International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 02 Issue 08 August 2022

Page No: 223-232

DOI: https://doi.org/10.47191/ijpbms/v2-i8-01, Impact Factor: 5.542

Development and Validation of UV and RP-HPLC Methods for Simultaneous Estimation of Mirabegron and Solifenacin Succinate in Their Pharmaceutical Dosage Form

Jenisha Patel[1], Grishma Patel[2], Dhananjay Meshram[3]

[1, 2, 3] Pioneer Pharmacy Degree College, Ajwa-Nimeta road, Vadodara-390019, Gujarat, India

ABSTRACT

Recently, a new formulation containing Mirabegron (MB) and Solifenacin succinate (SFS) has been approved for the management of over active bladder. However, only one analytical method has been reported for the simultaneous determination of both the analytes. Therefore, the current study was design to develop simple UV derivative spectroscopic and rapid RP-HPLC methods for simultaneous determination of MB and SFS. The chromatographic separation of MB and SFS was performed using Phenomenex Kinetex C_{18} (150mm × 4.5 mm × 5 µm) analytical column. A mixture of Water: Acetonitrile (20:80%v/v) was consider as mobile phase, at a flow rate of 1ml/min and at detector wavelength 225nm. A linear response was observe over the concentration range 2.5-12.5 µg/ml and 0.5-2.5 µg/ml respectively. The first order derivative method was develop by derivatisation of the zero absorption spectra for the first absorption spectra. The Zero crossing point of MB and SFS at 221 nm and 266 nm was obtain respectively. Beer's law is obey in the concentration range of 7.5-20 µg/ml and 1.5-4 µg/ml for MB and SFS with correlation coefficient (R2) of 0.9984 and 0.9993 respectively. Both the methods were validated in accordance to guidelines for linearity, precision, repeatability, limit of detection (LOD), Limit of Quantification (LOQ), accuracy and robustness. Further, both the methods were validated and compared statistically using Student's-t-test and employed for the concurrent estimation of MB and SFS in formulations. The proposed methods were simple, accurate, precise, and rapid. Therefore, they can be use for regular quality control of MB and SFS formulations and dissolution studies as well.

KEYWORDS: Mirabegron, Solifenacin succinate, First Order Derivative method, RP-HPLC, Student's t-test.

ARTICLE DETAILS

Published On:
01 August 2022

Available on:
https://ijpbms.com/

INTRODUCTION:

Recently, a new formulation containing mirabegron (MB) and solifenacin succinate (SFS) has been approved for the management of over active bladder. Combination of the beta-3 adrenoreceptor agonist mirabegron and the counter muscarinic specialist Solifenacin succinate may further develop adequacy in the treatment of overactive bladder (OAB) while decreasing the anti-muscarinic side effects. Mirabegron is a beta- 3 adrenergic agonist. The chemical name is 2-(2-amino-1, 3-thiazol-4-yl)-N-[4-(2-[[(2R)-2-hydroxy-2-phenylethyl]amino]ethyl]phenyl]acetamide having empirical formula $C_{21}H_{24}N_4O_2S$ and molecular weight 396.5 g/mol. The structural formula of Mirabegron is

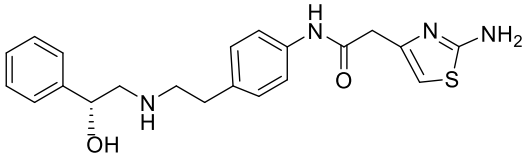

Figure 1 : Structure of Mirabegron

It has CAS number 223673-61-8. It has a (4.2) pKa1 and pKa2 (8.0). Mirabegron is a white powder. It is practically insoluble in water. It is soluble in methanol and dimethyl sulfoxide. It has 138-140ºC. It is classify as Class 3 biopharmaceutical classification system (high solubility and low permeability)[1]. Solifenacin Succinate is an anti-muscarinic selective M3 / anti-cholinergic drugs. The chemical name is [(3R)-1-azabicyclo [2.2.2] octan-3-yl] (1S)-1-phenyl-3,4-dihydro-1H-isoquinoline-2- carboxylate; butane dioic acid having empirical formula $C_{23}H_{26}N_2O_2$; $C_4H_6O_4$ and molecular weight 480.6 g/ml. The structural formula of Solifenacin Succinate is:

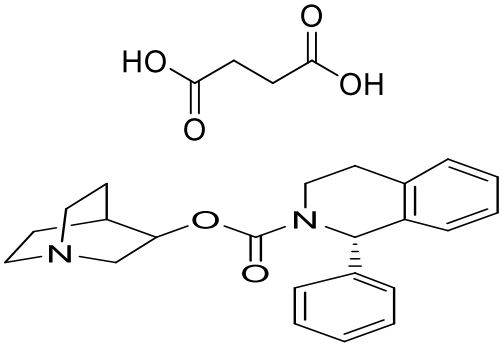

Figure 2: Structure of Solifenacin Succinate

It has CAS number 242478-38-2. It has a pKa (8.0). Solifenacin Succinate (SFS) is a white powder or crystals. It is freely soluble in water. It is also soluble in methanol. It has 134-136ºC. It is classified as Class 1 biopharmaceutical classification system (high solubility and high permeability [2]. RP-HPLC, UPLC, HPTLC and spectrophotometric methods for the estimation of MB in combination with other drugs are reported [3-17]. The literature survey revealed the report of LC-MS/MS, HPLC, LCMS and spectrophtometric methods for estimation of SFS [18- 40]. Literature survey reveals that only one HPTLC method available for simultaneous estimation of MB and SFS in combined dosage form [41].

