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Short communication

Chiral separation of tamsulosin by capillary electrophoresis

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Abstract

Enantiomers of (±) 5-[2 (*R*,*S*)-{[2-(*o*-ethoxyphenoxy) ethyl] amino} propyl]-2-methoxy-benzenesulfonamide (tamsulosin, drug frequently used in the treatment of prostate diseases) were separated by capillary electrophoresis (CE). An acidic background electrolyte (BGE) with sulfated-β-cyclodextrin (S-β-CD) was used to create a chiral separation environment. Baseline separation of the isomers was achieved during 5 min using cathodic electro-osmotic flow (EOF) (countercurrent mode). The quantification limits were 5.3×10^{-6} mol l^{-1} for *R*-isomer and 5.7×10^{-6} mol l^{-1} for *S*-isomer. The R.S.D. values of peak area were 0.54% for *R*-isomer and 0.75% for *S*-isomer. The results achieved enable determination of 0.5% of optical impurity.

Keywords: Tamsulosin; Sulfated-β-cyclodextrin; Capillary electrophoresis; Chiral separation

1. Introduction

(±) 5-[2 (*R*,*S*)-{[2-(*o*-ethoxyphenoxy) ethyl] amino} propyl]-2-methoxy-benzenesulfonamide (tamsulosin) hydrochloride exists in two enantiomeric forms (Fig. 1) but only *R*-isomer is the pharmaceutically active component. It has an antagonistic effect on alpha 1A adrenoreceptors in prostate [1,2]. Tamsulosin is used for treatment of bladder outlet obstruction of patients who suffer from benign prostate hyperplasia. Fast quantification of the tamsulosin enantiomers is very important for quality control of the synthesis procedure as well as for pharmacological and pharmacokinetic studies.

Determination of tamsulosin in blood plasma has been performed by high performance liquid chromatography (HPLC) [3–8] and by radioimmunoassay [9,10]. Macek et al. published L–L extraction procedure of tamsulosin from alkalised plasma with butyl acetate and re-extraction of the drug to the acidic phosphate buffer [8]. The limit of quantification was 0.4 ng ml^{-1} using 1 ml of plasma. However, these methods allow only achiral determination of tamsulosin.

Zhang et al. [11] developed an HPLC method for resolution of tamsulosin enantiomers using Tris (3,5-dimethylphenylcarbamate) cellulose as a chiral stationary phase and Qi et al. [12] developed HPLC method for resolution of tamsulosin enantiomers and its synthetic intermediates using Chiralcel OD-R column. Both chiral separations of *R*,*S*-tamsulosin by liquid chromatography which were published are time consuming and required chiral stationary phase for direct enantioseparation.

Capillary electrophoresis (CE) represents a powerful separation technique that is successfully utilized for separation of optical isomers as presented in many review papers [13–15]. The main advantages of this technique are high efficiency of separations, possibility to use new and

Abbreviations: α-CD, α-cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin; BGE, background electrolyte; CD(s), cyclodextrin(s); CE, capillary electrophoresis; CM-β-CD, carboxymethyl-β-cyclodextrin; CS(s), chiral selector(s); DM-β-CD, dimethyl-β-cyclodextrin; EOF, electro-osmotic flow; HP-β-CD, β-cyclodextrin; HPLC, high performance liquid chromatography; S-β-CD, sulfated-β-cyclodextrin; TRIS, tris(hydroxymethyl)aminomethane

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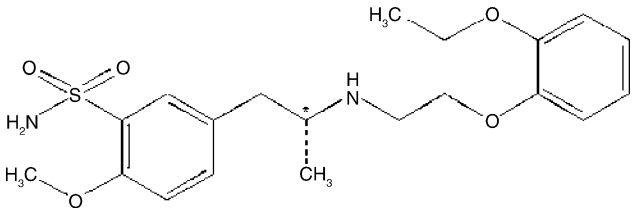

Fig. 1. Chemical structure of tamsulosin (chiral centre is marked by star).

expensive selectors, easy and fast method development. A wide choice and easy variation of chiral selectors (CSs) allow an effective search for suitable separation system that generates high-resolution values of pairs of enantiomers. The list of chiral selectors utilized in CE includes antibiotics, chiral crown-ethers, chiral micelles, proteins or cyclodextrins (CDs) and their derivatives. Cyclodextrins and their derivatives have been frequently used as chiral selectors in CE because of their applicability for separation of a wide range of structurally different compounds [16–18].

