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Original Article

DETERMINATION OF R-ISOMER IMPURITY OF PANTOPRAZOLE SODIUM IN A BULK DRUG SUBSTANCE BY NORMAL PHASE CHIRAL LIQUID CHROMATOGRAPHY METHOD

HEMSAGAR P. JADHAV

Department of Chemistry, Shri Jagdish Prasad Jhabarmal Tibrewala University, Vidyanagari, Jhunjhunu 333001 Rajasthan, India
Email; hemsagar_p@rediffmail.com

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ABSTRACT

Objective: To develop novel, simple and rapid R-isomer impurity determination of Pantoprazole sodium by normal-phase high-performance liquid chromatographic method as per ICH guidelines.

Methods: The R-isomer and S-isomer were baseline resolved on a Chiralpak AD-H, (250 x 4.0 mm i.d, 5 µm) column using a mobile phase system containing n-Hexane: Ethanol: Trifluoroacetic acid (80:20:0.1 v/v/v.) at detector wavelength 290 nm and column temperature 20 °C. The chromatographic resolutions between R-isomer and S-isomer were found three. The developed method was extensively validated according to ICH guidelines.

Results: Good linearity was observed for R-isomer impurity over the concentration range of 200–2000 ng/ml, with the linear regression (Correlation coefficient R = 0.999) and proved to be robust. The limit of detection and limit of quantification of R-isomer was found to be 200 and 600 ng/ml, respectively for 20 µl injection volume. The percentage recovery of R-isomer was ranged from 97.0 to 102.0 in bulk drug samples of Pantoprazole sodium. Pantoprazole sodium sample solution and mobile phase were found to be stable for at least 48 hours. The proposed method was found to be suitable and accurate for the quantitative determination of R-isomer impurity in bulk drugs.

Conclusion: A novel, simple and rapid R-isomer impurity determination of Pantoprazole sodium by normal-phase high-performance liquid chromatographic method was developed and validated as per ICH guidelines.

The developed method can be used for the quantitative determination R-isomer impurity in bulk drug materials in pharmaceutical industry.

Keywords: Pantaprazole, Chiralpak AD-H, Chiral HPLC, Validation, Solution and mobile phase stability.

INTRODUCTION

The Pantoprazole sodium is widely used as proton pump inhibitors (PPIs). The drug irreversibly inhibits proton pump function, they block final step in acid production and effective inhibit acid secretion. Pantoprazole sodium is also used as anti ulcer. Pantoprazole sodium is racemic mixture of [+] S and [-]R-Pantoprazole sodium. Pantoprazole sodium chemically known as sodium 5-(difluoromethoxy)-2–[[(3, 4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole sesquihydrate, and chemical formula is $C_{16}H_{14}F_2N_3NaO_4S$, $1^1/_2$ H_2O, chemical structures show in (Figure1), It has been demonstrated in animal that the [+]S-form is biologically active.

Several different methods have been reported for qualitative and quantitative analysis of Pantoprazole sodium. These include Direct Determination of Pantoprazole Enantiomers in Human Serum by Reversed-Phase High-Performance Liquid Chromatography Using a Cellulose-Based Chiral Stationary Phase and Column-Switching System as a Sample Cleanup Procedure [1], Determination of the enantiomeric impurity in S-(-) pantoprazole using high performance liquid chromatography with sulfobutylether-beta-cyclodextrin as chiral additive [2], Direct enantiomeric separation of pantoprazole sodium by high performance liquid chromatography [3], Direct HPLC separation of enantiomers of pantoprazole and other benzimidazole sulfoxides using cellulose-based chiral stationary phases in reversed-phase mode [4], Comparisom of the effects pantoprazole enantiomers on gastric mucosal lesions and gastric epithelial cells in rats [5], Three-wavelength spectrophotometric method for simultaneous estimation of pantoprazole and domperidone in pharmaceutical preparations [6], Enantiomeric determination of pantoprazole in human plasma by multidimensional high-performance liquid chromatography [7], Simultaneous determination of naproxen sodium and pantoprazole sodium in bulk and pharmaceutical dosage form by validated ultra-violet spectrophotometric method [8], Validated chromatographic methods for the determination of process related toxic impurities in pantoprazole sodium [9].

