ELSEVIER

Journal of Chromatography B, 692 (1997) 61–65

JOURNAL OF CHROMATOGRAPHY B

Rapid liquid–liquid extraction of cocaine from urine for gas chromatographic–mass spectrometric analysis

Diana Garside[a], Bruce A. Goldberger[a,*], Kenzie L. Preston[b], Edward J. Cone[b]

[a]*Diagnostic Referral Laboratories, University of Florida College of Medicine, 2310 SW 13th Street, Gainesville, FL 32608, USA*
[b]*Addiction Research Center, National Institute on Drug Abuse, P.O. Box 5180, Baltimore, MD 21224, USA*

Received 6 May 1996; revised 24 September 1996; accepted 24 September 1996

Abstract

A novel, simple and economic liquid–liquid extraction method for isolating cocaine from urine was developed utilizing gas chromatography–mass spectrometry (GC–MS) for analysis and quantification. The use of a single nonpolar organic solvent allowed only nonpolar analytes to be extracted from the biological fluid, and consequently, no derivatization step was necessary before GC–MS analysis. Large numbers of specimens (>60) can be extracted in approximately 3 h with this procedure. The method is highly precise (C.V. <7%), accurate (>98%), sensitive (limit of detection of 5 ng/ml) and has a mean recovery of 48.8%.

Keywords: Cocaine

1. Introduction

A survey of the literature shows many methods for the extraction of cocaine from biological matrices including blood, plasma, saliva, urine, amniotic fluid and hair [1–19]. The procedures employ a variety of analytical instrumentation, the most prevalent being gas chromatography–mass spectroscopy (GC–MS). For example, Cone et al. [13] developed a sensitive assay for the measurement of cocaine, cocaethylene and six cocaine metabolites, and anhydroecgonine methyl ester in biological fluids. The assay employed solid-phase extraction (SPE), derivatization and detection by GC–MS. In an extension of this work, Wang et al. [14] developed a similar assay for the simultaneous measurement of cocaine, heroin and their metabolites in plasma, saliva, urine and hair. Another report, by Taylor and Le [20], described a fully automated robotic system developed for the extraction and derivatization of cocaine and benzoylecgonine in urine for GC–MS analysis. As part of a rapid screen for 100 basic drugs and metabolites including cocaine and benzoylecgonine from urine, Logan et al. [21] utilized SPE and high-performance liquid chromatography (HPLC) with diode array detection. Micellar electrokinetic capillary chromatography has also been applied to the separation of cocaine and its metabolites from extracts of biological fluids [22].

While early works describe liquid–liquid extraction (LLE) procedures, the advent of SPE has caused the later literature to be dominated by this technique as it provides very clean extracts, eliminates time-consuming filtration steps and avoids sample loss through emulsion formation. Most methods, regardless of technique, are designed to simultaneously

*Corresponding author.

PII S0378-4347(96)00459-8

extract cocaine and many of its metabolites including benzoylecgonine and ecgonine methyl ester. However, the need arises periodically in research and treatment protocols to accurately measure low concentrations of cocaine in the presence of high concentrations of cocaine metabolites. The presence of cocaine in urine can be used as a marker of recent cocaine use. Consequently, an extraction system that favors the recovery of cocaine and highly discriminates against cocaine metabolites, e.g., benzoylecgonine, would be useful.

We report a simple, rapid and sensitive, single-step LLE method for the isolation of cocaine from urine in the presence of high concentrations of benzoylecgonine utilizing GC–MS for detection and quantification. The use of a single nonpolar organic solvent provides a cost-effective method for the isolation of cocaine with the exclusion of the sometimes unwanted polar metabolites, particularly benzoylecgonine. In addition, since only cocaine and nonpolar metabolites are extracted, there is no need for derivatization prior to GC–MS analysis.

