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Development and Validation of a UPLC Method for Determination of Mirabegron in Tablet Dosage Forms

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Authors' contributions

This work was carried out in collaboration among all authors. 'All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Mirabegron is a drug that is used to treat an overactive bladder. Mirabegron activates the β3 adrenergic receptor in the bladder's detrusor muscle, causing muscular relaxation and bladder capacity expansion. A few measurement procedures have been discovered, but they are inconvenient and time-consuming. The current study developed a simple, exact, precise, and practical UPLC technique for determining the amount of Mirabegron in tablet dosage forms. For the verification of Mirabegron, a simple and specific UPLC approach is applied. On an Acquity BEH C18 (50*3.0mm. 1.7m), chromatographic separation was achieved using a versatile stage containing Potassium di hydrogen phosphate: Methanol(70:30) v/v with a detection wavelength of 254 nm. Mirabegron (r2 = 0.997) showed linearity in the 50-150 g/ml range. The amount of Mirabegron to be employed by the suggested technique matches the label exactly. The proposed technique was validated according to ICH guidelines and used to ensure that the correct amount of medication was present in the dosage form.

Keywords: UPLC; Mirabegron; potassium di hydrogen phosphate; methanol.

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1. INTRODUCTION

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

The molecular weight of Mirabegron is 396.506 g/mol, and its chemical formula is C21H24N4O2S. It's a medicine that's used to treat overactive bladder. It was developed by Astellas Pharma and approved by the FDA in July 2012 [1].

Mirabegron activates the β3-adrenergic receptor in the bladder's detrusor muscle, causing muscular unwinding and bladder capacity expansion [2].

UPLC is used to determine the amount/dose of Mirabegron present in the formulation [3,4]. Ultra Performance Liquid Chromatography's key advantage is that it offers higher sensitivity, resolution, and speeds up the analytical process. UPLC uses high pressure (less than 2.5 m) and high linear velocities for the mobile phases, resulting in shorter column lengths, lower solvent use, and time savings [5,6].

The stationary phase, which consists of particles less than 2.5 m, is responsible for the UPLC's operation. The Van Deemeter equation, which is an empirical formula that gives the link between linear velocity of flow rate and plate height, monitors the principle of this progression [6,7].

H=A+B/v+Cv

Where;

Constants A, B, and C
The linear velocity, or carrier gas flow rate, is denoted by v.

*The A word denotes "eddy" mixing and is independent of velocity. It is made up of the tiniest, most uniformly packed column particles.
*The B word refers to axial diffusion, or the natural tendency of molecules to diffuse. Because the effect of large flow rates is reduced, the term is divided by v.

* The C word refers to the kinetic resistance in the separation process due to equilibrium. The time lag associated in transitioning from the gas phase to the packing stationary phase and return is referred to as kinetic resistance. The molecule on the packing tends to lag behind the molecules in the mobile phase as the gas flow increases. As a result, term equals to or proportional to v.

As a result, the amount of throughput, the speed of analysis, can be increased without affecting the chromatographic performance. The introduction of UPLC requires the development of a new liquid chromatography apparatus is capable of improving separation performance (by lowering dead volumes) while remaining pressure-independent (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). The effectiveness is related to the length of the column, while the particle size is inversely proportional [7,8].

2. MATERIALS

2.1 Chemicals and Reagents

Madras Pharmaceuticals, Chennai, provided the standard medication of Mirabegron with a purity certificate of 99.97 ± 0.512. Tablets of MIRAGO 25mg (Extended Release Tablets) were obtained from apollo pharmacy in Hyderabad. Rankem provided the HPLC with acetonitrile, water, and methanol. Merck provided potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, and Analytical grade O-phosphoric acid.

A Schimadzu (UV-1800) double beam UV-Vis spectrophotometer with a 1cm quartz cuvette was connected to a PC that had UV probe 2.21 was installed [9,10].

The chromatographic separation was achieved using a UPLC-agilent 1290 infinite with quaternary solvent manager, an autosampler injector, and a light diode array detector, as well as the program Empower for data acquisition. The Acquity BEH C18 (50*3.0mm. 1.7m) is used for the stationary phase of chromatographic separation development, as well as optimization and technique validation. The mobile phase of potassium di hydrogen phosphate: Methanol(70:30) v/v was used for isocratic elution. The flow rate was changed from 0.5mL/min to 1mL/min. The temperature of the column was raised to 25°C, and samples were injected at a volume of 10L with a run period of 5 minutes at a temperature rise of 10°C and measured at a wavelength of 254nm [11,12]. For the UPLC technique, a Stock solution of Mirabegron 1g/mL was produced by dissolving 10mg of Mirabegron in 10mL of mobile phase.

Preparation of potassium di hydrogen phosphate: Potassium dihydrogen phosphate was prepared by dissolving 1.35 g of potassium dihydrogen orthophosphate in 100 mL of purified water.

