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Use of β-cyclodextrin in the capillary zone electrophoretic separation of the components of clandestine heroin preparations

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Abstract

The present paper describes the methodological optimization and validation of a capillary zone electrophoresis method for the rapid determination of heroin, secondary products and additives present in clandestine heroin samples, by using 20 m*M* β-cyclodextrins in phosphate buffer, pH 3.23. Applied potential was 15 kV and separation temperature was 24°C; detection was by UV absorption at 200 nm wavelength. Heroin samples were first dissolved in $CHCl_3$–MeOH (96:4, v/v) and injected by pressure (0.5 p.s.i., 3 s; 1 p.s.i.=6894.76 Pa) after evaporation of the organic mixture and reconstitution in aqueous buffer. Under the described conditions, phenylethylamine (internal standard), morphine, monoacetylmorphine, heroin, acetylcodeine, papaverine, codeine and narcotine were baseline resolved in less than 10 min. The limit of detection was better than 1 μg/ml for each analyte. The study of the intra-day and day-to-day precision showed, in terms of migration times, RSDs ≤0.71% and, in terms of peak areas, RSDs ≤3.2%. Also, the evaluation of linearity and analytical accuracy of the method provided good results for all the analytes investigated, thus allowing its application to real cases of seized controlled drug preparations.

Keywords: Forensic analysis; Heroin; Morphine; Codeine; Narcotine

1. Introduction

Heroin is one of the most abused drugs around the world and still represents an analytical challenge for forensic toxicologists. In 1998, over 700 kg of heroin were purchased undercover by the Anti-Narcotics Department of the Italian Police and about 11 000 persons were handed over to justice for crimes linked to illegal heroin detention and trafficking.

In terms of composition [1], clandestinely manufactured heroin preparations can be highly complex, reflecting the different quality of raw materials and reagents and non-standardized extraction and preparation procedures. In addition, before the final distribution to the ''street market'', heroin is added with various excipients and adulterants which further complicate the composition of the final preparation.

As a world wide illicit drug, heroin is included in most controlled drug screening programs, which require the qualitative and quantitative analysis of the active principle as well as adulterants, solvents, by-products and impurities present in the confiscated samples.

Currently, illicit heroin analysis is performed by

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high-performance liquid chromatography (HPLC) [2] and gas chromatography, often coupled to mass spectrometry (GC–MS) [3].

Although still relatively new in forensic analysis, capillary electrophoresis (CE) has rapidly spread as a powerful complementary technique to HPLC and GC into a wide array of analytical areas and particularly in pharmaceutical analysis. The great interest raised by this technique is undoubtedly due to its excellent separation efficiency, high mass sensitivity, minimal need of samples and solvents, but particularly to its high versatility in terms of separation modes, most of which are based onto peculiar physico-chemical principles and display different selectivities.

In the early 1990s, CE was introduced in the field of the screening analysis of controlled drugs and in the least decade its applications in this area have grown rapidly [4].

Weinberger and Lurie [5] first reported the simultaneous separation and determination of a wide variety of neutral and charged illicit drugs, as well as acid and neutral impurities and adulterants in clandestine preparations of heroin, by using micellar electrokinetic chromatography (MEKC). As an alternative to MEKC, Chee and Wan [6] used capillary zone electrophoresis (CZE) in plain phosphate buffer, pH 2.35, achieving the baseline separation of 17 basic drugs, including therapeutic and controlled substances. Since then, a relatively vast literature has been published in this field, for the analysis of heroin, opium, opiates, cocaine, amphetamines, ring substituted amphetamines, LSD and anabolic steroids [7–11], both in seized materials and biological samples (blood, urine and hair).

However, the close similarity and the high number of compounds which may be present in clandestine preparations of heroin pose challenging demands to the separation method, and not always CZE and/or MECK show sufficient selectivity.

Quite recently, Hudson et al. [12,13] suggested the use of CZE coupled to highly sensitive photodiode array detection (DAD) as an extremely useful tool for drug screening (in blood samples), improving the identification power by combination of analyte mobility data and UV spectral information.

On the other hand, in both CZE and MEKC separation modes, cyclodextrins (CDs), due to their ability to interact with the analytes by chirally selective complex formation, have been used to introduce additional (chiral) selectivity into the system, showing a great potential for the direct separation of a vast spectrum of enantiomeric compounds [14]. In forensic drug analysis, CDs have found application for the chiral resolution of amphetamines and related compounds (for examples see Refs. [15,16]).

