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Reversed-phase high-performance liquid chromatographic determination of cocaine in plasma and human hair with direct fluorimetric detection

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Abstract

A simple, but sensitive and specific, high-performance liquid chromatographic assay for cocaine with direct fluorimetric detection, particularly intended for the routine analysis of hair and blood samples, is described. Benzoylecgonine, eluting before cocaine in a completely resolved peak, is also detectable. Detection is based on the weak native fluorescence of cocaine and benzoylecgonine, depending on the benzene ring present in both molecules. Hair samples (20–200 mg) were incubated overnight in 2 ml of 0.25 *M* HCl at 45°C and extracted with a commercial liquid–liquid method; the dried residue reconstituted with 500 μl of 0.05 *M* NaH_2PO_4 (pH 5.2) was injected. Blood plasma samples (200 μl) were mixed with 150 μl of 0.1 *M* Na_2HPO_4 (pH 8.9) and extracted with 5 ml of chloroform–2-propanol (9:1); the organic phase was evaporated and the residue dissolved and injected as above. Isocratic reversed-phase liquid chromatography was carried out on a column (150 × 4.6 mm I.D.) packed with spherical 5-μm poly(styrene–divinylbenzene) particles; the mobile phase was 0.1 *M* potassium phosphate (pH 3)–methanol–tetrahydrofuran (70:25:5). The excitation and emission wavelengths were set at 230 and 315 nm, respectively. Under the described conditions, cocaine eluted in a symmetrical peak with a capacity factor of about 5. The limit of detection was about 1 ng/ml (0.2 ng injected), with a signal-to-noise ratio of 3. Using external standardization and partial loop filling, the intra-assay precision of the assay was characterized by R.S.D.s of 5.0 and 3.6% ($n = 6$) for cocaine concentrations of 10 and 100 ng/ml, respectively, and in inter-assay tests ($n = 6$) R.S.D.s of 7.5 and 5.2% were achieved for the same cocaine levels. The linearity of the method was fairly good in the concentration range 1.5–500 ng/ml ($r^2 = 0.9998$). Possible interferences from as many as 90 therapeutic and/or illicit drugs were excluded.

1. Introduction

Cocaine has long been the major abused illicit drug in the USA and, in recent times, it has also flooded into Europe [1]. Differently from opiates, fatal acute intoxications from cocaine are relatively rare, but it is still a frequently mentioned drug by emergency rooms in the USA [2]. In addition, as one of the most potent central nervous system stimulants, cocaine use is often related to road accidents and diverse violent crimes [3]; its use as doping agent has also been reported [4].

Several methods have been developed for the determination of cocaine and its metabolites in biological fluids (*e.g.*, serum, plasma, urine and vitreous humour) and in tissues, among which hair has recently received particular attention [5].

Notwithstanding problems posed by its poor

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stability [6,7] and rapid metabolism to biologically inactive hydrophilic products (ecgonine methyl ester, benzoylecgonine, ecgonine) [8], several immunometric methods (*i.e.*, radioimmunoassays, enzyme immunoassays, fluorescence polarization immunoassays) and chromatographic methods of analysis, as specified later, are now available. However, it is well known that most immunoassays, because of insufficient selectivity, are not suitable for the determination of cocaine in the presence of its metabolites; this is particularly important in clinical and forensic toxicology, if one considers that only cocaine (excluding the minor metabolite norcocaine) is biologically active and that its major metabolites have longer half-lives [8]. Further, results from immunoassays need, in principle, confirmation with alternative (chromatographic) techniques to achieve legal defensibility [9].

Several gas chromatographic (GC) methods for the determination of cocaine and its metabolites in biological fluids and tissues have been reported, using flame ionization [10] or, more effectively, nitrogen-selective [11–13] or electron-capture [13,14] detection. More recently, gas chromatography–mass spectrometry (GC–MS) has achieved wide acceptance, becoming almost a "gold standard" [15,16] (for a review, see ref. 17).

Tandem mass spectrometry [18] and ion-trap mass spectrometry [19] have also proved to be excellent, although very expensive, spectrometric tools for, mainly qualitative, investigations of cocaine in complex biological matrices, such as human hair.

