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Development and Validation of RP-HPLC Method for the Estimation of Mirabegron and Silodosin in Synthetic Mixtur

Parth Trivedi1 , Prof. Mitali Dalwadi2 , Dr. Umesh Upadhyay3
Student1 , Professor2, Principal3
Sigma Institute of Pharmacy, Bakrol, Vadodara, Gujarat, 390019, India.

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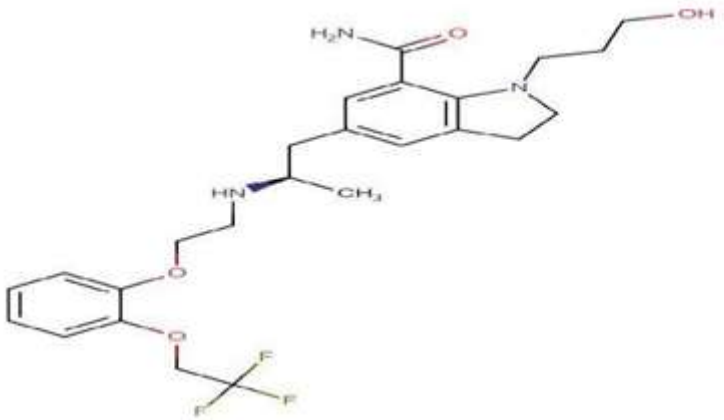
ABSTRACT:
A simple, rapid, accurate, precise specific and sensitive reverse phase high performance liquid chromatographic method has developed and validated for the simultaneous estimation of Mirabegron (MIR) and Silodosin (SIL) in synthetic mixture. The chromatographic separation was performed using Shimpack ODSC18 (4.6mmx250mm,5 um), utilizing a Mobile phase Acetonitrile: Methanol: water in the volume ratio 45:45:10v/v/vwere used. at Flow rate of 1 ml/min, injection volume 20µl with UV detection at 244nm. The retention time of Silodosin (SILO) 4.335 min and Mirabegron (MIRA) 6.690 min using RP-HPLC. The % RSD Value was found for the validation parameter that preciseness of the proposed method and is applicable for routine analysis for quantitative determination of SILO and MIRA. The LOD was found for SILO0.26 µg/ml, MIRA 0.40 µg/mlfor developed method respectively. The LOQ was found for SILO 0.79 µg/ml, MIRA1.22 µg/ml for developed method respectively. The result of analysis was validated according to ICH Q2 R1 Guidelines. This simple and precise method can be used of both drugs in quality control laboratories.

Keywords:SILODOSIN (SILO), MIRABEGRON (MIRA), reverse phase high performance liquid chromatography, validation.

I. INTRODUCTION TO DRUG

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



1-(3-hydroxypropyl)-5-[(2R)-2-[2-[2-(2,2,2 trifluoroethoxy)phenoxy]ethylamino]propyl]-2,3- dihydroindole-7-carboxamide

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

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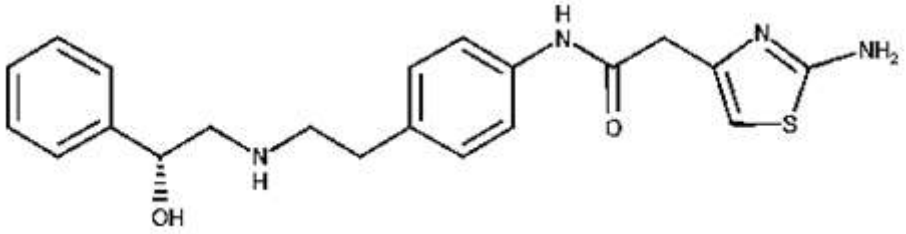
irritating (frequency, urgency, nocturia, urge urinary incontinence), and postmicturition (postvoid dribbling). Prostate contraction is that the main contributor to lower tract symptoms of benign prostate dysplasia. the sleek tone of the prostate is regulated by α1Aadrenoceptors, that area unit the foremost extremely expressed subtype of α1adrenoceptors within the human prostate tissue. it's been reported that blockade of α1A-adrenoceptors 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, noradrenaline and vasoconstrictive, results in the activation of phospholipase C and downstream signalling 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.

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



2-(2-amino-1,3-thiazol-4-yl)-N-[4-[2-[[(2R)-2-hydroxy-2-phenylethyl]amino]ethyl]phenyl] acetamide

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.

METHOD DEVELOPMENT AND VALIDATION
Selection of Diluent: Based on solubility, Silodosin (SILO) and Mirabegron (MIRA) was soluble in methanol. Hence, methanol was selected as diluent.

Preparation of Stock solution: Accurately weighed and transferred about 16 mg of Silodosin (SILO) and 100mg of Mirabegron (MIRA) in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of Silodosin (SILO) is 160 µg/ml and Mirabegron (MIRA) 1000 µg/ml. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Silodosin (SILO) is 16 µg/ml and Mirabegron (MIRA) 100 µg/ml. The optimum wavelength was selected for the estimation was 270 nm where both drugs give good absorbance.

