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Research Article

Development and Validation of Stability Indicating Analytical Method for the Simultaneous Estimation of Netarsudil and Latanoprost by RP-HPLC

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ABSTRACT

A simple, Accurate, Precise method was developed for the simultaneous estimation of the Netarsudil and Latanoprost in opthalmic solution dosage form. Chromatogram was run through Zodiacsil C18 150 x 4.6 mm, 5µ. Mobile phase containing 0.01N Ammonium acetate: Methanol taken with in the ratio 55:45 was pumped through column on a flow rate at 1.0ml/min. Buffer used in this method was Ammonium acetate. Temperature was maintained at 30ºC. Optimized wavelength selected was 225nm. Retention time of Netarsudil and Latanoprost were found to be 2.222 min and 2.706 min. %RSD of the Netarsudil and Latanoprost were and found to be 0.5 and 0.8 respectively. %Recovery was obtained as 100.09% and 100.5% for Netarsudil and Latanoprost respectively. LOD, LOQ values obtained from regression equations of Netarsudil and Latanoprost were 0.07, 0.22 and 0.03, 0.09 respectively. Regression equation of Netarsudil y = 33253x +578.8 and y =45497x + 399.2 of Latanoprost. Retention times were decreased and that runtime 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

INTRODUCTION

Netarsudil is approved by the FDA in March 2019 for use in combination with Latanoprost. Netarsudil is a Rhokinase inhibitor with norepinephrine transport inhibitory activity that reduces production of aqueous where it reduces elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. The combination drug is marketed by Aerie Pharmaceuticals under the brand Rocklatan (0.02%netarsudil &0.005% latanoprost opthalmic solution). Latanoprost is the first topical prostaglandin F2 alpha analogue used for glaucoma treatment. It can be administered once a day. 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

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pressure. The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product[4].

Literature survey revealed few analytical methods is reported for both the drugs in alone. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method with UV detection for the analysis of Netarsudil and Latanoprost in bulk samples and combined dosage formulation.

Objective: Following are the objectives of the present work:

- To develop a new stability indicating HPLC method for the simultaneous estimation of Netarsudil and Latanoprost and to develop the validated method according to ICH guidelines.
- To apply the validated method for the simultaneous estimation of Netarsudil and Latanoprost in pharmaceutical formulation.

EXPERIMENTAL

Chemicals and reagents: Netarsudil and Latanoprost pure drugs (API), combination Netarsudil and Latanoprost Opthalmic solution (ROCKLATAN), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer and Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Apparatus and chromatographic condition: The chromatographic separation was performed on a HPLC system (WATERS) Series Alliance e2695 Software EMPOWER- 2, integrated with Auto Sampler and 2998 PDA detector. The mobile phase was prepared freshly, filtered, sonicated be for use and delivered at a flow rate of 1.0 mL/min and the detector wavelength was set at 225 nm. The injection volume was 10 µL. Diluent used was Methanol and Water taken in the ratio of50:50.

Preparation of standard and sample solutions
Standard solution: Accurately weighed 10mg of Netarsudil, 2.5mg of Latanoprost transferred to 50ml volumetric flask and 10ml of diluent was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (200µg/ml Netarsudil, and 50µg/ml of Latanoprost)

Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Netarsudil and 5µg/ml of Latanoprost)

Sample Solution: 10 vials were weighed and was transferred into a 10 ml volumetric flask ,5ml of diluents was added and sonicated for10min, further the volume was made up with diluent and filtered by HPLC filters (20µg/ml Netarsudil, and 5µg/ml of Latanoprost). 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml Netarsudil, and 5µg/ml of Latanoprost).

Preparation of buffer: 0.01N Ammonium acetate Accurately weighed 0.77gm of Ammonium acetate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water to get 0.01N Ammonium acetate buffer.

Method validation
All of the analytical validation parameters for this proposed method were determined according to ICH guidelines[6]. Obtained validation parameters are presented in Table 1.

System suitability: All the system suitabilty parameters were within the range and satisfactory as per ICH guidelines (Table-2).

Specificity: Retention times of Netarsudil and Latanoprost were 2.222min and 2.706min respectively. We did not find any interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific Fig 6.

