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Research Article

Chiral separation and quantitation of cetirizine and hydroxyzine by maltodextrin-mediated CE in human plasma: Effect of zwitterionic property of cetirizine on enantioseparation

In the present study, a very simple CE method for chiral separation and quantitation of zwitterionic cetirizine (CTZ), as the main metabolite of hydroxyzine (HZ), and HZ has been developed. In addition, the effect of zwitterionic property of CTZ on enantioseparation was investigated. Maltodextrin, a linear polysaccharide, as a chiral selector was used and several parameters affecting the separation such as pH of BGE, concentration of chiral selector and applied voltage were studied. The best BGE conditions for CTZ and HZ enantiomers were optimized as 75 mM sodium phosphate solution at pH of 2.0, containing 5% w/v maltodextrin. Results showed that, compared to HZ, pH of BGE was an effective parameter in enantioseparation of CTZ due to the zwitterionic property of CTZ. The linear range of the method was over 30–1200 ng/mL for all enantiomers of CTZ and HZ. The quantification and detection limits ($S/N = 3$) of all enantiomers were 30 and 10 ng/mL, respectively. The method was used to quantitative enantioseparation of CTZ and HZ in spiked human plasma.

Keywords:
Cetirizine / Enantiomer / Hydroxyzine / Metabolite / Zwitterion
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1 Introduction

Cetirizine (CTZ), (*RS*)-2-[2-((4-chlorophenyl)phenylmethyl)-piperazine-1-yl]ethoxyacetic acid (Fig. 1), is a well-known second-generation long-acting H_1 antagonist. CTZ is a potent, well-tolerated and nonsedating antihistamine for treating the allergic rhinitis and chronic urticaria. Its H_1 antagonist activity is primarily due to levocetirizine (R-CTZ), the *R*-enantiomer of CTZ [1–3]. Thus, R-CTZ is considered to be a more active enantiomer, while dextrocetirizine, the *S*-enantiomer of CTZ, is the less active one. Three ionizable functions are present in the CTZ structure, namely a strong acid group ($pK_a = 2.93$), a strong basic group ($pK_a = 8.0$) and a weak basic group ($pK_a = 2.19$) [3]. Hydroxyzine (HZ), (*RS*)-2-[2-((4-chlorophenyl)phenylmethyl)-piperazine-1-yl] ethoxy ethanol, is a piperazine antihistamine (Fig. 1). It diminishes the main action of histamine by competitive reversible blockade histamine receptor sites H_1 in the tissues [4]. Racemic HZ can be administered orally or as an intramuscular injection. It is metabolized to a carboxylic acid in the liver through the oxidation of the alcohol moiety [4]. The main metabolite (45%) is CTZ. The pK_a values of HZ as a basic compound with two ionizable groups, for the tertiary amine group and the nitrogen heterocyclic, are 2.13 and 7.13, respectively [5]. Therefore, simple and economic approaches for the separation of these enantiomers are important.

A literature survey reveals that some methods have been reported for the enantioseparation of HZ by affinity electrokinetic chromatography (AEKC) [6], NMR spectroscopy [7], CEC [8], normal and reversed-phase nano-HPLC [9] and CE [10, 11]. In some of these methods, human serum albumin [6], and native [7] and anionic CDs [10, 11] were used as chiral selectors. Also, monolithic silica capillary column modified by coating of cellulose tris(3,5-dimethylphenylcarbamate) [8] and fused-silica capillaries packed with silica gel, which was modified by covalent attachment of poly-*N*-acryloyl-l-phenylalanine ethyl ester [9] as chiral columns, were used for enantioseparation of HZ by CE or nano-HPLC. Also, there are some reports on the enantioseparation of CTZ by MEKC [12], CE [13–17], NMR spectroscopy [18], HPLC [19–24], subcritical fluid chromatography [25, 26] and mass spectrometry [27] in the literatures. In these methods, clindamycin [12], glycogen [13], amylose [14] and native and derivatized CDs [15–17] were

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Abbreviations: CTZ, cetirizine; **HZ,** hydroxyzine; **MD,** maltodextrin; **R-CTZ,** levocetirizine

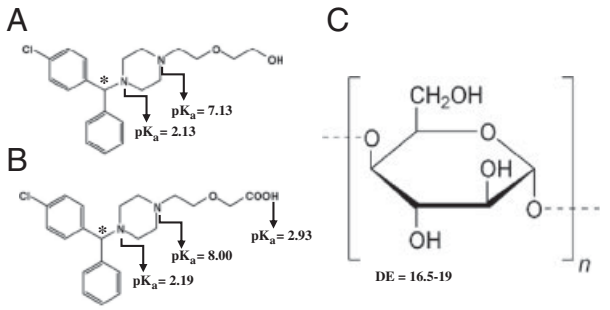

Figure 1. Chemical structure of (A) HZ, (B) CTZ and (C) MD. The asterisk denotes the chiral centre.

used as chiral selectors for enantioseparation of CTZ. In addition, amylose-based column as a chiral column was used for chiral separation of CTZ [25]. CTZ enantiomers were quantified in blood [14], plasma [19, 23] and urine [24] samples.

