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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SILODOSIN & MIRABEGRON IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A robust and sensitive reversed-phase HPLC method was developed and validated for simultaneous determination of silodosin and mirabegron in pharmaceutical formulations. Separation was achieved on a YMC-ODS column (200 × 4.6 mm, 5 µm) using a buffer:methanol (40:60, v/v) mobile phase at 1.0 mL/min on a Waters HPLC system, with UV detection at 227 nm, producing well-resolved, symmetric peaks. Validation following ICH Q2(R1) demonstrated excellent linearity (R^2 = 0.999) over 6.4–32 µg/mL for silodosin and 20–100 µg/mL for mirabegron. Mean recoveries were 98.25% and 98.64%, and assay results were 102.0% and 99.8%, indicating high accuracy. Precision (%RSD <2%) and recovery at 50%, 100%, and 150% levels (98–99%) confirmed method reproducibility and accuracy. LOD/LOQ values were 0.4/1.3 µg/mL for silodosin and 4.3/4.3 µg/mL for mirabegron, demonstrating good sensitivity. Robustness testing showed minor deliberate changes in chromatographic conditions did not affect performance.This validated RP-HPLC procedure is simple, precise, sensitive, and well suited for routine quality control of silodosin and mirabegron formulations.

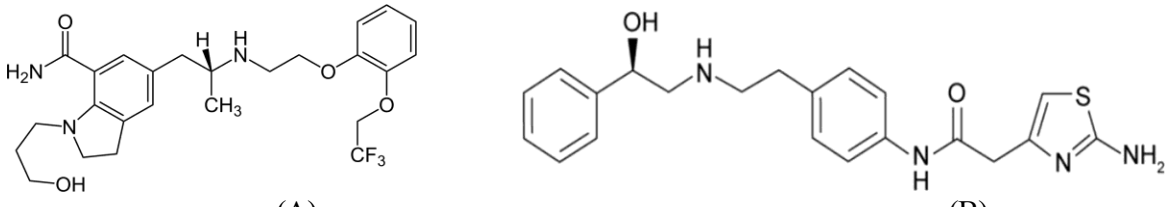
1. INTRODUCTION:

Silodosin and mirabegron are established pharmacotherapies for lower urinary tract disorders—primarily benign prostatic hyperplasia (BPH) and overactive bladder

(OAB), respectively[1]. BPH, a non-malignant enlargement of the prostate, commonly produces obstructive symptoms that impair urinary flow and quality of life in aging men[2]. Silodosin, a selective α1A-adrenoceptor antagonist, mitigates these obstructive symptoms by relaxing prostate and bladder-neck smooth muscle[3]. Mirabegron, a selective β3-adrenergic receptor agonist, targets storage dysfunction by relaxing the detrusor during filling, thereby increasing bladder capacity and reducing involuntary contractions[4]. Together, these agents provide complementary mechanisms that address both obstruction and storage symptoms in patients with overlapping BPH and OAB[5].

Regulatory approvals reflect their clinical value: silodosin (Rapaflo) received FDA approval for BPH in 2008, and mirabegron (Myrbetriq) was approved for OAB in 2012, with later indications expanded to include pediatric neurogenic detrusor overactivity[6]. Fixed-dose combinations (silodosin 8 mg with mirabegron 25–50 mg) and generic formulations are now available in regions such as India, underscoring widespread therapeutic adoption.

Because these drugs are frequently co-administered, there is a clear need for a rapid, selective, and sensitive analytical method for their simultaneous quantification in pharmaceutical products and biological matrices. UV spectrophotometry is limited by spectral overlap, whereas RP-HPLC offers superior resolution and selectivity. This study therefore develops and validates a reproducible RP-HPLC method for simultaneous estimation of silodosin and mirabegron in accordance with ICH Q2(R1), to support routine quality control and regulatory requirements



(A) (B)

Figure1: Chemical Structure A) Silodosin[8] and B) Mirabegron[9]

2. Experimental

2.1. Materials: .

HPLC-grade solvents, i.e., Acetonitrile, Methanol, and Water were supplied by Merck Pvt. Ltd., Mumbai, India. Solvents for analytical purposes with high purity level such as Ortho phosphoric acid and 30% w/v Hydrogen Peroxide (H2O2) were acquired from SD Fine-Chem Limited, Mumbai, India. The pure drug was received as a gift sample from MSN Labs Ltd Hyderabad.

