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Short Communication

Determination of opium alkaloids in opium by capillary electrophoresis*

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Introduction

It has always been a challenge to perform accurate measurements of the opium alkaloids and especially of morphine in opium and pharmaceuticals thereof. A major break through was achieved with the introduction of HPLC, and a number of HPLC methods for the determination of the alkaloids in crude opium and pharmaceuticals have been published [1–6]. With the introduction of capillary electrophoresis an additional principle of analysis based on another separation mechanism has been made available. A major problem with several of the HPLC methods is their inability to achieve a good separation of the alkaloids in a reasonable time as some of the alkaloids differ considerably in polarity and others like morphine, normorphine and codeine are very similar and exhibit relative short retention times in reversed-phase HPLC systems. Capillary electrophoresis may provide very high separation efficiency and, as the separation mechanism is based on mass over charge rather than on distribution (polarity) between phases, separation of the alkaloids within a short time may be obtained.

In three recent published papers [7–9] micellar electrokinetic chromatography (MEKC) of some opium alkaloids has been described using either sodium dodecyl sulphate (SDS) or cetyltrimethylammonium bromide (CTAB) as the surfactant, but in none of these papers is the separation of all major opium alkaloids described.

In this paper we present a method for the determination of the five major opium alkaloids in opium and drug formulations thereof.

Materials and Methods

Chemicals

6-Amino caproic acid (6-ACA), β-cyclodextrin (β-CD), heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD), γ-cyclodextrin (γ-CD) and polyoxyethylene-sorbitan monolaurate (Tween 20) were obtained from Sigma (St Louis, USA). Hydroxypropyl-β-cyclodextrin (HP-β-CD) and sodium dodecyl sulphate (SDS) were obtained from Aldrich (Steinheim, Germany). Sulphobutyl ether-IV-β-cyclodextrin (SB-β-CD) was obtained as a test sample from Isco, Inc. (Lincoln, USA). Acetic acid was obtained from Riedel-de Häen (Seelze, Germany) and dimethyl sulphoxide (DMSO) from Merck (Darmstadt, Germany). Morphine hydrochloride (102.0%, Ph.Eur.2nd Ed.), Pectyl

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Cough Mixture (ca. 1.8 mg opium/ml), Pectyl Strong Cough Mixture (ca. 2.5 mg opium/ml) and Opium Tincture (ca. 100 mg opium/g) were obtained from Nycomed DAK A/S (Copenhagen, Denmark). Noscapine and Nirvapon Comp. Mixture (containing 132 μg morphine hydrochloride/ml as well as some codeine, papaverine and noscapine salts) were obtained from Dumex A/S (Copenhagen, Denmark). Thebaine was obtained from Nomeco A/S (Copenhagen, Denmark). Papaverine hydrochloride and crude opium (Ph.Eur.2nd Ed.) from Mecobenzon A/S (Copenhagen, Denmark) and codeine hydrochloride from Nordisk Droge og Kemikalie A/S (Copenhagen, Denmark). Normorphine sulphamate was synthesized according to Rice and May [10].

Sample preparation

Appropriate amounts of morphine hydrochloride, normorphine sulphamate, codeine hydrochloride, thebaine and papaverine hydrochloride were dissolved in water–DMSO–1 M acetic acid (95:0.5:4.5 v/v/v) (solvent A). Noscapine was dissolved in methanol–water–DMSO–1 M acetic acid (25:70:0.5:4.5 v/v/v/v).

Crude opium (500 mg) was dissolved in 5 ml DMSO and diluted to 50.0 ml with 1 M acetic acid. After centrifugation, 1.0 ml supernatant was diluted to 20.0 ml with distilled water. All pharmaceuticals were diluted to an appropriate concentration with solvent A.

Apparatus

For the methods with cyclodextrins as additives, a HP^{3D} Capillary Electrophoresis System (Hewlett–Packard, Waldbronn, Germany) equipped with an on-column diode array detector (DAD) was used. A detection wavelength of 214 nm was used for all samples. The separation was performed in a fused-silica capillary (55 cm × 50 μm i.d.) (Polymicro Technologies, Phoenix, AZ, USA). The capillary was thermostated to 30°C by air. Samples were kept at ambient temperature in the autosampler and injected by applying a pressure of 5 kPa (50 mbar) for 3 s. A voltage of 30 kV was applied during analysis.

