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Chiral recognition of dapoxetine enantiomers with methylated-gamma-cyclodextrin: A validated capillary electrophoresis method

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ABSTRACT

The enantiomers of dapoxetine, a serotonin transporter inhibitor for the treatment of premature ejaculation have been separated by cyclodextrin modified capillary zone electrophoresis using uncoated fused-silica capillary. Over 20 cyclodextrins were screened as chiral selectors, investigating the stability of the inclusion complexes and enantioseparating properties. According to the preliminary experiments as chiral selector randomly methylated-γ-cyclodextrin was chosen. The basic chemical and instrumental parameters of enantioseparation as concentration of buffer, chiral selector and organic additive, pH, temperature and applied voltage were optimized afterwards using an orthogonal experimental design. Using this methodology not only the optimal parameter values for chiral separation (15 °C, +15 kV, 70 mM acetate, 20 v/v% MeOH, pH* = 4.5, 3 mM methylated-γ-CyD) but also the significance order of factors on resolution was determined. Applying these parameters an optimal resolution of 7.01 was achieved. The optimized method was then validated according to the ICH guideline Q2 (R1) with regard to repeatability, linearity range, LOD, LOQ, accuracy and robustness.

1. Introduction

Premature ejaculation (PE) is the most common male sexual disorder, and is estimated to affect up to 30% of men worldwide [1]. PE affects men of all ages equally from pubescent to elderly. Despite its high prevalence and well-known adverse effects on men's life quality, only recently has attention been focused on developing therapeutic strategies. As treatment of the condition numerous non-pharmacological (e.g. psychological and behavioral therapy, self-help techniques, mechanical aids) and pharmacological (topical anesthetics, selective serotonin reuptake inhibitors (SSRIs), type 5 phosphodiesterase inhibitors) approaches are in practice [2]. The potential of antidepressants to treat PE was first introduced by Ahlenius et al. in 1981 [3]. As SSRIs are intended for chronic use in the treatment of depression, they are designed to provide a constant systemic concentration with long-term administration and daily dosing. This application in the treatment of PE is associated with a number of undesirable side-effects, such as decreased libido and erectile dysfunction [4]. Therefore, an ideal SSRI compound treating PE should have rapid absorption and elimination to minimize the side-effects [5].

Dapoxetine (Dpx), (*S*)-N,N-dimethyl[3-(naphthalen-1-yloxy)-1-phenylpropyl]amine hydrochloride, Priligy®) is a novel short acting selective serotonin reuptake inhibitor that is being developed specifically as an on-demand oral treatment of PE with a unique physicochemical and pharmacokinetic profile [5]. Dpx attains its peak plasma concentration in about 1.5 h after dosing which is much faster than conventional SSRIs and by 24 h the plasma concentration decreases to approximately 5% of the peak concentration. These pharmacokinetic properties make Dpx an excellent candidate for on-demand dosing of PE. A wide range of synthetic procedures were developed to synthesize racemic and enantiopure Dpx [6–14]. The enantiomeric excess in these procedures were verified solely by chiral HPLC [8,11,13]. As the eutomer (*S*)-Dpx is 3.5 times more potent SSRI than (*R*)-Dpx [15] and most of the synthetic procedures bear the possibility of chiral contamination, a fast and reliable enantioseparating method is essential for the analysis of the compound. Moreover, this drug is also a target for adulteration as other active pharmaceutical ingredients (APIs) for the treatment of sexual disorders [16].

Enantiomeric purity and characterization of the enantiomers' biological activity are crucial requirements for novel chiral drug candidates. Therefore, enantioselective analytical methods are necessary to control the enantiomeric purity of pharmaceutical preparations. Undoubtedly, capillary electrophoresis has been

Abbreviations: API, active pharmaceutical ingredient; BGE, background electrolyte; Dpx, dapoxetine; DS, degree of substitution; EtOAc, ethyl acetate; PE, premature ejaculation; SSRI, selective serotonin reuptake inhibitor.

