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RP – HPLC method for the determination of Mirabegron in Pharmaceutical dosage form

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the determination of Mirabegron in bulk and Pharmaceutical dosage form. The separation was effected on a Waters ODS C_{18} column (150 mm x 3.9 mm;5µ) using a mobile phase mixture of buffer and acetonitrile in a ratio of 50:50 v/v at a flow rate of 1ml/min. The detection was made at 249 nm. The retention time of Mirabegron was found to be 2.502 min. Calibration curve was linear over the concentration range of 6.25-37.5 µg/ml of Mirabegron. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Keywords: Buffer, acetonitrile, Mirabegron, Tablets, Waters ODS C_{18} column, RP-HPLC.

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INTRODUCTION

Mirabegron(Figure1) (2-(2-amino-1, 3-thiazol-4-yl)-N-(4-(2-{(2R)-2-hydroxy-2-phenyl ethyl) amino} ethyl) phenyl) acetamide] is a potent and selective human β3-adernoceptor agonist is the first of a new class of compounds under development for the treatment of overactive bladder[1].Mirabegron activates β3-adernoceptors on the detrusor muscle of the bladder to facilitate filling of the bladder and urine storage[2]. A Literature survey reveals that only a method based on development and validation of LC-MS/MS methods for the determination of Mirabegron and its metabolites in human plasma and their application to a clinical pharmacokinetic study[3].The present investigation by the author describes a rapid, accurate and precise RP – HPLC method for the determination of Mirabegron from bulk sample and pharmaceutical dosage form. It is not official in any of the pharmacopoeia. The detector responses were linear in the concentration range of 6.25-37.5 µg/ml of drug. The method was validated as per ICH guidelines.

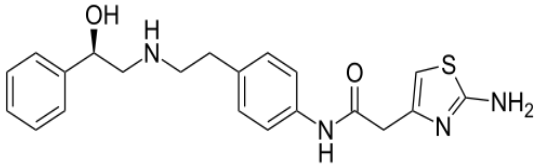


Figure 1: Structure of Mirabegron

MATERIALS AND METHOD

Chromatographic Conditions:

The determination was carried out on waters HPLC model 2695 equipped with UV Visible detector using data handling system-waters alliance empower two software. The column used in the development for determination is Waters ODS C_{18} (150× 3.9mm, 5 μ). The detector wavelength was set at 249 nm. A flow rate of 1ml/min was used for the determination of Mirabegron. The mobile phase composition was acetonitrile: potassium dihydrogen orthophosphate 0.01M in the ratio of 50:50 (v/v) and P^H adjusted to 5. The samples and standards were dissolved in the mobile phase and 20µl samples were injected into the HPLC system at the column temperature of 30ºC .HPLC grade acetonitrile, water were purchased from E.Merck Co; Mumbai, India, and Potassium dihydrogen phosphate AR grade purchased from SD Fine Chem. Mumbai, India.

Drug samples:

The Mirabegron reference and branded formulation was supplied by M/s Bio Leo Analytical Labs India Pvt. Ltd, Hyderabad, Andhra Pradesh, India.

Mobile phase

Accurately 1.36g of potassium dihydrogen phosphate was weighed out and dissolved in 1000ml of water. The solution was filtered through 0.45µ membrane filter and was degassed. A freshly prepared of buffer: Acetonitrile in a ratio of (50:50) V/V was used as the mobile phase. Mobile phase was used as diluent for preparing the working solution of the drug. The mobile phase was filtered through 0.05µ membrane filter and sonicated by using Power Sonicator, model no: 405, Hwashin Technology, Korea before use. The flow rate of the mobile phase was maintained at 1ml/min.The column temperature was maintained at 30ºC and the detection of the drug was carried out at 249nm.

Preparation of stock and working standard solution of Mirabegron

About 25mg of Mirabegron was weighed accurately and transferred into 100 ml volumetric flask, added 60 ml of diluent and the solution was sonicated to dissolve and dilute to the volume. Transferred 10 ml of the resulting solution into 100 ml volumetric flask and it was diluted with the mobile phase to get a working standard solution of 25 µg/ml of Mirabegron.

Linearity and Construction of Calibration Curve

The quantitative determination of the drug was accomplished by a standard method. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. Linearity of the peak area response was determined by taking measurement at Six concentrations Mirabegron working dilution of in the range of 6.25 -37.5µg/ml were prepared by taking suitable aliquots of working standard solution in different 100 ml volumetric flasks and diluting up to the mark with the mobile phase. 20µL quantity of the dilution was injected each time in to the column at a flow rate 1ml/min. The drug in the elutes was monitored at 249 nm.

