ISSN: 2347-6567

International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Vol. 14 | Issue 3| Jul- Sep- 2026

www.ijamscr.com

DOI : https://doi.org/10.61096/ijamscr.v14.iss3.2026.1185-1197

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Tafluprost and Timolol in Combined Tablet Dosage Form

G. Swapna*[1], Md Rahemath Pasha[1], Meesala Deepthipriya[1], Middela Siddu[1], Mohammed Faisal Khan[1], Mohammed Sohail[1], Dr. Kantlam[1]

[1]*Department of Pharmaceutical Analysis, Brilliant Grammar School Educational Society's Group of Institutions (Engineering And Pharmacy), Abdullapur (V), Abdullapurmet (M), Rangareddy (D), Hyderabad .*

*Corresponding Author: **G. Swapna***
Email: Swapnaguddeti88@gmail.com**.**

Published on: 04.07.2026
Published by: Futuristic Publications
2026| All rights reserved.

Creative Commons Attribution 4.0 International License.

Abstract: A simple, rapid, accurate, and precise Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Tafluprost and Timolol in combined tablet dosage form. Tafluprost is a prostaglandin F2α analogue used to reduce intraocular pressure, while Timolol is a non-selective beta-adrenergic receptor blocker widely used in the treatment of glaucoma. Accurate and reliable analytical methods are essential to ensure the quality, safety, and efficacy of such combination pharmaceutical products.

Chromatographic separation was achieved using a C18 column with a suitable mobile phase composition under optimized conditions. The analytes were detected at an appropriate wavelength where both drugs exhibited adequate absorbance. The developed method provided well-resolved peaks with satisfactory retention times, good peak symmetry, and acceptable system suitability parameters.

The method was validated according to ICH guidelines for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. The calibration curves were linear within the selected concentration range with correlation coefficients close to 1. The percentage recovery values indicated high accuracy, and low %RSD values confirmed good precision. The method proved to be robust against small deliberate variations in chromatographic conditions.

The validated RP-HPLC method was successfully applied for routine quantitative analysis of Tafluprost and Timolol in combined tablet dosage form and can be effectively used for quality control and stability studies.

Key Words: RP-HPLC, Tafluprost, Timolol, Method Development, Method Validation, Simultaneous Estimation, Combined Dosage Form, ICH Guidelines, Pharmaceutical Analysis, Quality Control.

1. INTRODUCTION

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

Method Development

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies

at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use" [1-2]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high-performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these shows unique selectivity. What is needed is a more informed decision-making process for column selection that may beused before the chromatographer enters the laboratory. The method of column selection presentedhere involves a minimal investment in time initially, with the potential of saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [3, 4]. The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization.[5] The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [6-8].

The degraded drug samples obtained are subjected to preliminary chromatographic separation to study the number and types of degradation products formed under various conditions [9]. Scouting experiments are run and then conditions are chosen for further optimization [10]. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development [11]. Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds. Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time. However, by operating at pH extremes (2-4 or 8-10), not only is there a 10–30-fold difference in retention time that can be exploited in method development but also the method can be made more robust which is a desirable outcome with validation in minutes [12,13]. Various steps for HPLC method development are given below.

Steps for HPLC method development

EXPERIMENTAL METHOD

INSTRUMENTS USED

HPLC	WATERS, Alliance 2695 separation module. Software: Empower 2,996 PDA detector.
pH meter	Lab India
Weighing machine	Sartorius
Volumetric flasks	Borosil

Pipettes and Burettes	Borosil
Beakers	Borosil
Digital ultra sonicator	Lab man

CHEMICALS USED:

Tafluprost	Saflutan
Timolol	Timoptic
Water and Methanol for HPLC	LICHROSOLV (MERCK)
Acetonitrile for HPLC	Merck
Phosphate buffer	Elab science

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Tafluprost and Timolol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.03ml of Tafluprost and 3.0ml of Timolol from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: 0.015 M phosphate buffer (50:50 v/v) respectively.

