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Method Development and Validation of Mirabegron in Bulk Drug and Pharmaceutical Dosage Form

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Abstract: *A new, simple, precise, accurate and reproducible Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for Simultaneous estimation of bulk and pharmaceutical formulations. Separation of Mirabegron was successfully achieve THERMO, C_{18}, 250X4.6mm, 5µm or equivalent in an isocratic mode utilizing 0.1M KH_2PO_4 : Methanol (60:40) at a flow rate of 1.0ml/min and eluate was monitored at 248nm, with a retention time of 3.684 minutes for Mirabegron. The method was validated and the response was found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Mirabegron. The values of the correlation coefficient were found to 0.999for Mirabegron. The Limit of Detection(LOD) and Limit of Quantification (LOQ) for Mirabegron were found to be 0.149 and 0.498 respectively. This method was found to be good percentage recovery were found to be 99 indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to International Council for Harmonisation(ICH) guidelines for Linearity, Accuracy, Precision, Specificity and Robustness.*

Keywords: *Mirabegron, High performance liquid chromatography, Validation.*

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I. Introduction

Analytical method development and validation play important role in the discovery, development and manufacture of pharmaceuticals. The Current Good Manufacturing Practice (CGMP) and Food Drug Administration (FDA) Guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior part (or) existing literature, using the same (or) quite similar instrumentation. It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC (High performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. Ultra Violet (**UV)** spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods[1].Most of the drugs can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures.On the literature survey, it was found that most of the analytical method available for the above mentioned drug is applicable for quantification in plasma samples, the most widely used method being liquid chromatography-mass chromatography. So it is felt that there is a need to develop accurate, precise analytical methods for the estimation of the drug in solid dosage formulation[2].

Aim istoanalyzeMirabegron by UV detection,High Performance Thin Layer Chromatography HPTLC, HPLC individually and combination with other drugs in bulk material and pharmaceutical forms. Objective of the present work is to development and validates a HPLC method development and validation Mirabegron of tablets. In the method development of Mirabegron Reverse phase High performance Liquid chromatography isincorporated.

Drug Profile

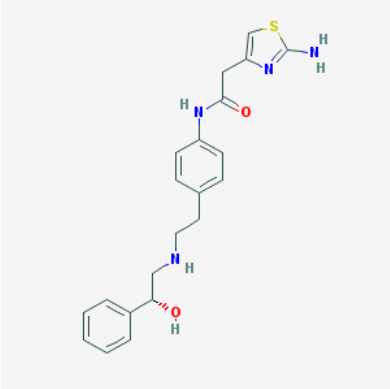
Drug **:** Mirabegron

Molecular formula **:**$C_{21}H_{24}N_4O_2S$

Molecular weight **:**396.509 g/mol

***IUPAC-Name*:**2-(2-amino-1,3-thiazol-4-yl)-N-[4-(2-{[(2R)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide

Structural formula **:**



Plan of Work

In order to develop a simple, reliable and an accurate method development and validation of Mirabegron in pharmaceutical dosage form by Reverse phase HPLC and validate the method for its repeatability and reproducibility.

Method Development

Four trials conducted, in all trials Flow Rate is 1.0ml/Min, Temperature: 25˚C, Volume 10µl, Run time 10min, Detector is PDA (photo diode array) but mobile phase and columns are different. Trails for 1 and 2 Mobile Phase: OPA: Methanol (60:40), for 3 and 4NaH_2PO_4: Methanol (60:40) andKH_2PO_4: Methanol (60:40) respectively. ***Column for Four trials:*** for trial 1 and 3 isWATERS, C18, 250x4.6mm, 5µm, for trial 2SUPELCO, C18, 250x4.6mm, 5µm, for trial 4 THERMO, C18, 250x4.6mm, 5µm. Observation for first 3 trials are Two peaks eluted but peak shape is not good, Reason: may be column not suitable, Corrective Action: change the column. But Observation for fourth trial is two peaks eluted and all the system suitability parameters are within the limit.Optimized Method Volume: 10µl, Detector: 280nm Mobile Phase: K_2HPO_4: Methanol (60:40), Column: THERMO, C18, 250X 4.6mm, 5µm, Flow Rate: 1.0ml/Min, Temperature: 25˚CObservation: Two peaks eluted and all the system suitability parameters are within the limit.

