IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Analytical Method Development And Validation For Simultaneous Quantification Of Tamsulosin HCL And Fexofenadine In Synthetic Mixture By HPLC"

Mr. Aniket Rajesing Girase*, Dr. Javesh Kashinath Patil,
P.S.G.V.P Mandal's College of Pharmacy Shahada (Maharashtra)

Corresponding Author:
Aniket Rajesing Girase* [1]
ORCID: https://orcid.org/0009-0002-6112-8457
Affiliation : Department of Pharmaceutical Quality Assurance P.S.G.V.P.M's College Of Pharmacy Shahada, Dist. Nandurbar, 425409

Co-Author :
Dr. Javesh Kashinath Patil [2]
Affiliation : Associate Professor Department of Pharmaceutical Quality Assurance P.S.G.V.P.M's College Of Pharmacy ,Shahada, Dist. Nandurbar, 425409

Abstract

This research focuses on creating and validating a HPLC method to concurrently measure Tamsulosin HCL and Fexofenadine in pharmaceutical products. The method development involved optimizing chromatographic conditions using a Symmetry C18 column and a mobile phase composed of Buffer, Methanol, and Acetonitrile (BMA, 50:10:40, pH 5 adjusted with OPA). These conditions resulted in well-defined peaks with satisfactory separation and symmetry for both substances. Validation of the method included assessing linearity over a concentration range of 4+1800 to 12+5400µg/ml for each compound, demonstrating strong correlation coefficients (R^2 > 0.999). Specificity testing confirmed that there were no interferences from excipients, ensuring precise quantification of the drugs. Accuracy studies showed recovery rates between 95-103%, indicating the method's reliability in determining drug concentrations. Precision studies revealed low %RSD values (<2%) for both intra-day and inter-day variability, indicating excellent repeatability and reproducibility. The method's sensitivity was assessed with LODs of 0.36µg/ml for Tamsulosin HCL and 163.75µg/ml for Fexofenadine, and LOQs of 1.08µg/ml and 496.20µg/ml, respectively. The developed RP-HPLC method was successfully applied to analyze a synthetic mixture containing Tamsulosin HCL and Fexofenadine, confirming its suitability for routine quality control

analysis in pharmaceutical formulations. Overall, this method provides a reliable approach for accurate and precise quantification of Tamsulosin HCL and Fexofenadine in complex matrices.

Keywords: RP-HPLC, Tamsulosin HCL, Fexofenadine, Method development, Method validation

Introduction

Analytical method development refers to the process of creating and optimizing procedures for analyzing samples to obtain accurate and reliable results. This process is crucial in various fields such as pharmaceuticals, environmental monitoring, food safety, and materials science. Analytical method development is the process of designing and optimizing an analytical method for the quantitative or qualitative determination of an analyte in a sample. The goal of analytical method development is to produce a method that is accurate, precise, reproducible, and robust.[1]

Key Steps in Analytical Method Development

a. **Defining Objectives**: Clearly establish the purpose and scope of the analysis.
b. **Selecting Analytical Techniques**: Choose appropriate techniques based on sample characteristics, analyte properties, and required sensitivity.
c. **Optimizing Parameters**: Fine-tune parameters such as pH, temperature, solvent composition, and detection wavelength to maximize sensitivity, selectivity, and precision.
d. **Validation and Verification**: Validate the developed method to ensure its reliability, accuracy, and reproducibility.
e. **Documentation**: Thoroughly document the method development process, including all experimental details and validation results.[2]

