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A Stability-indicating RP- HPLC technique for the determination of Netarsudil Mesylate in pharmaceutical bulk formulations.

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Netarsudil Mesylatein pharmaceutical dosage form. Chromatographic separation of Netarsudil Mesylate was achieved on Waters Alliance-e2695, by using Symmetry Shield RP18 (4.6×150mm,305µ) column and the mobile phase containing 0.1% FORMIC ACID ***& ACN*** *in the ratio of* ***40:60% v/v****. The flow rate was 1.0 ml/min; detection was carried out by absorption at* ***257nm*** *using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Netarsudil Mesylate were NLT 2000 and should not more than* ***2*** *respectively. % Relative standard deviation of peak areas of all measurements always less than* ***2.0.*** *The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Netarsudil Mesylate.*

Keywords: *RP-HPLC,* ***Netarsudil Mesylate****, Relative standard deviation*

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I. INTRODUCTION

In the pharmaceutical sector, quality control is vital to verify that the drug substance [1] and other raw materials are acceptable for use in the final medication product and to ensure that the drug product generated satisfies the highest quality standards needed by Food and Drug Administration [2] (FDA) (FDA). A full Quality Control team participates in all stages of medication production: from raw material and drug substance testing through intermediate and final testing.

The quality, safety, and effectiveness of modern human-use pharmaceuticals must all fulfil very high requirements. The availability of sufficient procedures for product quality control is required for the practical assessment of the aforementioned elements. As a result, the analytical procedures employed to determine the active drug and its associated contaminants in bulk drug material are put to a lot of work. In most cases, drug compounds are not provided in their natural state. Medicine is the whole pharmaceutical formulation [4] (dosage form) in which the active component (drug) [5] is mixed with additional chemicals (also known as excipients) [6] to make a convenient form of administration, such as tablet, capsule, injection and ointment.

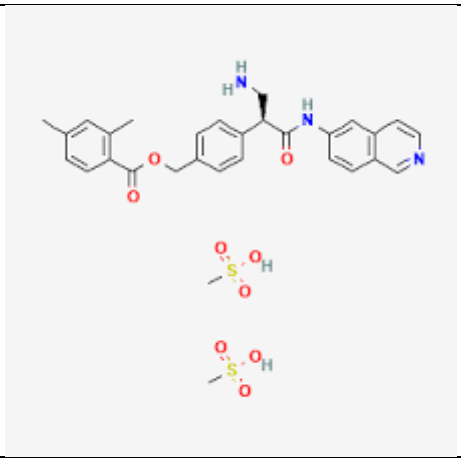
UV-spectral photometry and infrared spectroscopy are the most often used procedures in quality control (QC), as are colour reactions, melting point (range) measurements, titrations, and chromatography (such as TLC, HPLC, and GC). Microbiological testing is also well-established, as are tests such as residue on ignition, loss on drying, and others. In order to define a drug substance (DS) and its precursors, together with excipients and the final drug product, all of these methodologies are used (DP). Quality control is also carried out on main and secondary packaging materials, ensuring that they satisfy set standards. As a result, testing falls within the purview of quality control. In order to ensure quality, testing is just a means of managing it.

One of the most widely used analytical methods in the pharmaceutical sector is high-performance liquid chromatography, specifically reversible phase HPLC (RP-HPLC). It is commonly used in pharmaceutical quality control for assays and impurity analyses. There has been a rise in the importance of HPLC techniques' quality.

Durability and robustness should be tested early in the method development stage to guarantee long-term effectiveness of HPLC techniques in quality control testing. The time and resources necessary to redesign, revalidate, and retransfer analytical procedures if a non-robust or non-rugged approach is adopted might be considerable.

For the pharmaceutical and medical sectors, high-performance liquid chromatography is particularly important since it allows for both qualitative and quantitative examination.

Table-1: Drug profile of **Netarsudil Mesylate**

IUPAC name	bis(methanesulfonic acid); {4-[(1S)-2-amino-1-[(isoquinolin-6-yl)carbamoyl]ethyl]phenyl}methyl 2,4-dimethylbenzoate
Molecular Formula	$C_{30}H_{35}N_3O_9S_2$
Molecular Weight	645.7g/mol
Description	Netarsudil ophthalmic is used to treat glaucoma (a condition in which increased pressure in the eye can lead to gradual loss of vision) and ocular hypertension (a condition which causes increased pressure in the eye). Netarsudil is in a class of medications called rho kinase inhibitors
Solubility	Freely soluble in water and soluble in methanol.
Half life	16–17 hours
Molecular Structure	

II. MATERIALS AND METHODS

RP-HPLC Method Development for Netarsudil Mesylate:

Table 2: List of Apparatus used in HPLC

S.No	Name	Model	Manufacturer
1.	HPLC	ALLIANCE	Waters e 2695- Empower software2.0versions
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris
4.	UV/VIS spectrophotometer	-	UV-1700
5.	Pipettes, beakers and Burettes	-	Borosil
6.	Ultra sonicator	UCA 701	Unichrome
7.	Pump	Isocratic model	--

(b) Reagents & Chemicals

Table 3: List of chemicals used in HPLC Method

S.No	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Water (Milli Q)	HPLC	In house production
3.	Formic acid	HPLC	Analytical reagents
4.	Ortho Phosphoric acid	HPLC	Rankem

Determination of Working Wavelength (λ_{max}):
In estimation of the drug isobestic wavelength was used. Isobestic point is the wavelength where the molar absorptivity is the same for the substances that are inter convertible. So this wavelength was used in estimation of drug accurately.
The wavelength of maximum absorption of the solution of the drug in mixture of Acetonitrile and 0.1% formic acid (60:40) were scanned using PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% formic acid (60:40) as blank. The absorption curve shows isobestic point at 242 nm. Thus 242 nm was selected as detector wavelength for the HPLC chromatographic method.

