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Determination of opium alkaloids in crude opium using non-aqueous capillary electrophoresis

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Abstract

A method for the quantitative determination of the opium alkaloids morphine, codeine, thebaine, noscapine and papaverine in crude opium and in drug preparations based on non-aqueous capillary electrophoresis has been developed. The non-aqueous mode provides high separation selectivity and new possibilities for regulating the selectivity in capillary electrophoresis. The nature of the organic solvent, the acidity of the electrolytes as well as the temperature surrounding the capillary exhibit a major influence on the separation selectivity.

Keywords: Codeine; Morphine; Non-aqueous capillary electrophoresis; Opium alkaloids; Pharmaceutical analysis

1. Introduction

A capillary zone electrophoretic method for the determination of opium alkaloids in opium has recently been published [1]. The method was based on the use of aqueous buffers in non-coated fused-silica capillaries and either surfactants (micellar electrokinetic chromatography (MEKC)) or cyclodextrins (the "guest-host" complexation principle) were used for obtaining the necessary selectivity. In a few other papers [2-4] describing capillary electrophoresis of some of the opium alkaloids, MEKC has also been used with either sodium dodecyl sulphate or cetyltrimethylammonium bromide as the surfactant.

The use of non-aqueous capillary electrophoresis for the separation of drug substances [5-8] and small peptides [9] has recently been demonstrated. It was shown that selectivities, that were very difficult to obtain in aqueous buffers, even when using MEKC or complexing agents, were easily obtained when using the non-aqueous systems.

In this paper, highly selective non-aqueous capillary electrophoresis systems for the determination of opium alkaloids in opium are presented. No addition of surfactants or complexing agents to the electrophoresis medium is necessary.

2. Experimental

2.1. Chemicals

Dimethyl sulphoxide (DMSO), HPLC grade acetonitrile and sodium acetate were obtained from Merck (Darmstadt, Germany). Ammonium acetate, formamide and *N*-methylformamide (NMF) were obtained from Aldrich (Steinheim, Germany). *N,N*-dimethylacetamide (DMA) was obtained from Fluka Chemie AG (Buchs, Switzerland). Acetic acid and *N,N*-dimethylformamide (DMF) were obtained from Riedel-de Häen (Seelze, Germany. The

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methanol used was of HPLC grade and all chemicals were used without further purification.

Morphine hydrochloride (102.0%;, Ph. Eur., 2nd edn.) and opium tincture (approximately 100 mg opium per gram) were obtained from Nycomed DAK A/S (Copenhagen, Denmark). Codeine hydrochloride was obtained from Nordisk Droge og Kemikalie A/S (Copenhagen, Denmark), noscapine from Dumex A/S (Copenhagen, Denmark), thebaine from Nomeco A/S (Copenhagen, Denmark) and papaverine hydrochloride and crude opium (Ph.Eur., 2nd edn.) from Mecobenzon A/S (Copenhagen, Denmark). Normorphine sulfamate was synthesized according to the method of Rice and May [10].

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 was used for all samples unless otherwise stated. The separation was performed in a fused-silica capillary (64 cm × 50 μm i.d.; 55.5 cm to detector) obtained from Polymicro Technologies, Phoenix, AZ, USA. The capillary was thermostated to 25°C by air unless otherwise stated. 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 25 kV was applied during analysis.

Prior to use, the capillaries were rinsed with 1 M sodium hydroxide for 60 min, 0.1 M sodium hydroxide for 20 min, destilled water for 20 min and then 10 min with the final electrophoresis medium. Between analyses the capillaries were flushed for 2 min with the electrophoresis medium.

2.3. Sample preparation

The mixed test sample containing morphine, codeine, normorphine, thebaine, noscapine and papaverine, each at a concentration of 0.03 mg ml^{-1}, was prepared in methanol.

When investigating formamide, NMF, DMF, DMA and DMSO, the mixed test sample was prepared in the respective organic solvent.