A Waters Quanta 4000 Capillary Electrophoresis System (Milford, MA, USA) was used for studies with SDS and Tween 20 in a 6-ACA buffer (0.05 M, pH 4.0). Detection was performed by UV absorption at 214 nm. The separation was performed in a fused-silica capillary (60 cm × 75 μm i.d.) (Polymicro Technologies, Phoenix, AZ, USA).

Sample injection was accomplished by hydrostatic injection for 10 s. All analyses were performed using an applied voltage of 20 kV.

Data collection was performed using Turbochrom version 3.3 software (PE NELSON, Cupertino, CA, USA). 0.05 M 6-ACA (adjusted to pH 4.0 by glacial acetic acid) was used as background electrolyte.

Results and Discussion

Separations of small molecules with the same mass over charge is not possible in free solution capillary electrophoresis unless special reagents are added to the buffer. Also molecules with very similar molecular structure may be hard to separate.

In order to achieve separation of the opium alkaloids, of which morphine, codeine, normorphine and thebaine are similar in structure, two alternative principles involving addition of reagents to the electrophoresis buffer were investigated.

In micellar electrokinetic chromatography (MEKC), a surfactant is added to the buffer and at concentrations above the critical micellar concentration a lipophilic pseudo-phase is formed in the buffer. Two ionic solutes having the same electrophoretic migration rate but different distribution to the micellar pseudo-phase may thus be separated.

The "guest–host" principle involves complexation between the solutes and a component in the buffer. During complexation the solute will show a different migration rate from the uncomplexated solute molecules. Two solutes having the same migration rate in the buffer but different complexation constants with additives in the buffer may thus be separated. For this principle we have tried different cyclodextrins as additives.

MEKC

For separation of the opium alkaloids by the MEKC principle the nonionic surfactant Tween 20 and the anionic surfactant SDS were investigated using the 6-ACA buffer (0.05 M, pH 4.0). Separation of the six tested opium alkaloids (Fig. 1) in the plain buffer only resulted in two peaks (Fig. 2A), and it could be shown that normorphine, morphine, codeine and thebaine all had the same migration rates.

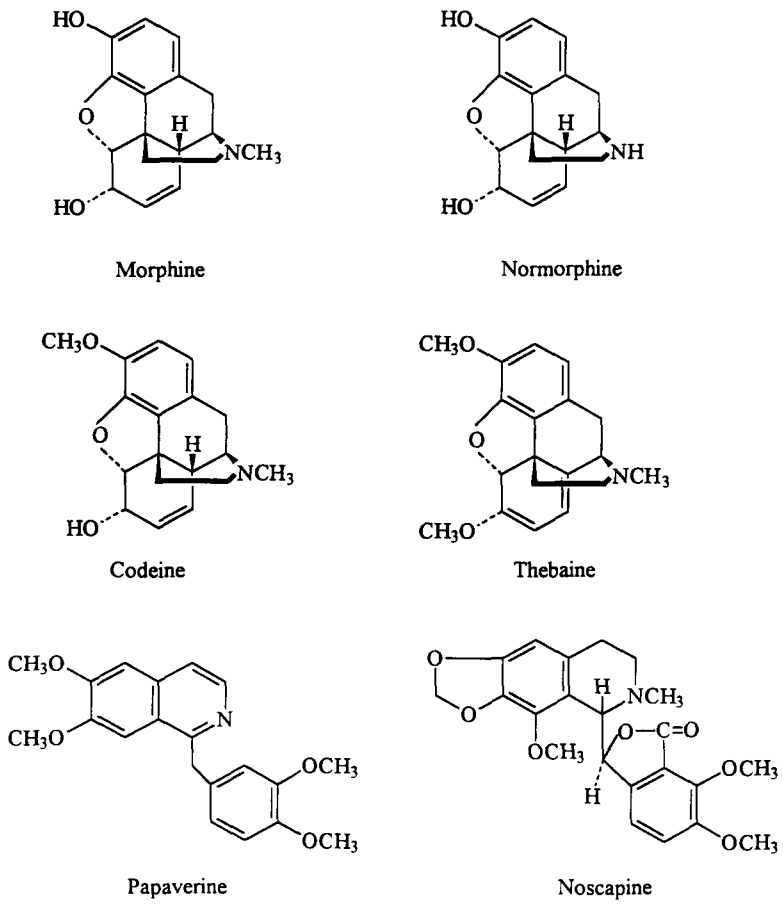

Figure 1
Structure of the 6 opium alkaloids.

