Development and validation of a UPLC method for determination of Mirabegron in tablet dosage forms.

Abstract:

Mirabegron is a drug used for the treatment of overactive bladder.Mirabegron activates the β_3 adrenergic receptor in the detrusor muscle in the bladder, which leads to muscle relaxation and an increase in bladder capacity. Several methods have found for quantification but those are not cost effective and time consuming. The present study developed simple, precise, accurate and cost effective UPLC method to determine mirabegron quantity in tablet dosage forms. A simple and selective UPLC method is described for the determination of mirabegron Chromatographic separation was achieved on a Acquity BEH C18 (50*3.0mm. 1.7µm) using mobile phase consisting Potassium di hydrogen phosphate: Methanol(70:30) v/v with detection of 254 nm. Linearity was observed in the range 50-150 µg /ml for mirabegron (r^2 = 0.997). The amount of drugs estimated by the proposed method was in good agreement with the label claim. The proposed method wasvalidated as per ICH guidelines and applied for the determination of cited drug in dosage form.

Key Words: UPLC, Mirabegron, Potassium di hydrogen phosphate, Methanol.

Introduction:

Mirabegron is chemically 2-(2-Amino-1,3-thiazol-4-yl)-*N*-[4-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide.

Mirabegron has molecular weight.:396.506 g/mol and molecular formula: $C_{21}H_{24}N_4O_2S$. is a drug for the treatment of overactive bladder. It was developed by Astellas Pharma and was approved in the United States in July 2012.[1]

Mirabegron activates the β_3 adrenergic receptor in the detrusor muscle in the bladder, which leads to muscle relaxation and an increase in bladder capacity.

The dose of Mirabegron present in the formulation were determined by using Ultra Performance Liquid Chromatography method. UPLC has greater sensitivity; resolution and speed of analysis. UPLC operates at high pressure than HPLC and fine particles i.e., less then 2.5 µm are used and mobile phases at high linear velocities decreases the length of column, reduces solvent consumption and saves time.[2]

The UPLC is based on the use of stationary phase consisting of particles less than 2.5 µm where as the HPLC column are typically filled with 3-5 µm particles. The principle of this evolution is governed by the Van Deemeter equation, which is an empirical formula that describes the relationship between linear velocity of flow rate and plate height.[3,4]

$H=A+B/v+Cv$

Where;

A, *B* and *C* are constants

v is the linear velocity, the carrier gas flow rate.

*The *A* term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform

The *B* term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by *v*.

* The *C* term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus term is proportional to *v*.

Therefore it is possible to increase throughput, and thus the speed of analysis without affecting the chromatographic performance. The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size.[5,6]

This technology has advantage of chromatographic principles to run seperations using packed column with similar particle size less than 2.5 µm are used with high flow rates speed gives superior resolution and sensitivity.

Materials and Methods:

Chemicals and Reagents: Drug standard of mirabegron were kindly supplied by Madras Pharmaceuticals, Chennai with certified purity of 99.97 ± 0.512.**MIRAGO** 25mg (Extended Release Tablets)Tablets were purchased from apollo pharmacy, Hyderabad. HPLC grade acetonitrile, water and methanol were obtained from Rankem. Analytical grade Potassium Dihydrogenortho phosphate, Dipotassium hydrogen orthophosphate and O-Phosphoric acid were obtained from Merck.

A Schimadzu (UV-1800) double beam UV-Vis spectrophotometer with 1cm quartz cuvette connected to a personal computer loaded with UV probe 2.21 software were used.

Chromatographic Method: Chromatographic separation was achieved by UPLC-agilent 1290 infinity with quaternary solvent manager, with autosampler injector and photo diode array detector, coupled with Empower software for data acruisition. Acquity BEH C18 (50*3.0mm. 1.7µm) was used as the stationary phase for the development of the chromatographic separation, optimization, and method validation.Isocratic elution was conducted using a mobile phase Potassium di hydrogen phosphate: Methanol(70:30) v/v. The flow rate was set at 0.5mL/min. Column temperature was adjusted at 25°C and samples were injected at 10µL injection volume with run time 5min at a temperature 10°C and determined at a wavelength of 254nm[8,9]. Mirabegron 1µg/mL stock solution was prepared for UPLC method by dissolving 10mg of Mirabegron in 100mL of mobile phase.

Preparation of potassium di hydrogen phosphate: 1.35 g of potassium dihydrogen orthophosphate was dissolved and made up to 100 ml with distilled water

Results and discussion:

UPLC Method Development:[7,8]The main target of the proposed UPLC method was to achieve separation of Mirabegronwithin short runtime. To determine the stationary phase (Acquity BEH C18 (50*3.0mm. 1.7µm)) column was chosen because it provided better peak symmetry. For

organic modifier, different ratio of Potassium di hydrogen phosphate: Methanolwere checked. It was found that Potassium di hydrogen phosphate found better resolution. Mobile phase ratio was found to be a mixture of Potassium di hydrogen phosphate: Methanol(70:30) v/v. Flow rate at 0.5mL/min was selected as optimum flow rate. The optimum wavelength for detection was 254nm. The retention time was 1.503 min respectively.According to the ICH guidelines, the system sustainability tests should be carried out prior to analysis. Several parameters were studied including: tailing factor, retention time, height equivalent to theoretical plates and RSD% of peak area for repetitive injections were studied. In all deliberately varied chromatographic conditions, the chromatogram of solution showed satisfactory resolution as shown in fig 1 and results shown in table 1.

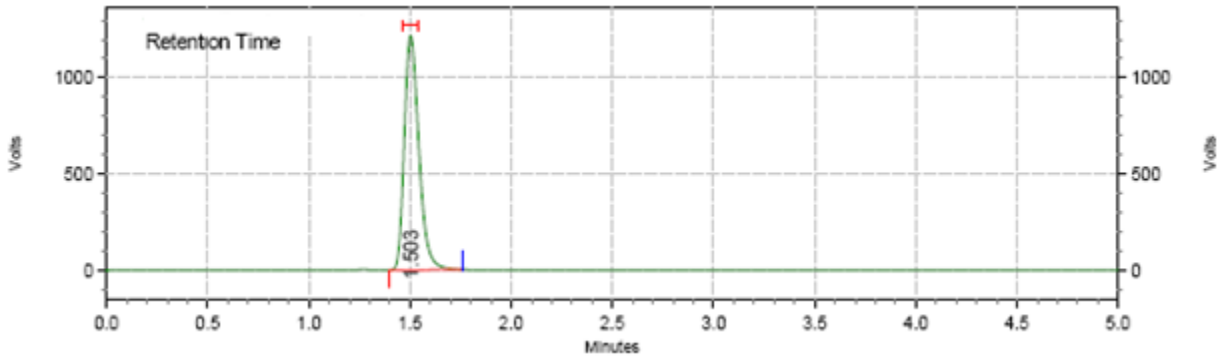

Fig 1.UPLC Chromatogram of MIRABEGRON

S.NO	Name	RT
1	Mirabegron	1.503

Table 1.UPLC Chromatogram of MIRABEGRON

Validation of Proposed Methods: The developed method was validated as per ICH guidelines.

