Liquid Chromatographic Determination of Cocaine, Benzoylecgonine, and Cocaethylene in Whole Blood and Serum Samples with Diode-Array Detection

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Abstract

An extraction with Bond Elut Certify solid-phase extraction (SPE) columns is developed for the isolation of cocaine, benzoylecgonine, and cocaethylene from whole blood and serum followed by reversed-phase liquid chromatography with diode-array detection. Two internal standards (2'-methylbenzoylecgonine and 2'-methylcocaine) with close structural resemblance to benzoylecgonine (a carboxylic acid) and to the two esters, cocaine and cocaethylene, are used in the analytical procedure. A thorough evaluation of this SPE and a comparison with different liquid–liquid extractions clearly show the superiority of the SPE. A linear response (correlation coefficient greater than 0.998) over a broad concentration range (0.025–5.0 µg/mL) is obtained. The sensitivity, specificity, precision (coefficients of variation less than 4.9% for within-day reproducibility and less than 5.3% for total reproducibility), and accuracy of the method are excellent for each analyte. Forensic blood samples from people suspected of cocaine abuse are analyzed and show the usefulness of the method, even for degraded postmortem samples.

Introduction

Cocaine is an alkaloid that is found in the plant *Erythroxylum coca* and is one of the most rapidly growing drugs of abuse; it is snorted, injected, or smoked as "free base" or "crack." Cocaine is rapidly and almost completely metabolized and deactivated to benzoylecgonine, ecgonine methyl ester, and ecgonine by esterases and spontaneous hydrolysis (1) (see Figure 1). Although benzoylecgonine, cocaine's main metabolite, has no pharmacological activity, it is of great toxicological interest; due to its long half-life (six times longer than cocaine), it remains detectable longer in the human body compared with the parent compound cocaine. Cocaethylene (2) is an active homologue of cocaine that arises through transesterification of cocaine following coconsumption of cocaine and alcohol. It shares many neurochemical and behavioral properties with cocaine and can reach significant blood concentrations.

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Numerous publications on the liquid chromatographic (LC) determination of cocaine and its metabolites have been published. Most publications are limited to the determination of cocaine, sometimes with benzoylecgonine and/or cocaethylene, in serum, plasma, or urine (3–10). Unfortunately, the forensic laboratory is often confronted with cases in which the only available specimen is hemolyzed and degraded blood. Moreover, a major problem concerning the determination in urine, in addition to this matrix's frequent unavailability, is the lack of a known, clear relationship between urinary concentration and toxicity or mortality. Supplementary information from the determination and quantitation of cocaine and its metabolites in blood proves extremely useful in these cases.

Several studies (11–15) have investigated the stability of cocaine and its metabolites in blood and biological fluids. Although they are not entirely consistent in their conclusions, they contain some useful experimental observations. An important conclusion of Isenschmid et al. (11) was that the benzoylecgonine concentration found in a blood sample is most likely constant in time and that the benzoylecgonine as such did not arise as a result of in vitro cocaine hydrolysis. However, Liu et al. (13) reported that the best procedures are those that detect both cocaine and benzoylecgonine because the total concentration (sum of cocaine and benzoylecgonine) in spiked blood samples kept at 16°C remained constant in their study. Brogan et al. (14) concluded that the concentration of cocaine in blood could be stabilized if the samples were promptly acidified and treated with pseudocholinesterase inhibitors such as organophosphates or sodium fluoride. For this reason, the addition of 2% sodium fluoride to blood and serum samples for cocaine analysis upon their arrival in the laboratory is recommended. The hydrolysis that is expected to take place between the time of death and the sampling at the autopsy remains a variable, yet it is beyond the control of any forensic toxicologist. We concluded that the analysis of cocaine exclusively should be avoided in judicial testing methods because failure to detect the parent drug in blood or urine may be the result of in vitro or postmortem hydrolysis.