Reversed-phase high-performance liquid chromatography (HPLC), inherently more compatible with biological matrices than GC, has also been applied to the determination of cocaine and its more polar metabolites, especially for emergency toxicology purposes. However, as almost all the methods reported in the literature use UV detection around 230 nm [20–32], some sensitivity and specificity problems still remain unresolved. This means that sample preparation needs special care.

The use of electrochemical detection, in principle more sensitive and selective, was first reported by Selavka *et al.* [33] and applied to plasma by Miller and DeVane [34], but the high potentials required limited both sensitivity and selectivity in practice.

Also the HPLC method with fluorescence detection (HPLC–FL) proposed by Roy *et al.* [35], although aimed specifically at the determination of the non-UV-absorbing metabolite ecgonine methyl ester, did not improve the sensitivity of cocaine determination and required a complex system of postcolumn ion-pair derivatization and in-line extraction.

Recently, capillary electrophoresis has also been reported as an alternative method for assaying drugs of abuse, including cocaine, in biological samples, but problems of sensitivity and reliability still exist [36,37].

The aim of this work was to develop a simple but very sensitive and specific HPLC assay for cocaine, based on the weak native fluorescence of the drug (related to its benzene ring), intended for application in forensic and clinical toxicology and, particularly, in the routine analysis of hair and blood samples.

2. Experimental

2.1. Reagents and standards

Toxi-tubes A (Analytical Systems, Laguna Hills, CA, USA) and Isolute Confirm HCX (IST, Hengoed, UK) were used for liquid–liquid and liquid–solid extraction, respectively.

Stock standard solutions of cocaine (Sigma, St. Louis, MO, USA) were prepared in methanol at 1 mg/ml and stored at −18°C. Working standard solutions at suitable concentrations were prepared every day in water or drug-free extracts of plasma or hair, as needed.

The chromatographic peak of benzoylecgonine was identified using standards of benzoylecgonine (about 10 μg dried on glass microfibre discs impregnated with silicic acid) enclosed in the Toxi Disc Library (Analytical Systems). Larger amounts of benzoylecgonine were obtained from cocaine by mild hydrolysis in 0.01 *M* borate buffer (pH 8.6) at room temperature; the conversion was monitored by this HPLC method and the benzoylecgonine peak was collected.

However, lacking a certified standard, no precise titration of benzoylecgonine was carried out.

Standards of 90 therapeutic drugs and drugs of abuse (10 μg each), supplied desiccated on glass microfibre discs impregnated with silicic acid, were obtained with the Toxi Disc Library.

Water and other solvents, all of HPLC grade, and chemicals (RPE grade) used for extraction and liquid chromatography were purchased from Carlo Erba (Milan, Italy).

Citrate–phosphate–dextrose solution (Sigma) was used as anticoagulant, according to the producer's guidelines (anticoagulant:whole blood ratio equal to 1.4:10).

2.2. HPLC instrumentation and analytical conditions

The isocratic HPLC system used consisted of a single-piston high-pressure pump (Model 302; Gilson, Villiers-le-Bel, France), a pulse damper (Model 802 C; Gilson), a manual injector (Model 7125; Rheodyne, Cotati, CA, USA) with a 200-μl loop and a double monochromator spectrofluorimeter (Model 821 FP; Jasco, Tokyo, Japan). The excitation and the emission wavelengths were routinely set at 230 and 315 nm, respectively. The detector signal was recorded with a data system (Model 620; Gilson).

The column (150 × 4.6 mm I.D.) was packed with spherical 5-μm poly(styrene–divinylbenzene) (Bio-Gel PRP 70-5; Bio-Rad Labs., Brussels, Belgium). The mobile phase, pumped at a flow-rate of 0.5 ml/min, was 0.1 *M* potassium phosphate (pH 3)–methanol–tetrahydrofuran (70:25:5).

Usually, 100 μl of sample were injected with partial loop filling but, when sensitivity was crucial, full-loop injections were accomplished. In this event, the loop was flushed with a sample volume at least three times greater than that of the loop itself (200 μl).

Lacking a suitable internal standard, external standardization was adopted for quantification.

Using a feature of the Jasco spectrofluorimeter, rough fluorescence spectra of 10 μg/ml cocaine dissolved in water and in 50 m*M* phosphate buffers in the pH range 1.5–9.2 were obtained. For this purpose, the flow cell of the instrument was filled with cocaine solutions using an intradermal syringe. The width of the excitation and emission slits was 18 nm.

