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Research Article

Development and Validation of New Analytical RP-HPLC Method for Simultaneous Estimation of Mirabegron and Solifenacin Succinate in Bulk and Marketed Pharmaceutical Dosage Form

Husna Kanwal Qureshi, Saniya Banu*

Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Goshamahal, Aghapura, Goshamahal Rd, near Nampally, Hyderabad, Telangana 500001.

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ABSTRACT

A New Accurate, precise, Validated Analytical Method was developed for the Estimation of Mirabegron and Solifenacin Succinate in bulk and marketed pharmaceutical Dosage Form by Using UV-Visible Spectroscopy and RP-HPLC method. In UV method the maximum absorption of Mirabegron and Solifenacin Succinate was found to be 236nm and 250nm respectively. The UV Method was validated as per the ICH Guidelines. In HPLC method, the chromatographic conditions were successfully developed for the separation of Mirabegron and Solifenacin Succinate by using Symmetry C18, 5 μ m (4.6mm x 150mm), flow rate was 1.0 ml/min, and mobile phase was Phosphate buffer (0.02M) pH-3.8, Methanol and Acetonitrile were taken in the ratio of 60: 20: 20 % v/v, detection wavelength was 260nm. The retention times of Mirabegron and Solifenacin Succinate were found to be 2.324mins and 4.314mins respectively. The % purity of Mirabegron and Solifenacin Succinate was found to be 99.8%. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Mirabegron and Solifenacin Succinate was found in concentration range of 12 μ g/ml-36 μ g/ml and 13 μ g/ml -39 μ g/ml and correlation coefficient (r^2) was found to be 0.9995 and 0.9998 respectively. The precision study was precise, robust, and repeatable. Hence the suggested UV and RP-HPLC method can be used for routine analysis of Mirabegron and Solifenacin Succinate in bulk and Pharmaceutical dosage form.

INTRODUCTION

Mirabegron is a monocarboxylic acid amide obtained by formal condensation of the carboxy group of 2-

*Corresponding Author: Saniya Banu

Address: Department of Pharmaceutical Analysis, Deccan School of Pharmacy, Goshamahal, Aghapura, Goshamahal Rd, near Nampally, Hyderabad, Telangana 500001

Email ✉: banusaniya15@gmail.com

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amino-1, 3-thiazol-4-ylacetic acid with the anilino group of (1R)-2-[[2-(4-amino phenyl) ethyl] amino]-1-phenyl ethanol. Used for the treatment of overactive bladder syndrome. It has a role as a beta-adrenergic agonist. It is a member of 1, 3-thiazoles, an aromatic amide, a member of ethanolamines and a monocarboxylic acid amide¹. Mirabegron is indicated for the treatment of overactive bladder (OAB) - with symptoms of urge urinary incontinence, urgency, and urinary frequency - either alone or in combination with Solifenacin. It is also indicated for the treatment of neurogenic detrusor over activity (NDO) in pediatric patients 3 years of age and older and weighing 35kg or more². Mirabegron is a medication used in the management of overactive bladder. It is in the sympathomimetic class of medications. It received approval from the US Food and Drug Administration (FDA) in June 2012 for treating overactive bladder with symptoms of urge urinary incontinence, urgency, and urinary frequency³. The IUPAC Name of Mirabegron is 2-(2-amino-1, 3-thiazol-4-yl)-N-[4-[2-[[[(2R)-2-hydroxy-2-phenyl ethyl] amino] ethyl] phenyl] acetamide. The Chemical Structure of Mirabegron is shown in follows

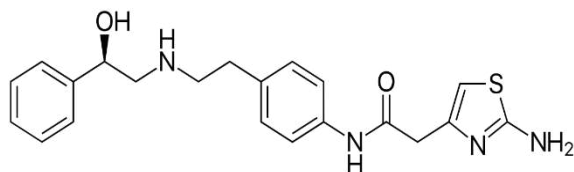


Figure 1: Chemical Structure of Mirabegron

Solifenacin is a member of isoquinolines. Solifenacin is a competitive muscarinic receptor antagonist

