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

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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  $\mu\text{g/mL}$ , the HPLC test technique was determined to be linear. Isocratic elution on a Restek C18 column (250 mm  $\times$  4.6 mm, 5 $\mu\text{m}$ ) 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 45°C, and the injection volume was 10  $\mu\text{L}$ . 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  $\mu\text{g/mL}$  and a limit of quantification (LOQ) of 0.049  $\mu\text{g/mL}$  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  $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}_2\text{S}$  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  $\beta_3$  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  $\alpha_1$  adrenergic receptors.

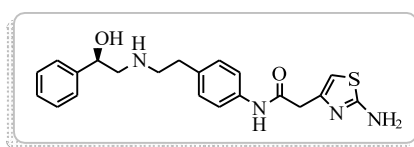


Fig. 1 Chemical structure of Mirabegron

Mirabegron is highly soluble in water, sparingly soluble in methanol, and practically insoluble in methylene chloride.<sup>6</sup> Analytical method development and validation play important role in the discovery, development and manufacture of pharmaceuticals.<sup>7-8</sup> A detailed literature survey reveals that only a few methods are reported previously to determine Mirabegron by isocratic RP-HPLC<sup>9-12</sup> and UV<sup>13</sup> spectrophotometry. LC- MS/MS is method for determination of Mirabegron in biological fluids and pharmaceutical dosage forms.<sup>14-16</sup>

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)

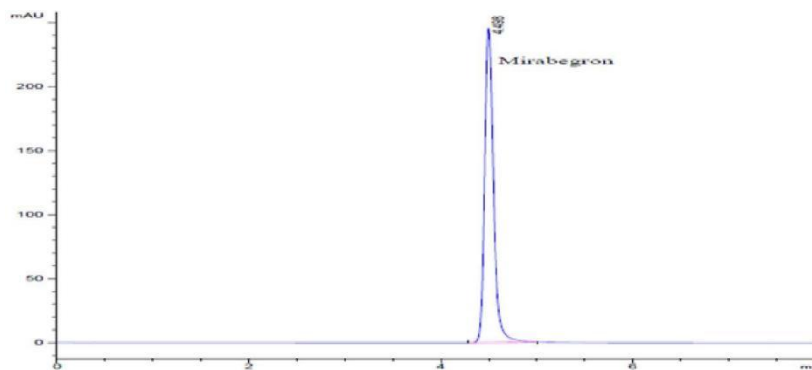


Fig. 2 System suitability chromatogram

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       |

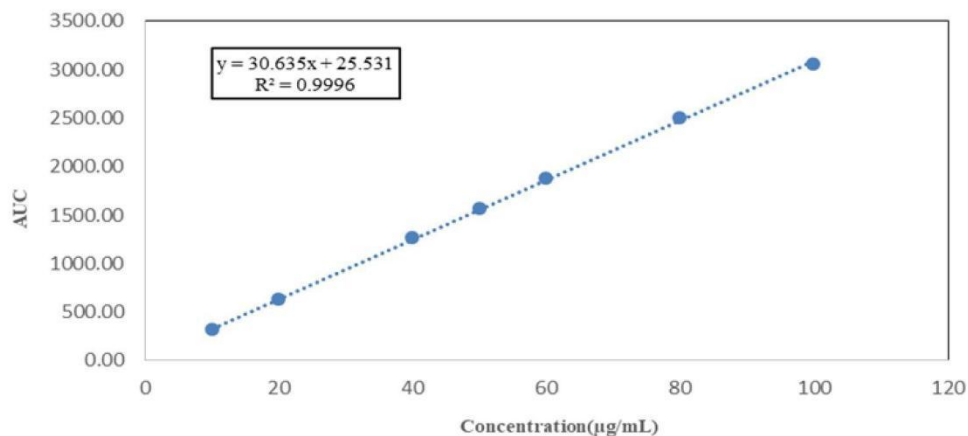


Fig. 3 Calibration curve for Mirabegron

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

#### **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 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 ( $\mu\text{g/mL}$ ) | 40      | 50      | 60      |
|------------------------------------|---------|---------|---------|
| <b>Day 1</b>                       |         |         |         |
| Mean peak area                     | 1236.51 | 1553.17 | 1825.59 |
| SD                                 | 0.80    | 0.38    | 0.38    |
| RSD %                              | 0.06    | 0.02    | 0.02    |
| <b>Day 2</b>                       |         |         |         |
| Mean peak area                     | 1237.47 | 1554.04 | 1825.28 |
| SD                                 | 0.45    | 0.49    | 0.53    |
| RSD %                              | 0.04    | 0.03    | 0.03    |
| <b>Day 3</b>                       |         |         |         |
| 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)

| 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. 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  $\times$  4.6 mm, 5 $\mu\text{m}$ ).

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 45°C. 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.

