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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NETARSUDIL & LATANOPROST BY RP-HPLC METHOD

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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 Std Agilent C18 150 x 4.6 mm, 5µ. Mobile phase containing Buffer KH2PO4: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1ml/min. Buffer used in this method was Potassium dihydrogen phosphate. Temperature was maintained at 30°C. Optimized wavelength selected was 220nm. Retention time of Netarsudil and Latanoprost were found to be 2.875min and 4.106min. %RSD of the Netarsudil and Latanoprost were and found to be 0.9 and 0.9 respectively. %Recovery was obtained as 100.46% and 100.20% for Netarsudil and Latanoprost respectively. LOD, LOQ values obtained from regression equations of Netarsudil and Latanoprost were 0.08, 0.25 and 0.04, 0.12 respectively. Regression equation of Netarsudil is y = 62952x + 4328.1 and y = 43163x + 510.88 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

Chemically Netarsudil (NTS) The medical condition glaucoma is a leading cause of progressive visual impairment and blindness across the world with primary open-angle glaucoma (POAG) being the major type of glaucoma. Elevated intraocular pressure (IOP) resulting from increased resistance to aqueous humor outflow is considered a major risk for the development and progression of POAG, , but various clinical studies have demonstrated that the reduction and tight control of IOP can delay or prevent POAG and the vision loss associated with it. Ordinary physiological 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. Structure of the NTS was shown in figure 1 (A).[1]

Chemically Latanoprost (LTP) Elevated intraocular pressure leads to an increased risk of glaucomatous visual field loss. The higher the intraocular pressure, the higher the risk of damage to the optic nerve and loss of visual field. Latanoprost selectively stimulates the prostaglandin F2 alpha receptor and this results in a decreased intraocular pressure (IOP) via the increased outflow of aqueous humor, which is often implicated in cases of elevated intraocular pressure. Possible specific mechanisms of the abovementioned increased aqueous outflow are the remodeling of the extracellular matrix and regulation of matrix metalloproteinases. These actions result in higher tissue permeability related to humor outflow pathways, which likely change outflow resistance and/or outflow rates. Structure of the LTP was shown in figure 1 (B).[2]

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two or three drugs.[5-9] But there is only very few HPLC methods are available for simultaneous estimation of NTS and LTP, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