MATERIALS AND METHODS

Instruments and Apparatus

A Shimadzu UV/Vis double beam spectrophotometer (model 1800) connected with Shimadzu UV-Probe 2.33 software was used for all spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-800 nm. The chromatographic analysis were carried out using Shimadzu LC 20 AD binary pump system equipped with UV detector with manual injector and Phenomenex Kinetex C18 column. Other instruments used were electronic balance and sonicator.

Chemical and Reagents

All the chemicals used were of A.R. grade and pure drug sample of Mirabegron was obatined from Swati Spentose Pvt., Vapi, Gujarat and pure Solifenacin succinate was gifted by Flax Laboratories, Mumbai, Maharashtra. Tablets of MB and SFS in combine dosage form with 25mg MB and 5 mg SFS label claim were purchase from local medical store.

For UV-Spectrophotometric method:

Determination of maximum wavelength

Wavelength of maximum absorption was determined by scanning 10 µg/ml solution of MB and SFS using UV spectrophotometer from 200 to 400 nm. This shows maximum absorbance at 247 nm and 210 nm for Mirabegron and Solifenacin Succinate respectively.

Preparation of standard stock solutions

Accurately weighed 100 mg of Mirabegron and 10 mg of Solifenacin succinate were transfer into separate 100 ml volumetric flask. To each flask, 5 ml methanol added and sonicated for 5 minutes. Then volume was make up to mark with distilled water. This will give primary stock solution containing 1000 µg/ml and 100 µg/ml concentration of Mirabegron and Solifenacin succinate respectively. From each flask take volume of 25 ml from MB solution and 5 ml from SFS solution and then the volume was made up to the mark with distilled water to make 250 µg/ml and 10 µg/ml (secondary stock) of Mirabegron and Solifenacin succinate. From the above secondary stock solution, different concentrations of the solution were prepared from the range of 7.5- 20 µg/ml for MB and 1.5-4 µg/ml for SFS of which volumes of were 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 were withdrawn each and transferred to 10 ml of volumetric flask and volume was made up to mark.

Preparation of sample solution for assay

Estimation of MB and SFS in dosage form, 20 tablets weighed individually, and an average weight of the tablets was calculated and triturated into fine powder. The powder equivalent to 25 mg MB and 5 mg SFS (350.6 mg) was

weighed accurately and transferred to 100 ml of volumetric flask dissolved in methanol and sonicate for 5 min and filter and diluted up to mark with distilled water to give 1º stock solution (250 µg/ml of MB 50 µg/ml of SFS). Further, dilute it to make the concentration of 12.5 µg/ml of MB 2.5 µg/ml of SFS analyzed for assay determination.

First order derivative method

Working standard solutions of Mirabegron and Solifenacin were prepared from the respective standard solution and were scanned in the UV range 200-400nm. The overlain spectra of Mirabegron and Solifenacin succinate were obtain. These absorption spectra of Mirabegron and Solifenacin succinate were converted into first order derivative spectra by using instrument mode (Δλ = 8 and scaling factor =10). From the overlain first order derivative spectra (Figure), zero crossing points of drugs selected for the analysis of another drug. The first wavelength selected was 221nm (zero crossing point of Mirabegron), where Solifenacin succinate showed considerable absorbance. The second wavelength selected was 266nm (zero crossing point of Solifenacin succinate), where Mirabegron showed considerable absorbance. Thus, the absorbance of the working solutions of MB and SFS were measured at 221nm (ZCP of MB) and 266nm (ZCP of SFS) respectively. The graph of absorbance vs concentration plotted at each wavelength and regression coefficient is calculated.