indicated to treat an overactive bladder with urinary incontinence, urgency, and frequency. It has a long duration of action as it is usually taken once daily⁴. Solifenacin was granted FDA approval on 19 November 2004. Solifenacin is a Cholinergic Muscarinic Antagonist. The mechanism of action of Solifenacin is as a Cholinergic Muscarinic Antagonist. Solifenacin is an anticholinergic and antispasmodic agent used to treat urinary incontinence and the overactive bladder syndrome⁵. Solifenacin has not been implicated in causing liver enzyme elevations or clinically apparent acute liver injury. The IUPAC Name of Solifenacin Succinate is [(3R)-1-aza bicycle [2.2.2] octan-3-yl] (1S)-1-phenyl-3, 4-dihydro-1H-isoquinoline-2-carboxylate; Butanedioic acid⁶. The Chemical Structure of Solifenacin Succinate is shown in following Figure-1.

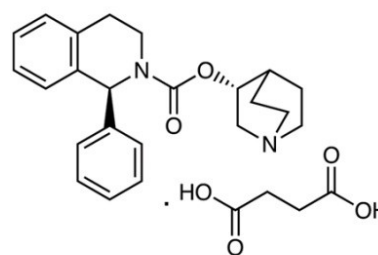


Figure 2: Chemical Structure of Solifenacin Succinate

MATERIALS AND METHODS

UV Experiment:

Table 1: List of Instruments

Sr. No.	Name of Instrument	Instrument Model	Name of Manufacturer
1	UV-Visible double beam spectrophotometer	UV 1800	Elico India
2	HPLC	1575	Hitachi LaChrome
3	Ultra Sonicator	-----	Entrech electronics limited
4	Digital pH Meter	-----	Lab India
5	Vacuum filtration kit	-----	Lab India

Table 2: List of Chemicals

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.99%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.



S.No.	Name	Specifications		Manufacturer/Supplier
3.	Dipotassium hydrogen phosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	orthophosphoric acid	99.9	L.R.	Sd fine-Chem ltd; Mumbai

Preparation of Standard Solution of Mirabegron

10 mg of Mirabegron was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 50 µg/ml of Mirabegron.

10 mg of Solifenacin Succinate was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 40 µg/ml of Solifenacin Succinate⁷.

HPLC Experiment:

Preparation of Standard Solution of Solifenacin Succinate

Table: 3: List of Equipments

S.No.	Instrument	Model No.	Software	Manufacturer's Name
1	HPLC Alliance	Waters 2695	Empower	Waters
2	UV Double Beam Spectrophotometer	UV 3000	UV Win 5	Lab India
3	Digital Weighing Balance	BSA224SCW	-	Sartorius
4	pH meter	AD102U	-	Lab India
5	Ultra Sonicator	SE60US	-	-
6	Suction Pump	VE115N	-	-

Table: 4: List of Chemical/Reagents

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium dihydrogen orthophosphate	Merck	A.R

Preparation of Phosphate buffer: (pH: 3.8):

Weighed 0.136 grams of KH_2PO_4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 3.8 with ortho phosphoric acid.

Preparation of Mobile Phase: A mixture of pH 3.8 Phosphate buffer 600 mL (60%), 200 mL of MEOH (20%) and 200 mL of Acetonitrile are taken and degassed in ultrasonic water bath for 15 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration⁸.

Diluent Preparation: Mobile phase is used as Diluent.

Preparation of the individual Mirabegron standard preparation: 10mg of Mirabegron working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of Diluent is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.



Preparation of the individual Solifenacin Succinate standard preparation: 10mg of Solifenacin Succinate working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of Diluent is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 10.0ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of Sample Solution: (Tablet) Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Mirabegron and Solifenacin Succinate (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it

completely and made volume up to the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

RESULTS AND DISCUSSION

UV Analysis

Method Development:

Selection of Wavelength:

The $\lambda_{\max}$ of the two ingredients i.e. Mirabegron & Solifenacin Succinate, were found to be 236 nm and 250 nm respectively in methanol as solvent system⁹. The isobestic point for the drugs was found at 250 nm.