Linearity and concentration range:[9]Aliquots equivalent to 50- 150 µg/mL of working solution (1mg/mL) of mirabegron were transferred in to 10mL volumetric flask and the volume was diluted with the mobile phase. The linearity values were summarized in table 2. The correlation coefficient R^2 was determined and was found to be 0.99 for MIRABEGRON were

given in table 3.The linearity graph shown in fig2 and the chromatograms are shown in fig 3-7

S. No.	Conc.(µg/ml)	Area
1	50	53446942
2	80	85170460
3	100	100756191
4	120	120169271
5	150	152596544

***Table 2*:** Linearity data of MIRABEGRON

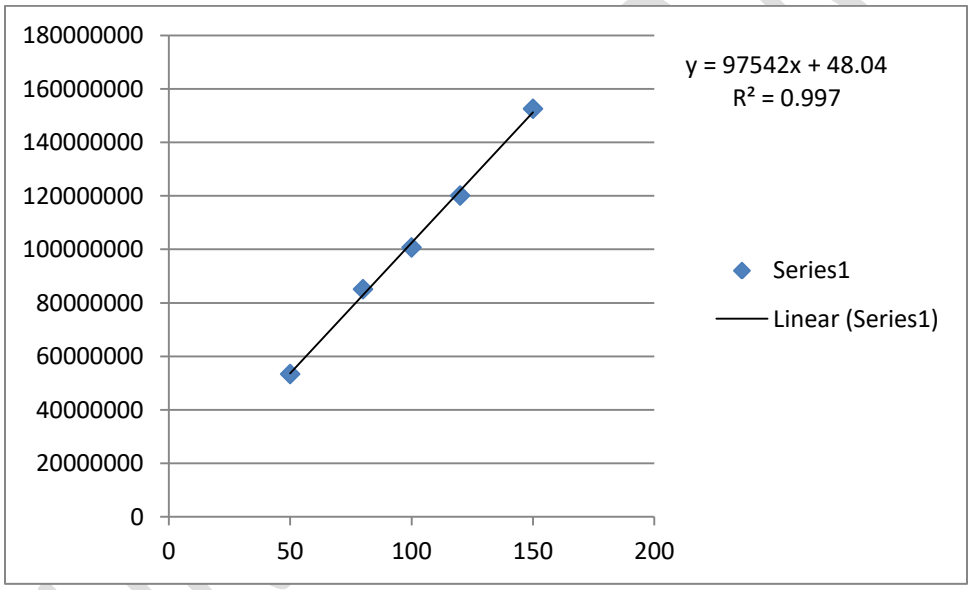

***Fig 2*:** Graph for Linearity data of MIRABEGRON

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

3	Intercept	48.04

***Table 3*:** Linearity results of Mirabegron.

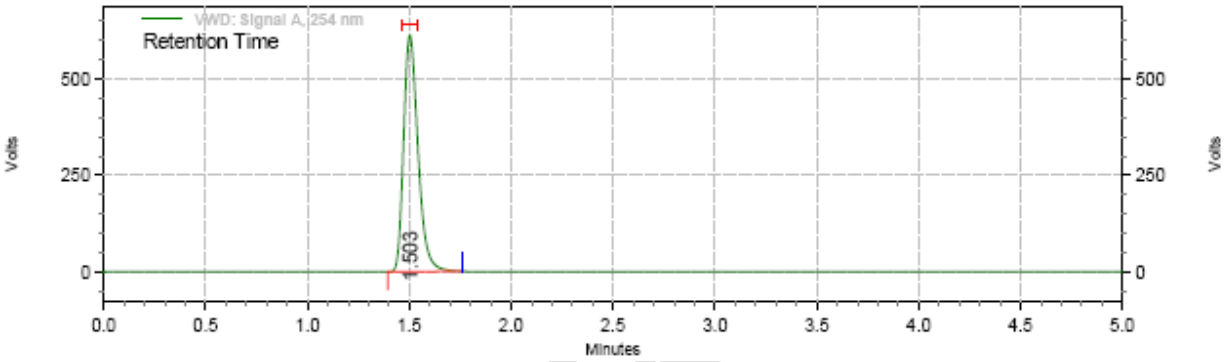

Fig. 3: Chromatogram of MIRABEGRON preparation-1

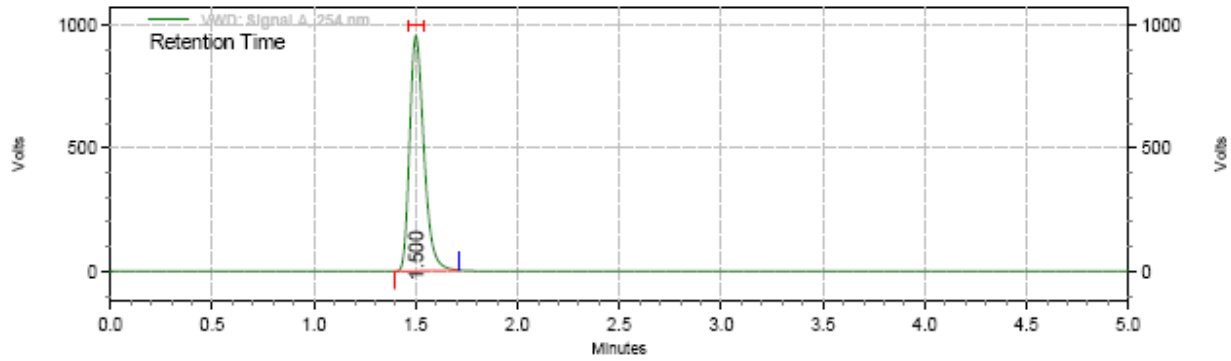

Fig. 4: Chromatogram of MIRABEGRON preparation-2

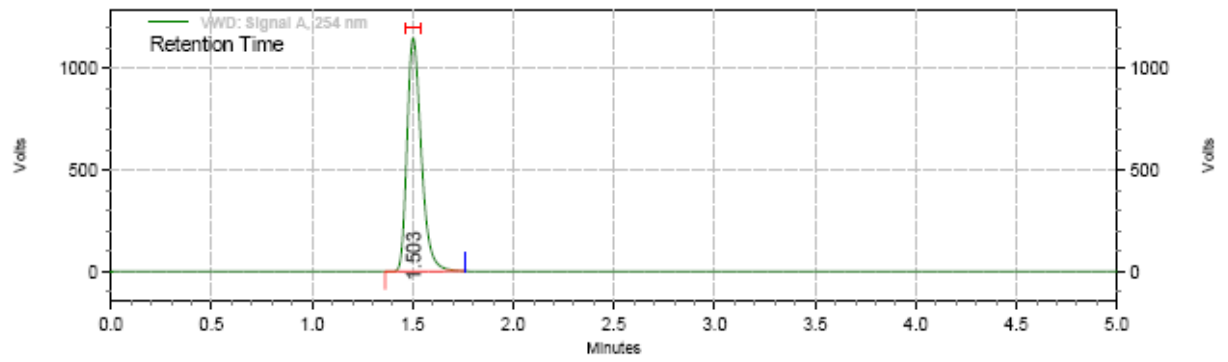

Fig. 5: Chromatogram of MIRABEGRON preparation-3

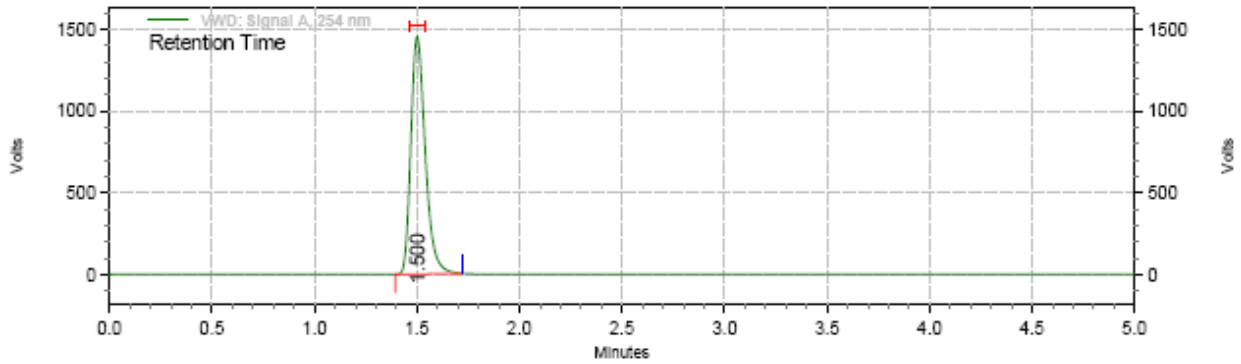

Fig.6: Chromatogram of MIRABEGRON preparation-4

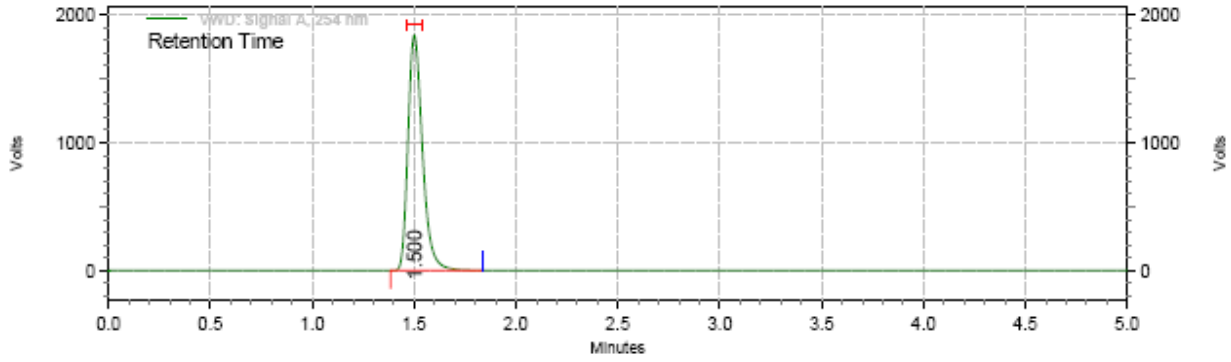

Fig.7: Chromatogram of MIRABEGRON preparation-5

System Suitability&Method precision:[10]The system suitability was evaluated by giving mirabegron injection five times and the chromatograms were recorded. The results were

summarised in table 4.The plate count and tailing factor results were found to be within the limits.The method precision chromatograms were recorded and the results were summarized in Table 5.

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.	-	-
SD	0.00154	26716.59	-	-
%RSD	0.103	0.082	-	-

