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Journal of Chromatography B, 752 (2001) 47–53

JOURNAL OF
CHROMATOGRAPHY B

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# Separation of olanzapine, carbamazepine and their main metabolites by capillary electrophoresis with pseudo-stationary phases

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Received 15 June 2000; received in revised form 6 September 2000; accepted 14 September 2000

**Abstract**

Conditions were worked out for the separation of carbamazepine, olanzapine, and their main metabolites carbamazepine 10,11-epoxide, 10-hydroxycarbamazepine, and desmethylolanzapine. The separation was based on electrokinetically driven methods in the capillary format. The main difficulty in separating these compounds is related to their different chemical classes. Whereas the carbamazepine members are amides, and are electrically neutral, the olanzapine members have aliphatic amino groups and are thus cationic under most experimental conditions. Different additives were applied as pseudo-stationary phases to implement selectivity. Poly(diallyldimethylammonium), PDADMA, is a polycationic replaceable and soluble polymer, that interacts mainly according to the polarisability of the analyte molecules. The MEKC principle was applied with the common SDS as micelle former. In both systems, only partial resolution of the analytes was obtained. The most favorable system consisted of a charged, oligomeric additive: full separation of all analytes within 4 min was achieved with heptakis-6-sulfato-$\beta$-cyclodextrin (7 m$M$) in 30 m$M$ borate buffer, pH 8.5. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Olanzapine; Carbamazepine

## 1. Introduction

Several decades after the introduction of the first neuroleptic, an improvement in the therapy for schizophrenia is still desirable. Classical neuroleptics were efficacious in some respects, but they showed total inefficacy against negative symptoms of schizophrenia and adverse extrapyramidal effects. In the last years new drugs, such as risperidone, clozapine and olanzapine (named ‘‘atypical antipsychotics’’) have been introduced for the treatment of psychotic disorders. They seem to be more effective since they can suppress positive and negative symptoms of schizophrenia and show less extrapyramidal effects [1,2].

In same cases even these new drugs do not completely fulfil the demands on the treatment of psychotic disorders. For that reason many new atypical antipsychotics were investigated in concomitance with the use of anticonvulsant, anxiolytic, serotonergic antidepressant and other agents to manage agitation.

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PII: S0378-4347(00)00514-4

Among these drugs, carbamazepine (5-carbamoyl-5H-dibenz[*b*,*f*]azepine), CBZ, which is typically used against some kinds of epilepsy and trigeminal neuralgia, is now widely administered for the treatment of the behavioural disorders [3,4], especially if associated with antipsychotic drugs like olanzapine. Olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3*b*][1,5]benzodiazepine), OLA, the most recent atypical antipsychotic drug to become commercially available, seems to be as effective as clozapine, but has not yet caused agranulocytosis [5] nor the severe constipation [6] sometimes associated with clozapine treatment.

The combination of carbamazepine and olanzapine produced good therapeutic results, but showed some unexpected pharmacokinetic interactions. In fact carbamazepine induces several P450 cytochromes including CYP3A4 and CYP1A2 that are involved in the olanzapine metabolism; in particular it decreases the half-life elimination and increases clearance and volume of distribution of olanzapine. This interaction seems to give no clinical effects since olanzapine has a wide therapeutic index and the changes in plasma concentration are within the 4-fold variation that occurs without concern for safety in a patient population [7,8].

Several techniques have been reported for the determination of olanzapine in biological fluids. They include gas chromatography [9,10] and HPLC with electrochemical [11–14] or UV detection [15]. For the determination of carbamazepine and its metabolites, HPLC [16,17] and MEKC [18] were described. However, to our best knowledge no methods have been described so far in the literature for the simultaneous determination of both drugs and their main metabolites.

It is the aim of this paper to develop an appropriate analytical method for the separation of olanzapine, carbamazepine and their main metabolites. Among several metabolites of carbamazepine we have chosen the 10,11-epoxide (CBZ-E) which is the main active one, and 10-OH-carbamazepine (CBZ-OH) [19]. With respect to OLA we selected desmethyl-olanzapine (D-OLA) as metabolite.

