

Development And Validation Of Stability Indicating UHPLC Method For The Quantitative Estimation Of Mirabegron And Solifenacin Succinate In Pharmaceutical Dosage Form

Mr. Manoj M. Kadam^{1*}, Dr. Ravindra P. Singh², Dr. Manoj S. Charde³

¹ Nims Institute of Pharmacy, Nims University Jaipur, Rajasthan, India 303121

² Department of Pharmaceutics, Nims Institute of Pharmacy, Nims University, Jaipur Rajasthan, India 303121

³ Government College of Pharmacy, Karad, 415110 Maharashtra E-mail: kadam.m.manoj@gmail.com

Corresponding author:

Mr. Manoj M. Kadam *

Nims Institute of Pharmacy, Nims University Jaipur, Rajasthan, India 303121 Email Id: kadam.m.manoj@gmail.com

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Abstract

A rapid stability-indicating UHPLC was developed and validated for the estimation of Mirabegron and Solifenacin combination in bulk and tablet dosage form using homologous mixture of mirabegron and solifenacin was injected into the Agilent-Poroshell (3 x 100 mm; 2.7 μ particle size) column and eluted using mobile phase as solvent A; 10mM ammonium formate (AF) and solvent B; methanol-acetonitrile (50:50 v/v) at 0.8ml/min flow rate for 18 mins. The proposed method was validated for the simultaneous determination of mifepristone and misoprostol in combined tablet dosage form. In comparison to previously reported methods, the performance of the proposed method was found to be rapid and cost-effective. The LOD 3.57, 1.75 μ g/ml and LOQ were 11.89 and 5.82 for mirabegron and solifenacin, respectively. These results signify that the selected wavelength 210 nm is more sensitive for mirabegron and Solifenacin. The forced degradation studies using UHPLC technique revealed the possible degradation of selected drugs; mirabegron and Solifenacin under the given stress conditions including effects of acid (0.1N HCl) alkali(0.1 N NaOH), hydrogen peroxide 3% H₂O₂, and thermal condition (45-60°C).As observed, upon exposure to all degradation conditions mirabegron was stable throughout the analysis similarly, upon exposure to all degradation conditions except NaOH degradation at +60°C solifenacin was significantly degraded and it was found almost 5% degradation. The developed and validated stability-indicating UHPLC method was suitable for quality control and drug analysis.

Key words: UHPLC, stability-indicating, tablet dosage form, forced degradation studies

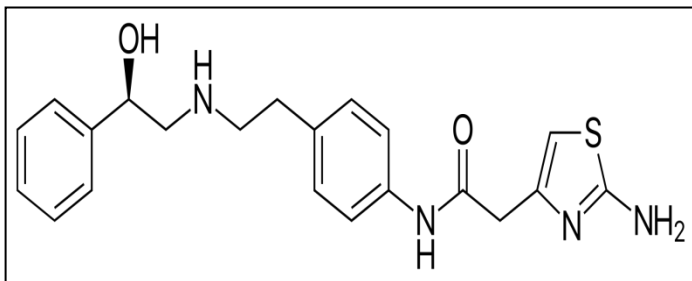
Introduction

Overactive bladder [OAB] is defined by the International Continence Society as urgency with or without urinary incontinence, usually associated with frequency and nocturia, is a multifactorial and common health disorder associated with detrimental effects on quality of life and huge economic burden and urge incontinence. It is estimated that in 2018 there were 546 million people suffering from OAB worldwide. It revealed that the prevalence of OAB worldwide is estimated as being greater in women than in men in 2008 (11.6% vs. 9.7%, respectively), 2013 (11.7% vs. 9.8%) and 2018 (11.9% vs. 10.0%)[1-2].

Mirabegron+Solifenacin is combination of Solifenacin (anti-cholinergic) and Mirabegron (beta-3 adrenergic agonist) medicine used to treat overactive bladder (OAB) with symptoms of urinary incontinence (urine leakage), urgent or frequent urination, increased night time urination, and dysuria (painful or difficult urination). Overactive bladder is a condition in which there is a problem with bladder function, which causes a sudden urge to urinate that, might be difficult to control[1-2].