Crude opium (500 mg) was dissolved in 5 ml of DMSO and diluted to 50.0 ml with acetonitrile containing ammonium acetate (25 mM)–acetic acid (1 M). After centrifugation at 18 000 *g* for 2 min, 1.00 ml of the supernatant was diluted to 20.0 ml with acetonitrile containing ammonium acetate (25 mM)–acetic acid (1 M).

Opium tincture (approximately 100 mg opium per gram) was diluted two-hundredfold with water, methanol or acetonitrile, respectively. Calibration standard solutions of morphine hydrochloride (0.05 mg ml^{-1}) were prepared in water or methanol, respectively.

3. Results and discussion

In order to explore the possibilities for obtaining the resolution of all peaks in opium, the influence of the nature of the organic solvent, the choice of electrolytes, the temperature surrounding the capillary and the content of water in the electrophoresis medium were investigated using six alkaloids (Fig. 1) as test solutes.

3.1. Organic solvent

The nature of the organic solvent used for the electrophoretic medium has a strong influence on the separation selectivity (Fig. 2).

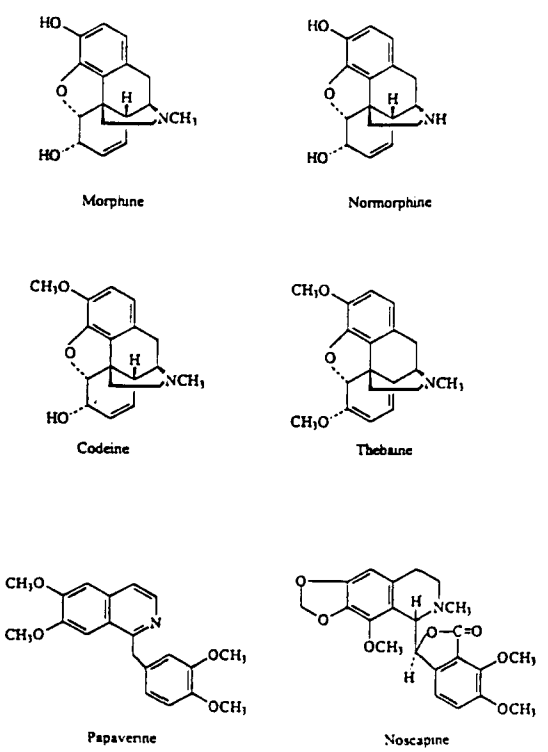

Fig. 1. Structures of the six test solutes.