Chou et al. [15] reported a CE method for enantioseparation of CTZ using a borate buffer (5 mM, pH 8.7) with 1% w/v sulfated β-CD as the chiral selector at 10 kV. Eeckhaut and Michotte [16] reported the CE separation of CTZ enantiomers with the phosphate buffer at pH 2.5 using heptakis (2,3-diacetyl-6-sulfato)-β-CD, triethanolamine and ACN as the chiral selector, co-ion and organic modifier, respectively. Mikuss et al. [17] described a CE enantioseparation of CTZ racemate, using dynamic coating of capillary with methylhydroxylethylcellulose in dual β-CDs and 25 mM morpholinoethanesulfonic acid (pH 5.2) as the chiral selector and buffer solution. As seen in these reports, the enantioseparation conditions were not very simple or reagents used were very expensive.

Maltodextrins (MDs) are complex malto-oligo and polysaccharide mixtures, which were obtained from starch by partial acid and/or enzymatic hydrolysis [28, 29]. When used in CE, MDs were found to enable highly efficient chiral separations of a broad range of acidic and basic compounds [28–30]. The mechanism of chiral recognition has been proven as a result of electrophoretic mobility and selectivity measurements using different buffer solutions and organic solvent additives [31]. Different interactions of chiral solutes with the helical structure of the MD emerge as the basis of the enantioselectivity. This notion is supported by ^{1}H and ^{13}C-NMR experiments [32]. The helical structure of the MD mimics the cavity responsible for chiral recognition by CDs.

The aim of this work was to develop a very simple CE method for simultaneous chiral separation of CTZ and HZ. During the method development, no literature data on simultaneous enantioseparation of CTZ and HZ with MD-mediated CE were available. CTZ is a zwitterionic compound and its enantioseparation behavior may be different from other acidic or basic compounds. Because there are some reports in the literatures that show ionic forms (cationic or anionic) of chiral compounds can interact with neural chiral selectors and it was found that ionic chiral selectors could separate enantiomers of neutral analytes [33]. However, zwitterionic compounds can be neutral while there can be positive and negative charges (ionic position) on the molecule simultaneously. Thus, this work wants to clear this obscurity: Do ionic positions on the analyte molecule affect the enantioseparation or total charges of a molecule affect the enantioseparation? In other words, charge neutralization by intramolecular interactions may come into play in zwitterionic compounds. Thus, for the evaluation of this hypothesis and comparison purpose, HZ, the parent drug of CTZ lacking an acidic group, was the best choice due to its structural resemblance with CTZ. In our study, at first, several parameters that influence the chiral separation were investigated. The optimized method was then validated for determination of CTZ and HZ enantiomers in spiked human plasma samples.

2 Materials and methods

2.1 Materials

All chemicals used in the analysis were of analytical grade. To prevent capillary blockage, all buffers were filtered through 0.45-μm filter membranes (Millipore, Bedford, MA, USA). Racemic CTZ, R-CTZ, HZ and *R*-enantiomer of HZ were obtained from Tofigh Daru Pharmaceutical Company (Tehran, Iran) and were used without further purification. MD with a dextrose equivalent (DE) of 16–19.5 was purchased from Fluka (Buchs, Switzerland). Analytical-grade H_3PO_4, $NaH_2PO_4.2H_2O$, Na_2HPO_4, NaOH, HCl and dichloromethane were purchased from Merck (Darmstadt, Germany). HPLC-grade water was obtained through a Milli-Q system (Millipore) and was used for preparation of all solutions. Phosphate buffers were prepared by mixing appropriate amounts of H_3PO_4 and NaH_2PO_4 solutions. Drug-free human plasma was obtained from Sina Hospital (Tehran, Iran).

2.2 CE equipment

CE was carried out using a Lumex Capel 105 (Ohiolumex, Twinsburg, Russia) equipped with a UV detector operated at 214 nm. The electrophoretic experiments were performed in an uncoated fused-silica capillary (Ohiolumex) 60 cm × 75 μm id (50 cm effective length). Throughout the studies, CE was performed at 25°C, at a constant potential of 20 kV. The current increased to approximately 120 μA after applying the separation potential. Prior to use, the capillary was conditioned for 20 min with 0.5 M HCl, 5 min with water, 30 min with 0.5 M NaOH and another 5 min with water. Additionally, the capillary was washed for 2 min with 0.5 M NaOH, 1 min with water and 2 min with the running buffer with positive pressure applied at the injection end before each run. Acquisition of electropherograms was computer-controlled by Chrom&Spec software version 1.5. The analytes were injected at the anodic end by applying pressure (60 mbar × 10 s).