3. RESULTS AND DISCUSSION

UPLC Method Development: [10,11] The main purpose of the described UPLC approach is to achieve Mirabegron separation in a short amount of time. Because it produces a greater peak of symmetry, the Acquity BEH C18 (50*3.0mm. 1.7m) column was chosen to calculate the stationary phase. Different amounts of potassium di hydrogen phosphate: methanol were tested for organic modifier. The mobile phase of potassium di hydrogen phosphate had greater resolution, and the mobile phase ratio was determined to be (70:30) v/v, with a flow rate of 0.5mL/min being chosen as the best flow rate. The detecting wavelength that worked best was 254nm. The average retention time was 1.503 minutes. The system sustainability tests should be performed before any analysis, according to the ICH recommendations. The tailing factor, retention time, and other factors were investigated.several factors were investigated, including tailing factor, retention period, height corresponding to theoretical plates, and RSD percent of peak area. The chromatogram of solution showed satisfactory resolution in all of the different chromatographic conditions, as shown in Fig. 1 and Table 1.

Table 1. UPLC Chromatogram of MIRABEGRON

S.NO	Name	RT
1	Mirabegron	1.503

Validation of Proposed Methods: The ICH guidelines were used to validate the developed method.

Linearity and concentration range: [12] The working solution of Mirabegron (1 mg/mL) was transferred to a 10 mL volumetric flask, and the volume was diluted with the mobile phase to form aliquots of 50-150 g/mL. Table 2 shows the linearity values. Table 3 shows the correlation coefficient R2 for MIRABEGRON, which was determined to be 0.99. Figs. 2 and 3&4 show the linearity graph and chromatograms, respectively.

System Suitability&Method precision: [13] The efficacy of the technology was determined by injecting Mirabegron five times and recording the chromatograms. Table 4 summarises the findings. The findings of the plate count and tailing factor were found to be within the recommendations' limits. The results of the procedure precision chromatograms were obtained and presented in Table 5.

Table 2. Linearity data of MIRABEGRON

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

Table 3. Linearity results of MIRABEGRON

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

Specificity: There were no peaks seen at the retention periods of Mirabegron in chromatograms of blank and placebo solutions.

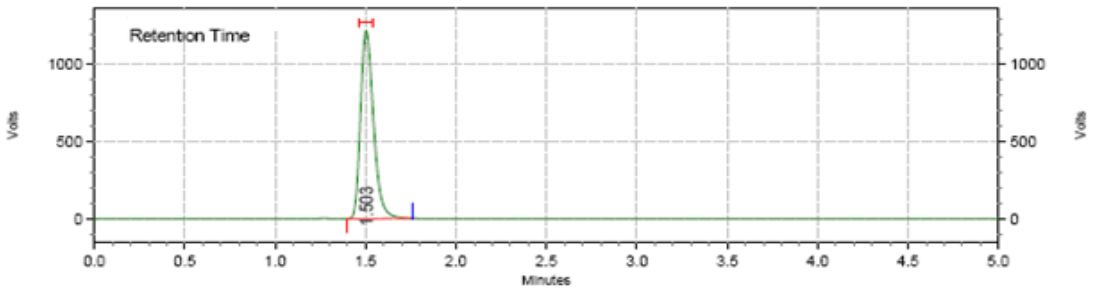

Fig. 1. UPLC Chromatogram of MIRABEGRON

It means that the peaks of the diluent or excipient do not interact with the Mirabegron peak. Figs. 3 and 4 depict the chromatograms.

Accuracy [14]: The proposed approach was put to the test by evaluating three different Mirabegron laboratory preparations in various ratios within the linearity range. Table 6 shows the values of mean percentage recoveries.

4. LIMIT OF DETECTION (LOD)& QUANTITATION (LOQ) [15,16]

Using the ICH standards, LOD and LOQ can be determined using the response's standard deviation and slope. Where, 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 [17,18]: The product's robustness was determined in accordance with the guidelines. Table 7 summarises the results achieved by deliberate change in process parameter.

Ruggedness: The ruggedness is investigated by calculating the analyst-to-analyst variation, which means that two separate analysts execute the ruggedness process according to the usual procedure. Table 8 summarises the outcomes of the technique, which is robust.

Analysis of Pharmaceutical dosage forms [18]**:** The Mirago 25 mg tablets were used to determine Mirabegron in medicinal dosages according to the defined standard procedure for the established process, with the findings indicating that there was no interference from excipients and good recoveries. Tables 9 and 10 summarised the findings.

