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ANALYTICAL METHOD DEVELOPMENT FOR THE ESTIMATION OF MIRABEGERON IN PURE AND ITS SOLID DOSAGE FORM BY UV-SPECTROSCOPY

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Abstract: Simple, economical, rapid, accurate and precise UV spectroscopic methods were developed for the determination of Mirabegron from its dosage forms. The method developed was based on the Calibration curve method, Area under curve method and Derivative spectroscopic method. Instrument used for the performing of analysis is UV -spectrophotometer Jasco (V-630). The absorption maxima of Mirabegron were found to be at 249 nm wavelength using 0.1N Hydrochloric acid as a solvent. For area under curve method, the wavelength selected was between the range of 234.8 to 259.6 nm whereas for first order derivative spectroscopic method, the wavelength was 208 nm. Linearity was found to be between 2.5 to 15µg/mL with correlation coefficient more than 0.999 for both methods. The LOD and LOQ for present method was further studied. The percent recovery was within the range of 100.04% to 100.25%. The developed methods were found to be accurate as well as precise with relative standard deviation less than 2% and developed method can be applicable for quantitate analysis of finished formulation of the drug. Hence developed method can be used for routine quantitative analysis of Mirabegron.

Keywords – Mirabegron, UV – spectroscopy, Area under curve method, First order derivative.

Introduction:

Spectroscopy is one of the most powerful tools available for the study of atomic and molecular structure and is used in analysis of a wide range of sample. Various methods are used for the estimation of drugs like calibration curve, Area under curve method, Derivative spectroscopic method, absorption ratio method, absorption correction method etc. [1,2,3] Mirabegron is a novel drug which is used for the overactive bladder syndrome disease several methods are developed for estimation of Mirabegron. Literature survey indicate that the drug has been estimated from bulk and marketed formulation by UV-spectroscopy including zero order method and Derivative spectroscopy methods which mainly used methanol or concentrated solution as a solvent. [4,5,6,7]. The proposed work represents three new simple, economical, and rapid UV-spectrophotometric methods for the quantification of Mirabegron in bulk and its tablets by using 0.1N Hydrochloric acid (cheaper solvent). The developed methods were tested for the accuracy, precision, robustness and sensitivity.

Drug profile - Mirabegron [8,9,10,11]

- Mirabegron is the first clinically available beta-3 agonist with approval for use in adults with Overactive bladder syndrome disease. Mirabegron was approved for medical use in the United States and in the European Union in 2012. In 2020, it was the 160th most commonly prescribed medication in the United States, with more than 3 million prescriptions It is available as a generic medication.

- **Chemistry of Mirabegron**:[12,13]

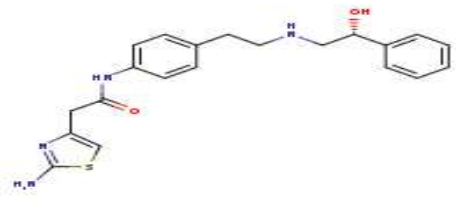

Figure 1 :Structure of Mirabegron

- **Chemical Name:** (R)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide.
- **Category:** Beta 3 - adrenergic agonist
- **Molecular Formula: -** $C_{21}H_{24}N_4O_2S$
- **Molecular weight: -** 396.506 g/mol
- **pka value: -** 4.5
- **Brand:** Mirago (Sun Pharma), Mirbeg (IPCA Laboratories Limited), Bladmir (Alembic Pharmaceuticals),
- vesi-beta (Cipla Limited)), lupin-Mira (Lupin Pharmaceuticals).
- **Pharmacokinetic Data**

  Bioavailability-29% - 35%

  Metabolism – Majorly in liver

  Elimination half-life – 50 hours Maximum plasma concentrations (C_{max}) at approximately 3.5 hours.

  Excretion - Urine and bile (90%)

MATERIALS AND METHODS

Chemical and Reagent:

Pharmaceutical grade Mirabegron standard was obtained as a gift sample. Solvents like Methanol AR Grade, Ethanol, Hydrochloric acid, N-Hexane, Double distilled water was used.

Instruments

UV-Spectrophotometer: Jasco V-630 and Shimadzu-1700 double beam

Sonicator: PCI Mumbai, Model No.3.5L 100H

Weighing balance: Shimadzu AUX 220 and Analytical Balance

Selection of solvent

The solubility of Mirabegron was determined in a variety of solvents as per Indian Pharmacopoeia standard 18. Solubility test was carried out in different polar and non-polar solvent. From the solubility studies 0.1 N Hydrochloric acid (use of cheaper solvent) was selected as a preferable solvent for the proposed methods.

