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Short Communication

Separation of codeine and its by-products by capillary zone electrophoresis as a quality control tool in the pharmaceutical industry

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ABSTRACT

Capillary zone electrophoresis can be used for the detection of by-products in purified codeine. Separation of the by-products thebaine and 6-methylcodeine illustrates that small geometrical differences can be exploited to achieve effective separation. Examples of the capillary zone electrophoresis and micellar electrokinetic chromatography of codeine-containing drug formulations are presented.

INTRODUCTION

Codeine (Fig. 1), an antitussive and mild analgesic, can be obtained from Indian opium or poppy straw and is additionally produced by methylation of morphine from the same sources [1]. To obtain pure codeine, various impurities, ranging from naturally occurring alkaloids (such as thebaine) to reaction side-products such as 6-methylcodeine and 3-O-(1,2-dichlorovinylmorphine) [2] must be removed. The purity of the codeine must then be ascertained.

Basic drugs can be analysed by HPLC [3] or TLC [4,5], but interactions with the stationary phases [3] can lead to tailed peaks. In electro-osmotically driven chromatography, with the stationary phase still present, tailing still occurs, as can be inferred from data presented by Verheij *et al.* [6]. As was demonstrated with another class of compounds, the hop acids [7], only the complete elimination of the stationary phase solves this kind of adsorption problem.

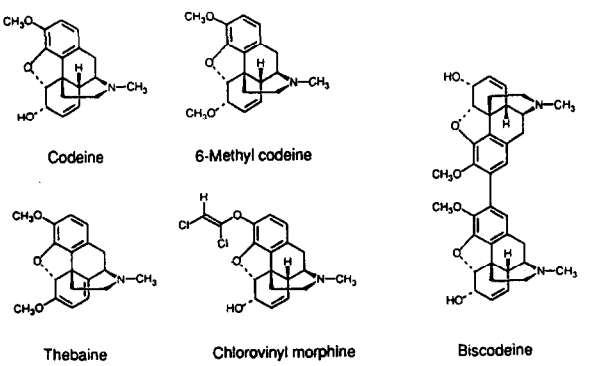

Fig. 1. Codeine and its by-products.

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Therefore, capillary zone electrophoresis (CZE) is a convenient alternative.

Several groups have reported the capillary electrophoretic separation of basic drugs [8–11] and alkaloids [12–14]. All of the work on codeine [8–10] has focused on its determination for forensic drug screening. This contribution deals with codeine in its pharmaceutical dosage form.

EXPERIMENTAL

Codeine was obtained from Sigma (St. Louis, MO, USA). The purified by-products of codeine and drug formulations were obtained from Slovakofarma (Hlohovec, Slovak Republic). The pure compounds were dissolved in separation buffer at the required concentration, except for the trace impurity analysis of codeine, for which 0.01 *M* hydrochloric acid was used as the sample solvent. The tablets (Kodynal, Spasmoveralgin, Alnagon) were crushed in a mortar and 65–150 mg of powder, equivalent to one-tenth of a tablet, were sonicated in 100 ml of separation buffer and filtrated through a 0.45-μm membrane filter (Schleicher & Schüll, Dassel, Germany). The Ipecarin drops were simply diluted 100 times with separation buffer. The actual concentrations of the substances are listed in the figure legends.

CZE was performed on a Quanta 4000 system (Millipore, Bedford, MA, USA). Fused-silica capillaries were 50 μm or 75 μm I.D., 375 μm O.D., 60 cm long, with the detection window at 53 cm.

Electropherograms were obtained with various buffers, all prepared with deionized water (Milli-Q, Millipore). Samples were introduced hydrodynamically with an elevation of 10 cm and an injection time of 5 or 10 s, analysed with an applied voltage of 17 or 20 kV and detected at 214 nm. After each run the column was rinsed with 0.05 *M* sodium hydroxide (1–3 min) and separation buffer for 3 min.