2.3. Sample preparation

Hair samples (20–200 mg), cut close to the scalp, were washed with two 20-ml volumes of 0.3% Tween 20 (Sigma) solution in water and then thoroughly rinsed with tap water. After drying at 37°C, the hair samples were cut manually into small fragments and incubated overnight in 2 ml of 0.25 *M* HCl at 45°C. The resulting mixtures were neutralized with equimolar amounts of 1 *M* NaOH and extracted twice into the organic phase with ready-to-use Toxi-Tubes A. The pooled organic layers were evaporated to dryness and the residue was usually reconstituted with 500 μl of 0.05 *M* NaH_2PO_4 (pH 5.2).

Blood bank plasma samples (with citrate–phosphate–dextrose) were assayed in addition to plasma and cadaveric blood from intoxicated subjects. The addition of NaF to blood to prevent cocaine hydrolysis was omitted because the plasma was soon extracted after blood centrifugation (at 4°C) or, otherwise, frozen at −20°C. Amounts of 200 μl plasma were mixed with 150 μl of 0.1 *M* Na_2HPO_4 (pH 8.9) and 5 ml of chloroform–2-propanol (9:1); after vortex mixing for 2 min and centrifuging at 3500 rpm (about 700 *g*) for 10 min, 4 ml of the organic phase were evaporated to dryness under air stream and the residue was dissolved as above.

With "dirty" biological matrices, such as some hair samples, a solid–liquid extraction (SPE) step was added to the liquid–liquid extraction. In this case, the extracts in phosphate buffer (pH 5.2) were loaded in "double-mechanism" (reversed-phase and cation-exchange) SPE cartridges (Isolute Confirm HCX, 80 mg), previously conditioned with 2 ml of methanol and 3 ml of 0.05 *M* NaH_2PO_4 (pH 5.2). After washing with 3 ml of 0.1 *M* HCl and 3 ml of methanol, cocaine was eluted with 3 ml of a methylene chloride–2-propanol (4:1) containing 2% of ammonia. The eluate was then evaporated to dryness and the

residue was usually dissolved in 500 μl of 0.05 *M* NaH_2PO_4 (pH 5.2).

Recovery studies were carried out by adding known amounts to blank plasmas or blank hair acid extracts, which were then processed according to the specific extraction procedures. The extracts were then injected and peaks measured *versus* the corresponding standards directly injected.

3. Results and discussion

3.1. Cocaine native fluorescence

Cocaine in water showed a fluorescence band with a maximum at 315 nm when irradiated at an excitation wavelength of 230 nm. No influence of pH on the fluorescence characteristics was observed.

3.2. HPLC determination

Under the described conditions, cocaine eluted with a capacity factor (k') of about 5. The adoption of a polymeric column, avoiding the silanol-related problems, common in the analysis of basic drugs such as cocaine, allowed us to obtain symmetric peaks (asymmetry factor = 1) without the use of any additives.

Benzoylecgonine also proved to be fluorescent and, according to its more polar nature, it eluted before cocaine in a symmetric peak, with k' = 3.0. However, because of the lack of a certified standard, poor extraction from aqueous solutions with our routine liquid–liquid methods and considering the scope of this work aimed at cocaine assay, its analytical optimization has been overlooked.

The efficiency of cocaine separation was about 20 000 plates/m.

The use of fluorescence detection, although based on the simple aromatic ring of cocaine, allowed an important increase in sensitivity in comparison with UV detection. In reality, the limit of detection (LOD) of the present method was about 1 ng/ml (0.2 ng injected), with a signal-to-noise ratio of 3. This result is more than five times better than the LODs recently reported using HPLC–UV detection [24–26,28, 29,34]. Only one HPLC–UV method, by Lampert and Stewart [23], gave a comparable sensitivity, but using a fixed-wavelength detector (cadmium lamp, 228 nm), which is inherently less selective. In addition, the instrument was fitted with a "laboratory-made preamplifier/analogue