In the literature, there is no method for the separation of R-isomer and S-isomer of Pantoprazole sodium in bulk drugs on Chiralpak AD-H (250 x 4.0 mm i.d, 5 µm) column by high performance liquid chromatography. Normal-phase chromatography is the most popular mode of liquid chromatography at present for separation of isomer. Briefly the major disadvantages of normal phase HPLC lie in the highly non-polar nature of the mobile phase, the possibility of column inactivation by water, contamination by polar compounds and lower potential in terms of selectivity

This report describes a normal phase LC method for the rapid separation of R-isomer and S-isomer Pantoprazole sodium on Chiralpak AD-H column. The developed HPLC method was validated for quantification of R-isomer in Pantoprazole sodium as per ICH guidelines.

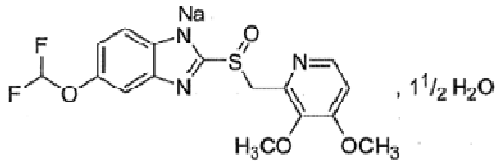

Fig. 1: Chemical structure of Pantoprazole sodium

MATERIALS AND METHODS

Chemicals

Pantoprazole sodium racemic, R-isomer and S-isomer were kindly gift by Enal lab Mumbai, Maharashtra, India. HPLC grade n-Hexane purchased from Merck, HPLC grade Absolute Ethanol purchased

from Hayman speciality products UK, HPLC grade Trifluoroacetic acid purchased from Sigma-Aldrich.

Equipment

A shimadzu 2010 series LC systems with UV detector and inbuilt auto injector were utilized for method development and validation. LC Solution software was used for data acquisition and system suitability calculations.

Sample preparation

Stock solutions of Racemic Pantoprazole sodium (0.1 mg/ml) were prepared by dissolving the appropriate amount of the substances inmobile phase. The analyte concentration of S-isomer was fixed as 0.1 mg/ml. working solutions of S-isomer and R-isomer were prepared in the mobile phase.

Chromatographic conditions

The chromatographic conditions were optimized on a Chiralpak AD-H (250 x 4.0 mm i.d, 5 µm) column. The mobile phase was n-Hexane: Ethanol: Trifluoroacetic acid (80:20:0.1 v/v/v). The flow rate was set at 1.0 ml/min. The column was maintained at 20 °C and the detection was carried out at a wavelength of 290 nm. The injection volume was 20 µl.

Validation of the method

Method reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between-days precision) of retention times and peak areas for R–isomer and S-isomer.

In order to determine the repeatability of the method, replicate injections (n=6) of a 0.1 mg/ml solution containing S-isomer spiked with R-isomer (0.5 %) was carried out. The intermediate precision was also evaluated over three days by performing six successive injections each day.

Limit of detection and limit of quantification of R-isomer

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

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

Linearity of R-isomer

Detector response linearity was assessed by preparing six calibration sample solutions of R-isomer covering from 200 ng/ml (LOQ) to 2000 ng/ml (200 ng/ml, 400 ng/ml, 600 ng/ml, 800 ng/ml,1000 ng/ml and 2000 ng/ml), prepared in mobile phase from R-isomer stock solution.

Regression curve was obtained by plotting peak area versus concentration using the least squares method. Linearity was checked for 3 consecutive days in the same concentration range from the same stock solution. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated.

Quantification of R-isomer in bulk sample

The Pantoprazole sodium bulk sample gift by Enal Lab Mumbai, showed the presence of 0.10 % 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.

The study was carried out in triplicate at 0.1, 0.2 and 0.5 percent of the Pantoprazole sodium target analyte concentration. The recovery of R-isomer was calculated from the slope and Y-intercept of the calibration curve.

Robustness

The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate, mobile phase composition and column temperature. To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between R-isomer and S-isomer 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 isomers, it was changed by 0.2 units from 0.8 ml/min to 1.2 ml/min. The effects of change in percent ethanol on resolution were studied by varying from-1 to+1 % while the other mobile phase components were held constant. The effect of column temperature on the resolution was studied at 15 ºC and 25 °C instead of 20 °C while the other mobile phase components were held constant.

Solution stability and mobile phase stability

Stability of Pantoprazole sodium in solution at analyte concentration was studied by keeping the solutions in tightly capped volumetric flask at room temperature on a laboratory bench for two days. Content of R-isomer was checked for six hours interval upto the study period.

Mobile phase stability was carried out by evaluating the content of R-isomer in Pantoprazole sodium sample solutions prepared freshly at six hours interval for two days. Same mobile phase was used during the study period.

