Validated Stability-indicating RP-HPLC Method Development for Determination of Netarsudil as Bulk Drug and in Ophthalmic Solution

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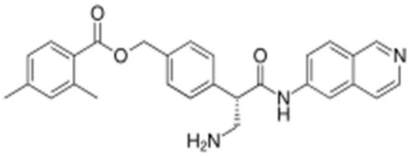
ABSTRACT

A new simple, accurate and precise stability-indicating RP-HPLC analytical method has been developed and validated for the quantitative analysis of Netarsudil as bulk drug and in ophthalmic solution. The objective of this study was to explore Netarsudil degradation behavior under ICH-recommended stress conditions, employing a newly designed stability indicating HPLC method and validating it. The method was developed on Agilent eclipse XBD C8 (150mm ×4.6 mm) column with Acetonitrile: 0.05 M Phosphate Buffer (pH 5) (80: 20, v/v) as mobile phase using simple gradient elution technique. The retention time was found to be 2.5 min. The developed procedure was successfully validated as per ICH Q2 (R1) guidelines. The developed method was found to be linear within the range of 2-10 µg mL^{-1} (R^2 =0.9905), precise as % R.S.D. for inter-day and intra-day precision were found to be < 2 %, accurate as % recovery values in accuracy study was found to be in range of 98-102 and sensitive as limit of detection and limit of quantitation were found to be 0.27 µg mL^{-1} and 0.84 µg mL^{-1}, respectively. Thus the proposed RP-HPLC method was found to provide a faster and cost effective quantitative control for routine analysis of Netarsudil as bulk drug and in ophthalmic solutions.

Keywords: Netarsudil, Stress degradation, Stability indicating, RP-HPLC

INTRODUCTION

Netarsudil, chemically, [4-[(2*S*)-3-amino-1-(isoquinolin-6-ylamino)-1-oxopropan-2-yl] phenyl] methyl 2, 4-dimethylbenzoate is used for the treatment of glaucoma. The Food and Drug Administration (FDA) in the United States approved a 0.02% ophthalmic solution for the lowering of elevated intraocular pressure in people with open-angle glaucoma or ocular hypertension in December 2017 [1].



A widespread literature survey shown that some analytical methods has been reported for quantitative analysis of Netarsudil mesylate. Several reverse phase high performance liquid chromatography (RP-HPLC) methods for estimation of Netarsudil mesylate either as single or in combination with other drugs in pure and pharmaceutical dosage forms are reported in the literature [2-5].

To finest of our information, reports in literature were not established for Netarsudil estimation in ophthalmic formulation with stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method. Therefore, the purpose of existing study is to develop and validate a suitable stability indicating RP-HPLC method for drug by performing degradation under various stress conditions like hydrolysis, oxidation, thermal and photolytic stress following ICH guidelines [6, 7].

MATERIALS AND METHODS

Chemicals and reagents

Active pharmaceutical ingradient (API) Netarsudil Mesylate was obtained as gift sample from Micro Labs Ltd. (Bengaluru, India). The pharmaceutical dosage form used in this study was NETALO eye drops (0.02 % w/v) was procured from local pharmacy. Methanol (HPLC Grade), Acetonitrile ((HPLC Grade), Potassium Dihydrogen Phosphate (AR Grade) were obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

HPLC instrumentation

The samples were analyzed using HPLC system (JASCO), model PU 2080 plus pump with Rheodyne sample injection port (20 µL). The study was performed using Agilent eclipse XBD C8 (4.6 mm×150 mm) column and detection was carried out with PDA detector (MD 2010) with Borwin chromatography software (version 1.5) and quantification at 247 nm wavelength. Mobile phase was optimized which contain Acetonitrile: 0.05 M Phosphate Buffer (pH 5) (80:

20, v/v) at the flow rate of 1 mL min^{-1}, employed in gradient mode. The study involved other instruments like UV-Visible Spectrophotometer (SHIMADZU UV-1780), Photo stability chamber (Newtronic- NEC103RSPI), Vacuum pump (JET-VAC-J1), hot air oven (Kumar Laboratory Oven).

