

SEPARATION OF TRAMADOL ENANTIOMERS BY CAPILLARY ELECTROPHORESIS USING HIGHLY SULFATED CYCLODEXTRINS

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ABSTRACT

In the pharmaceutical industry a continuing need for chiral resolution of drugs for various purposes and in diverse matrices exist. For these reasons, analysts may require a number of different separation systems capable of resolving a given pair of enantiomers. Highly sulfated cyclodextrins (HS-CDs) represent a relatively new class of chiral selectors in capillary electrophoresis (CE). In this investigation the use of HS-CDs as chiral selectors in CE for enantioseparation of tramadol, a highly potent analgesic, as the model drug and the influence of the type of selector and its concentration on enantiomeric resolution were studied. All of the available HSCDs (α , β and γ) could resolve tramadol enantiomers, but HS- γ -CD showed better resolution and a baseline resolution was achieved with this selector even at a concentration as low as 0.5% w/v. Additionally, effect of the buffer pH on the enantioresolution was studied. At low pH buffers, in which electroosmotic flow is low in CE, the negatively charged selector prevented the cationic tramadol to migrate out of the capillary even after a long analysis time of 60 minutes. However, at higher pH values (pH=7 or more), the electroosmotic flow is high enough to drag drug-selector complex toward the detector and a reasonable of the enantiomers of the drug was achieved.

Keywords: tramadol, enantiomers, separation, capillary electrophoresis

INTRODUCTION

The enantiomers of a racemic drug often show different pharmacokinetic and pharmacodynamic properties (1-4). As an analytical technique that is able to discriminate between enantiomers, capillary electrophoresis (CE) is a modern separation technique that provides rapid analysis with high efficiency and high resolution due to the use of high electric field and a variety of selective modes. The use of CE for the separation of chiral compounds may be dated by the work of Gassmann et al. in 1985 for the separation of dansyl amino acid (5). Cyclodextrins (CDs), including native CDs and their neutral or ionic derivatives, are by far the most common chiral selectors for the enantiomeric separation of chiral species by CE (6). Cationic and amphoteric CDs are less common, and only limited varieties are commercially available. Anionic CDs were originally used in CE for enantioseparation of neutral compounds. Among all of the anionic CDs, sulfobutyl ether and sulfated cyclodextrins in particular have received the most attention and have appeared to be the most effective charged chiral selectors. However, these anionic CDs are relatively heterogeneous and are the subject of much criticism because they may not provide reproducible results as chiral reagents. Highly

sulfated cyclodextrins (HS-CDs), are a new class of cyclodextrins for enantiomeric separation of chiral compounds by CE, which contain a substantially higher degree of sulfation and narrower heterogeneity than most of the sulfated CDs which have been used in the reported works (7,8). HSCDs are obtained as consistent products with reproducible performance as chiral selectors for CE-based enantioseparation of neutral, acidic and basic compounds (9).

Tramadol (trans - (\pm) - 2 -dimethylaminomethyl - 1-(3-methoxyphenyl)-1-cyclohexanol) is a chiral drug used as a highly potent analgesic (Fig 1).

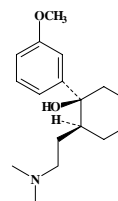


Figure 1. Chemical structure of tramadol

Although tramadol is currently marketed as the racemate, there has been considerable interest in the physiological properties associated with its individual enantiomers, namely (1S,2S)-(-)-tramadol and (1R,2R)-(+)-tramadol. Both

enantiomers have weak opiate activity, and the (-)-isomer inhibiting serotonin re-uptake, and the (+)-isomer inhibiting noradrenaline re-uptake. It has also been shown that the (+) tramadol is metabolized to the primary metabolite (+)-O-desmethyltramadol, which has significant opiate side effects (of the order of 100 times more than tramadol isomers by themselves). It is possible that further investigations in this field lead to a better understanding of the pharmacology of tramadol enantiomers, which could in turn allow improved pharmaceutical compositions to be identified (10). In the present work, application of HS-CDs as a chiral selectors in CE for enantioseparation of tramadol as a model drug, were studied. Three available HS-CDs (i.e. α , β and γ - HS-CD) were examined and the effects of other CE operational parameters, including pH of the running buffer, were evaluated.

