JOURNAL OF MASS SPECTROMETRY, VOL. 33, 183–186 (1998)

Chiral Separation of Local Anaesthetics by a Capillary Electrophoresis/Partial Filling Technique Coupled On-line to Micro-electrospray Mass Spectrometry

Emmy M. Jäverfalk,[1]* Ahmad Amini,[1] Douglas Westerlund[1] and Per E. Andrén[2,3]

[1] Analytical Pharmaceutical Chemistry, Uppsala University, P.O. Box 574, Biomedicum, S-751 23 Uppsala, Sweden

[2] Department of Pharmaceutical Biosciences, Uppsala University, P.O. Box 591, Biomedicum, S-751 24 Uppsala, Sweden

[3] Department of Chemistry, National Veterinary Institute, P.O. Box 7073, S-750 07 Uppsala, Sweden

A novel procedure for analysing chiral compounds is described in which a capillary electrophoresis/partial filling technique was coupled on-line to micro-electrospray mass spectrometry. Enantiomers of bupivacaine and ropivacaine were separated using methyl-β-cyclodextrin, a non-volatile buffer additive, as a chiral selector. To avoid introduction of the selector into the ion source, the capillary was coated with polyacrylamide to minimize the electroosmotic flow. Prior to sample injection, the capillary was partially filled with cyclodextrin dissolved in acetate buffer. When 30 kV was applied, the positively charged enantiomers migrated towards the mass spectrometer through the zone containing the neutral selector, which acted as a pseudo-stationary phase. A racemic mixture of bupivacaine and ropivacaine was successfully separated and an impurity of 0.25% of (*R*)-ropivacaine in (*S*)-ropivacaine could be detected. © **1998 John Wiley & Sons, Ltd.**

J. Mass Spectrom. Vol. **33**, 183–186 (1998)

KEYWORDS: capillary electrophoresis/mass spectrometry; partial filling technique; micro-electrospray; cyclodextrin; local anaesthetics

INTRODUCTION

The characterization and analysis of chiral drugs are important since each enantiomer may have different biological and toxicological effects. Therefore, investigations must be performed concerning the pharmacology, pharmacokinetics and purity of the individual drug enantiomers. Capillary electrophoresis (CE) is a separation technique that is useful in the analysis of chiral compounds. Speed, efficiency and simplicity are some of the advantages over the more commonly used liquid chromatographic techniques.

Coupling of on-line CE to mass spectrometry (MS) is becoming increasingly popular and is a powerful technique for the identification and structural characterization of compounds. Techniques that have been developed for CE are being modified to suit the special conditions that coupling to a mass spectrometer demands. The main purposes of the modifications are to minimize the introduction of non-volatile and interfering buffer components into the mass spectrometer in order to retain ionization efficiency and avoid contamination of the ion source. This can often be done by changing to a volatile buffer such as ammonium acetate, acetate or formate at low ionic strength. When micelles (micellar electrokinetic chromatography (MEKC)) or cyclodextrin are present in the background electrolyte, more sophisticated modifications are often necessary, such as a coupled-capillary set-up.[1,2] This system allows the zones of interest to be transferred from the first capillary, containing the selector dissolved in the buffer, to a second capillary that is connected to the mass spectrometer. Using buffers with low pH is another way of minimizing the introduction of selector, owing to decreased electroosmotic flow.[3]

A newly discovered technique to avoid the introduction of non-volatile selectors into the mass spectrometer is the partial filling technique. It was originally developed to avoid disturbances in UV detection by UV-absorbing proteins[4,5] and has been used for other selectors such as micelles,[6] cyclodextrins[7] and macrocyclic antibiotics.[8] Recently, MEKC was coupled to electrospray (ES) MS using the partial filling technique for the separation of peptides.[6] The present study shows for the first time that this technique can be used to perform chiral separations in CE/ESMS. Separation of enantiomers of local anaesthetics was achieved without introducing the chiral selector methyl-β-cyclodextrin into the mass spectrometer. This was done with a polyacrylamide-coated capillary which was partially filled with buffer containing cyclodextrin, prior to sample injection. The positively charged enantiomers

* *Correspondence to:* E. M. Jäverfalk, Analytical Pharmaceutical Chemistry, Uppsala University, P.O. Box 574, Biomedicum, S-751 23 Uppsala, Sweden.

