Analytical method development and validation for the estimation of mirabegron in pure and its solid dosage form by UV- spectrophotometric method

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ABSTRACT

A simple, economical, rapid, accurate, precise spectrophotometric method has been developed and validated according to ICH Guidelines for the Mirabegron as active pharmaceutical ingredient (API) by UV spectrophotometric method. Estimation of Mirabegron was achieved by UV method. After considering the solubility and stability, 1N Hcl was selected as solvent. The UV spectrophotometric λ value limit is 200-400. The absorption maxima of Mirabegron was found to be at 249 nm wavelength using 1N Hcl as a solvent. These solvents are color less. The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. Linearity range was found to be 3-15 $\mu\text{g/ml}$, with the correlation coefficient being more than 0.999. Slope (0.0387) and intercept (0.0079333), LOD (0.187 $\mu\text{g/ml}$) and LOQ (0.568 $\mu\text{g/ml}$) were calculated. The LOD and LOQ method was further studied by intraday and interday analysis. The relative standard deviation was found to be < 2%. The percentage recovery was within the range of 98% -105%, indicating that there is no significant interference from the other ingredients present in the formulation. The method can be applied for the routine analysis of Mirabegron as API in pharmaceutical preparation.

Keywords: Mirabegron; 1N Hcl; UV Spectrophotometry and Betmiga

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among others, is a medication used to treat overactive bladder^[4]. Its benefits are similar to other antimuscarinic medication such as solifenacin or tolterodine^[5]. In the United Kingdom it is less preferred to antimuscarinic medication such as oxybutynin^[6]. It is taken by mouth^[4]. Mirabegron, a selective β_3 -adrenoceptor (AR) agonist^[7], is a first in class drug for the treatment of OAB. Common side effects include high blood pressure, headaches, and urinary tract infections^[4]. Other significant side effects include urinary retention, irregular heart rate, and angioedema. It works by activating the β_3 adrenergic receptor in the bladder, resulting in its relaxation^{[4][6]}.

Mirabegron was approved for medical use in the United States in 2012^{[9][10][11]}. A month supply in the United Kingdom costs the NHS about £29 as of 2019^[9]. In the United States the wholesale cost of this amount is about 369 USD^[12]. In 2016 it was the 263rd most prescribed medication in the United States with more than a million prescriptions^[13, 14].

2-[2-Amino-1, 3-thiazol-4-yl]-N-[4-[2-{{[[2R]-2-hydroxy-2-phenylethyl] amino} ethyl] phenyl]acetamide

INTRODUCTION

Overactive bladder [OAB], defined by the International Continence Society as urgency with or without urinary incontinence, usually associated with frequency and nocturia^[1], is a multifactorial and common health disorder associated with detrimental effects on quality of life and huge economic burden^[2,3]. Mirabegron, sold under the brand name Myrbetriq

MATERIALS AND METHODS

Selection of Solvent

The solubility of Mirabegron was determined in a variety of solvents as per Indian Pharmacopoeia standards¹⁸. Solubility test was carried out in different polar and non-polar solvents from the solubility studies, 1N HCl was selected as suitable solvent for proposed method.

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving, accurately measured 10mg of Mirabegron in 1N HCl and the volume was made up to 10 ml in 10 ml volumetric flask (10 stock solution, 1000µg/ml).

Determination of Absorbance Maxima

1 ml of 10 stock solution was diluted to 10 ml with 1N HCl (20 stock solution, 100µg/ml). 1 ml of 20 stock solution was taken in 10 ml standard volumetric flask dilute to 10 ml with 1N HCl to get the concentration of 10µg/ml. The absorbance of resulting solution was measured against respective blank solution (1N HCl) in the UV region of 200-400 nm, which shows maximum absorbance at 249 nm.

Determination of concentration range

For preparation of different concentrations, aliquots of stock solution of suitable concentrations of Mirabegron were transferred into a series of 10 ml standard flasks and volumes were made up to mark with 1N HCl. Five different concentrations were prepared in the range of 3-15µg/ml and the absorbance were measured at 249 nm against solvent (1N HCl) blank. The obtained absorbance values are plotted against the concentrations of Mirabegron to get the calibration graph.