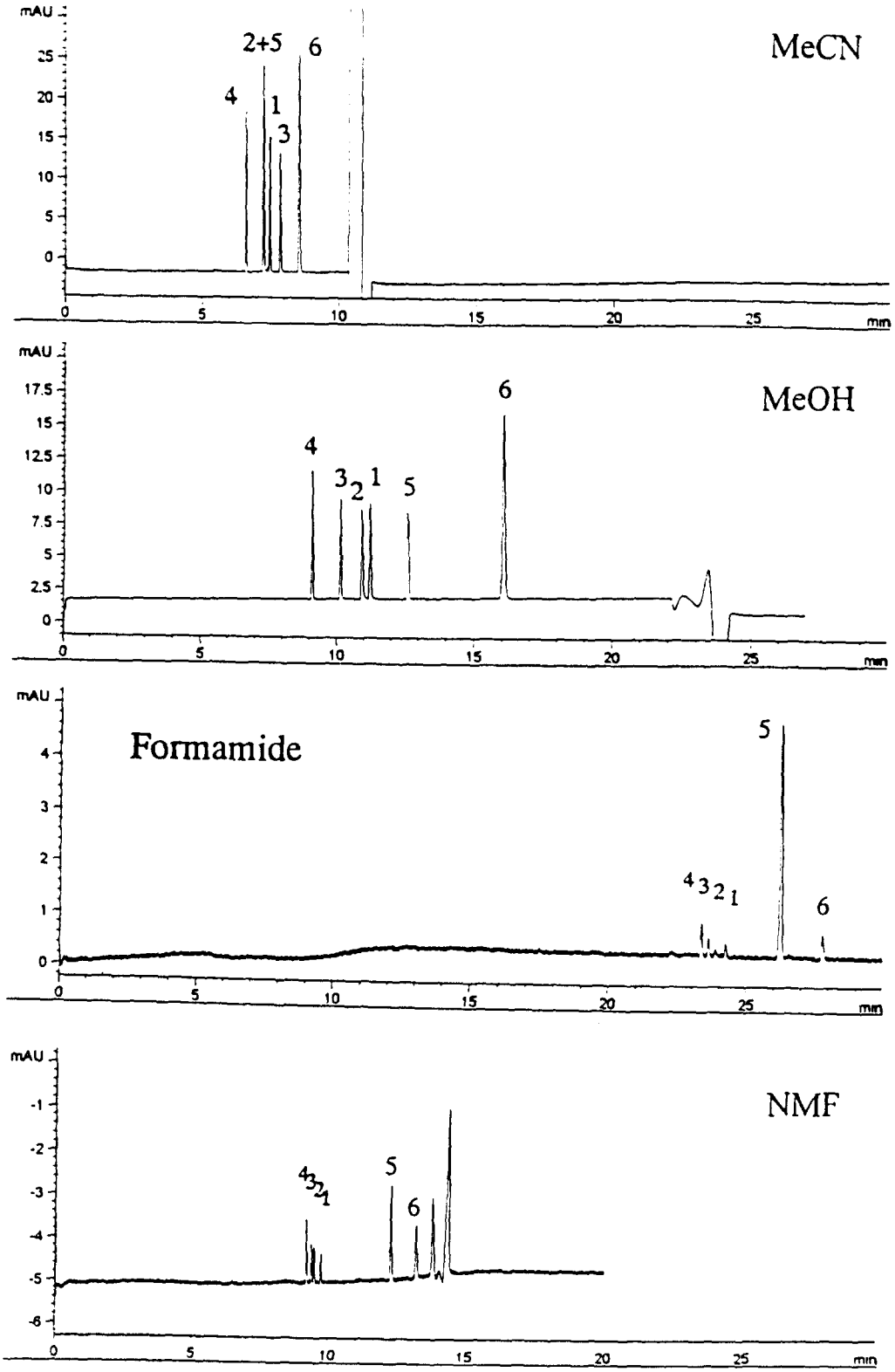

Fig. 2. Electropherograms of the test solutes using four different organic solvents. Conditions: capillary (to detector), 55.5 cm × 50 μm i.d.; temperature, 25°C; detection at (A,B) 214 nm, (C,D) 254 nm; electrophoresis medium, ammonium acetate (25 mM)–acetic acid (1 M) in acetonitrile (MeCN), methanol (MeOH), formamide or NMF as indicated on the figure; voltage, 25 kV. Peak identification: 1, morphine; 2, codeine; 3, normorphine; 4, thebaine; 5, papaverine; 6, noscapine.

While keeping the electrolyte (ammonium acetate (25 mM)–acetic acid (1 M)) constant, different organic solvents were investigated. In acetonitrile, methanol, formamide and NMF, high separation efficiency and high selectivity were obtained although a full separation of the six test solutes was not obtained in acetonitrile.

In DMF, DMA and DMSO (not shown) two or three of the alkaloids comigrated with the electroosmotic flow (EOF).

Detection may pose a problem in non-aqueous capillary electrophoresis. A significantly higher limit of detection is obtained when using formamide, NMF, DMF, DMA or DMSO, as the noise level increases due to the high UV absorbance of the organic solvent at lower wavelengths. When using methanol or acetonitrile, a detection wavelength at 214 nm may be used without problems.

The high EOF found when using acetonitrile and the selectivity differences between the test solutes obtained in acetonitrile and methanol lead to a more detailed study of the use of these two solvents. The gradual replacement of methanol with acetonitrile was investigated (Fig. 3). In changing the organic solvent from 100% acetonitrile through 25% methanol in acetonitrile and 75% methanol in acetonitrile to 100% methanol, improvements in selectivity were obtained. Full separation of all six test solutes and short migration times were obtained using 25% methanol in acetonitrile.

