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Development and Validation of UV Spectrophotometric Method for Estimation of Netarsudil Mesylate in Bulk and Pharmaceutical Formulations

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KEYWORDS

Eye Drops; HPLC; ICH Q2(R1); Netarsudil mesylate; UV Spectrophotometry

ABSTRACT:

A robust and precise UV Spectrophotometric method was developed and validated for the estimation of netarsudil mesylate in bulk and pharmaceutical formulations. Spectrophotometric detection was carried out at an absorption maximum (λ_{max}) of 243 nm using water as solvent. The proposed method exhibited excellent linearity over the concentration range of 4 to 12 µg/ml with a correlation coefficient of 0.9993. Validation was performed in accordance with ICH Q2(R1) guidelines, assessing parameters such as accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). The method demonstrated satisfactory accuracy with mean % recovery values ranging from 99.55-100.13%. The precision was measured in terms of % relative deviation, which was found to be consistently below 2. The LOD and LOQ were found to be 0.325 and 0.9852 µg/ml, respectively. The % amount of netarsudil mesylate estimated was 98.68% and was found to be in good agreement with the label claim. The developed method was successfully applied to the analysis of netarsudil mesylate in its marketed dosage form, showing no interference from common excipients. Further, results were compared with HPLC analysis of the drug in formulation and found no significant difference in assay. Owing to its simplicity, rapidity, and reliability, the method is well-suited for routine quality control and in-process analysis of neatrsudil in both bulk and finished pharmaceutical dosage form.

1. Introduction

When it comes to the analysis of pharmaceutical substances, UV techniques have many benefits. They are usually cost-effective, as they do not require costly reagents or complicated instrumentation. Furthermore, UV techniques are easy to use and rather quick, which makes them appropriate for regular quality monitoring in pharmaceutical contexts [1]. Pharmaceutical method validation is essential for assuring the precision, accuracy, and reliability of analytical data. It helps in confirming that the technique reliably yields results that accurately reflect the drug's concentration, which is crucial for patient safety and effectiveness. Once the UV method has been validated, researchers can use it with confidence for regulatory compliance and quality control, making sure the pharmaceutical product satisfies the necessary requirements [2].

Glaucoma is a group of eye diseases that are usually due to intraocular hypertension or increased pressure inside the eye, which damages the optic nerve and, if left untreated, can lead to blindness [3]. Glaucoma is the major cause of irreversible blindness and affects 60 million people worldwide currently. It is expected that by 2040, the cases will rise to more than 110 million, which will increase the demand for better treatments [4]. The chief therapeutic measure is to lower intraocular temperature, either by reducing the secretion of aqueous humour or by promoting its drainage. Lowering of intraocular temperature retards the progression of optic nerve damage even in normal/low intraocular temperature glaucoma [5].

Rho kinase is a serine/threonine protein kinase that plays a pivotal role in orchestrating the regulation of cellular morphology and dimensions through its modulatory effects on the cytoskeletal architecture [6].

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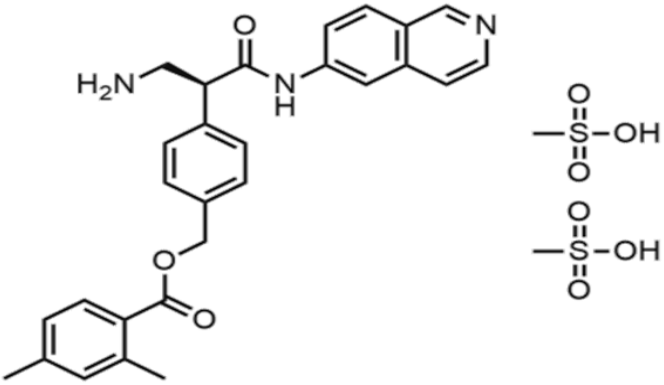
ROCK inhibitor, which directly targets the pathophysiology of the trabecular pathway to achieve a reduction in intraocular pressure (IOP). Netarsudil mesylate is a Rho kinase (ROCK) inhibitor that also exhibits norepinephrine transporter inhibitory activity, contributing to its dual mechanism in lowering intraocular pressure (IOP) [7]. Table 1 shows chemical and physicochemical characteristics of netarsudil mesylate.

