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Capillary electrophoresis for the investigation of illicit drugs in hair: determination of cocaine and morphine

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ABSTRACT

Toxicological analysis of hair is becoming a popular method for investigating past, chronic use of illicit drugs. Several analytical methods using immunometry, chromatography and mass spectrometry have been reported. In this work, capillary electrophoresis was first used for the determination of illicit drugs, such as cocaine and morphine, in the hair of heroin and cocaine users. After rapid washing, hair samples were incubated overnight in 0.25 *M* HCl at 45°C and the mixtures were extracted with ready-to-use Toxi-tubes A. The organic phase was evaporated and the residue dissolved in a suitable amount of electrophoresis buffer. Free zone capillary electrophoretic determinations of morphine, the main heroin metabolite, and cocaine were accomplished in 0.05 *M* borate buffer (pH 9.2) at a potential of 15 000 V, with UV detection at 214 and 238 nm, respectively. The use of the less selective wavelength of 200 nm allowed the simultaneous detection of both compounds. Efficient separations (up to 350 000 theoretical plates) and accurate and precise determinations (intra-day R.S.D.s in the range 3–5%) of cocaine and morphine in hair extracts were easily achieved. The analytical sensitivity was sufficient to determinate as little as 0.15 ng/mg of cocaine and morphine in hair using 100-mg samples. Interferences from more than 90 therapeutic drugs and drugs of abuse were excluded.

INTRODUCTION

Capillary electrophoresis (CE), after having been demonstrated to be a powerful tool in biopolymer separations, has rapidly expanded into the field of drug analysis [1,2]. CE possesses some unique characteristics that make it potentially valuable in the analysis of therapeutic and illicit drugs for pharmacological–pharmaceutical, clinical and forensic purposes.

First, CE is based on physical–chemical princi-

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ples substantially different from those in chromatography and other techniques used in pharmaceutical and toxicological analysis and thus has the potential to become an ideal "complementary" technique in analytical toxicology. An additional facet of CE is the need for minimum amounts of specimens, which makes it important especially when only minute amounts of sample are available, as often happens in forensic cases.

These unique features have prompted some forensic and clinical toxicologists to investigate possible applications of CE in analyses for drugs of abuse. A pioneering paper by Wernly and Thormann [3] demonstrated the effectiveness of micellar electrokinetic capillary chromatography (MECC) in the analysis of urines for common drugs of abuse and their metabolites (*e.g.*, opiates, benzoylecgonine, amphetamines and methaqualone). To the best of our knowledge, however, CE has never found application in the analysis of hair for drugs of abuse, in which this technique could display most of its advantages. This innovative approach to toxicological investigations, first proposed by Baumgartner *et al.* in 1979 [4], is now becoming a powerful means for demonstrating the chronic use of illicit drugs. An excellent overview of this subject has been published by Harkey and Henderson [5].

Several drugs undergoing chronic use become embedded in the hair at the follicle level, during the hair growth, and, lacking any metabolism in this structure, they remain fairly unaltered throughout the entire hair lifetime. The average hair growth being about 1 cm/month, the analysis of few centimetres of hair can provide information on the toxicological behaviour over several months before the collection of the hair sample. This is particularly important if one considers that usually drugs disappear from the blood in a few hours and from urine in a few days.

However, because, for aesthetic problems, only a few milligrams of hair can be collected, and as the concentration of drugs in the hair matrix is in the low ng/mg range, analytical sensitivity is a crucial point. At present, radioimmunoassay (RIA) is generally used for the preliminary screening and gas chromatography, gas chromatography–mass spectrometry, high-performance liquid chromatography (HPLC) or collisional activation mass spectrometry are used for confirmation of the results (for a review, see ref. 5). On this ground, CE, because of its high analytical efficiency and mass sensitivity, could become an important tool of investigation. In addition, its possible coupling with mass spectrometry should be taken into account in view of its possible use for forensic purposes.

The aim of this work was to test the performance of CE in the assay of hair for markers of cocaine and heroin use (*i.e.*, cocaine itself and morphine, the main metabolite of heroin) in comparison with the HPLC methods currently used in our laboratory.

EXPERIMENTAL

CE instrumentation and methods

A manual capillary electropherograph (Model 3850; Isco, Lincoln, NE, USA) equipped with an on-column UV detector and a split-flow injector was used. Bare silica capillaries (40 cm to the detector) of I.D. 50 μm were adopted, furnished by Isco. Separations were accomplished using constant potentials of 15 000 V in 0.050 *M* borate buffer (pH 9.2), with resulting currents no higher than 60 μA. The buffers were filtered through a 0.45-μm nylon 66 membrane (Alltech, Eke, Belgium) and deareated under reduced pressure (water pump) before use. After each injection the capillary surface was renewed by flushing with 0.1 *M* NaOH and rinsing with the working buffer. Injection was executed manually with a syringe through a splitter with a reported splitting ratio to the column of 1:830.