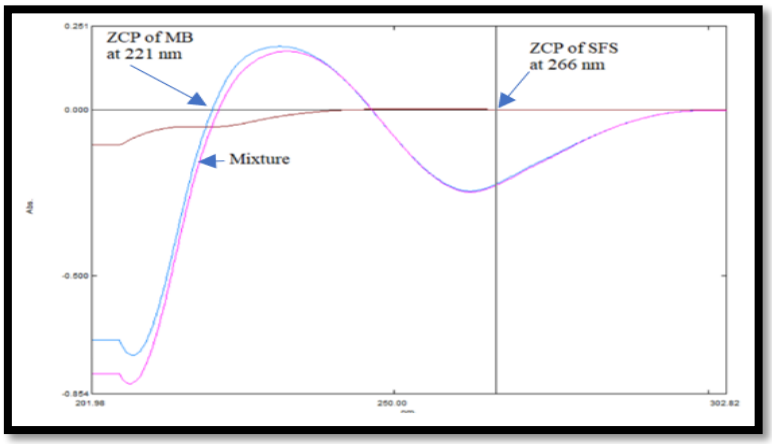

Figure 3: First order overlain of Mirabegron and Solifenacin succinate and mixture

For RP-HPLC method:

Determination of Wavelength

Mirabegron and Solifenacin succinate were scanned in UV range of 200-400nm in which both Mirabegron and Solifenacin succinate show reasonably good response at 225nm. So, 225 nm wavelength selected for the determination of Mirabegron and Solifenacin succinate.

Chromatographic conditions

The Mobile phase consists of Water: Acetonitrile (20:80%v/v), flowing through the column at a constant flow rate of 1.0ml/min. A Phenomenex Kinetex Column C18 (150mm × 4.5 mm × 5 µm) was used as stationary phase. By considering the parameter, 225nm selected as the detection wavelength for UV-Visible detector.

Preparation of solutions

100mg of Mirabegron and 10 mg of Solifenacin succinate were weighed accurately and transferred into separate 100 ml volumetric flask. To each flask 5 ml methanol was added and sonicated for 5 minutes. Then volume made up to mark with methanol. This will give 1000 µg/ml and 100 µg/ml (primary stock) concentration of Mirabegron and Solifenacin succinate respectively. From each flask take volume of 25 ml from MB solution and 5 ml from SFS solution and then the volume was made up to the mark with methanol to make 250 µg/ml and 10 µg/ml (secondary stock) of Mirabegron and Solifenacin succinate. From the above secondary solution the working solutions were prepared form the range of 2.5-12.5 µg/ml and 0.5-2.5 µg/ml for MB and SFS respectively.

Preparation of sample solution for assay

Estimation of MB and SFS in dosage form, 20 tablets weighed individually, and an average weight of the tablets was calculated and triturated into fine powder. The powder equivalent to 25 mg MB and 5 mg SFS (350.6 mg) was weighed accurately and transferred to 100 ml of volumetric flask dissolved in methanol and sonicate for 5 min and filter and diluted up to mark with distilled water to give 1º stock solution (250 µg/ml of MB 50 µg/ml of SFS). Further, dilute it to make the concentration of 10 µg/ml of MB 2 µg/ml of SFS analyzed for assay determination.

RP-HPLC method

Chromatography was performed on Shimadzu HPLC system equipped with UV Detector; using LC solution software. A manual loop injector valve with volume of 20µL. The chromatogram recorded at 225nm as both shows good response. During optimization separation method, Phenomenex Kinetex C18 column, (150mm × 4. 6mm × 5 µm) and the mobile phase composed of Acetonitrile and water were tested. After trying several mobile phases,

containing Water, Acetonitrile and Methanol the final mobile phase optimized was Water: Acetonitrile (20: 80) which gives better resolution and peak symmetry. Typical chromatogram obtained with final condition is shown in figure 4. The order was Solifenacin succinate (RT= 3.31min) and Mirabegron (RT= 5.82 min), at flow rate of 1ml/min.

Table 1. Optimized Chromatographic conditions

Chromatographic Conditions		**Results**
Elution		Binary Gradient
Column		Phenomenex kinetex C18 (150 × 4.6mm)
Mobile Phase composition (%v/v)		Water : Acetonitrile (20 : 80)
Flow rate (ml/min)		1.0ml/min
Detection wavelength (nm)		225nm
Injection volume		20µL
Run time		10 min
Retention time (min)	MB	5.82
	SFS	3.31

Validation Parameters

Linearity: Linearity was studied by analyzing five standard solutions (n=5) in the range of 7.5-20 µg/ml of MB and 1.5-4 µg/ml for SFS and range of 2.5-12.5 µg/ml for MB and 0.5-2.5 µg/ml for SFS in UV spectrophotometric and RP-HPLC method respectively. Calibration curves with concentration verses absorbance or peak area was plotted for each method and obtained data were subjected to regression analysis using least square method. Linearity of MB and SFS was established by ratios of drugs i.e. (5:1).

