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Miniaturised on-line solid-phase extraction for enhancement of concentration sensitivity in capillary electrophoresis

Maria Petersson, Karl-Gustav Wahlund, Staffan Nilsson*

Technical Analytical Chemistry, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

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Abstract

Two of the most important issues facing the technology of capillary electrophoresis (CE) are the limited concentration sensitivity and the applicability to biological samples. An enrichment technique using miniaturised on-line solid-phase extraction did enhance the concentration sensitivity by a factor of 7000 using UV-absorbance detection. The cationic drug terbutaline was used as a model compound. The extractor consisted of a short length (1–3 mm) of a capillary (200 μm I.D.) packed with C_{18} alkyl-diol silica (d_p 12 μm). This capillary was connected to a 50 μm I.D. separation capillary and glass-fibre filters retained the sorbent. Preparation and performance of the enrichment capillary is discussed and described in detail. The on-line enrichment procedure includes washing, wetting, conditioning, sorption, washing, filling and desorption. Then follows CE separation. The separation efficiency was excellent as 250 000 plates were obtained in a 58 cm enrichment capillary (550 000 plates m^{-1}). The concentration limit of detection for terbutaline in aqueous solution with on-line enrichment was 0.6 n*M* (10 min×140 kPa pressure injection) compared to 4.4 μ*M* (1 s×3.4 kPa pressure injection) without enrichment. The enrichment procedure was successfully adapted to high efficiency separation of terbutaline enantiomers using cyclodextrin as chiral selector. It was possible to enrich terbutaline from directly injected spiked plasma as well.

Keywords: Solid-phase extraction; Extraction methods; Pharmaceutical analysis; Enantiomer separation; Terbutaline; Basic drugs; Beta-agonists; Pre-concentration; Restricted-access media

1. Introduction

Capillary electrophoresis (CE) offers the high separation efficiency necessary in the analysis of complex mixtures. CE technology has advanced rapidly, but it is still insensitive in terms of concentration limit of detection (cLOD). Commercial CE systems most commonly offer UV-absorbance detection because of its on-line compatibility with fused-silica capillaries. The cLOD range using UV-absorbance detection is usually 10^{-6}–10^{-5} *M*. In pharmacokinetic studies drugs in body fluids often need to be determined in the low concentration range of 10^{-12}–10^{-9} *M*. Such applications thus present a major challenge for CE because some pre-concentration procedure giving enrichment factors of many thousand is needed. In addition, body fluids, especially plasma, can contain high (in the range of 70 g l^{-1}) concentrations of protein [1]. This can lead to variability of the electroosmotic flow (EOF) and to

*Corresponding author. Tel.: +46-46-222-8177; fax: +46-46-222-4525.

E-mail address: Staffan.Nilsson@TEKNLK.LTH.SE (S. Nilsson)

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band broadening due to analyte interaction with the surface-adsorbed protein. Proteins can also interfere with the detection of the desired analyte peaks. When analysing body fluids in CE, many users therefore precipitate the proteins using organic solvents like acetonitrile. Off-line solid-phase extraction (SPE) or ultrafiltration are more laborious but are also frequently used.

The small dimensions intrinsic to CE provide both advantages and limitations. The sample volume injected is, for example, normally limited to less than 25 nl (1% of the total capillary volume) to avoid band broadening. To improve the cLOD it is thus necessary to concentrate larger volumes (i.e., up to the 250 μl range) or to use sensitive detection methods like laser-induced fluorescence or amperometric detection. As one moves toward small sample volumes, sample handling and preparation steps become more difficult and the concentration step is therefore preferably done on-line although off-line approaches can be successful [2].

An on-line concentration approach is capillary isotachophoretic focusing. The concentrations of the sample components can be increased by several orders of magnitude [3,4]. However, capillary isotachophoresis (cITP) processes are vulnerable to the varying ion matrices commonly present in complex samples.

Another efficient on-line approach is stacking [5,6]. However, this requires samples of lower conductivity than the electrolyte. The sample can be injected either electrokinetically or hydrodynamically. The latter approach is normally limited to sample volumes less than the total capillary volume, which is 0.5–2.5 μl.

Analytes in complex matrices have been analysed in CE using a chromatographic pre-concentration approach. A small amount of sorbent, either an antibody [7], reversed-phase material [8–15] or hydrophobic adsorbing membranes [16], is placed on-line like an SPE microcartridge at the inlet end of the capillary. In those studies it seems that concentration factors of about 1000 could be obtained and that the separation efficiency was impaired as compared to regular CE without on-line SPE.

In this work we developed an SPE–CE methodology for the drug terbutaline with the objective of reaching high concentration factors and maintaining the high separation efficiency of CE.

Terbutaline (Bricanyl) is a β_2-receptor agonist widely used in the treatment of asthma. The drug is given as a racemate and in single-dose pharmacokinetic studies it is desirable to measure plasma concentrations of the enantiomers in the low nanomolar range. Terbutaline enantiomers have been determined in plasma down to 1 n*M* concentration using SPE and high-performance liquid chromatography (HPLC) with amperometric detection [17].

Our approach to detect low concentrations of terbutaline by CE is to use a reversed-phase restricted access sorbent for the on-line enrichment by SPE. The SPE unit is inserted at the inlet end of the capillary in a commercial CE instrument. Because terbutaline is a hydrophilic compound, and possessing a secondary amino group, this would test the performance of such an on-line extractor CE system. The restricted-access media have been introduced for SPE or coupled-column HPLC [18,19]. These bifunctional sorbents were developed for extraction and enrichment of low-molecular-mass hydrophobic compounds in proteinaceous fluids. Macromolecules such as proteins have limited access to the internal surface of the porous sorbent due to the small pore size. Furthermore, the sorbent external surface is non-adsorptive to proteins.

Results from investigations of the feasibility of the approach and performance of the system are presented and discussed in this study. Comparisons are made with results obtained in CE without on-line enrichment. Extensive effort was required in extractor construction and method development to achieve high concentration factors while simultaneously retaining the high separation efficiency of the capillary. The on-line enrichment procedure was adapted to separation of the terbutaline enantiomers by adding cyclodextrins. Initial work on application of the method to plasma samples is reported.

