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Mirabegron Quantitative Estimation in Extended-Release Tablets: A New RP-HPLC Method and Its Validation

Mehdi Rezaei, Ali Ramazani*

Department of Chemical and Life Sciences

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ABSTRACT

This work details and validates a sensitive and fast technique for RP-HPLC analysis of Mirabegron in Extended-Release tablets. In the concentration range of 10 to 100 µg/mL, the HPLC test technique was determined to be linear. Isocratic elution on a Restek C18 column (250 mm × 4.6 mm, 5µm) was used to successfully accomplish separation. The mobile phase was a mixture of acetonitrile and potassium dihydrogen phosphate at a ratio of 60:40 v/v. It was mixed at a flow rate of 1 mL/min and detected at 249 nm using UV light. The column oven was heated to 45ºC, and the injection volume was 10 µL. Recovery investigations confirmed the findings of the analysis. The recovery method's percentage was determined to be between 99.00 and 101.17%. A limit of detection (LOD) of 0.015 µg/mL and a limit of quantification (LOQ) of 0.049 µg/mL were determined. Every single one of the validation parameters fell inside the permissible range. The estimated quantity of Mirabegron in the tablets was determined using this proposed approach.

Keywords: Mirabegron Validation RP-HPLC Method Development

1. Introduction

Mirabegron, seen in Figure 1, is named chemically as 2-(2-amino-1, 3 thiazol-4-yl). The chemical formula and molecular weight of the compound in question are C21H24N4O2S and 396.509 g/mol, respectively. 1 As a beta-3 adrenergic agonist, mirabegron helps patients with OBS control their symptoms. 2 to 4 No evidence of acute liver damage or elevated liver enzymes has been found. Overactive bladder is its principal indication for use. 5 Using rat and human bladder strips precontracted with carbachol at low contraction tonus, mirabegron had a strong relaxant effect and elevated cyclic adenosine 3', 5'-monophosphate (cAMP) concentrations in a dose-dependent manner in the isolated tissues of the rats' bladders. It was recently shown that Mirabegron activates β3 adrenoceptors, resulting in the relaxation of prostate smooth muscle in both rabbits and humans in vitro. Additionally, the same group demonstrated that Mirabegron promotes relaxation of smooth muscles via the blocking of α1 adrenergic receptors.

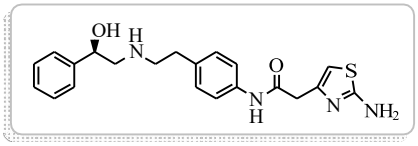

Fig. 1 Chemical structure of Mirabegron

Mirabegron is highly soluble in water, sparingly soluble in methanol, and practically insoluble in methylene chloride.[6] Analytical method development and validation play important role in the discovery, development and manufacture of pharmaceuticals.[7-8] A detailed literature survey reveals that only a few methods are reported previously to determine Mirabegron by isocratic RP-HPLC[9-12] and UV[13] spectrophotometry. LC- MS/MS is method for determination of Mirabegron in biological fluids and pharmaceutical dosage forms.[14-16]Hence there is a need to develop an RP-HPLC method for the estimation of Mirabegron in Extended-Release tablets. The present work reveals that RP-HPLC method is a simple, precise, accurate, rapid and economical for the assay of Mirabegron in Extended-Release tablets.

2. Results and discussion

2.1. System suitability study

The chromatographic parameters, such as peak area, retention time, theoretical plates and tailing factor were calculated. The peak symmetries were <1.5 and these values are according to the United States Pharmacopeia. Five replicates of a standard solution were injected to check the system suitability. All of the results are presented in (**Table 1**) and (**Fig. 2**).

2.2. Method Validation

All of the analytical validation parameters for the proposed method were determined according to the International

Conference on Harmonization (ICH) guidelines. Validation of method was divided into linearity and rang, precision, recovery, selectivity, robustness, limit of detection (LOD) and limit of quantification (LOQ) studies. The details of each section were followed.

2.3. Linearity and range

Linearity was checked by preparing standard solutions at seven different concentration levels of Mirabegron. A linear response was obtained in the concentrations 10, 20, 40, 50, 60, 80 and 100 µg/mL. The linear regression line was used to determine the linearity and concentration of the samples.