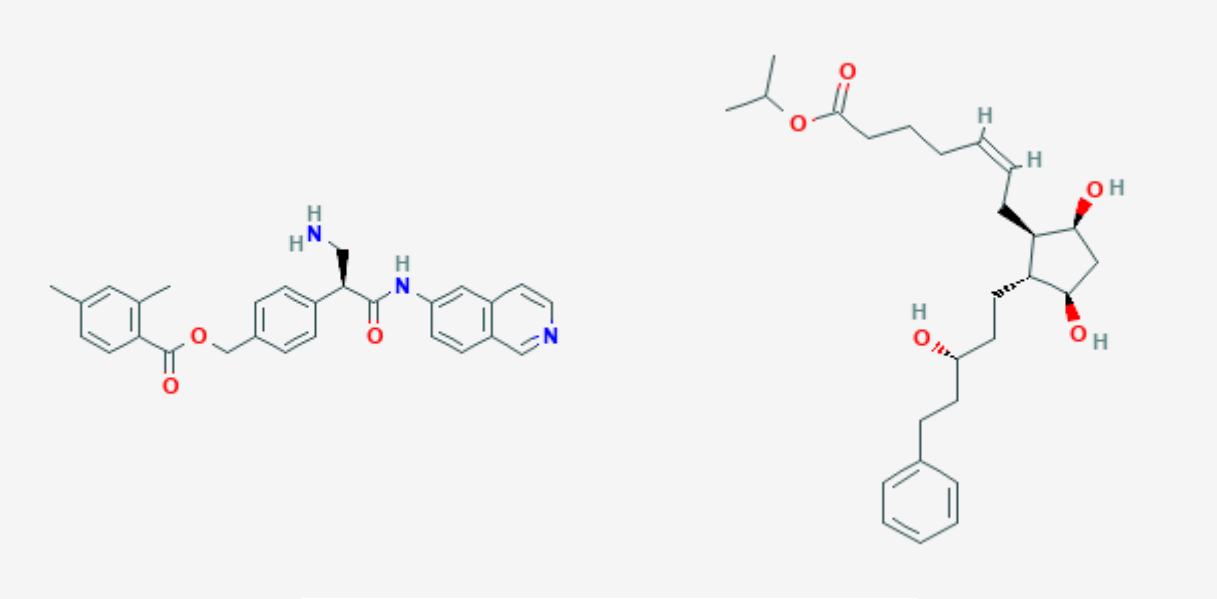

Figure 1: Structure of (A) Netarsudil (B) Latanoprost.

MATERIALS AND METHODS

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

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Agilent 150 C18 (4.6 x 150mm, 5µm). As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The Agilent C18 (4.6 x 150mm, 5µm) column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of 0.1N KH2PO4 and Acetonitrile was taken in the ratio of (60:40%v/v) mobile phase for the investigation with a flow rate of a 1 ml/min. The temperature was maintained at 10^0C. The injection volume was 10µl and the UV detection was achieved at 220nm. *Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0):* Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate 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 then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase: Buffer: Water - 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.

Preparation of mixture Standard stock solution: Accurately weighed 5mg of Netarsudil, 1.25mg of Latanoprost and transferred to individual 50ml 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. (100µg/ml of Netarsudil and 25µg/ml of Latanoprost).

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

Optimized chromatographic conditions

Column Used :Agilent 150 C18 (4.6 x 150mm, 5µm)
Mobile phase : 0.1N KH_2PO_4: Acetonitrile (60:40 v/v)
Flow rate : 1ml/min
Wavelength : 220.0 nm
Temperature : 10°C
Injection Volume : 10.0µl

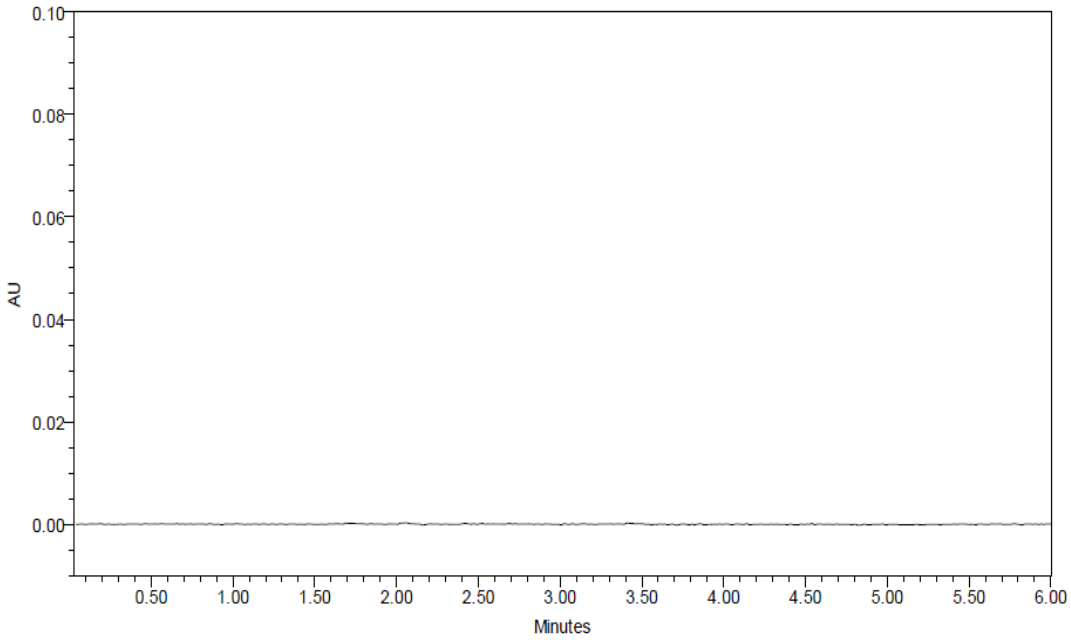

Figure 2: Blank chromatogram.

