RP-HPLC Method Development and Validation for the Quantitative Estimation of Mirabegron in Extended-Release Tablets

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ABSTRACT

In this study, we describe and validate a rapid and sensitive method for constitution of Mirabegron in the Extended-Release tablets by RP-HPLC. The assay method by HPLC was found to be linear in the concentration range of 10 to 100 µg/mL. Successful separation was achieved by isocratic elution on a Restek C18 column (250 mm × 4.6 mm, 5µm). The mobile phase was composed of buffer pH: 7.0 potassium dihydrogen phosphate and acetonitrile (60:40 v/v) at the flow rate of 1 mL/min using UV detection at 249 nm, column oven temperature 45ºC and injection volume 10 µL. The analytical results were validated by recovery studies. The percentage recovery method was found to be 99.00-101.17%. The LOD and LOQ were found to 0.015 µg/mL and 0.049 µg/mL. All the parameters of validation were in the acceptable range. This developed method was successfully applied for estimate the amount of Mirabegron in the tablets.

1. Introduction

The chemical name for Mirabegron (**Fig. 1**) is 2-(2-amino-1, 3 thiazol-4-yl)-N-[4-[2-[[(2R)-2-hydroxy-2-phenylethyl]amino]ethyl]phenyl]acetamide having a molecular formula of $C_{21}H_{24}N_4O_2S$ and a molecular weight of 396.509 g/mol.[1] Mirabegron is a beta-3 adrenergic agonist that is used for treatment of overactive bladder syndrome.[2-4] It has not been implicated in causing liver enzyme elevations or clinically apparent acute liver injury. Its primary use is in the treatment of overactive bladder.[5] Mirabegron dose-dependently increased cyclic adenosine 3', 5'-monophosphate (cAMP) concentrations in bladder tissues isolated from rats and showed a potent relaxant effect in isolated rat and human bladder strips precontracted with carbachol at low contraction tonus. Recently, Mirabegron was shown to relax in vitro human and rabbit prostate smooth muscle through activation of β_3 adrenoceptor. The same group also showed that Mirabegron encourages smooth muscle relaxation by α_1 adrenergic receptor blockade.
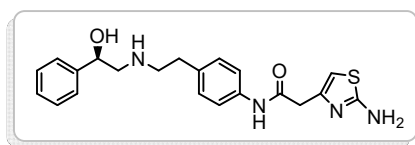

Fig. 1 Chemical structure of Mirabegron

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**)

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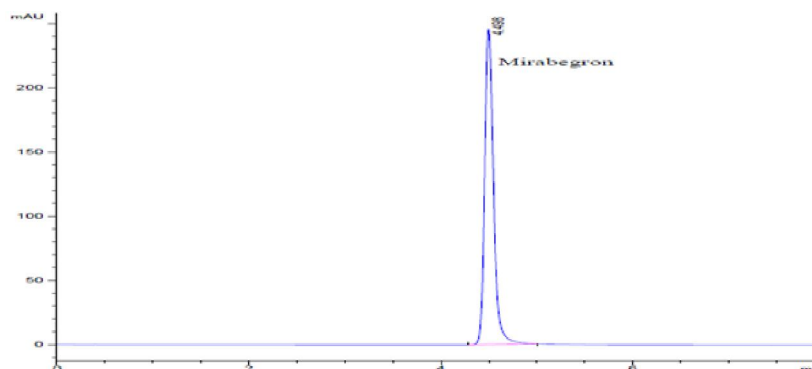*E-mail address*: aliramazani@znu.ac.ir (A. Ramazani)

Fig. 2 System suitability chromatogram

Table 1 System suitability parameters of standard chromatogram obtained to Mirabegron

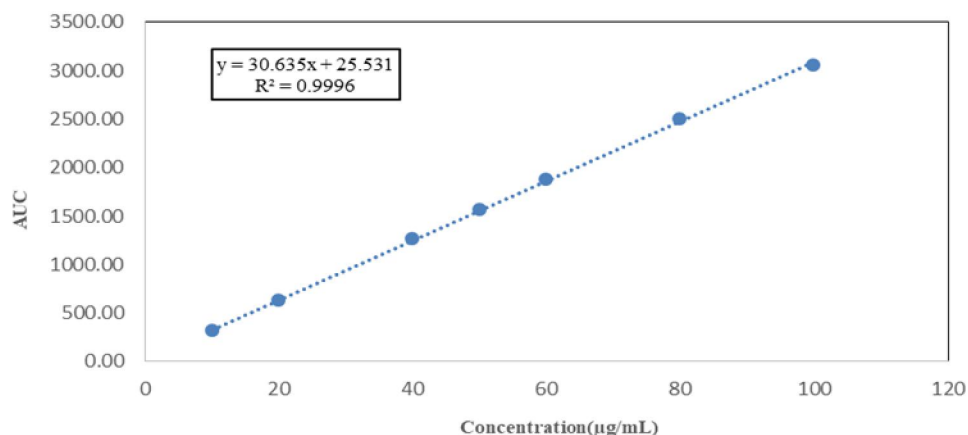| Standard	Retention time	Area	Theoretical plates	USP Tailing
1	4.576	1551.91	12448	1.25
2	4.574	1552.17	12420	1.28
3	4.579	1552.59	12462	1.28
4	4.578	1554.34	12445	1.27
5	4.579	1554.03	12469	1.28
Average	4.577	1553.01	12449	1.27
SD	0.0022	1.107	18.887	0.013
RSD %	0.047	0.071	0.152	1.025

