NOVEL RP-HPLC STABILITY-INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF MIRABEGRON AND SILODOSIN.

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ABSTRACT

A simple, accurate, precise, and stability-indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Mirabegron and Silodosin in bulk drug and tablet dosage forms. Method development was performed through systematic optimization of chromatographic parameters including column selection, mobile phase composition, pH, flow rate, and organic solvent ratio to obtain satisfactory resolution, peak symmetry, and analysis time. Effective chromatographic separation was achieved using a Platisil C18 column (250 × 4.6 mm, 5 µm) with a mobile phase comprising potassium dihydrogen phosphate buffer (pH 4.5) and methanol in the ratio of 30:70 v/v, delivered at a flow rate of 1.0 mL/min, with UV detection at 230 nm.The developed method was validated according to ICH guidelines for system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and assay. The method exhibited excellent linearity over the concentration range of 25–125 µg/mL for Mirabegron and 4–20 µg/mL for Silodosin, with correlation coefficients (R^2) of 0.999. Precision and intermediate precision studies showed %RSD values below 2%, confirming good repeatability and ruggedness of the method. Accuracy studies demonstrated percentage recoveries within the acceptable range of 98–102%.Forced degradation studies carried out under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed the stability-indicating nature of the method, as degradation products were well separated from the analyte peaks without interference. The developed RP-HPLC method was found to be robust, sensitive, and suitable for routine quality control analysis, assay determination, stability testing, and regulatory evaluation of Mirabegron and Silodosin in pharmaceutical formulations.

Keywords: RP-HPLC, Mirabegron, Silodosin, Method validation, Stability-indicating method, Forced degradation studies, Pharmaceutical analysis, ICH guidelines

1. INTRODUCATION OF SILODOSINE: Silodosin is an alpha-1 adrenergic receptor antagonist used to treat symptoms associated with benign prostatic hyperplasia (BPH

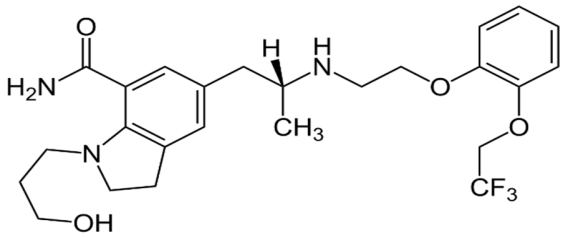

Fig no 1: Structure of Silodosin

Mechanism of Action: The pathologic process of benign prostate dysplasia isn't absolutely understood. it's believed to involve many pathways, as well as inflammation, apoptosis, and cellular proliferation. Most drug therapies aim to alleviate symptoms of benign prostate dysplasia, silodosin enclosed. Lower tract symptoms of benign prostate dysplasia area unit classified into 3 main groups: evacuation or hindering (hesitancy, slow stream, irregularity, incomplete emptying), storage or irritating (frequency, urgency, nocturia, urge urinary incontinence), and postmicturition (post void dribbling). Prostate contraction is that the main contributor to lower tract symptoms of benign prostate dysplasia. the sleek tone of the prostate is regulated by α1Aadrenoceptors, that area unit the foremost extremely expressed subtype of α1adrenoceptors within the human prostate tissue. it's been reported that blockade of α1Aadrenoceptors relieves bladder outlet obstruction. Blockade of α1D-adrenoceptors, another subtype found in prostate tissue, is believed to alleviate storage symptoms thanks to detrusor bodily function. α1- adrenoceptors area unit G protein coupled receptors: upon binding of its natural substance, nor adrenaline and vasoconstrictive, results in the activation of phospholipase C and downstream signaling molecules, as well as

vitamin B complex triphosphate and diacylglycerol. Ultimately, there's a rise in living thing metal levels and, consequently, sleek muscular contraction. Silodosin is associate antagonist of α1- adrenoceptors, with the very best property for the α1A-adrenoceptor subtype. By obstruction the α1A-adrenoceptor signalling pathway, silodosin promotes prostate and epithelial duct sleek

muscle relaxation, thereby up lower tract symptoms like evacuation. Silodosin additionally targets sensory nerves within the bladder bodily function and robust symptoms.