Mirabegron is a selective β_3 -adrenoceptor (AR) agonist, is a first in class drug for the treatment of OAB. Chemically it is name as (2-(2-amino-1,3-thiazol-4-yl)-N-[4-(2-((2R)-2-hydroxy-2-phenylethyl)amino) ethyl]phenyl]acetamide) having an empirical formula of $C_{21}H_{24}N_4O_2S$ and molecular weight of 396.5 gms/mol. It is a novel, effective and highly selective β_3 receptor agonists for the treatment of OAB. It was approved in the United States in July 2012. Astellas Pharma Inc. found it is an agonist of the human β_3 -adrenoceptor[1]. The activation of the β_3 adrenergic receptor in the distrust or can relax the muscle in the bladder and increase bladder capacity.

Fig. 1: Structure of Mirabegron

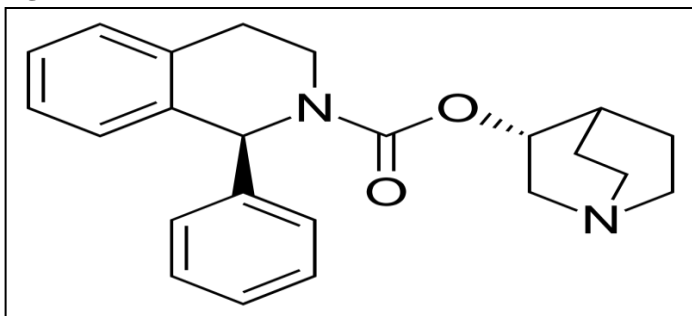


In the present study, the development of a sensitive and rapid method for estimating mirabegron in plasma was achieved and successfully applied to the pharmacokinetic study of mirabegron after oral and intravenous administration.

Solifenacin succinate (SOL) is a competitive muscarinic acetylcholine receptor antagonist used in the treatment of overactive bladder with or without urge incontinence. Chemically it is name as butanedioic acid(3R)-1-azabicyclo[2.2.2]octan-3-yl(1S)-1-phenyl-1,2,3,4 tetrahydro isoquinoline-2-carboxylate having an empirical formula of $C_{27}H_{32}N_2O_6$ and molecular weight of 480.5528 gms/mol.[3].

Solifenacin reduces the undesirable contractions of the bladder, thereby increasing the amount of urine that the bladder can hold. This decreases the urgency for urination Solifenacin is extensively metabolized in the liver. The metabolites observed as one pharmacologically active metabolite (4Rhydroxy solifenacin), and three pharmacologically inactive metabolites (N-glucuronide and the N-oxide and 4R-hydroxy-N-oxide of solifenacin) occurring at low concentrations in human plasma after oral dosing[3-4].

Fig. 2. Structure of Solifenacin



A review of the literature suggests that the quantification of Mirabegron and solifenacin in human plasma separately[R6], rat plasma [R1], pharmaceutical compounds [R7] was reported. These techniques were reported by using LC- MS/MS [3-6], HPLC [R7], HPTLC [R8] and UPLC [R9].

Survey shows that there is one RP-HPLC analytical testing method is available for Mirabegron and Solifenacin succinate in combination with lots of correctible data and one HPTLC method is available. But there is no UHPLC method reported for Mirabegron and Solifenacin succinate in combination pharmaceutical dosage form with column size 2.7 μ . The primary purpose of this work is to use UHPLC to simultaneously estimate Mirabegron and Solifenacin succinate in bulk and pharmaceutical dosage form[6-9].

Materials and Methods

Reagents and reference samples

The reference standards; mirabegron and solifenacin were obtained as a gift samples from Yarrow Pharma Ltd. Ammonium formate was purchased from Merck Ltd. (Mumbai-India) HPLC grade acetonitrile and deionised water were purchased from Merck (Mumbai, India). 0.20 μ and 0.45 μ nylon membrane filters were used and purchased from UltraChrom Innovatives Pvt. Ltd. (India). All other chemicals and reagents were used of HPLC grade.