The derivatized cyclodextrins can exhibit properties rather different from those of the native CDs. Derivatization of chiral selectors can be easily applied to improve selectivity of the enantioseparation, e.g. increased solubility, new possibilities for secondary bonding, different hydrophobic properties and size of the CD cavity, etc. Chargeable CDs can be also considerably helpful for CE method optimisation. Among advantages of the use of charged CDs as chiral selectors, the ability to enhance separation selectivity by using oppositely charged CDs should be emphasized [19,20]. Formation of inclusion-complexes with uncharged enantiomers enables their analysis in CE [21].

Furthermore, the movement of the chiral selector or the movement of the electro-osmotic flow (EOF) in the opposite direction to an analyte create separation conditions that are advantageous for chiral resolution. Such setup causes an increase of the mobility difference between free analyte and analyte in the complex with chiral selector (countercurrent mode) [22].

The aim of this work is to develop a rapid and efficient method for separation of tamsulosin enantiomers using sulfated-β-cyclodextrin (S-β-CD) as a chiral selector. This proposed simple method can be used for basic screening in production of tamsulosin-based pharmaceuticals.

2. Experimental

2.1. Chemicals

R,*S*-tamsulosin hydrochloride standards and tamsulosin hydrochloride product was provided by Farmak, a.s., Olomouc, Czech Republic. Acetic acid, sodium hydroxide and tris(hydroxymethyl)aminomethane (TRIS) (Sigma, St. Louis, MO, USA) were used as background electrolyte (BGE) components. Chiral cyclodextrins additives as α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), dimethyl-β-cyclodextrin (DM-β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), carboxymethyl-β-cyclodextrin (CM-β-CD) and sulfated-β-cyclodextrin sodium salt (S-β-CD, typical substitution 7–11 mol/mol) were also purchased from Sigma. All of the chemicals used were of analytical grade purity.

The standard solutions of *R*,*S*-tamsulosin and single enantiomers were prepared in 1×10^{-5} mol l^{-1} concentrations by dissolution of the standard compounds in the 10-times diluted background electrolyte without CDs.

2.2. Apparatus and conditions

The enantioseparations were performed on capillary electrophoresis system HP^{3D} CE (Agilent Technologies, Waldbronn, Germany) with a diode array detector, which was operating at 200 nm. Uncoated fused silica capillary (CACO-Sila Tubing and Optical Fibbers, Slovakia) of the total and effective lengths of 33 and 24.5 cm, respectively, and 50 μm i.d. × 365 μm o.d. was used in these experiments. The capillary was thermostated at 25 °C, the applied voltage was 20 kV (606 V cm^{-1}). The samples were injected by pressure 50 mbar 5 s^{-1}. The background electrolytes were prepared by dissolution of acetic acid in deionised water (18 MΩ cm^{-1}; Elga, Bucks, England) and pH was adjusted with sodium hydroxide or TRIS. Chiral selectors were added to the working electrolyte at the end.

The capillary was rinsed with 0.1 mol l^{-1} NaOH solution for 5 min, then with water (5 min) and 0.1 mol l^{-1} HCl (5 min) and again with water (5 min). Finally, the capillary was rinsed with working electrolyte containing chiral selector for 15 min. The last step was performed at the beginning of every working day. Between individual runs, the capillary was rinsed with the BGE containing the appropriate CS only for 10 min.

3. Results and discussion

3.1. Chiral separation

Considering the structures of tamsulosin enantiomers and cyclodextrins, we supposed that tamsulosin can interact with some of those chiral selectors and the interaction could be even stereoselective in some cases. In our preliminary experiments, the separations were performed using acetic-based background electrolyte (100 mM sodium acetate, pH 4.0) with addition of different cyclodextrins (α-, β-, γ-CD, hydroxypropyl-β-CD, dimethyl-β-CD, carboxymethyl-β-CD and sulfated-β-CD sodium salt). Tamsulosin migrates under such conditions as a cation, which is in the same direction as the electro-osmotic flow. The separations were performed in a homogenous electrolyte system, i.e. the chiral selector was in all separation compartments (inlet—capillary—outlet).

