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Determination of codeine and its metabolite in human urine by CE with amperometric detection

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Abstract

The amperometric detection of codeine and its metabolite, morphine following capillary zone electrophoretic separation is described. A carbon-disk electrode used as working electrode for two analytes exhibited a good response at 0.90 V (vs. Ag/AgCl). The optimal conditions of separation and detection were pH 8.4 phosphate buffer solution (0.06 mol l^{-1}), 12 kV for the separation voltage, sample injection at 12 kV for 10 s. The linear ranges were from 3.1×10^{-7} to 2.5×10^{-5} mol l^{-1} for codeine and 1.9×10^{-7} to 1.5×10^{-5} mol l^{-1} for morphine with a correlation coefficient of 0.9996 and 0.9999, respectively. The detection limits for codeine and morphine were 1.6×10^{-7} and 6.8×10^{-8} mol l^{-1}. The method developed was successfully applied to the determination of codeine and its metabolite, morphine in the human urine without any other sample pretreatment except filtration, and the results were satisfactory.

Keywords: Capillary zone electrophoresis; Amperometric detection; Codeine; Morphine; Metabolite

1. Introduction

Codeine and morphine are the main alkaloids in poppy seeds having pharmacological and toxicological activity. They are widely used as narcotic analgesic and antitussive drugs [1–4]. On the other hand, codeine is metabolized by *O*-demethylation to its active metabolite morphine [5]. Following codeine administration, morphine is only present in low concentrations in plasma and urine but contributes substantially to codeine's analgesic effect. Simultaneous determination of codeine and morphine in biological samples is a common practice in many laboratories involved in forensic and clinical toxicology. In addition, it is significative for pharmacological study to determine codeine and its metabolite in biological samples. The determination of drugs and metabolites in biological fluids is still a very challenging task. One of the largest difficulties is often the sample pretreatment step. Common procedures include liquid–liquid extraction, solid-phase extraction and protein precipitation often in combination with evaporation to dryness to preconcentrate samples.

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Various analytical methods have been reported for the determination of either single or both of them. High performance liquid chromatography (HPLC) with ultraviolet (UV) [6–8], fluorescence [9] or electrochemical detection [6,10,11], gas chromatography (GC) [12,13], and thin-layer chromatography (TLC) [14] have been used for this purpose. Among these methods, however, some are simple but lack sensitivity and specificity, and some are too laborious and time consuming due to pretreatment. Therefore, there is a growing need for the development of sensitive, rapid and simple (cheap) method for the analysis of them.

Recently, capillary electrophoresis (CE) has been rapidly developed in pharmaceutical and biomedical aspects [15–17]. The advantages of CE are related to the excellent separation efficiency, the high mass sensitivity, the minimal use of samples and solvents, the inexpensive capillary column and the possibility of using different detection systems. The separation and determination of codeine and morphine by CE with UV detection [18–20] has been described. Generally, electrochemical detection (ED) is highly sensitive, cheap, simple and convenient. It is useful for detecting low levels of codeine and morphine in biological fluids. The determination of codeine and morphine by means of CE with ED has not yet been reported.

In our study, we developed a method to separate and determine the two analytes by using CE with amperometric detection (CE-AD), which is more sensitive than the previous reports. Limits of detection, linearity and recoveries were presented. The method was directly applied to separate and determine codeine and morphine in human urine without any other pretreatment except filtration and the results were satisfactory.

2. Experimental

2.1. Apparatus

The CZE system with wall-jet amperometric detection assembly was constructed in the laboratory and was similar to that described previously [21]. Electrophoresis in the capillary was driven by a ± 30 kV high-voltage supplier (Shanghai Institute of Nuclear Research, China). The cyclic voltammetry was done on CHI630 Electrochemical System (CH Instruments, USA). The separations were proceeded in a 70 cm long, o.d. 360 μm, i.d. 25 μm, polyimide-coated fused silica capillary (Polymicro Technologies, Phoenix AZ). The injector electrode was kept at high positive voltage, the electrochemical cell for detection was kept at ground and samples were all injected electro-kinetically, applying 12 kV for 10 s.

The carbon disk electrode was constructed by using a 300 μm diameter pencil lead (produced by Mitsubishi Pencil Co., Ltd.) with one end winded by copper wire. A glass pipette of about 0.4 mm tip diameter drawn from a borosilicate glass tube (o.d. 0.4 cm, i.d. 0.2 cm) was cut to the desired length, ca. 18 cm. The unwired end of the pencil lead was introduced into the pipette until it protruded ca. 0.3 cm from the pipette tip. Non-conductive gel was applied at both ends of the pipette to seal the lead and copper wire. Prior to use, the surface of the carbon disk electrode was gradually polished with emery paper and 0.05 μm alumina powder, then ultrasonicated in de-ionized water, and finally carefully positioned opposite the capillary outlet with the aid of a micromanipulator to minimize the gap between the electrode tip and the capillary outlet.