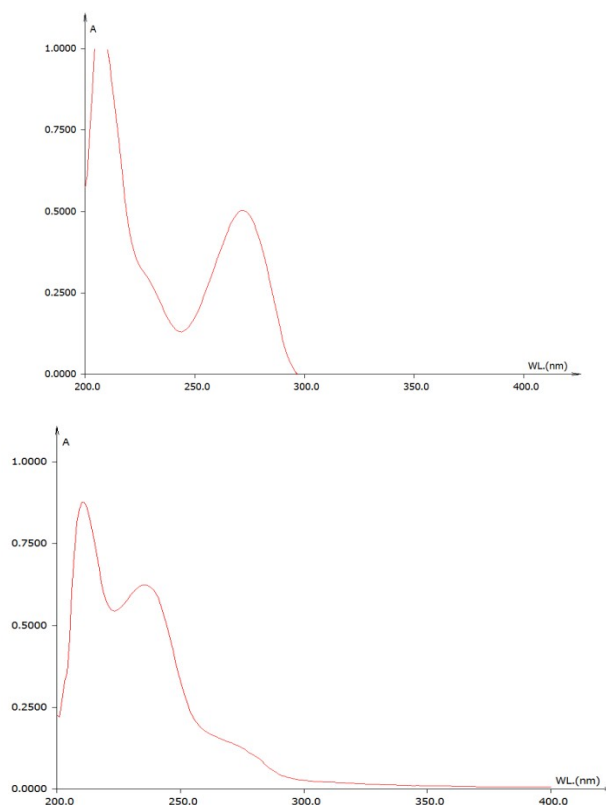


Figure: 3: Mirabegron (236nm) Fig-4:- Solifenacin Succinate (250nm)

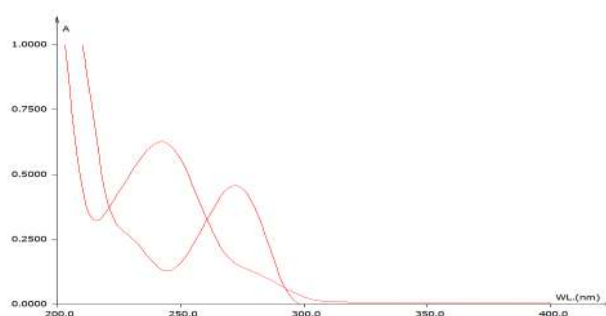


Figure: 5: Isobestic Point of Mirabegron & Solifenacin Succinate (265nm)

Validation of the Developed Method According to I.C.H Guidelines

Following parameters were taken into consideration for validation of proposed methods¹⁰:

1. Linearity

Preparation of Calibration Curve for Mirabegron & Solifenacin Succinate

Standard solutions of Mirabegron in the concentration range of 2 µg/ml to 80 µg/ml were obtained by transferring (0.2,1,2,3,4,5,6,7,8 ml) of Mirabegron stock solution (100 ppm) to the series of 10 ml volumetric flasks and standard solutions of Solifenacin Succinate in the concentration range of 5 µg/ml to 70 µg/ml were obtained by transferring (0.5,1,2,3,4,5,6,7 ml) of Solifenacin Succinate stock solution (100 ppm) to the series of 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 236 nm and 250 nm against the solvent system as blank and calibration curves were plotted. The Beer- Lambert's law is linear in concentration range of 2 to 80 µg/ml at 236 nm and 2 to 80 µg/ml at 250 nm for Mirabegron. The Beer- Lambert's law is linear in concentration range of 5 to 70 µg/ml at 236 nm and 5 to 70 µg/ml at 250 nm for Mirabegron¹¹. The results are shown in Table no. 5 and 6.

Table: 5: Absorptivities of Mirabegron

Conc. (µg/ml)	At 236nm
2	0.083
10	0.326
20	0.628
30	0.934
40	1.251
50	1.546
60	1.839
70	2.135
80	2.459

