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TO DEVELOP A NEW RP-UPLC METHOD FOR ESTIMATION OF MIRABEGRON IN PHARMACEUTICAL DOSAGE FORMS WITH FORCED DEGRADATION STUDIES

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ABSTRACT

A simple and selective UPLC method is described for the estimation of Mirabegron in tablet dosage forms. Chromatographic separation was achieved on a Waters Acquity C18 (50mm x2.1 mm ID) 1.8µm using mobile phase consisting of a mixture of 70 volumes of Potassium di-hydrogen phosphate and 30 volumes of methanol with detection of 254nm. The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. Linearity was observed in the range 50-150 µg /ml for Mirabegron (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

INTRODUCTION

Mirabegron is a drug for the treatment of overactive bladder with the symptoms of urge incontinence and urinary frequency. It activates the beta-3 adrenergic receptor in the detrusor muscle in the bladder which leads to muscle relaxation and an increase in the bladder capacity. It is chemically, 2-(2-Amino-1, 3-thiazol-4-yl) -*N*- [4-(2- { [(2*R*) - 2 – hydroxyl -2-phenylethyl]amino}ethyl)phenyl]acetamide and has the chemical formula and weight $C_{21}H_{24}N_4O_2S$ and 396.506 g/mol respectively. Literature review reveals that there are several analytical methods existing for individual dosage form containing Mirabegron, potassium di-hydrogen phosphate by HPLC.

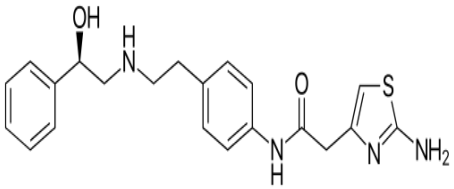

Fig.1 Structure of Mirabegron

There is no reported method of analysis by Ultra Performance Liquid Chromatography for determination of tablets containing Mirabegron, Potassium di-hydrogen phosphate. Hence UPLC method is selected in the present work and validated.

Materials and Methods:

Mirabegron sample is obtained from Madras Pharmaceuticals, Chennai, India. All reagents used were Rankem/AR grade, nylon membrane filters of 0.45µ pore size were used to filter the mobile phase and its components.

Instrumentation:

Analysis was carried out in waters acquity with binary UPLC pump equipped with PDA detector. Separation has been carried out using Acquity BEH C18 (50*3.0mm), 1.8 µ column.

Method Development

Various analytical development trials has been performed by using different chemicals and reagents, organic solvents at different pH ranges and strengths in different proportions of buffer and Organic solvents to obtain the peak with acceptable resolution and with good peak shape. Various stationary phases of multiple makes were used to check the chromatography with acceptable peak shape, tailing factor and plate count for reproducibility at 25^0C. Based on the observations and conclusions obtained from the number of chromatographic trials performed on UPLC, a particular set of chromatographic conditions were optimized to be suitable for estimation of the Mirabegron in the tablets. The optimized chromatographic conditions which are found to be suitable for the estimation of the Mirabegron are given below. Table No.1.

Preparation of Mobile Phase:

A mixture of 70 volumes of Potassium di-hydrogen phosphate and 30 volumes of Methanol. The mobile phase was sonicated for 10 min to remove gases and filtered using 0.45µm filters to remove all fine particles and gases.

Preparation of Potassium dihydrogen phosphate solution: 1.35 g of potassium dihydrogen orthophosphate was dissolved and made up to 100 ml with distilled water. 0.45µm filters to remove all fine particles and gases.

Preparations for Methodology:

Preparation of Mirabegron standard stock solution: 10 mg of MIRABEGRON was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Method Validation:

System suitability: It is assessed by injecting the six replicate into a system. Results are given in Table no.1 & 2.

Calibration curve: A linear relationship was evaluated across the range of the analytical procedure and demonstrated directly on the drug substance. The test results were evaluated by calculation of regression line by the method of least square. The respective component concentrations were given below.

Mirabegron – 50, 80, 100, 120, 150

All the above prepared solutions of respective individual component were analysed to calculate the correlation coefficient of the individual components.

Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated.

Sample stock preparation: 10 tablets of MIRAGO (25 mg extended release tablets) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solution of MIRABEGRON were prepared by dissolving weight equivalent to 10 mg of MIRABEGRON and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 10ug/ml of MIRABEGRON was made by diluting 1.0 ml to 10ml with mobile phase.

