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Enantiomeric separation of propranolol and selected metabolites by using capillary electrophoresis with hydroxypropyl-β-cyclodextrin as chiral selector

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Abstract

A capillary electrophoresis (CE) investigation of the enantiomeric separation of propranolol and some of its metabolites using CE was undertaken. Resolution of the enantiomers was achieved using hydroxypropyl-β-cyclodextrin (HP-β-CD) as the chiral selector. Parameters found to influence separation include cyclodextrin concentration, potential, pH and organic solvent/additive. It was observed that 17 m*M* HP-β-CD gave optimum separation over the concentration range used in this study, however different racemates appear to have best resolution at different CD concentration. The potential does not have a great effect on enantiomer resolution, but appears to cause relative metabolite migration times to alter such that separation is affected. Carrier pH affects both migration time, and enantiomer resolution and metabolite separation. Above pH 5 inferior results are obtained. This is the first report of enantiomeric resolution of propranolol metabolites using CE. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Pharmaceutical analysis; Propranolol; Beta-blockers

1. Introduction

Strict government regulations require that the composition of drug-containing products be known [1–3] and enantiomeric forms should also be identified. Different stereoisomers of drugs have different pharmacology and toxicological properties [2]. Propranolol is a β-adrenergic blocking agent; it is used in the treatment of hypertension and cardiovascular disorder [4–6]. The structures of propranolol and some of its metabolites studied in the present work are given in Fig. 1. The *S*-(−)-isomer is more potent than the *R*-(+)-isomer [5,7]. In Australia there has been reported cases where apparently propranolol has been used illegally in the horse and greyhound racing industry [6,8,9]. The drug causes the heart rate of the animal to slow down. In order to monitor this problem, a separation technique that will provide detection of propranolol and its metabolites is required. Administered propranolol may be metabolised to a variety of metabolites, such as hydroxyl compounds, and therefore traces of the parent drug propranolol may not be detected. Detection of the metabolites will provide proof of consumption of the parent compound. Only limited studies have been reported on the separation of propranolol metabolites

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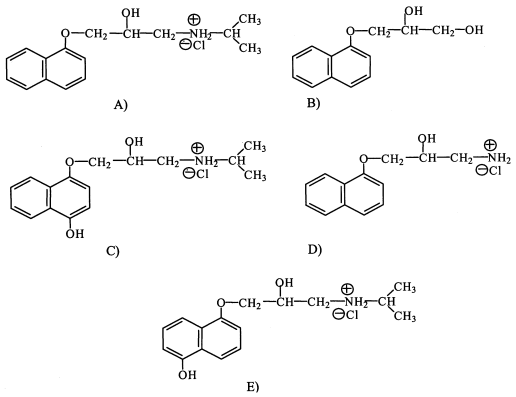

Fig. 1. The structures of propranolol and selected metabolites used in this study; (A) propranolol-HCl, (B) propranolol glycol, (C) 4′-hydroxypropranolol-HCl, (D) desisopropyl propranolol-HCl, (E) 5′-hydroxypropranolol-HCl.

by HPLC [4,6,10,11], GC [7,12] and GC–MS [4,6,8,9,12,13].

The popularity of CE as a separation technique has been established over recent years. With applications in the pharmaceutical industry now a major analysis area for CE, an increasing number of papers have been published [5,14–17]. The advantages of CE include its high efficiency, rapid method development, and low consumption of reagents. The CE technique may therefore be a powerful additional tool in the pharmaceutical chemist's arsenal of instrumental techniques.

Cyclodextrins (CDs) have been successfully utilised as chiral selectors in enantiomeric separations of chiral drugs and other compounds in CE [5,16,18]. CD is a cyclic oligosaccharide which can be described in simple molecular geometry terms as a truncated cylindrical cone [5,18–20]. It has a hydrophobic inner cavity and hydrophilic outer surface [5,18–20] (Fig. 2). Native β-CD has been widely used in the enantiomeric resolution of chiral compounds [5,21,24–27]. Due to the low solubility of the β-CD, derivatised forms have been synthesized. These include the amine, carboxylated, hydroxylated, methylated and sulfonated forms. The native and derivatised forms of β-CD have been widely used for drug chiral separation under conditions where the CD may be uncharged or charged [14,15,17,21,22]. Separation occurs via formation of a complex between the solute and the CD cavity.