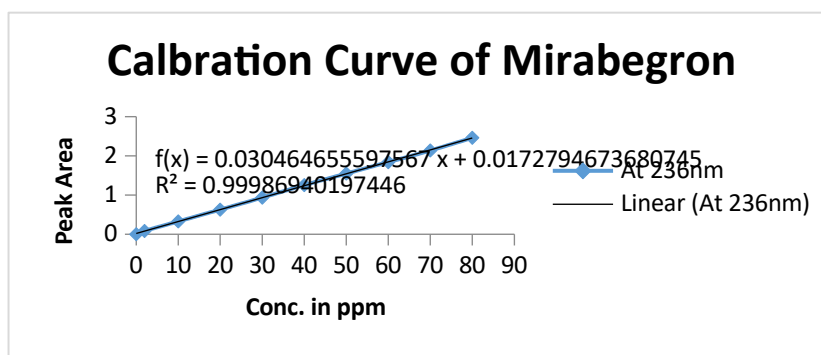


Figure: 6: Calibration Curve for Mirabegron at 236nm

Table: 6: Absorptivities of Solifenacin Succinate

Conc. (µg/ml)	At 250nm
5	0.161

Conc. (µg/ml)	At 250nm
10	0.302
20	0.581
30	0.887
40	1.176
50	1.471
60	1.749
70	2.041

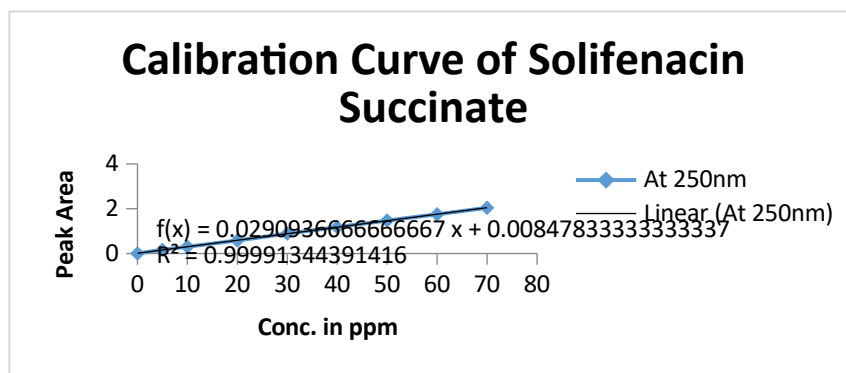


Figure: 7: Calibration Curve for Solifenacin Succinate at 250nm.

2. Range

Range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. It includes working range, linearity range and target range and 100% concentration or test concentration¹².

3. Accuracy

The results of analysis, obtained in three group containing three replicate experiments with API and different tablet dosage forms, had good agreement with the labelled amount of the drug¹³.

METHOD:

In nine different 10 ml volumetric flasks, 1 ml of the pre-analyzed tablet solution (100 µg/ml) was taken and added 1, 2, 3 ml of standard solution of bulk (API) mixture (100µg/ml) and the volume was made up to 10 ml with methanol. The results are shown in Table no.7.

Table: 7: Data of Recovery Studies

Mix.	WL. (nm)	Conc. of Tablet Sol ⁿ (ppm)	Std. Added (ppm)	Abs.	Amt. Found (mg)	% Recovery	Mean Recovery ± S.D.
1	236	2.079	1	0.569	4.066	99.35	MIR 99.383 ± 0.752
	250	9.996	10	0.678	19.987	99.91	
	236	2.079	1	0.568	4.050	98.55	
	250	9.996	10	0.677	19.964	99.68	MIR 99.782 ± 0.216
	236	2.079	1	0.570	4.081	100.10	
	250	9.996	10	0.679	20.01	100.140	
2	236	2.079	2	0.851	6.082	100.075	MIR 99.782 ± 0.216
	250	9.996	20	1.014	29.892	99.480	
	236	2.079	2	0.853	6.068	99.725	
	250	9.996	20	1.017	30.001	100.025	



Mix.	WL.	Conc. of	Std.	Abs.	Amt.	%	Mean Recovery
	236	2.079	2	0.852	6.097	100.450	
	250	9.996	20	1.015	29.915	99.595	
3	236	2.079	3	1.131	7.977	98.300	
	250	9.996	30	1.350	39.879	99.610	
	236	2.079	3	1.134	8.024	99.083	
	250	9.996	30	1.353	39.947	99.836	
	236	2.079	3	1.133	8.008	98.817	
	250	9.996	30	1.352	39.924	99.760	

4. Precision

Repeatability was assessed using: Six time repetition of target concentration 100 % that is (37.5µg/ml). Intermediate precision can be assessed by intra-day and inter day analysis¹⁴.

METHOD:

In the study of the repeatability precision which was conducted on the solution which has the concentration value 100 % of the target concentration (n = 6). The results are shown in Table-8.

