International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 22s, 2025
https://theaspd.com/index.php

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Dorzolamide and Natarsudil Dimesylate in Pharmaceutical Formulations

Mukesh Tiwari[1], Hemant Badwaik[2], Nitin Deshmukh[3], Mukesh Sharma[1], Madhulika Pradhan[1], Kushagra Nagori[1]
[1]Rungta College of Pharmaceutical Sciences and Research, Bhilai, CG, India
[2]Sri Shankaracharya Institute of Pharmaceutical Sciences and Research, Junwani, Bhilai, CG, India
[3]G.R.Y Institute of Pharmacy, Borawan, Khargone, MP, India
Corresponding author-*Dr. Madhulika Pradhan, Email-: madhulika.pradhan1@gmail.com

Abstract: *Dorzolamide hydrochloride and Natarsudil is a well-established fixed-dose combination for the treatment of glaucoma worldwide. The study focuses on validating and developing a precise, simple, and accurate stability-indicating RP-HPLC method for simultaneously estimating Dorzolamide and Natarsudil in pharmaceutical formulations. The chromatographic separation was achieved by using a Chomosil C_{18} column (250 × 4.5 mm, 5 µm) with acetonitrile: methanol: 0.01N Ammonium acetate buffer in a ratio of 45:35:20 v/v as a mobile phase at a constant flow rate of about 1.0 mL/min. The development and validation were carried out at a detection wavelength of 242 nm. We developed a robust RP-HPLC method, validated for linearity, precision, accuracy, specificity, and system suitability. The method demonstrated excellent linearity with correlation coefficient values r^2 was nearly 0.9989 and 0.9985 respectively with a linearity range 2.5-30 µg/mL for Dorzolamide and 2.5-30 µg/mL for Natarasudil. LOD and LOQ were found to be lower; hence, the method is sensitive. Percentage recovery was obtained at 99.97% and 99.99% for Dorzolamide and Natarasudil, respectively. In the case of precision, robustness and repeatability, RSD was found to be less than 2. The validated and developed RP-HPLC method offers an efficient and practical approach for simultaneously quantifying Dorzolamide and Natarsudil in pharmaceutical formulations, making it a valuable tool for quality control and pharmaceutical research.*
Keywords: *Dorzolamide, Natarsudil, RP-HPLC, Validation*

INTRODUCTION:

Glaucoma is still the most common cause of permanent blindness, affecting more than 60 million people globally (1). By 2040, the global glaucoma population is expected to reach over 110 million people, necessitating the development of innovative glaucoma treatments (2). It is estimated that the number of people with glaucoma will rise to 111.8 million in 2040, disproportionally affecting people living in Asia and Africa (3). Most patients require more than one topical intraocular pressure (IOP) lowering agent to achieve and maintain target IOP, especially after one year of glaucoma treatment. Given the asymptomatic nature of this chronic progressive disease, concomitant instillation with monotherapy has increased treatment complexity resulting in poor patient compliance and adherence (4).. Dorzolamide (DZL) hydrochloride, chemically (4S,6S)-4-(ethylamino)-6-methyl-5,6-dihydro-4H-thieno[2,3-b]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride (Figure1), is a carbonic anhydrase inhibitor, which is used for the treatment of glaucoma and ocular hypertension(3).

Netarsudil (NT) is chemically described as (4-((1S)-1-(Aminomethyl)- 2-(isoquinolin-6-ylamino)-2-oxoethyl) phenyl)methyl 2,4-dimethylbenzoate NT [Figure 2]. Its empirical formula is C28H27N3 O3 and its molecular weight is 453.54. Netarsudil is a Rhokinase inhibitor with norepinephrine transport inhibitory activity that reduces the production of aqueous where it reduces elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. Latanoprost is the first topical prostaglandin F2 alpha analogue used for glaucoma treatment(1).

Literature survey reveals there are several methods to estimate these drugs in single or in combination of two or three drugs. However, only very few HPLC methods are available for the simultaneous estimation of Dorzolamide and Natarsudil, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective is to improve the conditions and parameters, which should be followed in the development and validation processes(3).

