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A chiral enantioseparation generic strategy for anti-Alzheimer and antifungal drugs by short end injection capillary electrophoresis using an experimental design approach

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Abstract

The present study describes a generic strategy using capillary electrophoretic (CE) method for chiral enantioseparation of anti-Alzheimer drugs, namely, donepezil (DON), rivastigmine (RIV), and antifungal drugs, namely, ketoconazole (KET), Itraconazole (ITR), fluconazole (FLU), and sertaconazole (SRT) in which these drugs have different basic and acidic properties. Several modified cyclodextrins (CDs) were applied for enantioseparation of racemates such as highly sulfated α, γ CDs, hydroxyl propyl-β-CD, and Sulfobutyl ether-β-CD. The starting screening conditions consist of 50-mM phosphate-triethanolamine buffer at pH 2.5, an applied voltage of 15 kV, and a temperature of 25°C. The CE strategy implemented in the separation starts by screening prior to the optimization stage in which an experimental design is applied. The design of experiment (DOE) was based on a full factorial design of the crucial two factors (pH and %CD) at three levels, to make a total of nine (3^2) experiments with high, intermediate, and low values for both factors. Evaluation of the proposed strategy pointed out that best resolution was obtained at pH 2.5 for five racemates using low percentages of HS-γ-CD, while SBE-β-CD was the most successful chiral selector offering acceptable resolution for all the six racemates, with the best separation at low pH values and at higher %CD within 10-min runtime. Regression study showed that the linear model shows a significant lack of fit for all chiral selectors, anticipating that higher orders of the factors are most likely to be present in the equation with possible interactions.

KEYWORDS

azole derivatives, donepezil, enantiomers, experimental design, highly sulphated α,γ-cyclodextrins, rivastigmine

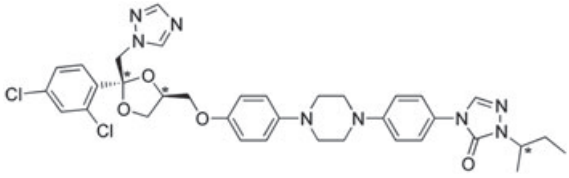
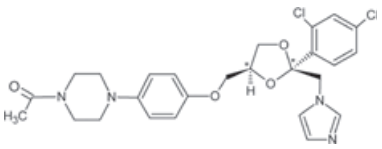
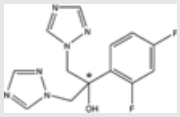
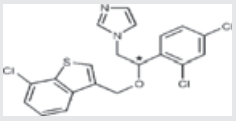
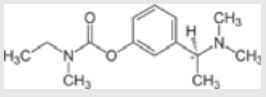
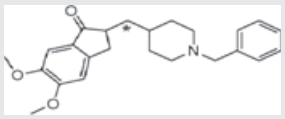
Abbreviations: BGE, Background electrolyte; CDs, Cyclodextrins; EOF, Electroosmotic flow; HS-CD, Highly sulfated Cyclodextrins; SBE- β-CD, Sulfobutyl Ether β- Cyclodextrin; TEA, Triethanolamine

1 | INTRODUCTION

Alzheimer disease is a neurodegenerative disorder characterized by progressive loss of memory followed by complete dementia which characterized by loss of memory and progressive deficits in different cognitive domains.[1] Rivastigmine (RIV) [(*S*)-*N*-ethyl-3-[(1-dimethyl-amino) ethyl]-*N*-methyl-phenyl-carbamate hydrogen tartrate] is a carbamate derivative of physostigmine which is able to react covalently with the active site of the enzyme.[2] The chemical structures of the drugs under study are shown in Table 1. Although its exact mechanism of action is still unknown, it is thought to exert its therapeutic effect by a reversible and noncompetitive acetylcholine esterase inhibitor, selectively inhibiting its activity in certain brain areas, also enhance choline acetyl transferase activity that stimulates acetylcholine synthesis.[3,4] A structural feature of DON is the presence of a chiral center adjacent to a carbonyl group which makes each enantiomer easily racemizable via keto-enol intermediate.[5] On the other hand, the antifungal drugs, azole compounds have been used for nearly 40 years. The compounds under investigation in this work are ketoconazole (KET), itraconazole (ITR), fluconazole (FLU), and sertaconazole (SRT); these azoles affect the synthesis of ergosterol because they are potent inhibitors of cytochrome P450 (CYP) of the fungus.[6] They have a broad and potent antifungal effect, but most of the azole antifungal drugs have side effects such as KET which limits its clinical applications due to the hepatic toxicity.[7] These azoles are chiral drugs although they are employed clinically as a racemic mixture 1:1 mixture of the dioxolane ring in *cis* configuration as shown in Table 1.

In the last years, special emphasis has been placed on the synthesis of enantiomerically pure compounds, particularly in the pharmaceutical industry enantiomers.[8] Enantioseparation of racemates is considered one of the attractive topics in the field of pharmaceutical analysis as optical purity control needs rapid and highly efficient analytical methods.[9] The FDA stated its policy statement for the method development and determination of chiral drugs: The stereoisomeric of a drug with a chiral center should be known as well as the composition of the material used in pharmacologic, toxicological and clinical studies.[10] DON enantiomers have shown different degrees of inhibition against acetylcholine in vivo, S-DON having a 2.2-fold higher affinity than R-DON while both RIV enantiomers have the same inhibitory activity.[11,12] S-KET was 2-4 fold more potent inhibitor of CYP450 than R-KET and therefore has stronger antifungal activity in vivo studies.[13] ITR has three chiral centers that give rise to eight

TABLE 1 Chemical structures for drugs under investigation

Acidic drugs	Basic drugs
 **Itraconazole**(pKa , 3.92)	 **Ketoconazole** (pKa, 6.75)
 **Fluconazole**(pKa , 2.03)	 **Sertaconazole** (pKa, 6.77)
	 **Rivastigmine** (pKa, 8.89)
	 **Donepezil** (pKa, 8.82)

stereoisomers. It was demonstrated that 2S, 4R, 2S-ITR and 2S, 4R, 2R-ITR possess the greatest antiangiogenic and antifungal activity.[14] Obviously, it was proven in some cases that the therapeutic effect is limited to one of the enantiomers. Furthermore, the pharmacologically inactive eutomer may cause undesirable side effects, in some cases, antagonist, and even toxic effect.[15] The method development strategy of enantiseparation in capillary electrophoresis (CE) is considered a time-consuming task, since finding the appropriate chiral selector is "trial and error" process and usually performed with a one-variable approach, in which the analyst examines the variables that can separate one from one influence while the other factors are constantly executed.[16] It is very hard to predict the selectivity of a chiral selector towards a certain enantiomer. So, the affinity of all selectors has to be examined one at a time. Nowadays, the design of experiment (DOE) is not considered fancy or complex to apprehend anymore. Many software tools and guided approaches are available to allow the multivariate approach which involves the simultaneous change of several variables. CE is suitable for the use of experimental design in which the experimental conditions could be varied from one experiment to another. Up to date, there are only a few studies that report the use of experimental designs in the field of chiral enantioseparation in CE.[17-23] The separation strategies are therefore valuable since they should propose a limited set of experimental conditions, which maximize the probability to separate numerous pharmaceuticals with different molecular structures and properties.[24] Literature survey revealed that reported techniques for estimation of the drugs under investigation were HPLC,[1,25-30] TLC,[31-33] CE,[34-43] and LC-MS/MS.[5,44-49]

This work was aimed to investigate the use of charged CDs for a generic strategy in order to achieve the separation of enantiomeric drug substances. Additionally, to explore the conditions rapidly at which the separation of the enantiomers is sufficiently good with acceptable analysis time. The initial set of experiments was performed to establish basic requirements and followed by a full factorial design for variable selection. This approach can also be considered as the first step for method optimization. It should be clear that the purpose of the DOE is not only to find the optimal separation but also to explore the experimental domain in such a way that a good opportunity to be implemented by a limited number of experiments.