***Table 4*:**Results for system suitability of mirabegron

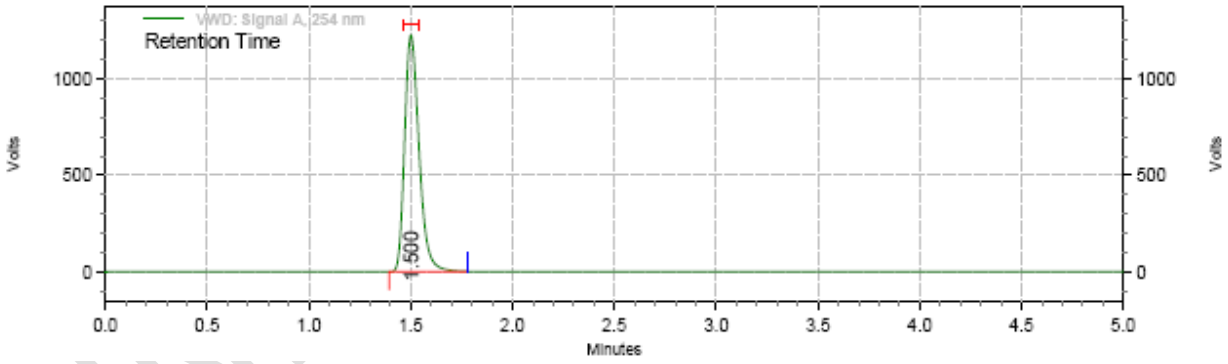

Fig. 8: Chromatogram of precision injection 1

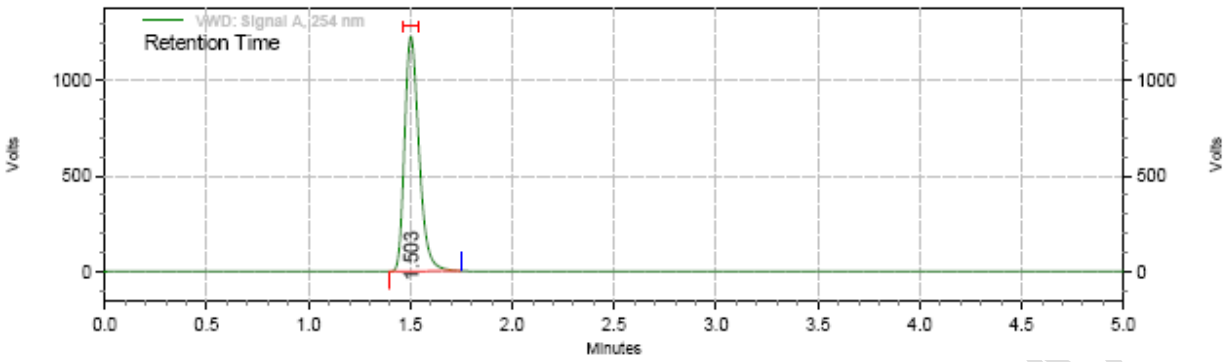

Fig. 9: Chromatogram of precision injection 2

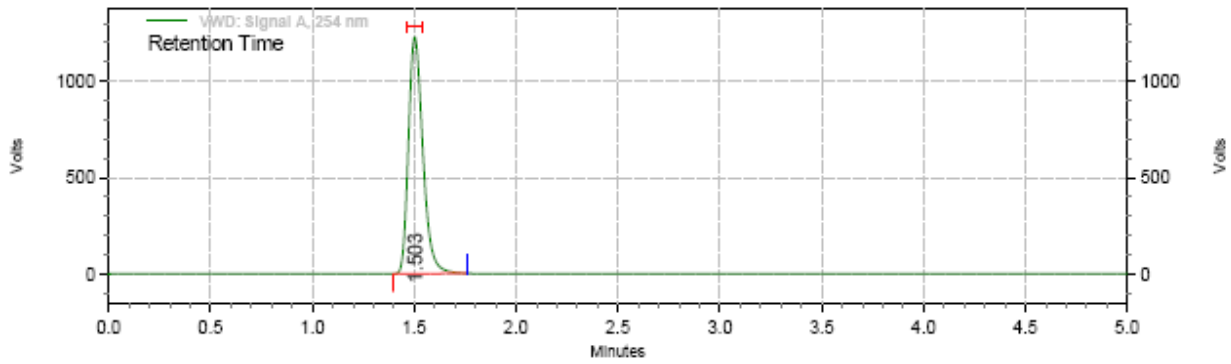

Fig. 10: Chromatogram of precision injection 3

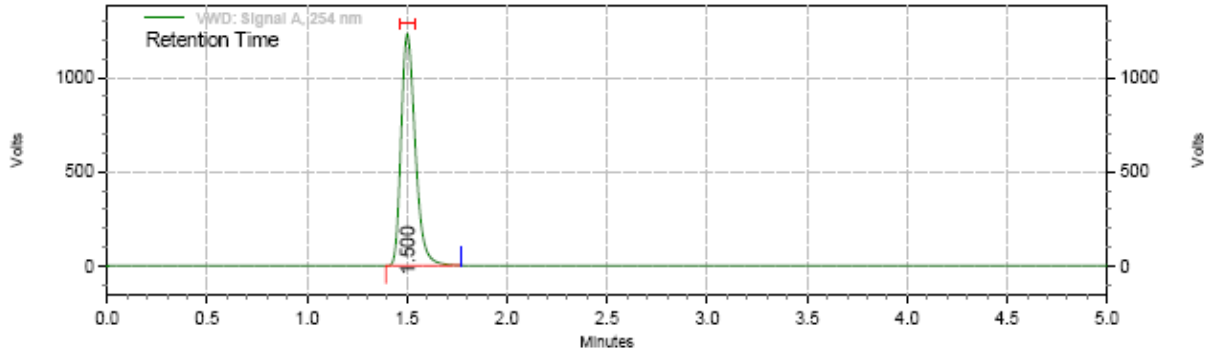

Fig. 11: Chromatogram of precision injection 4

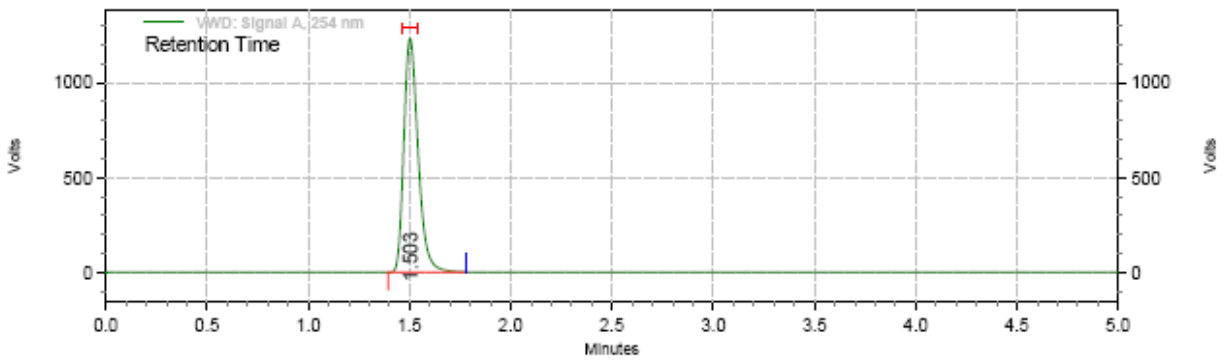

Fig. 12: Chromatogram of precision injection 5

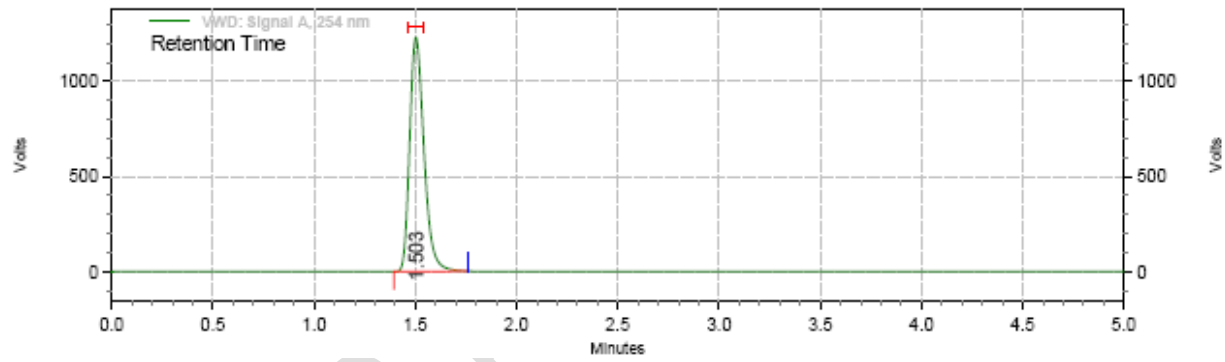

Fig. 13: Chromatogram of precision injection 6

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

