AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of a LC Method for the Enantiomeric Purity Determination of S-Mirabegron in a Pharmaceutical Drug Substance Using a Amylose Immobilised Based Chiral Stationary Phase

N. Rajan [1]*, K.Anverbasha[2]

1. Department of Chemistry, Sathyabama university, Chennai 600 119, India.

2. Department of Chemistry, C.Abdul Hakeem college, Melvisharam, 630 509, India.

ABSTRACT

A Chiral liquid chromatographic (LC) method was developed for the enantiomeric resolution of Mirabegron(R)-2-(2-aminothiazol-4-yl)-N-(4-(2-(2-hydroxy-2-phenyl ethyl) amino) ethyl) Phenyl acetamide, adrenoceptor agonist bulk drugs. The enantiomers of mirabegron were resolved on a Chiralpak IF (250mm x 4.6mm, 3.0µm) column using a mobile phase system containing n-Heptane, methyl tertiary butyl ether, methanol and ethanol amine in the ratio of (30:28:42:0.1*v/v/v/v*). The resolution between the enantiomers was found not less than 4.0. The presence of methyl tertiary butyl ether and ethanol amine in the mobile phase has played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. The developed method was extensively validated and proved to be robust. The limit of detection and limit of quantification of (S) enantiomer were found to be 0.2µgmL^{-1} and 0.6 µgmL^{-1} respectively for 15µl injection volume. The percentage recovery of (S) enantiomer was ranged from 98.5 to 102.5 in bulk drug samples of mirabegron. Mirabegron sample solution and mobile phase were found to be stable for at least 48hrs. The proposed method was found to be suitable and accurate for the quantitative determination of (S)-enantiomer in bulk drugs.

Keywords: Mirabegron, enantiomer

*Corresponding Author Email: rajanarcot@gmail.com

Received 16 November 2014, Accepted 22 November 2014

Please cite this article as: Rajan N *et al.*, Development and Validation of a LC Method for the Enantiomeric Purity Determination of S-Mirabegron in a Pharmaceutical Drug Substance Using a Amylose Immobilized Based Chiral Stationary Phase. American Journal of PharmTech Research 2014.

INTRODUCTION

Mirabegron [2-(2-amino-1,3-thiazol-4-yl)-N-[4-(2-[[2R)-2-hydroxy-2-phenylethyl] amino] ethyl) phenyl] acetamide (Figure-1) is a potent and selective human β3-adrenoceptor agonist and is the first of a new class of compounds under development for the treatment of overactive bladder [1]. Overactive bladder has been defined by the International Continence Society as "urgency, with or without urge incontinence, and usually with increased daytime frequency and nocturia, in the absence of local or metabolic factors" [2]. Mirabegron has a novel mechanism of action compared with the available therapeutic products for overactive bladder. β3-adrenoceptors have been shown to play a role in the relaxation of the urinary bladder detrusor smooth muscle [3]. Mirabegron activates β3-adrenoceptors on the detrusor muscle of the bladder to facilitate filling of the bladder and urine storage [4]. Mirabegron does not directly inhibit voiding bladder contractions, and may therefore represent a promising choice for the treatment of overactive bladder with or without lower urinary tract symptoms such as those seen with benign prostatic hypertrophy [1]. Few HPLC methods were reported in the literature for the quantitative determination of mirabegron in pharmaceutical dosage form by RP-HPLC [5], Development and validation of LC-MS methods for the determination of mirabegron and its metabolites in human plasma [6]. Mirabegron is produced as a single isomer and that the (S)-isomer (Figure 2) could be present as chiral impurity. In the literature, there is no reference for the enantiomeric separation of mirabegron in bulk drugs using high performance liquid chromatography. Enantiomers of racemic drugs often differ in pharmacokinetic behaviour or pharmacological action [7].The aim of this work was to optimize the chromatographic conditions in terms of temperature and mobile phase composition in order to separate, identify and quantify the enantiomer of mirabegron. The developed chiral HPLC method was reproducible and accurate for the quantitative determination of (S)-isomer in mirabegron.

