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Strategy for setting up single-capillary isotachophoresis–zone electrophoresis

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ABSTRACT

A strategy is described for the optimization of capillary zone electrophoresis (CZE) with isotachophoretic (ITP) preconcentration in a single-capillary set-up. CZE with indirect UV absorbance detection is used as a tool in setting up ITP–CZE. The electropherograms obtained with indirect detection give an immediate insight into the migration order of possible leading and terminating ions and of the analyte ions. Furthermore, information is obtained on the ionic composition of the sample matrix. The separation window in the ITP step can be chosen in such a way that a sample clean-up is achieved before zone electrophoresis is started. The described approach is applicable for setting up an ITP–CZE analysis in both the cationic and anionic modes. The usefulness of the strategy is illustrated by setting up the ITP–CZE of three cationic antimuscarinic drugs. Applying the developed strategy, combined with liquid–liquid extraction, concentrations of homatropine and scopolamine down to 100 ng/ml could easily be determined.

INTRODUCTION

One of the challenges in the application of capillary zone electrophoretic (CZE) separations in the analysis of biological samples is to achieve relevant determination limits. For a number of drugs this means that concentrations in the nanomolar range or lower have to be measured. To take full advantage of the separation power of CZE for trace analysis of biological samples, two approaches are considered to improve the determination limits. One is to increase the detection sensitivity. Several highly sensitive detection systems such as laser-induced fluorescence detection and electrochemical detection have been described [1–3]. However, these detection systems are only applicable to those analytes which show appropriate detection characteristics or can only be applied after a derivatization procedure. Especially for analyte concentrations at or below the nanomolar level, derivatization procedures are liable to involve artefact formation [4].

Another approach for improving the determination limits in CZE is to increase the sample loadability of the system. For reasons of separation efficiency, injection volumes in CZE are usually not higher than 1% of the total volume of the separation system. This means that the injection volumes are in the range 0.1–70 nl. Although absolute detection limits of femtomoles seem very impressive, the corresponding sample concentration detection limits are in the micromolar range, which is at least a factor 1000 away from trace analysis. One way to increase the loadability of the CZE system is to apply off-line concentrating sample pretreatment methods such as the use of extraction procedures in combination with an evaporation step. In these

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procedures millilitres of sample can be pretreated, concentrated and analysed.

Electrophoretic analyte focusing techniques provide an elegant way to increase the loadability in CZE. These focusing techniques, such as the coupling of isotachophoresis (ITP) with CZE and field amplified injection procedures, are based on the application of local differences in electrical field strength during the injection or the focusing step to permit stacking of the analyte ions [5–14]. The coupling of ITP with CZE has been described by several workers [5–10]. Improvements in determination limits by up to a factor of 1000 have been reported. In a recent paper we described the possibility of automated ITP–CZE for anionic separations in a single capillary using backpressure programming [15]. Sample volumes up to 55% of the total capillary volume were focused isotachophoretically and analysed in the CZE step. A hydrodynamic pressure was used for removal of the terminator buffer before the CZE was started. The ITP conditions prevented excessive zone broadening. The procedure was reproducible, quantitative and automated.

In this paper, a strategy is described for anionic and cationic separations using ITP–CZE in a single capillary. Four focusing procedures are described, two for each ionic mode. In both ionic modes the CZE run can take place in either the leading or the terminating buffer, depending on the configuration used. The choice of the leading and terminating buffer ions, which defines the actual separation window, is made after analyte and matrix analysis by CZE using indirect detection. Three antimuscarinic (atropinic) drugs, neostigmine, homatropine and scopolamine [16], were used as test compounds.

EXPERIMENTAL

Apparatus

An untreated fused silica capillary (100 μm I.D.) (SGE, Ringwood, Victoria, Australia) was used. A programmable injection system for capillary electrophoresis (PRINCE, Lauerlabs, Emmen, Netherlands) equipped with a reversible polarity power supply and possibility for pressurized and electrokinetic injection was used for the analyte focusing process.

On-capillary UV absorbance detection took place using a Spectra 100 UV–Vis detector (Spectra-Physics, Mountain View, CA, USA). The signal was registered on a BD 40 chart recorder (Kipp & Zn, Delft, Netherlands).

Chemicals

Scopolamine hydrobromide, homatropine hydrobromide and neostigmine bromide were obtained from Sigma (St. Louis, MO, USA), crystal violet, triethylamine (TEA) (99%), diethanolamine (DEtOHA) (99%), triethanolamine (TEtOHA) (97%), 2,4,6-collidine (2,4,6-trimethylpyridine) (99%) and pyridine (99%) from Janssen Chimica (Beerse, Belgium), phosphoric acid, tris(hydroxymethyl)aminomethane (Tris), lithium hydroxide, sodium hydroxide, potassium hydroxide and ammonium acetate from Merck (Darmstadt, Germany) and dichloromethane (99%) from J.T. Baker (Deventer, Netherlands). In all experiments deionized water obtained with a Milli-Q system (Millipore, Bedford, MA, USA) was used.

