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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF NELARABINE IN BULK AND IN INJECTION

Amit Kumar Nigam [1], Richa Gangwar [2], Pavankumar D Chopade [3], Ritika Arora [4], Abdul Kadir Jilani [5], Sindhu M [6], Navyashree C J [7], Rajeev Ranjan [8], Vinay Kumar Yanmandru*

1. Professor , Goel institute of Pharmaceutical Sciences near Indira canal Faizabad road Lucknow 226028
2. Assistant Professor , BIU college of Pharmacy, Bareilly International University Bareilly.
3. Assistant professor , Oriental college of pharmacy sanpada Navi Mumbai Maharashtra 400705
4. Research scholar, Department of Pharmaceutical Sciences, Sir J C Bose Technical Campus, Bhimtal Kumaun University 263136
5. Assistant Professor, School of pharmaceutical sciences, University of Science and Technology, Meghalaya, Ri-Bhoi, Techno City, Killing Road, Baridua, Meghalaya, 793101
6. Assistant professor, College-Gmit Bharathi nagara,Vtu,maddur taluk mandya pin code-571422
7. Assistant Professor , G. Madegowda Institute of technology, Bharathinagara Mandya -571422
8. Assistant Professor, DSPM University, Ranchi

Corresponding Author: Vinay Kumar Yanmandru

Email Id: winayky@gmail.com

Abstract:

An innovative, cost-effective reverse phase high performance liquid chromatography (RP-HPLC) approach has been devised to accurately and precisely determine the quantity of Nelaribine in both bulk and sterile dosage forms. The separation process was conducted using an Inertsil C18-ODS 3V column with dimensions of 250×4.6 mm and a particle size of 5 µm. Isocratic mode was employed, with a mobile phase consisting of a mixture of acetic acid pH-4.0 buffer and methanol in a ratio of 75:25 (v/v). The mobile phase was pumped into the column at a flow rate of 1.5 mL min-1. The eluent from the column was detected using a UV detector set at 260 nm. The whole duration of the experiment was 8 minutes, during which the column was kept at the ambient temperature. The observed retention time for Nelaribine was determined to be 3.99 minutes. The linearity of the standard curves was seen within the concentration range of 20-100 µg/ml, exhibiting a high coefficient of determination (R^2 = 0.9998). The results of the experiment indicated a percentage recovery ranging from

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100.0272% to 101.8%. Additionally, the relative standard deviation (RSD) was determined to be 0.3693%. The measured percentage content of a commercially available Nelaribine formulation was determined to be 100.20 %. The methodology was verified in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) recommendations. The suggested RP-HPLC technique has been validated by investigations, which have shown that it has characteristics of simplicity, specificity, rapidity, reliability, and reproducibility. Therefore, the approach presented in this study may be used for the regular analysis of Nelaribine in both bulk and injectable dose forms, specifically for quality control purposes.

Keywords: Nelarabine, Method development, Method validation and RP-HPLC

Introduction:

Nelarabineis a purine nucleoside analog that undergoes conversion to its equivalent arabinosylguanine nucleoside triphosphate, leading to the inhibition of DNA synthesis and the induction of cytotoxic effects. The medication undergoes metabolism to become the active 51-tri phosphate ara-GTP, which subsequently interferes with DNA synthesis and triggers apoptosis. Nelarabine is indicated for the treatment of T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma. The chemical name of Nelaribine is (2R,3S,4S,5R)-2-amino-6-(4-hydroxy-3-hydroxymethylbut-1-yl)oxane-3,4,5-triol.The compound -2-(2-amino-6-methoxy-9H-purThe compound in question is known as -(hydroxymethyl) oxolane-3,4-diol, with a chemical formula of $C_{10}H_{11}ClFN_5O_3$ and a molecular weight of 297.27 g/mol [1].

The aim of the present study was to establish a novel analytical approach for determining the concentration of Nelarabine injection using RP-HPLC. Additionally, the created technique was subjected to validation in accordance with the recommendations provided by the United States Pharmacopeia (USP) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [2,3]. The planned work encompasses a comprehensive strategy that comprises This study involves a comprehensive review of the existing literature on Nelarabine, focusing on its physico-chemical characteristics and analytical methodologies. This serves as the foundation for the formulation of a methodology. To conduct a solubility investigation for the analyte known as Nelarabine. The appropriate selection of a solvent for the purpose of quantitatively extracting an analyte from formulations. The solvent used should possess the desirable qualities of being

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easily accessible, cost-effective, and meeting the standards of analytical grade. The objective is to establish preliminary chromatographic conditions by carefully choosing an acceptable column and determining the optimal wavelength for UV detection [4-7]. This will facilitate the method's development and further optimization. In order to verify the accuracy and reliability of the analytical technique that has been established, it is necessary to conduct validation in accordance with the requirements outlined in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2B [8,9].

