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Short communication

A validated chiral LC method for the enantiomeric separation of Zolmitriptan key intermediate, ZTR-5

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Abstract

A new and accurate chiral liquid chromatographic method was described for the enantiomeric separation of ZTR-5 [(4*S*)-4-(4-aminobenzyl)-2-oxazolidinone, (*S*)-isomer], a key intermediate of Zolmitriptan in bulk drugs. The enantiomers of ZTR-5 were baseline resolved on a Chiralpak AD-H (250 mm × 4.6 mm, 5 μm) column using a mobile phase system containing hexane:ethanol (70:30, v/v). The resolution between the enantiomers was not less than four and interestingly distomer was eluted prior to eutomer. The limit of detection and limit of quantification of (4*R*)-4-(4-aminobenzyl)-2-oxazolidinone [(*R*)-isomer] were found to be 250 and 750 ng/ml, respectively, for 10 μl injection volume. The percentage recovery of (*R*)-isomer ranged from 92.0 to 105.6 in the bulk drug samples of ZTR-5. The validated method yielded good results regarding precision, linearity, accuracy and ruggedness. The proposed method was found to be suitable and accurate for the quantitative determination of (*R*)-isomer in bulk drug samples of ZTR-5.

Keywords: ZTR-5; Chiral HPLC; Chiralpak AD-H; Ruggedness; Validation; Accuracy

1. Introduction

Zolmitriptan (Zomig), a single enantiomer (4*S*)-4-[[3-[2-(dimethylaminoethyl)]-1H-indol-5-yl]methyl]-2-oxazolidinone is a novel serotonin 5-hydroxytryptamine receptor agonist that has shown, in an extensive clinical trial program, to be highly effective in the acute oral treatment of migraine with or without aura [1–6]. Zolmitriptan is synthesized as (*S*)-isomer, since it is pharmacologically more potent than (*R*)-isomer. Moreover (*R*)-isomer of Zolmitriptan is toxic in nature and the allowed limit of (*R*)-isomer (unrequired isomer) in Zolmitriptan bulk drug was 0.15% (w/w). ZTR-5 is a key starting material in the synthesis of Zolmitriptan and also chiral in nature. The chiral nature of Zolmitriptan is due to the presence of chiral moiety of ZTR-5 in the molecule. The content of (*R*)-isomer present in Zolmitriptan bulk drug mainly depends on the content of (*R*)-isomer present in ZTR-5. To our present knowledge no chiral HPLC methods were reported in the literature for the enantiomeric separation of ZTR-5. Therefore, it is felt necessary to develop a chiral LC method for the accurate quantification of unrequired enantiomer [(*R*)-isomer] of ZTR-5.

This report describes a chiral LC method for the enantiomeric separation of ZTR-5 using an amylose based chiral stationary phase, Chiralpak AD-H. The developed HPLC method was validated for optical purity assessment (determination of (*R*)-isomer in ZTR-5).

2. Experimental

2.1. Chemicals

ZTR-5 and (*R*)-isomer of ZTR-5 were supplied by Process Research Department of Dr. Reddy's Laboratories Limited,

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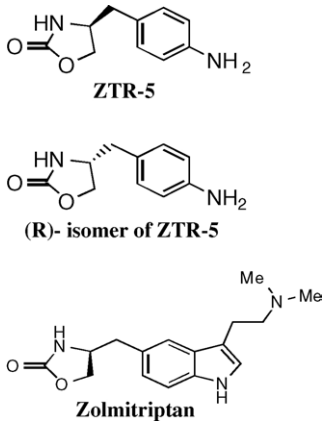

Fig. 1. Chemical structures of ZTR-5, (*R*)-isomer and Zolmitriptan.

Hyderabad, India. The chemical structures of ZTR-5, (*R*)-isomer of ZTR-5 and Zolmitriptan were presented in Fig. 1. HPLC grade hexane, isopropanol and ethanol were purchased from Merck (Germany).

2.2. Equipment

A Waters Alliance HPLC system equipped with 2695 separation module with built-in auto injector, 270852 thermostatic compartment and 996 photo diode array detector was utilized for method development and validation, located in Analytical Research department of Custom Pharmaceutical Services business unit, Dr. Reddy's Laboratories (Laboratory A). The second instrument, Waters LCM1 plus HPLC system equipped with 600 pump, 715 auto injector, 270852 thermostatic compartment and 486 tunable absorbance detector was utilized in ruggedness study, located in Analytical Research department of Discovery Research business unit, Dr. Reddy's Laboratories (Laboratory B). Millennium 32 chromatography manager software (Waters) was used for data acquisition and system suitability calculations.