3.2. pH*

Although pH is only defined in dilute aqueous solution, ionization in organic solvents with a reasonable dielectric constant is sufficient for performing capillary electrophoresis. The EOF as well as the separation selectivity are influenced (Fig. 4) when changing the electrolyte from a mixture of ammonium acetate–sodium acetate to ammonium acetate–acetic acid with increasing concentration. The changes in the EOF are most likely due to changes in the zeta potential at the inner capillary wall.

3.3. Temperature

A change in the temperature of the air surrounding the capillary has been shown to have a great influence on the selectivity when separating opium alkaloids in aqueous buffers [1]. The influence of changes of temperature from 10 to 40°C on the separation of components in a sample of crude opium was investigated in the non-aqueous mode using acetonitrile as the organic solvent (Fig. 5). Major changes in selectivity were observed and from the electropherograms of crude opium, 20°C was selected as the optimum temperature; however 40°C may also be used, as the known alkaloids at these two temperatures are separated from each other and from minor unknown alkaloids.

3.4. Water and other solvents for sample preparation

In plain aqueous buffer the five major opium alkaloids appear only as two peaks [1]. When investigating the influence of water on the separation it was found that gradual substitution of the acetonitrile in the non-aqueous buffer medium with water only gradually changes the selectivity of the system. Thus, small amounts of water do not harm the non-aqueous system, and it is possible even to introduce aqueous samples into the system with only very little change in selectivity and efficiency (Fig. 6). In order to study further the influence of the solvent used for sample preparation on the electrophoresis process, opium tincture was diluted two-hundredfold in water, methanol or acetonitrile, respectively. The samples were introduced into two different non-aqueous electrophoresis systems with acetonitrile or acetonitrile–methanol (25:75) as the organic solvent. Fig. 6 shows that the highest efficiency is obtained when using water as solvent for the sample preparation. The resolution between codeine and papaverine is partly lost when using acetonitrile (Fig. 6). When increasing the sample introduction time to 10 s, this becomes even more evident (Fig. 6). These phenomena are similar when using acetonitrile–methanol (25:75, v/v) as the solvent for the electrophoresis medium. Methanol as a solvent for sample preparation behaves intermediary to water and acetonitrile. The solvent effect on the efficiency is probably partly due to the differences in the dielectric constants of the solvents. The highest dielectric constant will give the highest ion mobility in the sample volume and thus introduce a stacking effect.

Thus, for quantitative analysis it is important that the sample and the calibration standard are dissolved in the same solvent. Also, differences in the amounts introduced into the capillary using an identical injection technique may be found when using different solvents, due to differences in the viscosities and vapour pressures of the solvents.

