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A NEW SIMPLE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF MIRABEGRON AND SOLIFENACIN SUCCINATE IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM DOSAGE FORM

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Abstract:

A novel, simple, specific, accurate, precise method development and validated for the simultaneous estimation of Mirabegron and Solifenacin Succinate by RP-HPLC in bulk and marketed formulation. A High-Performance Liquid Chromatography WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector with Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d. column, with mobile phase composition of Acetonitrile and Buffer pH-3.4 with OPA were taken in the ratio of 55:45% v/v/v was used. The flow rate of 1.0 ml min-1 and effluent was detected at 330 nm. The retention times of Mirabegron and Solifenacin Succinate were 2.285 and 3.135 minutes. Linearity was observed over the concentration range of 6-14µg/ml and 10µg/ml-30µg/ml for Mirabegron and Solifenacin Succinate respectively. The Limit of detection and limit of quantification of Mirabegron and Solifenacin Succinate were found to be 0.09ng ml-1 and 0.29ngml-1 & 0.1ng ml-1 and 0.3ngml-1 respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98% to 102%. The method was validated in terms of linearity, accuracy, precision, (repeatability, intermediate precision) specificity (by assay), robustness, and system suitability. Thus, the validated method can be successfully applied to routine analysis to regulate the quality. It also should be used for analytical research purposes.

Keywords: *Mirabegron and Solifenacin Succinate, RP-HPLC, Method Development, Validation.*

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INTRODUCTION:

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

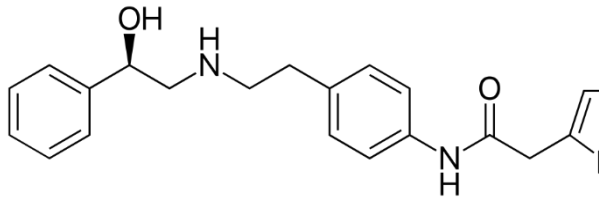

Fig-1: Chemical Structure of Mirabegron Etabonate

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 daily. 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 syndrome. Solifenacin has not been implicated in causing liver enzyme elevations or clinically apparent acute liver injury. Solifenacin tablets are indicated to treat an overactive bladder with urinary incontinence, urgency, and frequency. 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 acid. The Chemical Structure of Solifenacin Succinate is shown in follows

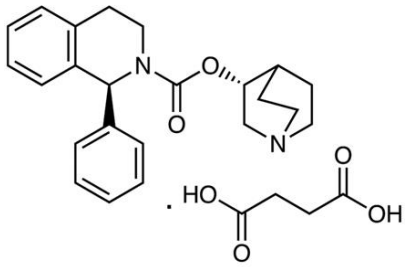

Fig-2: Chemical Structure of Solifenacin Succinate

The objective of the present work was to develop and validate a RP-HPLC method that is simple, quick, accurate, precise, selective, and reproducible for the measurement of Mirabegron and Solifenacin Succinate in bulk form and pharmaceutical dosage form[36-39].

The Rationale of the study is to develop a method which is simple, specific, performed well in terms of speed and more environmentally friendly because it uses less organic solvents.

EXPERIMENTAL

Method Development:

Table-1. Instruments used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Table-2. Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
4.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
5.	Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
8.	Hydrogen Peroxide	99.9%	L.R.	Sd fine-Chem ltd; Mumbai

Preparation of Standard Stock Solution of Mirabegron

Accurately weighed 10mg of Mirabegron and it was transferred to clean and dry 100 ml of volumetric flask and dissolved in acetonitrile: buffer (55:45) and made-up the volume to 100 ml with the same solvent system[7]. The final solution contained 100 µg per ml of Mirabegron solution.

Preparation of Standard Stock Solution of Solifenacin Succinate

Accurately weighed Solifenacin Succinate (10 mg) was transferred to a 100 ml volumetric flask, dissolved in Acetonitrile: buffer (55:45), and made up the volume to 100 ml with the same solvent system. The final solution contained 100 µg per ml of Solifenacin Succinate solution.