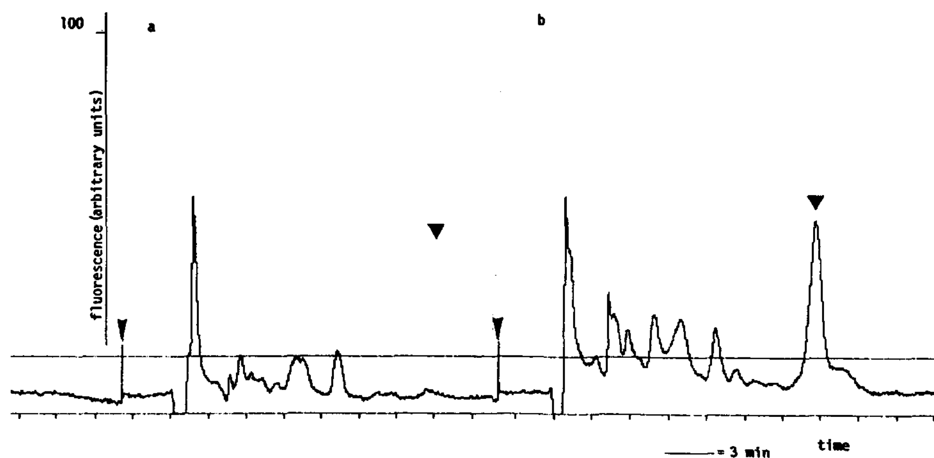

Fig. 1. Typical chromatograms of (a) extract (with Toxi-tubes) of blank hair (25 mg) and (b) hair sample (25 mg) from a very light cocaine user containing 0.2 ng/mg of cocaine (the cut-off limit is 0.1 ng/mg). Volume injected, 100 μl, corresponding to 1 ng of cocaine, in case b. The arrow heads indicate injections and triangles indicate cocaine retention time. Chart speed, 4 mm/min. For other analytical conditions, see text.

filter", which can hardly be reproduced in other laboratories.

Moreover, the main advantage of the proposed method is the possibility of dealing with biological matrices, such as plasma and hair, without complex sample pretreatments. In fact, a simple and rough liquid–liquid extraction was sufficient in the most instances for obtaining an extract suitable for injection, even at the highest sensitivity. Only in few cases did hair samples, which were still "dirty" after the liquid–liquid extraction, need further purification with SPE. Fig. 1 shows typical chromatograms of blank hair and hair from a cocaine user; in Fig. 2 chromatograms of blank plasma and spiked plasma, containing 10 ng/ml of cocaine, are depicted. In Fig. 3 the chromatogram of partially putrefied cadaveric blood (containing 1200 ng/ml of cocaine) from a subject who had died from intravenous overdosage of cocaine is shown (an important concentration of benzoylecgonine coexists with cocaine). All these samples were extracted with the simple liquid–liquid procedures reported for hair and plasma.

The effect of the application of SPE after Toxi-tube extraction to a very complex hair sample is shown in Fig. 4.

Owing to the high selectivity of the method, no suitable internal standard has yet been found.

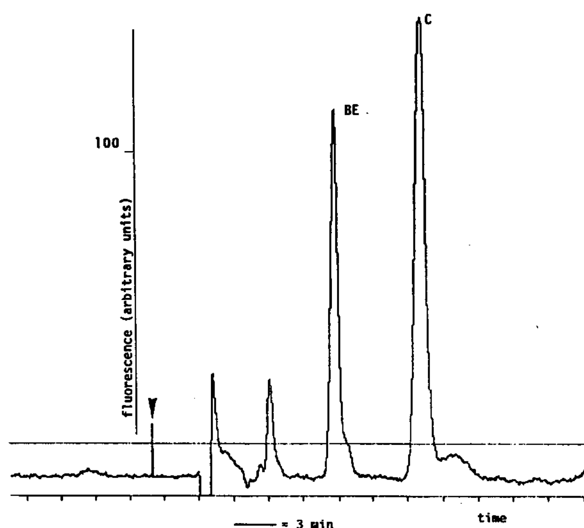

Fig. 3. Chromatogram of a sample of partially putrefied cadaveric blood (containing 1200 ng/ml of cocaine) from a subject who had died from an intravenous overdosage of cocaine (an important concentration of benzoylecgonine coexists with cocaine). Peaks: C = cocaine; BE = benzoylecgonine. Analytical conditions as in Fig. 1.

However, the use of the external standard method for quantification proved suitable for application in real cases.

