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Short communication

Capillary electrochromatography of basic compounds using octadecyl-silica stationary phases with an amine-containing mobile phase

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Abstract

The capillary electrochromatographic (CEC) analysis of basic compounds on octadecyl-silica stationary phases (Hypersil ODS and Spherisorb ODS I) was studied. A basic drug (fluvoxamine) and one of its possible impurities were used as test compounds. With an eluent of acetonitrile–phosphate buffer (pH 7.0), the compounds could be baseline-separated; however, broad and tailing peaks were obtained. To minimise detrimental interactions with residual silanol groups, the pH of the mobile phase was lowered to 2.5, but the plate numbers were still quite low ($<2.6\times10^4$ plates/m). Addition of a masking agent (hexylamine or triethylamine) to the mobile phase resulted in much better peak efficiencies (ca. 1×10^5 plates/m). Therefore, the influence of the amine concentration and pH of the mobile phase on the CEC performance (peak width, peak tailing, electroosmotic flow, selectivity) was investigated in detail. Highest efficiencies (2.8×10^5 plates/m) could be obtained with the Spherisorb column, while the Hypersil column offered a better selectivity. Furthermore, the results show that the residual silanol groups are (at least partly) responsible for the separation of the basic compounds and that the amount of injected sample has an unusually large effect on the peak efficiency. The usefulness of the system for impurity profiling was demonstrated with a mixture containing fluvoxamine and its stereoisomer (a possible impurity) at the 0.1% level. The general effectiveness of amine additives in CEC was illustrated by the separation of a mixture of five structurally different basic drugs yielding plate numbers in the 1×10^5–3×10^5 plates/m range. Comparison with capillary electrophoretic analysis revealed a unique selectivity of the CEC system which is based on both electrophoretic mobility and chromatographic partitioning.

Keywords: Electrochromatography; Stationary phases, CEC; Mobile phase composition; Amines; Basic compounds; Fluvoxamine

1. Introduction

Capillary electrochromatography (CEC) is a relatively new technique that has attracted much attention during the last years. In CEC the mobile phase is driven by an electric field through a capillary packed with a liquid chromatographic (LC) stationary phase. Solutes are separated according to their partitioning between both phases and, when charged, their electrophoretic mobility. In order to create a

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sufficient electroosmotic flow (EOF), classical reversed-phase stationary phases, such as Spherisorb ODS I and Hypersil ODS, are generally used [1,2]. These phases are not end-capped and, therefore, contain a relatively high amount of acidic silanol groups. As in CE, the EOF has a nearly flat profile resulting in reduced eddy diffusion leading to a high efficiency [3]. Moreover, there is no pressure drop over the packed bed allowing smaller particles to be used compared to LC, which enhances the efficiency even further.

CEC has been successfully used for the analysis of neutral drugs [4–8]. However, analysis of basic compounds often results in broad peaks and serious peak tailing. This tailing is caused by the same residual silanol groups that are essential for creating the EOF. Pharmaceuticals (and their impurities) often are basic, and, therefore, to extend the usefulness of CEC in pharmaceutical analysis it should be made applicable to a wide range of basic compounds. To overcome the problem of peak tailing, Smith and Evans [9] used cationic-exchange materials as stationary phases. Although high efficiencies were obtained, peak splitting occurred and the results were neither fully explained nor reproducible [1,10]. Zhang and El Rassi [11] used an in-house prepared octadecyl-sulfonated silica phase ('mixed-mode') to separate some nucleosides and bases. Recently, the groups of Gillott et al. [1] and Lurie et al. [12] showed that the CEC performance of basic compounds on conventional reversed-phase materials might be improved by adding a masking or competing amine to the mobile phase. Use of such amines is common practice in LC when non-end-capped octadecyl-silica (ODS) stationary phases are applied [13–16]. However, in CEC the use of masking amines is not self-evident since these might seriously slow down (or even reverse) the EOF and/or affect the ionic strength. Euerby and Lurie indicated the potential of amine additives in CEC, but their work was mainly focused on the simultaneous separation of basic, acidic and neutral compounds by CEC. Moreover, the influence of column type, column load, eluent pH and concentration of masking amine on the peak efficiency and selectivity was not or only briefly discussed.

In the present paper, the usefulness of amine-containing mobile phases in CEC on ODS columns is examined by the analysis of fluvoxamine (a primary amine) and its stereoisomer (a potential impurity). The basic drugs have been analysed at neutral and acidic pH, and the results obtained on two different ODS stationary phases are compared. The effect of the additives, hexylamine and triethylamine, on peak width and peak resolution is described and the allowable analyte load is studied. In addition, the applicability of the CEC system for impurity profiling and for the analysis of various basic drugs is investigated.