Upon reviewing the reported sample preparation procedures, we discovered that liquid–liquid extraction solvents require an

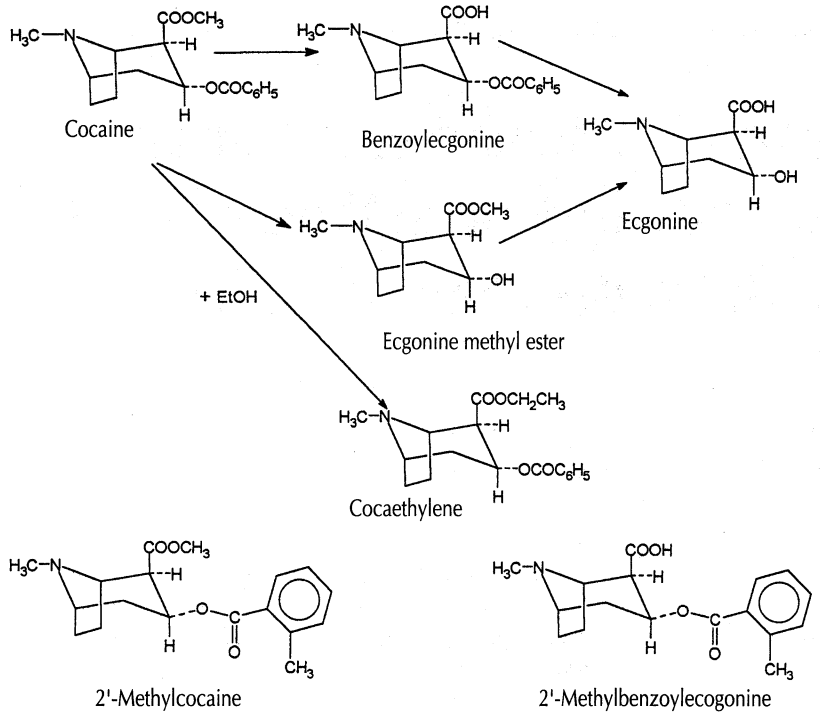

Figure 1. Chemical structure of cocaine and its main metabolites and of the two internal standards: 2'-methylcocaine and 2'-methylbenzoylecgonine.

Table I. Recovery Results (in %) of the Liquid–Liquid Extractions and SPE of Cocaine, Benzoylecgonine, and Cocaethylene and Their Internal Standards from Spiked Postmortem Whole Blood Samples*

Extraction solvent	BE	2'-Me-BE	Coc	CE	2'-Me-Coc
$CHCl_3$ – 2-propanol (95:5)	50	62	46	39	35
$CHCl_3$ – 2-propanol (90:10)	55	64	35	35	31
$CHCl_3$ – 2-propanol (80:20)	63	72	33	39	35
$CHCl_3$ – 2-propanol (70:30)	62	70	31	33	31
$CHCl_3$ – 2-propanol (60:40)	80	88	52	60	56
$CHCl_3$ – ethanol (95:5)	59	66	23	25	22
$CHCl_3$ – ethanol (90:10)	55	60	24	25	25
$CHCl_3$ – ethanol (80:20)	75	78	33	38	41
$CHCl_3$ – ethanol (70:30)	84	86	42	57	59
$CHCl_3$ – ethanol (60:40)	91	94	70	66	61
CH_2Cl_2 – 2-propanol (95:5)	41	54	31	33	33
CH_2Cl_2 – 2-propanol (90:10)	49	59	31	32	37
CH_2Cl_2 – 2-propanol (80:20)	45	56	30	35	35
CH_2Cl_2 – 2-propanol (70:30)	51	61	40	45	53
CH_2Cl_2 – 2-propanol (60:40)	52	62	48	54	61
CH_2Cl_2 – ethanol (95:5)	43	61	36	38	40
CH_2Cl_2 – ethanol (90:10)	53	66	55	45	51
CH_2Cl_2 – ethanol (80:20)	48	58	31	39	40
CH_2Cl_2 – ethanol (70:30)	51	61	40	45	53
CH_2Cl_2 – ethanol (60:40)	67	80	47	65	76
SPE	94	95	88	89	90

* Each extraction was carried out in triplicate. BE: benzoylecgonine, 2'-Me-BE: 2'-methylbenzoylecgonine, Coc: cocaine, CE: cocaethylene, 2'-Me-Coc: 2'-methylcocaine.

