ORIGINAL ARTICLE

Development and validation of a stability-indicating reverse-phase ultra-performance liquid chromatography method for the simultaneous determination of netarsudil and latanoprost in bulk and pharmaceutical formulation

G. Dharmamoorthy*[1], Dr. K. S. Nataraj[2], Dr. A. Krishna Manjari.Pawar[3]

[1]Research Scholar, Andhra University, Visakhapatnam, Andhra Pradesh, India, [2]Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India, [3]Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

Abstract

Objective: A new sensitive and simple stability-indicating reverse-phase ultra-performance liquid chromatography (RP-UPLC) method for the simultaneous estimation of netarsudil (NT) and latanoprost (LT) in bulk and pharmaceutical formulation. **Materials and Methods:** Chromatographic separation was achieved through BEH C18 column (50 mm × 2.8 mm i.d × 1.8 µm particle size) using water: methanol (70:30 v/v) mixture used as the mobile phase. The water ACQUITY Model UPLC system with TUV detector and EMPOWER version 2.0 software was monitored at detection wave length 220 nm on isocratic mode with flow rate 0.3 ml/min and the method was validated as per ICH guidelines. **Results and Discussion:** By applying the proposed method, the retention times of NT and LT were found to be 1.448 and 1.868 min, respectively, and the peak shapes were good. The resolution was found to be 3.8, which indicate good separation between the drug peaks. Quantitative linearity was obeyed in the concentration range of 2.5–15 µg/mL for NT and 0.625–3.75 µg/ml for latanoprost. The proposed stability-indicating RP-UPLC method has been developed and validated and found to be simple, specific, accurate, precise, and less time consuming. **Conclusion:** This method was successfully applied for the determination of NT and LT in their pharmaceutical formulation and hence can be used for the routine analysis of these drugs in combined dosage form.

Keywords: ICH guidelines, netarsudil and latanoprost, reverse-phase ultra-performance liquid chromatography, validation

INTRODUCTION

Netarsudil (NT)[1,2] is chemically described as (4-((1S)-1-(Aminomethyl)-2-(isoquinolin-6-ylamino)-2-oxoethyl) phenyl)methyl 2,4-dimethylbenzoate NT [Figure 1]. Its empirical formula is $C_{28}H_{27}N_3O_3$ and its molecular weight is 453.54. Latanoprost (LT) [Figure 2] is chemically described as isopropyl (Z)-7-((1R,2R,3R,5S)-3,5-dihydroxy-2-((3R)-3-hydroxy-5-phenylpentyl) cyclopentyl)-5-heptenoate. Its molecular formula is $C_{26}H_{40}O_5$ and its molecular weight is 432.5928. NT is an a rho kinase inhibitor with norepinephrine transport inhibitory activity that reduces production of aqueous which is used for the treatment of reducing elevated intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension and LT is a prodrug analog of prostaglandin F2 alpha that is used to treat elevated IOP. It was initially approved by the FDA in 1998. LT is the first topical prostaglandin F2 alpha analog used for glaucoma treatment.[3,4] It has been found

Address for correspondence:
G. Dharmamoorthy, Andhra University, Visakhapatnam, Andhra Pradesh, India. E-mail: dharmamoorthy111@gmail.com

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to be well-tolerated and its use does not normally result in systemic adverse effects like other drugs used to treat elevated IOP, such as timolol. Another benefit LT is that it can be administered once a day.

As far as literature is concerned, there are no reported methods for the determination of NT and LT in combined pharmaceutical dosage form using reverse-phase ultra-performance liquid chromatography (RP-UPLC) methods. All the UPLC methods lack stability-indicating nature. However, none of the reported analytical methods describe a stability-indicating method by UPLC for the simultaneous determination of NT and LT in a combined dosage form. To the best of our knowledge, this was the first report of a stability-indicating method for the simultaneous determination of both NT and LT in pharmaceutical dosage forms by UPLC. The present manuscript describes a simple, rapid, precise, and accurate isocratic reversed-phase stability-indicating UPLC method for the simultaneous determination of NT and LT in the same pharmaceutical dosage form and validated as per ICH guideline.[5-11]

MATERIALS AND METHODS

Instruments Used

UPLC used for the study was Waters ACQUITY separation module equipped with Waters TUV detector and EMPOWER version 2.0 software. The chromatographic separation was achieved on a BEH C18, 1.8 µm, 50 mm ×1.8 mm, i.d. column at a temperature of 300°C. Sartorius electronic analytical balance was used for weighing purpose and Orion digital pH meter was used to adjust the pH. Millipore vacuum filter was used to filter the mobile phase and Power Sonic 510 ultra-bath sonicator was used to remove the dissolved gases.