2 | MATERIALS AND METHODS

2.1 | Materials

All racemic drugs were purchased from Sigma Aldrich (St. Louis, Missouri). HS-α-CD and HS-γ-CD were purchased from Beckman Coulter (Fullerton, California). Hydroxypropyl-β-CD and sulfobutylether-β-CD were purchased from Cyclolab R&D (Budapest, Hungary). Orthophosphoric acid (85% w/w) and triethanolamine were obtained from Merck (Darmstadt, Germany). Ultra-pure water resistivity >18 MΩ cm^{-1} at 25°C and TOC < 5 ppb was obtained from Milli-Q UF-Plus system (Millipore, Bedford, Massachusetts).

2.2 | Instrumentation

A Beckman P/ACE MDQ Capillary Electrophoresis System equipped with a diode array detector (Beckman Coulter, Fullerton, California) with a BeckmanPACE station Version 1.1 used for data acquisition using fused-silica capillaries (Polymicro Technologies, Phoenix, Arizona) of 50-μm ID cut to total lengths 31.2 cm with an effective separation

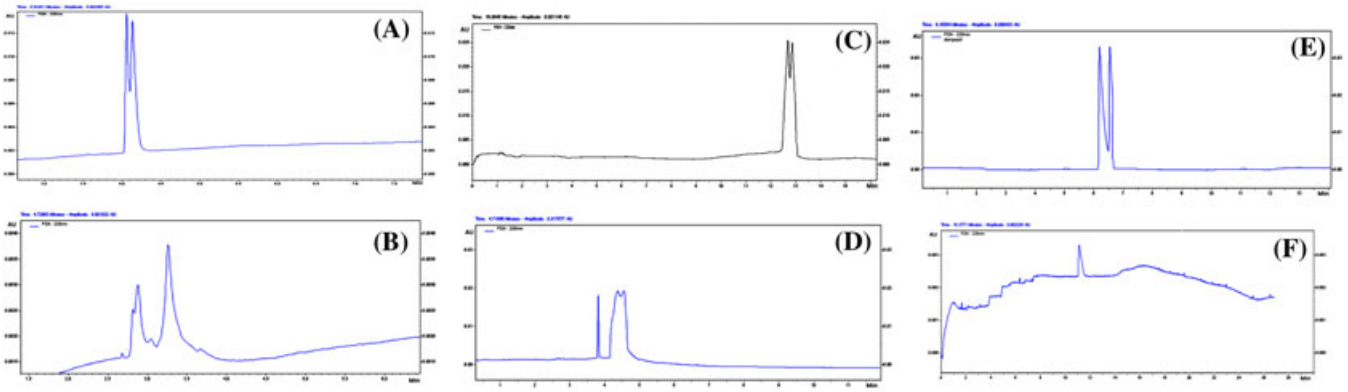


FIGURE 1 Representative CE electropherogram of the enantiomeric separation for A, Ketoconazole; B, Itraconazole; C, Flueconazole; D, Sertaconazole; E, Donepezil; F, Rivastigmine (100 μg mL^{-1}) for each drug under the following conditions: BGE consist of HS-γ-CD (10% m/v) in 50 mM phosphate buffer (pH 2.5), voltage 25 KV, temperature 25 °C and injection pressure 50 m bar for 10 sec

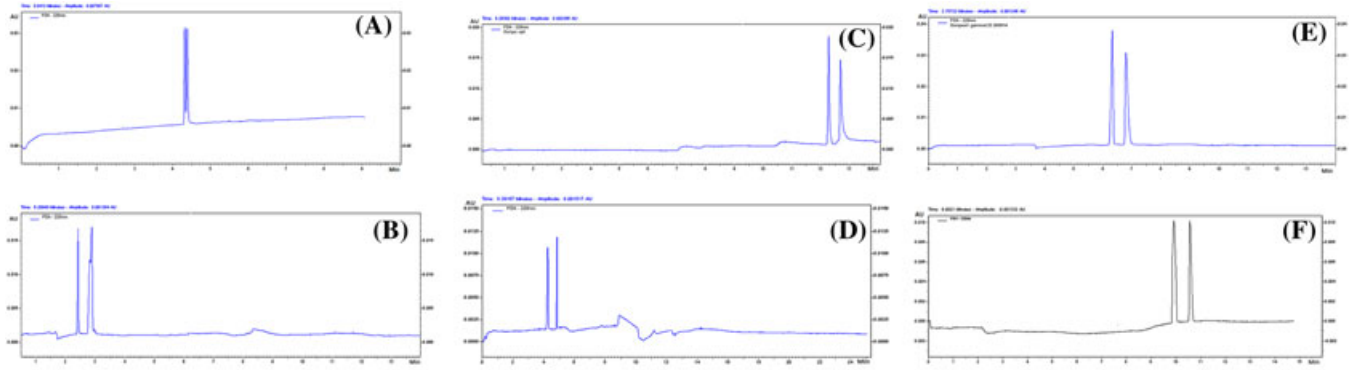


FIGURE 2 Representative CE electropherogram of the enantiomeric separation for A, Ketoconazole; B, Itraconazole; C, Flueconazole; D, Sertaconazole; E, Donepezil; F, Rivastigmine (100 µg mL^{-1}) for each drug under the following conditions: BGE consist of SBE (2.5% m/v) in 50 mM phosphate buffer (pH 2.5), voltage 30 KV, temperature 25 °C and injection pressure 50 m bar for 10 sec

TABLE 2A The 3^2 full factorial design and the measured response results (resolution) for investigated drugs

	Coded factors		Real factors		Rs											
	pH	%CD	pH	%CD	KET		ITR		FLU		SRT		DON		RIV	
Experiment					HS-γ-CD	HS-α-CD	HS-γ-CD	HS-α-CD	HS-γ-CD	HS-α-CD	HS-γ-CD	HS-α-CD	HS-γ-cd	HS-α-CD	HS-γ-CD	HS-α-CD
1	−1	−1	2.5	2.5	0.922	1.629	2.80	1.581	3.12	0.0	2.81	2.801	3.176	0.0	0.0	0.0
2	−1	1	2.5	10	0.899	1.165	1.81	1.141	3.31	0.0	0.321	2.998	3.176	0.451	0.0	0.0
3	1	−1	5.5	2.5	0.391	0.989	0.575	0.260	0.0	0.0	0.0	0.250	0.0	0.0	0.0	0.0
4	1	1	5.5	10	0.451	1.143	0.710	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	−1	0	2.5	5	0.999	1.014	4.80	1.528	3.33	0.0	2.80	3.541	3.67	0.341	0.0	0.0
6	1	0	5.5	5	0.400	2.832	0.790	0.391	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0	−1	4	2.5	0.687	1.116	1.11	0.789	1.81	0.0	1.11	0.671	0.421	0.930	0.0	0.0
8	0	1	4	10	0.790	4.010	1.81	0.451	0.0	0.0	1.09	0.377	0.40	2.90	0.0	0.0
9	0	0	4	5	0.780	4.297	0.987	0.991	0.0	0.0	1.537	0.689	0.342	3.467	0.0	0.0