Assay Result For Formulation: Label claim: Each tablet contains, Mirabegron-25mg, Average weight of each tablet is 260mg, Purity of working standards: Mirabegron: %purity-99.8%***Sample preparation*:**20 tablets were weighed and crushed, from the powdered tablets, weighed accurately about 260mg (25mg Mirabegron) into a 25ml volumetric flask and 25 ml of methanol was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with water. From the above solution 1 ml is taken and further diluted in 10ml volumetric flasks with methanol.

Standard preparation: Accurately weighed quantity of 25mg Mirabegron was taken in a 25ml volumetric flask and 25ml of Methanol was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with water. From the above solution 1 ml is taken and further diluted in 10 ml volumetric flasks with methanol.

Procedure: Separately injected both the standard (5 injections) and sample preparations (1injection) into the chromatographic system and recorded the peak area responses.

% percentage content =

$$\frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Concentration of Standard}}{\text{Concentration of Sample}} \times \frac{\text{Average Weight}}{\text{L.C}} \times P$$

II. Method Validation

System Suitability: Tailing factor for the Mirabegron should not be more than 2.0. Theoretical plates for the Mirabegron should not be less than 2000.

.**Specificity:** Solution of standard, sample, blank and placebo were prepared and injected into the HPLC. Acceptance criteria: Chromatogram of standard and sample should be identical with near Retention time but

blank should not show any peak at the retention time of analytepeak. There is no interference due to blank at the retention time of analyte. Hence the method is specific.

Linearity: Prepare a series of standard solutions and inject into HPLC. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for100% response.Acceptance criteria**:**Linearity regression coefficient of average peak area response plotted against concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.***Precision*:**Preparation of sample:Transfer the 160mg of sample into a 25ml of volume at flask and add 10ml of methanol and sonicate 20min and makeup with methanol. Transfer the above solution into 1ml into 10ml volume metric flask dilute to the volume with methanol.The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections.Acceptance criteria:The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention times is not more than 2.0.***Recovery/Accuracy*:**Recovery study can be performed in the concentration range of 80% to 120% of the target concentration of the test. Minimum 3 concentrations are recommended.Acceptance criteria: The average percentage recovery was between 98-102% and Relative standard deviation of these recovery concentrations was less than 2%.***Limit of Detection** and **Limit of Quantitation*:**The LOD and LOQ wascalculated by the use of signal to noise ratio. In order to estimate the LOD and LOQ value, the blank sample was injected six times and peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level. The LOQ was calculated as ten times the noise value gave the LOQ.

$$LOD = 3.0\ \sigma / S \qquad LOQ = 10\ \sigma / S$$

Where,σ = standard deviation of intercepts of calibration curves, S = mean of slopes of the calibration curves, the slope S may be estimated from the calibration curve of the analyte.***Robustness*:** Effect of variation in flow rate:Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow and ±2nm variation in wavelength. Evaluate the system suitability values as required by the test method for both flow rates and for both wavelengths. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked

III. Results And Discussion

System Suitability: Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.Specificity:The forced degradation study showed the method was highly specific, the chromatographic peak does not interfere with any other impurities**.** This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.Accuracy:MirabegronResults of accuracy study are presented in the table 1 and 2, fig 1.