***Table 5*:** Method precision results for Mirabegron

Specificity: A chromatograms of blank and placebo solutions had shown no peaks at the retention times of mirabegron. It indicates that diluent or excipient peaks do not interfere with the mirabegronpeak. The chromatograms are shown in fig 14 and fig 15

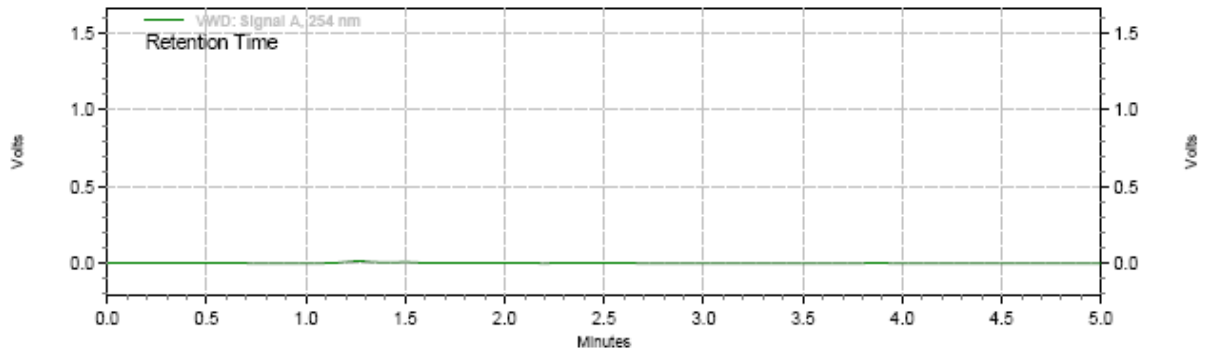

Fig 14:Chromatogram of Blank

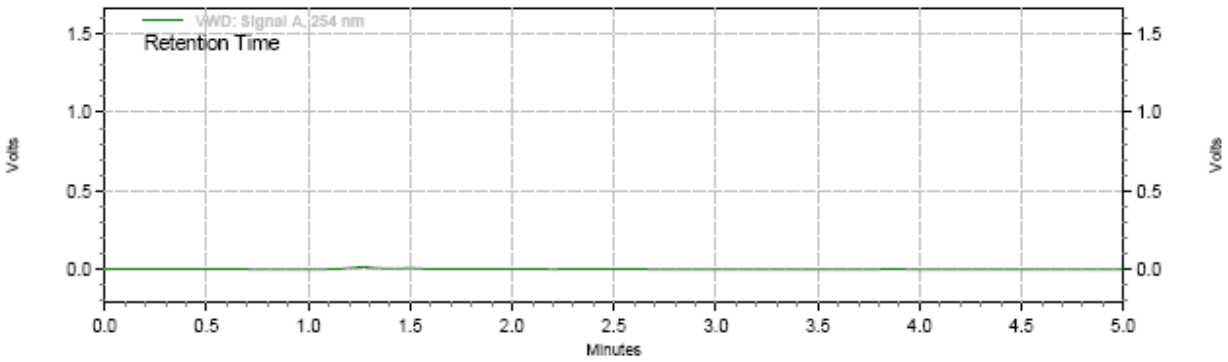

Fig 15:Chromatogram of Placebo

Accuracy[11]: The accuracy of the proposed method were determined by analyzing three different laboratory preparations of mirabegron in different ratios within the linearity range. The values of mean percentage recoveries were shown in table 6.