alcohol to improve the extraction efficiency of benzoylecgonine because of its high hydrophilic nature. The addition of alcohols, however, increases the recovery at the expense of the extracts' purity which negatively affects the detection limits (16). Solid-phase extractions (SPE) are considered to be a useful alternative for liquid–liquid extractions; the mixed-mode sorbents have become popular for the analysis of drugs of abuse (17). SPE has not yet been widely accepted for the isolation of drugs from whole blood in many forensic laboratories, perhaps because the SPE performed on these samples often causes clogging of the column and thus results in low recovery and reduced cleanliness of the resulting chromatograms. In this paper, we reported on our investigation of the liquid–liquid extraction of benzoylecgonine, cocaine, and cocaethylene from postmortem blood samples with different mixtures of chloroform or dichloromethane and 2-propanol or ethanol; we compared the results with those of SPE. An extraction with Bond Elut Certify solid-phase columns was developed for the isolation of cocaine and metabolites from whole blood and serum followed by reversed-phase high-performance liquid chromatography (HPLC) with diode-array detection (DAD). The results obtained with the SPE were irrefutably superior concerning both recovery of the compounds and quality of the resulting chromatograms. As was concluded in a study on the analysis of cocaine and metabolites in urine by HPLC with DAD (17), SPE can give reliable quantitative results if an adequate internal standard is used. Therefore, two internal standards (2'-methylbenzoylecgonine and 2'-methylcocaine) with close structural resemblance to benzoylecgonine (a carboxylic acid) and to the two esters, cocaine and cocaethylene, were used in the analytical procedure (see Figure 1).

Experimental

Apparatus

The LC unit was composed of a ternary low-pressure gradient pump (model 325) and an autosampler (model 460) with a 50-µL loop (Kontron Instruments, Milano, Italy). A DAD 440 linked to a Kromasystem 2000 data system (Kontron Instruments) was used for data acquisition and storage.

Reagents

All the reagents and products were of analytical grade and were from E. Merck (Darmstadt, Germany) unless stated otherwise.

HPLC-grade methanol and acetonitrile were from Romil Chemicals (Loughborough, UK), and HPLC-grade water was from Prosan (Ghent, Belgium). Ammonium hydroxide, 2-propanol, and dichloromethane were obtained from Aldrich Chemical (Milwaukee, WI). The SPE columns used were Bond Elut Certify (Varian, Harbor City, CA). The benzoylecgonine tetrahydrate, cocaethylene, and cocaine hydrochloride standards were from Makor Chemicals (Jerusalem, Israel). The internal standards 2'-methylbenzoylecgonine and 2'-methylcocaine were synthesized and characterized following an earlier described procedure (17).

Standard solutions

A stock standard solution of each compound (analytes as well as both internal standards) was prepared by dissolving 10 mg of the pure compound in 10 mL of acetonitrile. All solutions were stored in the dark at –20°C and were stable for at least one year. Working solutions were prepared by appropriate dilution of these stock standards in acetonitrile. For the internal standards, a single mixture containing both compounds was prepared at a concentration of 13 µg/mL. Similarly, the analyte standards were combined into a single set of seven different concentration levels (1, 2, 4, 10, 20, 50, and 100 µg/mL). They were all stored under the same conditions as the stock standard solutions and discarded after three months. All concentrations are expressed as that of the free base.

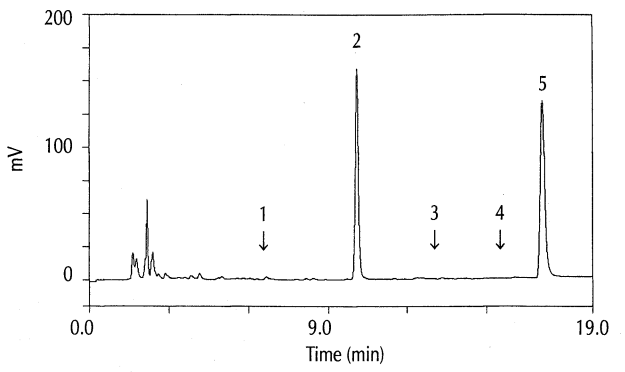

Figure 2. Chromatogram of a blank postmortem blood sample with peaks of 2'-methylbenzoylecgonine (2) and 2'-methylcocaine (5). Arrows indicate eluting position of benzoylecgonine (1), cocaine (3), and cocaethylene (4).

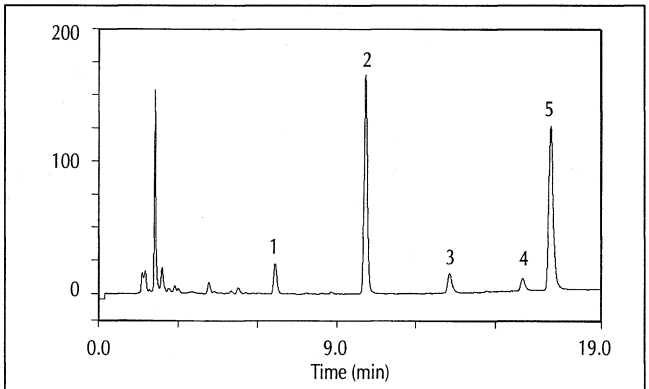

Figure 3. Chromatogram of a whole blood sample (the lowest point of the calibration graph) enriched with 0.05 µg/mL benzoylecgonine (1), 0.65 µg/mL 2'-methylbenzoylecgonine (2), 0.05 µg/mL cocaine (3), 0.025 µg/mL cocaethylene (4), and 0.65 µg/mL 2'-methylcocaine (5).

