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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MIRABEGRON AND SOLIFENACIN SUCCINATE

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Mirabegron and Solifenacin Succinate in pharmaceutical dosage form. Chromatographic separation of Mirabegron and Solifenacin Succinate was achieved on Waters Alliance e2695 by using Symmetry C18 (150 mm x 4.6 mm, 3.5µm) column and the mobile phase containing phosphate buffer pH 3.4 and acetonitrile in the ratio of 35:65% v/v. The flow rate was 1.0 mL/min and detection was carried out at 240 nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Mirabegron and Solifenacin Succinate were NLT 2000 and should not more than 2 respectively. % RSD of peak areas of all measurements was less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for simultaneous estimation of Mirabegron and Solifenacin Succinate in bulk and pharmaceutical formulation.

Keywords: Mirabegron, Solifenacin Succinate, HPLC and Validation.

INTRODUCTION

Mirabegron (Fig. 1) is a potent and selective sympathomimetic beta-3 adrenergic receptor agonist used to relax the smooth muscle of the bladder in the treatment of urinary frequency and incontinence[1]. Chemically it is 2-(2-amino-1,3-thiazol-4-yl)-*N*-[4-[2-[[(2*R*)-2-hydroxy-2-phenylethyl]amino]ethyl]phenyl]acetamide.

Mirabegron acts by the activation of beta-3 receptors relaxes detrusor smooth muscle during the storage phase of the urinary bladder fill-void cycle, which increases the bladder's storage capacity thereby alleviating feelings of urgency and frequency[2].

Solifenacin Succinate(Fig. 2) is a competitive muscarinic receptor antagonist indicated to treat an overactive bladder with urinary incontinence, urgency and frequency[3]. Chemically it is [(3*R*)-1-azabicyclo[2.2.2]octan-3-yl] (1*S*)-1-phenyl-3,4-dihydro-1*H*-isoquinoline-2-carboxylate; butane dioic acid. Solifenacin is a competitive muscarinic receptor antagonist. It has the highest affinity for M3, M1 and M2 muscarinic receptors. 80% of the muscarinic receptors in the bladder are M2, while 20% are M3. Solifenacin's antagonism of the M3 receptor prevents contraction of the detrusor muscle, while antagonism of the M2 receptor may prevent contraction of smooth muscle in the bladder[4].

Literature survey revealed that no HPLC method was reported so far for simultaneous estimation of Mirabegron and Solifenacin Succinatein combined pharmaceutical dosage form. Hence the objective of this method is to develop and validate a simple, specific, rapid, precise and accurate stability indicating RP-HPLC method

for the simultaneous estimation of Mirabegron and Solifenacin Succinatein combined pharmaceutical dosage form in accordance with ICH guidelines[5,6].

MATERIALS AND METHODS

Materials

Mirabegron and Solifenacin Succinate pure drugs (API) were procured from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. The marketed formulation of Mirabegron and Solifenacin Succinatetablets (MIRAGRON S 50 contains 50 mg of Mirabegron and 5 mg of Solifenacin Succinate) were procured from local market. Acetonitrile, potassium dihydrogen phosphate, trifluoro acetic acid and HPLC grade water were obtained from Rankem Chemicals Ltd., Mumbai, India.

Instrumentation

The analysis of drugs was carried out on Waters Alliance e 2695 separation module HPLC system with PDA Detectorat 240 nm on Symmetry C18 (150 mm x 4.6 mm, 3.5µm). The instrument is equipped with auto injector with 10 µL sample loop. A 10 µL hamilton syringe was used for injecting the samples. Data was analyzed by using Empower 2 software. A double-beam Schimadzu UV-1800 UV-Visible spectrophotometer was used for measuring absorbance for Mirabegron and Solifenacin Succinatesolutions. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Sartorius balance was used for weighing the materials.

Mobile phase

A mobile phase consisting of mixture of phosphate buffer (pH 3.4 adjusted with trifluoro acetic acid)and acetonitrile in the ratio of 35:65%v/v was prepared.

Diluent

Mobile phase used as diluent.

Preparation of standard stock solution

Accurately weighed and transferred 50 mg of Mirabegron, 5 mg of Solifenacin Succinate working standards into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute up to the mark with diluent (50 µg/mL of Mirabegron and5 µg/mL of Solifenacin Succinate).