2. Experimental

2.1. CE system

CEC and capillary electrophoretic (CE) experiments were performed on a Hewlett-Packard HP^{3D} CE system (Waldbronn, Germany) equipped with a diode array detector. CEC columns (33.0 cm×100 μm) had a packed-bed of 25 cm and were from Hewlett-Packard. Packing materials were either 3 μm Hypersil ODS or 3 μm Spherisorb ODS I. Prior to use, the columns were flushed with mobile phase for 30 min at 30 kV. During all analyses, the inlet and outlet vials were pressurised at 5 bar and a temperature of 20°C was maintained. Samples were injected by applying 5 kV for 1–10 s and analysed at 30 kV. For CE, a fused-silica capillary (54.2 cm×50 μm) was used, which, prior to use, was rinsed with 1 *M* NaOH (15 min), de-ionised water (15 min) and separation buffer (30 min). Samples were injected by applying 5 mbar for 2 s and analysed at 30 kV. Data were collected and interpreted at 200 nm using HP Chemstation software version 5.02. Plate number calculations were based on peak widths at half maximum. Asymmetry factors were calculated using peak widths at 10% of the peak height [17].

2.2. Chemicals

Fluvoxamine maleate (*E*-isomer), its *Z*-isomer were donated by Solvay Pharmaceuticals (Weesp, The Netherlands). A test mixture was prepared containing *E*-isomer (1.3×10^{-4} *M*), *Z*-isomer (0.72×10^{-4} *M*) and thiourea (1.0×10^{-3} *M*) as EOF

marker. Acetonitrile was purchased from Labscan (Dublin, Ireland), thiourea, prometazine hydrochloride, metamphetamine hydrochloride, propranolol hydrochloride, hexylamine and triethylamine from Sigma (St Louis, MO, USA). Sodium dihydrogenphosphate and phosphoric acid were from Merck (Darmstadt, Germany), lidocaine from Holland Pharmaceuticals Supply (Alphen a/d Rijn, The Netherlands) and oxyphenonium bromide from Ciba–Geigy (Basel, Switserland). All chemicals were of analytical grade. Deionized water was obtained from an Elga pure water system of Salm en Kipp (Breukelen, The Netherlands). A mixture of basic drugs was made dissolving metamphetamine (1.1×10^{-3} *M*), propranolol (6.6×10^{-4} *M*), lidocaine (6.9×10^{-4} *M*), oxyphenonium (4.7×10^{-4} *M*), prometazine (6.3×10^{-4} *M*) and thiourea (1.8×10^{-3} *M*) in water. All buffers were adjusted to the proper pH by addition of 1.0 *M* sodium hydroxide. The mobile phase for CEC was acetonitrile–25 m*M* phosphate buffer (pH 7.0 or 2.5) (60:40, v/v). Hexylamine or triethylamine was added to the buffers (before pH adjustment) in concentrations ranging from 0 to 15 m*M* leading to concentrations in the mobile phase of 0–6 m*M*. For CE the running buffer was 25 m*M* phosphate (pH 7.0 or 2.5) or the same as the CEC mobile phase.

3. Results and discussion

3.1. pH of the mobile phase

In order to evaluate the CEC system for basic compounds, a test mixture was made consisting of thiourea (EOF marker) and the anti-depressant fluvoxamine (*E*-isomer), its *Z*-isomer (a possible impurity). Using a mobile phase of pH 7.0, the test mixture was analysed on Spherisorb ODS I and Hypersil ODS. On both columns the isomers were baseline separated, but the peaks were unacceptably broad and strongly tailing. For example, for fluvoxamine on the Spherisorb column a plate number of 7.2×10^{3} plates/m and an asymmetry factor of 26 were found. At pH 7, the *Z*- and *E*-isomers (pK_a 9) are positively charged, but as was reported previously [18], *Z* and *E* cannot be separated by CE at pH 2.5–9.0. This demonstrates that the observed CEC separation is based on a chromatographic partition rather than electrophoretic principle. Moreover, the positively charged isomers migrated slower than thiourea, which indicates a relatively strong interaction of these basic compounds with the stationary phase.

