International Journal of Advanced Research in Engineering Technology & Science ISSN: 2349-2819
Impact Factor: 7.10
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Email: editor@ijarets.org Volume-11 Issue-11 November-2024 www.ijarets.org

UHPLC-BASED STABILITY-INDICATING METHOD FOR ACCURATE QUANTIFICATION OF MIRABEGRON AND SOLIFENACIN SUCCINATE IN PHARMACEUTICAL PRODUCTS

Akhilesh Yadav

Research Scholar, Glocal School of Pharmacy, The Glocal University

Mirzapur Pole, Saharanpur (U.P) India.

Dr. Fazlu Rehman

Research Supervisor, Glocal School of Pharmacy, The Glocal University

Mirzapur Pole, Saharanpur (U.P)

ABSTRACT

A fast stability-indicating UHPLC method was developed and validated for the quantification of Mirabegron and Solifenacin in both bulk and tablet formulations. A mixture of Mirabegron and Solifenacin was injected into an Agilent-Poroshell (3 x 100 mm; 2.7µ particle size) column and separated using a mobile phase consisting of solvent A: 10mM ammonium formate (AF) and solvent B: methanol-acetonitrile (50:50 v/v), at a flow rate of 0.8 mL/min for 18 minutes. The method was validated for the simultaneous determination of these compounds in combination tablet dosage forms. Compared to previously reported techniques, this method was found to be faster and more cost-effective. The limits of detection (LOD) for Mirabegron and Solifenacin were 3.57 and 1.75 µg/mL, respectively, while the limits of quantification (LOQ) were 11.89 and 5.82 µg/mL. The results demonstrated that the chosen wavelength of 210 nm was particularly sensitive for both drugs. Forced degradation studies conducted with the UHPLC method showed that both drugs were susceptible to degradation under certain stress conditions, including acid (0.1N HCl), alkali (0.1 N NaOH), hydrogen peroxide (3% H2O2), and thermal (45–60˚C) stress. Mirabegron remained stable under all conditions, whereas Solifenacin degraded significantly when exposed to 0.1 N NaOH at 60˚C, showing approximately 5% degradation. The validated stability-indicating UHPLC method is suitable for quality control and routine drug analysis.

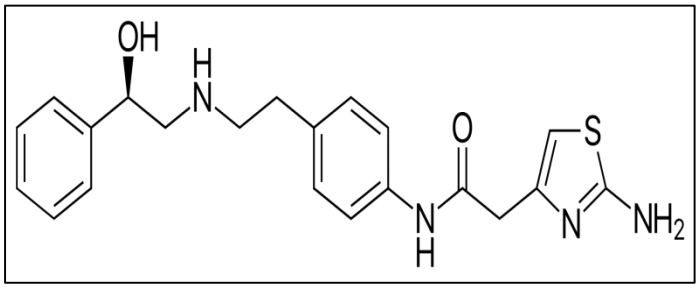
Key words: UHPLC, stability-indicating, tablet dosage form, forced degradation studies

INTRODUCTION

Overactive bladder (OAB), as defined by the International Continence Society, is characterized by urgency with or without urinary incontinence, often accompanied by frequency and nocturia. It is a common, multifactorial health condition that significantly impacts quality of life and imposes a considerable economic burden. OAB is also linked to urge incontinence. In 2018, it was estimated that 546 million people worldwide were affected by OAB. The prevalence of OAB is higher in women compared to men, with the rates in 2008 (11.6% vs. 9.7%), 2013 (11.7% vs. 9.8%), and 2018 (11.9% vs. 10.0%) showing this trend.The combination of Mirabegron and Solifenacin is used to treat OAB symptoms such as urinary incontinence, frequent or urgent

urination, increased nighttime urination, and dysuria (painful or difficult urination). OAB occurs when there is dysfunction in the bladder, leading to an uncontrollable urge to urinate. Mirabegron is a selective β3-adrenoceptor agonist and is the first drug of its kind for treating OAB. Chemically, it is known as (2-(2-amino-1,3-thiazol-4-yl)-N-[4-(2-{[(2R)-2-hydroxy-2-phenylethyl]amino} ethyl)phenyl]acetamide), with the empirical formula C21H24N4O2S and a molecular weight of 396.5 g/mol. It is a highly effective and selective β3 receptor agonist for OAB treatment, approved by the United States FDA in July 2012. Developed by Astellas Pharma Inc., Mirabegron activates the human β3-adrenoceptor, which helps relax bladder muscles and increase bladder capacity.