Experimental

Reagent and Chemicals Micro Labs Limited, located in Bengaluru, India, provided gift samples of Dorzolamide and Natarasudil for analytical purposes. The solvent used to include HPLC-grade acetonitrile was sourced from Merck Pvt. Ltd. The HPLC-grade water was collected from Milli-Q water obtained from the Micro Labs and HPLC-grade Methanol was procured from Merck Pvt. Ltd(4).

Methodology: Pharmaceutical analysis commonly uses simultaneous estimation using HPLC. It enables HPLC to determine the presence of many chemicals in a sample. Several techniques have been devised and proven effective for the simultaneous estimate of various components, including medications and their contaminants, in pharmaceutical formulations. Utilizing an appropriate column, mobile phase, and detection equipment, the simultaneous estimation technique by HPLC allows for the separation and quantification of the target substances (5).In pharmaceutical analysis, high-performance liquid chromatography (HPLC) is a great instrument for simultaneous estimation that offers confidence and specificity for the identification of chemical entities in pharmaceutical formulations (6).

Instruments:

The chromatographic separation was performed on an HPLC system (Young Lin) Series 3rd generation Acme HPLC Software Autochro-3000, integrated with **Gradient Pump** High Precision (< ± 0.1 %) Wide flow rate range (0.001 ~ 16.0 ml/min), With Vacuum Degasser & Mixer low-pressure gradient module and **UV/VIS Detector**. The mobile phase was prepared freshly, filtered, sonicated be for use, and delivered at a flow rate of 01.0 mL/min and the detector wavelength was set at 242 nm. The injection volume was 10 µL. The diluent used was acetonitrile: methanol: 0.01N Ammonium acetate buffer in a ratio of 45:35:20 v/v (7).

Preparation of standard and sample solutions. Standard solution:

Accurately weighed 50 mg of Dorzolamide and 50 mg of Netarsudil were transferred to a 50ml volumetric flask and 20ml of diluent was added to these flasks and sonicated for 10 minutes. The flask was made up with diluents and labeled as a Standard stock solution. (1000µg/ml Dorzolamide and 1000µg/ml of Netarsudil). 5 ml from each stock solution was pipetted out and taken into a 50ml volumetric flask and made up with diluent (100µg/ml Dorzolamide and 100µg/ml of Netarsudil) (8).

Sample Solution: 1ml of the formulation was taken and was transferred into a 100 ml volumetric flask,50ml of diluents was added and sonicated for10min, further the volume was made up with diluent and filtered by HPLC filters (222.6µg/ml Dorzolamide, and 228 µg/ml of Netarsudil). 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (22.8µg/ml Dorzolamide, and 22.6µg/ml of Netarsudil). As the concentration of Natarsudil is very low so 2 gm is added to the formulation. (External standard addition method. The known amount of standard was added. (6).

Validation Procedure

The analytical method was validated as per ICH Q2 (R1) (8) guidelines for parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), forced degradation and stability.

System Suitability: System suitability parameters were measured to verify the system performance. The parameters, including USP plate count, USP tailing, and %RSD are calculated and found to be within the limits.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excipients), which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Dorzolamide and Natarsudil (9).

Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies at three different concentration levels assessed accuracy. In each level, a minimum of three injections were given, and the amount of the drug present, percentage recovery, and related standard deviation were calculated(6).

Precision: The precision of an analytical method is the degree of agreement among individual test results. It was studied by analyzing multiple samples of a homogeneous sample. The precision of the present method was assessed in terms of repeatability and intra-day and inter-day variations. It was checked by analyzing the samples at different time intervals on the same day as well as on different days(7).

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The calibration

curve was constructed by plotting area under curve against respective concentration of Dorzolamide and Natarsudil(9).

Sensitivity: The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method (6).

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guidelines. Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.2ml/min), mobile phase minus (60B:40A), mobile phase plus (50B:50A), temperature minus (25°C) and temperature plus(35°C) were maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed, %RSD was within the limit(10).