2. Experimental

2.1. Chemicals

rac-Terbutaline was used as the sulphate (Astra Draco, Lund, Sweden). Water was prepared in a Milli-Q water purification system (Millipore, Bedford, MA, USA). Bovine plasma (anticoagulant trisodium citrate, lyophilised powder) and sodium

dodecyl sulphate [SDS; approx. 99% (GC)] was from Sigma (St. Louis, MO, USA). Potassium dihydrogenphosphate, tripotassium phosphate 7-hydrate, disodium tetraborate cryst., acetone, 2-propanol, sodium hydroxide solution (Combi-Titrisol 5 M), methanol and acetonitrile (both gradient grade) were obtained from Merck (Darmstadt, Germany). Ethanol (99.5%) was from Kemetyl (Stockholm, Sweden). Boric acid was from Riedel-de Haën (Seelze, Germany). A 40 m*M* potassium phosphate electrolyte was prepared by adding 40 m*M* KH_2PO_4 to 40 m*M* K_3PO_4 until the desired pH was reached. Dimethyl-β-cyclodextrin (Stadex, Malmö, Sweden) was dissolved in the electrolyte to a final concentration of 15 m*M*. The borate buffer was prepared by mixing 100 m*M* H_3BO_3 and 25 m*M* $Na_2B_4O_7$ to pH 9.0. The SDS was dissolved to 200 m*M* in the borate buffer. Before use all non-organic solutions were filtered through a 0.2-μm Dynagard filter tip (Microgon, Laguna Hills, CA, USA) and degassed by ultrasonication. Stock solutions of *rac*-terbutaline were dissolved in water, stored in a refrigerator, and diluted daily to the desired concentrations.

2.2. Construction of the enrichment capillary

Three fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of two different I.D.s inserted into each other were combined into a unit hereafter termed the enrichment capillary (Fig. 1). First, the capillary pieces were rinsed with 1 *M* sodium hydroxide followed by water, and then cleaned with acetone on the outside. A 6 cm capillary with an I.D. of 50 μm and an O.D. of 190 μm was inserted 2–3 mm into a 4 cm×200 μm I.D. capillary (320 μm O.D.) thus forming the inlet end of the enrichment capillary. The two capillaries were fixed with epoxy glue (Super epoxy, Plastic Padding, Gothenburg, Sweden). An end filter was made from a GF/D glass-fibre filter (Whatman, Maidstone, UK) by rotating the joined capillaries while pressing its 200 μm opening gently against the filter disc [20]. The filter was pushed further into the capillary with a 190 μm O.D. capillary, and compressed against the 50 μm I.D. capillary. The 200 μm I.D. capillary was then slurry packed using a Jasco Model 880-PU reciprocating pump (Tokyo, Japan) with methanol as the displacing medium. The packing pressure was 200–300 kg cm^{-2}. The packing material, which was suspended in acetone, consisted of alkyl-diol silica (ADS) with a d_p of 12 μm (gift from Professor K.-S. Boos, Munich, Germany) (ADS pre-columns are available from Merck (Darmstadt, Germany)). The internal pore surface was covered with stearoyl-(C_{18}) moieties and the outer surface with glyceryl residues. By a dilute slurry (1.5 mg ml^{-1}) and a small slurry reservoir (approx. 100 μl) the final length of the packed bed could be made as short as 1–3 mm. The second end filter was then put in place and the 200 μm I.D. capillary was shortened before the third capillary, i.e., the separation capillary (23–53 cm× 50 μm I.D.), was inserted 2–3 mm compressing the filter. The separation capillary was fixed with glue, and that completed the enrichment capillary.

2.3. CE apparatus

Enrichment and separation were done using a P/ACE 2050 CE instrument controlled by P/ACE Series 3.0 software. The resulting electropherograms were, unless otherwise stated, evaluated off-line using the System Gold version 7.11 software (Beckman, Fullerton, CA, USA). The UV-absorption detector was set at 200 nm. The separations were done at constant voltage, typically 10–20 kV with the cathode at the detection side. The capillary was thermostatted at 25 °C.

2.4. Enrichment and CE procedure

Enrichment capillaries were checked for leaks or blockage by a manual purge of methanol using a syringe and connecting tubing before installation into the P/ACE cartridge. The alignment of the separation capillary to the 100×200 detection aperture was carefully examined and adjusted under a light microscope. High- (20 p.s.i.=140 kPa) or low-pressure (0.5 p.s.i.=3.4 kPa) instrumental rinse facilities were used for pushing solutions of various kinds through the enrichment capillary. The time (and volume) necessary to fill the whole enrichment capillary with electrolyte using high pressure was determined and checked regularly by first filling the whole enrichment capillary with water and then filling it with electrolyte, varying the filling time. Voltage was then applied and the current measured. When the current obtained for different filling times reached a stable level the enrichment capillary was considered totally