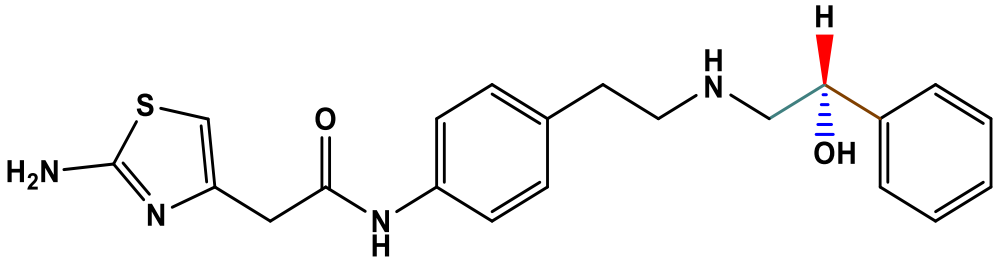

Figure 1 Chemical structure of Mirabegron

(R)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide

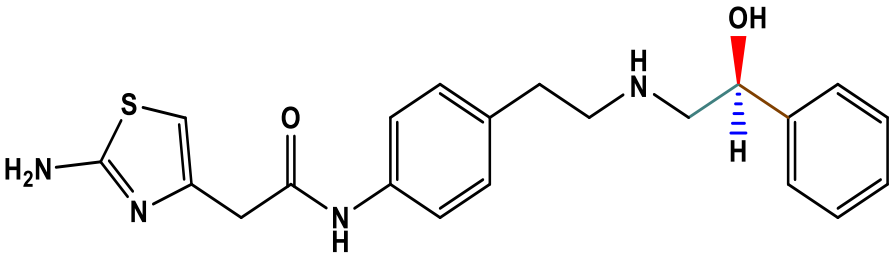

Figure 2 Chemical structure of S-Mirabegron

(S)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide

MATERIALS AND METHOD

Chemicals and Reagents

Mirabegron samples were obtained from the process research &development department of Hetero Drug, Hyderabad. chemical structure of mirabegron is presented in Figure 1. HPLC grade n-Heptane, methyl tertiary butyl ester were purchased from Merck (Darmstadt, Germany). Methanol were obtained from JT backer (Phillpsburg, USA); and ethanol amine was obtained from Acros organics (500 American Road, Morris Plains, USA), stock solution of analyte were prepared in mobile phase.

Chromatographic Conditions and Equipment

HPLC was performed with a Waters (Milford, MA, USA) system equipped with a binary solvent-delivery pump, an auto sampler, and a tunable photodiode array UV detector. Compounds were separated on a Chiralpak IF (250mm x 4.6mm, 3.0µm) column. The isocratic mobile phase composition was a mixture of n-Heptane, methyl tertiary butyl ether, methanol and ethanol amine in the ratio of (30:28:42:0.1*v/v/v/v*) which was pumped at a flow rate of 1.2mL/min. The temperature of the column was maintained at 30ºC and the eluent was monitored at a wavelength of 254nm. The injection volume was 15µL. The chromatographic parameters, including the retention factor (k), the separation factor (α), and the resolution (Rs) were selected to evaluate the separation of compounds. All the chromatographic results were repeated three times.

Sample solutions used for method development

The Standard solution of R- mirabegron bulk drug substance (2 mg mL^{-1}) was prepared by dissolved an appropriate amount of the drug in mobile phase and spiked with 0.15% of S-mirabegron. As a part of system suitability, two criteria were defined (i) resolution between R-mirabegron and S-mirabegron (ii) Tailing factor R-mirabegron. The system suitability results are summarized in Table-1.