MECC separations were carried out under conditions mostly resembling those published by Wernly and Thormann [3]. Briefly, 0.010 *M* borate buffer (pH 9.2) containing 0.050 *M* sodium dodecyl sulphate (SDS) was the background buffer and a potential of 20 000 V was applied. The capillary was the same as that described above. Wavelengths of 238 and 214 nm were chosen for the UV detection of cocaine and morphine, respectively, with a detector range of 0.005 AUFS. For calculating the net mobilities,

methanol was used as an electroosmotic flow marker.

Standards and sample preparation

Stock standard solutions of cocaine (Sigma, St. Louis, MO, USA) and morphine (Carlo Erba, Milan, Italy) were prepared from the respective hydrochloride salts in methanol to yield concentrations of 1 mg/ml and were stored at −18°C. Working standard solutions of suitable concentrations were prepared on the day of analysis by diluting the stock standard solution with the background buffer diluted 1:2 with water. Tetracaine and nalorphine, used as internal standards (I.S.), were purchased from Sigma.

Standards of therapeutic drugs and drugs of abuse, supplied dried on glass microfibre discs impregnated with silicic acid (Toxi Disc Library) and Toxi-tubes A were purchased from Analytical Systems (Laguna Hills, CA, USA).

The hair sample pretreatment has been fully described elsewhere [6] and it can be summarized as follows. Hair samples (25–100 mg), cut near the scalp, were washed with diethyl ether and 0.01 *M* HCl and then extracted by incubating overnight in 0.25 *M* HCl at 45°C. The incubation mixtures were neutralized with NaOH and extracted twice into the organic phase with ready-to-use Toxi-tubes A. The organic layer was then evaporated to dryness and the residue was reconstituted with 20 μl of the background buffer, previously diluted 1:2 with water, 5 or 10 μl of which were injected.

HPLC instrumentation and methods

Morphine and cocaine were assayed by HPLC using an isocratic instrument composed of a high-pressure pump (Model 880 PU; Jasco, Tokyo, Japan) and a six-port injection valve (Model 7125; Rheodyne, Cotati, CA, USA) with a 50-μl loop. For morphine, an amperometric detector (LC 4B/17A; BAS, West Lafayette, IN, USA) with a thin-layer cell and a glassy carbon working electrode (operated at 350 mV *vs.* an Ag/AgCl reference electrode) was used; for cocaine, a UV detector (Model 875 UV; Jasco) set at 235 nm was adopted.

The HPLC separation of morphine was carried out under conditions fully detailed elsewhere [7], using a Bio-Gel PRP 70-5 column (150 mm × 4.6 mm I.D.) (Bio-Rad RSL, Eke, Belgium), packed with 5-μm spherical particles of polystyrene–divinylbenzene, and a mobile phase of 0.050 *M* sodium phosphate (pH 9.5)–acetonitrile (85:15) at a flow-rate of 0.5 ml/min at 65°C.

Reversed-phase ion-pair liquid chromatographic assay of cocaine was accomplished according to Jatlow and Nadim [8], with minor changes. An ODS silica column (250 mm × 4.6 mm I.D.) packed with 5-μm Nucleosil 100 (Macherey–Nagel, Düren, Germany) was used with a mobile phase composed of 0.050 *M* phosphate buffer (pH 3.0)–acetonitrile (66:33), containing 0.2% of hexanesulphonic acid, at a flow-rate of 0.5 ml/min at room temperature.

RESULTS AND DISCUSSION

CE determination of cocaine

CE of cocaine under the described conditions results in a sharp and symmetrical peak migrating before the electroosmotic flow. In fact, cocaine (M_r 303.35), having only a tertiary amine as ionizable moiety with a p*K* of 8.7, is still partially ionized as a cation at the pH of the background buffer (9.2). Its net mobility (μ) was $0.42 \cdot 10^{-4}$ cm^2/V s under the adopted conditions; the dependence of μ on the pH of the buffer is shown in Fig. 1. The efficiency of separation was about 350 000 theoretical plates.