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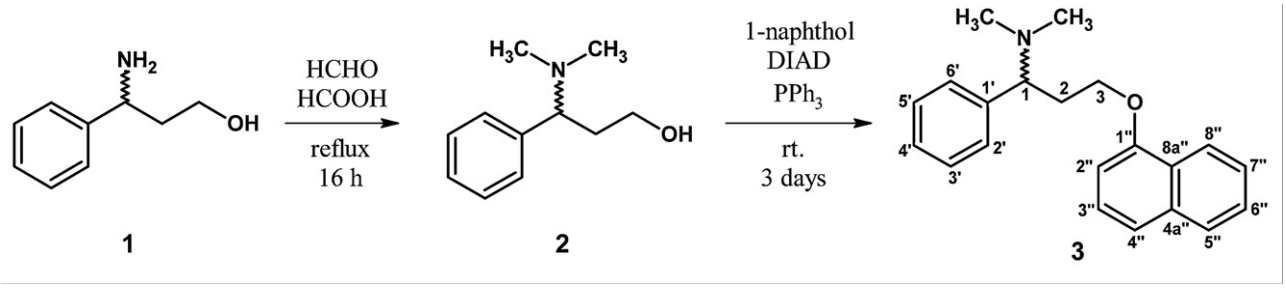

Fig. 1. Synthesis and numbering of dapoxetine (**3**).

widely used for chiral separation in terms of its high efficiency, rapid analysis time, high resolution, small sample volume requirement and less pollution [17]. Applying cyclodextrins (CyDs) and their derivatives as chiral selectors is one of the most commonly used methods in capillary zone electrophoresis [18]. Chiral recognition using CyDs is based on the different interaction affinity between the chiral selector and the analyte enantiomers. Native CyDs have truncated cone shapes with hydroxyl groups around its narrower and wider rims. The bulky aromatic moieties of dapoxetine (Fig. 1) make this API an excellent guest for cyclodextrin complexation. The surface hydroxyl groups of CyDs can be modified chemically, leading to CyDs with different properties. Modified CyDs accommodate a wide range of analytes owing to their increased solubility and additional stereoselective bondings [19]. In our work, several native and substituted, neutral and charged α-, β- and γ-cyclodextrin derivatives were applied as chiral selectors to separate the Dpx enantiomers.

Chiral CE method development strategy first involves the screening of various selectors. To obtain a satisfactory enantioresolution the optimization of the operating conditions are essential. The optimization strategy of changing one variable at a time requires a large number of independent runs and the interactions of variables cannot be observed either. Therefore, experimental designs are often employed for optimization in CE [20]. Orthogonal experimental design has been successfully applied in many scientific areas, which provided a simple, efficient, and systematic approach to optimize designs for performance, quality and cost. According to the statistical analysis, it allows a large number of factors to be screened and detects the significant effects from the many less important ones [21,22]. In this research, an orthogonal experimental design was carried out to investigate the effects of CyD concentration, temperature, applied voltage, buffer concentration, pH and amount of organic modifier added to the background electrolyte (BGE) on the enantioresolution.

To the best of our knowledge no capillary electrophoretic method has been published so far on dapoxetine, however cyclodextrin based chiral analysis of other SSRIs, such as sertraline, paroxetine, venlaflaxine and fluoxetine has already been carried out [23–31]. This study reports a robust, sensitive and validated method for the chiral separation of Dpx enantiomers via cyclodextrin complexation.