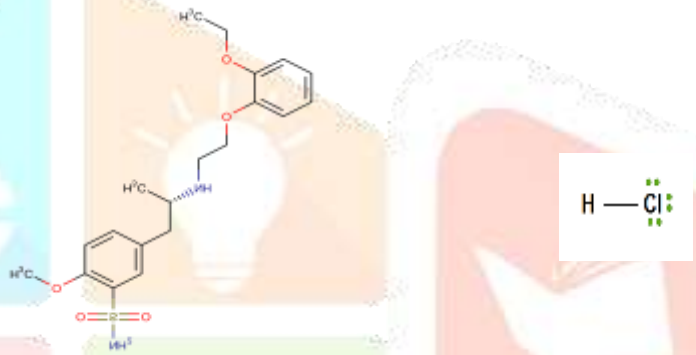
Factors Influencing Method Development

a. **Sample Matrix**: Characteristics of the sample matrix (e.g., complexity, interference) can affect method performance.
b. **Analyte Properties**: Physical and chemical properties of the analyte (e.g., polarity, stability) influence method selection and optimization.
c. **Instrumentation**: Availability and capabilities of analytical instruments impact method development choices.
d. **Regulatory Requirements**: Compliance with regulatory standards (e.g., FDA, EPA guidelines) may dictate method validation criteria.[3]

Tamsulosin HCL

Tamsulosin HCL, marketed under the brand names Flomax and Jalyn, is an alpha-1A and alpha-1B adrenergic receptor antagonist primarily indicated for the treatment of benign prostatic hyperplasia (BPH). Approved by the FDA in 1997, its chemical structure features a molecular weight of approximately 408.512 and a chemical formula of C20H28N2O5S, reflecting its selective action on prostate tissue.[4] Tamsulosin exerts its therapeutic effect by specifically blocking alpha-1A and alpha-1D adrenergic receptors, which are prevalent in the prostate gland. This targeted blockade results in the relaxation of prostate muscles, alleviating urinary symptoms associated with BPH such as urgency and hesitancy, while minimizing adverse effects like orthostatic hypotension. By focusing its action on these specific receptor subtypes, tamsulosin ensures localized treatment within the genitourinary tract, distinguishing it from broader alpha-blockers. [5]

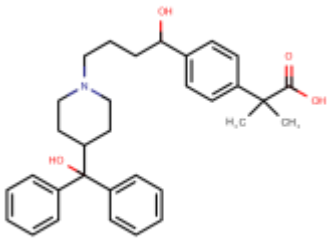
Fig No 1: Structure of Tamsulosin HCL



Fexofenadine

Fexofenadine belongs to the antihistamine category and is widely recognized under brand names such as Allegra and Wal-fex. Approved by the FDA in 2011, it possesses a chemical structure characterized by a molecular weight of about 501.6564 and a chemical formula of C32H39NO4.[6] Fexofenadine effectively manages allergy symptoms by antagonizing H1-histamine receptors, thereby preventing the release of inflammatory mediators responsible for allergic reactions. This mechanism involves stabilizing the inactive form of the H1 receptor, thereby impeding histamine binding and subsequent inflammatory responses. Unlike older antihistamines, fexofenadine offers the advantage of extended duration of action, up to 24 hours, facilitating convenient once-daily dosing and providing rapid relief from symptoms such as itching, sneezing, and runny nose. [7]

Fig No 2: Structure of Fexofenadine



These medications exemplify targeted pharmacological approaches tamsulosin through selective receptor blockade in prostate tissue and fexofenadine through specific antagonism of histamine receptors to effectively address distinct clinical conditions. Understanding their mechanisms of action and pharmacokinetic profiles is crucial for optimizing therapeutic outcomes and ensuring patient safety in clinical practice.

Experimental Work:

Working Standard & API: Standard Sample of Tamsulosin Hcl, 0.4Mg Capsule was Mfg by Cipla Pvt. Ltd. and fexofenadine 180 mg Oral tablet was Mfg. by Healing Pharma Pvt. Ltd.

Selection of mobile phase

A different solvent like acetonitrile, Methanol, Water is used based on the trial-and-error basis with the different ratios for the optimization of the mobile phase.

The final mobile phase was BMA (Buffer, Methanol, Acetonitrile) 50:10:40 % V/V pH-5 was adjusted by OPA. Potassium dihydrogen phosphate was used as a buffer.

