WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Method Development and Validation for the Estimation of Nelarabine Injection by Using RP-HPLC

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Nelarabine in Bulk and its pharmaceutical formulation. Separation was achieved with a Cosmicsil BDS C_{18} ((Make: Nomura chemicals (Japan); 150 x 4.6mm I.D; particle size 5 µm) Column and Trifluroacetic acid buffer (pH 2): Acetonitrile (90:10) as eluent at flow rate 1.0 ml/min and the Column temperature was 30°C. UV detection was performed at 248 nm and sample temperature was maintained at 5°C. The described method of Nelarabine is linear over a range of 12.5 µg/mL to 100 µg/mL. The method precision for the determination of assay was below 2.0 % RSD. The method enables accurate, precise, and rapid analysis of Nelarabine. It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

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

Introduction

Nelarabine is metabolized into ara-GTP, the metabolite accumulates in leukemic blasts and incorporates into DNA to exert its S phase-specific cytotoxic effects, leading to the induction of fragmentation and apoptosis. Administration of nelarabine in combination with adenosine deaminase inhibitors, such as pentostatin, is not recommended. The adverse drug reactions are neurologic, hematologic.In both intact and bile-cannulated animals, the predominant route of nelarabine elimination was by renal excretion. Fecal and biliary secretion presented only a minor route of elimination. Chemically it is (2R,3S,4S,5R)-2-(2-amino-6-methoxy-9H-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol. It is white or almost white crystaline powder. It is freely soluble in water and slightly soluble in methanol. The chemical structure of nelarabine is given in figure1.

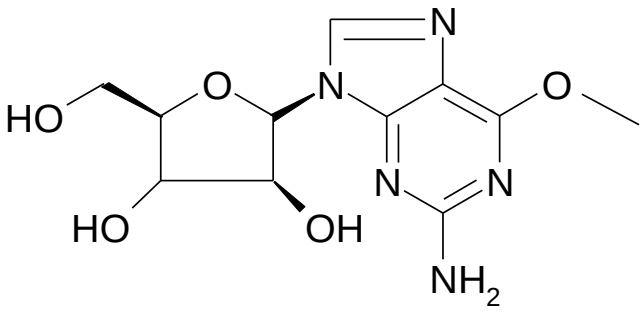

Figure 1. chemical structure of nelarabine

The objective of the proposed work was to develop new analytical method for the estimation of Nelarabine injection by RP-HPLC and to validate the developed method according to USP and ICH guidelines. The plan of the proposed work includes The extensive survey of literature for Nelarabine regarding their Physico-chemical properties and analytical methods. This forms the basis for the development of method. To undertake solubility study for the analyte i.e., Nelarabine. Selection of suitable solvent for quantitative extraction of analyte present in the formulations. The solvent should be readily available, economical and of analytical grade. To develop initial chromatographic conditions by selection of suitable column and appropriate wavelength in UV for detection and optimization of the method. To validate analytical method developed as per the ICH Q2B guidelines.

Materials and methods

Equipment

The Method development and Validation was carried out using Waters Alliance-HPLC system equipped with waters 1525 binary HPLC pump, 2695-separation module connected to 2996-photo diode array detector, and Waters 2707 auto sampler. The data was acquired by Empower® version 2. The other equipment used were Ascoset Electronic balance, ADWA pH meter, heating mantle. Ultrasonic bath was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV cross linker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used for photo degradation studies.

Chemicals and Reagents

Nelarabine working standard was kindly given as gift sample by Mylan labs Pvt. Ltd, Hyderabad. HPLC grade solvents include acetonitrile, water and methanol. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Ortho phosphoric acid, Triethyl amine and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic conditions

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module connected to 2996-photo diode array detector and the data was acquired by Empower® version 2. Separation was achieved using Cosmicsil adze C_{18}(150x4.6 mm) 5µm as a column with mobile phase of pH 3.0 buffer and Acetonitrile in the ratio of 90:10. The samples were analyzed using 10 µL injection volume, Flow rate was maintained at 1.0mL/min with runtime of 8 min and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs were achieved using PDA detector at 248 nm wavelength.

Preparation of standard solution:

An accurately weighed 31.25 mg of Nelarabine was taken in 50 ml volumetric flask .about 30ml of diluent was added , dissolved and volume was made to mark with diluent. From the above solution 2ml was withdrawn and transferred into 25 ml volumetric flask and volume was made to mark with diluent. (Concentration of Nelarabine 50 µg/ml).

Method Validation

The developed and optimized RP-HPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) in order to determine the system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and robustness.