The analytical method is based on electrophoresis in the capillary format. However, problems might arise due to the different chemical nature of the two classes of compounds present (Fig. 1). Carbamazepine and its metabolites are amides, and are thus very weak bases, with p$K_a$ values typically smaller than 0.5. It is obvious that these compounds cannot be protonated under conditions suitable for CE. Olanzapine and its metabolites, on the other hand, possess strongly basic aliphatic amino groups. It can be assumed that the p$K_a$ values of the aliphatic amines of the latter class of analytes are in the range around 9 or 10, thus leading to protonated ammonium ions at this pH of the BGE and below.

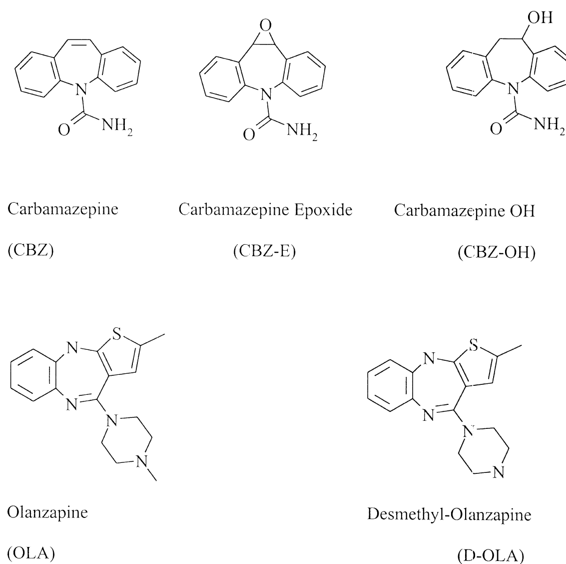


Fig. 1. Structural formulae of carbamazepine, olanzapine and the main metabolites.

We are confronted, thus, with the problem of the simultaneous separation of neutral and cationic analytes. Therefore different separation mechanism must be applied. Whereas normal CZE at low or moderate pH might be suitable for the olanzapine class, no possibility to resolve the neutral compounds exists under these conditions. Thus a system has to be found that, on the one hand, transports both, the neutral and the ionic sample compounds through the separation capillary and, on the other hand, enables association of the neutrals with charged additives for their separation. In the present case the transport medium is the EOF; note that the charged additive is normally, but not necessarily counter-migrating to the EOF.

We have investigated the separability of three

electrolyte systems consisting of different additives. The first, poly(diallyldimethylammonium), PDADMA, was found suitable for the separation of neutral compounds in a number of previous papers [20–22]. The second was SDS, applied above its CMC, the most common micelle-forming pseudo-stationary phase for MEKC. The third mechanism is based on a charged oligomer, heptakis-6-sulfato-$\beta$-cyclodextrin, applied mostly for the separation of chiral components (cf. e.g., Refs. [23,24]), but with separation ability for compounds similar to the present ones as well [25].

It should be pointed out that it was the primary goal of the present work to investigate conditions for the separation of the analytes, as a first, necessary step for their determination in real samples. An assay for the quantitation of the compounds in samples like biological fluids should concentrate on the appropriate sample pretreatment and concentration techniques, in order to avoid interference of matrix components with the analytes, on the one hand, and to reach those analyte concentrations which are needed for their appropriate detection. This latter topic is, however, not the goal of the present paper.

## 2. Experimental

### 2.1. Chemicals

The following pharmaceuticals were used: olanzapine and *N*-desmethyl olanzapine were kindly donated from Eli Lilly (Indianapolis, IN, USA). Carbamazepine, carbamazepine epoxide, and 10-OH-carbamazepine were from Novartis (Basel, Switzerland).

Sodium hydroxide, acetonitrile and methanol (all analytical grade), ortho-phosphoric acid 85% (w/w) and sodium dodecylsulfate were from Merck (Darmstadt, Germany), 35% (w/w) hydrochloric acid was from Loba Feinchemie (Fischamend, Austria). Poly-(diallyldimethyl) ammonium chloride of average molecular mass of 200–350 kDa (20%, w/w, solution in water), was obtained from Aldrich (Vienna, Austria). Heptakis-6-sulfato-$\beta$-cyclodextrin was from Regis Technologies (Morton Grove, IL, USA).

Water was doubly distilled from a quartz apparatus. All solutions were filtered (0.45 μm, Minisart RC25, Sartorius, Göttingen, Germany) prior to use.