However, the enhanced selectivity potentially offered by CDs with the introduction of complex formation interactions with the analytes has so far rarely been investigated in non-chiral environments. To our best knowledge, in the field of forensic drug analysis only Gong et al. [17] reported the use of β-cyclodextrin to modulate the separation of morphine, 6-monoacetylmorphine and heroin in a CZE method with chemiluminescence detection.

The present work describes the methodological optimisation and validation of a CZE method for the rapid and easy determination of heroin, impurities and additives in illicit heroin samples, based onto the addition of β-cyclodextrin to the running buffer to improve the separation power of the system. Also, a preliminary application of the optimised CZE conditions to achieve the simultaneous (chiral) determination of methadone, amphetamine, its methylenedioxy-derivatives and cocaine is reported.

2. Materials and methods

2.1. Standards and chemicals

The salts used for buffer preparation were of analytical reagent grade and the organic solvents ($CHCl_3$, MeOH) were of HPLC grade (Carlo Erba, Milan, Italy). Native β-cyclodextrins were furnished by Sigma–Aldrich (St. Louis, MO, USA). Standards of controlled drugs (morphine, monoacetylmorphine, heroin, acetylcodeine, papaverine, codeine, narcotine, etc.) were provided by Salars (Como, Italy). Phenylethylamine hydrochloride, used as the internal standard (I.S.), and other pure standards of therapeutic drugs were also purchased from Sigma–Aldrich. Milligram amounts of clandestine preparations of heroin were submitted for analysis to our laboratories by the Italian Police, as required by the laws on narcotics (D.P.R. 309/90).

2.2. Instrumentation and experimental conditions

A capillary electropherograph P/ACE 2100 (Beckman Coulter, Fullerton, CA, USA) fitted with a filter UV absorbance detector was used throughout the experiments. Control of the instrumentation, data acquisition and processing were performed with the software System Gold, version 8.1 (Beckman).

Uncoated fused-silica capillaries (Beckman Coulter), 37 cm (30 cm length to the detector×50 μm I.D.) were used. UV absorbance detection was monitored at 200 nm wavelength. CZE experiments were performed by using ''normal'' polarity (injection at the anode) at a constant voltage of 15 kV. The temperature of the capillary was kept constant at 24°C. The sample solutions were hydrodynamically injected at the anodic end of the capillary by using a positive pressure of 0.5 p.s.i. for 3 s (1 p.s.i.= 6894.76 Pa). New capillary conditioning was effected by rinsing with 0.1 *M* NaOH for 20 min applying a pressure of 20 p.s.i. at the injection end; in between individual runs the capillary was rinsed step-wise by deionized, distilled water (1 min), 0.1 *M* NaOH (3 min), distilled water (2 min) and the run buffer (4 min).

Under optimised electrophoretic conditions, separations were carried out using a running buffer composed of 0.1 *M* potassium dihydrogenphosphate and 0.1 *M* phosphoric acid (pH 3.23), containing 20 m*M* of β-cyclodextrin.

2.3. Sample preparation

Stock solutions of phenylethylamine hydrochloride, used as the I.S., were prepared at a concentration of 1 mg/ml in $CHCl_3$–MeOH (96:4; v/v) and stored at −20°C.