The poor peak shapes of the isomers at pH 7.0 are most likely due to the binding of these basic pharmaceuticals to the acidic silanol groups of the stationary phases. To reduce this detrimental interaction, the pH of the mobile phase was lowered to 2.5 and the isomer mixture was analysed again on the Spherisorb column (Fig. 1). With respect to pH 7, the EOF was reduced as indicated by the increased elution time of thiourea (from 3.0 to 7.2 min). At pH 2.5, both isomers migrated before the thiourea peak and a plate number of 2.6×10^{4} plates/m was calculated for fluvoxamine with an asymmetry factor of 5.8. These observations indicate that the interaction with the packing material is indeed decreased.

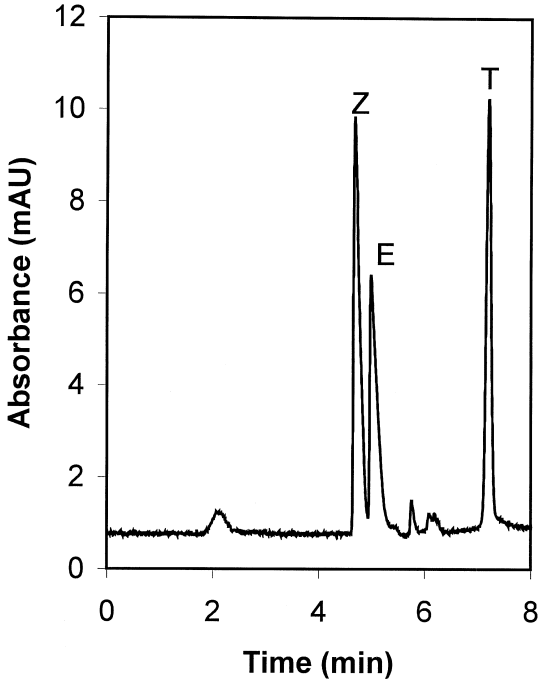

Fig. 1. CEC of test mixture. Mobile phase, acetonitrile–25 m*M* phosphate buffer (pH 2.5) (60:40, v/v); column, Spherisorb ODS I; injection time, 3 s. Peak denotation: E, fluvoxamine; Z, *Z*-isomer of fluvoxamine; T, thiourea.

However, the resolution of the *Z*- and *E*-isomers decreased from 2.1 to 1.3, suggesting that the silanol groups also play an important role in the separation of the basic compounds.

3.2. Type and concentration of amine additive

To further improve the peak shape of the basic compounds, the use of amine additives was investigated. Fig. 2 shows the separation of the *Z*- and *E*-isomers using the Hypersil and Spherisorb column with a mobile phase of acetonitrile–25 m*M* phosphate buffer (pH 2.5) (60:40, v/v) containing 6 m*M* hexylamine. Despite the low pH and the presence of the masking amine, there still was an appreciable EOF. In fact, the addition of the amine to the mobile phase had little effect on the EOF. Peak efficiencies of the basic isomers, however, considerably improved. For instance, on Spherisorb ODS I the plate number of the fluvoxamine peak increased from 2.6×10^4 plates/m (without hexylamine) to 1.0×10^5 plates/m (with hexylamine), while the asymmetry factor decreased from 5.8 to 2.9. From Fig. 2 it is clear that the peak efficiencies and symmetries are more favourable for the Spherisorb column, but the best resolution is obtained with the Hypersil column. This is probably related to the larger surface density of silanol groups on the Hypersil ODS phase which, as indicated by the thiourea peak, also generates a higher EOF.

Hexylamine (6 m*M*) was also tested with a neutral mobile phase. Similar to the CEC system at pH 7.0 without amine additive, the *Z*- and *E*-isomer now migrated after thiourea; however, the retention times of the basic compounds were decreased. Again, the hexylamine affected the EOF only slightly. More important, also at pH 7.0, the amine additive caused a considerable sharpening of the isomer peaks. For both columns reasonable plate numbers (typically $2–6\times10^4$ plates/m) were obtained. Still, at pH 2.5 the overall peak efficiencies were better and the analysis time was more favourable. Moreover, at low pH most acidic analytes will be protonated which enables their simultaneous analysis with neutral and