Drugs and Chemicals

NT (99.6%) and LT (99.7%) were kindly supplied by Spectrum Pharma Research Solutions, Hyderabad, India, and were used without further purification. Rocklatan 0.02% (NT)/0.005% (LT) mg ophthalmic solution was purchased from local market. Acetonitrile, potassium dihydrogen phosphate, orthophosphoric acid, sodium hydroxide, hydrochloric acid, and hydrogen peroxide were obtained from Merck (India). All reagents used were at least of analytical grade except acetonitrile which was grade. Milli-Q grade water was obtained following distillation in glass and passage through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

Preparation of Stock Solution

Accurately weighed 5 mg of NT and 1.25 mg of LT and transferred to individual 50 ml volumetric flasks separately. Three-fourth of diluents was added to both of these flasks and sonicated for 10 min. Flasks were made up with diluents and labeled as standard stock solution 1 and 2 (100 µg/ml of NT and 25 µg/ml of LT).

Preparation of Mobile Phase

Mobile phase was prepared by mixing water and water in the ratio of 70:30 v/v and sonicated for about 20 min.

Preparation of Placebo Solution

About 10 mg of the placebo powder was weighed accurately and transferred into a 100 mL volumetric flask and added 50 mL of mobile phase and the solution was sonicated for about 10 min. Then, the volume was made up to the mark with mobile phase and the solution was filtered through a 0.45 µm membrane filter. A 1 mL of the filtered solution was transferred to a 10 mL volumetric flask and the volume was made up to the mark with mobile phase.

Selection of Detection Wavelength

Both the drugs, NT and LT, were scanned in the wavelength region of 200–400 nm using TUV detector. It was found that both the drugs have shown good peak response at a detection wavelength of 220 nm. Therefore, 220 nm was selected as detection wavelength in the present study.

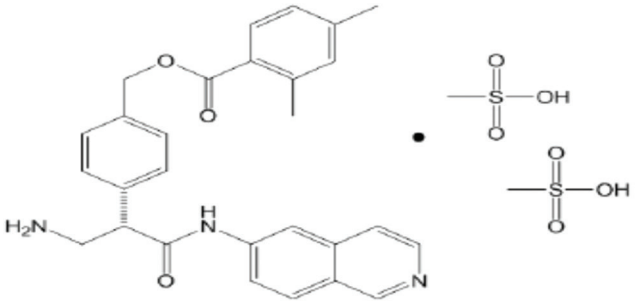

Figure 1: Chemical structure of netarsudil

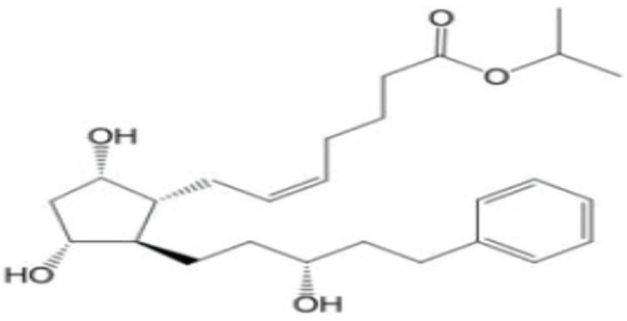

Figure 2: Chemical structure of latanoprost

Method Development

Method development was done by changing various, mobile phase ratios, buffers and columns, etc.

Optimized Chromatographic Conditions

Mobile phase: 70% water:30% methanol
Flow rate: 0.3 ml/min
Column: HSS C18 (2.6 × 50 mm, 1.8 µm)
Detector wavelength: 220 nm
Column temperature: 30°C
Injection volume: 1.0 mL
Run time: 5.0 min
Diluent: Water and methanol in the ratio of 50:50.