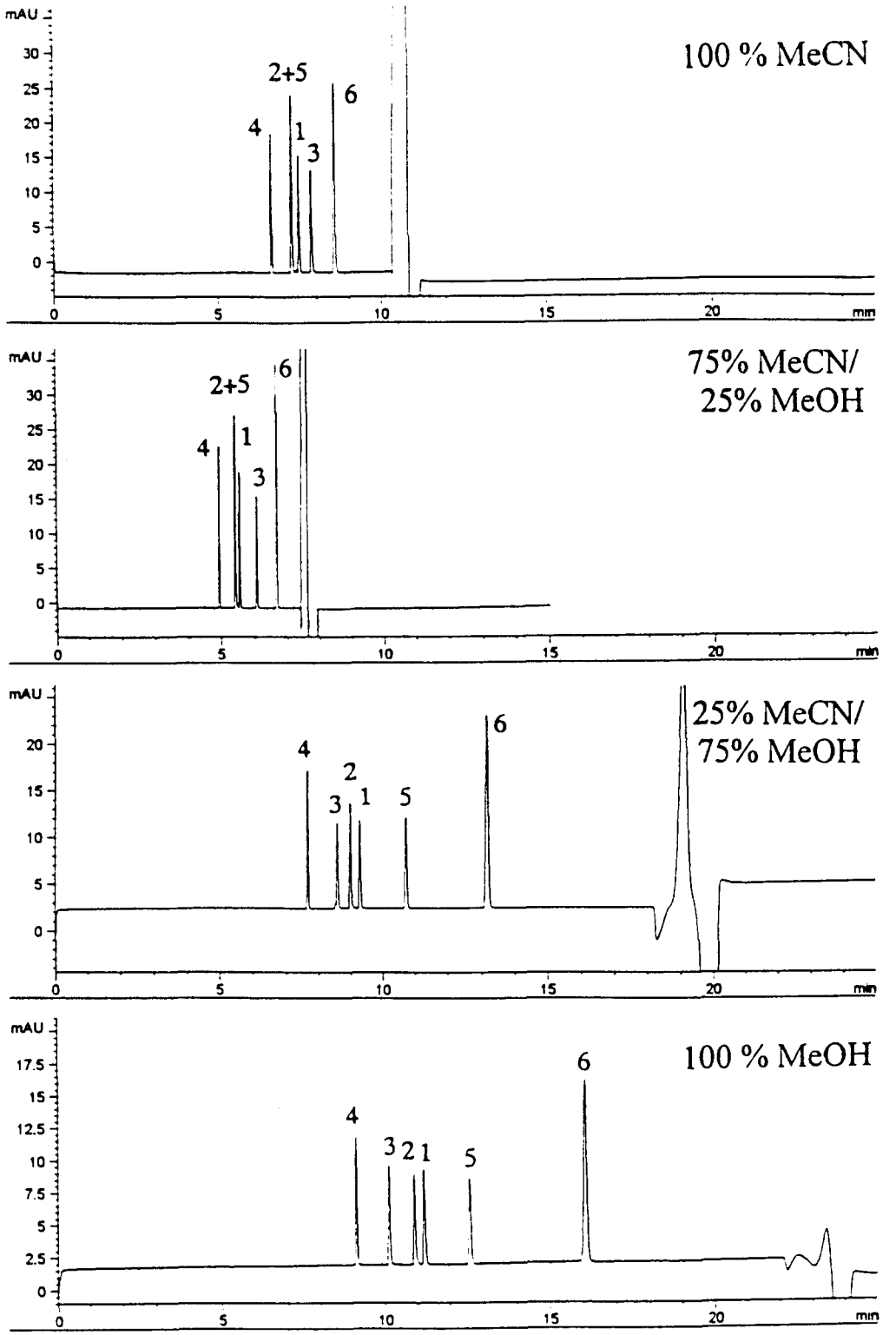

Fig. 3. Electropherograms of the test solutes in acetonitrile (MeCN), methanol (MeOH) and mixtures thereof. Conditions and peak identification as for Fig. 2.

4. Applications

The developed method may be used for the determination of the opium alkaloids in opium and in drug preparations. The content of morphine in crude opium and in opium tincture may, when taking the influence of the sample preparation solvent into consideration, be determined with the same accuracy and precision