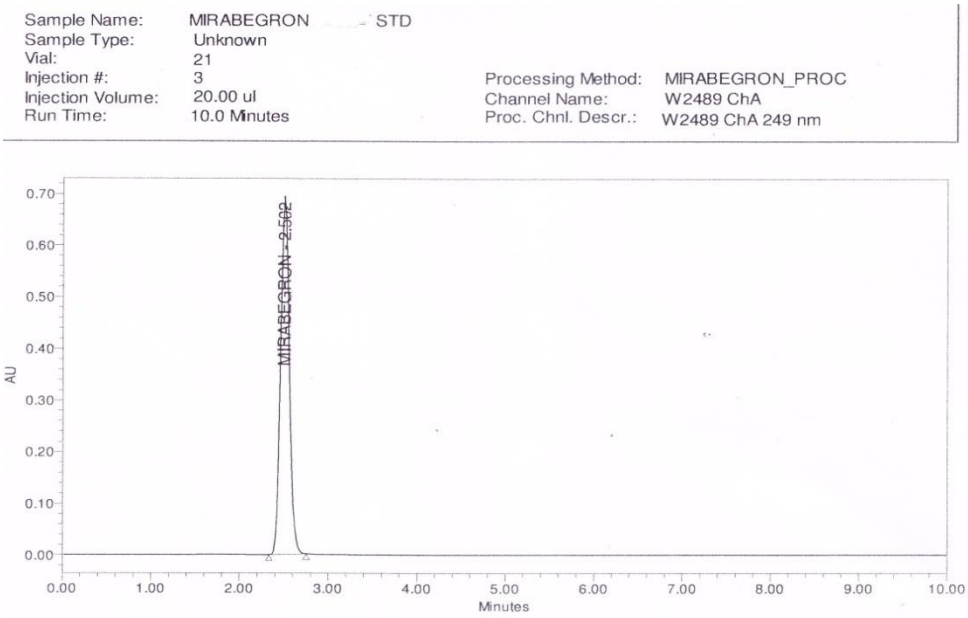
Analysis of Tablet

Twenty tablets of Mirabegron were weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 25mg was transferred into 100 ml volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100ml with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. From the above solution 10ml aliquot was taken into a separate 100ml volumetric flask and diluted up to the volume with the mobile phase and mixed well. The above solution (20µL) was then injected into the column.

RESULTS AND DISCUSSION

The goal of this study was aimed at developing a sensitive, precise and accurate HPLC method

for the analysis of Mirabegron in bulk drug and in pharmaceutical dosage form and forced degradation. In order to achieve optimum separation of the component peaks, mixtures of buffer: Acetonitrile in different combinations were tested as mobile phase on a Waters ODS C_{18} column (150 mm x 3.9 mm; 5µ) stationary phase. A mixture of buffer: Acetonitrile in a proportion of 50:50 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for Mirabegron was 2.502 min. (Figure 2)



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	MIRABEGRON	2.502	4703434	691755	100.00	1.13	3118

Figure 2: Chromatogram of Mirabegron Standard

To validate the RP-HPLC method, a series of tests were made using the most promising conditions. For linearity, a calibration curve was made and concentrations examined within the detection range of 6.25-37.5 µg/ml for Mirabegron the correlation coefficient was found to be 0.9999.The regression curve was constructed by linear regression fitting and its mathematical expression was Y = 19064X-7861 (Where Y gives peak area and X is the concentration of the drug). The regression characteristics are given in Table 1.The precision (expressed as the relative standard deviation (R.S.D)) for area under the curve (AUC) and retention times was determined for Mirabegron for repeated analysis (n=6). The R.S.D values obtained for retention times were 0.05% and for area under curve were 0.12%. The average R.S.D values for method precision obtained for AUC) and retention times were 0.26 % and 0.02% respectively. The assay values obtained by proposed method and the recovery experiment values obtained were performed by adding a fixed amount of drug to preanalyzed formulation summarized in Table 2.

Table 1: Optical and system suitability parameters

Parameters	values
Concentration range (µg/ml)	6.25 - 37.5
Slope (m)	19064
Correlation coefficient (r^2)	0.9999
Intercept (b)	-7861
Theoretical Plates (h)	3125
Tailing factor (T)	1.13

Table 2: Analysis of pharmaceutical formulation

Pharmaceutical Formulation	Labeled Amount (mg)	Amount found by Proposed method (mg)	Recovery by Proposed Method (%)*
Mirabegron	25	24.949	100.04

*Each value is a mean of three determinations at three different levels

Table 3: Robustness Study:Proposed conditions

Experiment	USP Tailing	% RSD
Linearity	1.10	0.12
Method Precision	1.13	0.26
Robustness Flow	1.12	0.26
Robustness Buffer	1.12	0.59