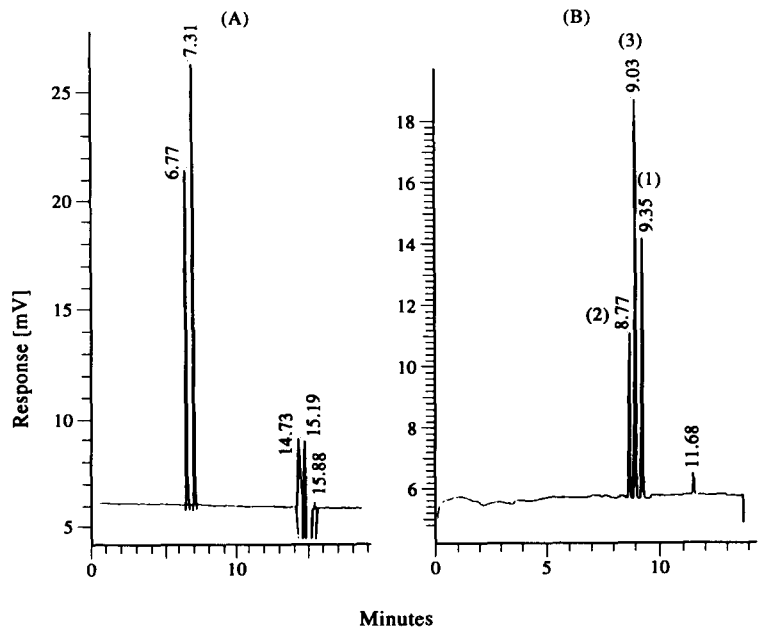

Figure 2
Electropherograms of six opium alkaloids in 6-ACA buffer (0.05 M, pH 4.0) (A); and in 0.05 M 6-ACA pH 4.0 with 12 mM SDS and 25 mM Tween 20 (B) added. Peak identity: normorphine (1), morphine (2) and codeine (3). Other conditions are given in the section Materials and Methods using the Waters apparatus.

The second peak corresponded to papaverine and noscapine. Addition of either of the two surfactants in the concentration range from 1 to 25 mM did not provide full separation of normorphine, morphine and codeine. Therefore, mixtures of the surfactants were investigated keeping the concentration of Tween 20 at 25 mM, as this concentration provided some separation of the three alkaloids. Several different concentrations of SDS were added to this buffer in order to provide the micelles with a negative charge and to vary this charge. High concentrations of SDS resulted in very long migration times for papaverine and noscapine. It was found that base-line separation of normorphine, morphine and codeine could be obtained using a medium concentration (12 mM) of SDS (Fig. 2B). However, due to the fact that thebaine, papaverine and noscapine have much higher affinity to the micellar pseudo-phase, which has a migration towards the anode, these solutes are seen as broad peaks long after the signal for the electroosmotic flow. If the principle of MEKC shall be used for the separation of all the opium alkaloids addition of cationic surfactants, which will result in reversal of the electroosmotic flow, may be a better choice of surfactant. An example of this has recently been published [9].

Cyclodextrins

Five different cyclodextrins have been investigated as "hosts" for separation of the opium alkaloids by the "guest–host" principle (Fig. 3). The different CDs provide very different selectivity. For further investigations we decided to use the DM-β-CD.

The concentration of the CD in the buffer has a significant influence on the selectivity. Especially the selectivity for thebaine and noscapine is altered and a concentration of 30 mM in the buffer was decided to be optimal with respect to time of analysis and peak capacity (Fig. 4).

Also the temperature surrounding the capillary influences the selectivity as well as the migration rates. Figure 5 shows that the separation of the major opium alkaloids and minor constituents in opium are best achieved at 30°C. At other temperatures some of the known or unknown solutes have the same migration rates.