Determination of Wavelength of Maximum absorbance for Mirabegron

Standard Mirabegron solution (1ml) was transferred to a separate 10 ml volumetric flask. The final volume was adjusted to 10 ml with the same mobile phase. The absorbance of the final solution was scanned in the range 400 to 220 nm against the mobile phase as blank[8].

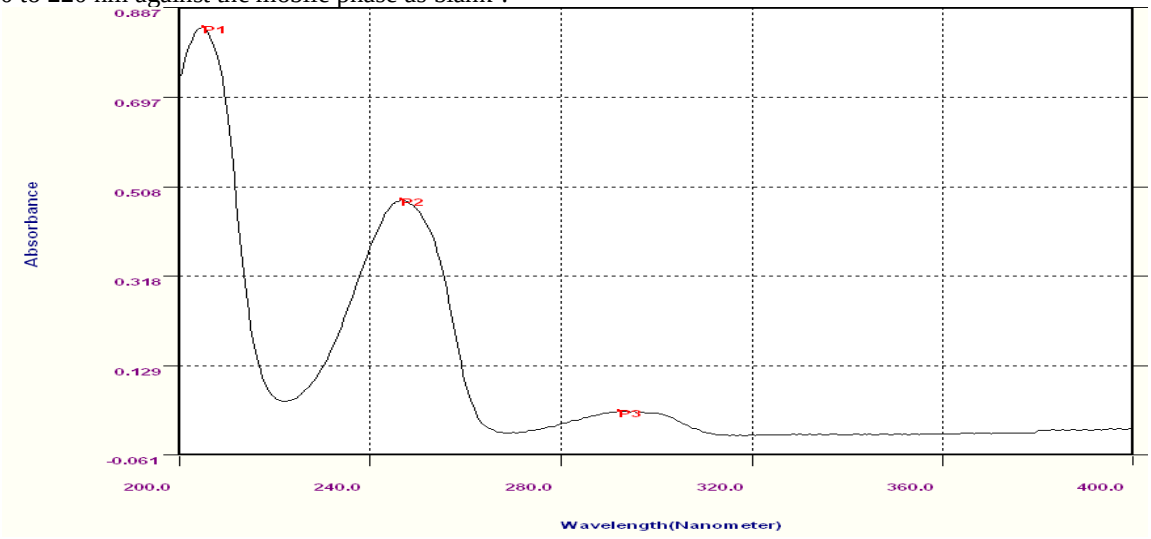

Fig-3: UV Spectrum of Mirabegron (292 nm)

Estimation of Maximum Wavelength for Solifenacin Succinate

First of all take 1ml of standard Solifenacin Succinate solution from the above standard solution (1 ml) was transferred to separate clean and dry of 10 ml volumetric flask. The final volume was adjusted to 10ml with same mobile phase (Solvent). The absorbance of the final resulted solution was scanned in the range 400 to 220 nm against solvent mixture as blank. The results are shown in following figure-4.

