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Short communication

Chiral capillary electrophoresis–electrospray mass spectrometry coupling with charged cyclodextrin derivatives as chiral selectors

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Abstract

Capillary electrophoresis–electrospray mass spectrometry (CE–ESI-MS) coupling using charged cyclodextrin (CD) derivatives as chiral selectors is described in this study. The self electrophoretic mobility of charged CDs in opposite direction to the analyte allows avoidance of the contamination of the ion source of ESI-MS with the CDs of the separation buffer in a simple way. The usefulness of this technique is illustrated for the CE separation and on-line MS detection of chiral pharmaceuticals. © 1998 Elsevier Science B.V.

Keywords: Capillary electrophoresis–mass spectrometry; Buffer composition; Enantiomer separation; Cyclodextrins, charged

1. Introduction

Capillary electrophoresis–mass spectrometry (CE–MS) coupling becomes increasingly important for the analysis of samples of a complex origin (biopharmaceutics, clinical, forensic, environmental samples, etc.). CE–MS combines the high separation efficiency of CE with the high sensitivity and informativeness of MS. The latter is especially important in CE where minute samples are analyzed and even a micropreparative fraction-collection for further analysis is exceptionally difficult and time-consuming.

The first on-line CE–MS coupling was reported by Olivares et al. [1]. Several review papers summarize the advantages and problems in this field [2–5]. Among various MS techniques electrospray ionization (ESI)-MS [6–8] seems to be one of the most suitable detection methods for CE [1,9–13].

Chiral separations belong to one of the important topics of CE applications [14–16]. Research efforts so far have been focused mainly on achieving and optimizing separations. Aspects of detection sensitivity, peak purity and identification were less addressed. However, these aspects become especially important when chiral CE is increasingly used for the analysis of samples of complex bioanalytical, clinical, environmental or forensic origin. Recently, an application of chiral CE–MS coupling has been reported for the analysis of chiral basic drugs terbutaline and ephedrine [17] as well as ropivacaine [18].

A problem in the coupling of high-performance liquid chromatography or CE with MS is a contamination of the ion source of MS with constituents of mobile phase or buffer. This problem is generally less severe in CE due to extremely low amounts of effluents [17]. Additionally, for some applications CE buffers can be selected in a way that they do not

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contain nonvolatile components (for instance formate, ammonium acetate, etc.). However, such manipulation is limited in chiral CE where a chiral selector is a necessary component of the background electrolyte. The chiral selector which appears in the ion source of the MS may compete with the analyte for an available charge. This results in a decrease of the sensitivity of the MS [17] as well as an increase in the baseline noise [18]. Sheppard et al. [17] consider the appearance of a small amount of CD-type chiral selectors in the ion source of ion-spray MS as not critical. However, Lamoree et al. [18] showed a detrimental effect of CD appearance in the ion source of ESI-MS. Thus, the use of a capillary connection and triple voltage switching has been proposed in order to avoid an appearance of CD in the ion source of MS. Another disadvantage of this technique is that a chiral selector must necessarily migrate behind the analyte. Otherwise it will be difficult to avoid the appearance of a chiral selector in the ion source of the MS system. This requirement limits the application of this technique.

In the present work a chiral CE–ESI-MS coupling is reported with charged CDs as chiral selectors. The use of a counter-migration principle of analyte and selector which has been previously reported for charged CDs [16,19] avoids in a simple way an appearance of a chiral selector in the ion source of the MS.

2. Experimental

2.1. Chemicals and reagents

Racemic tropic acid, chlorpheniramine maleate and mianserine hydrochloride were obtained from Sigma (Daisenhofen, Germany). Racemic dimethindene maleate was a gift from Zyma (Munich, Germany) and racemic etilefrine hydrochloride was a gift from Boehringer Ingelheim (Ingelheim, Germany). Stock solutions of 1 mg/ml of the racemic solutes were prepared in methanol, stored at 4°C and diluted to 50 μg/ml before use.

Acetic acid, ammonium acetate and methanol were from Merck (Darmstadt, Germany). Methacrylic acid 3-trimethoxysilylpropyl ester, tris-(hydroxymethyl)aminomethane, boric acid, ethylenediaminetetraacetic acid, acrylamide, sodium persulfate and N,N,N′,N′-tetramethylethylenediamine, all used for the preparation of polyacrylamide-coated capillaries [20], were purchased from Fluka (Buchs, Switzerland). Carboxymethyl ether of β-cyclodextrin (CM-β-CD) with an average substitution degree of 3.5 and 2-hydroxypropyltrimethylammonium salt of β-CD (TMA-β-CD) with an average substitution degree of 3.5 were gifts from Wacker Chemie (Munich, Germany). Sulfobutyl ether of β-CD (SBE-β-CD) with an average substitution degree of 4.0 was a gift from Professor J.F. Stobaugh (Center for Drug Delivery Research, University of Kansas, Lawrence, KS, USA).