Selection of Mobile Phase: The water, buffer, pH of the buffer, organic solvent, and buffer-to-solvent ratio were all factors in the mobile phase selection process. The HPLC technique selection is influenced by the sample's nature, physicochemical properties, molecular weight, and solubility. pH management necessitates the use of a buffer. The pH of the acidic component is kept low, while the pH of the base is kept high. Separation, peak purity, tailing factor, theoretical plate, and other parameters were used to optimize the mobile phase for HPLC system. Various mobile phases in various compositions and pH levels were tried to achieve a sharp peak of Silodosin (SILO) and Mirabegron (MIRA). Selection of Wavelength: An ideal wavelength is the one that gives Maximum response for the drugs that was to be detected. For selection of wavelength U.V spectrophotometer is used or using HPLC assisted with PDA detector, PDA detector UV spectra Silodosin (SILO) and

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Mirabegron (MIRA) were obtained. For High Performance Liquid Chromatography 270 nm was selected wavelength where both drugs show good absorbance.

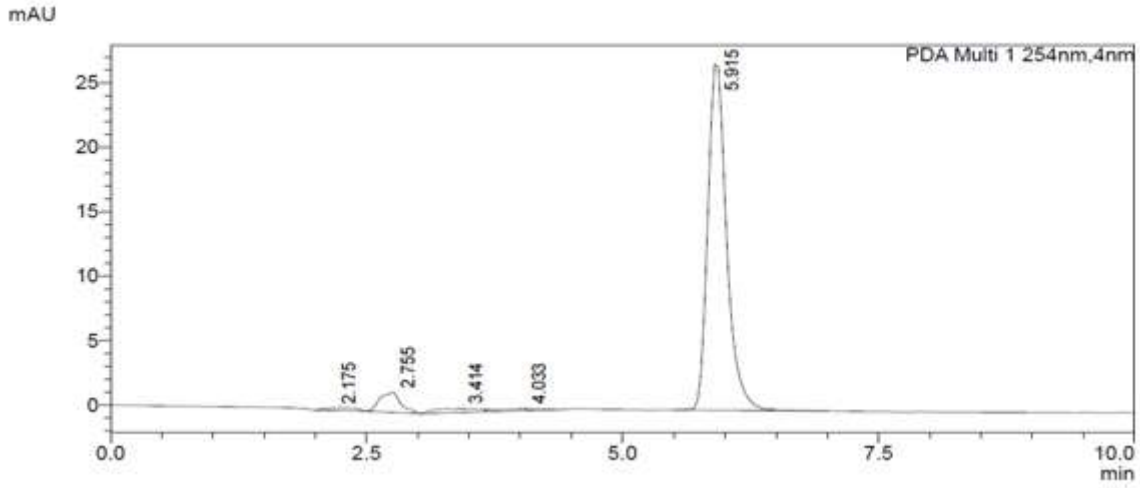
Preparation of Mobile Phase: Acetonitrile, Methanol, and water were filled in different mobile phase reservoir after filter and sonicate to degas the mixture. Mobile phase Acetonitrile: Methanol: water in the volume ratio 45:45:10 v/v/v were used. 7.2.6 Selection of column: Silodosin (SILO) and Mirabegron (MIRA) are polar in nature. So, C18 analytical column were selected for HPLC method. The column was used Shimpack ODS C18 column (250 mm × 4.6 mm, 5 µm) was used for the development of the method.

Preparation of standard stock solution: Preparation of Standard stock solution of Silodosin (SILO) Weigh accurately 16 mg of Silodosin (SILO) in 50 ml volumetric flask. Add 50 ml of methanol and sonicate to dissolve. Dilute up to the mark with methanol and mix well. The prepared solution having 160 µg/ml of SILO. Preparation of Standard stock solution of Mirabegron (MIRA) Weigh accurately 100mg of Mirabegron (MIRA) in 50 ml Volumetric flask. Add 50 ml of methanol and sonicate to dissolve. Dilute up to the mark with methanol and mix well. The prepared solution having 1000 µg/ml of MIRA. Preparation of Standard stock solution of Silodosin (SILO) and Mirabegron (MIRA) Transfer 10 ml of Silodosin (SILO) from their standard stock solution and 10 ml of Mirabegron (MIRA) from their standard stock solution in to 100 ml volumetric flask. Dilute up to the mark with methanol and mix well. The prepared solution has concentration 16ppm of Silodosin (SILO) and 100 ppm of Mirabegron (MIRA).