E-mail: emmy.javerfalk@farmkemi.uu.se

Contract/grant sponsor: Swedish Medical Research Council; *Contract/grant number:*11565.

Contract/grant sponsor: Thuring Foundation.

CCC 1076–5174/98/020183–04 $17.50

Received 13 June 1997
Accepted 13 November 1997

will then migrate towards the mass spectrometer through the zone containing the neutral selector, which will act as a pseudo-stationary phase, separating the enantiomers. The purpose of the study was to perform chiral separations using the principle of the partial filling technique in combination with MS, efficiently avoiding the introduction of selector into the ion source.

EXPERIMENTAL

Chemicals

Methyl-β-cyclodextrin (Sigma, St Louis, MO, USA) was dissolved in the background electrolyte that consisted of 50 mM (pH 3.0) acetic acid filtered through a Minisart N 0.45 μm membrane filter. Acetic acid was purchased from Merck (Darmstadt, Germany). Deionized water was used throughout the study. Reagents for coating the capillary were purchased from Bio-Rad Laboratories (Richmond, CA, USA), except for γ-methacryl-oxypropyltrimethoxysilane, which was obtained from Sigma. Ropivacaine and bupivacaine (Fig. 1) were kindly supplied by Astra Pain Control (Södertälje, Sweden). A racemic mixture of ropivacaine was made by mixing approximately equal amounts of the (*R*)- and (*S*)-enantiomers.

CE/MS instrumentation

A programmable injector for CE (Prince, Lauerlabs, Emmen, The Netherlands) was used. The inner surface of a fused-silica capillary (75 cm $\times$ 50 μm i.d.) was coated with non-cross-linked polyacrylamide by a procedure similar to the method developed by Hjertén.[9] Before each analysis, the CE capillary was rinsed with background electrolyte for 3 min. Acetate buffer containing methyl-β-cyclodextrin (100 mg ml^{-1}) was then injected by pressure (500 mbar), partially filling the CE capillary. This was followed by injection of 12 nl of sample by pressure (50 mbar). The sample was dissolved in water containing 10% background electrolyte to achieve stacking conditions. The inlet vial contained acetate buffer without any additives. The CE voltage during analysis was held at 30 kV. The voltage was switched off when the compounds had reached the detector after migrating through the selector solution. After the analysis, the selector was removed by rinsing with background electrolyte. The CE capillary was filled with new selector solution before the next analysis.

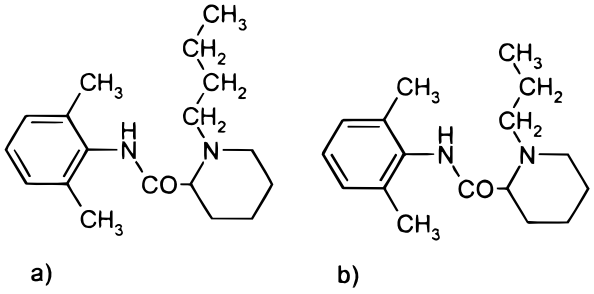

Figure 1. Structures of (a) bupivacaine and (b) ropivacaine.

Electrospray ionization was performed on a Finnigan TSQ7000 quadrupole mass spectrometer. The mass spectrometer was set in the selected reaction monitoring scan mode (MS/MS). The first mass analyser of the tandem mass spectrometer was set to transmit the parent ion mass of bupivacaine (*m/z* 288.9) or ropivacaine (*m/z* 245.0). The parent ions were dissociated by collision with Ar (1.5 mT) and the fragment ions were detected at *m/z* 140 and 125, respectively. The total scan time was 1 s and the second mass analyser was scanned over a narrow *m/z* range (0.3 units). To be sure that no selector was introduced into the ion source, the electrospray voltage was always turned off when the CE capillary was washed after an analysis.