Analysis of Formulation

Twenty tablets weighed and determined the average of each tablet, powdered tablet equivalent to 10 mg was transferred into 10 ml standard volumetric flask. The content was dissolved in 5ml of 1N HCl. This solution was sonicated for 5 mins and volume made up to 10ml. This solution was filtered through Whatmann filter paper number 40. One ml of the above solution was diluted to 10 ml with 1N HCl. Again 1ml of the solution was diluted to 10ml with 1N HCl in 10 ml std. volumetric flask and the absorbance of the solution was measured at 249nm and the amount was found by using slope and intercept from the calibration curve.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

Intraday and interday precision

A variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed. Intra-day precision was determined by analyzing Mirabegron for six time in the same day at 249 nm. Inter-day precision was determined by analyzing the drug daily once for six days at 249 nm.

Recovery studies

In order to study the accuracy, powder of Mirabegron was taken, and used to carry out the analysis. Recovery studies were carried out by addition of standard drug solution to the sample at 3 different concentration levels (50%, 100% and 150%) and the percentage recovery was determined by using the formula.

$$\text{Percentage Recovery} = \frac{\text{Amount of drug recovered}}{\text{Amount of drug added}} \times 100$$

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of intercept.

RESULTS AND DISCUSSION

A simple, precise, accurate and reproducible method was developed for the estimation of Mirabegron in bulk and tablet formulation. Mirabegron was procured from MSN Pvt, Hyderabad and melting point (138-140°C) was recorded to check the identification of the drug and was given (Table1).

Figure 1: Ultra violet absorption Spectrum of Mirabegron Using 1N HCl

Table 1: Identification of Drug by Melting Point

S.No	Identification No	Standard	Test
1	Melting Point	138-140°	140°

The solubility of Mirabegron was determined in a variety of solvent ranging from no polar to polar using an essentially a method of Scheffer and Higuchi. The drug was found to be very soluble in 1N HCl. Solubility profile of Mirabegron was given (Table 2). 10mg of Mirabegron raw material was accurately weighed and transferred into the 10ml volumetric flask and dissolved minimum quantity of 1N HCl and made up to 10ml with 1N HCl solution, resulting in 1000µg/ml of drug concentration and from this solution 1ml was

pipetted out into 10ml volumetric flask and made up to the mark with 1N Hcl solution resulting in 100µg/ml of drug concentration and from this

Table 2: Solubility profile

S.No	Solvent	Solubility (mg/µl)	Solubility Status
1.	1 N Hcl	200	Soluble
2.	Methanol	10	Very Soluble
3.	Ethanol	10	Very Soluble
4.	Butanol	8	Very Soluble
5.	CHCl ₃	50	Freely Soluble
6.	Dimethyl Formamide	10	Very Soluble

solution 1ml was pipette out into 10ml volumetric flask and made up to the mark with 1N Hcl solution. It was scanned in the range of 200-400nm and it showed constant λ_{max} at 249 nm and show in fig.1. Stability of the observance at λ_{max} 249 nm was also checked for up to 1 hr and 30 min. The linearity of the drug Mirabegron was found, its calibration curve was constructed and is shown in (Figure 2).

Figure 2: Calibration curve of Mirabegron by UV method using 1N Hcl

Table 3: Optical characteristics of Mirabegron by UV method

Parameters	Values
λ _{max} (nm)	249
Beers Law limits (µg / ml)	3-15
Regression equation (Y)	Y= 0.0387X+(-0.0079333)
Slope (m)	0.0387
Intercept (C)	0.0079333
Correlation coefficient (r²)	0.9992

The optical characteristics such as beer's law limits (3-15µg/ml), correlation coefficient (0.999), slope (0.0079333) and intercept (0.0387) were calculate and shown in (Table 3). Each tablet (Betmiga) containing 50 mg of Mirabegron was taken and the average weight of each tablet were found and powdered. The powdered tablet equivalent to 10 mg of Mirabegron was weighed and transfer into a 10ml volumetric flask, sufficient quantity 5ml 1N Hcl in sonicated for 5min and made up to the mark with 1N Hcl. The solution was filtered through whatmann filter paper 40. From clear solution, further dilution was made by using 1N Hcl solution, the amount of Mirabegron present in tablet formulation was found

(52.25). The amount found was in good agreement with the label claim and the results of analysis were shown in (Table 4).