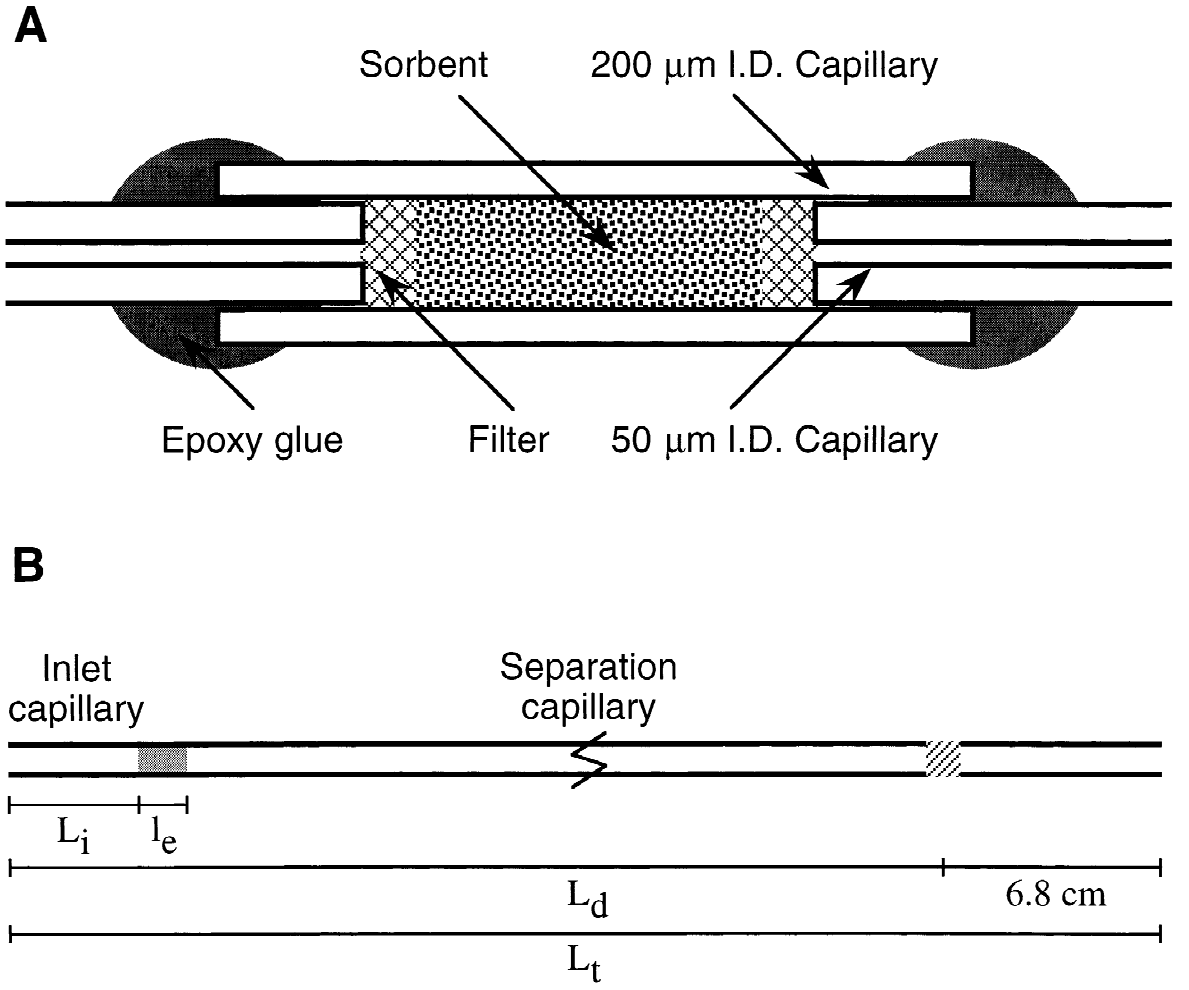

Fig. 1. Cross-section of (A) the extractor and (B) the enrichment capillary where L_t (28–58 cm) is the enrichment capillary total length, L_d (21.2–51.2 cm) is the length to the detector, L_i (5.4 cm) is the length of the inlet capillary and l_e (1–3 mm) is the extractor length.

filled with electrolyte. This time (and volume) was then taken as one enrichment capillary volume. Normally it took 0.6–0.7 min to fill a 58 cm enrichment capillary and 0.2–0.3 min to fill a 28 cm enrichment capillary.

The volumetric flow-rate through the enrichment capillaries was estimated by long (20 or 30 min) water rinses applied at a pressure of 140 kPa. The mass loss of the water-filled vial was used for calculating the volume that had passed through the capillary. The mean values of reverse and forward rinse are presented.

A typical operating procedure is presented in Fig. 2. Before sample injection, the enrichment capillary was washed with two enrichment capillary volumes of water from the inlet end, wetted with three enrichment capillary volumes of methanol, and conditioned with three enrichment capillary volumes of water using high pressure at the separation end. Samples were then injected by high pressure at the separation end. After sample injection, the extractor was washed as the enrichment capillary was filled with one enrichment capillary volume of electrolyte using high pressure at the separation end. The analyte was desorbed from the sorbent using less than 5% of one enrichment capillary volume of

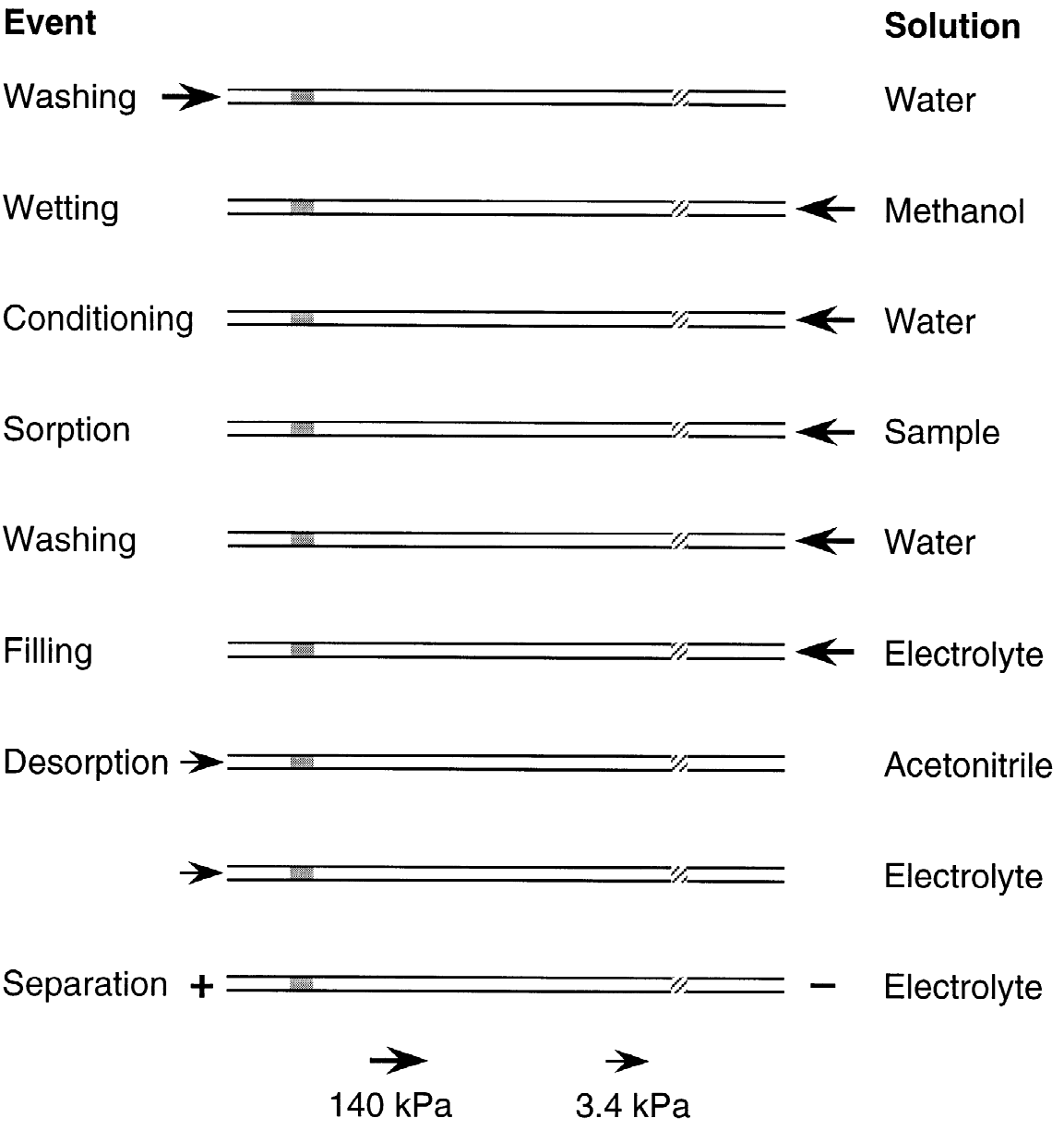

Fig. 2. Sample enrichment procedure for terbutaline dissolved in water. Arrows indicate flow directions. The post-sorption washing with water is optional, as the electrolyte filling, in which non-retained solutes are flushed out of the capillary, usually is enough for rather clean samples.