Molecular modelling calculations were performed on a MicroVAX II (Digital Equipment, Shrewsbury, MA, USA) with the MM2(85) Force Field program (MacroModel v3.0, W.C. Still, Columbia University, 1990).

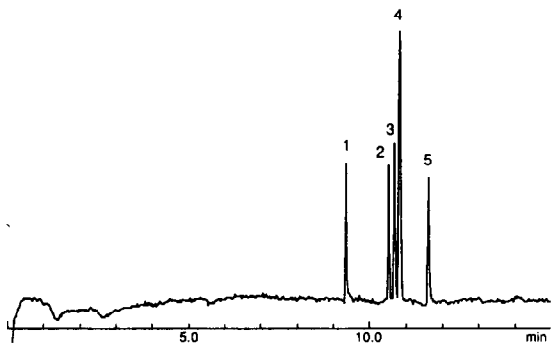

Fig. 2. CZE analysis of a synthetic mixture of codeine and its by-products. Buffer: 25 m*M* citrate, pH 4.2. Column: 60 cm (53 cm to detector) × 75 μm I.D. Applied voltage: 20 kV. Detection: 214 nm. Injection time: 5 s. 1 = Biscodeine; 2 = codeine; 3 = thebaine; 4 = 6-methylcodeine; 5 = 3-O-1,2-dichlorovinylmorphine.

RESULTS AND DISCUSSION

Fig. 2 shows an electropherogram of an artificial mixture of codeine and its by-products. The excellent separation of thebaine and 6-methylcodeine is remarkable as these compounds differ by only two hydrogen radicals. As the pK_a of thebaine is 6.05, charge effects are not expected to have a strong influence at pH 4.2. This is confirmed by examining the separation in the range pH 2.5–6.0 (Fig. 3). To avoid interferences from different ionic strengths in the pH scale, the buffers contained a large excess of sodium chloride. We did not attempt to measure electroosmotic flow at these low pH values, but by selecting a reference compound, in this case thebaine, and considering differences in observed mobility, the effect of electroosmosis is effectively neutralized (μ_{ep} = electrophoretic mobility, μ_{eo} = electroosmotic mobility)

$$\Delta\mu = (\mu_{ep,1} + \mu_{eo}) - (\mu_{ep,2} + \mu_{eo}) = \mu_{ep,1} - \mu_{ep,2} \quad (1)$$

No pH-related difference in electrophoretic mobility could be found between thebaine and 6-methylcodeine. The changes for bis-codeine are very likely related to the transition from the mono- to the diprotonated form. The small changes for codeine and 3-O-(1,2-dichlorovinylmorphine) are less well understood but

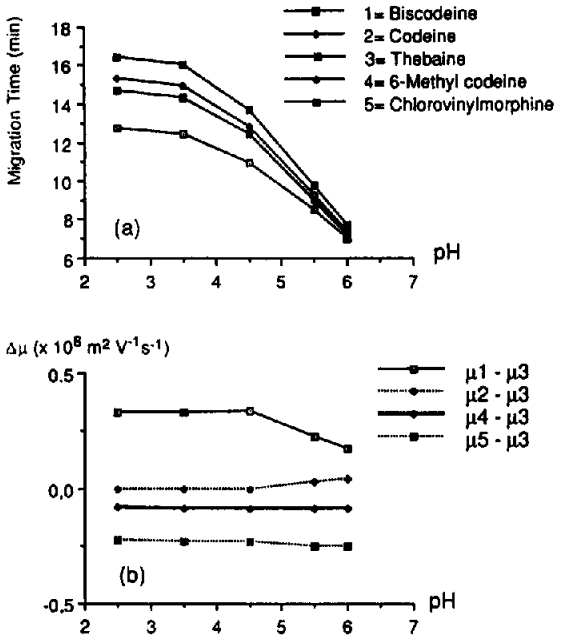

Fig. 3. Migration times (a) and mobility differences (b) in the pH range 2.5–6.0. Buffer: 100 m*M* NaCl with 10 m*M* phosphate (pH 2.5) or citrate (pH ≥ 3.5). Column: 60 cm (53 cm to detector) × 50 μm I.D. Applied voltage: 17 kV. (Codeine and thebaine are not well separated under these high-ionic-strength conditions.)

could be related to the presence of the allylic alcohol function, a common and distinctive feature of these structures.