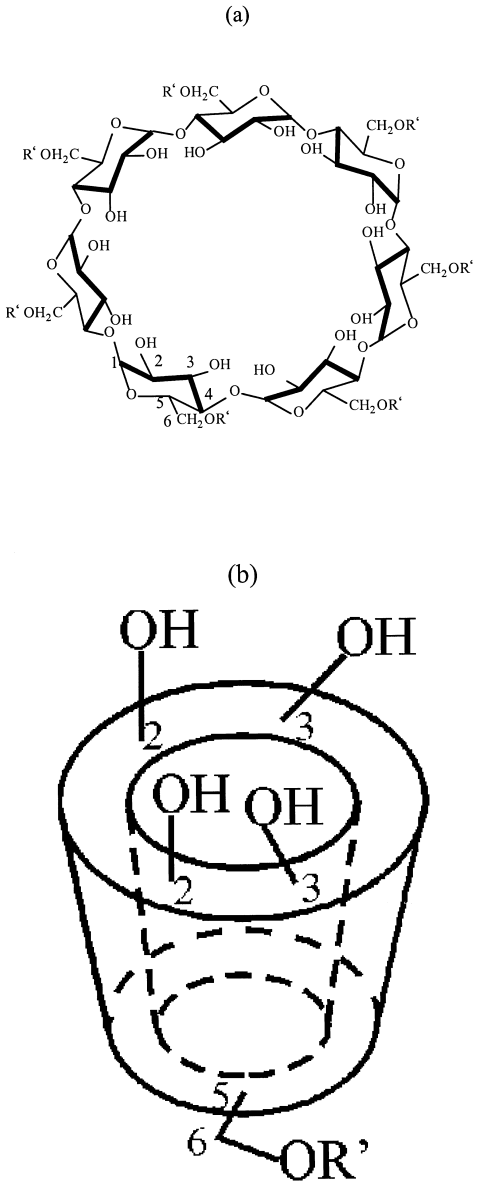

Fig. 2. (a) Structure of native β-cyclodextrin, (R′=H) and HP-β-CD [R′=$CH_2CH(OH)CH_3$]; (b) model of β-cyclodextrin as a truncated cylindrical cone.

Dalgliesh proposed a three-point rule for successful enantiomeric separations, where three types of interaction must occur between the solute and CD [23]. One of the interactions must be dependent on the steric geometry of the solute. The other two types of interaction can include electrostatic, dipole–dipole,

hydrophobic, attractive and repulsive interaction, and hydrogen bonding [22,23].

A variety of studies have been reported where CDs have been used for propranolol enantiomer resolution in CE. For example propranolol enantiomers have been resolved either in a mixture of basic drugs or on its own using native β-CD [5,21,24–27]. Previous work on propranolol resolution indicates that the degree of R_s varies significantly with the type of β-CD employed as chiral selector. Indicative resolutions reported range from 1.0 (β-CD) [5], 3.0 [carboxymethyl (CM)-β-CD] [5], 2.4 [hydroxypropyl (HP)-β-CD] [5], ≈1.8 [methyl (Me)-β-CD] [16], and <0.05 [sulfobutyl ether (SBE)-β-CD] [15]. Other approaches have employed cellulose as a chiral selector [28–30], and the chiral surfactant *N*-dodecoxycarbonylvaline [31,32] which was successfully used for resolution of propranolol using micellar electrokinetic chromatography (MEKC). Chiral separation of propranolol in nonaqueous media has been reported [33], where enantiomeric separation occurred via an ion pairing mechanism with (±)-camphorsulfonate as the ion pairing reagent.

Enantiomeric resolution of 4′-hydroxy propranolol (4′-OH-Pr) using HPLC was achieved via derivatisation of 4′-OH-Pr with a chiral derivatising agent 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [11]. GC and GC–MS has been used to analyse a mixture of propranolol metabolites from urine [4,7,8,12,13] although separation of the stereoisomers was not investigated.

The aim of this study was to separate the stereoisomers of propranolol and its synthetic metabolites in a mixture using cyclodextrin-modified free solution capillary electrophoresis (CD-FSCE) with HP-β-CD as the chiral selector. In order to distinguish between enantiomers being resolved, and different propranolol metabolites being separated, we will use resolution to refer to enantiomers and separation to refer to metabolites.

