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Research Article

New RP-HPLC Method Development & Validation for Simultaneous Estimation of Mirabegron & Silodosin as Per Ich Guidelines

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ABSTRACT

A simple, accurate, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and optimized for the simultaneous estimation of Silodosin and Mirabegron in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a SPURCIL C18 column with a mobile phase of ammonium acetate buffer and methanol (30:70 v/v) at pH 5.0, resulting in well-resolved peaks with acceptable retention times, good symmetry, and satisfactory system suitability parameters. The method exhibited excellent linearity with correlation coefficients greater than 0.999 for both drugs. Precision studies confirmed repeatability with %RSD values within acceptable limits, while accuracy studies demonstrated recovery values close to 100%, validating the reliability of the method. Sensitivity was established through low LOD and LOQ values, confirming its ability to detect and quantify trace levels effectively. Overall, the validated RP-HPLC method proved precise, accurate, linear, sensitive, and robust, meeting all standard guideline requirements. Its simplicity, cost-effectiveness, and time efficiency make it highly suitable for routine quality control analysis in pharmaceutical industries and research laboratories for combined dosage forms of Silodosin and Mirabegron.

INTRODUCTION

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RP-HPLC is one of the most commonly used analytical techniques for the separation and quantification of pharmaceutical compounds. It is based on the principle of partition chromatography, where the stationary phase is non-polar (typically C18 column) and the mobile phase is relatively polar. In RP-HPLC, compounds are separated based on their hydrophobic interactions with the stationary phase. More hydrophobic compounds exhibit longer retention times, whereas polar compounds elute faster. RP-HPLC is widely used for simultaneous estimation of drugs in combined dosage forms, making it an ideal choice for the present study involving Mirabegron and Silodosin. (3,4) . Mirabegron and Silodosin are often co-administered in the management of lower urinary tract symptoms (LUTS) associated with overactive bladder and benign prostatic hyperplasia. Their combination provides synergistic therapeutic benefits by targeting different mechanisms involved in urinary dysfunction.

Method Validation as per ICH Guidelines

Analytical method validation is a process used to confirm that the developed method is suitable for its intended purpose. According to ICH guidelines (ICH Q2(R1)), validation parameters include: (1,2)

Specificity: Ability to measure the analyte accurately in the presence of impurities, excipients, and degradation products.

Linearity: Ability to obtain test results that are directly proportional to the concentration of analyte within a given range

Accuracy: Closeness of agreement between the true value and the value found.

Precision: Degree of repeatability under normal operating conditions (intra-day and inter-day precision).

Limit of Detection (LOD) and Limit of Quantification (LOQ): Lowest concentration of analyte that can be detected or quantified with acceptable accuracy and precision.

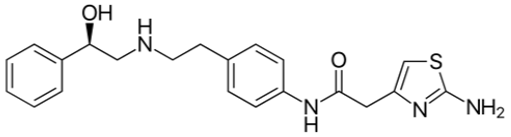
Robustness: Capacity of the method to remain unaffected by small variations in method parameters.

System Suitability Testing: Evaluation of system performance parameters such as resolution, tailing factor, and theoretical plates before analysis.

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical scale and includes geometric features such as molecular morphologies and distributions of species within a sample as well as single dimensional features such as percent composition and species identity.

2. Drug Profile

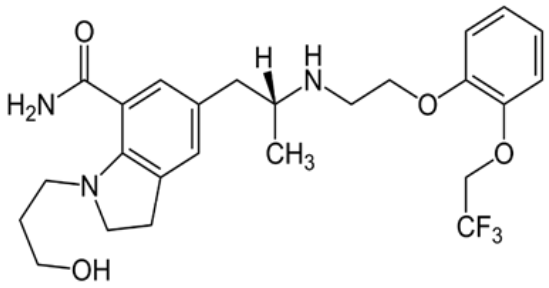
Molecular Structure Of Mirabegron



Molecular Formula	C25H32F3N3O4 (6,7)
Molecular Weight	495.53 g/mol (6)
IUPAC Name	2-(2-Amino-1,3-thiazol-4-yl)-N-[4-(2-oxo-1,2,3,4-tetrahydroquinazolin-7-yl)phenyl]acetamide (6)

ChemSpider ID	108879 (7)
Density	1.313g/cm^3 (7)
Boiling Point	690^0 (7)
Vapour Pressure	5.45E-20mmHg at 25°C (7)
Flash Point	371.1°C (7)
Refractive Index	1.68 (7)
LogP (Octanol/Water)	2.5 (6)
Generic Name	Mirabegron (5)
Brand Names	Myrbetriq (8,9)
Drug Category	Beta-3 adrenergic agonist (5)
Indications	Overactive bladder (OAB), Neurogenic detrusor overactivity (NDO) (8,9)
Pharmacology	Beta-3 adrenergic receptor agonist (5)
Potency	Moderate (5)
Tolerability	Generally well-tolerated (5)
Contraindications	Severe uncontrolled hypertension (9)
Adverse Effects	Hypertension, Nasopharyngitis, Urinary tract infection, Headache (8,9)
Avalilability	Prescription (9)
Mechanism of action	Mirabegron is a potent and selective agonist of beta-3 adrenergic receptors. 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. (5,8)

Molecular Structure Of Silodosine



Molecular Formula	C25H32F3N3O4 (11,12)
Molecular Weight	495.5 g/mol (11)
IUPAC Name	1-(3-Hydroxypropyl)-5-[2-(2-oxo-1,2-dihydroquinolin-3-yl)ethyl]-2,3-dihydro-1H-indol-7-yl trifluoromethyl ketone (11)
ChemSpider ID	5293683 (12)
Density	1.249±0.06 g/cm3(Predicted) (12)
Boiling Point	601.4±55.0 °C(Predicted) (12)