Materials & Method:

HPLC-grade solvents include acetic acid, water, and methanol. Analytical grade chemicals, namely sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, ortho phosphoric acid, and potassium dihydrogen phosphate, were procured from the supplier Qualigens.

High-performance liquid chromatography (HPLC) analysis was conducted using a Shimadzu HPLC system, which was equipped with an SPD M20A detector. The collected data was processed using LC Solution software. The separation process was conducted using an Inertsil C18-ODS 3V column, which had dimensions of 250×4.6 mm and a particle size of 5 µm. The mobile phase consisted of a pH 4.0 buffer and methanol, with a ratio of 75:25. The analysis of the samples was conducted with an injection volume of 20 µL. The flow rate was consistently maintained at 1.5 mL/min for a total duration of 8 minutes. The temperature was carefully controlled at 27°C during the whole analysis process. The drugs were detected and their purity was determined using a photodiode array (PDA) detector set at a wavelength of 260 nm.

Preparation of standard solution:

A precise measurement of 50 mg of Nelarabine was weighed and placed into a volumetric flask with a capacity of 50 ml. Roughly 30 mL of diluent was added to the solution, resulting in dissolution. The volume was then adjusted to the desired level by adding more diluent. In the aforementioned procedure, a volume of 2 mL was extracted and thereafter placed into a volumetric flask with a capacity of 25 mL. The remaining volume in the flask was then filled with a diluent to achieve the desired volume. The concentration of Nelarabine is 50 µg/ml.

Preparation of sample solution:

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Precisely transfer a volume of 2.0 mL of the sample solution into a 200 mL volumetric flask, ensuring no loss of solution occurs. To prepare the solution, include 120 ml of diluent and vigorously agitate to facilitate dissolution. Subsequently, proceed to dilute the solution to the desired amount using additional diluent, ensuring thorough mixing. A volume of 1 mL was extracted from the filtered solution and transferred into a 10 mL volumetric flask. The flask was then filled to a total capacity of 10 mL using a diluent.

The optimized chromatographic conditions:

The experiment is carried out using Inertsil C18-ODS 3V column, which had dimensions of 250×4.6 mm and a particle size of 5 µmat a Flow rate of 1.5ml/min using a detection wavelength of 260 nm, ambient column temperature was used, the injection volume is 20µl, run time of 08 minutes. A typical chromatogram is shown in Fig.No: 01.

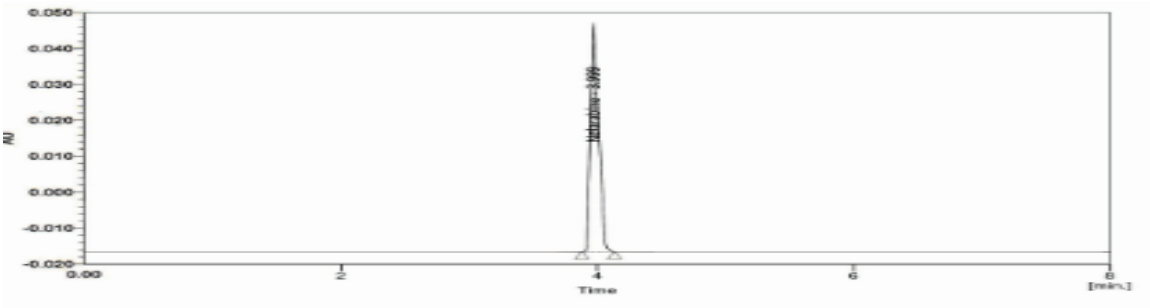

Fig.No. 01: Typical Chromatogram of Standard Nelarabine

Validation of Proposed method:

The technique that was developed underwent validation in accordance with the principles set out by the International Conference on Harmonization (ICH). This validation process included several aspects including system appropriateness, precision, specificity, forced degradation studies, linearity, accuracy, limit of detection, and limit of quantification.