Table 4: Quantification of formulation – Betmiga by UV method

S. No	Labeled amount	Amount found (mg)	Percentage obtained
1	50	52.25	104.5

The precision of method was confirm intraday and interday analysis. The analysis of formulation was carried out 6 times in the same day and daily once in the six consecutive days. The percentage of RDS value was found to be 0.0028 and 0.00306 for intraday and interday analysis of Mirabegron respectively. The reports of analysis were shown in (Table 5).

To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. The percentage recovery of Mirabegron sample was found to be 98% to 105%. The amount of the drug recovered from the formulation was very close to the expected value and the %RSD value also very low (0.055), this indicates that this method is very accurate. The recovery data was shown in Table 6. The limit of detection (0.187) and limit of quantification (0.568) was calculated by using the average value of slope and standard deviation of intercept.

CONCLUSION

Estimation of Mirabegron was achieved by UV method. After considering the solubility and stability, 1N Hcl was selected as solvent. Mirabegron 10µg/ml solution was prepared and scanned in the UV-region, from the spectra 249nm was selected as an analyzing wavelength.

Calibration curve was plotted by using concentration Vs absorbance. From the calibration curve it was found that Mirabegron obeys beer's law in the range of 3-15µg/ml, correlation coefficient (0.999), slope (0.0387) and intercept (0.0079333), LOD (0.187 µg/ml) and LOQ (0.568 µg/ml) were calculated. The percentage of Mirabegron in formulation was found to be 104.5% the precision of the method was studied by making repeated analysis. The LOD and LOQ method was further studied by intraday and interday analysis. The recovery studies were also carried out to ensure the accuracy of the method by adding known concentration of pure drug reanalyzed formulation.

A simple, rapid and accurate analytical method was developed for the determination of Mirabegron in bulk and tablet formulation by UV spectrophotometer. The method showed excellent sensitivity, accuracy and repeatability, which is evidence by low per-

Table 5: Precision of Mirabegron by UV-method

S.No	Amount (µg/ml)	Amount Found (µg/ml)	% Recovery	Average %	S.D	% RSD
1	9	8.99	99.8	99.2	0.0257	0.0028
2		8.903	98.9			
3		8.951	99.4			
4		8.836	98.1			
5		8.977	99.7			
6		8.946	99.4			

Table 6: Interday and Intraday analysis of Mirabegron by UV-method

S.No.	Interday*	Intraday*
	(Amount found in mg)	(Amount found in mg)
1	8.890	8.99
2	8.895	8.903
3	8.087	8.951
4	8.728	8.836
5	8.643	8.977
6	9.007	8.946
S.D.	0.0272	0.0257
% RSD	0.00306	0.0028

*Mean of six observations.

Table 7: Recovery studies for the Mirabegron by UV-method

S.No	% added	Amount (µg/ml)	Amount Added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% recovery	SD	%RSD
1	50%	8.86	4.5	13.3	4.44	98.5%	2.11	0.0205
2			4.5	13.1	4.24			
3			4.5	13.3	4.63			
4	100%		9	17.9	9.04	104.7%		
5			9	18.687	9.824			
6			9	18.27	9.41			
7	150%		13.5	22.35	13.49	104.96%		
8			13.5	22.63	13.77			
9			13.5	24.11	15.25			

centage relative standard deviation. The results obtained in recovery studies where indicating that there is no interference from the excipients used in the

formulation. Hence it is suggested that the proposed UV spectrometric can be effectively applied for the routine analysis of Mirabegron in bulk and formulation in quality control analysis.

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