Preparation of Solution

A. **Preparation of Standard stock solution of Tamsulosin HCL:** Dissolve 1 capsule containing 0.4 mg of tamsulosin HCl in a 10 ml flask. Add approximately 70% methanol and use ultrasound to aid dissolution. Top up the flask with more methanol to reach the mark, mix thoroughly, and filter through a 0.45µm Nylon Filter. The resulting solution will have a concentration of 40µg/ml.
B. **Preparation of Standard stock solution of Fexofenadine:** Crush a 180 mg fexofenadine tablet and put the powder into a 10 ml flask. Add approximately 70% methanol and use ultrasound to aid in dissolving the powder. Top up the flask with more methanol to reach the mark, mix well, filter the solution through a 0.45µm Nylon Filter, and you will achieve a solution with a concentration of 18,000 µg/ml.
C. **Preparation of standard solution of mixture of Tamsulosin HCL and Fexofenadine:** Take a 1 ml from 18000 µg/ml (Standard stock solution of Fexofenadine) and 1 ml from 40 µg/ml (Standard stock solution of Tamsulosin HCl) into a 10 ml volumetric flask. Add about 70% of the diluent to the flask and use ultrasonic waves to aid in dissolving. Then, fill the flask to its mark with diluent and ensure thorough mixing. This process will yield a solution with a concentration of 1800+4 µg/ml.

Assay Of synthetic Mixture

To Determine the concentration of Tamsulosin HCL and Fexofenadine in synthetic Mixture 10 ppm, 4500 ppm Accurately Weight and transfer into a 100 ml Volumetric Flask and Add Common Excipients used in marketed formulation. Then add 7o ml methanol and sonicate through 0.45µm Nylon Filter. Discarding First few ml of filtrate. Dilute 1 ml of the filtrate to 10 ml with diluents and mix well and sample analysed for assay containing 10µg/ml of Tamsulosin HCL and 4500µg/ml of Fexofenadine.

RESULT AND DISCUSSION

Method Development:

(OPTIMIZED CHROMATOGRAPHIC CONDITION)

BMA 50:10:40 pH 5 OPA

The conditions were Symmetry C18 Column (25cm×4.6mm, 5µm column, 270 nm) wavelength and Buffer: Methanol: Acetonitrile, 50:10:40 mobile phase.

Observation: Tamsulosin HCL and Fexofenadine eluted peak proper.

Conclusion: Method Approved

Fig No. 3 Chromatogram of Optimized Chromatographic Condition

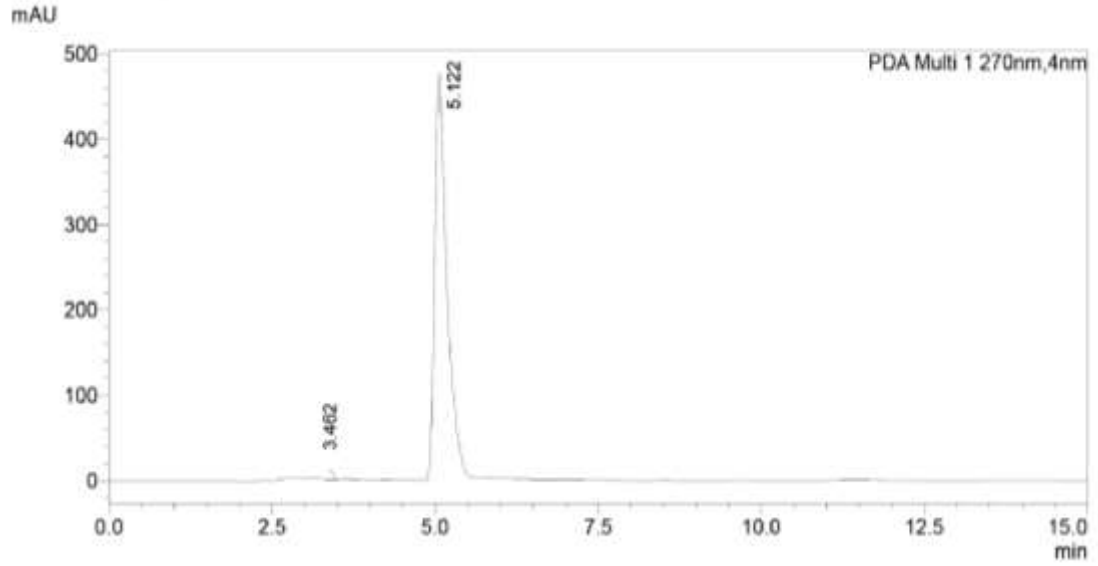


Table No 1: Optimization of HPLC chromatographic condition

Sr No.	Chromatographic Parameters	Optimized Condition
1	Column	Shimadzu Shim-pack VP-ODS C18 column (250mm × 4.6mm, 5µm)
2	Mobile phase	BMA 50:10:40 pH 5 OPA
3	Flow rate	1 ml/min
4	Temperature	25°C
5	Injection volume	10 µl
6	Detection wavelength	270 nm
7	Diluent	Methanol
8	Run time	15 min.
9	Retention time	3.462 min Tamsulosin HCL and 5.122 min Fexofenadine