The major metabolite of cocaine, benzoylecgonine, having an additional COOH moiety, was not extracted efficiently with the adopted liquid–

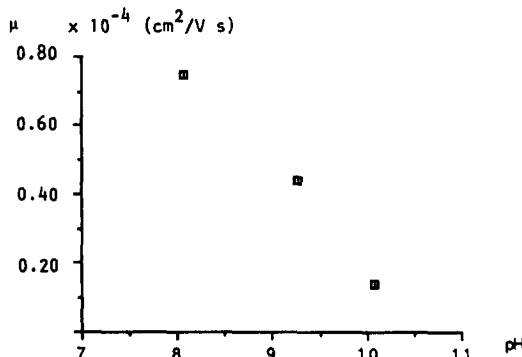

Fig. 1. Dependence of the net mobility (μ) of cocaine on the pH of the background buffer.

liquid extraction method and is a minor compound in the hair of cocaine users. Therefore, it was not investigated further. No interferences were observed from more than 90 therapeutic and illicit drugs at the level of 100 μg/ml, as shown in Table I, or from the "normal" constituents of hair matrix and common cosmetic treatments. In fact, the electropherograms of blank hair extracts were remarkably clean, allowing the injection of hair extracts reconstituted in very small volumes. This is particularly important because of the modest sensitivity, in terms of concentration, of CE with on-column UV detection. In fact, the limit of detection (LOD) was about 600 ng/ml (with a signal-to-noise ratio of 3), allowing the identification of levels of cocaine as low as 0.15 ng/mg in 100-mg hair samples. Fig. 2 shows the electropherograms of a blank hair sample (in which the addition of the I.S. has intentionally been omitted) and of a sample positive for cocaine at the level of 4.0 ng/mg (from a hair sample of 75 mg). Only a single peak is present in the blank hair electropherogram, which can be ascribed to the bulk of uncharged endogenous compounds co-extracted, which migrate all together at the velocity of the electroosmotic flow.

The determination of cocaine, notwithstanding an "acceptable" reproducibility of the manual split-flow injector in method development, needed the use of an I.S. in order to achieve the required degree of repeatability and accuracy for application to real samples. For this purpose, tetracaine, with $\mu = 0.22$, emerging close to cocaine but baseline resolved, was used at a level of 25 μg/ml.

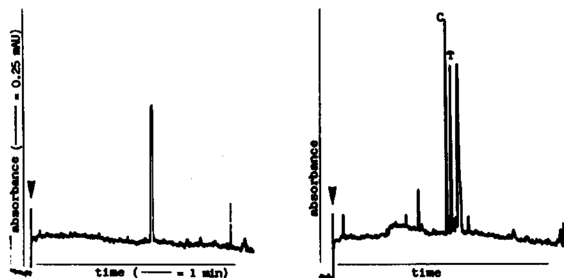

Fig. 2. Typical electropherograms of (left) a blank hair sample (in which the addition of the I.S. was intentionally omitted) and (right) hair (75 mg) from a cocaine user, containing cocaine at the level of 4.0 ng/mg [C = cocaine peak; T = tetracaine (I.S.) peak]. Chart speed, 0.5 cm/min; detection at 238 nm; other conditions are detailed in the text. The arrows indicate injections.

The linearity of the CE determination of cocaine in the range 0.78–100 μg/ml (corresponding to 0.16–20 ng/mg in 100-mg hair samples) is described by the equation $y = 0.0144 + 0.0747x$ ($r = 0.99990$); Tables II and III summa-

TABLE I

DRUGS INVESTIGATED IN ORDER TO EXCLUDE INTERFERENCES IN THE CE DETERMINATION OF COCAINE AND MORPHINE

No interferences were observed up to levels of 100 μg/ml.

Opiates and antagonists	Codeine, dextromethorphan, dihydrocodeine, diphenoxilate, ethylmorphine, hydrocodone, hydromorphone, meperidine, methadone, naloxone, oxicodone, papaverine, propoxyphene, terpin hydrate
Central nervous system active drugs	Amphetamine, amitriptyline, benztropine, carbamazepine, caffeine, chlorprothixene, chlorpromazine, cocaine, diazepam, diphenylhydantoin, doxepin, flurazepam, imipramine, loxapine, meprobamate, methamphetam, methaqualone, methylphenidate, nordiazepam, nortriptyline, phenmetrazine, phentermine, phencyclidine, phetidine, prazepam, protriptyline, strychnine, thioridazine, thiothixene, trifluperazine, triflupromazine
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