2. Materials and methods

2.1. Synthetic procedures

To synthesize the racemic dapoxetine (**3**) the literature procedure of (*S*)-dapoxetine reported by Torre et al. was followed with slight modifications (Fig. 1) [8]. 3-amino-3-phenylpropan-1-ol (**1**) (3.45 g, 23 mmol) was refluxed with formaldehyde (25 ml 35% aqueous solution) and formic acid (4 ml) for 16 h to obtain the crude racemic dimethyl derivative (**2**). Following the column chromatography purification using silica gel with ethyl acetate (EtOAc) to 20% MeOH/EtOAc gradient elution, 1.92 g pure (**2**) (yellow oil, yield 46%) was obtained. To the solution of **2** (1.00 g, 5.6 mmol in 80 ml dry tetrahydrofuran) 1-naphthol (1.70 g, 11.8 mmol) was added under nitrogen atmosphere. After cooling the mixture to 0 °C, triphenylphosphine (3.07 g, 11.7 mmol) and diisopropyl azodicarboxylate (2.2 ml, 11.2 mmol) were added sequentially. The solution was stirred at room temperature for 3 days. The solvent was removed under reduced pressure resulting in dark brown oil. The previously described purification using column chromatography with gradient elution of EtOAc to 10% MeOH/EtOAc was not sufficient. Thus, an additional column chromatography step was applied using 5% MeOH/CH_2Cl_2 to obtain the by-product free colorless oil **3** (0.85 g yield: 50%).

^{1}H NMR ($CDCl_3$, 600 MHz): δ(ppm): 8.23 (dm, 1H, J = 6.9 Hz, 8″-H), 7.77 (dm, 1H, J = 6.9 Hz, 5″-H), 7.47 (m, 1H, 6″-H), 7.46 (m, 1H, 7″-H), 7.37 (d, 1H, J = 8.3 Hz, 4″-H), 7.31 (m, 2H, 3′-H and 5′-H), 7.28 (m, 1H, 3″-H), 7.27 (m, 2H, 2′-H and 6′-H), 7.26 (m, 1H, 4′-H), 6.64 (d, 1H, J = 7.6 Hz, 2″-H), 4.06 (m, 1H, 3-H), 3.90 (m, 1H, 3-H), 3.60 (dd, 1H, J = 9.3, 5.4 Hz, 1-H), 2.63 (m, 1H, 2-H), 2.27 (m, 1H, 2-H), 2.25 (s, 6H, $N(CH_3)_2$).

^{13}C NMR ($CDCl_3$, 150 MHz): δ(ppm): 155.3 (C1″), 140.2 (C1′), 135.1 (C4a″), 129.3 (C2′ and C6′), 128.9 (C3′ and C5′), 128.1 (C5″), 128.0 (C4′), 127.0 (C6″), 126.5 (C3″), 126.3 (C8a″), 125.7 (C7″), 122.7 (C8″), 120.7 (C4″), 105.2 (C2″), 68.3 (C1), 66.3 (C3), 43.5 ($N(CH_3)_2$), 33.7 (C2).

HRMS calculated for $C_{21}H_{24}NO$ $[M+H]^+$ 306.1852, found 306.1842.

2.2. Chemicals, reagents

Racemic Dpx was synthesized by the authors as described in Section 2.1 as it was not commercially available at the time of the experiments. All reagents were obtained from commercial sources and used without purification. Compound **1** was prepared according to literature procedure [8]. Non-racemic Dpx was kindly provided by Vicente Gotor-Fernández and coworkers for the determination of enantiomer migration order in chiral separations. All the applied native CyDs and their derivatives (native α-, β-, γ-CyD, hydroxypropylated-α-, β-, γ-CyDs with various degrees of substitution (DS), methylated-β-, γ-CyDs with various DS, carboxymethylated-β-, γ-CyD, carboxyethylated-β-CyD, sulfopropylated-α-, β-, γ-CyD, sulfohydroxypropylated-γ-CyD and sulfobutylated-α- and β-CyD) were products of Cyclolab Ltd. (Budapest, Hungary). Acetic acid, NaOH and HCl used for the preparation of BGE and the analytical grade organic modifier acetonitrile and methanol were purchased from commercial suppliers. The NMR solvent $CDCl_3$ was obtained from Sigma. As EOF marker in CE experiments DMSO from Reanal (Budapest, Hungary) was used. Bidistilled Millipore water was used throughout this study.