2.3 Standard solutions and samples

Standard stock solutions of racemic CTZ (1.0 mg/mL) and racemic HZ (1.0 mg/mL) were prepared individually in HPLC grade water, stored at 4°C and protected from light. Stock solutions of CTZ and HZ were further diluted to obtain their working solutions. Then, they were diluted with water (or blank plasma) to yield final concentrations of 30, 50, 100, 250, 500, 1000 and 1200 ng/mL for each enantiomer to obtain calibration standard solutions. The calibration graphs of CTZ and HZ enantiomers were established with the peak area (mean of three injections) of each enantiomer as ordinate (y) versus the concentration of each enantiomer in ng/mL as abscissa (x). Three concentration levels (low, medium and high), as quality control samples, were prepared in blank plasma at concentrations of 50, 250 and 500 ng/mL for CTZ and HZ enantiomers.

2.4 Sample pretreatment for enantioseparation in water and plasma

A 2.0-mL aliquot of sample (calibration standard solutions or quality control samples) was pipetted into a 10-mL vial. Then, 4.0 mL of dichloromethane was added into the vial, then vortexed for 3 min. After mixing, the sample was centrifuged at 8000 rpm for 20 min. Thereafter, organic layer was separated, evaporated and reconstituted with 50 μL of deionized water. Then, the sample was transferred to a microinsert vial, which was placed into the sample tray of a CE system for analyses. The absolute recoveries, based on 100 ng/mL of each enantiomer spiked in blank plasma and water, were determined by comparing the calculated concentrations with the standard solutions.

2.5 Method validation

For investigation of matrix effect, dynamic linear range of method was examined in water and plasma samples. The intra- and inter-day precision and accuracy analyses of the each enantiomer were tested by analyzing three concentration levels (quality control samples) at 50, 250 and 500 ng/mL. The LOQ was defined by spiking the reference standard with decreasing concentrations of each enantiomer until the peak height was ten times the level of the baseline noise ($S/N = 10$). The LOD was determined by spiking the reference standards with decreasing concentrations of each enantiomer until the S/N was equal to 3.

3 Results and discussion

To obtain the optimum enantioseparation of the CTZ and HZ, effective experimental parameters, such as pH of BGE, chiral selector concentration, BGE concentration, capillary column temperature and applied voltage were optimized. In the course of operation, one of the mentioned parameters was varied while the others were kept constant. R-CTZ and *R*-enantiomer of HZ were used for identifying migration order of enantiomers in MD-mediated capillary electrophoresis. Results showed that *S*-enantiomer of CTZ migrated faster than *R*-enantiomer and S-form of HZ migrated before R-form in MD-mediated capillary electrophoresis.

3.1 Choice of pH and the effect of zwitterionic property of CTZ on the enantioseparation

Recently, physicochemical properties of the zwitterionic antihistamine CTZ were investigated by Chen [34] and acid–base behavior of CTZ was examined by Pagliara et al. [3]. In another work, ionization plots of CTZ and HZ were reported by Testa and co-workers [35]. Three ionizable functions are present in the CTZ structure, namely a strong acid group ($pK_a = 2.93$), a strong basic group ($pK_a = 8.0$) and a weak basic group ($pK_a = 2.19$) [3]. Thus, theoretically CTZ can be considered as a dication at pH < 2.19, as a cation at pH range of 2.19 to 2.93, as a zwitterionic compound (neutral) at pH range of 2.93 to 8.0 and as an anion at pH > 8.0. In fact, CTZ has four different forms (dication, cation, zwitterion and anion), but one or more than one form can exist in each pH and the percentage of each form is affected by pH. In the structure of HZ, two ionizable functions are present, namely a strong basic group ($pK_a = 7.13$) and a weak basic group ($pK_a = 2.13$) [5]. Thus, HZ can be considered as a dication at pH < 2.13, a cation at pH range of 2.13–7.13 and a neutral compound at pH > 7.13. In CZE, pH of BGE plays an important role in the enantioseparation of acidic or basic analytes because it determines the ionization extent of each individual analyte and the ionic state of capillary column wall when bare column is used [36]. Therefore, variation of BGE pH usually becomes a key strategy to optimize a separation. Thus, in this work, the effect of BGE pH on the enantioseparation of CTZ and HZ was investigated using 75 mM phosphate in the pH range of 1.8–10.0 containing 5.0% w/v of MD. Figure 2 shows that separation of CTZ enantiomers was obtained in two ranges of pH and verifies the zwitterionic property of CTZ. These two pH ranges were 1.8–4.5 and 8.0–8.5. With regard to CTZ pK_a values, in low pH range (pH ≤ 3.5), when CTZ has a cationic or dicationic form, complete enantiomer separation occurred. In the pH range of 4.0–7.5, CTZ was a zwitterionic compound and baseline separation was not obtained. In high pH range (pH ≥ 8.0), CTZ was an anionic compound and peak splitting ($R_s = 0.31$) was obtained. In comparison, HZ enantiomers were just separated at baseline in low pH range (pH < 5.0) and it was a cationic compound at pH < 7.13.