A potentiostat (ZF-3, Shanghai Second Component Factory, China) was used to supply a constant potential to the electrode in the electrochemical cell, which was composed of a platinum auxiliary electrode, a carbon disk working electrode and a Ag/AgCl (3 mol l^{-1} KCl) reference electrode. A picoammeter (WD-1, Shanghai Yanzhong Instrument Factory, China) was utilized to amplify the electrochemical current and a chart recorder (XWTD-164, Shanghai Dahua Instrument factory, China) was used to obtain the electropherograms.

2.2. Materials and procedures

Codeine and morphine were obtained from National Institute for the Control of Pharmaceutical and Biological Products. All the other chemicals

including carrier electrolytes were of analytical reagent grade and bought from local commercial sources. Double distilled water was used throughout.

An accurately weighed amount of codeine and morphine was dissolved separately with methanol to obtain stock solution with the concentration of 1.0×10^{-3} mol l^{-1}.

All experimental solutions were filtered through a polypropylene filter (0.22 μm), including urine samples, and degassed by ultrasonication prior to their use.

The capillary was prepared for 10 min with 0.5 mol l^{-1} NaOH before the first run and then for 10 min with 0.05 mol l^{-1} NaOH and for 10 min with the run buffer solution using a laboratory-built high-pressure system. After every fifth run, the capillary was washed with 0.05 mol l^{-1} NaOH and the run buffer solution each for 4 min to ensure that a constant electroosmotic flow was obtained.

The optimum standard separation conditions were voltage 12 kV (positive polarity), pH 8.4 phosphate buffer (PB) solution (0.06 mol l^{-1}), detection potential 0.90 V (vs. Ag/AgCl, 3 mol l^{-1} KCl), and sampling time 10 s at 12 kV. In addition, all experiments were performed at ambient temperature.

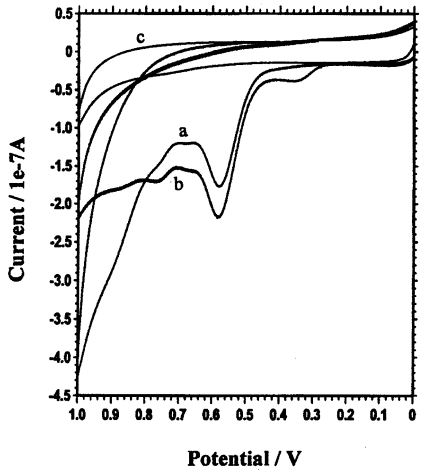

Fig. 1. The cyclic voltammorgrams of codeine and morphine at carbon disk electrode in 0.06 mol l^{-1} (pH 8.4) PB. Reference electrode: Ag/AgCl (3 mol l^{-1} KCl); auxiliary electrode: platinum wire. (a) 3.5×10^{-4} mol l^{-1} codeine; (b) 3.5×10^{-4} mol l^{-1} morphine; (c) blank solution. Scan rate: 100 mV s^{-1}.

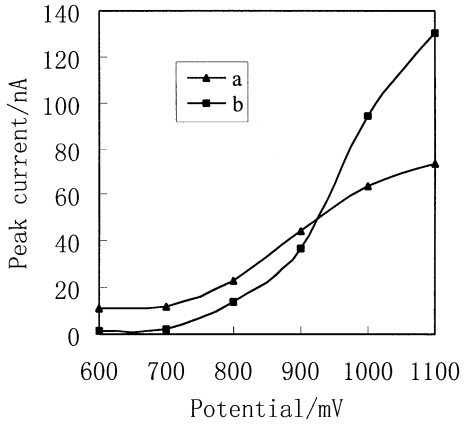

Fig. 2. The hydrodynamic voltammograms obtaining from the experiment for codeine and morphine: (a) codeine; (b) morphine. Fused-silica capillary: 25 μm i.d. × 70 cm; working electrode: 300 μm carbon disk electrode; separation medium: 0.06 mol l^{-1}, pH 8.4 PB; separation voltage: 12 kV; injection: 12 kV × 10 s.

