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# Use of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin in on-line capillary zone electrophoresis-mass spectrometry for the chiral separation of ropivacaine

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**Abstract**

The on-line coupling of chiral capillary zone electrophoresis (CZE) with mass spectrometry (MS) is described for the chiral separation of the basic drug ropivacaine using a coupled capillary system with the possibility of voltage switching. With this set-up, the introduction into the MS of the chiral selector heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) that has a detrimental effect on MS performance is avoided. In combination with MS detection, this coupled capillary set-up can be regarded as universally applicable for all CZE separations where the use of buffer additives that have negative influence on the MS is necessary.

*Keywords:* Mass spectrometry; Enantiomer separation; Chiral selectors; Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; Ropivacaine; Cyclodextrins

## 1. Introduction

Since it has been recognised that the enantiomers of a racemic drug display differences in pharmacological activity and toxicity, high demands have been placed on chiral purity control during various stages of development and manufacturing of these drugs. Due to the similarity of the physico-chemical properties of the enantiomers of a racemic compound, chiral separations are still a great challenge in analytical chemistry. High-performance liquid chromatography (HPLC) has been frequently applied because of the polarity and involatility of many drugs, but with the establishment of electromigration techniques that are capable of performing separations with high efficiency, more and more attention has been directed toward the field of capillary zone electrophoresis for chiral separations [1–5].

To obtain separation of the (*R*)- and (*S*)-enantiomer of a racemic compound by capillary zone electrophoresis, various buffer additives acting as chiral selectors have been described in literature, for example chiral micelles [6–8], proteins [9,10], crown ethers [11] and the most widely used cyclodextrins [12–15]. Lately, a lot of attention has been paid to the development of modifications of cyclodextrins, with the aim of selectivity enhancement as compared to the original cyclodextrins. The use of cyclodextrin

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polymers has been reported [16–18], just as the application of charged cyclodextrins [19–21].

For detection purposes in chiral separations, it would be ideal to be able to use mass spectrometry, for the reason that the identical masses of analytes that are just resolved in a chiral separation system are a very reliable indication of the presence of two enantiomeric compounds. In addition, the coupling of capillary zone electrophoresis to electrospray mass spectrometry has shown to be a very promising tool in analytical chemistry [22]. However, for capillary zone electrophoresis in combination with mass spectrometric detection, there is only a limited range of buffer systems that can be applied, because of the negative influence of the buffer additives on MS performance. To circumvent any problem inherent to the introduction of involatile buffer additives into the mass spectrometer, the design of a separation system capable of discarding specific buffer additives such as the cyclodextrins before mass spectrometric detection is advisable.

In this paper a coupled capillary system is described which has the potential of achieving chiral capillary zone electrophoretic separation of the basic drug ropivacaine with mass spectrometric detection, without the negative influence of essential buffer components on MS detection. This research has been realised in analogy to the on-line coupling of micellar electrokinetic chromatography (MEKC) to electrospray mass spectrometry reported earlier [23].

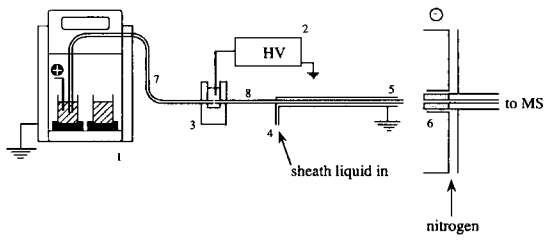


Fig. 1. Schematic representation of the experimental set-up for chiral CZE with MS detection: (1) programmable injector for capillary electrophoresis with internal high voltage power supply; (2) external high voltage power supply; (3) Plexiglass connection vial with electrode connection; (4) inlet for the sheath liquid; (5) grounded electrospray needle; (6) electrospray sampling capillary; (7) capillary for chiral CZE; (8) capillary for separation of the analytes from the chiral selector and transfer of the analytes to the electrospray tip.

## 2. Experimental

### 2.1. Experimental set-up

The experimental set-up is schematically represented in Fig. 1. For electrokinetic injection and power supply at the inlet of the chiral separation capillary a programmable injector for capillary electrophoresis (Prince Technologies, Emmen, the Netherlands) was used. For chiral separation and transfer of the analytes to the mass spectrometer, two 100 $\mu$m I.D., 170 $\mu$m O.D. capillaries (BGB Analytik, Zurich, Switzerland) were carefully aligned with their openings placed opposite each other in a connection vial with a small (0.5 ml) volume, thus creating an open liquid junction. The gap between the two capillaries was about 50 $\mu$m. The connection vial had an optional electrode connection to a Spellman CZE 1000R power supply (Plainview, NY, USA). The separation and the transfer capillary had a length of 500 mm and 400 mm respectively. The connection vial (see Fig. 2) consisted of two cylindrical pieces of Plexiglass and two silicon rings. The two cylinders could be screwed together with the silicon rings in between, while a hole (5 mm diameter) drilled in the upper and partly in the lower cylinder served as the chamber containing the liquid junction. The capillaries were placed between the silicon rings while the