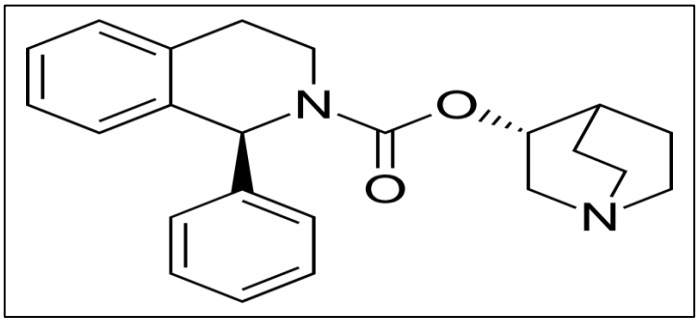
Fig. 1: Structure of Mirabegron



In this study, a sensitive and rapid method for quantifying Mirabegron in plasma was developed and successfully applied to a pharmacokinetic analysis of Mirabegron following both oral and intravenous administration.

Solifenacin succinate (SOL) is a competitive antagonist of the muscarinic acetylcholine receptor, used to treat overactive bladder with or without urge incontinence. Chemically, it is known as butanedioic acid (3R)-1-azabicyclo[2.2.2]octan-3-yl(1S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate, with the empirical formula C27H32N2O6 and a molecular weight of 480.5528 g/mol. Solifenacin works by reducing unwanted bladder contractions, increasing bladder capacity, and thereby decreasing the urgency to urinate. It undergoes extensive metabolism in the liver, producing one pharmacologically active metabolite (4R-hydroxy solifenacin) and three inactive metabolites (N-glucuronide, N-oxide, and 4R-hydroxy-N-oxide of solifenacin) that are found in low concentrations in human plasma following oral administration.

Fig. 2. Structure of Solifenacin



A review of the literature suggests that the quantification of Mirabegron and solifenacin in human plasma seperatly[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 2.7µ. 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 of Mirabegron and Solifenacin were kindly provided as gift samples by Yarrow Pharma Ltd. Ammonium formate was sourced from Merck Ltd. (Mumbai, India). HPLC-grade acetonitrile and deionized water were obtained from Merck (Mumbai, India). Nylon membrane filters with pore sizes of 0.20µ and 0.45µ were purchased from UltraChrom Innovatives Pvt. Ltd. (India). All other chemicals and reagents used were of HPLC grade.

Instrumentation

The high performance liquid chromatography (HPLC) of Shimadzu SCL-10AVP inbuilt with binary pump (LC-10ATVP), UV detector (SPD- 10AVP), Rheodyne 20µl 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 (2.7µm; 100 x 3 mm ID.) column was purchased from Agilent Ltd. (Mumabi, 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. 0.20µ and 0.45µ 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 µg/ml and 500 µg/ml) solution. Both samples were then sonicated for 2-5 minutes. Furthermore, as per the need, serial dilutions of homogenous mixture containing mirabegron (50 µg/ml) and solifenacin (5 µg/ml) was prepared and used for the determination of repeatability, precision and robustness.

