STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION OF NETARSUDIL AND LATANOPROST BY RP-HPLC AND ITS DEGRADATION

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Abstract
An accurate, rapid economical and simple, reliable method was developed and validated for the simultaneous estimation of Netarsudil and Latanoprost using RP-HPLC. In the proposed method efficient chromatographic separation was achieved using X-Bridge Phenyl column (150x4.6mm, 3.5µm) as a stationary phase and acetonitrile: buffer (30:70v/v, 2.5g of Octane-1-Sulphonic acid in 1lt of water adjust pH-2.5 with Ortho Phosphoric Acid) as a mobile phase with a flow rate of 1ml/min and UV detection at 228nm. Chromatography was carried out isocratically at ambient temperature and the run time was approximately 10min. In this method a good linearity was observed in the range of 0.0063-0.0938µg/ml and 0.0013-0.0188µg/ml with the limit of detection (s/n=7, 4) respectively. The proposed method was validated as per ICH guidelines.
Key words: Netarsudil, Latanoprost, RP-HPLC.

INTRODUCTION

Netarsudil (trade name Rhopressa) is a drug for the treatment of glaucoma [1, 2]. In the United States, the Food and Drug Administration has approved a 0.02% ophthalmic solution for the lowering of elevated intraocular pressure [3, 4] in patients with open-angle glaucoma or ocular hypertension [5].

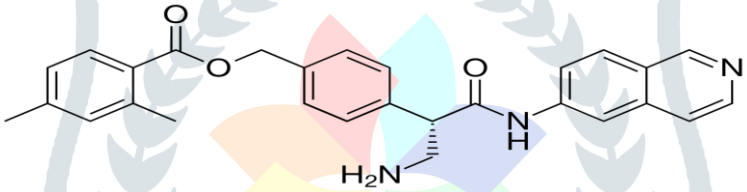

Fig. 1: Chemical Structure of Netarsudil

Latanoprost, sold under the brand name Xalatan among others, is a medication used to treat increased pressure inside the eye. This includes ocular hypertension and open angle glaucoma. It is applied as eye drops to the eyes. Onset of effects is usually within four hours, and they last for up to a day. Common side effects include blurry vision, redness of the eye, itchiness [6] and darkening of the iris. Latanoprost is in the prostaglandin [7] analogue family of medication. It works by increasing the outflow of aqueous fluid from the eyes through the uveoscleral tract [8]. Latanoprost approved for medical use in the United States in 1996. It is on the World Health Organization's [9, 10] list of essential medicines [10, 11] the most effective and safe medicines needed in a health system [13, 14]. Latanoprost is available as a generic medication [15, 16]. In 2016 it was the 79th most prescribed medication in the United States with more than 9 million prescriptions.

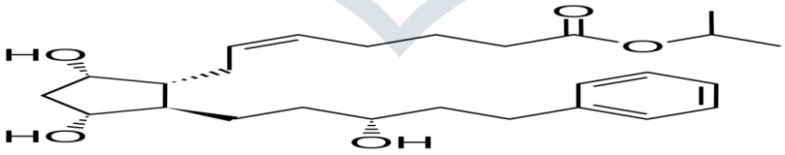

Fig. 2: Chemical Structure of Latanoprost

Different chromatographic methods were studied in an attempt to optimize simple, reliable and sensitive and an accurate method for the estimation of studied compounds in bulk and pharmaceutical dosage forms. But literature search reveals that there was no HPLC method for the simultaneous estimation of these drugs has been reported so far. The purpose of the present work therefore was to development a fast, economical, sensitive and confirmation of Netarsudil and Latanoprost in bulk and pharmaceutical dosage forms.

EXPERIMENTAL

Chemicals:

Acetonitrile, Octane-1-Sulphonic acid, Ortho Phosphoric Acid and Water (HPLC grade) were purchased from Merck (India) Ltd. Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of Netarsudil, Latanoprost as reference standards were procured from Glenmark pharmaceuticals private Ltd., Andheri (E), Mumbai, India (99.7-99.9% purity).

