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A validated chiral LC method for the determination of Zolmitriptan and its potential impurities

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Abstract

A new, accurate and reliable chiral HPLC method was developed for the determination of Zolmitriptan, (4*S*)-4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl] methyl]-2-oxazolidinone an antimigraine agent and its potential impurities namely (4*R*)-4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl] methyl]-2-oxazolidinone [(*R*)-enantiomer] and (4*S*)-4-(4-aminobenzyl)-2-oxazolidinone (Imp-1) in pharmaceutical formulations and in bulk drugs. HPLC separation was carried out by normal phase chromatography with a mobile phase composed of hexane:isopropanol:methanol:diethylamine in the ratio (75:10:15:0.1, v/v/v/v) pumped at a flow rate of 1.0 ml/min on a Chiralpak AD-H column. Zolmitriptan and its potential impurities were baseline resolved in the optimized method. The presence of diethylamine in the mobile phase has played a key role in achieving chromatographic resolution between the enantiomers and also in enhancing chromatographic efficiency. The developed method was also found to be selective under exposed conditions UV light and 60 °C. The developed method was completely validated and proved to be robust. The values of the limit of detection (LOD) and limit of quantification (LOQ) of (*R*)-enantiomer and Imp-1 were 100, 250 ng/ml and 30, 1000 ng/ml, respectively, for 10 μl injection volume. The validated method yielded good results regarding selectivity, linearity, precision, accuracy and ruggedness. Zolmitriptan sample solution and mobile phase are found to be stable for at least 24 h. The proposed method was found to be suitable and accurate for the quantitative determination of Zolmitriptan and its impurities namely (*R*)-enantiomer and Imp-1 in bulk drugs and commercial formulations.

Keywords: Zolmitriptan; Chiral HPLC; Chiralpak AD-H; Validation; Zolmitriptan impurities; Solution and mobile phase stability

1. Introduction

Zolmitriptan (zomig), a single enantiomer (4*S*)-4-[[3-[2-(dimethylamino)ethyl]-1*H*-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 [1,2]. It works by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. Zolmitriptan mimics this action of serotonin by directly stimulating the serotonin receptors in the brain and it relieves the pain of migraines.

A few techniques had been reported in the literature for Zolmitriptan quantification in plasma samples: coulometric detection, liquid chromatography/tandem mass spectroscopy and liquid chromatography with fluorescence detection [3–5].

So far to our present knowledge no chiral HPLC methods were reported in the literature for the enantiomeric separation of Zolmitriptan and accurate quantification of Zolmitriptan

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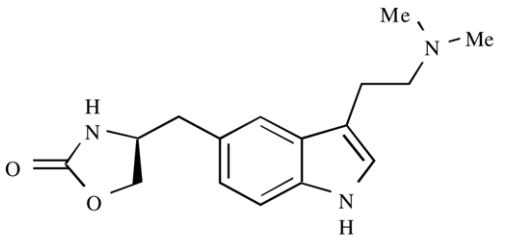
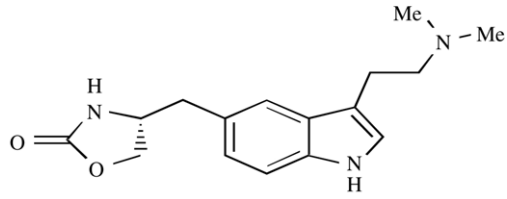
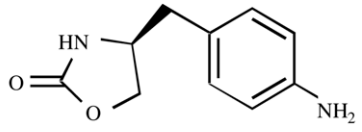


Zolmitriptan



(R)- enantiomer



Imp-1

Fig. 1. Chemical structure of Zolmitriptan, (*R*)-enantiomer and Imp-1.

and its potential impurities namely (*R*)-enantiomer and Imp-1 in bulk drugs and in pharmaceutical formulations.

Separation of enantiomers has become very important in analytical chemistry, especially in the pharmaceutical and biological fields, because some stereoisomers of racemic drugs have quite different pharmacokinetic properties and different pharmacological or toxicological effects [6,7].