System suitability

System suitability parameters were evaluated to verify system performance. 20 µL of standard solution was injected five times into the chromatograph, and the chromatograms were recorded. Parameters such as number of theoretical plates and peak tailing were determined.

Specificity

The specificity of the analytical method was established by injecting the solutions of diluent (blank), placebo, working standards and sample solution individually to investigate interference from the representative peaks.

Precision

Repeatability/ method precision was performed by injecting six replicates of same concentrations of Nelarabine, calculated % assay and %RSD. Reproducibility/ Ruggedness/ Intermediate precision was performed using different analysts and a different instrument in the same laboratory.

Accuracy

Accuracy of the proposed method was determined using recovery studies by spiking method. The recovery studies were carried out by adding known amounts (80%, 100% and 120%) of the working standard solutions of Nelarabine to the pre-analysed sample. The solutions were prepared in triplicates to determine the accuracy.

Linearity

Linearity was evaluated by analyzing different concentrations of the standard solutions of Nelarabine. Five working standard solutions ranging between 12.5µg/mL-100µg/mL were prepared and injected. The response was a linear function of concentration over peak area and were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient.

Limit of detection and Limit of quantification: Limit of detection (LoD) and limit of quantification (LoQ) of Nelarabine were determined by calibration curve method. Solutions of doripenem were prepared in linearity range and injected (n = 3).

Robustness

To examine the robustness of the developed method, experimental conditions were deliberately changed, resolution, tailing factor, and theoretical plates of Nelarabine peaks were evaluated. To study the outcome of the flow rate on the developed method, it was changed ± 0.2mL/minute. The effect of column temperature on the developed method was studied at ± 5°C, organic phase composition in mobile phase was changed ±10% and pH of the buffer is changed ±0.2. In all the above varied conditions, the composition of aqueous component of the mobile phase was held constant.

Results and Discussion

System Suitability

Table no.1: System suitability results for Nelarabine

Name	Inj	RT (min)	Area (µv*sec)	USP Plate Count	USP Tailing
Nelarabine	1	4.003	862111	7450	1.024
Nelarabine	2	4.002	862109	7511	1.024
Nelarabine	3	3.995	862116	7600	1.021
Nelarabine	4	4.001	862120	7490	1.020
Nelarabine	5	4.000	862105	7919	1.024
Mean			862112		
% RSD			**0.3**		

% RSD should not be more than 2.0 % for area .Tailing factor should not be more than 2 %. Plate count should not be less than 3000. It was observed from the data tabulated above that all the system suitability parameters meet the predetermined acceptance criteria as per the test method and indicates the suitability of the selected system.

Specificity:

The specificity of the method was evaluated by injecting blank, Standard Solution and the sample solution prepared as per the proposed method to check for interference, if any, at the retention time of nelarabine peak from any peak due to blank. It was found that there was no interference of blank at the nelarabine peak RT.

