Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Method Development and Validation for Concurrent Quantification of Netarsudil and Lantanoprost in Bulk and Injection form by RP-HPLC Method

Durga Pani Kumar Anumolu[1*], Veera Shakar Pulusu[2], Syed Sara Afreen[4], Lakshmi Madhuri M [3], Swathi Naraparaju[3], Mamatha Gashikanti[1].

[1*,] Gokaraju Rangaraju College of Pharmacy, Department of Pharmaceutical Analysis, Osmania University, Hyderabad, Telangana-500090, India
[2] Ohio University, Department of Chemistry &Biochemistry, Athens, OH, USA-45701

[3] Gokaraju Rangaraju College of Pharmacy, Department of Pharmaceutical Chemistry, Osmania University, Hyderabad, Telangana-500090, India

***Corresponding author:** Dr Durga Panikumar Anumolu
*Associate Professor, HOD, Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Hyderabad Telangana-500090, India. panindrapharma05@gmail.com

Cite this paper as: Durga Pani Kumar Anumolu, Veera Shakar Pulusu, Syed Sara Afreen, Lakshmi Madhuri M, Swathi Naraparaju, Mamatha Gashikanti (2024) Method Development and Validation for Concurrent Quantification of Netarsudil and Lantanoprost in Bulk and Injection form by RP-HPLC Method. *Frontiers in Health Informatics, 13* (3), 9640-9650

Abstract
For the simultaneous determination of the dosage forms of Latanoprost and Netarsudil in ophthalmic solution, a straight forward, precise, and accurate approach was designed. Chromatogram was developed using Phenomenex C18 150 x 4.6 mm, 5µ, Mobile phase containing Water: Methanol taken in the ratio 55:45 pumped through column at a flow rate of 1.0 mL/min with Potassium dihydrogen phosphate buffer at Optimized wavelength 290 nm. Temperature was maintained at 30°C.selected was. Retention time of Netarsudil and Latanoprost were found to be 2.588 min and 3.221 min. The developed method was validated by System Suitability, specificity, selectivity, accuracy, precision, accuracy, robustness and LOQ – LOD as per ICH Q2 (R1) guidelines. The regression equation of Netarsudil is y = 11999x + 1319.8 and y = 13329x + 380.64 of Latanoprost. The method developed was suitable for routine qualitative analysis of Latanoprost and Netarsudil in pharmaceutical dosage forms. Retention times were decreased, and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in industries.

Key Words*: Netarsudil, Latanoprost, RP-HPLC, Linearity, Accuracy,*

Introduction

Netarsudil is a medication used in the treatment and management of glaucoma and ocular hypertension. Netarsudil is classified as a rho-kinase inhibitor. Primary open-angle glaucoma is characterized by

progressive and irreversible optic neuropathy with a loss of optic nerve fibers. Netarsudil is helps lower high pressure in the eye and reduces the risk of vision loss. Ordinary physiological Elevated Intraocular pressure (IOP) results from aqueous humor produced by the ocular ciliary body and its outflow through two main outflow pathways: the conventional (trabecular) and the unconventional (uveoscleral) pathways. Latanoprost is a prodrug analog of prostaglandin F2 alpha that is used to treat elevated intraocular pressure (IOP). It was initially approved by the FDA in 1998. Latanoprost is the first topical glaucoma treatment. It has been found to be well tolerated and its use does not normally result in systemic adverse effects like other drugs used to treat elevated intraocular pressure, such as Timolol. Another benefit latanoprost is that is can be administered once a day. Based on literature review (1-11), few analytical HPLC methods were available for Simultaneous quantification of Netarsudil and Lantanoprost in pharmaceutical dosage forms , but that methods were showing run time somewhat more which indicates consumption of organic solvents.So keeping this point into consideration ,we attempt this work to develop and validate the RP-HPLC method with short retention time.