Chromatographic conditions

20 µl of freshly prepared homologous mixture of mirabegron and solifenacin was injected into the Agilent-Poroshell (3 x 100 mm; 2.7µ 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/mins 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

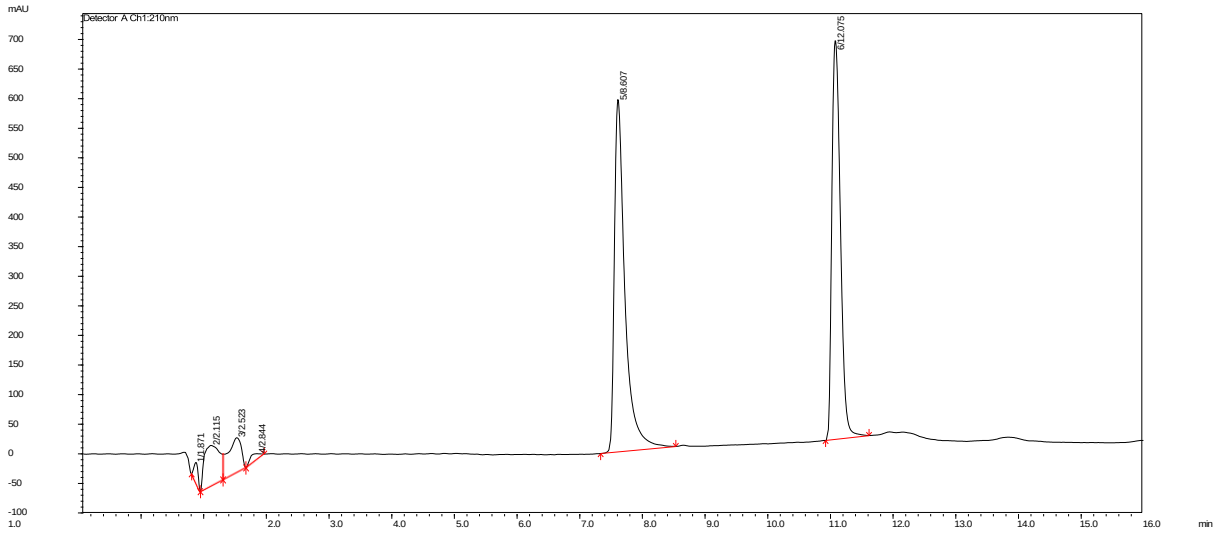


Table 1. Developed chromatogram in tabular form

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 (Rs) and theoretical plates (N), tailing factor/peak asymmetry (As) 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 µg/ml) of

standard stock solution of mirabegron and 500ppm (500 µg/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 µg/ml for mirabegron and 2.5-7.5 µg/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 (R2) was 0.999. The R2 value conformed to ICH recommendations.

Table 2. Linearity of Mirabegron

Name of Drug; Mirabegron		
Sr. No.	**Concentration (µg.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

