RP-HPLC method development and validation for the simultaneous estimation of Mirabegron and Solifenacin succinate in pharmaceutical dosage form

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Abstract:

A liquid chromatographic technique for determining the combination of Mirabegron and Solifenacin succinate has been developed and validated. The isocratic reverse phase method was used to obtain effective chromatographic separation on a Cosmosil C18 (4.6ID X 250 mm, 5m) column. The mobile phase employed was Methanol: Water (40:60), the pH was balanced to 3.0 by ortho phosphoric acid. The flow rate was maintained at 0.8 mL/min and elute was monitored at 223 nm. A linear response was observed over the concentration range 10-50 µg/mL (R2 =0.999) Mirabegron of and the concentration range 10-50 µg/mL (R2 =0.999) of Solifenacin. The limit of quantitation (LOQ) and limit of detection (LOD) for Mirabegron were 0.26 and 0.81 µg/mL and for Solifenacin were 0.05 and 0.1 µg/mL, respectively. The technique was proven effectively in compliance with ICH guideline Q2. It was observed that the percentage recovery was more than 99.0 %. The method was found to be accurate, precise, linear, specific, sensitive, rugged, robust and stability indicating.

Keywords: Mirabegron, Solifenacin succinate, validation, accuracy, HPLC

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1. Introduction

1.1 Mirabegron:2-(2-amino-1,3-thiazol-4-yl)-N-[4-[2-[[(2R)-2-hydroxy-2 phenylethyl]amino]ethyl]ph enyl] acetamide are used beta-3 adrenergic agonist. Clinically it is used to treat overactive bladder [1]. It is an alternative to antimuscarinic drugs for this indication. When Mirabegron is administered orally to healthy volunteers, the absorption rate reaches a maximal concentration after around 3.5 hours. By administering 25 mg of medication. By providing a dosage of 50 mg, the absolute bioavailability increases to 29 % while the relative bioavailability increases to 35 %.

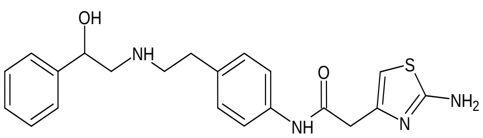

Fig 1: Mirabegron

1.2 Solifenacine succinate: [(3R)-1azabicyclo[2.2.2]octan-3-yl] (1S)-1-phenyl-3,4-dihydro1H-isoquinoline-2-carboxylate; butanedioic acid are used alpha-1 adrenergic blocker. Clinically it is used to treat overactive bladder. SOL is a competitive M3 receptor antagonist and its peak plasma concentration of 40.6 ng/mL was reached 3–8 hr after long-term oral administration. "SOL has 98% of plasma protein binding to α 1 acid glycoprotein of human plasma and is highly distributed to peripheral tissues. SOL has 90 % bioavailability and a long half-life of 48–68 hr" [2].

A review of the literature suggests that the quantification of Mirabegron and solifenacin in human plasma. [3-6], rat plasma [7,8], pharmaceutical compounds [9-12] was reported. These techniques were reported by using LC-

MS/MS [3-6], HPLC [2,9,10,,14],UPLC [15] and HPTLC [16,17].

Survey shows that there is only one analytical testing method is available for Mirabegron and Solifenacin succinate combination by HPTLC, But there is no any HPLC method reported for Mirabegron and Solifenacin succinate in combination pharmaceutical dosage form. The primary purpose of this work is to use RP-HPLC to simultaneously estimate Mirabegron and Solifenacin succinate in bulk and pharmaceutical dosage form.

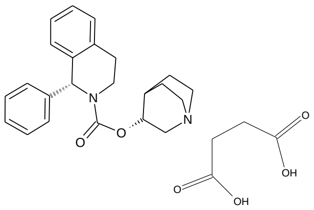

Fig 2: Solifenacine succinate

2. Materials and Methods :

Reagents and Chemicals : Cleanchem laboratories LLP Mumbai provided the active pharmaceutical ingredient Mirabegron and Solifenacin succinate as a gift sample. Orthophosphoric acid, methanol, and acetonitrile and triethylamine were obtained from Hexon laboratories. Commercially available, Mirabgron S 25 Tablet , were manufactured by STERIS Healthcare Pvt Ltd, India. Each tablet is labelled to contain 25 mg MIR and 5 mg SOL.