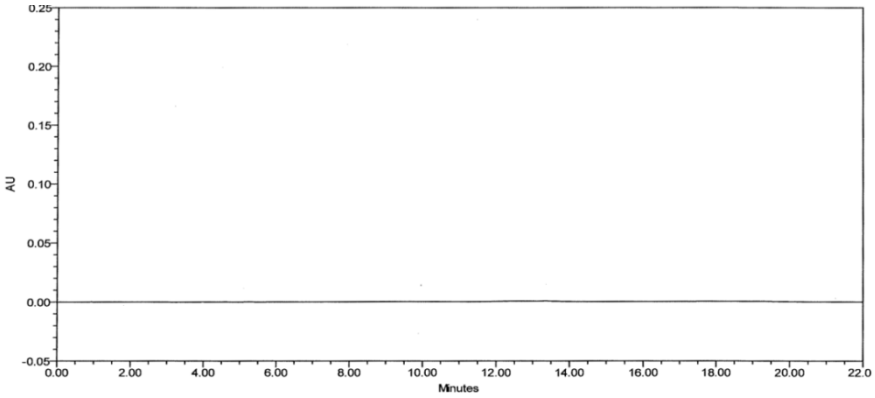

Fig. No. 2: Chromatogram of Blank

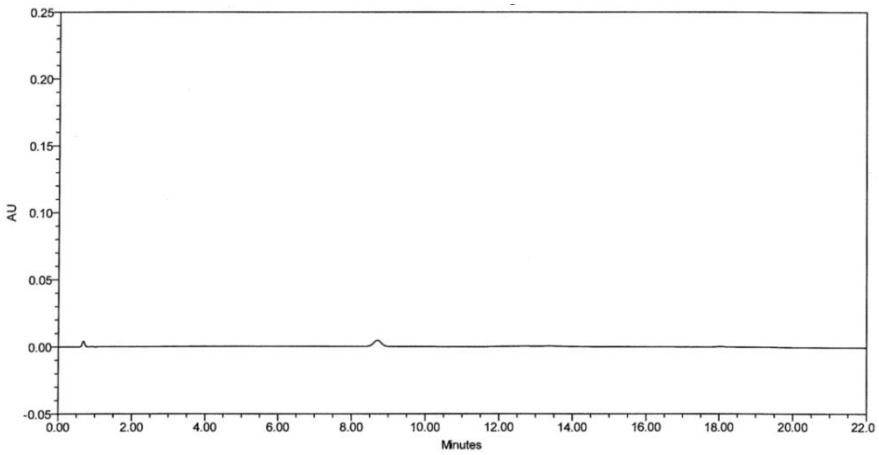

Fig. No. 3: Chromatogram of Placebo

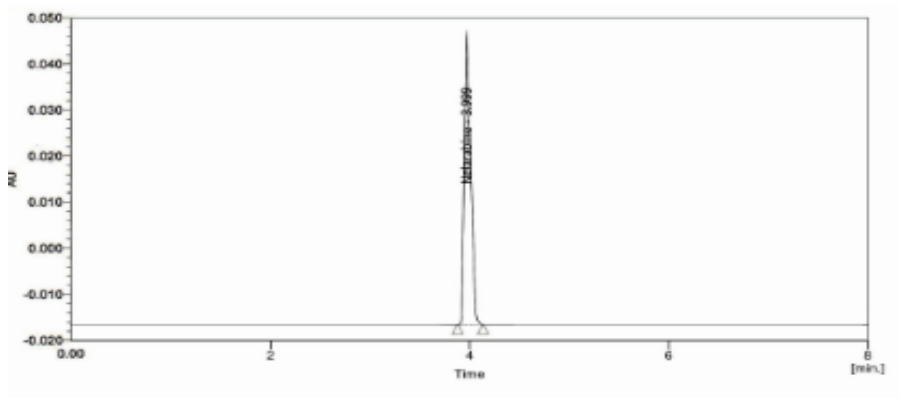

Fig. No. 4: Chromatogram of Standard

Precision

% RSD should not be more than 2.0% for Area. It is observed from the data tabulated, that the % RSD of the peak responses as peak area was found to be within acceptance criteria indicating an acceptance level of precision for system precision studies.

Table no 2: System Precision for Nelarabine

Nelarabine		
Inj.	**RT (min)**	**Area (µV*sec)**
1	4.000	8649928
2	4.002	862976
3	4.000	861861
4	3.999	854970
5	3.995	857275
6	4.000	859723
Mean		860288
SD		1515.56
% RSD		0.17

Method Precision

The % RSD of Nelarabine injection from the six units should be not more than 2.0 %. From the above results, it was concluded that the method is precise.

Table no. 3 : Method Precision Results for Nelarabine

Nelarabine		
Inj.	**RT (min)**	**Area (µV*sec)**
1	4.000	862976
2	4.002	854928
3	4.000	861861
4	3.999	859723
5	3.995	857275
6	4.000	854970
Mean		860289
SD		3712.45
% RSD		0.43

Accuracy

The mean percentage recovery of the Nelarabine at each level should be in the range of 99.0 – 101.0%.

The results were found within acceptance criteria. Hence the method is accurate throughout the selected range.

Table.no. 4 Accuracy data for Nelarabine

Concentration of Spiked Sample	**Amount added(ppm)**	**Amount found(ppm)**	**% Recovery**	**Statistical Analysis of % recovery**	
80% Sample	40	39.96	99.90	Mean	99.87
	40	39.85	99.62	SD	0.2411
	40	40.04	100.10	%RSD	0.24
100% Sample	50	49.93	99.86	Mean	99.96
	50	50.06	100.12	SD	0.1363
	50	49.96	99.92	%RSD	0.13
120% Sample	60	59.98	99.96	Mean	98.95
	60	59.08	98.46	SD	0.8693
	60	59.07	98.45	%RSD	0.87

Linearity:

The linearity of an analytical procedure is to elicit the test results are directly proportional to concentration. This is well understood by plotting a graph with peak area vs concentration.

Carried out the linearity studies for Nelarabine each at different concentration levels of 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml. Correlation coefficient should be not less than 0.9990. The Correlation coefficient Nelarabine was found to be **0.999**, which indicates that the peak responses are linear. This concluded that the method was linear throughout the range selected.

