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Comparison of aqueous and non-aqueous capillary electrophoresis for quantitative determination of morphine in pharmaceuticals

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Abstract

Two capillary electrophoresis methods involving aqueous and non-aqueous electrophoresis media, respectively, have been compared for the quantitative determination of morphine in pharmaceutical preparations. In the aqueous system the separation from other opium alkaloids was achieved using 2,6-di-*O*-methyl-β-cyclodextrin as an additive to the electrophoresis buffer. In the non-aqueous system no other additives than the electrolytes were necessary in order to achieve separation of the opium alkaloids. The two methods have been partially validated and compared with a currently used high-performance liquid chromatography method. From the overall point of view the validations show that the three methods are equivalent in performance and that they are appropriate for the purposes they are intended for. © 1997 Elsevier Science B.V.

Keywords: Morphine; Aqueous capillary electrophoresis; non-aqueous capillary electrophoresis; Opium alkaloids; 2,6-Dimethyl-β-cyclodextrins

1. Introduction

The accurate and precise quantitative determination of morphine in complex matrices has always been a difficult task due to the high polarity of the molecule. Therefore, methods where only few unit processes are used in the sample preparation and where no extraction steps are involved are to be preferred. High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are therefore methods to be preferred. In HPLC reversed-phase separation mechanism is the technique of choice in order to avoid extraction to organic solvents. However, special techniques like ion-pair chromatography are needed to obtain sufficient retention of the polar substance and in general high selectivities are obtained in the separation from other opium alkaloids resulting in very long analysis times. In CE morphine is easily separated from the electroosmotic flow by adjusting the pH but selectivity towards other opium alkaloids is poor in plain

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buffers due to similarities in mass over charge ratios. The selectivity may be enhanced by adding either surfactants or cyclodextrins. Another way to enhance selectivity is to use non-aqueous electrophoresis media [1].

Recently, a number of papers dealing with capillary electrophoretic separation of opium alkaloids have appeared in the literature [2–6]. For these separations aqueous buffers with different additives like sodium dodecyl sulphate, cetyltrimethylammonium bromide or cyclodextrins have been used. However, one of the systems [6] was based on non-aqueous capillary electrophoresis, and it has been questioned whether such a system where volatile solvents are used as a major part of the electrophoresis medium are stable and repeatable enough to be used for quantitative measurements with a reasonable coefficient of variation.

In this paper the validation of the method based on the non-aqueous electrophoresis medium is compared with the previously published validation of the method based on an aqueous buffer with 2,6-dimethyl-β-cyclodextrin added [5], and both capillary electrophoresis methods are compared with a currently used HPLC method.

2. Experimental

2.1. Chemicals

6-Amino caproic acid (6-ACA) and heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD), were obtained from Sigma (St. Louis, USA). Acetic acid was obtained from Riedel-de Häen (Seelze, Germany) and dimethyl sulphoxide (DMSO), HPLC grade acetonitrile and sodium acetate from Merck (Darmstadt, Germany). Ammonium acetate was obtained from Aldrich (Steinheim, Germany). The methanol used was of HPLC grade and all chemicals were used without further purification.

Morphine hydrochloride (102.0%, Ph. Eur. 2nd edn.), Pectyl Cough Mixture (ca. 1.8 mg ml^{-1} opium), Pectyl Strong Cough Mixture (ca. 2.5 mg ml^{-1} opium) and Opium Tincture (ca. 100 mg g^{-1} opium) were obtained from Nycomed DAK (Copenhagen, Denmark). Noscapine and Nirvapon Comp. Mixture (containing 132 μg ml^{-1} morphine hydrochloride as well as some codeine, papaverine and noscapine salts) were obtained from Dumex (Copenhagen, Denmark). Thebaine was obtained from Nomeco (Copenhagen, Denmark). Papaverine hydrochloride from Mecobenzon (Copenhagen, Denmark) and codeine hydrochloride from Nordisk Droge og Kemikalie (Copenhagen, Denmark). Normorphine sulphamate was synthesized according to Rice and May [7].

2.2. Apparatus

An 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 (bandwidth: 16, Ref: off) was used for all samples. The separation was performed in a fused-silica capillary (64 cm × 50 μm i.d.; 55.5 cm to detector) (Polymicro Technologies, Phoenix, AZ). The capillary was externally thermostated to 30°C for the aqueous system and to 25°C for the non-aqueous system 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 (60 μA) was applied during analysis using the aqueous system and 25 kV (14 μA) was applied during analysis using the non-aqueous system.

2.3. Electrophoresis media

Aqueous system: 0.05 M 6-ACA (adjusted to pH 4.0 by glacial acetic acid) with 30 mM DM-β-CD added.

Non-aqueous system: acetonitrile–methanol (25:75, v/v) with 25 mM ammonium acetate and 1 M acetic acid added.

