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Enantiomeric resolution of doxazosin mesylate and its process-related substances on polysaccharide chiral stationary phases

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Abstract

High-performance liquid chromatographic methods for separation of racemic doxazosin mesylate and its synthetic precursors on polysaccharide based stationary phases viz., amylose tris-(3,5-dimethylphenylcarbamate) (Chiralpak AD-H) and cellulose tris-(3,5-dimethylphenylcarbamate) (Chiralcel OD-H) were developed. The base line separation with $R_s > 1.50$ was obtained using a mobile phase containing *n*-hexane–alcohol–0.1% diethylamine (ethanol, 1-propanol and 2-propanol) in various proportions. The effect of concentration of the alcoholic modifiers on the resolution was studied. A good separation was achieved on amylose based Chiralpak AD-H column when compared with cellulose based Chiralcel OD-H. The effects of structural features of the solutes and solvents on discrimination between the enantiomers were examined. The detection was carried out at 240 nm with UV detector while identification by polarimetric detector connected in series. The method was suitable not only for process development of doxazosin mesylate but also determination of enantiomeric purity of bulk drugs and pharmaceuticals.

Keywords: Anti-hypertensive; Doxazosin mesylate; Synthetic precursors; Enatiomeric purity; Polysaccharide based stationary phases

1. Introduction

In the recent past, drug sterioisomerism has acquired increasing importance not only amongst the pharmaceutical chemists but also those involved in drug development and quality control. The determination of enantiomeric purity has become an important issue in the analysis of chiral drugs. This is mainly because of the fact that only one of the enantiomers fully contributes to the desired therapeutic activity while the other may be an unwanted by product [1,2]. Control of the unwanted enantiomers in the drug substances is necessary and it must be determined at the levels prescribed by the regulatory authorities [3]. In case of racemates, the drug enantiomers must be investigated in pharmacokinetic and dynamic models to justify the choice on a scientific basis demonstrating that there are no side effects.

Doxazosin mesylate (DXZN), (±)-1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(2,3-dihydro-l,4-benzodioxin-2-yl)carbonyl]piperazine monomethane sulfonate, is a selective α_1-adrenergic blocker belonging to quinazoline family of drugs and marketed as a racemate for therapeutic applications [4]. It is used in the treatment of hypertension either alone or in combination of diuretics and β-adrenergic-receptor-antagonists [5]. More recently, it has proven to be effective in the treatment of benign prostatic hyperplasia BPH [6]. Pre-clinical studies have suggested that the *S*-(+) enantiomer of DXZN would offer both reduced side effects (e.g. asthma, dizziness), and improved efficacy over the racemate for the treatment of BPH [7]. Determination of its quality is quite important not only for safety but also to prove its efficacy for the benefit of the patients who ultimately receives it in different dosages during the treatment of hypertension.

A thorough literature search has revealed that several differential pulse-polargraphic [8,9], square-wave voltametric [10,11], UV-spectrophotometric [12] and HPLC [13–16] methods have been reported for the determination of doxazosin mesylate in biological fluids. However, for analysis of pharmaceuticals only a limited number of HPLC [17], HPTLC [18,19] and voltametric [20] methods are available. The degradation studies of DXZN under a variety of stress conditions were studied with UV and HPLC [21,22]. DXZN enantiomers have been previously separated on cellulase silica [23] and α_1-acid glyco-

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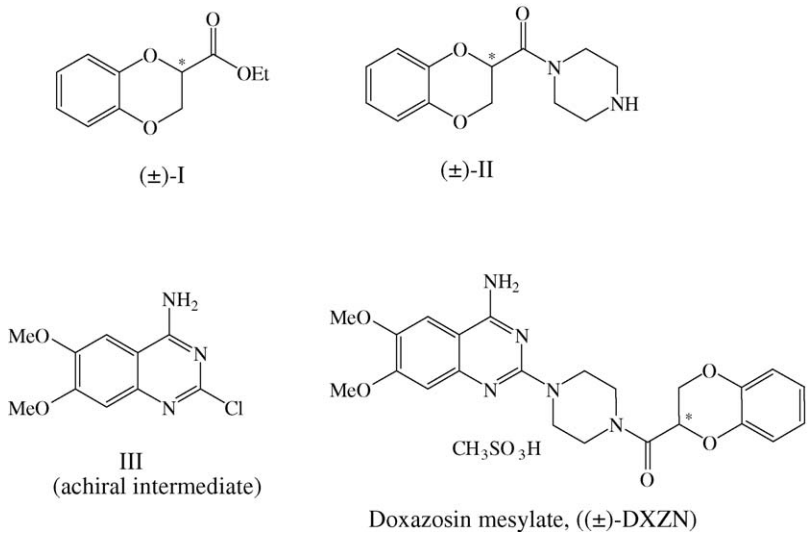

Fig. 1. Chemical structures of doxazosin mesylate and its synthetic chiral I, II and achiral III intermediate.

protein as chiral stationary phases [24]. Native and substituted β-cyclodextrins were used as mobile phase additives in RP-HPLC [25]. LC coupled with tandem mass spectrometry was used to determine the enantiomers of DXZN on Chiralpak AD column in biological fluids [7,26]. However, these methods do not cover the resolution of the synthetic precursors [23–26] of DXZN, which is highly needed not only for process development and control but also to optimize the quality of DXZN in finished products.