Linearity: The calibration curve was constructed by plotting response factor against respective concentration of Netarsudil and latanoprost. The plots of peak area Vs respective concentration of Netarsudil and Latanoprost were found to be linear in the range of 5-30µg/mL and 1.25-7.5µg/mL with coefficient of correlation (r^2) 0.999 for two drugs. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Netarsudil and Latanoprost were given in Fig. 4 andFig.5.

Precision: From a single volumetric flask of working standard solution six injections were given. A study was carried out for Repeatability under same operating conditions and also for intermediate precision with the same analyst on the different day for six sample preparations. Average

area, standard deviation and %RSD were calculated for two drugs in both methods. results obtained are presented in Table 4. As the limit of precision was less than "2" in both methods ,the system precision was passed in these methods.

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 100.09% and 100.57% for Netarsudil and Latanoprost respectively. The obtained results are presented in Table3.

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. The limit of detection (LOD) and limit of quantification (LOQ) were found to 0.07µg/ml and 0.22µg/ml for Netarsudil and 0.03µg/ml and 0.09µg/ml for Latanoprost. The LOD and LOQ showed that the method is sensitive for Netarsudil and Latanoprost.

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 (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60B:40A), mobile phase plus (50B:50A), temperature minus (25^{o}C) and temperature plus(35^{0}C) were maintained and samples were injecetd in duplicate manner. System suitabilty parameters were not much affected and all the parameters were passed, %RSD was within the limit.

Assay: (ROCKLATAN), bearing the label claim Netarsudil 0.2mg, Latanoprost 0.05mg. 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

RESULTS AND DISCUSSIONS

Optimization of Chromatographic conditions :A suitable chromatographic method was developed for estimation of Netarsudil and Latanoprost in opthhalmic dosage forms .optimization chromatographic conditions were developed after changing various parameters such as the mobile phase ,injection volume ,flow rate etc .The final analysis was performed by using 55% 0.01N Ammonium acetate : 40% Methanol at a flow rate of 1ml/min .samples were analysed at 225 nm detector wavelength and at an injection volume of 10µl using Zodiasil C18(4.6 x 150mm ,5µm) with run time of 5min.The proposed method was optimized to give sharp peak with good resolution, minimum tailing effect and theoretical plate count for Netarsudil and Latanoprost, optimized chromatogram was obtained as shown in Fig.

Stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Netarsudil and Latanoprost remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table4.

Acid degradation studies: To 1 ml of stock s solution Netarsudil and Latanoprost bromide, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^{0}c. The resultant solution was diluted to obtain 20µg/ml & 5µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Fig.7.

Base degradation studies: To 1 ml of stock solution Netarsudil and Latanoprost, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}c. The resultant solution was diluted to obtain 20µg/ml & 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Fig. 8

Peroxide degradation sample: To 1 ml of stock solution of Netarsudil and Latanoprost, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^{0}c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml & 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of oxidative degradation was given in Fig. 9

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60^{o}C. For HPLC study, the resultant solution was diluted to 20µg/ml & 5µg/ml solution and 10µl were injected into the system and chromatogram were recorded to assess the stability of the sample. The typical

chromatogram of neutral degradation studies given in Fig10.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105ºC for 6h to study dry heat degradation For HPLC study the resultant solution was diluted to 20µg/ml &5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of dry heat degradation was given in Fig 11

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 200µg/ml & 50µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml & 5µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of thermal degradation was given in Fig 12.

CONCLUSION

Chromatographic conditions used are stationary phase Zodiacsil C18 (150 x 4.6mm ,5µ). Mobile phase 0.01N Ammonium acetate: Methanol in the ratio of 55:45 and flow rate was maintained at 1ml/min, detection wavelength was 225 nm, column temperature was set at 30ºC and diluent was methanol: water in ratio 50:50, conditions were finalized as optimized method.

System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, R^2 value was found to be 0.999.

Precision was found to be 0.2 for repeatability and intermediate precision. LOD and LOQ obtained for Netarsudil and Latanoprost were 0.07, 0.22 and 0.03,0.09 respectively. Assay of opthalmic dosage form was carried out % Assay found to be 100.57 %and 100.43% was present.

Degradation studies of Netarsudil and Latanoprost were done, in all conditions purity threshold was more than purity angle and within the acceptable range. 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.