The optimum pH for the chiral separation will give important information on the interaction between MD

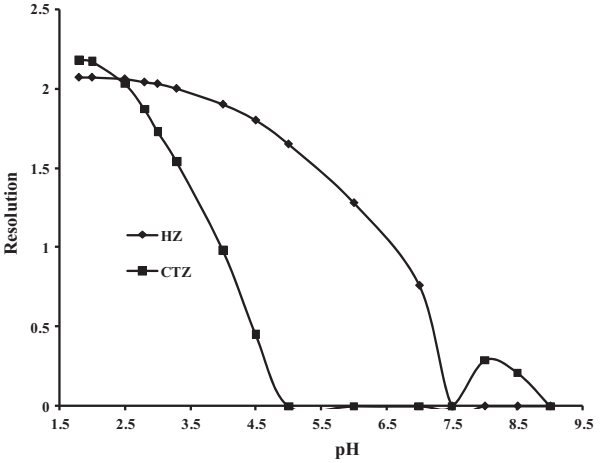

Figure 2. Influence of the BGE pH on the enantiomer resolution. Experimental condition – capillary: 60 cm (50 cm effective length) × 75 μm id; detection: 214 nm; applied voltage: 20 kV; temperature: 25°C; injection: 60 mbar × 10 s; separation solution: 75 mM phosphate solution containing 5% w/v MD; concentration of each enantiomer is 15.0 μg/mL.

and these compounds. Most of the polysaccharides' enantiorecognition can be attributed to their ability to form multiple interactions such as hydrogen bonding, ionic and hydrophobic [37]. For neutral polysaccharides, two primary interactions are thought to be hydrogen bond and hydrophobic interaction [38]. In this work, since MD is a neutral polysaccharide, the hydrogen bonding existing between the amino groups and carboxyl group in analytes and the hydroxyl groups in MD is probably expected to play a major role for the enantiorecognition. Since the protonation degree of amino group and dissociation of carboxyl group are dependent on pH, the optimum pH will point to the most compatible state of drugs for binding to MD. Thus, obtained results in this work show that ionization of CTZ and HZ plays an important role in the interaction between their ionic forms and MD. CTZ has cationic form in pH $\leq$3.5 and complete enantioseparation of these compounds was obtained in pH$\leq$3.5, whereas CTZ is a zwitterion in pH $>$ 3.5 and an anion in pH$\geq$8.0 and enantioseparation did not occur. With regard to HZ, as the pH increased from 1.8 to 7.0, the resolution decreased and there is no enantioseparation of HZ in the pH$\geq$7.5, due to the neutralization of HZ in the pH $>$ 7.13 and increasing of EOF. These results show that for enantioseparation, the interaction between cationic form of mentioned enantiomers and MD is preferred to anionic (about CTZ) and neutral form. In other words, complete enantioseparation occurred in MD-mediated CE when analytes (CTZ and HZ) were cationic compounds.

The effects of buffer pH on the migration times of the CTZ and HZ could be complex. Changes in pH can affect the migration times in three ways: First, a decrease in pH leads to change in the net charge on the ionizable analytes. A second contribution to the increased migration time may be due to the reduction of EOF at low pH. Finally, varied pH will affect the intensity of the interaction between the chiral selector and analyte, which results in change of migration times. The results in Table 1 indicate that the increase of BGE pH from 1.8 to 3.0 results in increasing the CTZ and HZ enantiomers migration times because of decreasing the protonation degree (changing of dication to cation form). However, increasing BGE pH (from 3.0 to 9.0) results in decreasing migration times due to increasing of EOF. Therefore, rest experiments were performed at the optimum pH values of 2.0 for CTZ and HZ.