For UV Spectrophotometric method

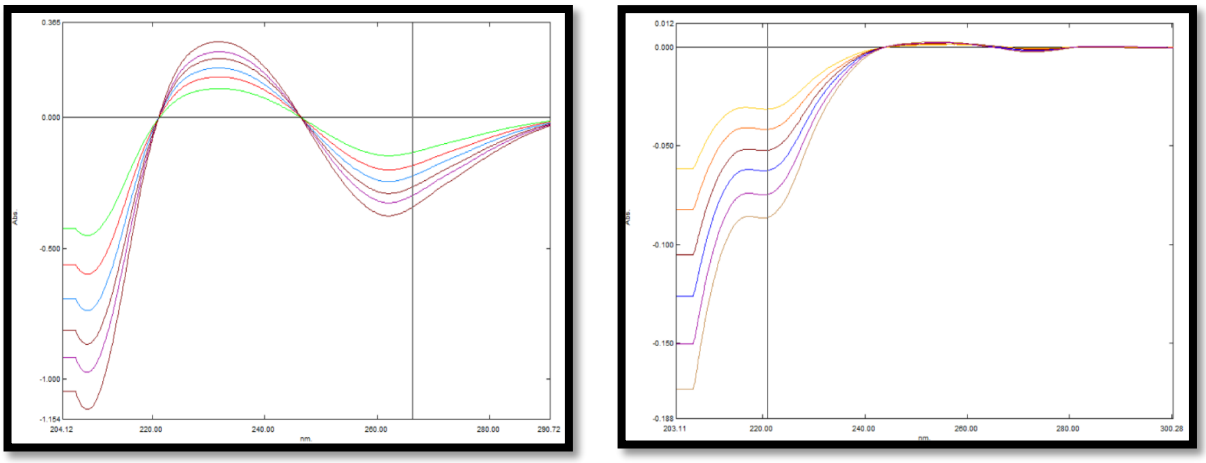

Figure 4: First order derivativ spectrum of MB(7.5-20 µg/ml) and SFS(1.5-4µg/ml)

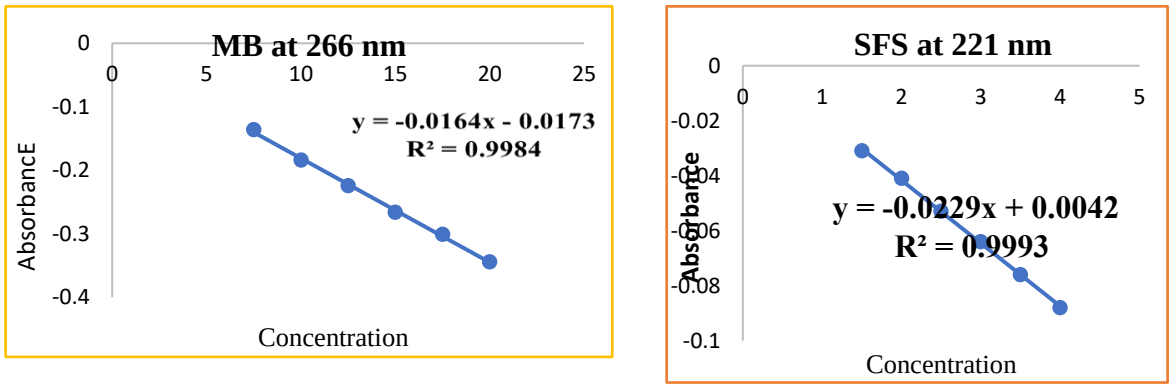

Figure 5: Calibration curve of MB and SFS

For RP-HPLC method

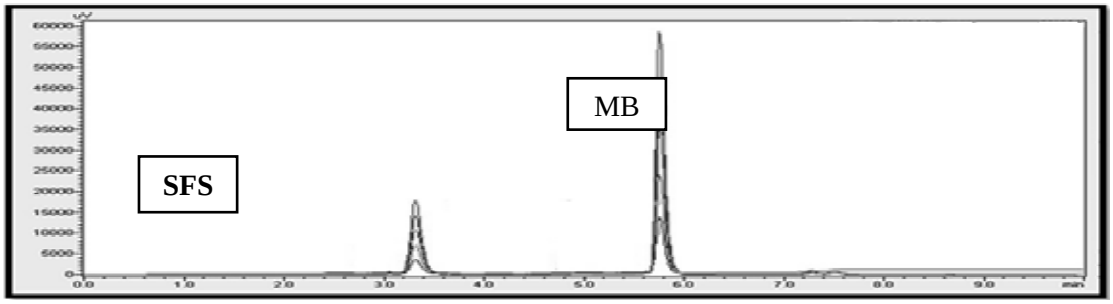

Figure 6: Overlain Chromatogram of MB(2.5-12.5 µg/ml) and SFS(0.5-2.5 µg/ml)