2.2. CE

CE separations with UV detection (CE–UV) were carried out in a fused-silica capillary of 75 cm total and 60 cm effective length using a Grom capillary electrophoresis system 100 (Herrenberg, Germany). A 10 m*M* acetic acid–ammonium acetate buffer, pH 3.5 containing 0.2 mg/ml CM-β-CD was used as separation medium. The applied voltage was 30 kV. The samples were introduced hydrostatically (10 cm) during 5–10 s at the anodic end of the capillary and detected at the cathodic end of the capillary at 210 nm.

CE separations of the basic analytes with MS detection (CE–MS) were performed in a fused-silica capillary of 44 cm×50 μm I.D. using 10 m*M* acetic acid–ammonium acetate buffer, pH 3.5. A voltage of 20 kV was applied by a Grom capillary electrophoresis system 100 high voltage supply. The current generated was 2–5 μA and 8–20 μA in achiral and chiral runs respectively. The samples were introduced hydrostatically in the same way as described for CE–UV above. The cathodic end of the separation capillary was protruded to the ion-spray tip where a voltage of 2.6 kV was maintained.

The acidic analyte tropic acid was resolved to the enantiomers in a polyacrylamide-coated capillary of the same dimensions as for the cationic compounds. The coated capillary was used in order to avoid an adsorption of TMA-β-CD on the fused-silica capillary wall and consequent generation of a strong anodic electroosmotic flow (EOF) [21]. A 10 m*M* acetic acid–ammonium acetate buffer, pH 5.0, was

used. The sample was introduced at the cathodic end of the capillary and the CE voltage was 16 kV. The voltage on the ion-spray tip was −2.6 kV.

For MS detection a LCQ spectrometer (Finnigan, Branford, CT, USA) equipped with an electrospray interface was used in the positive ion mode for the detection of the basic compounds and in the negative ion mode for the detection of the tropic acid. All electropherograms were detected in a full scan mode and plotted as the single ion tracks. A sheath liquid [methanol–water–acetic acid (50:49:1) for the basic compounds and methanol–water–ammonia (50:48:2) for the tropic acid] was delivered at a flow-rate of 6.0 μl/min with a syringe pump.

3. Results and discussion

The counter-migration separation mode (Fig. 1) relies on the opposite direction of migration of an analyte and a chiral selector. The first chiral separation with negatively charged SBE-β-CD as a chiral selector involving this mechanism has been described in Ref. [19]. This technique seems to be especially useful in those separations where an appearance of any chiral selector at the point of detection must be avoided due to possible signal interference or contamination of a detector. The separation of the enantiomers of the sympathomimetic drug etilefrine with 3 mg/ml CM-β-CD is shown in Fig. 2. A baseline separation with sharp peaks (N_1=112 000/m, N_2=87 000/m) could be observed within 5 min. No significant additional band-broadening due to the extra capillary CE–MS connection or due to disturbance of flow profile or any baseline problems were observed either before or after detection of the analyte peaks.

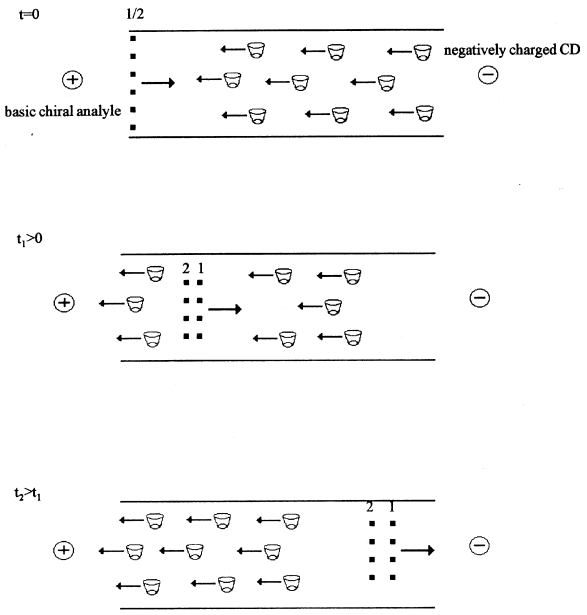

Fig. 1. Schematic representation of the counter-migration principle of a basic analyte and a negatively charged CD.