The available literature reveals various methods for estimation of netarsudil mesylate including HPLC, LC-MS, etc. Raju and Reddy [8] have used LC-Q-TOF-MS/MS to identify and structurally characterize novel hydrolytic degradation products of netarsudil, and in silico toxicity prediction was also incorporated to assess the potential safety degradation risks. To build upon this, Gayatri Devi and colleagues [9] have developed an RP-HPLC method for netarsudil mesylate in bulk drug, extolling the method's precision, linearity, and suitability for routine quality control. Additionally, Kumar et al. [10] have also developed a stability-indicating RP-HPLC method that permitted the simultaneous quantification of netarsudil and its process-related impurities, with effective separation under forced degradation. All these methods are very tedious, time consuming and costly requiring lots of organic solvent which also have environment concern.

The current study was aimed to develop and validate a simple, reliable, and economical UV spectrophotometric method for netarsudil mesylate and its pharmaceutical preparations. This study seeks to formulate a method that is fast and reliably executes all the steps of validation as per ICH Q2(R1) concerning accuracy, precision, linearity, and reproducibility. With the rising clinical application of netarsudil mesylate for managing ocular hypertension and open-angle glaucoma, having a simple UV method would significantly streamline the process of routine quality control in resource-strained settings as opposed to costly methods.

Table 1: Drug profile

Common name	Netarsudil Mesylate
Category	Rho Kinase Inhibitor
Chemical Structure	
IUPAC name	[4-[(2*S*)-3-amino-1-(isoquinolin-6-ylamino)-1-oxopropan-2-yl]phenyl]methyl 2,4-dimethylbenzoate;methanesulfonic acid
Chemical formula	$C_{30}H_{35}N_3O_9S_2$
Molecular weight	645.7 g/mol
CAS number	1422144-42-0
Category	Rho-associated protein kinase 2 inhibitor
Solubility	Very soluble in ethyl alcohol and methyl alcohol. Freely soluble in water

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Marketed formulations	Netalo eye drops (0.02%), Rhopressa eye drops (0.02%)

2. Materials and Methods

Materials

Netarsudil mesylate was provided as kind gift sample from Falx Laboratories Pvt. Ltd., Vadodara. All chemicals and reagents of analytical grade and were purchased from Loba Chemie Pvt. Ltd. Mumbai. The water used was deionized and double-distilled. Marketed Eye drop formulation containing 0.02% of netarsudil mesylate was purchased from a local drug store in Nadiad city.

Instrumentation

A double-beam UV-Visible spectrophotometer (Shimadzu UV-1900i, Kyoto, Japan), equipped with 1 cm path length quartz cells and a fixed slit width of 1 nm, was used for all spectroscopic measurements. The instrument offers high wavelength accuracy (±0.1 nm) and features automatic wavelength calibration and correction. Data acquisition and processing were performed using LabSolutions UV-Visible software (Shimadzu, Japan) running on a Windows-based PC. Spectral scanning was conducted over the wavelength range of 400–200 nm at a medium scanning speed.

Preparation of stock solutions of Netarsudil mesylate for UV

A standard stock solution of Netarsudil mesylate was prepared by dissolving accurately weighed 50 mg of the drug in a 50 mL volumetric flask, shaking and dissolving, and diluting up to the mark with distilled water. For the preparation of diluted stock solution, 1mL of stock solution was withdrawn and transferred into a 10 mL volumetric flask and diluted up to the mark with distilled water to produce 100µg/mL.

For the preparation of working standard solutions of netarsudil mesylate, appropriate volumes of diluted stock solution were withdrawn and transferred into a series of 10 mL volumetric flasks, and the solutions were diluted up to the mark with distilled water to obtain concentrations ranging from 4-12 µg/mL [11]. All working solutions were scanned using distilled water as blank in the range of 200-400 nm to determine the wavelength of maximum absorption (λmax) of the drug. Further, the absorbance in triplicate at estimated λmax was measured for each working solution and calibration curve was plotted by taking average absorbance on Y and concentration on X.

Preparation of stock solutions for HPLC

Accurately weighed 50 mg of netarsudil mesylate was transferred into a 50 mL clean dry volumetric flask containing HPLC water and sonicated to dissolve it completely and make volume up to the mark with the same solvent. Further, the working solutions were prepared by taking appropriate volume of above stock solutions and diluted up to 10 ml in volumetric flask to get desired concentration (50 to 150 µg/mL) of netarsudil mesylate solution [9].