Optimization of Column:

The method was performed with various columns like Thermosil RPC18 column (4.5 x 100 mm, 5.0 µm). was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	35ºC
Column	:	Thermosil RPC18 (4.5 x 100 mm, 5.0 µm)
Mobile phase	:	Acetonitrile: 0.015 M phosphate buffer (50:50 v/v)
Flow rate	:	1ml/min
Wavelength	:	220nm
Injection volume	:	10 µl
Run time	:	8 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Phosphate buffer pH 3.9:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.9.

Preparation of mobile phase:

Accurately measured 500 ml (50%) of Acetonitrile and 500ml of 0.015 M phosphate buffer were mixed and degassed in digital ultrasonicated for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Chromatogram (Standard)

Mobile phase : Acetonitrile: 0.015 M phosphate buffer (50:50 v/v, pH 3.5)

Column : Thermosil RPC18 (4.5 x 100 nm, 5.0 µm)

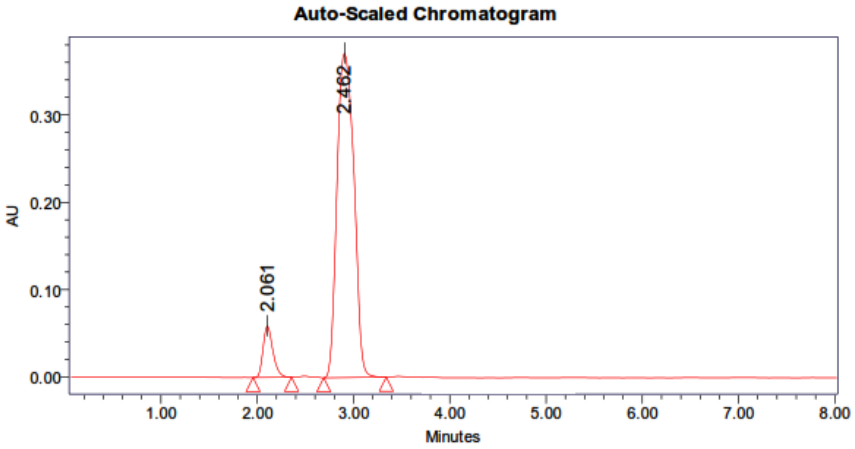
Flow rate : 1 ml/min

Wavelength : 220 nm

Column temp : 35ºC

Injection Volume : 10 µl

Run time : 8minutes



Optimized Chromatogram

Table: peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Timolol	2.061	247392	58952	1.2	7243
2	Tafluprost	2.462	3530866	371748	1.1	3389

Observation: From the above chromatogram it was observed that the Timolol and Tafluprost peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

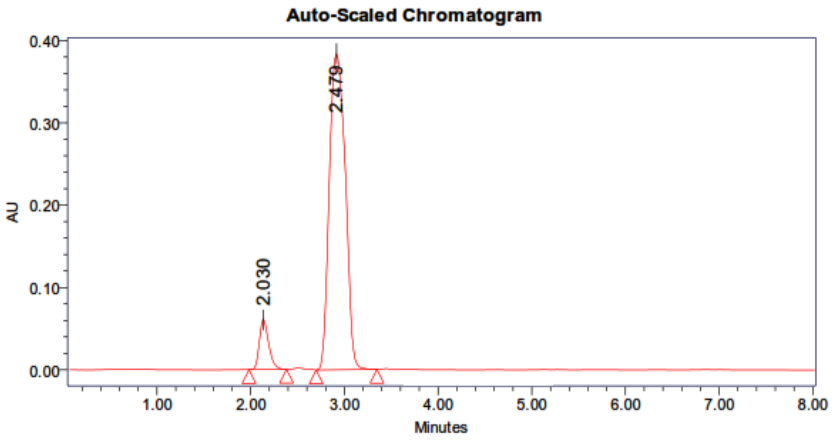


Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Timolol	2.030	240019	60878	1.2	7246
2	Tafluprost	2.479	3544380	384304	1.1	3375

Acceptance criteria:

- 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.