Optimization Of Mobile Phase Composition:

Based on review of literature, several mobile phases were selected on the basis of solubility of Silodosin (SILO) and Mirabegron (MIRA vcf) in the solvents. Various solvents and mixtures of solvents was tried using methanol, acetonitrile, HPLC grade water and phosphate buffer of different pH and their combinations. And the best result was obtained by using Acetonitrile: Methanol: water in the volume ratio 45:45:10 v/v/v (Trial 6) having good peak shape and resolution of greater than 2 as well as theoretical plate more than 2000. Flow rate was 1 ml/min monitor at 270nm. Stationary phase was Shimpack ODS C18 column 25 cm (4.6 mm x 250mm, 5 um) and Injection Volume was 10 µl. Retention time of Silodosin (SILO) 4.335 min and Mirabegron (MIRA) 6.690 were obtained. So, optimization of mobile phase for HPLC method includes various trials as summarized

Tirales



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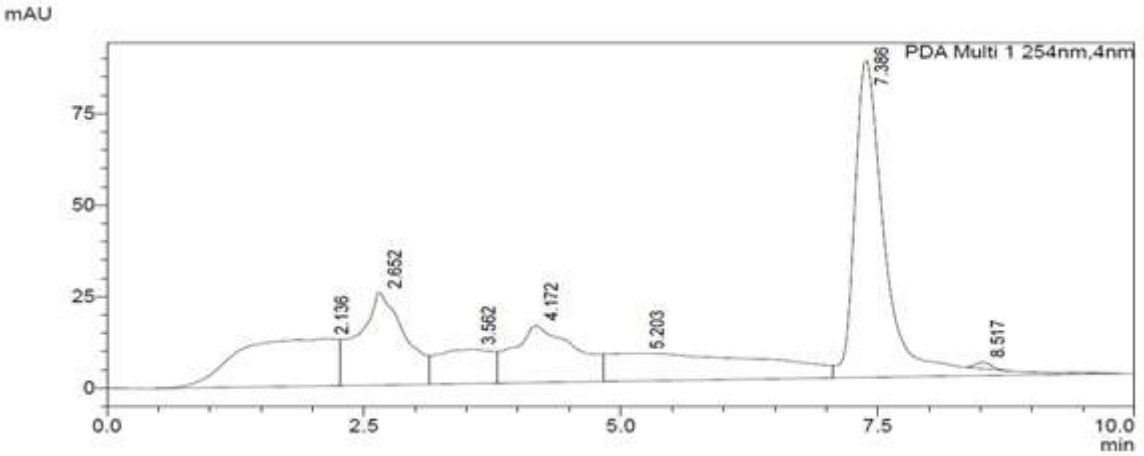

Figure 1 Trail 1: HPLC Chromatogram of SILO and MIRA (8 ppm and 50 ppm)

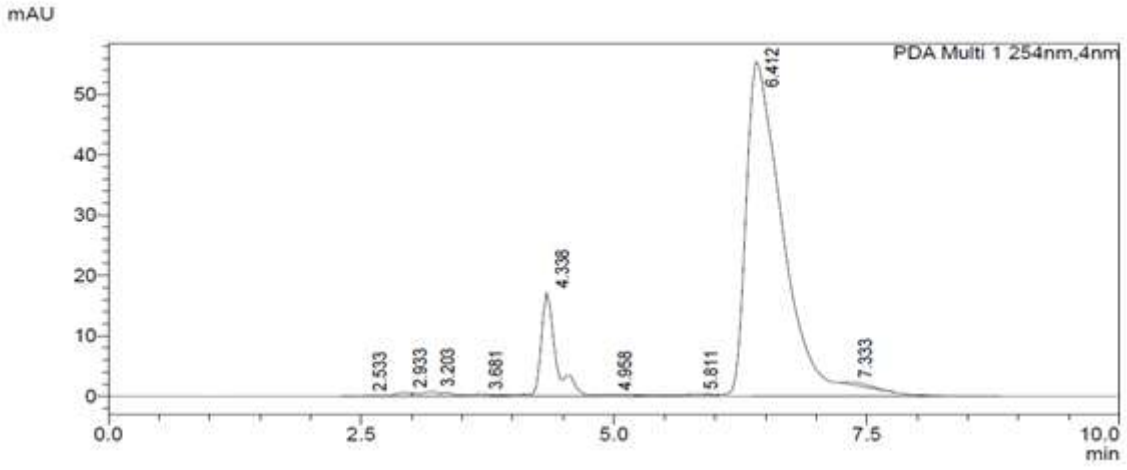

Figure2Trail2:HPLCChromatogramofSILOandMIRA(8ppmand50 ppm)