TABLE 2B The 3^2 full factorial design and the measured response results (resolution) for investigated drugs with SBE-β-CD

	Coded factors		Real factors		*Rs* (SBE-β-CD)					
Experiment	pH	%CD	pH	%CD	KET	ITR	FLU	SRT	DON	RIV
1	−1	−1	2.5	2.5	1.95	1.11	1.51	1.91	2.98	3.31
2	−1	1	2.5	10	3.67	1.92	1.81	2.33	3.59	4.98
3	1	−1	8.5	2.5	0.0	0.0	0.0	0.0	0.0	0.43
4	1	1	8.5	10	0.0	0.0	0.0	0.0	0.0	0.31
5	−1	0	2.5	5	0.95	0.79	0.89	0.99	1.14	2.87
6	1	0	8.5	5	0.0	0.0	0.0	0.0	0.0	0.41
7	0	−1	5.5	2.5	0.34	0.22	0.65	0.91	0.97	0.98
8	0	1	5.5	10	0.88	0.45	0.78	1.87	1.70	1.21
9	0	0	5.5	5	0.65	0.35	1.03	1.32	1.00	0.99

length 21.0 cm was employed. The detection wavelength was fixed at 220 nm.

2.3 | Instrumental conditions

The separations were performed using 50-mM phosphate-triethanolamine buffer (pH 2.5) with varying concentrations of CDs. The activation and preconditioning step for a new capillary were performed by rinsing with 1.0 M NaOH, 0.1 M NaOH, ultra-pure water, and finally BGE solution. Each preconditioning step was performed at a pressure of 60 psi for 10 min at temperature 20°C. After each run, the capillary was first washed with 0.1 N NaOH for 3 min. Then, it was rinsed for 2 min with phosphate buffer (pH 2.5) and equilibrated for 2 min with chiral selector solution. At the end of the day, the capillary was flushed with 0.1 M NaOH for 5 min and finally with water for 10 min.

2.4 | Analysis procedures

Stock solutions of 1.0 mg mL^{-1} of the racemic investigated drugs were prepared in the least amount of methanol and diluted with water/BGE solution for method development studies. Standard solutions of 100 μg mL^{-1} of each

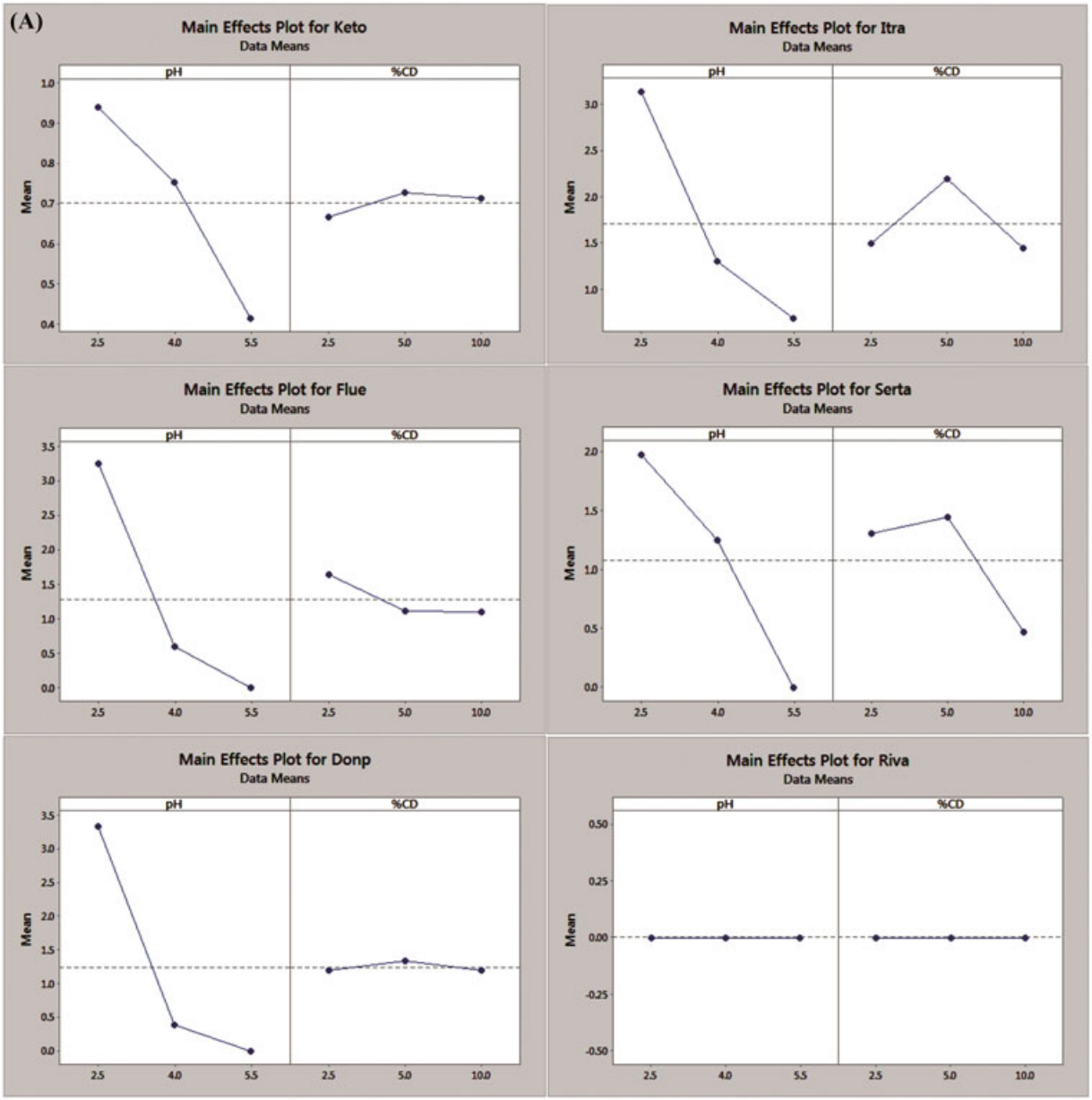


FIGURE 3 A, Main effect plot for the effect of pH and %CD on the resolution of racemates using HS-γ-CD. B, Contour plots for the resolution of six racemates on HS-γ-CD. The X-axis shows the pH and the Y-axis shows the %CD