Validation Parameters:

System Suitability:

To guarantee the optimal performance of the analytical system, it is important to set the parameters for system appropriateness. The conventional procedure included quantifying around 10 mg of Nelarabine, which was thereafter introduced into a 100 ml volumetric flask. Following that, a volume of 50 ml of diluent was added to aid in the process of dissolution,

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and the resulting mixture was vigorously agitated for a period of about 10 minutes. Ultimately, the solution's volume was modified to the intended magnitude with the addition of more diluent. The findings indicate that the relative standard deviation (% RSD) is below 2%, the plate count exceeds 5000, and the peak symmetry is below 1.2. The findings are recorded and organized in Table 1.

Table No. 01: System Suitability Data

Injection Number	Retention Time	Peak Area	Plate Count	Peak Symmetry
1	3.99	664658	9578	1.012
2	3.98	664578	9645	1.125
3	4.01	664875	9612	1.054
4	4	664287	9854	1.023
5	4.01	664878	9321	1.028
6	3.99	664859	9642	1.085
Average	4.00	664689		
Standard Deviation	0.01	234.14		
% RSD	0.3030	0.0352		

Linearity:

Aliquots of 0.20, 0.40, 0.60, 0.80, and 1.0 ml were extracted from a stock solution with a concentration of 100 µg/ml of Nelaribine. These aliquots were then diluted with a diluent until reaching the desired volume. The resulting concentrations fell within the range of 20-100 parts per million (ppm) for Nelaribine. Duplicate injections of each sample, with a volume of 20 µl, were performed for every concentration level. A calibration curve was then generated by graphing the peak area against the corresponding drug concentration. In the range of investigation, a linear correlation was discovered between the peak area and

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concentration. The measurements and calibration curve were shown in Figure 2 and the data in Table 1.

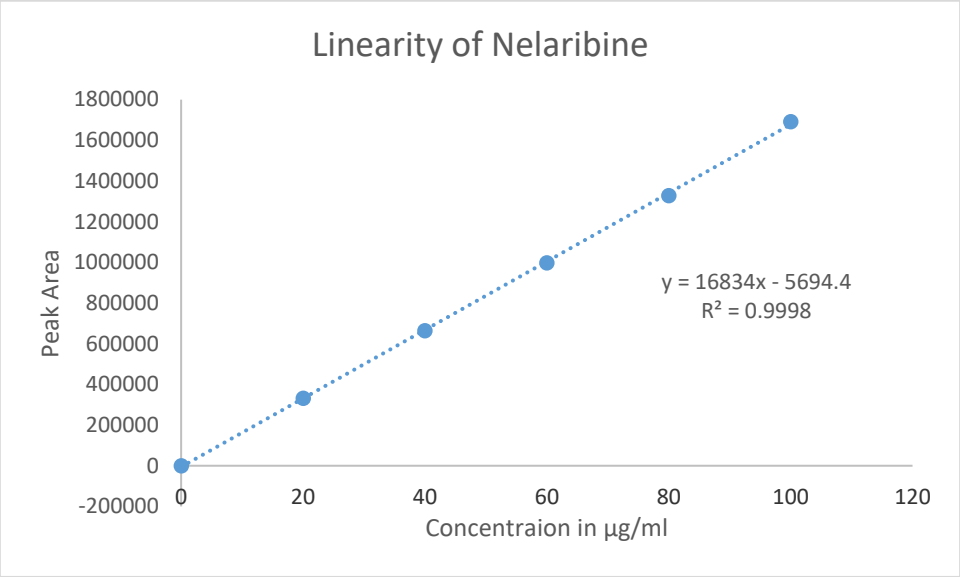

Fig.No. 02: Linearity Plot of Nelarabine

Table 2: Linearity data

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	20	332277
3	40	664658
4	60	997854
5	80	1329245
6	100	1691888
Slope		16834
Intercept		-5694.4
Regression		0.9998

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Precision:

Approximately 05milligrammes of Nelarabine wasweighed and placed into a volumetric flask with a capacity of 100 millilitres. Subsequently, 50 millilitres of a suitable diluent were added to the flask in order to facilitate dissolution. The mixture was allowed to dissolve for a duration of 10 minutes. The volume was then increased to 100 ml by adding a diluent. The standard solution was made in six duplicates, and the resulting data was recorded in a tabular format in Table No.03.

Table 3: Precision data

Sample Preparation No.	Assay (%)
1	100.12
2	100.32
3	99.98
4	99.78
5	100.52
6	100.47
Mean	100.20
SD	0.29
RSD (%)	0.2896

Accuracy:

In order to assess the validity of the suggested methodology, recovery experiments were conducted at three different levels: 50%, 100%, and 150% of the target concentration, in accordance with the principles set out by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The recovery research was conducted in triplicate at each level. Table 4 presents the findings of the recovery investigations.