Using Toxi-tubes, the average overall recovery of the method, from 100-mg blank hair samples

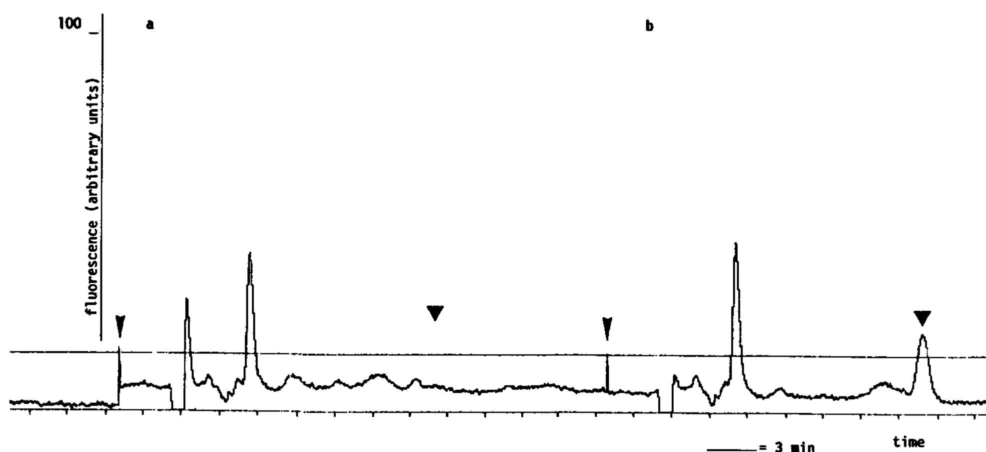

Fig. 2. Typical chromatograms of (a) extract (liquid–liquid method) of blank plasma and (b) plasma spiked with 10 ng/ml of cocaine (100 μl of extract injected, corresponding to 0.4 ng of cocaine). The arrow heads indicate injections and triangles indicate cocaine retention time. Analytical conditions as in Fig. 1.