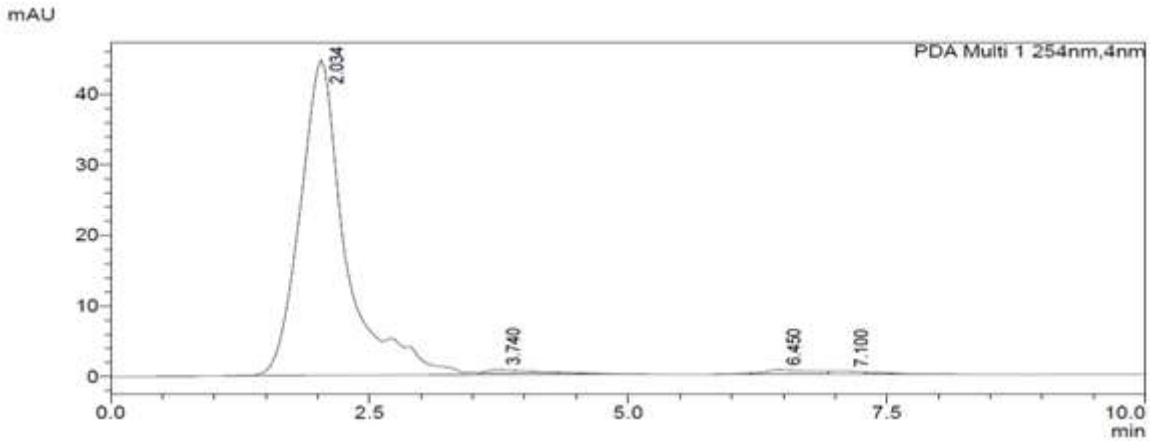

Figure 3 Trail 3 HPLC Chromatogram of SILO and MIRA (8 ppm and 50 ppm)

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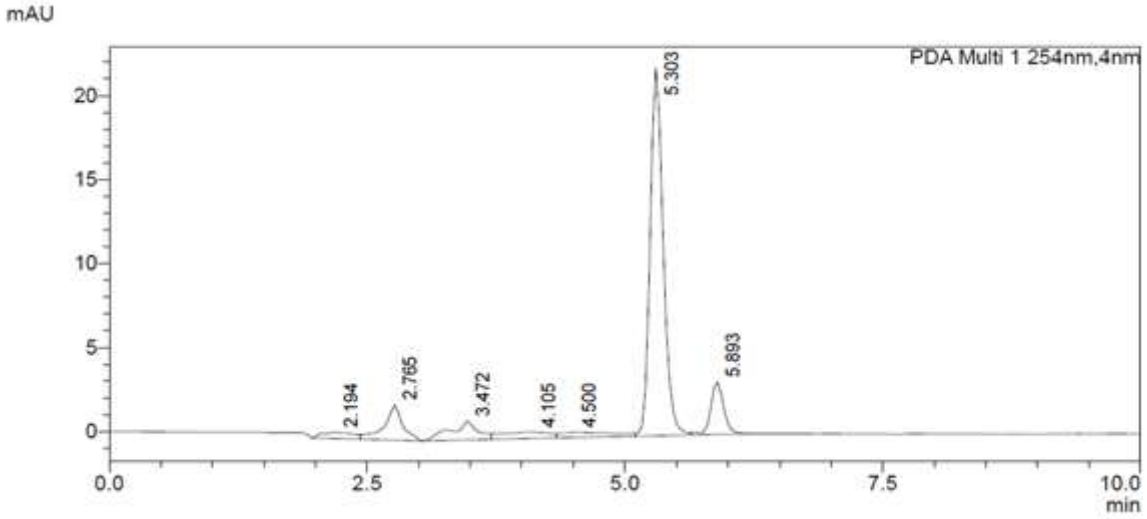

Figure 4 Trail 4: HPLC Chromatogram of SILO and MIRA (8 ppm and 50 ppm)

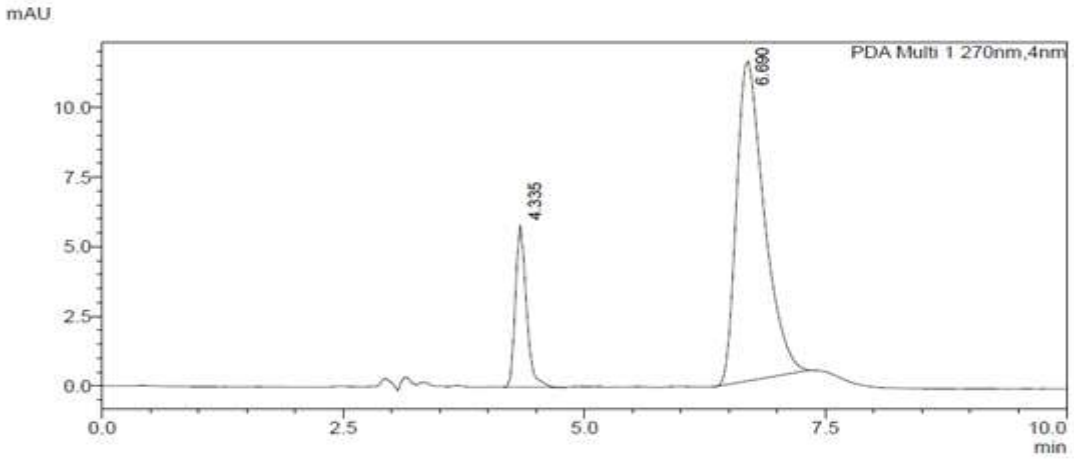

Figure 5 Trail 5: HPLC Chromatogram of SILO and MIRA (8 ppm and 50 ppm)