Vapour Pressure	2.58E-15mmHg at 25°C [12]
Flash Point	317.5±31.5 °C [12]
Refractive Index	1.552 [12]
LogP (Octanol/Water)	3.5 [11]
Generic Name	Silodosin [10]
Brand Names	Rapaflo, Silodyx, Urorec [13,14]
Drug Category	Alpha-1 adrenergic receptor antagonist [10]
Indications	Benign prostatic hyperplasia (BPH) [13,14]
Pharmacology	Alpha-1A adrenergic receptor antagonist [10]
Potency	High [10]
Tolerability	Moderate tolerability [10]
Contraindications	Severe renal impairment, Severe hepatic impairment, Concomitant use with strong CYP3A4 inhibitors [14]
Adverse Effects	Retrograde ejaculation, Dizziness, Diarrhea, Orthostatic hypotension, Headache, Nasopharyngitis, Nasal congestion [13,14]
Availability	Prescription [14]

Mechanism of action	1. Relaxation of smooth muscle: Silodosin binds to α1A-adrenergic receptors in the smooth muscle of the prostate gland and bladder neck, causing relaxation of these muscles. 2. Increased urine flow: The relaxation of smooth muscle in the prostate gland and bladder neck leads to an increase in urine flow and a decrease in urinary hesitancy. 3. Relief of BPH symptoms: By relaxing the smooth muscle in the prostate gland and bladder neck, silodosin helps to relieve the symptoms of benign prostatic hyperplasia (BPH), such as difficulty urinating, weak urine flow, and frequent urination. [10,13]

3. Materials And Methods

Table no 1: List of Proposed Materials:

S.No.	Chemicals/standards and reagents	Grade	Make	Used for the estimation of drugs
1	Phosphate buffer	HPLC	Qualigens	1.Mirabegron & silodosin
2	Acetic acid	HPLC	Qualigens	1. Mirabegron & silodosin
3	Water	HPLC	Qualigens	For all drugs
4	Acetonitrile	HPLC	Qualigens	For all drugs
5	Methanol	HPLC	Rankem	For all drugs

Table no. 2: Equipments and instruments used in the study:

S.No.	Equipment	Model/Type	Manufacturer
1	Electronic Balance	SAB2032	SCALETEC

2	Ultra-Sonicator	SE60US	LABMAN SCIENTIFIC INDIA
3	Thermal Oven	i-THERM A17782	DWARAKA SCIENTIFIC
4	pH Meter	ORION STAR A111	THERMOSCIENTIFIC
5	Filter Paper	0.45 microns	MILLIPORE
6	HPLC System	WATERS 2690 SEPARATION MODULE	WATERS

Optimization of Column:

SPURCIL C18 (4.6*250mm, 5µ) (DIKMA) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions

Equipment : High performance liquid chromatography equipped with Auto Sampler and PDA detector

Column : SPURCIL C18 (4.6*250mm, 5µm) (DIKMA)

Buffer : Ammonium acetate

PH : 5.0

Mobile phase :30% buffer: 70% Methanol

Flow rate : 1.0 ml per min

Wavelength : 230 nm

Injection volume : 20 □l

Run time : 10 min.

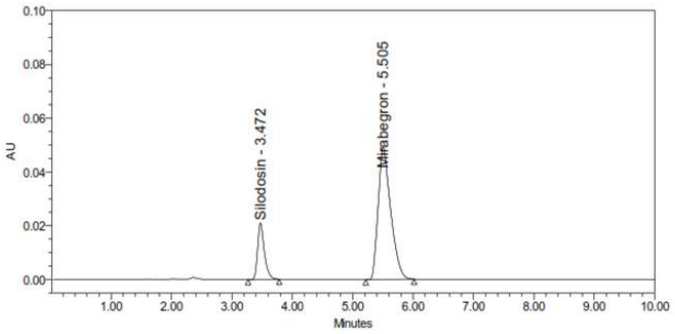


Table no 3:

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	Resolution	USP tailing	USP plate count
1	Silodosin	3.472	327896	602157	5.2	1.10	5147
2	Mirabegron	5.505	15478	20157		1.02	4278

3.1 Method Validation Parameters:

3.1.1assay:

Standard Solution Preparation:

Accurately weigh and transfer 6.4mg of Silodosin and 20mg Mirabegron working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (24ppm Silodosin &75ppm Mirabegron).

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 6.4mg of Silodosin and 20mg Mirabegron equivalent weight of the sample into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (24ppm Silodosin &75ppm Mirabegron).

Procedure:

Inject 10 mL of the standard, sample into the chromatographic system and measure the areas for the Silodosin and Mirabegron peaks and calculate the % Assay by using the formulae.

3.1.2. Linearity:

Preparation Of Stock Solution:

Accurately weigh and transfer 6.4mg of Silodosin and 20mg Mirabegron working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (8ppm of Silodosin and 25ppm Mirabegron):

0.25 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (16ppm of Silodosin and 50ppm Mirabegron):

0.5 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (24ppm of Silodosin and 75ppm Mirabegron):

0.75 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

3.1.3. Precision:

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

3.1.4. Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

3.1.5. Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 3.2mg of Silodosin and 10mg Mirabegron working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 6.4mg of Silodosin and 20mg Mirabegron working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (24ppm Silodosin &75ppm Mirabegron).