Relatively low peak efficiencies have been observed for some analytes in CE–ESI-MS. In order to study a possible contribution of CE–ESI-MS coupling to band-broadening in more detail the separation of the mianserine enantiomers was performed in CE–MS and in CE–UV. As shown in Fig. 3 no significant difference was observed between CE–MS and CE–UV. In particular the plate numbers were N_1=11 000/m, N_2=9000/m and N_1=9300/m, N_2=4830/m in CE–MS and CE–UV, respectively.

The enantioseparations of other two basic drugs are shown in Fig. 4. CM-β-CD in a concentration of 0.2 mg/ml was used as a chiral selector in both cases. Almost baseline or good baseline separations were obtained in both cases with acceptable peak efficiencies. No baseline disturbances were observed due to penetration of CM-β-CD in the ion-spray source of MS.

To verify the suitability of other charged CDs in the same CE–ESI-MS mode the separation of mianserine enantiomers has been performed also with SBE-β-CD. The peaks were somewhat broader but still of acceptable efficiency with this chiral selector (Fig. 5). SBE-β-CD was used in two times lower w/v (0.1 mg/ml) concentration compared with CM-β-CD.

An important requirement for a successful use of the technique proposed in Ref. [18] is that a chiral analyte has a higher apparent mobility compared to the selector. This strictly limits the application of this technique with neutral chiral selectors and anionic analytes. However, the counter-migration technique proposed in this study can also be used for anionic chiral compounds (Fig. 6). The enantioseparation of tropic acid was performed using 8.0 mg/ml TMA-β-

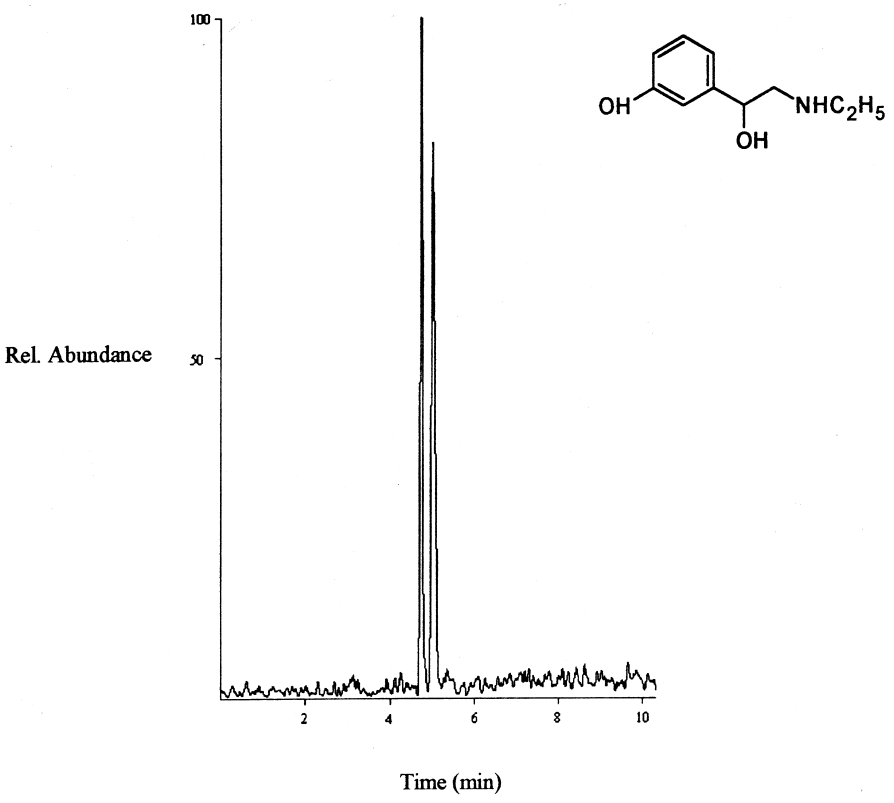

Fig. 2. CE–ESI-MS electropherogram of (±)-etilefrine plotted in the single ion track (m/z=181.7–182.7) mode with 3 mg/ml CM-β-CD as a chiral selector (pH 4.3). Other separation and detection conditions as in the text.