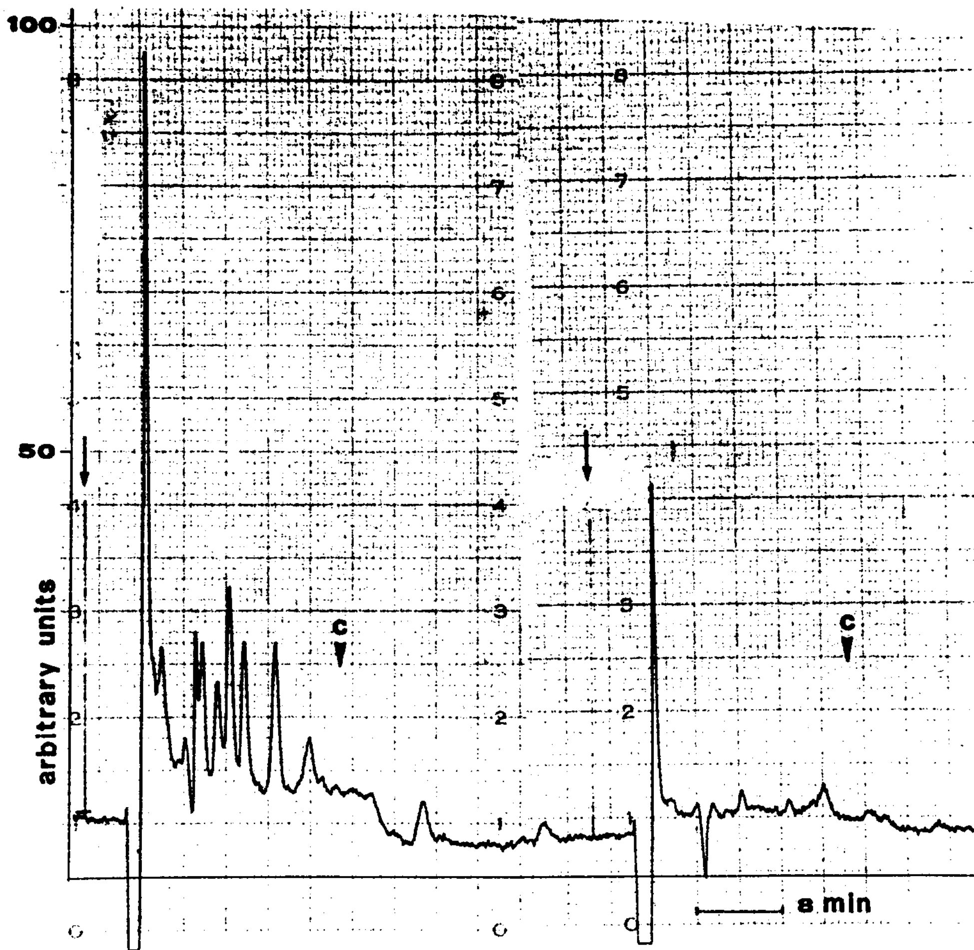

Fig. 4. Chromatograms of the same "dirty" blank hair sample (100 mg, 100 μl of extract injected) extracted only with Toxi-tubes (left) and with Toxi-tubes followed by SPE (Isolute Confirm HCX, 80 mg) (right). Arrows indicate injections; c indicates the elution time of cocaine. Chart speed, 2.5 mm/min. For other analytical conditions, see text.

with 50 ng of cocaine added, was 94.9% with a relative standard deviation (R.S.D.) of 2.2% ($n = 10$).

With the chloroform–2-propanol extraction, the recovery of cocaine from blank plasma samples spiked with the drug to a final concentration of 100 ng/ml was 101.0% (R.S.D. = 4.8%; $n = 10$) and 98.5% (R.S.D. = 3.1%; $n = 10$) for a concentration of 500 ng/ml.

For economic reasons, we used SPE only in series with one of the two liquid–liquid methods, when specifically needed. With this two-step procedure, the recovery of cocaine from hair extracts (spiked as above) was 91.0% (R.S.D. = 2.9%; $n = 6$) and 95.2% (R.S.D. = 5.2%; $n = 6$) from plasma (cocaine concentration 100 ng/ml).

Unfortunately, with both of the liquid–liquid methods presented the recovery of benzoylecgonine, tested in the concentration range 90–540 ng/ml, was unsatisfactory, varying from 13 to 27% with the Toxi-Tube method and from 15 to 23% with the chloroform–2-propanol method. On the other hand, with SPE the recovery of benzoylecgonine was very good, as reported by other workers [15,35], being 93.8% (R.S.D. = 2.9%; $n = 6$) for a level of 100 ng/ml.

Notwithstanding the better performance of SPE with benzoylecgonine, for our purposes we retained the liquid–liquid extractions because these methods allowed the HPLC determination of morphine in the same extracts [38], which with SPE would have required a specific protocol.

The linearity of the method for cocaine was fairly good in the concentration range 1.5–500 ng/ml (corresponding for hair to 0.015 and 5 ng/mg, respectively), being described by the equation $y = 0.395x - 0.028$, $r^2 = 0.9998$ (where x = cocaine concentration in ng/ml and y = fluorescence response).

The intra-assay precision was characterized by R.S.D.s of 5.0 and 3.6% ($n = 6$) for cocaine concentrations of 10 and 100 ng/ml, respectively; in inter-assay tests ($n = 6$), R.S.D.s of 7.5 and 5.2% were achieved for the same cocaine levels in plasma. A comparable precision was also observed in the analysis of hair extracts.

The use of full-loop injection allowed the precision to be increased to R.S.D.s down to less than 3% intra-assay and 4% inter-assay; however, in order to ensure reproducible full-loop filling, a volume of sample at least three times larger had to be used.

Possible interferences were studied by injecting mixtures of common therapeutic and illicit drugs contained in the Toxi Disc Library (Table 1) at a concentration of 20 μg/ml, with the fluorimeter at a sensitivity range allowing the determination of 2 ng/ml of cocaine. Under these conditions, none of the 90 drugs gave any significant peak eluting at the retention time of cocaine. Of course, as the test was carried out *in vitro*, we could not exclude possible interferences from drug metabolites.