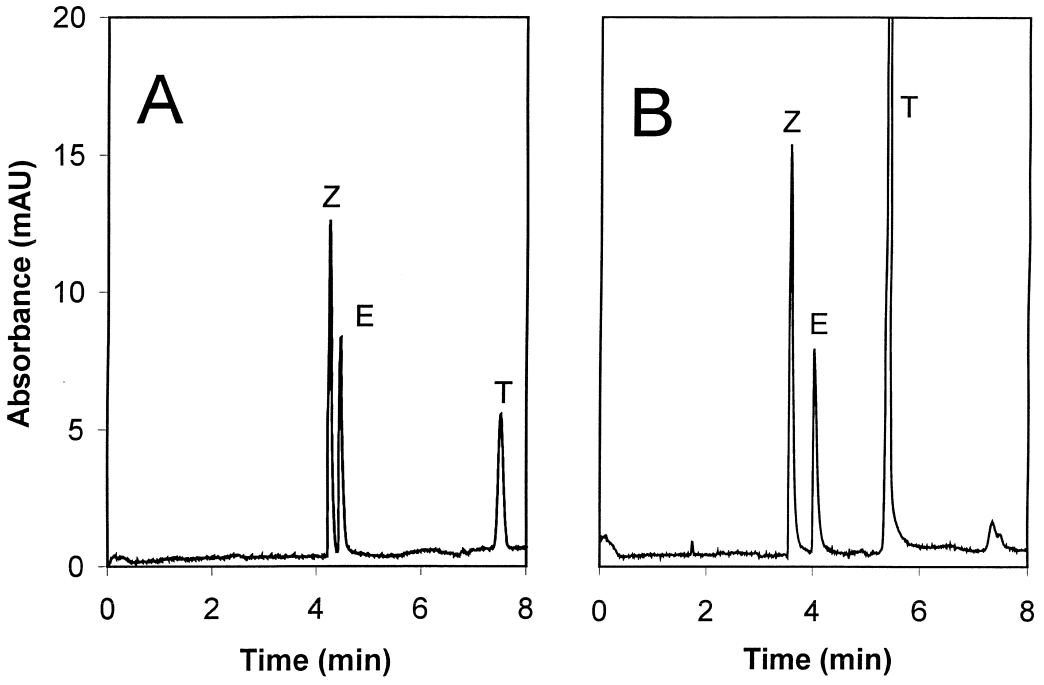

Fig. 2. CEC of test mixture. Mobile phase, acetonitrile–25 m*M* phosphate buffer (pH 2.5) (60:40, v/v) containing 6 m*M* hexylamine; column, Spherisorb ODS I (A) or Hypersil ODS (B); injection time, 3 s. For peak denotation, see Fig. 1.

basic components [12]. The Spherisorb column in combination with an acidic mobile phase was used in the experiments described below.

To study the effect of an amine additive in more detail, the concentration of hexylamine in the mobile phase was varied between 0 and 6 m*M*. Increase of the concentration resulted in a drastic improvement of the plate numbers of the basic test compounds (Fig. 3). However, at the same time the retention time difference between the isomers was reduced and, consequently, the gain in resolution was marginal. Nevertheless, sharper peaks lead to a larger peak capacity, which is important when unknown components are analysed. The hexylamine concentration was limited to 6 m*M* to prevent excessive Joule heating induced by the high buffer concentration that would be needed to maintain the amine-containing mobile phase at a low pH. As an alternative an amine additive, triethylamine, was also tested in concentrations ranging from 0 to 6 m*M*. Again, the plate numbers of the *Z*- and *E*-isomers increased with increasing amine concentration, but the gain was less than for hexylamine. Furthermore, a concentration of 6 m*M* triethylamine caused extreme baseline noise and, therefore, was not useful. In further experiments, a mobile phase containing 6 m*M* hexylamine was used.

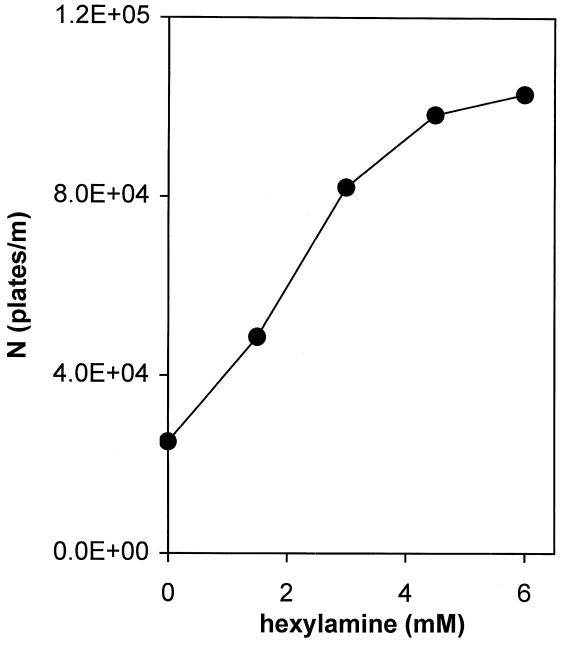

Fig. 3. Plate number of fluvoxamine as a function of the concentration hexylamine in the mobile phase. Mobile phase, acetonitrile–25 m*M* phosphate buffer (pH 2.5) (60:40, v/v); column, Spherisorb ODS I; injection time, 3 s.