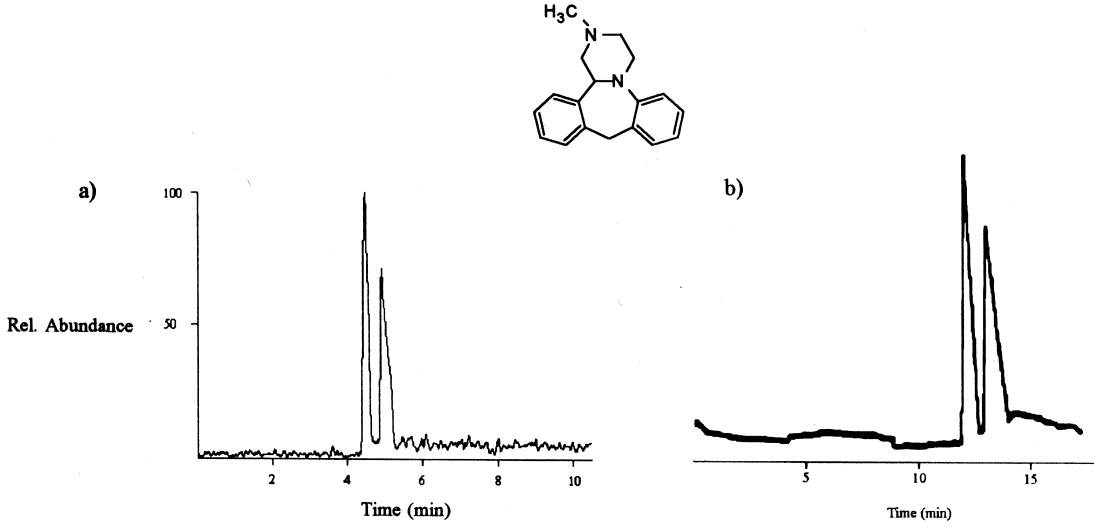

Fig. 3. CE–ESI-MS electropherogram of (±)-mianserine plotted in the single ion track (m/z=264.9–265.9) mode (a) and CE–UV electropherogram (b) with 0.2 mg/ml CM-β-CD as a chiral selector. Other separation and detection conditions as in the text.

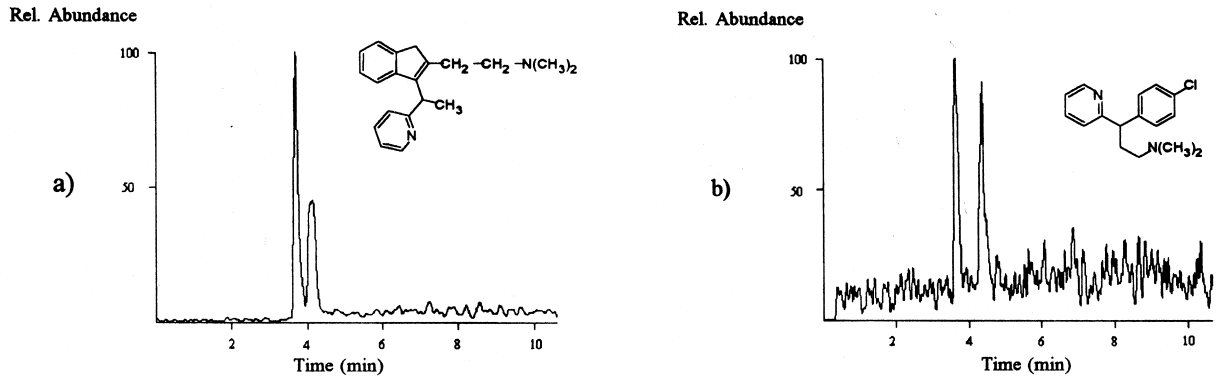

Fig. 4. CE–ESI-MS electropherogram of (±)-dimethindene plotted in the single ion track (m/z=292.5–293.5) mode (a) and (±)-chlorpheniramine (m/z=275.3–276.3) (b) with 0.2 mg/ml CM-β-CD as a chiral selector. Other separation and detection conditions as in the text.

CD as the chiral selector in a polyacrylamide-coated silica capillary in this case.

One important advantage of MS detection is its potential in peak purity testing and identification. Fig. 7 represents a full mass scan of the chiral separation of the dimethindene enantiomers (Fig. 4a) in the mass range m/z=50–1850. The results confirm that both peaks are enantiomers of dimethindene.