2. Materials and methods

2.1. Chemicals

Petroleum ether, ethyl acetate and acetonitrile (Fisher Scientific, Pittsburgh, PA, USA) were reagent grade solvents. Cocaine and d_3-cocaine were purchased from Radian Corporation (Austin, TX, USA). Buffer (pH 8.0±0.1) was prepared from a mixture of 0.1 *M* potassium phosphate and 10 *M* potassium hydroxide.

2.2. Standard preparation

All stock and working solutions were prepared in acetonitrile with volumetric glassware, and were stored at −20°C when not in use. Standard curves, prepared in blank urine with authentic drug standards, consisted of eight concentrations in the range of 25–4000 ng/ml. Each batch included four control samples, with two each at target concentrations of 250 and 750 ng/ml.

2.3. Liquid–liquid extraction

Urine (1 ml) was pipetted into a glass centrifuge tube containing phosphate buffer (pH 8; 2 ml) and internal standard, d_3-cocaine (25 μl of a 10 ng/μl solution). Petroleum ether (2 ml) was added and the mixture was vortex mixed for approximately 10 s. The two phases were separated by high speed centrifugation for 15 min. The organic phase (top layer) was transferred to a glass culture tube with a Pasteur pipette and dried under a stream of nitrogen at 60°C in a TurboVap (Zymark, Hopkinton, MA, USA). The residue was reconstituted with ethyl acetate (50 μl), vortex mixed and transferred to an autosampler vial for analysis by GC–MS.

2.4. Instrumentation

GC–MS analyses were performed with a Hewlett-Packard 5890 Series II Plus gas chromatograph and a Hewlett-Packard 7673 automatic liquid sampler interfaced with a Hewlett-Packard 5972 mass selective detector (Little Falls, DE, USA). A cross-linked 95% dimethyl 5% diphenylpolysiloxane capillary column (Restek Rtx-5; 15 m×0.25 mm I.D., 0.1 μm film thickness; Bellefonte, PA, USA) and a 2 mm I.D. silanized fused-silica liner were used. Injections (2 μl) were made in the splitless mode with a purge time of 0.50 min. Ultra-pure grade helium was used as the carrier gas, programmed at a constant rate of 1.0 ml/min. The injector port and transfer line temperatures were 250°C and 290°C, respectively. The oven temperature was maintained at 150°C for 0.5 min, and then programmed at 25°C/min to 250°C, with a hold at 250°C for 2.5 min.

The mass selective detector was operated in the selected ion monitoring mode. The following ions were monitored at a dwell time of 20 ms (ions in italics were used for quantification): [$^2H_3$]-cocaine: *m/z 185*, 275 and 306; cocaine: *m/z 182*, 272 and 303. Cocaine was identified based on comparison of retention time and ion ratio with the corresponding values of standards assayed in the same run. Ion ratios were calculated by dividing the area of the confirming ion by the area of the quantitative ion. Quantification of cocaine was based upon the ratio of the integrated ion area to the corresponding deuterated internal standard analogue.

The MS was tuned daily with perfluorotributylamine with ions m/z 69, 219 and 502. The electron multiplier was operated between 400–600 eV relative to the tune value. Routine maintenance of the GC–MS included clipping the GC column and replacing the injection port liner and gold-plated seal.

3. Results

Urine specimens were extracted by LLE and analyzed by GC–MS for cocaine. Fig. 1 represents the chromatogram of a 100 ng/ml cocaine standard (Panel A), and an authentic specimen containing cocaine at a concentration of 3068 ng/ml (Panel B). The simplicity of the method allowed large numbers of specimens (>60) to be extracted in a short period of time. Once reconstituted in ethyl acetate, the extracts were stable for approximately 12–18 h. The length of an typical batch was approximately 10 h. The limit of detection ($S/N>5$) for the method was approximately 5 ng/ml. The standard curve was linear over a range of 25–4000 ng/ml. Within-run and between-run accuracy and precision data of the assay using control samples is shown in Table 1. All control values were within 20% of the target concentration.