Due to the chiral nature of the drug it is felt necessary to develop a chiral LC method for the enantiomeric separation and accurate quantification of unwanted enantiomer ((*R*)-enantiomer) of Zolmitriptan. Interestingly other potential impurity of Zolmitriptan namely Imp-1 was also well separated from Zolmitriptan and (*R*)-enantiomer in the developed method. This paper deals with method validation of Zolmitriptan and its potential impurities.

2. Experimental

2.1. Chemicals

Zolmitripatn, (*R*)-enantiomer and Imp-1 were kindly supplied by Process Research Department of Dr. Reddy's Laboratories Limited, Hyderabad, India and the chemical structures were given in Fig. 1. HPLC grade hexane, isopropanol and methanol from Merck (Germany) were used to prepare the mobile phase, together with diethylamine from Rankem (India) of reagent grade quality. Zolmitriptan tablets (Zomig, 5 mg) were purchased from Astra Zeneca, USA.

2.2. Equipment

A Waters Alliance HPLC system equipped with 2695 separation module with inbuilt auto injector, 270852 thermostatic compartment and 996 photo diode array detector was utilized for method development and validation which was 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 which was 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. Photo diode array detector was used for determining peak purity.

2.3. Sample preparation

Stock solutions of (*R*)-enantiomer, Imp-1 (250 μg/ml) and Zolmitriptan (5 mg/ml) were prepared by dissolving the appropriate amount of the substance in mobile phase. The analyte concentration of Zolmitriptan was fixed as 0.5 mg/ml. Working solutions of Zolmitriptan, (*R*)-enantiomer and Imp-1 were prepared in mobile phase.

2.4. Chromatographic conditions

The chromatographic conditions were optimized using a amylose based chiral stationary phase Chiralpak AD-H (250 mm × 4.6 mm, 5 μm, Daicel make) which was safeguarded with a 1 cm long guard column. The mobile phase was hexane:isopropanol:methanol:diethylamine (75:10:15:0.1, v/v/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 225 nm. The injection volume was 10 μl. Cellulose based chiral stationary phases Chiralcel OD-H and Chiralcel OJ-H (Daicel make) were also employed during method development. 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. System suitability test

System suitability test is an integral part of chromatographic methods and is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed [8]. The system suitability test results of

Table 1
System-suitability report

Column name	Compound	k	R_S	N	T	$\propto$
Chiralpak AD-H	(*R*)-enantiomer	4.9	–	5054	1.4	
	Zolmitriptan	7.2	5.4	4610	1.5	1.5
	Imp-1	18.8	16.6	7730	1.4	2.6

$n = 3$ determinations; k, capacity factor; R_S, USP resolution; N, number of theoretical plates (USP tangent method); T, USP tailing factor; $\propto$, enantioselectivity.

the chiral LC method on Chiralpak AD-H are presented in Table 1.

2.5.2. Selectivity

Method selectivity was challenged by forced degradation of Zolmitriptan sample under UV light (254 nm) and heat (60 °C) for 10 days. Content of (*R*)-enantiomer and Imp-1 were checked in Zolmitriptan sample exposed under light and heat on each day upto the study period. The exposed samples were tested for peak purity using photo diode array detector.

2.5.3. Precision

Precision of the method is the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample.

The precision of the method was checked by analyzing nine replicate samples of Zolmitriptan (at the analyte concentration, i.e. 0.5 mg/ml) spiked with 0.15% (750 ng/ml) of (*R*)-enantiomer, 0.5% (2500 ng/ml) of Imp-1 and calculating the percentage relative standard deviation.

2.5.4. Limit of detection and limit of quantification of (R)-enantiomer and Imp-1

The limit of detection (LOD) and the limit of quantification (LOQ) for (*R*)-enantiomer and Imp-1 were determined at a signal-to-noise ratio of 3 and 10 [9]. LOD and LOQ were achieved by injecting a series of dilute solutions of (*R*)-enantiomer and Imp-1.

The precision of the developed LC method for (*R*)-enantiomer and Imp-1 at limit of quantification was checked by analyzing six test solutions of (*R*)-enantiomer and Imp-1 prepared at LOQ level and calculating the percentage relative standard deviation. The accuracy of the method was checked for (*R*)-enantiomer and Imp-1 at LOQ level by analyzing three replicate samples of Zolmitriptan (0.5 mg/ml) spiked with (*R*)-enantiomer and Imp-1 at LOQ level and calculating the percentage recovery.