Chromatographic conditions:
During the selection of chromatographic conditions, numbers of trails were carried out and the best trail was selected for optimized method.

Preparation of standard stock solution:
Accurately weighed and transferred the 10 mg of Netarsudil Mesylate working standard into a 10 ml clean dry volumetric flask added diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipetted 1 ml of the above stock solutions into a 10 ml volumetric flask and diluted up to the mark with diluent. (100ppm of Netarsudil Mesylate)

Sample Solution Preparation:
Accurately weighed and transferred 0.2 ml of Netarsudil Mesylate sample into a centrifuge tube clean dry volumetric flask added 0.8 ml of diluent and sonicated to dissolve it completely

Table-4: Optimization of chromatographic condition

Column	Symmetry Shelid RP18 (4.6×150, 3.5µ)
Mobile phase ratio	Acetonitrile: 0.1% formic acid (60:40)
Detection wavelength	242 nm
Flow rate	1ml/min
Injection volume	10µl
Run time	5min
Observation	This method is suitable for validation

The Netarsudil Mesylate peak was observed at 2.271 min with peak area 2459162, tailing factor 1.06. This trial was optimized.

Preparation of Mobile Phase: Mobile phase was prepared by mixing 0.1% formic acid and ACN taken in the ratio 40:60. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of Diluent: Mobile phase was used as a diluent.

Procedure:
Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Netarsudil Mesylate peak and calculate the %Assay by using the formulae.

SYSTEM SUITABILITY:
Tailing factor for the peak due to Netarsudil Mesylate in Standard solution should not be more than 2.0
Theoretical plates for the Netarsudil Mesylate peak in Standard solution should not be less than 2000.

Formula for Assay:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where: AT = average area counts of test (sample) preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
DS = Dilution of working standard in ml.
DT = Dilution of test (sample) in ml.
WT = Weight of test (sample) taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

III. Method Validation Summary:

Specificity:
Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific**.**

LINEARITY:

Preparation of stock solution:

Accurately weighed and transferred 10mg of Netarsudil Mesylate working standard into a 10 ml clean dry volumetric flask added diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Concentrations of 25ppm,50ppm,75ppm,100ppm,125 and 150 ppm of Netarsudil Mesylate were taken and tested for linearity.

Procedure:

Injected each level into the chromatographic system and measured the peak area.

Plotted a graph of peak area versus concentration (concentration on the X-axis and Peak area on Y- the axis) and calculate the correlation coefficient.

Range:

The Range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated with precision, accuracy and linearity

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

Precision

Precision is the degree of repeatability of an analytical method under normal operating conditions. Precision is of 3 types

1. System precision
2. Method precision
3. Intermediate precision (Inter-day precision)

To guarantee that the analytical system is functioning correctly, its accuracy is tested using a standard chemical compound. The peak area and percentage drug of six determinations are assessed, and the relative standard deviation (RSD) is computed.

A single batch sample should be tested six times to ensure procedure accuracy. Find out whether a procedure produces the same results with each batch. Six replicates of the sample analysis and a % RSD calculation are shown below.

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 100ppm of Netarsudil Mesylate).

Acceptance Criteria:

The % RSD for the absorbance of six replicate injection results should not be more than 2%.

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate varied from 0.9 ml/min to 1.1 ml/min.

Standard solution 100ppm of Netarsudil Mesylate was prepared and analysed using the varied flow rates along with method flow rate.On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

The variation of Organic Phase ratio: Standard solution of 100ppm of Netarsudil Mesylate was prepared and analysed using the varied in mobile phase ratio.

DEGRADATION STUDIES:

Acid degradation: Pipetted 0.2 ml of above solution into a 1ml Centrifuse tube and 0.1 ml of 1N HCl was added and leave it for 15 min. After 15 min neutralized with 1 N NaOH and made up to 1ml with diluent. Filter the solution with 0.45 microns syringe filters and placed in vials.

Alkali degradation:Pipetted 0.2 ml of above solution into a 1ml Centrifuse tube and 0.1 ml of 1N NaOH was added and leave it for 15 min. After 15 min neutralized with 1 N HCl and made up to 1ml with diluent. Filtered the solution with 0.45 microns syringe filters and placed in vials.

Peroxide degradation : Pipetted 0.2 ml of above solution into a 1ml Centrifuse tube and 0.1 ml of 10% H_2O_2 was added and leave it for 15 min. After 15 min made up to 1ml with diluent. Filtered the solution with 0.45 microns syringe filters and placed in vials.

Reduction degradation : Pipetted 0.2 ml of above solution into a 1ml Centrifuse tube and 0.1 ml of 10% Sodium Bisulphate solution was added and leave it for 15 min. After 15 min made up to 1ml with diluent. Filtered the solution with 0.45 microns syringe filters and placed in vials.