Instrumentation

The high performance liquid chromatography (HPLC) of Shimadzu SCL-10A_{VP} inbuilt with binary pump (LC-10AT_{VP}), UV detector (SPD-10A_{VP}), Rheodyne 20 μ l loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC-Solution software was used to interpret the HPLC reports. Agilent Poroshell (2.7 μ m; 100 x 3 mm ID.) column was purchased from Agilent Ltd. (Mumbai, India) was used throughout the analysis. Digital weighing balance (ME-204) purchased from Mettler-Toledo (USA), ultra-sonicator Labman[®] purchased from UltraChrom Ltd, India. Digital pH meter from Mettler-Toledo was purchased from (Mumbai-India). 50 μ micro-syringe was purchased from Hamilton USA. 0.20 μ and 0.45 μ nylon membrane filters were purchased from Phenomenex[®] Mumbai, India.

Selection of solvent and wavelength

Both mirabegron and solifenacin are soluble in methanol; partially soluble in water and acetonitrile. Hence, standard stock solution of both mirabegron and solifenacin was prepared in methanol-acetonitrile-water (80:10:10% v/v). Both mirabegron and solifenacin shows maximum UV absorbance at 210 nm wavelength; hence, it was selected throughout the HPLC-UV analysis.

Preparation of stock standard solutions

Exactly, 50 mg of mirabegron and 5 mg solifenacin standards were weighed separately and dissolved in methanol-water-acetonitrile (8:1:1,v/v) to get 5000 ppm and 500 ppm(5000 μ g/ml and 500 μ g/ml) solution. Both samples were then sonicated for 2-5 minutes. Furthermore, as per the need, serial dilutions of homogenous mixture containing mirabegron (50 μ g/ml) and solifenacin (5 μ g/ml) was prepared and used for the determination of repeatability, precision and robustness.

Chromatographic conditions

20 μ l of freshly prepared homologous mixture of mirabegron and solifenacin was injected into the Agilent-Poroshell (3 x 100 mm; 2.7 μ particle size) column and eluted using mobile phase as solvent A; 10mM ammonium formate (AF) and solvent B; methanol-acetonitrile (50:50 v/v) at 0.8ml/min flow rate for 18 mins. Separation was carried out at room temperature and monitored at 210 nm wavelength.

Fig. 3. Developed chromatogram of mirabegron and solifenacin in 10mM AF

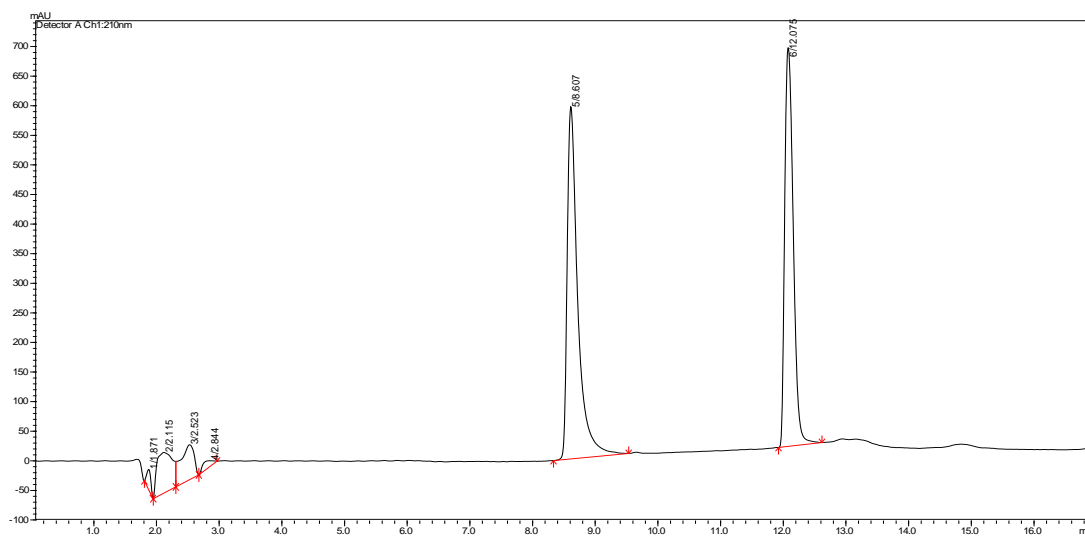


Table 1. Developed chromatogram in tabular form

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.871	147797	33463	0.9346	3421.595	--	0	1.054