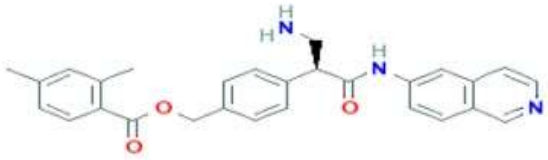

Fig-1 Chemical structure of Netarsudil

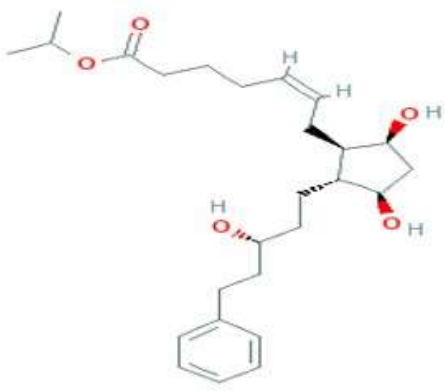

Fig -2 Chemical structure of Latanoprost

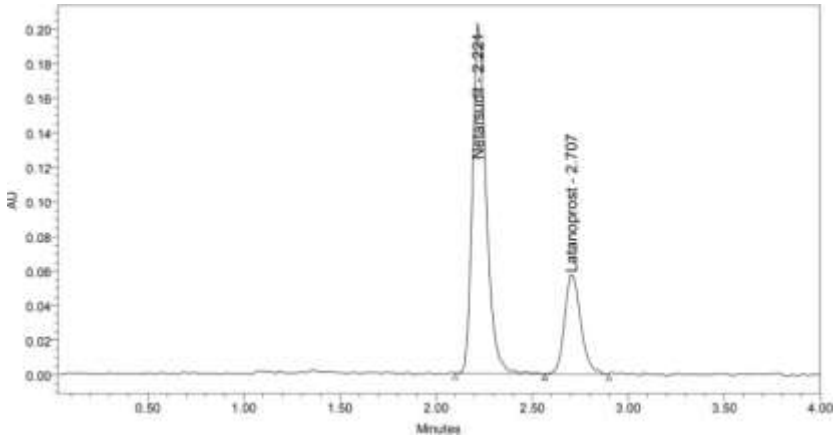

Fig -3 Optimized Chromatogram of Netarsudil and Latanoprost

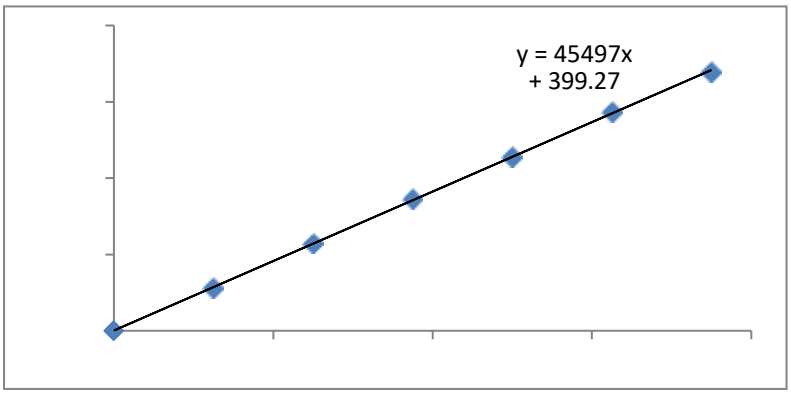

Fig. 4: Calibration curve of Netarsudil

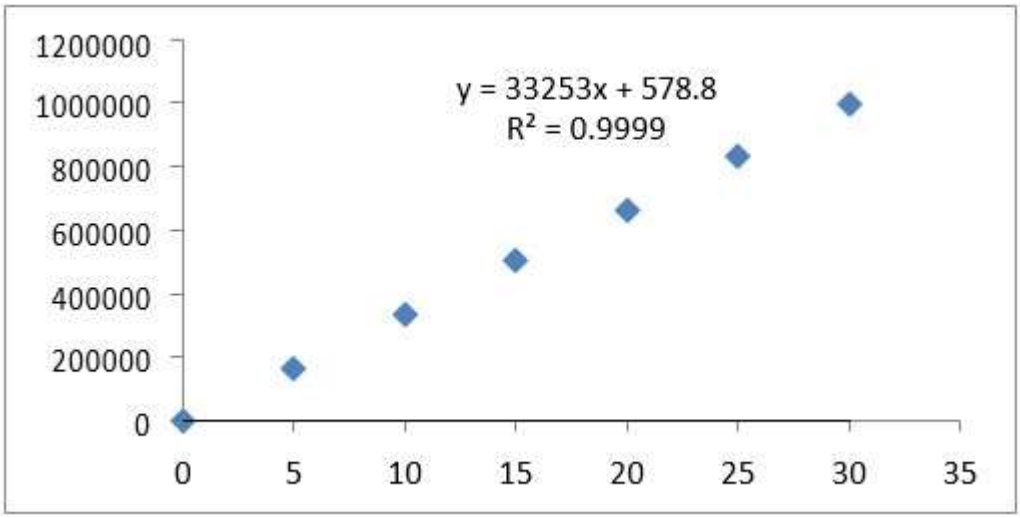

Fig. 5: Calibration curve for Latanoprost

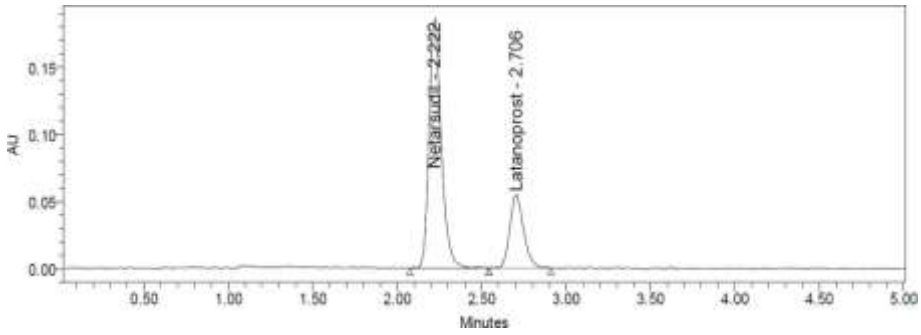

Fig. 6: Typical chromatogram of Netarsudil and Latanoprost

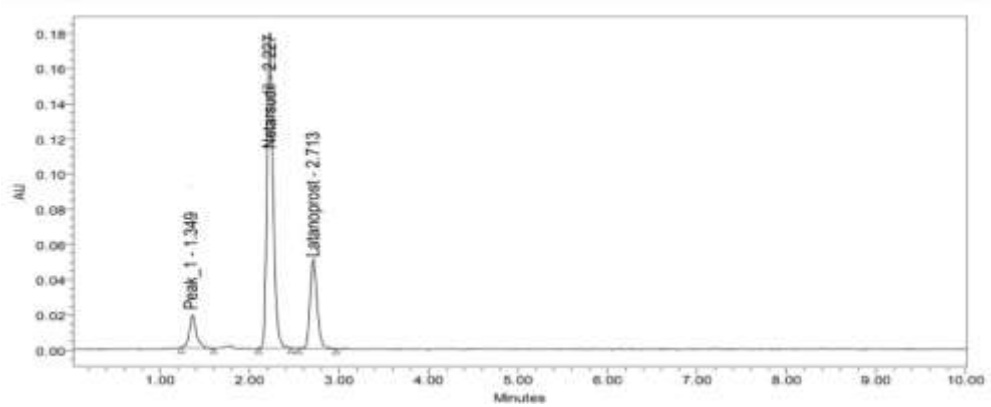

Fig. 7: Acid degradation chromatogram of Netarsudil and Latanoprost

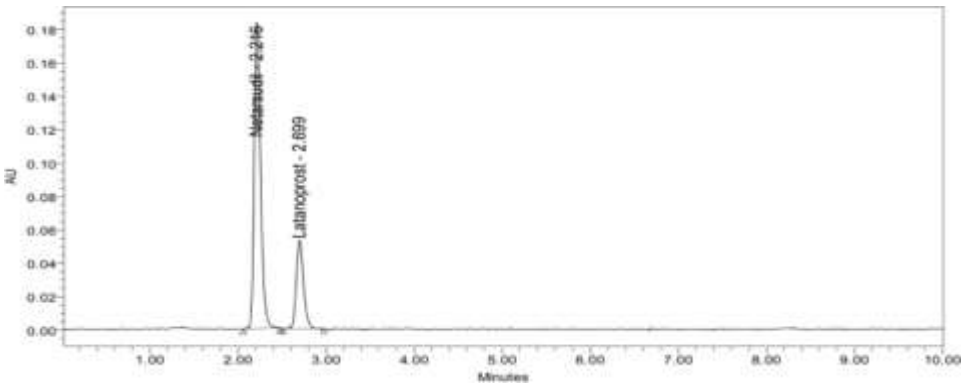

Fig. 8: Base degradation chromatogram of Netarsudil and Latanoprost

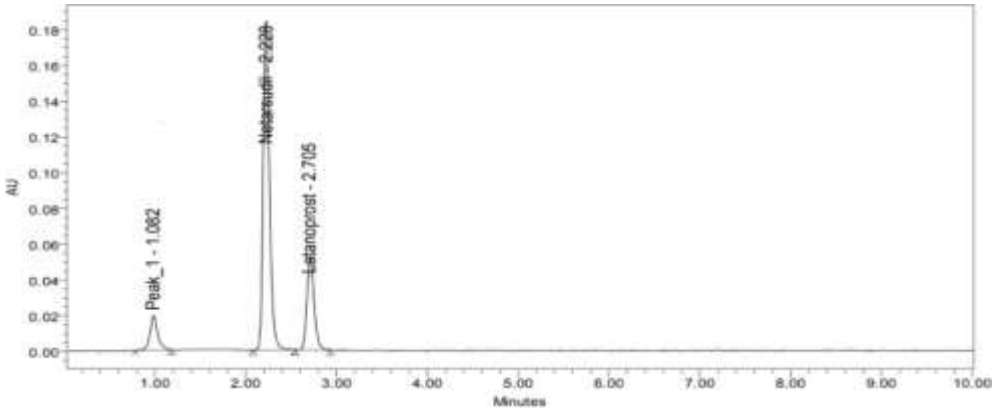