2.3. Instrumentation

All NMR experiments were carried out on a 600 MHz Varian DDR NMR spectrometer equipped with a 5 mm inverse-detection gradient (IDPFG) probehead. Standard pulse sequences and processing routines available in VnmrJ 2.2 C/Chempack 4.0 were used for structure identifications. The probe temperature was maintained at 298 K and standard 5 mm NMR tubes were used.

The exact mass of Dpx was determined with an Agilent 6230 time-of-flight mass spectrometer. Samples were introduced by the Agilent 1260 Infinity LC system, the mass spectrometer was operated in conjunction with a JetStream source in positive ion mode. Reference masses of m/z 121.050873 and 922.009798 were used to calibrate the mass axis during analysis. Mass spectra were processed using Agilent MassHunter B.02.00 software.

The CE experiments were carried out on a HP^{3D} capillary electrophoresis system equipped with a photodiode array detector and controlled by the HP^{3D} CE ChemStation software. An untreated fused-silica capillary of 50 μm i.d., with a total length of 64.5 cm (56 cm effective length) purchased from Agilent was used for the separation. Conditioning of new capillaries was conducted by flushing with 1 M NaOH for 30 min followed by 0.1 M NaOH and buffer for 60 min each. Samples were injected hydrodynamically by applying 50 mbar pressure for 4 s. The detection wavelength was set to 215 nm. The capillary temperature varied from 15 to 35 °C and voltage was applied from 15 kV to 30 kV. Prior to all runs the capillary was preconditioned by rinsing with water (2 min), 0.1 M NaOH (1 min), water (1 min) and BGE (2 min).

2.4. Preparation of BGE and sample

Dapoxetine hydrochloride has a pK_a of 8.6 [32]. For CE measurements acetate buffers (30 mM, 50 mM and 70 mM) at pH 4.5, 5.5, and 6.5 were applied, pH was adjusted by addition of 1 M NaOH or 1 M HCl. BGEs were prepared by dissolving the desired amounts of both the organic modifier (0–20 v/v%) and chiral selector in the appropriate acetate buffer. In the case of preliminary experiments the cyclodextrin concentration varied from 1 to 32 mM. Stock solution of Dpx (0.5 mg/ml) was prepared in MeOH and a fivefold dilution was used for the measurements. Spiked samples were prepared by adding 20 (v/v%) of non-racemic Dpx (enriched in (*S*)-isomer) solution to the racemate.

3. Results and discussion

3.1. Preliminary experiments

For the identification of the suitable CyD for chiral separation a wide range of single CyD systems were investigated at various concentration levels. As BGE, 50 mM pH 5.5 acetate buffer was used. The temperature of the capillary was maintained at 25 °C, 30 kV voltage was applied and no organic modifier was added to the BGE. The separation potential of all CyDs was investigated at three concentrations (4 mM, 8 mM and 12 mM for β-CyD and 8 mM, 16 mM and 32 mM for other CyDs, respectively). The binding constants of the inclusion complexes were determined using the *x*-reciprocal linearization method [33]. The resolution (R_S) was applied as the secondary response function when evaluating the performance of the separation system and was calculated as:

$$R_S = \frac{2(t_R - t_S)}{w_R + w_S}$$

where t_R and t_S stand for the migration times and w_R and w_S for the extrapolated peak width of the enantiomers at the baseline. All preliminary experiments were run in triplicate, thus both complex stabilities and resolution values were determined based on three parallel experiments.