The calibration curve was developed by plotting concentration of Mirabegron on X-axis and their respective area under the curve (AUC) on Y-axis. The calibration curve is shown in (Fig. 3)

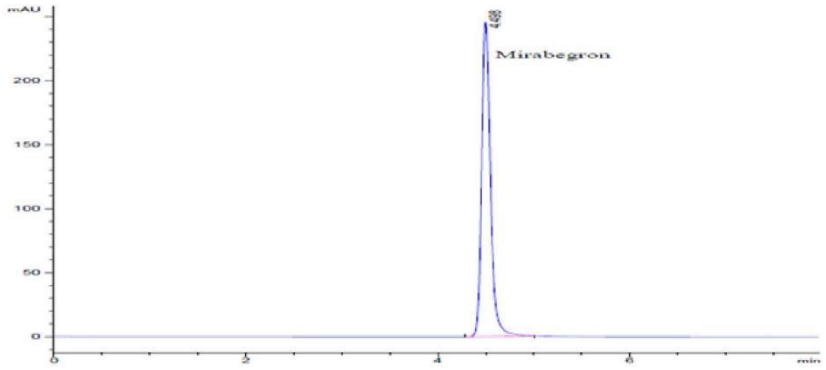

Fig. 2 System suitability chromatogram

Table 1 System suitability parameters of standard chromatogram obtained to Mirabegron

Standard	Retention time	Area	Theoretical plates	USP Tailing
1	4.576	1551.91	12448	1.25
2	4.574	1552.17	12420	1.28
3	4.579	1552.59	12462	1.28
4	4.578	1554.34	12445	1.27
5	4.579	1554.03	12469	1.28
Average	4.577	1553.01	12449	1.27
SD	0.0022	1.107	18.887	0.013
RSD %	0.047	0.071	0.152	1.025

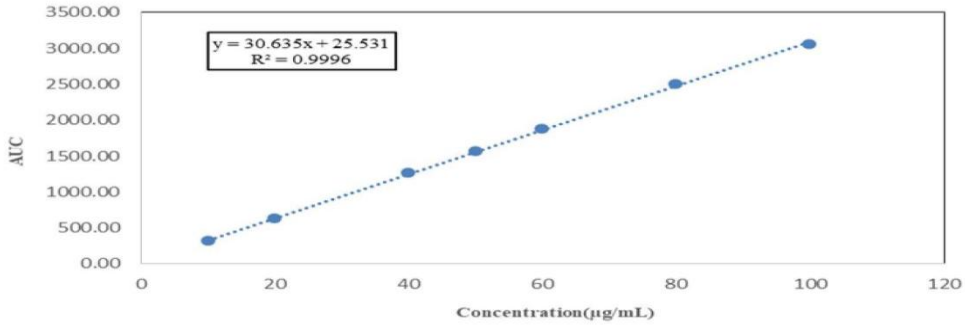

Fig. 3 Calibration curve for Mirabegron

2.4. Precision

analyzed at the three different levels (80%, 100%, and 120 %) in triplicate by the explained method. The recovery studies were

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations 40, 50 and 60 µg/mL for Mirabegron three times on the same day. The intermediate precision of the method was checked by repeating studies on three different days (**Table 2**).

2.5. Recovery studies

Recovery of analytical procedure refers to the closeness of a measured value to a standard or known value. The recovery was

carried out by adding a known amount of pure drug Mirabegron at three different levels to placebo. From the amount of Mirabegron found, percentage recovery was estimated. The results obtained are shown in (**Table 3**).

2.6. Selectivity

Its ability to accurately and specifically measure the analyte of interest without interferences from the blank and other ingredients in the matrix was defined. **Fig. 4** shows the noninterference excipients with Mirabegron peak.

2.7. Detection and quantitation limits

The detection limit of an individual analytical procedure is the lowest amount of substance in a sample that can be distinguished but not necessarily quantified as an exact value. The quantitation limit of an individual analytical the procedure is the lowest

amount of substance in a sample that can be quantitatively determined with acceptable precision and accuracy. Signal-to- noise ratios were of 3:1 and 10:1 was obtained for the LOD and LOQ. The LOD and LOQ were found to be 0.015 µg/mL and 0.049µg/mL.

Table 2 Intermediate precision on three different days

Concentration (µg/mL)	40	50	60
Day 1			
Mean peak area	1236.51	1553.17	1825.59
SD	0.80	0.38	0.38
RSD %	0.06	0.02	0.02
Day 2			
Mean peak area	1237.47	1554.04	1825.28
SD	0.45	0.49	0.53
RSD %	0.04	0.03	0.03
Day 3			
Mean peak area	1239.87	1556.23	1827.46
SD	1.35	1.78	0.35
RSD %	0.11	0.35	0.02

Table 3 Determination of percentage recovery method (n=3)

Level of addition (%)	Amount of pure drug added (mg)	Amount of pure drug Recovered (mg)	Recovery%	Mean recovery%	SD	RSD%
80	40	39.6	99.00	100.32	1.161	1.157
100	50	50.4	100.80			
120	60	60.7	101.17			

3. Conclusion

The purpose of this research was to create and test an Extended-Release tablet formulation of Mirabegron using the RP-HPLC technology. When it comes to determining Mirabegron, the suggested HPLC approach is quick, sensitive, accurate, easy, and straightforward. Whether in bulk or tablet form, it may be dependably used for regular quality control testing. The analysis validation parameters for the suggested approach were all established in accordance with the standards set forth by the International Conference on Harmonization (ICH).