IV. Results And Discussion

Determination of Working Wavelength (λ_{max}):

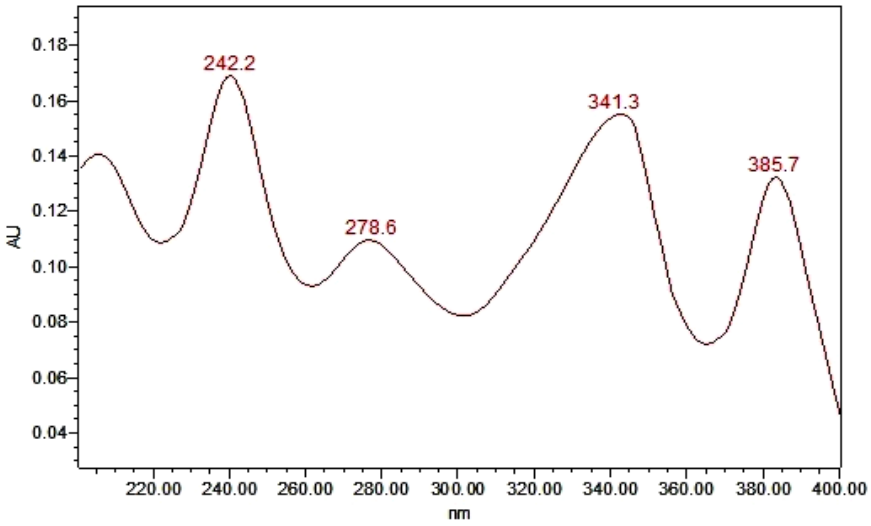


Figure 1: PDA-Spectrum of Netarsudil Mesylate

Table-5: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Instrument used	Waters HPLC with auto sampler and UV detector.
Injection volume	10µl
Mobile Phase	Acetonitrile and 0.1% formic acid (60:40)
Column	Symmetry ShieldRP18 (4.6×150,3.5)
Detection Wave Length	242nm
Flow Rate	1 mL/min
Runtime	5min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

Table-6: Chromatogram of Trial-9

S.No	Name	Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Netarsudil Mesylate	2.271	2459162	100.00		1.06	2620

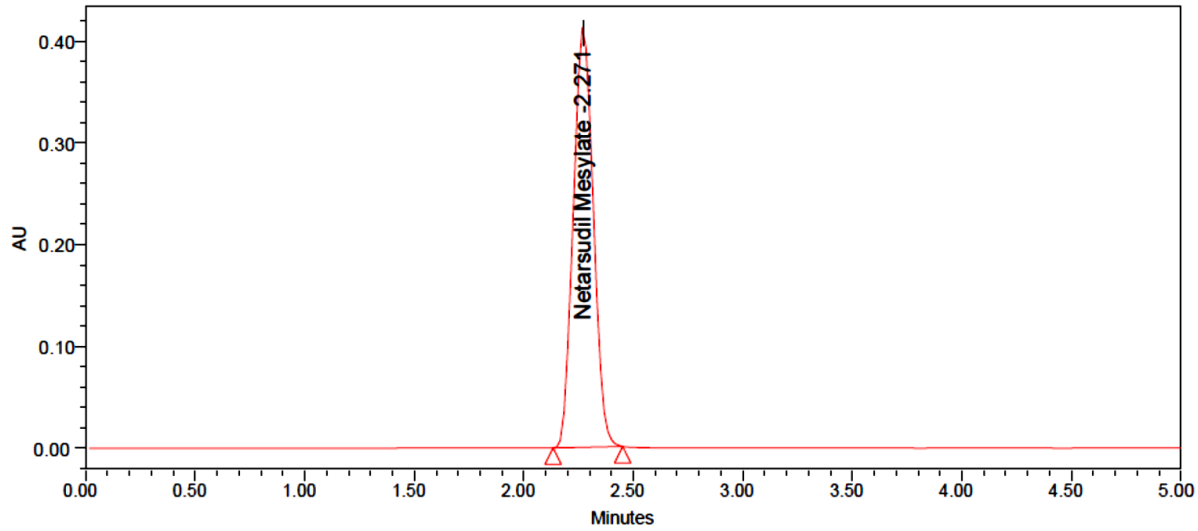


Figure 2: Chromatogram of standard *Netarsudil Mesylate*

Specificity:

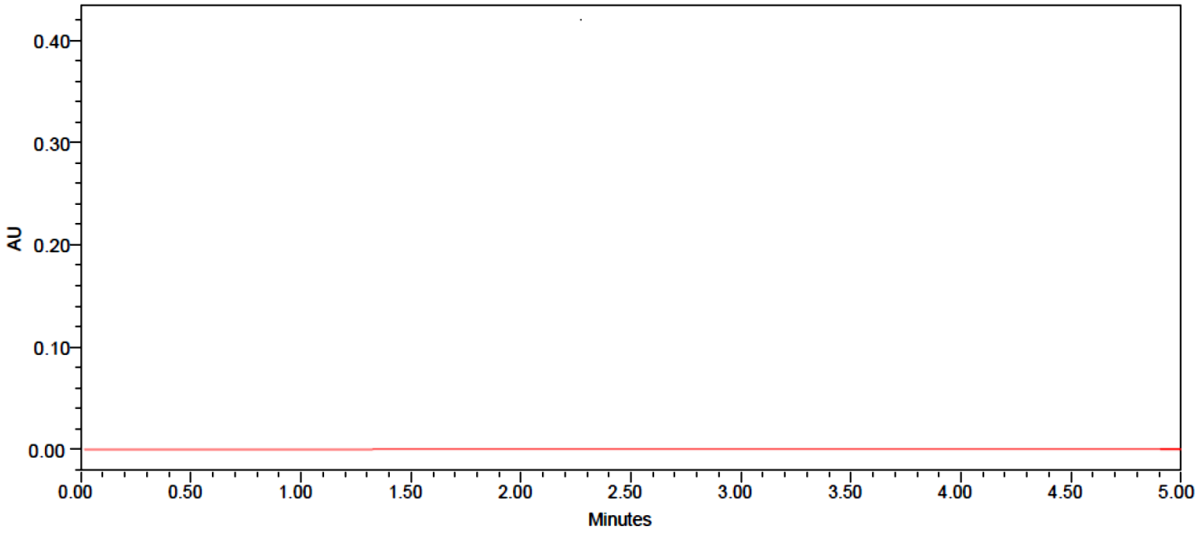


Figure 3: **Chromatogram of blank**

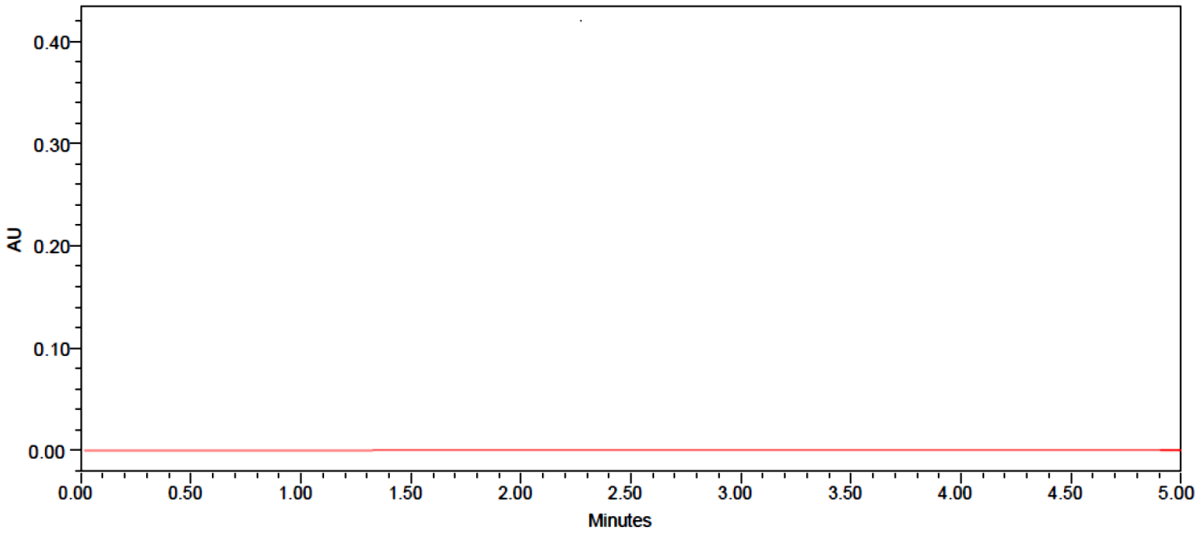


Fig No.4: Chromatogram of placebo

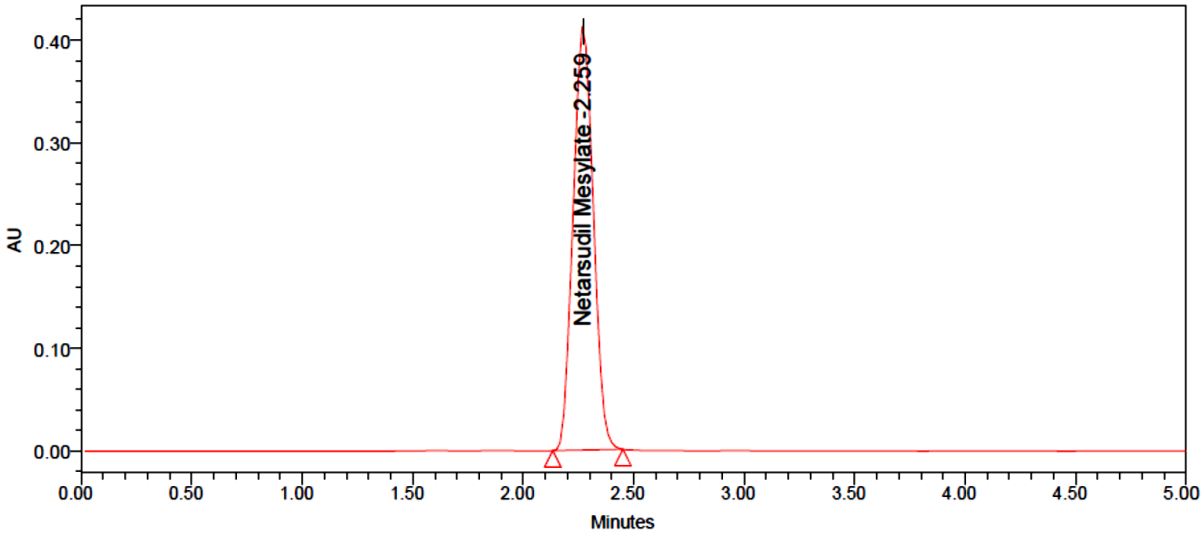


Figure 5: Chromatogram of Sample

Table-7: Assay of Netarsudil Mesylate

Brand	Drug	sample area	Average Area	Std. wt (mg)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
-	Netarsudil Mesylate	2419546	2441224	10	0.028	99.9	0.1	100.01
		2462901						

Acceptance Criteria:
The % assay should be within the range of 98-102%
Observation: The % assay was found to be within the range.