The recovery of cocaine from urine was based upon comparison of extracted standards to unextracted standards. The mean determination across 5 separate days was 48.8% (range=40.4–58.8%, standard deviation=20.8%). Although the recovery of cocaine was moderate, adequate sensitivity was achieved and further optimization was not necessary. In addition to the studies described here, an additional experiment was performed to demonstrate the absence of benzoylecgonine in the cocaine extracts. Urine fortified with 5000 ng/ml of benzoylecgonine, and extracted by the method described and assayed by GC–MS as the trimethylsilyl derivative, was negative for benzoylecgonine. Further, an authentic urine specimen known to contain 3068 ng/ml of

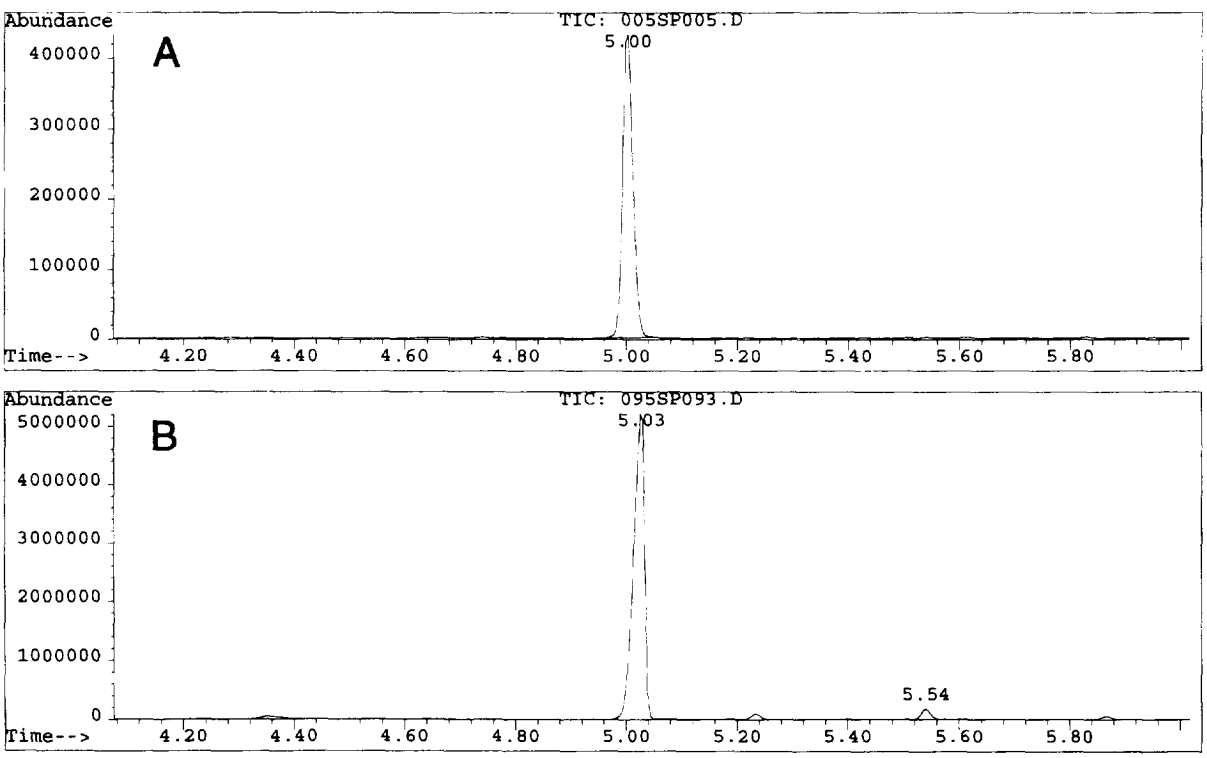

Fig. 1. Panel A: chromatogram of a 100 ng/ml cocaine standard prepared in urine; Panel B: an authentic urine specimen containing cocaine at a concentration of 3068 ng/ml.