1.2 INTRODUCATION OF MIRABEGRONE:- Mirabegron is a beta-3 adrenergic agonist used to treat overactive bladder and neurogenic detrusor over activity.

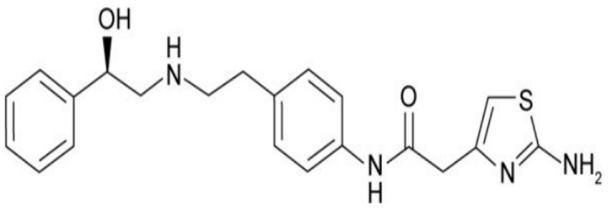

Fig no 2: Structure of Mirabegron

Mechanism of action:- Mirabegron may be a potent and selective agonist of beta-3 adrenergic receptors. The activation of beta-3 receptors relaxes detrusor swish muscle throughout the storage section of the vesica fill-void cycle, that will increase the bladder's storage capability thereby assuaging feelings of urgency and frequency

2. EXPERIMENTAL WORK:

Materials and Methods: The APIs of Mirabegron and Silodosine were obtained as gift samples from MSN Drugs Pvt Ltd. Silotrif -M50 tablets having the label claim Mirabegron 50mg, , Silodosin 8mg were taken from the local market. HPLC grade solvents like Acetonitrile, Water and Methanol and AR grade chemicals like Potassium Dihydrogen phosphate and Orthophosphoric acid were obtained from Rankem.

Instruments: Analysis was carried out by using Microprocessor UV Visible Double Beam, Waters Alliance 2690 HPLC embedded with empower 2 software and UV detector. The other instruments used were Analytical weighing Balance, Ultrasonicator, pH Meter, Hot air oven, Vortex meter.

Diluent: Based up on the solubility of the drugs, diluent was selected, MEOH: KH2PO4 PH 4.5 taken in the ratio of (700:300ml).

Preparation of Standard stock solutions:

Accurately weigh and transfer 25 mg of **Mirabegron**and 4 mg **Silodosin** working standard into a 25 ml clean dry volumetric flasks 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 each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. (75ppm**Mirabegron**and 12ppm **Silodosin**)

Sample Solution Preparation

Accurately weighed and transfer 35mg of Tablet Powder i.e. equivalent weight to 25 mg of **Mirabegron**and 4 mg **Silodosin** working standard into 25 ml clean dry volumetric flasks 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 each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents.

3. RESULTS AND DISCUSSION:

Several trials has been taken for the proper optimization of RP HPLC method by changing different ratio of buffer and mobile phase and columns.

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Instrument used : High performance liquid chromatography equipped with Auto Sampler and PDA or UV detector

Temperature : Ambient

Column : PlatisilC18 Column, (250×4.6mm, 5µm)

Mobile phase : 30% KH2PO4 PH 4.5: 70% MEOH

Flow rate **:** 1.0ml /min

Wavelength : 230 nm

Injection volume : 20 µl

Run time : 10 min.