System Suitability Test

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

Table No 2: System suitability parameter of optimized condition

Peak	Retention time	Area	Theoretical plate	Height	Tailing factor	Resolution (USP)
Tamsulosin HCL	3.462	117694	12212	9574	1.058	3.658
Fexofenadine	5.122	5834962	22295	467702	1.251	

Method Validation

[8-13]

Linearity

Linearity studies were performed on different working standard solution 4+1800 to 12+5400µg/ml for Tamsulosin HCL & Fexofenadine. The peak area for each concentration was recorded and then its linearity was analyzed by plotting the calibration curve.

Fig No 4: Linearity Data Comparison

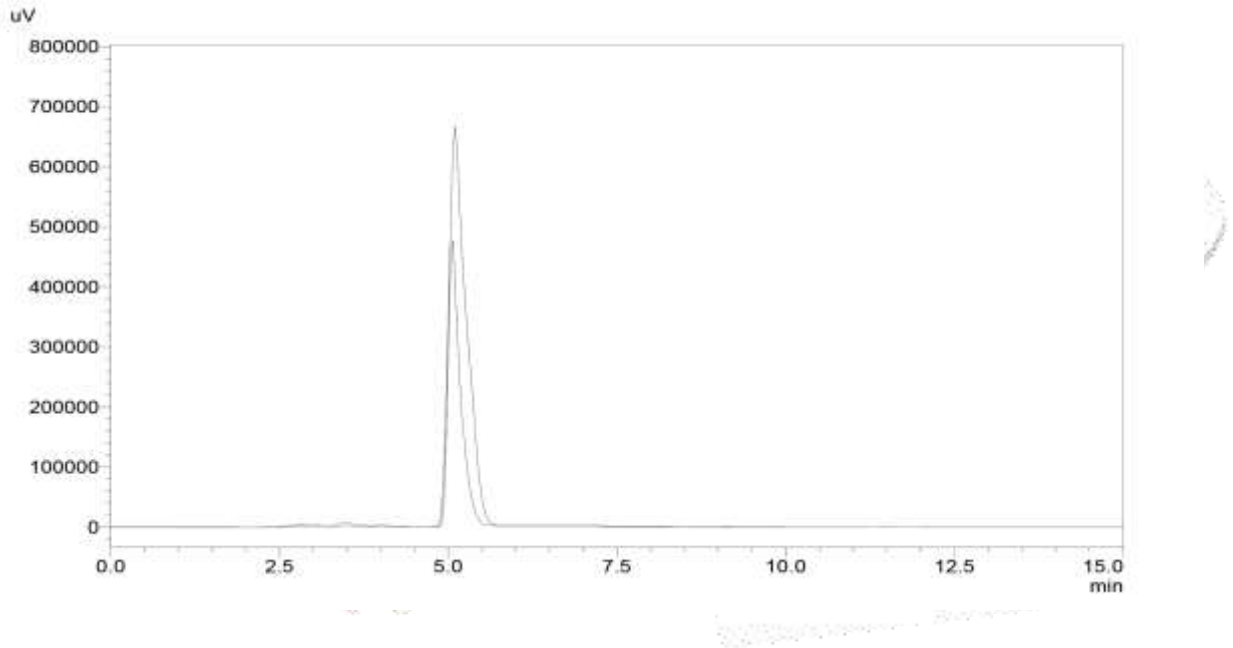
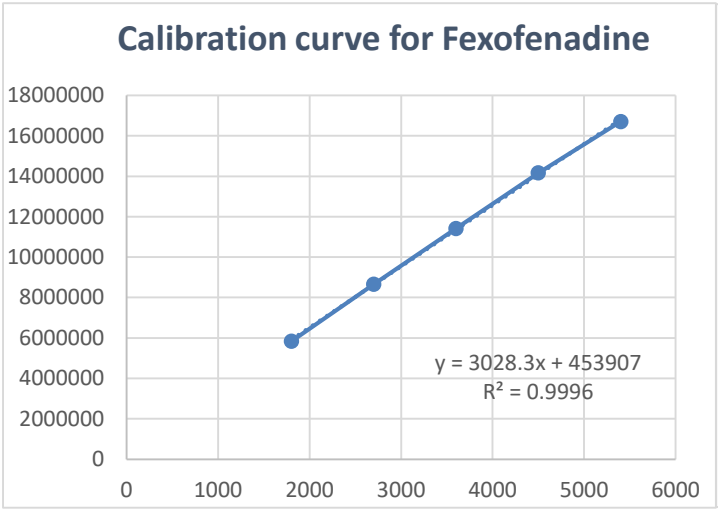
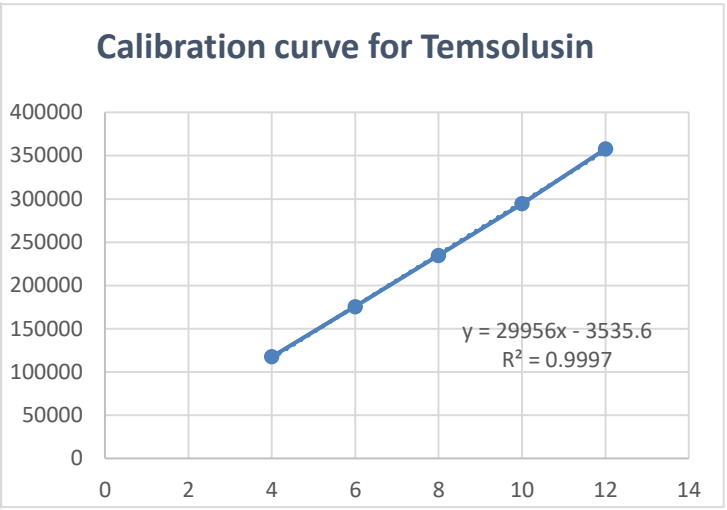