Table 1: Parameter for solvent selection

Sr. No	Solvent	Solubility status
1.	0.1N HCl	Freely soluble
2.	Methanol	Soluble
3.	Ethanol	Very soluble
4.	Water	Insoluble
5.	N-Hexane	Soluble

Preparation of Standard stock solution A.

Standard stock solution was prepared by dissolving; accurately measured ~10.0mg of Mirabegron in 0.1N Hydrochloric acid and the volume was made up to mark in 100 mL volumetric flask. **(Standard stock solution, A - 100 µg/mL).**

Preparation of working standard solution B.

From the above Standard Stock Solution, A 1.0 mL of Solution dilute up to the mark in 10.0 mL volumetric Flask. **(Working standard solution B -10 µg/mL).**

Selection of appropriate wavelengths for analysis of Mirabegron

Method I: (Zero order) The above working standard solution, B (10 µg/mL) solution was scanned in the UV range 400–200 nm; Mirabegron shows a maximum absorbance at 249.0 nm.

Method II: (First order derivative UV-spectrophotometry using amplitude), the zero-order absorption spectrum of Mirabegron was derivatized in first order and the amplitudes was recorded at 208.0 nm.

Method III: (Area under Curve): From the zero-order spectrum of Mirabegron, the AUC between a wavelength range 234.8 to 259.6 nm was considered for the analysis.

The selection of wavelengths in all methods is shown in **Figure 2**.

Preparation of Calibration Curve

Appropriate dilutions of working standard solution A were made to get final concentration in the range of 2.5-15 µg/mL. Absorbance and area under curve were measured for each prepared solution at above selected wavelengths. The calibration curve was plotted between concentration and absorbance/AUC, have correlation coefficient 0.999 and 0.9996 respectively **(Figure 3**).