• Instruments: The HPLC column used was Cosmosil C18 (4.6ID X 250 mm, 5m) column using isocratic reverse phase technique. The mobile phase employed was Methanol: Water (40:60), the pH was adjusted to 3.0 by ortho phosphoric acid. The flow rate was maintained at 0.8 mL/min and elute was monitored at 223 nm.Pharmaceutical Accurately weighed 10 mg of Mirabegron and Solifenacin succinate was transferred to 10 ml volumetric flask separately , dissolved in water and methanol , and volume was then made up to the mark with water (1000 ppm) and further diluted to 25 ppm, 50 ppm, 75ppm, 100 ppm, 125 ppm for Mirabegron and 5 ppm, 10 ppm, 15 ppm, 20 ppm,25 ppm for Solifenacin succinate

Dosage Form Solutions Preparation: Sample concentration was prepared in accordance with the new formulation. Mirabegron S25. Weighing a synthetic combination containing 25 mg Mirabegron and 5 mg Solifenacin succinate in a 100 mL volumetric flask, adding 25 mL of methanol, mixing thoroughly to dissolve and making up the volume with methanol, sonicating for 15 minutes, and filtering through a 0.25 m membrane filter. Pipette 1 mL of the above mentioned solution into a 10 mL volumetric flask, dilute with mobile phase to the mark, and inject into the HPLC system for analysis.

Chromatographic Conditions: The mobile phase used was mixture of methanol and water in the ratio of (40:60 v/v) isocratic elution at a flow rate of 0.8 mL/min and the injection volume was 10 μL. The analytical column used was Cosmosil C18 (4.6ID X 250 mm, 5m) at ambient temperature. The detection was carried out at a wavelength of 223 nm for a run time of 10.98 min. Mirabegron and Solifenacin succinate reported retention times of 4.41min and 7.34 min, respectively.

Result and Discussion:

Optimization of the chromatographic conditions: The initial literature search indicated that many HPLC methods are available for individual drugs. Based on literature search, attempts were made to develop a simple method which has less retention time and higher selectivity, top priority was given for complete separation of Mirabegron and Solifenacin succinate. Mirabegron and Solifenacin succinate are hydrophilic compounds that are nearly soluble in water and easily soluble in methanol. In earlier investigations, several mobile phases were tried until significant resolution between two compounds was achieved. Various trails were carried out utilising various amounts of ortho phosphoric acid buffer with varying pH with methanol, water, and acetonitrile. The mobile phase containing ortho phosphoric acid pH 3 containing methanol and water (40:60 v/v) (adjusted to pH 3): (isocratic programme) was found to be satisfactory and gave symmetric and well resolved peak for Mirabegron and solifenacin succinate.

Validation: The method was validated successfully in compliance with ICH guideline Q2 (R1). The method was validated, and the criteria were linearity, range, accuracy, precision, LOQ, and LOD.

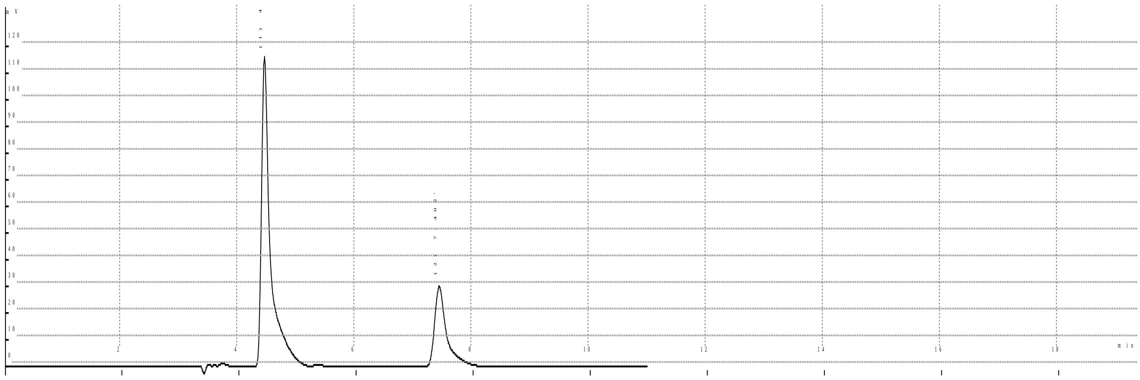

Fig 3: Chromatographic separation of standard mirabegron and solifenacin succinate