2	2.115	1197567	68825	7.5732	235.859	0.718	0.13	--
3	2.523	905054	59230	5.7234	529.257	0.825	0.348	--
4	2.844	145563	10308	0.9205	1616.705	0.89	0.52	0.865
mirabegron	8.607	7009617	595330	44.3274	14469.74	20.248	3.599	2.111
solifenacin	12.075	6407667	673511	40.5208	36758.9	12.889	5.452	1.466

System suitability studies

The homogenous mixture of freshly prepared stock solution of equal concentration of mirabegron (50 ppm) and solifenacin (5 ppm) were injected 6 times to determine the closeness of results achieved for relative standard deviation (RSD) in percentage; The calculated values should always less than 2%. Moreover, other system suitability parameters including, retention or capacity factor (k'), resolution (R_s) and theoretical plates (N), tailing factor/peak asymmetry (A_s) and separation factor were tested and evaluated.

Method validation studies

The proposed UHPLC method for the simultaneous quantification of Mirabegron and Solifenacin was validated as per the ICH guidelines. The validation parameters like repeatability, precision (interday/intermediate), robustness/ruggedness, linearity/calibration, force degradation and accuracy studies were tested and evaluated and found they are in accordance with the ICH guidelines.

Sample preparation for Linearity/Calibration studies

The linearity/calibration studies of HPLC-DAD method represents its ability to explicit the results that should proportional to the known concentration of studied analytes. The sample solution 5000 ppm (5000 $\mu\text{g/ml}$) of standard stock solution of mirabegron and 500ppm (500 $\mu\text{g/ml}$) of standard stock solution of solifenacin was made separately, followed by mixing both stock solutions to get 50 ppm and 5 ppm of each. Subsequently, serial dilutions of six different concentrations ranging between 25–150ppm and 2.5-15ppm were made respectively for Mirabegron and Solifenacin. Each sample is ultrasonicated and then analysed as per the chromatographic condition in section Furthermore, the calibration curve (linearity graph) was plotted by calculating the peak area against known concentration. Therefore, over the known concentrations of mirabegron and solifenacin their corresponding area were found highly proportional since as noted their regression coefficients (R^2) were close to 1.

RESULTS AND DISCUSSION:

Linearity and Range:

At concentration ranges of 25-75 $\mu\text{g/ml}$ for mirabegron and 2.5-7.5 $\mu\text{g/ml}$ for solifenacin, linearity was evaluated. In each case, a strong linear relationship was observed between concentration and peak area. The relationship is described by the linear equations $y = 43633x - 18350$ for mirabegron and $y = 12255x + 6446,4$ for solifenacin. Where X represents the drug concentration and Y the peak area. In every instance, the regression coefficient (R^2) was 0.999. The R^2 value conformed to ICH recommendations.

Table 2. Linearity of Mirabegron

Name of Drug; Mirabegron		
Sr. No.	Concentration ($\mu\text{g.mL}^{-1}$)	Area
1	25	3122833
2	50	6155456
3	75	9412654
4	100	12284329