2. Experimental

2.1. Apparatus

Experiments were performed on the Prince CE (Lauerlabs, Netherlands). A BAS UV-116A variable-wavelength detector (W. Lafayette, IN, USA) was used. Detection was performed at either 210 or 214 nm. Fused-silica capillary columns (uncoated) of 50 μm I.D×363 μm O.D., with total length of 69.3 cm and effective length of 52.8 cm were used as the separation capillary (Supelco, Bellefonte, PA, USA). A detection window was created by burning off the polyimide coating on the capillary. All electropherograms were carried out at ambient laboratory temperature of 20°C. Data were collected using BAS data acquisition software. Electropherograms were converted in Microsoft Excel as text files and then imported into Origin 4.1 Graphics Program. A Hanna HI 8519 pH meter (Hanna Instruments, Woonsocket, RI, USA) with HI 1332B refillable double junction, combination pH electrode was used to measure the pH.

2.2. Chemicals

β-CD and HP-β-CD (degree of substitution≈1) were purchased from Aldrich (Milwaukee, MI, USA). Propranolol hydrochloride (Pr-HCl), desisopropylpropranol hydrochloride (DIP-HCl), 4′-hydroxypropranolol hydrochloride (4′-OH-Pr-HCl), 5′-hydroxypropranolol hydrochloride (5′-OH-Pr-HCl) and propranolol glycol (Pr-glycol) as racemic mixtures were synthesised in this department. Standards of *R*-(+)- and *S*-(−)-Pr-HCl were obtained from Aldrich. HPLC grade methanol was purchased from BDH (Poole, UK), phosphoric acid (85%, AR grade) and triethanolamine (LR grade) from Ajax Chemicals (Sydney, Australia).

2.3. Buffer and sample preparations

100 m*M* phosphoric acid buffer was prepared in Milli-Q water (Millipore, Milford, MA, USA) and the pH adjusted with triethanolamine. HP-β-CD was added to the buffer after adjustment of pH to 3.0 unless stated otherwise. No organic modifier was added unless indicated. Stock solutions of 0.1 mg/ml propranolol and metabolites were prepared in 100% methanol. The stock sample was diluted with buffer containing the HP-β-CD giving a final concentration of 0.05 mg/ml. All solutions were filtered with

0.45-μm syringe filters (Microfiltration Systems, CA, USA).

The capillary column was treated with 1 *M* NaOH, then Milli-Q water, followed by the carrier electrolyte at the start of each working day. Prior to each analysis the column was rinsed with Milli-Q water and then background electrolyte.

Hydrodynamic injections of samples were carried out at 50 mbar for 1 s. An applied voltage of 20 kV was used for analysis, unless stated otherwise, with the injection end, the anode.

3. Results and discussion

CD–FSCE was used as the method of separation, where the CD chiral selector is added to the carrier electrolyte. In this method enantiomer resolution occurs via interaction of the solute with the CD cavity. The differential affinity between the enantiomers for the hydrophobic cavity, arising from the steric orientation of the solute structure which allows the formation of hydrogen bonding with the hydroxyl group or other functionalities on the CD rim, provides a mechanism for resolution of the enantiomers [14,22,34]. The interaction of Pr-HCl and its metabolites with the CD molecule is presently being studied using the Molecular Modeling program Hyperchem. Native β-CD did not yield enantiomer resolution for most of the metabolites, however a resolution of 0.61 and 0.94 was found for 5′-OH-Pr-HCl and Pr-HCl respectively. Hence an alternative CD, HP-β-CD was investigated. Through coinjection of authentic individual standards of *R*-(+)- and *S*-(−)-Pr-HCl, the *S*-(−)-isomer was found to migrate out of the column before the *R*-(+)-isomer when using HP-β-CD. This suggests that the *R*-(+)-Pr-HCl interacted more strongly with the HP-β-CD than the *S*-(−)-isomer did. 100 m*M* phosphoric acid buffer was used throughout, with triethanolamine used to adjust the pH of the solution. HP-β-CD was added to give the required concentration for each buffer studied. Over the pH range used the CD was neutral. The above buffer composition resulted in very slow reversed electroosmotic flow (EOF), especially at the low pH used [5]. The EOF marker was not observed, and neither was the Pr-glycol metabolite (under these conditions it will be expected to be neutral), since they had very long migration times. Samples were dissolved in methanol as it was found to improve peak shape with no peak tailing observed. The effect of a number of experimental parameters on propranolol and its metabolites' separation and chiral resolution are discussed further below.