Application

The technique using 6-ACA buffer (0.05 M, pH 4.0) with 30 mM of DM-β-CD added as the electrophoretic buffer has been used for the simultaneous determination of the concentrations of morphine, codeine, papaverine, thebaine and noscapine in four different drugs

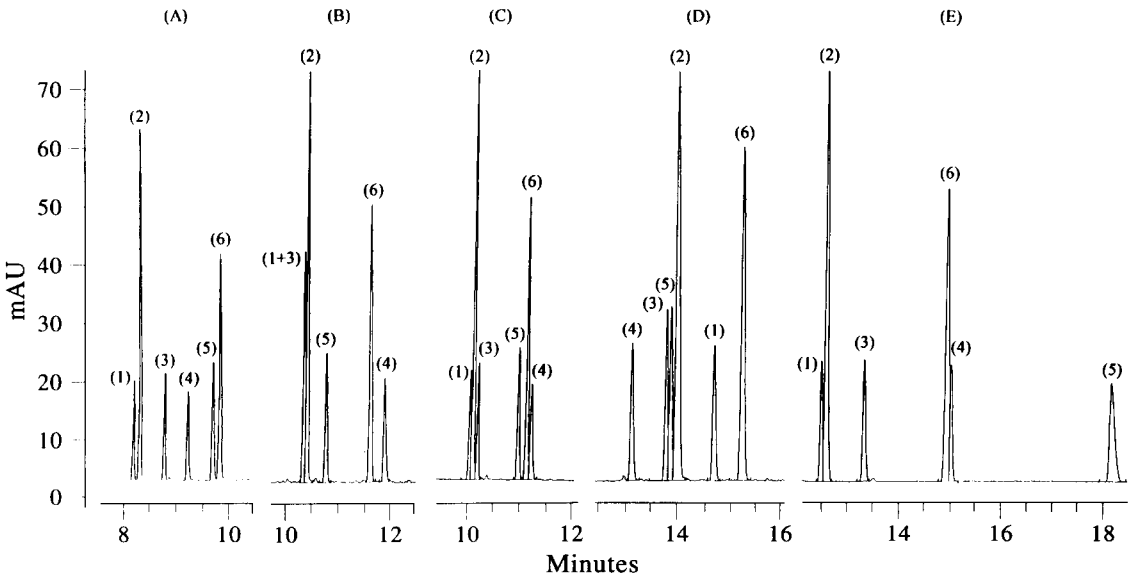

Figure 3
Electropherograms of six opium alkaloids in 6-ACA buffer (0.05 M, pH 4.0) with either 30 mM DM-β-CD (A), 20 mM TM-β-CD (B), 20 mM HP-β-CD (C), 30 mM γ-CD (D) or 5 mM SB-β-CD (E) added. Peak identity: normorphine (1), morphine (2), codeine (3), papaverine (4), thebaine (5) and noscapine (6). Other conditions are given in the section Materials and Methods using the HP^{3D} apparatus.

