DETERMINATION OF MIRABEGRON FOR QUALITY CONTROL TESTS IN PHARMACEUTICAL DOSAGE FORM

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Abstract

An accurate, precise, and highly selective spectrophotometric method was determined the estimation of mirabegron for quality control tests in pharmaceutical dosage forms. The method involves the measurement of absorbance at a wavelength of 251 nm. Validation was performed according to ICH guidelines. Beers law obeyed in the concentration range of 2-14 µg/mL, were found to be linear. It showed good reproducibility and recovery with a relative standard deviation of less than 2 %. The limit of detection and quantification was found to be 0.15 and 0.48 µg/mL. Thus, the proposed method was found to be specific in the presence of other excipients, precise and accurate quality control tools for routine analysis of mirabegron in pharmaceutical dosage form.

Keywords: Mirabegron, Spectrophotometry, Quality control, ICH, Validation, Beers law

INTRODUCTION

Overactive bladder is a common condition, which significantly affects people's quality of life. Anticholinergic medications used for treatment for overactive bladder effectively for many years. Mirabegron is a novel, orally active, first-in-class potent β3 adrenoceptor agonist, to be approved for the use in overactive bladder. It was found to low side effects comparable to other anticholinergic medications[1]. It has poor solubility profile in water but freely soluble in dimethylsulfoxide, sparingly soluble in ethanol, slightly soluble in methanol, and acetonitrile. It is rapidly absorbed after oral administration, has a t_{max} of 3–4 hours, the half-life of 40 hours and a bioavailability of 35% at the 50 mg dose. Mirabegron is highly lipophilic and is metabolized in

the liver by cytochrome p450. A total of 55% is excreted in urine and 34% recovered in feces, both in its unchanged form[2].

Literature review According to the literature, it was found that few analytical methods such as HPLC[3, 4], stability-indicating method[5,6], Biological fluids[7], Thin-layer chromatography[8], LC-MS/MS[9] were reported for mirabegron. UV spectrophotometry is one of the analytical techniques used in pharmaceutical analysis. It is characterized as simple, rapid, and cheap, which promotes its wide application for the analysis of mixtures containing two or more components in pure form and in the pharmaceutical formulation without chemical pretreatment or using sophisticated equipment[10]. To our knowledge, no simple study related to the UV spectrophotometric method for the estimation of mirabegron has been reported in the literature. Therefore, there is a challenge to develop the UV spectrophotometric method for estimation of mirabegron. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate, economical, and precise UV spectrophotometric method for the estimation of mirabegron in the pharmaceutical dosage form. The development of methods for the analysis of drugs from excipients, adding to getting significant data from the system or turning the investigative technique greener.

MATERIALS AND METHODS

Mirabegron was supplied were procured from Mylan laboratories (Hyderabad, India) and stored at 10 ºC. Commercially available bladmir 25 mg was purchased from a local pharmacy. Methanol, acetonitrile, potassium dihydrogen phosphate, and sodium hydroxide were obtained from Merck Chemicals. Water used during the entire study was purified using a Milli-Q water purification system. Spectral and absorbance measurements were made on a UV visible spectrophotometer (Model-UV 1800) with 10 mm matched pair of quartz cell and spectral bandwidth of ± 2nm.

Preparation of standard solutions

The mirabegron reference standard solution (100 µg/mL) was prepared by accurately weighing 10.00 mg of mirabegron reference in a 100.00 mL volumetric flask. The volume was completed with methanol and sonicated for 20 min. The above solution was diluted in a 100 mL volumetric flask with methanol to obtain a final solution containing 10.00 µg/mL.

Preparation of Phosphate Buffer pH 6.8

It was prepared by adding 22.4 mL of 0.1M sodium hydroxide to 60 mL of 0.02M potassium dihydrogen orthophosphate solution and sufficient water was added to produce to 200 mL.

Preparation of sample solutions

To analyze the concentration of mirabegron tablets, 10 tablets of each sample were independently weighed and triturated to get a homogenous blend. An amount of powder equivalent to 25 mg was transferred to 100.00 mL volumetric flask. The volume was completed with methanol. The resulting solution was sonicated for 10 min to facilitate proper solubilization. Aliquots of this solution were as needs be diluted with a phosphate buffer (pH 6.8), to obtain a working solution concentration of 50.00 µg/mL. All solution was filtered through a hydrophilic film of 0.45 µm.

The calibration curve was constructed by analyzing six different concentrations of standard solution, prepared on the same day. The concentration ranges of 2-14 µg/mL. All determination was conducted in triplicate.

Determination of maximum absorption

From the standard solution (15 µg/mL) approximately 3.5 mL was taken and scanned from 200 to 400 nm with UV spectrophotometer. The methanol was used as blank. Mirabegron presented maximum absorption at 251 nm.