Preparation of sample solutions

Eye drop containing 0.02% of netarsudil mesylate was used for preparation of sample solutions of marketed pharmaceutical formulation. From the eye drops, 0.3 mL (60 µg) was withdrawn and diluted up to the mark in 10 mL volumetric flask with distilled water to get the resultant solution of 6 µg/mL, which was used for analysis of the formulation by UV [12]. Further, 4 mL (80 µg) of the eye drops was diluted up to the mark in 10 mL volumetric flask with distilled water to get the resultant solution of 80 µg/mL, which was used for analysis of formulation by HPLC [9].

Method Validation

The present study was conducted to obtain a new, affordable, cost-effective, and convenient method for spectroscopic determination of netarsudil mesylate in bulk and eye drop formulation. The method was validated for the parameters like linearity, accuracy, precision, and robustness as per ICH guidelines.

Linearity

The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of analyte in samples within a given range [13]. This was determined using a calibration graph using increasing amounts of standard solutions (4-12 µg/mL). Calibration curves were constructed, and the proposed method was evaluated by its correlation coefficient and intercept value calculated in the corresponding statistical study. Characteristic parameters for the regression equation of the method

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were obtained by least squares treatment of the results, and these parameters were used to confirm the good linearity of the method.

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

The Limit of detection (LOD) is the lowest concentration of analyte that can be qualitatively detected under stated experimental conditions. The limit of quantification (LOQ) is the lowest concentration that can be quantitatively measured under the given experimental conditions. Both the detection limit and quantification limit can be established visually, using signal-to-noise ratios, or by using the data from the standard deviation and slope of the calibration curve. The LOD and LOQ were calculated based on signal-to-noise ratio using the following equations [13,14].

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

Where, σ = Standard deviation of all responses; S= Slope of the calibration curve

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

Where, σ = Standard deviation of all responses; S = Slope of the calibration curve

Intraday and Inter-day Precision

Precision is the degree of agreement between the results of the same quantity. In other words, it is the reproducibility of the result. Intraday precision is the reproducibility of the results within a single day when the same procedure is repeated at different time intervals under the same experimental conditions. On the other hand, if the same procedure is repeated over multiple days to check the reproducibility of results over time is called inter-day precision. The precision is expressed as the standard or relative standard deviation (%RSD) [13].

Intraday precision was determined by performing three repeated analysis of the five standard solutions of drug (4, 6, 8, 10µg/mL) on the same day, under the same experimental conditions. Inter-day precision of the method was assessed by carrying out the analysis of standard solutions on three different days in the same laboratory, under the same experimental conditions. Measurement of absorbance was in triplicate, and the mean, standard deviation, and relative standard deviation (% RSD) were determined in order to assess the precision of the method.

Repeatability

It is the measure of the precision over a short duration of time. It is done under the same experimental conditions as usually given for an analytical method [13]. The repeatability was checked by scanning and measurement of the responses of the standard solution (8 µg/mL) and the test solution (8 µg/mL) without changing the parameters of the proposed method. The procedure was repeated six times, and % RSD was calculated.

Accuracy

Accuracy is described as the degree of agreement of test results with the true value. It is carried out to determine the suitability and reliability of the proposed method [13]. Accuracy was determined by calculating the % recovery of netarsudil mesylate from the formulation prepared by the standard addition method, in which a known amount of standard solution of drug at 0%, 80 %, 100 %, and 120 % levels was added to the pre-analyzed sample solution of 6 µg/ml of netarsudil mesylate. The procedure was performed in triplicate, and the recovered amounts of netarsudil mesylate were calculated at each level, and the % recovery was reported [15].

Robustness

It is a capability of analytical method to produce the results with the acceptance criteria when small but deliberate variations are made in the method parameters [13]. Here, the deliberate changes were made in the detection wavelength by ± 0.5nm and ± 1nm from the actual λmax. The absorbance of the test solution (8 µg/ml) at ± 0.5nm and ± 1nm wavelengths was measured. The results were then analyzed to determine whether there is a change in absorbance with minor changes in the λmax.