Appropriate working standard solutions were pre-

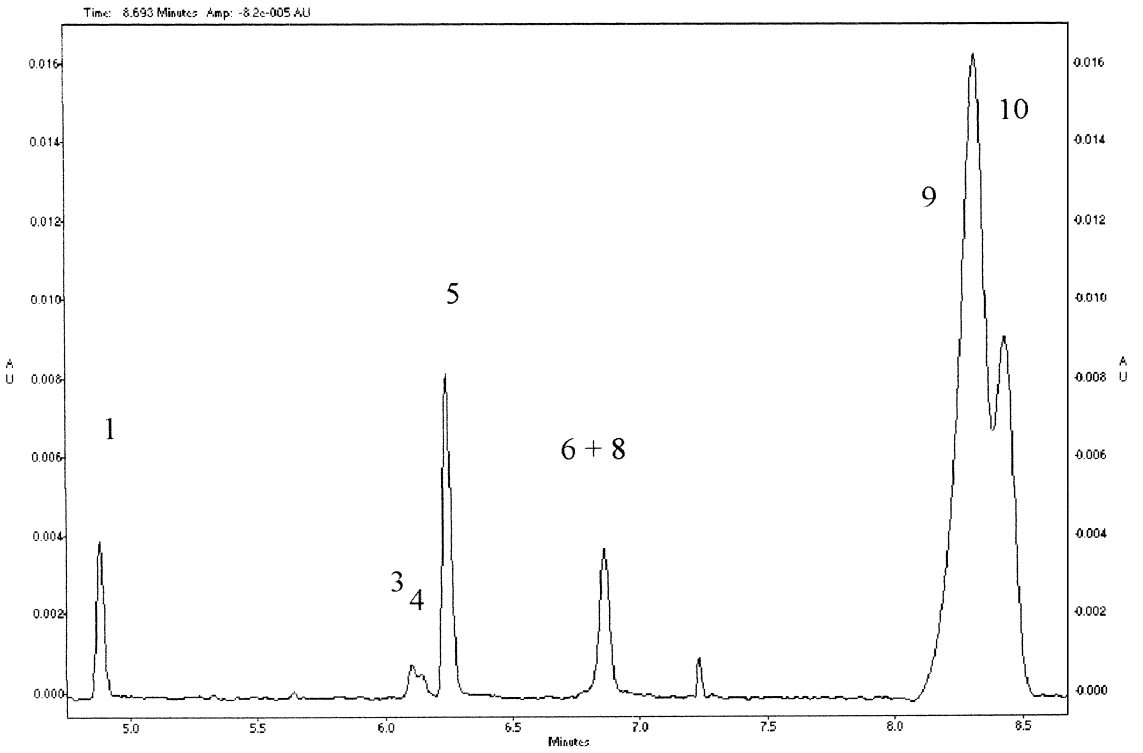

Fig. 1. Typical electropherogram from a real clandestine preparation of heroin containing: (1) phenylethylamine (I.S.); (3) monoacetylmorphine; (4) acetylcodeine; (5) heroin; (6) papaverine; (8) narcotine; (9) caffeine; (10) paracetamol. Analytical conditions: injection, pressure application 0.5 p.s.i. for 3 s; capillary, uncoated fused-silica, 37 cm×50 μm I.D.; buffer, 0.1 *M* potassium phosphate (pH 6.38); potential 7 kV; detection, UV absorbance at 200 nm. For other conditions see Section 2.2.

pared by dissolving 1 mg of each analyte in 2 ml of the internal standard solution. Illicit heroin samples were prepared by dissolving 20 mg in 2 ml of the I.S. solution. The resulting mixtures were kept at room temperature for 30 min and then filtered. Aliquots of 50 μl of the filtered organic phase were evaporated under a stream of N_2; the dried residues were reconstituted in 1 ml of diluted running buffer in water (1:9) and then hydrodynamically injected.

3. Results and discussion

Pure standards and illicit samples were easily prepared by usual procedures including dissolution in organic solvents, filtration, to remove particulates present in the raw material, and desiccation under nitrogen. Reconstitution of the residue with aqueous electrophoretic buffer did not give any problem.

In a preliminary stage of method development, we used plain phosphate buffer (0.1 *M* KH_2PO_4/K_2HPO_4) at pH 6.38 as the CZE background electrolyte and at a constant voltage of 7 KV. A typical electropherogram from an illicit heroin sample is shown in Fig. 1. Under these analytical conditions the analytes migrated in the following order: I.S., monoacetylmorphine (MAM), acetylcodeine, heroin, narcotine+papaverine, caffeine, paracetamol. While heroin was baseline resolved, MAM, acetylcodeine, narcotine, papaverine, caffeine and paracetamol were only partially separated, probably because of the high electroosmotic flow (EOF) generated by voltage application at this pH. Also the suppression of the EOF by working at a frankly acidic pH of 2.38 did not provide an acceptable separation, because heroin and papaverine co-migrated (data not shown), suggesting the insufficiency of the plain CZE separation mechanisms based onto the mass-to-charge ratio to provide enough resolution power between so closely related compounds.