3. Results and discussion

3.1. Selection of potentials applied to the working electrode

Because the potential applied to the working electrode greatly affects the electrode response of analytes, it is very necessary to select a suitable potential when AD acts as a detection method in CE. The electrochemical behaviors of codeine and morphine at the disk carbon electrode were initially investigated using cyclic voltammetry in 0.06 mol l^{-1} PB solution (pH 8.4). Typical cyclic voltammograms for the analytes are shown in Fig. 1. In blank pH 8.4 phosphate solution, no anodic peak is observed in the potential range of 0.00–1.00 V. After the separate addition of codeine and morphine to solution, two anodic peaks are observed at around 0.58 V. From Fig. 1, we can see that codeine and morphine can be oxided when the working potential is above 0.60 V. Fig. 2 illustrates the hydrodynamic voltammograms for codeine and morphine. When the potential was lower than +0.70 V, the peak

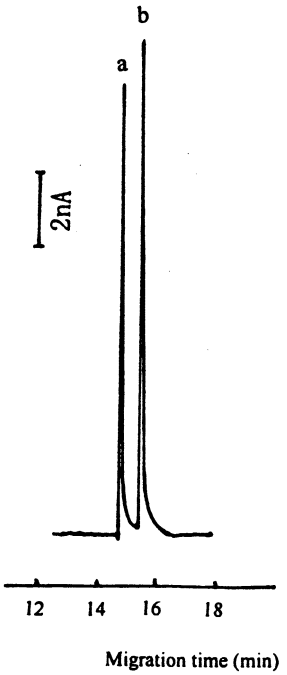

Fig. 3. Typical electropherograms obtained in the chosen optimum conditions for codeine and morphine: (a) codeine (3.0×10^{-6} mol l^{-1}); (b) morphine (1.6×10^{-6} mol l^{-1}). Working potential, +0.90 V (vs. Ag/AgCl); other conditions as in Fig. 2.

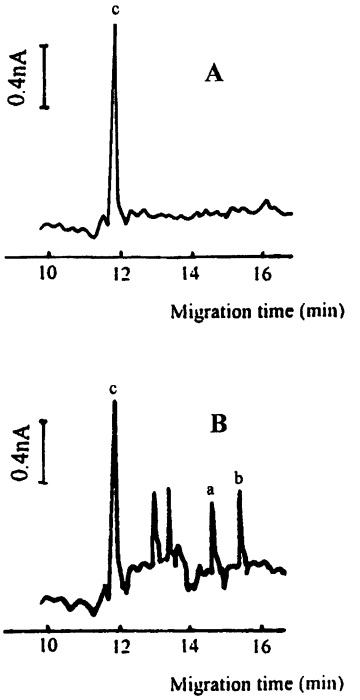

Fig. 4. Electropherograms of codeine and morphine in urine. (A) Blank urine. (B) Urine after oral administration. (a) Codeine; (b) morphine; working potential, +0.90 V (vs. Ag/AgCl); other conditions as in Fig. 2.

currents for the two analytes were relatively small. While the potential was greater than +0.70 V, the response currents of codeine and morphine increased with the increase of the potential applied to the working electrode. At the same time, the base current increased too. Considering the detection sensitivity of the studied analytes and the baseline noise, +0.90 V was chosen as the optimum working potential.

3.2. Effect of the concentration and pH of the running solution

Two buffers, namely phosphate and borate were tested. The experimental results showed that

Table 1
The regression equations and the detection limits

Analyte	Regression equation C (mmol l^{-1}); I (nA)	Correlating coefficient	Linear range (mol l^{-1})	Detection limit[a] (mol l^{-1})
Codeine	$I = 4730.1C - 0.4726$	0.9996	3.1×10^{-7} -2.5×10^{-5}	1.6×10^{-7}
Morphine	$I = 7979.6C - 0.3782$	0.9999	1.8×10^{-7} -1.5×10^{-5}	6.8×10^{-8}

CZE-AD condition as in Fig. 3.
[a] Detection limits were estimated based on three-time ratio of signal to noise.

Table 2
Analytical results of urine sample ($n = 5$)

Analyte	Found amount (mol l^{-1})	RSD (%)
Codeine	2.0×10^{-6}	3.26
Morphine	4.4×10^{-7}	4.00

CZE-AD conditions as in Fig. 4. The average value of five times repetitive injections of urine sample.

under the same conditions, phosphate gave the better separation. With this buffer, the analysis of codeine and morphine was carried out in 16 min. The peak currents of the two analytes in phosphate system were higher than in borate system. And a peak tailing occurred in the borate buffer, which lead to an incomplete separation. So the PB was chosen in our experiments.

For the PB, the dependence of the migration times and the peak currents of codeine and morphine on pH was examined with PB in the pH range 7.4–9.1 and the results demonstrated that there were better separations for codeine and morphine at a pH 8.4. In addition, the effect of the concentration of the running solution was examined using 0.02, 0.04, 0.06 and 0.08 mol l^{-1} pH 8.4 PB solution. The results indicated that with an increase of the concentration of phosphate, the migration time of the two analytes also increased. This is because the ionic strength of PB solution increased with an increase of its concentration, which resulted in a decrease of the electroosmotic flow in the capillary. In addition, with an increase of concentration of PB, the electric current in the capillary increased, which would lead to peak shape broadening. Considering the sensitivity, the time of analysis and the resolution, 0.06 mol l^{-1} PB (pH 8.4) was chosen as the running solution in our experiments.