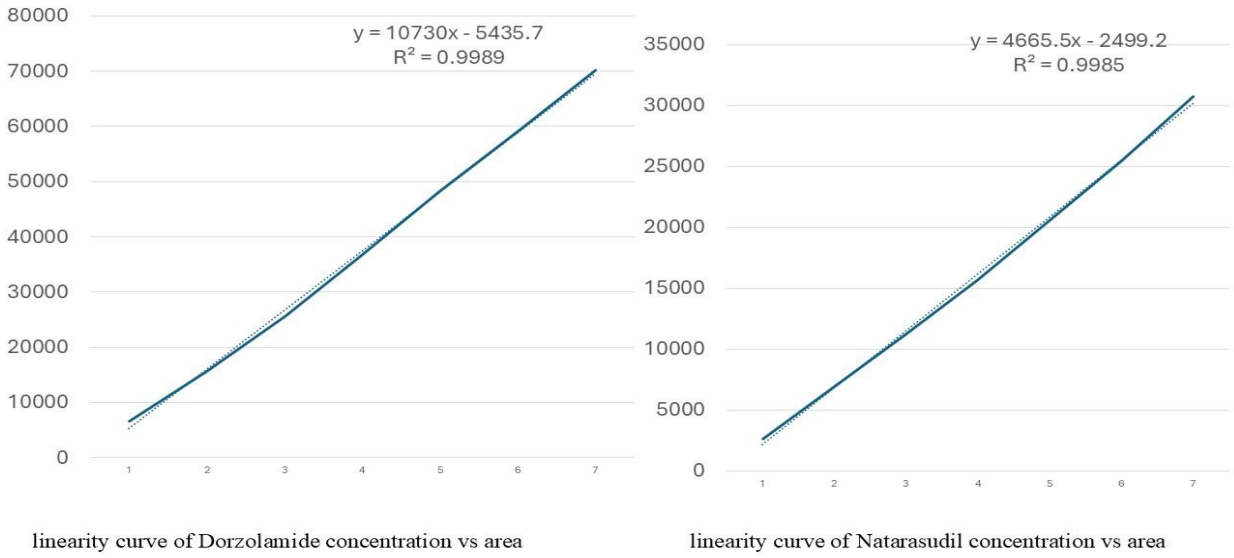
Assay: Formulation bearing the label claim Dorzolamide 22.26mg/ml, Natarsudil 0.28mg/ml. An assay was performed with the above formulation. Average % Assay for Netarsudil and Latanoprost obtained was 100.57% and 100.43% respectively. Obtained results are presented in table

RESULT AND DISCUSSION

Linearity: The plots of peak area Vs respective concentration of Dorzolamide and Natarsudil were found to be linear in the range of 2.5-30µg/mL and 2.5-30µg/mL with a coefficient of correlation (r^2) of 0.9989 and 0.9985 for Dorzolamide and Natarsudil, respectively. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Dorzolamide and Natarsudil are given in Fig 1

Table 1: Linearity of Dorzolamide and Natarasudil

S. No	**Dorzolamide**		**Natarasudil**	
	Concentration	AUC	Concentration	AUC
1	2.5	6554.4	2.5	2613.4
2	5	15712.4	5	6932.2
3	10	25651.1	10	11244.3
4	15	36832.7	15	15664.6
5	20	48300.3	20	20514.7
6	25	59114.6	25	25446.4
7	30	70212.5	30	30725.6
	Mean	37482.57	Mean	16163.03
	SD	23191.64	SD	10086.54



linearity curve of Dorzolamide concentration vs area

linearity curve of Natarasudil concentration vs area

Figure:1 linearity curve of Dorzolamide and Natarasudil concentration vs area

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Table 2: RESULTS OF ACCURACY EXPERIMENT USING SPIKED PLACEBO METHOD FOR DORZOLAMIDE

Level %	Amount of drug spiked (mg)	Found (mg)	Recovery % (n=3)
10	2.89	2.85	98.61
20	5.0	4.99	99.8
30	7.05	6.98	99.0
SD			0.606658
%RSD			0.611941

TABLE 3: RESULTS OF ACCURACY EXPERIMENT USING SPIKED PLACEBO METHOD FOR NATARASUDIL

Level %	Amount of drug spiked (mg)	Found (mg)	Recovery % (n=3)
2.5	1.09	1.1	100.9
5.0	1.67	1.68	100.5
10	2.94	2.92	99.3
		SD	0.832666
		%RSD	0.830728

Precision

The precision of this method was assessed in terms of intraday (repeatability) and interday (intermediate precision) variations. The intraday studies were determined by performing six repeated analysis of the sample solution of Dorzolamide and Natarsudil on the same day under the same laboratory by studying the analysis with different analyst and different instrument. The method is highly precise as % RSD values were found to be ≤2% Good recoveries (98-100%) of the drug were obtained at each added concentration, indicating that the method was accurate. The results and chromatograms were furnished below.