Estimation of netarsudil mesylate in bulk

The standard stock solution of netarsudil mesylate was prepared by dissolving 50 mg of the drug in 50 mL volumetric flask using distilled water. From this, 1 mL was withdrawn and transferred into 10 mL volumetric flask, and the volume was made up to the mark with distilled water to give 100 µg/mL. The working solution

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was prepared by withdrawing 0.6 mL from the above solution and was diluted to the mark with water to achieve a 6 µg/mL concentration. This resultant solution was scanned in the range of 200-400 nm. The spectra were recorded at λmax 243 nm. The % recovery of the drug was calculated from the linear regression equation [15].

Estimation of netarsudil mesylate in pharmaceutical formulation

For analysis of formulation, 0.3 mL of netarsudil mesylate eye drops was taken in a 10 mL volumetric flask, and the volume was made up to the mark with distilled water to give a 200 µg/mL concentration. From this, 0.3 mL was taken and transferred to a 10 mL volumetric flask, and the volume was made up to the mark with distilled water to give an 6 µg/mL concentration. The resultant solution was scanned in the range of 200–400 nm. The spectrum was recorded at λmax 243 nm. The % recovery of the drug was calculated from the linear regression equation [15]. Further, the sample of netarsudil mesylate eye drops containing 80 µg/mL of drug was analysed using HPLC and concentration was calculated with the help of HPLC calibration curve of drug.

3. Results and Discussion

The UV absorption spectrum for netarsudil mesylate was recorded in distilled water in the range of 200-400nm, and the drug showed maximum absorbance at 243 nm as shown in figure 1. This method was validated as per ICH guideline.

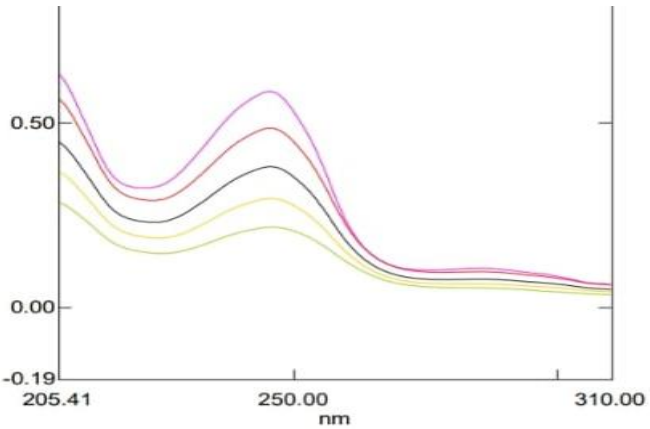

Figure 1: Overlay UV spectra of netarsudil mesylate (4-12 µg/mL)

Table 2 reveals the data of absorbance at 243 nm for various concentration of drug. As the value for %RSD for all are less than 2%, precision of data obtained with triplicate readings can be assumed.

Table 2: Linearity data of netarsudil mesylate by UV

Concentration (µg/mL)	Absorbance	
	Mean ± SD	%RSD
4	0.135 ± 0.0024	1.02
6	0.224 ± 0.0024	1.06
8	0.331 ± 0.0051	1.53
10	0.432 ± 0.0043	0.99
12	0.527 ± 0.0029	0.56

Linearity of netarsudil mesylate is shown in Figure 2. The drug showed a good correlation coefficient (R^2 =0.9983) in the concentration range of 4-12 µg/mL.

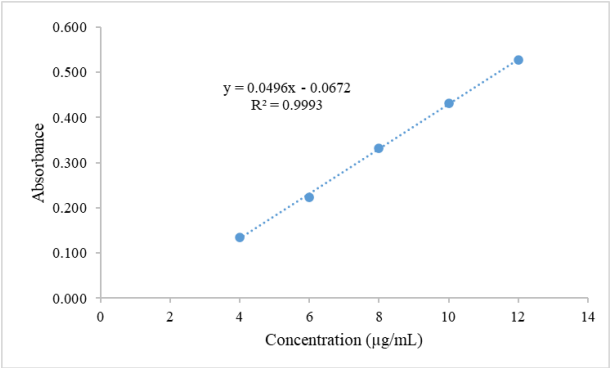

Figure 2: Standard calibration curve for netarsudil mesylate using UV spectrophotometry at 243nm.

Table 3 shows linear regression parameters for UV analysis of netarsudil mesylate along with regression co-efficient and equation.