2.3. Sample preparation

Stock solutions of (*R*)-isomer (250 μg/ml) and ZTR-5 (2.5 mg/ml) were prepared by dissolving the appropriate amounts of the substances in ethanol. The analyte concentration of ZTR-5 was fixed as 0.5 mg/ml. Working solutions of ZTR-5 and (*R*)-isomer were prepared in mobile phase.

2.4. Chromatographic conditions

The chromatographic conditions were optimized using an amylose based chiral stationary phase Chiralpak AD-H (250 mm × 4.6 mm, 5 μm, Daicel make) that was safeguarded with a 1 cm long guard column. The mobile phase was hexane:ethanol (70:30, v/v). The flow rate was set at 1.0 ml/min. The column was maintained at 25 °C and the detection was carried out at a wavelength of 240 nm. The injection volume was 10 μl. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

2.5. Validation of the method

2.5.1. Precision

The method precision was checked by analyzing six replicate sample solutions of ZTR-5 (at the analyte concentration, i.e. 0.5 mg/ml) spiked with 0.5% (2500 ng/ml) of (*R*)-isomer and calculating the percentage relative standard deviation of area.

2.5.2. Limit of detection and limit of quantification of (R)-isomer

The limit of detection, defined as lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal-to-noise ratio [7]. The limit of quantitaion, defined as lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated at 10 times the signal-to-noise ratio [7]. The limit of detection (LOD) and limit of quantification (LOQ) were achieved by injecting the series of dilute solutions of (*R*)-isomer.

The precision of the method was checked for (*R*)-isomer at limit of quantification by analyzing six test solutions of (*R*)-isomer prepared at LOQ level and calculating the percentage relative standard deviation of area.

2.5.3. Linearity of (R)-isomer

Detector response linearity was checked by preparing five calibration sample solutions of (*R*)-isomer at concentrations 750, 1250, 2500, 3750 and 5000 ng/ml, prepared in mobile phase from (*R*)-isomer stock solution.

Linear regression curve was obtained by plotting peak area versus concentration, using the least squares method.

2.5.4. Quantification of (R)-isomer in ZTR-5 bulk sample

The ZTR-5 bulk sample, provided by Process Research Department of Dr. Reddy's Laboratories, showed the presence of 0.07% of (*R*)-isomer. Standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of (*R*)-isomer in bulk drug samples [8].

The study was carried out in triplicate at 0.4, 0.5 and 0.6% of the ZTR-5 target analyte concentration. The recovery of (*R*)-isomer was calculated from the slope and *Y*-intercept of the calibration curve, drawn in the concentration range of 750–5000 ng/ml (slope and *Y*-intercept values obtained in the linearity study).

2.5.5. Ruggedness

The ruggedness of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under variety of normal test conditions such as different labs, different analysts, different instruments and different lots of reagents. The precision experiments carried out

in Section 2.5.1 were again carried out in laboratory B using a different instrument.

3. Results and discussion

3.1. Method development

The aim of this work is to separate the enantiomers of ZTR-5 and accurate quantification of (*R*)-isomer. The racemic mixture was prepared by physical mixing the equal portions of ZTR-5 and (*R*)-isomer (0.1 g of each sample). A 0.5 mg/ml solution of racemic mixture prepared in ethanol was used during the method development. To develop a rugged and suitable LC method for the enantiomeric separation of ZTR-5, different mobile phases and stationary phases were employed. Three different chiral columns were employed during method development namely Chiralcel OD-H, Chiralpak AD-H and Chiralcel OJ-H of Daicel. All the columns chosen were of 250 mm length, 4.6 mm internal diameter and 5 μm particle size. The mechanism of separation in direct chiral separation methods is the interaction of chiral stationary phase (CSP) with analyte enantiomers to form a short lived, transient diastreomeric complexes [9]. Various experiments were conducted, to select the best stationary and mobile phase that would give optimum resolution and selectivity for the enantiomers. Baseline chromatographic resolution was achieved on all the columns using a mobile