organic solvent injected by low pressure at the inlet end. (A 30 s×3.4 kPa injection corresponds to a volume of 30 nl as calculated for a 50 μm×58 cm capillary without extractor.) The desorbing solvent was pushed further into the inlet capillary by a second low-pressure injection of electrolyte. Based on the estimated volume of the enrichment capillary and its known filling time, the desorbing solvent was stopped past the extractor. Electrophoretic separations were then carried out in the normal fashion.

The experiments were carried out in duplicate. The cLOD was defined by the signal-to-noise ratio equal to two ($S/N=2$) where the noise was taken as the peak-to-peak noise.

The EOF in the enrichment capillary was estimated using water as marker and compared to that in a capillary without extractor. Both capillaries were rinsed with methanol, water and electrolyte. When using reversed polarity (detection at the anode) a small volume of water was injected electrokinetically from the ordinary outlet end (length to the detector 6.8 cm). Using this injection end prevented the marker from passing the extractor, which could have impaired the measurement. A separation voltage was then applied and the time measured for the water dip to migrate to the detection window. These migration time data were evaluated manually.

A 50 μm×58 cm capillary without extractor was used for making enantiomer separation comparisons. It was filled from the separation end with 40 m*M* potassium phosphate (pH 6.4)×0.2 min×140 kPa pressure followed by 15 m*M* dimethyl-β-cyclodextrin in 40 m*M* potassium phosphate (pH 6.4)×0.5 min×140 kPa pressure. The sample was dissolved in the potassium phosphate buffer and injected for 3 s by 3.4 kPa pressure. The separation voltage was 14 kV.

3. Results and discussion

3.1. The enrichment capillary characteristics

The extractor (Fig. 1) was made small enough to be used in a commercial instrument. It was easily placed inside a Beckman CE cartridge. Once installed into the CE instrument, the enrichment capillaries required no manual handling and they could be used for more than 200 runs without deterioration in performance.

The flow was 1.7–2.0 μl min^{-1} through a 58 cm long enrichment capillary using 140 kPa pressure. This is 70–80% of the flow in a capillary without extractor. To increase sample throughput, as volumes up to 100 μl are encountered, an instrument with a large variable pressure range or a removable extractor for off-line loading [21] can be used.

By constructing the extractor from fused-silica capillaries, visual inspection of the packed bed, filters and seals, etc., under a light microscope was enabled. The compressed glass-fibre filters were estimated to be less than 100 μm in length. Sintered frits were not used, as they tend to be longer. To reduce the dead volumes in the filters and the shift in internal diameter, the smallest available outer diameter of the inlet and separation capillaries was chosen, and the extractor internal diameter was selected so that they were inserted snugly. The best accessible shift was from 200 to 50 μm.

Any failure of the enrichment capillaries was due to breakage of the capillary ends, leakage of liquid through the glue joint, or clogging. Clogging increases the filling time which is crucial as it affects the volumes passing through the enrichment capillary under the constant pressure applied. The high-pressure packing of the extractor should reduce settling, yet the filling time increased slightly during consecutive runs.

The sorbent to be used in SPE is governed by the application, i.e., the properties of analyte and sample matrix. Terbutaline in plasma has been extracted on various sorbents [22]. A few different reversed-phase sorbents were tested and a restricted-access medium, C_{18} alkyl-diol silica [19] was found suitable for the CE on-line enrichment. However, the fused-silica separation capillary may not be regenerated with sodium hydroxide as this would deteriorate the silica-based sorbent.

3.2. Concentration limit of detection of terbutaline with on-line enrichment

A cLOD of 0.6 n*M* was obtained for terbutaline with on-line enrichment, following the general procedure illustrated in Fig. 2, using a 28 cm enrichment capillary with a 4 mm extractor (Fig. 3A and B). This is more than 7000-times lower than without enrichment and stacking (Fig. 3C). The cLOD was determined using 1 and 10 n*M* terbutaline solutions in water (injection 10 min×140 kPa). The terbutaline peak height showed a linear relationship versus terbutaline concentration. The separation efficiency was about 2/3 of that obtained without enrichment and stacking.

The initial concentration factor (ratio between sample and desorption volume) was here 1000.