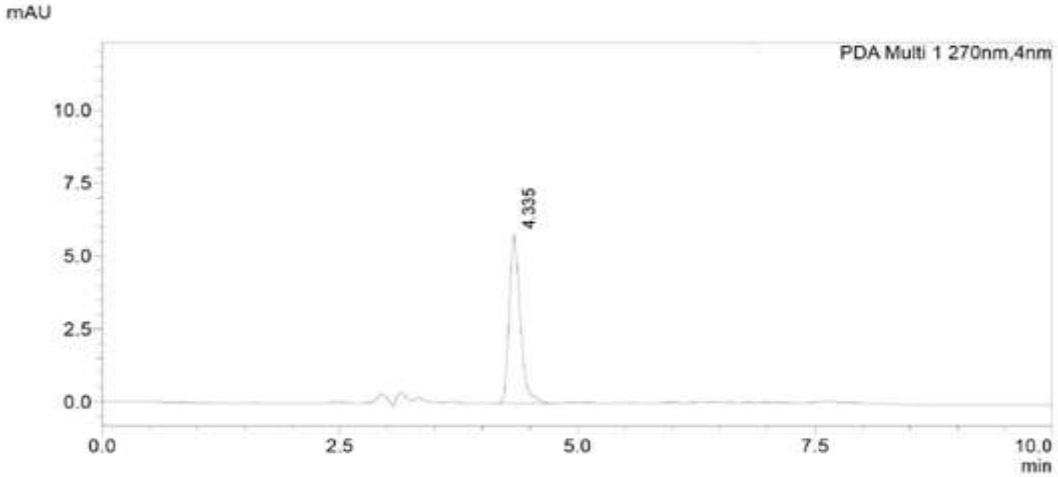

Figure 6 Trail 6: HPLC Chromatogram of SILO and MIRA (4 ppm and 25 ppm) Mobile Phase: Acetonitrile: Methanol: water in the volume ratio 45:45:10

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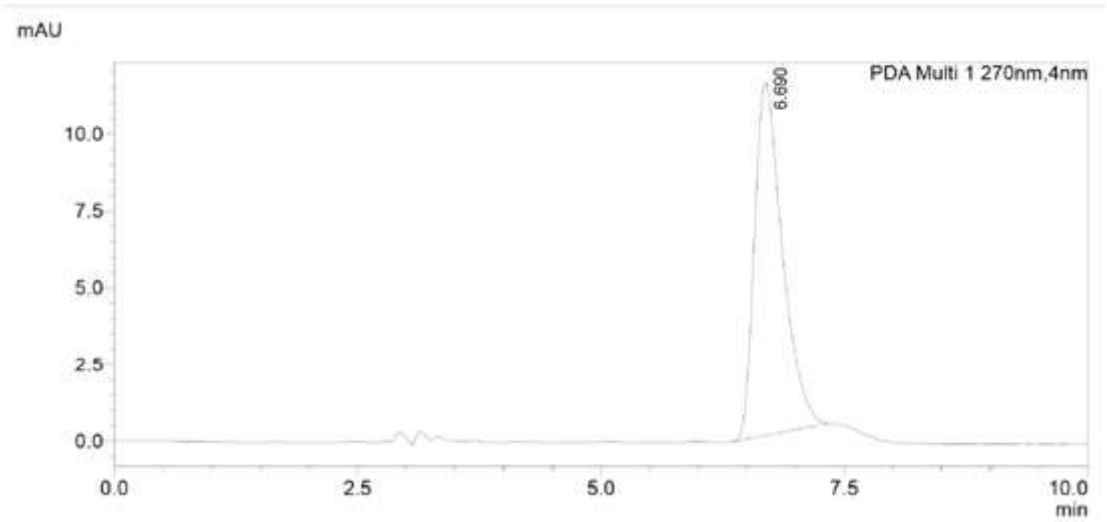

Figure 7 HPLC Chromatogram of SILO (4 ppm) Mobile Phase: Acetonitrile: Methanol: water in the volume ratio 45:45:10 v/v/v

Figure 8 HPLC Chromatogram of MIRA (25 ppm) Mobile Phase: Acetonitrile: Methanol: water in the volume ratio 45:45:10 v/v/v