Table No 3: Linearity of Tamsulosin HCL & Fexofenadine

Tamsulosin HCL		Fexofenadine	
Conc.	**Area**	**Conc.**	**Area**
4	117694	1800	5834962
6	175642	2700	8652443
8	234878	3600	11418631
10	294540	4500	14167505
12	357804	5400	16704577





Specificity

There is no interference of excipients in the peak of Tamsulosin HCL and Fexofenadine it is shown by comparing chromatogram of blank, standard Solution.

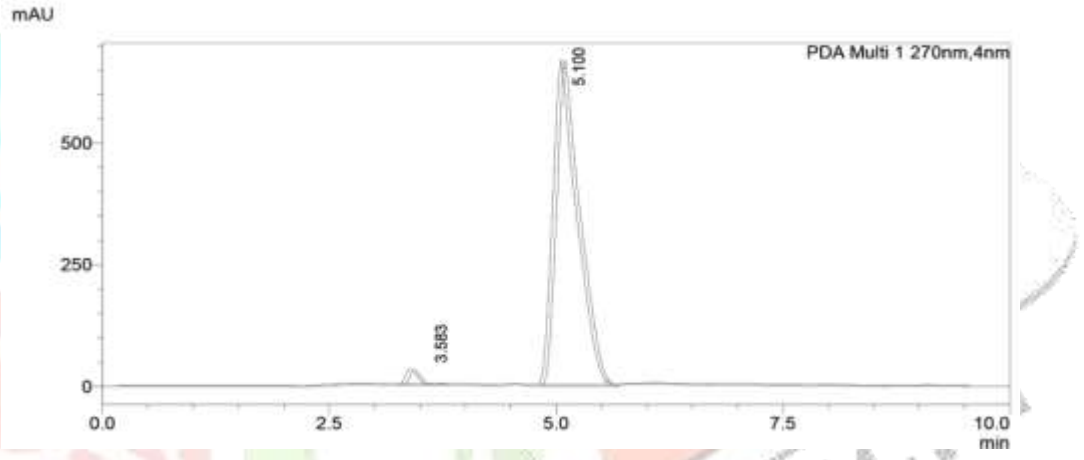


Fig No 5: Chromatogram of Drug with excipients

Accuracy

Accuracy was performed at three levels (50%,100%, and 150%). Percentage recovery for Tamsulosin HCL & Fexofenadine was found in a range of 95-103%.