RESULTS AND DISCUSSION

Method development

The aim of this work is to separate the R-isomer and S-isomer of Pantoprazole sodium using normal phase HPLC within the short run time. In the previous reported work, there is no method for the separation of R-isomer and S-isomer of Pantoprazole sodium in bulk drugs on Chiralpak AD-H (250 x 4.0 mm i. d, 5 µm) column by high performance liquid chromatography. The analysis of Pantoprazole sodium sample using normal phase is time consuming. A 0.1 mg/ml solutions of isomeric mixture prepared in mobile phase were used in the method development. To develop a rugged and suitable LC method for the separation of Pantoprazole sodium isomer, different mobile phases and stationary phases were employed in an attempt to separate the isomer of Pantoprazole sodium. Various experiments were conducted to select the best stationary and mobile phases that would give optimum resolution and selectivity for the two isomer. The chromatographic separation was achieved using a Chiralpak AD-H (250x4.0 mm i. d, 5µm) column using a mobile phase system containing n-Hexane: Ethanol: Trifluoroacetic acid (80:20: 0.1 v/v/v).

The flow rate of the mobile phase was 1.0 ml/min. At 20ºC column temperature, the peak shape of Pantoprazole sodium was found symmetrical.

In the optimized method, the typical retention times of R-isomer and S-isomer of Pantoprazole sodium were about 8.1 and 9.1 minutes respectively. The isomeric separation of Pantoprazole sodium is shown in system suitability chromatogram (fig. 2). Typical HPLC chromatogram of Pantoprazole sodium bulk sample (100 µg/ml) spiked with R-isomer (0.5 %) shown in (fig. 3).

Validation results of the method

The system suitability test results are presented in (Table1). In the repeatability study, the relative standard deviation (RSD) was better than 0.5 % for the retention times of the isomers, 1.0 % for Pantoprazole sodium peak area and 3.0 % for R-isomer peak area (table 2). In the intermediate precision study, results show that RSD values were in the same order of magnitude than those obtained for repeatability (table 2).

The limit of detection (LOD) and limit of quantification (LOQ) concentrations were estimated to be 200 and 600 ng/ml 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 the limit of quantification was less than 3 % RSD (table 2).

Good linearity was observed for R isomer over the concentration range of 200–2000 ng/ml (Correlation coefficient R = 0.999). Linearity was checked for R–isomer over the same concentration range for three consecutive days. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was 2.3 and 1.2 respectively (table 2).

The standard addition and recovery experiments were conducted for R isomer in bulk samples in triplicate at 0.1, 0.2 and 0.5 percent of analyte concentration. Recovery was calculated from the slope and Y-intercept of the calibration curve obtained in linearity study and percentage recovery was ranged from 97.0 to 102.0 (table 3).

The chromatographic resolution of R-isomer and S-isomer of Pantoprazole sodium peaks was used to evaluate the method robustness under modified conditions. The resolution between R-isomer and S-isomer of Pantoprazole sodium was greater than 2.5 under all separation conditions tested (table 4), demonstrating sufficient robustness.

No significant change in the R-isomer content was observed in Pantoprazole sodium sample during solution stability and mobile phase stability experiments. Hence Pantoprazole sodium sample solution and mobile phase is stable for at least 48 hours.