The CE/ESMS interface used was of a co-axial capillary arrangement similar to that described by Smith *et al.*[10] The electrospray probe consisted of a fused-silica capillary (460 µm o.d., 320 μm i.d.) surrounding the CE capillary. The end of the outer capillary was drawn in a flame to obtain a tapered spray needle with a diameter of $\sim$230 µm where the CE capillary protruded. The tip of the CE capillary protruded about 0.25 mm from the opening of the outer capillary. The sheath liquid was introduced into the fused-silica capillary through a stainless-steel tee situated about 20 cm behind the probe tip. The tee also provided for the high-voltage connection with the sheath liquid. The sheath liquid was a mixture of methanol and water (50:50, v/v) to which 0.25% acetic acid was added. The flow rate was 3 µl min^{-1}. When using the partial filling technique, the sheath liquid is the only liquid that is electrosprayed into the mass spectrometer, since no liquid comes out of the CE capillary, only migrating ions.

RESULTS AND DISCUSSION

Separation of racemic mixtures of local anaesthetics

The analysis of chiral compounds using MS demands that the enantiomers are fully resolved since they have the same mass. Methyl-β-cyclodextrin is known as a useful selector for enantioseparation of local anaesthetics in CE.[7,11] Acetic acid (50 mM; pH 3.0) was chosen as the background electrolyte. Low pH is preferable since it will further decrease the electroosmotic flow and keep the polyacrylamide coating stable.

It was possible to separate a mixture containing racemic mixtures of bupivacaine and ropivacaine by filling the capillary partly with methyl-β-cyclodextrin (Fig. 2). The amount of each enantiomer injected was $\sim$620 fg (bupivacaine 2.1 fmol and ropivacaine 2.2. fmol), which corresponds to a concentration of 50 ng ml^{-1}.

To examine the length of the capillary that had to be filled with selector, different injection times of the cyclodextrin solution were investigated. In Fig. 3 the separation of ropivacaine on injecting cyclodextrin for (a) 1 min (30 cm of the capillary filled with selector) and (b) 2 min (60 cm of the capillary filled with selector) is shown. The resolution increases with increasing length of the

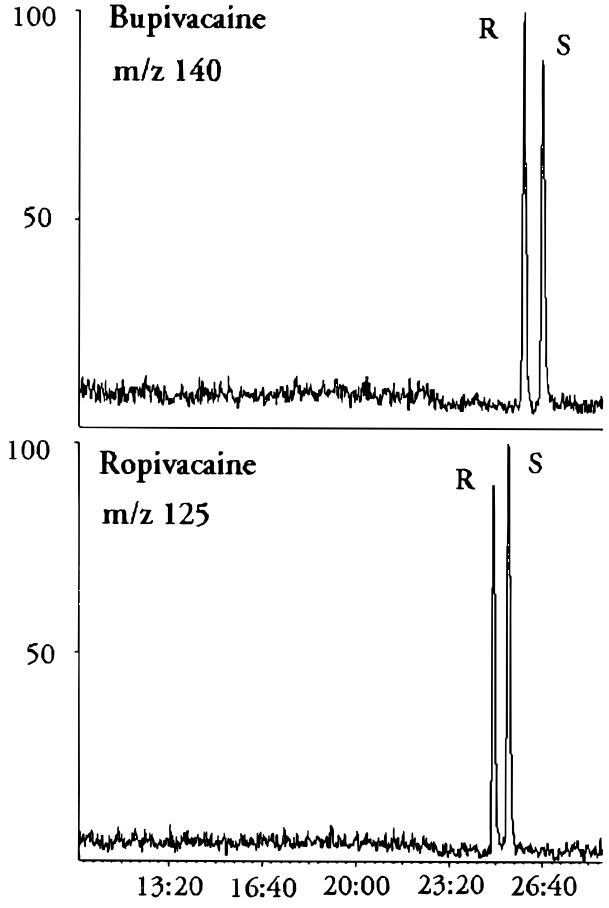

Figure 2. Mass electropherogram of the chiral separation of racemic bupivacaine and ropivacaine. The amount injected was 620 fg of each enantiomer, which corresponds to a concentration of 50 ng ml^{-1}. Methyl-β-cyclodextrin (100 mg ml^{-1}) was injected using 500 mbar pressure for 2 min.

zone containing selector. The long migration times are mostly due to the extremely low electroosmotic flow and the relatively long separation capillary needed to connect the CE instrument to the MS. The detection limits were found to be <1.1 fmol (25 ng ml^{-1}) of each enantiomer.