The results showed that the binding constants of the complexes varied from 30 to 700 M^{-1}. The most stable complexes were formed with methylated-β-CyD (DS ∼ 12) ($K = 510 \pm 10\ M^{-1}$), methylated-γ-CyD (DS ∼ 12) ($K_S = 360 \pm 20\ M^{-1}$ and $K_R = 590 \pm 40\ M^{-1}$) and sulfobutylated-β-CyD (DS ∼ 4) ($K_S = 600 \pm 10\ M^{-1}$ and $K_R = 700 \pm 20\ M^{-1}$). Six of the applied CyDs resulted in partial or baseline enantioseparation: hydroxypropylated-γ-CyD (DS ∼ 3), methylated-γ-CyD (DS ∼ 12), permethylated-β-CyD (DS ∼ 21), carboxymethylated-γ-CyD (DS ∼ 3), sulfopropylated-β-CyD (DS ∼ 2), and sulfobutylated-β-CyD (DS ∼ 4). Applying these CyDs further experiments were carried out with the single variation optimization of the cyclodextrin concentration. Hydroxypropylated-γ-CyD, sulfobutylated-β-CyD and sulfopropylated-β-CyD had poor enantioselectivity and $R_S = 1.00$ could not be exceeded with either of these selectors. Carboxymethylated-γ-CyD and permethylated-β-CyD proved to be promising selectors as the resolutions of $R_S = 1.38 \pm 0.09$ and $R_S = 1.54 \pm 0.07$ were detected at optimal CyD concentrations, respectively. However, based on the results of preliminary experiments methylated-γ-CyD (DS ∼ 12) was chosen as the final chiral selector. The optimal concentration of this selector (3 mM) resolved the enantiomers with the highest resolution of $R_S = 3.32 \pm 0.06$. The complex with a higher binding constant was formed with (*R*)-Dpx in this case, thus this enantiomer migrated second. Based on these results 1, 3 and 5 mM CyD concentrations were chosen for the orthogonal experimental design. Separations with the highest resolution achieved applying hydroxypropylated-γ-CyD (DS ∼ 3), permethylated-β-CyD (DS = 21) and methylated-γ-CyD (DS ∼ 12) are depicted in Fig. 2.

The complex stabilities and capillary electrophoresis-based chiral separations of other structurally related SSRIs – most commonly fluoxetine – have already been thoroughly investigated. Binding constants of fluoxetine with β-, methylated-β-, hydroxypropylated-β- and sulfobutylether-β-CyD were studied by Piperaki et al. and were found to vary from 100 to 1000 M^{-1} [24], which is in good correlation with our results as stability of Dpx–CyD complexes were found to be between 30 and 700 M^{-1}. The fluoxetine enantiomers were successfully separated using β- ($R_S = 0.62$), methylated-β- ($R_S = 0.32$), hydroxypropylated-β- ($R_S = 0.59$) and sulfobutylether-β-CyD ($R_S = 1.71$) by Piperaki et al. [24] and baseline separation was published with dimethylated-β-CyD by Desiderio et al. [25]. The highest resolution was reported by Rudaz et al., as highly sulfated-γ-CyD separated the isomers with a resolution of 22.5 [31]. In our study, methylated, hydroxypropylated and several charged CyDs – including sulfobutylether-β-CyD – were also proved to have chiral separation potential for Dpx enantiomers.

The application of organic modifiers in the separation method can significantly influence the resolution by changing the viscosity of the BGE and the stability of the inclusion complex [22,34]. During the preliminary experiments, the effect of two organic additives, methanol and acetonitrile were investigated. While addition of methanol improved the resolution, acetonitrile had an unfavorable effect on peak shape, thus methanol was chosen for further optimization at 0–10–20 v/v% ratios.

The ionic strength of the BGE, which can be significantly influenced by variation of buffer concentration, has a great impact on the separation. On the basis of preliminary experiments 30, 50 and 70 mM acetate buffers were applied for the optimization. The pH of the BGE has a great significance as well and it was chosen according to the pK_a of the analyte. In the orthogonal experimental design BGEs of pH 4.5, 5.5 and 6.5 were used. As in this pH range the analyte is practically fully protonated, changing the pH merely affects the velocity of the EOF. Consequently, solely achiral forces take part