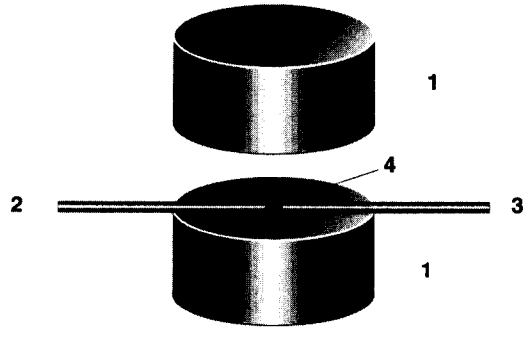


Fig. 2. Detail of the schematic representation of the connection vial with the coupled capillaries: (1) cylindrical piece of plexiglass; (2) first capillary for chiral separation; (3) second capillary for transfer of the analytes to the electrospray tip; (4) electrode which is placed slightly out of center in order to prevent interruption of the electric field lines due to bubble formation.

two cylinders were screwed together. The capillaries are held aligned by means of a 3 mm piece of PTFE tubing (not shown in Fig. 2) in which the capillaries are inserted. The PTFE tubing is perforated at the location of the liquid junction to ensure free buffer contact. This connection vial design is slightly different from the design reported for the coupling of MEKC with MS [23].

For mass spectrometric detection, an SSQ 710 single-quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA), equipped with an electrospray interface (Analytica, Branford, CT, USA) was used in the positive ion mode. Electrical contact at the tip of the electrospray needle was established via a sheath liquid, delivered at a flow-rate of 2.2 $\mu$l min$^{-1}$ by a Model 2400 syringe pump (Harvard Apparatus, Edinbridge, UK). Nitrogen was used as drying gas. The electrospray tip was held at ground potential, while the electrospray counterelectrode was set at −3.4 kV. The $[M+H]^+$ ion corresponding to the protonated analyte ($m/z$ 275.2) was monitored in the single ion detection mode.

### 2.2. Chiral capillary zone electrophoresis and electrophoretic transfer of the analytes to the MS

Chiral separation of the analyte was achieved using a buffer that consisted of formic acid (1 m*M*, pH 2.85), to which heptakis(2,6-di-*O*-methyl)-$\beta$-cyclodextrin (50 m*M*) was added as the chiral selector. These buffer conditions were derived from a chiral separation system for several vacaines described by Sänger [24], where UV detection was applied. For our purposes, we carried out preliminary UV experiments with a Spectra 100 UV–Vis absorbance detector (Spectra-Physics, Mount View, CA, USA) at a wavelength of 208 nm.

The buffer used for the electrophoretic transfer of the analytes to the mass spectrometer consisted of 1 m*M* formic acid (pH 2.85). In this buffer no organic modifier has been employed. The sheath liquid was a mixture of formic acid (1 m*M*, pH 2.85) and acetonitrile (1:3, v/v).

### 2.3. Chemicals

Pure (*R*)- and (*S*)-ropivacaine hydrochloride monohydrate (chemical structure represented in Fig. 3) were kindly provided by ASTRA Pain Control (Södertälje, Sweden). The chiral selector heptakis(2,6-di-*O*-methyl)-$\beta$-cyclodextrin (DM-$\beta$-CD) was obtained from Avebe (Foxhol, Netherlands). Formic acid and acetonitrile were purchased from Baker (Deventer, Netherlands). Throughout the experiments, deionized water was used, obtained with a Milli-Q system (Millipore, Bedford, MA, USA). The samples were dissolved in 1 m*M* formic acid adjusted to pH 2.85.

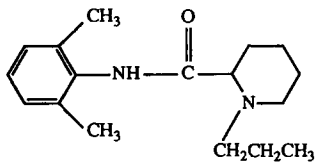


Fig. 3. Chemical structure of ropivacaine.

