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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF NETARSUDIL AND LATANOPROST IN BULK AND OPHTHALMIC SOLUTIONS

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of netarsudil and latanoprost in ophthalmic solutions. Chromatogram was run through Agilent C18 150 x 4.6 mm, 5µ. Mobile phase containing 0.01N potassium dihydrogen ortho phosphate: methanol taken in the ratio 55:45 was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 30ºC. Optimized wavelength selected was 220nm. Retention time of netarsudil and latanoprost were found to be 2.589min and 3.221min. %RSD of method precision for netarsudil and latanoprost were found to be 0.7 and 1.4, respectively. % Recovery was obtained 99.86 % and 100.47% for netarsudil and latanoprost, respectively. LOD & LOQ values obtained from regression equations of netarsudil and latanoprost were 0.07, 0.02 and 0.21, 0.07 respectively. Regression equation of netarsudil is y = 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.

KEYWORDS: Netarsudil, Latanoprost, RP-HPLC.

INTRODUCTION

Netarsudil is a drug for the treatment of glaucoma. Netarsudil a rho kinase inhibitor with nor epinephrine transport inhibitory activity that reduces production of aqueous. As of December 18, 2017 the FDA approved Aerie Pharmaceutical's Rhopressa (netarsudil ophthalmic solution) 0.02% for the indication of reducing elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. Acting as both a rho kinase inhibitor and a nor epinephrine transport inhibitor, netarsudil is a novel glaucoma medication,[16] in that it specifically targets the conventional trabecular pathway of aqueous humor outflow to act as an inhibitor to the rho kinase and nor epinephrine demonstrate.

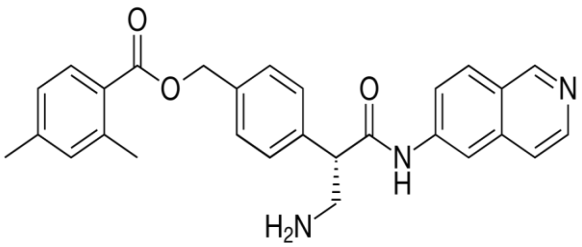

Figure 1: Chemical structure of netarsudil.

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 prostaglandin in F2 alpha analog used for glaucoma treatment.[17] 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 it can be administered once a day.

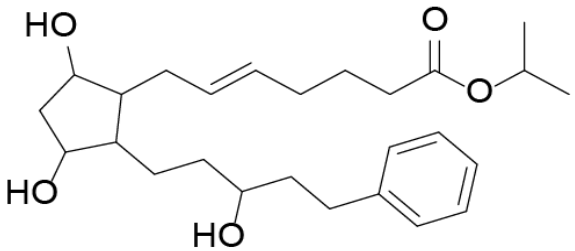

Figure 2: Chemical structure of latanoprost.

The literature survey revealed that there are a few HPLC and spectroscopic methods available for the determination of netarsudil and latanoprost in combination (18) with other drugs. In view of non-availability of HPLC methods for the selected

combination, as per literature, author has aimed to develop a new HPLC method for simultaneous estimation of netarsudil and latanoprost in combined pharmaceutical dosage form.

Experimental

Chemicals and reagents: Netarsudil and Latanoprost pure drugs (API), Combination netarsudil and latanoprost ophthalmic solution (ROCKLATAN), Distilled water, acetonitrile, phosphate buffer, methanol, potassium dehydrogenate ortho phosphate buffer, ortho-phosphoric acid. All the above chemicals and solvents are from rankem.

Equipments: Electronics balance-denver, p^H meter - BVK enterprises, India 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 bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of netarsudil and latanoprost solutions.

Chromatographic condition: The mobile phase used was 0.01N potassium dihydrogen ortho phosphate and methanol in the gradient mode employing flow rate at 1 ml/min. The analytical column Inspire C18 (4.6 x 150 mm, 5 µm). The detection was carried out at a wavelength of 220 nm with a run time of 5 min. Water and acetonitrile in the ratio of 50:50 v/v used as diluent.

Preparation of solutions

Preparation of buffer solution: 0.01N potassium dihyrogen ortho phosphate

Accurately weighed 1.36gm of potassium dihyrogen ortho phosphate in a 1000ml of volumetric flask then 900ml of milli-Q water was added and degassed. Finally made up the volume with water then p^H is adjusted to 3.5 with dil. ortho phosphoric acid solution.

Preparation of mobile phase: A mixture of the above 0.01N potassium dihydrogen ortho phosphate buffer and methanol was used as mobile phase and used in the gradient mode

The 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 solution: Accurately weighed 5mg of netarsudil, 1.25mg of latanoprost and transferred to individual 50ml volumetric flasks separately. $3/4^{th}$ of diluent was added to both of the flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as standard stock solution 1and 2. (100µg/ml of netarsudil and 25µg/ml of latanoprost)

Preparation of sample solution: Solution from 10 vials were pooled into a beaker, then 10 ml of this solution was further diluted with diluent to get a final injected solution and filtered by using HPLC filters (100µg/ml of netarsudil and 25µg/ml of latanoprost).