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 9.6mg of Silodosin and 30mg Mirabegron working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Silodosin and Mirabegron and calculate the individual recovery and mean recovery values.

3.1.6. Limit Of Detection:

Preparation of Silodosin and Mirabegron solution:

Preparation of 0.18µg/ml solution:

Accurately weigh and transfer 6.4mg of Silodosin working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 075ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 0.15 ml of the above stock solution into a 20ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 0.02µg/ml solution:

Accurately weigh and transfer 20mg Mirabegron working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 0.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent

3.1.7. Limit Of Quantification:

Preparation Of Silodosin And Mirabegron Solution:

Preparation Of 0.62µg/Ml Solution:

Accurately weigh and transfer 6.4mg of Silodosin working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 0.52ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation of 0.07µg/ml solution:

Accurately weigh and transfer 20mg Mirabegron working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 0.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Further pipette 0.9 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

3.1.8. Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied at 0.9 ml/min to 1.1 ml/min.

Standard solution 6.4µg/ml of Silodosin and 20µg/ml Mirabegron prepared and analysed using the varied flow rates along with method flow rate.

b) The Organic composition in the Mobile phase was varied from 63% to 77%

Standard solution 6.4µg/ml of Silodosin and 20µg/ml Mirabegron was prepared and analysed using the varied Mobile phase composition along

with the actual mobile phase composition in the method.

4. Results And Discussion

Trial 1

Chromatographic device : High performance liquid chromatography equipped with AutoSampler & PDA

Thermal profile : Room

Packed column : spursilC8,(150×3.0mm,3□m)

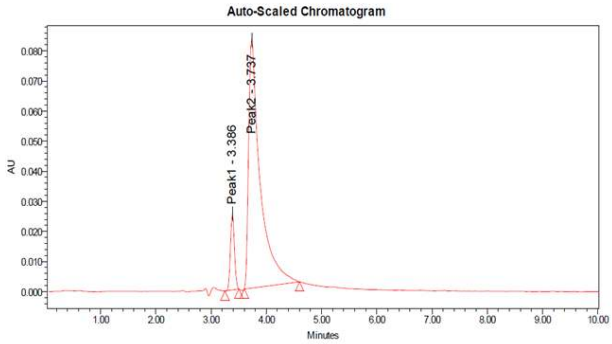
Mobilephase : 60%Methanol:40%OPAPH-4

Elution rate : 0.9mlpermin

λmax : 230nm

Injection load : 10□l

Elution duration : 10min.



Trial 2

Chromatographic device : High performance liquid chromatography equipped with AutoSampler & PDA

Thermal profile : Room

Packed column :PlatisilC18,(150×4.6mm,3□m)

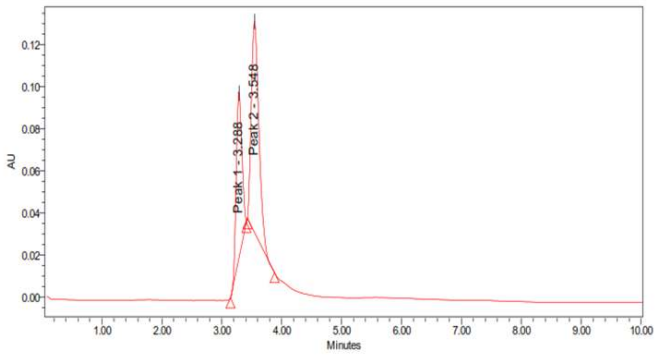
Mobile phase : 80%Methanol:20%OPAPH-4.5

Elution rate : 1.0mlpermin

λmax : 230nm

Injection load : 10□l

Elution duration : 10min.



Trial 3

Chromatographic device : High performance liquid chromatography equipped with AutoSampler & PDA

Thermal profile : Room

Packed column : Platisil,(250×4.6mm,5□m)

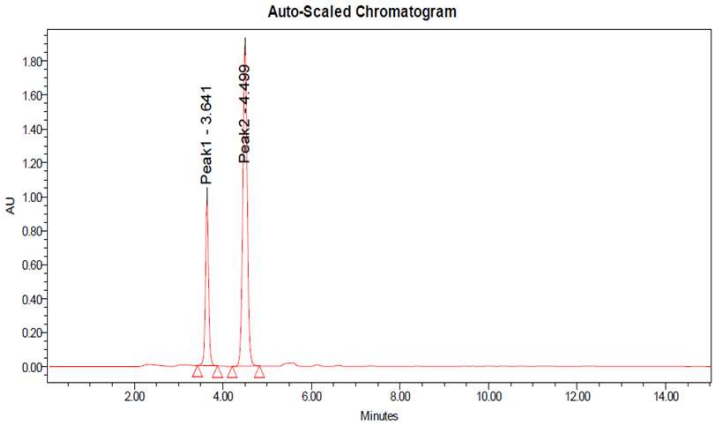
Mobilephase : 70%Methanol:30%OPAPH-4.5

Elution rate : 0.9mlpermin

λmax : 230nm

Injection load : 10□l

Elution duration : 14min.



System Suitability:

Equipment : High performance liquid chromatography equipped with Auto Sample and PDA detector

Column : SPURCIL C18 (4.6*250mm, 5µm) (DIKMA)

Buffer : Ammonium acetate

PH : 5.0

Mobile phase : 30% buffer: 70% Methanol

Flow rate : 1.0 ml per min

Wavelength : 230 nm

Injection volume : 20 □l

Run time : 10 min.