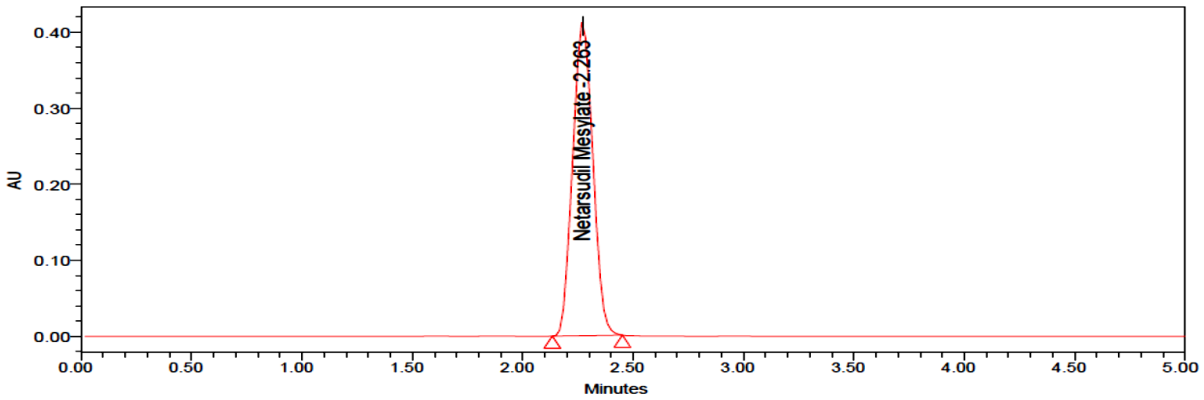


Figure 6: Chromatogram of Assay-1

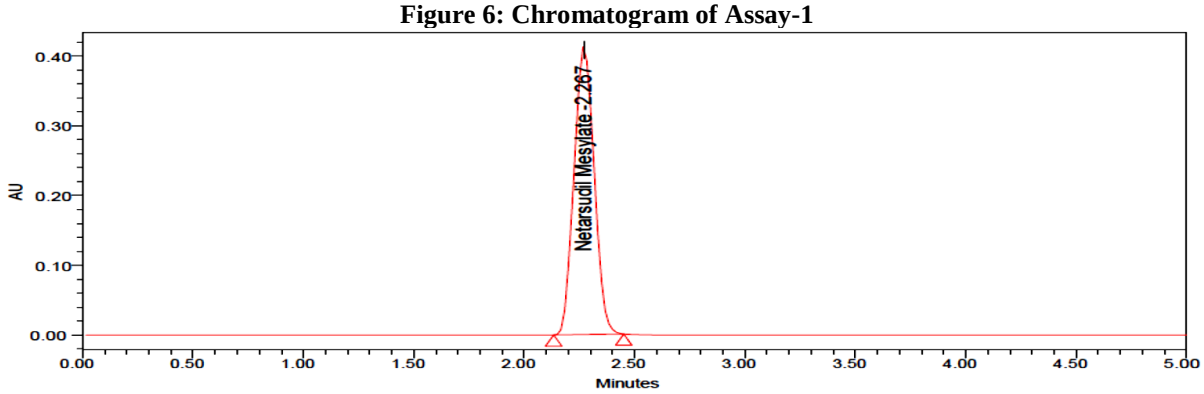


Figure 7: Chromatogram of Assay-2

ANALYTICAL METHOD VALIDATION (HPLC)

The method was validated for its linearity range, accuracy, precision, and specificity. Method validation was carried out as per ICH guidelines.

Linearity:

Table-8: Results of linearity for Netarsudil Mesylate

S.NO	Netarsudil Mesylate	
	Conc.(µg/ml)	Peak area
1	25	631124
2	50	1245605
3	75	1847242
4	100	2460358
5	125	3066123
6	150	3684515
Regression equation	y = 244832.80x +11606.43	
Slope	24483.28	
Intercept	11606.43	
R^2	0.99998	

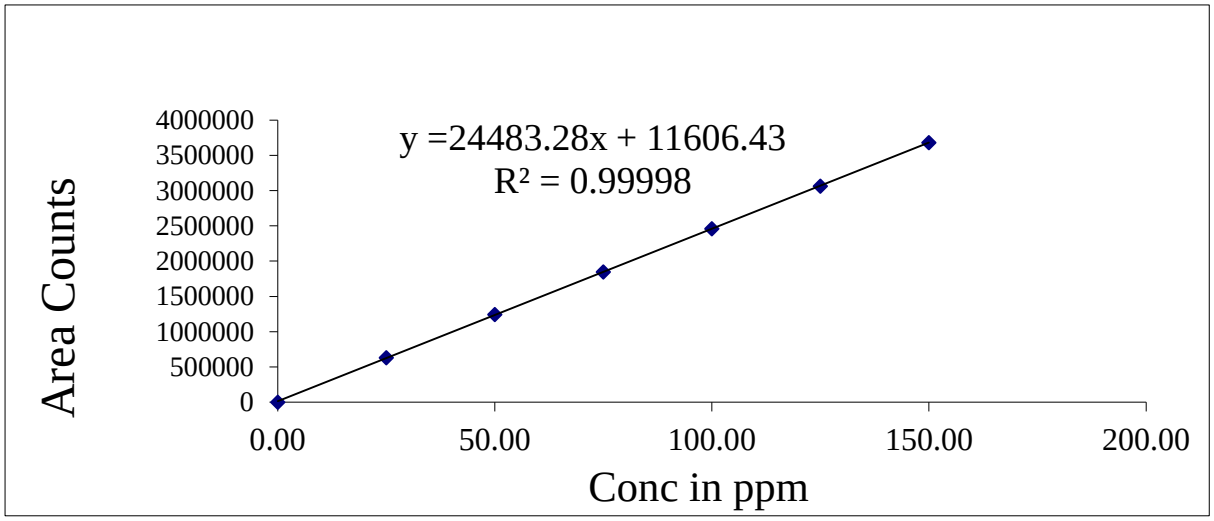


Figure 8: Calibration curve for Netarsudil Mesylate at 242 nm

Precision:

In a method precision investigation, six distinct standard solutions with **Netarsudil Mesylate** concentrations of 100 µg/ml each are produced and injected into an HPLC system. It was discovered that the **Netarsudil Mesylate** %assay was 100%. Peak areas, from which mean, SD, and %RSD values were derived, were determined. Table 7 below contains these outcomes.