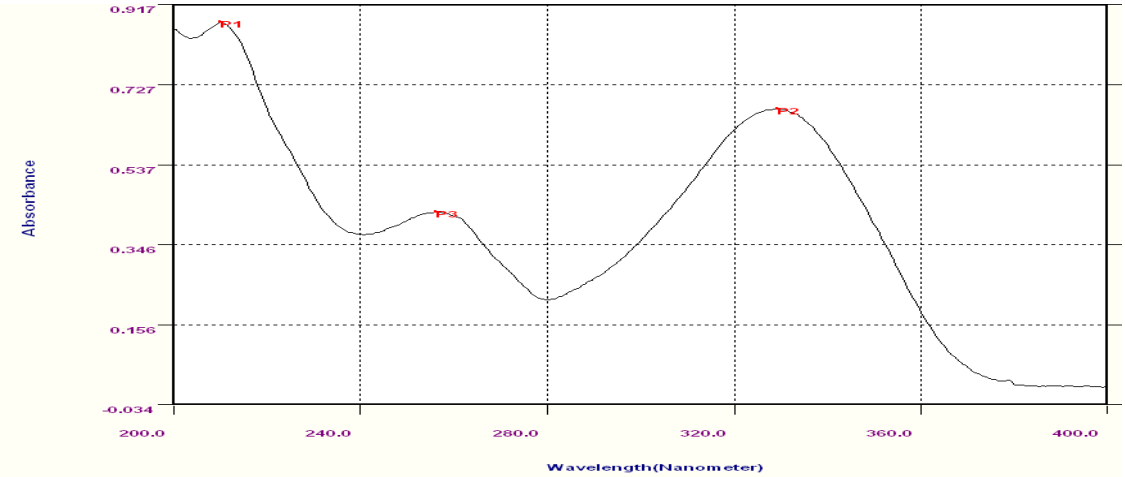

Figure-4: UV Spectrum of Solifenacin Succinate (330 nm)

Development of Analytical Method by HPLC:

Selection of Wavelength:

The λ_{max} of the two ingredients i.e. Mirabegron and Solifenacin Succinate, were found to be 292 nm and 330 nm respectively in methanol as solvent system. As the two drugs have almost near absorption max & at 330 nm Mirabegron shows more intensity as compared to Solifenacin Succinate at 292 nm, 330 nm has been chosen as the common absorption maximum for HPLC analysis[9].

Preparation of Standard Solution of Mirabegron

Weighed accurately 10mg of standard Mirabegron and transferred it into a clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve in 100 ml of volumetric flask. The final volume was made up to the mark with the same solvent. The final solution contained about 100 µg/ml of Mirabegron.

Preparation of Standard Solution of Solifenacin Succinate

First 10 mg of Solifenacin Succinate was weighed accurately and transferred into a clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve it in the mobile phase. The final volume was made up to the mark with the same solvent[10]. The final solution contained about 100 µg/ml of Solifenacin Succinate.

Initialization of the Instrument

The HPLC instrument was switched on. First, the column was washed with the HPLC grade water for 45 minutes. After washing the column the column is saturated with the mobile phase in 45 minutes. The mobile phase was run to find the peaks or identification of peaks. After 20 minutes the standard drug solution was prepared and injected in the HPLC system[11].

Different Chromatographic Conditions used and their Optimizations

The various HPLC chromatographic conditions are used to find the optimum chromatographic condition for the best elution of drugs in the mixture.

Preparation of Mobile Phase

The mobile phase can be prepared by taking acetonitrile: Phosphate Buffer and maintained pH-3.4 with diluted orthophosphoric acid (55:45). The resulted Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath. The final obtained mobile phase was pumped through the selected column and maintained at a flow rate of 1.0 ml/min[12].

Optimized Chromatographic Conditions:

Table-3: Optimized Chromatographic Condition

Mobile phase	**Acetonitrile: Buffer pH-3.4 with OPA (55:45% v/v)**
Wavelength	330 nm
Flow rate	1.0 ml/ min.
Auto Sampler Temperature	Ambient
Injection Volume	20µl
Run time	6 min.
Column	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
Column Temperature	Ambient