Stock solutions of olanzapine reference standard and *N*-desmethyl olanzapine were prepared by dissolving suitable amounts of powdered sample in slightly acidic solution (pH~2, hydrochloric acid) and treated in an ultrasonic bath for 15 min. Stock solutions of carbamazepine, carbamazepine epoxide and 10-OH-carbamazepine were prepared by dissolving suitable amount of powdered sample in water or pure acetonitrile.

All stock solutions had a concentration of 1 mg/ml and were stored at −18°C for 1 month at most and the standard solutions were prepared by dilution with double-distilled water and stored at 4°C at most for a week.

The following background electrolytes were used: (i) formic acid–sodium formate, total concentration 20 m*M*, pH 4; PDADMA (1%, w/w) as pseudo-stationary polymeric phase; (ii) boric acid–sodium borate, total concentration 30 m*M*, pH 8.5; 25 m*M* SDS; (iii) boric acid–sodium borate, total concentration 30 m*M*, pH 8.5; varying concentrations of heptakis-6-sulfato-$\beta$-cyclodextrin.

### 2.2. Equipment

#### 2.2.1. PDADMA system

CE measurements were carried out with an instrument (270A-HT, Applied Biosystem, Perkin-Elmer, Foster City, CA, USA) using an uncoated fused-silica capillary (Supelco, PA, USA) with a total length of 42.0 cm and effective length of 22.0 cm (I.D. 50 μm). The sample was injected into the capillary by pressure (165 mbar) for 1 s. The instrument was operated between −5 and −25 kV with currents typically less than 45 μA. Detection was at 190 nm (detector placed at the anode side of the capillary). The data were recorded and processed with a dual-channel interface (970 A, PE Nelson, Cupertino, CA, USA).

#### 2.2.2. MEKC and cyclodextrin systems

Measurements were carried out with an instrument (P/ACE System 2100, Beckman Instruments, San Ramon, CA, USA) using an uncoated fused-silica capillary (Supelco) with a total length of 26.8 cm and effective length of 20.1 cm (I.D. 50 μm). The

sample was injected into the capillary by pressure (35 mbar) for 1 s. The instrument was operated between +10 and +20 kV with currents typically less than 35 μA. Detection was at 200 nm (detector placed at cathode side of capillary).

### *2.3. Procedure for capillary preparation and handling*

#### *2.3.1. PDADMA system*

Before use, the capillary was rinsed for 10 min with deionised water and filled with 4% (w/w) solution of PDAMDA with a syringe to establish a coating of the polymer at the wall (note that the concentration of PDADMA in the BGE for separation was only 1%). The capillary was stored overnight to enable a better coating. After each run the capillary was rinsed with water (2 min) and with background electrolyte (2 min).

#### *2.3.2. MEKC and cyclodextrin systems*

Before use and every 30 runs, the capillary was rinsed for 10 min with deionized water, 10 min with 0.1 mol/l sodium hydroxide, 5 min with 1 mol/l sodium hydroxide, 10 min with 0.1 mol/l sodium hydroxide and 30 min with water. After each run the capillary was rinsed with water (2 min) and with background electrolyte (2 min).

## 3. Results and discussion

### *3.1. PDADMA as pseudo-stationary phase*

It is known that PDADMA, like other cationic polyelectrolytes, modifies the surface of the capillary due to adsorption of a positively charged layer that reverses the zeta potential and results in a stable, reproducible EOF directed towards the anode [26]. When added to the BGE, it may interact as pseudo-stationary phase with neutral compounds, retaining their migration and introducing separation selectivity thus. This is based on the formation of positively charged associates, which move against the EOF (towards the anode), and leads to mobility, $\mu_i$, of neutral analytes, $i$, expressed by

$$\mu_i = \mu_{\mathrm{eof}} \frac{1}{1+k_i'} + \mu_{\mathrm{add}} \frac{k_i'}{1+k_i'} \quad (1)$$

where subscripts eof and add indicate the mobility of the EOF and the additive, respectively, and $k_i'$ is the capacity coefficient, or retention factor, of the analyte, $i$. The capacity coefficient is, as in chromatography, the mass distribution coefficient, describing the partition of the analyte between the polymeric pseudo-stationary phase and the "free" solution. Note that for small analytes and large polymers we can assume that the mobilities of the polymer and that of the analyte–polymer associate are equal.