Equipment:

Water alliance-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

Chromatographic conditions:

Chromatographic separation was carried out in isocratic mode at room temperature using a X-Bridge Phenyl (150x4.6mm, 3.5µm) column. The mixture of buffer (2.5g of Octane-1-Sulphonic Acid in 1lt of water sonicated to dissolve adjusted the pH-2.5 with OPA): acetonitrile 70:30v/v at a flow rate of 1ml/min was used as a mobile phase. The injection volume was 10µl and eluent was monitored at 240nm using PDA detector. The run time was 10min and each of the studied component was quantified by using total peak height.

Preparation of buffer pH:

Simple, economical and proper acidic buffer was selected for the estimation of the current drugs in their combined dosage forms. 2.5g of Octane-1-Sulphonic acid was weighed accurately and transferred in to 1lt beaker and made up the volume up to the mark with HPLC grade water. The pH 2.5 was adjusted with ortho phosphoric acid.

Selection of Mobile Phase:

The mobile phase was set by injecting different ratios of buffer and acetonitrile. The selected mobile phase ratio was70:30v/v of buffer: acetonitrile. The selected mobile phase has given a sharp peaks with low tailing factor i.e. <2.0 and also plate count will be less than 3,000.

Selection of wavelength

The absorption spectra of solution of each Netarsudil and Latanoprost were scanned over the range 200-400nm using photodiode spectrophotometer and the spectra were recorded. Netarsudil and Latanoprost having lowest label claim and shows absorbance at wavelength 228nm, at which the two drugs showed good absorbance, was selected as a detection wavelength.

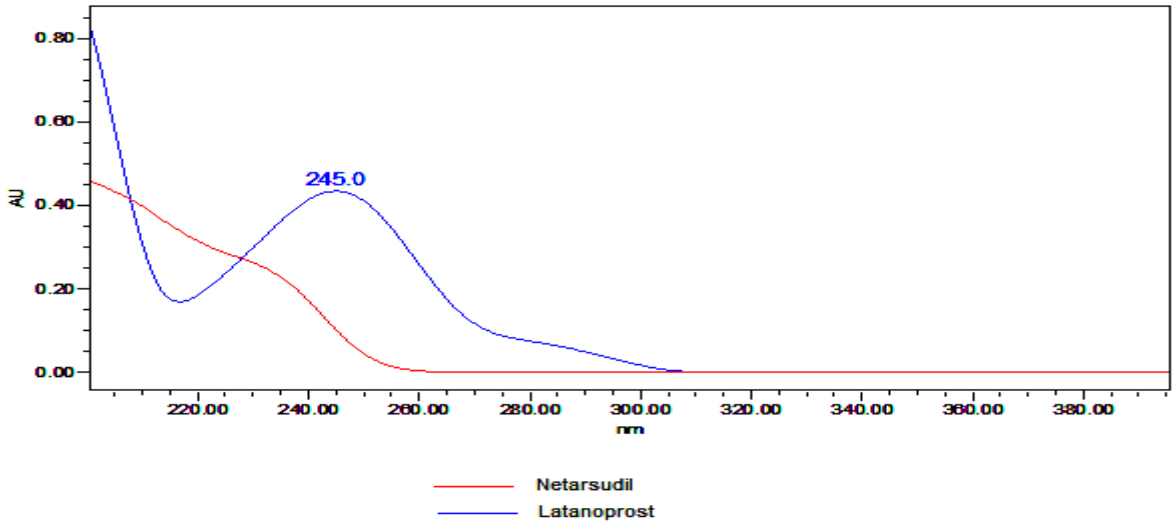

Fig. 3: PDA Spectrum for Netarsudil and Latanoprost

Preparation of Diluent:

Mobile phase is used as a diluent.

Preparation of Standard Stock Solution

Standard stock solution-A: Accurately weighed about 6.25mg of Netarsudil (working standard) and transferred to a 100ml volumetric flask. Then they were dissolved in 70ml of diluent and sonicated for about 10min with intermittent shaking and diluted up to the mark with diluent. Further dilute 1ml of the above solution to 100ml with diluent.