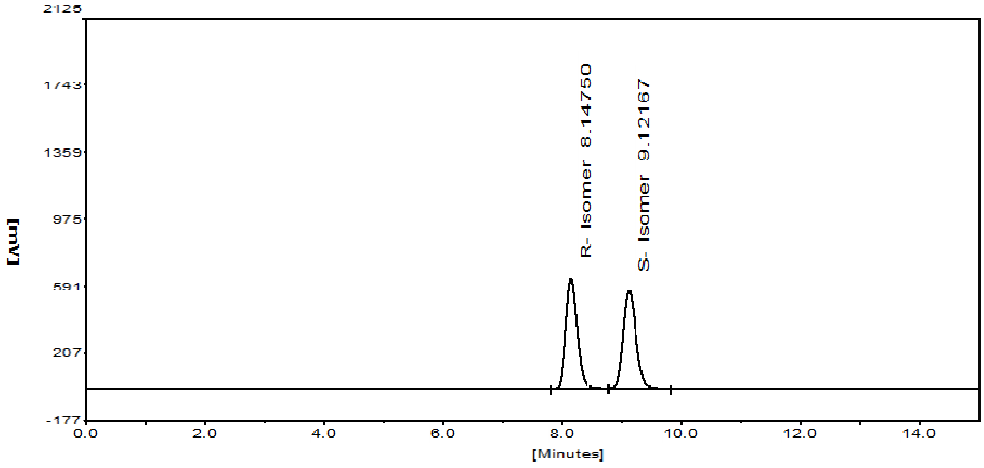

Fig. 2: Typical HPLC chromatogram of system suitability

Table 1: System-suitability report

Compound (n=3)	Rt	R_S	N	T
R-isomer	8.1	-	6500	1.0
S-isomer	9.1	3.0	5100	1.1

n =3 determinations

R_S-USP resolution, N-number of theoretical plates (USP tangent method), T-USP tailing factor

Table 2: Validation results of the developed reverse phase method

Validation parameter	Results
Repeatability (n=6, % RSD)	
Retention time (R-isomer)	0.5
Retention time (S-isomer)	0.3
Area (R-isomer)	3.0
Area (S-isomer)	1.0
Intermediate precision (n=18, % RSD)	
Retention time (R-isomer)	0.3
Retention time (S-isomer)	0.4
Area (R-isomer)	2.8
Area (S-isomer)	0.8
LOD-LOQ (R-isomer)	
Limit of detection (ng/ml)	200
Limit of quantification (ng/ml)	600
Precision at LOQ (% RSD)	2.9
Linearity (R-isomer)	
Calibration range (ng/ml)	200-2000
Calibration points	6
Correlation coefficient	0.999
Slope (% RSD)	2.3
Intercept (% RSD)	1.2

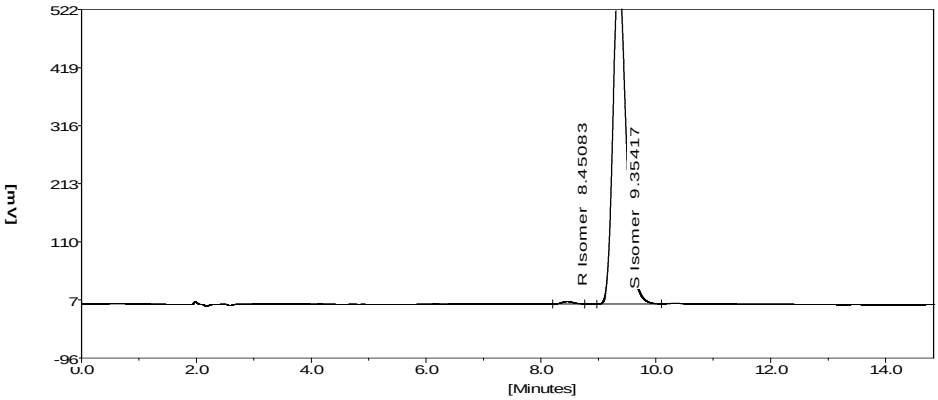

Fig. 3: Typical HPLC chromatogram of pantoprazole sodium bulk sample (100 µg/ml) spiked with R-isomer (0.5 %)

Table 3: Recovery results of R-isomer in bulk drugs

Added (ng) (n=3)	Recovered (ng)	% Recovery	%RSD
1005	975	97.0	2.1
2017	2057	102.0	2.8
5010	5060	101.0	2.5

Table 4: Robustness of the method

Parameter	USP resolution between R-isomer and S-isomer of Pantoprazole sodium
Flow rate (ml/min)	
0.8	3.1
1.0	3.0
1.2	2.9
Column temperature (°C)	
15	3.1
20	3.0
25	2.8
Ethanol percentage in mobile phase	
19	3.0
20	3.0
21	2.9

CONCLUSION

A novel, simple and rapid R-isomer determination of Pantoprazole sodium using normal-phase n-Hexane: Ethanol: Trifluoroacetic acid (80:20: 0.1 v/v/v) mobile phase by high-performance liquid chromatographic method was developed and validated as per ICH guidelines. The method validation was carried out by using Chiralpak AD-H, (250 x 4.0 mm i. d, 5 µm) column. The developed method can be used for the quantitative determination of R-isomer in bulk drug materials in the pharmaceutical industry.

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CONFLICT OF INTERESTS

Declared None

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