Selection of analytical wavelength

A solution of 10 µg mL^{-1} was prepared from standard stock solution (1000 µg mL^{-1}) and scanned over 200-400 nm in UV Spectrophotometer. The maximum absorbance was shown at 247 nm. Hence it was selected as analytical wavelength.

Stock solution and working standard preparation

An accurately 10 mg of Netarsudil mesylate weighed and transferred to a 10 mL volumetric flask, and the volume was made up to 10 mL with methanol to get standard stock solution having concentration 1000 µg mL^{-1} which was further diluted to acquire final working standard solution of concentration 100 µg mL^{-1}.

Assay of marketed formulation

For the assay of marketed formulation, 1 mL of the marketed sample solution was pipetted out using a volumetric pipette and transferred to a 10 mL of volumetric flask and diluted with methanol to get the concentration of 4 µg mL^{-1}. After setting the chromatographic conditions, the sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of drug present was estimated from the calibration curve. The % assay was found to be 99.97 ± 1.53 (mean ± S.D).

Stress degradation studies

Stress degradation studies were carried out to confirm the stability by exposing the bulk drug to various stress conditions for varying periods of time, using various strengths of reagents recommended by ICH. The studies were carried out at 100 µg mL^{-1} concentration. The hydrolytic studies were carried out by treatment of stock solution of drug separately with 0.1 N HCl and 0.1 N NaOH at room temperature for 2 h. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 8 µg mL^{-1}. The oxidative degradation was carried out in 6 % H_2O_2 at room temperature for 2 h and sample was diluted to obtain 8 µg mL^{-1} solution. Thermal stress degradation was performed by keeping drug in oven at 60ºC for period of 6 h. Photolytic degradation studies were carried out by exposure of drug to UV and fluorescence light.

RESULTS AND DISCUSSION

Method optimization

Analytical method development for estimation of Netarsudil mesylate was started with preliminary trials using HPLC grade methanol, acetonitrile and water mixed in different proportions as mobile phase. The development was initiated through use of mobile phase which contained methanol: 0.05 M Phosphate buffer, acetonitrile: 0.1 M Phosphate buffer in diverse ratios like (50: 50, v/v), (60: 40, v/v), 70:30, v/v). Finally solvent mixture composing Acetonitrile: 0.05 M Phosphate buffer (pH 5) (80: 20, v/v) was selected as an optimum mobile phase. The mobile phase utilized offered excellent resolution along with sharp and well resolved peak without any tailing. Short retention time with improved baseline stability as well as low noise level was achieved. The retention time was found to be 2.5 ± 0.04 by the use of the proposed method. The optimized chromatographic conditions are summarized in Table 1. The representative chromatogram of the standard solution is shown in Figure 1.

Table 1: Summary of optimized chromatographic conditions

Parameters	Conditions used
Stationary Phase	Agilent eclipse XBD C8(4.6 mm×150 mm)
Mobile Phase	Acetonitrile and 0.05 M Phosphate buffer (80: 20, v/v)
Flow Rate	1 mL min^{-1}
Run Time	5 min
RT (min)	2.5 ± 0.04
Asymmetry	1.54
Plates (N)	>2464