5	125	15162872
6	150	17948736

Fig. 4. Calibration curve of Mirabegron

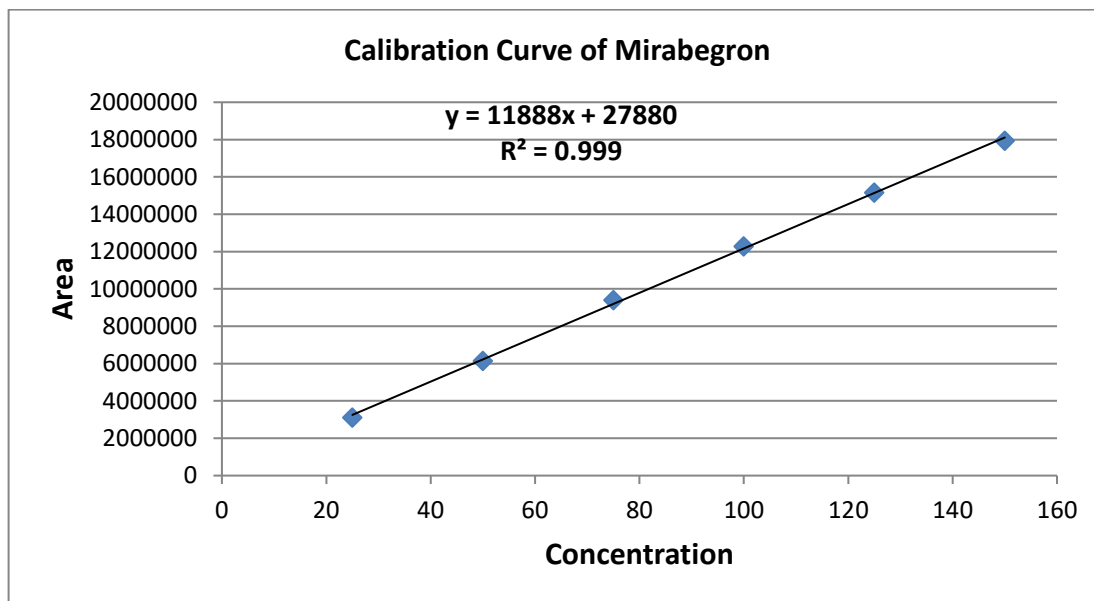
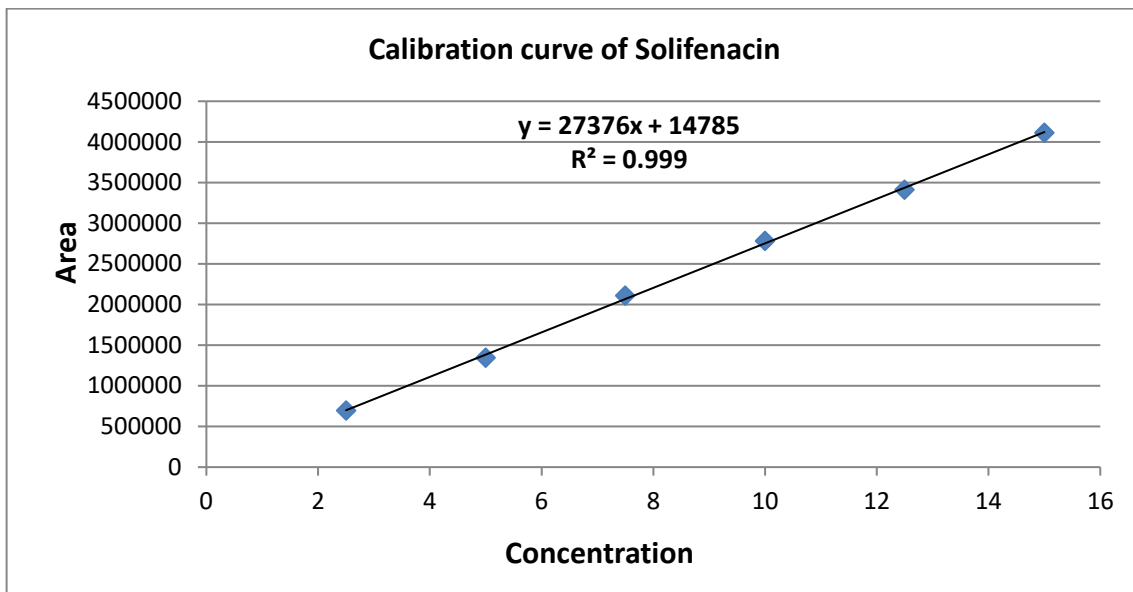


Table 3. Linearity of Solifenacin

Name of Drug; Solifenacin		
Sr. No.	Concentration ($\mu\text{g.mL}^{-1}$)	Area
1	2.5	697528
2	5	1345892
3	7.5	2108535
4	10	2783475
5	12.5	3412535
6	15	4113458

Fig. 5. Calibration curve of Solifenacin



Precision studies for mirabegron and solifenacin

The precision of HPLC method represents its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions. As displayed in Table 4 & 5 for intermediate variability for precision studies, this method is significantly precise over the testing range of mirabegron and solifenacin. Moreover, the peak area of all studied samples was also correlated with selected concentration since as observed their percentage relative standard deviation (RSD) was less than 2%. Thus it reflects, the proposed method has acceptable precision with minimum variations and can be applicable for routine analysis.