Samples

Samples were from forensic sources. Blood samples (serum and whole blood) were investigated whenever there was suspicion of cocaine use (a positive result for urine, known drug use, needle tracks). Upon the arrival of the blood samples to the laboratory, 2% NaF was immediately added. Samples were stored at –20°C and analyzed within one week after arrival. Calibration curve standards were treated in a similar way as unknown samples and prepared in blank postmortem blood or serum samples (2 mL) from healthy individuals not taking any medication. To each calibration sample aliquot, 100 µL of the internal standard working solution and 100 µL of an appropriate working standard dilution were added. These samples were then allowed to equilibrate for 15 min at room temperature.

SPE method validation

Standard curves were prepared in blank postmortem blood or blank serum as previously described over a concentration range of 0.05–5.0 µg/mL for cocaine and benzoylecgonine and 0.025–2.5 µg/mL for cocaethylene. For each curve, seven different concentration levels, equally distributed over the concentration range, were used. Peak height ratios between benzoylecgonine and its internal standard and between cocaine and cocaethylene and their internal standard were plotted against the concentration of each compound.

The recovery of the three compounds was assessed. Three individual extracts and three replicates of the compounds, directly prepared in the eluent, were injected onto the column. The assay recovery for each compound was determined using the following equation: mean peak height of the extract/mean peak height of the direct injection × 100.

Precision was evaluated by analyzing aliquots from a blank whole blood or serum pool spiked with the tested compounds at three different concentrations on the same day (eight replicates, within-day reproducibility) and over separate days (eight replicates, total reproducibility).

To supervise the overall accuracy, two positive control sam-

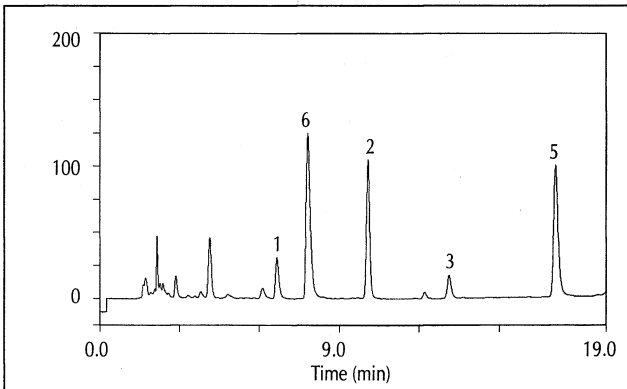

Figure 4. Chromatogram of a real postmortem whole blood sample. Peaks: benzoylecgonine (1), 2'-methylbenzoylecgonine (2), cocaine (3), 2'-methylcocaine (5), and 3,4-methylenedioxy-*N*-ethylamphetamine (6).

ples were independently prepared using different solutions and different volumetric material than those used to prepare calibration standards. They were extracted and analyzed with each batch of samples, and their quantitative results related to the spiked concentrations.

The detection limit was determined by analyzing decreasing concentrations of the compounds in whole blood. It was established as the lowest concentration at which the ultraviolet (UV) spectrum could be recognized by the system with a matching factor greater than 900 on a scale of 0–1000. The quantitation limit was defined as the lowest point of the calibration graph.

Isolation from forensic blood samples

Liquid–liquid extraction

To 2 mL whole blood, 100 µL of internal standard working solution, 1 mL of a 1M K_2CO_3 buffer (pH 9.5) and 8.0 mL of a mixture of dichloromethane or chloroform and 2-propanol or ethanol in different proportions (95:5, 90:10, 80:20, 70:30, and 60:40) were added. Samples were mixed at a rotary mixing device (20 min) and centrifuged (20 min, 2500 rpm). The organic layer was transferred into a second tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The dry residue was dissolved in 200 µL of the HPLC eluent A (see Chromatographic conditions section), and a 50-µL aliquot was injected into the HPLC.

SPE

Whole blood. We used a modification of a procedure for the toxicological analysis of whole blood as described by Chen et al. (18) and Zweipfenning et al. (19). Blood (2 mL) was sonicated for 5 min; 3 mL of water, 2 mL of a phosphate buffer (0.1M, pH 6.0, 6.805 g KH_2PO_4 was dissolved in 500 mL HPLC water, and the pH was adjusted with 1.0M KOH), and 100 µL of the internal standard solution were added. After vortex mixing for 30 s, sonicating for 15 min, and centrifuging for 10 min (3000 rpm), the supernatant was used for SPE.

