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A NOVEL STUDY ON RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF NETARSUDIL AND LATANOPROST IN API ANDPHARMACEUTICAL DOSAGE FORM

Dr. K. Padmalatha, D. Vijaya Durga* and N. Jagadeeswari

Department of Pharmaceutical Analysis, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada-521108.

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***Corresponding Author**
D. Vijaya Durga
Department of Pharmaceutical Analysis, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada-521108.

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 Agilent C18 150 x 4.6 mm, 5µ. Mobile phase containing KH2PO4: Acetonitrile taken in the ratio 55:45 was pumped through column on a flow rate at 1.0ml/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 2.189min and 2.860min. %RSD of the Netarsudil and Latanoprost were and found to be 0.8 and 1.1 respectively. %Recovery was obtained as 100.25% and 100.11% for Netarsudil and Latanoprost respectively. LOD, LOQ values obtained from regression equations of Netarsudil and Latanoprost were 0.24, 0.09 and 0.72, 0.26 respectively. Regression equation of Netarsudil is y = 26436x + 3132.7. and y = 39041x + 2230.6 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: Latanoprost, Netarsudil, RP-HPLC.

INTRODUCTION

Netarsudil

Netarsudil is freely soluble in water, methanol, sparingly soluble in dimethyl formamide, and practically insoluble in dichloromethane and heptane. The chemical name of netarsudil is: (S)-4-(3-amino-1-(isoquinolin-6-yl-amino)-1-oxopropan2-yl) benzyl 2, 4-dimethylbenzoate dimesylate. Its chemical structure is:

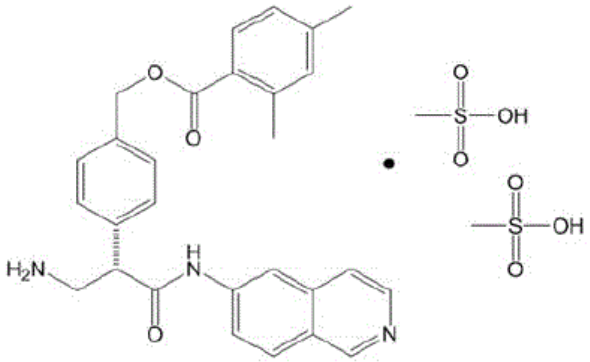

Fig. 1: Structure of netarsudil.

Latanoprost

Latanoprost is very soluble in acetonitrile and freely soluble in acetone, ethanol, ethyl acetate, isopropanol, methanol, and octanol. It is practically insoluble in water. The chemical name of latanoprost is: isopropyl-(Z)-7[1R, 2R, 3R, 5S) 3, 5-dihydroxy-2-[(3R)-3-hydroxy-5phenylpentyl] cyclopentyl]-5-heptenoate. Its chemical structure is:

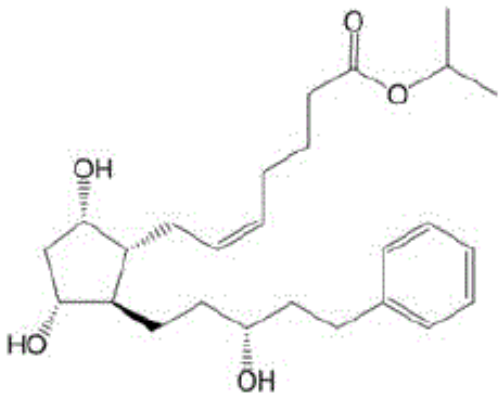

Fig. 2: Structure of latanoprost.

MATERIALS AND METHODS

Materials

- Netarsudil and Latanoprost pure drugs (API), Combination Netarsudil and Latanoprost Opthalmic 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.

Instruments

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultra sonicator-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.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50 as diluent.

Preparation of Standard stock solutions: Accurately Weighed and transferred 10mg of Netarsudil, and 2.5mg of Latanoprost working Standards into a 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents (200µg/ml Netarsudil, and 50 µg/ml of Latanoprost).

Preparation of Standard working solutions (100% solution): 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (20µg/ml Netarsudil, and 5 µg/ml of Latanoprost).

Preparation of Sample 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 (20 µg/ml Netarsudil, and 5 µg/ml of Latanoprost)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20 µg/ml Netarsudil, and 5 µg/ml of Latanoprost).

Construction of calibration curve

Six linear concentrations of Netarsudil (5-30µg/ml) and Latanoprost (1.25-7.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Netarsudil was y = 26436x + 3132.7.and of Latanoprost was y = 39041x + 2230.6. Correlation coefficient obtained was 0.999 for the two drugs.