Changed conditions

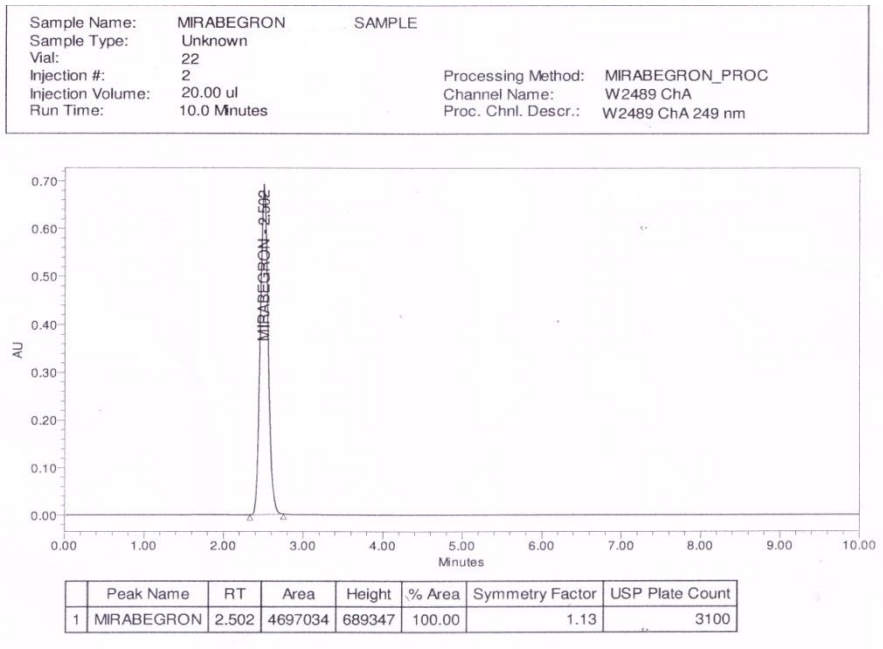
Experiment	USP Tailing	% RSD
Robustness Flow (+5%)	1.10	0.20
Robustness Flow (-5%)	1.13	0.09
Robustness Buffer (+5%)	1.15	0.57
Robustness Buffer (-5%)	1.12	0.13

Table 4: Forced Degradation

Condition	Time (hours)	Retention time (min)	Area	Retention time of additional degradation peak (min)	% Degradation	% of Active drug Present after Degradation
Control Sample	00	2.503	4706364	------	------	100
Acid Degradation	06	2.511	1506827	1.965 3.386 3.860	18.611 0.030 0.064	81.295
Alkaline Degradation	06	2.511	1311143	1.966 2.883 3.044 3.878	18.351 0.019 0.059 3.991	77.580
Thermal Degradation	08	2.513	2103343	1.969 3.419	4.157 18.101	77.742
Photolytic Degradation	8	2.511	1498513	1.965 3.386 3.860	18.931 0.030 0.064	80.974

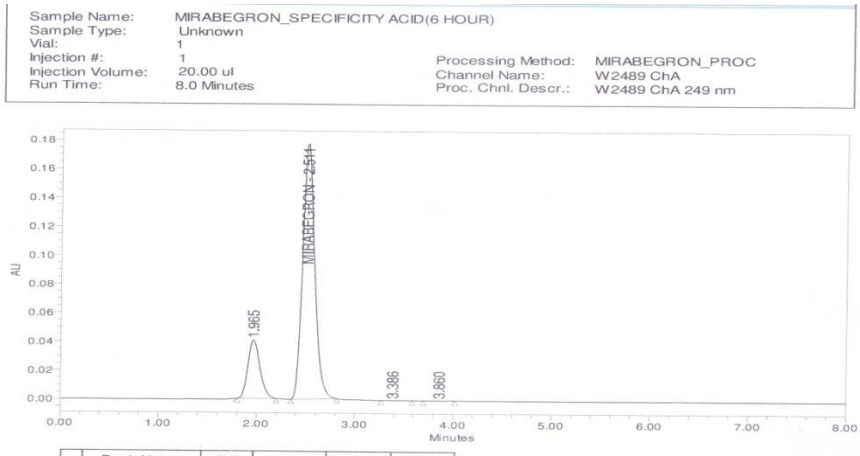
The absence of additional peaks indicated non-interference of common excipients used in the tablets (Figure 3). The stability of sample was checked by forced degradation in different

conditions and the percentage of degradation was calculated. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH, Photolytic and Thermal degradation at 105ºC. The results of specificity data for degradation study are given in Table 4 (figure 4-7). The following values indicate that the any other impurity is not merging with the main peak. The reliability of the method was determined by made small deliberate variations in method parameters and the RSD values obtained given in Table 3, an indication of its reliability on normal usage. The LOD and LOQ values as obtained by the proposed method were found to be 2.06 and 6.27 µg/mL indicates the sensitivity of the method