Separation of enantiomeric impurities

Chiral analysis of ropivacaine is of great importance since only the (*S*)-enantiomer is used when ropivacaine is given as a drug. The amount of (*R*)-enantiomer should be as low as possible. Using the same method as above it was possible to detect an impurity of 0.25% of (*R*)-ropivacaine present in (*S*)-ropivacaine (Fig. 4). The amount of (*R*)-ropivacaine injected was 310 fg (1.1 fmol) and that of (*S*)-ropivacaine 120 pg (450 fmol). About 66 cm of the capillary was filled with cyclodextrin.

Using a longer CE capillary makes it possible to increase further the separation between the enantiomers since the amount of cyclodextrin can be increased. This will be useful when even lower impurities of the (*R*)-enantiomer are to be determined and higher concentrations of the (*S*)-enantiomer must be injected. Increasing the amount of (*S*)-ropivacaine makes the peak wider

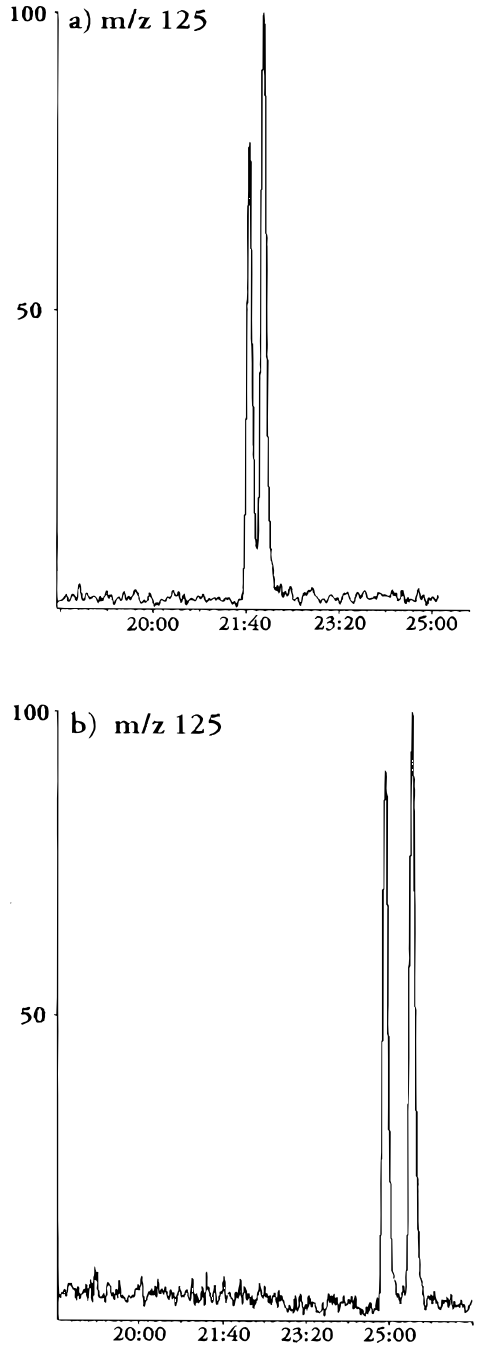

Figure 3. Mass electropherogram showing the effect of different amounts of cyclodextrin on the resolution. Methyl-β-cyclodextrin (100 mg ml^{-1}) was injected using 500 mbar pressure for (a) 1 and (b) 2 min. The amount of ropivacaine injected was 620 fg (2.2 fmol) of each enantiomer.

and it will tend to overlap the small (*R*)-enantiomer peak if the separation is not increased.

Partial filling technique

We used cyclodextrin as a chiral selector. However, previous studies have shown that introduction of large amounts of cyclodextrin into the mass spectrometer gave an increased noise level and unstable operation.[2] During this study we observed no decrease in sensitivity or increased noise level and no traces of cyclodextrin