2.2 Instruments:

The apparatuses and software used in this study include High-Performance Liquid Chromatograph (HPLC) from WATERS comprising Empower software, 2690 separation module, and UV detection. UV3000+ UV/Vis Spectrometer by LABINDIA was used for spectroscopic studies. A pH meter called Adwa–AD1020 was used to measure the pH. An AFCOSET ER-200A weighing machine was used for weighing. Moreover, the commonly used glasswares like pipette, burette, and beaker made from Borosil were used in experimental work.

2.3 Preparation of Solutions:

Preparation of NaH_2PO_4:

Sodium dihydrogen phosphate (NaH2PO4) buffer solution is prepared by dissolving 6.4gm of Phosphate buffer in 1000ml volumetric flask and diluted with HPLC water. Adjust the pH of the solution as required using either acid or base solutions.

Preparation of mobile phase:.

Dissolve accurately measured quantities of NaH2PO4 (as per above preparation) and Methanol in ultrasonic water bath for five minutes, after which filtration is carried out under vacuum using a 0.45µ filter.

Stock solution of Silodosin and Mirabegron:

Weight accurately and transfer 8 mg of Silodosin and 25 mg of Mirabegron working standards into a 25ml clean and dry volumetric flask. Dissolve it with diluent and sonicate it until dissolved to mark the volume. (Stock solution)

Preparation of working standard solution

Weight accurately and transfer 8 mg of Silodosin and 25 mg of Mirabegron working standards into a 25ml clean and dry volumetric flask. Dissolve it with diluent and sonicate it until dissolved to mark the volume with same solvent.

2.4 Methodology

An optimized RP-HPLC method was established for the simultaneous estimation

of Silodosin and Mirabegron. HPLC analysis was carried out using Waters HPLC system equipped with an YMCODS column (200 x 4.6 mm, 5µm). Mobile phase consisting of buffer and methanol in 40:60 v/v ratio was run at 1.0 mL/min flow rate through the analytical system. UV detector was employed for the detection of Silodosin and Mirabegron at 227 nm wavelength. Both standards and sample solutions were prepared by dissolving in the mobile phase. The method was validated following the ICH guidelines for linearity, precision, accuracy, specificity, robustness, and stability studies under different stresses like acidic, alkaline, oxidative, thermal, and photo-degradation. The proposed method showed outstanding reproducibility and specificity that made it reliable for the quantitative estimation of Silodosin and Mirabegron in their pharmaceutical formulations.

Method Validation

The analytical method was validated using ICH Q2 (R1) guidelines taking into account system suitability, specificity, accuracy, precision, linearity, robustness, LOD, and LOQ as the validation parameters.

System suitability

To measure the system suitability appropriate characteristics of theoretical plates, retention time, tailing factor, and percentage RSD, prepared standard solution were made and injected six times.

Accuracy

Accuracy can be defined as the closeness of the measurement value to the true or real value of that measurement. Accuracy was checked with the help of recovery studies. Accuracy studies are carried out at three different levels, i.e., 50%, 100%, and 150%. The concentration of the analyte is taken at these three different levels, and then the respective chromatogram is recorded.

Exactly weigh and transfer 8mg of Silodosin and 25mg of Mirabegron into a 25ml volumetric flask, add 25ml of Diluent and dissolve it thoroughly by sonication. (Stock solution). Further, take 0.6ml of the above stock solution in a 10ml volumetric flask and make up the volume with Diluent (19.2ppm Silodosin & 60ppm Mirabegron).

Specificity

Specificity is defined as the capacity of a method to identify an analyte unequivocally in the presence of other compounds such as degradation products, impurities, excipients, etc.

The specificity was evaluated by injecting blank, placebo, sample into the system. All of the samples were prepared as per the

developed method. For identifying the analyte, chromatograms were recorded, and retention times from sample were compared with those of standard. No interference was observed from blank, placebo, or known impurities at retention time of Silodosin and Mirabegron peaks

Precision

Precision is the measurement of the degree to which various outputs of the same quantity agree with one another. This test can be carried out on the same day as well as over three consecutive days to test intra-day and inter-day precision. The RSD% of the peak area and retention time were determined.