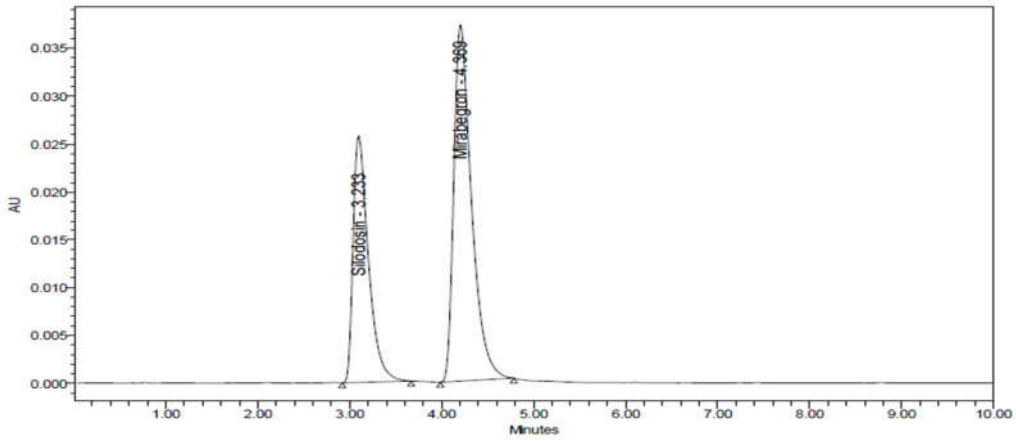

Fig 3: Optimized Chromatogram for Mirabegron and Silodosin

3.1 SYSTEM SUITABILITY: The system suitability parameters were determined by injecting standard Solutions of 20µg/ml of Mirabegron and 10µg/ml of Silodosin six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should be not more than 2%.

Table 1: System Suitability results for Mirabegron and Silodosin

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	Resolution	USP tailing	USP plate count
1	**Silodosin**	3.233	1970425	235874	4.15	1.11	3451
2	**Mirabegron**	4.389	3089845	379874		1.22	7145

Observation:

- Excellent peak symmetry
- Satisfactory resolution
- USP Tailing, resolution, and plate count within limits.
- Method validated as efficient, robust, and suitable for routine analysis

3.2 LINEARITY:

The linearity range was found to lie from 4µg/ml to 20µg/ml of Silodosin and 25µg/ml to 125µg/ml of Mirabegron . Calibration curves were plotted for both the standard drug solutions by taking concentration on X-axis and peak areas on Y-axis. The obtained data were subjected to regression analysis using least squares method. Chromatograms are shown below.

Table 2: Linearity results for Mirabegron and Silodosin

MIRABEGRON		SILODOSIN	
Conc (µg/mL)	**Area**	**Conc (µg/mL)**	**Area**
4	644127	25	1109510
8	1307412	50	2048412
12	1961021	75	3084125
16	2615540	100	4099651
20	3195142	125	5112453
4	644127	25	1109510