Prior to use the capillaries were rinsed with 1 M sodium hydroxide for 60 min, 0.1 M sodium hydroxide for 20 min, distilled water for 20 min and then 10 min with the final electrophoresis medium. Between analyses the capillaries where

Fig. 1. Electropherograms of six opium alkaloids in (A) 6-ACA buffer (0.05 M, pH 4.0) with 30 mM DM-β-CD added and in (B) acetonitrile–methanol (25:75, v/v) with 25 mM ammonium acetate and 1 M acetic acid added. Peak identity: normorphine (3), morphine, codeine (2), papaverine (5), thebaine (4) and noscapine (6). Other conditions are given in Section 2. Furthermore, the structures of the 6 major opium alkaloids are given.

flushed for 2 min with the electrophoresis medium. The electrophoresis medium in the vials holding the running buffers were replaced every five to six runs.

2.4. Sample preparation

Morphine hydrochloride calibration standard was dissolved in water–DMSO–1 M acetic acid

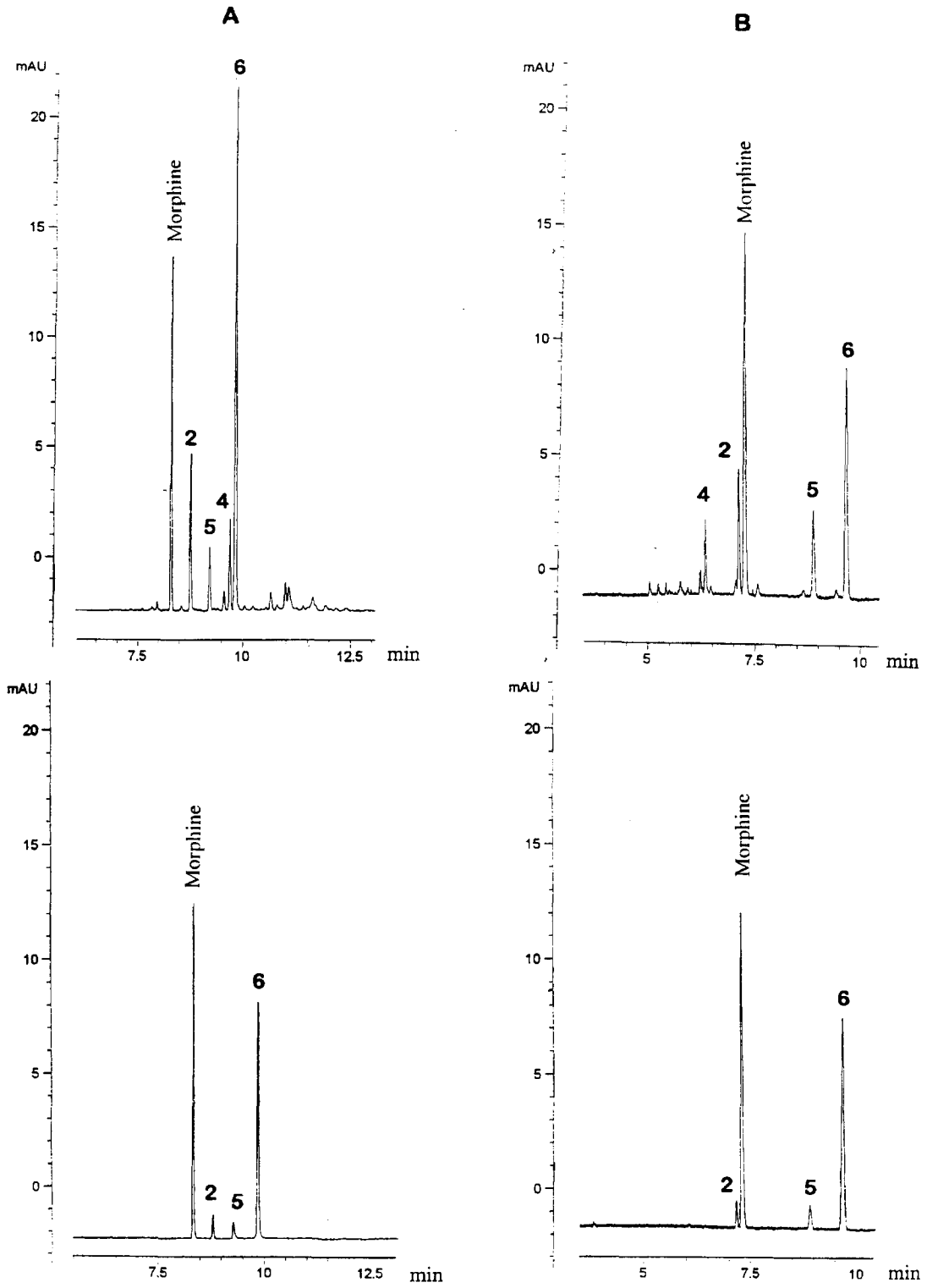

Fig. 2. Electropherograms of Pectyl Strong Cough Mixture (above and Nirvapon Comp. Mixture (below) in aqueous (A) and non-aqueous (B) media, respectively. Peak identity: as in Fig. 1. Other conditions are given in Section 2.