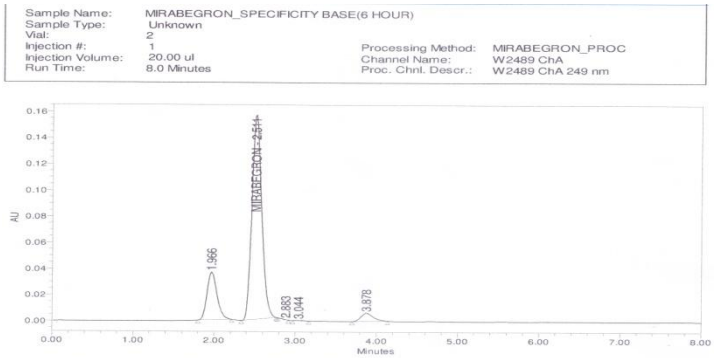
	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	MIRABEGRON	2.502	4697034	689347	100.00	1.13	3100

Figure 3: Chromatogram of Mirabegron Sample



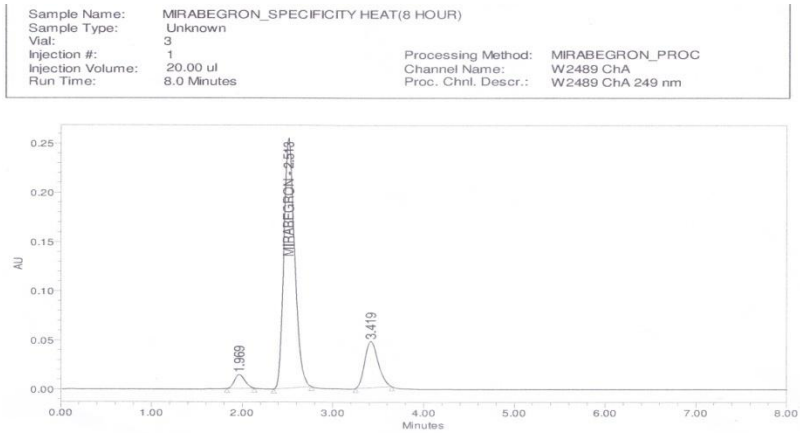
	Peak Name	RT	Area	Height	% Area
1		1.965	344951	40758	18.611
2	MIRABEGRON	2.511	1506827	177746	81.295
3		3.386	564	56	0.030
4		3.860	1181	121	0.064

Figure 4: Acid Degradation Chromatogram of Mirabegron



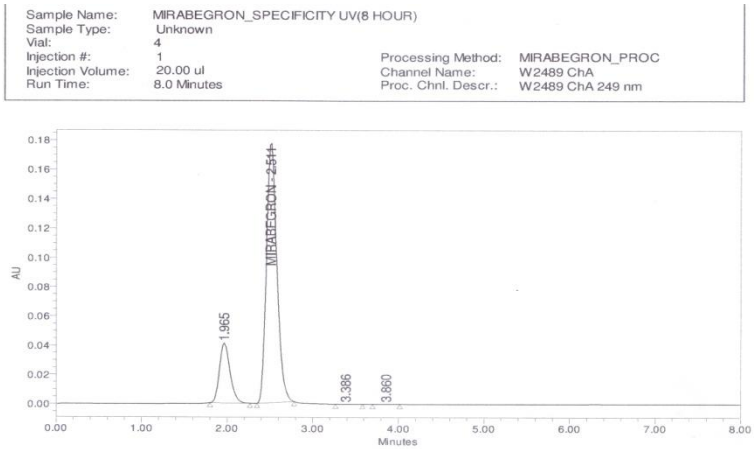
	Peak Name	RT	Area	Height	% Area
1		1.966	310142	36419	18.351
2	MIRABEGRON	2.511	1311143	156424	77.580
3		2.883	319	-86	0.019
4		3.044	1005	138	0.059
5		3.878	67442	6345	3.991

Figure 5: Base Degradation Chromatogram of Mirabegron



	Peak Name	RT	Area	Height	% Area
1		1.969	112461	14134	4.157
2	MIRABEGRON	2.513	2103343	254207	77.742
3		3.419	489727	47355	18.101

Figure 6: Thermal Degradation Chromatogram of Mirabegron



	Peak Name	RT	Area	Height	% Area
1		1.965	350347	40921	18.931
2	MIRABEGRON	2.511	1498513	177525	80.974
3		3.386	564	56	0.030
4		3.860	1181	121	0.064

Figure 7: Photolytic Degradation Chromatogram of Mirabegron

CONCLUSION

A RP-HPLC method was developed for the determination of tablets which is simple, quick, specific and reliable. The results indicate that the described method can be used for quantitative analysis of the compound with short analysis time.

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