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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF MIRABEGRAON BY RP-HPLC METHOD

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ABSTRACT

A novel very rapid, sensitive, reverse phase High Performance Liquid Chromatography (RP-HPLC) technique was developed for the quantitative estimation of mirabegraon in bulk and tablet dosage form..It was resolved by using a mobile phase Potassium di-hydrogen phosphate: acetone in the ratio (40:60 v/v) at a flow rate of 1.0 mL/min. using UV - Visible detector at the wavelength of 243 nm for quantification. Efficient separation was achieved for mirabegraon on used Cosmosil C_{18} (100 × 2.1 mm, 5µm). The retention time mirabegraon of was 2.754min. The calibration graphs were linear and the method showed excellent recovery for tablet dosage form. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness.

KEY WORDS: Mirabegraon, HPLC, new method development, validation

1. INTRODUCTION

Mirabegron is a potent and selective agonist for beta-3 adrenergic receptors. Once beta-3 receptors are activated, the detrusor smooth muscle relaxes to allow for a larger bladder capacity. At higher doses (200 mg), there is a potential for mirabegron to activate beta-1 and beta-2 adrenergic receptors.2-(2-amino-1,3-thiazol-4-yl)-N-[4-[2-[[(2R)-2-hydroxy-2- phenylethyl]amino]ethyl]phenyl]acetamide[1].

ISSN 2454-9940

www.ijasem.org

Vol 19, Issue 3, 2025

The structure of the drug was shown in figure 1[2]. It is highly soluble in water, sparingly soluble in methanol, and practically insoluble in methylene chloride. ThePKa value of drug was 6.2 [3].

Literature survey reveals the availability of various analytical methods for the analysis of Mirabegron in biological samples by RP-HPLC.[4-6] and few Spectrophotometric methods are available for estimation of in bulk and pharmaceutical dosage form.[7,8].There is only one RP-HPLC method is also available for this Mirabegron formulation. [9] The reported RP-HPLC method was not economical in terms of mobile phase composition, flow rates and less efficient. Hence there is a need to develop an RP-HPLC method for the estimation of Mirabegron in the tablet formulations. The aim of the present analytical research is to develop simple, precise, accurate, rapid and economical RP-HPLC method for the assay of Mirabegron in tablet formulation.

Structure of drug

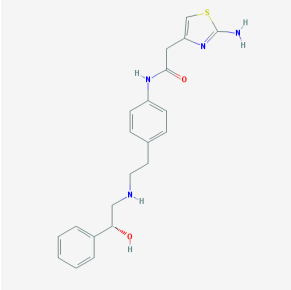

Figure 1: Structure of mirabegraon

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www.ijasem.org

Vol 19, Issue 3, 2025

2. EXPERIMENTAL DETAILS

2.1 Materials and Reagents: Mirabegron Working Standard was procured from Aurobindo laboratories, Hyderabad, India. Commercially Mirabegron available purchased from local pharmacy. Acetonitrile HPLC Grade and Ortho phosphoric acid AR grade were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

2.2 Chromatographic conditions: Chromatography separation was performed on LC Solution HPLC with UV detector. The output signal was monitored and processed using Chrome- work station HPLC V4.0 software. The chromatographic column used Cosmosil C_{18} (100 × 2.1 mm, 5µm). The mobile phase of Potassium di-hydrogen phosphate: acetone in the ratio (40:60 v/v) at a flow rate of 1.0 mL/min. The detection was monitored at the Wavelength of at 249 nm nm. The injection volume was 20.0 µL and the chromatographic runtime of 6 min was used.

2.3 Preparation of solutions

2.3.1 Preparation of Phosphate buffer: Weighed 7.0 grams of Potassium di hydrogen phosphate into a 1000 mL beaker, dissolve and diluted to 1000mL with milli pore water. Adjusted the pH to 4.0 with ortho phosphoric acid.