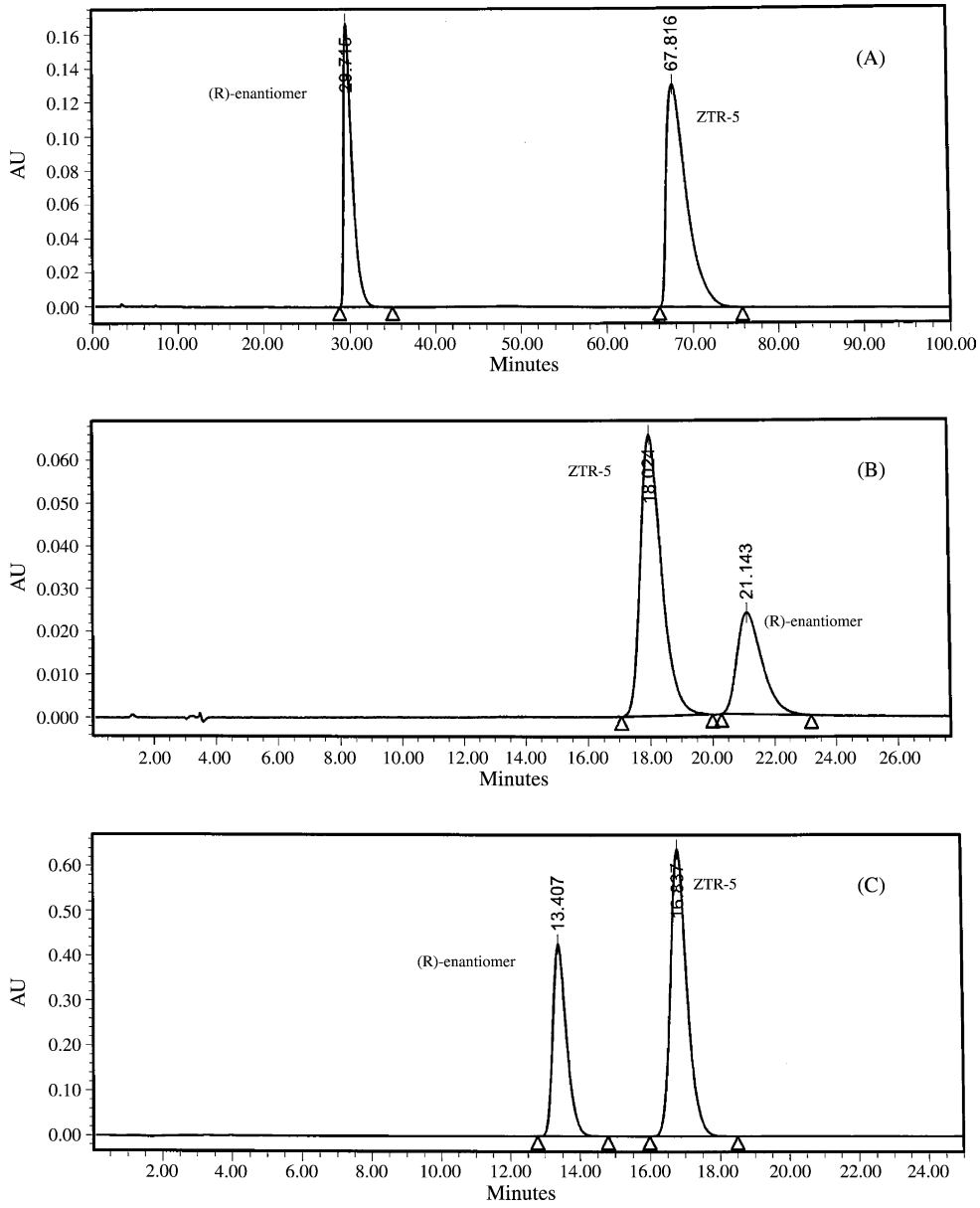

Fig. 2. Enantiomeric separation of racemic ZTR-5 on (A) Chiralcel OJ-H, (B) Chiralcel OD-H and (C) Chiralpak AD-H columns; mobile phase composed of hexane:ethanol (70:30, v/v); flow rate: 1.0 ml/min; UV-240 nm; column temperature: 25 °C.

phase system containing hexane:isopropanol (70:30, v/v) and the enantiomer peaks were found to be broad. Replacement of isopropanol by ethanol in the mobile phase has improved the peak shape of enantiomers of ZTR-5. Using the mobile phase system containing hexane:ethanol (70:30, v/v), distomer was eluted prior to eutomer in Chiralcel OJ-H and Chiralpak AD-H columns where as elution order of enantiomers was reversed on Chiralcel OD-H column. The resolution between the enantiomers was about 2, 4 and 12 on Chiralcel OD-H, Chiralpak AD-H and Chiralcel OJ-H columns using the above mobile phase. Chiralpak AD-H column was selected as a compromise between resolution and run time.