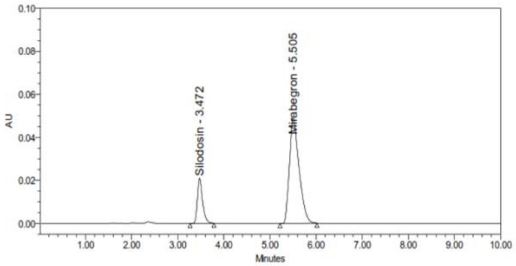


Figure 1: Chromatogram for system suitability

Table 4: Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	Resolution	USP tailing	USP plate count
1	Silodosin	3.472	15752	602157	5.2	1.10	5147
2	Mirabegron	5.505	96123	20157		1.02	4278

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

4.2 Validation Parameters:

4.2.1 Assay:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

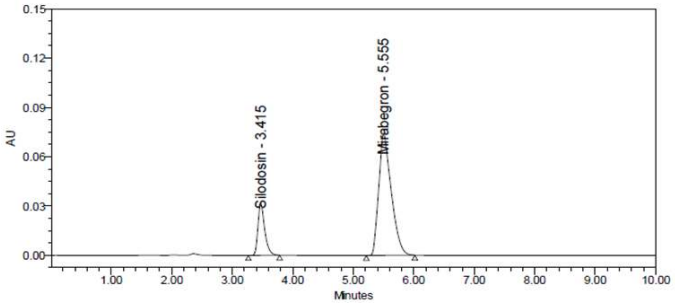


Figure 2: Chromatogram for Standard

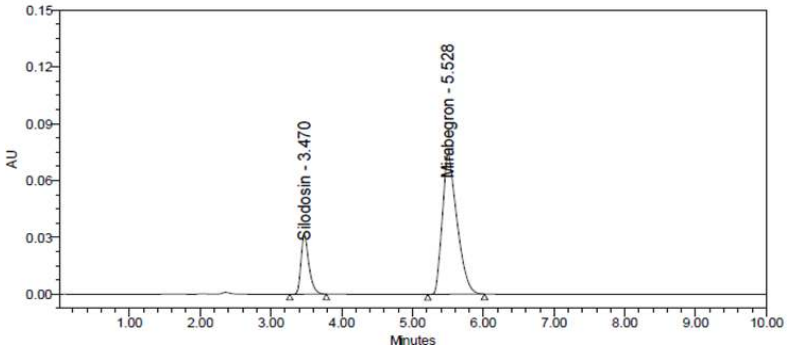


Figure 3: Chromatogram for Sample

S.No	Name (STD)	RT(min)	Area (µV sec)	Height (µV)	Resolution	USP tailing	USP plate count
1	**Silodosin**	**3.415**	**15812**	13536	**4.7**	**1.03**	**3050**
2	**Mirabegron**	**5.555**	**97321**	18461		**1.07**	**6928**
S.No	**Name (Sample)**	**RT(min)**	**Area (µV sec)**	**Height (µV)**		**USP tailing**	**USP plate count**

1	Silodosin	3.470	15752	13568	5.01	1.21	3026
2	Mirabegron	5.528	96123	18483		1.05	6922

Table 5: Results of Assay for Silodosin and Mirabegron

	Label Claim (mg)	% Assay
Silodosin	8 mg	99.0 %
Mirabegron	25mg	98.1%

4.2.2 LINEARITY:

The linearity range was found to lie from 10µg/ml to 50µg/ml of Silodosin and Mirabegron and chromatograms are shown below.