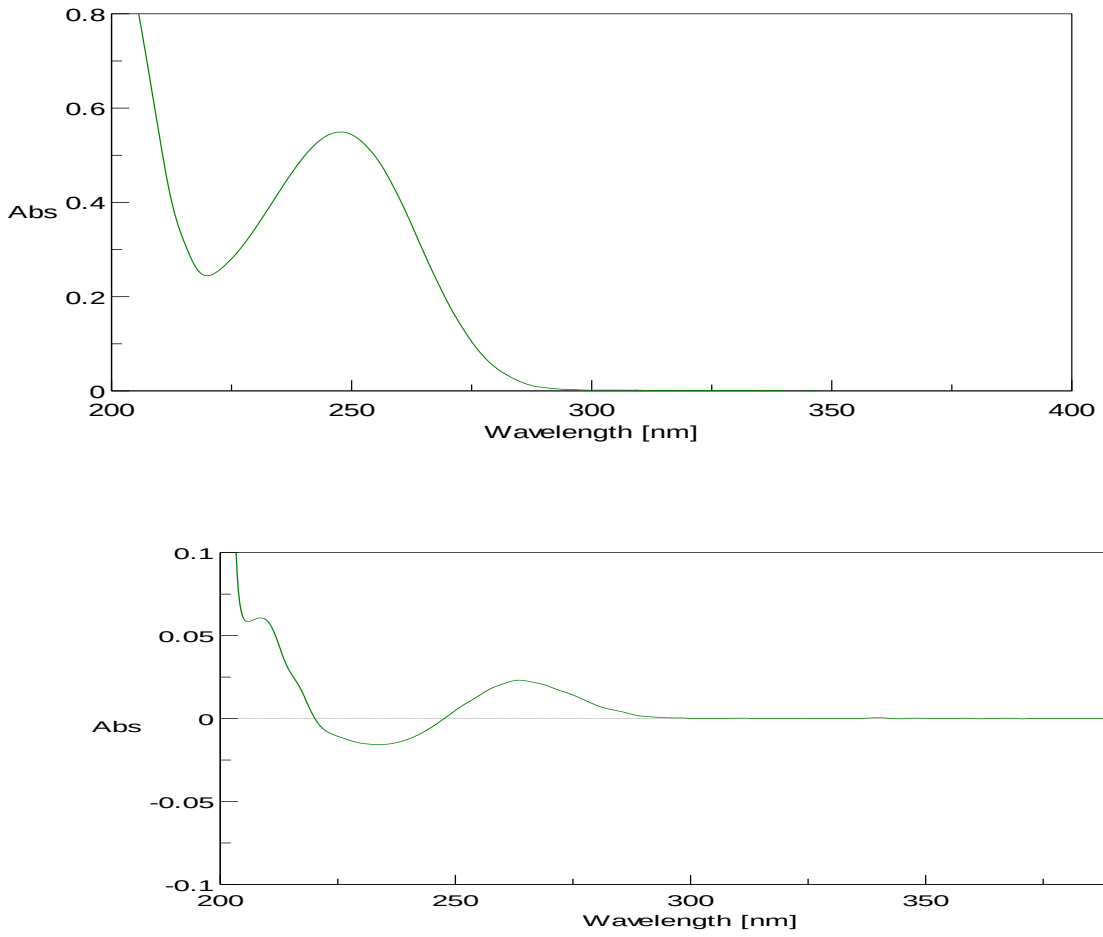

Figure 2: (a) Zero order spectrum of Mirabegron showing AUC between selected wavelengths,