TABLE 4 Method precision data

Conc. Of Dorzolamide in µg/ml	AUC	% RSD	Conc. Of Natarsudil in µg/ml	AUC	% RSD
10	25651.1	0.3265	10	11244.3	0.4170
	25504.2			11203.5	
	25702.5			11198.9	
	25698.2			11298.5	
	25674.3			11305.7	
	25745.6			11220.5	

TABLE 5 Intermediate precision data

Conc. Of Dorzolamide in µg/ml	AUC	% RSD	Conc. of Natarsudil in µg/ml	AUC	% RSD
10	25751.6	0.1693	10	11354.5	0.4699
	25694.3			11303.8	
	25712.4			11275.3	
	25675.7			11398.6	
	25794.3			11408.9	
	25702.4			11320.1	

Sensitivity: LOD and LOQ

LOD and LOQ were separately determined by calibration curve method. LOD and LOQ of the compounds were determined by injecting progressively lower concentrations of standard solutions using the developed RP-HPLC method. The LOD values for Dorzolamide and Natarsudil were found to be

7.33 µg/ml and 7.13 µg/ml respectively. The LOQ values were found to be 22.2 µg/ml and 21.63 µg/ml respectively.

Robustness

As per ICH norms, small but deliberate variations were made in the method parameters such as a change in the flow rate (±0.2), organic content in the mobile phase (±10%), the wavelength of detection (±5) and pH (±0.2) to check the method capacity to remain unaffected. The robustness of the method was evaluated by observing the effect of the modified parameters on retention time, tailing factor, area, and percentage content. The degree of reproducibility of the results which were obtained by small deliberate variations has proven that the method is robust.

Table 6: result of Robustness Study

S. No	Parameters	Variations	Assay % of Dorzolamide	Assay % of Natarasudil
1	Flow rate (±20% of the set ratio)	At 0.8 ml/min	99.12	99.27
		At 01.2 ml/min	99.32	99.43
2	Mobile phase ratio ((±2% of the set ratio)	At buffer 68%	98.97	99.64
		At buffer 72%	99.68	99.08

Table 7 Stability of standard and formulation of Dorzolamide and Natarasudil

Time Interval	Standard Dorzolamide		Formulation		Standard Natarasudil		Formulation	
	Standard Peak Area	% Difference	Standard Peak Area	% Difference	Standard Peak Area	% Difference	Standard Peak Area	% Difference
O	59114.6	.....	59034.5	.....	2613.4	~~~	2584.3	~~~
30 days	59096.2	0.03	59001.4	0.05	2598.2	0.58	2534.7	1.91
60 days	59091.3	0.04	58971.8	0.10	2584.3	1.11	2501.2	3.21
90 days	59079.1	0.06	58931.1	0.17	2572.4	1.56	2484.5	3.86

Table 8 Assay result of the formulation

Drug	Lebel Strength in mg/ml	% Assay
Dorzolamide	22.26	99.84
Natarsudil	0.28	100.4

Analysis of Dorzolamide and Natarsudil in ophthalmic dosage form

The response of sample solutions was measured at 242 nm for quantitation of Dorzolamide and Natarsudil by the method described above. The amount of Dorzolamide and Natarsudil present in the sample solution was determined by applying peak area values to the calibration graph's regression equation. The results of the assay were given in Table 3