Table-1 System suitability Results

S.No	Parameter	Results
1	The resolution between S-Mirabegron and R-Mirabegron	3.8
2	The Tailing factor of R-Mirabegron from system Suitability solution	1.14

Specificity

Degradation conditions employed were UV light (200 Watt hours/m^2), Visible light (1.2 million lux hours), thermal exposure at 60ºC, acid hydrolysis with 0.2N HCl, base hydrolysis with 0.1N NaoH, water hydrolysis, and oxidation degradation using 3%H_2O_2. Peak purity testing was carried out on the stressed samples of mirabegron by using the PDA detector.

RESULTS AND DISCUSSION

Method Development and Optimization

The objective of this study was to separate the enantiomers of mirabegron and accurately quantify the (S)-mirabegron. To develop the suitable chiral HPLC method for the separation of the enantiomers of mirabegron, different mobile phases and stationary phase were employed. For this, different chiral columns were used, chiralcel OJ-H, chiral-AD-H, The enantiomeric separation for mirabegron was not achieved by using chiralcel OJ-H using n-Heptane and methanol, (70:30 *v/v*), This procedure shows that the listed results are certainly not the best results that could be obtained working with the studied polymeric CSPs. Further,there was an indication of separation on amylose tris (3-chloro-4-methylphenylcarbamte) immobilized column chiral pak IF using a mobile phase consisting of n-Heptane and methanol (75:25 *v/v*), but the peak shape was poor. Methyl tertiary butyl ester serves as a competitive binding agent for the cyclodextrin cavity, there by displacing the analyte more readily and effectively than other mobile phase components, methyl tertiary butyl ester has been added to the mobile phase. Better separation was achieved on the chiral pak IF- column (resolution between enantiomers was found to be >2.5) using the mobile phase n-Heptane, methyl tertiary butyl ester and methanol (30:28:42, *v/v/v*), This combination provided the desired separation in a reasonable amount of time. To further improve the peak shape and column efficiency, the organic modifier ethanol amine was added into the mobile phase. Ethanolamine used in the mobile phase that may help to block any active sites and improve peak symmetry. Introduction of ethanolamine in the mobile phase enhanced the chromatographic efficiency, peak symmetry. Good separation with peak shape was achieved on chiralpak IF column between enantiomers of Mirabegron using the mobile phase system n-Heptane, methyl tertiary butyl ester, methanol and ethanolamine in the ratio of (30:28:42:0.1, *v/v/v/v*). Figure 3 shows a typical chromatogram of enantiomeric separation of mirabegron on Chiralpak IF column.

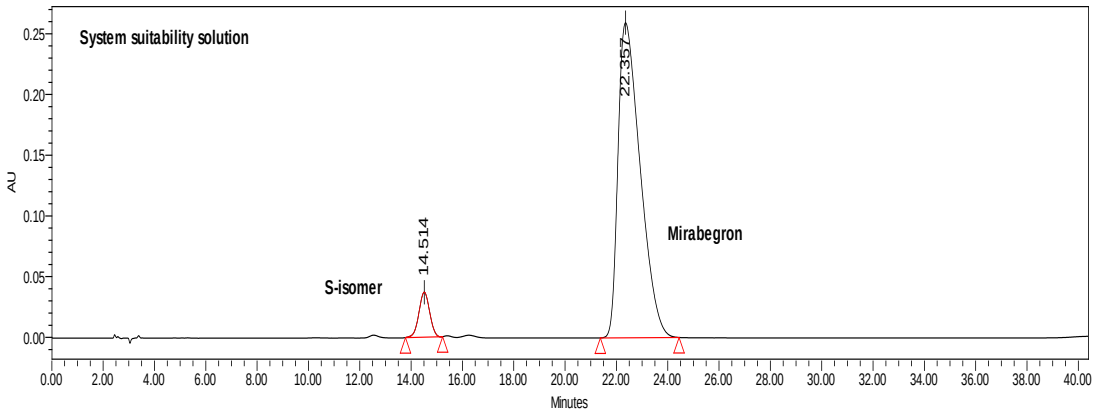

Figure-3: A typical chromatogram of system suitability solution (Chiralpak IF column with dimensions 250mm x 4.6mm, 3.0µm), flow rate 1.2mL min^{-1}, wavelength 254nm, column oven temperature 30ºC).