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1 ml/min for 5 minutes to equilibrate the column at ambienttemperature. Chromatographic separation was achieved by injecting a volume of 10µl of standard into inspire C18 (4.6 x 150 mm, 5 µm) column, the mobile phase of composition 0.01N potassium dihydrogen ortho phosphate buffer and methanol in the gradient mode was allowed to flow through the column at a flow rate of 1ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in Table 1 and figure 3.

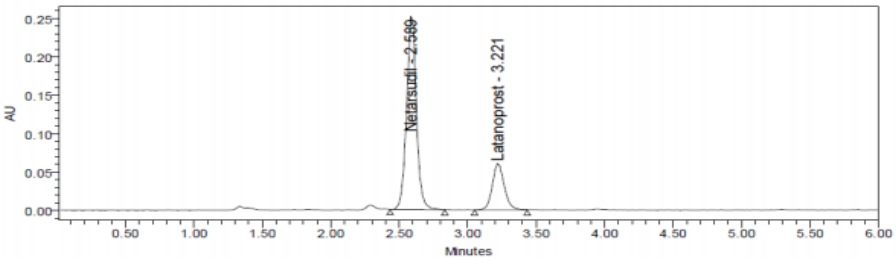

Figure 3: Standard chromatogram

Table 1: System suitability parameters.

Parameters	Netarsudil	Latanoprost
Retention time (min)	2.589	3.244
USP Plate count	6700.33	6821
USP Tailing	1.05	1.10

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine netarsudil and latanoprost simultaneously in their pharmaceutical dosage form. The result obtained for netarsudil and latanoprost was comparable with the corresponding labeled amounts and they were shown in Table-2 and figure-4.

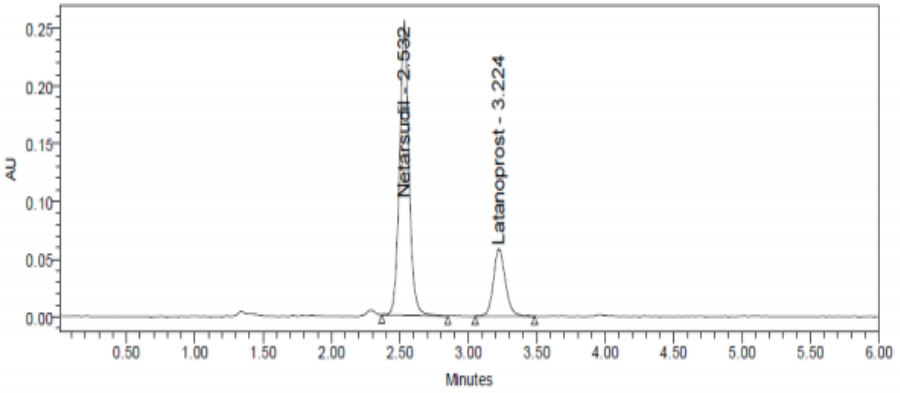

Figure 4: Sample chromatogram.

Table 2: Assay results for netarsudil and latanoprost.

	Label Claim (mg)	% Assay
Netarsudil	5	99.12±0.88
Latanoprost	1.25	99.54±0.46

Method validation

The method was validated in accordance with ICH guidelines.[8]

Linearity: Linearity of the method was studied by injecting six concentrations of the drugs in triplicate prepared in the range of 2.5-15µg/ml for netarsudil and 0.625-3.75µg/ml for latanoprost into the HPLC system. Linear graphs were plotted by using the peak areas against concentration in µg/ml from which the correlation coefficients, slopes and *Y*-intercepts of the calibration curves were determined. The results were shown in Table 3 and figure 5 & 6.

Table 3: Linearity results for netarsudil and latanoprost.

Parameters	Netarsudil	Latanoprost
Concentration range(µg/ml)	2.5-15	0.625-3.75
Correlation coefficient	0.9997	0.9995
Intercept	11999	13329
Slope	1319.8	380.64