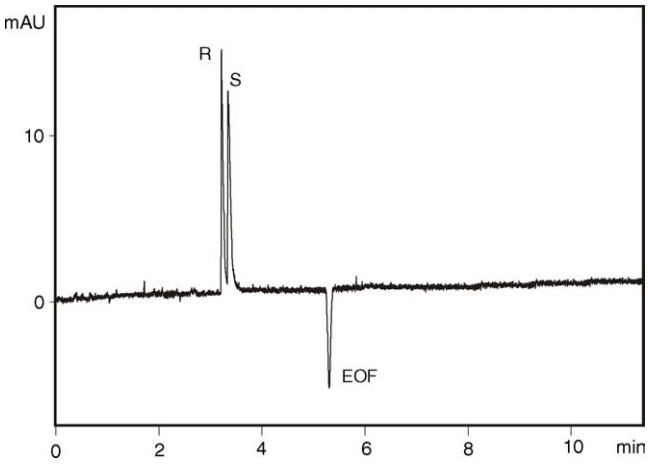

Fig. 2. Electropherogram of the sample solution containing racemic mixture of tamsulosin enantiomers. Conditions: 100 mM acetate/TRIS pH 4.0 with 0.1% S-β-CD, +20 kV.

Native CDs and neutral CD derivatives were not able to separate the two enantiomers in the acetic-based background electrolyte. Partial enantioseparation of *R*,*S*-tamsulosin was obtained only with CM-β-CD but the resolution value 0.53 was not sufficient for quantification of the individual enantiomers.

Using S-β-CD as the CS, the enantiomers were baseline separated within 5 min (Fig. 2). Then, the separation conditions were studied in detail. The effect of the S-β-CD concentration, of the concentration of acetic acid in the BGE and the influence of the co-ions and the counterions on the chiral separation were investigated.

The addition of S-β-CD to the acetic-based BGE, 100 mM sodium acetate, pH 4.0, was studied in a narrow range of concentration from 0.1 to 0.5% (w/v), in which no change of buffer pH was observed after the addition of the S-β-CD.

The resolution of the tamsulosin enantiomers increased with increasing concentration of S-β-CD in the BGE (Fig. 3). S-β-CD migrates in the opposite direction to the *R*,*S*-tamsulosin and to the migration of the electro-osmotic flow (countercurrent mode). The good separation ability of S-β-CD was ascribed to enhanced hydrogen bonding of the analyte with the sulfonic acid group of the chiral selector, possible ion-pair interactions and use of the countercurrent separation mode. The migration order of the enantiomers was *R*-tamsulosin before *S*-tamsulosin. The migration order was the same in whole studied concentration range of S-β-CD. Peak distortion (both peaks were tailing) was observed with increasing concentration of S-β-CD. For this reason, the best concentration of S-β-CD was 0.1% (w/v).

Fig. 4 shows the influence of the concentration of S-β-CD on migration of both enantiomers. When the concentration of S-β-CD was higher than 0.25% (w/v), the resulting migration of enantiomers was slower than migration of the EOF. Characteristics of observed peaks (such as theoretical plate number, resolution, selectivity and mobility of electro-

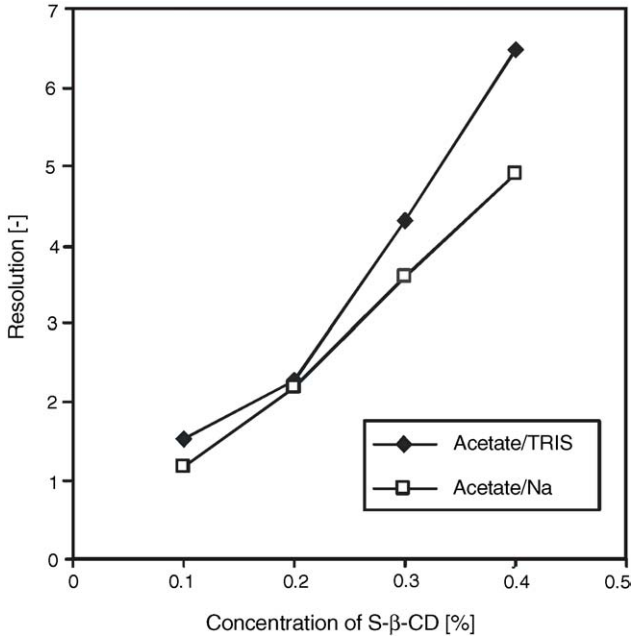

Fig. 3. Influence of concentration of S-β-CD on chiral resolution of tamsulosin enantiomers. Conditions: 100 mM acetate/TRIS or 100 mM acetate/Na, pH 4.0, with addition of appropriate amount of chiral selector.