Results of Method Validation studies

The validation study was conducted according to the ICH (International conference on Harmonization) guidelines[8]. The validated method parameters were LOD, LOQ, Accuracy, Precision, Linearity, Range, Ruggedness and Robustness.

Sensitivity

Sensitivity was determined by establishing the Limit of Detection (LOD) and Limit of Quantification for S-mirabegron estimated at a signal to noise ratio of 3:1 and 10:1 respectively, by injecting a seriesof dilute solutions with known concentrations. The detection limit (LOD) of the S-mirabegron was found about 0.2µgmL^{-1} and the quantification limit (LOQ) was about 0.6µgmL^{-1} respectively. The precision study was also carried out at LOQ level by injecting six individual preparation of S-mirabegron. Calculated the % RSD for the areas of S-enantiomer. The precision values at LOQ concentration for S-enantiomer was below 5.0%. The recovery at LOQ concentration level for S-enantiomer was 98.5 – 102.5%.

Precision

Precision is the closeness of agreement among measurements from multiple sampling of homogenous sample under the recommended conditions. The precision of the analytical method was estimated by calculating repeatability and time-dependent intermediate precision at each concentration level. The relative standard deviation (RSD) values were calculated from the estimated concentrations. As can be seen from Table 3, the RSD values were found to be less than 5.0%, illustrating the excellent precision of the proposed method. Figure 4 shows a typical chromatogram spiked sample solution at 0.15% level.

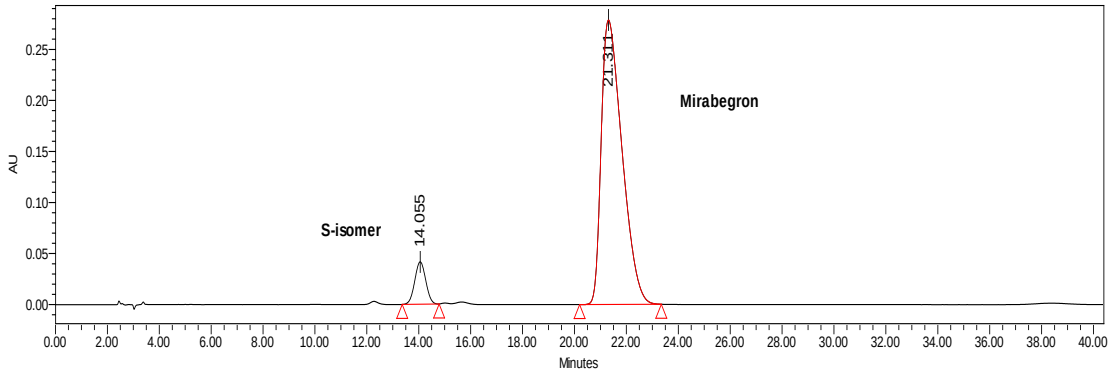

Figure-4: A typical chromatogram of Mirabegron sample solution (Chiralpak IF column with dimensions 250mm x 4.6mm, 3.0µm), flow rate 1.2mL min^{-1}, wavelength 254nm, column oven temperature 30ºC).

Accuracy

Accuracy refers to the closeness of agreement between the test results and the accepted value, namely the conventionally true value. The accuracy of the analytical method takes into account the total error, i.e both systematic and random errors, related to the test results. The upper and lower β-expectation tolerance limits expressed in relative bias (%) as a function of the introduced concentrations are presented in Table -2. The difference tolerance limits of the mean relative bias did not exceed the acceptance limits for each concentration level. Therefore, the developed method can be considered as accurate over the whole concentration range investigated.