Standard Stock Solution-B: 12.5mg of Latanoprost was weighed accurately and transferred to a 100ml volumetric flask and dissolved in 70ml diluent and sonicated to dissolve and diluted to volume with the diluent. Further dilute 0.1ml to 100ml with diluent.

Preparation of Standard Solution

Transferred 1ml of standard stock solution-A and 1ml of standard stock solution-B were transferred into 10ml volumetric flask and diluted upto the mark with diluent.

Preparation of Sample solution

Carefully transfer 1ml of the sample solution into 10ml volumetric flask and add 5ml of diluent and sonicated to dissolve and diluted upto the mark with the diluent. And it was filtered through 0.45µ nylon syringe filter.

Procedure for Analysis:

A steady baseline was recorded by the optimized chromatogralphic conditions. It was stabilized for about 30min and successive aliquots of the standard solution of the same concentration were injected and chromatogram was recorded until the reproducibility of the peak areas was satisfactory. This procedure was repeated using the sample solution so that duplicate injection of the sample solution was bracketed by injection of the standard solution.

The response factor of the standard peak and sample peak was obtained and the amount of each drug in the sample was determined. This procedure was repeated six times. The concentration of each drug in the triple component dosage form was calculated using the formula,

$$\text{Concentration of drug} = \frac{\text{Response factor of the samlple x Concentration of standard}}{\text{Response factor of the standard}}$$

Validation Procedure

The analytical method was validated as per ICH Q2 (R1) [17] guidelines for the 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 Netarsudil and Latanoprost.

Accuracy:

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

Precision:

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

Linearity and range:

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

LOD and LOQ:

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined based on the calibration curves. The LOD and LOQ for Netarsudil and Latanoprost were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD and LOQ were calculated as 3.3s/n and 10s/n respectively as per ICH guidelines, where s/n indicates signal-to-noise ratio.

Stress degradation:

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q_1A (R_2). The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were perdormed by different types of stress conditions to obtain the degradation of about 20%.

Robustness:

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (±0.2ml/min), wavelength (±5nm), variation in pH (±0.2), organic content in the mobile phase (±10%). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

Stability:

Analytical solution was prepared and injecting into the HPLC system at periodic intervals of 0h to 24h at 6h intervals depending on the instrument utilization and sequence of injection.

Results and Discussion

The current study was designed to develop a simple, precise and rapid analytical RP-HPLC method, which can be used for the analysis of assay method for simultaneous estimation of Netarsudil and Latanoprost in bulk and pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. To optimize mobile phase, various combinations of buffer (2.5g of Octane-1-Sulphonic acid in 1lt water adjust pH-2.5 with OPA): acetonitrile were tried for Netarsudil and Latanoprost and the final working mobile phase is Octane-1-sulphonic buffer and acetonitrile in composition of 70:30v/v. Mobile phase for each drug was selected based on its polarity. Detection was carried out in several wavelengths in order to obtain enough sensitivity for the two APIs in smaller proportion (Netarsudil and Latanoprost). At last, the wavelength 228nm, at which the two drugs showed good absorbance, was selected as a detection wavelength. The flow rate was 1.0ml/min, which is critical as it affects the peak symmetry parameters. The retention time for Netarsudil and Latanoprost were 2.648 and 3.151min respectively. The proposed method is validated in accordance with the ICH guidelines with all of the results within the limits. In this method a good linearity was observed in the range of 0.0063-0.0938µg/ml and 0.0013-0.0188µg/ml respectively.