Stability

The standard stock solutions of mirabegron concentration 7.0 µg/mL were subjected to heat treatment on 40-50 ºC and absorbance was measured. The absorbance for 40 ºC for 1 h was the same while for 50 ºC, the absorbance was decreasing which was indicative that the drug is stable at 40 ºC but at 50 ºC mirabegron solution unstable.

Method validation

The analytical method was validated with respect to parameters such as linearity, precise, accurate, limit of detection, quantification, and ruggedness.

RESULTS

The application of all the proposed methods for quality control can be easily performed for the analysis of mirabegron in pharmaceutical dosage forms. ICH guidelines[11] are followed to ensure their suitability for the intended use. The UV spectrum shown in fig 1, indicated that the compound

absorbed maximum at 251 nm with a molar absorptivity (ε) of 3.18 x 10^5 L Mol^{-1} cm^{-1}. This wavelength can be used to develop and validate a UV-spectrophotometric method for determination of the mirabegron. In addition, no interference from the diluent in mirabegron near λmax was verified. Phosphate buffer (pH 6.8) was considered a suitable diluent for validating the proposed method, since it showed no interferences in the analysis, thus supporting the reproducibility of the results.

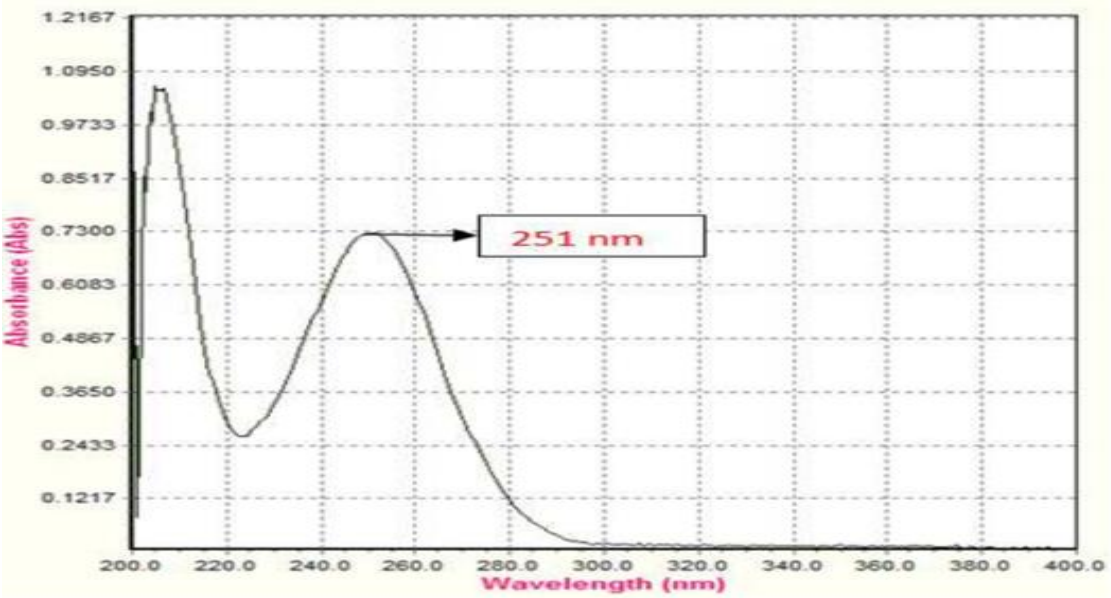

Fig. 1: UV spectrum of mirabegron (10µg/mL)

Linearity

Mirabegron was analyzed by proposed the UV spectrophotometric method in tablets. The calibration curve showed linearity over a concentration range from 2-14 µg/mL. Linearity was established by least-squares linear regression analysis of the calibration curve. Absorbance was plotted versus respective concentrations (Fig. 2, Table I). The correlation coefficients of the curve obtained with the linear regression method were 0.9982. It obeyed beer's law in these concentration ranges [12, 13].

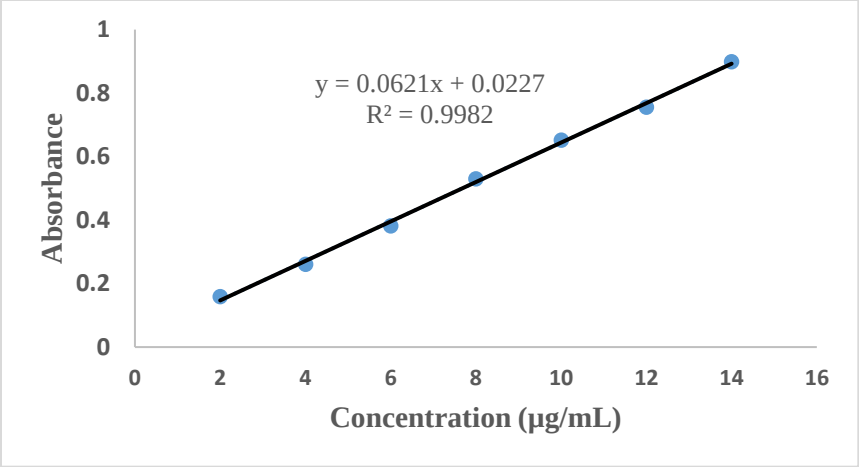

Fig.2: Calibration curve of mirabegron

Table I: Result of regression analysis of mirabegron.