4.1 Repeatability

Table: 8: Data of Repeatability of Absorbances

S. No.	Conc. µg/ml	Wavelength (nm)	Abs.	Mean ± S.D.	% RSD
1	(MIR 1 + SOL 10) µg/ml	236	1.067	MIR	SOL
		250	1.272	7.547 ± 0.033	0.437
2		236	1.065		
		250	1.212		
3		236	1.066		
		250	1.272		
4		236	1.068	MIR	SOL
		250	1.274	37.553 ± 0.036	0.096
5		236	1.067		
		250	1.273		
6		236	1.064		
		250	1.270		

5. Limit of Detection and Limit of Quantification

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as¹⁵:

$$\text{L.O.D.} = 3.3 (\text{SD/S}) \text{ \& L.O.Q.} = 10 (\text{SD/S})$$

Where, SD = Standard deviation of the response and S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the Analyte.

CONCLUSION:

The LOD was found to be 0.2682 µg/ml and 0.2542 µg/ml and LOQ was found to be 0.7698 µg/ml and 0.6365 µg/ml for Mirabegron and Solifenacin Succinate respectively which represents that sensitivity of the method is high.

Estimation of Mirabegron and Solifenacin Succinate in Pharmaceutical dosage form

Twenty tablets were taken and the I.P. method was followed to determine the average weight.

METHOD:



Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 120 mg of drugs were transferred to 100 ml volumetric flask, and mixed with 70 ml of methanol and solution was sonicated for 10 minutes there after volume was made up to 100 ml with same solvent system. The solution was filtered through Whatman filter paper. Then 10 ml of the above filtrated solution was diluted to 100 ml with the solvent system. From this stock solution (1ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system¹⁶.

The absorbances of these solutions were measured at 236 nm and at 250 nm using methanol as blank.

CONCLUSION:

The amount of drug in tablet was found to be 49.563 mg/tab for Mirabegron and 4.642 mg/tab for Solifenacin Succinate respectively.

HPLC Analysis:

Method Development:

Optimized Chromatographic Method:

Optimized Chromatographic Conditions:

Column : Symmetry C18, 5 μ m (4.6mm x 150mm)

Mobile phase ratio: Phosphate buffer (0.02M) pH-3.8:

Methanol: Acetonitrile (60:20:20%v/v)

Detection wavelength : 260nm

Flow rate : 1.0ml/min

Injection volume : 20 μ l

Column temperature : Ambient

Auto sampler temperature: Ambient

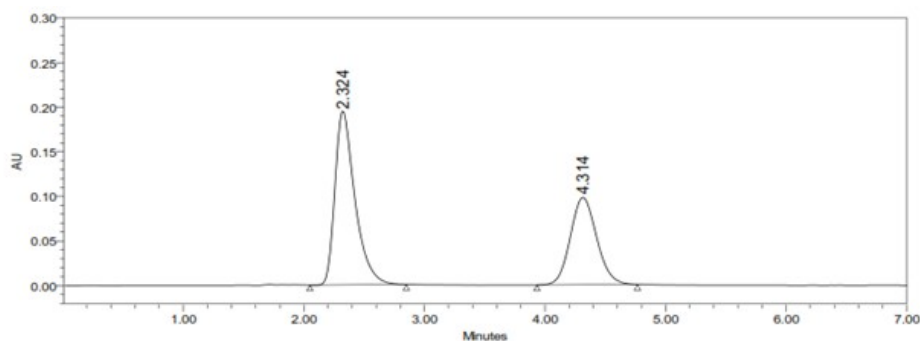


Figure: 8: Optimized Chromatographic Condition

Validation of Analytical Method:

System Suitability: System Suitability was the checking of a system to ensure system performance before or

during the analysis of unknowns. Parameters such as tailing factor, resolution, plate count and reproducibility are determined and compared against the specification suitable for the method¹⁷.