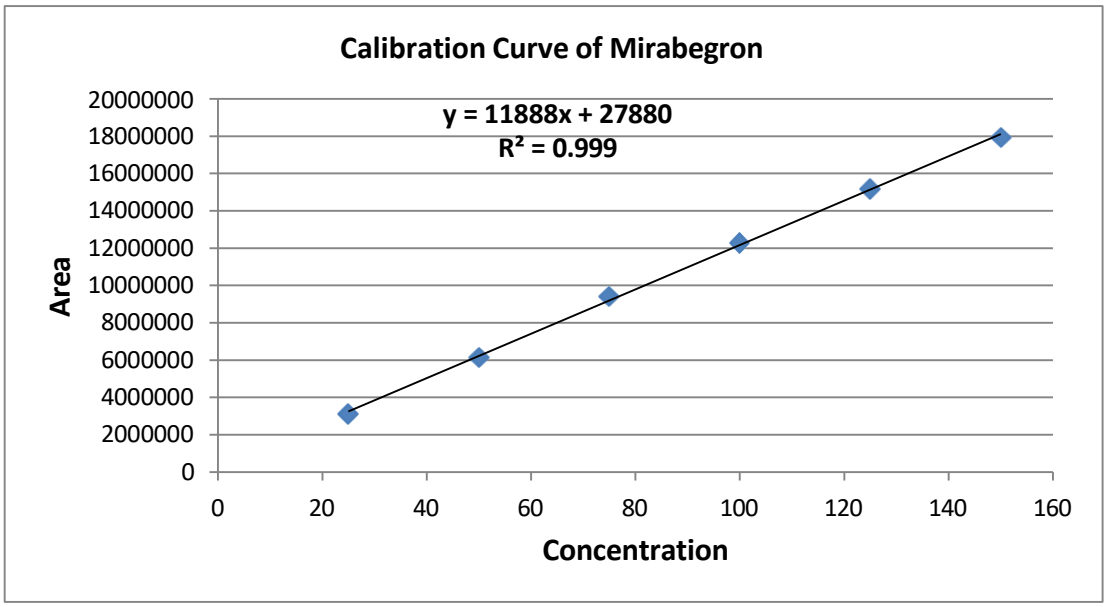
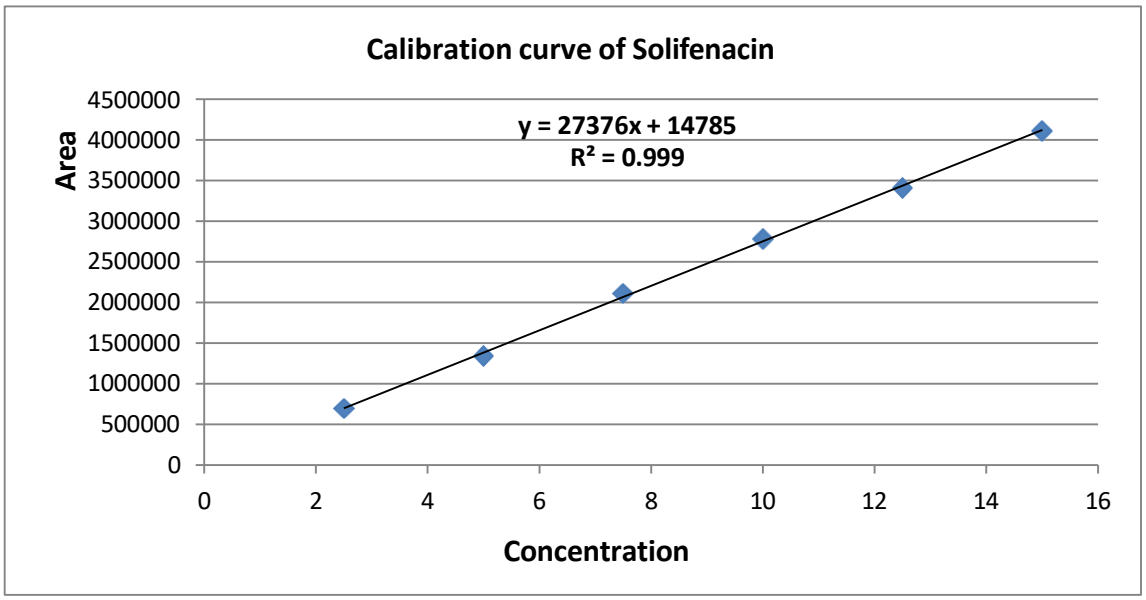


Table 3. Linearity of Solifenacin

Name of Drug; Solifenacin		
Sr. No.	**Concentration (µg.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 ± 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 ± 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 (Rs), tailing factor (Tf), 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 (0.8ml ± 0.1 ml/minutes), organic modifier as solvent B in gradient(solvent B±2% ml), and wavelength (210 ±2nm. 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 (Rs). 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 ±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±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	107.98
				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% H_2O_2), 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