Table 2 Accuracy Results

Level of Accuracy	R-Mirabegron	Isomer added (mgmL-1)	Isomer Recovered (mgmL-1)	% of Isomer Recovered (n=3)
50%	1	0.0015	0.0016	102.5
	2	0.0015	0.0014	
	3	0.0015	0.0016	
100%	1	0.0030	0.0029	101.8
	2	0.0030	0.0031	
	3	0.0030	0.0032	
150%	1	0.0045	0.0043	98.5
	2	0.0045	0.0045	
	3	0.0045	0.0044	

Linearity

A linearity test solution for chiral method was prepared by diluting the S-Mirabegron to the required concentrations. The solutions was prepared at six concentrations levels. From LOQ to 150% of the 0.15% level of S-Mirabegron (i.e., LOQ, 50%, 75%, 100%, 125% and 150%) was

subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The correlation coefficient for S-Mirabegron was more than 0.999. The coefficient of determination (R^2) obtained for S-Mirabegron was within the acceptance criteria. The results are depicted in Table-3. Figure-5 shows a typical chromatogram of S-Mirabegron at 0.15% level.

Table 3 LOD, LOQ, Regression and Precision Data

Parameter	R-Mirabegron	S-Enantiomer
LOQ (µg/mL)	0.4µg/mL	0.2µg/mL
LOD (µg/mL)	0.12 µg/mL	0.6 µg/mL
Regression equation (y)		
Slope (b)	93264	116825
Intercept (a)	-0.08	154
Correlation coefficient	0.9992	0.9997
Y-intercept at 100% level	-0.12%	3.16%
Precision [(RSD (%)]	3.4	4.1
Intermediate precision(%RSD)	4.2	3.8

Linearity range is LOQ 150% with respect to 2mg/mL Mirabegron for Impurities

#six determinations using 0.15% spiked solution of impurities to analyte.

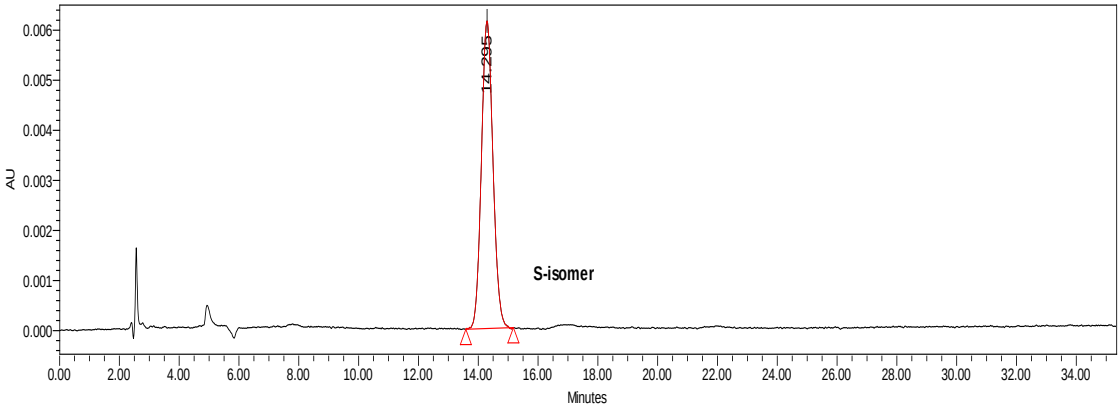

Figure-5: A typical chromatogram of S-Mirabegron at 0.15% level in Zoom condition (Chiralpak IF column with dimensions 250mm x 4.6mm, 3.0µm), flow rate 1.2mL min^{-1}, wavelength 254nm, column oven temperature 30ºC).

Robustness

To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolutions between enantiomers was evaluated. The flow rate of the mobile phase was 1.2 mL/min. To study the effect of flow rate on resolution of enantiomers, it was changed 0.2 units from 1.0 and 1.4 mL/min. The effect of change in percentage of N-Heptane on

resolution was studied by varying from -10% to +10% while the other mobile phase components were held constant, as stated in chromatographic condition section. The effect of column temperature on resolution was studied at 25ºC and 35 ºC instead of 30 ºC while other chromatographic conditions were held constant, as stated in chromatographic condition section. In all the deliberately varied chromatographic conditions (flow rate, column temperature, mobile phase), the resolution between enantiomers was found to be more than 2.5. The results are depicted in Table-4.