Table No 4: Accuracy data for Tamsulosin HCL

Level	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	% Recovery
50%	4	2	6	175642	5.74	95.76
	4	2	6	174469	5.70	95.10
	4	2	6	175125	5.72	95.47
	4	4	8	235878	7.75	96.95

100%	4	4	8	239875	7.88	98.62
	4	4	8	233798	7.68	96.08
150%	4	6	10	297251	9.80	98.05
	4	6	10	291239	9.60	96.04
	4	6	10	296120	9.76	97.67

Table No 5: Statistical Validation of Recovery Studies for Tamsulosin HCL

Level of Recovery (%)	% Mean Recovery	Standard Deviation	% RSD
50%	95.44	0.02	0.34
100%	97.22	0.10	1.33
150%	97.25	0.11	1.10

Table No 6: Accuracy data for Fexofenadine

Level	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	% Recovery
50%	1800	900	2700	8652443	2707.30	100.27
	1800	900	2700	8665422	2711.59	100.43
	1800	900	2700	8569145	2679.79	99.25
100%	1800	1800	3600	11418631	3620.75	100.58
	1800	1800	3600	11423512	3622.36	100.62
	1800	1800	3600	11654289	3698.57	102.74
150%	1800	2700	4500	14242121	4553.12	101.18
	1800	2700	4500	14595050	4669.66	103.77
	1800	2700	4500	14398823	4604.86	102.33

Table No 7: Statistical Validation of Recovery Studies for Fexofenadine

Level of Recovery (%)	% Mean Recovery	Standard Deviation	% RSD
50%	99.98	17.25	0.64
100%	101.31	44.47	1.22
150%	102.43	58.39	1.27

Precision

For the precision study, RSD was found to be less than 2. Precision data are given in the table below.

Table No 8: Intraday and Interday Precision data for Tamsulosin HCL & Fexofenadine

Precision	Intraday precision			Interday precision	
Drugs	**Conc.**	**Mean area ± SD**	**%RSD**	**Mean area ± SD**	**%RSD**
Tamsulosin HCL	4	116148.89±1783.64	1.54	117548.67±286.16	0.24
	8	237316.58±4417.21	1.86	237033.93±4204.96	1.77
	12	357810.67±3134.01	0.88	359744.42±1696.58	0.47
Fexofenadine	1800	5806291.58±25954.34	0.45	5847363.00±15186.59	0.26
	3600	11536889.04±117831.34	1.02	14378676.13±134458.33	0.94
	5400	16520176.33±164523.60	1.00	16463787.67±213333.86	1.30

Table No 9: Repeatability study of Tamsulosin HCL & Fexofenadine

	Tamsulosin HCL			Fexofenadine	
Conc.	**Mean area ±SD**	**%RSD**	**Conc.**	**Mean area ± SD**	**%RSD**
4	116611±1354.55	1.16	1800	5839587±26620.55	0.46
6	175431±1122.06	0.64	2700	8692240±118066.82	1.36
8	236482±2069.45	0.88	3600	11469807±94610.10	0.82
10	295457±2664.59	0.90	4500	14393004±176865.41	1.23
12	355324±4715.45	1.33	5400	16358976±247406.96	1.51

LOD and LOQ

LOD was found to be 0.36 and 163.75µg/ml for Tamsulosin HCL & Fexofenadine respectively. LOQ was found to be 1.08 and 496.20µg/ml for both drugs respectively.

Robustness

Deliberate changes in different parameters like flow rate, Wavelength are applied, and the relative standard deviation of peak area was found to be less than 2.