2.5.5. Linearity of Zolmitriptan, (R)-enantiomer and Imp-1

Linearity for Zolmitriptan was evaluated by determining six working solutions of Zolmitriptan ranging from 250 to 1000 μg/ml, (250, 375, 500, 625, 750 and 1000 μg/ml), prepared in mobile phase from Zolmitriptan stock solution.

Linearity for (*R*)-enantiomer was evaluated by determining six working solutions of (*R*)-enantiomer ranging from 250 ng/ml (LOQ) to 1500 ng/ml (0.3%) (250, 500, 750, 1000, 1250 and 1500 ng/ml), prepared in mobile phase from (*R*)-enantiomer stock solution.

Linearity for Imp-1 was evaluated by determining six working solutions of Imp-1 ranging from 1000 ng/ml (LOQ) to 5000 ng/ml (1.0%) (1000, 2000, 2500, 3000, 4000 and 5000 ng/ml), prepared in mobile phase from Imp-1 stock solution.

The peak area and concentration of Zolmitriptan, (*R*)-enantiomer and Imp-1 were subjected to regression analysis to calculate calibration equation and correlation coefficient. Linearity was checked for three consecutive days in the same concentration range from the same stock solutions. The percentage relative standard deviation of the slope and *Y*-intercept of the calibration curves was calculated.

2.5.6. Quantification of Zolmitriptan, (R)-enantiomer and Imp-1 in bulk sample

The accuracy of the developed LC method for Zolmitriptan was carried out in triplicate at 80% (400 μg/ml), 100% (500 μg/ml) and 120% (600 μg/ml) of target analyte concentration in bulk drug samples. The recovery was calculated from slope and *Y*-intercept of the calibration curve obtained in Section 2.5.5.

The Zolmitriptan bulk sample, provided by Process Research Department of Dr. Reddy's Laboratories, showed the absence of distomer and Imp-1. Standard addition and recovery experiments were conducted to determine accuracy of the present method for the quantification of (*R*)-enantiomer and Imp-1 in bulk drug samples.

The quantification study for (*R*)-enantiomer and Imp-1 were carried out in triplicate at 0.12, 0.15, 0.18% and 0.5, 0.75, 1.0% of the Zolmitriptan target analyte concentration, respectively. The recoveries of (*R*)-enantiomer and Imp-1 were calculated from the slope and *Y*-intercept of the calibration curves obtained in Section 2.5.5.

2.5.7. Quantification of Zolmitriptan, (R)-enantiomer and Imp-1 in formulation

Zomig are film-coated tablets contain 2.5 and 5 mg of Zolmitriptan and the excipients present in Zomig are lactose anhydrate, microcrystalline cellulose, sodium startch glycollate, magnesium sterate, iron oxide and titanium dioxide. Sixty tablets of zomig (5 mg) were finely ground using agate mortar and pestle. The ground material which was equivalent to 250.0 mg of the active pharmaceutical ingredient (Zolmitriptan) was extracted into mobile phase in a 100-ml volumetric flask by vortex mixing followed by ultrasonication. The resultant mixture was filtered through 0.45 um

membrane filter. The filtrate was used as stock solution for preparing the accuracy test solutions.

The accuracy of the developed LC method for Zolmitriptan was carried out in triplicate at the same concentration levels as described in Section 2.5.6 in formulation samples. The recovery was calculated from slope and *Y*-intercept of the calibration curves obtained in Section 2.5.5.

Standard addition and recovery experiments were conducted for (*R*)-enantiomer and Imp-1 in Zolmitriptan formulation (Zomig tablets) in triplicate at the same concentration levels as described in Section 2.5.6. The recoveries of (*R*)-enantiomer and Imp-1 were calculated from the slope and *Y*-intercept of the calibration curves obtained in Section 2.5.5.

2.5.8. 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 same experiments carried out in precision study (Section 2.5.3) were again carried out using a different analyst in Laboratory B and the percentage of R.S.D. was calculated.