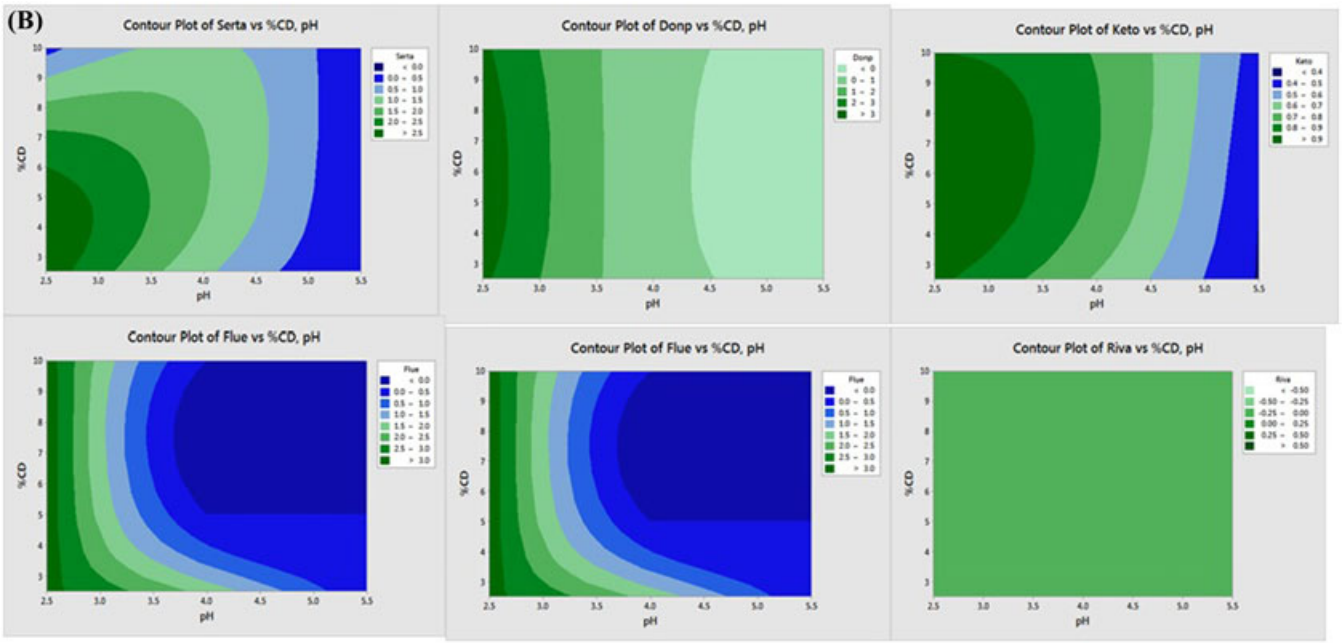


FIGURE 3 Continued

compound was obtained by appropriate dilution with BGE solution.

2.5 | Short-end injection

The samples were injected at the end of the capillary near to the detector; this could be performed by applying negative pressure. For successful separation when using the short-end of the capillary, the CE equipment was operated in the positive polarity mode which means that the detection electrode was the cathode.

2.6 | Long-end injection

Long-end injection or conventional injection means the injection of the analytes was performed at the end of the capillary away from the detector using positive pressure. Thus, the electrophoretic separation was done using negative polarity voltage in which the capillary must be thermostatted as the short-end was outside the capillary cartilage.

2.7 | Experimental design

DOE and optimization were performed using Minitab17 software (State College, Pennsylvania). The screening stage aims to achieve rapid and acceptable separations for all compounds under investigation. Screening strategy is based on the use of highly sulfated (HS-α-CD) and (HS-γ-CD). The design of experiment was based on full factorial design of the crucial two factors (pH and %CD) at three levels, to obtain total nine (3^2) experiments with high, intermediate, and low values for both factors.

3 | RESULTS AND DISCUSSION

Enantioseparation of racemates is a unique challenge. In a symmetric environment, the physical and chemical properties of optical encounters (except the rotation of polarized light) are all identical.[21,50] The drugs under investigation are basic and acidic drugs possessing pKa values ranging from 2.1 to 8.9 for weak acids and bases as shown in Table 1. Protonation of the piperidinic nitrogen of DON and the tertiary amino group of RIV were obtained at acidic pH values. Since ionoselective selective interaction (the dissociated form complexes with the CD) is expected to occur at low pH values where the analytes are in their charged form. So pH ranging from 2.5 to 5.0 was selected to represent the levels of pH in the DOE. Modified CDs were more preferable than natural ones because they provide a number of advantages in terms of solubility, increased cavity depth, and the occurrence of additional combinations that can interact and stabilize the CD-drug complex.[50] For screening phase, it is preferable to start by HS-CDs when developing new chiral enantioseparation strategy. HS-CDs have a strong electrophoretic mobility toward the positive electrode in the CE environment that may be due the negatively charged groups. In addition, HS-CDs with phosphate-TEA buffer (pH 2.5) have been selected to enhance solubility and to generate mobility by protonation of the amine buffers.

Adding TEA to the buffers helps to suppress the EOF in order to increase the enantioselectivity. Under these conditions, the basic compounds (which are strongly cationic at low pH) would interact with the hydrophobic cavity as well sonically with the negatively charged sulfates. At pH from 2.5 to 3, zwitterionic analytes will be positively charged and behave similarly like basic compounds, while acidic compounds will be primarily protonated and interact with the hydrophobic cavity of the HS-CDs. Compounds comprising two rings in their chemical structures (KET, SRT, DON, and ITR) have highly enantiomeric resolution values upon using HS-CDs presumably due to higher affinity and better fit in the hydrophobic cavity of the CD. It was observed that a high affinity for enantioseparation of all analytes occurred when performed at low CD concentrations, while drugs having large chain substituent tend to show the lowest resolution (RIV, FLU). This could be attributed to the steric hindrance that may be produced by those chains which does not allow the molecule to be quite fit with the outer negatively charged CDs. However, experiments should be performed in the order: HS-γ-CD then HS-α-CD as shown Figure 1.

After the resolution (Rs) for each drug has been calculated, some optimization steps were needed except for cases where Rs exceeded 1.5 meaning baseline acceptable separation. While in case of $0 < Rs < 1.5$ after screening, where the peaks are only partially separated, some optimization steps