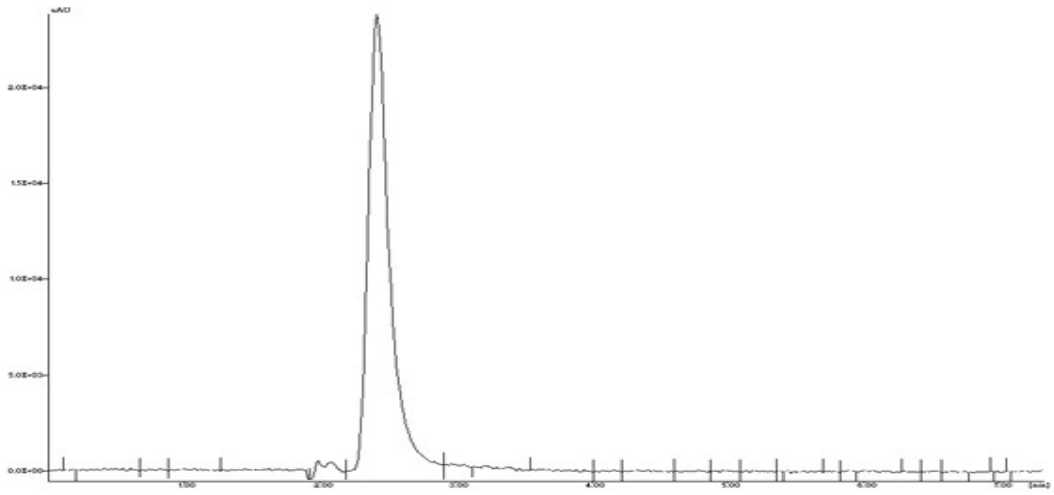


Figure 1: Chromatogram of standard solution (10 µg mL^{-1}, RT = 2.5 ± 0.04 min)

Result of stress degradation studies

When stress degradation experiments were performed and subjected to chromatographic analysis, it was found that Netarsudil was stable to photolytic conditions but substantial degradation was obtained under acid, alkali, oxidative and thermal conditions. The stress condition applied on the active drug substance showed assay loss of drug with decrease in the peak area and without appearance of any degradation peaks under acid, alkali, oxidative and thermal conditions. Figures 2-4 represents the chromatogram of acid, alkali and peroxide induced degradation, while Figure 5 illustrates the chromatogram of thermal degradation. The findings of degradation studies along with % degradation and % of drug recovered are summarized in Table 2.

Table 2: Stress degradation studies

Stress conditions	% Recovery	% Degradation
Acid hydrolysis (0.1 N HCl, Kept at RT for 2 h)	86.21	13.79
Base hydrolysis (0.1 N NaOH, Kept at RT for 2 h)	80.73	19.27
Oxidative degradation (6 % H_2O_2, Kept at RT for 2 h)	73.26	26.74
Thermal degradation (60º C for 6 h)	81.11	18.89
Photolytic degradation (UV light, 200 watt h) square meter^{-1})	99.42	----