In our present investigation, the direct separation of the enantiomers of (±)-ethyl 2,3-dihydro-1,4-benzodioxin-2-carboxylate (I), (±) *N*-(2,3-dihydro-1,4-benzodioxin-2-carbonyl) piperazine (II) and doxazosin mesylate (DXZN) (Fig. 1) on amylose tris-(3,5-dimethylphenylcarbamate) and cellulose tris-(3,5-dimethylphenylcarbamate) was studied. The chiral recognition mechanism and the influence of the mobile phase solvents and the temperature on separation of DXZN and its process-related intermediates were investigated. The optimum separation was obtained on Chiralpak AD-H column containing amylose tris-(3,5-dimethylphenylcarbamate) as a stationary phase with *n*-hexane:ethanol:2-propanol:diethylamine (70:23:7:0.1, v/v/v/v) as mobile phase and UV-detector at 240 nm. The individual enantiomers were identified based on their optical rotation using a polarimeric detector, which was connected to UV in series. Further, the method was applied for quantitative determination of related intermediates in process samples and reaction monitoring during the synthesis of DXZN.

2. Experimental

2.1. Chromatograph

The HPLC system composed of two LC-10AT VP pumps, an SPD-10AVP UV detector, Rheodyne injector (7725i, Cotati, USA), a DGU-12A degasser and SCL-10A VP system controller (all from Shimadzu, Kyoto, Japan). Chiralcel OD-H, Chiralpak AD-H columns (25 cm × 4.6 mm i.d.; particle size 5 μm) were connected to guard column Chiralcel OD-H and Chiralpak AD-H (1 cm × 4.6 mm; 5 μm), respectively, used for separation (Daicel Chemical Industries Ltd., Tokyo, Japan). The chromatographic and the integrated data were recorded using HP-Vectra (Hewlett-Packard, Waldron, Germany) computer system.

2.2. Chemicals and materials

All reagents were of analytical-reagent grade unless stated otherwise. HPLC-grade *n*-hexane (Merck, India), ethanol, 1-propanol, 2-propanol and diethylamine (DEA) (Spectrochem, Mumbai, India) were used. Samples of doxazosin (DXZN) and process related substances viz., (±)-ethyl 2,3-dihydro-1,4-benzodioxin-2-carboxylate (I) and (±)-*N*-(2,3-dihydro-1,4-benzodioxin-2-carbonyl) piperazine (II) were collected during the process development work and used.

2.3. Chromatographic conditions

The mobile phase was *n*-hexane:ethanol:2-propanol:DEA (70:23:7:0.1, v/v/v/v). Before delivering into the system, it was filtered through 0.45 μm, PTFE filter and degassed using vacuum. The analysis was carried out under isocratic conditions using a flow rate of 1.0 ml/min. Chromatograms were recorded at 240 nm using a SPD-10AVP UV detector. The *dextro* (+) and *levo* (−) enantiomers were identified by polarimetric detector (IBZ Messtechnik GmbH, Hannover, Germany) detector based on their optical rotations (optical rotation range, 250; average, 10 and offset is 50).

2.4. Analytical procedures

Solutions (500 μg/ml) of DXZN, I and II were prepared by dissolving each compound in the minimum amount of methanol and diluting with the mobile phase. The stock solu-

tions were adequately diluted to obtain required concentrations to study the accuracy, precision, limit of detection and limit of quantitation.

3. Results and discussion

Fig. 1 shows the chemical structures of DXZN and its synthetic precursors generally used in the manufacturing processes. Compound I has one stereogenic center in the molecule, which on condensation with piperazine forms II, further it couples with the 4-amino-2-chloro-6,7-dimethoxyquinazoline substrate (III) to give a racemic product of DXZN. In the present investigation, the main aim was to develop a HPLC method for enantioselective separation and determination of DXZN and the synthetic precursors shown in Fig. 1.