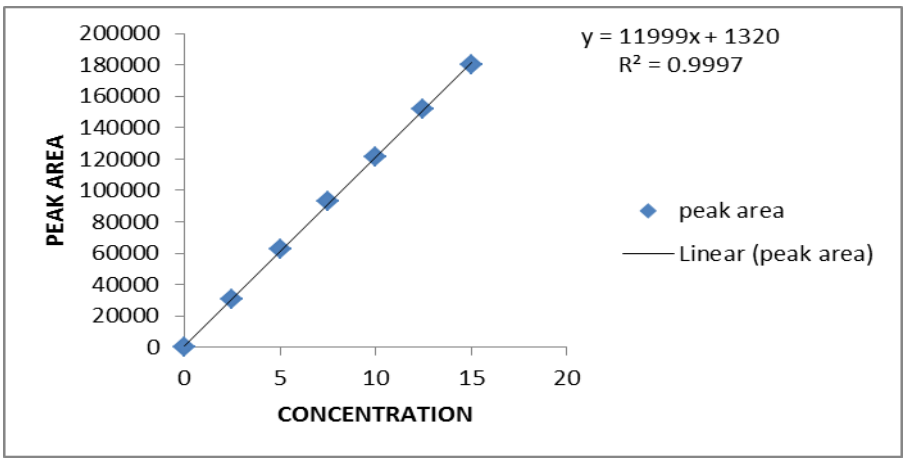

Figure 5: Linearity graph for netarsudil.

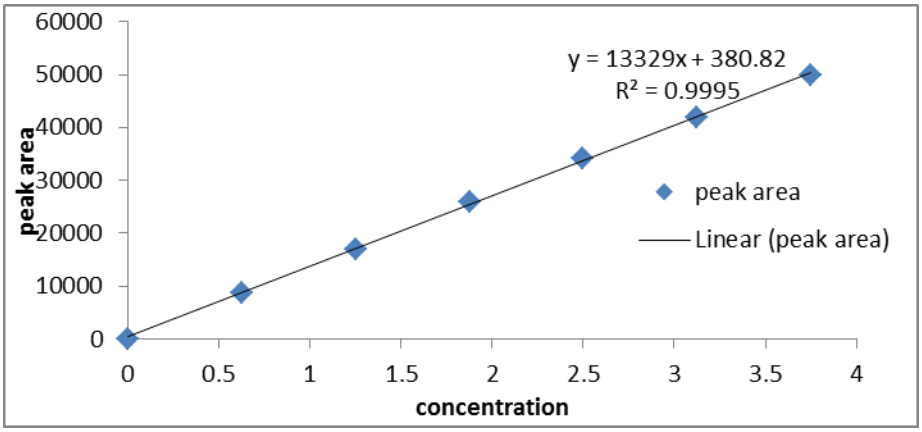

Figure 6: Linearity graph for latanoprost.

Accuracy: The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed sample. Percent recovery was calculated by comparing the area with pre analysed sample. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from pre analysed sample area. The results were shown in Table-4 and Table-5.

Table 4: Accuracy results for netarsudil.

% Level	Amount Spiked (µg/mL)	% Recovery	Mean % Recovery
50%	**5**	**98.86**	**99.86%**
	5	**100.54**	
	5	**100.06**	
100%	**10**	**100.45**	**99.86%**
	10	**100.04**	
	10	**100.35**	
150%	**15**	**98.34**	**99.86%**
	15	**98.11**	
	15	**100.04**	

Table 5: Accuracy results for latanoprost.

% Level	Amount Spiked (µg/mL)	% Recovery	Mean % Recovery
50%	**1.25**	**99.46**	**100.47%**
	1.25	**101.63**	
	1.25	**100.14**	
100%	**2.5**	**98.75**	**100.47%**
	2.5	**99.85**	
	2.5	**100.42**	
150%	**3.75**	**100.83**	**100.47%**
	3.75	**100.89**	
	3.75	**102.23**	

Precision: For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The sample was injected in six replicate for intermediate precision and six replicate for precision. The peak area for injections was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed HPLC method was found to be precise. The Precision results were shown in Table-6 and Intermediate Precision were shown in Table-7.

Table 6: Precision results for netarsudil and latanoprost.

Injection	Netarsudil	Latanoprost
Injection 1	120822	35848
Injection 2	120912	36210
Injection 3	122079	35221
Injection 4	122699	35813
Injection 5	122491	34806
Injection 6	122444	35863
Average	121908	35627
Standard deviation	831.1	513.4
% RSD	0.7	1.4

Table 7: Intermediate Precision results for netarsudil and latanoprost.

S. NO.	Area of Netarsudil	Area of Latanoprost
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

Robustness: Robustness of the method was checked by making deliberate changes in chromatographic conditions like mobile phase ratio (±10%), and flow rate (5 ml/min). It wasobserved that there were no marked changes in system suitability parameters, which demonstrated that the developed HPLC method is robust.

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method

CONCLUSION

In the present work a new, accurate, precise and robust HPLC method was developed and validated for simultaneous estimation of netarsudil and latanoprost in pharmaceutical dosage form in accordance with the ICH Guidelines. The method gives good resolution between both the compounds with a short analysis time (5 min). Linearity is observed in the concentration range of 2.5-15µg/ml for netarsudil and 0.625-3.75µg/ml for latanoprost. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the netarsudil and latanoprost in combined dosage form without any interference from excipients.

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