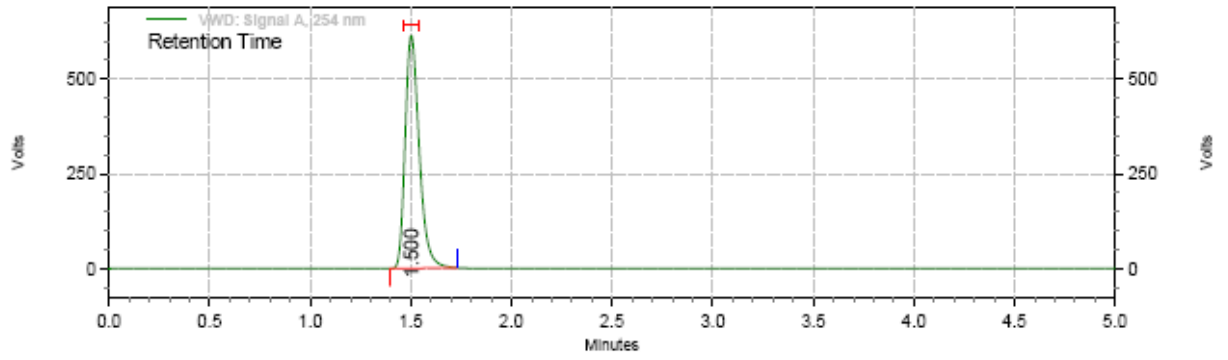

Fig. 16: Chromatogram of 50% recovery (injection 1)

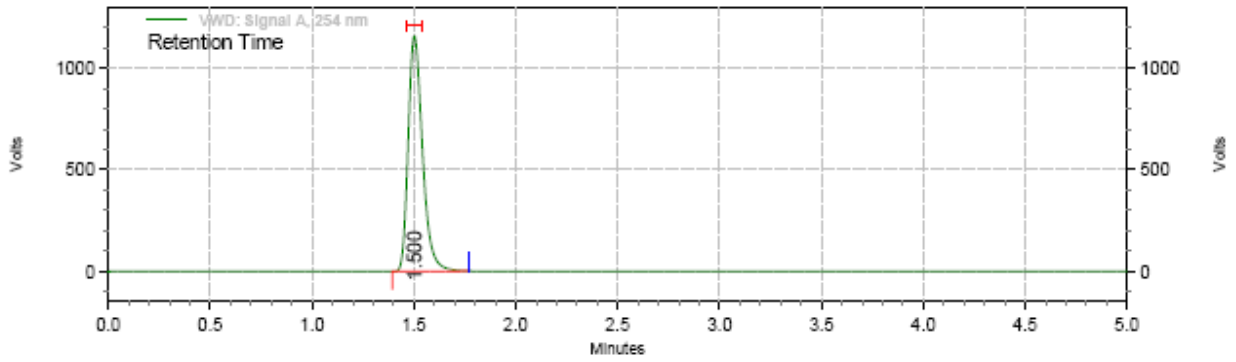

Fig. 17: Chromatogram of 100% recovery (injection 1)

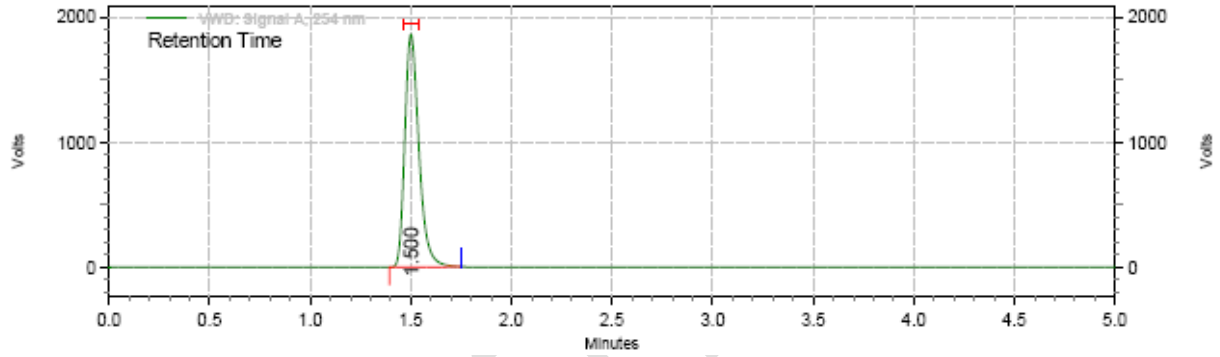

Fig. 18: Chromatogram of 150% recovery (injection 1)

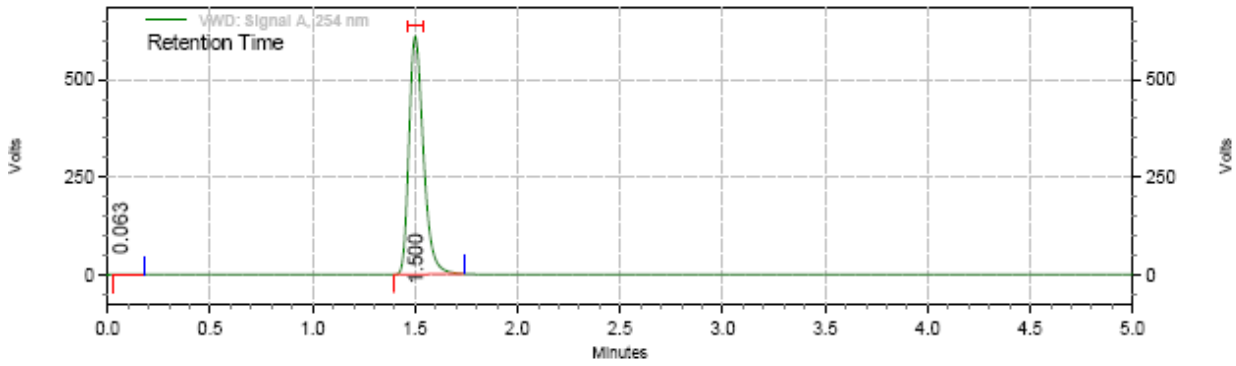

Fig. 19: Chromatogram of 50% recovery (injection 2)