Table 1
Accuracy and precision of cocaine determinations

	n	Concentration (ng/ml)		Accuracy (%)	Precision	
		Target	Measured mean (range)		S.D. (ng/ml)	C.V. (%)
Within-run	6	250	246.50 (232–261)	98.6	10.11	4.10
	6	750	762.83 (743–780)	101.7	14.82	1.94
Between-run	85	250	250.06 (210–277)	100.0	15.09	6.04
	85	750	755.31 (623–865)	100.7	49.80	6.59

cocaine and approximately 400 000 ng/ml benzoylecgonine was extracted by the method described (for cocaine) and by SPE (for benzoylecgonine). The chromatogram shown in Fig. 1, Panel B demonstrates no apparent sample matrix or cocaine metabolite interference. Fig. 2 illustrates the magnitude of benzoylecgonine present.

4. Discussion

A simple, rapid and sensitive, single-step LLE method was developed for determination of cocaine in urine. To achieve optimal assay sensitivity and reduce potential interference from matrix components, selected ion monitoring was utilized as the mode of detection. Since polar cocaine analytes were not extracted, derivatization was not required prior to GC–MS analysis.

Many of the procedures described in the literature utilize laborious, multi-step extraction procedures. Generally, the matrix is mildly alkalinized (approximately pH 9), extracted with an organic solvent and back-extracted into an acid fraction before final extraction into a second organic solvent. One multi-step LLE procedure boasts that as many as 60 plasma samples can be extracted in a day [12]. With the new single-step method reported in this paper, over 60 urine samples can be extracted by one person in approximately 3 h.

One of the earliest reports of the LLE of cocaine from plasma utilized ether, acetic acid and hexane [23]. Other solvent systems used to isolate cocaine from plasma and serum are dichloromethane [24], isoamyl alcohol–hexane solution [25] and chloroform–isopropanol solution [12]. The latter two procedures used hydrochloric acid and sulfuric acid, respectively, to facilitate extraction. Thompson et al. [15] utilized toluene, sulfuric acid and ethyl acetate to isolate cocaine from saliva. More recently, Dawling et al. [11] used *n*-butyl acetate in a single-step extraction of cocaine from mildly basic serum,

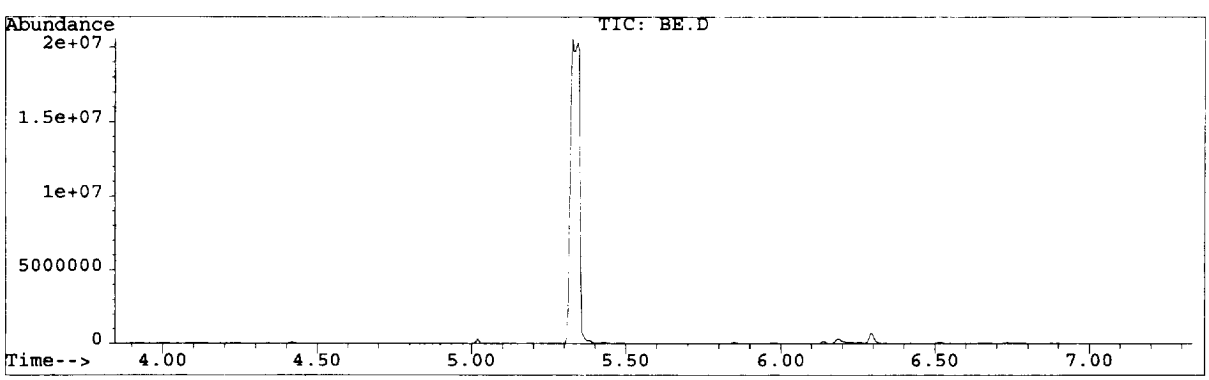

Fig. 2. Chromatogram of the trimethylsilyl derivative of benzoylecgonine isolated as a 1:10 dilution from the specimen shown in Fig. 1B.

plasma or blood samples. To the best of our knowledge, no protocol has been described specifically for the extraction of cocaine only from urine.