Table-9: Standard results for Netarsudil Mesylate by RP-HPLC method

Injection	Area for Netarsudil Mesylate
Injection-1	2459162
Injection-2	2433584
Injection-3	2447715

Injection-4	2438640
Injection-5	2429551
Injection-6	2428795
Average	2439575
Standard Deviation	11860.569
%RSD	0.49

Table-10: Method Precision for Netarsudil Mesylate by RP-HPLC method

Parameter	Area for Netarsudil Mesylate
Method precision-1	2421541
Method precision -2	2450185
Method precision -3	2473161
Method precision-4	2440535
Method precision -5	2419774
Method precision -6	2421628
Average	2437804
Standard Deviation	21270.139
%RSD	0.87

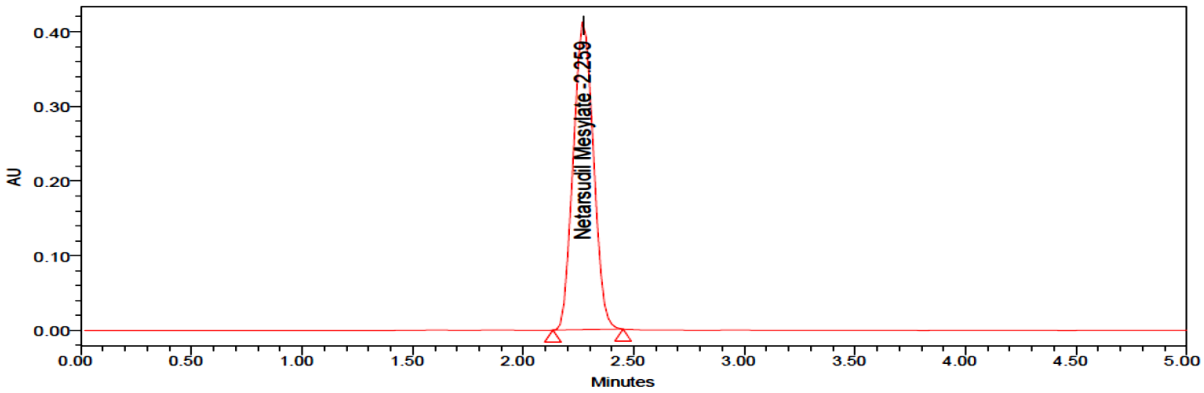


Figure 9: Chromatogram of Method Precision

Acceptance Criteria: The % RSD for the area of six standard injection results should not be more than 2%**.**

Robustness:

Table-11: Robustness results of Netarsudil Mesylate by RP-HPLC

Parameter	Netarsudil Mesylate					
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change (mL/min)	Less flow(0.9ml)	3.352	2856412		1.08	2849
	Actual(1ml)	2.271	2459162		1.06	2617
	More flow(1.1ml)	1.998	2226953		1.01	2566
Organic Phase change	Less Org (54:46)	3.299	2628462		1.12	2705
	Actual(60:40)	2.278	2433584		1.05	2632
	More Org (66:34)	1.996	2184854		1.05	2598

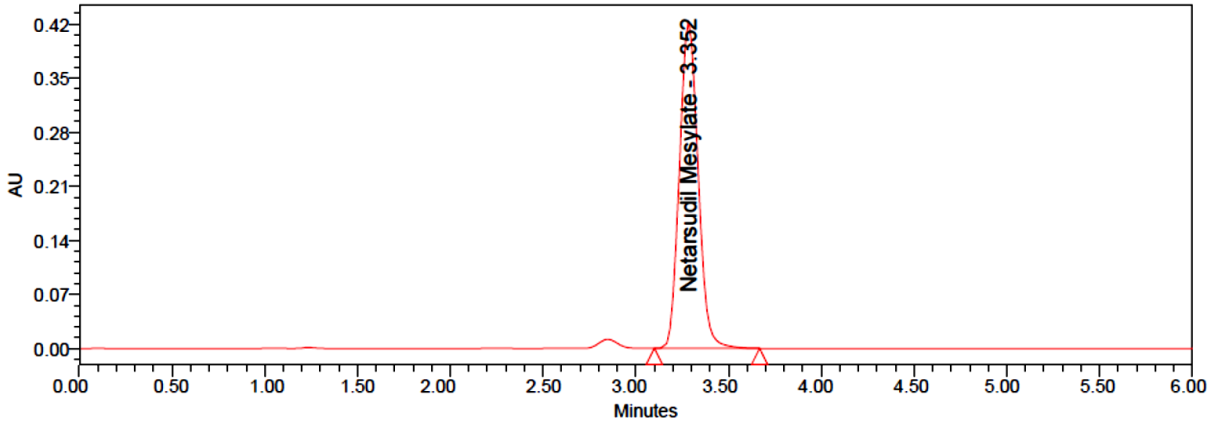


Figure 10: Chromatogram for less flow rate (0.9 ml)

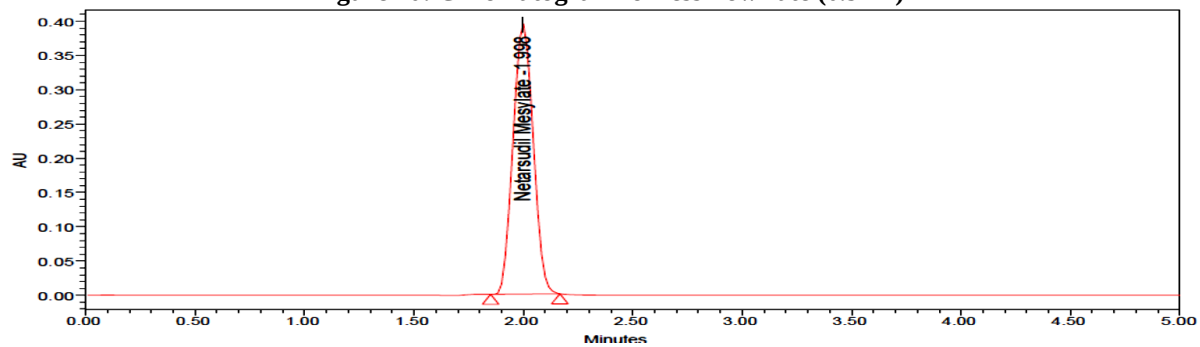


Figure 11: Chromatogram for more flow rate (1.1mL)