Serum. Water (3 mL), 0.1M phosphate buffer (2 mL, pH 6.0), and 100 µL of the internal standard solution were added to 2 mL serum. After 30 s of vortex mixing, this mixture was used for SPE.

SPE procedure. The extraction cartridges were conditioned with methanol (3 mL) followed by 3 mL phosphate buffer (0.1M, pH 6.0). The sample was slowly applied (at approximately 1 mL/min) to the column, which was then washed with HPLC-grade water (3 mL), 0.1M hydrochloric acid (3 mL), methanol (3 × 3 mL), and acetonitrile (3 × 3 mL). The cartridges were finally eluted with 2.0 mL of dichloromethane–2-propanol–25% ammonium hydroxide (80:20:2, v/v/v). The eluent was evaporated to dryness at 40°C under a gentle stream of nitrogen. The dry residue was dissolved in 100 µL of the HPLC eluent A (see Chromatographic conditions section), and a 50-µL aliquot was injected into the HPLC.

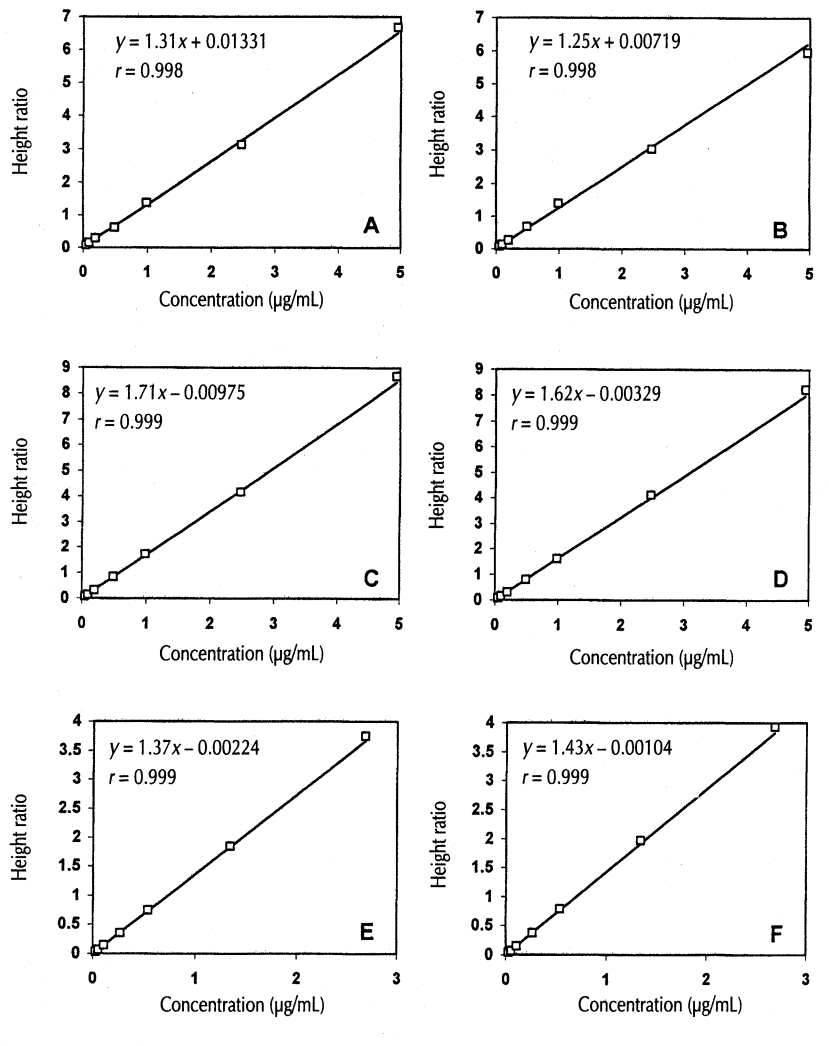

Figure 5. Typical calibration curves for benzoylecgonine in whole blood (A) and serum (B), cocaine in whole blood (C) and serum (D), and cocaethylene in whole blood (E) and serum (F).