In our preliminary experiments, Chiralpak AD-H and Chiralcel OD-H columns were selected. Polar modifiers such as 2-propanol, 1-propanol and ethanol in different combinations of *n*-hexane–0.1% DEA were tried. The enantioselectivity and resolution data of I, II and DXZN are summarized in Tables 1 and 2 for Chiralpak AD-H and Chiralcel OD-H columns, respectively. The UV-absorption spectra of the enantiomers were identical for compounds I (213, 272, 363 nm), II (208, 220, 275 nm) and DXZN (214, 246, 331 nm). The detection was made at 240 nm, which was the absorption maximum of DXZN. Compounds I and II also have some absorption at this wavelength.

Table 1
Chromatographic parameters: retention factor (k), resolution (R_s) and selectivity (α) of I, II and DXZN on Chiralpak AD-H

Compound	Parameter	% of 2-Propanol in *n*-hexane (v/v/)					
		10	20	25	30	35	40
I	k_1	0.90	0.64	0.60	0.54	0.42	0.24
	k_2	0.90	0.64	0.60	0.54	0.42	0.24
	R_s	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	α	1.00	1.00	1.00	1.00	1.00	1.00
II	k_1	5.04	2.75	–	1.37	–	0.50
	k_2	5.37	2.91	–	1.43	–	0.50
	R_s	0.58	0.47	–	0.41	–	n.r.
	α	1.07	1.06	–	1.04	–	1.00
DXZN	k_1	–	–	–	3.24	2.42	1.97
	k_2	–	–	–	5.13	3.86	3.14
	R_s	–	–	–	6.26	5.16	4.89
	α	–	–	–	1.58	1.60	1.59
		% of 1-Propanol in *n*-hexane (v/v)					
I	k_1	–	0.78	–	0.60	0.53	–
	k_2	–	0.87	–	0.66	0.58	–
	R_s	–	0.63	–	0.38	0.21	–
	α	–	1.12	–	1.10	1.09	–
II	k_1	–	3.23	–	1.53	1.19	–
	k_2	–	3.54	–	1.68	1.30	–
	R_s	–	1.05	–	0.72	0.62	–
	α	–	1.11	–	1.10	1.09	–
DXZN	k_1	–	6.80	–	4.81	–	2.98
	k_2	–	10.50	–	7.28	–	4.42
	R_s	–	5.26	–	4.57	–	4.03
	α	–	1.54	–	1.51	–	1.48
		% of Ethanol in *n*-hexane (v/v)					
I	k_1	–	0.94	0.90	1.08	–	1.03
	k_2	–	1.22	1.15	1.35	–	1.27
	R_s	–	2.03	1.92	1.83	–	1.85
	α	–	1.30	1.28	1.25	–	1.23
II	k_1	–	5.30	4.32	3.97	–	2.97
	k_2	–	6.90	5.44	4.89	–	3.67
	R_s	–	3.16	2.88	2.48	–	2.53
	α	–	1.30	1.26	1.23	–	1.26
DXZN	k_1	–	–	–	7.83	6.20	4.85
	k_2	–	–	–	8.06	6.45	5.03
	R_s	–	–	–	n.r.	0.28	0.45
	α	–	–	–	1.03	1.04	1.04

n.r.: not resolved; '–': experiment not performed.

Table 2
Chromatographic parameters: retention factor (k), resolution (R_s) and selectivity (α) of I, II and DXZN on Chiralcel OD-H

Compound	Parameter	% of 2-Propanol in *n*-hexane (v/v)							
		5	10	15	20	25	30	35	40
I	k_1	2.60	2.24	1.90	–	1.49	1.35	1.58	1.21
	k_2	3.06	2.48	2.03	–	1.54	1.35	1.58	1.21
	R_s	2.03	1.78	0.98	–	0.25	n.r.	n.r.	n.r.
	α	1.30	1.11	1.07	–	1.03	1.00	1.00	1.00
II	k_1	–	–	–	–	4.37	3.44	2.98	2.57
	k_2	–	–	–	–	5.84	4.56	3.94	3.39
	R_s	–	–	–	–	4.50	3.93	3.55	3.38
	α	–	–	–	–	1.34	1.33	1.32	1.32
DXZN	k_1	–	–	–	–	–	–	13.83	10.85
	k_2	–	–	–	–	–	–	14.26	11.49
	R_s	–	–	–	–	–	–	0.54	0.86
	α	–	–	–	–	–	–	1.03	1.03
		% of 1-Propanol in *n*-hexane (v/v)							
I	k_1	1.81	1.43	1.19	1.07	0.99	–	–	0.81
	k_2	2.19	1.70	1.38	1.24	1.13	–	–	0.91
	R_s	3.41	2.56	2.24	2.01	1.83	–	–	1.41
	α	1.20	1.18	1.16	1.15	1.14	–	–	1.12
II	k_1	–	–	4.40	3.18	2.51	2.00	–	–
	k_2	–	–	4.59	3.36	2.68	2.01	–	–
	R_s	–	–	0.21	0.45	0.57	0.60	–	–
	α	–	–	1.04	1.05	1.06	1.07	–	–
DXZN	k_1	–	–	–	–	–	10.02	–	5.64
	k_2	–	–	–	–	–	10.02	–	5.64
	R_s	–	–	–	–	–	n.r.	–	n.r.
	α	–	–	–	–	–	1.00	–	1.00
		% of Ethanol in *n*-hexane (v/v)							
I	k_1	1.70	1.37	–	1.45	–	0.95	–	0.44
	k_2	1.81	1.42	–	1.55	–	0.95	–	0.44
	R_s	0.94	0.41	–	0.96	–	n.r.	–	n.r.
	α	1.06	1.04	–	1.07	–	1.00	–	1.00
II	k_1	–	–		5.07	–	2.08	0.74	–
	k_2	–	–		6.06	–	2.17	0.74	–
	R_s	–	–		2.65	–	0.32	n.r.	–
	α	–	–		1.20	–	1.04	1.00	–
DXZN	k_1	–	–	–	18.36	–	8.50	–	5.66
	k_2	–	–	–	18.36	–	8.50	–	6.01
	R_s	–	–	–	n.r.	–	n.r.	–	0.54
	α	–	–	–	1.00	–	1.00	–	1.06