Extraction of spiked plasma and urine samples

The extraction procedure used is similar to that described for atropine [17]. Stock solutions of 1 mg/ml scopolamine hydrobromide, homatropine hydrobromide and neostigmine bromide were prepared in water and kept frozen (253 K) until used. Dilutions of the drugs were added to 225 μl of untreated blank plasma and urine from healthy volunteers until a final volume of 250 μl. The pH was increased by adding 50 μl of 1 mol/l lithium hydroxide solution. The samples were extracted by adding 600 μl of dichloromethane followed by vortex mixing for 1 min. The samples were centrifuged for 5 min at 1000 *g*. The upper layer was discarded and 500 μl of the lower layer were transferred into another vial and evaporated to dryness under reduced pressure using a Speedvac SVC 100H evaporation centrifuge (Savant Instruments, Farmingdale, NY, USA). After evaporation to dryness, the residues were dissolved in 500 μl of terminating buffer for ITP–CZE or CZE buffer for CZE analysis.

Analyte focusing

The analyte focusing procedure for the three antimuscarinic drugs consisted of five steps (Fig. 1A).

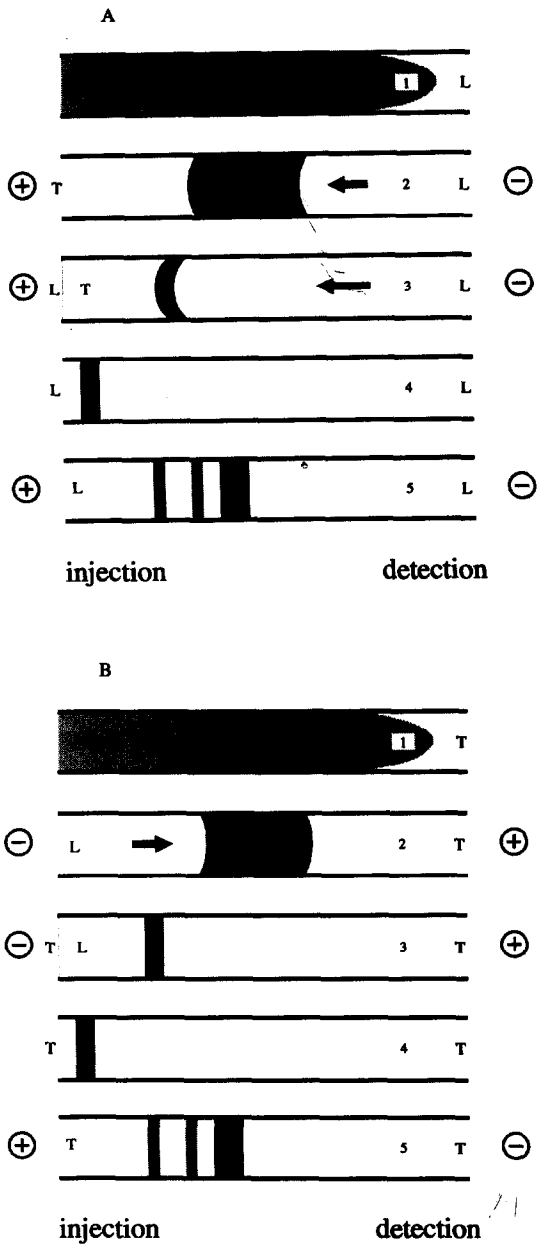

Fig. 1. Schematic representation of the ITP–CZE procedure for cations, (A) using the leading buffer (L) as CZE background electrolyte in combination with a negative backpressure (arrows) and (B) using the terminating buffer (T) as CZE background electrolyte and a positive backpressure. A reversal of the voltage is necessary in procedure B.

Step 1. After filling the capillary and the ITP cathode vial with leading buffer, the capillary is loaded with sample.

Step 2. The sample vial is replaced by the anode vial containing terminating buffer. A hydrodynamic backpressure is applied to counter balance the migration velocity of the analyte ions.

Step 3. When the focusing process is completed, the voltage is switched off and the vial containing terminating buffer is replaced with a vial containing CZE buffer which is the same as the leading buffer. The voltage is turned on with a similar hydrodynamic backpressure but with a lower voltage than used in step 2. As a result, the highly concentrated plug of analyte cations moves into the direction of the capillary inlet.