Chromatographic conditions

Chromatographic separation was achieved on a Hypersil BDS C_{18} column (150 × 4.6 mm, 5-µm particle size) protected by a guard column of the same packing (7.5 × 4.6 mm, 5-µm particle size). Both columns were from Alltech (Deerfield, IL). The mobile phase was a 0.045M solution of ammonium acetate in HPLC-grade water (80%), methanol (10%), and acetonitrile (10%) (eluent A) or in methanol (40%), acetonitrile (40%), and HPLC-grade water (20%) (eluent B). Before use, both eluents were filtered through Nylon 66 (0.2-µm pore size) filters (Alltech). A linear gradient from 100 to 47.2% A within 19 min was used, which was then held for 2 min. The pump flow rate was 1 mL/min. After completion of the chromatographic run, the pump was programmed to regain its initial conditions within 1 min, and 5 min

Table II. Precision Evaluation for the Analysis of Cocaine and its Metabolites*

Compound	Concentration added (µg/mL)	Coefficients of variation (%) Within-day reproducibility Whole blood	Total reproducibility Whole blood	Within-day reproducibility Serum	Total reproducibility Serum
Benzoylecgonine	0.05	1.6	3.9	2.3	5.3
	1.0	1.5	4.1	4.4	4.8
	5.0	2.3	4.7	3.6	3.5
Cocaine	0.05	4.9	4.1	3.5	4.9
	1.0	1.0	3.3	3.8	4.1
	5.0	2.0	4.7	2.8	5.2
Cocaethylene	0.025	3.4	1.9	1.8	3.8
	0.5	1.0	2.6	3.1	4.9
	2.5	1.7	4.8	4.2	4.6

* Eight replicates.

Table III. Accuracy Results Obtained for the Analysis of Cocaine and its Metabolites

Compound	Matrix analyzed	Concentration added (ug/mL)	Accuracy result (%)
Benzoylecgonine	Whole blood	0.165	101.1
	Whole blood	1.65	103.1
	Serum	0.165	98.8
	Serum	1.65	100.4
Cocaine	Whole blood	0.164	101.4
	Whole blood	1.64	103.7
	Serum	0.164	100.0
	Serum	1.64	100.6
Cocaethylene	Whole blood	0.0893	102.6
	Whole blood	0.893	102.3
	Serum	0.0893	98.6
	Serum	0.893	99.7

reconditioning time was allowed before the next injection. The detector was set to collect a spectrum every 21 ms (over the 220–400 nm range). Display wavelengths were 236 and 280 nm with a bandwidth of 4 nm, and the chromatographic channel at 236 nm was used for the final chromatogram construction. The identity of each compound was established by comparing the retention times and UV spectra in real samples with those obtained after the injection of standards.

Results and Discussion

Balíková and Večerková (5) experimentally confirmed that benzoylecgonine can be simultaneously extracted with cocaine from neutral or basic media with chloroform or dichloromethane. The efficiency of such an extraction is advantageously enhanced by the addition of alcohols (most often 2-propanol or ethanol) to the extraction solvent. The same conclusions can be drawn from a review (16) that summarized several procedures in which cocaine and benzoylecgonine were simultaneously determined. The various extractions were all based on the same principle: the samples were buffered at an alkaline pH followed by extraction with chloroform–2-propanol, phloroform–ethanol, dichloromethane–2-propanol, or dichloromethane–ethanol in different proportions.

In search of an efficient extraction method for the determination of cocaine and benzoylecgonine in whole blood, we explored the suitability and potentials of different liquid–liquid extractions. We extracted spiked, forensic blood samples (1 µg/mL of each compound and of the two internal standards, in triplicate) with different mixtures of dichloromethane–chloroform and ethanol–2-propanol after buffering at pH 9.5. Our main interest in this experiment concerned extraction recovery and the degree of sample cleanup as evidenced by the resulting chromatograms. The LC chromatograms we obtained after these liquid–liquid extractions were all essentially unacceptable. In some chromatograms, the peaks were barely discernable; the better chromatograms still contained numerous interfering and late-eluting peaks preventing qualitative or quantitative interpretation. Recovery results from these experiments are presented in Table I. As expected, chromatogram quality was inversely related to the obtained recovery for our analytes of interest. The recoveries were unsatisfactory for all of the mixtures, particularly considering the demand for simultaneous cocaine and benzoylecgonine assessment. For that reason, we did not investigate an additional cleanup step of the liquid–liquid extracts because this would only result in extra loss of compounds, which would result in an even lower recovery and consequently less reliable quantitative data.

Therefore, we decided to switch to SPE. The recovery results (shown in Table I) and the chromatograms of a blank postmortem blood sample (shown in Figure 2), a spiked whole

blood sample (the lowest point of the calibration curve, Figure 3), and a real postmortem blood sample (Figure 4) proved the superiority of our extraction.