Table: 9: Data of System Suitability Test for Mirabegron

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing	Resolution
1	Injection 1	2.327	946257	5245	1.3	8.6
2	Injection 2	2.328	946325	5326	1.2	8.7
3	Injection 3	2.319	946859	5124	1.3	8.9
4	Injection 4	2.320	945875	5296	1.3	8.6
5	Injection 5	2.323	946396	5248	1.2	8.9
6	Injection 6	2.328	946548	5295	1.3	8.7
Mean			946376.7			
S.D			325.8936			



S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing	Resolution
%RSD			0.034436			

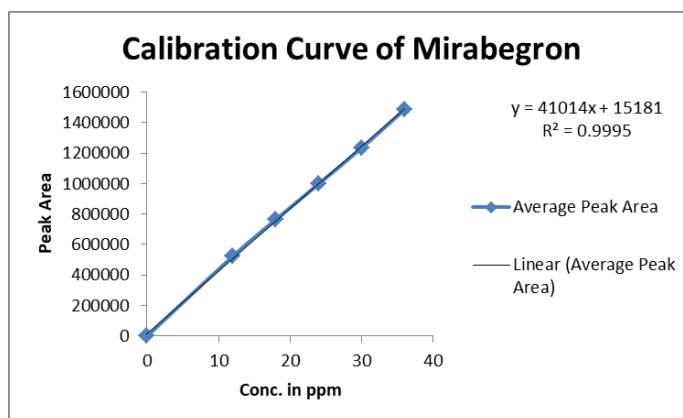
Table: 10: Data of System Suitability Test for Solifenacin Succinate

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	4.331	112543	3854	1.4
2	Injection 2	4.341	111652	3965	1.5
3	Injection 3	4.299	112854	3874	1.3
4	Injection 4	4.313	111485	3698	1.5
5	Injection 5	4.325	113526	3785	1.4
6	Injection 6	4.341	112985	3965	1.6
Mean			112507.5		
S.D			795.4945		
%RSD			0.707059		

Linearity: Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line¹⁸.

Table: 11: Chromatographic Data for Linearity Study of Mirabegron

Concentration $\mu\text{g/ml}$	Average Peak Area
12	523864
18	764875
24	999874
30	1235658
36	1488542

**Figure: 9: Calibration Curve of Mirabegron****Table: 12: Chromatographic Data for Linearity Study of Solifenacin Succinate**

Concentration $\mu\text{g/ml}$	Average Peak Area
13	65698

Concentration $\mu\text{g/ml}$	Average Peak Area
19.5	98254
26	128587
32.5	160648
39	191874

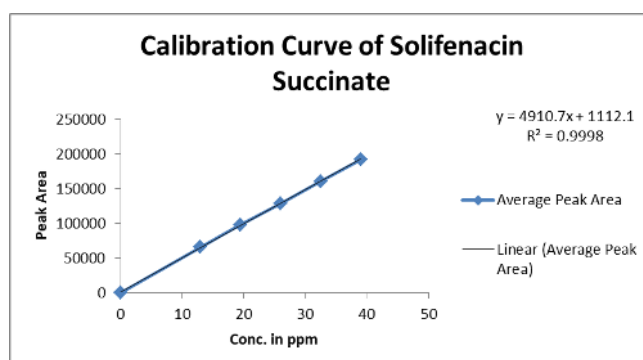


Figure: 10: Calibration Curve of Solifenacin Succinate

Accuracy: The accuracy was performed for this 50%, 100%, 150% respectively. The % recovery was method by conducting the recovery studies were carried out by adding three different concentration level found and tabulated in 13 and 14 to be in the range 98-102%¹⁹.

Table: 13: Accuracy Results of Mirabegron

%Concentration (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	508367	12	12.024	100.200%	100.150%
100%	999100.3	24	23.989	99.954%	
150%	1496200.3	36	36.110	100.305%	

Table: 14: Accuracy Results of Solifenacin Succinate

%Concentration (at specification Level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	65093.67	13	13.029	100.223%	100.280%
100%	129339.3	26	26.111	100.426%	
150%	178242.7	39	36.070	100.194%	

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions²⁰.