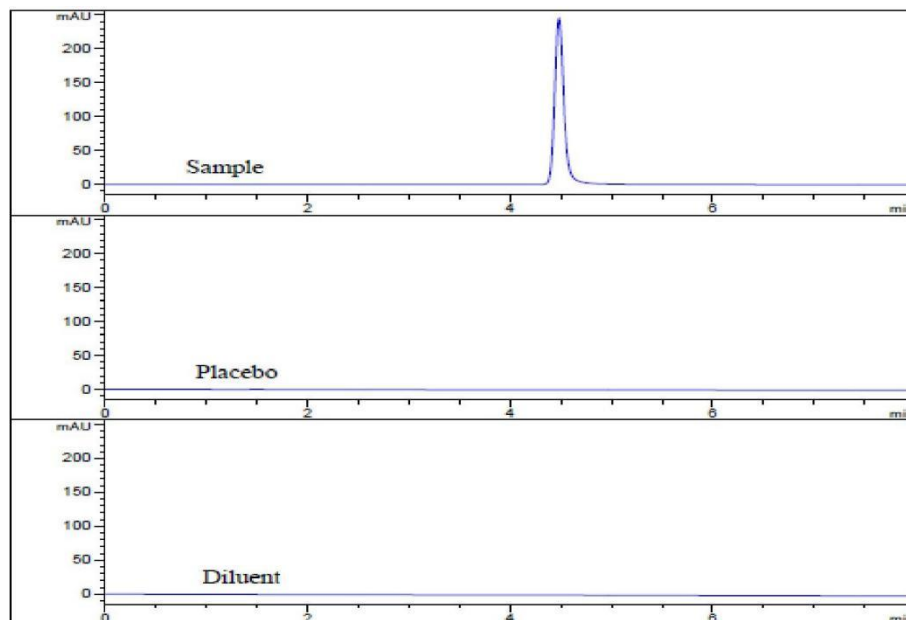


Fig 4. Diluent, placebo and sample solution chromatogram

Table 4 Robustness testing (n=5)

| Parameters                   | Area    | Retention time | Theoretical plates | Tailing factor |
|------------------------------|---------|----------------|--------------------|----------------|
| <b>A: Flow rate (mL/min)</b> |         |                |                    |                |
| 0.8                          | 1947.96 | 5.780          | 14173              | 1.28           |
| 1.2                          | 1299.67 | 3.861          | 11370              | 1.22           |
| <b>B: Temperature (°C)</b>   |         |                |                    |                |
| 40                           | 1563.07 | 4.682          | 12140              | 1.25           |
| 50                           | 1565.77 | 4.612          | 13391              | 1.21           |
| <b>C: Mobile phase pH</b>    |         |                |                    |                |
| 6.8                          | 1562.95 | 4.494          | 12472              | 1.29           |
| 7.2                          | 1554.02 | 4.793          | 12869              | 1.28           |
| <b>D: % of Acetonitrile</b>  |         |                |                    |                |
| 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  $\mu\text{m}$  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  $\mu\text{m}$  PTFE filter.

#### 4.6. Sample preparation

Twenty pills were measured and ground into a powder. A 50 ml volumetric flask was used to dilute the tablet powder, which contained 25.0 mg of Mirabegron. The solution was filtered after being sonicated for 15 minutes to achieve a concentration of 500  $\mu\text{g/mL}$ . To achieve a final concentration of 50  $\mu\text{g/mL}$  of Mirabegron, 2 mL of

this solution was further diluted with the mobile phase. This solution should be filtered using a 0.45 µm PTFE filter.

published the results.

#### References

1. Therapeutic Advances in Urology, edited by E. Sacco and R. Bientinesi, 2012, 4, 315-324.
2. European journal of drug metabolism and pharmacokinetics, 2. K. Konishi, D. Tenmizu, S. Takusagawa. 17, 43, 1-9.
3. Journal of Pharmacology and Experimental Therapeutics, 2007, 321, 642-647, T. Takasu et al., M. Ukai, S. Sato, T. Matsui, I. Nagase, T. Maruyama, M. Sasamata, K. Miyata, H. Uchida, O. Yamaguchi.
4. C. Von Pirquet, in Munchen Medical Week Reports, 1906, 53, 1457-1458.
5. The journal of urology published an article by V.W. Nitti, S. Auerbach, N. Martin, A. Calhoun, M. Lee, and S. Herschorn in 2013, volume 189, pages 1388-1395.
6. In Clinical Therapeutics (2012), 34, 2144-2160, W. Krauwinkel et al. discuss the following: M. Schaddelee, C. Eltink, J. Meijer, G. Strabach, S. van Marle, V. Kerbusch, and M. van Gelderen.
7. In the article "RSC Advances. 2015, 5, 31024-31038" written by P.D. Kalariya, M. Sharma, P. Garg, J.R. Thota, S. Ragampeta, and M.K. Talluri, the authors discuss....
8. Eighth, in the Journal of Chromatographic Science (2015), 53, 1361-1365, F. Zhou, Y. Zhou, Q. Zou, L. Sun, and P. Wei wrote the paper.
9. Journal of Pharmaceutical Technology and Research, 2012, 2, 565-571, C.N. Bhimanadhuni and D.R. Garikapati.
10. Journal of Pharmacy Research, 2017, 11, 682-685, G.R. Paisa, et al.
11. In the Indo-American Journal of Pharmaceutical Research (2016), pages 6880-6887, R. Spandana, R.N. Rao, and L.S.S. Reddy published an article.
12. In the 2017 edition of the World Journal of Pharmacy and Pharmaceutical Sciences, G.R. Babu, G.V. Kumar, M. Kalyani, M. Roshna, P.J. Rani, P.V. Kumar, and S. Ajay published an article with the DOI number 6, 912-925.
13. In Der Pharmacia Lettre (2016), 8, 96-103, P. Ravisankar, S. Vidya, D. Nithya, and P.S. Babu authored the article.
14. In the 2016 issue of the Journal of Liquid Chromatography & Related Technologies, authors S. Parsha, Y. Ravindra Kumar, M. Ravichander, L. Prakash, and B. Sudharani reported on pages 178-194.
15. In the 2014 edition of the American Journal of Pharmatech Research, the authors Rjan and Anverbasha discuss the topic at length, with the page numbers 2249-3387.
16. In the 2012 edition of the Journal of Chromatography B, R. Van Teijlingen, J. Meijer, S. Takusagawa, M. Van Gelderen, C. Van Den Beld, and T. Usui