Table 4 Robustness

Description	USP Tailing	USP Resolution
Column flow: 1.0mL/min	1.2	3.2
Column flow: 1.4mL/min	1.3	3.8
Column Temperature: 25ºC	1.1	3.4
Column Temperature: 35ºC	1.2	3.5
Organic ratio : 110%	1.1	2.8
Organic ratio: 90%	1.2	3.1

Solution stability and mobile phase stability

The stability of the mirabegron drug in solution during analysis was determined by repeated injection of samples during the course of experimentation on the day of preparation, and also storage for five days under laboratory conditions (25±1ºC) and under refrigeration (5±3ºC). From stock solution (S)-mirabegron were prepared by suitable dilution with diluent to furnish a final concentration of (S)-mirabegron. The samples were analyzed immediately, and after 1, 3 and 5 days.

Results of Forced Degradation studies

Degradation was not observed in a mirabegron bulk sample during thermal exposure, acid, base, oxidative and photo degradation. But, the peak shape is drastically affected by the acid, base and peroxide. Peak purity test results confirmed that the mirabegron peak was homogeneous and pure in all the analysed stress samples.

CONCLUSION

In this paper, a chiral LC method with chiralpak IF as CSP in nonpolar organic solvent chromatography mode, was developed to determine the enantiomeric purity of S-mirabegron in a mirabegron drug substance. The method development shows the reversal of mirabegron enantiomers elution order according to organic modifier ethanol amine. The method was completely validated according to the strategy based on the accuracy profiles. Good performance with respect to selectivity, precision, accuracy, linearity and robustness. The limit of quantification

(0.6µgmL^{-1}) and detection (0.2µgmL^{-1}) and the analysis time make the method suitable for rapid quality control of the enantiomeric purity of mirabegron in comparison with the existing methods.

REFERENCES

1. T.Takasu, M.Ukai, S. Sato T.Matsui, I.Nagase, T.Maruyama, M. Sasamata, K. Miyata, H. Uchida, O, Yamaguchi, J. Pharmacol, ExpTher, 2007; 321; 642.
2. P. Abrams, Urology, 2003; 62; 28.
3. A.Nergardh, L.O.Boreus, A.SNaglo, Acta Pharmacol. Toxicol, 1977; 40: 14.
4. O.Yamaguchi, C.R.Chapple, Neurourlol, Urodyn, 2007; 26: 752.
5. Chusena Narasimharaju Bhimanadhuni, Devala Rao Garikapati, RP-HPLC method for the determination of Mirabegron in Pharmaceutical dosage form, 2012; 6: 565-571.
6. Raymond van Teijlingen, John Meijer, Shin Takusagawa, Marcel van Gelderen, Cas van den Beld, Takashi Usui, Development and validation of LC-MS/MS methods for the determination of mirabegron and its metabolites in human plasma and their application to a clinical pharmacokinetic study, 2012; 887: 102-111.
7. C.G.Sahajwalla, New Drug Development, vol.141, Marcel Dekker, Inc, New York, 2004:421-426.
8. ICH Q2 (R1), Validation of analytical procedures, text and methodology, USFDA-Federal Register, USA, 2006.
9. Ermer J, Validation in pharmaceutical analysis, part 1; an integrated approach, J pharm Biomed Anal, 2001; 24: 755-767.
10. Ermer J, Ploss HJ, Validation in pharmaceutical analysis, part-II; Central importance of precision to establish acceptance criteria and for verifying and improving the quality of analytical data. J Pharm Biomed Anal 2005;37:859-870.

AJPTR is

- **Peer-reviewed**
- **bimonthly**
- **Rapid publication**

Submit your manuscript at: editor@ajptr.com