Table 1
Validation of two CE method for the determination af morphine (expressed as morphine hydrochloride) in pharmaceuticals

	Linearity (used range) (μg/ml)	Limit of detection (μg ml^{-1})	Repeatability (% R.S.D., $n=6$)	Recovery of morphine (%) from Nirvapon Comp. Mixture			
				Added (μg)	Found (μg)	Amount in sample found by CE	Recovery (%)
Morphine (aqueous system)	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	99.9
Morphine (non-aqueous system)	15.1–300.1	0.2	2.0	37.5	158.0	118.7	101.2
				75.0	195.5	118.7	101.0
				150.0	270.5	118.7	100.7

The methods are based on an aqueous system using DM-β-CD as additive to the buffer or using a non-aqueous electrophoresis medium. Nirvapon Comp. Mixture has been used for the determination of repeatability and recovery. Recovery is calculated as: measured amount (added amount + amount in sample)$^{-1} \times 100\%$.

(95:0.5:4.5 v/v/v) (solvent A). All pharmaceuticals were diluted to an appropriate concentration with solvent A. The test mixture sample containing morphine, codeine, normorphine, thebaine, noscapine and papaverine each at a concentration of 0.07 mg ml^{-1} was prepared in solvent A for the non-aqueous and aqueous systems (0.25 mg ml^{-1} morphine in the test mixture used for the aqueous system).

3. Results and discussion

Separations of small molecules with the same mass over charge ratio 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 either involving addition of DM-β-CD to the electrophoresis buffer or using a non-aqueous electrophoresis medium were investigated. Two independent methods have been developed [5,6] giving full separation of the major opium alkaloids but with large differences in selectivities (Fig. 1). The separation principle in the aqueous system is based upon different complexation of the six opium alkaloids and the cyclodextrin, the complexes having less electrophoretic migration due to their higher mass.

In the non-aqueous system no additives are used and the separation is therefore based on differences in charge and in solvation. It is well known that the pK_a values of compounds are strongly dependant on the solvent used and the solvation will of course change dependant on the solvent in use.

3.1. Validation

The two CE methods developed have been used for the determination of the concentrations of morphine in four different drug formulations

Table 2
The amount of morphine (expressed as morphine hydrochloride) found in three drug formulations by HPLC (figures obtained by Nycomed DAK using their validated HPLC method) and the two CE methods, respectively

	Pectyl cough mixture (mg ml^{-1})	Pectyl strong cough mixture (mg ml^{-1})	Opium tincture (mg ml^{-1})
Label claim	0.20		
HPLC method	0.20	0.29	11.6
Aqueous CE	0.20	0.31	11.4
Non-aqueous CE	0.20[a]	0.36	11.9

The figures are means of two determinations.
[a] The assay was biased by interfering peaks as the cough mixture contains liquorice and other ingredients.

marketed in Denmark. Fig. 2 shows electropherograms of Pectyl Strong Cough Mixture and Nirvapon Comp. Mixture.

The two methods have been validated using one of the cough mixtures for precision and recovery (accuracy) (Table 1). The linearity of the calibration curves for morphine was determined by analysing six concentration levels in the range given 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 was estimated by comparing the results for morphine in three different samples with the results found by Nycomed DAK using their validated HPLC method (Table 2). Figures in Table 2 are mean values of two determinations, and therefore no S.D. are given. It is noted that the results were obtained in separate laboratories at separate times with separate calibration standards. Furthermore, the recoveries for the methods were 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 % R.S.D. of the peak areas. The limit of detection were estimated as three times the signal to noise ratio.

It should be emphasized that the precision obtained here using volatile non-aqueous electrophoresis media is not obtainable using any CE instrumentation. In order to obtain reproducible results it is important that the evaporation of the sample solvent and the electrophoresis medium is eliminated by using closed vials. Otherwise, the solvent will evaporate quickly and thereby change the composition of the electrophoresis medium and or the sample.

4. Conclusion

Two methods for the determination of morphine in drug preparations based on capillary electrophoresis using either the 'guest-host' complexation principle by addition of DM-β-CD to the electrophoresis buffer or the non-aqueous approach have been developed. The methods have been validated and the results obtained for both methods are comparable to those obtained using HPLC. In general the selectivity can easily be altered using non-aqueous CE just by using different organic solvents or by using mixtures of these. For these reasons non-aqueous CE should be considered when choosing a separation technique (HPLC or CE) for a given separation problem.

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