Table 3: Linear regression parameters for analysis of netarsudil mesylate by UV

Parameter	Value
Wavelength	243 nm.
Concentration range	4-12 µg/ml
Regression equation	y = 0.0496x - 0.0672
Regression coefficient (R^2)	R^2 =0.9993
Average of slope	0.04962
Standard deviation of intercept	0.00489

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The linear regression equation was found to be y = 0.046x - 0.0338. This indicates that the method can produce consistent results across the linearity range, validating the method for quantitative analysis of netarsudil mesylate.

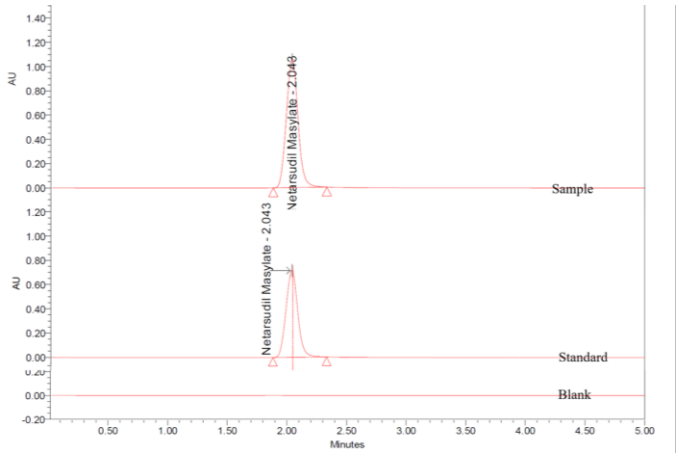

Figure 3: HPLC chromatograms of netarsudil mesylate in sample and standard solutions.

Figure 3 depicts HPLC chromatogram of the netarsudil mesylate in standard solution and sample solution indicating RT value of 2.043.

Table 4 and figure 4 represents the data for calibration curve of drug by HPLC method. For HPLC method the linearity was observed in the concentration range of 50-150 µg/mL (Figure 4).

The lower limit of detection (LOD) and lower limit of quantification (LOQ) for the proposed UV method were found to be 0.325 µg/mL and 0.985 µg/mL, respectively. This demonstrates the method's high sensitivity for detecting and quantifying netarsudil mesylate even at low concentrations. Repeatability was checked by measuring the responses of standard solution (8 µg/mL) and test solution (8 µg/mL) under the same conditions over a short time duration. To ensure reproducibility, the same procedure was performed in six replicates, and the % RSD was found to be less than 2, as shown in table 4. This demonstrates the excellent repeatability of the developed method over a short period.

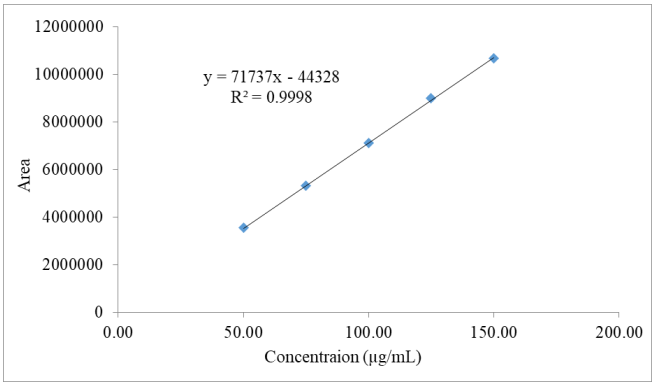

Figure 4: Standard calibration curve for netarsudil mesylate using HPLC.

Table 4: Repeatability studies

Sample	Absorbance Mean ± SD (n=6)	%RSD
Standard (8 µg/ml)	0.332 ± 0.0054	1.62
Test (8 µg/ml)	0.326 ± 0.0032	0.98

The precision is measured in terms of % relative deviation, which was found to be less than 2, as shown in table 5. This indicates that this method is precise for the determination of netarsudil mesylate drug in bulk and dosage form. In other words, this method is reproducible and can be used for routine quantitative analysis of netarsudil mesylate.