Table1: Method Development Trial

Trial No.	Condition	Observation
1	Column: C 18 (250 mm x 4.6 mm),5 µm MobilePhase:Acetonitrile:waterinthevolume ratio 50:50 v/v Flow Rate: 1 ml/min Wavelength:270nm InjectionVolume:10µl	Peak splittingand only one peak observed
2	Column:C 18 (250mm x4.6mm),5 µm Mobile Phase: Methanol: water in the volume ratio 50:50 v/v Flow Rate: 1 ml/min Wavelength: 270 nm Injection Volume: 10 µl	Peak splitting was observed and only one peak observed
3	Column: C 18 (250mm x4.6mm),5µm Mobile Phase: Acetonitrile: Methanol: water in the volume ratio 20:30:50 v/v/v Flow Rate: 1 ml/min Wavelength: 270 nm Injection Volume: 10 µl	Peak shape is not proper alsopeakbroadeningis observed and tailing is observed.
4	Column: C 18 (250mm x4.6mm),5µm Mobile Phase: Acetonitrile: Methanol: water in the volume ratio 40:40:20 v/v/v Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 10 µl	No Resolution isobserved and tailing is observed at 2nd peak.and peak shape was not proper.
5	Column: C 18 (250mm x4.6mm),5µm Mobile Phase: Acetonitrile: Methanol: water in the volume ratio 50:30:20 v/v/v Flow Rate: 1 ml/min Wavelength: 254 nm Injection Volume: 10 µl	Shorter retention time of Drug and peak tailingwas observed, resolution good
6	Column:C 18 (250mm x4.6mm),5 µm Mobile Phase: Acetonitrile: Methanol: water in the volume ratio 45:45:10 v/v/v Flow Rate: 1 ml/min Wavelength: 270 nm	Retention time of Drug is less than 10 minutes and peak shape was proper, resolution good

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	Injection Volume: 10 µl	

Optimization of RP-HPLC chromate graphic condition

Table 2 Optimization condition

Sr. No.	Chromatographicparameter	OptimizeCondition	
1	FlowRate	1 ml/min	
2	DetectionWavelength	270nm	
3	MobilePhasecomposition	Acetonitrile: Methanol: water in the volume ratio 45:45:10 v/v/v	
4	Column	C18(250mm×4.6mm×5µm)	
5	InjectionVolume	10µl	
6	Retentiontime(min)	Silodosin(SILO)	Mirabegron(MIRA)
		4.335min	6.690min

SYSTEM SUITABILITY TEST:-

The system suitability parameters were calculated and all system suitability parameter are within the acceptable range.

Table 3 system suitability test

Parameter	Silodosin(SILO)	Mirabegron(MIRA)
RetentionTime(min)	4.335min	6.690min
Resolution	0.00	5.728
Theoreticalplate	34273	14162
SymmetricFactor	1.31	1.49

METHOD VALIDATION:

Linearity:

Linear responses were analyzing 6 independent level of calibration curve in the range of 4–32ppm (4,8, 16, 24, 32) for SILO and 25–200ppm (25, 50, 100, 150, 200)for MIRA. The data for linearity has shown in table 6.8for Silodos in (SILO)and Mirabegron (MIRA). The calibration curve for Silodosin (SILO) and Mirabegron (MIRA) was given in fig.

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Table 4Linarity

Silodosin(SILO)			Mirabegron(MIRA)		
Conc.	**PeakArea**	**RSD**	**Conc.**	**PeakArea**	**RSD**
4	46848.00	1.29	25	235469	0.49
8	93314.03	1.52	50	505045	1.00
16	186322	0.78	100	934412	0.77
24	284922	1.37	150	1457633	0.49
32	366312	0.71	200	1908754	1.16

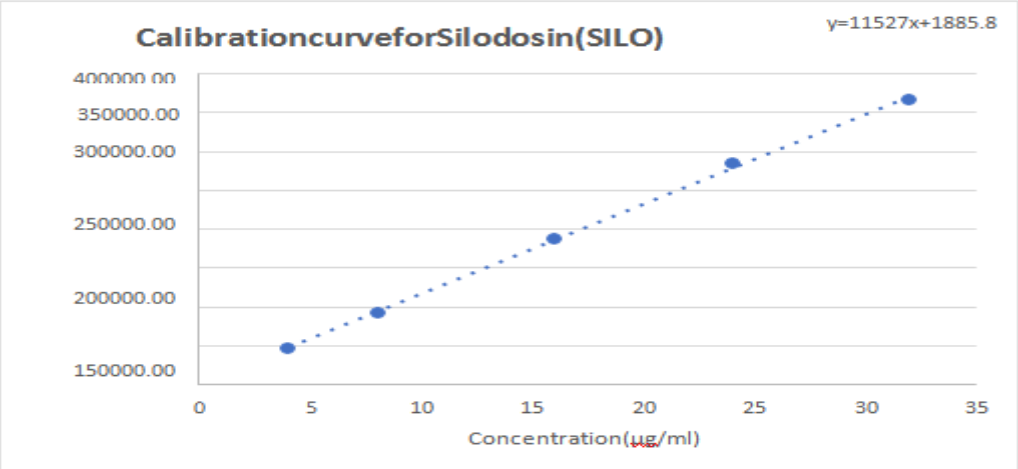
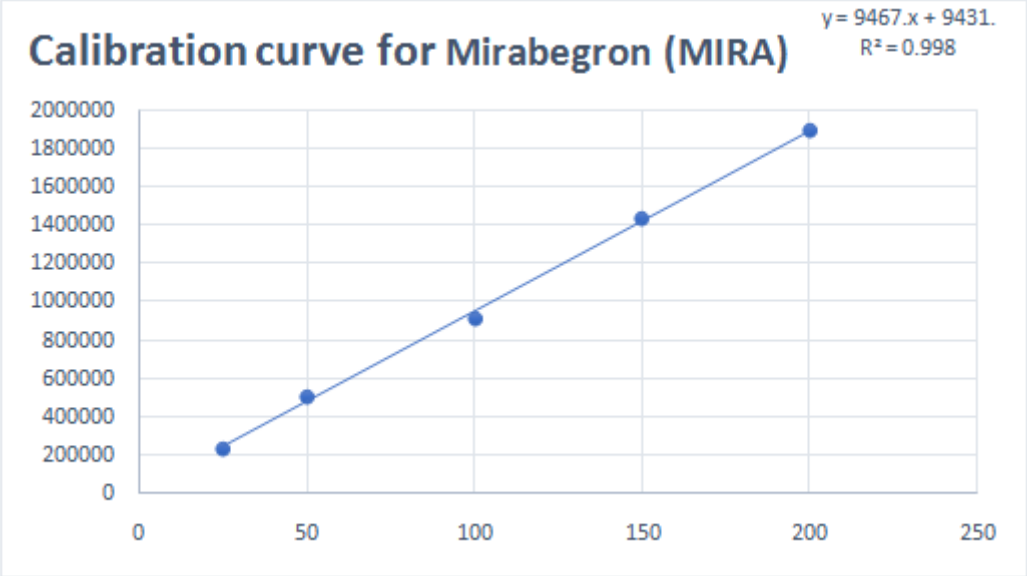