(b) First order derivative spectrum

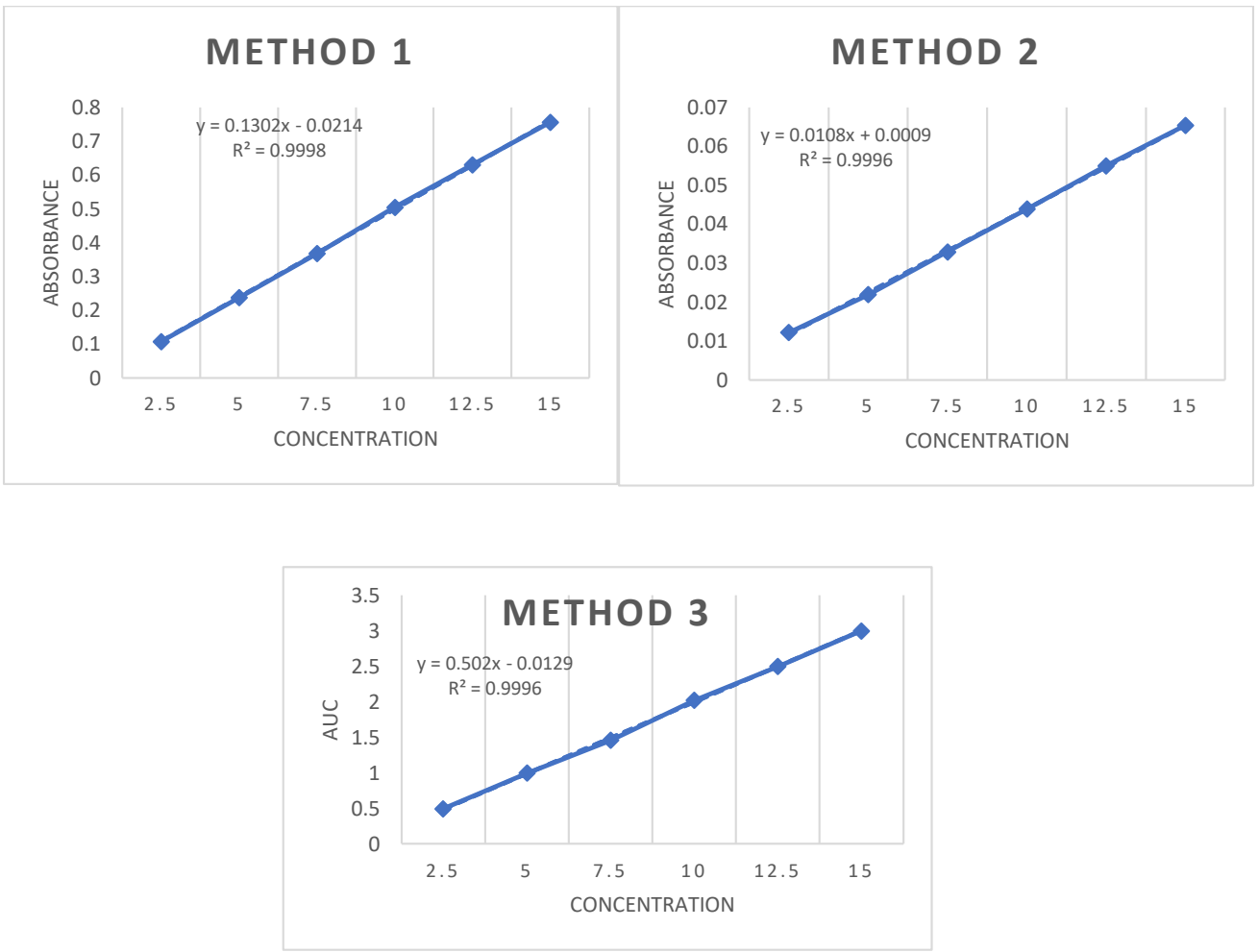

Figure 3: Beer's Lambert's plots for proposed three methods

Accuracy and precision of proposed Methods

Linearity*:*

An analytical procedure's capacity to produce test results that are directly proportional to the concentration (quantity) of an analyte in the sample within a specified range is known as linearity. For linearity study, six solutions of Mirabegron of different concentration was prepared and analysed for proposed methods and the obtained data was utilized to plot calibration curves.

Accuracy:

Recovery study is one of the parameters of accuracy. Weighed the pre-analysed tablet powder equivalent to ~10.00 mg; a known amounts of pure drug were added at different levels i.e., 80-120 %. The resultant solutions were then re-analysed by the developed methods. At each concentration, each sample was analysed thrice at each level to check repeatability.

Precision:

The analytical method's precision describes how closely a set of measurements obtained by multiple sampling of the same homogenous sample under the required circumstances coincide. The precision of the methods can be studied as; intra-day variation, inter-day variation studies. Intra-day study was carried out by analysing the 10 µg/mL of sample for three times in the same day while in inter-day study same solution analysed for five different days.

Limit of Detection (LOD) And Limit of Quantification (LOQ) of Proposed Methods.

Limit of Detection is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of an analyte in a sample that can be determined. LOD and LOQ were obtained from the slope and the standard deviation of the intercept from three calibration curves determined by a linear regression line as defined by ICH.

Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$LOD = 3.3 * (N/S) \quad \ldots\ldots 1$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$LOD = 10 * (N/S) \quad \ldots\ldots 2$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Ruggedness:

Ruggedness of proposed methods was performed to examine effect of non-procedure related factors such as variation in instruments and analysts. For this study, Mirabegron (10 µg/mL) was analysed by proposed methods using two different analyst and two different UV-spectrophotometers (Jasco V-630 and Shimadzu-1700) retaining similar experimental conditions.

RESULTS AND DISCUSSION

Though Mirabegron was found to be soluble in various organic solvent like Methanol, Acetonitrile etc. its solubility and stability in 0.1N HCL [13] can be utilised to develop economical UV methods. All developed methods obeyed Beer's-lambert's law for Mirabegron in the concentration range of 2.5-15 µg/mL, in 0.1N HCl, with correlation coefficient around the 0.999. In order to test the appropriateness of the developed methods to the pharmaceutical formulation, an assay of Mirabegron tablets was performed at working concentration. The recovery study was carried out at three different levels 80-120 %.

Analysis of marketed Tablets

The percentage amounts of Mirabegron estimated from tablet formulation using Method I-III was found to be 100.04, 100.10 and 100.25 respectively. The % amount estimated from tablet formulation indicates that there was no interference from excipients present in it. **(Table 2)**

Validation Data of Proposed Methods

Developed methods was studied for the validation parameter like linearity, accuracy, precision, ruggedness and sensitivity.

Accuracy Data of proposed Method

The solutions were re-analysed by proposed methods; results of recovery studies were reported in Table 2. The % RSD value was found to be less than 2 indicate that the methods were accurate **(Table 3).**

$$Percentage\ Recovery = \frac{Total\ drug\ estimated - Amount\ contributed}{Amount\ of\ pure\ drug\ added} * 100 \quad \ldots\ldots 3$$

Precision Data of Proposed Methods

The precision of the method was expressed in terms of % Relative Standard Deviation (RSD). The obtained results showed reproducibility of the assay. The % RSD values were found within limit, indicates that the developed methods were precise. (**Table 1)**

Linearity of Proposed Methods

From the linear regression data, it is clear that the calibration curves showed good linear relationship over the concentration ranges. The calibration curves were shown in **Figure 3**.

Ruggedness Data of Proposed Methods:

The results of ruggedness study were found in the acceptable range with % RSD values less than 2 by all the methods as shown in **(Table 4a, 4b, 4c, 4d).** The results showed no statistical differences between different operators and instruments suggesting that the developed methods were rugged.