3.3. Effect of separation voltage and sampling

The dependence of the migration velocities of the solute on the applied electrical field strength was examined for various voltages. The experiment results showed that with an increase of the separation voltage, migration time decreased and the peak shapes became sharper, but the electric current in the capillary increased obviously. A too high electric current in the capillary will lead to peak shape broadening. In order to obtain higher separation efficiency and to save analysis time, 12 kV was used as the separation voltage.

The amount of sampling was also tested by changing the sampling time for 5, 8, 10 12 and 14 s at 12 kV. The peak current was relatively higher for longer injection times. When the injection time was more than 10 s, the height of the peak current changed slowly, but the peak exhibited a significant broadening. In our experiments, 10 s was chosen as the sampling time.

The suggested analytic conditions were therefore: working potential 0.90 V (vs. Ag/AgCl, 3 mol l^{-1} KCl), separation voltage 12 kV, sampling time 10 s at 12 kV, and a 0.06 mol l^{-1} PB solution at pH 8.4.

Under the selected optimum conditions, the electropherogram for the mixture of codeine and morphine is shown in Fig. 3. It is clear that the two analytes are baseline separated in 16 min.

Table 3
The results of the determination of recoveries in this method ($n = 5$)

Analytes	Sample (mol l^{-1})	Added amount[a] (mol l^{-1})	Found amount (mol l^{-1})	Recovery (%)	RSD (%)
Codeine	4.00×10^{-7}	8.00×10^{-7}	11.78×10^{-7}	97.2	3.86
	4.00×10^{-7}	1.20×10^{-6}	1.53×10^{-6}	94.2	2.99
Morphine	8.8×10^{-8}	2.00×10^{-7}	2.80×10^{-7}	96.0	2.47
	8.8×10^{-8}	5.00×10^{-7}	6.00×10^{-7}	102.4	1.65

CZE-AD conditions as in Fig. 4.
[a] The concentrations after dilution.

3.4. Linearity and detection limits

Under the optimized conditions, a series of concentrations of codeine and morphine were tested to determine the linearity for the two analytes at the carbon disk electrode in CZE-AD. The linear range, regression equation, correlation coefficient and detection limits are listed in Table 1. Good linear relationships between peak heights and the concentrations of the two analytes were obtained in the concentration range of 3.1×10^{-7}–2.5×10^{-5} mol l^{-1} ($r = 0.9996$) for codeine and 1.9×10^{-7}–1.5×10^{-5} mol l^{-1} ($r = 0.9999$) for morphine. Based on a signal to noise ratio of 3, the detection limits for codeine and morphine were 1.6×10^{-7} and 6.8×10^{-8} mol l^{-1}, respectively. The results are satisfactory. Reproducibility was determined by making five repetitive injections codeine and morphine. The precisions of peak currents (in terms of relative standard deviation (RSD)) ($n = 5$) were found to be 3.59% and 1.18% for codeine and morphine, respectively.

3.5. Application and recovery

Urine sample was obtained from a healthy volunteer after oral administration of 20 ml cough syrup, which contains codeine phosphate. And it was filtered through a polypropylene filter (0.22 μm). Take 2 ml urine sample, and dilute to 10 ml, then do CZE-AD experiment according to the procedure described in Section 3.4. Fig. 4 shows the electropherograms of the urine sample containing codeine and morphine. We identified peak a as codeine and peak b as morphine by spiking each of the two analytes of the standards into the sample. Additionally, there were some other oxidation current peaks existed in the electropherogram. It was some other active substances in the cough syrup. But all these peaks did not interfere with the determination of codeine and morphine. The quantitative analytical results are summarized in Table 2. It showed that the RSD was less than 4.00%. The concentrations of codeine and morphine determined in urine sample were 2.0×10^{-6} and 4.4×10^{-7} mol l^{-1}, respectively. Recovery experiments were performed in order to study the accuracy of the method. Known amounts of codeine and morphine were added to human urine samples under the same condition as stated above. The experimental data are shown in Table 3. The average recoveries ranged between 94.2% and 102.4%, and the RSD were less than 3.86% ($n = 5$).

4. Conclusions

The method described above shows that it is rapid, selective, of lower expense and is easy to handle for the separation and determination of codeine and its metabolite morphine in human urine. It is useful for detecting low levels of drugs in biological fluids. Because of the excellent separation efficiency, there was no interference in the sample analysis in spite of its complicated composition. It cannot only be used for pharmacokinetic and pharmacology studies but also for monitoring clinical intoxication cases and drug abuse. We believe that this method should allow therapeutic and forensic applications, too.

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