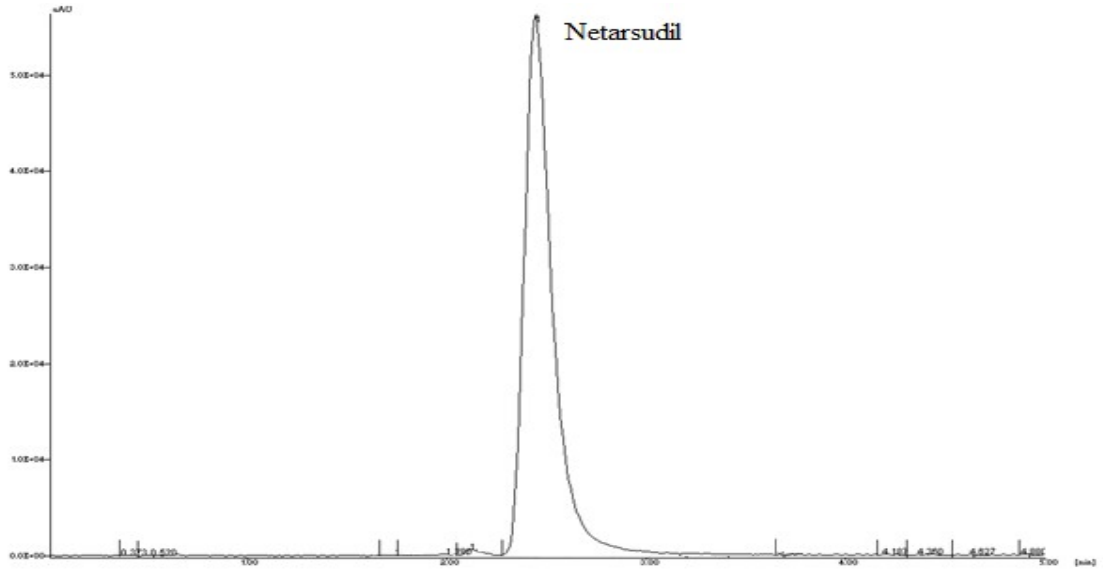


Figure 2: Chromatogram after treatment with 0.1 N HCl Kept at RT for 2 h

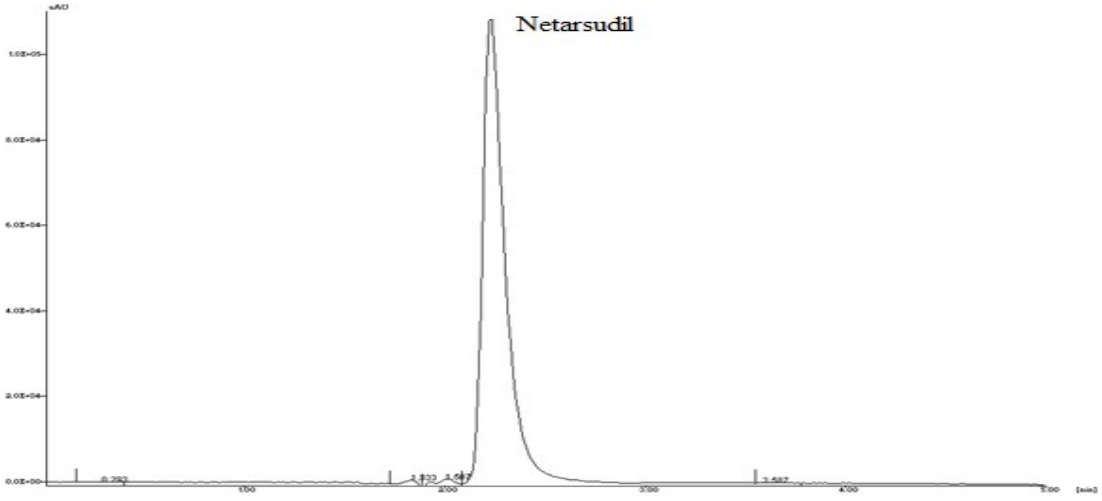


Figure 3: Chromatogram after treatment with 0.1 N NaOH Kept at RT for 2 h

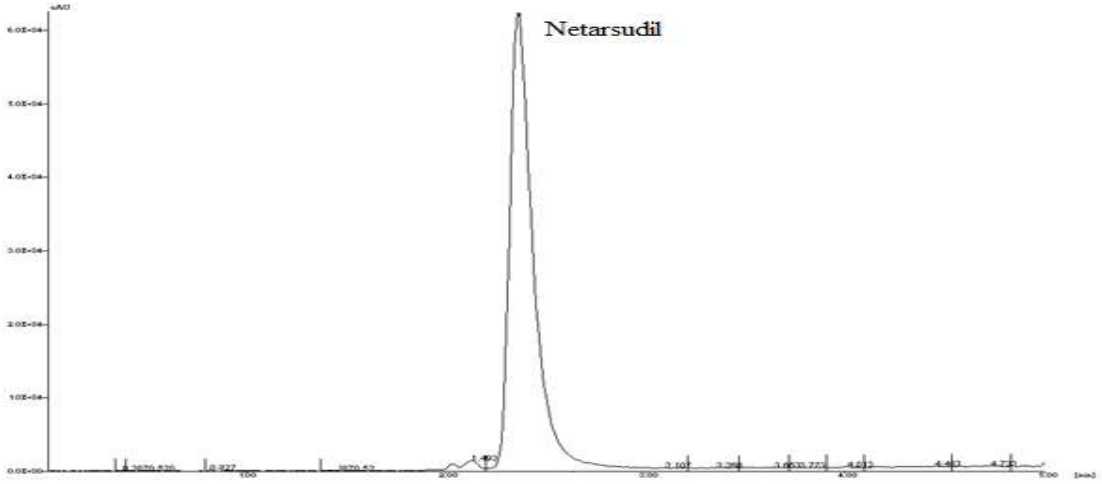


Figure 4: Chromatogram after treatment with 6 % H_2O_2 Kept at RT for 2 h

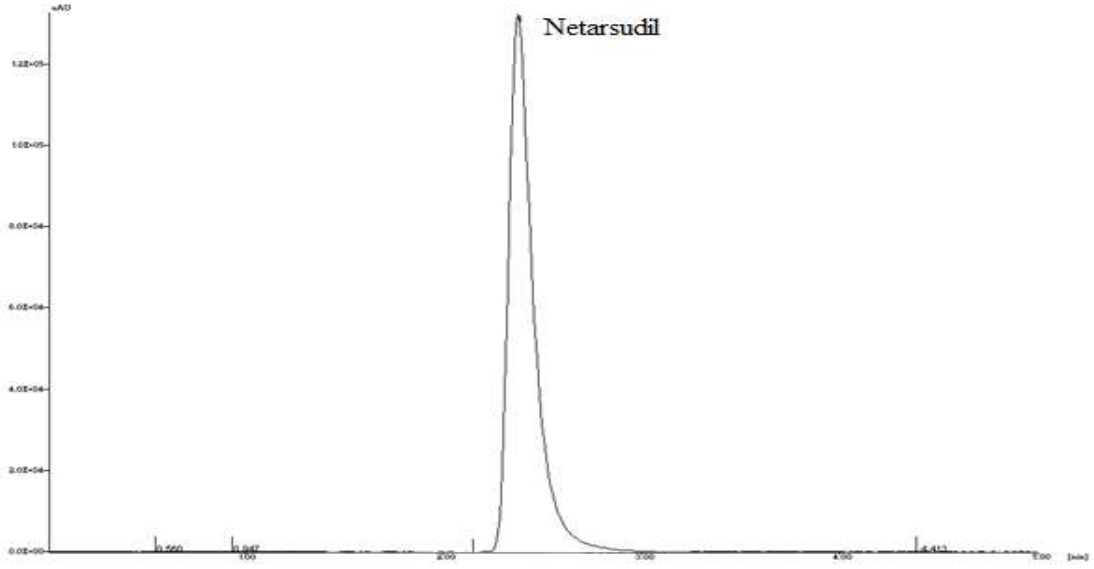


Figure 5: Chromatogram after exposure to heat at 60ºC for 6 h

Analytical method validation

The method was validated as per ICH guidelines for linearity and range, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.

Linearity and Range

The linearity of method was evaluated by linear regression analysis. From the stock solution, aliquots of 0.2, 0.4, 0.6, 0.8, 1 mL were taken in 10 mL volumetric flasks and diluted with methanol to get final concentration in the range of 2-10 µg mL^{-1}. A good linear relationship as indicated by correlation coefficient value 0.990 observed for Netarsudil over the range of 2-10 µg mL^{-1}. Calibration curve was constructed by plotting obtained peak area against drug concentration to prove the linearity. The slope and intercept value for calibration curve was found as; y=122589x+186109. The calibration curve in the concentration range of 2-10 µg mL^{-1} is represented in Figure 6.