Association of the analytes with the polymer is caused mainly by interactions based on the polarisation of the analyte, as demonstrated by linear free energy relationship [21]. Carbamazepine with the π electrons conjugated over three rings in the molecule might be well suited for such interaction, probably better than the metabolites, where the double bond in the central, seven-ring has reacted with oxygen forming the epoxide or the OH group, respectively. This degree of interaction is reflected in Fig. 2, showing the result of the application of 1% (w/w) PDADMA as pseudo-stationary phase. The metabolites are indeed less retarded than CBZ, leading to an at least partial separation of CBZ from its two metabolites. However, resolution of the two metabolites is not obtained.

The cationic analytes OLA and D-OLA should not interact with the polyelectrolyte due to electrostatic repulsion. Therefore it can be expected that these two compounds migrate with mobility that is the sum of their own electrophoretic, effective mobility, and the mobility of the EOF. Their effective mobilities, measured at the pH of the BGE without additive are +23 and +22 mobility units [$10^{-9}$ $m^2$ $V^{-1}$ $s^{-1}$]. They should exhibit therefore migration times of 4.5 and 4.7 min in the BGE with 1% PDADMA, and should be well separable. Indeed they are separated, as can be seen from Fig. 2. Surprisingly, their migration times are much larger than expected, namely about 14 and 17 min, which means that their mobilities (that of the EOF subtracted) are as small as −7.3 and −6.0 units, respectively. Taking the mobility of the EOF as −41 units, effective mobilities of +34 and +35 units for OLA and D-OLA, respectively, are calculated from the electropherog-

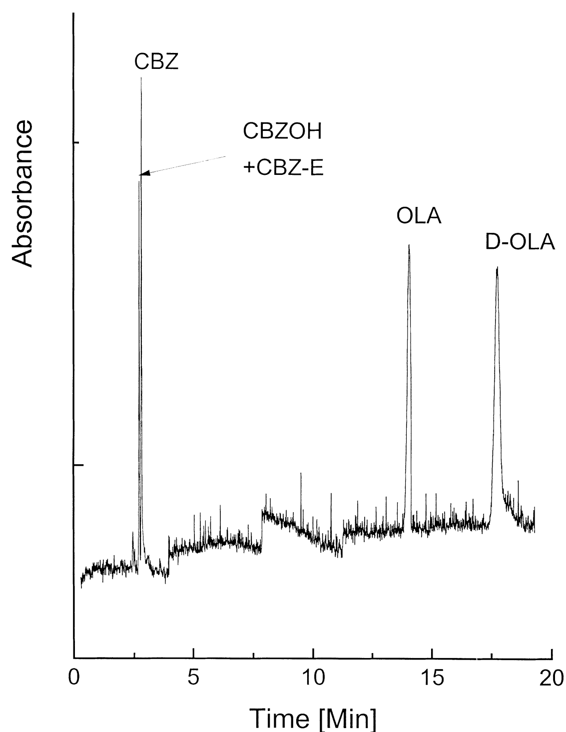


Fig. 2. Electropherogram of the separands with PDADMA (1%, w/w, added to the BGE) as pseudo-stationary polymeric phase. BGE: 20 m*M* formate, pH 4. Electrophoretic conditions: capillary, total length 42 cm, effective length 22 cm, I.D. 50 μm; voltage, −15.0 kV; current, 45 μA; detection, 190 nm.

ram shown in Fig. 2. It follows that not only the sequence of migration reverses upon addition of 1% PDADMA, but the mobilities are also much larger than those observed in the system at the same pH, but without polymeric additive. The reason for this effect is somewhat unclear. This effect cannot stem from the difference on the ionic strength between the additive-free and the additive-containing systems, because the smaller ionic strength in the BGE without additive would lead to an even higher mobility.

In contrast to these two analytes, CBZ and its metabolites are not separated, seemingly due to the too low capacity factors obtained for these analytes under the given conditions. A possibility to increase the retention of the latter analytes lays in an increase of the concentration of PDADMA, as mentioned above, thus increasing the phase ratio. This approach, however, would result in an increase of the migration time of OLA and D-OLA into a range that is not favourable for analysis. Therefore it follows that this system is not well suitable for determination of the analytes.