Table 4; Interday (intermediate) Precision data of Mirabegron

Drug Name: Mirabegron				
Sr. No.	Concentration (ppm)	Area	Mean \pm SD	%RSD
DAY 1	50 PPM	6126515	2746458.0214	0.31
	50 PPM	6165307		
	50 PPM	6146504		
DAY 2	50 PPM	5907488	3239817.8354	1.79
	50 PPM	5929568		
	50 PPM	5908250		
DAY 3	50 PPM	6248788	3433132.4569	0.46
	50 PPM	6294322		
	50 PPM	6260901		
Range of % RSD				0.82-1.10

Table 5. Interday (intermediate) Precision data of Solifenacin

Drug Name: Solifenacin

Sr. No.	Concentration (ppm)	Area	Mean \pm SD	%RSD
DAY 1	10 PPM	2783475	1522421.74253	0.55
	10 PPM	2760746		
	10 PPM	2794241		
DAY 2	10 PPM	2696525	1480858.1396	0.91
	10 PPM	2726574		
	10 PPM	2687621		
DAY 3	10 PPM	2736573	1509885.6388	0.80
	10 PPM	2756571		
	10 PPM	2776576		
Range of % RSD				0.30 -1.26

Robustness for the chromatographic method

Robustness of any HPLC method represents its ability to remain unaffected by small but deliberate changes in certain separation factors to ascertain its reliability during routine HPLC analysis. The variation in separation factors such as effect of temperature, flow rate, wavelength, column length, stationary phase particle size, pH, organic modifier composition in mobile phase and injection volume have been considered. The effects of all these variables over changes in retention pattern including effects on capacity/retention factor (k'), resolution (R_s), tailing factor (T_f), separation factor, theoretical plates (N) and peak area can be monitored.

In this method, robustness studies was established by making deliberate changes in flow rate ($0.8\text{ml} \pm 0.1\text{ ml/minutes}$), organic modifier as solvent B in gradient(solvent B $\pm 2\%$ ml), and wavelength ($210 \pm 2\text{nm}$). Variation in flow rate and organic modifier have made slight changes in retention pattern like increase in flow rate and organic modifier have reduce the retention time, retention factor and resolution whereas decreasing the same variables have marginally extended the retention time, capacity/retention factor (k'), resolution (R_s). As noted, these variations have not made any significant changes in theoretical plates and tailing factor of all selected drugs.

The robustness studies for simultaneous estimation of mirabegron and solifenacin were almost unchanged which clearly depicts that the proposed HPLC method obliged all minimum requirements led by the ICH guidelines.

Repeatability studies

Implementing the procedure under chromatographic condition of experimental section, the homologous mixture of 50 ppm and 5ppm of each drug is selected analytes was injected six times with similar procedure within a same day. The% RSD was calculated and found it is less than 2% for mirabegron (1.52%) and solifenacin (0.51%).

Table 6. Repeatability data of mirabegron and solifenacin

S. No.	Drug Name;Mirabegron	Drug Name:Solifenacin
	Peak Area; Conc. 50 ppm	Peak Area; Conc. 5 ppm
1	6326515	1309698
2	6365307	1360746
3	6346504	1384241
4	6325327	1396388

5	6382048	1313943
6	6120093	1361096
Mean	6310965.667	1354352
STD. DEV.	96071.90672	35703.24743
(%)RSD	1.52	0.51

Accuracy studies of marketed formulation

Percentage drug accuracy of three different concentrations; 80%, 100% and 120% (injected thrice) to estimate the Mirabegron and Solifenacin from marketed formulation[Mirago-S 50 Tablet] and results obtained have been reported in Table 7 Alternatively, accuracy can also be studied by applying the calibration curve.

As resulted, the achieved drug recovery of both mirabegron and Solifenacin were in the range of 101.40-102.98and 101.42-108.25, respectively. As recommended by International conferences of Harmonization (ICH) guidelines the drug recovery should be within the range of $100 \pm 10\%$ and the RSD in percentage were quite less than 2%. Hence, the calculated drug recoveries for simultaneous estimation of Mirabegron and Solifenacin represents the drug recovery were in the acceptance limit given by ICH guidelines.