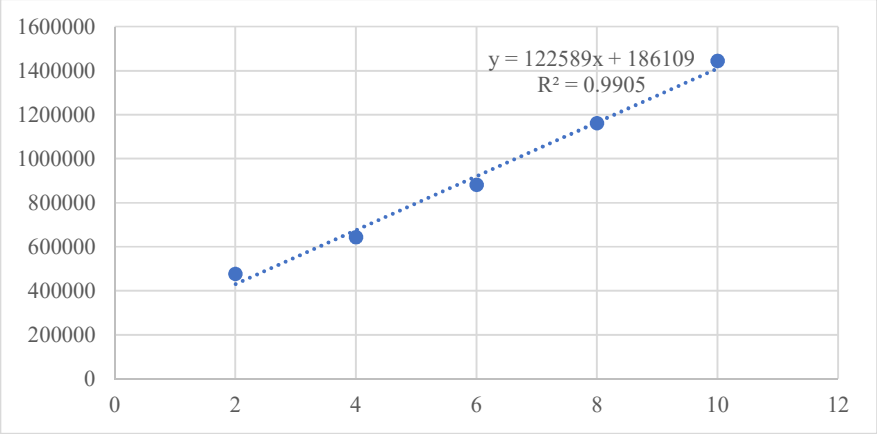


Figure 6: Calibration curve for Netarsudil

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Intra-day and inter day precision study were carried out by estimating the corresponding responses on the same day and on next day for 3 different concentrations (4, 6, 8 µg mL^{-1}) in triplicate. The % R.S.D. values obtained for Intraday and Interday variations were found to be < 2 which indicated that method is precise. The results obtained for intraday and inter-day precision studies are shown in Table 3 and 4, respectively.

Table 3: Intraday precision studies

Injected Concentration (µg mL^{-1})	Average Area	Recovered concentration (µg mL^{-1})	% R.S.D.*
4	670370	3.94	0.71
6	914438	5.93	1.26
8	1156624	7.91	0.97

* Average of three determinations

Table 4: Interday precision studies

Concentration (µg mL^{-1})	Average Area	Recovered concentration (µg mL^{-1})	% R.S.D.*
4	670598	3.95	0.87
6	920446	5.95	0.95
8	1167954	7.88	0.59

* Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 0.27 µg mL^{-1} and 0.84 µg mL^{-1} respectively.

Accuracy

Recovery studies were performed to confirm accuracy of developed method by standard addition method which involved addition standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 4 µg mL^{-1}. The results indicated accurateness of developed method for estimation of drug in ophthalmic formulation.

Table 5: Recovery studies

Drug	Basic sample concentration (µg mL^{-1})	Concentration added (µg mL^{-1})	Concentration found (µg mL^{-1})	% Recovery±R.S.D.*
Netarsudil	4	2	06.01	100.32±1.20
	4	4	07.93	99.17±0.61
	4	6	09.89	98.92±1.29

*Average of three determinations, R.S.D. is relative standard deviation

Specificity

It was determined by peak purity profiling studies in order to demonstrate that the chromatographic peak is not attributable to more than one component. The ability of developed method to separate the drug from excipients present in the formulation proved the specificity of method.