3.3. Injection solvent and volume

In the above experiments it was observed that the plate numbers of the test compounds were higher when water was used as injection solvent instead of mobile phase. This phenomenon is probably caused by stacking, which occurs for ionic species when the conductivity of the sample plug is relatively low. The basic drugs are charged in solution and, therefore, it is favourable to use an injection solvent with a low ionic strength. The injection time appeared to have a considerable effect on the peak efficiency. When the injection time was varied between 1 and 6 s (at a constant voltage of 5 kV) the plate numbers of the basic test compounds gradually decreased from 2.8×10^5 to 3.4×10^4 plates/m. Such a substantial decrease was not observed for neutral compounds. By varying the injection time and sample concentration systematically it was revealed that the band broadening was related to the amount of base injected (mass overloading). The effect is most likely due to the limited number of available silanol groups, which in essence are responsible for the interaction of the basic compounds with the stationary phase. Such mass overloading of basic compounds has also been observed in LC [19]. Fig. 4 illustrates the excellent results that can be obtained with small sample injections (1 s at 5 kV; 0.27 pmol of drug injected), yielding a nice base-line separation and very sharp peaks for both isomers. Obviously, a decrease of the injection volume also leads to a lower detectability. Therefore, when in CEC ODS phases are used for basic compounds, the injection conditions often will be a compromise between efficiency and detection limit.

3.4. Applicability

The potential of the CEC system (Spherisorb ODS I with a hexylamine-containing eluent) for impurity profiling was explored by analysing an aqueous solution of fluvoxamine (5 mg/ml) with its *Z*-isomer

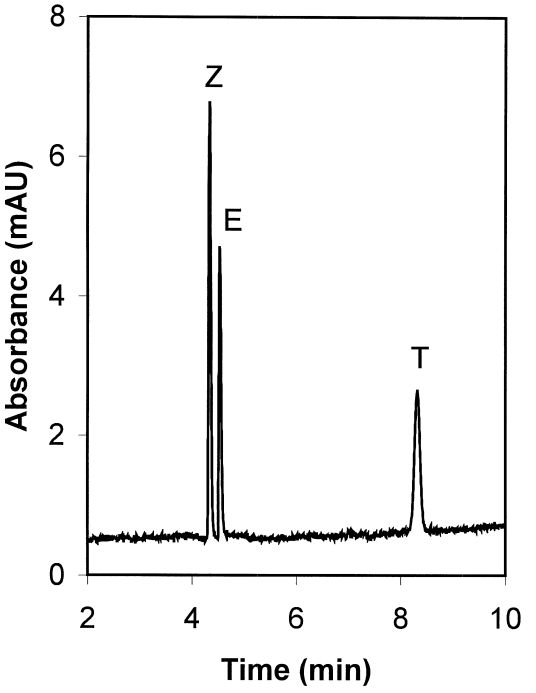

Fig. 4. CEC of test mixture. Mobile phase, acetonitrile–25 m*M* phosphate buffer (pH 2.5) (60:40, v/v) containing 6 m*M* hexylamine; column, Spherisorb ODS I; injection time, 1 s. For peak denotation, see Fig. 1.

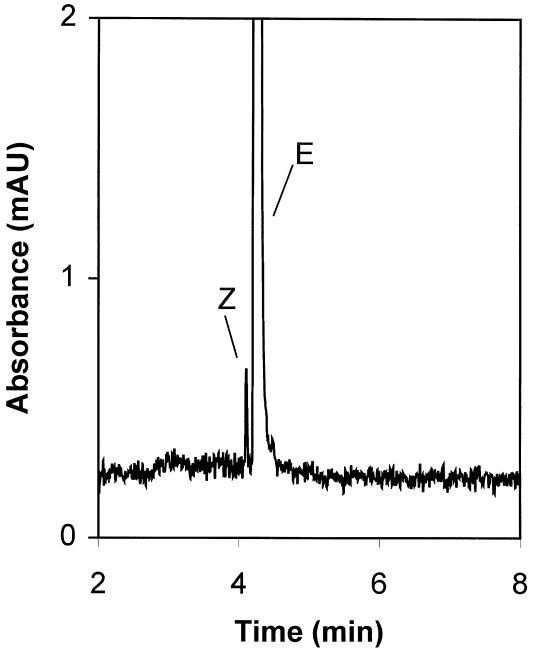

Fig. 5. CEC of a mixture containing 5 mg/ml fluvoxamine and 0.005 mg/ml *Z*-isomer. Mobile phase and column as in Fig. 4; injection time, 2 s. For peak denotation, see Fig. 1.