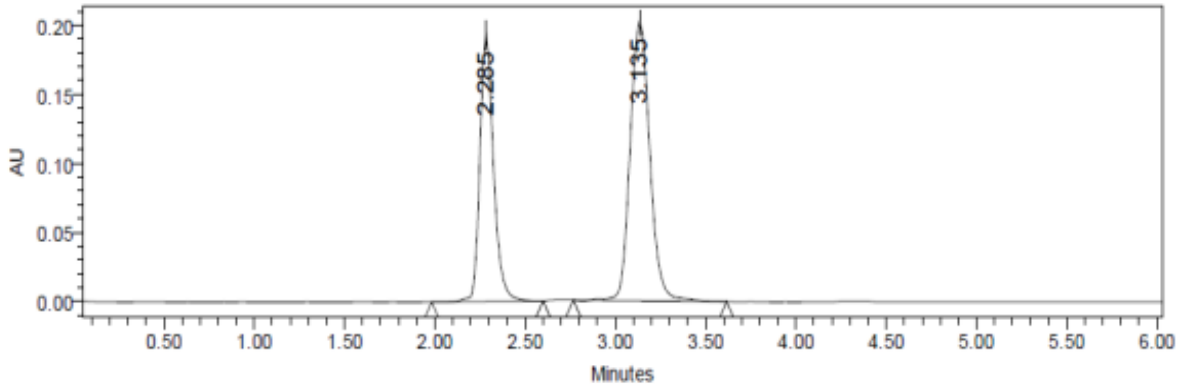

Fig-5: Optimized Chromatographic Condition

ANALYTICAL METHOD VALIDATION

1. Accuracy[13-15]:

Recovery study:

Accuracy: For 80%

Table-4: Data of Recovery Studies for Mirabegron

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.039	348673	100.487	Mean= 100.633%
S_2 : 80 %	8	8.046	348945	100.575	S.D. = 0.182066
S_3 : 80 %	8	8.067	349745	100.837	% R.S.D.= 0.180921

Table-5: Data of Recovery Studies for Solifenacin Succinate

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	16	15.991	989572	99.943	Mean= 100.1577%
S_2 : 80 %	16	16.143	998756	100.893	S.D. = 0.654939
S_3 : 80 %	16	15.942	986589	99.637	% R.S.D.= 0.653908

For 100%

Table-6: Data of recovery studies for Mirabegron

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 100 %	10	9.862	419823	98.62	Mean= 99.95%
S_2 : 100 %	10	9.993	424941	99.93	S.D. = 1.340112%
S_3 : 100 %	10	10.130	430295	101.3	R.S.D.= 1.340782

Table-7: Data of recovery studies for Solifenacin Succinate

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 100 %	20	19.995	1231734	99.975	Mean= 100.795%
S_2 : 100 %	20	20.158	1241569	100.79	S.D. = 0.822511%
S_3 : 100 %	20	20.325	1251694	101.62	R.S.D.= 0.816024

FOR 120%

Table-8: Data of recovery studies for Mirabegron

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 120 %	12	12.115	507788	100.958	Mean= 100.9717%
S_2 : 120 %	12	12.179	510262	101.491	S.D. = 0.512637
S_3 : 120 %	12	12.056	505468	100.466	% R.S.D.= 0.507703

Table-9: Data of recovery studies for Solifenacin Succinate

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 120 %	24	24.335	1494218	101.395	Mean= 100.805%
S_2 : 120 %	24	24.204	1486312	100.85	S.D. = 0.613739
S_3 : 120 %	24	24.041	1476398	100.170	% R.S.D.= 0.608837

2. Precision

The precision of the analytical developed method was studied by analysis of multiple sampling of homogeneous (same) sample. The precision expressed as standard deviation (SD) or relative standard deviation (% RSD). The precision of the method can be analyzed by the intermediate precision. It includes the intra-day and inter-day variation[16-18].

2.1. Repeatability

The precision of each method was achieved separately from the peak areas obtained by actual estimation of 5 injections of fixed homogenous sample concentrations of Mirabegron & Solifenacin Succinate respectively. The % relative standard deviation for the Mirabegron and Solifenacin Succinate was calculated[19].

Table-10: Results for Repeatability of Mirabegron & Solifenacin Succinate

Concentration of Remo + Tene in ppm	Rt of Remo	Peak area of Remo	Rt of Tene	Peak area of Tene
10 +10	2.264	3303800	3.132	951802
10 +10	2.246	3349883	3.132	958267
10 +10	2.264	3353514	3.129	954481
10 +10	2.246	3384162	3.113	952151
10 +10	2.280	3390496	3.113	952308
AVG	**2.26**	**3356371**	**3.1238**	**953801.8**
S.D.	**0.014353**	**34463.10324**	**0.009935**	**2709.017**
% RSD	**0.635075**	**1.026796598**	**312.38**	**0.284023**

4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-11, % RSD < 2%) the developed RP-HPLC method for the analysis of Solifenacin Succinate (API)[20-21].