The influence of mobile phase composition, structure of the analytes and temperature on the separation was studied.

3.1. Influence of mobile phase composition

Several kinds of mobile phase compositions were investigated by changing the nature and percentage of alcohol. Baseline separation ($R_s > 1.50$) was obtained for all the compounds on Chiralpak AD-H, but not for DXZN on Chiralcel OD-H.

On Chiralpak AD-H, the retention times and retention factors, k, of all the three compounds were increased with decreasing percentage of ethanol in mobile phase, while α was unaffected. The increase in R_s was less than 25% for compounds I and II, but DXZN was not resolved. Similar trend was observed when 1-propanol was used. These results were consistent with the decreasing ability of the solvent to displace the solute from CSP, due to decrease in solvent polarity [27]. As illustrated in Figs. 2 and 3 the change in the mobile phase modifier from ethanol to 1-propanol resulted in decrease of k, whereas the enantioselectivity remained constant and the resolution decreased for I, II. In case of DXZN, both enantioselectivity and resolution (R_s 5.26) were increased at 20% of 1-propanol significantly.

When 1-propanol was substituted by 2-propanol, a great decrease in the retention for all compounds was observed. The α and R_s values were slightly increased for DXZN (Fig. 4), where as a significant decrease was noted for II, while compound I was unresolved. The observed changes in retention and stereoselectivity were likely due to the steric differences between the two solvent molecules, which may result in quite different chiral surfaces on the stationary phases [28,29].

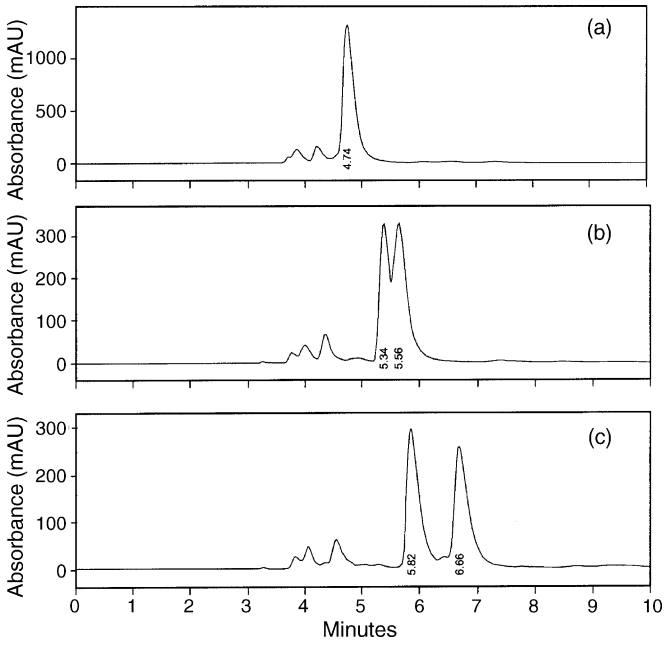

Fig. 2. Effect of the polar modifier (a) 2-propanol (30%), (b) 1-propanol (20%) and (c) ethanol (20%) on resolution of compound I on Chiralpak AD-H.