Preparation of sample solution

Accurately weighed and transferred156 mg of sample into a 100 mLclean dry volumetric flask, add diluent and sonicate it up to 30 min to dissolve and centrifuge for 30 min to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter (Stock solution).Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute up to the mark with diluent (50 µg/mL of Mirabegronand 5 µg/mL of Solifenacin Succinate).

Procedure

The column was maintained at a temperature of 25^0C. The run time was set at 6 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. Inject 10 µL of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed.

METHOD VALIDATION

Linearity

Several aliquots of standard solutions of Mirabegron and Solifenacin Succinatewere taken in six different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations were in the range of 12.5-75 µg/mL for Mirabegronand 1.25-7.5 µg/mL for Solifenacin Succinate. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 240 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of drug in tablet dosage form.

Precision

Precision for Mirabegron and Solifenacin Succinate was determined in terms of system precision, repeatability and intermediate precision. Every sample was injected six times. The measurements for peak areas were expressed in terms of % RSD.

Accuracy
The accuracy of the method was assessed by recovery studies of Mirabegron and Solifenacin Succinate at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Mirabegron and Solifenacin Succinate. Each level was repeated three times. The % recovery of Mirabegron and Solifenacin Succinate were calculated.

System suitability
The system suitability parameters like retention time, theoretical plate count, tailing factor and resolution were evaluated by six replicate analysis of Mirabegron and Solifenacin Succinate and compared with standard values. The acceptance criteria for theoretical plates number (N) at least 3000 per each peak, tailing factors not more than 2.0 and % RSD of peak areas not more than 2% for Mirabegron and Solifenacin Succinate.

Limit of detection and limit of quantitation
The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of Mirabegron and Solifenacin Succinateusing the developed HPLC method. LOD and LOQ were estimated from signal-to-noise ratio.

Robustness
The robustness of the method was determined by making small deliberate changes in method like variation of flow rate, mobile phase ratio and temperature.

Assay
Standard preparations are made from the bulk drug and sample preparations are made from formulation. Both standard and sample solutions were injected in six homogeneous samples. 10 µL of sample solution was injected into the chromatographic system and measure the peak areas of Mirabegron and Solifenacin Succinate and calculate the % assay by using the formula. The results were compared with the label claim of Mirabegron and Solifenacin Succinatein tablet dosage form.

DEGRADATION STUDIES
Accurately weigh and transfer 50mg of Mirabegron and 5mg of Solifenacin Succinate working standards into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Acid degradation
Pipette 5 mL of above solution into a 50 mL volumetric flask and 3 mL of 1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1 N NaOH and make up to 50 mL with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Alkali degradation
Pipette 5 mL of above solution into a 50 mL volumetric flask and add 3 mL of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1N HCl and make up to 50 mL with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Peroxide degradation
Pipette 5 mL above stock solution into a 50 mL volumetric flask, 1 mL of 3% v/v of hydrogen peroxide added in 50 mL of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal degradation
Pipette 5 mL of stock solution transferred into 50 mL volumetric flask, to this add 1 mL of 10% sodium bisulphate and kept in 105°C for 1 hr. For HPLC study, the resultant solution was diluted and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photolytic degradation
The photochemical stability of the drug was also studied by exposing the sample solution to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted and 10 µLwere injected into the system and the chromatograms were recorded to assess the stability of sample.

Hydrolytic degradation
Stress testing under neutral condition was studied by refluxing the drug in water for 1 hr at a temperature of 60ºC. For HPLC study, the resultant solution was diluted and 10 µL were injected into the system and the chromatograms

were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop a simple, novel, accurate, precise and reproducible method for simultaneous estimation of Mirabegron and Solifenacin Succinate in tablet dosage form using Symmetry C18 (150 mm x 4.6 mm, 3.5 µm) column in isocratic mode with mobile phase composition of potassium dihydrogen phosphate (pH 3.4 adjusted with trifluoro acetic acid) and acetonitrile in the ratio of 35:65% v/v resulted in peak with maximum separation, good shape and resolution. A flow rate of 1.0 mL/min gave an optimum signal-to-noise ratio with reasonable separation time. Total run time was 6 minutes. The drug components were measured with UV detector at 240 nm. The results of optimized chromatographic conditions were shown in Table 1.