### 3.2. MEKC with SDS

More pronounced interaction with the lipophilic carbamazepine and its metabolites can be expected with the lipophilic core of the SDS micelles, leading to stronger retention and better resolution, as compared with the weak interaction found in case of PDADMA. This is indeed observed, as can be seen from Fig. 3. The three compounds (CBZ, CBZ-OH and CBZ-E) are well separated, and elute far after the EOF (indicated by a negative peak at 2 min). OLA and D-OLA, which have positive charge even at the high pH of the experiment (pH is 8.5) show even stronger interaction, on the one hand due to

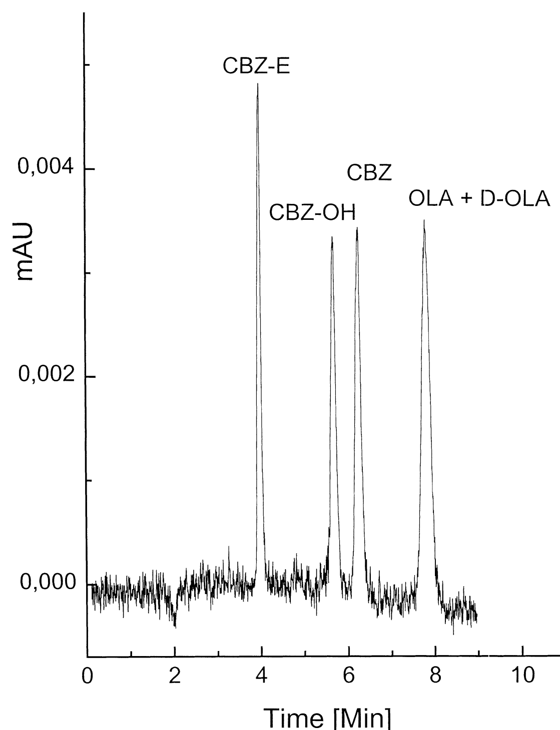


Fig. 3. MEKC of the separands in a system containing 25 m*M* SDS in BGE (30 m*M* borate buffer, pH 8.5). Electrophoretic conditions: capillary, total length 26.8 cm, effective length 20.1 cm, I.D. 50 μm; voltage, +10.0 kV; current, 12 μA; detection, 200 nm.

their lipophilic character, on the other hand due to electrostatic attraction by the charged micelle. However, selectivity is seemingly lost for these two compounds, and the same extent of partitioning is observed: they exhibit too similar capacity factors as to be resolved. This is a behaviour not unexpected for cationic analytes in such SDS system [27]. For this reason another additive was investigated, which combines more possibilities for selectivity than those described so far.

### *3.3. Separation of complexes with heptakis-6-sulfato-β-cyclodextrin*

As a result of the application of PDADMA and SDS as additives it was seen that interactions based on electrostatic attraction and on polarisation or lipophilicity do not suffice for separation of the analytes under the given conditions. Therefore an additional variable for selectivity manipulation is introduced, namely the differences of the analytes in matching sterically into the cavity of a cyclodextrin derivative. The additive must be charged in order to introduce a migration vector different from the EOF since three of the five analytes are neutral. Heptakis-6-sulfato-β-cyclodextrin was found in previous work to be a favourable additive for such purpose [25]. The effect of this additive on retention cannot easily be predicted for the analytes under consideration due to the mixed mechanism involved. However, increasing the concentration of heptakis-6-sulfato-β-cyclodextrin increases retention (Fig. 4). The most retained compound is CBZ at all concentrations, which is somewhat surprising as this result indicates that the electrostatic interaction between the positively charged analytes OLA and D-OLA are not dominating the retention mechanism. The charge numbers of these two analytes, on the other hand, might not be high enough at the pH of the BGE to balance the more favourable steric conditions for CBZ. From Fig. 4 it can also be deduced that at heptakis-6-sulfato-β-cyclodextrin concentrations higher than 3 m*M*, separation of all compounds should be obtained. This is seen from Fig. 5, where clear resolution of all analytes is established within less than 4 min. This system is thus found to be the most favourable for separation of the analytes. The reproducibility of the migration times is best in this system, with a (relative) span less than 1% for all