Step 4. When the concentrated analyte ions approach the capillary inlet the hydrodynamic backpressure is switched off and the CZE (step 5) is started.

RESULTS AND DISCUSSION

In each ionic mode of ITP–CZE the CZE step can take place in either the leading or the terminating buffer, resulting in four analyte focusing procedures (Figs. 1 and 2). The ITP–CZE procedure which will be applied for the cationic test compounds as given in Fig. 1A will now be discussed. The differences from the other three procedures will be indicated rather than discussing all procedures in detail. The procedure for ITP–CZE of anions as given in Fig. 2B has been described in detail elsewhere [15].

Analyte focusing

When during the focusing step (step 2) a positive voltage is applied at the capillary inlet, the analyte cations will migrate in the direction of the cathode because of the electrophoretic and electroosmotic mobility. A hydrodynamic pressure is applied to counterbalance the migration velocity of the analyte ions. In contrast to conventional ITP, the analyte ions do not move into the capillary during the focusing step but are concentrated and fixed at a position in the capillary by the hydrodynamic backpressure. The terms positive and negative backpressure are

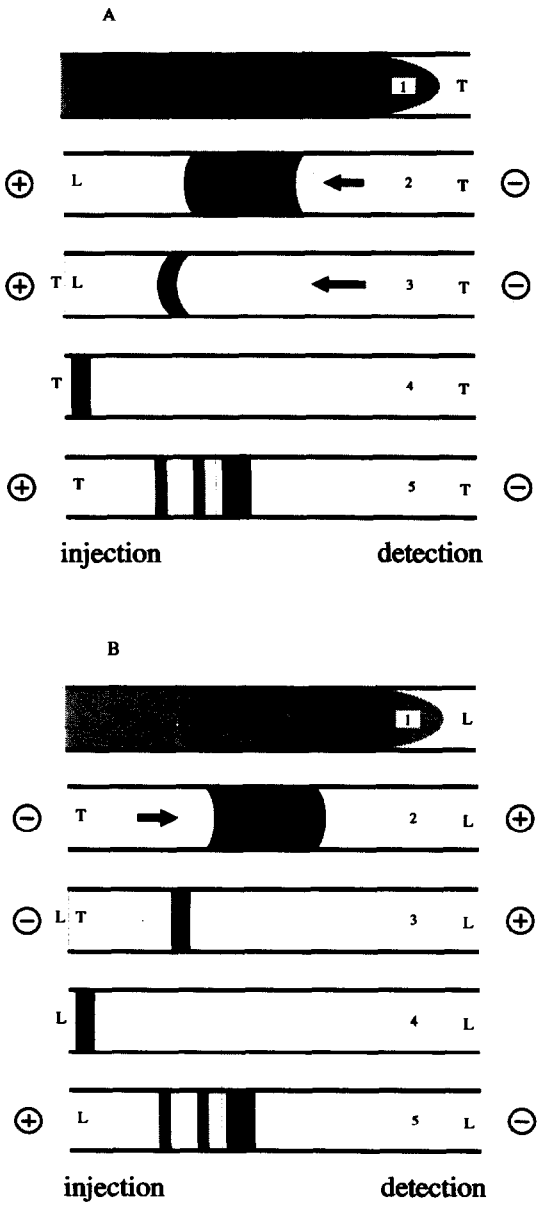

Fig. 2. Schematic representation of the ITP–CZE procedure for anions (A) using the terminating buffer (T) as CZE background electrolyte in combination with a negative backpressure (arrows), and (B) using the leading buffer (L) as CZE background electrolyte and a positive backpressure. A reversal of the voltage is necessary in procedure B.

used to describe the direction in which the hydrodynamic backpressure is applied: a positive backpressure induces a flow in the direction of the capillary outlet and a negative backpressure results in a flow in the opposite direction.

In step 3, the highly concentrated plug of analyte cations is moving hydrodynamically in the direction of the capillary inlet. Zone broadening is counteracted by the isotachophoretic conditions that still exist. The plug of terminating buffer that is still in the capillary is removed at the capillary inlet.

The most critical point in the procedure is the timing of the moment to switch off the hydrodynamic backpressure (step 4) and to start the CZE (step 5). This is done at the moment the analytes are about to leave the capillary. When the CZE step is started too early, the remaining plug of terminating ions causes an inhomogeneous electric field and influences the efficiency and migration times in the CZE step. When the CZE step is started too late the analyte ions have left the capillary. In the first few runs a visible dye is used for precise timing of the moment that the sample ions are near the capillary inlet. When reproducible migration times in the focusing step are obtained, the procedure can be automated and the dye is no longer needed. Timing is also possible by monitoring the increase in current during step 3. The plug of terminating buffer raises the total resistance of the capillary, which means that at constant voltage the current increases until the terminating buffer has left the capillary.