RESULTS AND DISCUSSION

In this trail by increasing the water solution, NT and LT were eluted with good peak shape here the representive chromatogram present in the Figure 3. As per ICH guidelines, all system suitability parameters were within a limit. Hence, these methods were optimized and to be validated.

Method Validation

System suitability

System suitability test was carried out on a freshly prepared standard solution containing 10.0 µg/mL of NT and 2.5 µg/mL of LT. A 2.0 µL of this solution was injected 6 times into the UPLC system under optimized chromatographic conditions. Parameters that were studied to evaluate the suitability of the system were number of theoretical plates, asymmetric factor, resolution, and retention time. The values of the system suitability are shown in Tables 1 and 2.

Specificity

Blank and placebo interference

A study to establish the interference of blank and placebo was conducted. Analysis was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of blank and placebo solutions had shown no peaks at the retention times of NT and LT. This indicates that the excipients used in the formulation do not interfere in the estimation of NT and LT.

Preparation of degradation samples for specificity study

To establish whether the analytical method and the assay were stability-indicating, Rocklatan ophthalmic solution and pure active pharmaceutical ingredient of both NT and LT was stressed under various conditions such as acid, base, thermal, oxidative, and photolytic conditions to conduct forced degradation studies. As these drugs are freely soluble in acetonitrile, it was used as a solvent and diluent in all the forced degradation studies.

Table 1: System suitability of netarsudil

S. No.	Peak response	Retention time	Theoretical plates	Asymmetric factor
1.	176,593	1.448	3110	1.32
2.	175,775	1.449	3045	1.34
3.	178,859	1.449	3173	1.31
4.	176,721	1.451	3070	1.32
5.	179,755	1.452	3077	1.33
Mean	178,654	1.452		
SD	177,726	1.448		
%RSD	1572.4	1.449		

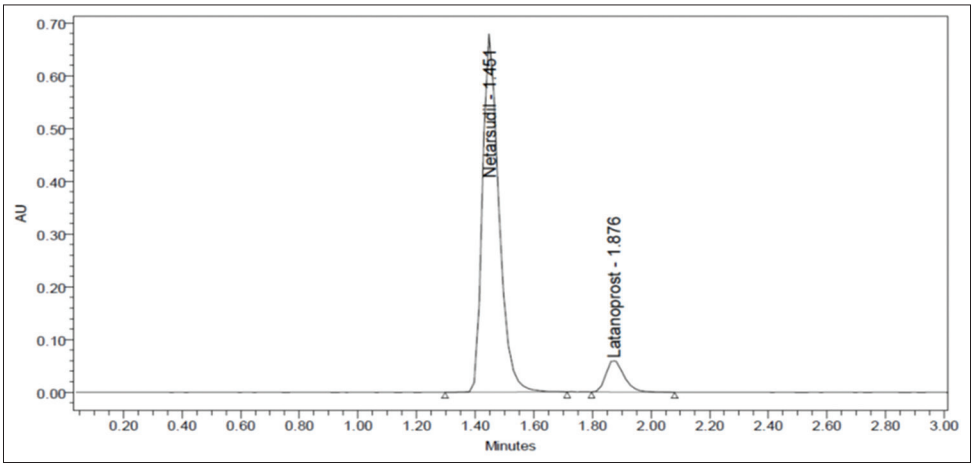

Figure 3: Optimized chromatogram

Linearity and Range

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. A series of working standard solutions were prepared in 10 mL calibrated volumetric flasks by appropriate dilution of the stock solution with mobile phase to obtain a concentration range of 2.5–15 µg/ml for NT and 0.625–3.75 µg/ml for LT, respectively. Each solution was injected 3 times and the chromatograms were recorded. Calibration curves for both the drugs were plotted by taking average peak area on the Y-axis and the concentration on the X-axis. The linearity data and the calibration curves are presented in Tables 3 and 4 and Figures 4 and 5.

Precision

The intraday and interday precision study of the proposed method was carried out by finding the responses of single concentration of NT (10 µg/mL) and LT (2.5 µg/mL). The results were interpreted by statistical analysis by calculating the %RSD values and the results are presented in Tables 5 and 6.