Parameters	Value
Beer's law limit (µg/mL)	2-14
Correlation coefficient	0.9982
Regression equation	y = mx + C
Slope	0.0621
Intercept	0.0227

Precision

The assay was determined by repeatability (intraday) and intermediate precision (inter-day) of mirabegron at concentrations of 7.0 µg/mL. Determinations were performed with six replicates on the sample day. The precision is expressed as a relative standard deviation (RSD) among responses. The method was considered as precise, hence the statistical evaluation was found to be less 2 % . The results were tabulated in Table II.

Table II: Precision values of mirabegron

S.No	Concentration (µg/mL)	Absorbance	
		Intra-day	Inter-day
1	7	0.456	0.463
2		0.443	0.475
3		0.454	0.482
4		0.461	0.467

5	0.453	0.469
6	0.467	0.473
Mean	**0.455**	**0.471**
SD	**0.0080**	**0.006**
% RSD	**1.77**	**1.417**

Accuracy

The accuracy of the method was evaluated by the standard addition method at 3 levels. A standard quantity equivalent to 50%, 100%, and 150% is to be added to the sample. Results within the range of 99.29-100.74% ensure an accurate method as well as indicate of non-interference with the excipients (Table III).

Table III: Recovery data of standard solutions added to the samples analyzed by using the proposed spectrophotometric method

Pharmaceutical Dosage Form	Fortified theoretical concentration (µg/mL)	Found experimental concentration (µg/mL)[a]	Recovery (%)	
			Result	Average[b]
Tablets (25 mg)	3.5	3.52	100.74	100.10 ± 0.74
	7	6.94	99.29	
	10.5	10.52	100.29	

[a]Average of three determinations; [b]95% of confidence interval level (t-Distribution)

Ruggedness

It is a measure of reproducibility of test results under normal and expected operational conditions from analyst to analyst and instrument to instrument. Appropriate concentrations of mirabegron were analyzed using different UV spectrophotometry equipment on different days and by a different analyst to obtain various regression equations and coefficients were obtained. % RSD was calculated using regression coefficients obtained on different days, instrument and analyst and values should be less than 2%. Sample analysis and data processing resulted in % RSD values found to 0.95.

Limit of detection and Quantification

The LOD and LOQ were found to be 0.1594 and 0.4830 µg/mL respectively, which indicates the sensitivity of the methods.

Estimation of mirabegron

The developed UV method was successfully applied for the estimation of mirabegron content in BLADMIR tablet 25 mg. Average percent assay of mirabegron tablet was found to be 99.20% (Table IV).

Table IV: Determination of active ingredients in tablets

Sample	Label claim	Amount Found mg Tab*	% Labelled Claim*
Mirabegron	25	24.82	99.20

*Average of three determinations

DISCUSSION

Mirabegron is a UV-absorbing molecule with chromophores in the structure that absorb at a specific wavelength. This circumstance was successfully employed for their quantitative determinations using the UV spectrophotometric method. The objective of the analytical procedure is to govern the validation characteristics which need to be evaluated and should consider the list of validation characteristics are: Linearity, Accuracy, Precision, LOD and LOQ. The linear regression data for the calibration plot indicated an excellent linear relationship between absorbance and concentration over a wide range. The spectral analysis showed the λmax of Mirabegron to be 251nm. It complied with Beer's law within the concentration range of 2–14 g/mL and determined the regression analysis of the calibration curves and the optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity. The validated mean % recovery as per the ICH guidelines. The evaluation of the accuracy of the method was performed by the standard addition method. The precision (measurements of intraday and interday) results showed good reproducibility with a percentage relative standard deviation (% RSD) is below 2.0. It indicated that the method is highly precise. The optimal parameters were tabulated in Table V. The results obtained were satisfactory and agreed with the ICH guidelines.

Table V: Optical parameter for mirabegron

Parameter	**Method**
Diluent	Phosphate buffer pH 6.8
Linearity	2-14 µg/mL
Wavelength (nm)	251
Molar extension coefficient (Litre/mole/cm)	3.18×10^5
Sandel's sensitivity (µg/cm^2/0.001 absorbance unit	0.0125
Slope	0.0621

Intercept		0.0227
Correlation coefficient		0.9982
Precision	Intra-day	1.77
	Inter-day	1.41
Accuracy (% Recovery)		100.10
Assay (%)		99.20

CONCLUSION

A spectrophotometric method for quantifying mirabegron in formulation samples has been developed and validated. The assay is selective, precise, accurate, and linear over the concentration range studied. Mirabegron can be estimated as low as 0.4830 µg/mL in the formulation. It could be precisely quantified and LOD was approximately 0.1594 µg/mL in the formulation. In summary, the proposed method can be used for drug analysis in routine quality control.

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