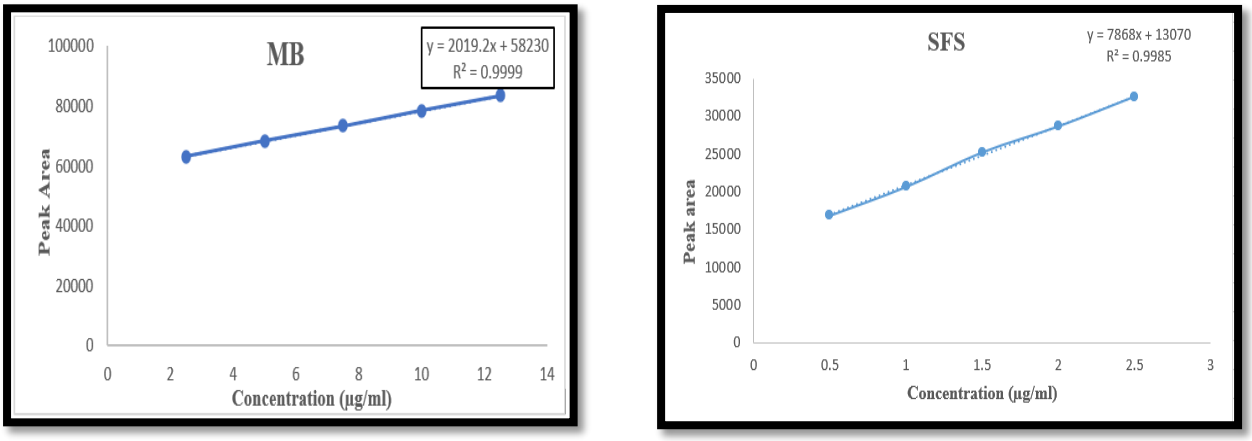

Figure 7: Calibration Curve of MB and SFS

Table 2. Statistical data for the regression equation of the proposed method

Parameters	First Order Derivative method		RP-HPLC method	
	MB	SFS	MB	SFS
Linearity range (µg/ml)	7.5-20	1.5-4	2.5-12.5	0.5-2.5
Regression equation	y= -0.0164x-0.0173	y=-0.0229x +0.0042	y= 2019.2x + 58230	y= 7868x + 13070
Slope	-0.0164	-0.0229	2019.2	7868
Intercept	0.0173	0.0042	58230	13070
Correlation coefficient (R^2)	0.998	0.999	0.999	0.998
Limit of Detection (µg/ml)	0.170	0.304	0.61	0.06
Limit of Quantification (µg/ml)	0.484	0.922	1.85	0.20

Precision: The precision of an analytical method expresses the closeness of agreement between a series of measurement which are obtained by performing multiple samplings of the same homogenous sample under the given conditions of the method. Here, the intraday and inter-day precision was determined. For that three concentration having lower, upper and middle limits of both the drugs were taken and analysed three times on the same day for intra-day precision and on 3 different days for interday precision at the same concentration level. The %RSD (relative standard deviation) of the results was calculated.

Table 3. Precision data of MB and SFS by UV-spectrophotometric method

Conc. (µg/ml)	Intraday	Interday
	Mean Absorbance ± RSD (n=3)	Mean Absorbance ± RSD (n=3)
MB		
7.5	-0.134 ± 0.74	-0.134 ± 0.74

15	-0.265 ± 0.37	-0.265 ±0.37
20	-0.343 ± 0.29	-0.343 ± 0.29
SFS		
1.5	-0.031 ± 1.83	-0.031 ± 1.82
3	-0.063 ± 1.58	-0.063 ± 1.54
4	-0.086 ± 1.74	-0.087 ± 1.74

Table 4. Precision data of MB and SFS by RP-HPLC method

Conc. (µg/ml)	**Intraday**	**Interday**
	Mean Absorbance ± %RSD (n=3)	**Mean Absorbance ± %RSD (n=3)**
MB		
2.5	63823 ± 0.68	63937 ± 0.68
7.5	73760 ± 0.62	73593 ± 0.62
12.5	83242 ± 0.54	82968 ± 0.54
SFS		
0.5	17016 ± 0.95	16948 ± 0.66
1.5	24993 ± 0.42	25094 ± 1.09
2.5	32696 ± 0.50	32650 ± 0.63

Accuracy: The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added the pre-analysed sample mixture at 80%, 100% and 120% levels. The recovery studies were carried out and the % recovery and % RSD of the % recovery were calculated and given in Table 5 for UV-spectrophotometric method and in Table 6 for RP-HPLC method.

Table 5. Accuracy data of Mirabegron and Solifenacin succinate by UV Spectrophotometric method

Drug	**%Spiked**	**Std. Conc.spiked (µg/ml)**	**Conc. recovered(µg/ml)**	**%Recovery (n=3)**	**%RSD**
MB	80	6	6.04	100.5	0.63
	100	7.5	7.56	100.8	0.40
	120	9	8.87	98.50	0.35
SFS	80	1.2	1.18	98.80	0.99
	100	1.5	1.49	99.20	1.16
	120	1.8	1.80	99.90	0.98

Table 6. Accuracy data of Mirabegron and Solifenacin succinate by RP-HPLC method

Drug	**%Spiked**	**Std. Conc.spiked (µg/ml)**	**Conc. recovered(µg/ml)**	**%Recovery (n=3)**	**%RSD**
MB	80	4	4.06	101.5	0.38
	100	5	5.07	101.4	0.81
	120	6	6.07	101.1	0.17
SFS	80	0.8	0.79	100.3	0.84
	100	1	1.00	100.1	0.76
	120	1.2	1.19	99.60	0.69

System Suitability Parameters

For RP-HPLC method, the system suitability test was performed to verify the suitability of chromatographic system for intended analysis. The test was performed by three replicate injections of standard solution for Mirabegron and Solifenacin succinate and system suitability parameters were

determined, for their retention time, theoretical plates, asymmetric factor and resolution. The results are given in the Table 7 which are within the accpetable limits.