The procedures for ITP–CZE using negative backpressure (Figs. 1A and 2A) differ from those using positive backpressure (Figs. 1B and 2B) in that in step 3 the backpressure is increased to mobilize the focused sample zone in the direction of the capillary inlet. In step 4 the backpressure is reduced to zero and the CZE run is started without reversal of the voltage. This means that the migration order of ions is the same in the ITP and CZE steps. In the procedures with reversal of the voltage, a reversal of the migration order of the analyte ions also takes place when switching from ITP to CZE. This phenomenon did not affect the efficiency or resolution in ITP–CZE with respect to CZE separations of relatively clean samples [15].

ITP as sample clean-up for CZE

The choice of the buffer system determines the extent to which the ITP focusing step can be used in sample clean-up for the CZE analysis. In

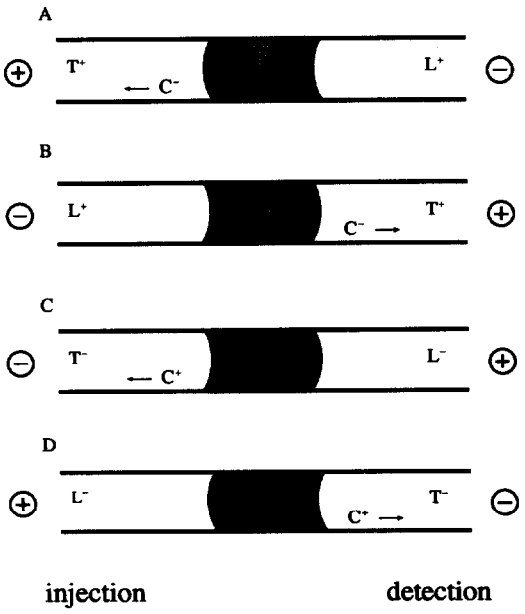

Fig. 3. The migration direction of the neutral species and counter ions (C^+, C^-) in the focusing step (Figs. 1 and 2, step 2) of the ITP–CZE procedure. Situations A and C are favourable with respect to sample clean-up.

Fig. 3 a summary is given of the different focusing steps in ITP–CZE. The migration direction of the ions with a lower mobility than the terminating ions is either in the direction of the capillary inlet or in the direction of the detector. Obviously, with respect to sample clean-up, the cationic mode with negative backpressure (Figs. 1A and 3A) and the anionic mode with positive backpressure (Figs. 2B and 3C) are favourable. In both instances the ions with a lower mobility than the terminating ions, including the neutral species and the counter ions, are removed and diluted in the inlet vial during the focusing step.

The focusing step is completed when all ions move isotachophoretically under steady-state conditions [18]. The more complex a sample is with respect to ion composition and ionic strength, the more time it takes to reach a true isotachophoretic state. Therefore, a sample pretreatment before the ITP–CZE step that results in a decrease in ionic strength of the sample matrix will reduce the focusing time in ITP–CZE. In the case of large variations in the composition of the sample matrix (*e.g.*, urine), a pretreatment step is likely to improve both the reproducibility and the efficiency in ITP–CZE. These aspects will be demonstrated below, where a strategy is given and discussed for setting up an ITP–CZE system for the three cationic test compounds.

Choice of CZE buffer

The first step in the optimization procedure for ITP–CZE will always be the optimization of the CZE step. Several papers are available describing CZE separations and methods for optimization [1–3]. However, in ITP–CZE the CZE buffer will also be a leading (or terminating) buffer. This will influence the choice of the background electrolyte for CZE.

With respect to sample clean-up it is favourable to use the leading buffer as the background electrolyte in CZE. Another aspect in favour of using the leading buffer concerns the band broadening caused by conductivity differences between the sample zones and the background electrolyte [19]. When a highly concentrated sample zone after the focusing step is switched to zone electrophoresis, band broadening takes place because of differences in conductivity of the sample zone and the background electrolyte [15]. The sample zone itself disturbs the homogeneity of the electric field in the electrophoresis tube. Zone broadening because of differences in conductivity is described by the equation

$$dz = \frac{L}{\kappa} \cdot d\kappa \qquad (1)$$

where $d\kappa$ is the conductivity difference between the sample zone and the background electrolyte, κ is the conductivity of the background electrolyte, L is the electrophoretic migration distance and dz is the zone broadening due to the conductivity difference [19]. Because the conductivity of the leading buffer is always higher than that of the analyte ions, the band broadening will be less when the leading buffer is used as the background electrolyte than when the terminating buffer is used as the background electrolyte in the CZE step, for similar values of $d\kappa$.