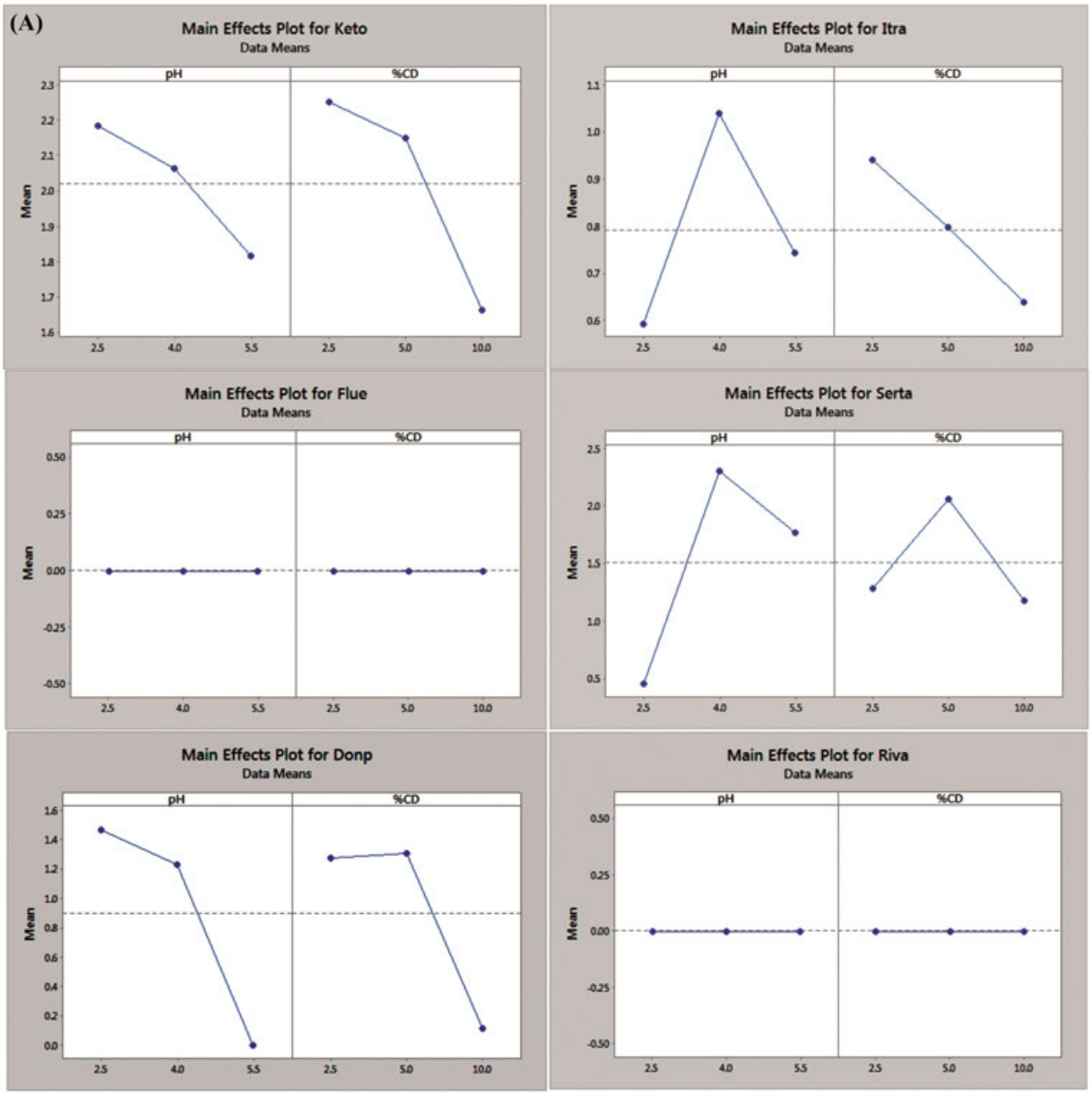


FIGURE 4 A, Main effect plot for the effect of pH and %CD on the resolution of racemates on HS-α-CD. B, Contour plots for the resolution of 6 racemates on HS-α-CD. The X-axis shows the pH and the Y-axis shows the %CD

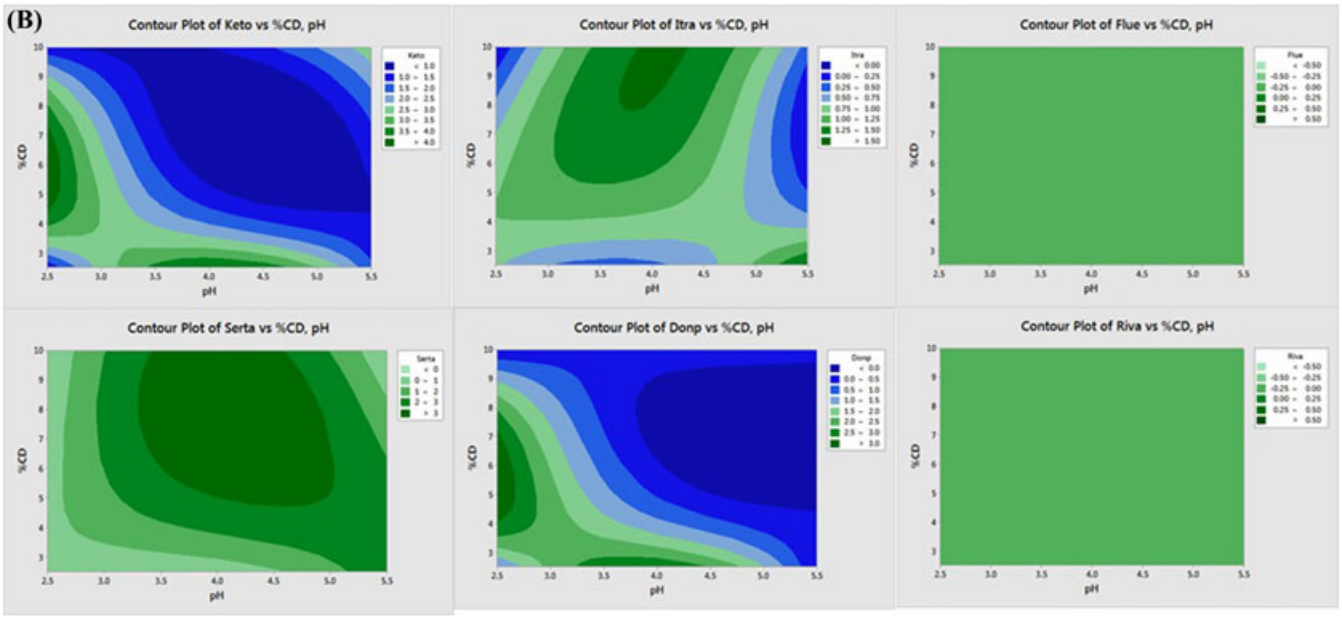


FIGURE 4 Continued

were performed to increase the resolution. Finally, when Rs = 0 after screening, a limited number of additional experiments are done; however, if separation does not improve, mixtures of HS-CDs with neutral CDs where tried because they generally yield to better results. However, it was found that SBE-β-CD led to the best enantioeparation in most of the investigated drugs as shown Figure 2.

3.1 | Modeling the separation

Factorial experiments are based on varying all factors simultaneously at a limited number of factor levels, where the most influential factors, their ranges of influence, and factor interactions are not yet known. DOE allows experiments to be performed over the whole range of the factor space. They show a high degree of accuracy in exchange with a minimum experimental effort, enabling factor interactions to be detected. The concentration of CDs and pH are considered the most crucial parameters that influence in the chiral separation for drugs under investigation. The influence of two parameters, each at three levels, on the resolution of investigated drugs was studied. Nine experiments were carried out in duplicate (3^2) with high, low, and intermediate values for both factors. The full factorial design and their responses (Rs) for these compounds are shown in Table 2A, B. For each factor, three effects can be estimated, for the intervals between the levels [1,0], [0,-1], and [1,-1]. Only two of those three effects are independent calculated (Equations 1 and 2

$$E_{x[1,\ 0]} = \frac{\sum Y(1)}{N/3} - \frac{\sum Y(0)}{N/3}, \quad (1)$$

$$E_{x[0,\ -1]} = \frac{\sum Y(0)}{N/3} - \frac{\sum Y(-1)}{N/3}, \quad (2)$$

in which ΣY^{1}, ΣY(0), and ΣY(-1) represent the sum of the responses were the factor x is at level 1, 0, and −1, respectively, and N is the number of design experiments. However, the separation also depends on other factors such as the EOF, the ionic strength and the co-ion of the BGE, the presence of additives such as TEA. These are secondary parameters, which were not examined in the experimental design, and kept constant.

3.2 | Hydroxy propyl-β-CD (HP-β-CD)

For acidic and basic compounds, lower neutral CD concentrations were expected to be optimal because of their lower affinity for the charge selector. Almost no peaks were detected for HP-β-CD (50-100 mM) and no baseline separations were obtained. It was found that screening with neutral CDs does not result in sufficient separation; it is advised to investigate with charged CDs. Here, negative polarity is applied since the EOF is very low.