Table 1: Accuracy data forMirabegron

S.NO	Accuracy Level	Injection	Sample area	RT
		1	1283106	3.651
1	50%	2	1286610	3.649
		3	1281331	3.643
		1	2567461	3.638
2	100%	2	2567572	3.645
		3	2562984	3.638
		1	3843235	3.637
3	150%	2	3843992	3.639
		3	3846597	3.639

Table 2: Accuracy (%recovery) results of Mirabegron

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
		1	130	50.000	49.89	100	
		2	130	50.000	50.03	100	100
1	50%	3	130	50.000	49.82	100	
		1	260	100	99.83	100	
		2	260	100	99.84	100	
2	100%	3	260	100	99.66	100	100
		1	390	150	149.44	100	
3	150%	2	390	150	149.47	100	
		3	390	150	149.57	100	100

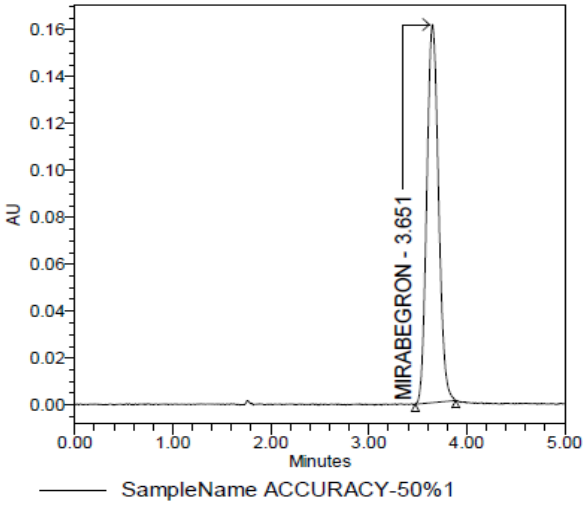


Fig 1: Typical chromatogram for Accuracy 50 **%**

The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 100.00% for Mirabegron. All the results indicate that the method is highly accurate**.**Precision:Results of variabilityPercentage relative standard deviation (% RSD) of peak areas was calculated for various run. %RSD was found to be less than 2% which proves that method is precise**.** Linearity: A linear relationship between peak areas versusconcentrations was observed for Mirabegron in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for Mirabegronwhich prove that the method is linear in the range of 50% to 150%.

Table 3: Linearity data for Mirabegron

s.no	**Conc (µg/ml)**	**RT**	**Area**
1.	50	3.639	1289412
2.	75	3.647	1929880
3.	100	3.646	2569767
4.	125	3.642	3206987
5.	150	3.646	3840345
Correlation coefficient (r^2)			0.999