Linearity was obtained in the range of 12.5-75 µg/mL for Mirabegron and 1.25-7.5 µg/mL for Solifenacin Succinate. The correlation coefficient (r^2) was found to be 0.999 for both Mirabegron and Solifenacin Succinate respectively. The regression equation of the linearity plot of concentration of Mirabegron over its peak area was found to be y=50445.58x+22410.75, where x is the concentration of Mirabegron(µg/mL) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Solifenacin Succinate over its peak area was found to be y=52000.03x+969.18, where x is the concentration of Solifenacin Succinate (µg/mL) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results was shown in Table 2 and the calibration curves were shown in Fig. 3 and Fig. 4.

The % RSD for system precision, repeatability and intermediate precision for Mirabegron were found to be 0.61%, 0.87% and 0.48% respectively (limit % RSD<2.0%). The % RSD for system precision, repeatability and intermediate precision for Solifenacin Succinate were found to be 0.51%, 0.52% and 0.50% respectively (limit % RSD<2.0%) and hence the method is precise. The precision data of Mirabegron and Solifenacin Succinate were furnished in Table 3, 4 and 5.

The mean % recovery of the drugs Mirabegron and Solifenacin Succinate were found to be 99.98% and 100.15% respectively and the high percentage of recovery of Mirabegron and Solifenacin Succinate indicates that the proposed method is highly accurate. The results of accuracy studies of Mirabegron and Solifenacin Succinate were shown in Table 6 and Table 7.

The retention times for the drugs Mirabegron and Solifenacin Succinate was 2.332 minutes and 4.879 minutes respectively. The number of theoretical plates calculated for Mirabegron and Solifenacin Succinate was 5482 and 6628 respectively. The tailing factor for Mirabegron and Solifenacin Succinate was 1.16 and 1.09 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Mirabegronwere found to be 1.5 µg/mL and 4.5 µg/mL; 0.15 µg/mL and 0.5 µg/mL for Solifenacin Succinate respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 8.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Mirabegron and Solifenacin Succinate were found to be within the acceptable limits.

Validated method was applied for the simultaneous estimation of Mirabegron and Solifenacin Succinatein commercial tablet dosage forms. The % Assay of Mirabegron and Solifenacin Succinate were found to be 100.64% and 99.4% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Mirabegron and Solifenacin Succinate by the proposed HPLC method. The assay results are shown in Table 9.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Mirabegron and Solifenacin Succinate standard were shown in Fig. 5. All the degradation products formed during forced

degradation studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating**.** The results of the degradation studies are presented in Table 10.

CONCLUSION

The present method was proposed for the simultaneous estimation of the Mirabegron and Solifenacin Succinate by using RP-HPLC in tablet dosage form is found to be simple, accurate, rapid and precise. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be applied in regular quality control tests in industries.