A problem commonly encountered with LLE procedures is the formation of emulsions, making separation of the aqueous and organic phases difficult. It was found with this procedure that very little, if any, emulsion formed and it was easily removed by the high-speed centrifugation step. SPE, most often used for the extraction of cocaine and its analytes, is unnecessary and would require more time and expense for this application. The use of a single, nonpolar solvent, combined with buffer enabled cocaine to be extracted with the exclusion of its polar analytes including benzoylecgonine.

References

[1] I.M. Roy, T.M. Jefferies, M.D. Threadgill and G.H. Dewar, J. Pharm. Biomed. Anal., 10 (1992) 943.
[2] M. Balikova and J. Vecerkova, J. Chromatogr. B, 656 (1994) 267.
[3] N.C. Jain, D.M. Chinn, R.D. Budd, T.S. Sneath and W.J. Leung, J. Forensic Sci., 22 (1977) 7.
[4] S.J. Mule and G.A. Casella, J. Anal. Toxicol., 12 (1988) 153.
[5] J.E. Wallace, H.E. Hamilton, H. Schwertner, D.E. King, J.L. McNay and K. Blum, J. Chromatogr., 114 (1975) 433.
[6] M. Nishikawa, K. Nakajima, M. Tatsuno, F. Kasuya, K. Igarashi, M. Fukui and H. Tsuchihashi, Forensic Sci. Int., 66 (1994) 149.
[7] K.L. Peterson, B.K. Logan and G.D. Christian, Forensic Sci. Int., 73 (1995) 183.
[8] L. Virag, S. Jamdar, C.R. Chao and H.O. Morishima, J. Chromatogr. B, 658 (1994) 135.
[9] R.E. Aderjan, G. Schmitt, M. Wu and C. Meyer, J. Anal. Toxicol., 17 (1993) 51.
[10] I.R. Tebbett and Q.W. McCartney, Forensic Sci. Int., 39 (1988) 287.
[11] S. Dawling, E.G. Essex, N. Ward and B. Widdop, Ann. Clin. Biochem., 27 (1990) 478.
[12] P. Jacob, B.A. Elias-Baker, R.T. Jones and N.L. Benowitz, J. Chromatogr., 417 (1987) 277.
[13] E.J. Cone, M. Hillsgrove and W.D. Darwin, Clin. Chem., 40 (1994) 1299.
[14] W.L. Wang, W.D. Darwin and E.J. Cone, J. Chromatogr. B, 660 (1994) 279.
[15] L.K. Thompson, D. Yousefnejad, K. Kumor, M. Sherer and E.J. Cone, J. Anal. Toxicol., 11 (1987) 36.
[16] L. Jain, W. Meyer, C. Moore, I. Tebbett, D. Gauthier and D. Vidyasagar, Obstet. Gynecol., 81 (1993) 787.
[17] J.A. Sandberg and G.D. Olsen, J. Chromatogr., 525 (1990) 113.
[18] O.Q. Casanova, N. Lombardero, M. Behnke, F.D. Eyler, M. Conlon and R.L. Bertholf, Arch. Pathol. Lab. Med., 118 (1994) 988.
[19] D. Garside and B.A. Goldberger, in P. Kintz (Editor), Drug Testing in Hair, CRC Press, 1996.
[20] R.W. Taylor and S.D. Le, J. Anal. Toxicol., 15 (1991) 276.
[21] B.K. Logan, D.T. Stafford, I.R. Tebbett and C.M. Moore, J. Anal. Toxicol., 14 (1990) 154.
[22] W. Thormann, S. Lienhard and P. Wernly, J. Chromatogr., 636 (1993) 137.
[23] A.N. Masoud and D.M. Krupski, J. Anal. Toxicol., 4 (1980) 305.
[24] D.M. Ruiz and M.M. Gallego, Vet. Hum. Toxicol., 30 (1988) 413.
[25] L.P. Hackett, L.J. Dusci and K.F. Ilett, J. Anal. Toxicol., 11 (1987) 269.