## 3. Results and discussion

For the coupling of a chiral CZE separation system to the mass spectrometer, a strategy was developed to avoid introduction of cyclodextrin that is essential to achieve the desired separation but simultaneously creates problems when sprayed directly into the MS. Analogous to the coupling of micellar electrokinetic chromatography to mass spectrometry [23] a coupled capillary set-up with the possibility of voltage switching during the various stages of the separation has been developed. With this system, the whole analysis can be subdivided into three separate steps. In the first step, the chiral separation of the enantiomers is effected in the first capillary (see Fig. 1), while in the second step a zone that has been heartcut from the first capillary migrates into the second capillary. The third step can be regarded as the transfer of the analytes to the electrospray tip for introduction into the mass spectrometer, while at the same time the cyclodextrin used as the chiral selector is separated from the enantiomers due to their differences in charge.

In Table 1, the consecutive actions in the voltage switching procedure are outlined for each stage of the analysis. The inlet vial and the first capillary are filled with formic acid (1 m*M*, pH 2.85) containing 50 m*M* DB-$\beta$-CD, while the connection vial and the

Table 1
Time intervals

| | Inlet vial | Connection vial | Electrospray tip | Time interval (min) |
|---|---|---|---|---|
| Chiral separation | + 8 kV | −2 kV | ground | 0–14 |
| Heartcut | +15 kV | floating | ground | 14–18 |
| Transfer | ground | +5 kV | ground | >18 |

second capillary are filled with a buffer that consists only of formic acid (1 m*M*, pH 2.85). It should be noted that during the voltage switching procedure, no buffer switching in the connection vial takes place, which is a simplification compared to the more complicated procedure used for the coupling of MEKC to MS [23].

After injection of the sample, which is done electrokinetically during 6 s at 5 kV with the connection vial held at ground potential, the chiral separation in the first capillary is started by applying the voltages to the vials as stated in Table 1. During this step, no chiral separation buffer enters the second capillary because of the potential difference between the connection vial and the electrospray tip. When after 14 min the zone that contains the enantiomers has reached the end of the chiral separation capillary, the voltages on the inlet and connection vial are switched off, and the connection vial is physically disconnected from the power supply, so that no electrical split occurs during the introduction of the heartcut zone of interest into the second capillary. The voltage on the inlet vial is switched on again, and the zone containing the analytes and the DM-$\beta$-CD migrates into the second capillary. When after 4 min the whole zone of interest has entered the second capillary, the voltage on the inlet vial is switched off, and a positive voltage is applied to the connection vial. Under the influence of the applied electric field, the resolved analytes are separated from the DM-$\beta$-CD due to the differences in charge. The positively charged enantiomers reach the electrospray tip first and are sprayed for MS detection. After detection of the enantiomers, the analysis is stopped in order to prevent introduction of DM-$\beta$-CD into the MS.

A factor worth considering is the composition of the buffer in the second capillary and the composition of the sheath liquid. Throughout the experiments a totally aqueous buffer was used for transfer of the enantiomers to the electrospray tip. Initially, methanol was chosen as the organic modifier in the sheath liquid, but problems arose with regard to the maintenance of the electrical contact at the spray tip. This phenomenon was attributed to bubble formation that occurred as a result of the heat that was generated during the exothermic mixing of the aqueous buffer with the methanol. Therefore, acetonitrile showing endothermic mixing was chosen as the organic modifier in the sheath liquid, and bubble formation at the spraytip was not observed any more.

Furthermore, due to the difference in potential between the electrospray tip and the connection vial during the chiral separation, an electroosmotic flow in the direction of the connection vial is established between these two, resulting in introduction of the acetonitrile containing sheath liquid into the second capillary. Because of changes in the potential related to the presence of acetonitrile, the neutral cyclodextrin and the charged enantiomers may exhibit short migration times in the transfer step.

In Fig. 4, the mass electropherogram that is obtained after chiral separation of a 1:1 mixture of (*R*)- and (*S*)-ropivacaine according to the procedure described above is represented. Mass spectrometric data acquisition is started simultaneously with the chiral separation in the first capillary. The injected amount was 230 pg of each enantiomer. The noise at $t=19$ min that is observed in the mass electropherogram arises from the switching of the voltages from the inlet vial to the connection vial. As shown, mass spectrometric detection of the two enantiomers is possible with good resolution, which opens up opportunities for monitoring the enantiomeric purity during drug development and manufacturing.