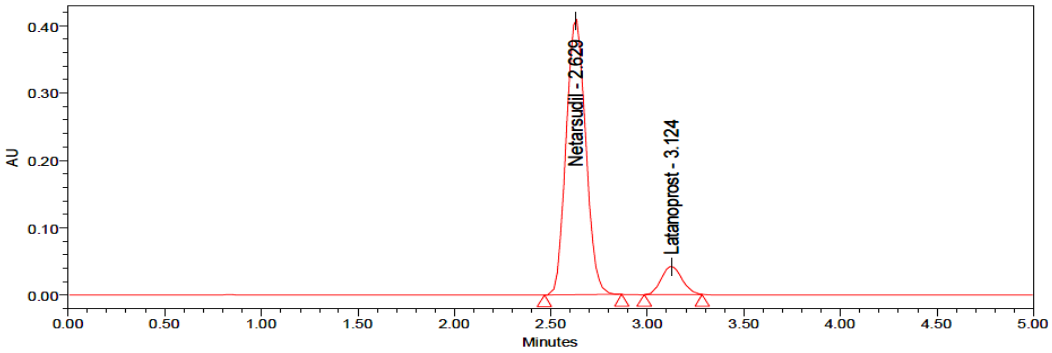

Fig. 4: Typical chromatogram of Standard

Method Validation tests

System Suitability

The HPLC system was stabilized for 60min to get a stable base line. Six replicate injections of the mixture containing 0.0625µg/ml of Netarsudil and 0.0125µg/ml of Latanoprost were assessed to check the system suitability. The system suitability parameters were evaluated from six replicate injections. The study concludes that the suitability of the HPLC system being used and results were summarized below.

Table 1: System suitability data

System suitability Parameters	Acceptance criteria	Drug Name	
		Netarsudil	Latanoprost
% RSD	NMT 2.0	0.21	0.14
USP Tailing	NMT 2.0	1.08	1.09
USP Plate count	NLT 3000	3126	3958

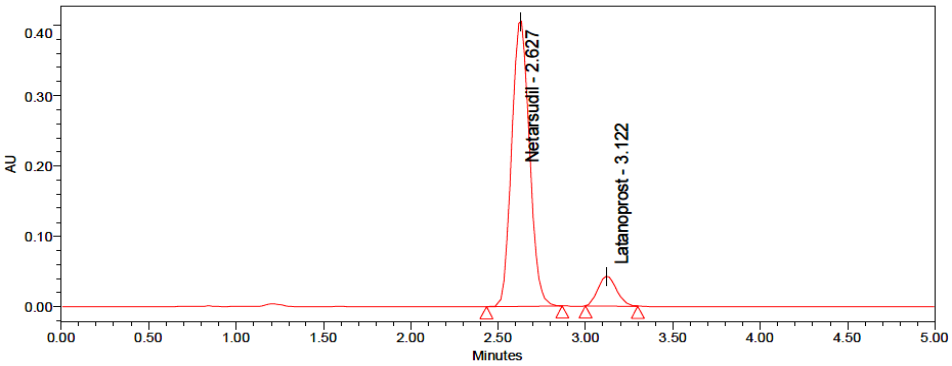

Fig. 5: Chromatogram of system suitability

Specificity

There was no interference from blank at the retention time of Netarsudil and Latanoprost. Hence the method is specific.