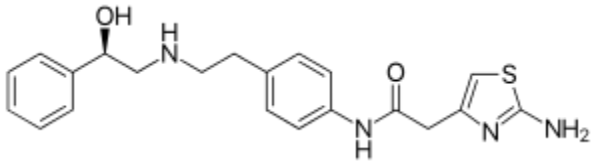

Fig. 1: Chemical structure of Mirabegron

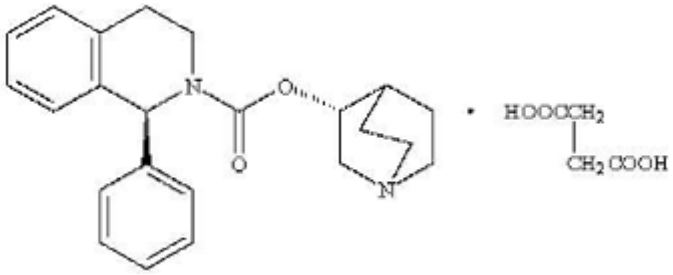

Fig. 2: Chemical structure of Solifenacin Succinate

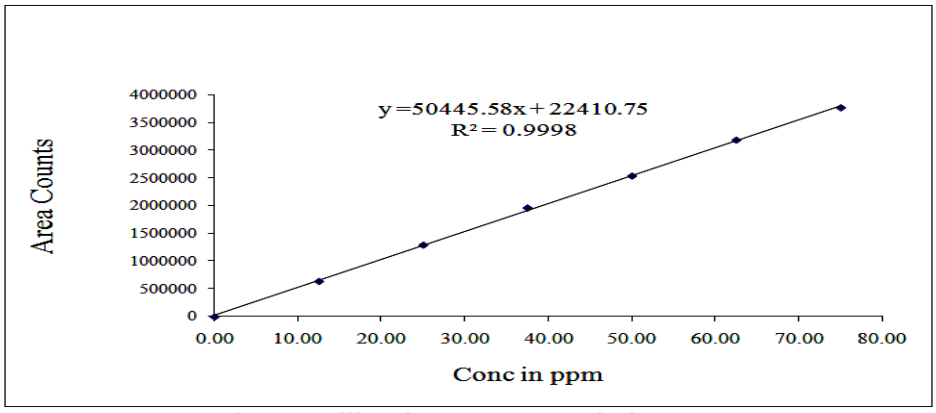

Fig. 3: Calibration curve for Mirabegron

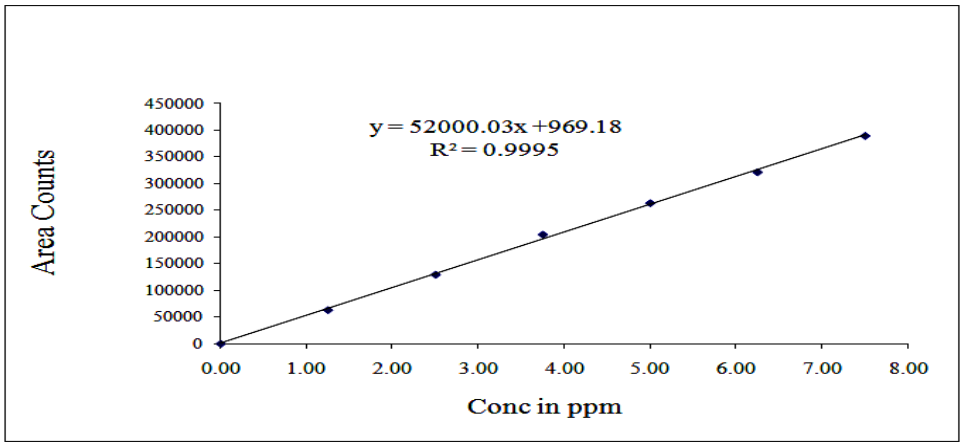

Fig. 4: Calibration curve for Solifenacin Succinate

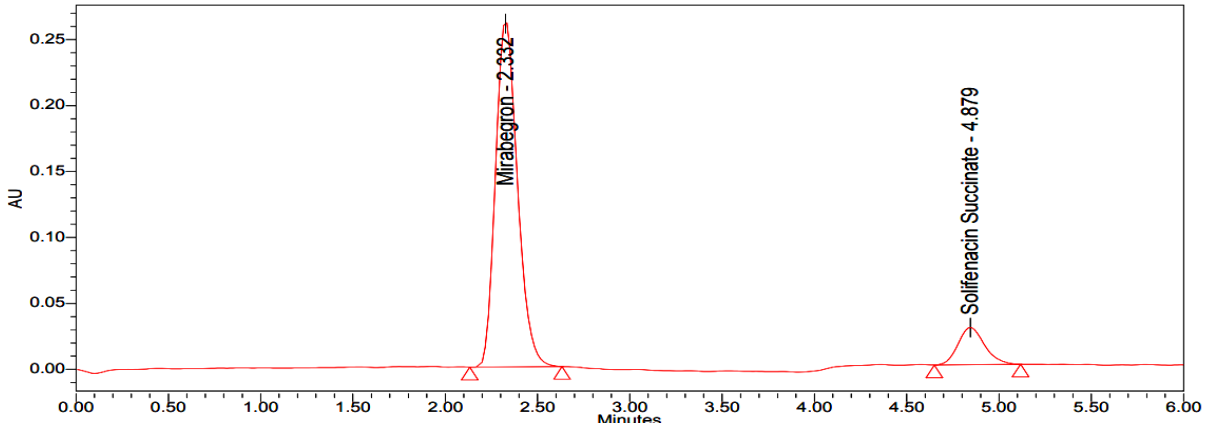

Fig. 5: Chromatogram of Mirabegron and Solifenacin Succinate

Table 1: Optimized chromatographic conditions

Parameter	Condition
Mobile phase	Phosphate buffer pH 3.4:Acetonitrile (35:65% v/v)
Diluent	Mobile phase
Column	Symmetry C18 (150 mm x 4.6 mm, 3.5 µm)
Column temperature	25^0C
Wave length	240 nm
Injection volume	10 µL
Flow rate	1.0 mL/min.
Run time	6 min.

Table 2: Linearity results of Mirabegron and Solifenacin Succinate

S. No.	Concentration of Mirabegron (µg/mL)	Peak area	Concentration of Solifenacin Succinate(µg/mL)	Peak area
1	12.50	639653	1.25	63123
2	25.00	1295299	2.50	129437
3	37.50	1963147	3.75	204437
4	50.00	2541563	5.00	263524
5	62.50	3188539	6.25	321632
6	75.00	3770639	7.50	389632

Table 3: System precision data of Mirabegron and Solifenacin Succinate

S. No.	Peak area of Mirabegron	Peak area of Solifenacin Succinate
1	2537472	262893
2	2545965	264560
3	2512625	261950
4	2530410	263591
5	2505364	265786
6	2520205	264268
Mean	2525340	263841
SD	15397.15	1343.09
% RSD	0.61	0.51

Table 4: Repeatability data of Mirabegron and Solifenacin Succinate

S. No.	Peak area of Mirabegron	Peak area of Solifenacin Succinate
1	2555679	264319
2	2512432	262247
3	2573387	264054
4	2537485	263138
5	2522066	264055
6	2541679	266359
Mean	2540455	264029
SD	22136.168	1377.222
% RSD	0.87	0.52