The combination of sonication and dilution of the blood was found to be an effective solution for the problem of obstruction of the solid-phase columns. The blood sample was sonicated to disrupt the red blood cells and facilitate drug release. It was also diluted with water and phosphate buffer to reduce the viscosity of the sample, adjust the pH, and lower the ionic strength of the sample. No protein precipitation step was included because we found that this resulted in a lower recovery of cocaine and its metabolites. In order to remove particulate matter, the diluted blood was centrifuged. We obtained the highest concentrations of cocaine and benzoylecgonine in real postmortem blood samples when they were sonicated twice: once before adding the water and phosphate buffer and once afterwards. For the overall SPE, we also found that in order to obtain a good recovery in the higher concentration ranges, SPE columns containing 300 mg of sorbent were crucial and that only an intensive column wash including methanol and acetonitrile resulted in sufficiently clean chromatograms, particularly for degraded postmortem samples. The presence of an alcohol (2-propanol) in the elution mixture of the SPE columns was not considered hazardous with respect to transesterfication, resulting in the eventual formation of "cocaisopropylene." Two studies (20,21) established that such a transesterification between cocaine and an alcohol never occurs spontaneously. It is mediated by esterases and occurs solely in the presence of liver homogenates.

The calibration curves were linear over the specified ranges. They were constructed using weighted linear regression in an effort to account for data heteroscedasticity. The concentration of cocaine and its two metabolites in unknowns was calculated from this regression line. Good correlations (greater than 0.998) were obtained for all the compounds in both matrices, and the y-intercepts for all the curves were virtually zero (see Figure 5).

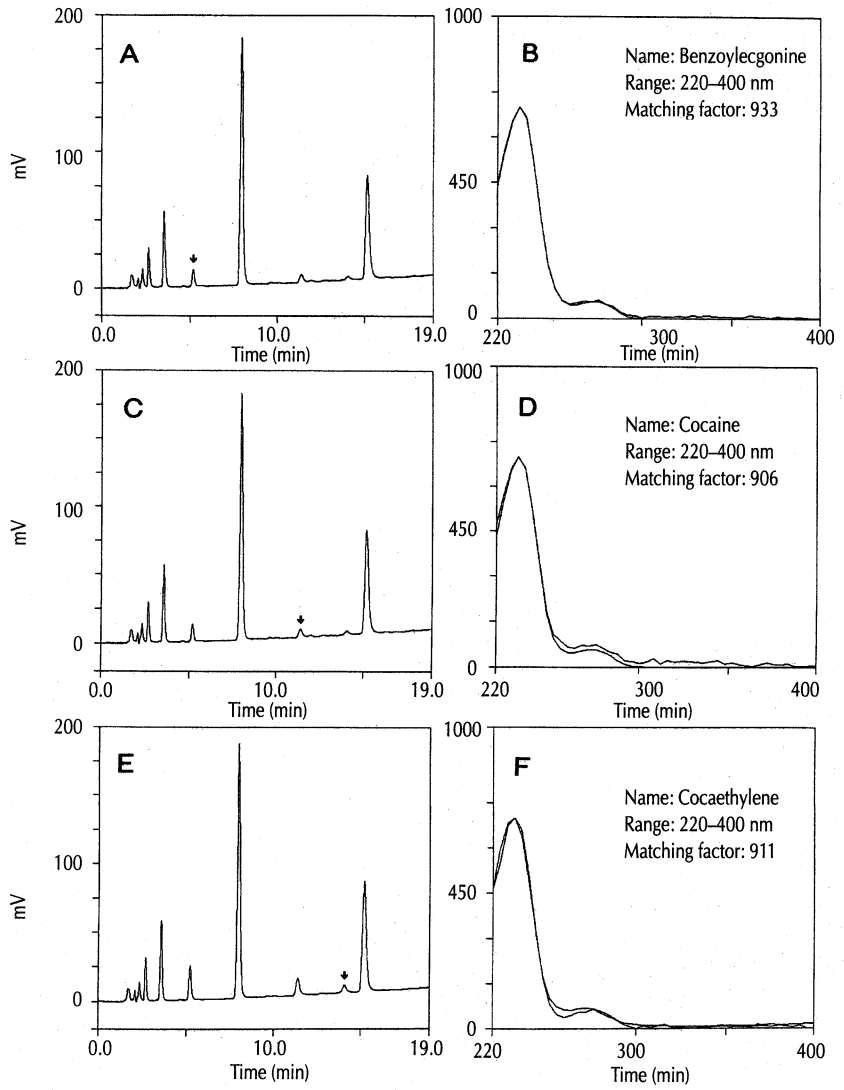

Figure 6. Chromatograms of serum samples enriched with 0.02 µg/mL (the detection limit) of the various analytes with the corresponding UV spectra. Benzoylecgonine (A and B), cocaine (C and D), and cocaethylene (E and F). Respective UV spectra (see arrow) are matched with a reference spectrum (matching factor scale, 0–1000).