3.1. Effects of HP-β-CD concentration

An optimum HP-β-CD concentration of about 17 m*M* was found for maximum resolution (Fig. 3). Increasing the HP-β-CD concentration to 23 m*M* decreased resolution and caused the migration time for propranolol and its metabolites to increase (Fig. 4), most likely due to the increase in viscosity of the carrier electrolyte. Guttman and Brunet found similar trends for the resolution of propranolol using HP-β-CD as the chiral selector at pH 3 [20]. The net separation of metabolites also decreases as HP-β-CD concentration increases.

3.2. Effects of pH

Carrier electrolyte pH had some influence on both resolution and separation. As pH increases from 3 to 5, enantiomer resolution does not change markedly. At about pH 6 there is a resolution decrease for 4′-OH-Pr-HCl and 5′-OH-Pr-HCl, but Pr-HCl is still adequately resolved. Upon increasing pH, Pr-HCl and 4′-OH-Pr-HCl peaks eluted closer to one another, as did 5′-OH-Pr-HCl and a DIP-HCl impurity, hence metabolite separation decreased as shown in Fig. 5. At higher pH, all of the compounds eluted faster due to increasing EOF, with EOF

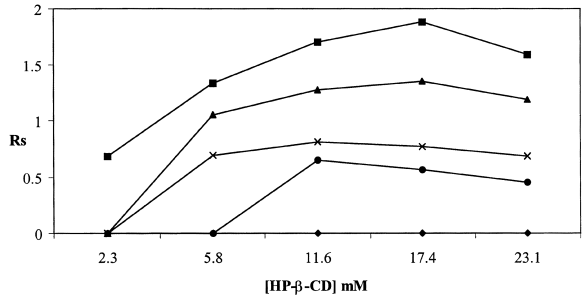

Fig. 3. Effect of the concentration of HP-β-CD on the resolution of propranolol and its metabolites; (♦) DIP-HCl, (■) Pr-HCl, (▲) 4′-OH-Pr-HCl, (×) DIP-HCl impurity [1].

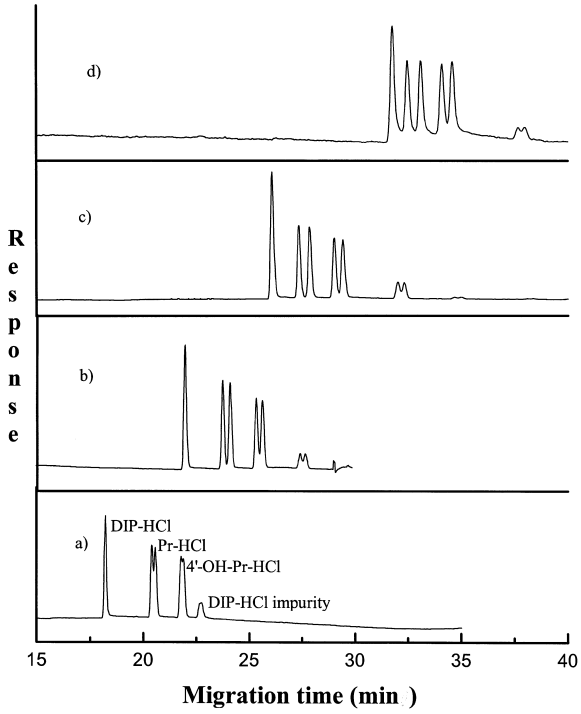

Fig. 4. Electropherograms of the separation of propranolol and its metabolites with varying concentration of HP-β-CD. Conditions used: buffer composition; (a) 100 m*M* phosphoric acid–triethanolamine (pH 3.05) containing 2.3 m*M* HP-β-CD, (b) buffer composition as in (a) but with 5.8 m*M* HP-β-CD, (c) with 11.6 m*M* HP-β-CD, and (d) with 23.1 m*M* HP-β-CD.