Accurately weigh and transfer 8mg of Silodosin and 25mg Mirabegron working standards into a 25ml clean dry volumetric flask, add diluent, and sonicate till dissolved to make volume up to the mark with the same solvent (stock solution). Pipette 0.6ml of this solution into a 10ml volumetric flask and add diluent to make it up to the mark (19.2ppm Silodosin &60ppm Mirabegron).

Linearity and range

It is the capability of the analytical procedure to provide results that are proportional to the concentrations of analytes in a predetermined range.

Accurately weigh and transfer 8mg of Silodosin and 25mg Mirabegron working standards into a 25ml clean dry volumetric flask, add diluent, and sonicate till dissolved to make volume up to the mark with the same solvent (stock solution).

LOD and LOQ

The signal-to-noise ratio was fixed at 3:1 for LOD and 10:1 for LOQ in the calculations used for LOD and LOQ determination.

Accurately weigh and transfer 8mg of Silodosin working standard into a 25ml clean dry volumetric flask; add Diluents and sonicate to dissolve it completely and dilute it to mark with the same solvent (Stock solution). Then further transfer 1ml, 1ml, 1.25ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. (0.4ppm)

Accurately weigh and transfer 25mg Mirabegron working standard into a 25ml clean dry volumetric flask; add Diluents and sonicate to dissolve it completely and dilute it to mark with the same solvent (Stock solution). Then further transfer 1ml, 1ml, 4.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. (4.3ppm)

Robustness

The robustness of the analytical procedure is a measure of its capacity to remain

unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usuage. Small variations in analytical method parameters may include temperature (+-50C), buffer pH (+-0.5), ion strength of buffers, additions to MP, flow rate (+-0.2ml/min), wavelength (+-2nm).

The standard solution of 19.2µg/ml of Silodosin and 60µg/ml of Mirabegron were injected by changing the conditions of chromatography. No significant changes have been noticed in parameters such as resolution, tailing factor, asymmetry factor, and plate count.

3. RESULTS AND DISCUSSION

Using HPLC, the area was determined after each of five injections of the standard solution. The five replicate injections "peak areas" percent relative deviation (%RSD) was computed, and it was discovered to be within the predetermined bounds. In figure 1, the chromatogram with the five injections is displayed

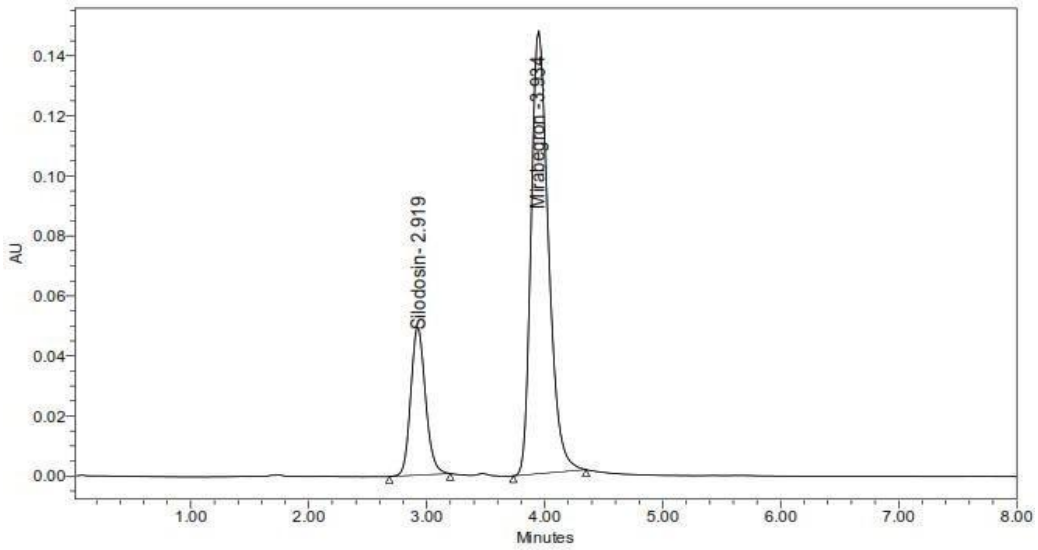


Fig.2. Optimized chromatogram of Silodosin and Mirabegron

Inference of optimized chromatogram:

From the results of method development trails, it is evident that mobile phase composition, pH and column selection are very important factors which affect the process of chromatographic separation. The initial results without use of buffer were not satisfactory whereas the use of buffer made a significant improvement.