Table 4. Recovery Studies

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Level	Amount found (mg/ml)	Amount added (mg/ml)	Recovery (%)	Mean (%)
Level-1 (50%)	25.25	25	101	101.24
	25.23	25	100.92	
	25.45	25	101.8	
Level-2 (100%)	50.01	50	100.02	100.42
	50.5	50	101	
	50.12	50	100.24	
Level-3 (150%)	75.45	75	100.6	100.70
	75.48	75	100.64	
	75.65	75	100.87	
Mean			100.56	
SD			0.37	
% RSD			0.3693	

Robustness of method:

A research investigation was undertaken to ascertain the impact of purposeful modifications in the optimum chromatographic parameters, such as the composition of the mobile phase, flow rate, wavelength and mobile phase pH. The impact of these modifications on the system suitability characteristics, such as the tailing factor and number of theoretical plates, % RSD as well as on the assay, was investigated. In order to maintain consistency, just one condition was manipulated while all other factors were held constant. The obtained findings were determined to fall within the permissible thresholds, hence indicating the method's specificity. The findings of the investigation on the robustness of the experiment are shown in Table 5.

Table 5: Method Robustness

Condition	% RSD	Tailing Factor	% Recovery	No. of Theoretical

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				plates
1) Change in Flow rate				
Normal Condition (1.5 ml per minute)	0.05	1.11	99.78	9858
Flow rate (1.2 ml per minute)	0.12	1.07	100.12	9678
Flow rate (1.7 ml per minute)	0.25	1.25	100.25	9214
2) Change in minor component in the mobile phase				
Normal Condition (Buffer : Methanol) (75 : 25))	0.24	1.25	99.63	9632
(Buffer : Methanol) (60 : 40))	0.32	1.58	99.12	9456
(Buffer : Methanol) (90 : 10))	0.48	1.45	100.78	9785
3) Change in Wave Length				
Normal: Wave Length 260 nm	0.12	1.45	99.12	9856
Wave Length 255 nm	0.25	1.85	99.45	9564
Wave Length 265 nm	0.65	1.42	100.25	9245
4) Change in Ph				
Normal: pH 4.0	0.12	1.45	99.45	9945
pH 4.5	0.78	1.12	100.25	9678
pH 3.5	0.98	1.56	100.47	9325

Ruggedness:

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Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, exhibits the ruggedness of developed analytical method and results are presented in Table 6.

Table 6. Method Ruggedness

Sample Preparation No.	**Assay (%)**
1	100.45
2	100.78
3	101.23
4	99.89
5	99.45
6	99.98
Mean	100.30
SD	0.65
RSD (%)	0.6478
Difference between method precision and intermediate precision assay	**0.26**

Conclusion:

The primary objective of this work was to establish a high-performance liquid chromatography (HPLC) technique that is both sensitive and capable of providing precise and accurate results for the analysis of Nelarabine in both its bulk drug form and in pharmaceutical dose forms. The C18 column was used at a flow rate of 1.5 ml/min. The mobile phase consisted of a mixture of acetic acid buffer solution at pH 4 and methanol in a

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volumetric ratio of 75:25. The wavelength of detection was measured to be 260 nm. The experimental setup included the use of a Shimadzu HPLC system, which was outfitted with an SPD M20A detector. The retention times were determined to be 3.99 minutes. The validation of the analytical technique was conducted in accordance with the requirements established by the International Council for Harmonisationof Technical Requirements for Pharmaceuticals for Human Use (ICH), specifically ICH Q2b. The study yielded a correlation coefficient (r2) of 0.9998, indicating a strong positive relationship between the variables. The recovery percentage ranged from 101.8% to 100.02%, suggesting a high level of accuracy in the measurement process. The relative standard deviation (RSD) for precision, determined by repeat injection, was found to be 0.3693, indicating a moderate level of variability in the results. The accuracy research showed a high level of precision, robustness, and repeatability.

The investigation focused on evaluating the system suitability characteristics using six replicates of a standard solution of the medicine. The estimated values were found to fall within the predetermined acceptance requirements. The tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP) are within acceptable ranges. Therefore, the author asserts that the HPLC technique suggested in this study exhibits sensitivity and reproducibility when used for the measurement of Nelarabine in pharmaceutical dosage forms. Additionally, the approach has a quick analysis time.

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