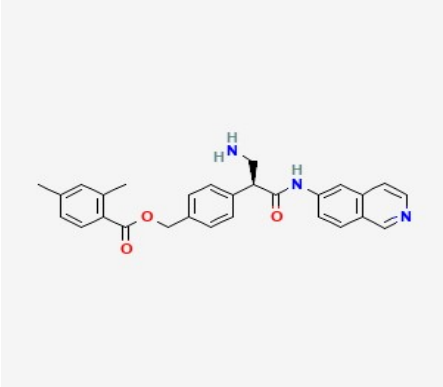

Figure 1:Structure of Netarsudil

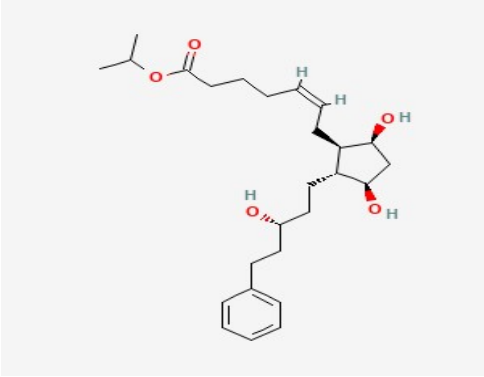

Figure 2:Structure of Lantanoprost

Experimental procedure

Materials:

Netarsudil and Latanoprost pure drugs (API), Combination Netarsudil and Latanoprost Ophthalmic solution (ROCKLATAN) Distilled water, Acetonitrile (HPLC grade), Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer,Ortho-phosphoric acid (AR grade) .All the above chemicals and solvents were from Rankem.

Instruments:

Electronics Balance-Denver, pH meter -BVK enterprises, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps,Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special band width of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Netarsudil and Latanoprost solutions.

Method development

The HPLC method was developed for the simultaneous estimation of reference standards of Netarsudil and Latanoprost

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50 as diluent.

Preparation of Standard stock solutions: Accurately weighed 5 mg of Netarsudil, 1.25 mg of Latanoprost and transferred to individual 50 mL volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (100 µg/mL of Netarsudil and 25 µg/mL of Latanoprost)

Preparation of Standard working solutions (100% solution): 1 mL from each stock solution was pipetted out and taken into a 10 mL volumetric flask and made up with diluent. (10 µg/mL Netarsudil of and 2.5 µg/mL of Latanoprost).

Preparation of Sample stock solutions: 10 vails were weighed and was transferred into a 10 mL volumetric flask, 5 mL of diluents was added and sonicated for 10 min, further the volume was made up with diluent and filtered by HPLC filters (100 µg/mL of Netarsudil and 25 µg/mL of Latanoprost)

Preparation of Sample working solutions (100% solution): 1 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent.(10 µg/mL of Netarsudil and 2.5 µg/mL of Latanoprost)

Validation

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Netarsudil (10 ppm) and Latanoprost (2.5 ppm) and the solutions were injected 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 not be more than 2%.

Specificity:

Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Sample stock solutions: 10 vails were weighed and was transferred into a 10 mL volumetric flask, 5 mL of diluents was added and sonicated for 10 min, further the volume was made up with diluent and filtered by HPLC filters(100 µg/mL of Netarsudil and 2.5 µg/mL of Latanoprost)

Preparation of Sample working solutions (100% solution): 1 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent.(10 µg/mL of Netarsudil and 2.5 µg/mL of Latanoprost)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 5 mg of Netarsudil, 1.25 mg of latanoprost and transferred to individual 50 mL volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (100 µg/mL of Netarsudil and 2.5 µg/mL of Latanoprost). Linearity studies were performed on the different concentration ranging from 25 %-150% of the target formulation. Concentration ranges from 2.5 -15 µg/mL of Netarsudiland 0.625- 3.75 µg/mL of Latanoprost.

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 5 mg of Netarsudil, 1.25 mg of Latanoprost and transferred to individual 50 mL volumetric flasks separately. 3/4th of diluents was

added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2.

Preparation of 50%,100%, 150% Spiked Solution: 0.5 mL,1.0 mL 1.5 mL, of sample stock solutions were taken into a 10 mL volumetric flasks , to that 1.0 mL from each standard stock solutions were pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102

Robustness:

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature were made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9 mL/min), Flow plus (1.1 mL/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was 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.

LOD sample Preparation: 0.25 mL each from two standard stock solutions was pipetted out and transferred to two separate 10 mL volumetric flasks and made up with diluents. From the above solutions 0.1 mL each of Netarsudil, Latanoprost, solutions respectively were transferred to 10 mL volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25 mL each from two standard stock solutions was pipetted out and transferred to two separate 10 mL volumetric flask and made up with diluent. From the above solutions 0.3 mL each of Netarsudil, Latanoprost, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

Degradation studies:

Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different condition like acid, alkali and oxidative, photolytic, thermal and neutral analysis.