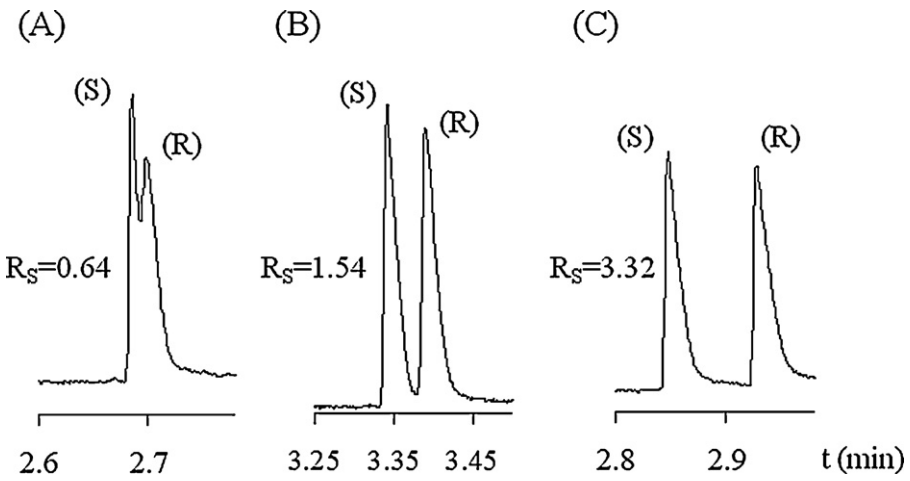

Fig. 2. Highest enantioresolutions achieved at optimal concentrations of hydroxypropylated-γ-CyD (A, C_{CyD} = 3.75 mM), permethylated-β-CyD (B, C_{CyD} = 9.50 mM) and methylated-γ-CyD (C, C_{CyD} = 3.00 mM).

in altering the resolution values by application of buffer differing in concentration and pH in this case.

Applied voltage and temperature also influence the chiral separation, therefore need to be optimized. The voltage affects the velocity of the electroosmotic flow, the analytes and the applied chiral selectors as well, thus greatly affects the migration times and the Joule heating. Generally higher applied voltage results in higher separation efficiency by peak-shape improvement. This favorable effect of high applied voltage is counteracted by the increasing Joule heating. The temperature of the capillary has effect on the viscosity of the BGE and stability of the inclusion complex. During the optimization temperatures of 15, 25 and 35 °C and voltages of 15, 20 and 25 kV were used.

3.2. Method development and optimization

The development of an effective methodology to investigate and optimize significant factors is of great importance. The single variation method is an easy way to optimize the separation parameters, however, it neglects that the parameters affect each other. Thus, orthogonal experimental design was applied for the optimization, providing not only more information but also making it possible to achieve the optimal experimental conditions in relatively low number of experiments. It is also a useful tool to discriminate the significant factors from the investigated ones. There were six experimental factors chosen as variable parameters: temperature (15, 25 and 35 °C), applied voltage (15, 20 and 25 kV), concentration of chiral selector methylated-γ-CyD (DS ~ 12) (1, 3 and 5 mM), MeOH concentration (0, 10 and 20 v/v%), BGE concentration (30, 50 and 70 mM) and pH (4.5, 5.5 and 6.5). A standard orthogonal table L_{18} (6^3) was used for the design of the experiments, in which 18 stands for the total number of experiments, while 3 represents the number of levels and 6 stands for the number of investigated parameters. All experiments were carried out in triplicate. The interactions among various factors examined were neglected in the screening step. As a response, resolution values were recorded in each experiment. The experimental parameters of the individual runs and the resolution values are summarized in Table 1. The R_S varied from 1.21 to 6.45 in these experiments.

A range analysis was carried out to clarify the significance of each factor on the resolution. In Table 1 K1–K3 stand for the average resolution value under every level of a variable, e.g. in the temperature column K1 is the average resolution achieved at 15 °C. Range value (R) displays the difference between the maximal and

Table 1
Orthogonal experiments design chart, six variables, and three levels.