On Chiralcel OD-H, the retention factors obtained were slightly greater than on Chiralpak AD-H. The resolutions data for compounds I, II and DXZN is shown in Table 2. A decrease in the polar modifier (ethanol) in the mobile phase resulted in an increase in both k and α but R_s increased for I and II. When ethanol was substituted by 1-propanol, a slight increase in both k and α for I, with R_s 3.45 at 5% 1-propanol. Where as II was resolved partially, while DXZN was unresolved. Use of 2-propanol instead of ethanol resulted in an increase in k for all solutes. This increase in retention could be associated with

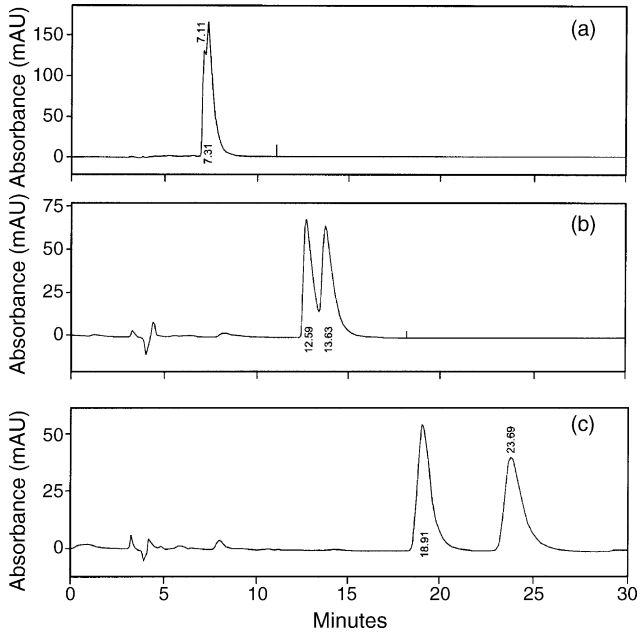

Fig. 3. Effect of the polar modifier (a) 2-propanol (30%), (b) 1-propanol (20%) and (c) ethanol (20%) on resolution of compound II on Chiralpak AD-H.

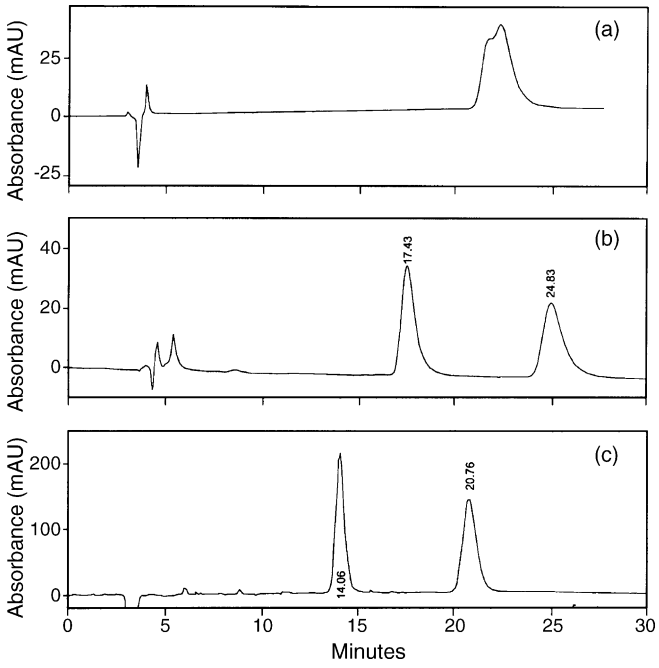

Fig. 4. Effect of the polar modifier (a) ethanol (35%), (b) 1-propanol (20%) and (c) 2-propanol (30%) on resolution of DXZN on Chiralpak AD-H.

an increase in selectivity (α) and resolution (R_s) for compounds I and II. These results are consistent with decreasing ability of 2-propanol to displace the solute from binding sites as the chiral cavity might change its geometry and/or size, according to the kind of alcohol modifier employed [30,31].

In addition to the solvent effects, the chiral cavity of CSP plays an important role in discrimination of enantiomers. The carbamate residue on Chiralpak AD-H and Chiralcel OD-H columns generally induces dipole–dipole and hydrogen bonding interactions through the carbonyl oxygen and –NH groups [32,33]. The possible interactions are between the carbonyl oxygen of the analytes (I, II and DXZN) and the –NH groups of CSP. As the chemical derivatization of both amylose (Chiralpak AD-H) and cellulose (Chiralcel OD-H) is the same, the difference in chiral recognition of amylose and cellulose derivatives must be due to the different configuration of the glucoside residue and higher order structure [34,35].