4. Materials and Method

4.1. Reagents and chemicals

We acquired the Mirabegron standard from Zhejiang Pharmaceutical Company in China, which has a purity level of 99.7 percent. The Puris equipment employed LC-grade water, and the tested samples were from Astellas Pharma Europe's BetmigaTM brand. It was from Merck Company that we acquired the acetonitrile and potassium dihydrogen phosphate analytical grades.

4.2. Analytical and Instrumental Settings

One such HPLC system is responsible for the development of the Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) technique. An auto sampler was used to conduct the HPLC studies using an Agilent 1260 Infinity Quaternary LC. The Restek C18 column (250 mm à 4.6 mm, 5µm) was used for the separation. Chemstation software was used for data analysis. A UV/VIS detector set at 249 nm was used to keep an eye on the analysis. A mobile phase of 60:40 v/v of potassium dihydrogen phosphate and buffer pH: 7.0 was used in the isocratic elution mode of the high-performance liquid chromatography (HPLC). A temperature of 45ºC was used for this study. The elution flow rate was one milliliter per minute. The sample solution, reference solution, and mobile phase were all sonicated using an ultrasonic.

4.3: Solutions Preparation

The mobile phase, standard solution, and sample solution are the three components that make up the solution preparation portion. The specifics of every part were adhered to.

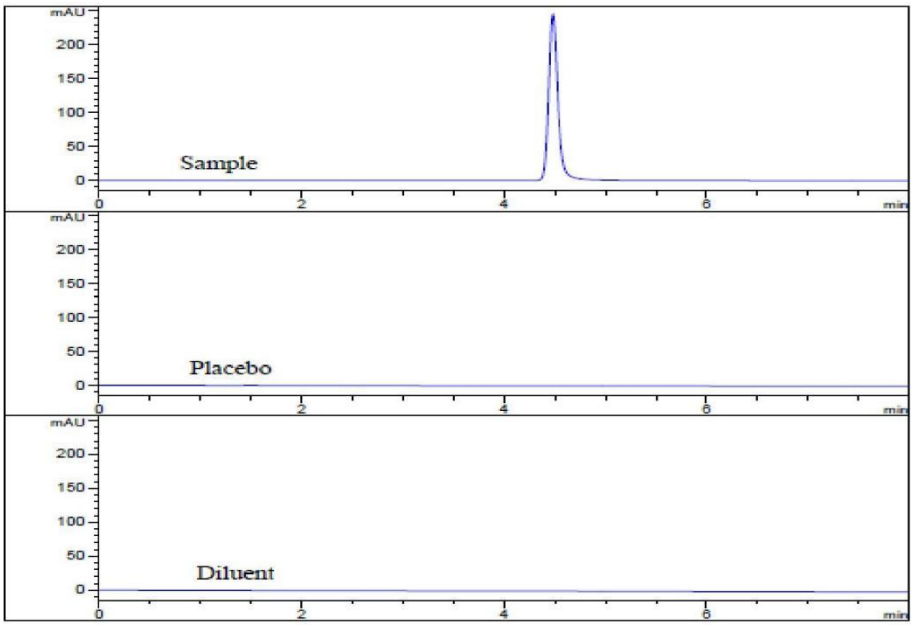

Fig 4. Diluent, placebo and sample solution chromatogram

Table 4 Robustness testing (n=5)

Parameters	Area	Retention time	Theoretical plates	Tailing factor
A: Flow rate (mL/min)				
0.8	1947.96	5.780	14173	1.28
1.2	1299.67	3.861	11370	1.22
B: Temperature (ºC)				
40	1563.07	4.682	12140	1.25
50	1565.77	4.612	13391	1.21
C: Mobile phase pH				
6.8	1562.95	4.494	12472	1.29
7.2	1554.02	4.793	12869	1.28
D: % of Acetonitrile				
39.2	1557.39	4.782	12696	1.26
40.8	1569.98	4.522	12397	1.26

4.4 Buffer preparation

Dissolved 1.36 g potassium dihydrogen phosphate in 1000 mL of water. We used a sodium hydroxide solution to bring the pH down to 7.0. Filtrated this solution via 0.45 µm nylon membrane and degas it.

4.5. Preparation of Standard solution

Accurately weighed and placed around 25.0 mg of Mirabegron reference standard into a 50 mL volumetric flask. Blended with mobile phase until diluted to volume. Pipetted out 2mL of this solution into a 20 mL volumetric flask and make up to mark with the mobile phase. This solution was filtered by
0.45 µm PTFE filter.

4.6. Sample preparation

Twenty pills were measured and ground into a powder. After measuring out 25.0 milligrams of Mirabegron tablet powder, it was transferred to a 50 milliliter volumetric flask and diluted with mobile phase. Sonicated for 15 minutes and filtered so as to yield solution with concentration 500 µg/mL. 2 mL this solution was further diluted with the mobile phase to reach the final concentration 50 µg/ml of Mirabegron. Filtrate this solution using 0.45 µm PTFE filter.

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