Experiment	T (°C)	U (kV)	c_{CyD} (mM)	c_{org} (v/v%)	c_{BGE} (mM)	pH	R_S
1	15	15	1	0	30	4.5	4.92
2	15	20	3	10	50	5.5	5.74
3	15	25	5	20	70	6.5	6.45
4	25	15	1	10	50	6.5	4.46
5	25	20	3	20	70	4.5	5.81
6	25	25	5	0	30	5.5	4.01
7	35	15	3	0	70	5.5	3.68
8	35	20	5	10	30	6.5	2.35
9	35	25	1	20	50	4.5	2.39
10	15	15	5	20	50	5.5	4.61
11	15	20	1	0	70	6.5	4.31
12	15	25	3	10	30	4.5	4.27
13	25	15	3	20	30	6.5	2.55
14	25	20	5	0	50	4.5	3.26
15	25	25	1	10	70	5.5	2.74
16	35	15	5	10	70	4.5	3.03
17	35	20	1	20	30	5.5	1.21
18	35	25	3	0	50	6.5	2.16
K1	5.05	3.88	3.34	3.72	3.22	3.95	
K2	3.81	3.78	4.04	3.77	3.77	3.67	
K3	2.47	3.67	3.95	3.84	4.34	3.71	
R	2.58	0.21	0.7	0.12	1.12	0.28	

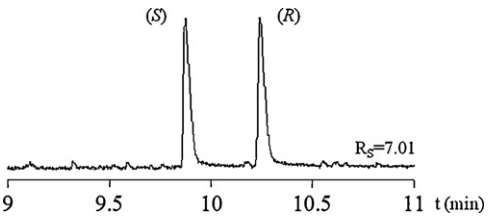

Fig. 3. Highest resolution of Dpx enantiomers achieved with the optimized parameters (3 mM selector, 70 mM acetate buffer, pH* = 4.5, 20 v/v% methanol, 15 °C, 15 kV) of a methylated-γ-CyD system. Migration order was determined by spiking the sample with non-racemic analyte.

minimal value of the three levels ($K_{max} - K_{min}$ for each parameter). The higher range value indicates the greater influence of the parameter on the separation.

Based on the results of the range analysis, the order of significance levels is as follows: temperature, buffer concentration, CyD concentration, pH, applied voltage and concentration of organic modifier. The variance analysis based on the results revealed that none of the investigated parameters had significant effect on the resolution value, thus none of the parameters needed to be optimized further. It must be clarified that conclusions drawn from the significance of the investigated parameters are only valid in the applied range.

According to the orthogonal experimental design data the optimal response is achieved at 15 °C applying 15 kV voltage using a BGE of 70 mM acetate with 20 v/v% MeOH pH* set to 4.5 including 3 mM methylated-γ-CyD. Applying these parameters an optimal resolution of 7.01 ± 0.11 was achieved (Fig. 3).

3.3. Method validation

After systematic optimization of the BGE and capillary parameters, the validation of the chiral CE method was carried out by studying the repeatability, linearity range, LOD, LOQ, accuracy and robustness following the ICH Q2 (R1) guideline [35].

3.3.1. Repeatability

The repeatability of migration times and peak areas of (*R*)- and (*S*)-Dpx were determined in eight parallel measurements. The concentrations of the enantiomers were kept constant at 90 μg/ml during these runs. The RSD of migration times were 2.63% and 2.67%, the RSD of peak areas were 3.72% and 4.06%, while the relative standard deviation of resolution was 3.74%. Repeatability was additionally checked at 25 μg/ml and 250 μg/ml enantiomer concentrations in five parallel measurements. The RSD percents of migration times, corrected peak areas and enantioresolutions were found to be below 5.00% in all cases. Based on these data the method has good repeatability.

3.3.2. Linearity range

The linearity of (*R*)- and (*S*)-Dpx was investigated at nine concentration levels of the racemic sample ranging from 2 μg/ml to 1000 μg/ml for each enantiomer. The calibration curves were linear over the concentration range from 5 μg/ml to 1000 μg/ml for both isomers. The correlation coefficients (r^2) of the calibration line were over 0.999 for the (*S*) and over 0.998 for the (*R*) isomer. The linear equations for (*R*) and (*S*) were $y_R = 0.4069x + 4.0362$ and $y_S = 0.3773x + 4.0137$ (x represents the concentration of the racemic sample in μg/ml; y represents the peak area of the enantiomer).