Figure 9 Calibration Curve of 4–32ppm (4,8,16,24,32) for SILO



Figur 10 Calibration Curve of 25–200ppm (25, 50, 100, 150, 200) for MIRA

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Accuracy:

Accuracy of method was carried out at three levels (50 %, 100 % and 150 %). %Recovery for Silodosin (SILO) was found to be in range of 97.23 – 102.60 %, while for and Mirabegron (MIRA) it was found to be in range of 97.10 - 102.86 % are shown in Tables

Table 5 accuracy

Leve 1 (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	% Recovery
Silodosin(SILO)						
0	8	0	8	93689.00	7.96	99.55
50	8	4	15	137747.0 0	11.79	98.22
100	8	8	16	186411.6 7	16.01	100.05
150	8	12	20	235747.0 0	20.29	101.44
Mirabegron(MIRA)						
0	50	0	50	489793.2 2	50.74	101.47
50	50	25	75	722201.6 7	75.28	100.38

Precision:

For precision RSD was found to be less than 2 revels that the proposed method is acceptable shown in Table

Table 6 Data of Silodosin (SILO) and Mira begron(MIRA)

Silodosin(SILO)			**Mirabegron(MIRA)**		
Sr. No	Conc. (µg/ml)	Area	Sr. No	Conc. (µg/ml)	Area
1	16	185172	1	100	944412
2	16	188781	2	100	917545
3	16	185735	3	100	937545
4	16	184725	4	100	922515
5	16	185775	5	100	925351
6	16	186745	6	100	914510
Average	186156		Average	926980	

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SD	1454.37	SD	11684.21
% RSD	0.78	RSD	1.26

Table 7 Intraday and Interday precision of method

Silodosin(SILO)				
Conc.	**Intradayprecision**		**Interdayprecision**	
	PeakArea (Mean±SD)^n	%RSD	PeakArea (Mean±SD)^n	%RSD
4	46657.33±333.75	0.72	47272.00±816.83	1.73
16	186896.00±1809.88	0.97	185748.33±1010.26	0.54
32	366353.00±2123.74	0.58	366270.33±3511.88	0.96
Mirabegron(MIRA)				
25	236898.67±1332.12	0.56	236058.00±1039.13	0.44
100	926500.67±8481.86	0.92	920792.00±5622.13	0.61
200	1900247.00±17449.98	0.92	1913486.33±31179.26	1.63

LOD and LOQ

LOD & LOQ of Silodosin (SILO) and Mirabegron (MIRA) were determined by equation according to ICH guideline calculation of these was given inTable

Table 8 LOD and LOQ

Drug	**Silodosin(SILO)**	**Mirabegron(MIRA)**
Limit of detection(LOD)	0.26µg/ml	0.40µg/ml
Limit of quantification(LOQ)	0.79µg/ml	1.22µg/ml

Robustness:

Robustness study for Silodosin (SILO) and Mirabegron(MIRA)

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Table 9 Robustness

1	Changeinthe Flow Rate	Silodosin (SILO)	0.9 ml/min	93668.33±1477.56	1.58
			1.1 ml/min	91671.00±512.56	1.21
		Mirabegron (MIRA)	0.9 ml/min	522663.67± 8270.57	1.58
			1.1 ml/min	496012.67± 3273.50	0.66
2	Change in wavelength	Silodosin (SILO)	240nm	92672.33±485.04	0.52
			248nm	93964.67±1138.77	1.21
		Mirabegron (MIRA)	240nm	512348.67± 7513.06	1.47
			248nm	519179.67± 6836.21	1.32
			35,25,40	93770.67±1588.52	1.69
3	Changeinmobile phase ratio	Silodosin (SILO)	35,30,35	93692.33±1503.19	1.60
		Mirabegron (MIRA)	35,25,40	506017.33± 3273.84	0.65
			35,30,35	509017.67± 3959.67	0.78