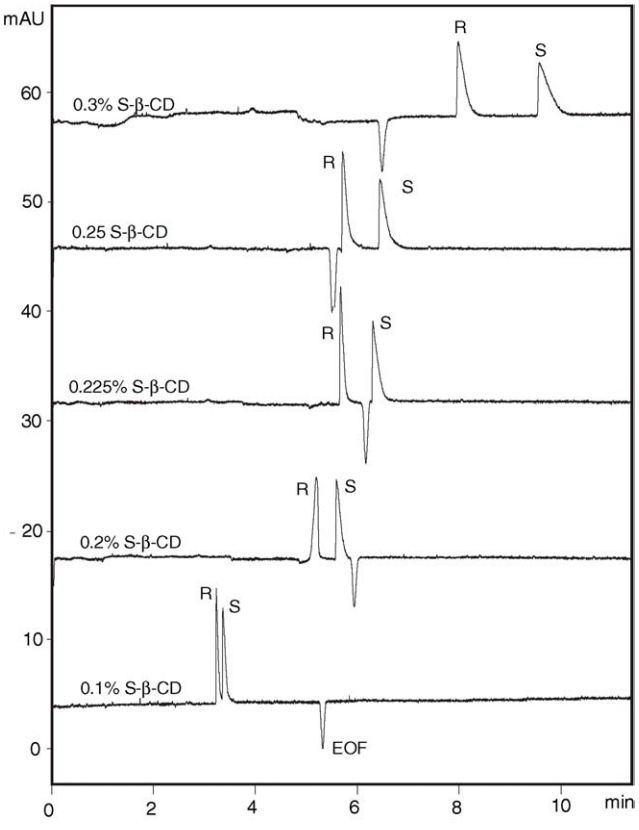

Fig. 4. Electropherograms of racemic mixture of tamsulosin enantiomers. Influence of the concentration of chiral selector on migration behaviour of both enantiomers. Conditions: 100 mM acetate/TRIS, pH 4.0, with addition of appropriate amount of chiral selector, +20 kV.

Table 1
Characteristics of chiral separation of *R*,*S*-tamsulosin

Concentration of S-β-CD (%)	Resolution, R_S (−)	Selectivity, α (−)	Plate number, TP/m (−), (*R*-tamsulosin)	Plate number, TP/m (−), (*S*-tamsulosin)
0.1	1.5	1.03	105170	72258
0.2	2.2	1.08	53300	60776
0.225	2.4	1.10	127785	57636
0.25	3.2	1.13	78473	43836
0.3	4.1	1.20	55864	31573

Concentration of S-β-CD (%)	μ_{EOF}[a] ($m^2\,V^{-1}\,s^{-1}$)	$\mu_{effective}$ (*R*-tamsulosin) ($m^2\,V^{-1}\,s^{-1}$)	$\mu_{effective}$ (*S*-tamsulosin) ($m^2\,V^{-1}\,s^{-1}$)
0.1	1.266×10^{-8}	8.190×10^{-9}	7.380×10^{-9}
0.2	1.139×10^{-8}	1.65×10^{-9}	9.3×10^{-10}
0.225	1.093×10^{-8}	9.5×10^{-10}	-2.4×10^{-10}
0.25	1.220×10^{-8}	-3.7×10^{-10}	-1.72×10^{-9}
0.3	1.039×10^{-8}	-1.947×10^{-9}	-3.355×10^{-9}

Conditions: 100 mmol l^{-1} acetate/Tris, pH 4.0, with addition of appropriate amounts of S-β-CD.
[a] Electro-osmotic flow was measured by negative distortion of baseline and the confirmation of it was provided by mesityloxide as neutral marker.

osmotic flow and effective mobility of both enantiomers) are shown in Table 1.

The cationic mode (concentration of S-β-CD lower than 0.25% (w/v), normal polarity) is suitable for determination of low concentrations of *R*-tamsulosin because this enantiomer elutes first while peaks tailing does not allow good determination of *S*-enantiomer in very low concentration levels.

We used untreated fused silica capillary and thus the electro-osmotic flow can have very strong influence to chiral separation, as we mentioned above. EOF is in opposite direction to migration direction of S-β-CD. The mobilities of electro-osmotic flow were not affected significantly with increasing amount of S-β-CD in studied concentration range (see values of mobilities of EOF and effective mobilities of both enantiomers in Table 1).