Table 2: Results of % estimation from tablet

Sr. No	Weight of Mirabegron (mg)	% Label Claim		
		M-1	M-2	M-3 (AUC)
		249 nm	208 nm	234.8 to 259.6 nm
1.	254.70	99.98	100.1	100.37
2.	254.71	99.88	99.65	100.3
3.	254.73	100.28	99.88	100.39
4.	254.70	100.08	100.56	100.33
5.	254.69	100.28	100.33	99.86
6.	254.65	99.78	100.1	100.37
	Mean	**100.04**	**100.10**	**100.25**
	SD	**0.0222**	**0.3589**	**0.2207**
	%RSD	**0.02**	**0.35**	**0.22**

Table 2: Results of recovery study

Sr. No	Total Amt. of drug estimated			Amt. of pure drug added			% Recovery		
	M-1	M-2	M-3	M-1	M-2	M-3	M-1	M-2	M-3 (AUC)
	249 nm	208 nm	234.8 to 259.6 nm	249 nm	208 nm	234.8 to 259.6 nm	249 nm	208 nm	234.8 to 259.6 nm
1.	18.01	18.02	18.03	8.01	8.02	8.03	100.5	101.0	101.5
2.	20.03	20.01	20.00	10.03	10.01	10.00	100.7	100.2	100.0
3.	22.08	22.01	22.06	12.17	12.13	12.14	101.3	100.1	101.0
						Mean	**100.83**	**100.43**	**100.83**
						SD	**0.0041**	**0.0049**	**0.0076**
						% RSD	**0.41**	**0.49**	**0.75**

Table 4a): Result of analyst-to-analyst variation

Sr. No	Analyst	Wt. of powder taken (mg)	%Label Claim		
			M-1	M-2	M-3 (AUC)
			249 nm	208 nm	234.8 to 259.6 nm
1.	Analyst - 1	254.7	99.88	100.33	100.05
2.	Analyst - 2		100.08	100.33	100.02
Mean			**100.03**	**100.255**	**100.02**
SD			**0.1707**	**0.5499**	**0.0384**
% RSD			**0.17**	**0.54**	**0.03**

Table 4 b): Result of instruments-to-instruments variation

Sr. No	Instruments	Wt. of powder taken (mg)	%Label Claim		
			M-1	M-2	M-3 (AUC)
			249 nm	208 nm	234.8 to 259.6 nm
1.	Shimadzu-1600	254.7	100.08	100.56	100.01
			99.88	100.79	100
			100.98	100.01	99.99
2.	Jasco v- 630	254.7	100.78	100.33	100.38
			100.18	99.88	100.19
			99.98	100.1	100.1
		Mean	**100.38**	**100.31**	**100.11**
		SD	**0.4743**	**0.3768**	**0.1700**
		%RSD	**0.47**	**0.37**	**0.16**

Table 4 c): Results of Intraday study

Sr. No	Time (hr)	Wt. of powder taken (mg)	%Label Claim		
			M-1	M-2	M-3 (AUC)
			249 nm	208 nm	234.8 to 259.6 nm
1.	0	254.7	101.08	100.1	100.15
2.	1		100.88	100.33	100.12
3.	2		100.78	99.88	100.13
4	3		101.97	100.56	100.15
		Mean	**101.17**	**100.21**	**100.13**
		SD	**0.5428**	**0.2930**	**0.0150**
		%RSD	**0.53**	**0.29**	**0.01**

Table 4 d): Results of Interday study

Sr. No	Day	Wt. of powder taken (mg)	%Label CSlaim		
			M-1	M-2	M-3 (AUC)
			249 nm	208 nm	234.8 to 259.6 nm
1.	1	254.7	100.68	100.79	100.14
2.	2		100.98	100.33	100.07
3.	3		101.78	101.02	100.05
4	4		101.97	101.7	100.01
5	5		100.38	101.47	100.04
		Mean	**101.15**	**101.06**	**100.06**
		SD	**0.6913**	**0.5443**	**0.0486**
		%RSD	**0.68**	**0.53**	**0.04**

CONCLUSION:

Developed UV-Spectrophotometric methods for the determination of Mirabegron were based on Calibration curve, Derivative and Area under curve techniques. The methods were found to be economic, simple, sensitive, accurate, and precise. Hence, can be used successfully for routine quantitative analysis of Mirabegron from its formulation.

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