Finally, using a leading buffer as background electrolyte for CZE is convenient with respect to current monitoring for the precise timing of the moment to switch from ITP to CZE (Figs. 1 and 2, step 4). Because at the time of switching the capillary is filled with the background electrolyte

for CZE the current will be high (typically in the microampere range) when using a leading buffer as the background electrolyte for CZE. When a terminating buffer is used as the background electrolyte for CZE the current will be considerably lower at the time of switching (typically in the high nanoampere range). Fluctuations in the current caused by ripples in the power supply make precise timing difficult, especially in the latter instance.

Addition of selectivity-enhancing compounds to the background electrolyte is allowed as long as those additives are non-ionic (*i.e.*, non-ionic detergents, non-ionic complex-forming agents, organic modifiers). With ionic additives care must be taken that they do not disturb the ITP conditions.

The ionic strength of the CZE buffer should be chosen so that the concentrating properties in the ITP step are high, but not so high that the analyte precipitates (*e.g.*, 5–100 mmol/l). The pH of the CZE buffer affects the electroosmotic flow-rate, which influences the efficiency of the ITP step. A high electroosmotic flow-rate will result in a mixing of zones in ITP. Therefore, at high pH additives are used that reduce the electroosmotic flow-rate, *e.g.*, hydroxypropylmethylcellulose (HPMC).

In conventional CZE the background electrolyte should have a buffering capacity to maintain a constant pH during separation. In ITP and ITP–CZE the counter ion should have this buffering capacity. Under steady-state conditions in ITP the analyte ions are focused between the leading and terminating ions. The counter ions are present throughout the separation tube. This means that the best way to buffer the system is by means of the counter ions.

The most important parameters in the initial conditions of the CZE background electrolyte will be the choice of pH, the buffering counter ions and the additives used. The choice of the leading and terminating ions can be made after studying the ITP separation window. In the ITP–CZE analysis of the cationic test compounds the migration order of the analyte, leading and terminating ions was not changed on switching from triethylamine to sodium as leading ion. However, changing the pH, the percentage of organic modifier or buffering counter ion might affect the migration order.

ITP separation window

One of the advantages in ITP–CZE is that most of the matrix constituents can be removed in the focusing step. The choice of the leading and terminating ions determines the focusing window. All compounds outside the separation window in the ITP step migrate under zone electrophoretic conditions. When maximum sample clean-up is desired a system is chosen where the CZE step takes place in the leading buffer. Complete removal of all counter ions and neutral species from the capillary is then expected (Figs. 1A, 2B, 3A and 3C).

However, matrix ions with a higher mobility than that of the leading ions in this instance migrate into the separation capillary even during the focusing step. When such ions are present in the matrix at a high concentration then it is advisable to trap them within the ITP window or to use these ions as leading ions. Otherwise, these ions may cause an inhomogeneity in the electrical field during the CZE run. For the optimization of ITP–CZE it is therefore necessary to have information on the ionic composition of the sample matrix. A conventional CZE system with indirect UV absorbance detection [20] is particularly suitable as a screening method for the main matrix components (Fig. 4).

Ions with a higher mobility than the leading ions giving a strong signal in the detection system may cause a drift in the baseline. These ions should also be trapped within the ITP separation window. Because of their detection properties these ions cannot be used as leading ions in a conventional detection system. In that particular event a leading ion with a higher mobility should be used.

With respect to sample clean-up the terminating ion should be chosen so that the mobility is just below that of the analyte ion with the lowest mobility.

Choice of the leading and terminating buffer

Several reviews on ITP are available that detail buffer systems and ionic mobilities of

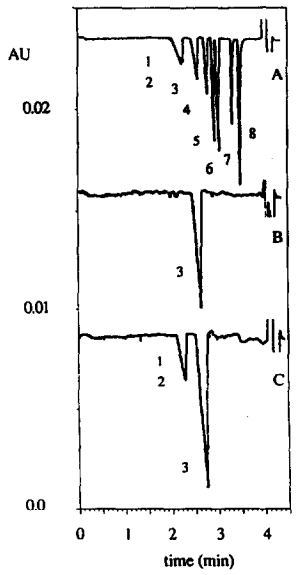

Fig. 4. Electropherograms of (A) a mixture of cations (2.5 mmol/l in background electrolyte), (B) human plasma (diluted 1:19 in background electrolyte) and (C) human urine (diluted 1:19 in background electrolyte). A voltage of 25 kV (4.7 μA) was applied over the 900 × 0.1 mm I.D. separation capillary. Indirect UV absorbance detection took place at 550 mm from the injection end at 200 nm using a 10 mmol/l collidine buffer adjusted to pH 8.0 with phosphoric acid. Injection was performed (A) hydrodynamically using 10 mbar for 6 s or (B, C) electrokinetically at 6 kV for 6 s. The cationic mixture consisted of ammonium (1), potassium (2), sodium (3), lithium (4), TEA (5), DEtOHA (6), Tris (7) and TEtOHA (8). At 4 min a system peak appeared together with the electroosmotic flow marker (acetonitrile).