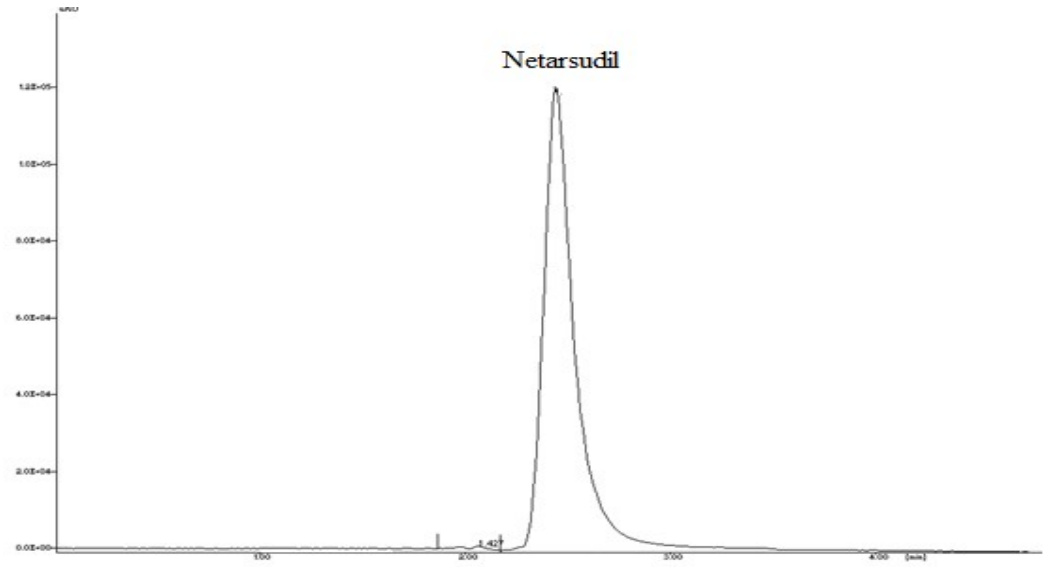


Figure 7: Chromatogram of marketed formulation (4 µg mL^{-1}, RT = 2.4 ± 0.02 min)

Robustness

Method robustness was evaluated by making intentional variations in optimized conditions. The parameters altered were change in mobile phase composition (± 1% Acetonitrile), flow rate (± 0.1 mL min^{-1}). The deliberate variations made during study demonstrated that peak areas were not affected due to small alterations made in experimental parameters as well as values of R.S.D. were observed within the limit demonstrating robustness of the method.

CONCLUSION

The RP-HPLC method developed for the analysis of Netarsudil in ophthalmic preparations is stability indicating, precise, accurate and with a short run time. The results of stress testing undertaken according to the ICH guidelines revealed susceptibility of drug to hydrolytic, oxidative, thermal stress conditions and stability under photolytic stress conditions. The method was fully validated showing satisfactory data for all the method validation parameters tested. The method developed would serve as a versatile analytical tool suitable for the analysis of the drug in the tested formulation and would be of interest for QC and clinical monitoring laboratories.

ACKNOWLEDGEMENTS

The authors express their gratitude to Micro Labs Limited, (Bengaluru, India) for the gift sample of pure Netarsudil. Thanks are also extended to Dr. Ashwini Madgulkar, Principal, AISSMS College of Pharmacy for providing research facilities to carry out the research work.

REFERENCES

1. https://en.wikipedia.org/wiki/Netarsudil (Accessed on 03/02/2023)
2. Kunala A, Gummadi S. Simultaneous estimation of netarsudil and latanoprost by RP-HPLC-PDA. International Journal of Pharmaceutical Quality Assurance and Pharmaceutical Analysis, 2022; 13(3): 309-314.
3. Padmalatha K, Vijaya Durga D, Jagadeeswari N. A novel study on RP-HPLC method development and validation for estimation of netarsudil and latanoprost in API and pharmaceutical dosage form. World Journal of Pharmaceutical Research, 2021; 10(12): 1624-1633.
4. Venkata Ramana L, Devanaboyina N, Vijaya Lakshmi G. Method development and validation for simultaneous estimation of netarsudil and latanoprost by RP-HPLC Method. EJPMR, 2020; 7(7): 888-894.
5. Dharmamoorthy G, Nataraj KS, Krishna A, Pawar M. Development and validation of a reverse-phase ultra-performance liquid chromatography method for the simultaneous determination of netarsudil and latanoprost in bulk and pharmaceutical formulation. International Journal of Green Pharmacy, 2020; 14 (2): 179-185.
6. ICH Guideline Q2A (R1), Validation of analytical procedure, text and methodology Q2 (R1) (2005)
7. ICH Guideline Q1A (R2), Stability testing of new drug substances and products Q1A (R2) (2003)