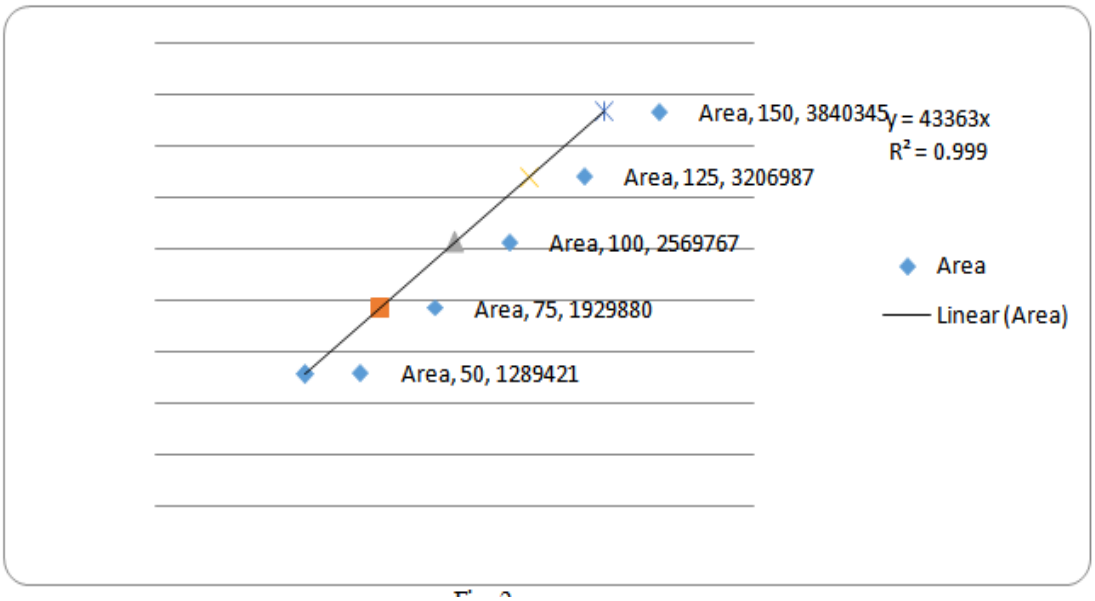


Fig: 2

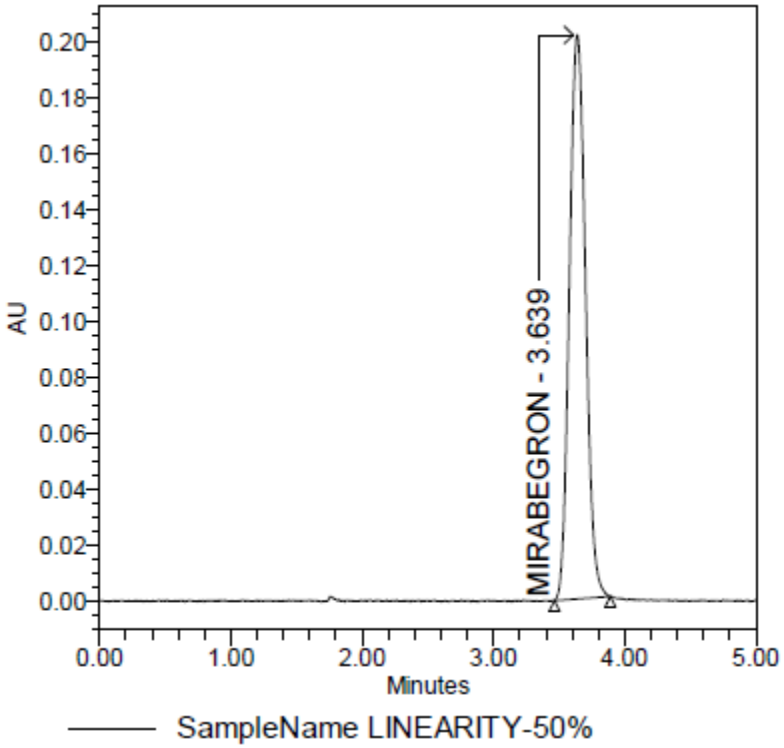


Fig 3: Chromatogram representing linearity 1

Robustness: The results of Robustness of the present method had shown that changes made in the Flow and Temperature did not produce significant changes in analytical results which were presented in the table. As the changes are not significant we can say that the method is Robust.

Table 4: Robustness data for Mirabegron

parameter	RT	Theoretical plates	Tailing Factor
Decreased flow rate(0.8ml/min)	4.673	5397	1.11
Increased flow rate(1.2ml/min)	2.982	3160	1.18
Decreased temperature(20^0c)	3.613	3854	1.11
Increased temperature(30^0c)	3.627	4016	1.10

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Limit of Detection:Minimum concentration of standard component in which the peak of the standard gets merged with noise called the LOD. Limit of Quantification: Minimum concentration of standard component in which the peak of the standard gets detected.

LOD = 3.3* σ/S LOD for Mirabegron = 0.149, LOQ = 10*σ/SLOQ for Mirabegron= 0.498

Where;σ = standard deviation S = slope

IV. Conclusion

The study is focused to develop and validate HPLC methods for estimation of Mirabegron in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery,from the specificity studies it was found that the developed methods were specific for Mirabegron..

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