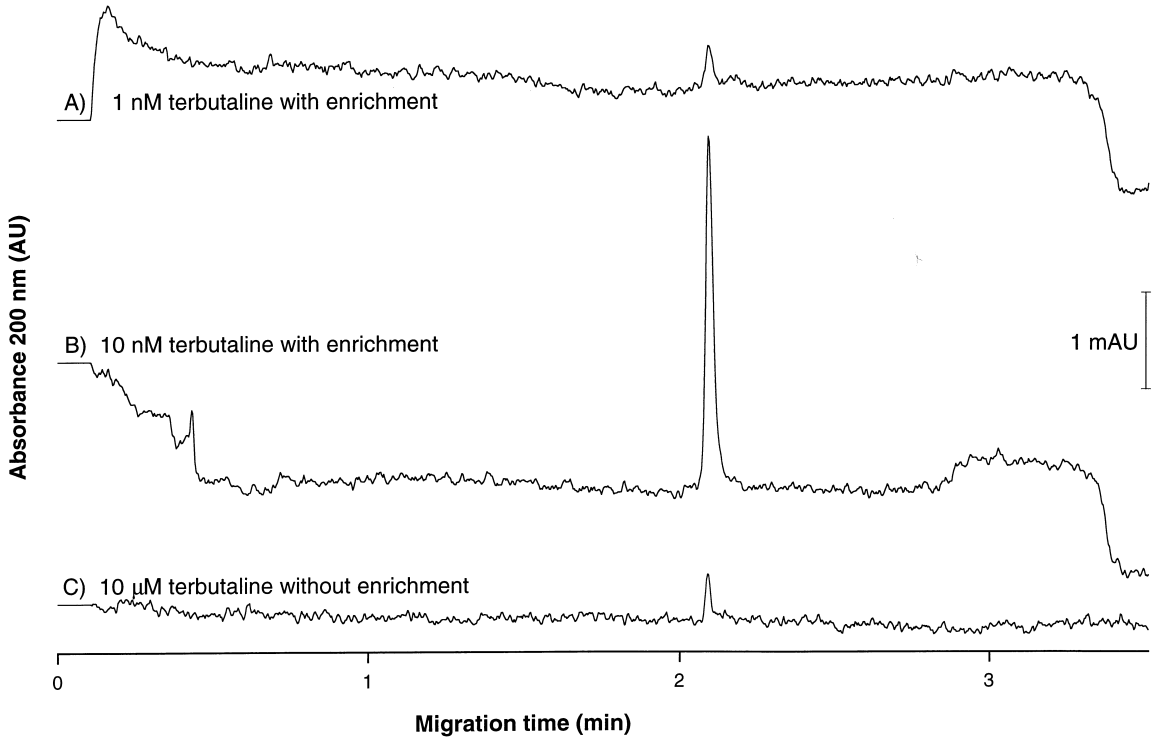

Fig. 3. CE of (A) 1 n*M* and (B) 10 n*M* terbutaline solutions with on-line enrichment and (C) 10 μ*M* terbutaline solution without on-line enrichment, demonstrating a 7000-fold enhancement of concentration sensitivity. Enrichment capillary: L_t 28.0 cm, L_d 21.2 cm, L_i 5.3 cm, l_e 4 mm, wash: water×0.6 min×140 kPa, wetting: methanol×1.2 min×140 kPa, conditioning: water×1.2 min×140 kPa, injection: (A) 1 n*M* and (B) 10 n*M* terbutaline in water×10.0 min×140 kPa, wash/filling: 40 m*M* potassium phosphate (pH 6.5)×0.3 min×140 kPa, desorption: acetonitrile×24 s×3.4 kPa followed by 40 m*M* potassium phosphate (pH 6.5)×2.2 min×3.4 kPa, voltage: 10 kV, detection wavelength: 200 nm, temperature: 25°C.

Stacking in the organic solvent can 10-fold the analyte concentration. A final concentration factor in the range of 10 000 can thus be achieved. This is showed by the observed concentration factor of 7000 in this study.

In biomedical applications, sample volumes are often limited to a few millilitres which is in the same range as the desorption volume for a normal-sized off-line SPE cartridge [23]. The achievable concentration factor is thus low. The CE on-line enrichment still offers high concentration factors even if the sample volume is in the microlitre range because the desorption volume is in the nanolitre range.

3.3. Concentration limit of detection of terbutaline without on-line enrichment

For terbutaline, the cLOD was 4.4 μ*M* without on-line enrichment (Fig. 3C). The cLOD was determined using a 10 μ*M* terbutaline solution in 40 m*M* potassium phosphate buffer (pH 6.5) injected for 1 s at 3.4 kPa in a 28 cm capillary. The plate number was 78 000 (370 000 plates m^{-1}) at the 360 V cm^{-1} used. The detectability using UV-absorbance detection depends on the molar absorptivity of the analyte and the common cLOD is in the low micromolar range.

3.4. Breakthrough time for terbutaline

The breakthrough injection time for a 110 n*M* terbutaline solution in water was 12 min on a 2 mm extractor in a 28 cm capillary, as longer injection times did not further increase terbutaline peak height. This corresponds to a sample volume of about 45 μl.

The extractor breakthrough (retention) volume is a

critical parameter controlling both the amount of sample in a defined solvent and the volume that can be applied to the cartridge before the analyte starts to be eluted. The hydrophilic compound terbutaline has an apparent partition coefficient of 0.03 (*n*-octanol–water, pH 6.5) [24]. With more hydrophobic analytes the breakthrough volume can be much increased and the cLOD considerably lowered.

3.5. Post-sorption washing

Post-sorption washing with water and electrolyte, respectively, was investigated (Fig. 4). The terbutaline peak height dramatically decreased using electrolyte washing times longer than 0.1 min (excluding the 0.7 min filling time). Washing with water, on the other hand, gave no decrease in peak height. Obviously, the electrolyte caused some desorption of terbutaline. It is thus important to investigate the post-sorption washing conditions to avoid the risk of losing less hydrophobic analytes.

To avoid desorption of terbutaline, the electrolyte volume passing through the extractor was minimised by making the sample injection and the electrolyte filling from the separation end. In addition, injection from the separation end resulted in slightly higher plate numbers than injection from the inlet end.

3.6. Electroosmotic flow

The EOF mean value of four enrichment capillaries (both unused and used ones) was $2.5 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} in 40 m*M* potassium phosphate buffer (pH