MATERIALS AND METHODS

Materials

Tramadol powder was kindly gifted by Pars Minoo Pharmaceutical Company (Tehran, Iran) and was used as received. (1S,2S)-(-)-Tramadol was supplied by from Noor Educational and Research Institute as gift. Highly sulfated cyclodextrins were obtained from Beckman Coulter, Inc. (Fullerton, CA) as 20% w/v solution in water. All of the chemicals used were reagent grades or better, and were used without further purification. Buffers were prepared from sodium dihydrogen phosphate, di-sodium hydrogen phosphate and ortho phosphoric acid (Merck). Buffers pH were measured using a Model 691 digital pH meter (Metrohm, Herisau, Switzerland) calibrated with aqueous standards immediately prior to use. HS-CDs were added to the buffer solution at proper concentration before adjustment of the pH of the final solution. Tramadol sample solutions were made by serial dilutions of a stock solution of the drug in methanol-water (50-50 v/v).

CE Instrument and conditions

All experiments were performed on a Biofocus 3000 (BioRad, Hercules, CA, USA) instrument equipped with an on-column diode array UV absorbance detector. Data acquisition and control were preformed using Biofocus 3000 operating software version 6.0 (BioRad Laboratories, Hercules, CA, USA) for Windows 95 on a Pentium II personal computer. Untreated fused silica capillaries Biocap, Hercules, CA, USA) with an inner diameter of 75 μ m and a total length of 40 cm (32 cm to the detector) were used. In all experiments, the capillary was thermostatted at 20°C. Samples were introduced by hydrodynamic

injection (10 psi.sec) at the anodic end of the capillary and detected at 200 nm. Separations were performed at 10 kV, which was experimentally determined to be within the linear portion of the Ohm's plot.

RESULTS AND DISCUSSION

Type of Selector

Stereoselective analysis of tramadol enantiomers by CE using some neutral and anionic CDs, including carboxymethyl-beta-cyclodextrin, methyl-beta-cyclodextrin and sulfobutyl ether beta-cyclodextrin, as chiral selectors has been reported (12-16).

Well characterized HS-CDs with a degree of substitution of 12 have recently been introduced by Beckman (11). These CDs are available in α -, β - and γ - formats. One of the main advantages of these chiral selectors is that they contain a substantially higher degree of substitution (i.e. sulfation) and narrower heterogeneity, unlike other anionic CDs (such as sulfated- or sulfobutyl ether CDs) which are relatively heterogenous and may not provide reproducible results as chiral selectors. HS-CDs are negatively charged over the entire pH range and, therefore, move in the opposite direction of the electroosmotic flow. Compounds like tramadol contain a positively charged moiety (protonated amine groups) under the buffer pH below 9.0 and consequently move in opposite direction of the chiral selector. This countercurrent movement enhances the enantiomeric separation window which in turn, results in remarkably high resolutions. The racemic amines are generally well resolved by the use of HS-CDs with short analysis times. It is indicative of strong affinity due to the electrostatic attraction of the positively charged amine moiety with sulfate groups of HSCDs (16,17).