On these grounds, we investigated the effect of cyclodextrins, as complex forming agents, which

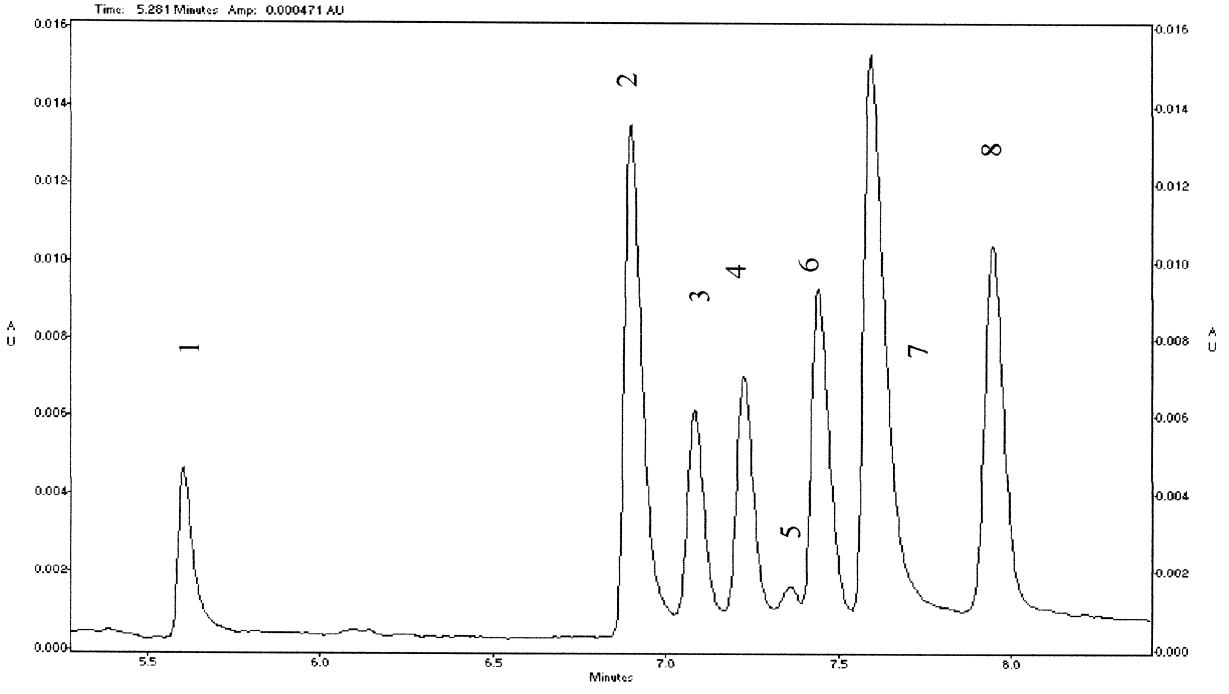

Fig. 2. Typical electropherogram of the separation of a standard mixture containing: (1) phenylethylamine (I.S.); (2) morphine; (3) monoacetylmorphine; (4) heroin; (5) acetylcodeine; (6) papaverine; (7) codeine; (8) narcotine. Analytical conditions: injection, pressure application 0.5 p.s.i. for 3 s; capillary, uncoated fused-silica, 37 cm×50 μm I.D.; buffer, 0.1 *M* potassium phosphate (pH 3.23), 20 m*M* β-cyclodextrin; potential 15 kV; detection, UV absorbance at 200 nm. For other conditions see Section 2.2.

could introduce additional selectivity criteria based on their interactions with the analytes, including dipole–dipole and hydrophobic interactions, H-bonding, steric hindrance. We adopted a low pH in order to minimise the EOF thus allowing analytes to interact with cyclodextrins in the buffer for adequate time. Also, at a fairly acidic pH the charge on the alkaloid analytes is kept constant despite minor fluctuations of the buffer pH.

Fig. 2 shows the baseline separation of a standard mixture containing phenylethylamine (I.S.), morphine, monoacetylmorphine, heroin, acetylcodeine, papaverine, codeine and narcotine which was achieved with a running buffer composed of 0.1 *M* potassium phosphate, pH 3.23, with 20 m*M* β-cyclodextrin added.

Under these analytical conditions, structurally related opiates, displaying the same net positive charge at the running pH, migrate not only according to their molecular masses (which often are very similar), but also to the complex interactions they establish with the uncharged complexing agents — cyclodextrins — resulting in an increased resolution power of the system.