3.3 | Highly sulfated gamma-CD (HS-γ-CD)

While there was no separation for RIV at any pH and %CD, a study of the main effect showed that %CD was of little effect on the resolution of KET and DON, with higher effect on the resolution of FLU and has maximal effect on the resolution of SRT and ITR. The best separation was obtained at intermediate values of %CD for those

two racemates (ITR and SRT). On the other hand, pH has pronounced effect on Rs of all drugs (except RIV as previously mentioned), the higher the pH, the lower the Rs. Best Rs is obtained at pH 2.5 for all five racemates as shown in Figure 3A**.** Contour plots clearly show that max. Rs were obtained at low pH values. %CD has a variable effect on Rs but is generally best when kept at low values with different thresholds as shown in Figure 3B. At a desirability (>0.6) the optimum conditions for all drugs were pH = 2.5 and %CD = 2.5, except for KET were Rs was best at 10 %CD. For the various drugs under investigation accuracy of prediction lies indifferent confidence intervals and has different desirability in terms of whether was maximum Rs achieved for the various drugs.

3.4 | Highly sulfated alpha-CD (HS-α-CD)

While there was no separation for RIV or FLU, at any of the studied levels of all factors, the main effect study showed that for DON, the Rs improved at low values of pH and low or intermediate values of %CD. For KET, the Rs improved at low values of pH and %CD. For ITR, intermediate pH shows better resolution than low and high values, and at low %CD. For SRT, intermediate

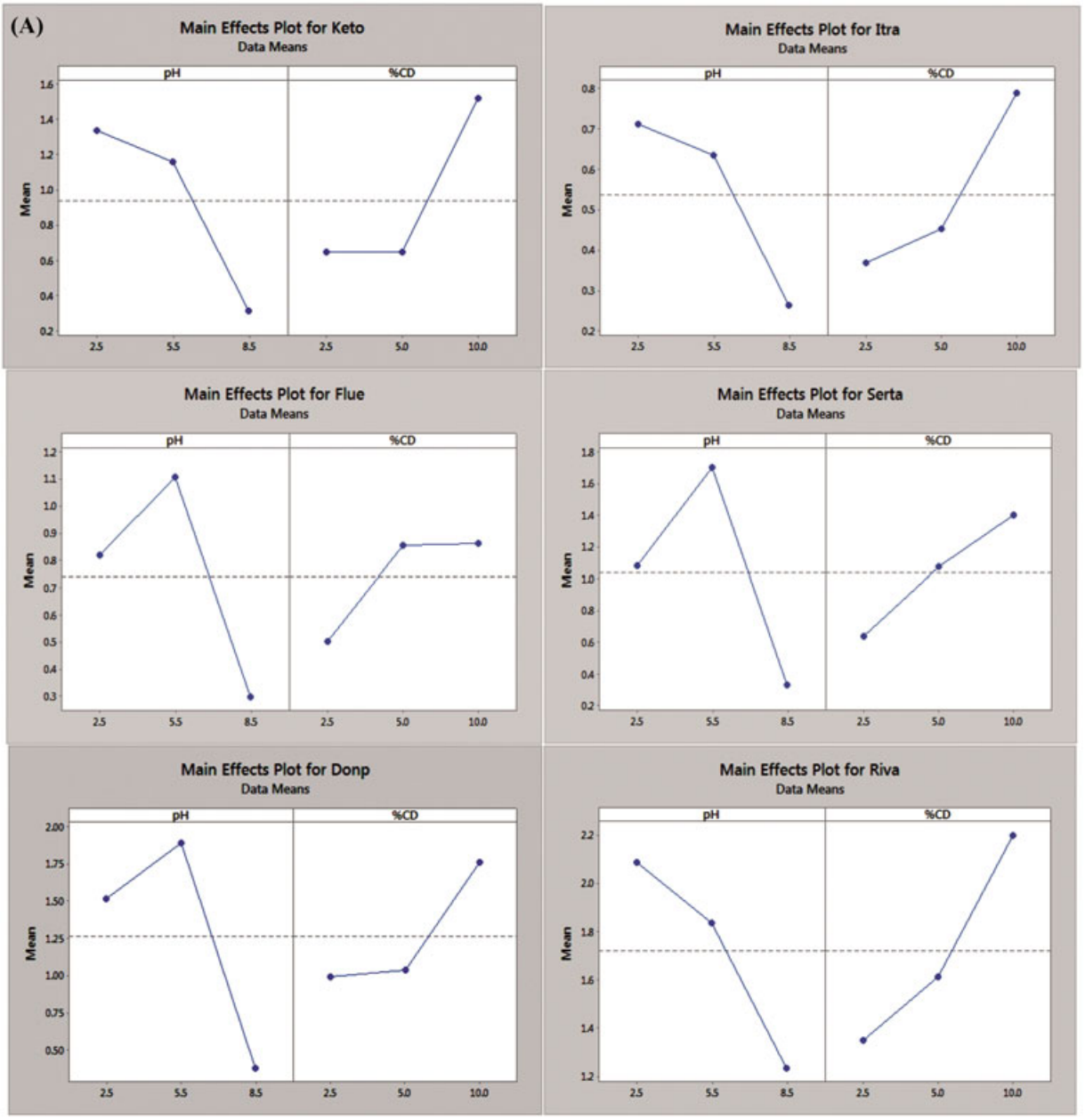


FIGURE 5 A, Main effect plot for the effect of pH and %CD on the resolution of racemates on SBE-β-CD. B, Contour plots for the resolution of 6 racemates on SBE-β-CD. The X-axis shows the pH and the Y-axis shows the %CD