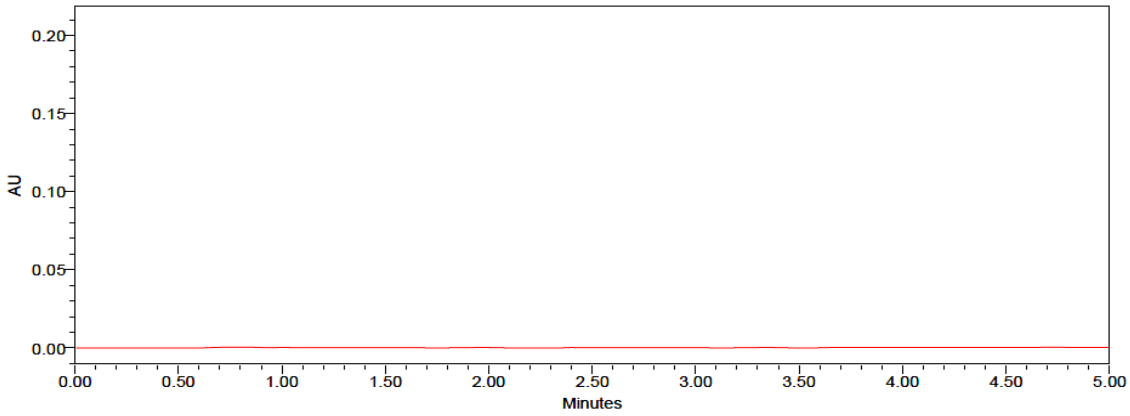

Fig. 6: Chromatogram of Blank

Linearity

Linearity was determined by plotting a calibration curve of peak area against their respective concentration. From this calibration curve it was found that the curve was linear in the range of 0.0063-0.0938µg/ml for Netarsudil and 0.0013-0.0188µg/ml for Latanoprost. The regression equation for calibrationcurve was Y= 45585409x+9335.5 for Netarsudil and Y=24230862x+929.06 for Latanoprost respectively.

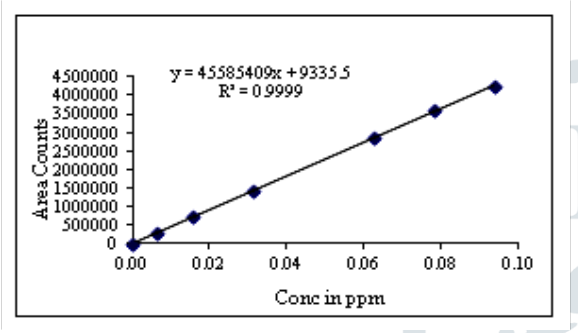

Fig. 7: Linearity plot for Netarsudil

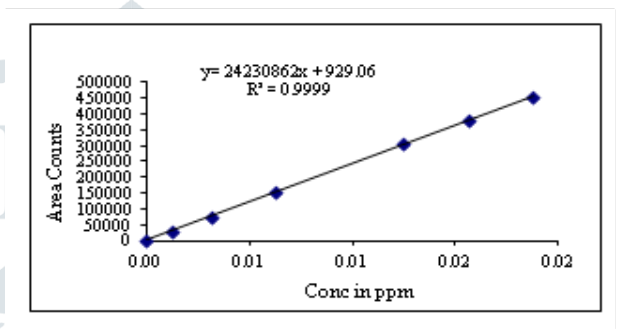

Fig. 8: Linearity plot for Latanoprost

Accuracy

The accuracy of the method was performed by calculating the recovery experiments at three levels (50%, 100% and 150%). APIs with concentration 0.0313, 0.0625 and 0.0938µg/ml of Netarsudil and 0.0063, 0.0125 and 0.0188µg/ml of Latanoprost were prepared. The test solution was injected three times for each spike level and assay was performed as per the test method. The recovery results were close to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviation were calculated. Recovery values demonstrated that the method was accurate within the desired range. The results are summarized below.

Table 2: Results of Accuracy

Accuracy	Amount of Netarsudil drug µg/ml	Recovery solution (area) mAU	% drug Recovery	Amount of Latanoprost drug µg/ml	Recovery solution (area) mAU	% drug Recovery
50%	0.0313	1438621	100.2	0.0063	150304	101.1
100%	0.0625	2839925	99.9	0.0125	303496	100.7
150%	0.0938	4283201	100.5	0.0188	456253	99.6