Interestingly, while β-cyclodextrin was effective in providing increased resolution, α- and γ-cyclodextrin did fail. Since they only differ in the dimensions of the inner cavity of their molecules, these data suggest that analyte complexation inside cyclodextrin is the real phenomenon occurring in such situation.

The resolution power of the electrophoretic buffer improved neatly with the increase of concentration of β-cyclodextrin until the limit of 20 m*M*, above which the solubility of the additive was problematic. Hence, this concentration was adopted in the optimised method.

Fig. 3 shows the electropherogram obtained from the injection of a sample of a clandestine preparation of heroin under the same analytical conditions (the same sample had previously been analyzed with a routine gas chromatographic method). The baseline

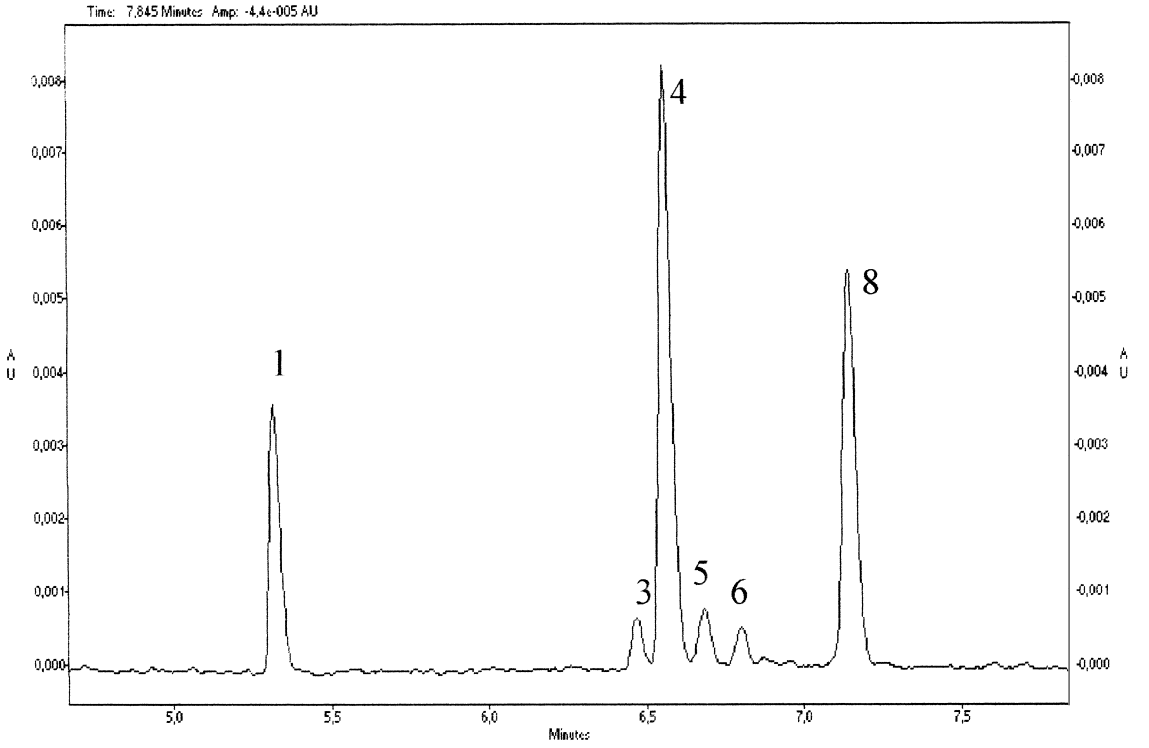

Fig. 3. Typical electropherogram from a real heroin illicit preparation containing: (1) phenylethylamine; (3) monoacetylmorphine; (4) heroin; (5) acetylcodeine; (6) papaverine; (8) narcotine. Analytical conditions as in Fig. 2.

separation of the peaks allowed the qualitative and quantitative determination of all the relevant components. Furthermore, the method proved to be useful for the simultaneous (and potentially chiral) separation of morphine, codeine, amphetamine, its methylenedioxy-derivatives contained in ''ecstasy'' [3,4-methylenedioxymethamphetamine (MDMA), 3-4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDE)], methadone and cocaine, as shown in the electropherogram depicted in Fig. 4. The few overlapping peaks (narcotine and an enantiomer of amphetamine and cocaine and an enantiomer of MDE) correspond to compounds that are very unlikely present in the same sample and which can be easily distinguished on the basis of different UV spectra.