VALIDATION

Blank:

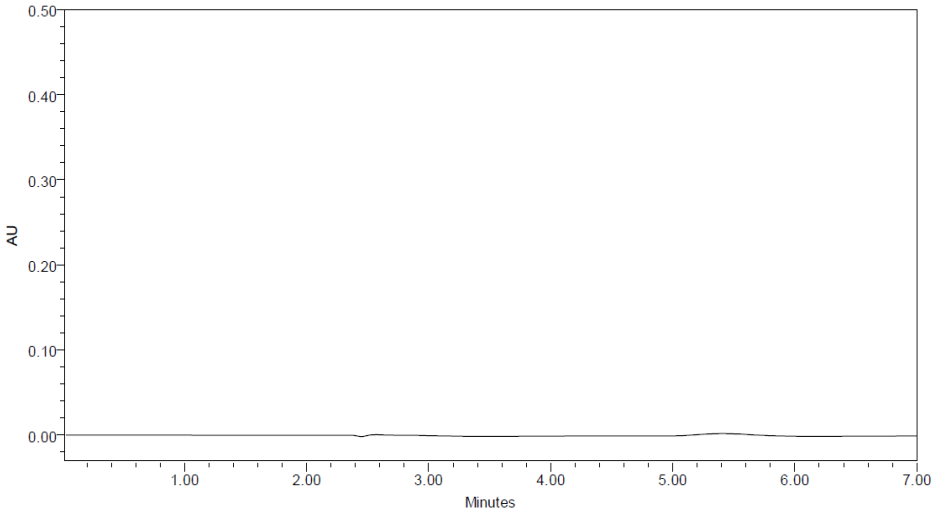


Fig: Chromatogram showing blank (mobile phase preparation)

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Timolol and Tafluprost in drug product.

Assay (Standard):

Table: Results of system suitability for Timolol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Timolol	2.048	246713	73455	11318	1.1
2	Timolol	2.074	245617	78152	7105	1.2
3	Timolol	2.071	245830	78146	8974	1.2
4	Timolol	2.069	240552	78242	7087	1.2
5	Timolol	2.070	245725	77705	5124	1.2
Mean			244887.4			

Std. Dev			2462.26			
% RSD			1.005466			

Acceptance criteria:

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

Table: Results of system suitability for Tafluprost

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tafluprost	2.446	3363754	636862	8484	1.1
2	Tafluprost	2.490	3326434	641486	7889	1.0
3	Tafluprost	2.489	3345949	638081	7846	0.9
4	Tafluprost	2.488	3336621	617725	6772	0.9
5	Tafluprost	2.490	3355244	631710	6884	0.9
Mean			3345600			
Std. Dev			14753.43			
% RSD			0.44098			

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.

Assay (Sample):

Table: Peak results for Assay sample

S. No	Name	Rt	Area	Height	USP Tailing	USP plate count
1	Timolol	2.068	244102	89282	1.2	5949
2	Tafluprost	2.489	3357566	576562	1.0	6866
3	Timolol	2.070	240052	88021	1.2	5861
4	Tafluprost	2.491	3371663	576999	1.0	6808
5	Timolol	2.067	243230	88882	1.2	5879
6	Tafluprost	2.489	3364001	570315	1.0	6823

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Timolol and Tafluprost in pharmaceutical dosage form was found to be 100.2 %.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Timolol:

Concentration µg/ml	Average Peak Area
1	88442
2	165724
3	242754
4	315906
5	396371