2.5.9. Robustness

The capability to remain unaffected by small but deliberate variations in the method parameters was studied in order to anticipate the problems, which may arise during the regular application of the developed method. To determine robustness of the method experimental conditions were purposely altered and chromatographic resolution between Zolmitriptan and (*R*)-enantiomer was evaluated.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution of enantiomers, it was changed by 0.2 units from 0.8 to 1.2 ml/min.

To study the effect of percentage organic modifier (diethylamine) on the resolution of enantiomers, it was carried out at 0.2 and 0.3% concentration of diethylamine in mobile phase while the other mobile phase components were held constant as stated in Section 2.4. The effect of change in percent methanol on resolution was studied by varying from −1 to +1% while the other mobile phase components were held constant as stated in Section 2.4. The effect of column temperature on resolution was studied at 20 and 30 °C instead of 25 °C while the other mobile phase components were held constant as stated in Section 2.4. The percentage of R.S.D. of resolution between Zolmitriptan and (*R*)-enantiomer was calculated under all separation conditions tested.

2.5.10. Solution stability and mobile phase stability

Stability of Zolmitriptan sample solution spiked with (*R*)-enantiomer at specification level (0.15%), was studied by keeping the solution in tightly capped volumetric flask at room temperature on a laboratory bench for 24 h. Content of (*R*)-enantiomer was checked for 6 h interval upto the study period.

Mobile phase stability was carried out by evaluating the content of (*R*)-enantiomer in Zolmitriptan sample solutions spiked with (*R*)-enantiomer at specification level, which were prepared freshly at 6 h interval for 24 h. Same mobile phase was used during the study period.

3. Results and discussion

3.1. Method development

The objective of this study was separation and accurate quantification of Zolmitriptan and its potential impurities (*R*)-enantiomer and Imp-1 in bulk drug and formulation samples. A 0.5 mg/ml solution of racemic mixture prepared in mobile phase and spiked with Imp-1 was utilized in the method development. 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. Firstly, hexane:isopropanol (75:25, v/v) was used as mobile phase. Zolmitriptan enantiomers were coeluted on Chiralcel OJ-H column where as poor separation was observed on the Chiralcel OD-H column while using the above mobile phase. Baseline chromatographic resolution (USP resolution about 3) was achieved on Chiralpak AD-H column using the above mobile phase and Imp-1 was found to be well resolved from Zolmitriptan enantiomers. But enantiomer peaks were found to be very broad (USP tailing >3.5) under the above mobile phase conditions. Further trials were continued only on Chiralpak AD-H column due to good resolution obtained on the column. Secondly, hexane, isopropanol and methanol mixture (75:10:15, v/v/v) was tested for improving the peak shapes of enantiomers. Introduction of methanol in the above mobile phase has improved the peak shapes of the enantiomers (USP tailing about 2.8), but the resolution between the enantiomers was only 1.8. Interestingly introduction of diethylamine in the mobile phase has played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. With the mobile phase consisting of hexane:isopropanol:methanol:diethylamine (75:10:15:0.1, v/v/v/v), the typical retention times of (*R*)-enantiomer, Zolmitriptan and Imp-1 were about 5.9, 8.2 and 19.8 min, respectively, and the resolution between the enantiomers was not less than five (Fig. 2). The USP tailing factor for Zolmitriptan and its potential impurities was about 1.5 in the developed method.

The chiral stationary phase (CSP) 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 and polar carbamate group on the CSP.