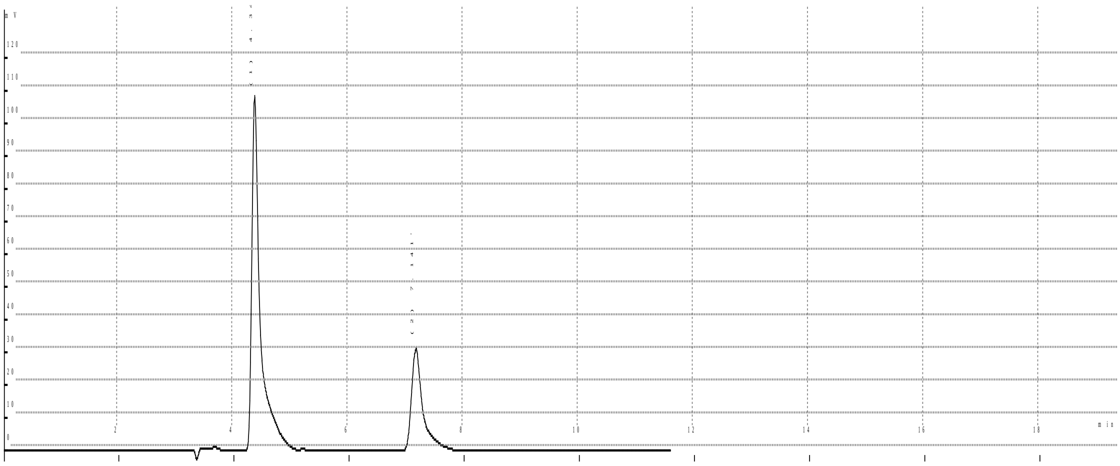

Fig 4: Chromatographic separation of formulation mirabegron and solifenacin succinate

Linearity and Range: Linearity test solutions of concentration range of 25-125 µg/ml and 5-25 µg/ml for Mirabegron and Solifenacin succinate respectively. The calibration curves were constructed by plotting peak areas versus their corresponding concentrations. The slope,*Y*-intercept, and correlation coefficient of the calibration curve were calculated. The correlation coefficient was found to be 0.999 and the calibration curve for Mirabegron and Solifenacin succinate is given in Figure 5 and 6 respectively

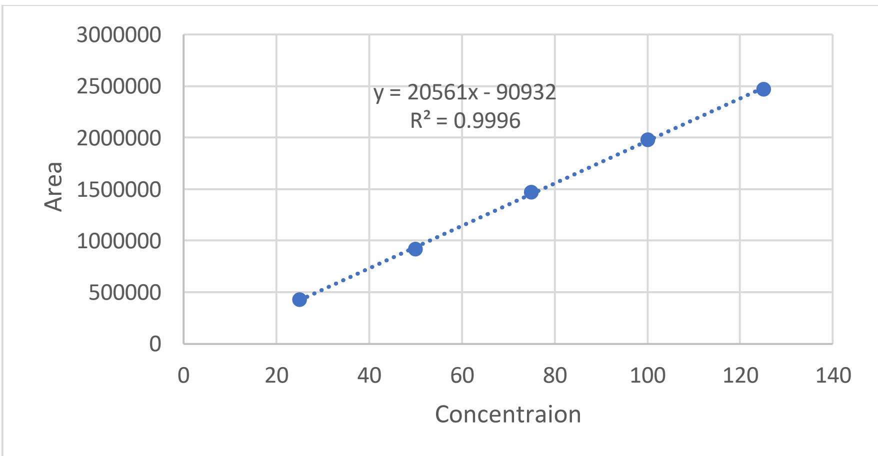

Fig 5: Calibration curve of Mirabegron

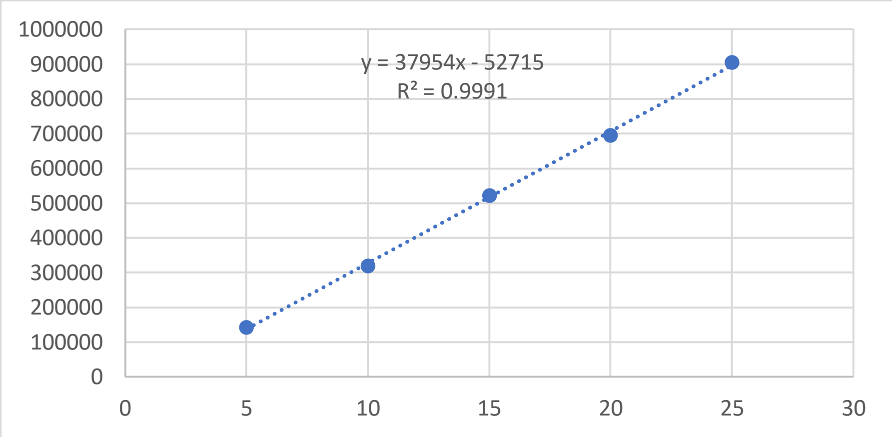

Fig 6: Calibration curve of Solifenacin succinate

Precision: Precision was determined by injecting five replicate injections of Mirabegron and Solifenacin succinate at standard concentrations under the same chromatographic parameters, and the percent RSD was calculated. The percent RSD shows that the proposed procedure may be repeated. The %RSD for assay of Mirabegron Solifenacin succinate was found to be 0.46 % and 0.54 % respectively. The results are shown in Table 1 and Table 2 respectively.