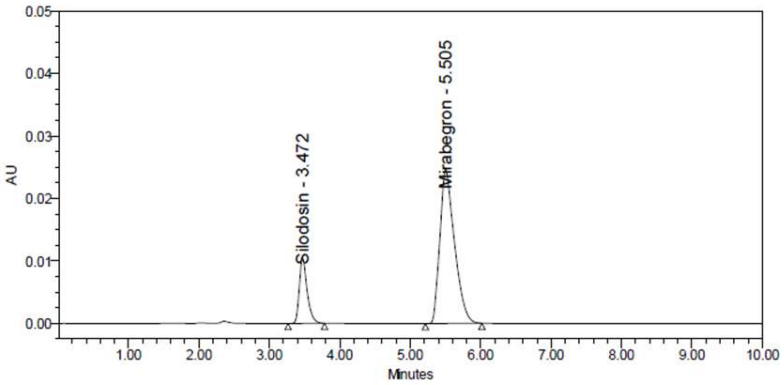


Figure 4: Chromatogram for linearity-1

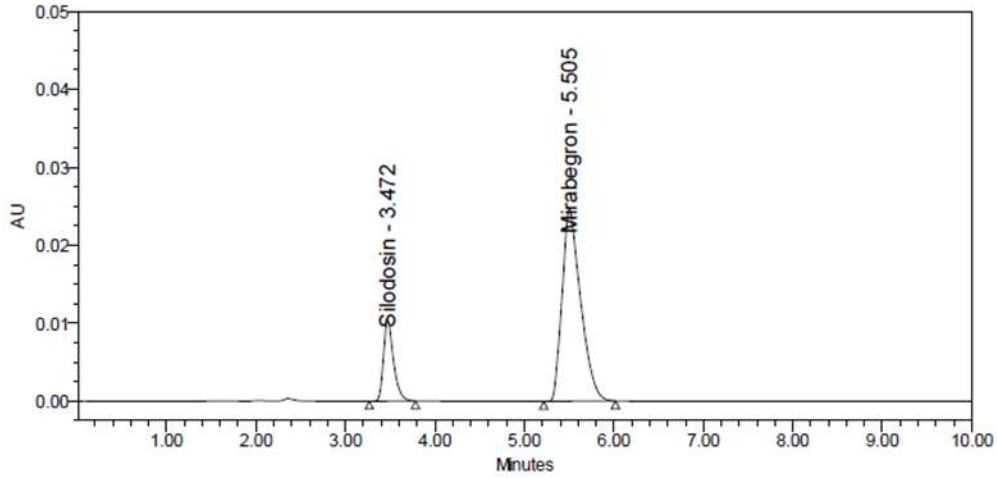


Figure 5: Chromatogram for linearity-2

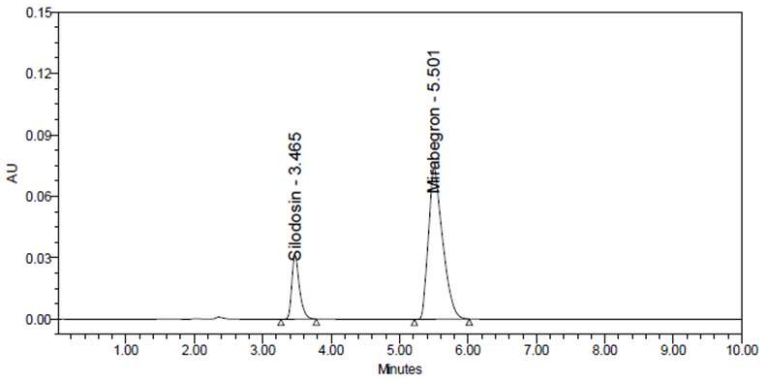


Figure 6: Chromatogram for linearity-3

Table 6: Area of different concentration of Silodosin and Mirabegron

S. No	Silodosin	
	Concentration (µg/ml)	Area
1	8	5163
2	16	10384
3	24	15699

S. No	Mirabegron	
	Concentration (µg/ml)	Area
1	25	33642
2	50	64395
3	75	98953

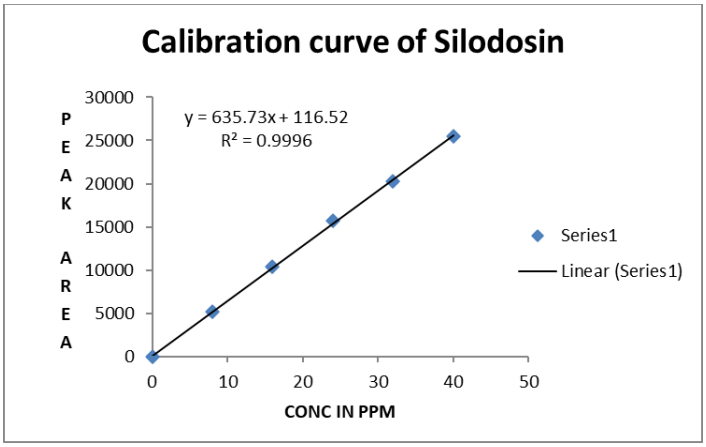


Figure 7: Calibration graph for Silodosin

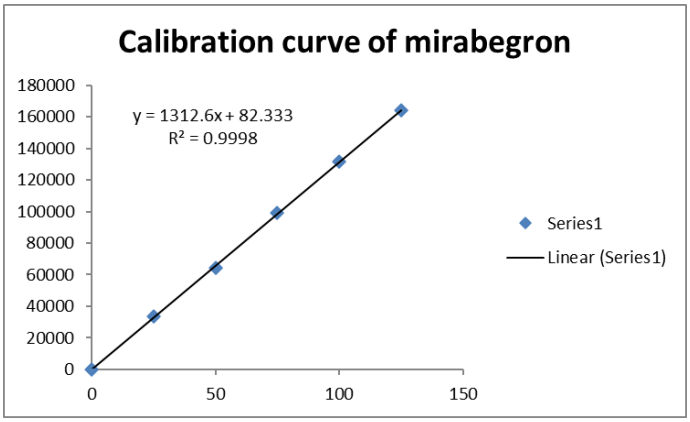


Figure 8: Calibration graph for Mirabegron

Table 7: Analytical performance parameters of Silodosin and Mirabegron

Parameters	Silodosin	Mirabegron
Slope (m)	203.43	4101.8
Intercept (c)	116.52	82.333
Correlation coefficient (R^2)	0.9996	0.9998

Acceptance criteria:

Correlation coefficient (R2) should not be less than 0.999

• The correlation coefficient obtained was 0.999 which is in the acceptance limit.