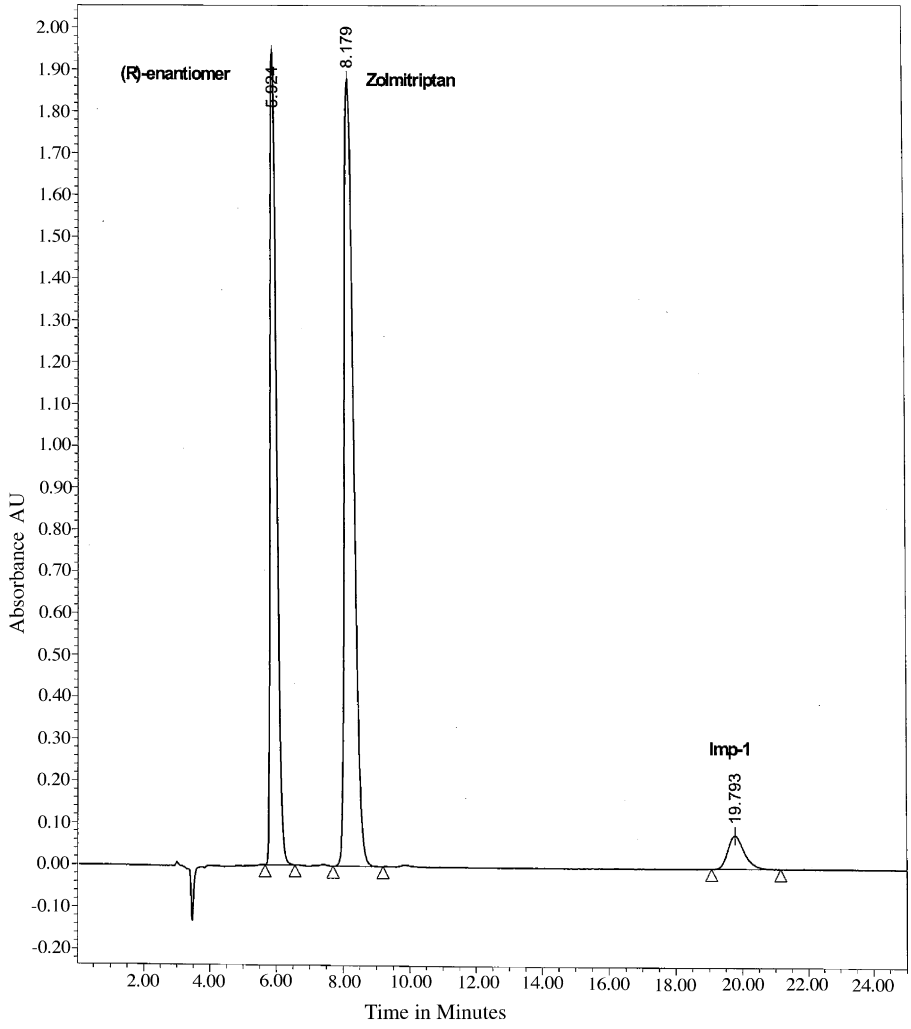


Fig. 2. Typical HPLC chromatogram of racemic Zolmitriptan in the presence of Imp-1.

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 Zolmitriptan. In addition, the dipole–dipole interactions can occur between the C=O group on the CSP and the C=O group on the Zolmitriptan. Wainer et al. have reported that solutes having aromatic functionalities could provide additional stabilizing effect to the solute-CSP complex by insertion of the aromatic portion of the solute into the chiral cavity. In our case, this type of stabilization effect may also exist due to the presence of the aromatic functionality in Zolmitriptan. Chiral discrimination between the enantiomers is due to the difference in their steric fit in 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 Zolmitriptan. 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 Zolmitriptan on Chiralpak AD-H column is believed to be due to the same reason.

3.2. Validation results of the method

In the case of stress by UV light (254 nm) and heat (60 °C), it was observed that rigorous stress of Zolmitriptan sample did not cause any significant degradation and change in the (*R*)-enantiomer and Imp-1 content for 10 days study period. The proposed chromatographic conditions were found to be selective to the Zolmitriptan sample subjected to the applied stress conditions. Peak purity was obtained for Zolmitriptan by overlay of the spectra captured at the apex, up slope and down slope using photo diode array detector and no interference was noted for Zolmitriptan in stress samples. Hence the developed method is stability indicating and found to be selective.

In the precision study, the relative standard deviation of analysis repeatability for Zolmitriptan, (*R*)-enantiomer and

Imp-1 was found to be 0.5, 5 and 3%, respectively, indicating the good precision of the method.

The limit of detection (LOD) and limit of quantification (LOQ) concentrations were calculated to be 100, 250 ng/ml and 300, 1000 ng/ml, respectively, for (*R*)-enantiomer and Imp-1, when a signal-to-noise ratio of 3 and 10 was used as the criteria. The method precision for (*R*)-enantiomer and Imp-1 at limit of quantification was less than 9% R.S.D. The percentage recoveries of (*R*)-enantiomer and Imp-1 at limit of quantification were 95 and 97 in the spiked Zolmitriptan samples.