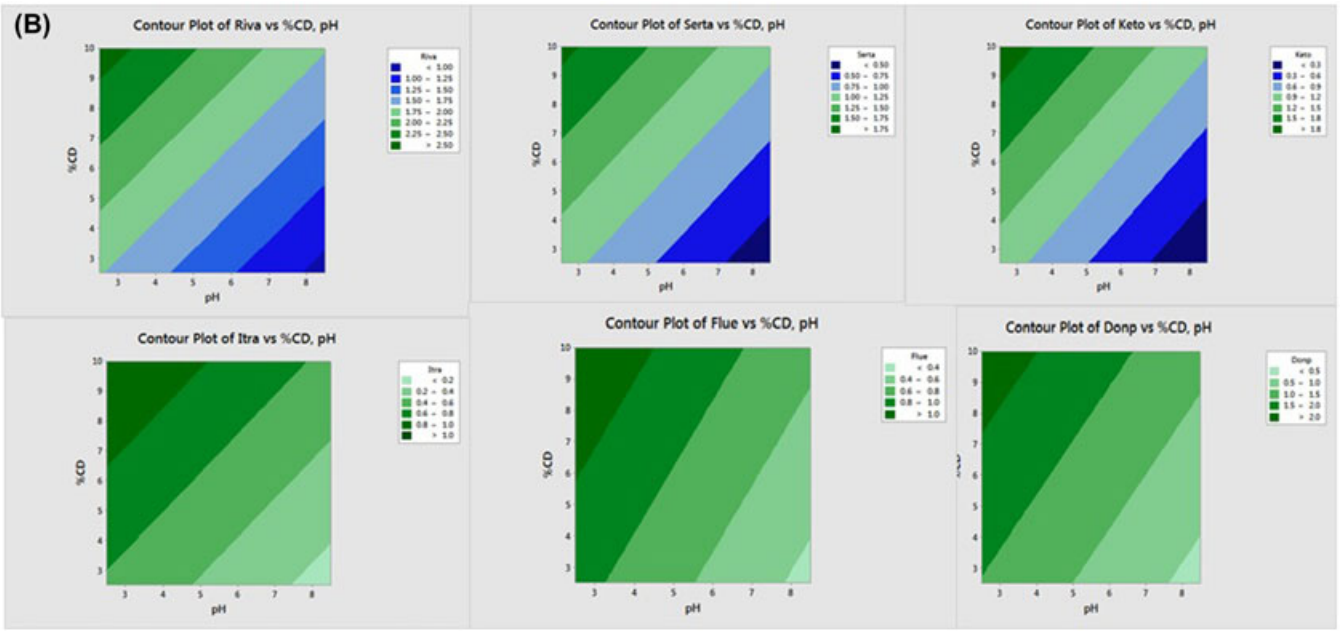


FIGURE 5 Continued

values of pH and of %CD show the best resolution as shown in Figure 4A. Contour plots control of %CD and pH is critical in case of KET and DON where Rs drops to very low values (<0.5) at pH higher than 4.0 if %CD over than 5% (blue areas). A very limited space in the design (dark green) offers an acceptable resolution for these two drugs; either at intermediate %CD coupled with low pH or vice versa. SRT has a better design space with respect to the large robust area (dark green) at intermediate values of %CD and pH. FLU and RIV racemates did not resolve at any of the studied conditions as shown in Figure 4B.The optimum conditions %CD was 2.5 for all drugs, while it varied for the pH. In the case of KET and DON, pH optimally is 2.5 to achieve good resolution while it should be 5.5 for SRT and ITR. The accuracy of estimation lies in different confidence intervals and has different desirability in terms of whether was maximum Rs achieved for the various drugs.

3.5 | Sulfobutyl ether β-cyclodextrin (SBE-β-CD)

SBE-β-CD was the most successful chiral selector offering Rs for all the six racemates. In all cases, pH and %CD had a dramatic effect on the separation. The worst separation was always obtained at high pH values and at low %CD as shown in Figure 5A. The contour plots interestingly show similar resolution pattern two groups of drugs: for RIV, SRT and KET, at one hand, and ITR, FLU, and DON on the other hand as shown in Figure 5B. In all cases, the best resolution occurs at the upper left-hand side of the space, at lowest pH and highest %CD. Those two factors are more critical to RIV, SRT, and KET than the others since Rs < 0.5 cannot be prevented at low %CD coupled with high pH. Consequently, the optimization plot proved again that the best conditions of pH and %CD for all drugs were 2.5 and 10, respectively. For the various drugs under study, accuracy of prediction lies indifferent confidence intervals and have different desirability in terms of whether was maximum Rs achieved for the various drugs.

4 | CONCLUSION

Design of experiment–based screening and optimization strategies for the separation of six racemic compounds were studied using four different chiral selectors and had been successfully applied using full factorial design approach (3^2) that provide high efficiencies with analysis time less than 10 min. The selection of the experimental factors to be fixed and of others to be varied is considered crucial step for the success of this CE strategy. Among HP-β-CD, HS-γ-CD, HS-α-CD, SBE- β-CD, the later was unique leading to good resolution for all six racemates at low pH values and higher %CD. In addition, the response surfaces obtained with this chiral selector were able to visually classify the behavior of the enantiomers according to the enatiomeric resolution pattern into two main groups; the first comprises RIV, SRT, and KET, and the second ITR, FLU, and DON. However, the regression results indicate that the linear model shows a significant

lack of fit for all chiral selectors, anticipating that higher orders of the factors are most likely to be present in the equation with possible interactions. For future work, this strategy will be tested for the development of enantiomeric separations of other acidic and basic drugs under the same experimental conditions. One improvement that we should investigate is if a central point will be added to the two-level designs can help to find good conditions occurring at intermediate levels.

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REFERENCES

1. Radwan MA, Abdine HH, Al-Quadeb BT, Aboul-Enein HY, Nakashima K. Stereoselective HPLC assay of donepezil enantiomers with UV detection and its application to pharmacokinetics in rats. *J Chromatogr B Biomed Sci Appl*. 2006;830:114-119.
2. Brunton LL, Parker KL. *Goodman & Gilman's Manual of Pharmacology and Therapeutics*. McGraw-Hill Companies, Inc: CA, USA; 2008.
3. Muller T. Rivastigmine in the treatment of patients with Alzheimer's disease. *Neuropsychiatr Dis Treat*. 2007;3:211-218.
4. Sugimoto H, Yamanishi Y, Iimura Y, Kawakami Y. Donepezil hydrochloride (E2020) and other acetylcholinesterase inhibitors. *Curr Med Chem*. 2000;7:303-339.
5. Matsui K, Oda Y, Nakata H, Yoshimura T. Simultaneous determination of donepezil (aricept) enantiomers in human plasma by liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl*. 1999;729:147-155.
6. Crego AL, Marina ML, Lavandera JL. Optimization of the separation of a group of antifungals by capillary zone electrophoresis. *J Chromatogr A*. 2001;917:337-345.
7. Khoza S, Moyo I, Ncube D. Comparative hepatotoxicity of fluconazole, ketoconazole, itraconazole, terbinafine, and griseofulvin in rats. *J Toxic*. 2017;1:1-9.
8. Aboul-Enein HY, Wainer IW. *The Impact of Stereochemistry on Drug Development and Use: Chemical Analysis series*. New York, USA: John Wiley & Sons; 1997.
9. Maier NM, Franco P, Lindner W. Separation of enantiomers: needs, challenges, perspectives. *J Chromatogr A*. 2001;906:3-33.
10. FDA, Policy. Statement for the development of new stereoisomeric drugs. Washington, DC avialble at https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122883.htm; 1992.
11. Lu J, Wan L, Zhong Y, et al. Stereoselective metabolism of donepezil and steady-state plasma concentrations of S-donepezil based on CYP2D6 polymorphisms in the therapeutic responses of Han Chinese patients with Alzheimer's disease. *J Pharmacol Sci*. 2015;129:188-195.
12. Bolognesi ML, Bartolini M, Cavalli A, et al. Design, synthesis, and biological evaluation of conformationally restricted rivastigmine analogues. *J Med Chem*. 2004;47:5945-5952.
13. Dilmaghanian S, Gerber J, Filler S, Sanchez A, Gal J. Enantioselectivity of inhibition of cytochrome P450 3A4 (CYP3A4) by ketoconazole: testosterone and methadone as substrates. *Chirality*. 2004;16:79-85.
14. Shi W, Nacev BA, Bhat S, Liu JO. Impact of absolute stereochemistry on the antiangiogenic and antifungal activities of itraconazole. *ACS Med Chem Lett*. 2010;1:155-159.
15. Tomaszewski J, Rumore MM. Stereoisomeric drugs: FDA's policy statement and the impact on drug development. *Drug Dev Ind Pharm*. 1994;20:119-139.
16. Francotte ER. Enantioselective chromatography as a powerful alternative for the preparation of drug enantiomers. *J Chromatogr A*. 2001;906:379-397.
17. Dejaegher B, Vander Heyden Y. The use of experimental design in separation science. *Acta Chromatographica*. 2009;21:161-201.
18. Dejaegher B, Durand A, Vander HY. *Experimental design in method optimization and robustness testing*. New Jersey, USA: John Wiley & Sons; 2010.
19. Ali I, Aboul-Enein HY, Gupta VK. *Nanochromatography and Nanocapillary Electrophoresis: Pharmaceutical and Environmental Analyses*. Hoboken, USA: John Wiley & Sons; 2009.
20. Ali I, Suhail M, Al-Othman ZA, Alwarthan A, Aboul-Enein HY. Enantiomeric resolution of multiple chiral centres racemates by capillary electrophoresis. *Biomed Chromatogr*. 2016;30:683-694.
21. Al-Othman ZA, Al-Warthan A, Alam SD, Ali I. Enantio-separation of drugs with multiple chiral centers by chromatography and capillary electrophoresis. *Biomed Chromatogr*. 2014;28:1514-1524.
22. Ali I, Al-Othman ZA, Al-Warthan A, Asnin L, Chudinov A. Advances in chiral separations of small peptides by capillary electrophoresis and chromatography. *J Sep Sci*. 2014;37:2447-2466.
23. Ali I, Al-Othman ZA, Al-Warthan A, A-Eh Y. Recent trends in chiral separations by nano liquid chromatography and nano capillary electrophoresis. *Curr Chromatogr*. 2014;1:81-89.
24. Ates H, Mangelings D, Vander Heyden Y. Fast generic chiral separation strategies using electrophoretic and liquid chromatographic techniques. *J Pharm Biomed Anal*. 2008;48:288-294.
25. Huang Q, Zhang K, Wang Z, Wang C, Peng X. Enantiomeric determination of azole antifungals in wastewater and sludge by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2012;403:1751-1760.