3.2 Effect of MD concentration

The effects of MD concentration on the enantioseparation of CTZ and HZ were investigated using 75 mM phosphate buffer solution (pH 2.0) over a concentration range of 1.0–15.0% w/v. The concentration of MD was limited to 15% considering the high viscosity of the solutions. The migration times of all the enantiomers increased with increasing concentration of MD. Increases in the drug–chiral selector complex adduct and in viscosity of the BGE were the main causes of the retarded migration for enantiomers. For CTZ and HZ enantiomers, the resolution increased as MD concentration rose, but reached maximum at 5.0% w/v MD addition. Figure 3A shows the resolution of CTZ and HZ enantiomers with different MD concentrations. At higher concentration, the resolution decreased slightly due to saturated complexation between the enantiomers and chiral selector. These results corresponded well with those derived from the Wren and Rowe model concerning the existence of the maximum in resolution at certain concentration of chiral

Table 1. Effect of pH on the migration time (min) and resolution of enantiomers

pH	HZ			CTZ		
	t_S [a)]	t_R [b)]	R_s	t_S	t_R	R_s
1.8	28.07	28.51	2.07	32.11	33.07	2.18
2.0	28.72	29.39	2.07	32.63	33.65	2.17
2.5	38.21	39.05	2.06	47.21	48.07	2.03
2.8	44.07	44.97	2.04	56.65	57.31	1.87
3.0	43.78	44.69	2.03	63.78	64.36	1.73
3.3	41.55	42.51	2.0	68	68.48	1.54
4.0	35.43	36.07	1.9	67.3	67.63	0.98
4.5	31.65	32.04	1.8	59.24	59.52	0.45
5.0	28.31	28.71	1.65	52.0	52.0	0
6.0	22.23	22.56	1.28	29.83	29.83	0
7.0	14.62	14.81	0.76	18.14	18.14	0
7.5	11.2	11.2	0	15.34	15.34	0
8.0	8.11	8.11	0	24.36	24.57	0.38
8.5	7.21	7.21	0	21.65	21.83	0.29
9.0	6.27	6.27	0	20.14	20.14	0

a) t_S is the migration time of S-form of enantiomer.
b) t_R is the migration time of R-form of enantiomer.

selector [39, 40]. Considering successful separation and appropriate analysis time, a MD concentration of 5.0% w/v was chosen for the target compounds.

3.3 Effect of BGE concentration

The effect of BGE concentration on enantioseparation is complicated, which principally includes EOF, Joule heating and the adsorption of analyte on the inner surface of capillary column. An increase in ionic strength leads to better enantioseparation owing to the decrease of the above-mentioned adsorption. However, further increase in the BGE concentration is usually unfavorable for the chiral separation due to the effect of Joule heating. The effects of BGE concentration were investigated by using 25–120 mM phosphate solution (pH 2.0, 5.0% w/v MD) for CTZ and HZ. Results are presented in Fig. 3B. It was obvious that the migration times of all the enantiomers tended to increase as the BGE concentration rose, which could be interpreted as a result of a decrease of EOF mainly. For CTZ and HZ, as the BGE concentration increased, the resolution first increased due to the enhanced CTZ-MD complexation or narrower peak, and then decreased because of Joule heating leading to peak broadening. Further increase in the BGE concentration was limited by the concomitant rise in electric current. Therefore, BGE concentrations of 75 mM were used in the following experiments.

3.4 Effect of applied voltage

In general, the effect of applied voltage on the enantioseparation includes four aspects: efficiency of electrophoresis, Joule heating, and the electrophoretic velocities of EOF and analyte, which affect the time of interaction between the chiral selector and analyte. The effects of applied voltage on the enantioseparation of CTZ and HZ were investigated using 75 mM phosphate solution (pH 2.0, 5.0% w/v MD) in the range of 12–25 kV. The migration times of enantiomers increased as the applied voltage declined. This could be attributed to the decreased electrophoretic velocities of analytes and EOF as well as the increased chiral selector and analyte interaction. Furthermore, as the applied voltage declined in the studied range of 12–25 kV, the R_S of the drugs at first tended to increase with the interaction time increasing, and then decreased because lower voltage brought lower capillary column efficiency (Fig. 3C). Taking account of high R_S and short analysis time, applied voltage of 20 kV was determined to be the optimum for the chiral separation.