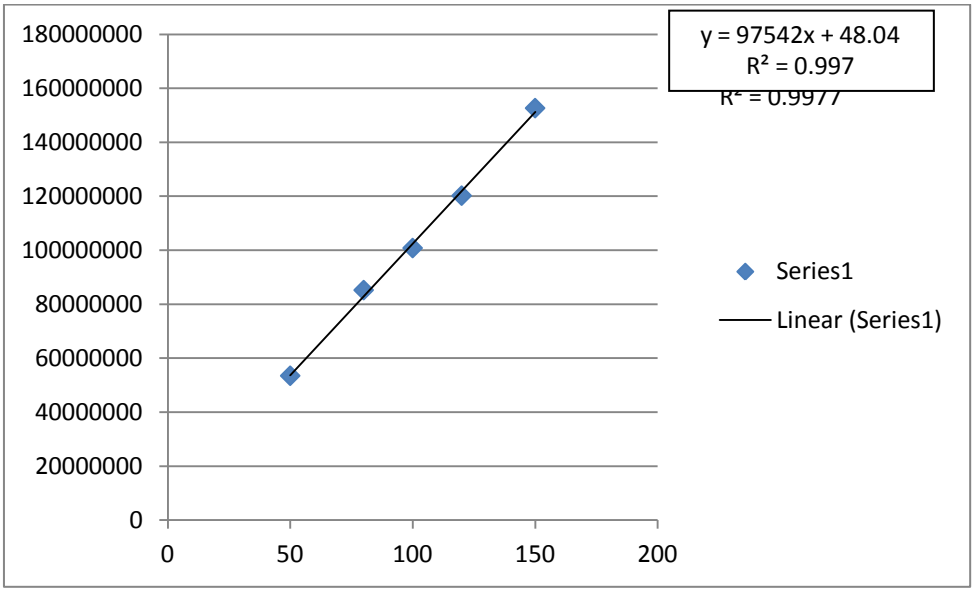

Fig. 2. Graph for linearity data of Mirabegron

Table 4. 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
15	1.497	103574883	2230	1.28
Mean	1.497	104351664.2	-	-
SD	0.00154	26716.59	-	-
%RSD	0.103	0.082	-	-

Table 5. Method precision results for Mirabegron

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

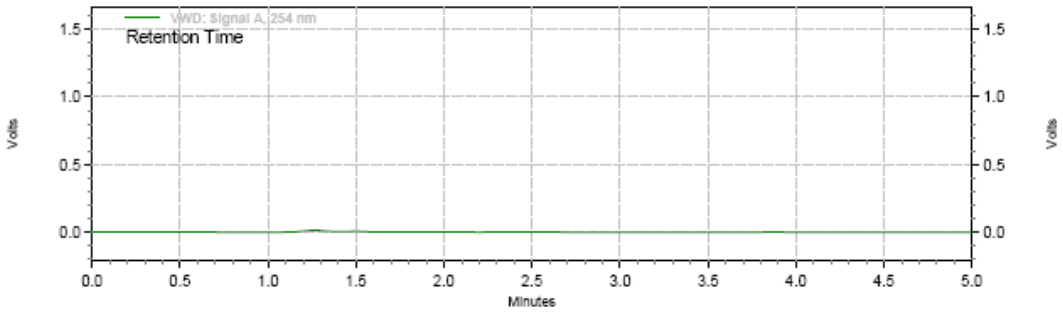

Fig. 3. Chromatogram of Blank

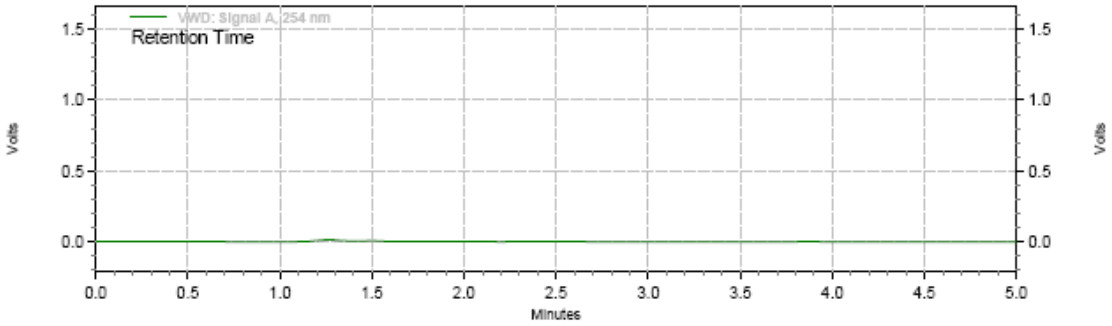

Fig. 4. Chromatogram of Placebo

Table 6. Results for recovery of Mirabegron

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

Table 7. Results for Robustness of Mirabegron

Chromatographic changes		Theoretical Plates	Tailing factor	% RSD
Flow rate	0.4	3431	1.69	0.2
(mL/min)	0.6	2571	1.66	0.08
Temperature(°C)	25	3055	1.76	0.03
	35	3082	1.78	0.1

Table 8. Ruggedness Results of MIRABEGRON

MIRABEGRON	%Assay
Analyst 01	99.6
Anaylst 02	100.52
% RSD	0.57

Table 9. Results of Mirago dosage form

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 10. Results of assay

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

5. CONCLUSION

The newly developed UPLC method for estimating Mirabegron pharmaceutical dosage form has created the way for a new era of accurate, quick, and precise findings. The medicine in consideration was examined, and it was discovered that it has no interactions with the excipients. According to the percent recovery (50-150), percent mean recovery (100.1), and percent RSD (0.57) values, the suggested approach is highly sensitive, precise, and accurate. The advantage of implementing this UPLC method in the assay of this antiviral medicine, based on the results, is that we can achieve results with a reasonable degree of accuracy and precision with little sample preparation, low cost, and time effectiveness.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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