Table 10. Robustness study of Tamsulosin HCL & Fexofenadine (Wavelength)

Drugs	Wavelength	Mean area ±SD	%RSD
Tamsulosin HCL	266	174420.24±1688.32	0.97
	270	175006.95±701.56	0.40
	274	174426.17±2435.16	1.40
Fexofenadine	266	8565612.25±88649.94	1.03
	270	8594439.62±50369.00	0.59
	274	8566139.37±145834.33	1.70

Table 11. Robustness study of Tamsulosin HCL & Fexofenadine (Flow rate)

Drugs	Flow rate	Mean area ±SD	%RSD
Tamsulosin HCL	0.9ml/min	175295.62±2896.81	1.65
	1ml/min	175006.95±701.56	0.40
	1.1ml/min	174718.04±1931.48	1.11
Fexofenadine	0.9ml/min	8634269.42±111324.83	1.29
	1ml/min	8594439.62±50369.00	0.59
	1.1ml/min	8563228.86±135893.75	1.59

Assay of synthetic mixture

A synthetic mixture containing 10mg of Tamsulosin HCl and 4500mg of Fexofenadine was analyzed by this developed method, it shows that there was no interference of excipients in chromatograph of drugs

Fig. No 6: Chromatogram Assay of synthetic mixture

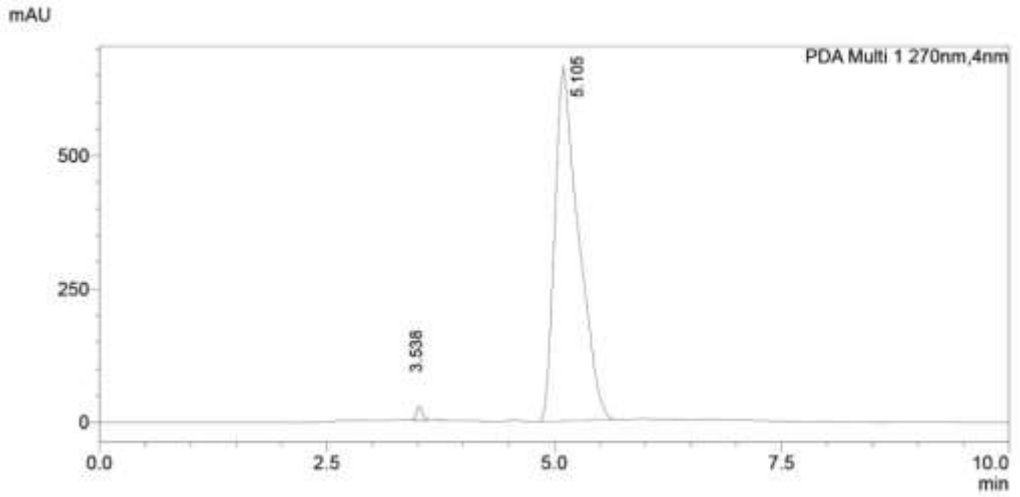


Table No 12: Assay of synthetic mixture

Drugs	Dose strength	Conc. found	%Assay
Tamsulosin HCL	10µg	9.71µg/ml	97.08%
Fexofenadine	4500µg	4583.75µg/ml	101.86%

Conclusion

This study presents a robust HPLC method that was developed and validated following ICH Q2(R1) guidelines to quantify and separate Tamsulosin HCL and Fexofenadine. The method showed excellent linearity across concentration ranges of 4-12µg/ml for Tamsulosin HCL and 1800-5400µg/ml for Fexofenadine, achieving regression values greater than 0.999. Accuracy assessments fell within acceptable ranges, with Tamsulosin HCL ranging from 95.44% to 97.25% and Fexofenadine from 99.98% to 102.43%. The method demonstrated low LOD and LOQ values, indicating high sensitivity, and precision studies confirmed consistent intraday and interday results with RSD values consistently below 2%. Robustness testing validated the method's reliability under deliberate variations in parameters such as wavelength and flow rate. Application of the method to a synthetic mixture confirmed accurate quantification without interference from excipients. In summary, this validated HPLC method provides a dependable analytical tool for ensuring quality control and conducting pharmaceutical analysis of Tamsulosin HCL and Fexofenadine in complex samples.

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