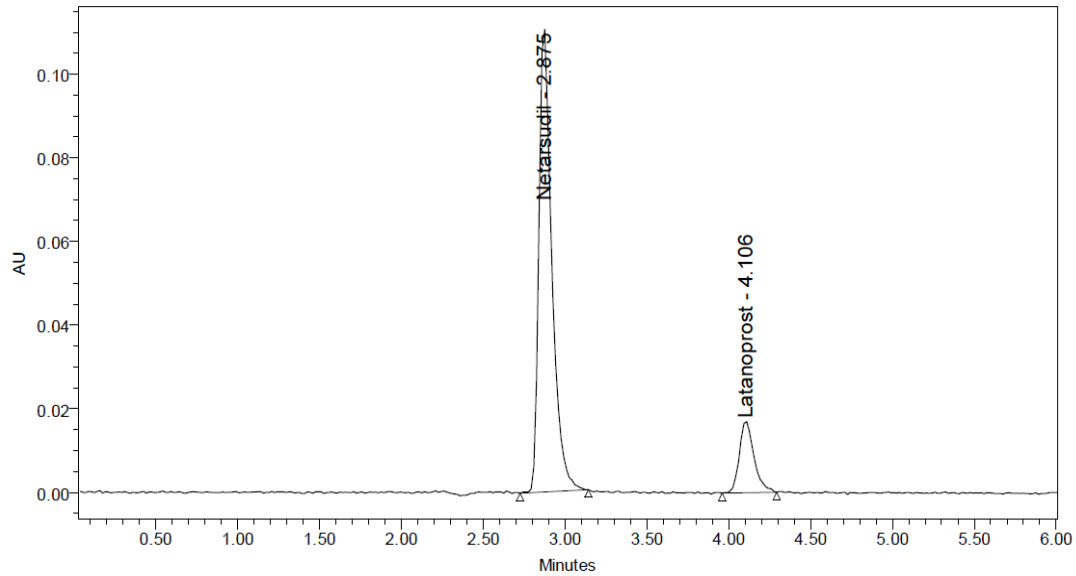

Figure 3: Chromatogram of standard mixture of NTS & LTP.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Netarsudil	2.876	632678	1.26	4	8000
2	Latanoprost	4.106	108096	1.30	3.9	10693

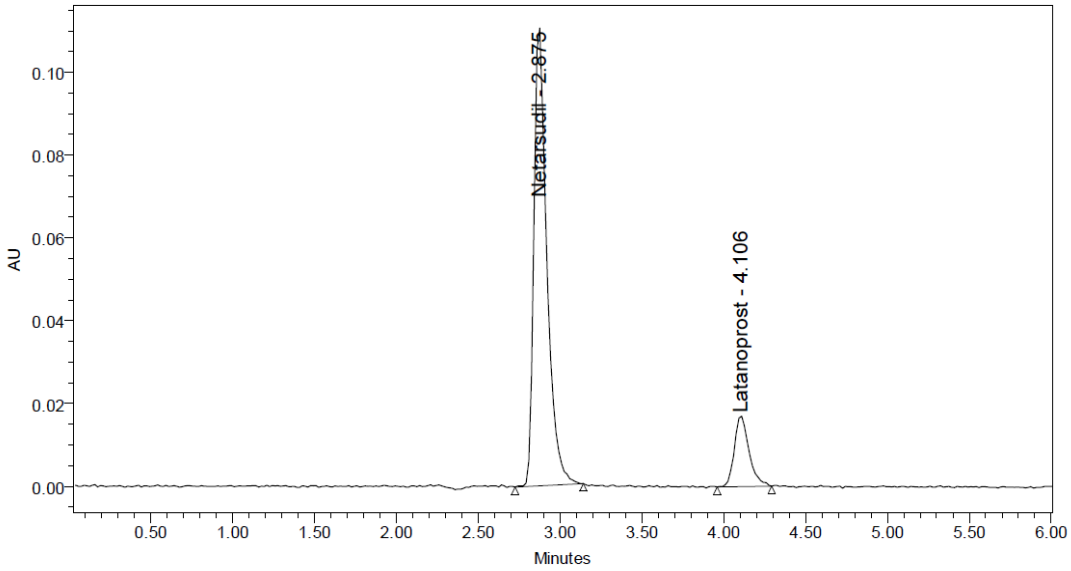

Figure 4: Chromatogram of sample mixture of NTS & LTP.