Under the optimized separation conditions, analytical precision was evaluated intra-day and day-to-day, in terms of both migration times and peak areas, using pure standards of morphine, MAM, heroin, acetylcodeine, papaverine, codeine, and narcotine. Mixtures of pure standards were chosen because of the high differences in terms of relative concentrations of components which can be found in real samples. The I.S. was introduced exclude the variability dependent on fluctuations of the EOF. A concentration of 50 μg/ml of each analyte was used to check the repeatability, with six repeats in intra-day precision tests, which was repeated on six consecutive days. The results of the reproducibility study are displayed in Table 1. The intra-day relative standard deviations (RSDs) for migration times were <0.17%, and in day-to-day experiments were <0.71%. In terms of peak area reproducibility, RSDs were always <3.2%. Results from real samples were also extremely reproducible as it is shown in Table 2.

Analytical linearity of the method was also studied for all analytes in the range 0.62–4.39 mg/ml. Linear regression equations were calculated by using

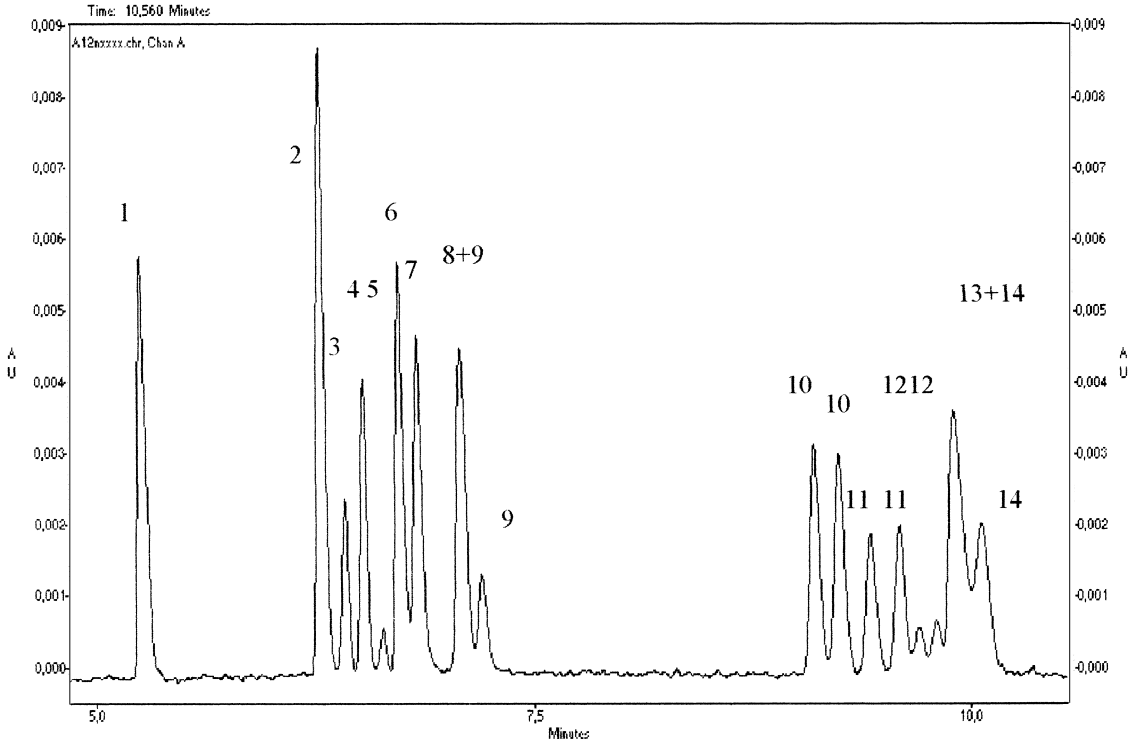

Fig. 4. Electropherogram from the same illicit heroin preparation spiked with: (2) morphine; (7) codeine; (9) D,L-amphetamine; (10) D,L-MDA; (11) D,L-MDMA; (12) D,L-methadone; (13) D,L-MDE; (14) cocaine. Analytical conditions as in Fig. 2.