Fig. 9: Peroxide degradation chromatogram of Netarsudil and Latanoprost

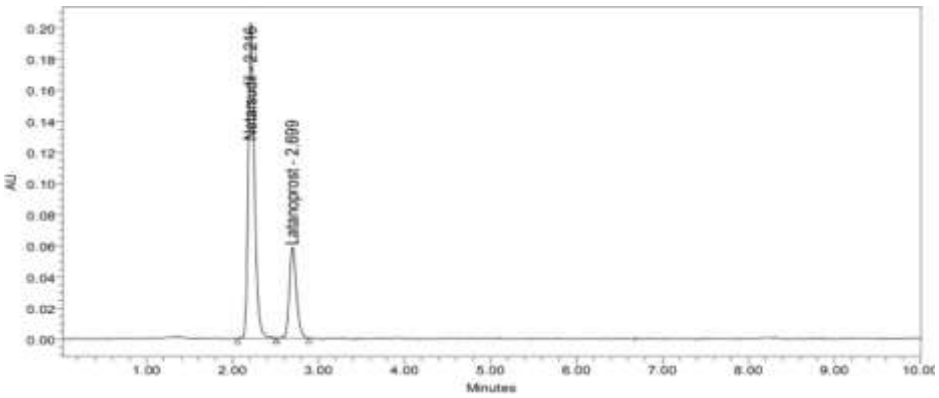

Fig. 10: Water degradation chromatogram of Netarsudil and Latanoprost

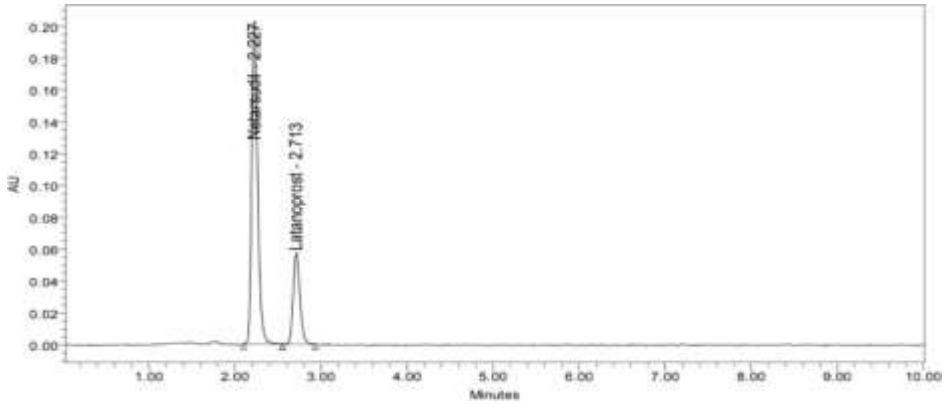

Fig. 11: Thermal degradation chromatogram of Netarsudil and Latanoprost

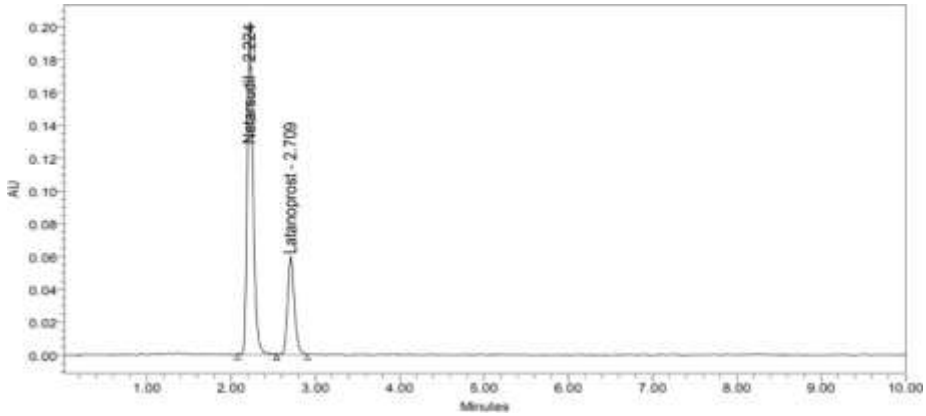

Fig 12:UV degradation chromatogram of Netarsudil and Latanoprost

Table-1 Optimized Chromatographic conditions

Parameters	**Conditions**
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	55%0.01N Ammonium acetate : 45%Methanol
Flow rate	1ml/min
Column	Zodiacsil C18 (4.6 x 150mm, 5µm)
Injection Volume	10µl
Run time	5min
Diluent	Water :Acetonitrile in the ratio 50:50
Retention Time	Netarsudil-2.221min ,Latanoprost-2.707

Table 2: System suitabilty parameters of Netarsudil and Latanoprost