TABLE II

ACCURACY OF COCAINE AND MORPHINE DETERMINATIONS

Drug	Expected concentration (μg/ml)	Observed concentration (mean ± S.D., n = 6) (μg/ml)	Recovery (%)
Cocaine	50.0	49.1 ± 2.07	98
	10.0	9.3 ± 0.30	93
Morphine	10.0	9.5 ± 0.38	95
	2.5	2.1 ± 0.11	84

TABLE III

PRECISION OF DETERMINATION AND MIGRATION TIMES OF COCAINE AND MORPHINE

Precision	Drug	Mean concentration (n = 6) (μg/ml) ± R.S.D. (%)	Mean migration time (n = 12) (min) ± R.S.D. (%)				
			E.O. flow	Cocaine	Tetracaine	Morphine	Nalorphine
Intra-day	Cocaine	9.3 ± 3.2% 49.1 ± 4.2%	8.18 ± 0.31%	7.40 ± 0.91%	7.69 ± 0.92%		
	Morphine	9.5 ± 4.0% 2.1 ± 5.2%	8.18 ± 0.31%			9.26 ± 0.96%	9.46 ± 1.12%
Inter-day	Cocaine	10.2 ± 5.7% 49.7 ± 7.0%	8.09 ± 0.93%	7.32 ± 2.26%	7.55 ± 2.18%		
	Morphine	10.1 ± 7.2% 2.4 ± 8.3%	8.09 ± 0.93%			9.06 ± 2.96%	9.26 ± 2.90%

rize the accuracy and precision data, respectively.

CE determination of morphine

Morphine, separated under the described conditions, gave a symmetrical peak migrating after the electroosmotic flow. Morphine (M_r 285.33), having both a tertiary amine and a phenolic group (pK 8.1 and 9.85, respectively) on the whole behaves as an anion at the pH of the background buffer (9.2). The anionic mobility of morphine was $\mu = 0.44 \cdot 10^{-4}$ cm^2/V s under the adopted separation conditions; the dependence of μ on the pH of the buffer, reflecting its amphoteric character, is shown in Fig. 3. The efficiency of separation was about 150 000 theoretical plates.

As for cocaine, no interferences were observed from the compounds listed in Table I. The electropherograms of blank hair extracts did not show interfering peaks at the migration time of morphine. The LOD for morphine was about 600 ng/ml (with a signal-to-noise ratio of 3),

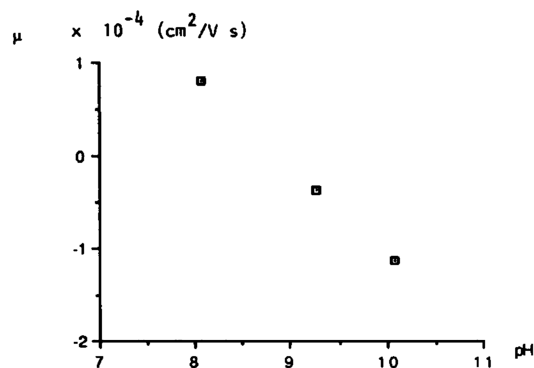

Fig. 3. Dependence of the net mobility (μ) of morphine on the pH of the background buffer.

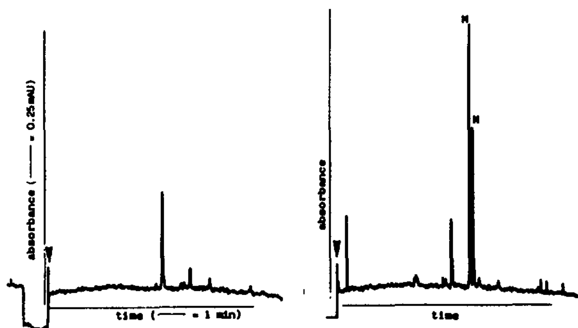

Fig. 4. Typical electropherograms of (left) a blank hair sample (in which the addition of the I.S. was intentionally omitted) and (right) hair (75 mg) from a heroin user, containing morphine at the level of 3.0 ng/mg [M = morphine peak; N = nalorphine (I.S.) peak]. Chart speed, 0.5 cm/min; detection at 214 nm; other conditions are detailed in the text. The arrows indicate injections.