26. Hamdy DA, Brocks DR. Nonlinear stereoselective pharmacokinetics of ketoconazole in rat after administration of racemate. *Chirality*. 2009;21:704-712.
27. Hamdy DA, Brocks DR. A stereospecific high-performance liquid chromatographic assay for the determination of ketoconazole enantiomers in rat plasma. *Biomed Chromatogr*. 2008;22:542-547.
28. Thienpont A, Gal J, Aeschlimann C, Félix G. Studies on stereoselective separations of the "azole" antifungal drugs ketoconazole and itraconazole using HPLC and SFC on silica-based polysaccharides. *Analusis*. 1999;27:713-718.
29. Srinivasu MK, Rao BM, Reddy BS, Kumar PR, Chandrasekhar KB, Mohakhud PK. A validated chiral liquid chromatographic method for the enantiomeric separation of rivastigmine hydrogen tartarate, a cholinesterase inhibitor. *J Pharm Biomed Anal*. 2005;38:320-325.
30. Barth T, Conti R, Pupo MT, Okano LT, Bonato PS. Chiral HPLC analysis of donepezil, 5-O-desmethyl donepezil and 6-O-desmethyl donepezil in culture medium: application to fungal biotransformation studies. *Anal Bioanal Chem*. 2012;404:257-266.
31. Saysin S, Liawruangrath B, Liawruangrath S. High-performance thin-layer chromatographic determination of ketoconazole in pharmaceutical formulations. *J Cosmet Sci*. 2010;61:367-376.
32. Abdel-Moety EM, Khattab FI, Kelani KM, AbouAl-Alamein AM. Chromatographic determination of clotrimazole, ketoconazole and fluconazole in pharmaceutical formulations. *Farmacoterapia*. 2002;57:931-938.
33. Radosław J, Ekiert Krzek J, Rzeszutko W. Evaluation of a TLC densitometric method for analysis of azole antifungal agents. *Chromatographia*. 2008;67:995-998.
34. Velikinac I, Cudina O, Jankovic I, Agbaba D, Vladimirov S. Comparison of capillary zone electrophoresis and high performance liquid chromatography methods for quantitative determination of ketoconazole in drug formulations. *Farmacoterapia*. 2004;59:419-424.
35. Castro-Puyana M, Crego AL, Marina ML. Enantiomeric separation of ketoconazole and terconazole antifungals by electrokinetic chromatography: rapid quantitative analysis of ketoconazole in pharmaceutical formulations. *Electrophoresis*. 2005;26:3960-3968.
36. Wei Y, Li J, Zhu C, Hao A, Zhao M. 2-O-(2-hydroxybutyl)-beta-cyclodextrin as a chiral selector for the capillary electrophoretic separation of chiral drugs. *Anal Sci*. 2005;21:959-962.
37. Castro-Puyana M, Crego AL, Marina ML, Garcia-Ruiz C. Enantioselective separation of azole compounds by EKC. Reversal of migration order of enantiomers with CD concentration. *Electrophoresis*. 2007;28:2667-2674.
38. Breadmore MC, Thormann W. Capillary electrophoresis evidence for the stereoselective metabolism of itraconazole in man. *Electrophoresis*. 2003;24:2588-2597.
39. Jimidar MI, Van Ael W, Shah R, Redlich D, De Smet M. Fast method development and rapid analysis using a screening approach for enantiomeric separations in capillary electrophoresis. *J Capill Electrophor Microchip Technol*. 2003;8:101-110.
40. Lucangioli SE, Tripodi V, Masrian E, Scioscia SL, Carducci CN, Kenndler E. Enantioselective separation of rivastigmine by capillary electrophoresis with cyclodextrines. *J Chromatogr A*. 2005;1081:31-35.
41. Kavalirova A, Pospisilova M, Karlicek R. Enantiomeric analysis of rivastigmine in pharmaceuticals by cyclodextrin-modified capillary zone electrophoresis. *Anal Chim Acta*. 2004;525:43-51.
42. Gotti R, Cavrini V, Pomponio R, Andrisano V. Analysis and enantioresolution of donepezil by capillary electrophoresis. *J Pharm Biomed Anal*. 2001;24:863-870.
43. Wan Ibrahim WA, Arsad SR, Maarof H, Sanagi MM, Aboul-Enein HY. Chiral separation of four stereoisomers of ketoconazole drugs using capillary electrophoresis. *Chirality*. 2015;27:223-227.
44. Bhatt J, Subbaiah G, Kambli S, et al. A rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the estimation of rivastigmine in human plasma. *J Chromatogr B Biomed Sci Appl*. 2007;852:115-121.
45. Pommier F, Frigola R. Quantitative determination of rivastigmine and its major metabolite in human plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl*. 2003;784:301-313.
46. Thomas S, Shandilya S, Bharati A, Paul SK, Agarwal A, Mathela CS. Identification, characterization and quantification of new impurities by LC-ESI/MS/MS and LC-UV methods in rivastigmine tartrate active pharmaceutical ingredient. *J Pharm Biomed Anal*. 2012;57:39-51.
47. Arumugam K, Chamallamudi MR, Gilibili RR, et al. Development and validation of a HPLC method for quantification of rivastigmine in rat urine and identification of a novel metabolite in urine by LC-MS/MS. *Biomed Chromatogr*. 2011;25:353-361.
48. Shah HJ, Kundlik ML, Pandya A, et al. A rapid and specific approach for direct measurement of donepezil concentration in human plasma by LC-MS/MS employing solid-phase extraction. *Biomed Chromatogr*. 2009;23:141-151.
49. Apostolou C, Dotsikas Y, Kousoulos C, Loukas YL. Quantitative determination of donepezil in human plasma by liquid chromatography/tandem mass spectrometry employing an automated liquid-liquid extraction based on 96-well format plates. Application to a bioequivalence study. *J Chromatogr B Biomed Sci Appl*. 2007;848:239-244.
50. Beesley TE, Scott RPW. *Chiral Chromatography*. New York, USA: John Wiley & Sons; 1998.

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