Repeatability:

Obtained five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table: 15: Results of Repeatability for Mirabegron:

S. No.	Peak Name	Retention time	Area (μV*sec)	Height (μV)	USP Plate Count	USP Tail-ing	Resolution
1	Mirabegron	2.321	946253	155465	5326	1.36	8.25
2	Mirabegron	2.317	947845	154578	5246	1.37	8.26
3	Mirabegron	2.323	945867	155845	5478	1.35	8.34
4	Mirabegron	2.322	948572	155698	5425	1.38	8.37
5	Mirabegron	2.324	949857	154857	5326	1.36	8.39
Mean			947678.8				
Std. Dev			1649.66				



S. No.	Peak Name	Retention time	Area ($\mu\text{V} \cdot \text{sec}$)	Height (μV)	USP Plate Count	USP Tailing	Resolution
%RSD			0.174074				

Table: 16: Results of Repeatability for Solifenacin Succinate:

S. No.	Peak Name	Retention time	Area ($\mu\text{V} \cdot \text{sec}$)	Height (μV)	USP Plate Count	USP Tailing
1	Solifenacin Succinate	4.304	111563	13254	3869	1.42
2	Solifenacin Succinate	4.300	111254	13425	3852	1.43
3	Solifenacin Succinate	4.308	111672	13254	3896	1.45
4	Solifenacin Succinate	4.310	112654	13265	3962	1.42
5	Solifenacin Succinate	4.314	113123	13154	3874	1.48
Mean			112053.2			
Std. Dev			795.2614			
%RSD			0.709718			

Intermediate Precision²¹:

Table: 17: Results of Intermediate Precision for Mirabegron

S.No.	Peak Name	RT	Area ($\mu\text{V} \cdot \text{sec}$)	Height (μV)	USP Plate Count	USP Tailing	Resolution
1	Mirabegron	2.328	956325	156325	5246	1.35	8.24
2	Mirabegron	2.326	958741	157854	5367	1.38	8.26
3	Mirabegron	2.327	957542	156986	5265	1.34	8.47
4	Mirabegron	2.326	956895	158547	5384	1.39	8.29
5	Mirabegron	2.331	957486	156985	5297	1.35	8.34
Mean			957397.8				
Std. Dev.			899.5091				
% RSD			0.093954				

Table: 18: Results of Intermediate Precision for Solifenacin Succinate

S.No.	Peak Name	Rt	Area ($\mu\text{V} \cdot \text{sec}$)	Height (μV)	USP Plate count	USP Tailing
1	Solifenacin Succinate	4.335	121231	13458	3896	1.52
2	Solifenacin Succinate	4.336	121457	13674	3785	1.54
3	Solifenacin Succinate	4.334	123142	13485	3969	1.58
4	Solifenacin Succinate	4.337	121325	13958	3859	1.57
5	Solifenacin Succinate	4.340	123654	13875	3789	1.59
Mean			122161.8			
Std. Dev			1145.733			
% RSD			0.937882			

Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate ($\pm 0.1\text{ml/min}$), Temperature ($\pm 2^\circ\text{C}$), Wavelength of detection ($\pm 5\text{nm}$) & acetonitrile content in mobile phase ($\pm 2\%$) studied to determine the robustness of the

method are also in favour of (Table-19 & 20, % RSD < 2%) the developed RP-HPLC method for the analysis of Mirabegron (API)²².

Table: 19: Result of Method Robustness



Change in Parameter	% RSD
Flow (1.1 ml/min)	0.96
Flow (0.9 ml/min)	0.84
Temperature (27°C)	0.81
Temperature (23°C)	0.94
Wavelength of Detection (265 nm)	0.56
Wavelength of detection (255 nm)	0.17

Table: 20: Result of Method Robustness

Change in Parameter	% RSD
Flow (1.1 ml/min)	0.58
Flow (0.9 ml/min)	0.64
Temperature (27°C)	0.72
Temperature (23°C)	0.91
Wavelength of Detection (265 nm)	0.86
Wavelength of detection (255 nm)	0.78

Limit of Detection (LOD) & Limit of Quantification (LOQ):

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as²³:

$$\text{L.O.D.} = 3.3 (\text{SD/S}) \text{ \& L.O.Q.} = 10 (\text{SD/S})$$

Where, SD = Standard deviation of the response & S = Slope of the calibration curve

CONCLUSION:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.377µg/ml & 4.174µg/ml respectively for Mirabegron. The LOD was found to be 1.079µg/ml and LOQ was found to be 3.272µg/ml for Solifenacin Succinate which represents that sensitivity of the method is high.

Estimation of Mirabegron & Solifenacin Succinate in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally the

weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra Sonicator, Then the final volume was make up to the mark with the mobile phase²⁴. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicate to degas the mobile phase (Solvent system). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase).