Table 7. System suitability parameters

Parameters	Data obtained	
	MB	SFS
Retention time (Rt) ± SD	5.80 ± 0.04	3.29 ± 0.05
Area ± SD	77914 ± 889.5	28645 ± 299.0
Theoretical Plates per column (N) ± SD	6795 ± 27.6	2964 ± 48.6
Symmetry factor/ Tailing factor ± SD	1.14 ± 0.02	1.12 ± 0.04
Resolution	11	

Robustness: For the robustness of the analytical method ,changed the ratio of mobile phase, flow rate and wavelength. To study the effect of the flow rate, it was changed to 0.2 units i.e. 0.8 and 1.2 ml/min. The effect of ratio of mobile phase was studied by changing 2 units i.e. 22:78 and 18:82. The change in wavelength ± 2nm. The results of robustness are summarized in Table 8

Table 8. Robustness data for MB and SFS

Conditions	Mirabegron			Solifenacin Succinate		
	Peak Area (n=3)	Theoretical Plates (n=3)	Tailing Factor (n=3)	Peak Area (n=3)	Theoretical Plates (n=3)	Tailing Factor (n=3)
Flow rate 0.8ml/min	76741	6767	1.23	28489	2874	0.96
Flow rate 1.2ml/min	75537	6781	1.25	28645	2908	0.97
MobilePhase water:ACN (18:82)	77493	6785	1.25	27568	2899	0.69
MobilePhase water:ACN (22:78)	77418	6778	1.27	28873	2936	1.03
Wavelength 223nm	77244	6734	1.27	29240	2832	0.96
Wavelength 227nm	76578	6764	1.26	29156	2937	0.97

The robustness of the method demonstrates that the method developed is robust. The study suggested that all the parameters have no significant influence on the determination. Result indicate that the selected factors remained unaffected by small variation of these parameters.

Analysis of Mirabegron and Solifenacin succinate in marketed formulation

Table 9. Assay Mirabegron and Solifenacin succinate by UV- spectrophotometric method

Drug	Label claim (mg)	Conc. found (µg/ml)	% Assay (n=5)	Content Found (mg)
MB	25	12.54	100.3 ± 1.07	25.2
SFS	5	2.50	100.1 ± 1.15	5.01

Table 10. Assay of Mirabegron and Solifenacin succinate by RP-HPLC method

Drug	Labelclaim (mg)	Conc. found (µg/ml)	% Assay (n=5)	Content Found (mg)
MB	25	10.11	101.1	25.2
SFS	5	2.00	100.1	5.02

Statistical Comparison using Student's T-test:

The Student's t-test (also called T test) used to compare the means between two groups and there is no need of multiple comparisons as unique *P* value was observe. It is use to test whether mean difference between two groups is statistically significant. T test are three types i.e one sample *t*-test, independent sample *t*-test and paired sample *t* test.

Table 11. *T-test* method for MB and SFS

Source of Variation		P value	t (critical)
Between groups	MB	0.1896	2.77644
	SFS	0.915	2.776

From the Statistical Comparison using Student's T-test it could be concluded that P value was found to be smaller compared to t_{crit} value, therefore there were no significant different groups.

CONCLUSION

Two simple analytical procedures were established for the concurrent determination of MB and SFS from laboratory mixed solutions and tablets without prior separation. The UV spectroscopic method was economical and eco-friendly as water has been used as a solvent. The optimized chromatographic technique was simple, rapid, precise, and robust for the concurrent quantification of MB and SFS from solid dosage forms. Based on the result, percentage of recovery shows that the method is free from interference of the excipients used in the formulation and can be employed for routine analysis of these two drugs in combined dosage form and from the Statistical Comparison using Student's T-test it could be concluded that P value was found to be smaller compared to t_{crit} value, therefore there were no significant different groups.

REFERENCES

I. Yadav P, Chhalotiya U, Patel K, Kachhiya H and Shah D, "Quantification of a β-adrenergic receptor agonist drug Mirabegron in presence of degradants by high performance thin layer Chromatography." Anal. Chem. Lett. 2021, 11(4), 512-522.

II. Bharathi T and Bhadre G, "RP-HPLC method development and validation for the quantitative determination of Potential Impurities of Mirabegron." Am. J. Pharm. Health. Res. 2021, 9(1), 2-9.

III. Suryawanshi R, Shaikh S and Patil S, "RP-HPLC method Development and Validation for the Estimation of Mirabegron in Bulk and Dosage Form." J. Drug. Deliv. Ther. 2020, 10(1), 31-38

IV. Yadav P, Chhalotiya U, Patel K and Tandel J, "Quantification of a β-adrenergic receptor drug Mirabegron by stability Indicating LC method and UV-Visible Spectroscopic Method in bulk and pharmaceutical dosage form." Chemical Methodologies. 2020, 4, 340-358.