Effect of the pH

Tramadol has a pK_a 9.41 and is positively charged in buffers of pH below 9.0 due to the protonation of its side chain amino group (15). In acidic buffers (i.e. a pH range of 2.5-5.0) electroosmotic flow is relatively low. Thus, anionic selectors which move in the opposite direction of the electroosmotic flow, retard migration of tramadol toward the detector (14). In case of HS-CDs, it seems that the interaction of tramadol with the selector at acidic pH is strong enough to prevent the analyte to come out of the capillary, even after a long analysis time of 60 minutes. Therefore, a higher pH buffer in which electroosmotic flow is high enough to push selector- tramadol complex toward the detector, was selected. A suitable buffer like phosphate buffer of pH 7.0 was therefore employed for enantioseparation of

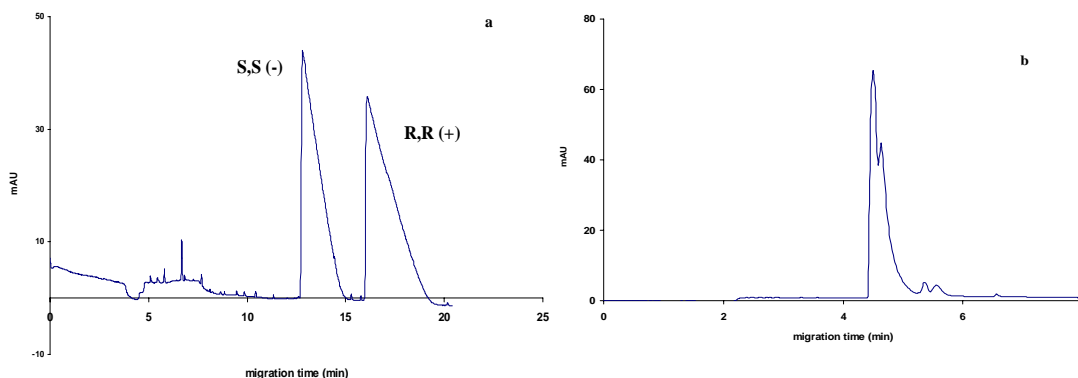


Figure 2. Resolution of tramadol enantiomers using (a) HS- β CD, and (b) HS- α CD, both at 2% w/v concentration. Other conditions: phosphate buffer 25 mM at pH 7.0, voltage 10 kV, temp. 20°C, detection wavelength 200 nm.