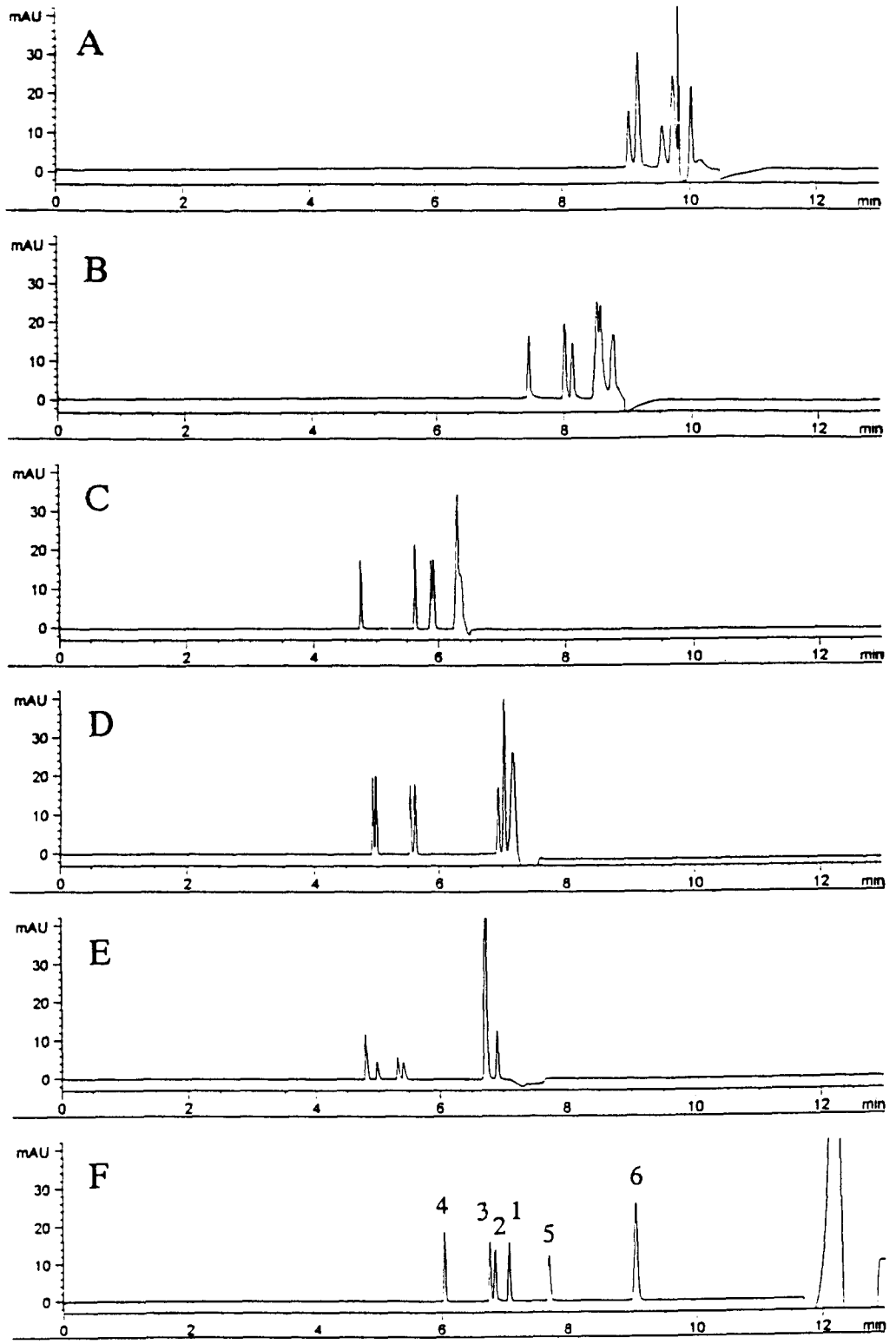

Fig. 4. Electropherograms of the test solutes using basic and acidic electrolytes in acetonitrile–methanol (1:1, v/v). Electrophoresis media (all in acetonitrile–methanol (1:1, v/v) containing 25 mM ammonium acetate: (A) 100 mM sodium acetate; (B) 50 mM sodium acetate; (C) no additives; (D) 100 mM acetic acid; (E) 250 mM acetic acid; (F) 1 M acetic acid. Other conditions and peak identification as for Fig. 2. No attempts were made to identify the peaks in electropherograms A, B, C, D and E.