From molecular modelling calculations, it follows that there is a geometric difference between thebaine and 6-methylcodeine (Fig. 4), related to the position of the double bonds. As the electrophoretic mobility is determined not only by the charge but also by friction in the aqueous medium, a property determined by the effective radius, geometric differences can lead to separation. Generally, such geometric differences are amplified through selective complexation with, for example, cyclodextrins [15], but this example shows that they can lead to separation by their own virtue. This type of geometry-controlled separations has also recently been reported for *cis–trans* isomers by Chadwick and Hsieh [16].

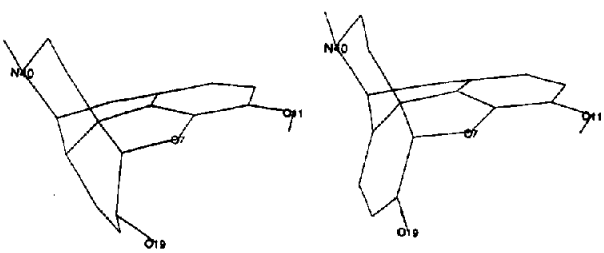

Fig. 4. Geometrical difference between 6-methylcodeine (left) and thebaine (right) according to MM2 calculations (for clarity, H atoms have been omitted).

Up to 1% impurities are allowed in codeine [5]. However, for the determination of codeine in pharmaceutical formulations [17], we require that the level of by-products in the standard be less than 0.05%. Fig. 5 illustrates that these levels can effectively be detected in a codeine sample. Although satisfactory for our purpose, this is very likely not the ultimate limit. Altria [18] demonstrated better signal-to-noise ratios at the 0.02% impurity level by using wide-bore (180 μm) capillaries, but at the expense of an increased analysis time. The recently introduced capillaries with bubble cells [19], having a threefold increase in capillary internal diameter at the detection location, could provide better sensitivity without the need to reduce the separation voltage.

This type of analysis, CZE in acid medium, can also be used for the determination of codeine in drug formulations (Fig. 6a and b), but, depending upon the nature of the accompanying drugs, other separation conditions may be necessary. The presence of neutral compounds necessitates the use of micellar electrokinetic chromatography (Fig. 6b and c).

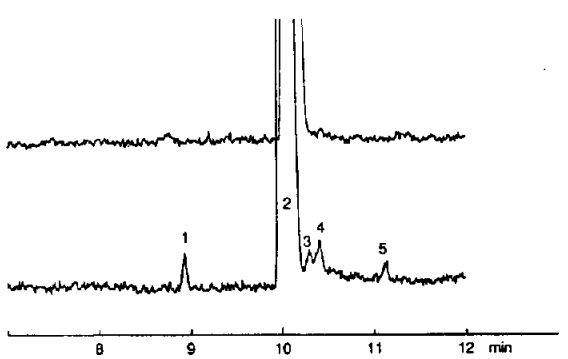

Fig. 5. CZE analysis of codeine (a) and codeine spiked with 0.05% by-products (b). Conditions and compounds as in Fig. 2, except for the injection time, which is 10 s. The codeine concentration is 10 000 ppm.