2.3.2 Preparation of mobile phase: Mixed a mixture of above buffer 400mL (40%) and 600 mL of acetonitrile (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

2.4 Preparation of the mirabegron Standard and sample Solution:

2.4.1 Standard Solution Preparation:. Accurately transferred 10mg of Mirabegron working standard into a 10 mL volumetric flask and about 7 mL of diluent added then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent(Stock

solution). Further pipetted 0.5 mL of the above stock solution into a 10mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter

2.4.2 Sample Solution Preparation: Accurately transferred the sample equivalent to 10 mg of Mirabegron into a 10 mL volumetric flask. About 7 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45µm filter. Further pipetted 5 mL of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter.Further pipetted 3 mL of the above stock solution into a 10mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

2.5 Method validation

2.5.1 Precision: The precision of the method was evaluated by carrying out six independent asses of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

2.5.2 Intermediate Precision/Ruggedness:

2.5.2.1 Intra-day precision: The precision of the assay method was evaluated by carrying out six independent assays Mirabegron (50,100, 150% i.e. 5.0, 10.0, 15.0µg/mL.) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

2.5.2.2 Intermediate precision (inter-day): Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the six samples of Mirabegron against qualified reference standard. The percentage of RSD of six assay values was calculated. The %RSD for the area of six replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

2.5.3 Accuracy: Recovery of the assay method for Mirabegron was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Each solution was injected thrice (n=3) into HPLC system and the average peak area was

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www.ijasem.org
Vol 19, Issue 3, 2025

calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

2.5.4 Linearity: Test solutions were prepared from stock solution at 5 concentration levels (10, 20, 30, 40 and 50 µm/mL). The peak area vs. concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.)

2.5.5 Limit of Detection (LOD) Limit of Quantification (LOQ): LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations

2.5.6 Robustness: To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies.

3. RESULTS

3.1 Method development: Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, Phosphate buffer and acetonitrile) were evaluatedin order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method.At 40:60 (buffer:ACN) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at40:60 (buffer:ACN) in an isocratic mobile phase flow rate. The typical chromatogram obtained for from final HPLC conditions are depicted in Figure2.

ISSN 2454-9940

www.ijasem.org

Vol 19, Issue 3, 2025

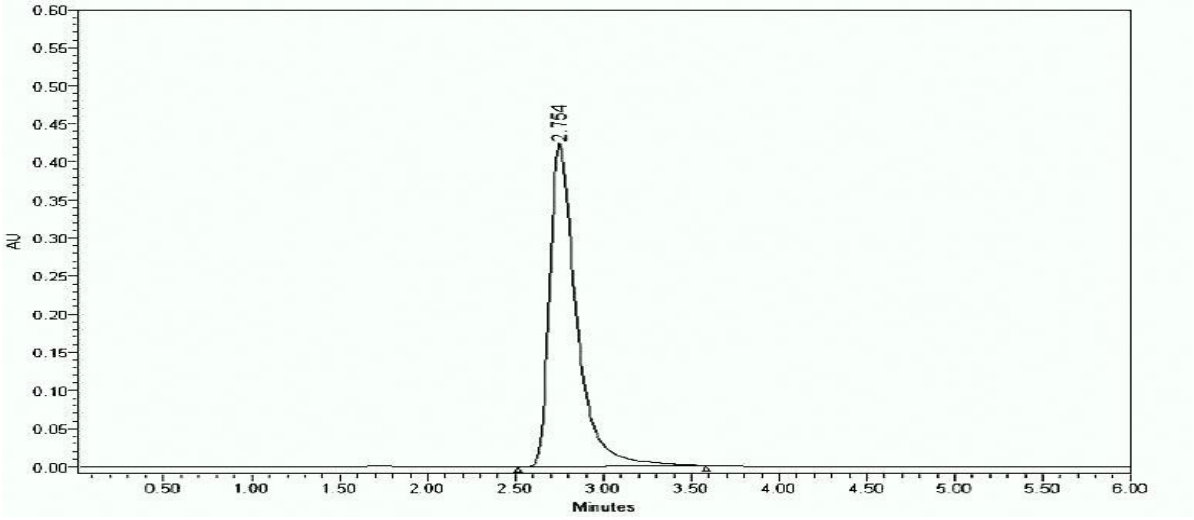

Figure 2: typical chromatogram of mirabegron by proposed method

3.2 Method validation: Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ , robustness and sensitivity as follows.

3.2.1 System suitability: The system suitability results for the proposed HPLC method are Tailing factor Obtained from the standard injection is 1.4.Theoretical Plates Obtained from the standard injection is 7582.4. The results proved that the optimized HPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