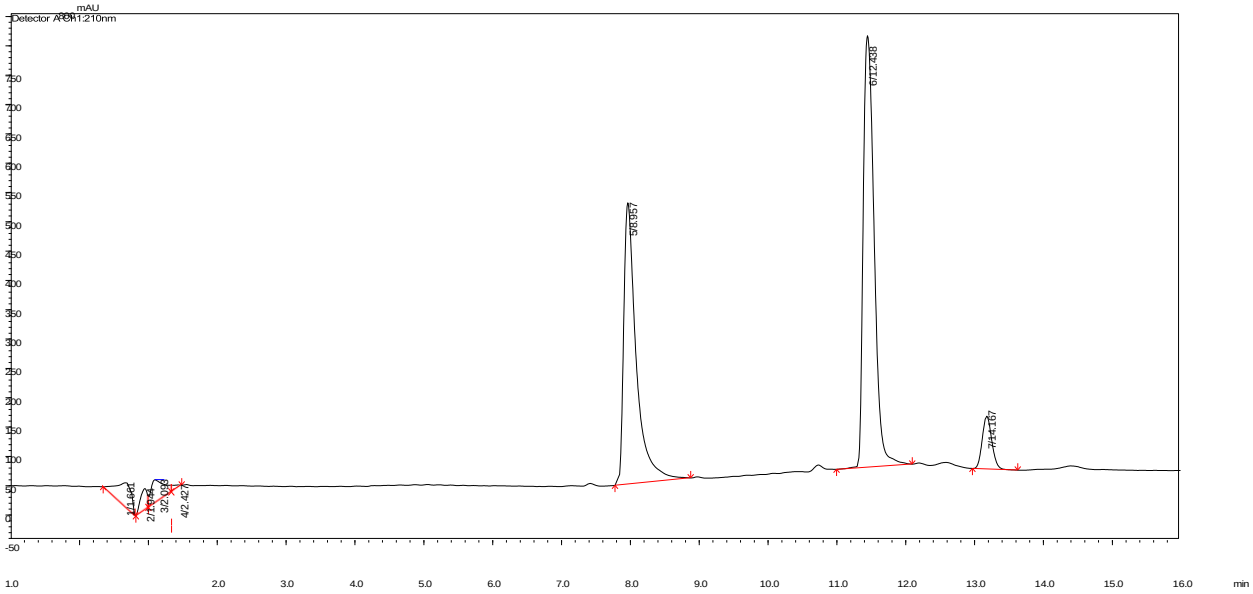


Table 9. Force degradation studies of Mirabegron

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% H2O2) + Room Temp.	8.6	8.91	0 degradation	0.13%