VALIDATION

The above optimized chromatographic method has been validated for the assay of NTS & LTP using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Netarsudil and Latanoprost (NTS & LTP)drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Netarsudil (5mg)+ Latanoprost (1.25mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Netarsudil (5mg)+ Latanoprost (1.25mg) respectively. The % RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Netarsudil and Latanoprost. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for NTS was found to be y = 62952x + 4328.1 (slope, intercept and correlation coefficient were found to be 62952, 4328.1 and 0.999 respectively) and linear over beer's range of 2.5-15µg/ml. The regression equation for LTP was found to be y = 43163x + 510.88 (slope, intercept and correlation coefficient were found to be 43163, 510.88 and 0.999 respectively) and linear over beer's range of 0.625-3.75 µg/ml. Linearity graph of NTS & LTP were shown in Figure 5 & 6 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. % RSD of system precision for Netarsudil and Latanoprost were and found to be 0.9 and 0.6 respectively. %RSD of method precision for Netarsudil and Latanoprost were and found to be 0.9 and 0.5 respectively. % recovery was obtained as 100.46% and 100.20% for Netarsudil and Latanoprost respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug NTS & LTP and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 98.91% and 98.65% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for NTS & LTP was found to be 0.08µg/ml and 0.04µg/ml respectively. LOQ for NTS & LTP was found to be 0.25µg/ml and 0.12µg/ml respectively. Summary of all the validation parameter shown in table 3.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 1: Linearity table for NTS & LTP.

Netarsudil		Latanoprost	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
2.5	169159	2.5	169159
5	319482	5	319482
7.5	475186	7.5	475186
10	630141	10	630141
12.5	789603	12.5	789603
15	951690	15	951690

Table 2: System precision table of NTS & LTP.

S. No	Area of Netarsudil	Area of Latanoprost
1.	629278	108432
2.	635873	108606
3.	636722	109178
4.	639643	108536
5.	624980	106479
6.	629571	107342
Mean	632678	108096
S.D	5577.6	992.0
%RSD	0.9	0.9

Table 3: summary of validation data of NTS & LTP.

Parameters		**Netarsudil**	**Latanoprost**	**LIMIT**
Linearity Range(µg/ml)		2.5-15µg/ml	0.625-3.75 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		62952	43163	
Intercept(c)		4328.1	510.88	
Regression equation (Y=mx+c)		y = 62952x + 4328.1	y = 43163x + 510.88	
Assay (% mean assay)		98.91%	98.65%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.9	0.6	NMT 2.0%
Method precision %RSD		0.9	0.5	NMT 2.0%
Accuracy%recovery		100.46%	100.20%	98-102%
LOD		0.08	0.04	NMT 3
LOQ		0.25	0.12	NMT 10
Robustness	**FM**	0.7	1.1	% RSD NMT 2.0
	FP	1.2	1.7	
	MM	0.6	0.4	
	MP	1.2	1.6	
	TM	1.7	0.8	
	TP	1.0	0.9	