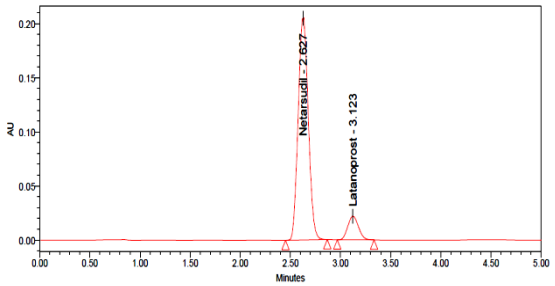

Fig. 9: Chromatogram of Accuracy 50%

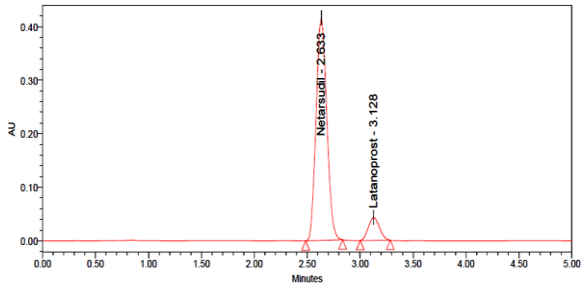

Fig. 10: Chromatogram of Accuracy 100%

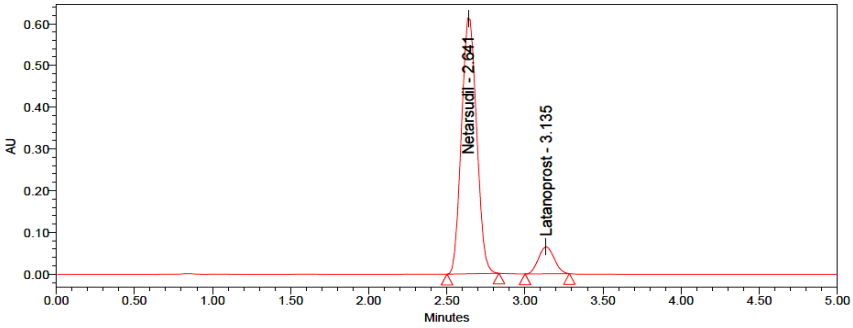

Fig. 11: Chromatogram of Accuracy 150%

Precision

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 Netarsudil and Latanoprost 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 3: Method precision data

Cocentration of Netarsudil drug µg/ml	Area mAU	%RSD	Cocentration of Latanoprost drug µg/ml	Area mAu	%RSD
0.0625	2857694	0.16	0.0125	307903	0.40
	2869618			306948	
	2865051			308919	
	2844807			302971	
	2872570			307586	
	2874662			309714	

Table 4: Intermediate precision data

Concentration of Netarsudil drug µg/ml	Area mAU	%RSD	Concentration of Latanoprost drug µg/ml	Area mAu	%RSD
0.0625	2854025	0.5	0.0125	304303	0.34
	2861457			306496	
	2843183			305178	
	2853887			307618	
	2876320			305550	
	2922192			312263	

LOD and LOQ

LOD and LOQ were separately determined by calibration curve method [18]. 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 Netarsudil and Latanoprost were found to be 0.000625µg/ml and 0.000125µg/ml respectively. The LOQ values were found to be 0.00625 and 0.00125µg/ml respectively.

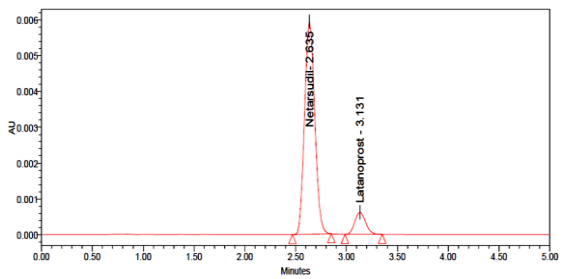

Fig. 12: Chromatogram of LOD

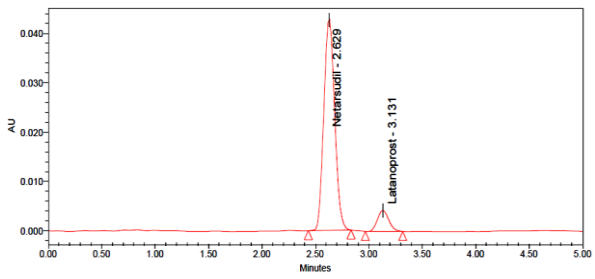

Fig. 13: Chromatogram of LOQ

Forced Degradation

The proposed analytical method can be used for release and stability studies for effective evaluations and can be considered as stability indicating method. The forced degradation study was carried out according to the ICH requirements include acid, base, hydrogen peroxide, reduction, thermal and photolytic degradation. From the chromatograms, it is evident that the selected drugs were stable under the applied stress conditions though the degraded peaks were observed. Results were shown in table.