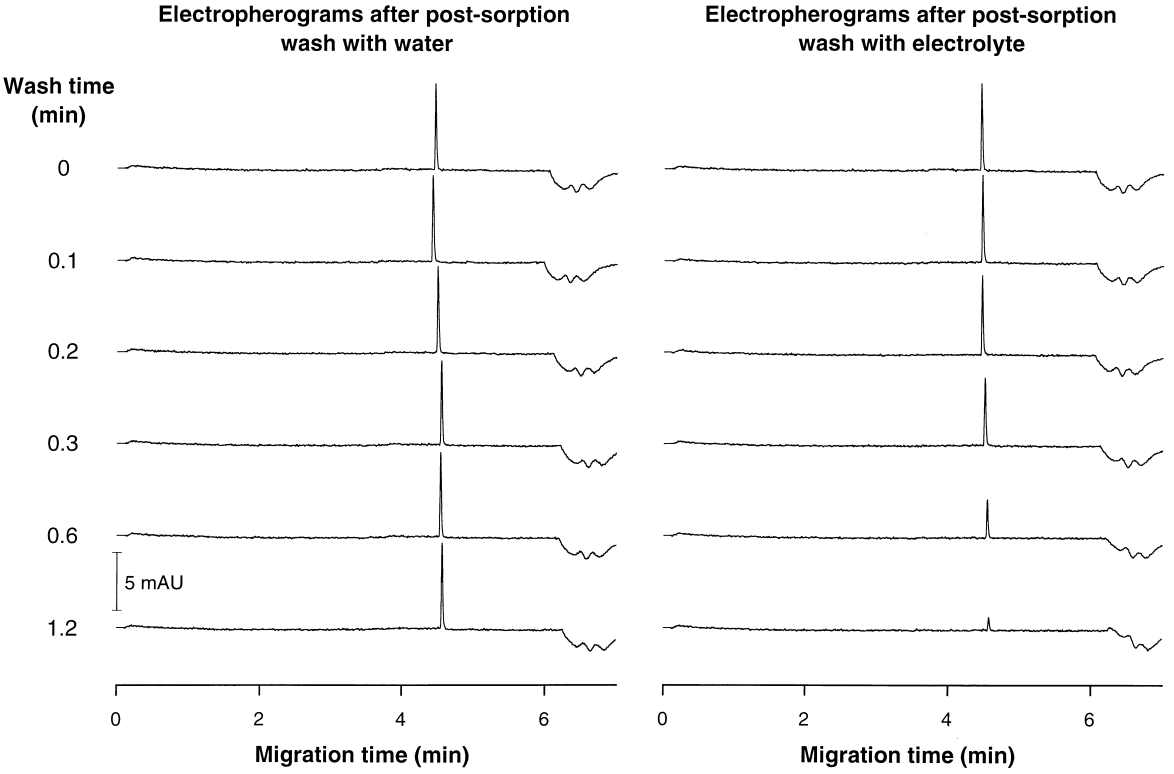

Fig. 4. CE of terbutaline with on-line enrichment showing the effect of increasing post-sorption washing times with water or electrolyte. Enrichment capillary: L_t 58.0 cm, L_d 51.2 cm, L_i 5.4 cm, l_e 1.25 mm, wash: water×1.4 min×140 kPa, wetting: methanol×2.1 min×140 kPa, conditioning: water×2.1 min×140 kPa, injection: 100 n*M* terbutaline in water×4 min×140 kPa, wash: water or 40 m*M* potassium phosphate (pH 6.4)×0–1.2 min×140 kPa, filling: 40 m*M* potassium phosphate (pH 6.4)×0.7 min×140 kPa, desorption: acetonitrile×30 s×3.4 kPa followed by 40 m*M* potassium phosphate (pH 6.4)×3.0 min×3.4 kPa, voltage: 20 kV, detection wavelength: 200 nm, temperature: 25°C.

6.4) which agrees with the value obtained in the capillary without extractor. The relative average deviation from the mean was 17% for the enrichment capillaries. Within each enrichment capillary the relative average deviation from the mean was below 2% ($n=6$).

The magnitude of EOF has been reported to be reduced in the presence of a solid-phase C_{18} material [25,26]. To stabilise the net transport toward the detector, pressure can be used in addition to the separation voltage. This approach was not necessary in the present study.

3.7. Desorption and separation

The enrichment of terbutaline followed by CE separation gave 250 000 plates (550 000 plates m^{-1}), Fig. 5. This is a superior efficiency as compared to other SPE–CE enrichment procedures [8–15].

The volume of a 52 cm×52 μm separation capillary is about 1 μl and the volume of the empty extractor capillary is about 30–90 nl. The desorbing solvent volume required was about half of the empty extractor volume as the corrected terbutaline peak area reached a maximum and remained constant using acetonitrile or methanol injections longer than 20–40 s at 3.4 kPa injection pressure. A larger desorption volume than the ideal 1% of the separation capillary volume was thus applied.

To reduce band broadening a good desorbing solvent should cause complete desorption in a small volume and have optimal electrophoretic properties (maximal stacking and minimal disturbance of EOF).

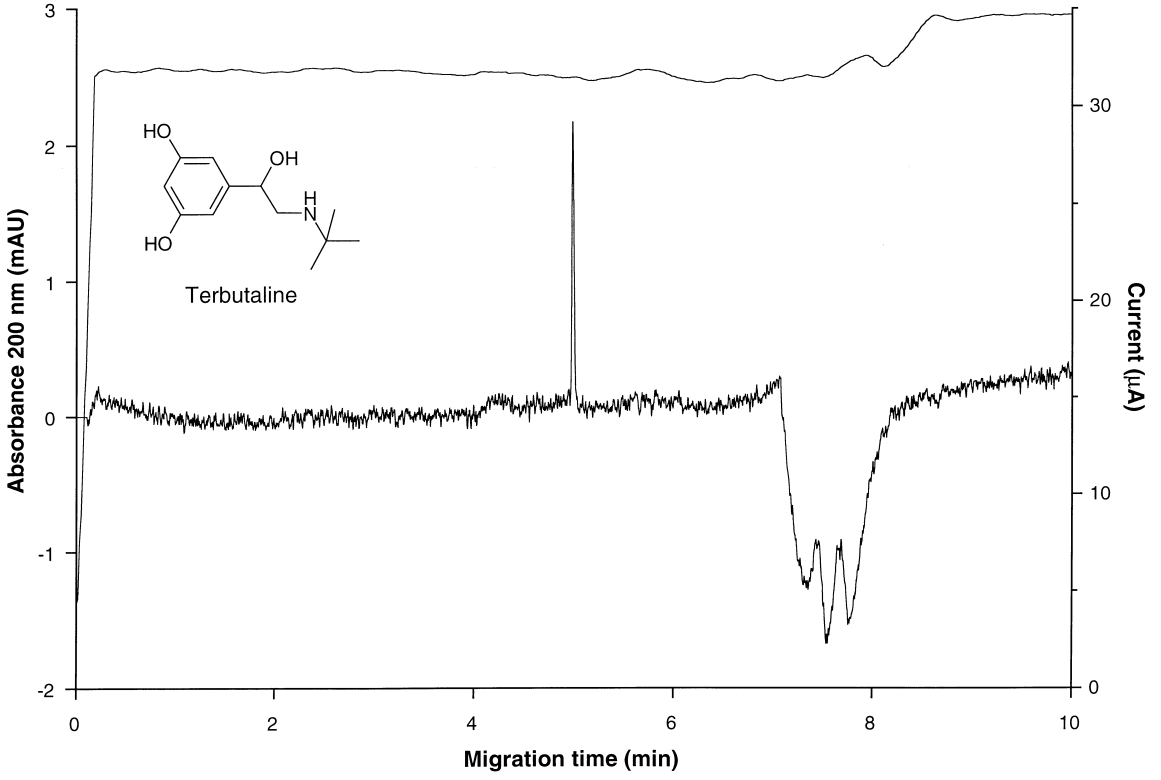

Fig. 5. Typical electropherogram of terbutaline demonstrating that high separation efficiency (250 000 plates) was maintained during the on-line enrichment procedure. Enrichment capillary: L_t 58.0 cm, L_d 51.2 cm, L_i 5.4 cm, l_e 1.25 mm, wash: water×1.4 min×140 kPa, wetting: methanol×2.1 min×140 kPa, conditioning: water×2.1 min×140 kPa, injection: 100 n*M* terbutaline in water×1 min×140 kPa, wash/filling: 40 m*M* potassium phosphate (pH 6.4)×0.7 min×140 kPa, desorption: acetonitrile×30 s×3.4 kPa followed by 40 m*M* potassium phosphate (pH 6.4)×3.0 min×3.4 kPa, voltage: 20 kV, detection wavelength: 200 nm, temperature: 25°C.