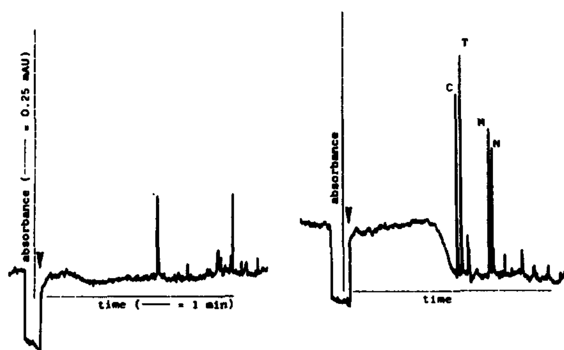

Fig. 5. Electropherograms of (left) a blank hair sample and (right) a hair extract spiked with cocaine (C), tetracaine (T), morphine (M) and nalorphine (N), to simulate a concentration of about 4 ng/mg of each analyte in hair. Chart speed, 0.5 cm/min; detection at 200 nm; other conditions are detailed in the text. The arrows indicate injections.

allowing the identification of levels of morphine as low as 0.15 ng/mg in 100-mg hair samples. Fig. 4 shows the electropherograms of blank hair (without the I.S.) and of a sample positive for morphine at a level of 3.0 ng/mg (from a hair sample of 100 mg).

For the determination, nalorphine, which was baseline resolved from morphine, was used as the I.S. at the level of 15 μg/ml.

The linearity of the CE determination of morphine in the concentration range 0.62–100 μg/ml is described by the equation $y = 0.0461 + 0.1295x$ ($r = 0.99980$). Tables II and III give the accuracy and precision data for both concentration and migration times.

Simultaneous determination of cocaine and morphine

Because of the different UV absorption maxima of cocaine and morphine, the simultaneous determination of the two analytes was not possible at either of the wavelengths used. UV detection at 200 nm, where both cocaine and morphine absorb, could overcome this problem, as shown in Fig. 5. Unfortunately, determinations at this low and inherently less selective wavelength may suffer from interferences. For this reason, detection at 200 nm is to be used only for screening purposes and positive samples should be reassayed at the more specific wavelengths.

Comparison with HPLC

The results of the CE determination of cocaine and morphine in eight real samples were compared with the results given by HPLC on the same extracts. Good correlation coefficients were observed ($r = 0.96$ for morphine and 0.99 for cocaine), but CE showed at least a three times higher productivity than HPLC (Fig. 6). No deterioration of the capillary was observed over several months.

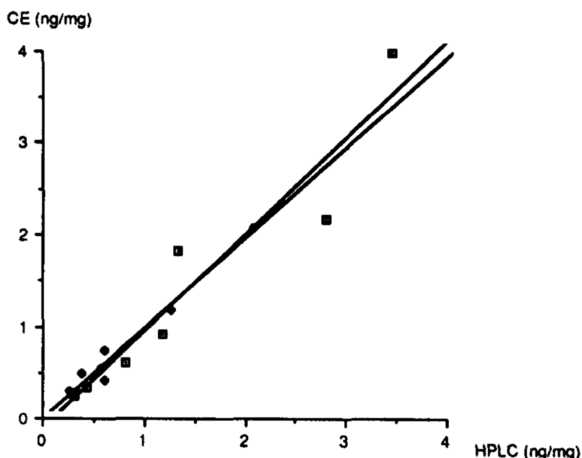

Fig. 6. Correlation between HPLC and CE in the determination of cocaine and morphine observed in the cross-assay of eight hair extracts from opiate and/or cocaine users. □ = Morphine ($y = -0.1018 + 1.0479x$, $r = 0.96$); ◆ = cocaine ($y = 0.0025 + 0.9764x$, $r = 0.99$).

CONCLUSION

Although the most common approach to the CE analysis of drugs is MECC [3,9], the present study demonstrates that free zone CE is suitable for the determination of charged drugs, such as cocaine and morphine, in rough extracts of human hair. For this purpose, capillary zone electrophoresis proved superior to MECC because the latter technique, although able to provide excellent separations of drugs of abuse, produced, in our hands, complex electropherograms when real hair extracts were injected, with several small peaks potentially interfering with those of the analytes of interest (data not shown). This was probably due to the inherent ability of MECC to "separate" also uncharged molecules, which in this particular instance apparently represent most of the co-extractives from the hair matrix.

On the other hand, free zone CE showed excellent selectivity for the analytes of interest, being suitable also for the determination of cocaine and morphine, meeting the sensitivity, accuracy and precision requirements for a technique suitable for use in forensic work. On-line scanning UV detection, although only preliminarily studied, seems an interesting feature, providing CE analyses with valuable additional information on peak purity and identity [3]. In conclusion, CE can be considered as a powerful technique suitable for wide use in forensic and clinical toxicology. It can be considered not only complementary to liquid and gas chromatography for confirmatory purposes, but could also, because of the possibility of automation, its ruggedness and negligible reagents costs per test [2], be interesting for screening analyses.

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