4.2.3 Precision:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

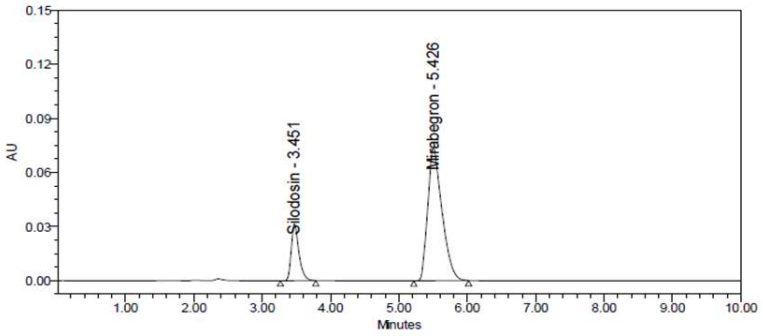


Figure 9: Chromatogram for Precision -1

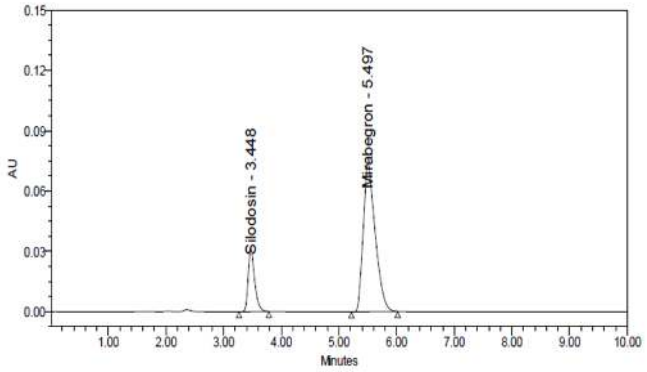


Figure 10: Chromatogram for Precision -2

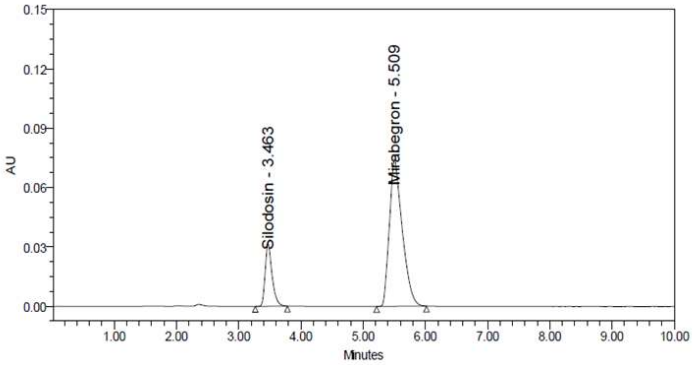


Figure 11: Chromatogram for Precision -3

Table 8: Results of Precision for Silodosin and Mirabegron

Injection	Silodosin Area	Mirabegron Area
Injection-1	15765	97453
Injection-2	15763	97685
Injection-3	15742	97932
Average	15753.17	97514
Standard Deviation	28.63157	300.6779
%RSD	0.2	0.3

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

4.2.4 Intermediate Precision (ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

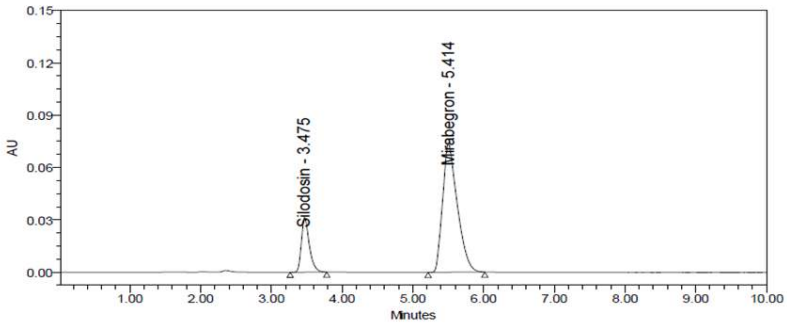


Figure 12: Chromatogram for ID Precision -1

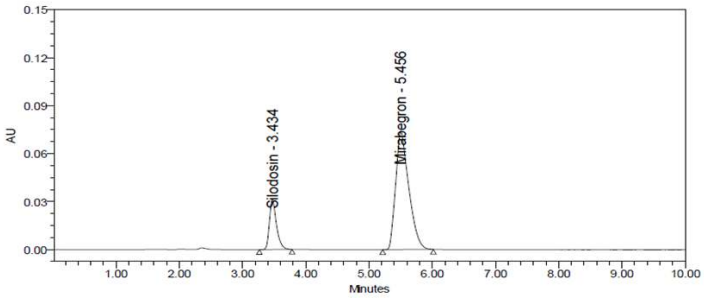


Figure 13: Chromatogram for ID Precision -2

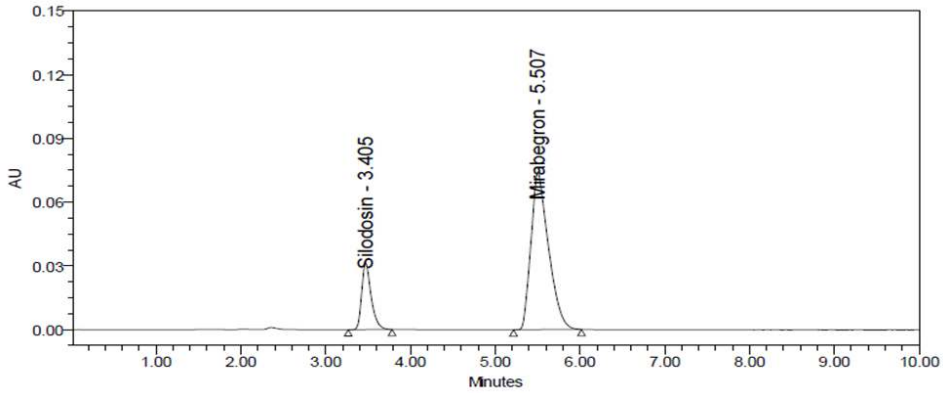


Figure 14: Chromatogram for ID Precision

Table 9: Results of Intermediate precision for Silodosin and Mirabegron

Injection	Silodosin Area	Mirabegron Area
Injection-1	15832	97236
Injection-2	15841	96652
Injection-3	15803	96142
Average	15837.17	96557.5
Standard Deviation	21.4515	453.3607
%RSD	0.1	0.5