Table 5: Data for precision studies

Concentration (µg/mL)	Interday		Intraday	
	Absorbance Mean ± SD (n=3)	%RSD	Absorbance Mean ± SD (n=3)	%RSD
4	0.136 ± 0.00252	1.855	0.136 ± 0.00252	1.846
6	0.225 ± 0.00252	1.117	0.219 ± 0.00231	1.056
8	0.333 ± 0.00404	1.212	0.332 ± 0.00416	1.253
10	0.434 ± 0.00100	0.230	0.434 ± 0.00819	1.886
12	0.526 ± 0.00416	0.792	0.527 ± 0.00153	0.290

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The recovery study was carried out by the addition of a known number of standard solutions to the samples at levels 80, 100, and 120 % and analyzed by the developed method, in triplicate. The proposed method was confirmed to be accurate as the mean % recovery was found between 99.55±0.414 to 100.13±0.838, with % RSD less than 2, as shown in table 6. This indicates that the method is accurate and has minimum matrix interference. Thus, the method can be used for accurate quantification of netarsudil mesylate in the formulation.

Table 6: Data for recovery from marketed formulation

Spike (%)	Concentration of sample (µg/mL)	Concentration of std. spiked (µg/mL)	Total Concentration of drug taken (µg/mL)	% Recovery Mean ± SD	%RSD
80	6	4.8	10.8	100.13 ± 0.838	0.837
100	6	6.0	12.0	99.66 ± 0.553	0.555
120	6	7.2	13.2	99.55 ± 0.414	0.416

Table 7: Estimation of netarsudil mesylate in marketed formulation

Sample	UV				HPLC			
	Conc. Taken (µg/mL)	Conc. Found* (µg/mL)	% Assay	%RSD	Conc. Taken (µg/mL)	Conc. Found* (µg/mL)	% Assay	%RSD
Bulk	6	5.91 ± 0.0263	98.63 ± 0.438	0.444	80	79.33 ± 0.6470	99.17 ± 0.809	0.816
Eye Drops	6	5.92 ± 0.0294	98.72 ± 0.491	0.497	80	79.07 ± 0.6623	98.84 ± 0.829	0.839

To study the robustness, the UV method was evaluated by making deliberate changes in the detection wavelength by ±0.5 nm and ±1 nm from the actual λ_{max}. The %RSD was found to be 0.633% and 0.630%, respectively. This result indicates that the method developed is robust to minor changes in wavelength. The low % RSD indicates that the method is robust and is not affected by any small changes in method parameters. The robustness of method was also determined by using different sources of distilled water for the preparation of working solution of the drug. The value of RSD for detection of netarsudil mesylate was found 0.72% (< 2%) revealed robustness of the method.

For estimation of netarsudil mesylate in bulk, the spectrum was recorded and absorbance was measured at 243 nm. The amount of drug was calculated using UV calibration equation and was found between 98.34 and 99.45% as shown in the table 7. Similar procedure was applied for estimation of netarsudil mesylate in pharmaceutical formulation and the amount of drug was found between 98.21 and 99.45% (table 7).

Summary of the parameters for developed UV method are revealed in table 8. Overall, the developed UV method is simple, accurate and sensitive enough to be employed in estimation of netarsudil mesylate in bulk as well as pharmaceutical formulations.

Table 8: Summary table for UV method

Parameters	Results
Wavelength (λ_{max})	243 nm
Beer's law limit (µg/mL)	4-12
Regression equation	y = 0.0496x - 0.0672
Correlation coefficient	r^2 = 0.9993
Repeatability	%RSD < 2
Intraday and Interday precision	%RSD < 2
Limit of detection (µg/mL)	0.325
Limit of quantification (µg/mL)	0.985
Robustness	%RSD < 2

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4. Conclusion

The UV-spectrophotometric method has been developed to quantify netarsudil mesylate in bulk as well as pharmaceutical dosage form. The method was validated in terms of linearity, precision, accuracy, robustness, LOD, and LOQ. Beer's law was obeyed over the concentration range of 4-12 µg/mL. The sensitivity parameters LOD and LOQ were found to be 0.325 µg/mL and 0.9852 µg/mL, respectively. Further, the comparison of analysis of drug with developed method and HPLC have shown similar precision and accuracy. According to ICH guideline, the method meets the acceptance criteria and was found to be simple, accurate, precise, reproducible, robust, and linear over the concentration range studied. Thus, the developed UV method be applied for routine analysis of netarsudil mesylate in bulk and pharmaceutical formulations.

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