Methanol, ethanol, 2-propanol and acetonitrile were tested as desorbing solvents. The largest terbutaline corrected areas were obtained using acetonitrile and methanol, followed by ethanol and 2-propanol. Compared to methanol, acetonitrile resulted in higher peak height, higher plate number, shorter migration times and a more stable current.

From the observed corrected areas it can be assumed that acetonitrile and methanol were more efficient desorbing solvents for terbutaline than ethanol and 2-propanol. Stacking is supported to be more efficient in acetonitrile than in methanol as it showed the highest terbutaline peak heights and plate numbers. In addition, acetonitrile probably affected EOF less as it produced the shortest migration times. Stacking is favoured in high electrophoretic mobility and high electric field strength conditions in the sample zone. This can be achieved when the sample medium is of lower conductivity and lower viscosity (fulfilled for both methanol and acetonitrile) than the separation electrolyte [27].

The current increased (Fig. 5) when the solvent plug left the capillary and the final current generated was the same as in a capillary without extractor. The enrichment capillaries were vulnerable to current failure, probably due to the formation of bubbles, as it often caused an increased filling time. Moderate field strengths were therefore used.

Not only the analyte of interest can be enriched but also other components present in the water, electrolyte, and sample. This was observed as a large detector signal sometimes recorded at the migration time for neutral compounds. As terbutaline migrates faster than the EOF this was not a problem. To avoid interferences, pretreatment of the electrolyte by SPE can be used [12].

3.8. Terbutaline enantiomer separation

The enrichment procedure was tested in the separation of terbutaline enantiomers. Baseline separation was achieved (R_s=1.6) using 15 m*M* dimethyl-β-cyclodextrin electrolyte, acetonitrile as the desorbing solvent and partial filling (Fig. 6). The separation efficiency was 300 000 plates. The higher separation efficiency that acetonitrile provides, compared to methanol, was necessary to resolve the enantiomers. The separation capillary was partially filled with cyclodextrin electrolyte, as there was a possibility that the enriched solutes were eluted when the sorbent was flushed with electrolyte containing the cyclodextrin. This was demonstrated by total filling experiments where the terbutaline peak heights decreased almost 50% as compared to partial filling.

For comparison, enantiomer separation was also done in a capillary without extractor. The capillary was partially filled with the cyclodextrin electrolyte. The resolution (R_s=2.2) and plate number (470 000) was higher than with on-line enrichment. The sample contained *rac*-terbutaline at a concentration of 10 μ*M* of each enantiomer.

The CE experimental parameters for separation of terbutaline enantiomers have been investigated thoroughly before [28,29]. Neutral β-cyclodextrins, both underivatised and derivatised, are good enantioselective agents for the resolution of terbutaline. Because terbutaline migrates as a cation below pH 9.5, resolution is greatest when the EOF is reduced, either by using a coated capillary or by using an electrolyte with low pH value. The enrichment procedure was tested at low electrolyte pH (3.0) but the separation efficiency was dramatically lower than at higher pH values, and highly irreproducible migration times were obtained. The enrichment procedure was therefore done at a higher pH than optimal for the enantiomer separation.

3.9. Plasma samples

The feasibility of enhancing cLOD of terbutaline also in plasma samples was studied in initial experiments. The enrichment was successful (Fig. 7). The terbutaline migration time was higher than in runs without plasma, probably because of a decrease in EOF. Fouling of the capillary wall with plasma protein during the analysis was expected though.

The use of coated capillaries, like poly(vinyl alcohol) (PVA), can decrease problems involved with protein adsorption. However, PVA capillaries are not available in the dimensions required. Washing with sodium hydroxide is excluded due to incompatibility with the silica-based sorbent. Alternatives, then, are the use of polymer-based sorbents [30] or a detergent such as SDS [31].

An SDS washing step can easily be included using the existing enrichment capillaries. A five capillary