Fig. 3 Calibration curve for Mirabegron

2.4. Precision

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 analyzed at the three different levels (80%, 100%, and 120 %) in triplicate by the explained method. The recovery studies were 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	50	50.4	100.80	100.32	1.161	1.157
120	60	60.7	101.17			

3. Conclusion

The aim of this study was to carry out development and validation of Mirabegron by RP-HPLC method in the Extended-Release tablets. The proposed HPLC method is rapid, sensitive, precise, simple and accurate for the determination of Mirabegron. It can be reliably adopted for routine quality control analysis in the bulk and tablet dosage forms. All of the analytical validation parameters for the proposed method were determined according to the International Conference on Harmonization (ICH) guidelines.

4. Materials and Method

4.1. Reagents and chemicals

The standard of Mirabegron (purity, 99.7%) was obtained from Zhejiang Pharmaceutical Company, CHINA. Tested samples with brand name Betmiga™ belonged to the Astellas Pharma Europe and LC-grade water has been used by the Puris instrument. Acetonitrile and potassium dihydrogen phosphate analysis grade were purchased from Merck Company.

4.2. Instrumental and Analytical conditions

The Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed on an HPLC system. The HPLC analyses were carried out on Agilent 1260 Infinity Quaternary LC equipped with an auto sampler. The separation carried out on Restek C18 column (250 mm × 4.6 mm, 5μm).

Data were analyzed by using Chemstation software. The analysis was monitored with UV/VIS detector at 249 nm. The HPLC was operated in an isocratic elution mode with the mobile phase consisting of buffer pH: 7.0 potassium dihydrogen phosphate and acetonitrile (60:40 v/v). This analysis was done at the 45ºC. The flow rate of elution was 1.0 mL/min. An ultrasonic was used for the sonication of the mobile phase, standard solution, and sample solution.

4.3. Preparation of Solutions

Preparation of solution section is divided into the preparation of mobile phase, standard solution, and sample solution. The details of each section were followed.
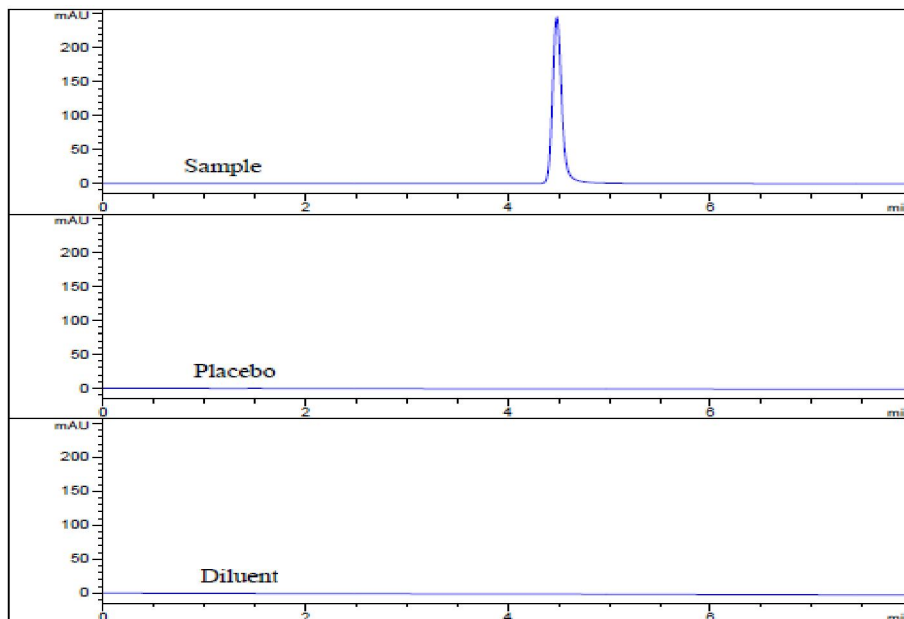
Fig 4. Diluent, placebo and sample solution chromatogram

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. Adjusted the pH to 7.0 with sodium hydroxide solution. Filtrated this solution through 0.45 μm nylon membrane and degas it.

4.5. Preparation of Standard solution

Accurately weighed and transferred about 25.0 mg of Mirabegron reference standard into a 50 mL volumetric flask. Dissolved and diluted to volume with mobile phase. Pipetted out 2mL of this solution into a 20 mL volumetric flask and make up to mark with the mobile phase. Filtrated this solution through 0.45 μm PTFE filter.

4.6. Sample preparation

Twenty tablets were weighed and powdered. Tablet powder equivalent to 25.0 mg of Mirabegron was weighed and transferred to 50 ml volumetric flask and diluted with mobile phase. Sonicated for 15 minutes and filtered so as to get solution having concentration 500 μg/mL. 2 mL this solution was further diluted with the mobile phase to get the final concentration 50 μg/ml of Mirabegron. Filtrate this solution through 0.45 μm PTFE filter.

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