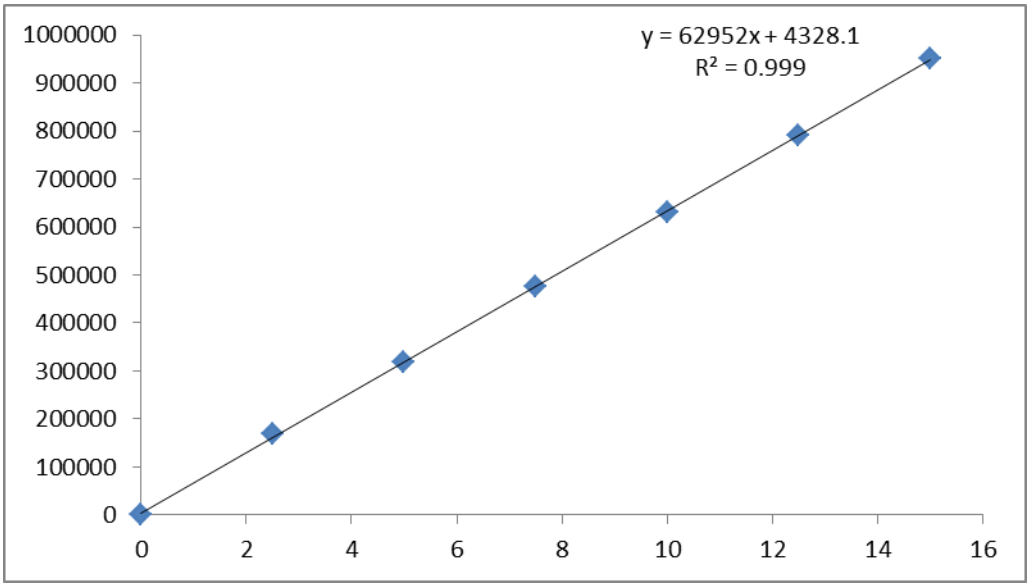

Fig No 7: Linearity curve of Netarsudil.

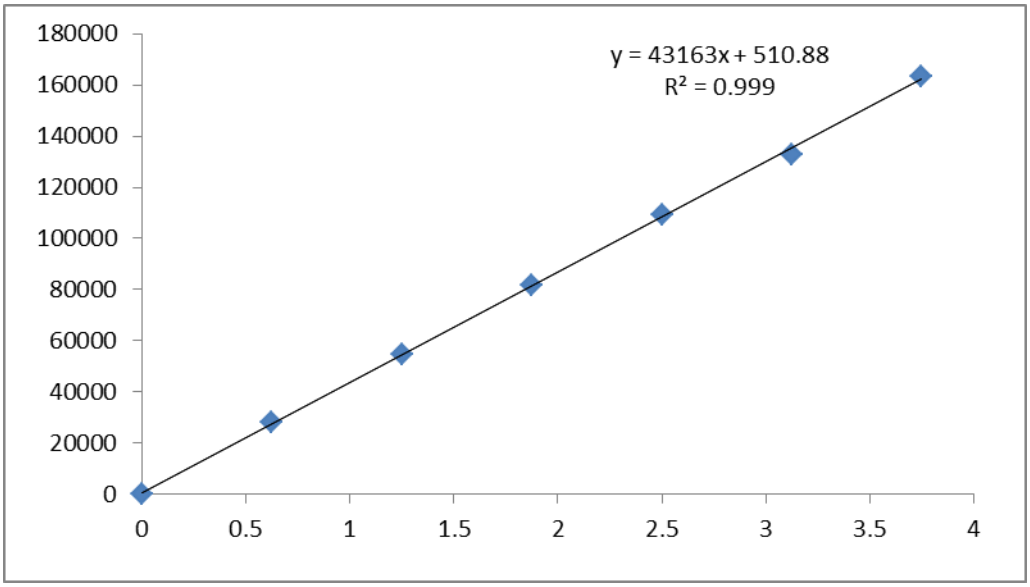

Fig No 8: Linearity curve of Latanoprost.

Table no 4: Degradation data of NTS & LTP.

Type of degradation	Netarsudil			Latanoprost		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	608519	95.80	4.20	102690	94.81	5.19
Base	594165	93.54	6.46	101982	94.16	5.84
Peroxide	606959	95.55	4.45	103275	95.35	4.65
Thermal	622703	98.03	1.97	105632	97.53	2.47
Uv	629320	99.07	0.93	107204	98.98	1.02
Water	631873	99.07	0.93	107449	99.20	0.80

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Netarsudil and Latanoprost in Tablet dosage form was developed and the proposed method as suitable for routine analysis of NTS & LTP.

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