Table 1
Drugs investigated in order to exclude interferences in cocaine determination

Type	Drugs			
Opiates and antagonists	Codeine	Dextromethorphan	Dihydrocodeine	Diphenoxilate
	Ethylmorphine	Hydrocodone	Hydromorphone	Meperidine
	Morphine	Methadone	Naloxone	Oxicodone
	Papaverine	Propoxyphene	Terpin hydrate	
Central nervous system active drugs	Amphetamine	Amitriptyline	Benztropine	Carbamazepine
	Caffeine	Chlorprothixene	Chlorpromazine	Diazepam
	Diphenylhydantoin	Doxepin	Ethinamate	Flurazepam
	Imipramine	Loxapine	Meprobamate	Methamphetam.
	Methaqualone	Methylphenidate	Nordiazepam	Nortriptyline
	Pentobarbital	Phenmetrazine	Phentermine	Phencyclidine
	Phenobarbital	Phenytoin	Phetidine	Prazepam
	Protriptyline	Secobarbital	Strychnine	Thioridazine
	Thiothixene	Trifluperazine	Triflupromazine	Amobarbital
	Aprobarbital	Butabarbital	Barbital	
Miscellaneous	Acetaminophen	Atropine	Benzoylecgonine	Carisoprodol
	Chlorpheniramine	Cimetidine	Diphenhydramine	Disopyramide
	Doxylamine	Emetine	Erythromycin	Glutethimide
	Hydrocortisone	Hydroxyzine	Lidocaine	Methapyrilene
	Methocarbamol	Nicotine	Orphenadrine	Pentazocine
	Phenacetin	Pyrilamine	Phenolphthalein	Phenylpropanola.
	Propranolol	Procaine	Procainamide	Pseudoephedrine
	Quinine	Salicylamide	Spironolactone	Triamterene
	Triexyphenidyl	Trimeprazine	Trimetobenzamide	Trimethoprim

No interferences were observed up to levels of 20 μg/ml of each drug.

4. Conclusions

In the investigations of drugs of abuse, high costs, low productivity and/or insufficient sensitivity of the chromatographic methods (TLC, GC, GC–MS) customarily adopted for confirmation of the results from immunometric screening assays are often disadvantages. These limits are crucial in epidemiological surveys, in which high sensitivity and low cost per analysis, together with a high degree of automation, are mandatory features. Also, the traditional chromatographic approaches show limits in clinical toxicology, requiring rapidity, simplicity and ruggedness.

The HPLC–FL method here reported is, to the best of our knowledge, the first based on the direct fluorescence of the cocaine molecule. It is suitable also for the determination of benzoylecgonine. The sensitivity achieved for cocaine is very good, being higher than that reported with GC–MS [15]; the accuracy and precision are comparable to those of other chromatographic methods and the sample pretreatment needed is very simple. In fact, the selectivity of fluorescence detection permitted cocaine to be determined also in the presence of the many UV-absorbing coextractives, which the rapid but rough extraction procedures adopted do not eliminate. Owing to its simplicity, the method is rugged and intrinsically susceptible to automation.

Hence the present HPLC–FL method seems to be important for the determination of cocaine and, tentatively, benzoylecgonine not only in forensic and clinical toxicology, but also in epidemiological surveys on the spreading of drugs of abuse based on hair testing, a purpose for which it is currently applied in our laboratory.