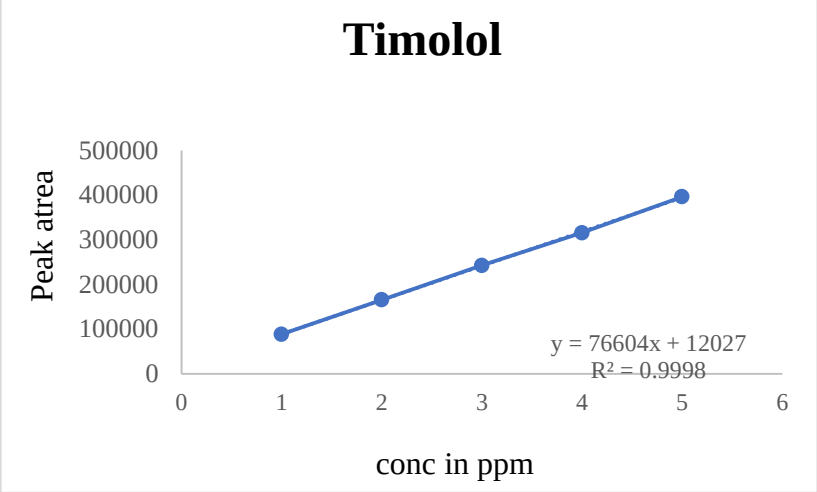


Fig 6.3.4: calibration graph for Timolol

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Timolol is a straight line.

Y = mx + c

Slope (m) = 76604

Intercept (c) = 12027

Correlation Coefficient (r) = 0.9998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.9998, and the intercept is 12027. These values meet the validation criteria.

Tafluprost

Concentration µg/ml	Average Peak Area
100	1131032
200	2245302
300	3355282
400	4429382
500	5623754

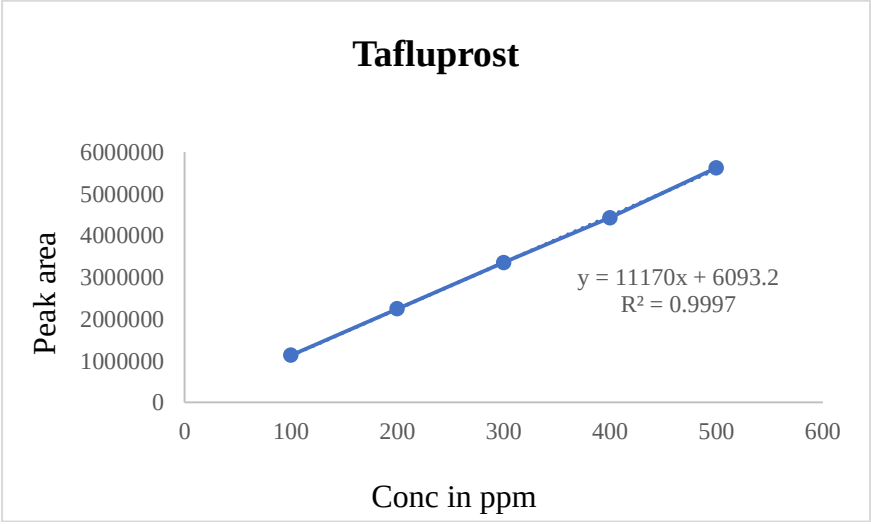


Fig 6.3.4: calibration graph for Tafluprost

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Tafluprost is a straight line.

Y = mx + c

Slope (m) = 11170

Intercept (c) = 6093.2

Correlation Coefficient (r) = 0.9997

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.9997 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.9997, and the intercept is 6093. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table: Results of repeatability for Timolol:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Timolol	2.065	249684	12079	5343	1.0
2	Timolol	2.064	249696	12068	5473	1.2
3	Timolol	2.064	246325	11949	5473	1.1
4	Timolol	2.065	249816	11811	5389	1.1
5	Timolol	2.067	249892	11735	5180	1.0
Mean			249082.6			
Std. Dev			1543.964			
% RSD			0.61986			

Acceptance criteria:

- %RSD for sample should be NMT 2

Table: Results of method precession for Tafluprost:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tafluprost	2.486	3233700	59095	6654	1.2
2	Tafluprost	2.484	3241323	57552	6524	1.3
3	Tafluprost	2.482	3245927	57213	6440	1.3
4	Tafluprost	2.483	3245927	57096	6411	1.4
5	Tafluprost	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Acceptance criteria:

- %RSD for sample should be NMT 2

Intermediate precision:

Day 1:

Table: Results of Intermediate precision for Timolol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Timolol	2.066	242721	11323	5272	1.21
2	Timolol	2.066	240155	11564	5168	1.16
3	Timolol	2.066	240945	11887	5310	1.14
4	Timolol	2.065	240385	11938	5275	1.19
5	Timolol	2.069	249920	11652	5078	1.10
6	Timolol	2.067	240820	11750	5225	1.17
Mean			243991			
Std. Dev			4641.97			
% RSD			1.5			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision for Tafluprost

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tafluprost	2.477	3325309	54143	6149	1.25
2	Tafluprost	2.478	3323780	53740	6127	1.21
3	Tafluprost	2.483	3328190	54791	6607	1.28
4	Tafluprost	2.486	3329035	55098	6769	1.28
5	Tafluprost	2.489	3325968	52379	6709	1.30
6	Tafluprost	2.483	3327725	54779	6756	1.36
Mean			3326668			
Std. Dev			1985.641			
% RSD			0.059689			

Acceptance criteria:

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

Day 2:

Table: Results of Intermediate precision Day 2 for Timolol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Timolol	2.067	249499	11594	5240	1.2
2	Timolol	2.069	240991	11357	5130	1.2
3	Timolol	2.068	240431	11878	5136	1.2
4	Timolol	2.069	241330	11748	5267	1.2
5	Timolol	2.067	240519	11830	5222	1.2
6	Timolol	2.067	240470	11475	5982	1.2
Mean			242206.7			
Std. Dev			3590.034			
% RSD			1.48222			

Acceptance criteria:

- % RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision for Tafluprost

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tafluprost	2.485	3426979	53353	6700	1.3
2	Tafluprost	2.484	3446641	54454	6563	1.3
3	Tafluprost	2.496	3430606	53532	6855	1.3
4	Tafluprost	2.484	3430952	55157	6864	1.3
5	Tafluprost	2.490	3431676	56223	6942	1.3
6	Tafluprost	2.490	3429187	58578	6644	1.3
Mean			3433812			
Std. Dev			7041.409			
% RSD			0.205061			

Acceptance criteria:

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

6.3.4: ACCURACY:

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

The accuracy results for Timolol

% Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124675.7	15	15.1	101%	100.4%
100%	242006.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

The accuracy results for Tafluprost

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696259	18.75	18.71	99.8%	99.2%
100%	3351661	37.5	37.2	99.4%	
150%	4975094	56.25	55.47	98.6%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Timolol:

=3.3 × 12027/76604
=0.51µg/ml

Tafluprost:

=3.3 × 6093.2/11170
=1.80µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ=10\times\sigma/S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Timolol:

=10×12027/76604
= 1.57µg/ml

Tafluprost:

=10 × 6093.2/11170
= 5.45µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Timolol and Tafluprost. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Timolol and Tafluprost 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

TABLE: RESULTS FOR ROBUSTNESS

TIMOLOL:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
Less organic phase	445628	2.165	4709	1.2
More organic phase	69404	1.967	5590	1.4

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

TAFLUPROST:

Parameter used for sample analysis	**Peak Area**	**Retention Time**	**Theoretical plates**	**Tailing factor**
Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
Less organic phase	3965572	2.590	5550	1.0
More organic phase	527708	2.390	6273	1.0