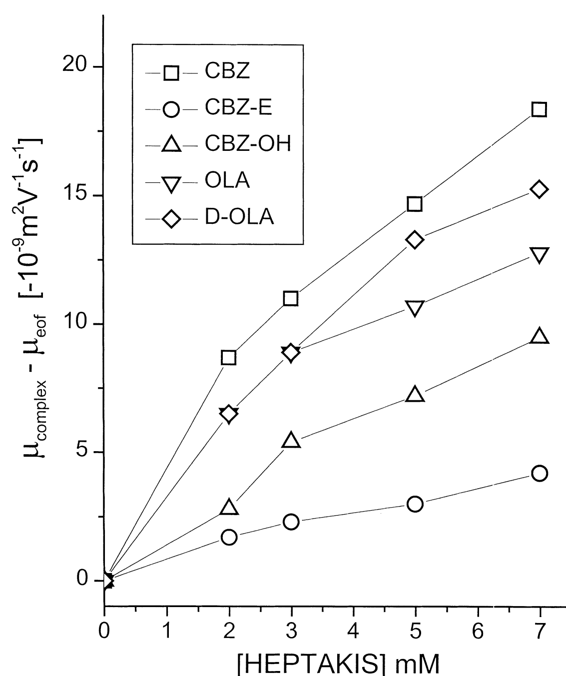


Fig. 4. Complex mobility as a function of heptakis-6-sulfato-β-cyclodextrin (heptakis) concentration from 2 to 7 m*M*. BGE, 30 m*M* borate buffer, pH 8.5.

analytes. This is in marked contrast to the SDS system with a typical span of 5%, and that in the PDADMA system with nearly 10% span between the smallest and the largest migration time of a particular analyte (measured for three to five repeat runs).

## 4. Conclusion

In the present paper the aspect of selectivity was investigated for analytes of different chemical nature: charged and electrically neutral compounds. Separation of the analytes can be carried out within less than 4 min with heptakis-6-sulfato-β-cyclodextrin used as a charged additive. However, these favourable conditions were obtained with pure reference compounds as analytes. For real samples, e.g., stemming from biological fluids (explicitly not treated in this paper) other problems might occur, which might be overcome by special pre-treatment procedures. Critically, we expect that the separation method based on electrokinetic transport might be sensitive to a number of sample-dependent variables

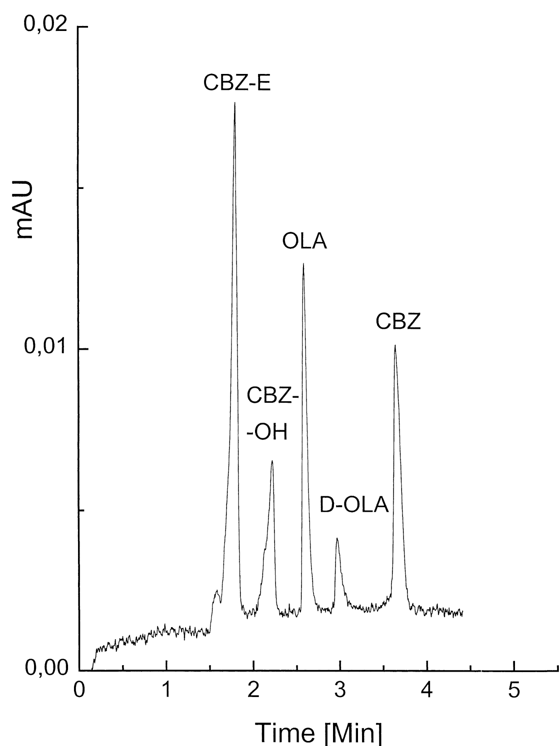


Fig. 5. Separation of the analytes with 7 m*M* heptakis-6-sulfato-$\beta$-cyclodextrin as complexation agent. BGE, 30 m*M* borate buffer, pH 8.5. Electrophoretic conditions: capillary, total length 26.8 cm, effective length 20.1 cm, I.D. 50 µm; voltage, +18.0 kV; current, 39 µA; detection, 200 nm.

for biological fluid analysis. One is connected to the contamination of the silica wall by adsorbed matrix components, thus modifying the electroosmotic flow. Another depends on the composition of the solvent. It is well known that organic solvents like methanol or acetonitrile affect complex formation with cyclodextrins when these solvents are constituents of the background electrolyte. However, interferences can be observed even for the case that they are present in the sample solvent only (data not shown). Although we have demonstrated that the analytes of interest can be resolved successfully in a single run, we are aware that the experimental conditions necessary for real samples might be more crucial.

## Acknowledgements

The authors acknowledge financial support from an ERASMUS project (European Union) for G.I.

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