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged

4.2.5 Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

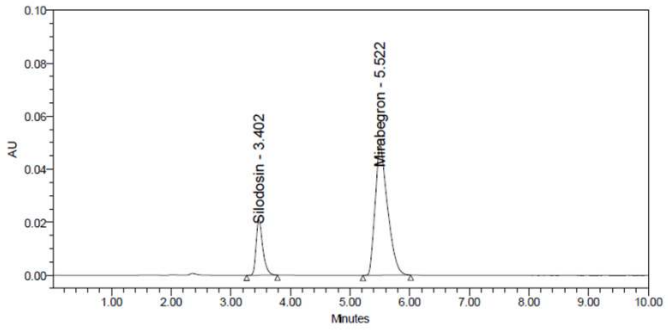


Figure 15: Chromatogram for Accuracy 50%

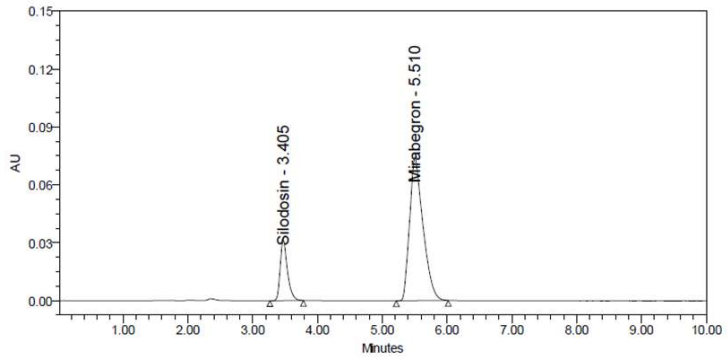


Figure 16: Chromatogram for Accuracy 100%

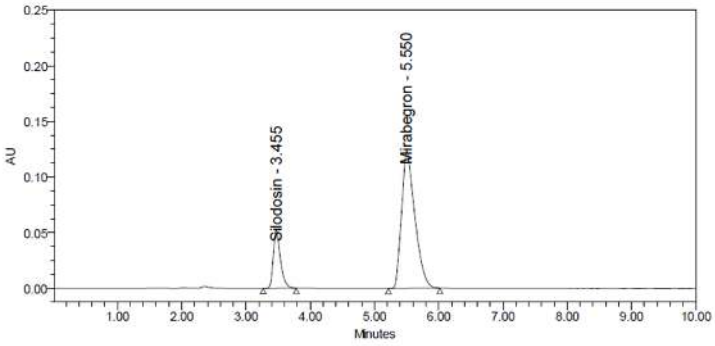


Figure 17: Chromatogram for Accuracy 150%

Table 10: Accuracy (recovery) data for Mirabegron:

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	48362	**10**	**9.92**	**99.2**	
100%	96123	**20**	**19.71**	**98.6**	
150%	145658	**30**	**29.87**	**99.58**	**99.1**

Accuracy (recovery) data for Silodosin:

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	7966	**3.2**	**3.2**	**100.6**	
100%	15752	**6.4**	**6.4**	**99.4**	
150%	23452	**9.6**	**9.5**	**98.7**	**99.6**

4.2.6 Limit Of Detection For Silodosin And Mirabegron

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

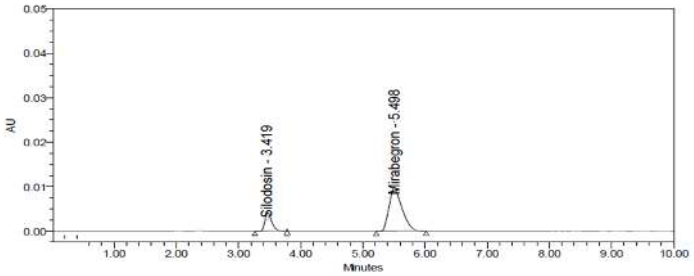


Figure 18: Chromatogram of Silodosin and Mirabegron showing LOD

Table 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc.
Silodosin	**53**	**147**	**2.77**	**0.18µg/ml**
Mirabegron	**53**	**528**	**9.96**	**0.07µg/ml**

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit.

4.2.7 Limit Of Quantification For Silodosin And

Mirabegron

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

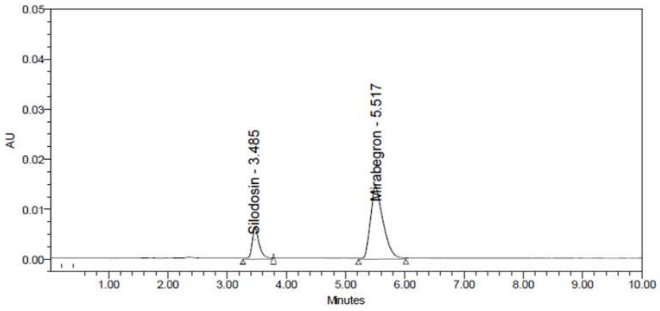


Figure 19: Chromatogram of Silodosin and Mirabegron showing LOQ

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc.
Silodosin	**53**	**521**	**9.83**	**0.62µg/ml**
Mirabegron	**53**	**528**	**9.96**	**0.07µg/ml**

- Signal to noise ratio shall be 10 for LOQ solution
- The result obtained is within the limit.