V. Panchmarthy R, Kishore K, Babji B and Sulthana S, "Analytical method development and validation for the determination of Mirabegron in pharmaceutical dosage form by RP-HPLC." Int. J. Pharm. Sci. Res. 2020, 11(5), 2223-2228.

VI. Badike S, Nerusula A and Boyina S, "Analytical method development and validation for the estimation of Mirabegron in Pure and its solid dosage form by UV-spectrophotometric method." Int. J. Res. Pharm. Sci. Tech. 2020, 1(4), 146-150.

VII. Ramazani A and Rezaei M, "RP-HPLC method development and validation for the quantitative estimation of Mirabegron in extended-release tablets." J. Med. Chem. Sci. 2018, 1, 36-40.

VIII. Saipriya P, Kumar M, Marakatham and Kanduri V, "To develop a new RP-HPLC method for the estimation of Mirabegron in Pharmaceutical dosage forms with forced degradation studies." Global Trends Pharm. Sci. 2018, 9(3), 5692-5701.

IX. Jyothsna M, Ahmed R, Ramesh T, Syed A and Kranthi R, "Method development and validation of Mirabegron in bulk drug and pharmaceutical dosage form." ISOR Journal of Pharmacy and Biological Sciences. 2018, 13(1), 78-83.

X. Raveendra G, Kumar V, Kalyani M, Roshna Md, Jeevana R, Kumar V and Ajay S, "Stability-indicating simultaneous estimation of Vildagliptin

and Mirabegron in bulk and pharmaceutical dosage form by UV-visible spectroscopy." World. J. Pharm. Pharm. Sci. 2017, 6(5), 912-925.

XI. Mounika B, Srikanth L and Venkatesha A, "Determination and validation of RP-HPLC method for the estimation of Mirabegron in tablet dosage form." Int. J. Curr. Pharm. Res. 2017, 9(5), 140-151.

XII. Nageswara Rao R, Madhuri D, Reddy S, Rani K, Tejaswini P and Gandla K, "development and validation of derivative spectrophotometric method for estimation of Mirabegron in bulk and tablet dosage form." World. J. Pharm. Res. 2017, 6(14), 760-767.

XIII. Spandana R, Nageswara Rao R and Reddy S, "Analytical method development and validation for the estimation of Mirabegron in bulk and pharmaceutical dosage form." Indo. Am. J. Pharm. Res. 2016, 6(11), 6880-6887.

XIV. Panchumarthy R, Vidya V, Durga N and Srinivasa P, "Validated UV-spectrophotometric method for quantitative determination of Mirabegron in bulk and pharmaceutical dosage form." Der. Pharmacia. Lettre. 2016, 8(14), 96-103

XV. Zhou F, Zhou Y, Zou Q, Sun L and Wei Ping, "Liquid chromatographic separation and thermodynamic investigation of Mirabegron Enantiomers on a Chiralpak AY-H column." Journal of Chromatographic Science. 2015, 1-5.

XVI. Tanuja A, Ganapaty S and Varanasi M, "Stability indicating RP-HPLC method development and validation for the determination of Solifenacin Succinate in bulk and its pharmaceutical formulation." Res. J. Pharm. Tech. 2021, 14(7), 1-16.

XVII. Srinivasa R, Kumar H, Chiranjeevi P and Rao V, "simultaneous estimation of Solifenacin succinate and Tamsulosin Hydrochloride in Combined dosage form by using first order derivative spectrophotometric method." Indian. J. Pharm. Sci. 2021, 83(2), 331-335.

XVIII. Bhavana V, Kumar H Srinivasa R and Prasad K, "RP-HPLC method for estimation of Solifenacin Succinate in API and tablet dosage form." Asian. J. Pharm. Anal. 2019, 1-7.

XIX. Reddy H, Reddy R, Park Y, Reddy H, Park S and Cho W. "Stability-Indicating HPLC method for Quantification of Solifenacin Succinate & Tamsulosin Hydrochloride along with its impurities in tablet dosage form." American Journal Analytical Chemistry. 2016 7, 840-862.

XX. Attia A, Eman Y, Mohamed G and Ahmed H, "liquid chromatographic determination of Solifenacin Succinate, Flavoxate Hydrochloride and Tolterodine Tartate in Bulk Drugs and their pharmaceutical dosage forms." J. Chil. Chem. Soc. 2016, 61, 2772-2776.

XXI. Damle M and Rokade P, "Development and validation of stability indicating HPTLC method for determination of Solifenacin Succinate as bulk drug and in tablet dosage form." Int. J. Pharm. Sci. Drug Res. 2016, 8(4), 218-222.