Results and discussion:

Optimized chromatographic conditions:

Parameters	conditions
Mobile phase:	55% water: 45% methanol
Flowrate:	1ml/min
Column:	Phenomenex C18 (4.6×150mm, 5µm)
Detector wavelength:	220 nm
Column temperature:	30^0C
Injection volume:	10 µL
	5minwater and acetonitrile in the ratio 50: 50

Results: In this trial both peaks were eluted, and all the system suitability parameters were within the limits.

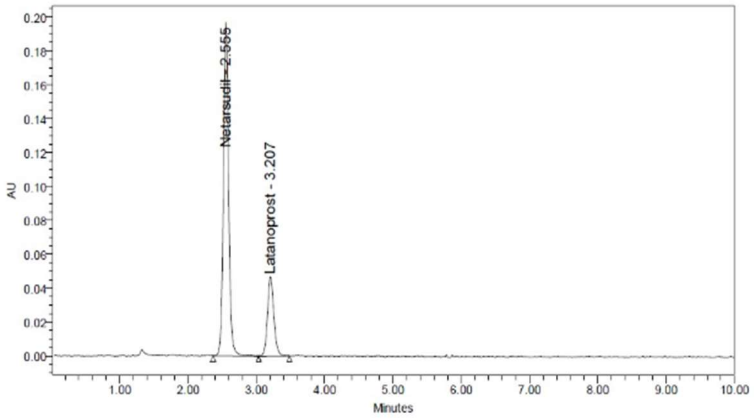

Figure 3: Optimized Chromatogram of Netarsudil and Lantanoprost

Observation: Netarsudil and Latanoprost were eluted at 2.555 min and3.207 min respectively

System Suitability: According to ICH guidelines plate countshould be more than 2000, tailing factor should be less than 2 and resolution must be more than 2.

Table 1: System suitability parameters of Netarsudil and Lantanoprost

S.No.	**Netarsudil**			**Latanoprost**			
Inj	RT (min)	USP plate count	Tailing	RT (min)	USP plate count	Tailing	Resolution
1	2.535	6881	1.1	3.221	6672	1.11	4.3
2	2.543	6600	1.0	3.223	6779	1.09	4.3
3	2.544	6604	1.0	3.224	6849	1.14	4.7
4	2.548	6823	1.1	3.224	6925	1.12	4.7
5	2.584	6810	1.1	3.233	6916	1.10	4.9
6	2.589	6484	1.1	3.244	6785	1.10	4.8

Specificity:

Retention times of Netarsudil and Latanoprost were 2.589 min and 3.221 min respectively.

Linearity:

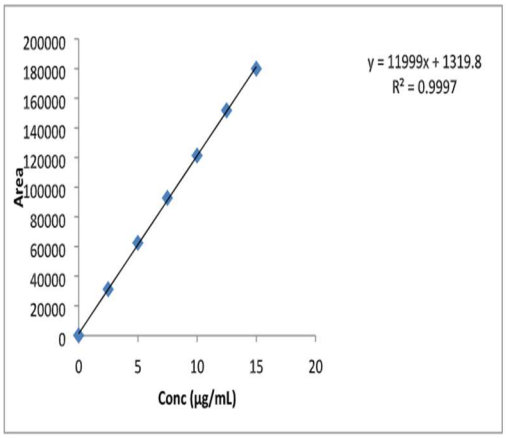

Figure 4: Calibration curve of Netarsudil

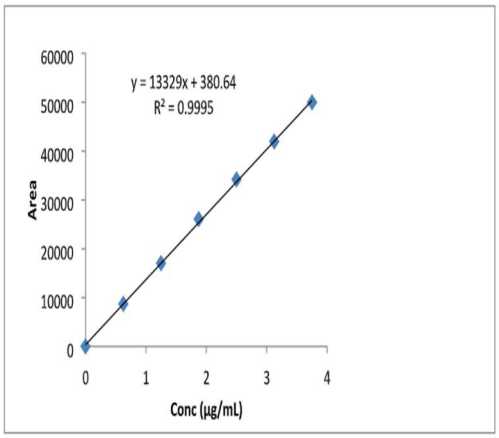

figure 5: Calibration curve of Latanoprost

Precision:

. %RSD obtained as 0.7%and 1.4% respectively for Netarsudil andLatanoprost .