In the optimized method the typical retention times of (*R*)-isomer and ZTR-5 were 13.4 and 16.8 min, respectively. The enantiomeic separation of ZTR-5 on Chiralcel OD-H, Chiralcel OJ-H and Chiralpak AD-H columns was shown in Fig. 2. The system suitability test results were presented in Table 1.

The chiral stationary phase (CSP) present in Chiralpak AD-H column is tris(3,5-dimethylphenyl carbamate) amylose derivative coated on silica gel. The separation of enantiomers on Chiralpak AD-H column could be due to the interaction between the solute enantiomers and polar carbamate group (–HN–C=O) on the CSP. The carbamate group on the CSP can interact with solute enantiomers through hydrogen bonding using the C=O and NH groups which are present in both CSP and ZTR-5. In addition, the dipole–dipole interactions can occur between the C=O group on the CSP and the C=O group on the ZTR-5. Chiral discrimination between the enantiomers is due to the difference in their steric fit in

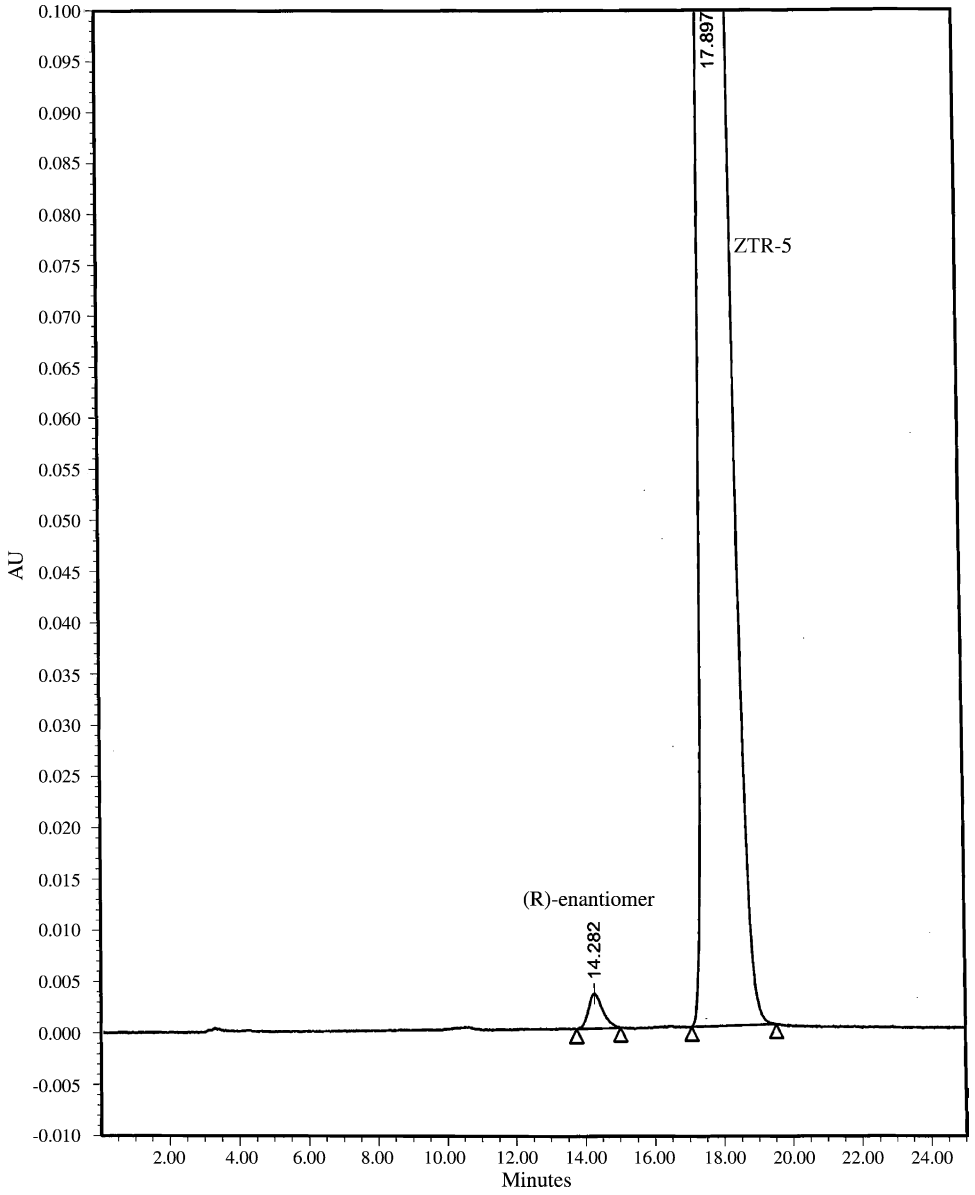

Fig. 3. Typical HPLC chromatogram of ZTR-5 bulk sample (0.5 mg/ml) spiked with (*R*)-isomer (0.5%).