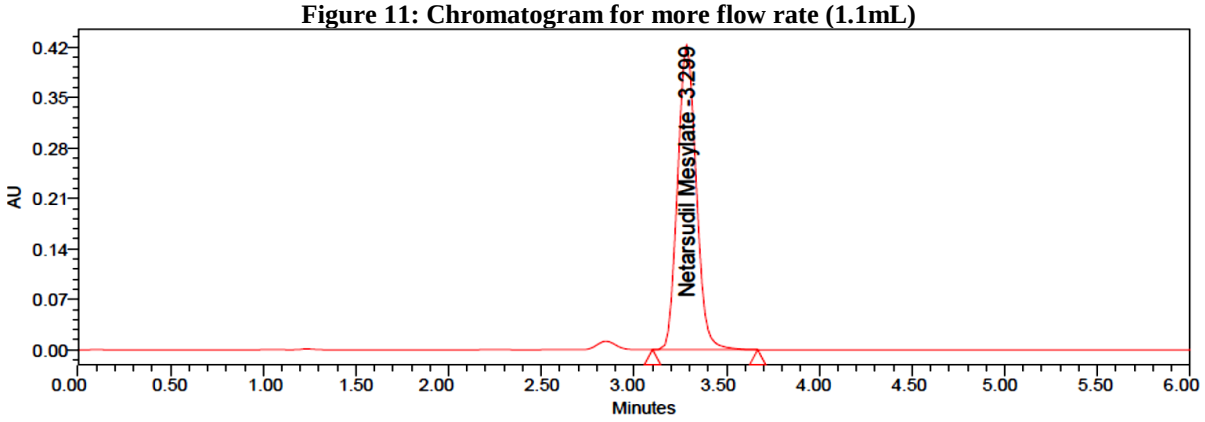


Figure 12: Chromatogram for less Organic Phase (54:46)

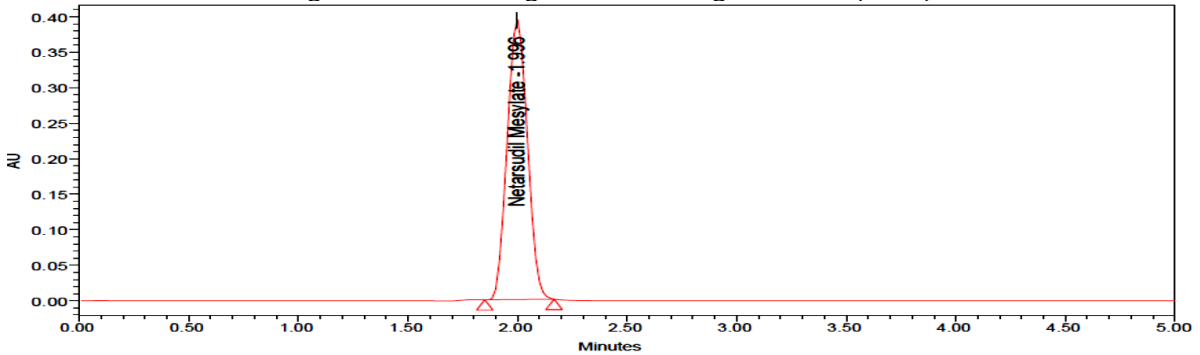


Figure 13: Chromatogram for more Organic Phase (66:34)

DEGRADATION STUDIES:

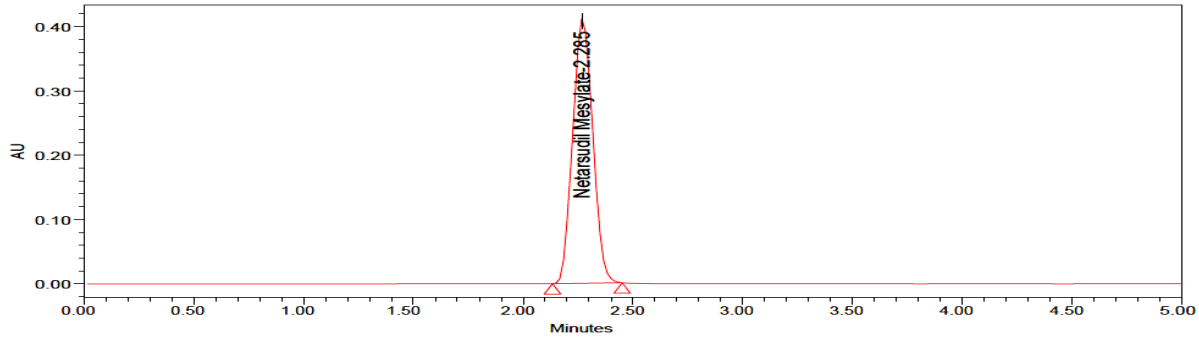


Figure 14: Chromatogram of Control degradation

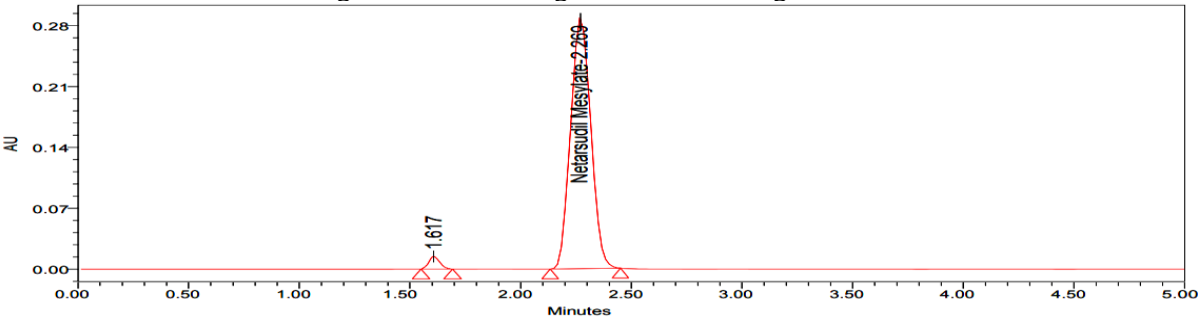


Figure 15: Chromatogram of Acid degradation

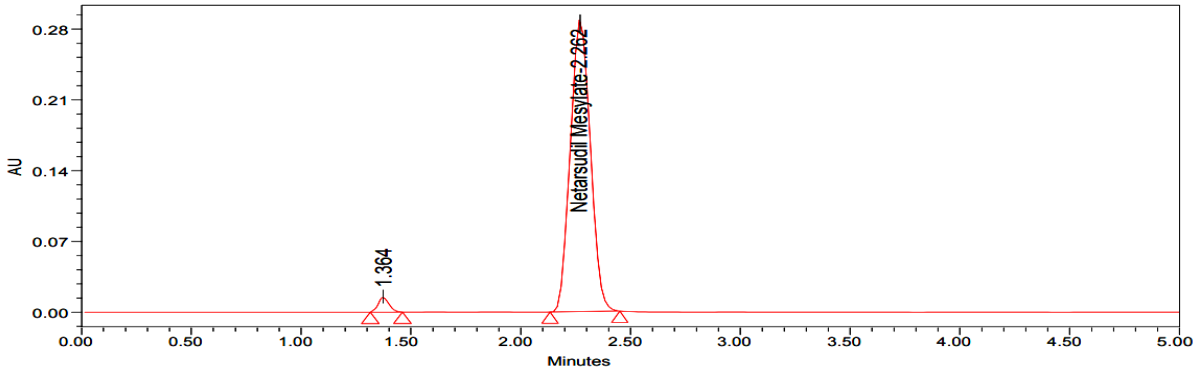


Figure 16: Chromatogram of Alkali degradation

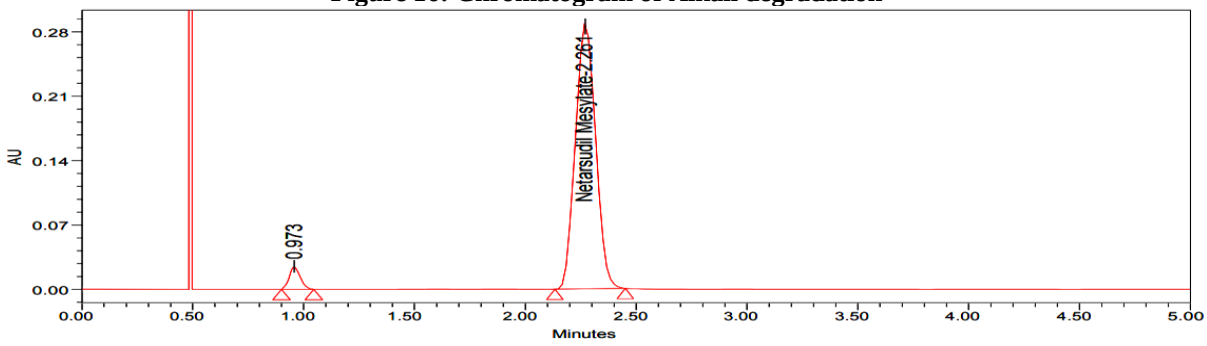


Figure 17: Chromatogram of Peroxide degradation

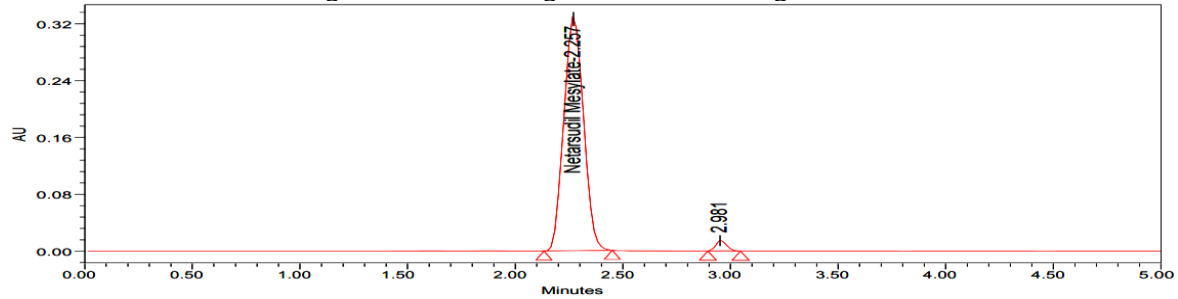


Figure 18: Chromatogram of of Reduction degradation

Table-12: Forced Degradation results for Netarsudil Mesylate

Results: % Degradation results	Netarsudil Mesylate	
	Area	% Degradation
Control	2438147	0.1
Acid	1755230	12.0
Alkali	1740136	11.6
Peroxide	1715403	13.4
Reduction	1887452	8.7

V. CONCLUSION:

The developed HPLC method for the estimation of selected drug is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.

The sample recoveries were in good agreement with their respective label claims and they suggested non interference of formulation excipients in the estimation and can be used in laboratories for the routine analysis of selected drugs.

Since the system validation parameters of HPLC method used for estimation of selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Netarsudil Mesylate.

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