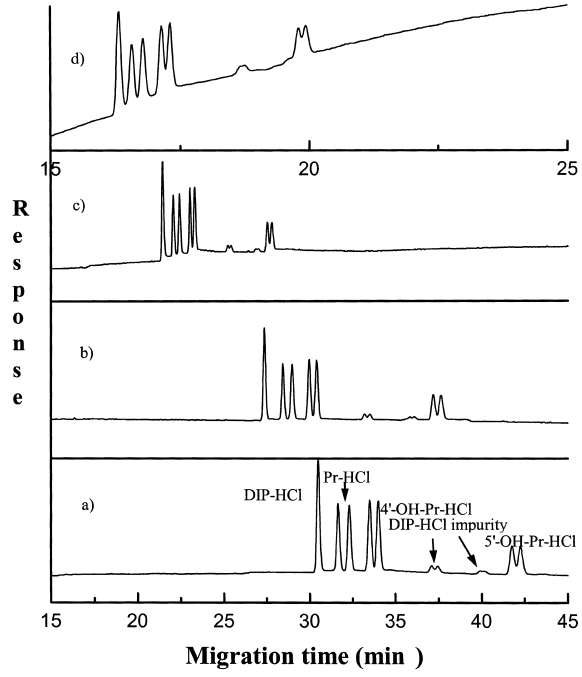

Fig. 5. Influence of pH on the resolution and separation of propranolol and its metabolites. Conditions used: buffer composition; 100 m*M* phosphoric acid–triethanolamine, containing 17.4 m*M* HP-β-CD at; (a) pH 3.05, (b) pH 4.05, (c) pH 5.08, and (d) pH 6.08.

becoming more significant at pH>4 [35,36]. At low pH there is more time for the solute to interact with the CD as a result of increased time spent in the column. Changes in the degree of ionization of the solute may cause changes in interaction with the CD, especially if structural or charge changes alter the manner in which solute binds to the cyclodextrin and thus affects the chiral interaction such as may be operative under the Dalgleish three-point interaction rule. An optimum pH of 3–4 was found for the resolution/separation of propranolol and its metabolites using HP-β-CD. At pH 6 the baseline was found to be less stable (Fig. 5d).

3.3. Effects of potential

On increasing potential, migration time decreased and overlapping of some analyte peaks occurred. For instance increasing potential from 25 to 30 kV caused 4′-OH-Pr-HCl to move toward Pr-HCl while 5′-OH-Pr-HCl moved closer to one of the DIP-HCl impurity peaks (results not shown). At 30 kV, the *S*-(−)-Pr-HCl peak and the *R*-(+)-4′-OH-Pr-HCl peak co-eluted (peak assignment based on the elution order found for (+)- and (−)-Pr-HCl with HP-β-CD reported above). Hence enantiomer resolution of each individual metabolite was still good, but separation of metabolites was less favourable. The 5′-OH-Pr-HCl peak also was not separated from the impurity peak of DIP-HCl.

An operating potential of 20 kV gave the best resolution for Pr-HCl, 4′-OH-Pr-HCl, 5′-OH-Pr-HCl and the DIP-HCl impurity peak. Decreasing the potential to 15 kV or increasing to 30 kV lead to a slight reduction in resolution (R_s). However, potential did not have a strong effect on R_s unlike CD concentration and pH of the background electrolyte. An optimum potential of 20 kV was indicated.

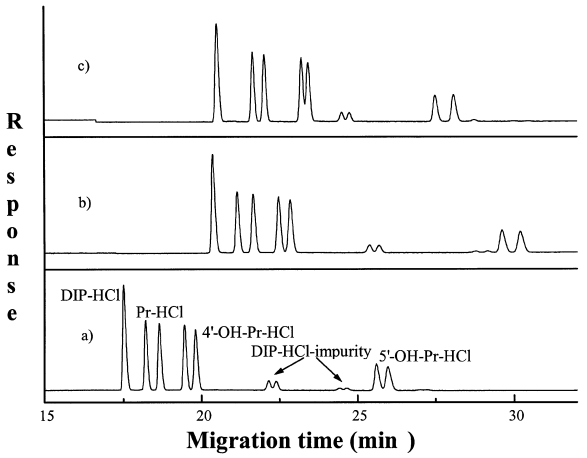

Fig. 6. Electropherograms of the separation of propranolol and its metabolites at different methanol levels. Instrument used: ABI CE system (model 270A-HT). Conditions used: capillary; 64.9/43.1 cm×50 μm I.D. (uncoated), *V*=25 kV, vacuum injection at 5 in. Hg for 1 s (1 in. Hg=3386.379 Pa), buffer composition: 100 m*M* phosphoric acid–triethanolamine at pH 3.05, containing 17.4 m*M* HP-β-CD with; (a) 0% MeOH, (b) 10% MeOH, and (c) 30% MeOH.