compounds which are most helpful in setting up an ITP–CZE separation [18,21]. The ionic mobility of a compound is defined as the electrophoretic mobility of the compound when it is fully ionized. For ions such as sodium and chloride tabulated values can be used without correction for pH. For weak acids and bases the effective electrophoretic mobility can be calculated using the relationship between the pK_a, pH and ionic mobility [21]. This relationship is given by the equation

$$\mu_{HA} = \mu_{A^-} \cdot \frac{1}{1 + \frac{[H^+]}{K_a}} \quad (2a)$$

for compounds with one pK_a, where μ_{A^-} is the ionic mobility and K_a the protolysis constant. For dibasic acids this equation becomes

$$\mu_{H_2A} = \frac{\mu_{HA^-} + \mu_{A^{2-}} \cdot \frac{K_{a_2}}{[H^+]}}{\frac{[H^+]}{K_{a_1}} + 1 + \frac{K_{a_2}}{[H^+]}} \quad (2b)$$

where K_{a_1} and K_{a_2} are the dissocation constants for a dihydric acid and the ionic mobilities are given by $\mu_{A^{2-}}$ and μ_{HA^-}.

Eqns. 2a and 2b correct for the fraction of the compound that is not in the ionic form. Only the ionized fraction contributes to the electrophoretic mobility of an ion. Using the conditions in Fig. 4A for Tris the effective mobility is calculated to be $1.55 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} (migration time 3.40 min, electroosmotic flow marker 4.05 min, electrical field strength 25 kV per 90 cm, at pH 8.0). According to eqn. 2a, effective mobility of $1.48 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} is expected, which is close to the measured effective electrophoretic mobility (the tabulated value for the ionic mobility of Tris is $2.95 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} [21] and the pK_a of Tris is 8.0). In the same system sodium, which is always fully ionized, has an effective electrophoretic mobility of $5.05 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}, which is approximately the same as the ionic mobility [18], as expected.

As shown in Fig. 6, a CZE system applying indirect detection can be used for selecting suitable leading and terminating ions in ITP–CZE. Especially in those instances where additives to the buffer are used, tabulated values are not always available. The electropherogram gives a good insight into the migration order of the leading, terminating and sample ions. To make a good comparison possible it is necessary that the indirect detection system is similar to the chosen CZE buffer in the ITP–CZE analysis with respect to additives, counter ion and pH.

Based on data obtained by the indirect detection system, the final choice of leading and terminating ions can be made. For plasma and urine samples sodium is most likely the main cationic compound, which is confirmed by the electropherograms in Fig. 4. Therefore, when the mobility of the analyte ions is lower, sodium can be used as the leading ion.

Application of ITP–CZE to spiked plasma and urine samples

The first step in the ITP–CZE analysis of the three antimuscarinic test compounds in spiked plasma and urine was the optimization of the CZE system. At pH 5.0 the compounds were baseline separated (Fig. 5). Therefore, an acetate buffer adjusted to pH 5.0 was chosen as the background electrolyte for CZE. To permit a high loadability of the system, a separation capillary of 900 × 0.1 mm I.D. was used. The current at 25 kV was reduced by adding 50% methanol to the buffer. To reduce the CZE run times the detector was placed at 550 mm from the capillary inlet. This offered the additional advantage of allowing the application of a negative hydrostatic backpressure by raising the electrode vial at the capillary outlet.