Table 5: Intermediate precision data of Mirabegron and Solifenacin Succinate

S. No.	Peak area of Mirabegron	Peak area of Solifenacin Succinate
1	2518427	264138
2	2546859	260955
3	2544021	262547
4	2528633	263659
5	2550492	261242
6	2536589	263348
Mean	2537504	262648
SD	12183.097	1310.382
% RSD	0.48	0.50

Table 6: Accuracy results of Mirabegron

% Concentration level	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery	% Mean recovery
50 %	25	25.09	100.36	99.98%
100%	50	50.01	100.02	
150%	75	74.69	99.58	

Table 7: Accuracy results of Solifenacin Succinate

% Concentration level	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery	% Mean recovery
50 %	21	20.85	98.8	100.15%
100%	42	41.7	101.8	
150%	63	63.33	99.86	

Table 8: System suitability parameters of Mirabegron and Solifenacin Succinate

S. No.	Parameters	Mirabegron	Solifenacin Succinate
1	Linearity (µg/mL)	12.5-75.0	1.25-7.50
2	Correlation coefficient	0.999	0.999
3	Retention time (min.)	2.332	4.879
4	Resolution	--	10.28
5	Tailing factor	1.16	1.09
6	Theoretical plates (N)	5482	6628
7	LOD (µg/mL)	1.5	0.15
8	LOQ (µg/mL)	4.5	0.5

Table 9: Assay results of Mirabegron and Solifenacin Succinate

Formulation		Label claim	Amount found	% Assay
MIRAGRON S 50	Mirabegron	50 mg	50.32 mg	100.64%
	Solifenacin Succinate	5 mg	4.97 mg	99,4%

Table 10: Degradation results for Mirabegron and Solifenacin Succinate

S. No.	Degradation condition	Mirabegron		Solifenacin Succinate	
		Peak area	% Degradation	Peak area	% Degradation
1	Acid	2204763	12.7	235670	10.6
2	Alkali	2172274	14.0	227563	13.7
3	Peroxide	2102715	16.8	222113	15.8
4	Thermal	2263289	10.4	260184	1.4
5	Photolytic	2496377	1.2	261412	0.9
6	Hydrolytic	2525530	1.6	263806	0.5

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