Table 7. Accuracy data of mirabegron and solifenacin

Drug Name: mirabegron			Drug content: 50 mg		Marketed formulation: Mirago-S 50 Tablet			
Std. conc. (%)	Std. (ppm)	Peak area	Drug (%)	Drug (ppm)	Peak area	Avg. peak area	Drug Rec. (%)	
100%	50 ppm	6287230	80	40	5211491	5159361	102.57	
				40	5107231			
			100	50	6365307	6376208.5		101.42
				50	6387110			
			120	60	7483611	7457017		99.21
				60	7430423			
Drug recovery Range (%) as per ICH = $100 \pm 10\%$							99.21 % - 102.57%	

Table 8. Accuracy data of mirabegron and solifenacin

Drug Name: solifenacin			Drug content: 5 mg		Marketed formulation: Mirago-S 50Tablet		
Std. conc. (%)	Std. (ppm)	Peak area	Drug (%)	Drug (ppm)	Peak area	Avg. peak area	Drug Rec. (%)
100%	5 ppm	134533	80	4	109778	105988.5	98.47
				4	102199		
			100	5	138330	145324.5	

			5	152319			
		120	6	171192	169701	105.11	
			6	168210			
		Drug recovery Range (%) as per ICH = 100±10%				98.47% - 107.98%	

Force degradation studies

The forced degradation studies using UHPLC technique revealed the possible degradation of selected drugs; mirabegron and Solifenacin under the given stress conditions including effects of acid (0.1N HCl) alkali(0.1 N NaOH), hydrogen peroxide 3% H₂O₂), and thermal condition (45-60°C).

As observed, upon exposure to all degradation conditions mirabegron was stable throughout the analysis (Fig 6. and Table 9).similarly, upon exposure to all degradation conditions except NaOH degradation at +60°C solifenacin was significantly degraded and it was found almost 5% degradation (Figure 7).

Figure 6; Effect of 45° Conc. analysis of mirabegron and solifenacin

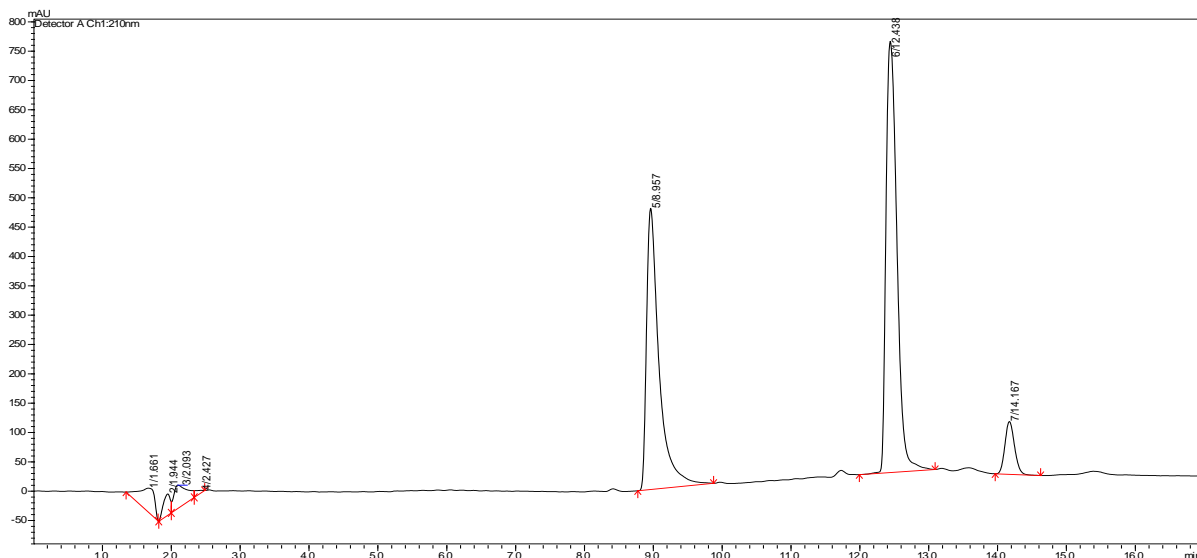


Table 9. Force degradation studies of Mirabegron

Conditions:	t _R (mins)	t _R after degradation	Degradants no.	Degradation (%)
Thermal (45-60°C) + 12 Hrs	8.6	8.61	0 degradants	0.00%
Acid (0.1NHCl) + 45-60°C + 12 Hrs.	8.6	9.19	0 degradants	1.00%
Base (0.1NNaOH) + 60°C + 12 Hrs.	8.6	8.95	0 degradants	0.43%