Clearly, the timing of the events requires some caution in the sense that analytes will be lost if no attention is paid to that aspect. For example, if the

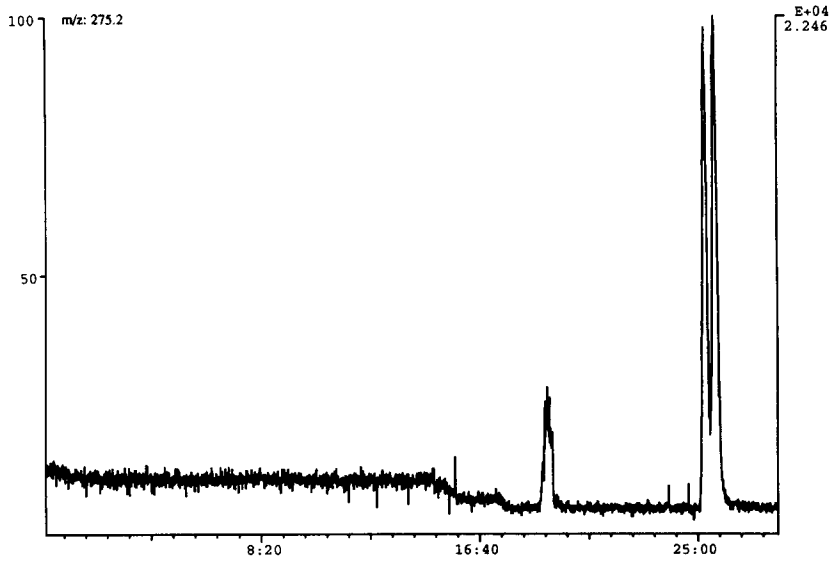


Fig. 4. Mass electropherogram obtained after chiral separation of ropivacaine according to the procedure described in the text. The injected amount of each enantiomer was 230 pg.

chiral separation in the first capillary is carried out for too long, the analytes will migrate towards the electrode at earth in the connection vial and are lost for introduction into the second capillary, so that finally no analytes will be detected. The second event, which is the introduction of the heartcut from the first into the second capillary, is less critical with regard to the timing. The time for heartcutting the zone of interest has to be long enough to ensure migration of the enantiomers into the second capillary. Practically, once the enantiomers have entered the second capillary, the voltage is applied over the transfer capillary only with an electrode in the connection vial, in order to minimize the amount of chiral selector introduced into the second capillary. Very important for the coupling of chiral CZE to MS by means of a coupled capillary set-up is the necessity to stop the analysis after detection of the enantiomers has taken place. If this is ignored, the neutral DM-$\beta$-CD reaches the electrospray tip on the electroosmotic flow, and will be introduced into the MS.

In Fig. 5 the mass electropherogram that is obtained after chiral separation of a 1:3 mixture of (*R*)- and (*S*)- ropivacaine is shown. The injected amount of (*S*)-ropivacaine was 230 pg. The noise at $t=19$ min was ascribed to voltage switching. It can be concluded from this figure, where the analysis is not stopped after detection of the enantiomers, that the introduction of the chiral selector seriously hampers mass spectrometric detection of any compound. This can be concluded from the increased noise level that is observed even when a single ion corresponding to the enantiomers is monitored. The increased noise level is probably due to electrospray instability arising from the presence of an excess of cyclodextrin. After this experiment, the electrospray interface had to be cleaned thoroughly, because no successful measurements could be carried out any more. The end of the electrospray sampling capillary and the tubelens were covered with a layer of DM-$\beta$-CD. From this observation, it is clear that direct coupling of chiral CZE to MS with the introduction of a constant flow of cyclodextrin in the CZE effluent is likely to raise problems and that this is not advisable for long term stable operation of the mass spectrometer. However, direct coupling of chiral CZE to MS has also been described using ionspray with a different MS setup [25].