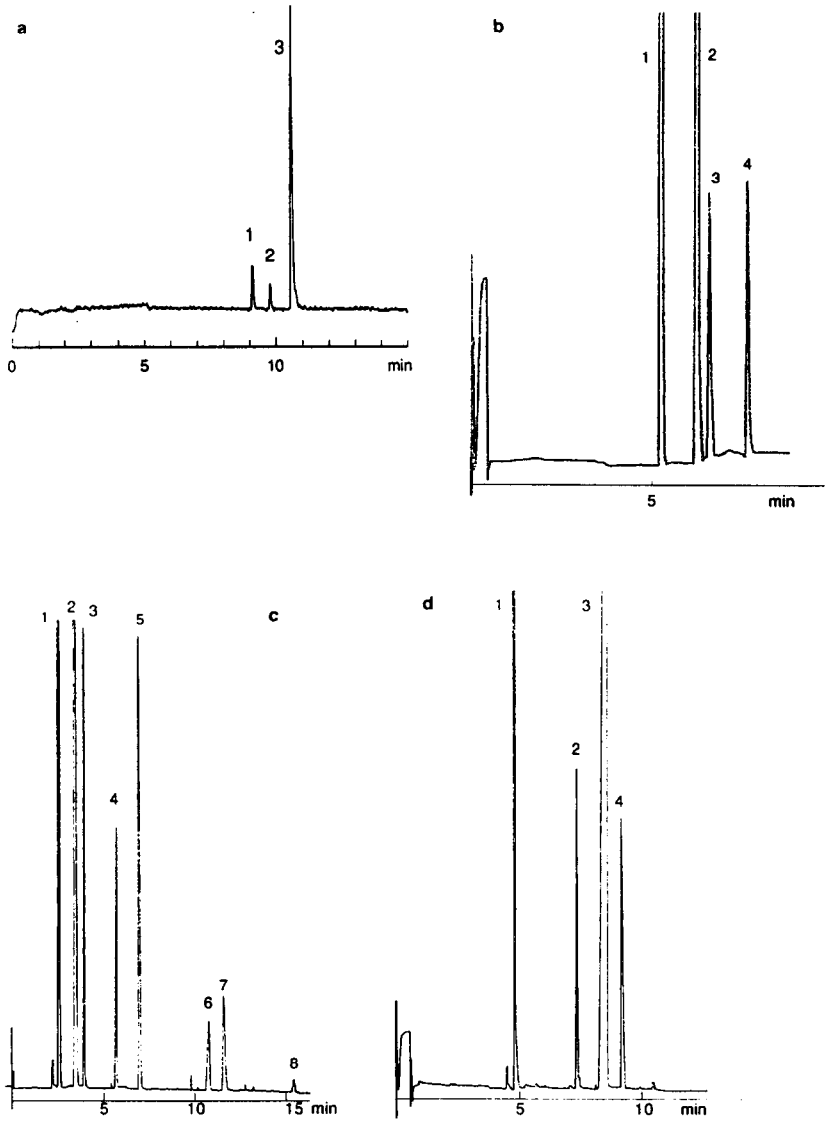

Fig. 6. Examples of the analysis of codeine-containing drug formulations. The concentrations reflect the relative amounts in the formulation. (a) Kodynal. Same conditions as in Fig. 2. 1 = Emetine (1 ppm); 2 = ephedrine (20 ppm); 3 = codeine (40 ppm). (b) Ipecarin. Buffer: 12.5 m*M* citrate, pH 6.5. Other conditions as in Fig. 2. 1 = Ephedrine (130 ppm); 2 = codeine (93 ppm); 3 = emetine (1 ppm); 4 = pilocarpine (15 ppm). (c) Spasmoveralgin. Buffer: 40 m*M* SDS in 10 m*M* phosphate, pH 8.5. Other conditions as in Fig. 2. 1 = Caffeine (50 ppm); 2 = aminophenazone (150 ppm); 3 = bromoisovalum (250 ppm); 4 = phenobarbital (20 ppm); 5 = codeine (15 ppm); 6 = ephedrine (5 ppm); 7 = papaverine (30 ppm); 8 = antropine methylbromide (0.5 ppm). (d) Alnagon. Same conditions as in (c), except for the addition of 15% acetonitrile to the buffer. 1 = Caffeine (80 ppm); 2 = phenobarbital (20 ppm); 3 = aspirin (380 ppm); 4 = codeine (20 ppm).

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