An optimized chromatogram resulted in excellent separation with sharp and symmetrical peaks when using potassium dihydrogen phosphate buffer (pH 3.5) with methanol and C18 column. The retention

times were consistent, and system suitability parameters were well within acceptance criteria.

Therefore, the optimized chromatogram gives high resolution with good peak symmetry, and reproducibility, indicating that the developed method is suitable for routine quantitative analysis.

Specificity:

The specificity of the RP-HPLC method was confirmed by analyzing the blank, standard and sample chromatograms.

Table.1: Specificity results of Silodosin and Mirabegron

S.No	Analyte	Type	RT (min)	Resolution	USP Tailing	USP plate count
1	**Silodosin**	Standard	2.919	-	1.06 – 1.28	3400–3500
2	**Silodosin**	Sample	2.910	-	1.06 – 1.28	3400–3500
1	**Mirabegron**	Standard	3.145	>2.0	1.32 – 1.36	3100–3240
2	**Mirabegron**	Sample	3.328	>2.0	1.32 – 1.36	3100–3240

Inference: No interference was observed at the retention times of the analytes, confirming that the method is highly specific.

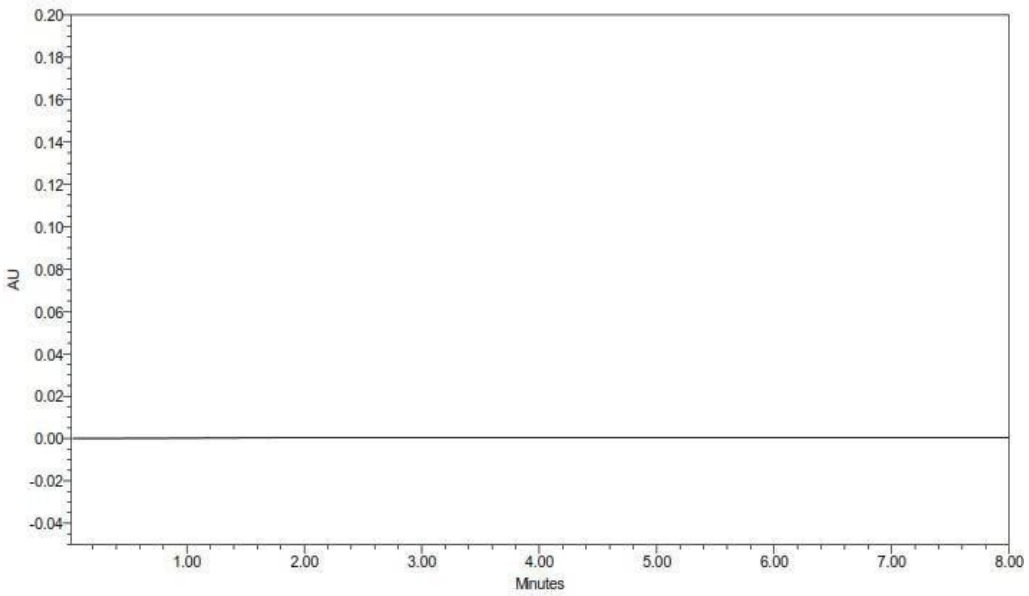


Fig.3. Chromatogram of Blank

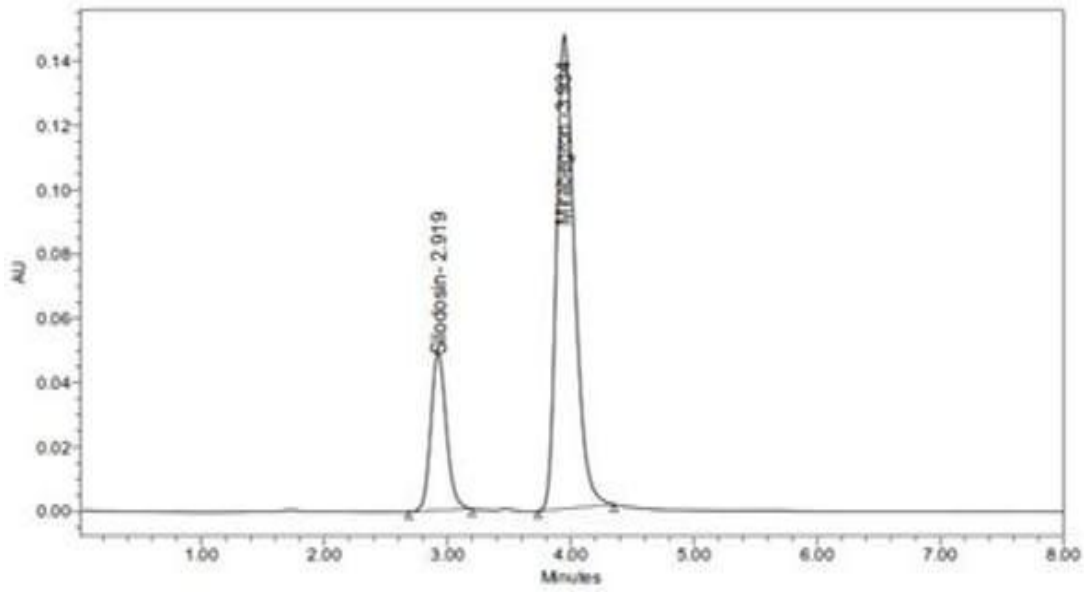


Fig.4. Chromatogram of Standard

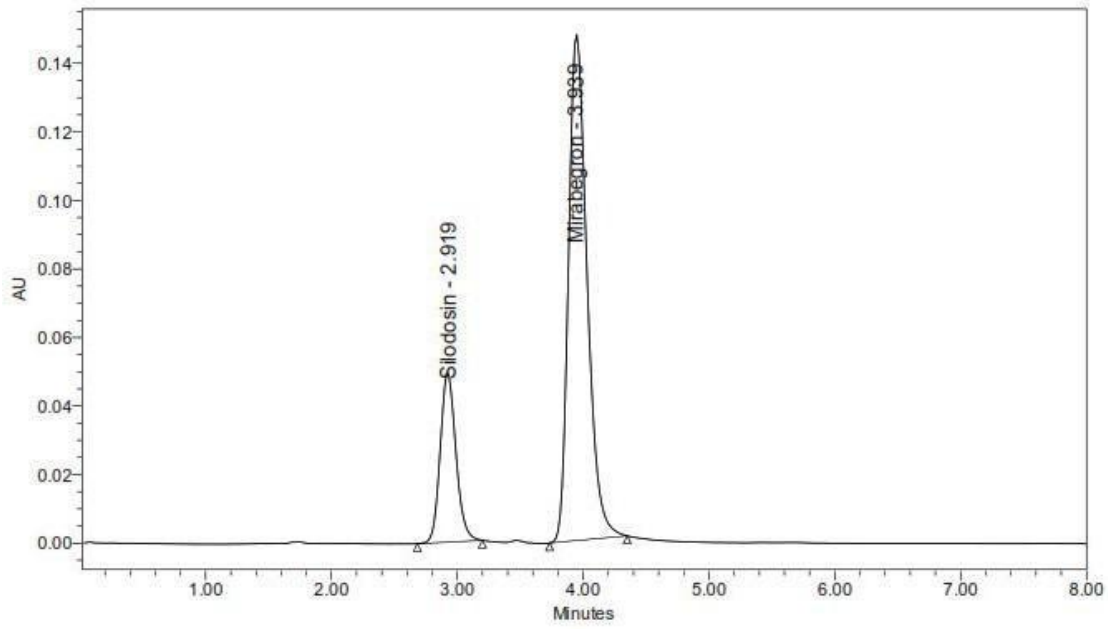


Fig.4. Chromatogram of Sample

System Suitability:

System suitability tests are conducted by injecting the prepared standard solution six times and measuring parameters such as theoretical plates, retention time, tailing factor, and %RSD. All the results shown in table 2.

Table.2: Results of System suitability

S. No	Name	RT (min)	Area (µV sec)	Height (µV)	Resolution	Tailing Factor	Theoretical plate count
1	**Silodosin**	2.923	12867	13536	4.7	1.03	3050
2	**Mirabegron**	3.948	24966	18461		1.07	6928

Linearity:

It has been observed that the linearity range for Silodosin is between 6.4µg/ml to 32µg/ml for Silodosin and while for Mirabegron is from 20µg/ml to 100µg/ml, indicating good linearity of the method and the results & calibration curve grapgh are shown below Table 3 ad figure 6 (a&b).