(a possible impurity) at the 0.1% level (Fig. 5). The fluvoxamine is overloaded and elutes as a rather broad peak. Nevertheless, the *Z*-isomer is still baseline separated from the *E*-isomer and can easily be detected, illustrating the good resolution that can be obtained with the used CEC system. It appeared also possible to simultaneously separate another possible impurity of fluvoxamine (a neutral ketone) which, as can be expected, eluted after the EOF marker (result not shown).

In order to evaluate the general usefulness of the CEC system for basic compounds, a second test mixture, comprised of several relevant pharmaceuticals (secondary, tertiary and quartenary amines) was analysed (Fig. 6). All basic components elute before thiourea and are nicely separated with plate numbers ranging from 1.2×10^5 (oxyphenonium) up to 3.0×10^5 plates/m (metamphetamine). These results show that the peak-sharpening effect of the amine additive is quite general and that the CEC system can provide a satisfactory selectivity, peak capacity and efficiency for basic drugs. When analysed with CE using the amine-containing mobile phase as background electrolyte, all basic drugs eluted in a time interval of 1 min and could not be fully separated. This observation indicates that in the present CEC system, chromatographic partition contributes considerably to the separation of the positively charged drugs. The mixture of basic drugs was also analysed with CE under conventional conditions using a phosphate buffer (pH 2.5 and 7.0), but again the drugs could not be completely separated. More interestingly, the peak order observed in CE differed from the order obtained with CEC using an amine-containing eluent. In other words, for basic pharmaceuticals the CEC system offers a different selectivity than CE.

4. Conclusions

It is demonstrated that CEC with ODS stationary phases can be used for the efficient separation of basic pharmaceuticals. Prerequisite for good peak

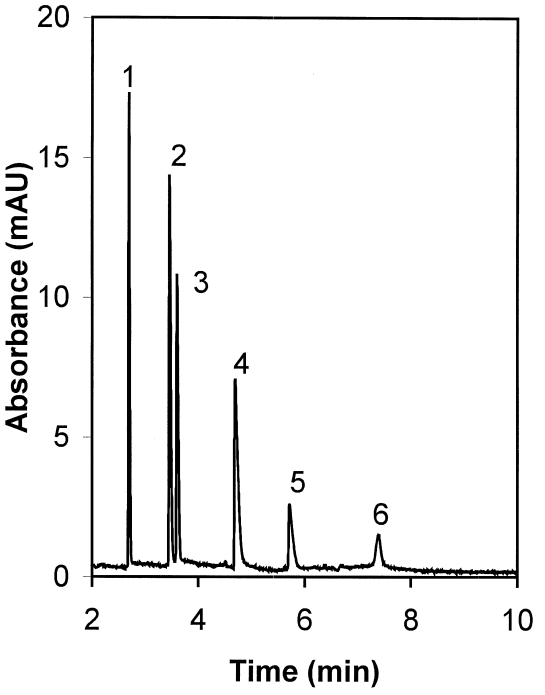

Fig. 6. CEC of a mixture of five basic drugs. Mobile phase and column as in Fig. 4; injection time, 2 s. Peak denotation: (1) methylamphetamine; (2) lidocaine; (3) propranolol; (4) prometazine; (5) oxyphenonium; (6) thiourea.

shapes of the basic compounds is the addition of a masking amine to the mobile phase and the injection of relatively small amounts of sample. With an acidic mobile phase containing 6 m*M* hexylamine, there still is an appreciable EOF and plate numbers of up to 3.0×10^5 plates/m can be obtained. The selectivity of the CEC system is good allowing the separation of fluvoxamine from its Z-isomer, but also allowing the separation of a wide range of basic pharmaceuticals. Although for a good CEC performance of basic compounds, masking of the silanol groups is essential, their presence also seems to be vital for the separation of the bases. In conclusion, the main merits of CEC in the analysis of basic drugs are its high efficiency when compared with LC, and its unique selectivity with respect to both LC and CE. In the near future, real-life applications have to further prove the value of CEC in pharmaceutical analysis next to other separation techniques.

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