Table 1
System-suitability report

Column name	Compound ($n = 3$)	k	R_S	N	T	α
Chiralcel OD-H	ZTR-5	17.0	2.2	3374	1.2	
	(*R*)-isomer	20.1		3269	1.4	1.2
Chiralcel OJ-H	(*R*)-isomer	28.7	12.4	2871	1.3	
	ZTR-5	66.8		6681	1.8	2.3
Chiralpak AD-H	(*R*)-isomer	12.4	4.3	5722	1.3	
	ZTR-5	15.8		6239	1.2	1.3

$n = 3$ determinations; k, capacity factor; R_S, USP resolution; N, number of theoretical plates (USP tangent method); T, USP tailing factor; α, Enantioselectivity, $\alpha = k_2/k_1$ (k_1 and k_2 are capacity factor values of enantiomer peaks).

the chiral cavities [10]. While the cellulose based Chiralcel OD-H column had the same derivitisation group (3,5-dimethylphenyl carbamate) as its amylose-based counterpart (Chiralpak AD-H), it showed different chiral recognition abilities for the enantiomers of ZTR-5. Okamoto and co-workers attributed the difference in chiral recognition ability between Chiralcel OD-H and Chiralpak AD-H columns to the conformational difference between them. So the chiral discrimination between the enantiomers of ZTR-5 on Chiralpak AD-H column is believed to be due to the same reason. The separation of enantiomers on Chiralcel OJ-H column may be due to the dipole–dipole interactions between the C=O group on the CSP and the C=O group on the ZTR-5. The reverse enantiomer elution order between the ester type Chiralcel-OJ and the cellulose type Chiralcel-OD columns could be due to the alteration of steric environment of the chiral cavities.

3.2. Validation results of the method

In the precision study, the relative standard deviation (R.S.D.) was better than 0.5% for ZTR-5 peak area and 4% for (*R*)-isomer peak area, indicating good precision of the method.

The limit of detection and limit of quantification concentrations were estimated to be 250 and 750 ng/ml, respectively, for (*R*)-isomer when a signal-to-noise ratio of 3 and 10 was used as the criteria. The method precision for (*R*)-isomer at limit of quantification was less than 7% R.S.D.

Good linearity was observed for (*R*)-isomer over the concentration range of 750–5000 ng/ml and the correlation coefficient of the calibration curve was 0.997.

The standard addition and recovery experiments were conducted for (*R*)-isomer in bulk drug samples in triplicate at 0.4, 0.5 and 0.6% of analyte concentration and the percentage recovery was ranged from 92.0 to 105.6 (Table 2).

A HPLC chromatogram of spiked (*R*)-isomer at 0.5% level in ZTR-5 bulk drug sample was shown in Fig. 3.

In the ruggedness study, the relative standard deviation was better than 0.6% for ZTR-5 peak area and 5.3% for (*R*)-isomer peak area. The results show that R.S.D. values were in the same order of magnitude than those obtained for repeatability. This confirms the ruggedness of the method.

Table 2
Recovery results of (*R*)-isomer in bulk drug sample

Added (ng) ($n = 3$)	Recovered (ng)	Recovery (%)	R.S.D. (%)
2002	1842	92.0	3.9
2503	2643	105.6	5.2
3003	3072	102.3	4.1

$n = 3$ determinations.

4. Conclusion

A new and accurate normal phase chiral LC method was described for the determination of (*R*)-isomer in ZTR-5, a key intermediate of Zolmitriptan. Cellulose based chiral stationary phases namely Chiralcel OD-H, Chiralcel OJ-H and amylose based chiral stationary phase Chiralpak AD-H was found to be selective for the enantiomers of ZTR-5. The method validation was carried out due to the better chromatographic results obtained on Chiralpak AD-H column. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently used for the quantitative determination of chiral impurity ((*R*)-isomer) in bulk materials.

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