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References

[1] G. Barrio Anta, in F. Bruno (Editor), *Cocaine Today: its Effects on the Individual and Society*, Publication No. 44, United Nations Interregional Crime and Justice Research Institute (UNICRI), Rome, 1992, pp. 144–149.
[2] *Annual Emergency Room Data, 1990, Series I, No. 10-A, Publication No. (ADM) 91-1839*, Department of Health and Human Services, Washington, DC, 1991.
[3] J. Goldstein, in F. Bruno (Editor), *Cocaine Today: its Effects on the Individual and Society*, Publication No. 44, United Nations Interregional Crime and Justice Research Institute (UNICRI), Rome, 1992, pp. 238–245.
[4] S.C. Chan, G.A. Torok-Both, D.M. Billay, P.S. Przybylski, C.Y. Graden, K.M. Papp and J. Petruzelka, *Clin. Chem.*, 37 (1991) 1289–1296.
[5] M.R. Harkey and G.L. Henderson, in R.C. Baselt (Editor), *Advances in Analytical Toxicology*, Vol. II, Year Book Medical, Chicago, 1989, pp. 298–329.
[6] S.M. Fletcher and V.S. Hancock, *J. Chromatogr.*, 206 (1981) 193–195.
[7] Y. Liu, R.D. Budd and E.C. Griesemer, *J. Chromatogr.*, 248 (1982) 318–320.
[8] R.C. Baselt, *Disposition of Toxic Drugs and Chemicals in Man*, Biomedical Publications, Davis, CA, 2nd ed., 1982, pp. 193–198.
[9] B.S. Finkle, *Clin. Chem.*, 33(B) (1987) 13B.
[10] J.C. Valentour, V. Aggarwal, M.P. McGee and S.W. Goza, *J. Anal. Toxicol.*, 2 (1978) 134–137.
[11] P.I. Jatlov and D.N. Bailey, *Clin. Chem.*, 21 (1975) 1918–1921.
[12] B.H. Dvorchik, S.H. Miller and W.P. Graham, *J. Chromatogr.*, 135 (1977) 141–148.
[13] M.J. Kogan, K.G. Verebey, A.C. DePace, R.B. Resnick and S.J. Mulé, *Anal. Chem.*, 49 (1977) 1965–1969.
[14] J.I. Javaid, H. Dekirmenjian, J.M. Davis and C.R. Schuster, *J. Chromatogr.*, 152 (1978) 105–113.
[15] M.R. Harkey, G.L. Henderson and C. Zhou, *J. Anal. Toxicol.*, 15 (1991) 260–265.
[16] M.R. Moeller, P. Fey and S. Rimbach, *J. Anal. Toxicol.*, 16 (1992) 291–296.
[17] E.J. Cone and W.D. Darwin, *J. Chromatogr.*, 580 (1992) 43–61.
[18] D.A. Kidwell, *J. Forensic Sci.*, 38 (1993) 272–284.
[19] O. Curcuruto, F. Guidugli, P. Traldi, A. Sturaro, F. Tagliaro and M. Marigo, *Rapid Commun. Mass Spectrom.*, 6 (1992) 434–437.
[20] P.I. Jatlow, C. Van Dyke, P. Barash and R. Byck, *J. Chromatogr.*, 152 (1978) 115–121.
[21] M.A. Evans and T. Morarity, *J. Anal. Toxicol.*, 4 (1980) 19–22.
[22] A.N. Masoud and D.M. Krupski, *J. Anal. Toxicol.*, 4 (1980) 305–310.
[23] B.M. Lampert and J.T. Stewart, *J. Chromatogr.*, 495 (1989) 153–165.

[24] J.A. Sandberg and G.D. Olsen, *J. Chromatogr.*, 525 (1990) 113–121.
[25] P. Jatlow and H. Nadim, *Clin. Chem.*, 36 (1990) 1436–1439.
[26] P. Bouis, G. Taccard and U.A. Boelsterli, *J. Chromatogr.*, 526 (1990) 447–459.
[27] B.K. Logan and D.T. Stafford, *J. Forensic Sci.*, 35 (1990) 1303–1309.
[28] C.E. Lau, F. Ma and J.L. Falk, *J. Chromatogr.*, 532 (1990) 95–103.
[29] C.E. Lau, *J. Chromatogr.*, 582 (1992) 167–172.
[30] A.K. Larsen and I.R. Tebbett, *J. Forensic Sci.*, 37 (1992) 636–639.
[31] P.R. Puopolo, P. Chamberlin and J.G. Flood, *Clin. Chem.*, 38 (1992) 1838–1842.
[32] L.J. Murphey, G.D. Olsen and R.J. Konkol, *J. Chromatogr.*, 613 (1993) 330–335.
[33] C.M. Selavka, I.S. Krull and I.S. Lurie, *Forensic Sci. Int.*, 31 (1986) 103–117.
[34] R.L. Miller and C.L. DeVane, *J. Chromatogr.*, 570 (1991) 412–418.
[35] I.M. Roy, T.M. Jefferies, M.D. Threadgill and G.H. Dewar, *J. Pharm. Biomed. Anal.*, 10 (1992) 943–948.
[36] P. Wernly and W. Thormann, *Anal. Chem.*, 63 (1991) 2878–2882.
[37] F. Tagliaro, C. Poiesi, R. Aiello, R. Dorizzi, S. Ghielmi and M. Marigo, *J. Chromatogr.*, 638 (1993) 303–309.
[38] F. Tagliaro, G. Carli, F. Cristofori, G. Campagnari and M. Marigo, *Chromatographia*, 26 (1988) 163–167.