3.2.2 Precision: The % R.S.D. of Sitagliptin assay during the method precision was found to be 0.45%, indicating good precision of the method. The results are summarized in table 1.

Table 1- Results of precision

Injection	Area
Injection-1	4796667

ISSN 2454-9940
www.ijasem.org
Vol 19, Issue 3, 2025

Injection-2	4712916
Injection-3	4721422
Injection-4	4771493
Injection-5	4750737
Average	4750647
Standard Deviation	34749.6
%RSD	0.73%

3.2.4 Limits of detection (LOD) and quantification (LOQ): LOD and LOQ for Sitagliptin were 0.049 and 0.15µg/ml, respectively. Since the LOQ and LOD values of Sitagliptin are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

3.2.5 Accuracy:Percentage recovery of Sitagliptin samples ranged from 100.0% to 101.2% and the mean recovery is 100.5%, showing the good accuracy of the method. The result is shown in Table 3.

Table 3 - Results of Accuracy

Injection	**Area**
Injection-1	4796667
Injection-2	4712916
Injection-3	4721422
Injection-4	4771493
Injection-5	4750737

Average	4750647
Standard Deviation	34749.6
%RSD	0.73%

3.2.6 Linearity: The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e.10 - 50 µg/ml for three times, and the correlation coefficient obtained was 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte.

3.2.7 Robustness: In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 20 -60 µg/ml, (n=3). It can be concluded that the variation in flow rate and the variation in 10% Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% and change in the Mobile phase ±10%. The results are summarized in table 4.

Table- 4- Results of Robustness

Injection	**Area**
Injection-1	4796667
Injection-2	4712916
Injection-3	4721422
Injection-4	4771493
Injection-5	4750737
Average	4750647
Standard Deviation	34749.6
%RSD	0.73%

ISSN 2454-9940
www.ijasem.org
Vol 19, Issue 3, 2025

3.2.7 Application of the developed method to commercial mirabegraon tablets: When the developed method was used to analyze a commercial brand of Sitagliptin tablet formulation, the mean recovery of five replicates was 99.69 % with % R.S.D. of 0.45. The % recovery value indicates non-interference from the excipients present in the dosage form.

DISCUSSION:

Method development and optimization: The main aim of the developedmethodwas to achieve separation and quantification of mirabegraon using an isocratic mobile phase with HPLC system. Developing a HPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of mirabegraon was adequate at 249 n nm.The initial trial was conducted using HPLC and chromatographic separation was obtained onCosmosil C_{18} (100 × 2.1 mm, 5µm).The mobile phase was buffer 400mL (40%) and 600 mL of acetonitrile (60%) at a flow rate of 1.0 ml/min. While developing the HPLC method, basic chromatographic conditions such as the used Cosmosil C_{18} (100 × 2.1 mm, 5µm) column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the HPLC column, its stability at the lower pH was taken into consideration to preserve the long life of the column. Most commercial C_{18} columns are not stable at lower pH on the longer run, thus shortening their life span. Column was found to be more suitable and stable at this pH. The peak was sharp and acceptable. The flow rate also is scaled down from 2.0 to 1.0 ml/min. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for mirabegraon which eluted at around 2.754min min giving a total run time of 6 min.

ISSN 2454-9940

www.ijasem.org

Vol 19, Issue 3, 2025

4. CONCLUSION: The new, isocratic RP-HPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 2.754min min allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of Sitagliptin in tablet formulations.

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