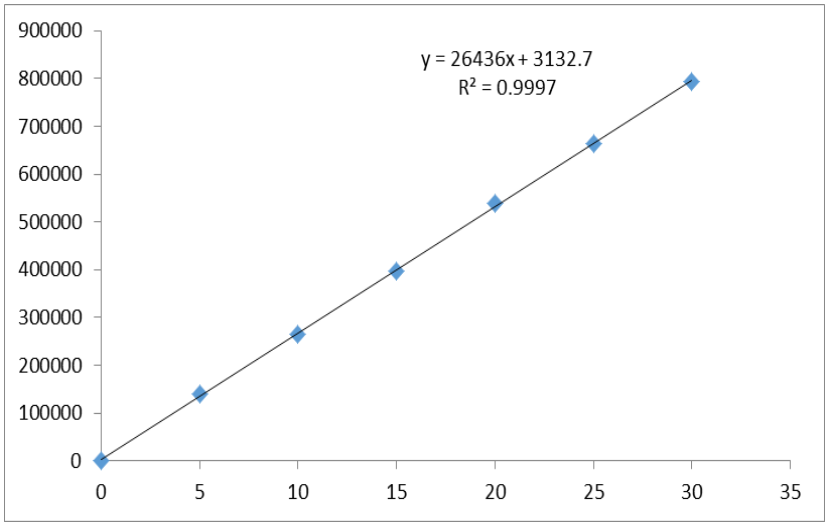

Fig. 3: Calibration curve of Latanoprost.

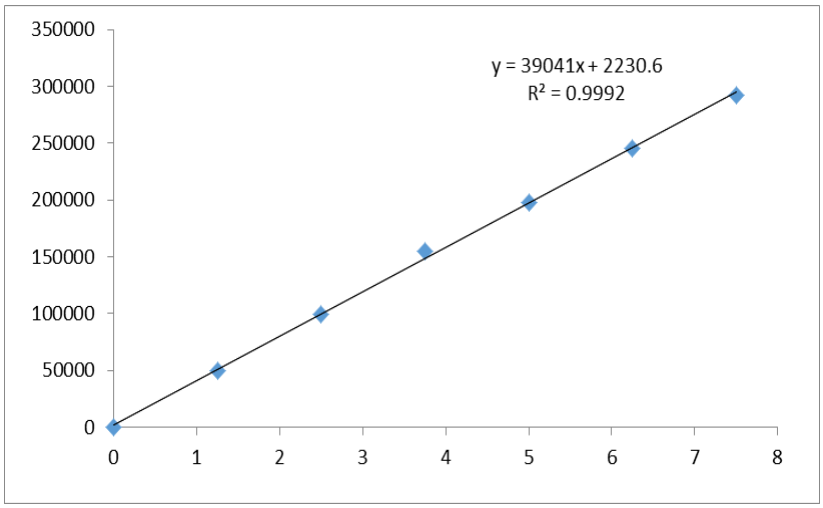

Fig. 4: Calibration curve of Netarsudil.

METHOD VALIDATION

Validation is a process of establishing documented evidence, which provides a high degree of assurance that is a specific activity will consistently produce the desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated according to ICH guidelines for various parameters like Range, Linearity, Precision, Accuracy, Robustness, Ruggedness, LOD, LOQ, and Sensitivity.

Range

The range is an interval between the upper and lower concentration analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision accuracy and linearity.

Linearity

The linearity of a technique is a measure of how well an adjustment plot of reaction versus focus approximates a straight line. Linearity can be evaluated by performing single estimations at a few analyte fixations. The information is then prepared utilizing a direct slightest squares relapse. The subsequent plot incline, capture and relationship coefficient give the coveted data on linearity.

Accuracy

The exactness of estimation is characterized as the closeness of the deliberate an incentive to the genuine esteem. In a technique with high precision, an example (whose "genuine esteem" is known) is investigated and the deliberate esteem is indistinguishable to the genuine esteem. Normally, exactness is spoken to and controlled by recuperation considers.

Precision

The precision of estimation is characterized as the closeness of the deliberate an incentive to the genuine esteem. In a technique with high precision, a specimen (whose "genuine esteem" is known) is broke down and the deliberate esteem is indistinguishable to the genuine esteem. Normally, exactness is spoken to and dictated by recuperation thinks about. There are three approaches to decide exactness:

1. Repeatability is the precision of a method under the same operating conditions over a short period of time.
2. Intermediate precision is the agreement of complete measurements (including standards) when the same method is applied many times within the same laboratory.
3. Reproducibility examines the precision between laboratories and is often determined in collaborative studies or method transfer experiments.

Robustness

Robustness of an analytical procedure is the capacity to remain unchanged by small but deliberate changes in parameters.

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines.

Limit of Detection: It is the lowest amount of the drug in a sample that can be detected, but not necessarily quantitated.

$$LOD = (3.3\times\sigma)/S$$

Where S= standard deviation

Limit of Quantification: It is an amount of analyte that can be quantitated with a specified limit of and precision.

$$LOQ = (10\times\sigma)/S$$

LINEARITY

Six linear concentrations of Netarsudil (5-30µg/ml) and Latanoprost (1.25-7.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Netarsudil was y = 26436x + 3132.7.and of Latanoprost was y = 39041x + 2230.6. Correlation coefficient obtained was 0.999 for the two drugs.