Accuracy

The accuracy of the method was determined by calculating recoveries of NT and LT by the method of standard addition. Known amount of NT and LT was added to the placebo at 50%, 100%, and 150% levels. About 50%, 100%, and 150% level solutions were prepared 6 times by appropriate dilution. All the prepared solutions were filtered through 0.35 µm syringe filters and filled in separate UPLC vials. A 2.0 µL of each of these solutions were injected into the UPLC system and the chromatograms were recorded using optimized chromatographic conditions. The mean% recovery values and the %RSD values were calculated and given in Tables 7 and 8.

Robustness

Robustness of the method was studied by deliberately altering the composition of organic phase by ±10%, pH by ±0.1, flow rate by ±0.1 mL, and column temperature by ±5°C. No marked changes were observed in the system suitability parameters and the results are presented in Table 9.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD was determined based on signal-to-noise ratios and was determined using an analytical response of 3 times the background noise. The LOD for both NT and LT was found to be 0.09 µg/ml and 0.01 µg/ml, respectively.

The LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections. The % RSD for these studies was <0.54%. The LOQ that produced the requisite precision and accuracy was found to be 0.27 µg/ml and 0.3 µg/ml for NT and LT, respectively.

Table 2: System suitability of latanoprost

S. No.	Peak response	Retention time	Theoretical plates	Asymmetric factor	Resolution
1.	17,827	1.868	4324	1.30	3.8
2.	17,961	1.870	4261	1.32	3.8
3.	18,137	1.870	4085	1.36	3.7
4.	17,686	1.882	4523	1.27	3.8
5.	17,894	1.887	4215	1.34	3.8
Mean	18106	1.895			
SD	17,935	1.868			
%RSD	170.9	1.870			

Table 3: Linearity data for netarsudil

S. No.	Linearity level	Concentration (µg/mL)	Peak response	Regression analysis
1.	LOQ	2.5	43,783	Regression equation: y=17,648x+941.7
2.	25%	5	89,228	
3.	50%	7.5	136,030	
4.	75%	10	178,548	
5.	100%	12.5	222,953	R^2=0.999
6.	125%	15	262,564	
7.	150%	2.5	43,783	

Table 4: Linearity data for latanoprost

S. No.	Linearity level	Concentration (µg/mL)	Peak response	Regression analysis
1.	LOQ	0.625	4385	Regression equation: y=7030x+67.03
2.	25%	1.25	8801	
3.	50%	1.875	13,477	
4.	75%	2.5	17,942	
5.	100%	3.125	21,750	R^2=0.999
6.	125%	3.75	26,386	
7.	150%	0.625	4385	

Table 5: Intraday and interday precision data for netarsudil

S. No.	Concentration (µg/mL)	Intraday precision		Interday precision	
		Mean±SD	%RSD	Mean±SD	%RSD
1.	10	9.994±0.116	1.2	9.24±0.09	1.0

SD: Standard deviation

Table 6: Intraday and interday precision data for latanoprost

S. No.	Concentration (µg/mL)	Intraday precision		Interday precision	
		Mean±SD	%RSD	Mean±SD	%RSD
1	2.5	2.48±0.006	0.7	2.22±0.12	1.4

SD: Standard deviation

Table 7: Accuracy data for netarsudil

% Spike level	Amount added (mg)	Amount recovered (mg)	% recovery	% mean recovery±SD	%RSD
50%	5	4.956	99.11	99.00±0.5	0.43
	5	5.037	100.74		
	5	4.964	99.29		
100%	10	9.913	99.13	99.26±0.18	0.16
	10	9.944	99.44		
	10	9.922	99.22		
150%	15	15.008	100.05	100.06±0.05	0.28
	15	15.053	100.35		
	15	14.968	99.79		

Table 8: Accuracy data for latanoprost

Spike level (%)	Amount added (mg)	Amount recovered (mg)	% recovery	% mean recovery± SD	% RSD
50	1.25	1.239	99.13	98.95±0.08	0.39
	1.25	1.231	98.51		
	1.25	1.240	99.22		
100	2.5	2.459	98.36	100.98±0.16	0.23
	2.5	2.518	100.74		
	2.5	2.511	100.42		
150	3.125	3.137	100.38	99.91±0.05	0.64
	3.125	3.100	99.19		
	3.125	3.130	100.17		