3.4. Effects of added methanol

The effect on resolution of added methanol to the carrier electrolyte was investigated. Figs. 6 and 7 show that on addition of methanol, resolution of Pr-HCl, 4′-OH-Pr-HCl, 5′-OH-Pr-HCl and impurity of DIP-HCl were affected as below. With 10% methanol, resolution of 5′-OH-Pr-HCl was greatly enhanced. No other significant effects were observed for the other compounds at this level. The addition of 30% methanol had an impact on the R_s of 4′-OH-Pr-HCl, Pr-HCl and the impurity peaks of DIP-HCl, giving a reduced resolution, while for 5′-OH-Pr-HCl, R_s was still similar to that obtained with 10% methanol. A plot of the effect of methanol on resolution is given in Fig. 7. The reason for these observations could be explained in terms of the effect of organic solvent on the binding constant between solute and CD cavity. It has been reported that as the % methanol increases the equilibrium constant for the formation of an inclusion complex decreases [24,37,38] and interaction of solute with the CD is less favoured [37,38]. Interestingly, for 5′-OH-Pr-HCl the opposite was observed. Migration times increased for the Pr-HCl and its metabolites as the methanol content was altered from 0 to 10%. 4′-OH-Pr-HCl appears to be much more affected by methanol with respect to extent of the resolution compared to the other solutes. Except for 5′-OH-Pr-HCl, increasing the methanol content to 30% resulted in an increase of migration time of the other compounds (Fig. 6c), with 4′-OH-Pr-HCl having the greatest migration time increase. The increase in migration with the addition of methanol could be the result of a decrease in EOF and an increase in viscosity of the carrier electrolyte [36].

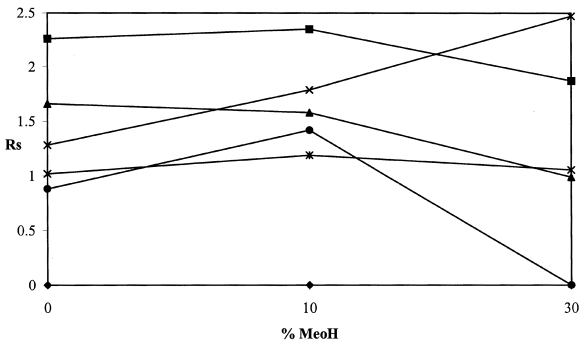

Fig. 7. Effect of methanol level on resolution of propranolol and its metabolites; (♦) DIP-HCl, (■) Pr-HCl, (▲) 4′-OH-Pr-HCl, (×) 5′-OH-Pr-HCl, (*) DIP-HCl impurity [1], (●) DIP-HCl impurity [2]. Same conditions as in Fig. 6.

3.5. MEKC

A brief comparison of CD–FSCE with MEKC was undertaken since the elution of the neutral metabolite was not achieved using the former technique. It was anticipated that the Pr-glycol might elute more readily when included into the lipophilic core of the migrating micelle. MEKC generally gave reverse elution order for the series of compounds. Using phosphate buffer with 32 m*M* sodium dodecyl sulfate (SDS) and 17.4 m*M* HP-β-CD as the chiral selector at a pH of 9.3, the order of elution found was as follow; Pr-glycol<5′-OH-Pr-HCl<DIP-HCl<4′-OH-Pr-HCl<Pr-HCl. Under the conditions employed there was little indication of enantiomer resolution and so the use of MEKC was not as promising as FSCE. Whilst other studies have reported successful enantiomeric resolutions with

MEKC, such as studies in which a chiral surfactant was used, the present work on the drug molecules suggests that ready enantiomeric resolutions in FSCE cannot be extrapolated to MEKC conditions. Further work will be required to determine why this is the case.

4. Conclusion

Enantiomeric separation of propranolol and most of its metabolites using CD–FSCE was accomplished through using modified β-CD (HP-β-CD). An optimum level of CD and pH was found which produced maximum resolution when using this chiral selector. Organic solvent had a strong influence on resolution of 4′-OH-Pr-HCl, with a decrease in resolution at the 30% addition level.

Enantiomeric resolution of DIP-HCl was not observed throughout this study, and Pr-glycol was not readily analysed due to its long migration time under the FSCE conditions employed. Current study is being carried out to find a CD chiral selector and operating conditions that will allow enantiomeric resolution of all the propranolol metabolites; charged CDs may well offer this advantage.

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