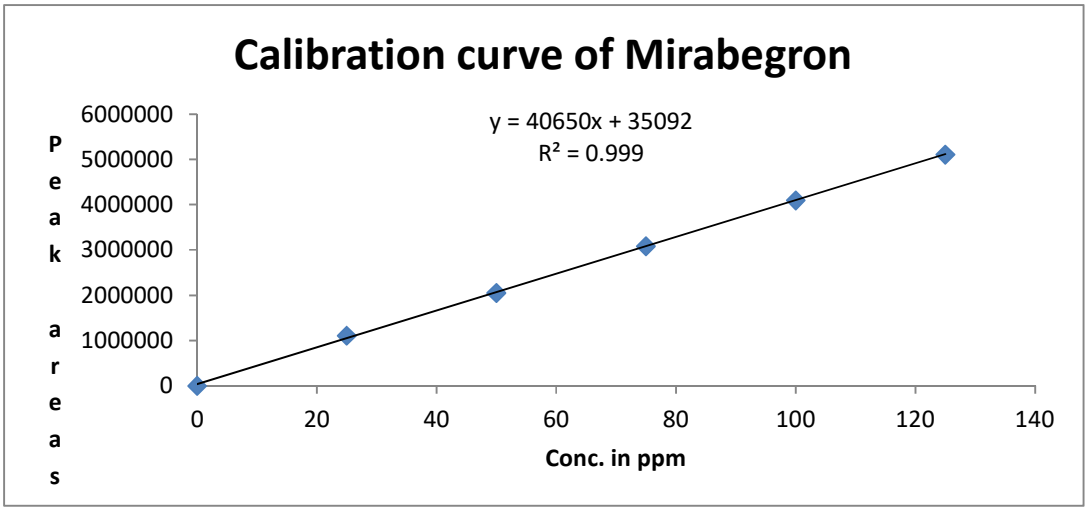

Fig no 4: Calibration curve of Mirabegron

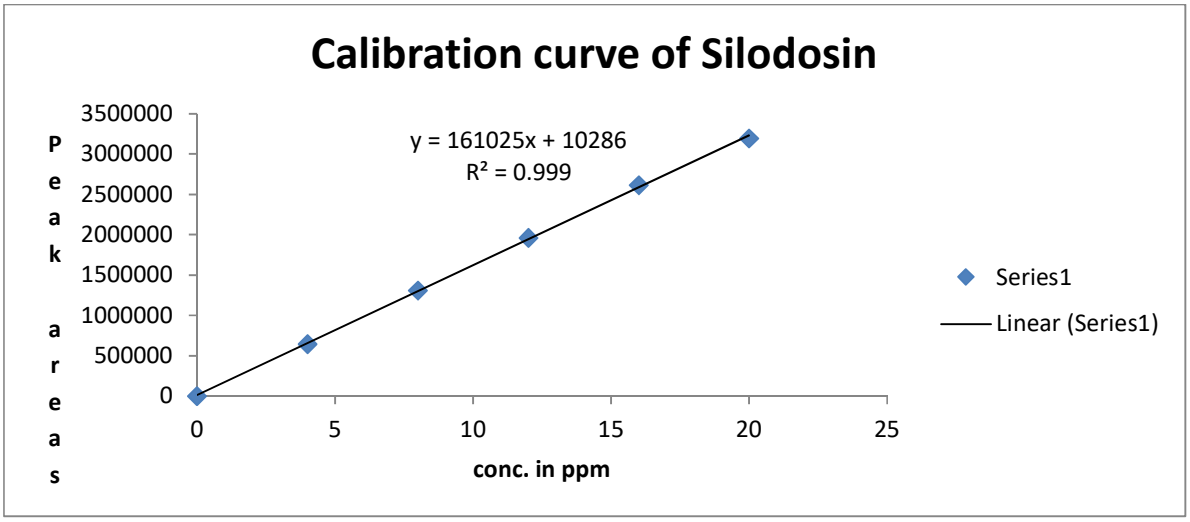

Fig no 5: Calibration curve of Silodosin

Acceptance criteria:

- Correlation coefficient (R^2) should not be less than 0.999
- The correlation coefficient obtained was 0.999 which is in the acceptance limit.

3.3 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.

Table 3: Precision results for Mirabegron and Silodosin

Injection	Silodosin Area	Mirabegron Area
Injection-1	1847512	2951472
Injection-2	1854271	2955124
Injection-3	1852147	3011245
Injection-4	1836214	2999651
Injection-5	1855214	2955210

Injection-6	1833214	2963214
Average	1846428.667	2972652.667
Standard Deviation	9502.884313	25951.83246
%RSD	0.5	0.9

Acceptance criteria:

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

3.4 ACCURACY: Analytical recovery tests were performed by using ICH guidelines to verify the recovery of a method that was developed and to study the interference of formulation excipients. The results of the recovery study and its statistical data are reveal in Table no. 6&7, which shows that the designed technique has the highest accuracy. The recovery rate was 99.00% and 99.20% for Mirabegron and Silodosin respectively

Table 4: Accuracy (recovery) data for Silodosin and Mirabegron

%Concentration (at specification Level)	**Area***	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	**983245**	**2**	**1.99**	**99.5**	**99.0**
100%	**1968471**	**4**	**3.98**	**99.6**	
150%	**2904125**	**6**	**5.88**	**98.0**	

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1527452	12.5	12.31	98.5	99.2
100%	3091254	25	24.92	99.7	
150%	4628512	37.5	37.3	99.5	