On the other hand, the increasing of concentration of S-β-CD affected the resulting effective charge of transient diastereomeric complex of enantiomers with S-β-CD and thus the effective charge of it was gradually changed from positive charge to negative one (see Fig. 4).

Follows from this point of view, the time for both enantiomers which were in interaction with S-β-CD was increased with increasing concentration of chiral selector and the effective mobilities of both enantiomers are relative changing the charges to the mobility of EOF. In the preliminary experiments, the separation was performed using sodium acetate buffer, pH 4.0, with additions of S-β-CD. As a co-ion can also positively influence the separation process in CE, we also investigated this parameter. Background electrolyte with TRIS co-ion provided better repeatability of migration times of both enantiomers and slightly low magnitude of the electro-osmotic flow. Also, the resolution values in the acetate/TRIS-based buffers were higher than in the sodium acetate ones. Therefore, TRIS was used instead of sodium co-ion in the following measurements.

In the next step, the effect of pH on the enantioseparation was examined. In the whole studied pH range (pH 2.0–4.5), the chiral separation was possible but the best chiral resolution was obtained at pH 4.0. On the other hand, the analysis time at the concentration of the chiral selector (0.1%, w/v), which is necessary for baseline chiral separation, was lower at pH 4.0 than in a more acidic BGE.

As the final step, we investigated the influence of the counter ion concentration of the BGE in the concentration range from 10 to 125 mmol l^{-1}. With increasing concentration of acetic acid in the BGE, the values of chiral resolution slightly decreased, but the efficiency increased.

The optimised conditions for enantioseparation of *R*,*S*-tamsulosin were: 100 mmol l^{-1} acetate/TRIS, pH 4.0, with 0.1% of S-β-CD.

3.2. Repeatability

Repeatability of migration times (R.S.D. values, $n = 5$) was 1.23 and 1.42% for *R*-enantiomer and *S*-enantiomer, respectively. The repeatability (R.S.D. values, $n = 5$) for peak areas was 0.54% for *R*-enantiomer and 0.75% for *S*-enantiomer. The R.S.D. values of peak height for the two enantiomers were 0.89 and 1.05% for *R*- and *S*-isomer, respectively.

3.3. Recovery

Recovery of both enantiomers at two concentration levels was studied. First, we added 5×10^{-5} mol l^{-1} of the individual enantiomers to the solution, which contained 1×10^{-6} mol l^{-1} of each enantiomer. The recoveries were 97.5% for *R*-isomer and 96.3% for *S*-isomer. The R.S.D. values ($n = 5$) were 1.25 and 1.46% for *S*-isomer and *R*-isomer, respectively.

Then, 2×10^{-5} mol l^{-1} of each enantiomer was added to the solution of 1×10^{-6} mol l^{-1} of the individual enantiomers. The recoveries were 95.1% for *R*-isomer and 94.5% for *S*-isomer. The R.S.D. values ($n = 5$) were 1.56 and 1.89% for *R*- and *S*-isomer, respectively.