ASSAY OF SYNTHETIC MIXTURE:
Synthetic mixture of Silodosin (SILO) and Mirabegron (MIRA) containing 8mg and 50mg when analysed using the developed method, showed 100.80% assay for Silodosin (SILO) and 98.96 % assayfor Mirabegron (MIRA). Chromatogram was given in fig no.6.25 and % assay wasgiven in Table,

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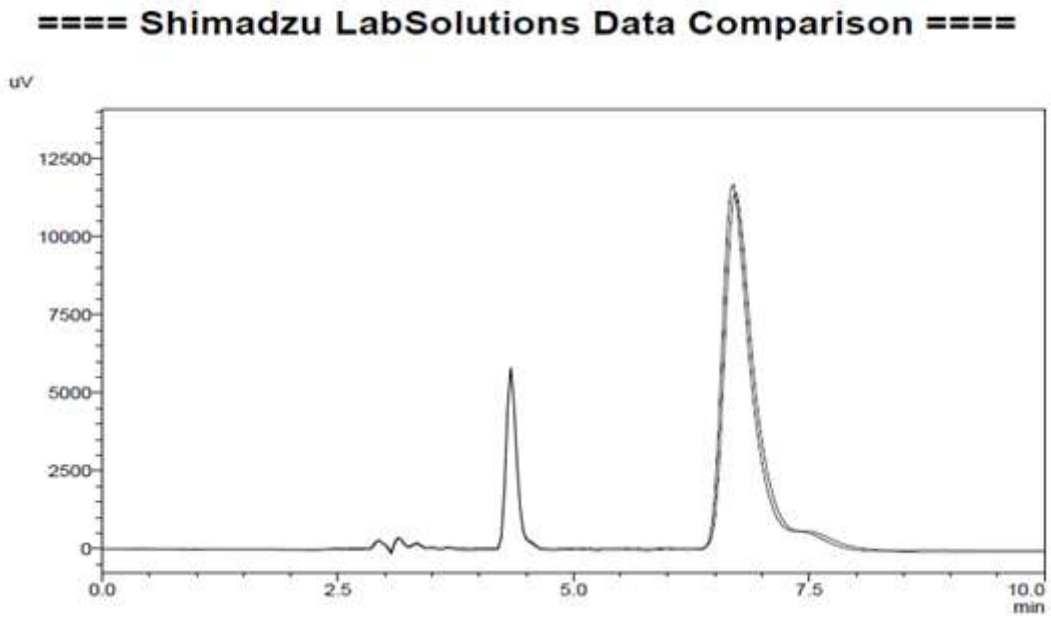

Figure 11

Analysis of Synthetic mixture

Table 10 analysis synthetic mixture

Drugs	Conc.	%Assay
Silodosin(SILO)	8mg	100.80±1.75
Mirabegron(MIRA)	50mg	98.96±0.54

II. CONCLUSION

In the present study, a specific, precise, accurate and robust first derivative UV spectroscopic method for analysis of Silodosin (SILO) and Mirabegron (MIRA) was developed and validated according to ICH guidelines. The zero-crossing point of Mirabegron (MIRA) and Silodosin (SILO) in their first derivative spectra was selected as wavelength for development of method. Theywerefoundto be248 nm and 264.40 nm Zero crossing point of Mirabegron (MIRA) and Silodosin (SILO).

In the present study, a specific, precise, accurate and robust high-performance liquid chroma to graphic method for analys is of Silodosin (SILO) and Mirabegron (MIRA) was developed and validated according to ICH guidelines. The HPLC column C185µm, 250 × 4.6 mm, was used for developed method and mobile phase Acetonitrile: Methanol: water in the volume ratio 45:45:10 v/v/v and flow rate 1ml/min. Detection Wavelength is 270nm. Retention time of Silodosin (SILO) 4.335 min and Mirabegron (MIRA) 6.690 min were obtained.

Developed method was validated as per the ICH Q2R1 guideline. Summary of validation parameter is shown in Table.

Table 11

Sr. No.	Parameter	Silodosin(SILO)	Mirabegron(MIRA)
1	Linearity	4-32µg/ml	25-100µg/ml
2	Correlation coefficient	0.9991	0.9988

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3	Accuracy(% Recovery)	98.22 %-101.44 %	98.13 %-102.48 %
4	Repeatability	%RSD found shouldbeless than 2	
5	Intradayprecision(RSD)	%RSD found shouldbeless than 2	
6	Interday precision(RSD)	%RSD found shouldbeless than 2	
7	Robustness	%RSD found shouldbeless than 2	
8	LOD(µg/ml)	0.26 µg/ml	0.40 µg/ml
9	LOQ(µg/ml)	0.79 µg/ml	1.22 µg/ml

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