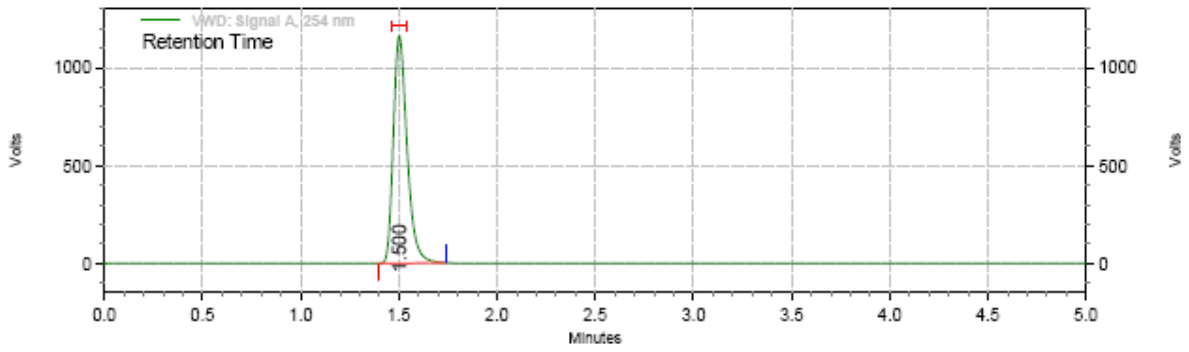

Fig. 20: Chromatogram of 100% recovery (injection 2)

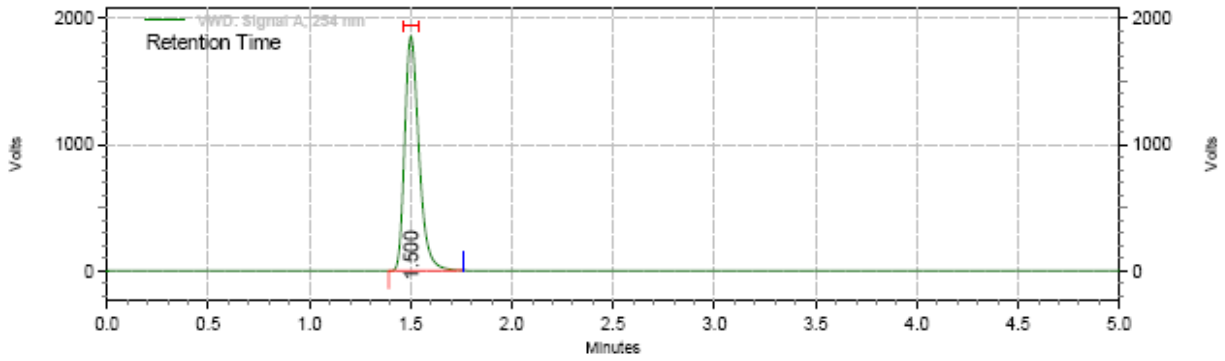

Fig. 21: Chromatogram of 150% recovery (injection 2)

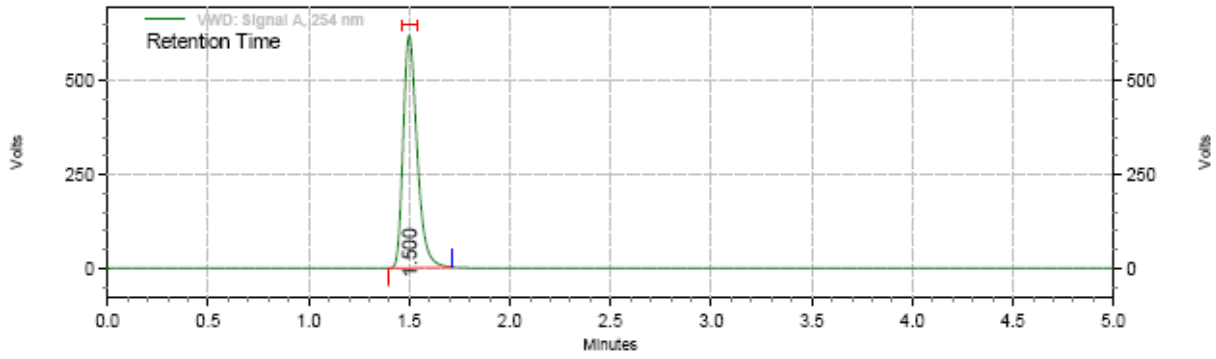

Fig. 22: Chromatogram of 50% recovery (injection 3)

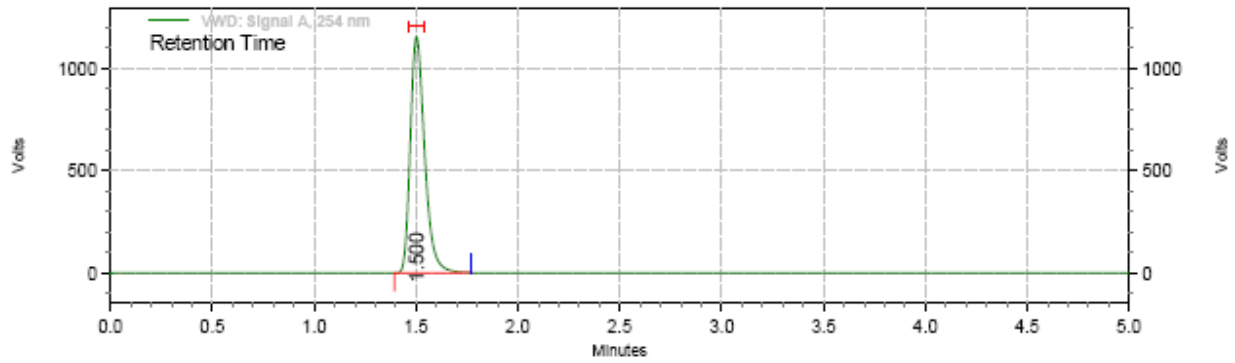

Fig. 23: Chromatogram of 100% recovery (injection 3)

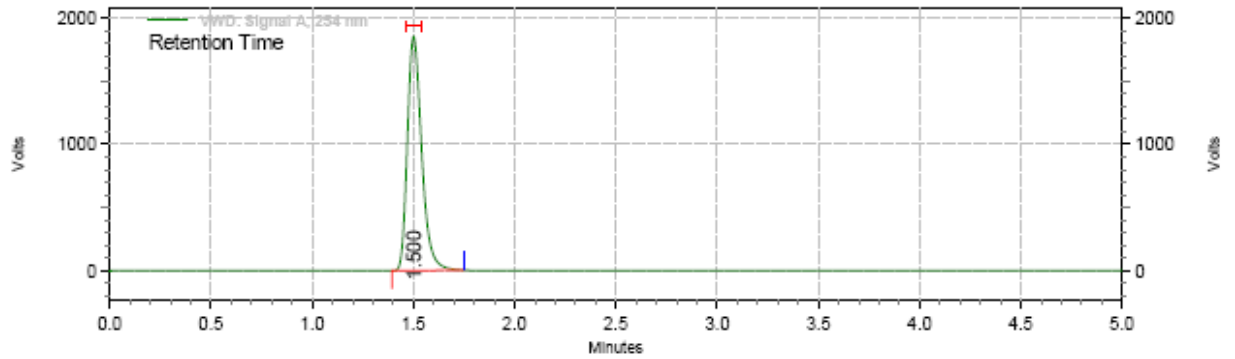

Fig. 24: Chromatogram of 150% recovery (injection 3)

%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	

***Table 6*:** Results for Recovery of Microbegron

LIMIT OF DETECTION (LOD)& QUANTITATION (LOQ):[12,13]

According to ICH guidelines LOD and LOQ can be calculated using the standard deviation of the response and the slope. LOD = 3.3* σ/S and LOQ = 10* σ/S. Where, σ = the standard deviation of the response and S = the slope of the calibration curve. LOD and LOQ of the drug were found to be 0.11µg/mL and 0.34µg/mL respectively.