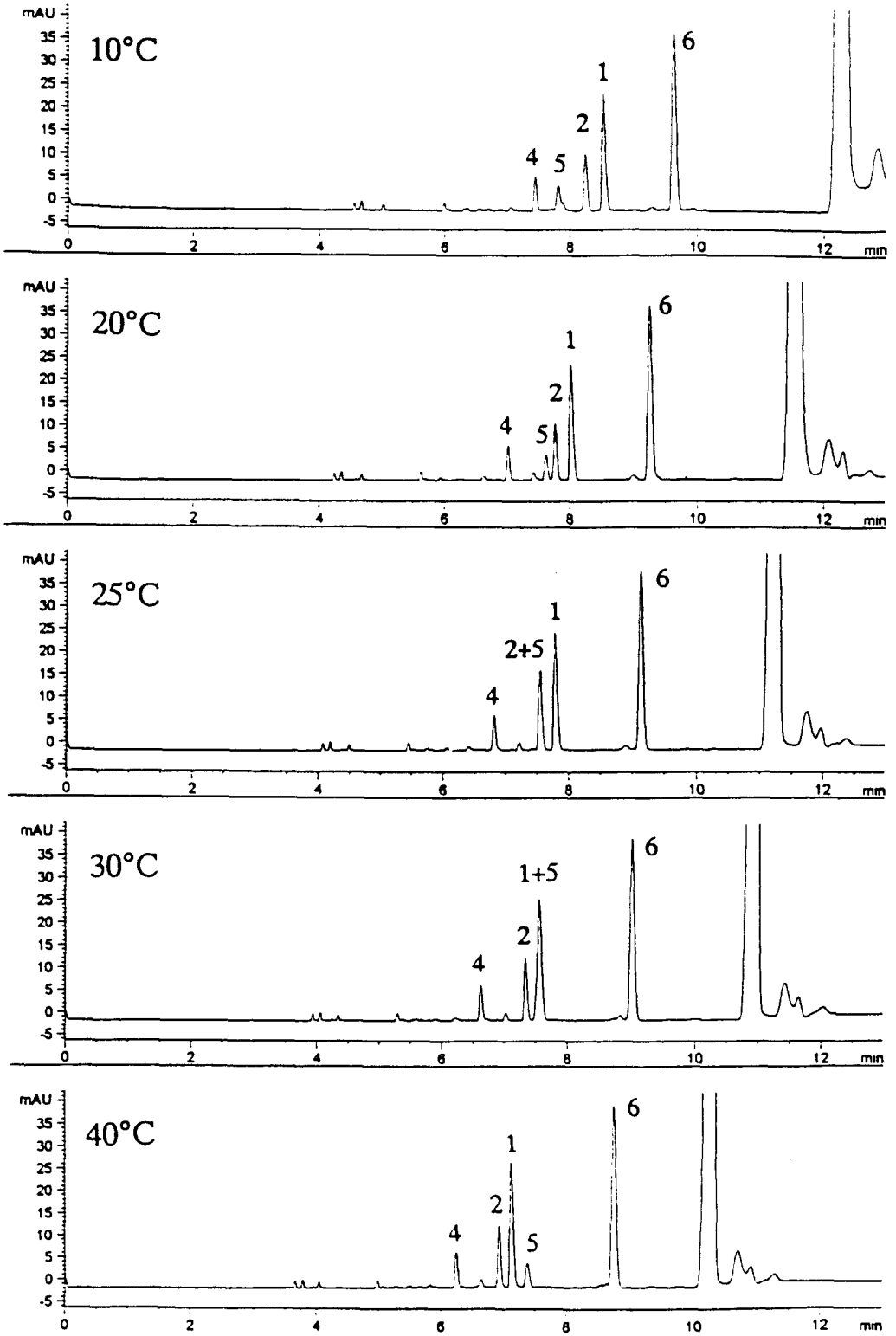

Fig. 5. Electropherograms of crude opium at different temperatures surrounding the capillary. Electrophoresis medium, acetonitrile containing ammonium acetate (25 mM)-acetic acid (1 M). Other conditions and peak identification as for Fig. 2.

as was obtained previously in aqueous capillary electrophoresis systems [1]. No full validation of the method has been performed as this was not the intention in the present work.