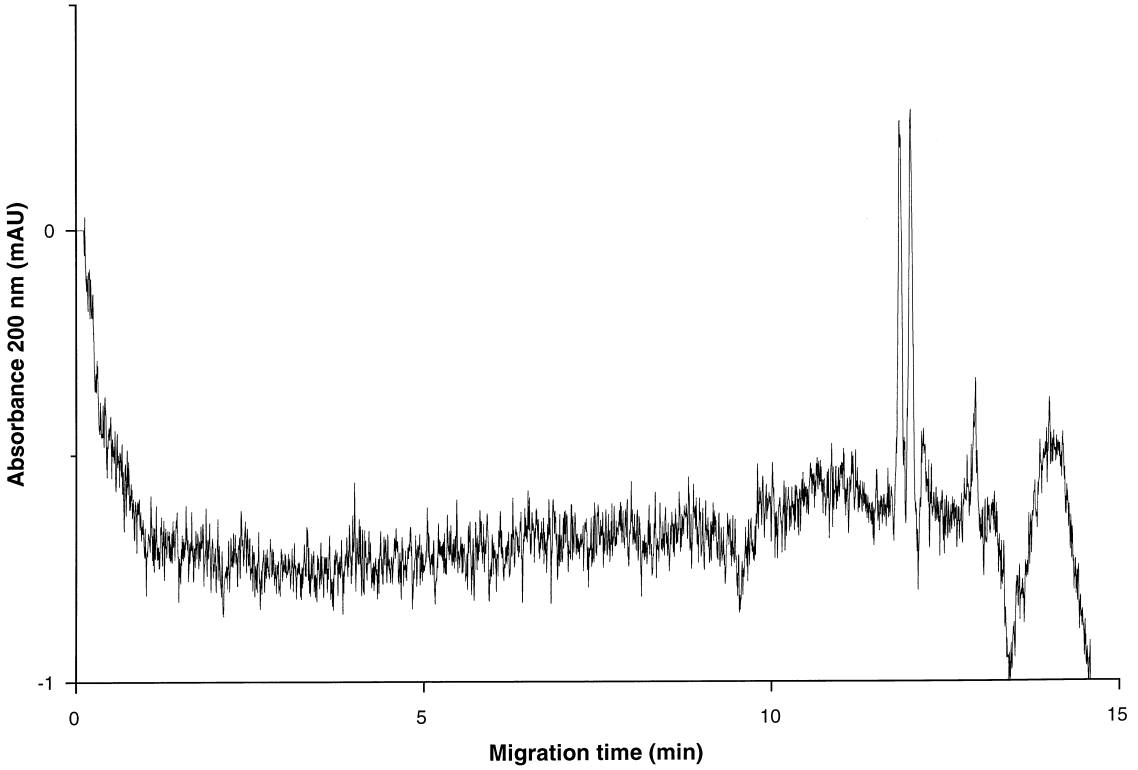

Fig. 6. Terbutaline enantiomer separation with on-line enrichment. A resolution of 1.6 and a separation efficiency of 300 000 plates were obtained. Enrichment capillary: L_t 58.0 cm, L_d 51.2 cm, L_i 5.5 cm, l_e 2.5 mm, wash: water×1.6 min×140 kPa, wetting: methanol×2.4 min×140 kPa, conditioning: water×2.4 min×140 kPa, injection: 100 n*M* *rac*-terbutaline in water×1.0 min×140 kPa, wash/filling: 40 m*M* potassium phosphate (pH 6.4)×0.1 min×140 kPa, 15 m*M* dimethyl-β-cyclodextrin in 40 m*M* potassium phosphate (pH 6.4)×0.7 min×140 kPa, desorption: acetonitrile×40 s×3.4 kPa followed by 15 m*M* dimethyl-β-cyclodextrin in 40 m*M* potassium phosphate (pH 6.4)×4.0 min×3.4 kPa, voltage: 14 kV, detection wavelength: 200 nm, temperature: 25°C.

volume wash with 200 m*M* SDS in 100 m*M* sodium borate buffer (pH 9.0) was added before the methanol wetting step. The methanol wetting step was increased in time to ensure that the SDS was removed. This procedure showed no negative effects on the on-line enrichment.

When using plasma samples, the SDS wash removed high-absorbing species as the detector signal initially raised and then levelled out on a lower and reproducible level, but not as low as before the first plasma injection. After the enrichment capillary had been exposed to plasma once, the terbutaline migration time was nearly constant if the SDS wash was used. However, partially clogging of the enrichment capillary was observed after repeated plasma injections.

4. Conclusions

The SPE–CE methodology realises the use of UV-absorbance detection in the low nanomolar range. The sample was concentrated from microlitre to nanolitre volumes. Thus it meets the demands for application to bioanalysis. Concentration factors of three to four orders of magnitude were reached for the hydrophilic drug terbutaline. With more hydrophobic analytes, even higher concentration factor can be achieved. The high separation efficiency characteristic for CE was reached and stacking in the desorbing solvent was essential. Optimisation of the post-sorption washing and desorbing conditions was crucial. To solve the problems associated with plasma protein adsorption, for example by the use of

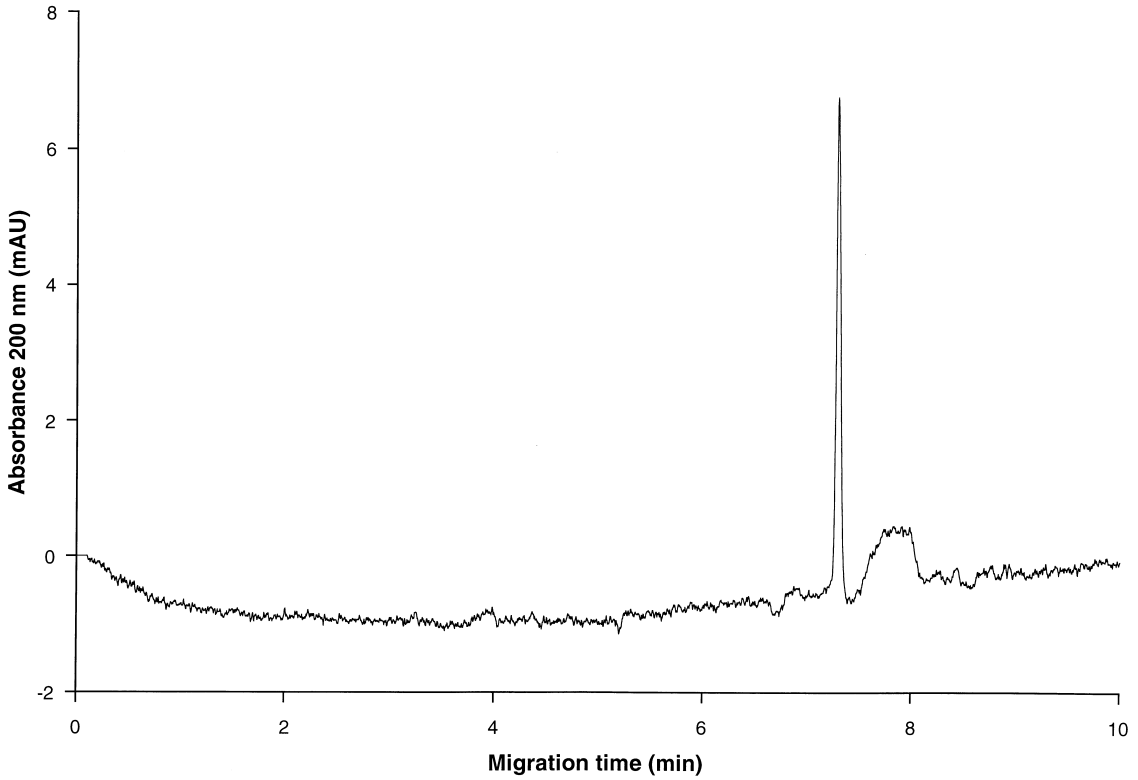

Fig. 7. Direct injection of terbutaline in plasma with on-line enrichment. The enrichment capillary had not been subjected to plasma samples before this run. Enrichment capillary: L_t 58.0 cm, L_d 51.2 cm, L_i 5.4 cm, l_e 1.25 mm, wash: water×1.4 min×140 kPa, 200 m*M* SDS in 100 m*M* sodium borate (pH 9.0)×3.5 min×140 kPa, wetting: methanol×7.0 min×140 kPa, conditioning: water×2.1 min×140 kPa, injection: 2 μ*M* terbutaline in water–bovine plasma (3:1)×0.1 min×140 kPa, wash: water×1.4 min×140 kPa, filling: 40 m*M* potassium phosphate (pH 6.4)×0.8 min×140 kPa, desorption: acetonitrile×30 s×3.4 kPa followed by 40 m*M* potassium phosphate (pH 6.4)×3.0 min×3.4 kPa, voltage: 20 kV, detection wavelength: 200 nm, temperature: 25°C.

coated capillaries, comes next. The methodology is favoured by the rapid development of CE instruments having variable high-pressure facilities, and in the future robotics.

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