Additionally, caution is required when heartcutting

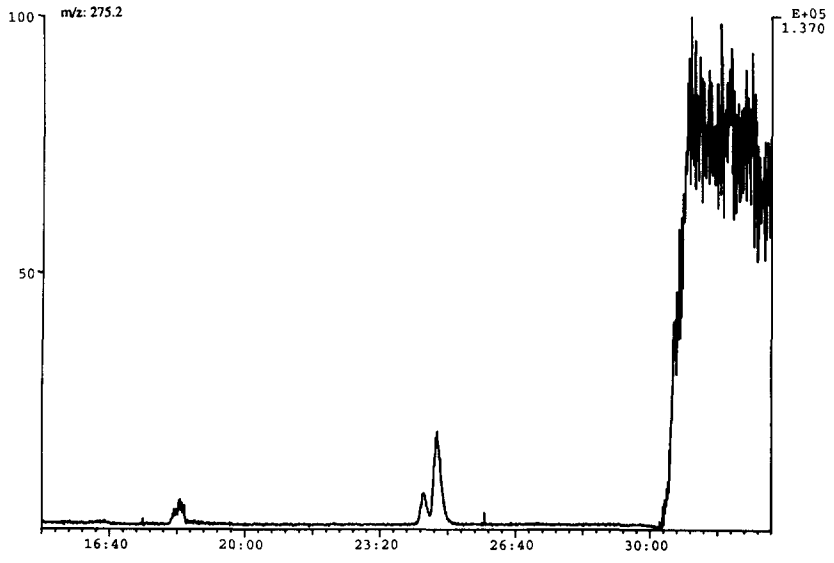


Fig. 5. Mass electropherogram obtained after chiral separation of a 1:3 mixture of (*R*)- and (*S*)-ropivacaine. The injected amount of (*S*)-ropivacaine was 230 pg. The analysis was not stopped directly after detection of the enantiomers, which resulted in an increased noise level that was presumably caused by the entry of DM-β-CD into the MS.

a very large zone from the chiral separation capillary and introducing it into the second capillary for transfer to the MS. When the analytes at the back of the zone migrate too slowly to overtake the cyclodextrin at the front of the zone, problems with detection as described above may be expected. One way to avoid problems of this kind is the use of (negatively) charged cyclodextrins [19–21], that have a lower migration velocity than the neutral cyclodextrins and therefore offer a longer time for enantiomer detection.

In the pharmaceutical field, chiral analysis is of great importance especially during manufacturing, purification and quality control of pharmaceutical compounds. Thus, apart from sensitivity, the ability of the chiral CZE–MS system to distinguish between the (*R*)- and (*S*)-enantiomer of a compound present in different concentrations should be considered. In Fig. 6 the mass electropherogram of (*R*)- and (*S*)-ropivacaine present in a ratio of 1:20 is shown. For routine use in chiral compound analysis, this ratio does not suffice at present, because for this purpose ratios of 1:100 should be obtained. However, with this system it can be deduced from the mass electropherogram that a ratio of 1:50 can be distinguished, which offers a good starting point if further optimisation of the system is needed.

## 4. Conclusions

With the coupled capillary system which was developed analogous to the MEKC–MS system described earlier [23] we were able to perform the chiral separation of ropivacaine with subsequent MS detection of the enantiomers. Obviously, the coupling of a chiral CZE system to mass spectrometry offers great opportunities in chiral analysis, because of the requirement of mass spectrometric detection in chiral separations paired to the necessity of enantiomeric separation for discrimination between signals of (*R*)- and (*S*)-enantiomers in mass spectrometry.

The experimental set-up used for this research was essentially the same as that used for the coupling of MEKC with MS. Therefore, the system can be regarded as universally applicable for CZE sepa-

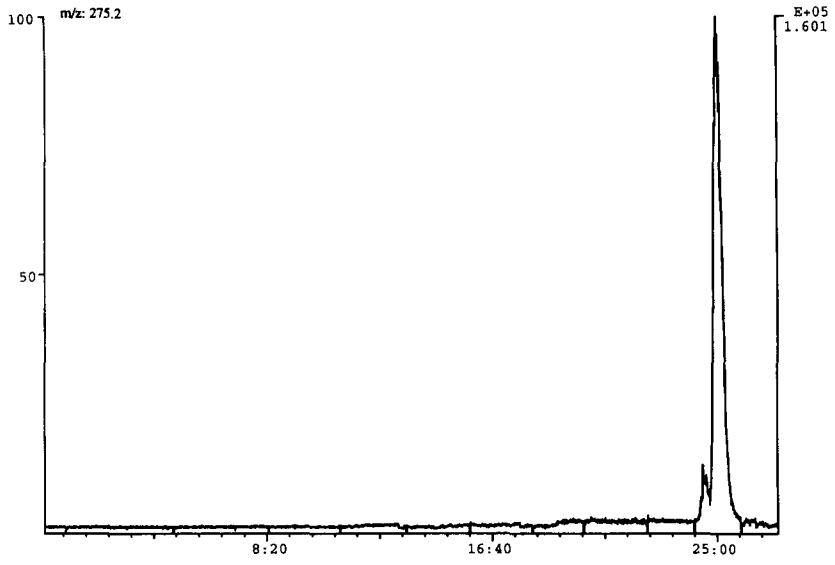


Fig. 6. Mass electropherogram obtained after chiral separation of a 1:20 mixture of (*R*)- and (*S*)-ropivacaine. The injected amount of (*S*)-ropivacaine was 230 pg.

rations where buffer additives that have a detrimental effect on the mass spectrometer's performance have to be used in order to obtain a proper separation, such as MEKC and chiral CZE. The coupled capillary set-up combined with appropriate voltage switching procedures opens up possibilities for all kinds of complicated buffer systems that are necessary for difficult separations.

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