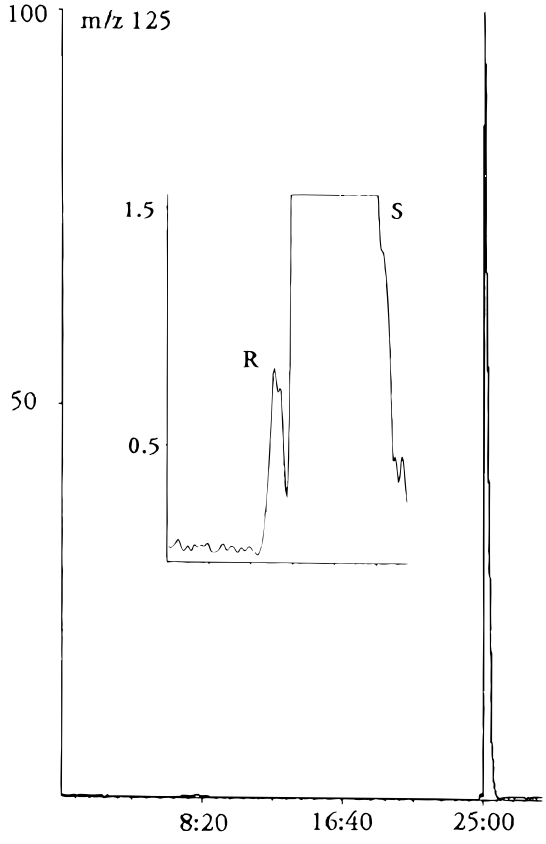

Figure 4. Mass electropherogram of the chiral separation of a mixture containing 0.25% of (*R*)-ropivacaine in (*S*)-ropivacaine. The amount of (*R*)-ropivacaine injected was 310 fg (1.1 fmol) and that of (*S*)-ropivacaine 120 pg (450 fmol). Methyl-β-cyclodextrin (100 mg ml^{-1}) was injected for 2.2 min by pressure (500 mbar), filling approximately 66 cm of the CE capillary.

could be seen when scanning in the higher mass region. This confirms that the partial filling technique is a suitable procedure to avoid the introduction of non-volatile selectors from the CE capillary into the mass spectrometer.

The partial filling technique has other advantages, e.g. its simplicity, no extra modifications of either the CE or MS equipment are necessary and the method is easy to automate. Also, the consumption of the often expensive selectors is extremely low since the inlet vial does not have to contain any selector, only pure buffer. For example, using the partial filling technique it is possible to perform 7300 analyses from 1 g of selector compared with only 50 analyses using a normal CE method without partial filling. The separation can easily be regulated by changing the length of the selector zone and the selector concentration, i.e. the amount of selector is the main parameter.[12] The method can be used for other purposes besides chiral separations and MEKC, such as affinity-CE/MS for protein[13] and receptor[14] binding studies and enzymatic microreactors in CE/MS. The drawbacks are often the long migration times, due to the low electroosmosis, and the fact that only charged compounds can be analysed.

Acknowledgements

This study was supported by the Swedish Medical Research Council, grant No. 11565, and the Thuring Foundation. We thank Salumeh Bastami for coating the capillaries.

REFERENCES

1. M. H. Lamoree, U. R. Tjaden and J. van der Greef, *J. Chromatogr.* **712**, 219 (1995).
2. M. H. Lamoree, A. F. H. Sprang, U. R. Tjaden and J. van der Greef, *J. Chromatogr.* **742**, 235 (1996).
3. R. L. Sheppard, X. Tong, J. Cai and J. D. Henion, *Anal. Chem.* **67**, 2054 (1995).
4. L. Valtcheva, J. Mohammad, G. Pettersson and S. Hjertén. *J. Chromatogr.* **638**, 263 (1993).
5. Y. Tanaka and S. Terabe, *J. Chromatogr.* **694**, 277 (1995).
6. K. Koezuka, H. Ozaki, N. Matsubara and S. Terabe, *J. Chromatogr. B* **689**, 3 (1997).
7. A. Amini and U. Paulsen-Sörman, *Electrophoresis* **18**, 1019 (1997).
8. T. J. Ward, C. Dann, III and A. P. Brown, *Chirality* **8**, 77 (1996).
9. S. Hjertén, *J. Chromatogr.* **347**, 191 (1985).
10. R. D. Smith, C. J. Barinaga and H. R. Udseth, *Anal. Chem.* **60**, 1948 (1988).
11. C. Sänger-van de Griend, K. Gröningsson and D. Westerlund, *Chromatographia* **40**, 263 (1996).
12. A. Amini, C. Pettersson and D. Westerlund, *Electrophoresis* **18**, 950 (1997).
13. A. Amini and D. Westerlund, *Anal. Chem.*, accepted.
14. Y.-H. Chu, D. Kirby and B. Karger, *J. Am. Chem. Soc.* **117**, 5419 (1995).