Table 3: Results of Linearity by RP- HPLC method

S.No	Silodosin		Mirabegron	
	Concentration(µg/ml)	Area	Concentration(µg/ml)	Area
1	6.4	4289	20	8322
2	12.8	8278	40	15644
3	19.2	12867	60	24966
4	25.6	17156	80	33288
5	32	21445	100	41610

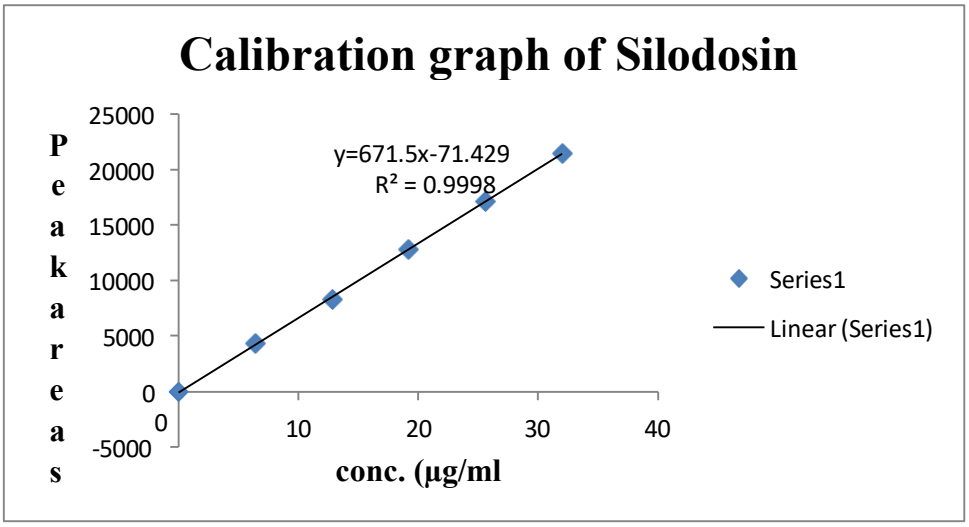


Figure 6.a: Calibration graph for Silodosin

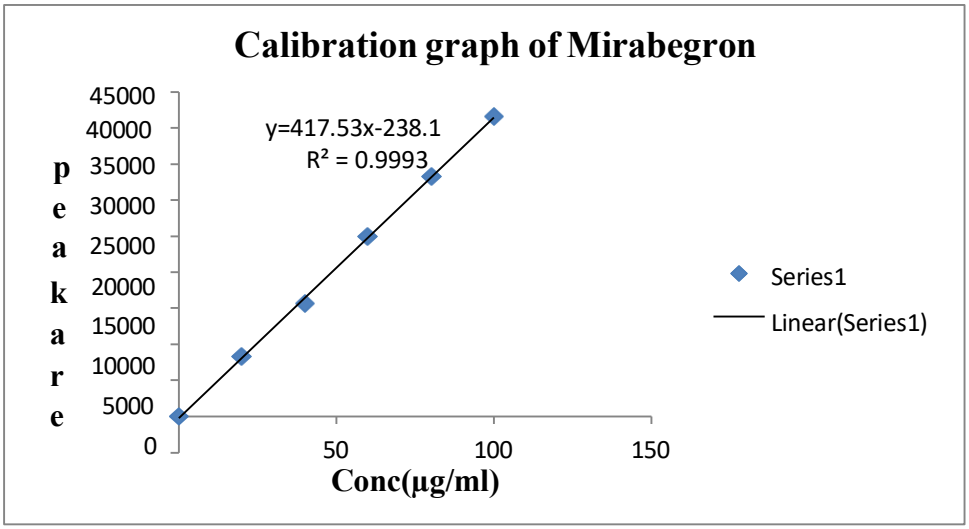


Figure 6.b: Calibration graph for Mirabegron

Precision:

The Precision of the method was performed on both sample solutions (Silodosin & Mirabegron). The system precision results indicate consistent peak areas for Silodosin and Mirabegron with a %RSD obtained is within the limits, indicating good repeatability and precision of the analytical method. The corresponding results are shown below.