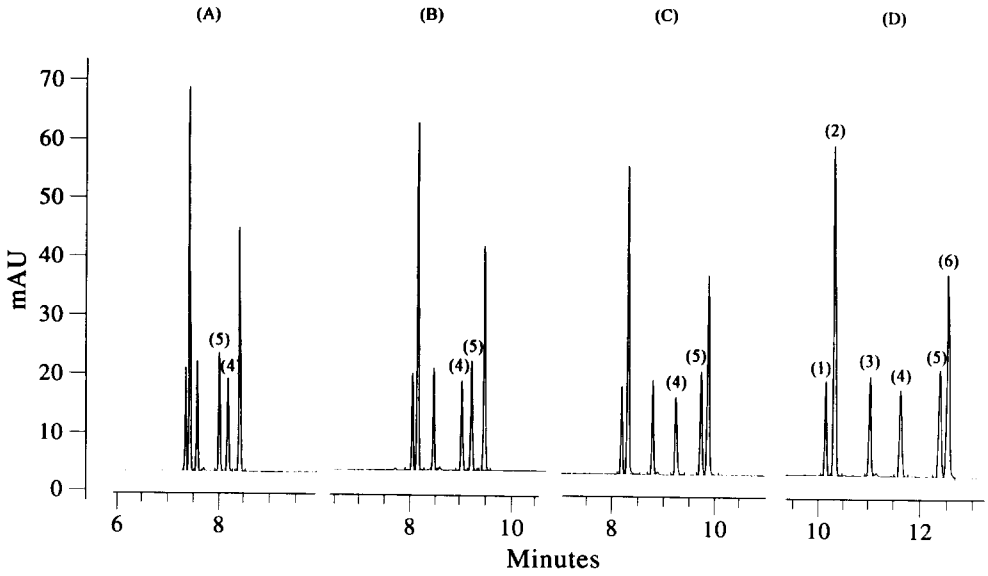

Figure 4
Electropherograms of six opium alkaloids in 0.05 M 6-ACA pH 4.0 with either 10 mM DM-β-CD (A), 20 mM DM-β-CD (B), 30 mM DM-β-CD (C) or 40 mM DM-β-CD (D) added. Peak identity: As Fig. 3.

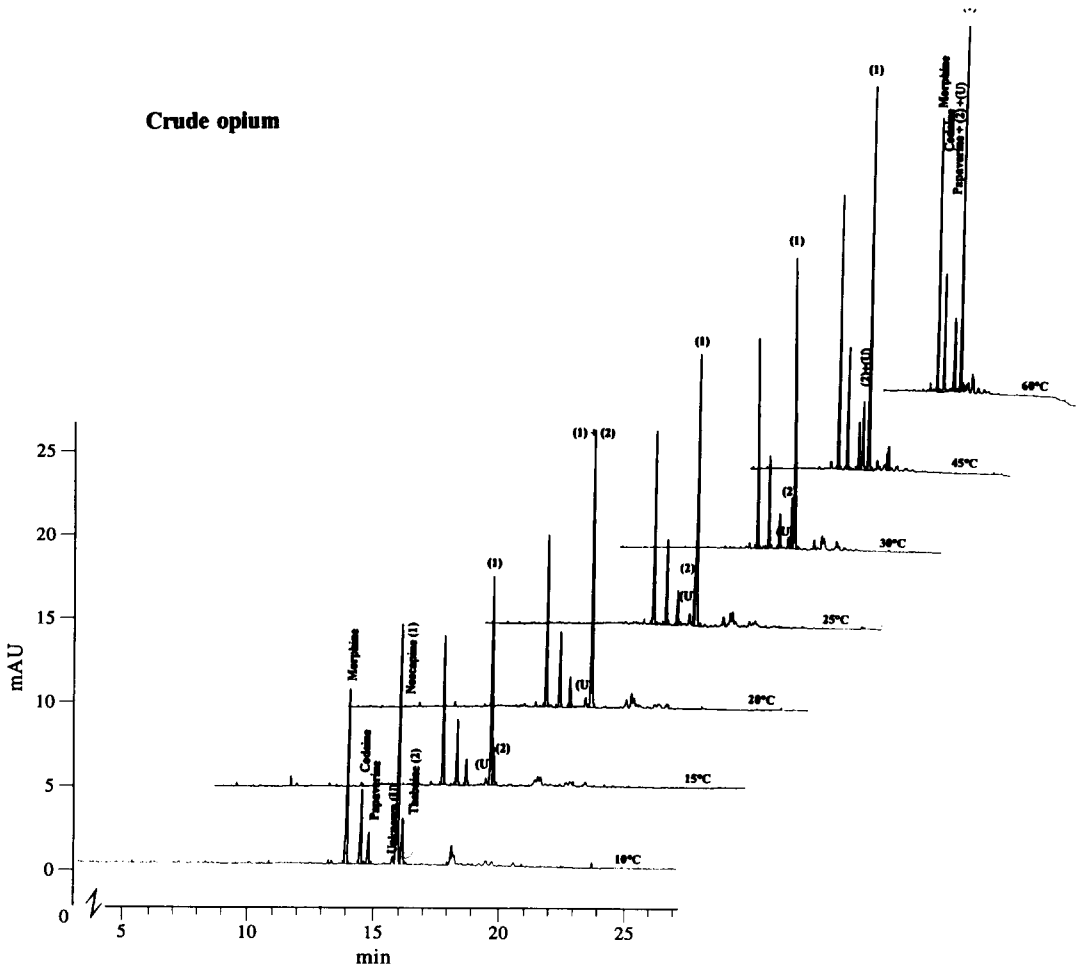

Figure 5
Capillary electrophoresis of crude opium at different temperatures surrounding the capillary. Other conditions are given in the section Materials and Methods using the HP^{3D} apparatus.