Table 2: System precision table of Netarsudil and Lantanoprost

S.No	Area of Netarsudil	Area of Lantanoprost
1	120822	35848
2	120912	36210
3	122079	35221
4	122699	35813
5	122491	34806
6	122444	35863
Mean	121908	35627
S.D	831.1	513.4
%RSD	0.7	1.4

Accuracy:

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.86% and 100.47% for Netarsudil and Latanoprost respectively.

Table 3: Accuracy table of Latanoprostand Netarsudil

% Level	Amount spiked (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery	Amount spiked (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery
Latanoprost					**Netarsudil**			
50%	1.25	1.24	99.46	100.47%	5	4.94	98.86	99.86%
	1.25	1.27	101.63		5	5.03	100.54	
	1.25	1.25	100.14		5	5.00	100.06	
100 %	2.5	2.47	98.75		10	10.04	100.45	
	2.5	2.50	99.85		10	10.10	101.04	
	2.5	2.51	100.42		10	10.03	100.35	
150 %	3.75	3.78	100.83		15	14.75	98.34	
	3.75	3.78	100.89		15	14.87	99.11	
	3.75	3.78	100.23		15	15.01	100.04	

Repeatability:
%RSD were calculated for two drugs and obtained as 0.7% and 0.8% respectively for Netarsudil and Latanoprost. As the limit of Precision was less than "2" the system precision was passed in this method

Table 5: Repeatability table of Netarsudil and Lantanoprost

S.NO	Area of Netarsudil	Area of Latanoprost
1	121065	35964
2	121332	35635
3	122547	35602
4	121900	35442
5	120843	35138
6	120248	35431
Mean	121323	35535
S.D	810.4	274.1
%RSD	0.7	0.8

Intermediate precision:
Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day ofthe sample preparation and obtained areas were mentioned in the abovetable. Average area,

standard deviation and %RSD were calculated for two drugs and obtained as 1.1% and 1.2% respectively for Netarsudil and Latanoprost. As the limit of Precision was less than "2" the system precision was passed in this method

Table 6: Intermediate precision table of Netarsudil and Lantanoprost

S.no	Area of Netarsudil	Area of Lantanoprost
1	118127	34885
2	119853	34298
3	120942	35393
4	122239	34594
5	120564	34938
6	119656	35303
Mean	120230	34902
S.D	1381.9	415.5
%RSD	1.1	1.2

Sensitivity:

Table 7: Sensitivity table of Netarsudil and Latanoprost

Molecule	LOD (ug/mL)	LOQ (ug/mL)
Netarsudil	0.07	0.21
Lantanoprost	0.02	0.07

Robustness:

Robustness conditions like Flow minus (0.9 ml/min), Flow plus(1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A),temperature minus (25°C) and temperature plus(35°C) was maintained, and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD waswithin the limit.

Table 8: Robustness data for Netarsudil and Latanoprost

S.no	Condition	%RSD OF Netarsudil	%RSDof Lantanoprost
1	Flow rate (-) 0.9 ml/min	0.8	1.0
2	Flow rate (+) 1.1 ml/min	0.9	1.0
3	Mobile phase (65B:35A)	0.1	0.9
4	Mobile phase (+)55B:45A	0.6	0.6
5	Temperature (-)	0.9	1.5

	25°C		
6	Temperature (+) 35°C	0.4	1.4

Assay: (ROCKLATAN), bearing the label claim Netarsudil 0.2 mg, Latanoprost 0.05 mg. Assay was performed with the above formulation. Average % Assay for Netarsudil and Latanoprost obtained was 99.70% and 99.70%respectively

Table 9: Assay data of Netarsudil and Latanoprost

s.no	**Standard area**	**Sample area**	**% Assay**	**Standard Area**	**Sample Area**	**%Assay**
Netarsudil				**Latanoprost**		
1	120822	121065	98.91	35848	35964	100.74
2	120912	121332	99.13	36210	35635	99.82
3	122079	122547	100.12	35221	35602	99.73
4	122699	121900	99.59	35813	35442	99.28
5	122491	120843	98.73	34806	35138	99.43
6	122444	120248	98.24	35863	35431	98.25
Avg	121908	121323	99.12	35627	35535	99.54
Stdev	831.1	810.4	0.66	513.4	274.1	0.8
%RSD	0.7	0.7	0.7	1.4	0.8	08.

