylecgonine	74.85	0.19	0.25
Lidocaine	350.24	1.48	0.42
Cocaine	245.06	0.76	0.31
Norcocaine	505.17	0.40	0.08
Ethylcocaine	528.93	0.41	0.08
Norethylcocaine	664.24	1.47	0.22

[a]Listed in order of elution.
[b]Values represent mean of five replicate injections of 250 ng ml^{-1}.
[c]Calculated as 100 × (standard deviation/mean).

Table 4. HPLC assay capacity factor

Compound[a]	Mean[b]	Standard deviation	Relative standard deviation[c]
Benzoylecgonine	1.23	0.003	0.24
Lidocaine	2.62	0.008	0.31
Cocaine	4.04	0.008	0.20
Norcocaine	4.79	0.002	0.04
Ethylcocaine	6.41	0.003	0.05
Norethylcocaine	7.30	0.009	0.12

[a]Listed in order of elution.
[b]Values represent mean of five replicate injections of 250 ng ml^{-1}.
[c]Calculated as 100 × (standard deviation/mean).

analyte peak to that of the internal standard peak. Minimum detectable concentrations of each standard solution were 1 ng ml^{-1} for cocaine, 2.5 ng ml^{-1} for ethylcocaine and 5 ng ml^{-1} for benzoylecgonine, norcocaine, norethylcocaine and lidocaine.

Table 1 documents the system precision for each compound. Studying the assay precision lends some insight into the degree of reproducibility of the method. The assay was found to be most precise for cocaine, norcocaine and ethylcocaine, producing relative standard deviations of 0.95, 2.83 and 2.84, respectively. The relative standard deviations for assay precision for norethylcocaine, benzoylecgonine and lidocaine were 5.19, 6.51 and 6.84, respectively. Table 2 depicts the assay resolution for each compound. Studying the resolution of the assay gives a measure of the degree of separation of each compound relative to each other. All of the compounds studied were well resolved from the solvent front. Benzoylecgonine was the earliest peak eluted in the analysis and the closest to the solvent front, however it was well resolved from it. All other compounds eluted at later times and were separated from each other, including the *N*-dealkylated metabolites of cocaine and ethylcocaine. Table 3 documents the calculated theoretical plate counts in order to study the effect of each compound on the analysis column. Table 4 reports the study of the capacity factor for cocaine, its metabolites and lidocaine. Benzoylecgonine had the lowest capacity factor value, then lidocaine, cocaine, norcocaine and ethylcocaine, and norethylcocaine with the highest.

The mobile-phase conditions reported were found to be optimum for achieving adequate peak resolution and separation. During assay development, it was found that lidocaine's retention time was very sensitive to small changes in pH. Resolution of cocaine, norcocaine, ethylcocaine and norethylcocaine was dependent on both pH and concentration of the organic component. Benzoylecgonine was most effected by changes in the concentration of the organic component and least affected by pH:

Although other methods such as radioimmunoassay (RIA) and gas chromatography/mass spectrometry (GC/MS) can be employed for the analysis of these compounds, HPLC offers some advantages. The high level of sensitivity of the method described allows quantitation of low analyte concentrations without employing radioisotopes. Also, the methodology is fast, simple to use and cost effective.

The application of this method employed an animal model. However, the analytical technique described in this report is sensitive enough for the quantitative and qualitative determination of these compounds in biological fluids and tissues even following abuse of these substances only on one occasion.

REFERENCES

1. N. L. Benowitz, Clinical pharmacology of cocaine. *Pharm. Toxicol* **72**, 3–12 (1993).
2. R. K. Siegel, Cocaine smoking. *J. Psychoactive Drugs* **13**, 271–343 (1982).
3. NIDA Notes, National Institute of Drug Abuse, Vol. **5(5)** (1991).
4. Drug Abuse Warning Network (DAWN) Annual Data, US Department of Health and Human Services, Vol. **1(9)** (1990).
5. A. Y. Ghali, J. J. Lindenthal, W. Deckel, R. Bansil and M. S. Abdel-Rahman, Patterns of cocaine abuse in an inner city emergency psychiatric service setting. *Med. Law* **8**, 165–170 (1989).
6. B. F. Grant and T. C. Harford, Concurrent and simultaneous use of alcohol and cocaine: results of a national survey. *Drug Alcohol Depend.* **25**, 97–104 (1990).
7. P. Jatlow, J. D. Elsworth, C. W. Bradberry, G. Winger, J. R. Taylor, R. Russell and R. H. Roth, Cocaethylene: a neuropharmacologically active metabolite associated with concurrent cocaine–ethanol ingestion. *Life Sci.* **48**, 1787–1794 (1991).
8. J. K. Brown and M. H. Malone, Status of drug quality in the street-drug market—an update. *Clin. Toxicol.* **9**, 145–168 (1976).
9. R. W. Derlet, T. E. Albertson and R. S. Tharratt, Lidocaine potentiation of cocaine toxicity. *Ann. Emergency Med.* **20**, 135–138 (1991).
10. S. Z. Mehanny and M. S. Abdel-Rahman, Teratogenic effect of cocaine and diazepam in CF1 mice. *Teratology* **43**, 11–17 (1991).
11. C. E. Lau, Determination of cocaethylene, cocaine and their metabolites in rat serum microsamples by high-performance liquid chromatography, and its application to pharmacokinetic studies in rodents. *J. Chromatogr.* **582**, 167–172 (1992).
12. J. A. F. de Silva and C. Puglisi, Determination of medazepam (Nobrium), diazepam (Valium) and their major biotransformation products in blood and urine by electron capture gas–liquid chromatography. *Anal. Chem.* **42**, 1725–1736 (1970).
13. The United States Pharmacopeia XXII, 1710–1712, US Pharmacopeial Convention, Inc., Rockville, MD (1990).