Table 9: Robustness study data of the proposed method

Optimum conditions	Modifications	Retention time		Asymmetric factor		Theoretical plates		Resolution
		NT	LT	NT	LT	NT	LT	
Mobile phase composition (buffer: ACN) (60:40 v/v)	65:35	1.524	1.968	1.23	1.14	3544	5038	3.9
	55:45	1.260	1.624	1.26	1.12	2833	4443	3.6
Column temperature (30°C)	25	1.458	1.912	1.29	1.28	3273	5035	4.2
	35	1.449	1.860	1.28	1.30	3354	4744	3.9
Flow rate (1.5 mL/min)	0.2	1.441	1.812	1.27	1.33	3449	4493	3.5
	0.4	1.463	1.941	1.29	1.36	3281	4491	4.3

NT: Netarsudil, LT: Latanoprost

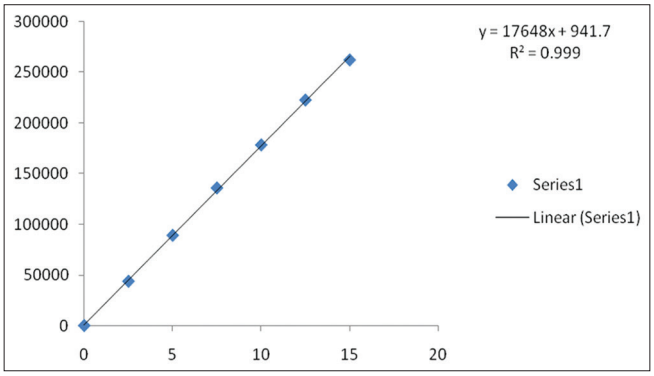

Figure 4: Calibration curve of netarsudil

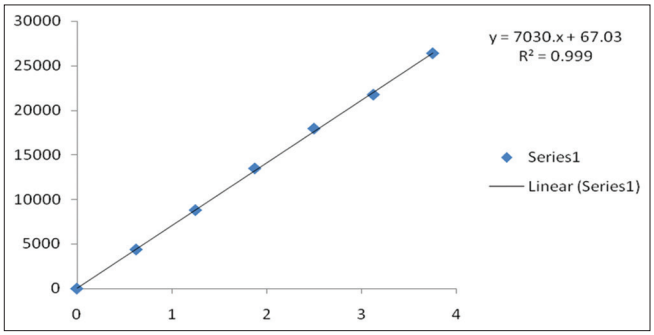

Figure 5: Calibration curve of latanoprost

DISCUSSION

By applying the proposed method, the retention times of NT and LT were found to be 1.448 and 1.868 min, respectively, and the peak shapes were good. The resolution was found to be 3.8, which indicates good separation between the drug peaks. Quantitative linearity was obeyed in the concentration range of 2.5–15 µg/mL for NT and 0.625–3.75 µg/ml for LT, respectively. The regression equations of concentration of NT and LT over their peak areas were found to be $y = 17{,}732x+1038.2$ ($R^2=0.999$) and $y = 7030.2x+67.036$ ($R^2=0.999$), respectively, where x is the concentrations and y is the peak area of NT and LT (µg/mL).The lower % RSD values of intraday and interday precision study have shown that the method is precise. The higher % recovery values indicate that the method is accurate. The limits of detection were found to be 2.4 µg/ml and 0.9 µg/ml for NT and LT, respectively. The limits of quantification were found to be 0.27 µg/ml and 0.03 µg/ml for NT and LT, respectively. Both LOD and LOQ indicate the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that the excipients used in ophthalmic formulation and the degradation products from the drug substances did not interfere with the estimation of the drugs by the proposed stability-indicating RP-UPLC method.

CONCLUSION

Thus, in the proposed study, a stability-indicating RP-UPLC method has been developed and validated and found to be simple, specific, accurate, precise, and less time consuming. This method was successfully applied for the determination of NT and LT in their pharmaceutical formulation and hence can be used for the routine analysis of these drugs in combined dosage form.

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