Good linearity (correlation coefficient $R = 0.999$) was observed for Zolmitriptan, (*R*)-enantiomer and Imp-1 over the concentration ranges tested, with the linear regression equations $y = 23065472x + 423$, $y = 4220x + 220$ and $y = 11246x + 124$, respectively. Linearity was checked for Zolmitriptan and potential impurities over the same concentration ranges for three consecutive days. The percentage relative standard deviation of the slope and *Y*-intercept of the calibration curves for Zolmitriptan, (*R*)-enantiomer and Imp-1 were 1.2, 8; 2.5, 12 and 3.2, 13, respectively. The results show that good correlation existed between the peak area and concentration.

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

Added (ng)	Recovered (ng)	% Recovery	% R.S.D.
607	634	104.4	3.6
759	730	96.2	4.2
910	929	102.1	6.6

$n = 3$ determinations.

Table 3
Recovery results of Imp-1 in bulk drug sample

Added (ng)	Recovered (ng)	% Recovery	% R.S.D.
2500	2358	94.3	3.9
3750	3945	105.2	4.6
5000	5170	103.4	5.2

$n = 3$ determinations.

The percentage recovery of Zolmitriptan in bulk drug samples was ranged from 98.5 to 101.2. The percentage

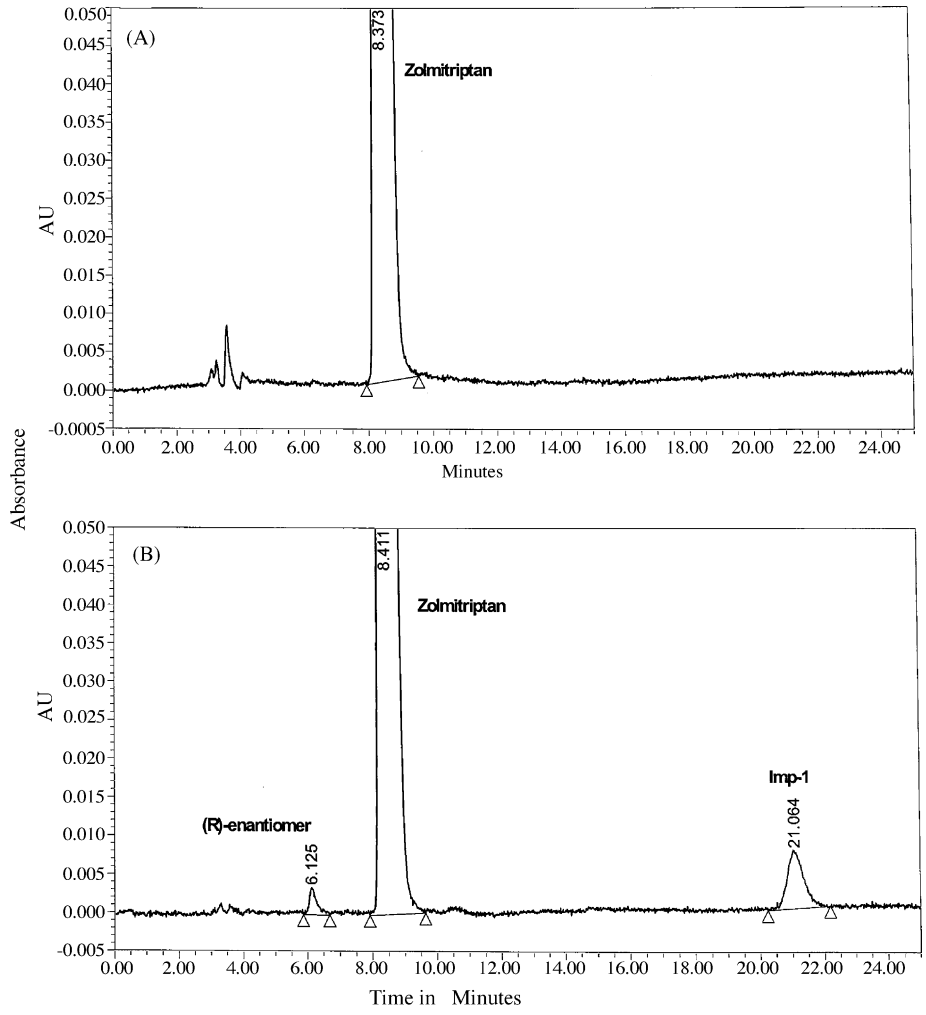


Fig. 3. LC chromatogram of (A) Zolmitriptan formulation and (B) Zolmitriptan formulation spiked with 0.15% of (*R*)-enantiomer and 1.0% of Imp-1 in the developed method.