Fig. 7. Effect of 0.1N NaOHon analysis of mirabegron and solifenacin

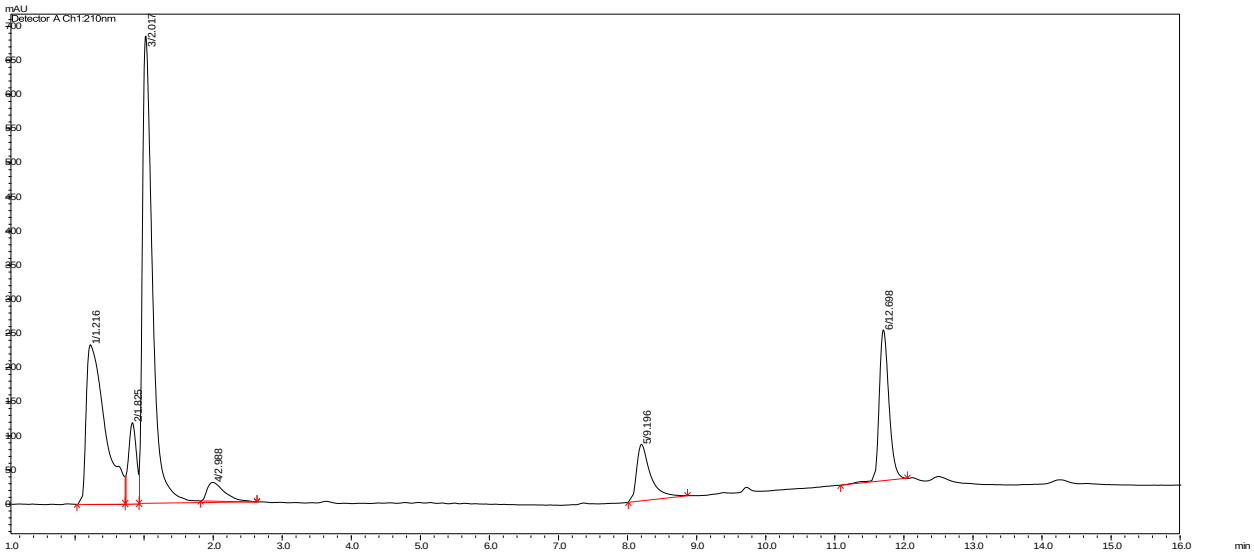


Table 10. Force degradation studies of Solifenacin

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_2O_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:

In accordance with ICH guidelines, a UHPLC method for the simultaneous quantification of Mirabegron and Solifenacin in tablet dosage form was developed and validated. Linearity was assessed within concentration ranges of 25-75 µg/mL for Mirabegron and 2.5-7.5 µg/mL for Solifenacin. The linear relationships were

expressed by the equations y = 43633x - 18350 for Mirabegron and y = 12255x + 6446.4 for Solifenacin, where X represents drug concentration and Y denotes the peak area. In all cases, the regression coefficient (R2) was 0.999, aligning with ICH guidelines. The method developed is simple, sensitive, rapid, linear, rugged, precise, robust, and specific.

REFERENCE

1. Badike Kuruva Suresh et al., Analytical method development and validation for the estimation of mirabegron in pure and its solid dosage form by UV- Spectrophotometric method (2024) Int. J. Res. Pharm. Sci & Tech., 1(4), 146-150
2. Lingdi Chen, Yu Zhang Determination of Mirabegron In Rat Plasma By UPLC–Ms/Ms After Oral And Intravenous Administration, Rev Assoc Med Bras 2022; 65(2):141-148
3. Hari Kishan Reddy Ganthi, Raveendra Reddy, So Jin Park, Stability Indicating HPLC Method for Quantification of Solifenacin Succinate & Tamsulosin Hydrochloride along with Its Impurities in Tablet Dosage Form, American Journal of Analytical Chemistry, 2016, 7, 840-862
4. Rihana P. Shaik, Srinivasa B. Puttagunta, Chandrasekar K. Bannoth, Analytical Method Development and Validation of Solifenacin in Pharmaceutical Dosage Forms by RP-HPLC, Hindawi Publishing Corporation ISRN Analytical Chemistry Volume 2014, Article ID 132024, Page1- 5.
5. International Committee on Harmonization (ICH) (2005) Validation of Analytical Procedures: Text and Methodology Q2 (R1). IFPMA, Geneva
6. Van Teijlingen R, Meijer J, Takusagawa S, van Gelderen M, van den Beld C, Usui T. Development and validation of LC-MS/MS methods for the determination of mirabegron and its metabolites in human plasma and their application to a clinical pharmacokinetic study. J Chromatogr B Anal Technol Biomed Life Sci. (2012) 887– 888:102–11.
7. Ganesh Andhale, Manish Shankarpure, Jyoti Kadam, Madhuri Shelar, Soniya Singh, RP-HPLC method development and validation for the simultaneous estimation of Mirabegron and Solifenacin succinate in pharmaceutical dosage form, Neuro Quantology September 2022 Volume 20, Issue 9, 2652-2659.
8. Tantawy MA, Weshahy SA, Wadie M, Rezk MR. Stability-indicating HPTLC method for the simultaneous detection and quantification of alfuzosin hydrochloride, solifenacin succinate along with four of their official impurities. Microchem J. (2024) 157.
9. Kalariya PD, Sharma M, Garg P, Thota JR, Ragampeta S, Talluri MVNK. Characterization of stress degradation products of mirabegron using UPLC-QTOFMS/MS and in silico toxicity predictions of its degradation products. RSC Adv (2015)5(39):31024–38