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

SUMMARY

The present study focused on the development and validation of a simple, rapid, precise, and accurate RP-HPLC method for the simultaneous estimation of Tafluprost and Timolol in combined tablet dosage form. Tafluprost, a prostaglandin analogue, and Timolol, a non-selective beta-adrenergic blocker, are widely used in combination therapy for the management of glaucoma and ocular hypertension. Accurate quantification of these drugs in pharmaceutical formulations is essential to ensure product quality, safety, and therapeutic efficacy. An optimized Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed using a suitable C18 column with an appropriate mobile phase composition. The chromatographic conditions were adjusted to obtain well-resolved, symmetrical peaks with acceptable retention times for both drugs. Detection was carried out at a selected wavelength where both analytes showed adequate absorbance. The developed method was validated according to ICH guidelines. The method demonstrated good specificity, with no interference from excipients. Linearity was observed within the selected concentration range with correlation coefficients close to unity. The method showed acceptable accuracy, with percentage recovery within prescribed limits. Precision studies indicated low %RSD values, confirming repeatability and intermediate precision. The limits of detection (LOD) and quantification (LOQ) were found to be satisfactory, indicating adequate sensitivity. Robustness studies confirmed that small deliberate variations in chromatographic conditions did not significantly affect the results. The validated method was successfully applied for the quantitative estimation of Tafluprost and Timolol in combined tablet dosage form.

CONCLUSION

A simple, economical, accurate, and precise RP-HPLC method was successfully developed and validated for the simultaneous estimation of Tafluprost and Timolol in combined tablet dosage form. The method fulfilled all validation criteria as per ICH guidelines, including specificity, linearity, accuracy, precision, sensitivity, and robustness. The developed method is suitable for routine quality control analysis of Tafluprost and Timolol in pharmaceutical formulations. Furthermore, due to its reliability and reproducibility, the method can be effectively employed in regulatory submissions and stability studies.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Brilliant Grammar School Educational Society's Group of Institutions (Engineering and Pharmacy), Abdullapur (V), Abdullapurmet (M), Rangareddy (D), Hyderabad., for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Pharma Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

BIBLIOGRAPHY

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. BraithWait and F.J. Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL: http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahaj Walla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. D. H. Shewiy, E. Kaale, P. G. Risha, B. Dejaegher, J. S. Verbeke, Y. V. Heyden, Journal Pharmaceut. Biomed. Anal, 66,2012,11-23.

9. M. D. Rockville, General Tests, Chapter 621 – Chromatography SystemSuitability, United States Pharmacopeial Convention (USP), USP 31,2009.
10. FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Centre for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER),2000.
11. Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modelling in HPLC method development. J Adv Res, 3**,** 2012,53-63.
12. Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. Lc Gc N. Am, **75,** 2002, 49-50**.**
13. Snyder LR, Kirkland JJ, Glajach JL. X. In Practical HPLC Methods Development.John Wiley, New York, 295**,** 1997, 643-712.
14. Swartz M, Murphy MB. New Fronties in Chromatography. Am Lab**,** 37**,** 2005, 22-27.
15. Dolan JW. Peak tailing and resolution. Lc Gc N. Am, 20**,** 2002, 430-436.
16. Chan CC, Leo YC, Lam H. Analytical method validation and InstrumentPerformance Validation. Vol-I, Wiley Interscince, 2004.
17. Shabir GA, Lough WJ., Arain SA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. J Chromatograph RT, 30**,** 2007, 311-333.
18. Miller JN, Miller JC, Statistics and chemometrics for analytical chemistryHarlow, Pearson Prentice Hall, 2005, 263.
19. Huber L. Validation of HPLC methods. Bio Pharm. Int, 12**,** 1999, 64-66.
20. European-commission Quality control procedures for pesticide residues analysis.Report numberSANCO/10232/2006.
21. ICH Q2 (R1), Validation of Analytical Procedures: Text andMethodology. International Conference on Harmonization, IFPMA, Geneva, 2005.
22. https://pubchem.ncbi.nlm.nih.gov/compound/Amlodipine
23. https://en.wikipedia.org/wiki/ Amlodipine
24. https://www.drugbank.ca/drugs/DB00999
25. https://pubchem.ncbi.nlm.nih.gov/compound/Hydrochlorothiazide