3.5 LIMIT OF DETECTIONAND 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

Table 5: Sensitivity result of silodosin and mirabegron

DRUG	LOD	LOQ
Mirabegron	0.01µg/ml	0.03µg/ml
Silodosin	0.03µg/ml	0.12µg/ml

3.6 DEGRADATION STUDIES

Acid degradation:

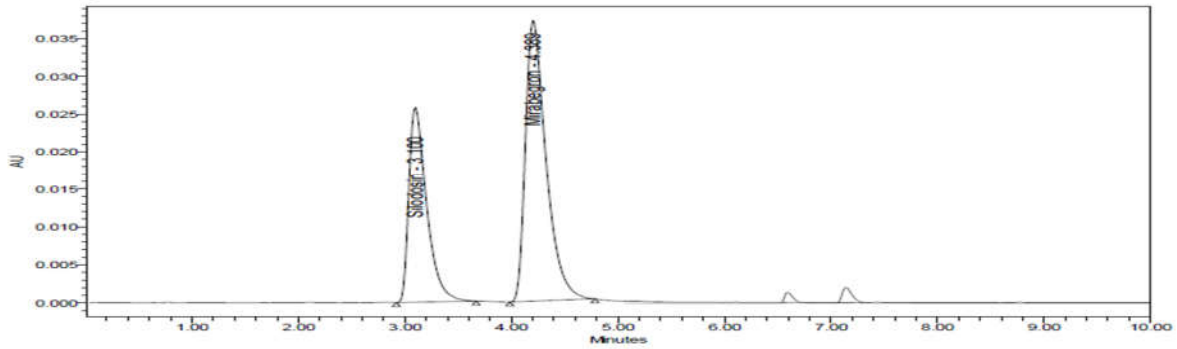

Fig no 6: chromatogram of silodosin and mirabegron

Base Degradation:

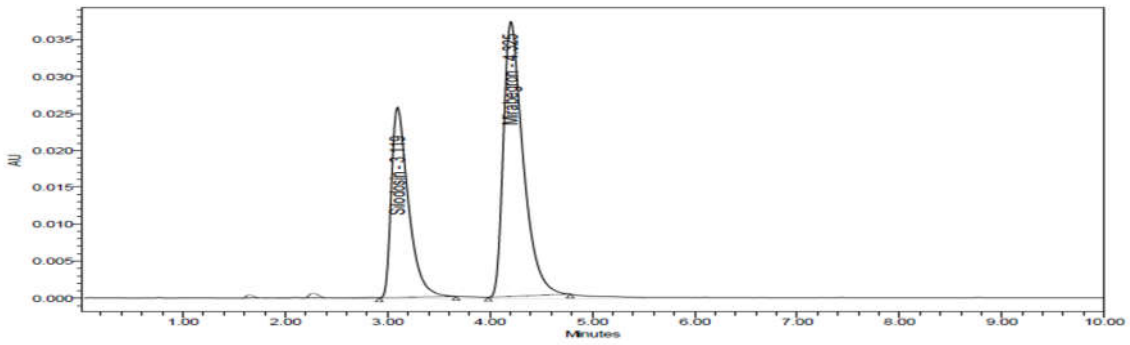

Fig no 7: chromatogram of silodosin and mirabegron

Peroxide Degradation

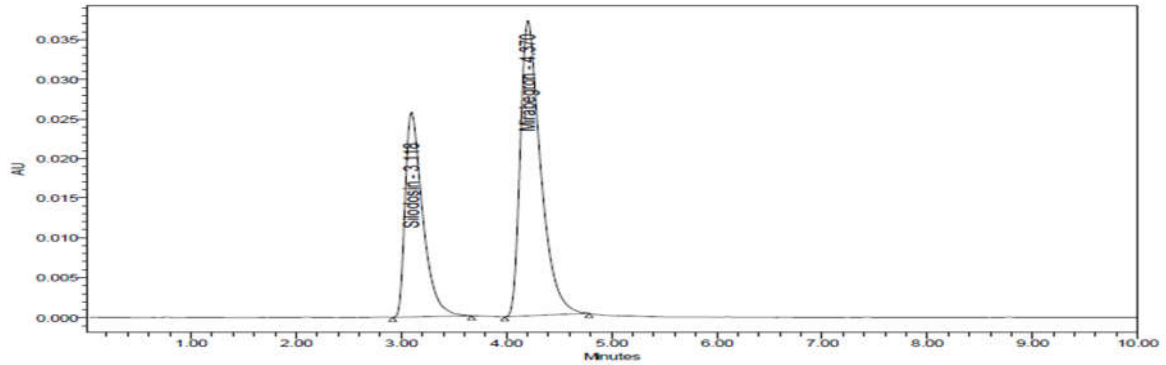

Fig no 8: chromatogram of silodosin and mirabegron

Photo Degradation

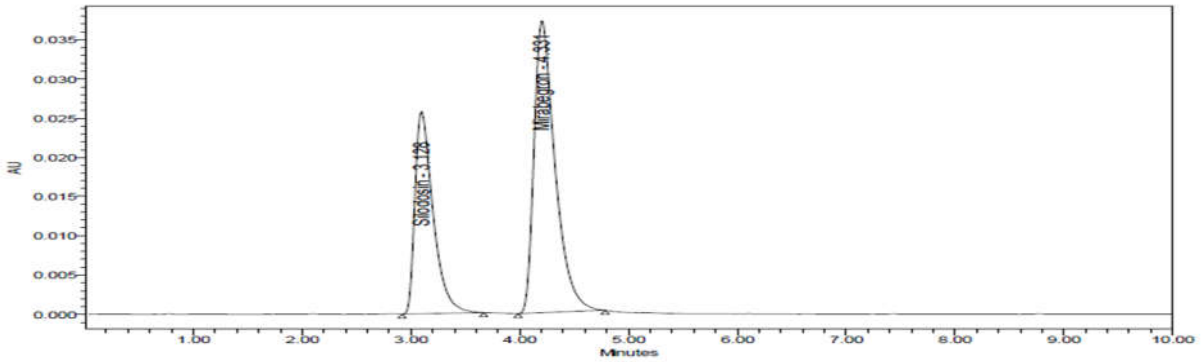

Fig no 9: chromatogram of silodosin and mirabegron

Degradation Studies:
Table 6: Degradation results of silodosin and mirabegron

TYPE OF DEGRADATION	MIRABEGRON		SILODOSIN	
	Area	% Degradation	Area	% Degradation
STANDARD	3095417	-----	1972147	-----
ACID	2854123	7.80	1854247	5.98
BASE	2985471	3.55	1785214	9.48
PEROXIDE	2899654	6.32	1801245	8.67
THERMAL	3011245	2.72	1924268	2.43
PHOTO	2899651	6.32	1852142	6.08

4. CONCLUSION

The current study effectively developed a new, accurate, and stability-indicating RP-HPLC. technique for the simultaneous measurement of Rizatriptan and Meloxicam in pharmaceutical and bulk dose forms. The efficacy of the approach was confirmed by the chromatographic settings being tuned to produce sharp, symmetrical, well-resolved peaks with short retention periods. Excellent linearity, accuracy, precision, robustness, and specificity were shown in validation trials conducted in compliance with ICH Q2 (R1) requirements; recovery was within acceptable bounds (98–102%) and %RSD values were less than 2%. The method's strong sensitivity was demonstrated by the low LOD and LOQ values.
For routine quality control, stability testing, and assay analysis of Meloxicam and Rizatriptan in mixed formulations, the suggested method proved to be straightforward, quick, economical, and dependable. Additionally, the technique can be expanded for bioanalytical or dissolution studies with minor modifications, contributing to improved analytical quality assurance in pharmaceutical research.

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