3.5 Effect of cartridge temperature on resolution

Capillary temperature control is extremely important for reproducibility of the analyses. When current passes along a capillary, part of the electrical energy is converted into Joule heating. Change in temperature can change the viscosity of the buffer and then the mobility of the analytes

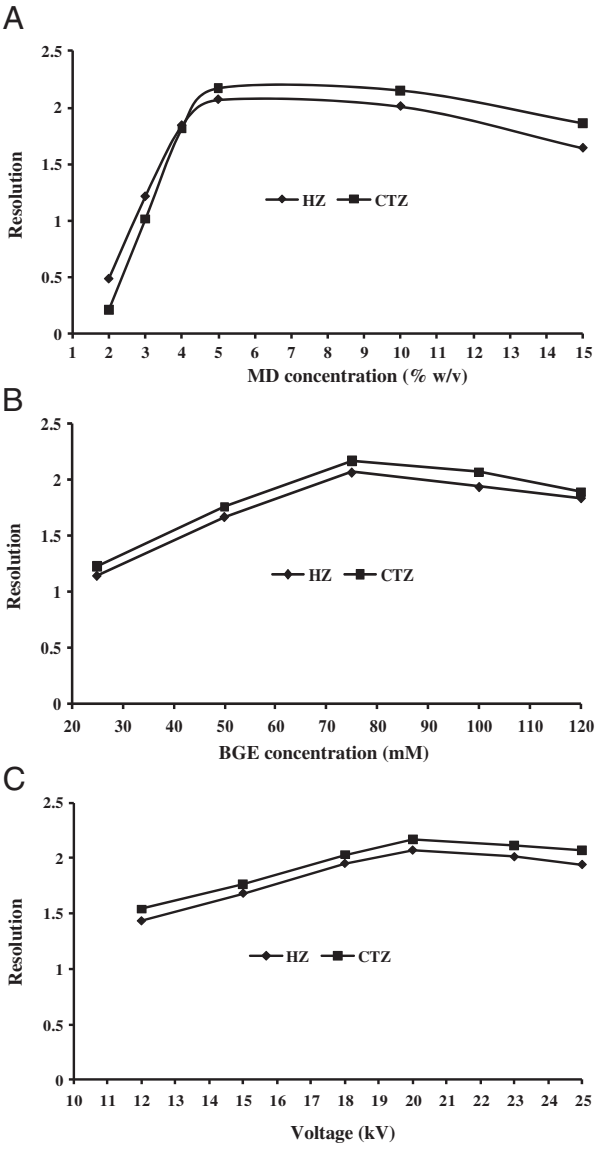

Figure 3. Effect of (A) MD concentration, (B) BGE concentration and (C) applied voltage on resolution of CTZ and HZ enantiomers. CE conditions were the same as in Fig. 2.

Table 2. Effect of cartridge temperature on migration time (min) and resolution of enantiomers

Temperature (°C)	HZ			CTZ		
	t_S	t_R	R_s	t_S	t_R	R_s
15	41.45	42.30	2.34	45.32	46.17	2.48
20	32.24	33.01	2.11	37.13	37.93	2.21
25	28.72	29.39	2.07	32.63	33.65	2.17
30	23.81	24.27	1.94	27.58	28.38	2.01
35	21.11	21.33	1.61	24.98	25.42	1.74

that can affect the migration times and consequently the resolution of the analytes [41]. Temperature may also influence the kinetics of the inclusion complex with the chiral selector [42]. The resolutions of mentioned enantiomers decreased slightly with an increase in the temperature from 15 to 35°C. These data are summarized in Table 2. The migration times decreased as the cartridge temperature increased. However, at temperatures lower than 20°C, it was noticed that the CE instrument was not as efficient in controlling the temperature and that equilibration time was rather long. A convenient operational temperature of 25°C was thus selected for the analyses. Figure 4A shows the typical electropherogram in optimized condition.