The recovery for our SPE (Table I) was very good. The SPE recovery in serum was in concordance with the recoveries found in whole blood and was equally good. Table II presents the obtained reproducibility data. Coefficients of variation were not higher than 5.3% for any of the three compounds. These results clearly indicated that the reproducibility suited the purpose of the analytical method. In the accuracy evaluation of our method (Table III), we found values between 98.6 and 103.7% (determined at two concentrations, i.e., 0.150 and 1.50 µg/mL for benzoylecgonine and cocaine and 0.090 and 0.900 µg/mL for cocaethylene in whole blood and serum). The detection limit as defined in the experimental section was 0.02 µg/mL (Figure 6). The detection limit would have been much lower if the more common signal-to-noise definition was used. We restricted ourselves to the more stringent criteria of spectral recognition in order to preserve, even in the lowest concentration range, the double identification potential of retention time and UV spectra. The quantitation limit, defined as the lowest point of the calibration graph with an acceptable reproducibility (less than or equal to 6%), was 0.05 µg/mL for benzoylecgonine and cocaine and 0.025 µg/mL for cocaethylene.

We examined 80 compounds, prescription drugs, and drugs of abuse for interference (data not given). Only three compounds (the bacon, bromazepam, and propranolol) partially overlapped with one of our compounds of interest (17). Fortunately, the UV spectra of the interfering substances were distinctly different from those of either analyte under investigation, which allowed a

Table IV. Results in Cases that Tested Positive for Cocaine

Case	Matrix	Benzoylecgonine (µg/mL)	Cocaine (µg/mL)	Cocaethylene (µg/mL)	Other drugs involved
Postmortem	Whole blood	3.850	1.201	0.031	cannabinoids, ethanol
Postmortem	Whole blood	0.672	undetectable	undetectable	opiates, benzodiazepines, cannabinoids
Postmortem	Whole blood	0.428	undetectable	undetectable	3,4-methylenedioxy-*N*-ethylamphetamine, benzodiazepines, cannabinoids
Postmortem	Whole blood	0.297	0.093	0.062	opiates, benzodiazepines, ethanol
Postmortem	Whole blood	1.371	0.298	undetectable	opiates
	Serum	1.362	0.286	undetectable	
Postmortem	Whole blood	0.261	undetectable	undetectable	opiates, amphetamines
Postmortem	Whole blood	0.465	undetectable	undetectable	opiates
Postmortem	Whole blood	0.630	0.053	undetectable	benzodiazepines, cannabinoids
	Serum	0.691	0.058	undetectable	
Judicial	Whole blood	0.288	undetectable	undetectable	benzodiazepines, cannabinoids methadone
Judicial	Whole blood	0.915	undetectable	undetectable	benzodiazepines
Judicial	Whole blood	2.53	0.46	undetectable	opiates, benzodiazepines

reliable differential diagnosis.

To evaluate the practical application of the method, whole blood and/or serum samples from postmortem or judicial cases (33 samples) were analyzed whenever cocaine use was suspected. The obtained results of the cases that tested positive (11 samples) are summarized in Table IV together with a list of other drugs, which the overall toxicological screening indicated were taken in combination.

Conclusion

An in-depth evaluation of liquid–liquid extraction compared with SPE for the quantitative evaluation of cocaine and metabolites clearly showed the superiority of the latter approach. To that end, we have developed and thoroughly validated such an SPE procedure, combined with LC analysis. As can be seen from the various results, it excelled in every way when compared with liquid–liquid extraction-based methods. The obtained validation data showed that it can provide the analytical or forensic toxicologist with sound quantitative results. This was made possible by the combination of a carefully elaborated SPE, the use of internal standards that perfectly compensate for possible problems and eventual losses in the extraction, and a robust chromatographic method. We are convinced that this method is also a step in the right direction toward finding a distinct correlation between toxic effects and whole blood or serum concentrations of cocaine and its metabolites.

Acknowledgments

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