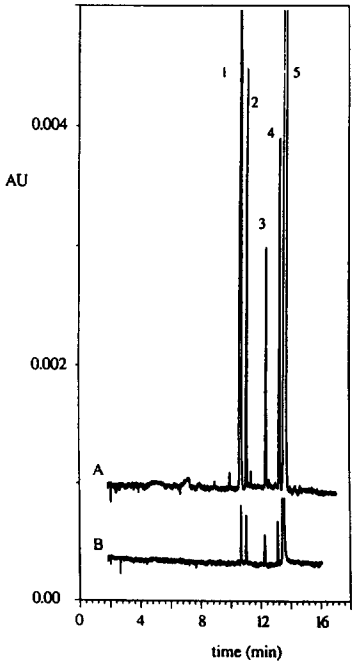

Fig. 5. (A) ITP–CZE of 280 nl and (B) CZE of 28 nl of the three test compounds neostigmine (2), homatropine (3), scopolamine (4) and of the dye crystal violet (5). Peak 1 is an unidentified system peak. In both instances a concentration of a 1 μg/ml of the test compounds and 10 μg/ml for the dye was injected. The leading buffer was 10 mmol/l TEA in 50% methanol adjusted to pH 5.0 with acetic acid. The terminating buffer consisted of 10 mmol/l β-alanine in 50% methanol adjusted to pH 5.0 with acetic acid. UV absorbance detection took place at 550 mm from the injection end at 200 nm. The detector settings were the same for both electropherograms. ITP–CZE was performed as described in Fig. 1A.

The second step was the selection of the ITP separation window. Depending on the separation window set by the leading and terminating ions, ITP can be used for sample clean-up for CZE. Therefore, an indirect detection system was used to study the main matrix components in urine and plasma (Fig. 4). Conditions for the indirect detection of urine and plasma were chosen so that semi-quantitative information was obtained rapidly. The electropherograms of urine and plasma were compared with an electropherogram of a mixture of cations. The main cationic component in urine and plasma was sodium, as expected. Therefore, sodium was chosen as a leading ion in the ITP–CZE method. Especially urine contains a considerable concentration of cations with higher mobility such as potassium and ammonium ions (Fig. 4C). These ions did not disturb the final ITP–CZE analysis, otherwise one of these ions could be chosen as the leading ion.

The choice of β-alanine as terminating ion was made in a similar way using indirect detection. However, to make a good comparison with the migration order in ITP–CZE possible, the conditions of the indirect system were carefully chosen. The buffer consisted of acetate at pH 5.0 in 50% methanol. The only difference to the conditions of the CZE background electrolyte in the final ITP–CZE system was the use of pyridine as UV-absorbing co-ion instead of sodium. Fig. 6 clearly demonstrates the separation window between the leading and terminating ions.

Depending on the separation window, the complexity of the matrix and the analyte concentration, ITP [22–24] and ITP–CZE can be used for the analysis of plasma and urine samples without any or with minor pretreatment (*e.g.*, filtration). In Fig. 7 the electropherograms obtained after ITP–CZE of 10 μg/ml antimuscarinic drugs in urine with the without pretreatment are shown. For trace analysis it is unlikely that the selectivity in ITP–CZE without an additional pretreatment will be sufficient. Interferences in the CZE analysis will by definition

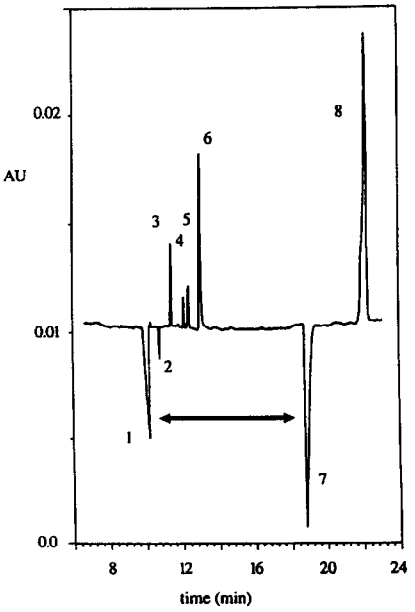

Fig. 6. Representation of the ITP separation window (arrow) using CZE with an indirect detection system. The background electrolyte consisted of 10 mmol/l pyridine in 50% methanol adjusted to pH 5.0 with acetic acid. The migration order of neostigmine (3), homatropine (4), scopolamine (5) and crystal violet (6) can be easily determined with respect to possible leading (1, TEA) and terminating (7, β-alanine) ions. Peak 2 is an unidentified system peak and peak 8 is pyridine. For electrophoretic conditions, see Fig. 4.