3.3.3. LOD and LOQ

LODs and LOQs of each isomer of Dpx were calculated at signal to noise ratio, 3:1 and 10:1, respectively. The LODs and LOQs for (*R*)- and (*S*)-Dpx were 1.5 μg/ml and 5.0 μg/ml.

3.3.4. Accuracy

The method accuracy was assessed as recovery obtained when spiking the test solutions (250 μg/ml racemate) with known concentrations of the racemic sample (10, 25 and 50 μg/ml). The recovery values for both enantiomers were over 95% (*R*: 96.68%, *S*: 95.58%) with RSD lower than 3% (*R*: 1.60%, *S*: 2.50%).

3.3.5. Robustness

During the robustness testing, the analysis method must prove to be able to remain unaffected by small, but deliberately introduced variations in method variables. If the variables have significant effect on the responses, one should change the method or control experiment procedure more strictly. For the test sample containing 0.1 mg/ml racemic Dpx was used. As response functions peak areas, migration times and resolution were obtained. The robustness of the method was studied with the widely accepted Plackett–Burman statistical experimental design to minimize the number of experiments. The factor interactions can be negligible due to the small variations in the factor levels [22,36].

In the test, six variables which might affect the results were investigated on six responses. The method variables were investigated at the upper (+) and lower (−) values with regard to the nominal one which is the optimal value in the procedure. The six variables included the CyD concentration, buffer concentration, pH, organic additive concentration, temperature and voltage. Two

Table 2
The Plackett–Burman experimental design applied for robustness testing of the optimized method.

Experiment	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	Dummy-1	Dummy-2
1	3.2	72	4.4	22	14	14	−1	1
2	2.8	68	4.4	22	16	16	−1	1
3	2.8	68	4.4	18	14	14	−1	−1
4	3.2	72	4.6	18	16	16	−1	1
5	3.2	68	4.6	18	14	14	1	1
6	2.8	68	4.6	22	16	14	1	1
7	3.2	68	4.4	18	16	16	1	−1
8	3.2	68	4.6	22	14	16	−1	−1
9	2.8	72	4.4	18	14	16	1	1
10	2.8	72	4.6	22	14	16	1	−1
11	3.2	72	4.4	22	16	14	1	−1
12	2.8	72	4.6	18	16	14	−1	−1

[a] CyD concentration (mM).
[b] BGE concentration (mM).
[c] pH.
[d] MeOH concentration (v/v%).
[e] Temperature (°C).
[f] Applied voltage (kV).

dummy factors were included in the design to estimate the experimental error. The dummy factor is an imaginary variable of which the change between the levels does not represent a physical change in the method [37]. The experimental designs are given in Table 2.

According to the statistical testing of the results, it was found that none of the six studied variables had a significant effect on either the peak areas, or time migration times or the resolution of Dpx isomers. The test thus confirmed the proper robustness of the method.

4. Conclusions

Cyclodextrin-hosted diastereomeric complexation and concomitant enantioseparation of dapoxetine enantiomers and characterization of their inclusion complexes were carried out. The apparent binding constants and enantiomeric resolution of the isomers with 22 various CyDs were determined. The parameters of the most promising system, using randomly methylated-γ-CyD as chiral selector was further optimized in terms of CyD, buffer and organic modifier concentration, pH, temperature and applied voltage. For the testing of parameters orthogonal experimental design was used, thus the significance of the factors was also studied. The highest enantioresolution ($R_S = 7.01 \pm 0.11$) was achieved using a pH* 4.5 BGE of 70 mM acetate buffer with 20 v/v% MeOH, including 3 mM methylated-γ-CyD at 15 °C applying 15 kV voltage. The method was afterwards validated according to the proper ICH guideline.

Conflict of interest

The authors have declared no conflict of interest.

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