Intermediate Precision:. The technique was validated by measuring precision on the same instrument with the same chromatographic parameters on various days. Even when conducted on multiple days, the percent RSD of solifenacin succinate was shown to be less than 2. The method is said to be precise with respect to the criteria of the intermediate precision.

S. No.	Precision	Intermediate Precision
1	1470776	1470776
2	1460784	1460784
3	1459001	1459001
4	1468949	1467703
5	1463802	1469160
6	1448311	1457887
Average	1461937	1464218
% RSD	0.55 %	0.38 %

Table 1: Precision data Mirabegron

S. No.	Precision	Intermediate Precision
1	522077	522077
2	521802	521802
3	515472	515472
4	520418	521373
5	515976	517530
6	517046	519811
Average	518798	519811
% RSD	0.58 %	0.51 %

Table 2: Precision data Solifenacin succinate

Accuracy: The recovery analysis was performed at three levels of 50 %, 100 %, and 150 % by standard addition method to evaluate the method's quality and applicability. By injecting the samples, the percent recoveries for Mirabegron and Solifenacin succinate were estimated and determined to be within limits. the results are given in Table 4

Spike Level	Area	Added	Found	% Recovery	Mean	SD	% RSD
80	427466	40	40.1	100.25			
80	420945	40	39.9	99.75	100.41	0.62	0.76
80	419658	40	40.5	101.25			
100	1470776	50	50.2	100.4			
100	1460784	50	49.9	99.8	100.33	0.41	0.50
100	1459001	50	50.4	100.8			
120	2467027	60	60.1	100.1			
120	2488795	60	60.3	100.5	99.64	0.94	1.16
120	2456533	60	59.3	98.33			

Table 3: Accuracy data Mirabegron

Spike Level	Area	Added	Found	% Recovery	Mean	SD	% RSD
80	142888	08	8.11	101.3			
80	141703	08	7.98	99.75	100.1	0.87	1.07
80	142494	08	7.94	99.25			
100	522077	10	10.1	101			
100	521802	10	9.93	99.3	99.9	0.77	0.95
100	515472	10	9.94	99.4			
120	904396	12	12.12	101			
120	894276	12	11.97	99.75	100.08	0.65	0.80
120	907983	12	11.94	99.50			

Table 4: Accuracy data Solifenacin succinate

Robustness: The results of systematic alterations in flow rate, column oven temperature, mobile phase composition, wave length, and chromatograms have been observed. in table 5

Conc.	Area	Mean	SD	% SD
20	914651			
20	906537	911582	4403.37	0.48304675
20	913559			

Table 5: Robustness studies for Mirabegron change in wavelength

Conc.	Area	Mean	SD	% SD
20	914651			
20	912402	918481	8655.38	0.94235786
20	928391			

Table 6: Robustness studies for Mirabegron change in pH

Conc.	Area	Mean	SD	% SD
20	319468			
20	314329	318443	3709.28	1.16481811
20	321532			

Table 7: Robustness studies for Solifenacin succinate change in wavelength

Conc.	Area	Mean	SD	% SD
20	319468			
20	313427	317177	3273.91	1.03220323
20	318635			

Table 8: Robustness studies for Solifenacin succinate change in pH

LOD and LOQ: Preparation of calibration curve from the serial dilutions of standard was repeated for three times. The limit of detection & limit of quantification was calculated by using average value of slope and standard deviation of intercept and listed in table no. 9.

Drug	LOD (μg/Ml)	LOQ (μg/mL)
Mirabegron	0.26	0.81
Solifenacin succinate	0.25	0.46

Table 9: LOD and LOQ studies for Mirabegron and solifenacin succinate

Conclusion: The current work refers to the development and validation of the most accurate, precise, and robust reverse phase high performance liquid chromatography (RP-HPLC) method for estimating mirabegron and solifenacin succinate in pharmaceutical dosage form in accordance with the ICH Q2 (R2) parameters. The percentage of recovery indicates that the method is free from interference of the excipients used in the formulation. As a result, the proposed method can be used on a regular basis for analysis of Mirabegron and Solifenacin succinate.

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