Oxidation (3-6% H ₂ O ₂) + Room Temp.	8.6	8.91	0 degradation	0.13%
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Fig. 7. Effect of 0.1N NaOH on analysis of mirabegron and solifenacin

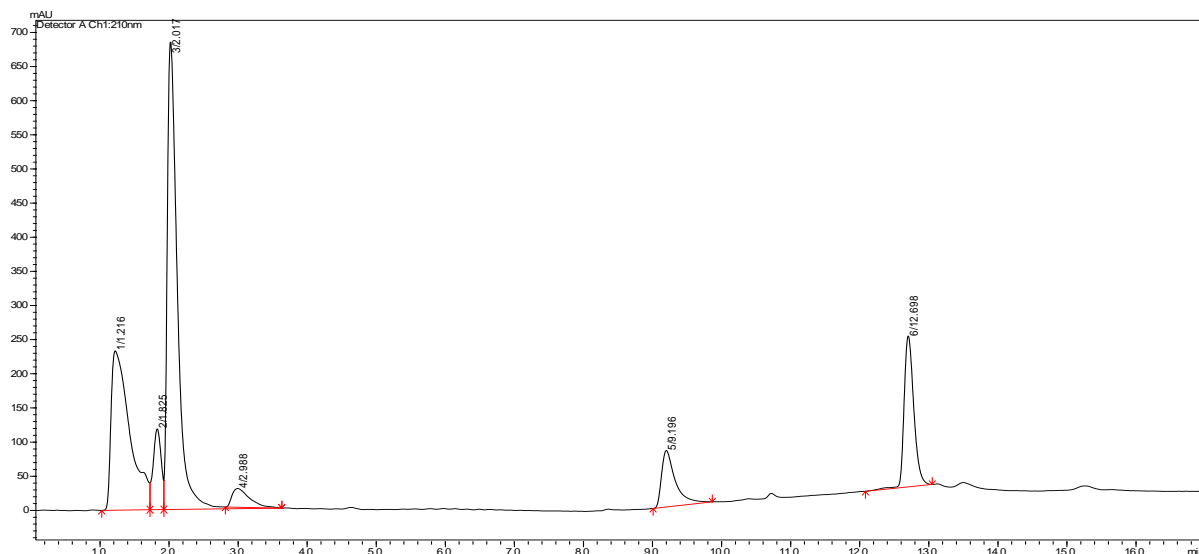


Table 10. Force degradation studies of Solifenacin

Conditions:	t _r (mins)	t _r after degradation	Degradants no.	Degradation (%)
Thermal (45-60°C) + 12 Hrs	12.07	12.22	1 degradant	0.27%
Acid (0.1NHCl) + 45-60°C + 12 Hrs.	12.07	12.69	0 degradants	0 %
Base (0.1NNaOH) + 60°C + 12 Hrs.	12.07	14.43	1 degradants	5.15%
Oxidation (3-6% H ₂ O ₂) + Room Temp.	12.07	12.37	0 degradation	0 %

LOD and LOQ

Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the regression equation. As observed, the LOD 3.57, 1.75 µg/ml and LOQ were 11.89 and 5.82 for mirabegron and solifenacin, respectively. These results signify that the selected wavelength 210 nm is more sensitive for mirabegron and Solifenacin. Thus, the proposed method can be used for the routine UHPLC analysis of either individual or simultaneous analysis of selected drugs from pharmaceutical drugs or biological fluids.

Conclusion:

According to ICH guidelines, an UHPLC method for the simultaneous estimation of mirabegron and solifenacin in tablet dosage form was developed and validated. At concentration ranges of 25-75 µg/ml for mirabegron and 2.5-7.5 µg/ml for solifenacin, linearity was evaluated. The relationship is described by the linear equations $y = 43633x - 18350$ for mirabegron and $y = 12255x + 6446,4$ for solifenacin. Where X

represents the drug concentration and Y the peak area. In every instance, the regression coefficient (R²) was 0.999. The R² value conformed to ICH recommendations. The developed method is simple, sensitive, rapid, linear, rugged, precise, robust, and specific.

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