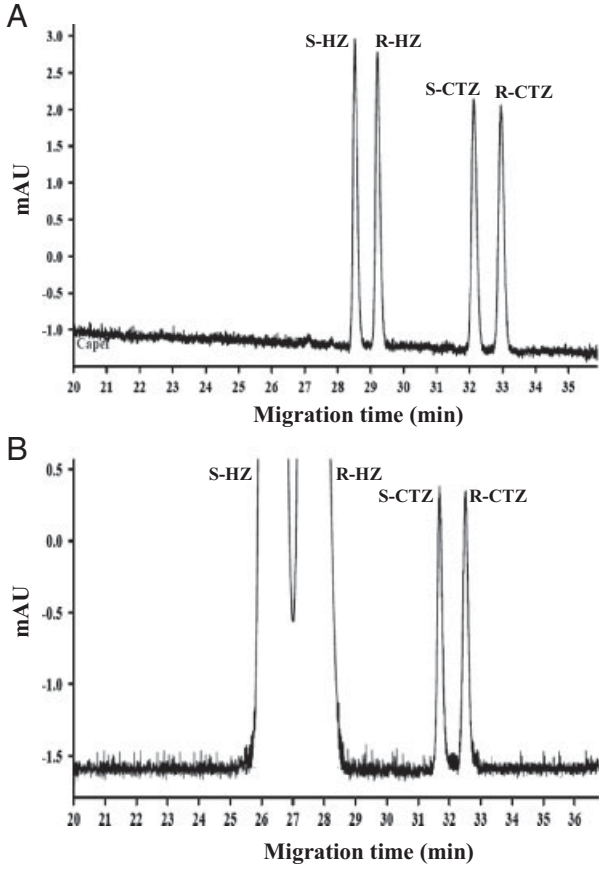

Figure 4. (A) A typical electropherogram obtained in optimized condition (concentration of each enantiomer is 5.0 μg/mL), (B) Enantioseparation of CTZ enantiomers (100 ng/mL) in the presence of high concentration of HZ (10 μg/mL). CE conditions were the same as in Fig. 2.

3.6 Method validation

The method was validated with respect to particular parameters including linearity, LOD and LOQ, precision, accuracy, recovery and selectivity [43, 44]. The equation of the calibration plots was established by linear regression of the peak area versus enantiomer concentration for all enantiomers. The standard curves for all enantiomers were linear from 30 to 1200 ng/mL. The LOQ ($S/N = 10$) was estimated to 30 ng/mL for all enantiomers, while the limit of detection ($S/N = 3$) was 10 ng/mL for all enantiomers utilizing UV detection at 214 nm (Table 3). The intra- and inter-day precision and accuracy data for each enantiomer were assessed by using standard solutions prepared to produce solutions of three different concentrations (50, 250 and 500 ng/mL). Repeatability or intra-day precision was investigated by injecting five replications of each concentration and inter-day precision was assessed by injecting the same samples over four consecutive days. Intra- and inter-day precision extractions varied between 3.7 and 11.7% for all enantiomers (Table 4). In each case, the percent relevant error and accuracy were calculated and found to be less than 8.2% for each enantiomer (Table 4). For investigation of extraction procedure recovery, known amount of each enantiomer was added to the water or blank plasma sample and diluted to yield concentration of 100 ng/mL for each enantiomer. These samples were extracted as the previously explained extraction procedure and analyzed by optimized MD-mediated CE method. The results are shown in Table 3. The resultant RSDs for this study varied between 5.9 and 8.1% for CTZ enantiomers and between 4.8 and 7.1% for HZ enantiomers. The selectivity of the proposed method

Table 3. Validation parameters from pure water and blank plasma samples

Sample		Linear equation	R^2	LOQ[a)]	LOD[a)]	Linearity[a)]	Recovery[b)] %, RSD
Water	S-HZ	$Y = 0.19X - 1.04$	0.999	30	10	30–1200	96.2, 5.1
	R-HZ	$Y = 0.19X - 0.79$	0.999	30	10	30–1200	95.9, 4.8
	S-CTZ	$Y = 0.16X - 0.72$	0.999	30	10	30–1200	97.3, 5.9
	R-CTZ	$Y = 0.16X - 0.58$	0.998	30	10	30–1200	96.7, 6.1
Plasma	S-HZ	$Y = 0.18X - 0.97$	0.995	30	10	30–1200	94.7, 7.1
	R-HZ	$Y = 0.18X - 0.84$	0.993	30	10	30–1200	94.1, 6.7
	S-CTZ	$Y = 0.15X - 0.68$	0.993	30	10	30–1200	93.7, 7.8
	R-CTZ	$Y = 0.15X - 0.56$	0.995	30	10	30–1200	93.1, 8.1

a) Concentration is based on ng/mL.
b) Recovery was obtained for 100 ng/mL of each enantiomer ($n = 3$).

Table 4. Precision and accuracy for the analysis of cetirizine and hydroxyzine enantiomers in plasma

Actual concentration (ng/mL)		HZ			CTZ		
		Concentration found (mean ± SD)	RSD (%)	RE (%)	Concentration found (mean ± SD)	RSD (%)	RE (%)
Intra-day[a)]							
S-form	50	52.1 ± 4.2	8.1	+4.0	46.7 ± 5.1	10.9	−6.6
	250	244.2 ± 10.4	4.3	−2.3	243.8 ± 11.3	4.6	−2.5
	500	493.3 ± 18.3	3.7	−1.3	507.5 ± 19.8	3.9	+1.5
R-form	50	52.0 ± 4.7	9.7	+4.0	45.9 ± 5.2	11.3	−8.2
	250	243.8 ± 9.8	4.0	−2.5	243.1 ± 10.9	4.5	−2.8
	500	491.9 ± 19.7	4.0	−1.6	506.8 ± 21.3	4.2	+1.4
Inter-day[b)]							
S-form	50	46.6 ± 5.1	10.9	−6.8	46.6 ± 5.3	11.4	−6.8
	250	256.9 ± 11.3	4.4	+2.8	243.1 ± 11.9	4.9	−2.8
	500	491.9 ± 19.7	4.0	−1.6	490.6 ± 21.7	4.4	−1.9
R-form	50	46.4 ± 4.9	10.6	−7.2	46.1 ± 5.4	11.7	−7.8
	250	256.1 ± 10.8	4.2	+7.8	241.4 ± 11.3	4.7	−3.4
	500	489.8 ± 20.1	4.1	−2.0	491.0 ± 22.5	4.5	−1.8