Table 5: Results of forced degradation studies

Stress condition/duration/solution	Degradation (Netarsudil)	Degradation (Latanoprost)
Acid degradation (0.2N, 0.1N, 1h)	11.7	19
Alkaline degradation (0.2N, 0.1N, 1h)	14.5	18.4
Oxidative degradation (5% H_2O_2, 80°C, 30min)	12.4	14.7
Reduction degradation (10% $NaHSO_4$, 80°C, 15min)	18.1	15.4
Thermal degradation (Solid sample, 80°C, 3h)	15.3	18
Photolytic degradation (sample expose sun light 6h)	12.8	18.2

Robustness

As per ICH norms, small but deliberate variations were made in the method parameters such as change in the flow rate (±0.2), organic content in the mobile phase (±10%), 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, 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: Results of Robustness studies

Change in parameter	% RSD for Netarsudil	% RSD for Latanoprost
Flow (0.8ml/min)	0.57	0.5
Flow (1.2ml/min)	0.43	0.15
Organic phase (+10%)	0.42	0.15
Organic phase (-10%)	0.3	0.99
Wavelength (245nm)	0.45	0.21
Wavelength (235nm)	0.26	0.47

Stability

To assess the stability of sample solutions, they were analysed initially to 24h at different intervals of time at room temperature. No significant degradation was observed during this period and the % deviation was not more than 5.0%, suggesting that the solutions were stable for at least 24h, which was sufficient for the whole analytical procedure. Results are furnished below.

Table 7: Results of stability studies

Stability	% Lable claim Netarsudil	% Deviation Netarsudil	% Lable claim Latanoprost	% Deviation Latanoprost
Initial	100.4	0.00	102.5	0.00
6 Hr	100.4	0.00	102.3	-0.20
12 Hr	99.1	-1.29	101	-1.46
18 Hr	98	-2.39	99.9	-2.54
24 Hr	100.4	0.00	102.5	0.00

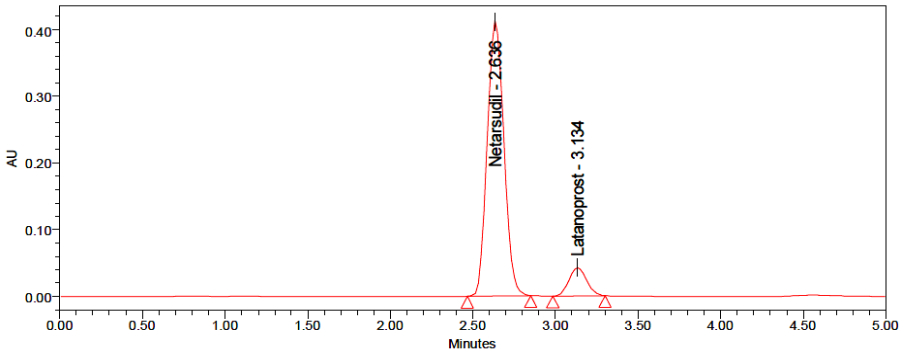

Fig. 14: Chromatogram for Stability Initial

Conclusion

In this study a novel, simple, rapid, economical, sensitive and easily available HPLC method was developed for the simultaneous determination of Netarsudil and Latanoprost in bulk and sample dosage form. The main advantages of this method over the previously reported HPLC methods are its availability, shorter run times, low price, accessibility, sensitivity, reliability and reproducibility. These properties are important when a large number of samples are to be analysed. The validation of all the parameters like linearity, accuracy, specificity, robustness, stability was done and found to be within the acceptance criteria. The RSD values for all parameters were found to be less than 2, which indicates the validity of method and results obtained by this method are in fair agreement. SO the proposed method could be easily being applied for the routine analysis and pharmaceutical formulations of Netarsudil and Latanoprost in quality control laboratories without any preliminary separation.

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