It seems also possible to avoid the negative effect of CDs in CE–MS coupling by working with low pH buffers with diminished EOF. However, as reported in Ref. [18] this problem was significant even in buffers with a pH as low as 2.85. Additionally, high pH buffers are favorable for certain chiral separations in CE. Another alternative could be the use of coated capillaries without an EOF. However, the neutral analytes will remain outside the scope of CE–MS coupling in this case.

In conclusion, chiral CE–ESI-MS coupling seems to be an attractive technique and may substantially broaden the application potentials of chiral CE in biopharmaceutical and clinical analysis as well as for samples of environmental and forensic interest. The counter-migration principle allows avoidance in a

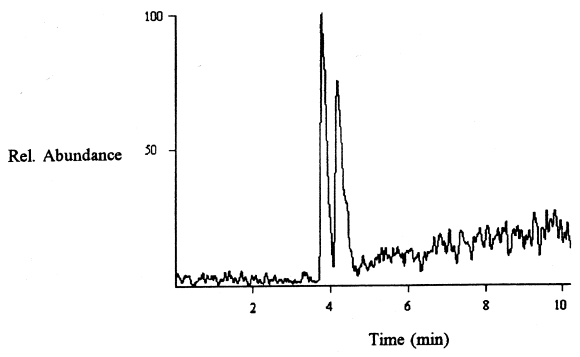

Fig. 5. CE–ESI-MS electropherogram of (±)-mianserine plotted in the single ion track (m/z=264.9–265.9) mode with 0.1 mg/ml SBE-β-CD as a chiral selector. Other separation and detection conditions as in the text.

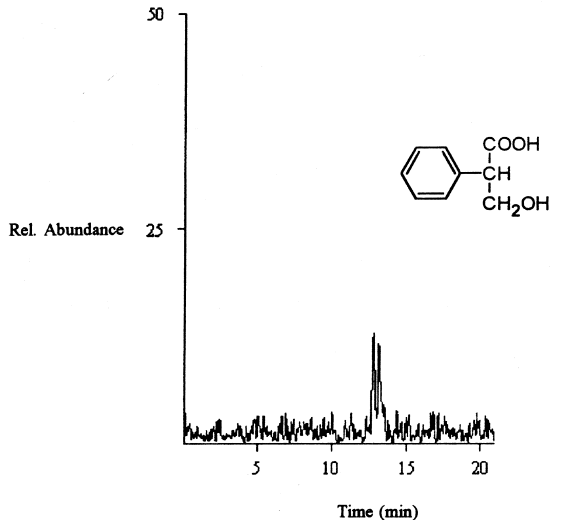

Fig. 6. CE–ESI-MS electropherogram of (±)-tropic acid plotted in the single ion track (m/z=164.7–165.7) mode with 8 mg/ml TMA-β-CD as a chiral selector. Other separation and detection conditions as in the text.

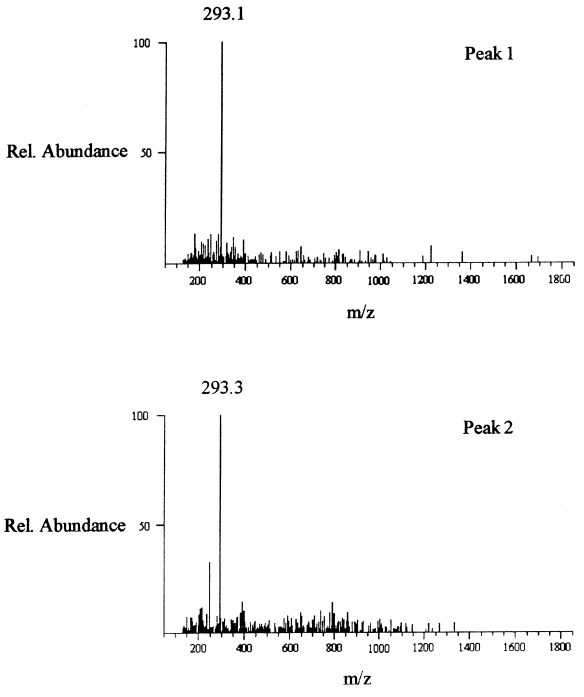

Fig. 7. Full-scan ESI-MS spectra of peaks 1 and 2 of the electropherogram in Fig. 4a.

simple way of any negative effects of chiral selector appearance in the MS. This technique can be applied to other charged chiral selectors such as macrocyclic antibiotics, charged chiral micelles, peptides and charged polymers.

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