Table-11: Result of Method Robustness Test

Change in Parameter	% RSD
Flow (1.1 ml/min)	1.05
Flow (0.9 ml/min)	0.67
Temperature (27^0C)	0.58
Temperature (23^0C)	0.61
Wavelength of Detection (332 nm)	0.38
Wavelength of detection (328 nm)	0.17

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-12, % RSD <2%) the developed RP-HPLC method for the analysis of Solifenacin Succinate (API)[22].

Table-12: Result of Method Robustness Test

Change in Parameter	% RSD
Flow (1.1 ml/min)	0.09
Flow (0.9 ml/min)	0.07
Temperature (27^0C)	0.28
Temperature (23^0C)	0.74
Wavelength of Detection (332 nm)	0.86
Wavelength of detection (328 nm)	0.67

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

The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

$$L.O.D. = 3.3 (SD/S).$$

$$L.O.Q. = 10 (SD/S)$$

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Result & Discussion

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.29 µg/ml respectively for Mirabegron[23].

The LOD was found to be 0.1 µg/ml and LOQ was found to be 0.3 µg/ml for Solifenacin Succinate which represents that sensitivity of the method is high[24].

System Suitability Parameter

It is an integral part of so many analytical procedures. The parameters are based on the idea that the equipment, electronics, analytical operations and the samples to be analyzed constitute as an integral system which can be examined. Finally system suitability test parameters are established[25-26]. The obtained data is shown in the following table-13.

Table-13: Data of System Suitability Parameter

S. No.	Parameter	Mirabegron	Solifenacin Succinate
1	Retention time	2.264	3.129
2	Theoretical plates	3154	3635
3	Tailing factor	1.02	1.25
4	Area	3061716	925851
5	Resolution	6.9	

The system suitability parameters were found to be within the specified limits for the proposed method.

6. Assay of Mirabegron & Solifenacin Succinate in 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 upto the mark with the mobile phase[27]. 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[28].

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[29]. The obtained data are shown in Table 14.

ASSAY:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

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)[30]. The results which are obtained are following:

Table-14: Assay of MIRABEGRON & SOLIFENACIN SUCCINATE Tablets

Brand Name of Tablets	Labelled Amount of Drug (mg) Mirabegron/Solifenacin Succinate	Mean (±SD) Amount (mg) Found by the Proposed Method (n=6)	Mean (± SD) Assay (n = 6)
Megatas Plus 50 Combikit Tablets from Intas Pharmaceuticals Ltd	50/5	49.658 (± 0.368)/4.675 (± 0.478)	99.574 (± 0.574) /99.749 (± 0.695)

Observation: The assay of Megatas Plus 50 Combikit Tablets containing Mirabegron was found to be 49.658 (± 0.368) and Solifenacin Succinate was found to be 4.675 (± 0.478) and the % purity of the Mirabegron and Solifenacin Succinate was found to be 99.574 (± 0.574) /99.749 (± 0.695).

Stability Studies

The results of the stress studies indicated the **specificity** of the method that has been developed. Mirabegron and Solifenacin Succinate were stable only in photolytic stress conditions and little bit in thermal stress conditions[31-35]. The results of forced degradation studies are given in the following Table-15.

Table-15: Results of Forced Degradation Studies of Mirabegron and Solifenacin Succinate API

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	76.52	23.48	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	79.37	20.63	100.00
Thermal Degradation (50 ^{0}C)	24Hrs.	90.41	9.59	100.00
UV (254nm)	24Hrs.	99.21	0.79	100.00
3% Hydrogen peroxide	24Hrs.	81.62	18.38	100.00

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. 0First of all, maximum absorbance was found to be at 330nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d. because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The

flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetonitrile and Buffer pH-3.4 with OPA was taken in the ratio of 55:45% v/v/v was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Methanol and water were selected because of maximum extraction sonication time was fixed to be 15min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6.0min because analyze gave peak around 2.285min and 3.135min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range of 6-14µg/ml of the Mirabegron and 10-30µg/ml of the Solifenacin Succinate target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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