3.2. Optimization of chromatographic conditions

On Chiralpak AD-H, compounds I and II were resolved when 20% ethanol was used as organic modifier, but DXZN was not resolved. It was resolved when 30% of 2-propanol was used in the mobile phase. The effect of mixture of 2-propanol and ethanol in the mobile phase was studied (Table 3). It could be seen from Table 3, all the three compounds have baseline resolution ($R_s > 1.50$) with the mobile phase consisting of *n*-haxane–ethanol–2-propanol–DEA (70:23:7:0.1, v/v/v/v). Typical HPLC chromatogram showing the enantiomeric separation of a synthetic mixture of I, II and DXZN is shown in Fig. 5. The optical rotation [*dextro* (+) or l*evo* (−)] of individual enantiomers of each compound was monitored using polarimetric detector, which was connected in series with UV.

Table 3
Resolution of DXZN its chiral intermediates on Chiralpak AD-H using polar organic modifiers

Eluent (a[a], v/v)	Compound	k_1	k_2	R_s	α
60:20:20:0.1	I	0.67	0.67	n.r.	1.00
	II	1.38	1.48	0.47	1.07
	DXZN	3.15	4.29	4.23	1.36
65:25:10:0.1	I	0.75	0.84	0.68	1.12
	II	2.18	2.37	0.72	1.08
	DXZN	5.36	6.83	4.74	1.27
70:20:10:0.1	I	0.74	0.80	0.43	1.08
	II	2.15	2.28	0.44	1.06
	DXZN	5.78	7.67	4.82	1.30
70:25:5:0.1	I	0.78	0.94	1.38	1.21
	II	3.03	3.67	2.04	1.21
	DXZN	8.40	9.32	2.22	1.11
70:23:7:0.1	I	0.75	0.91	1.54	1.21
	II	2.89	3.54	2.70	1.22
	DXZN	7.99	9.04	2.43	1.13
70:22:8:0.1	I	0.74	0.84	0.78	1.14
	II	2.4	2.70	0.96	1.13
	DXZN	6.39	8.05	3.33	1.26
80:10:10:0.1	I	0.88	0.88	n.r.	1.00
	II	3.08	3.32	0.75	1.08
85:5:10:0.1	I	0.97	0.97	n.r.	1.00
	II	4.26	4.61	0.86	1.08

[a] a: *n*-hexane:ethanol:2-propanol:diethylamine [DEA]; flow rate:1.0 ml/min.

The retention data and sign of optical rotations are recorded in Table 4.

3.3. Influence of analyte structures

On comparing the structural changes in compounds I and II, it could be seen that the substitution of piperazine in place of ethoxy group could result in an increase in retention factor, selectivity and resolution on Chiralpak AD-H. Since compound II contains secondary amine, it has stronger ability to form a hydrogen bond leading to higher retention factor when compared to compound I. Similarly the substitution of 6,7-dimethoxy (2-piperazinyl)-quinazoline ring on I, gives DXZN, which might be responsible for formation of not only stronger hydrogen bond but also π–π interactions of different magnitude between the substituted phenyl moieties of carbamate and

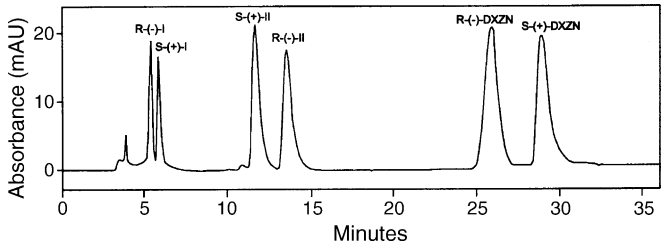

Fig. 5. HPLC chromatogram showing enantiomeric separation of a synthetic mixture of DXZN and its synthetic precursors I and II on Chiralpak AD-H using *n*-hexane:ethanol:2-propanol:diethylamine (70:23:7:0.1, v/v/v/v) as a mobile phase.

Table 4
Retention data of I, II and DXZN enantiomers on Chiralpak AD-H column

Component	t_R	k	R_s	T_f	Elution sequence
R-(−) I	5.38	0.79		1.17	(−)<(+)
S-(+) I	5.80	0.93	1.80	1.12	
R-(−) II	13.55	3.51	2.70	1.12	(+)<(−)
S-(+) II	11.68	2.89		1.17	
R-(−) DXZN	25.88	7.62		1.05	(−)<(+)
S-(+) DXZN	28.86	8.62	2.80		1.20

T_f: tailing factor; t_R retention time; eluent:*n*-hexane:ethanol:2-propanol:diethylamine (70:23:7:0.1, v/v/v/v), flow rate, 1.0 ml/min; detection at 240 nm; (+), (−) isomers were identified by polarimetric detector.

the quinazoline ring leading to still higher retention factors and resolution compared to compounds I and II. These effects could be clearly seen from the data recorded in Table 1. Similar observations were also noted on Chiracel OD-H for compounds I and II.