Robustness[14]**:** The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table 7.

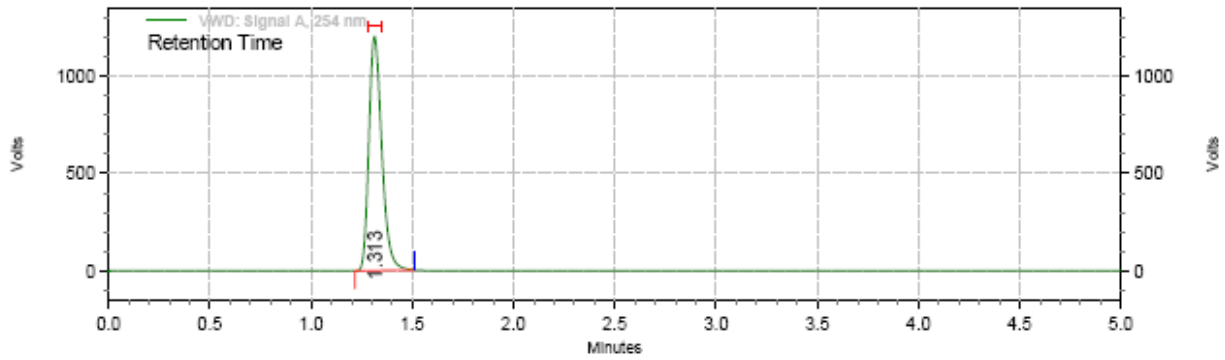

Fig. 25: Chromatogram of flow rate from 0.5mL/min0.6mL/min.

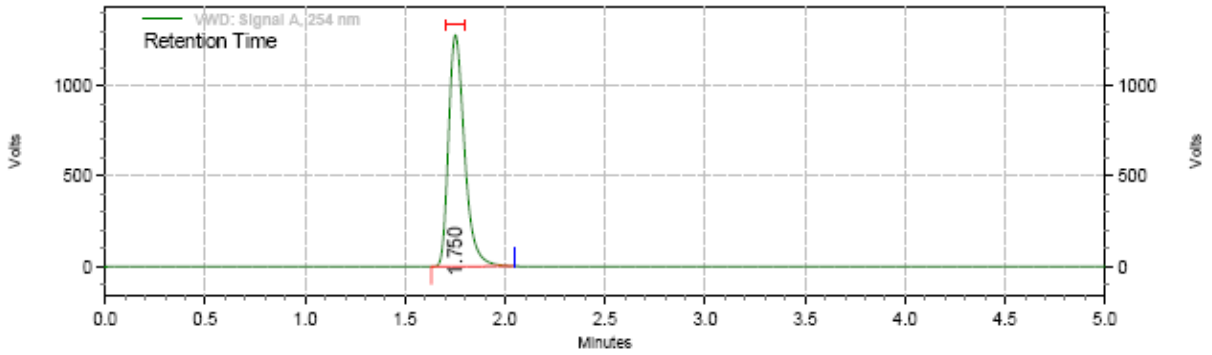

Fig. 26: Chromatogram of flow rate from 0.5mL/min to 0.4mL/min.

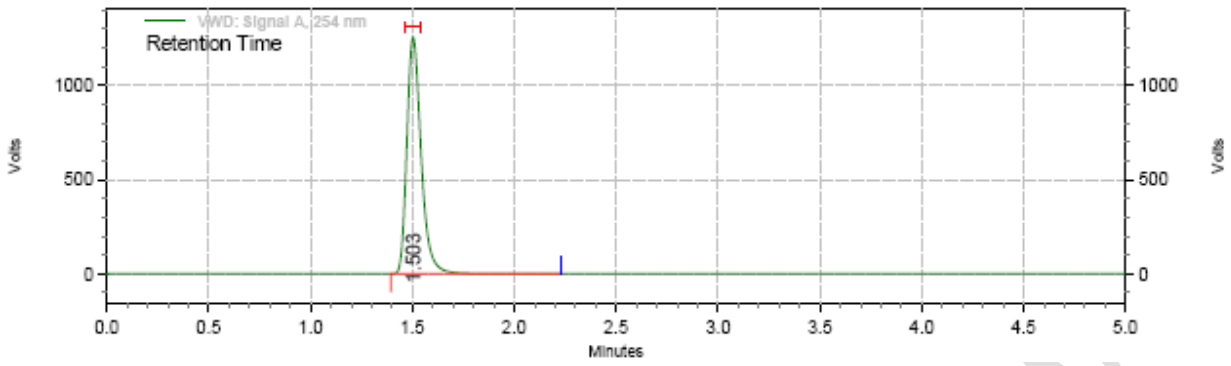

Fig. 27: Chromatogram of Temperature Variation from 30°C to 35°C

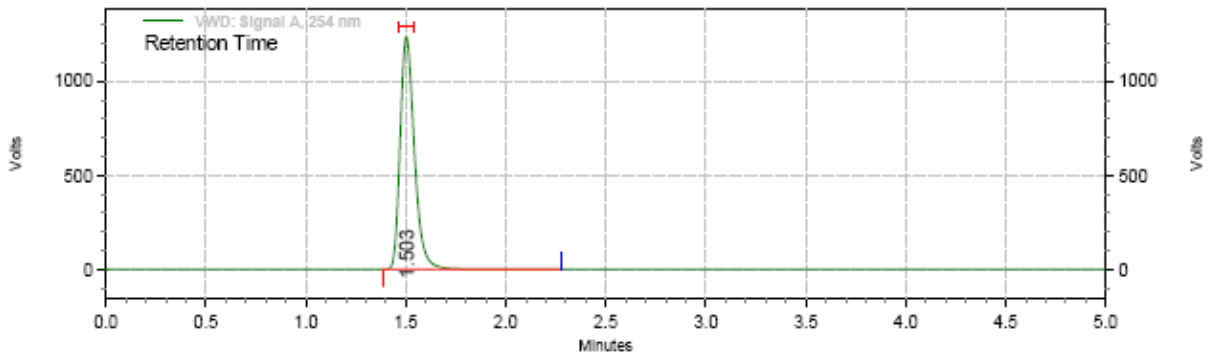

Fig. 28: Chromatogram of Temperature Variation from 30°C to 25°C

Chromatographic changes		**Theoretical Plates**	**Tailing factor**	**% RSD**
Flow rate (mL/min)	0.4	3431	1.69	0.2
	0.6	2571	1.66	0.08
Temperature	25	3055	1.76	0.03

(°C)	35	3082	1.78	0.1

***Table 7*:** Results for Robustness of mirabegron

Ruggedness: The ruggedness of the method was studied by the determining the analyst-to-analyst variation by performing the Assay by two different analysts.The method is rugged and the results are summarized in table 8.