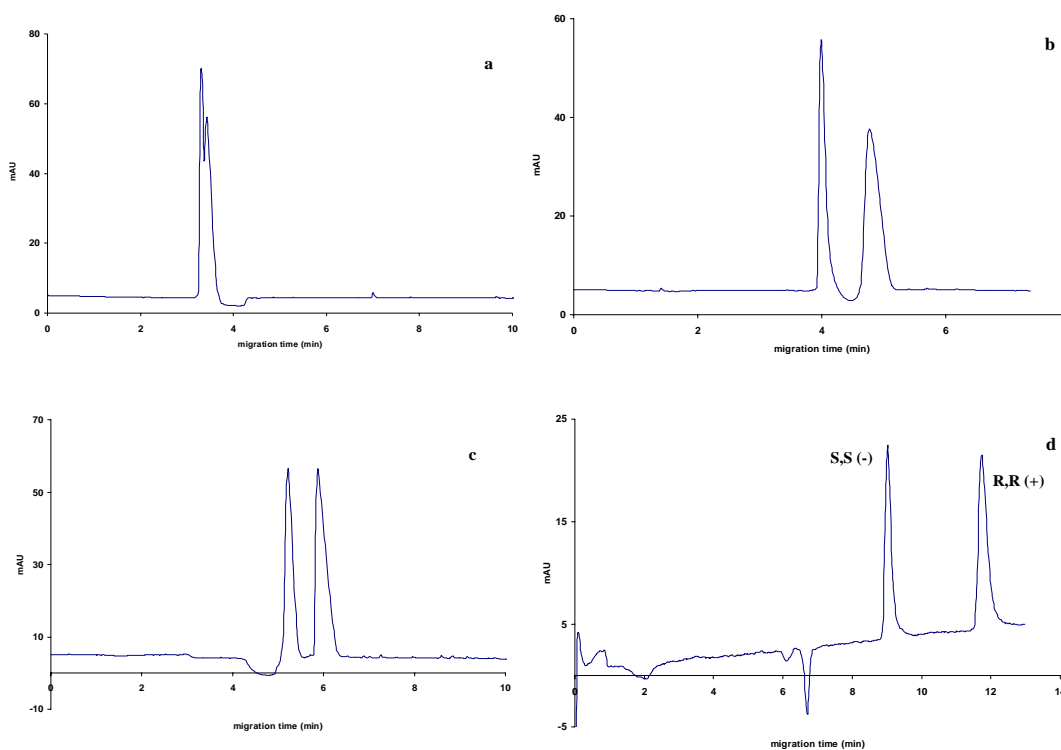


Figure 3. Resolution of tramadol enantiomers using HS- γ CD at concentrations of (a) 0.25%, (b) 0.5%, (c) 1.0% and (d) 2.0% w/v. Other conditions as in Fig. 2.

tramadol. At pH 7.0 tramadol is almost completely protonated, which promotes formation of the selector-tramadol complex. Also, buffer of pH 7.0 provides strong electroosmotic flow which allows detection of tramadol enantiomers.

Type and concentration of HS-CD

To investigate the capability of HS-CDs for separation of tramadol enantiomers, phosphate buffers with different concentrations of α -, β - and γ - forms of the selector between 0.25-5% w/v

were prepared at pH 7.0. Tramadol enantiomers are capable to interact with the three HS-CDs, but at different strengths (8). Longer migration time of tramadol which was observed when HS- β CD was employed, indicates stronger selector-tramadol complex (Fig. 2a). Conversely, shorter migration time observed with HS- α CD is indicative of weaker complex of the selector and the drug (Fig. 2b). This also may be deduced from the lower resolution between tramadol enantiomers with α -selector. Only at high concentration of HS- α CD,

the two enantiomers could be resolved. In the case of HS- β CD, despite the stronger interaction between the selector and the enantiomers, baseline resolution may be achieved only at higher concentration of the selector. Also, strong interaction between tramadol enantiomers and HS- β CD caused broadening of both peaks of enantiomers.

The enantioseparation was achieved with lower concentration of HS- γ CD (i.e. 2%w/v), compared to 5% w/v concentration for α form (Fig. 3). Lower peak broadening and faster analysis time observed with HS- γ CD compared to HS- β CD indicates weaker interaction between two enantiomers with HS- γ CD. But the difference between strength of the drug-selector for two enantiomers is high enough with HS- γ CD to cause a baseline resolution of two enantiomers.

The effect of concentration of the selector is also shown in Fig.3. As concentration of the selector is increased, better resolution of the enantiomers, and consequently better peak shapes were obtained.

The order of migration for tramadol enantiomers are the same for 3 selectors, and is indicative of stronger interaction between R,R (+) enantiomer with chiral selectors as it migrates slower than the S,S (-) isomer.

Other CE Conditions

The effect of phosphate buffer (6-8) of pH 7.0 concentration on the resolution of the enantiomers over a range of 10-50 mM was evaluated. No

significant changes were observed in resolution of the enantiomers. Thus, buffer concentration was kept at the minimum to avoid excess Joule heating associated with higher buffer concentrations. Separations were performed at 10 kV, which was experimentally determined to be within the linear portion of the Ohm's plot.

CONCLUSION

HS-CDs were shown to afford separation of tramadol enantiomers at pH 7.0. Better resolution at shorter time and lower selector concentration was obtained with HS- γ CD. In the case of HS- β CD although this selector has strong complex constant with enantiomers but because difference between the two complexation constants of enantiomers are low, more time and selector concentration are needed for baseline resolution. The interaction of tramadol with HS-CDs in acidic buffers does not allow detection of the enantiomers.

ACKNOWLEDGEMENTS

The authors appreciate Food and Drug Quality Control Lab of Ministry of Health of Iran for providing the CE instrument to perform this research. They also wish to thank Pars Minoo Pharmaceutical Company for kind gifting of tramadol and Noor Educational and Research Institute for kind gifting (1S,2S)-(-)-tramadol enantiomer.

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