Degradation studies:

Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different condition like acid, alkali and oxidative, photolytic, thermal and neutral analysis.**Degradation data**

Type of degradation	**Netarsudil**			**Lantanoprost**		
	AREA	**%RECOVERED**	**%DEGRADED**	**AREA**	**%RECOVERED**	**%DEGRADED**
Acid	116378	95.08	4.92	34018	95.29	4.71
Base	116953	95.55	4.45	34357	96.24	3.76
Peroxide	117642	96.11	3.89	34519	96.70	3.30
Thermal	119804	97.88	2.12	34979	97.99	2.01
UV	120341	98.32	1.68	35160	98.49	1.51
Water	121353	99.15	0.85	35457	99.32	0.68

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netarsudil and Latanoprost Tablet dosage form.Retention time of Netarsudil and Latanoprost were found to be 2.588min and 3.221min. %RSD of the Netarsudil and Latanoprost were and found tobe 0.7 and1.4 respectively. %Recovery was obtained as 99.86% and100.47% for Netarsudil and Latanoprost respectively. LOD, LOQ values obtained from regression equations of Netarsudil and Latanoprost were0.07, 0.02 and 0.21, 0.07 respectively. Regression equation of Netarsudil isy = 11999x + 1319.8.and y = 13329x + 380.64 of Latanoprost .Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

References

1. Agarwal Ankit, (2013) Method development and its validation for quantitativesimultaneous determination of latanoprost, timolol and benzalkonium chloridein ophthalmic solution by RP-HPLC, Journal of Drug Delivery &Therapeutics; 3(2), 26-30.

2. Alessio Zammatar, (2011) Development and validation of a liquidchromatography/electrospray ionization tandem mass spectrometry method forthe quantification of latanoprost free acid in rabbit aqueous humor and ciliarybody, Journal of Mass Spectrometry 46(11):1168-74.

3. Jigar Mehtal,(2010) A versatile LC method for the simultaneous quantification of latanoprost, timolol and benzalkonium chloride and related substances inthe presence of their degradation products in ophthalmic solution, Analytical Methods.

4. McLoughlin D.A., Olah T.V., and Gilbert J.D., (1997) A direct technique for the simultaneous determination of 10 drug candidates in plasma by Liquid Chromatography atmospheric pressure chemical ionization mass spectrometryinterfaced to a prospect solid-phase extraction system. J. Pharm. Biomed.Anal. ; 15: 1893–1901

5. Michael E. Swartz, (2005) UPLC An Introduction and Review Waters Corporation, Milford, Massachusetts, USA, Journal of Liquid Chromatography & Related Technologies, ; 28: 1253–1263.

6. Pratikeshkumar Patel, Bioanalytical Method Development and Validation for Latanoprost Quantification in Pharmaceutical Opthalmic MicroemulsionFormulation by RP-HPLC, J Anal Bioanal Tech 6:284.R.V Rele, (2011).

7. Simultaneous RP HPLC determination of Latanoprost and Timolol Maleate in combined pharmaceutical dosage form, J. Chem. Pharm.Res., 3(1):138-144.

8. Swartz M. E. (MAY 2005) , Ultra Performance Liquid Chromatography(UPLC): An Introduction, Separation Science Re-Defined, LCGC Supplement,p. 8-11.Swartz M., (2005) Ultra Performance Liquid Chromatography. LCGC. ; 23(1):46–53.

9. U.satyanarayana, (June-2016) Reversed-phase HPLC method development and validation of latanoprost in ophthalmic solution and identification of unknown impurities by LC-MS/MS, International Journal Of Pharmacy &Technology IJPT | Vol. 8 | Issue No.2 | 13941-13940.
10. Van Deemter JJ, Zuiderweg EJ, Klinkenberg (1956) A Longitudinal diffusion and resistance to mass transfer as causes of non ideality in chromatography .Chem. Eng. Sci.; 5: 271–289.

11. Wu N., Lippert J.A. And Lee M.L., (2001) "Practical aspects of ultra highpressure capillary liquid chromatography,,J. Chromatogr., A 911: 1–12.Website information: Acquity columns. www.waters.com/acquitycolumns