Method Precision: The Precision of the method was determined by sample preparation. Calculated % of assay using formula

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation
AT: Peak area due to assay preparation
WS: Standard Weight of Mirabegronin mg
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation
DS: Dilution of standard preparation
P: Purity of Mirabegron
AV: Average weight of tablets in mg
LC: Labelled claim of Mirabegron

Forced Degradation study

Peroxide Degradation: Sample solution of Mirabegron (10µg/ml) and 1 ml of 20% H_2O2 was mixed. The resultant solution 10 µl was injected into the system and the chromatograms were recorded to evaluate the degradation of sample.

Photolytic Degradation: The photochemical stability of the drug was studied by exposing the 50µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days. For UPLC study, the resultant solution 10µl was injected into the system and the chromatogram were recorded to assess the stability of sample.

Acidic Degradation: Take 1 tablet, powdered and place in a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N HCL then kept in oven at 60^0c for 1 hour then cool and add 1 ml of 0.1N NaOH it then make up the volume up to 50ml with mobile phase, then place the sample in the vial and measure the chromatogram.

Alkaline Degradation: Take 1 tablet, powdered and place in a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N NaOH then kept in oven at 60^0c for 1 hour then cool it and add 1 ml of 0.1N HCl then make up the volume up to 50ml with mobile phase, then place the sample in the vial and measure the chromatogram.

Thermal Degradation: Sample solution of Mirabegron (10µg/ml) was placed in oven at **105^0C** for 6 hr to study dry heat degradation. For UPLC study, the resultant solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSIONS:

Method Optimization: The developed method was optimized after many trials. The optimized method developed on C18(50mm x 2.1mm ID), 1.8µm as stationary phase. Using phosphate di-hydrogen buffer and methanol in the ratio 75:30 %v/v as mobile phase. The column temperature was maintained constantly at 35ºC. Mobile phase pumped with a flow rate of 0.5ml/min and injection volume is 10µl.

Table no. 1: Chromatographic conditions

Mobile phase	Potassium di-hydrogen phosphate : Methanol (75:30) %v/v
Column	Waters AcquityC18(50mm x2.1 mm ID) 1.8µm
Flow rate	0.5ml/min
Column temperature	35°C
Sample temperature	15°C
Wavelength	254 nm
Injection volume	10µl
Run time	5 min
Retention time	1.503 min (Mirabegron)

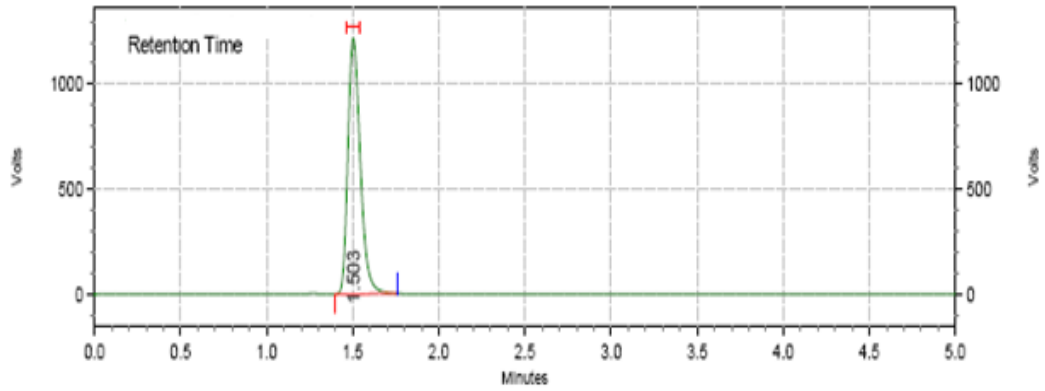

Fig no.1: chromatogram of optimized condition

Table no. 2: System Suitability

Results for system suitability of MIRABEGRON

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.497	105011137	2197	1.32
2	1.497	104237044	2209	1.28
3	1.497	104909445	2213	1.28
4	1.497	104025812	2216	1.32
5	1.497	103574883	2230	1.28
Mean	1.497	104351664.2	-	-
SD	0.00154	26716.59	-	-
%RSD	0.103	0.082	-	-

Table no. 3: Linearity

S.No	Parameter	MIRABEGRON
1	Correlation coefficient	0.997
2	Slope	97542
3	Intercept	48.04