Table 1: Linearity table for Netarsudil and Latanoprost.

Netarsudil		Latanoprost.	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
5	140631	1.25	49927
10	264545	2.5	99709
15	396060	3.75	154891
20	539493	5	198287
25	663562	6.25	245174
30	793402	7.5	292455

PRECISION

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.8% and 1.1% respectively for Netarsudil and Latanoprost. As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability

Table: Repeatability table of Netarsudil and Latanoprost.

S. No	Area of Netarsudil	Area of Latanoprost
1.	529170	197285
2.	538745	202582
3.	530617	202229
4.	526243	199711
5.	533007	199428
6.	538512	201097
Mean	532716	200389
S.D	5077.5	1985.7
%RSD	1.0	1.0

Table: Inter-day study.

%RSD	Netarsudil	Latanoprost
	0.4	1.6

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 100.25% and 100.11% for Netarsudil and Latanoprost respectively.

Table: Accuracy table of Netarsudil.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	10	9.88	98.80	100.25%
	10	10.09	100.95	
	10	10.01	100.10	
100%	20	20.23	101.14	
	20	19.86	99.32	
	20	19.87	99.34	
150%	30	30.11	100.37	
	30	30.09	100.29	
	30	30.20	100.65	

Table 6.6: Accuracy table of latanoprost.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	2.5	2.49	99.71	100.11%
	2.5	2.53	101.12	
	2.5	2.52	100.88	
100%	5	5.00	99.93	
	5	5.00	99.92	

	5	5.05	101.04	
150%	7.5	7.45	99.28	
	7.5	7.46	99.53	
	7.5	7.47	99.56	

ROBUSTNESS

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°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table: Robustness data for Netarsudil and Latanoprost.

S.no	Condition	%RSD of Netarsudil	%RSD of Latanoprost
1	Flow rate (-) 0.9ml/min	0.3	0.5
2	Flow rate (+) 1.1ml/min	1.9	1.5
3	Mobile phase (-) 60B:40A	1.1	1.3
4	Mobile phase (+) 50B:50A	1.2	1.4
5	Temperature (-) 25°C	0.6	0.7
6	Temperature (+) 35°C	0.8	1.8

SENSITIVITY

Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines. They are calculated by checking absorbance using solvent and calculate using formulae and the results are shown in below table.

Table: LOD & LOQ.

Limit of Detection		Limit of Quantification	
Netarsudil	**Latanoprost**	**Netarsudil**	**Latanoprost**
0.24 µg/mL	0.09 µg/mL	0.72 µg/ml	0.26µg/mL

RESULTS AND DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, LOD, and LOQ. Beers law obeyed over the concentration range of 5-40 µg/mL, using regression analysis the linear obtained for Netarsudil was y = 26436x + 3132.7.and of Latanoprost was y = 39041x + 2230.6. Correlation coefficient obtained was 0.999 for the two drugs. The precision results show % RSD were calculated for two drugs.% RSD obtained as 0.8% and 1.1% respectively for Netarsudil and Latanoprost. The accuracy and mean %Recovery was obtained as 100.25%

and 100.11% for Netarsudil and Latanoprost respectively. The robustness and ruggedness studies reveal that the method is enough robust and rugged. The LOD, LOQ values indicate that the method is more sensitive. There was no interference observed from the excipients present in the formulation, indicated that the method is specific. The% RSD values in all the parameters were within the acceptable limit (<2%) All the characteristics of the method are represented in the below table.

Table: Results of validated parameters.

Parameters		**Results**	
		Netarsudil	**Latanoprost**
Linearity Range(µg/ml)		5-30µg/ml	1.25-7.5 µg/ml
Regression coefficient		0.999	0.999
Slope(m)		26436	39041
Intercept(c)		3132.7	2230.6
Regression equation (Y=mx+c)		y = 26436x + 3132.7	y = 39041x + 2230.6
Assay (% mean assay)		100.02%	100.19%
Specificity		Specific	Specific
System precision %RSD		0.8	1.1
Method precision %RSD		1.0	1.0
Accuracy %recovery		100.25%	100.11%
LOD		0.24	0.09
LOQ		0.72	0.26
Robustness	**FM**	0.3	0.5
	FP	1.9	1.5
	MM	1.1	1.3
	MP	1.2	1.4
	TM	0.6	0.7
	TP	0.8	1.8

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netarsudil and Latanoprost in Opthalmic solution dosage form. Retention time of Netarsudil and Latanoprost were found to be 2.189min and 2.860min. %RSD of the Netarsudil and Latanoprost were and found to be 0.8 and 1.1 respectively. %Recovery was obtained as 100.25% and 100.11% for Netarsudil and Latanoprost respectively. LOD, LOQ values obtained from regression equations of Netarsudil and Latanoprost were 0.24, 0.09 and 0.72, 0.26 respectively. Regression equation of Netarsudil is y = 26436x + 3132.7 and y = 39041x + 2230.6 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.

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