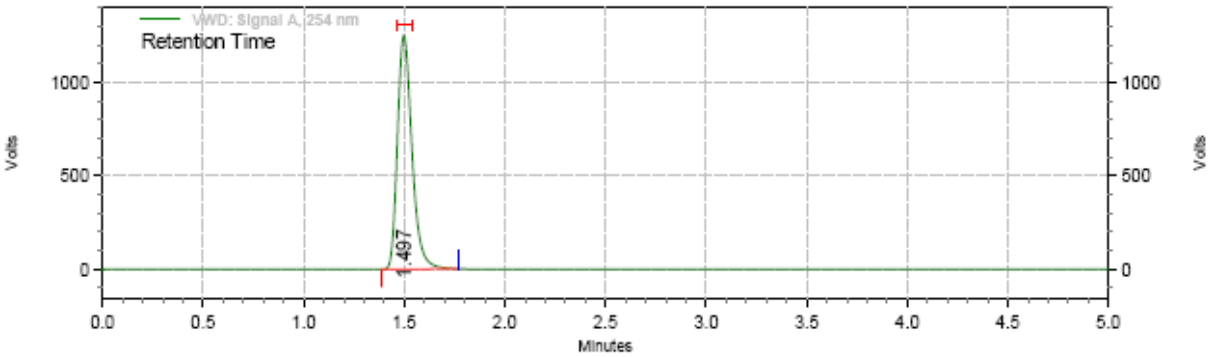

Fig. 29: Chromatogram of Analyst 01 standard preparation

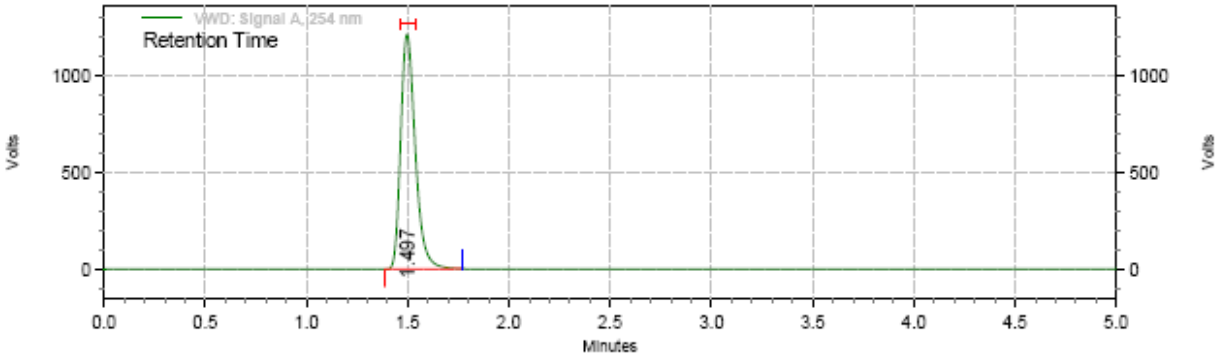

Fig. 30: Chromatogram of Analyst 01 sample preparation

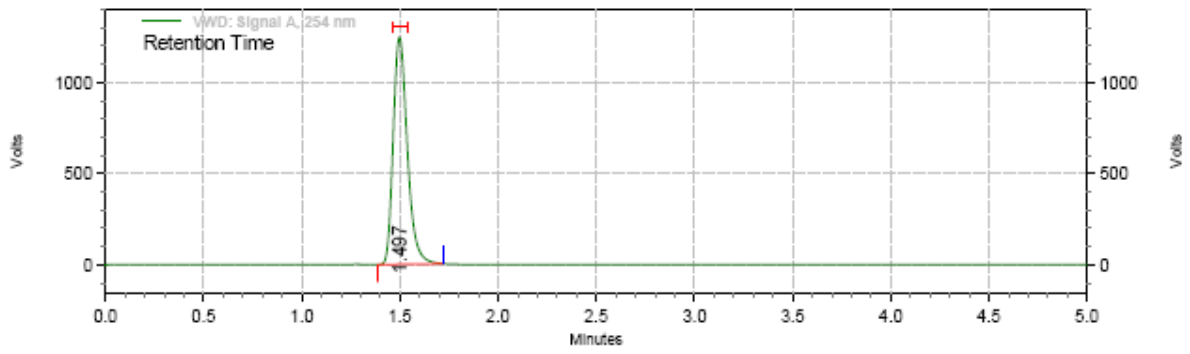

Fig. 31: Chromatogram of Analyst 02 standard preparation

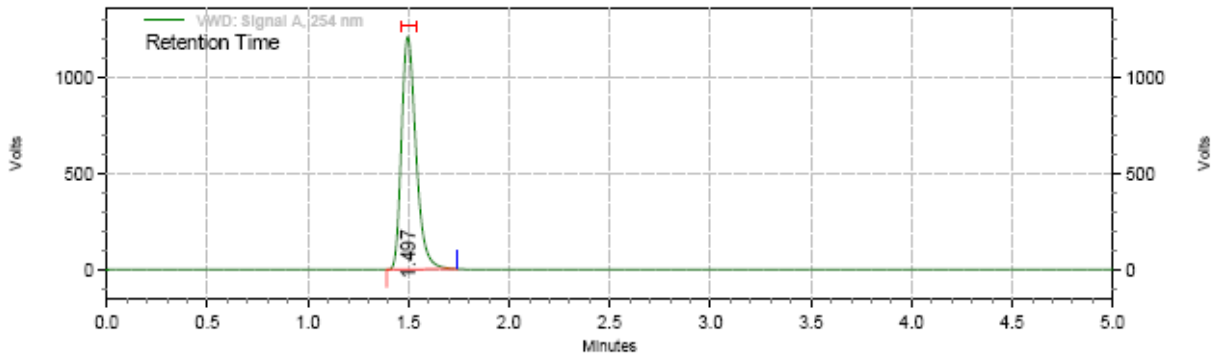

Fig. 32: Chromatogram of Analyst 02 sample preparation

MIRABEGRON	**%Assay**
Analyst 01	99.6

Anaylst 02	100.52
% RSD	0.57

***Table 8*:** Ruggedness Results of mirabegron

Analysis of Pharmaceutical dosage forms[15]**:** The proposed method was applied for the determination of mirabegron in pharmaceutical dosage form mirago 25mg tablets, without interference from the excipients and good recoveries were obtained by applying the standard addition technique. The results were summarized in table 9 and 10.

MIRABEGRON		
	Standard Area	**Sample Area**
Injection-1	102102014	102262822
Injection-2	102433207	101950528
Injection-3	101915558	101670222
Injection-4	101721781	101998023
Injection-5	101795941	101853817
Average Area	101993700.2	101947082.4
Assay(%purity)	**99.95**	

***Table 9*:** Results of Mirago dosage form

Drug	**Label claim(mg)**	**Amount found(mg)**	**% Assay**
MIRABEGRON	25	24.5	98

***Table 10*:**Results of assay

CONCLUSION:A new precise, accurate, rapid method has been developed for the estimation of Mirabegron pharmaceutical dosage form by UPLC. The cited drug was analyzed without any interference from excipients indicates the selectivity of the method. The proposed method is highly sensitive, precise and accurate as indicated from % recovery, % mean recovery and % RSD values respectively. From the results it indicates that the UPLC method is applicable to assay of this antiviral drug with minimum sample preparation, cost and time effectiveness with satisfactory level of accuracy and precision. Hence it is successfully applied for the quantification of API content in the commercial formulations ofMirabegron in educational institutions and Quality control laboratories. UPLC method is economic, faster, consumes less mobile phase than HPLC it indicates it is faster and eco-friendly.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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