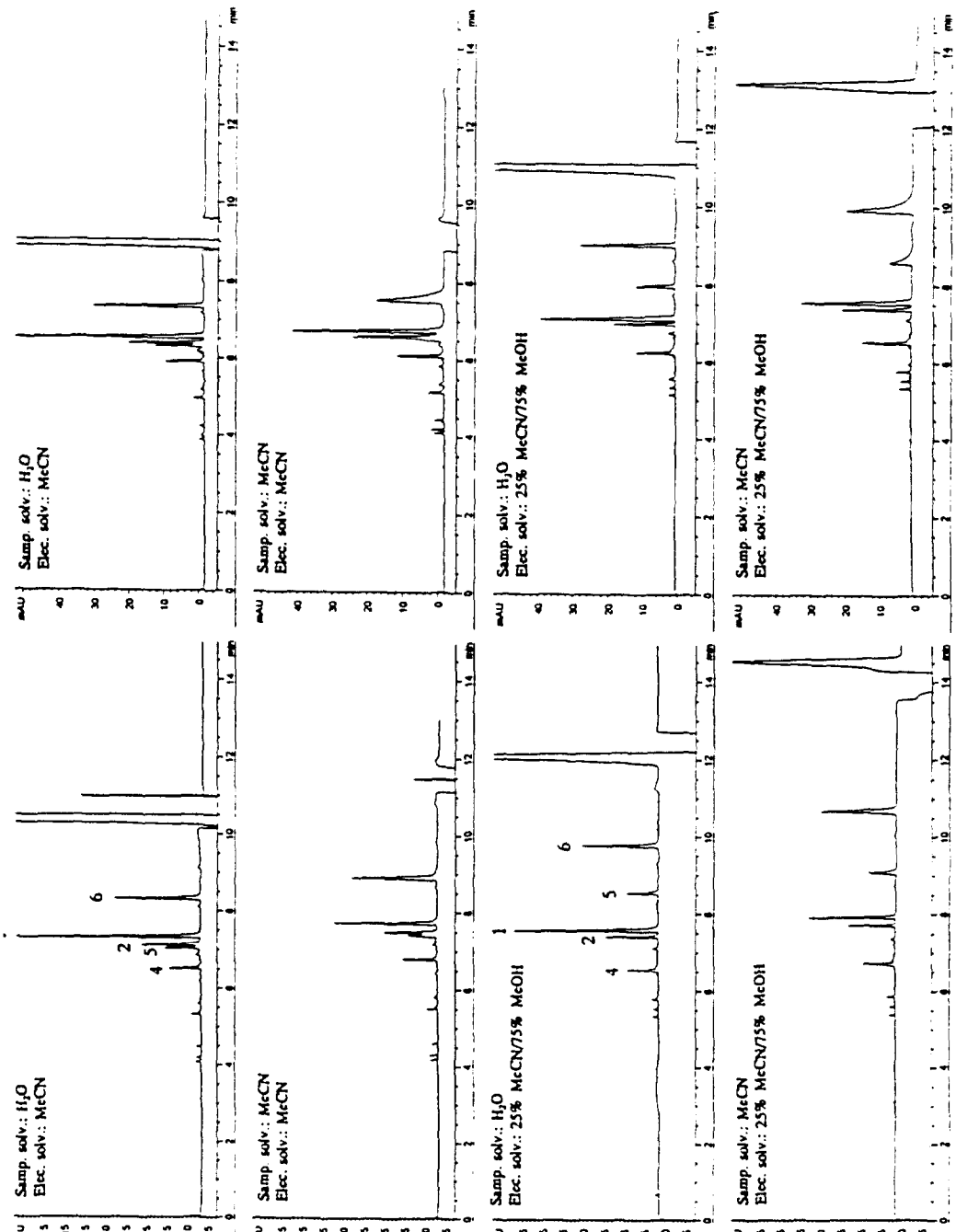

Fig. 6. Electropherograms of opium tincture diluted two-hundredfold in different solvents (indicated on the figure) obtained in two different electrophoresis systems. Left row: injection time, 3 s; right row: injection time, 10 s. Temperature, 20°C; electrolyte, ammonium acetate (25 mM)–acetic acid (1 M) in organic solvent (given on the figure as Elec. solv.). Other conditions and peak identification as in Fig. 2.

5. Conclusions

Non-aqueous capillary electrophoresis has in this and in previous papers [5-9] been shown to be a very powerful separation technique. Extended possibilities for the separation of very similar substances are provided due to solvent selectivities (controlled by dielectric constants and polarities) and extended pH ranges. The technique will definitely find widespread applications in the separation of low molecular weight substances (e.g. drug substances) and in bioanalysis.

Acknowledgements

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