The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. The obtained data are shown in Table 21.

Assay % =

$$\frac{\text{AT} \times \text{WS} \times \text{DT}}{\text{P} \times \text{AS} \times \text{DS} \times \text{WT}} \times 100$$

----- x -----x ----- x -----x Average weight = mg/tab

$$\frac{\text{AT} \times \text{WS} \times \text{DT}}{\text{P} \times \text{AS} \times \text{DS} \times \text{WT}} \times 100$$

Where:

AT = Test Preparation Peak Area

AS = Standard preparation Peak Area

WS = Working standard weight taken in mg

WT = Sample weight taken in mg

DS = Standard solution dilution

DT = Sample solution dilution

P = Working standard percentage purity

The assay was performed as explained in the previous chapter (Above). The results which are obtained are following²⁵:



Table: 21: Assay of Mirabegron & Solifenacin Succinate Dosage Form

Brand Name of Dosage forms	Labelled Amount of Drug (mg) Mirabegron/Solifenacin Succinate	Mean ($\pm$ SD) amount (mg) found by the proposed method (n=6)	Mean ($\pm$ SD) Assay (n = 6)
Megatas Plus 50 Combikit (Intas Pharmaceuticals Ltd)	50mg / 5mg	49.586 ($\pm$ 0.06) /4.686 ($\pm$ 0.07)	99.865 ($\pm$ 0.245) /99.658 ($\pm$ 0.354)

Stability Studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was

exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient²⁶⁻²⁷. The results were shown in figures-22 and 23.

Table: 22: Results of Forced Degradation Studies of Mirabegron

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	946124	0	100%	100%
2	Acidic	903311.889	4.525	95.475	100%
3	Basic	910710.578	3.743	96.257	100%
4	Oxidative	893670.885	5.544	94.456	100%
5	Thermal	907947.896	4.035	95.965	100%
6	Photolytic	940030.961	0.644	99.356	100%

Table: 23: Results of Forced Degradation Studies of Solifenacin Succinate

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	111541	0	100%	100%
2	Acidic	107459.714	3.659	96.341	100%
3	Basic	108837.246	2.424	97.576	100%
4	Oxidative	107932.648	3.235	96.765	100%
5	Thermal	105581.364	5.343	94.657	100%
6	Photolytic	109739.612	1.615	98.385	100%

SUMMARY AND CONCLUSION**Summary of UV Method:**

The standard solutions of Mirabegron and Solifenacin Succinate in Methanol (10 μ g/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Mirabegron and Solifenacin Succinate was found to be at 236 nm and 250nm respectively. Therefore, 265nm was selected as Isobestic Point of Mirabegron and Solifenacin Succinate for the present study. The calibration curve of Mirabegron and Solifenacin Succinate was found to be linear in the range of 2-80 μ g/ml at 236 nm and 250nm respectively. Therefore, it was clear that Mirabegron and Solifenacin Succinate can be determined without

interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk (Standard) drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of Mirabegron and Solifenacin Succinate standard concentration. Three samples were prepared for each recovery level. The solutions were then analysed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.



The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

Summary of HPLC Method:

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for simultaneous analysis of Mirabegron and Solifenacin Succinate different chromatographic conditions were applied & the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In HPLC method, the chromatographic conditions were successfully developed for the separation of Mirabegron and Solifenacin Succinate by using Symmetry C18, 5 μ m (4.6mm x 150mm), flow rate was 1.0 ml/min, and mobile phase was Phosphate buffer (0.02M) pH-3.8, Methanol and Acetonitrile were taken in the ratio of 60: 20: 20 % v/v, detection wavelength was 260nm. The retention times of Mirabegron and Solifenacin Succinate were found to be 2.324mins and 4.314mins respectively. The % purity of Mirabegron and Solifenacin Succinate was found to be 99.8%. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Mirabegron and Solifenacin Succinate was found in concentration range of 12 μ g/ml-36 μ g/ml and 13 μ g/ml-39 μ g/ml and correlation coefficient (r^2) was found to be 0.9995 and 0.9998 respectively.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Mirabegron and Solifenacin Succinate it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 μ l were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay & stability which can help in the simultaneous analysis of Mirabegron and Solifenacin Succinate in different formulations.

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