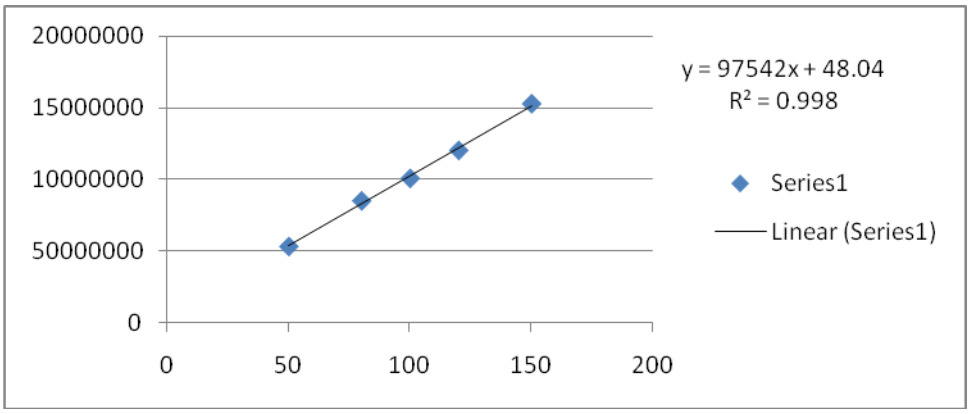

Fig.No.2 Linearity graph of MIRABEGRON

Table no. 4: Accuracy - Recovery results for MIRABEGRON

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	% Mean Recovery
50%	50	50.52	99.1	100.1
100%	100	98.45	101.7	
150%	150	149.15	99.5	

***** Mean of three observations

Specificity: Placebo solution was prepared and it was injected and the chromatogram was recorded.

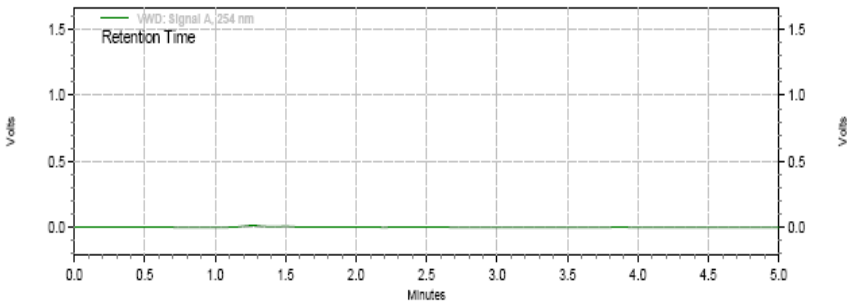

Fig.No.3 (a) Chromatogram of Blank

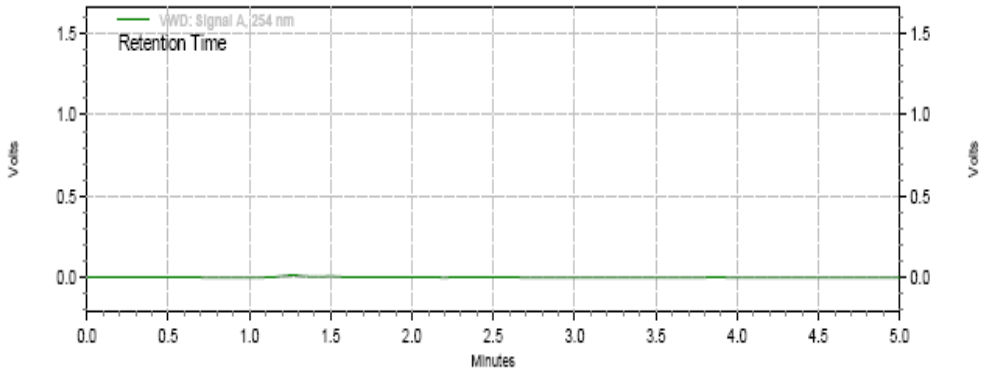

Fig.No.3 (b) Chromatogram of Placebo

Table no. 5: Precision

MIRABEGRON		
S. No.	Retention time	Area
1	1.343	32519212
2	1.343	32538125
3	1.343	32521241
4	1.343	32546751
5	1.343	32504681
6	1.347	32471581
avg	1.343	32516931.83
stdev	0.0016	26716.59
%RSD	0.12	0.082

Degradation studies: Peroxide degradation:

Fig.No.4

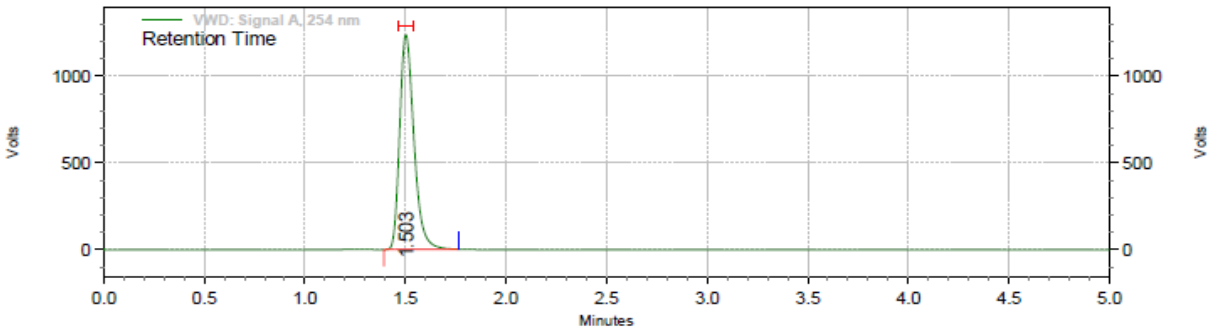

Table.No.6

S.NO	Name	RT	Area	TP	TF	R_S
1	Mirabegron	1.503	102246690	2852	1.02	-

Photolytic degradation: Fig.No.5

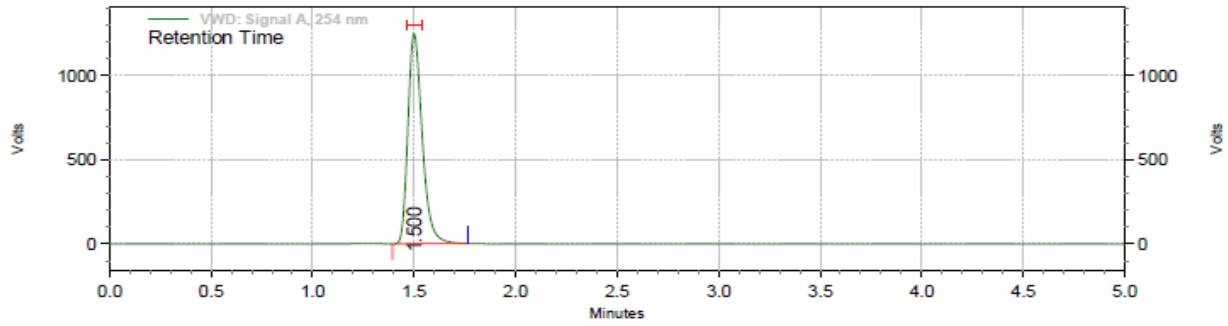

Table.No.7

S.NO	Name	RT	Area	TP	TF	R_S
1	Mirabegron	1.500	102307092	2752	1.06	-

Acidic Degradation:

Fig.No.6

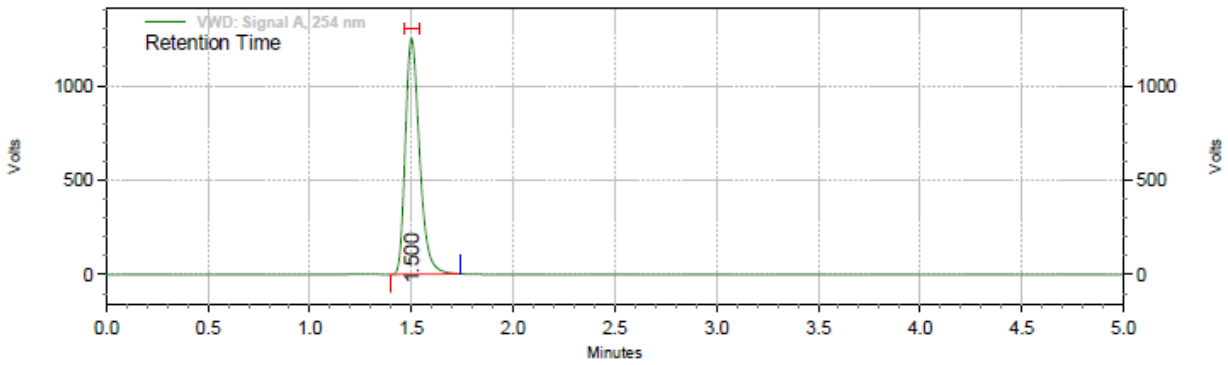

Table.No.8

S.NO	Name	RT	Area	TP	TF	R_S
1	Mirabegron	1.500	101775661	2850	1.10	-

Alkaline Degradation:

Fig.No:7

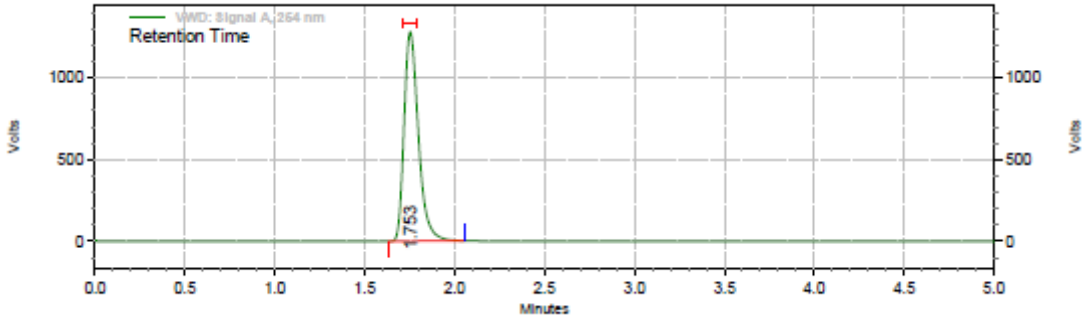

Table.No.9

S.NO	Name	RT	Area	TP	TF	R_S
1	Mirabegron	1.753	119028417	2817	1.19	-

Thermal Degradation:

Fig.No.8

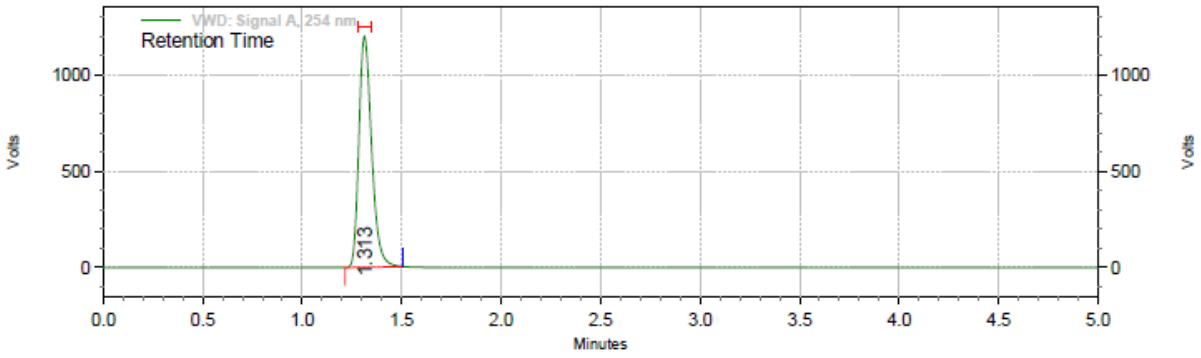

Table.No.10

S.NO	Name	RT	Area	TP	TF	R_S
1	Mirabegron	1.313	89391954	2775	1.11	-

System suitability: All system suitability parameters were passed which include the theoretical plates, tailing factor, resolution for Mirabegron. Table No.02.

Linearity: The best fit line was obtained with regression coefficient between the peak area vs concentration. Results are given below .Table No.03 and Fig No.2

Specificity: It was evaluated by injecting blank and placebo along with drug product, no interference was found at the components respective retention timings. Chromatogram depicted below Fig No.3a and 3b.

Method Precision: The Precision of the method was determined by injecting Mirabegron with sample solution 6 times respectively. Method precision was expressed in terms of % RSD. Results are given in table no.05.

Accuracy: Prepared accuracy at 3 levels in triplicate at 50%, 100% and 150% with matrix and achieved satisfactory results and at each level of recovery was calculated. Results are given in table No.04.

CONCLUSION:

A new precise, accurate, rapid method has been developed for the estimation of Mirabegron in pharmaceutical dosage form by RP-UPLC. The optimum wavelength for the determination of Mirabegron was selected at 254 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but Potassium di-hydrogen phosphate: Methanol (70:30) %v/v) was selected as good peak symmetry and resolution between the peaks was observed. The Retention timewas found to be 1.503. The retention time for the drug observed was considerably less compared to the retention time obtained for the drug in the other mobile phase. The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 50-150 µg/ml. From linearity the correlation coefficient R^2 value was found to be 0.998. The proposed UPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The percentage recovery was found to be 100.1 % shows that the proposed method is highly accurate. Hence the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulation of Mirabegron in Educational institutions and Quality control laboratories.

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