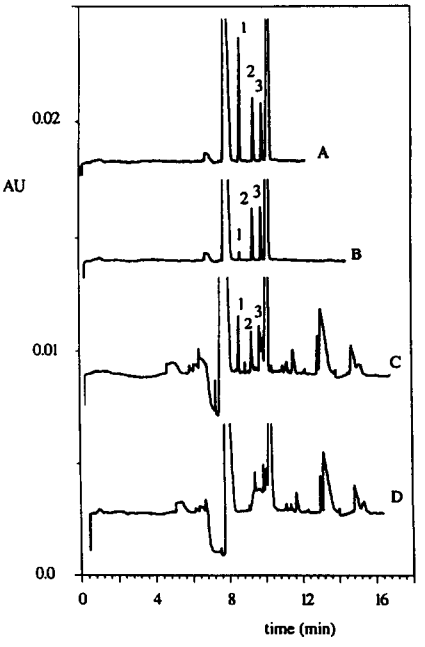

Fig. 7. ITP–CZE of 92 nl (10 μg/ml) of neostigmine (1), homatropine (2) and scopolamine (3), (A) in buffer, (B) in urine after dichloromethane extraction and (C) in urine without any pretreatment, in comparison with (D) blank urine. ITP–CZE was performed as described in Fig. 1A. Sodium acetate (10 mmol/l) at pH 5.0 in 50% methanol was used as the leading buffer and as the CZE background electrolyte. The termination ion was β-alanine. During the focusing step (Fig. 1A, step 2) a voltage of 10 kV and a backpressure of 22 mbar were applied. For the hydrodynamic mobilization of the sample zones (Fig. 1A, step 3) the backpressure was increased to 30 mbar at the same voltage. The focusing step took 5.5 min for runs A and B and 15 min for C and D.

migrate within the separation window in the ITP step because the separation principle in both electrophoretic modes is the same. The use of spacer ions in the ITP step may enhance the selectivity of the final analysis, but in the case of comigrating zones in CZE the ITP step will not give additional selectivity.

The selectivity is considerably enhanced by applying a liquid–liquid extraction with dichloromethane prior to the ITP–CZE step (Figs. 7–9). As can be seen in Fig. 7B, the extraction recovery for neostigmine is low. Scopolamine and homatropine can be measured at a concentration level of 100 ng/ml in plasma (Fig. 9) and urine (Fig. 8) after extraction. Although it is likely that the determination limits can be lowered using a concentration step by dissolving the evaporated extract in a smaller volume, no attempts were made to optimize the method.

CONCLUSIONS

Two ITP–CZE procedures for cationic and two ITP–CZE procedures for anionic separations are given. A strategy is described for setting up an ITP–CZE analysis. Information on the migration order of leading ions, terminating ions and analyte ions can be obtained using CZE with indirect UV absorbance detection. Furthermore, information on the ionic composition of the matrix can be obtained. Indirect detection permits tuning of the ITP separation window when ITP is used for sample clean-up for CZE.

The applicability of the described strategy is

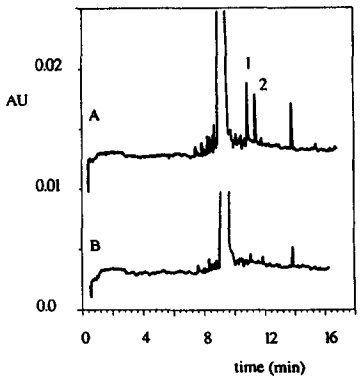

Fig. 8. ITP–CZE of 1.8 μl of (A) 100 ng/ml homatropine (1) and scopolamine (2) in urine and (B) blank urine after dichloromethane extraction. ITP–CZE conditions as in Fig. 7 except for the backpressure in the focusing step (Fig. 1A, step 2), which was 30 mbar at a voltage of 15 kV. The voltage was decreased to 10 kV at a constant backpressure to mobilize the focused analyte zones (Fig. 1A, step 3) towards the capillary inlet. The focusing step took 25 min before the CZE step was started at 25 kV. Crystal violet was no longer used as a dye. The current in step 3 increased from 0.1 to 3.1 μA.

demonstrated for the optimization of ITP–CZE of homatropine, scopolamine and neostigmine in spiked urine and plasma samples. Depending on the analyte concentrations plasma and urine samples can be analysed without pretreatment. However, because the separation mechanisms in both ITP and CZE are based on the same principle, an additional sample pretreatment will be needed in the case of comigrating matrix interferences. The coupling of a sample pretreatment based on separation mechanism other than electrophoresis in combination with the tremendous concentrating properties of ITP makes the highly efficient separation power of CZE applicable for trace analysis.

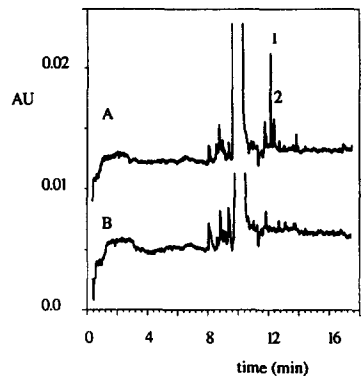

Fig. 9. ITP–CZE of 1.8 μl of (A) 100 ng/ml homatropine (1) and scopolamine (2) in plasma and (B) blank plasma after dichloromethane extraction. ITP–CZE conditions as in Fig. 8.

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