a) Intra-day data were based on five replicate analyses.
b) Inter-day data were based on four consecutive days.

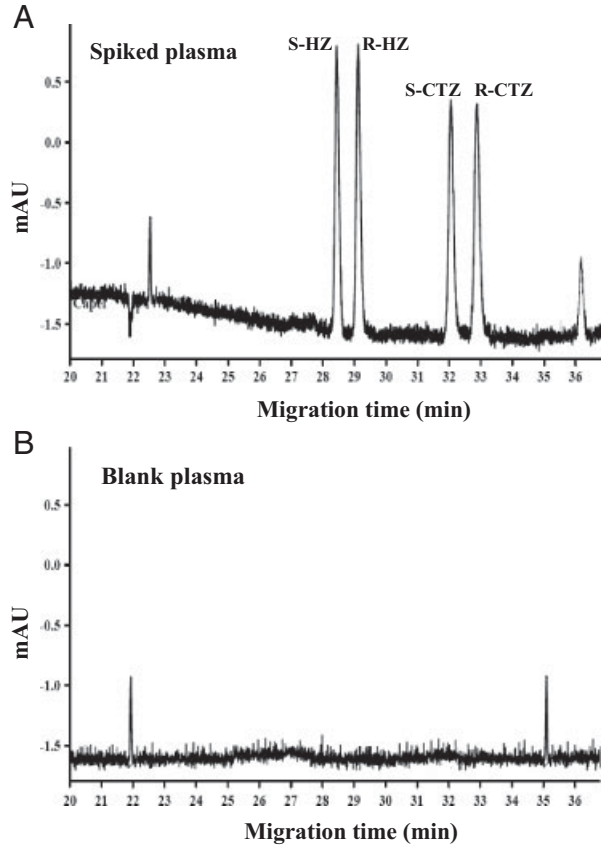

Figure 5. Electropherograms obtained from spiked and drug-free human plasma (spiked sample solution: 100 ng/mL of each enantiomer), and CE conditions were same as in Fig 2.

was tested on the enantioseparation of CTZ enantiomers as metabolite of HZ in the presence of high-concentration HZ (Fig. 4B). Under MD-mediated CE conditions, complete separation of CTZ enantiomers from the HZ enantiomers was obtained.

3.7 Determination of CTZ and HZ enantiomers in plasma

For investigation of matrix effect, linear range of method was examined in blank plasma and results showed that plasma and water have the same linear ranges for CTZ and HZ enantiomers (30-1200 ng/mL). The LOQ ($S/N = 10$) was estimated to 30.0 ng/mL for all enantiomers, while the limit of detection ($S/N = 3$) was 10.0 ng/mL for all enantiomers in plasma samples. The validation results are summarized in Table 3. In addition, the obtained results showed that there was no discrimination between extractions of enantiomers from plasma. The typical electropherograms of the plasma sample are shown in Fig. 5. There is no interference with enantiomers in plasma sample after liquid–liquid extraction in the proposed method. The therapeutic concentrations of CTZ and HZ in human plasma are 311–1000 and 100–280 ng/mL, respectively [45, 46]. Thus, obtained results showed that optimized chiral method in this work could cover plasma concentration of mentioned enantiomers.

4 Concluding remarks

In this work MD, as a linear polysaccharide, has been first proven to be a chiral selector for the simultaneous enantioseparation of CTZ and HZ enantiomers using CE. The method described here represents a very simple and efficient analytical method for the enantioseparation of CTZ and HZ enantiomers. BGE pH was the primary parameter in optimizing the

enantioseparation. The results showed that the interaction of cationic form of enantiomers with chiral selector for enantiomer separation is more effective than the interaction of anionic and neutral forms. The best separation results were obtained with 75 mM phosphate solution (pH 2.0) containing 5.0% w/v MD for the mentioned enantiomers. The proposed CE method provides a high degree of enantioseparation of CTZ and HZ enantiomers in plasma samples; therefore, it could be suitable for routine use and pharmacokinetic studies.

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