3.4. Effect of temperature

The effect of temperature was investigated in the range of 25–40 °C for compounds I, II and DXZN as a potential factor affecting the enantioselectivity. Higher temperatures led to a decrease in retention factors, selectivity and resolution on both the columns. For example, on Chiralpak AD-H, the parameters k, α, R_s for DXZN were 3.14, 1.53, 5.89 and 2.47, 1.50, 4.63 at 25 °C and 40 °C, respectively.

The variation in $\ln k$ and $\ln \alpha$ versus $1/T$, according to the van't Hoff model, showed a linear relationship with $r^2 > 0.995$ showing no conformational changes on the stationary phase. The enantioselective interactions involved in the separations were unchanged at these temperatures [31]. The thermodynamic parameters were determined from the slope and intercepts of the linear relations (Table 5). Negative ΔH° indicated that it was energetically more favorable for the solute to be in the stationary phase. Negative $\Delta S^{\circ *}$ also indicated an increase in the order of the chromatographic system as the solute transferred from mobile phase to the stationary phase. The calculated ΔH°, ΔS° and $\Delta\Delta H^\circ$, $\Delta\Delta S^\circ$ were negative, indicating that the transfer of solute from the mobile phase to the stationary phase and the separation, respectively, were enthalpy-driven.

Table 5
Thermodynamic parameters for compounds I, II and DXZN on Chiralpak AD-H and Chiralcel OD-H Columns

Compound	CSP	$\Delta\Delta H^\circ$ (k J mole^{-1})	$\Delta\Delta S^\circ$ (k J mole^{-1})	$\Delta\Delta G^\circ$ (k J mole^{-1})
I	AD-H	−0.56	−0.22	−0.50
	OD-H	−0.75	−0.72	−0.54
II	AD-H	−4.83	−14.29	−0.57
	OD-H	−0.78	−4.55	−2.13
DXZN	AD-H	−2.96	−6.13	−1.14

$\Delta\Delta H^\circ = -R(\text{slope } k_2 - \text{slope } k_1)$; $\Delta\Delta S^\circ = R(\text{intercept } k_2 - \text{intercept } k_1)$; $\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ$ (calculated at 298K; R gas constant, 8.314 J mole^{-1} K^{-1}).

Table 6
Recovery of I and II enantiomers from DXZN as determined by HPLC

	Nominal % of enantiomer spiked to DXZN				
	50	75	100	125	150
Amount added (μg/ml)	12.50	18.75	25	31.25	37.50
% Recovery (±R.S.D.%)[a]					
R-(−)-I	97.25 ± 1.83	98.52 ± 1.05	99.81 ± 0.62	98.60 ± 1.25	98.20 ± 0.55
S-(+)-I	97.50 ± 1.75	97.85 ± 1.05	98.92 ± 0.45	99.54 ± 0.36	99.40 ± 0.62
R-(−)-II	98.87 ± 1.30	99.50 ± 0.72	98.96 ± 1.50	99.43 ± 0.92	99.64 ± 0.46
S-(+)-II	98.92 ± 1.15	98.65 ± 0.58	98.78 ± 1.36	99.29 ± 0.84	98.92 ± 0.53

[a] $n = 3$ (triplicate determinations).

Table 7
Linearity, LOD and LOQ

Enantiomer	Range (μg/ml)	Regression equation	r^2	LOD (μg/ml)	LOQ (μg/ml)
R-(−)-I	12.5–50	$y = 5057x + 585$	0.9997	2.25	6.85
S-(+)-I	12.5–50	$y = 5303x - 532$	0.9996	2.32	7.18
R-(−)-II	12.5–50	$y = 5034x + 645$	0.9998	2.01	6.09
S-(+)-II	12.5–50	$y = 4963x + 1651$	0.9998	1.69	5.14
R-(−)-DXZN	12.5–50	$y = 489222x + 14286$	0.9995	0.53	1.62
S-(+)-DXZN	12.5–50	$y = 50897x + 25174$	0.9999	0.59	1.80

3.5. Validation

3.5.1. Precision

The precision of the method was checked by six ($n = 6$) injections of 10 μg/ml solutions of I and II spiked to DXZN. The R.S.D. (%) of retention time and peak areas were calculated. The range of R.S.D. (%) was from 0.13 and 0.60, respectively.

3.5.2. Accuracy

The recoveries of *R*-(−) and *S*-(+) enantiomers of I and II were assessed by spiking I and II to DXZN at five different levels ranging from 12.5 to 37.5 μg/ml. The recovery range and R.S.D. (%) for each enantiomer were 97.2–99.64% and 0.36–1.83%, respectively (Table 6).