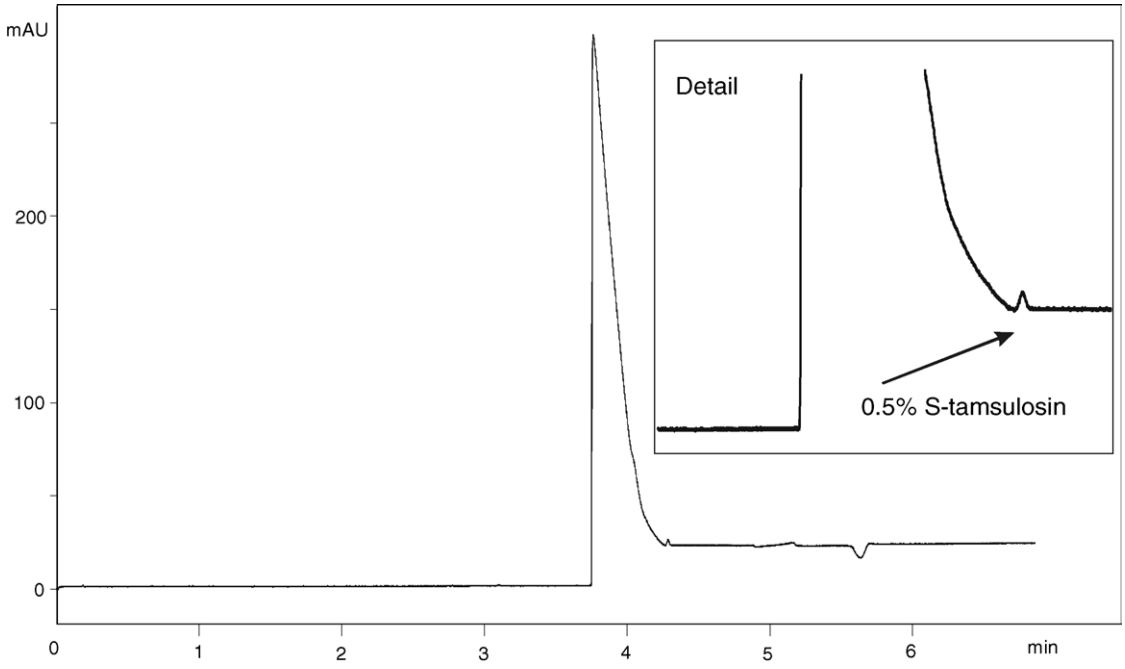

Fig. 5. Electropherogram of sample solution containing *R*-isomer 1×10^{-2} mol l^{-1} (4 mg ml^{-1}) and *S*-isomer 5×10^{-5} mol l^{-1} (0.02 mg ml^{-1}). Conditions: 100 mM acetate/TRIS, pH 4.0, with 0.1% of S-β-CD, +20 kV.

3.4. Determination of optical impurity

Asymmetrical peaks (both tailing and fronting) can cause problems if determination of optical impurities has to be performed. In order to illustrate the power of the method, a solution containing 1×10^{-2} mol l^{-1} (4 mg ml^{-1}) of *R*-tamsulosin and 5×10^{-5} mol l^{-1} (0.02 mg ml^{-1}) of *S*-tamsulosin, as optical impurity, was injected. Under the optimised conditions (see above), the method could quantify 0.5% as optical impurity besides the main component. The electropherogram shows separation of the sample solution containing 0.5% of *S*-isomer as optical impurity besides the main isomer (Fig. 5).

Analysis of the real tamsulosin product, in which the concentration of tamsulosin product was 0.01 mg ml^{-1} (2.45×10^{-6} mol l^{-1}) is shown in Fig. 6. The analysis shows that in real tamsulosin sample was detected only *R*-isomer.

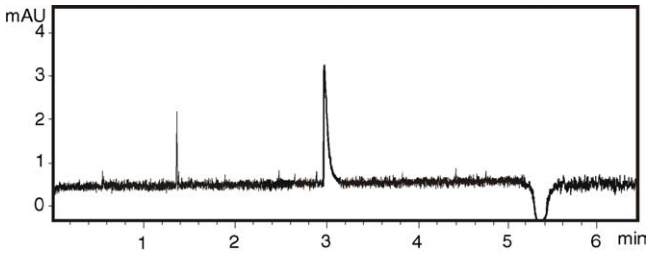

Fig. 6. Electropherogram of real tamsulosin product containing 0.01 mg ml^{-1} (2.45×10^{-6} mol l^{-1}) of tamsulosin. Conditions: 100 mM acetate/TRIS, pH 4.0, with 0.1% of S-β-CD, +20 kV.

3.5. Calibration

The concentration range used for calibration was from 6×10^{-6} to 6×10^{-5} mol l^{-1}. Calibration standard solutions were prepared by dissolution of the standard compounds in the 10-times diluted background electrolyte without CDs.

The linear regression equations were: $y = 1.06 \times 10^3 x - 6.86$ ($R^2 = 0.997$) and $y = 1.03 \times 10^3 x - 4.67$ ($R^2 = 0.995$) for the *R*- and *S*-enantiomer, respectively. All analyses were carried out in triplicates.

3.6. Limits of detection and limits of quantifications

The detection limits were 1.6×10^{-6} mol l^{-1} for *R*-isomer and 1.7×10^{-6} mol l^{-1} for *S*-isomer (S/N = 3). The limits of quantification were 5.3×10^{-6} mol l^{-1} for *R*-isomer and 5.7×10^{-6} mol l^{-1} for *S*-isomer (S/N = 10).

4. Conclusions

The method for chiral separation of tamsulosin enantiomers was developed. The method is simple, fast and effective. It can be used for determination of both enantiomers and applied for quality control and fast screening metabolic studies. The countercurrent flow of negatively charged S-β-CD to oppositely charged analytes allows chiral separation with small amounts of the chiral selector.

Acknowledgement

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