Table 4
Recovery results of (*R*)-enantiomer in commercial formulation

Added (ng)	Recovered (ng)	% Recovery	% R.S.D.
607	571	94.1	7.3
759	782	103.0	3.1
910	968	106.4	5.2

$n = 3$ determinations.

Table 5
Recovery results of Imp-1 in commercial formulation

Added (ng)	Recovered (ng)	% Recovery	%R.S.D.
2500	2670	106.8	4.5
3750	3570	95.2	5.6
5000	5165	103.3	6.5

$n = 3$ determinations

recovery of (*R*)-enantiomer and Imp-1 in bulk drug samples of Zolmitriptan was ranged from 96.2 to 104.4 and 94.3 to 105.2, respectively (Tables 2 and 3).

The percentage recovery of Zolmitriptan in formulation samples was ranged from 98.2 to 101.8. The percentage recovery of (*R*)-enantiomer and Imp-1 in formulation samples of Zolmitriptan was ranged from 94.1 to 106.4 and 95.2 to 106.8, respectively (Tables 4 and 5). The unspiked and spiked chromatograms of the (*R*)-enantiomer (0.15%) and Imp-1 (1.0%) of target analyte concentration in formulation sample is shown in Fig. 3.

In the ruggedness study, the relative standard deviation of area for Zolmitriptan, (*R*)-enantiomer and Imp-1 was found to be 0.4, 4.3 and 2.3%, respectively. 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.

The chromatographic resolution of Zolmitriptan and (*R*)-enantiomer peaks was used to evaluate the method robustness under modified conditions. Sufficient resolution for Zolmitriptan and (*R*)-enantiomer was obtained under all separation conditions tested, demonstrating sufficient robustness (Table 6). The percentage of R.S.D. of resolution between Zolmitriptan and (*R*)-enantiomer was less than 10 in all altered chromatographic conditions.

Table 6
Robustness of the chiral LC method

Parameter	USP resolution between Zolmitriptan and (*R*)-enantiomer	% R.S.D.
Flow rate (ml/min)		
0.8	5.9	9.3
1.0	5.4	
1.2	4.9	
Column temperature (°C)		
20	5.7	6.5
25	5.4	
30	5.0	
Methanol percentage in mobile phase		
14	5.6	4.7
15	5.4	
16	5.1	
Diethylamine percentage in mobile phase		
0.1	5.4	1.1
0.2	5.5	
0.3	5.5	

Table 7
Solution stability and mobile phase stability results of Zolmitriptan–Chiral LC method

Interval (h)	% (*R*)-enantiomer (solution stability)	% (*R*)-enantiomer (mobile phase stability)
0	0.15	0.15
6	0.16	0.14
12	0.15	0.14
18	0.14	0.15
24	0.15	0.15

No significant change in the (*R*)-enantiomer content was observed in Zolmitriptan sample during solution stability and mobile phase stability experiments (Table 7). Hence Zolmitriptan sample solution and mobile phase are stable for at least 24 h.

4. Conclusion

A new, accurate and selective normal phase chiral LC method was described for the determination of Zolmitriptan and its potential impurities namely (*R*)-enantiomer and Imp-1. Chiralpak AD-H, an amylose based chiral stationary phase was found to be selective for the enantiomers of Zolmitriptan and Imp-1. The introduction of diethylamine in the mobile phase has played an important role on improving chromatographic efficiency and enantiomeric resolution. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the quantitative determination of Zolmitriptan, chiral impurity ((*R*)-enantiomer) and Imp-1 in pharmaceutical formulations and in-process materials.

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