3.5.3. Linearity

The linearity of peak area versus concentration was studied from 12.50 to 50 μg/ml for *R*-(−) and *S*-(+) enantiomers of DXZN, I and II. The data were subjected to statistical analysis using a linear-regression least-squares method. The calibration curves were found to be linear with correlation coefficients $r^2 > 0.9995$ and the results shown in Table 7.

3.5.4. Limit of detection (LOD) and quantitation (LOQ)

The limits of detection (LOD) and quantitation (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratio of 3 for LOD and 10 for LOQ, respectively. The LOD and LOQ values were determined by measuring the magnitude of analytical background by injecting mobile phase and calculating the signal-to-noise ratio for each enantiomer by injecting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. The results are given in Table 7

The optimized conditions viz.; *n*-hexane:ethanol:2-propanol:DEA (70:23:7:0.1, v/v/v/v) as mobile phase was used for the enantioselective determination of impurities I and II in DXZN.

Table 8
Results of analysis of process samples and bulk drugs of Doxazosin mesylate

Sample	I (%)		II (%)		DXZN (%)	
	R-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)
Reaction mix1	0.02	0.02	0.28	0.26	49.70	49.72
Reaction mix2	0.03	0.02	0.43	0.42	49.54	49.56
Reaction mix3	0.05	0.05	0.35	0.33	49.61	49.61
Bulk drugs						
Batch1	nd	nd	nd	nd	49.96	49.98
Batch2	nd	nd	nd	nd	49.97	49.98
Batch3	nd	nd	nd	nd	49.99	49.98

Average of three determinations ($n = 3$); nd: not detected.

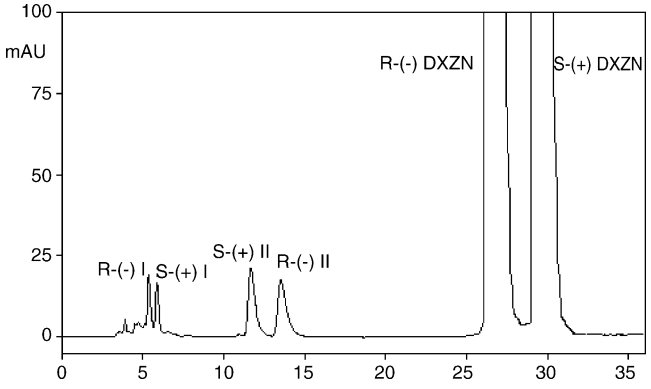

Fig. 6. HPLC chromatogram of DXZN spiked with 0.5% of each enantiomer I and II, obtained on Chiralpak AD-H using mobile phase consisting of *n*-hexane:ethanol:2-propanol:DEA (70:23:7:0.1, v/v/v/v), at a flow rate of 1.0 ml/min and detection at 240 nm.

Bulk drugs as well as the batch samples of synthetic process of DXZN were analyzed by the developed method and the results are recorded in Table 8. It could be seen from Table 8 that the batch samples contain enantiomeric impurities I and II in the range of 0.02–0.05% and 0.26–0.43%, respectively. However, these impurities were found to be below detection limits in bulk drug samples. A typical HPLC chromatogram shows DXZN spiked with 0.5% of each enantiomer I and II is shown in Fig. 6.

4. Conclusions

The resolution of DXZN and its synthetic intermediates on Chiralpak AD-H and Chiralcel OD-H columns was studied. Chiralpak AD-H column containing tris-(3,5 dimethylphenylcarbamate) as a stationary phase was found to be well adopted for the determination of I, II and DXZN when compared to Chiralcel OD-H. The effects of organic modifiers viz.; ethanol, 1-propanol, 2-propanol and temperature on selectivity and resolution was studied. The optimum separation was obtained on Chiralpak AD-H column with *n*-hexane:ethanol:2-propanol:diethylamine (70:23:7:0.1, v/v/v/v) as mobile phase and UV-detector at 240 nm. The separation was found to be an enthalpy driven process and the method was validated with respect to precision, accuracy, linearity, limit of detection and limit of quantitation. The separation of enantiomers of DXZN, I and II make the chromatographic method suitable for both qualifying optical purity and isolation of individual enantiomers. It is also useful for determination of chiral impurities during the process development and reaction monitoring during the synthesis of DXZN. The advantages of the developed method include (i) separation of the enantiomers of the synthetic precursors and doxazosin was achieved in a single run, (ii) the observed elution order *R*-(−) < *S*-(+) is beneficial for quantitative analysis, since the minor enantiomer eluted first, assuming that the *S*-(+) enantiomers is desired one and (iii) as the applied chiral stationary phase is available in bulk, it makes the chromatographic method up scalable for eventual (semi) preparative purification and production of the desired enantiomer (*S*-(+)).

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