Table 1
Validation of the CE method using DM-β-CD as additive to the buffer. Nirvapon Comp. Mixture has been used for the determination of repeatability and recovery. Recovery is calculated as: measured amount/(added amount + amount in sample) × 100%

				Recovery of morphine (%) from Nirvapon Comp. Mixture			
	Linearity (used range) (μg ml^{-1})	Limit of detection (μg ml^{-1})	Repeatability (% RSD, n = 6)	Added (μg)	Found (μg)	Amount in sample by CE	Recovery (%)
Normorphine	1.0–307	0.2					
Morphine	4.4–1300	0.3	1.1	31.2	154.9	123.7	100.6
				62.4	186.1	123.7	100.1
				124.8	248.5	123.7	99.0
Codeine	1.2–359	0.2					
Papaverine	1.3–377	0.2					
Thebaine	1.4–407	0.2					
Noscapine	1.4–430	0.08					

Table 2
The amount of morphine (expressed as morphine hydrochloride) found in three drug formulations by HPLC and CE, respectively. The figures are means of two determinations

	Pectyl Cough Mixture mg ml^{-1}	Pectyl Strong Cough Mixture mg ml^{-1}	Opium Tincture mg ml^{-1}
HPLC method	0.20	0.29	11.6
CE method	0.20	0.31	11.4

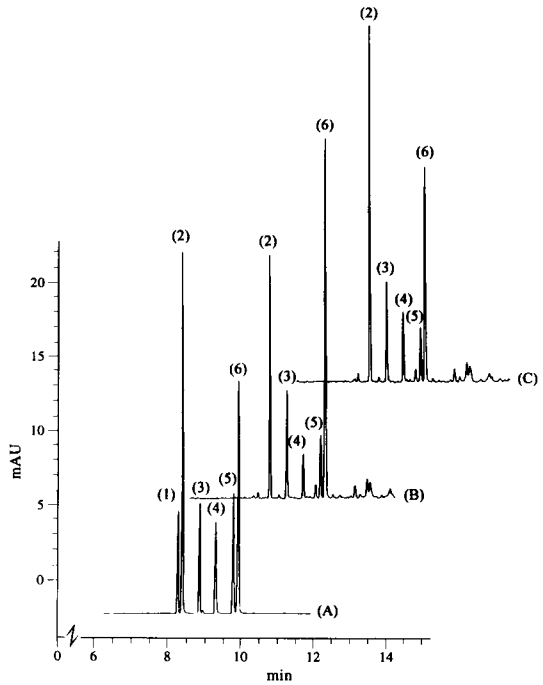

Figure 6
Electropherograms of the alkaloid standard mixture (A), crude opium (B) and Pectyl Strong Cough Mixture (C). Peak identity: as in Fig. 3. Other conditions are given in the section Materials and Methods using the HP^{3D} apparatus.

marketed in Denmark. Figure 6 show electropherograms of the standards, a sample of crude opium and a cough mixture.

The method has been validated using one of the cough mixtures for precision and recovery (Table 1). The linearity of each of the calibration curves for the six alkaloids was determined by analysing six concentration levels in the range given for each alkaloid in Table 1. Linear regression of peak area versus concentration gave straight lines with correlation coefficients in all cases >0.999. Accuracy of the method is estimated by comparing the results for morphine in three different samples with the results found by Nycomed DAK A/S using their validated HPLC method (Table 2). Figures in Table 2 are mean values of two determinations and, therefore, no standard deviations are given. Thus, the results were obtained in separate laboratories at separate time with separate calibration standards. Furthermore, the recovery for the method is determined (Table 1) by standard addition of morphine hydrochloride corresponding to 25, 50 and 100% of the nominal morphine concentration in the cough mixture determined by CE.

The precision expressed by the repeatability has been determined by injecting six separately prepared samples of the Nirvapon Comp. Mixture and calculating the % RSD of the peak areas. The limit of detection were estimated as three times the signal to noise ratio. No normorphine was detected in any of the four pharmaceuticals.

Conclusion

A method for the determination of the major alkaloids in opium and drug preparations thereof based on capillary electrophoresis using the "guest–host" complexation principle has been developed. The method has been validated and the results obtained are comparable to those obtained using HPLC.

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