S no	**Netarsudil**			**Latanoprost**			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.221	4951	1.24	2.708	5154	1.18	3.3
2	2.222	4721	1.24	2.706	5125	1.19	3.4
3	2.222	4814	1.28	2.707	5810	1.13	3.5
4	2.223	4847	1.28	2.708	5389	1.18	3.4
5	2.224	4826	1.29	2.708	5449	1.21	3.4
6	2.224	4779	1.27	2.709	5167	1.14	3.4

Table 3: Accuracy studies of Netarsudil and Latanoprost

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery	% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	10	9.93	99.28	100.09%	50%	2.5	2.52	100.87	100.57%
	10	9.95	99.54			2.5	2.48	99.03	
	10	10.01	100.07			2.5	2.49	99.66	
100%	20	19.97	99.87		100%	5	5.05	101.03	
	20	19.92	99.62			5	5.05	101.01	
	20	19.91	99.55			5	50.8	101.58	
150%	30	30.30	100.99		150%	7.5	7.53	100.36	
	30	30.24	100.80			7.5	7.57	100.95	
	30	30.31	101.04			7.5	7.55	100.62	

Table 4:Repeatability and Inter-day precision of Netarsudil and Latanoprost

Drug	**Sample Weight(mg)**	**Repeatability**		**Inter-day precision**	
		SD	**%RSD**	**SD**	**%RSD**
Netarsudil	10	5380.7	0.8	4894.5	0.7
Latanoprost	2.5	1105.8	0.5	2042.2	1.0

Table 5: Assay result of tablet dosage formulation

Drug	**Label strength (mg)**	**% Assay**
Netarsudil	0.2	99.41%
Latanoprost	0.05	100.08%

Table 6: Forced degradation studies of Netarsudil and Latanoprost

Type of degradation	**Netarsudil**		**Latanoprost**	
	%recovered	**%Degraded**	**%Recovered**	**% Degraded**
Acid	93.64	6.36	93.24	6.76
Base	95.21	4.79	95.54	4.46
Peroxide	95.81	4.19	95.89	4.11
Thermal	97.53	2.47	97.99	2.01
Water	99.52	0.48	99.45	0.55
UV	99.18	0.82	98.57	1.43

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