4.2.8 Robustness:

The standard and samples of Silodosin and Mirabegron were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

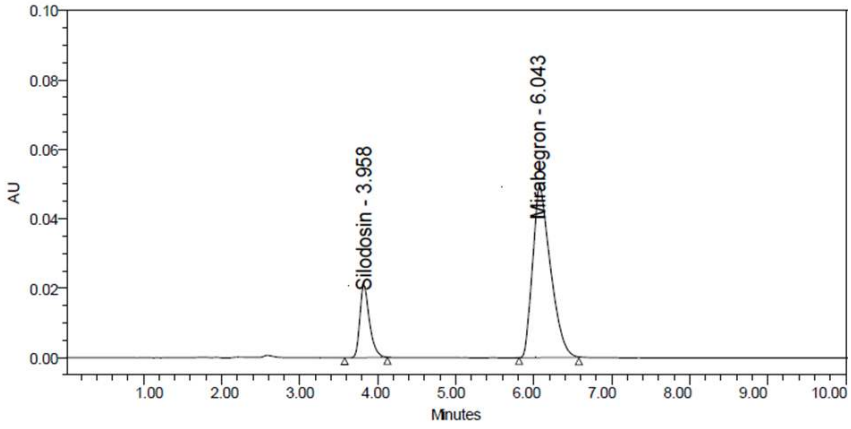


Figure 20: Chromatogram showing less flow

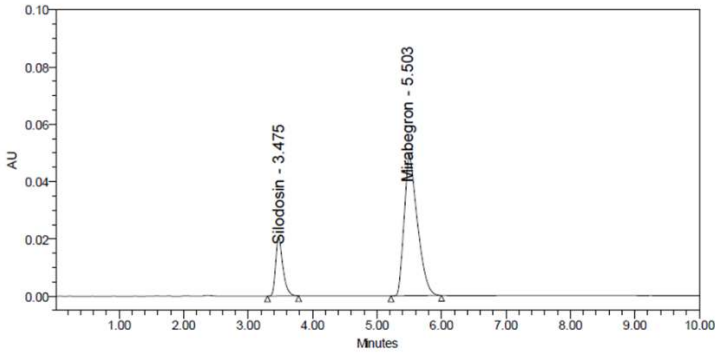


Figure 21: Chromatogram showing more flow

Variation of mobile phase organic composition:

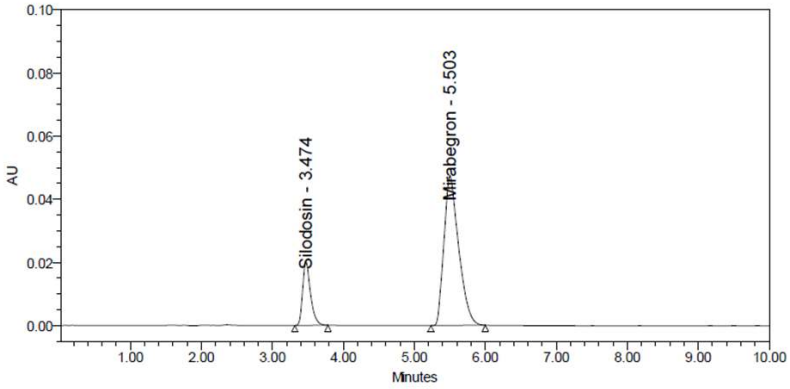


Figure 22: Chromatogram showing less organic composition

Figure 23: Chromatogram showing more organic composition

Table 13: Results for variation in flow for Silodosin and Mirabegron

S. No	Flow Rate (ml/min)	System Suitability Results of Silodosin	
		USP Plate Count	USP Tailing
1	**0.9**	3412	1.01
2	**1.0**	3489	1.03
3	**1.1**	3473	1.5

S. No	Flow Rate (ml/min)	System Suitability Results of Mirabegron	
		USP Plate Count	USP Tailing
1	**0.9**	5941	1.03
2	**1.0**	5986	1.02
3	**1.1**	5932	1.4

*** Results for actual flow (1.2 ml/min) have been considered from Assay standard**

Table 20: Results for variation in mobile phase composition for Silodosin and Mirabegron

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results of Silodosin	
		USP Plate Count	USP Tailing
1	**10% less(54ml)**	3412	1.01
2	***Actual(60ml)**	3489	1.03
3	**10% more(66ml)**	3473	1.5

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results of Mirabegron	
		USP Plate Count	USP Tailing
1	**10% less(54ml)**	5941	1.03
2	***Actual(60ml)**	5986	1.02
3	**10% more(66ml)**	5932	1.4

*** Results for actual Mobile phase composition have been considered from Accuracy standard.**

Acceptance criteria:

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

Summary:

A simple, accurate, and reproducible RP-HPLC method was successfully developed and optimized for the simultaneous estimation of Silodosin and Mirabegron in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a SPURCIL C18 column with a mobile phase consisting of ammonium acetate buffer and methanol (30:70 v/v) at pH 5.0, providing well-resolved peaks with acceptable retention times, good symmetry, and satisfactory system suitability parameters. The method demonstrated excellent linearity over the studied concentration ranges with correlation coefficients greater than 0.999. Precision studies showed %RSD values well within acceptable limits, confirming repeatability, while accuracy studies indicated recovery values close to 100%, demonstrating the reliability of the method. The method also exhibited good sensitivity with low LOD and LOQ values.

CONCLUSION:

The validated RP-HPLC method proved to be precise, accurate, linear, sensitive, and robust for the simultaneous determination of Silodosin and Mirabegron. All validation parameters including system suitability, precision, accuracy, linearity,

LOD, LOQ, and robustness were found to be within acceptable limits as per standard guidelines. The method is simple, cost-effective, and time-efficient, making it suitable for routine quality control analysis in pharmaceutical industries and research laboratories. Therefore, the developed method can be effectively applied for the estimation of Silodosin and Mirabegron in combined dosage forms with high reliability and reproducibility.

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