Table 4: Results of Precision for Silodosin and Mirabegron

S. No.	Silodosin		Mirabegron	
	Intraday precision Area	Interday precision Area	Intraday precision Area	Interday precision Area
1	12867	12867	24962	24966
2	12858	12958	24957	24987
3	12866	12866	24946	24940
4	12867	12864	24930	24855
5	12865	12865	24962	24978
6	12767	12767	24858	24873
Mean	12848.33	12864.5	24935	24933
Std Dev	39.98833	60.42764	40.03	56.14
%RSD	0.31	0.46	0.16	0.22

Sensitivity:

Limit Of Detection (LOD):

The signal-to-noise (S/N) ratio for Silodosin and Mirabegron was found to be 2.96. This indicates that the obtained signals are clearly detectable when compared to the baseline noise. Hence, the method demonstrates good sensitivity and the results are within the acceptable range.

Table 5.a: Showing results for Limit of Detection

Drug name	Baseline noise (µV)	Signal obtained(µV)	S/N ratio	Concentration
Silodosin	95	282	2.96	0.4µg/ml
Mirabegron	135	400	2.96	1.3µg/ml

Limit Of Quantification (LOQ):

LOQ analysis gives S/N ratio of 9.89 and 9.94 for Silodosin and Miraberon respectively, which is close the required limit of 10. The signals obtained are clearly higher than the baseline noise for both the drugs, indicating good quantification ability of the analytical method. Hence, the results are within the acceptable limits.

Table 5.b: Showing results for Limit of Quantification

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio	Concentration
Silodosin	95	940	9.89	1.3µg/ml
Mirabegron	135	1342	9.94	4.3µg/ml

Accuracy:

The accuracy study was carried out at 50%, 100% and 150% concentration levels, and the % recovery values were found to be within acceptable limits. Silodosin showed a mean recovery of 98.25%, while Mirabegron showed 98.64%. The results indicate and confirm that the method is accurate and reliable for analysis.

Table 6.a: Recovery results for Silodosin

% Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	6433	4	3.89	97.25	98.25
100%	12867	8	7.88	98.5	
150%	19300	12	11.89	99.0	