XXII. Saiyed N, Patel D, and Desai S, "Development and validation of first order derivative spectrophotometric method for estimation of Alfuzosin Hydrochloride and Solifenacin succinate in combined dosage form." Int. J. Pharm. Pharm. Res.2015, 2(4), 176-183.

XXIII. Hepsebah N and Kumar A, "Assay method development and validation for the estimation of Solifenacin succinate in Tablets by UV-Spectrophotometry." Indo. Am. J. Pharm. Sci. 2015, 2(6), 978-983.

XXIV. Mohan C, Hemalatha B, Shainy B, Vasundhara, Sandhya S and Kumar A, "A rapid RP-HPLC method development and Validation for the Quantitative estimation of Solifenacin Succinate in tablets." Int. J. Pharm. Pharm. Sci. 2014, 6(10), 201-204.

XXV. Srinivasa B, Shaik R, Bannoth C, Reddy C and Zayed B, "Bioanalytical method for quantification of Solifenacin in rat plasma by LC-MS/MS and its application to pharmacokinetic study." Journal of Analytical Science and Technology. 2014, 5(35), 1-8.

XXVI. Shaik R, Srinivasa P, Bannoth C and Reddy S, "Analytical method development and validation of Solifenacin in pharmaceutical Dosage forms by RP-HPLC." Analytical Chemistry. 2014, 1-5.

XXVII. Rakesh B, Bhargavi P, Reddy S and Kumar A, "UV spectrophotometric method development and validation for the quantitative estimation of Solifenacin Succinate in tablets." Int. J. Pharm. Pharm. Sci. 2014, 6(10), 190-193.

XXVIII. Annapurna M, Sowjanya G, Naidu N and Lohithasu D, "A validated Liquid Chromatographic method for the Determination of Solifenacin Succinate in tablets." Chem. Sci. Trans. 2014, 3(2), 602-607.

XXIX. Reddy R, Reddy S, Raman N, Reddy T and Rambabu C, "development and validation of specific stability indicating high performance liquid chromatographic methods for related compounds and assay of Solifenacin Succinate." Journal of Chemistry. 2013, 1-10.

XXX. Teja G, Dasu D, Srinivasa P and Ravishankar P. "Quantitative analysis of Solifenacin Succinate in pharmaceutical dosage form using UV-absorption

spectroscopy." J. Chem. Pharm. Sci. 2013, 6(3), 195-198.

XXXI. Israel D, Krishnachaitanya K and Gowrishankar D, "RP-HPLC method for the estimation of Tamsulosin and Solifenacin in Bulk and its Dosage forms." Int. J. Pharm. Pharm. Sci. Res. 2013, 4(11), 4343-4350.

XXXII. Raul S, Kumari R and Patnaik A, "A RP-HPLC method development and validation for the estimation of Solifenacin in Bulk and Pharmaceutical Dosage forms." Int. J. Bioassays. 2012, 210-213

XXXIII. Desai D, Patel G, Shukla N and S. Rajput, "Development and validation of stability-indicating HPLC method for Solifenacin Succinate: Isolation and Identification of Major base Degradation Product." Acta. Chromatographica. 2012, 24(3). 399-418.

XXXIV. Desai N, Syed H, Vasanthraju S and Karthik A and Udupa N, "Development and Validation of Stability indicating HPLC method for determination of Solifenacin in Bulk Formulations." Int. J. Pharm. Pharm. Sci. 2011, 3(1), 70-74.

XXXV. Singh L and Nanda S, "Spectrophotometric estimation of Solifenacin succinate in tablet formulations." Pharmaceutical Methods. 2011, 2(1), 21-24.

XXXVI. Macek J, Ptacek P and Klima J, "Determination of Solifenacin in human plasma by liquid chromatography-tandem mass spectrometry." J. Chromatogr. B. 2010, 878, 3327-3330.

XXXVII. Krishna S, Rao B and Rao S, "A validated rapid stability-indicating method for the determination of related substances in Solifenacin Succinate by Ultra-Fast Liquid Chromatography," Journal of Chromatographic Science. 2010, 48, 807-810.

XXXVIII. Yanagihara T, Aoki T, Soeishi Y, Iwatsubo T and Kamimura H, "Determination of Solifenacin succinate, a novel muscarinic receptor antagonist, and its major metabolite in rat plasma by semi-micro high performance liquid chromatography." J. Chromatogr. B. 2007, 859, 241-245.

XXXIX. Shah D, Tahilramani P, Patel V and Chhalotiya U, "High Performance thin layer chromatography method for the estimation of Mirabegron and Solifenacin Succinate used in the treatment of Overactive Bladder." Journal of Planar. Chromatography. 2019, 32(4), 323-327.

XL. ICH Q2(R1), Validation of Analytical Procedures: Text and Methodology, 2005.

XLI. Mishra P, Singh U, Pandey C, Mishra P and Pandey G, "Application of Student's t-test, Analysis of Variance, and Covariance." Ann. Card. Anaesth. 2019, 22, 407-411.