Table 1
Analytical precision expressed as intra-day and day-to-day RSD% of relative migration times and peak areas, using the I.S. ($n=6$)

	Relative intra-day RSD (%)		Relative day-to-day RDS (%)	
	Migration time	Peak area	Migration time	Peak area
Morphine	0.13	1.11	0.33	1.20
MAM	0.16	1.83	0.41	2.11
Heroin	0.17	2.12	0.37	2.63
Acetylcodeine	0.16	2.81	0.61	3.12
Papaverine	0.15	3.13	0.52	3.20
Codeine	0.13	2.82	0.71	3.03
Narcotine	0.13	2.30	0.69	2.71

the least-square method between analyte-to-I.S. peak area ratio and analyte concentration, with excellent correlation coefficients ($R^2=0.9997–0.9999$). The resulting equations are described in Table 3.

The limits of detection with a signal-to-noise ratio of 4 are shown in Table 4 and perfectly satisfy the usual analytical requirements applied for controlled drugs analysis in forensic laboratories.

Analytical accuracy was calculated by comparing the results from CZE of real samples with those from a gas chromatographic method currently used in our laboratory [18]. The results from both methods are displayed in Table 5.

4. Conclusions

In recent years, CE has gained popularity as a new analytical tool in forensic sciences and has been applied successfully to the analysis of controlled

Table 3
Linearity equations and correlation coefficients

	Linearity equations	R^2
Morphine	$y = 2.756x + 0.0085$	0.9998
MAM	$y = 2.7145x + 0.0456$	0.9997
Heroin	$y = 1.3002x + 0.012$	0.9999
Acetylcodeine	$y = 1.7478x - 0.0048$	0.9999
Papaverine	$y = 0.8091x - 0.0692$	0.9997
Codeine	$y = 1.7438x - 0.0062$	0.9999
Narcotine	$y = 0.7588x + 0.0195$	0.9998

Table 4
Limits of detection (LODs), $S/N>4$

Compound	LOD (ng/ml)[a]
Morphine	500
MAM	500
Heroin	500
Acetylcodeine	500
Papaverine	400
Codeine	500
Narcotine	700

[a] Hydrodynamic injection (3 s).

Table 5
Analytical accuracy for real samples by comparing CZE and GC results

	CE (%)	GC (%)
Monoacetylmorphine	1.66	1.71
Heroin	19.37	20.01
Acetylcodeine	1.64	1.69
Papaverine	0.57	0.58
Narcotine	7.84	7.98

Table 2
Repeatability of analysis in a real sample of clandestine heroin (three replicates per day) on five consecutive days (mean±SD of percent concentrations)

	Results in different days (%)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Monoacetylmorphine	1.31±0.03	1.21±0.03	1.20±0.02	1.19±0.03	1.23±0.03
Heroin	16.9±0.47	15.9±0.43	15.6±0.40	15.5±0.43	16.1±0.44
Acetylcodeine	1.09±0.03	1.02±0.03	1.01±0.03	1.00±0.03	1.04±0.03
Papaverine	0.53±0.01	0.49±0.01	0.48±0.01	0.48±0.01	0.50±0.01
Narcotine	6.61±0.16	6.22±0.15	6.10±0.16	6.08±0.15	6.30±0.16

drugs in both clandestine preparations and biological fluids. The main advantages of this technique are the high versatility in terms of separation modes and the possibility to modulate the selectivity of separation introducing additional and peculiar physico-chemical mechanisms.

The experimental work discussed in the present paper was directed to investigating the effect of cyclodextrins as complex forming agents for improving the separation of heroin, its secondary products and additives usually present in the clandestine preparations. It was demonstrated that β-cyclodextrin, usually applied for chiral separations, improves also the resolution of the closely related non-chiral substances.

The optimized analytical conditions provided excellent separation power and reproducibility, in terms of migration times, quantitation and linearity.

The additional possibility of on-line recording the UV spectra from the peaks offered by modern CE instrumentation increases the identification power and allows to better estimate the peak purity.

In conclusion, CZE again proves to be a sensitive, precise, easy, low cost and rugged tool for forensic analysis, which can be used as a complementary technique to traditional methodologies, or as a first choice technique in environments where HPLC and GC look inadequate (because of difficulties in supplying pure solvents and/or gases), as in developing countries.

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