Table 6.b: Recovery results for Mirabegron

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	12483	12.5	12.32	98.56	

100%	24966	25	24.68	98.72	98.64
150%	37449	37.5	36.99	98.64	

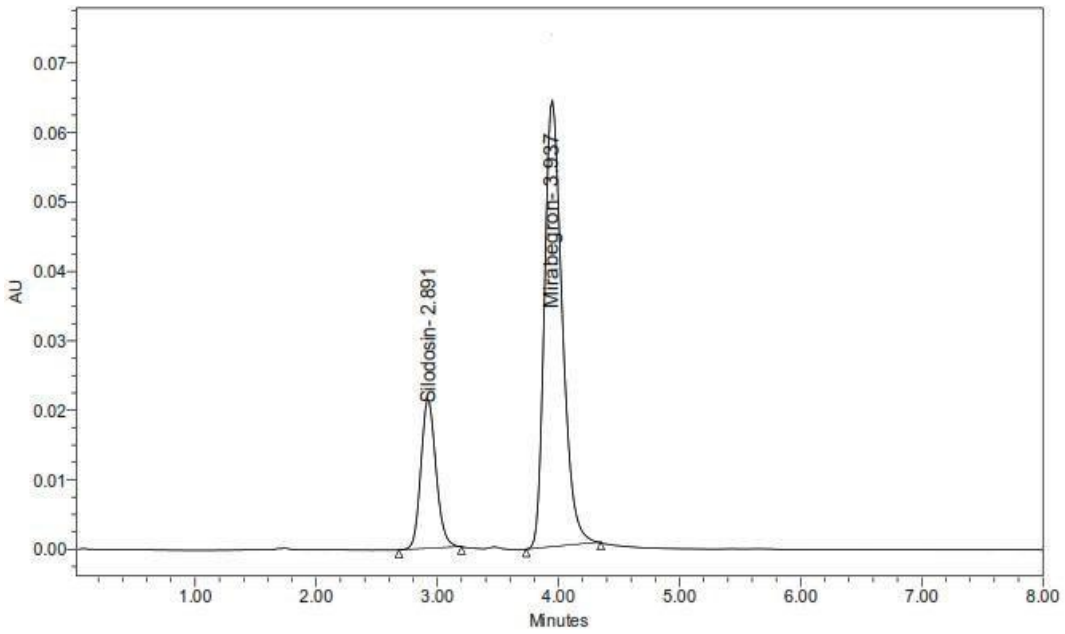


Fig.7. 50% Accuracy chromatogram of Silodosin and Mirabegron

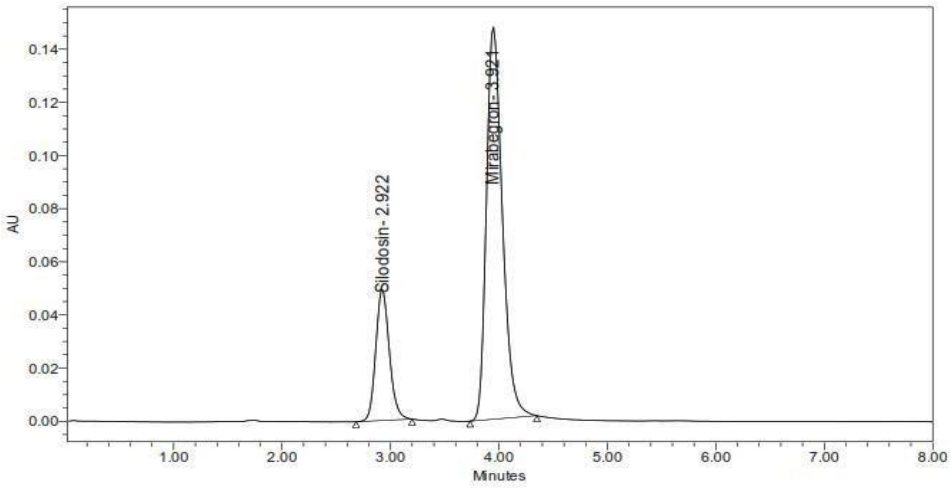


Fig.8. 100% Accuracy chromatogram of Silodosin and Mirabegron

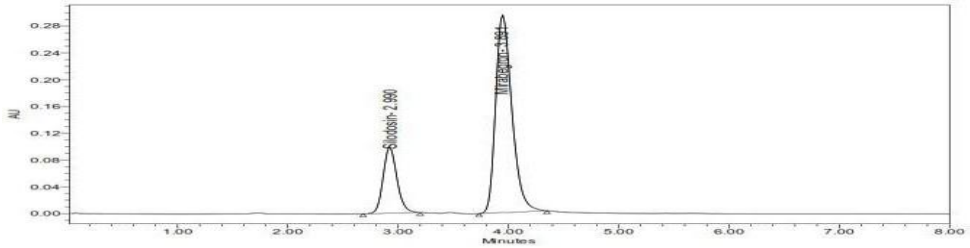


Fig.9. 150% Accuracy chromatogram of Silodosin and Mirabegron

Robustness:

The robustness study was performed by making slight variations in flow rate, and the system suitability parameters remained consistent. Silodosin and Mirabegron showed acceptable plate count and tailing factor values under all conditions. This indicates that small changes in method parameters do not significantly affect the results. Hence, the method is robust and reliable.

Table 6.a & 6.b displays the results of small modifications made to the analytical method's temperature and flow rate.

Table 7.a: Results of Robustness studies for Silodosin

S. No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3042	1.03
2	1.0	3050	1.03
3	1.2	3054	1.01

Table 7.b: Results of Robustness studies for Mirabegron

S. No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	6925	1.04
2	1.0	6928	1.07
3	1.2	6932	1.2

4. Conclusion

The newly developed RP-HPLC method for the simultaneous estimation of Silodosin and Mirabegron is precise, accurate and reliable, making it suitable for routine analysis in both bulk and pharmaceutical dosage forms. The technique is validated according to the ICH guidelines, and the results demonstrates high levels of linearity, recovery and precision. Thus, the proposed method offers an efficient and robust analytical tool for the quality control and routine analysis of Silodosin and Mirabegron.

5. Acknowledgement

I am very thankful to Director, JNTUA-OTPRI, Ananthapuramu for providing the laboratory facilities and chemicals to carryout entire research work.

6. Conflict of interest

We confirm that there are no conflicts of interest.

7. References:

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