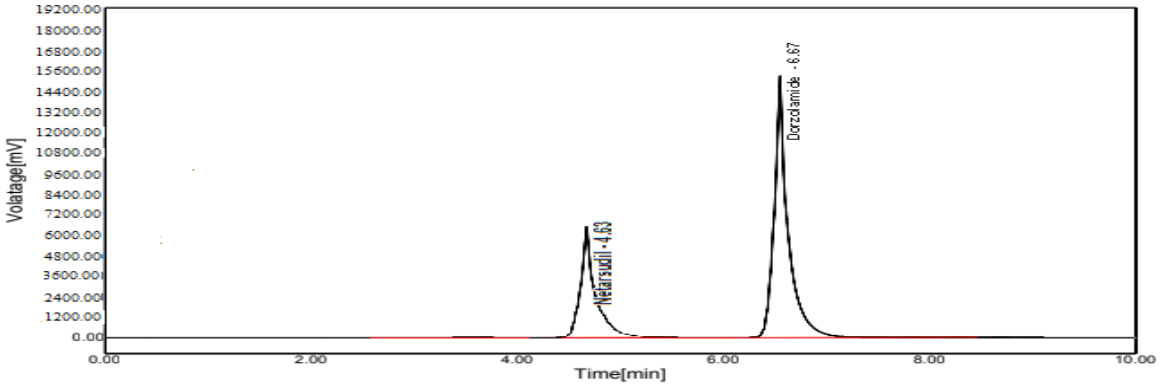


Figure 2 Calibration graph's regression equation of Dorzolamide and Natarsudil in ophthalmic dosage form

CONCLUSION:

The proposed method has the advantage of simplicity and convenience for the separation and quantification of Dorzolamide and Natarsudil in the combination. It can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to an environmentally friendly chromatographic procedure. The method is accurate, precise, rapid, and selective for the simultaneous estimation of Dorzolamide and Natarsudil in the Ophthalmic dosage form. Hence, it can be conveniently adopted for routine analysis.

REFERENCES

1. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology. 2014;121(11):2081–2090.
2. Weinreb RN, Khaw PT. Primary open-angle glaucoma. Lancet. 2004;363(9422):1711–1720.
3. Dasso L, Al-Khaled T, Sonty S, Aref AA. Profile of netarsudil ophthalmic solution and its potential in the treatment of openangle glaucoma: evidence to date. Clin Ophthalmol. 2018 Oct 4; 12:1939-1944.
4. Parrish RK, Palmberg P, Sheu WP., XLT Study Group. A comparison of latanoprost, bimatoprost, and travoprost in patients with elevated intraocular pressure: a 12-week, randomized, masked-evaluator multicenter study. Am J Ophthalmol. 2003;135(5):688-703.
5. P. Sai Sharanya, S. Shoba Rani. Development and Validation of Stability Indicating Analytical Method for the Simultaneous Estimation of Netarsudil and Latanoprost by RP-HPLC. World J Pharm Sci 2022; 10(01): 104-112;
6. G. Dharmamoorthy, Dr. K. S. Nataraj, Dr. A. Krishna Manjari.Pawar, Development and validation of a stabilityindicating reverse-phase ultra-performance liquid chromatography method for the simultaneous determination of netarsudil and latanoprost in bulk and pharmaceutical formulation, International Journal of Green Pharmacy • Apr-Jun 2020 • 14 (2) | 179-185.
7. Maithani, M. (n.d.). Simultaneous estimation of drugs by RP-HPLC.
8. P.K. Sahu, N.R. Ramisetti, T. Cecchi, S. Swain, C.S. Patro, J. Panda, An overview of experimental designs in HPLC method development and validation, J. Pharm. Biomed. Anal. 147 (2018) 590–611, https://doi.org/10.1016/J. JPBA.2017.05.006.
9. A.M. Sabir, M. Moloy, P.S. Bhasin, HPLC method development and validation: a review, Int. Res. J. Pharmacy 4 (4) (2016) 39–46, https://doi.org/10.7897/2230- 8407.04407.
10. Shweta Mishra, Ninama Surekha, Ashlesha Chauhan,2023, Development and validation of stability indicating RP-HPLC method for simultaneous estimation of silodosin and mirabegron in synthetic mixture, ELESVIER 0003-4509. https://doi.org/10.1016/j.pharma.2023.12.013