Table No. 5 Linearity Data For Nelarabine

s.no	**Concentration (µg/ml)**	**Are LINEARITY:- Inj 1**	**Area-Inj 1**	**Area-Inj 2**	**Avg Response**
1	12.5	215069	215074	215072	215071.66
2	25	430144.5	430147.6	430140	430144.33
3	50	860285	860291	860289	860288.33
4	75	1290432	1290429	1290425	1290428,33
5	100	1660578	1660582	1660575	1660578.33
	Correlation coefficient			**0.999**	
	Slope (m)			**16888x**	

Ruggeddness: (Intermediate Precision):

The United States pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. The % RSD of Nelarabine from the six sample preparations should be not more than 2.0 %.

Table 6: Data for Ruggedness

S. No	**Peak Area**	**Peak Area**
	Day-1	**Day-2**
1	8621100	8621111
2	8621133	8621109
3	8621190	8621116
4	8621101	8621119
5	8621109	8621125
6	8621160	8621100
Avg	8621132.6	8621113
SD	36.50	3.54
%RSD	0.04	0.4

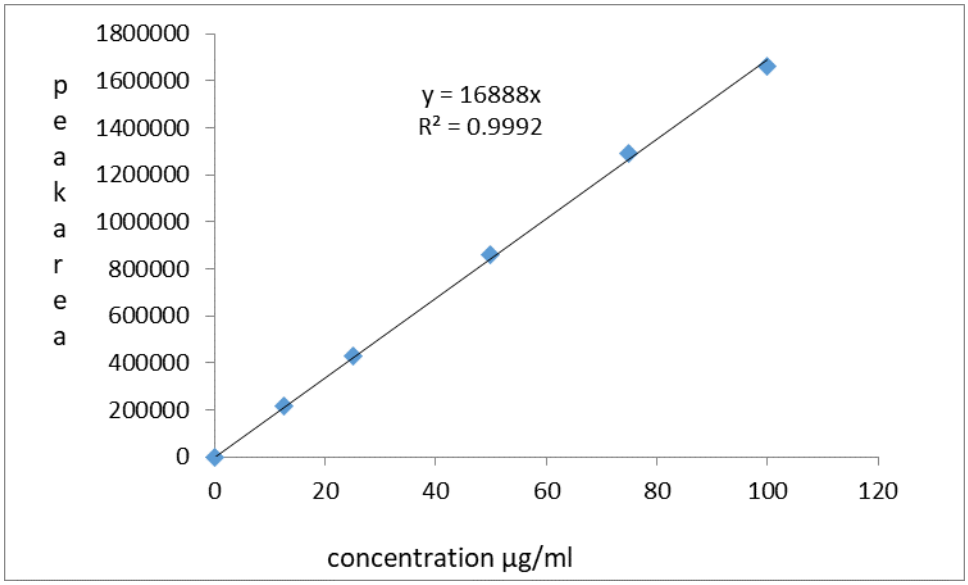

Fig no.5 linearity graph

Robustness

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the robustness of proposed analytical method. Change in column temperature, change in flow rate and change in column. All the system suitability parameters were well within the acceptance criteria. Hence, it was concluded that the method is robust**.**

Table no. 7: Peak Results for Robustness of Nelarabine

Parameter	**Flow Plus (-10%)**	**Flow Minus (+10%)**	**Column Temp High (+5°C)**	**Column Temp Low (-5°C)**	**Wave length Plus (+ 5nm)**	**Wave length (- 5nm)**
Area (Inj. 1)	7766784	949273	1032346	688231	877633	842944
Area (Inj. 2)	7765784	949270	1032500	688300	877280	842644
Average Area	7766284	949272	1032423	688266	877457	842944
% RSD	0.02	0.08	0.01	0.03	0.01	0.08

Conclusion:

High performance liquid chromatography is at present one of the most sophisticated tools of analysis. The estimation of Nelarabine was done by Reverse Phase HPLC. The mobile phase used consists of Buffer containing Trifluro acetic acid and mobile phase ratio of Trifluro acetic acid: acetonitrile (90:10). A C_{18} column containing Octadecyl silane (ODS) chemically bonded to porous silica particles (150 × 4.6 mm, 5µm particle size) was used as the stationary phase. The detection was carried out using UV detector set at 248 nm. The solutions are chromatographed at a constant flow rate of 1.0 ml/ min. The retention time for Nelarabine was around 3.99 min.

The quantitative estimation was carried out on the injection using RP HPLC. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2.0 %, indicating the accuracy and precision of the method. The percentage recoveries vary from 99.0 – 101.0 % for Nelarabine. The results obtained on the validation parameters met the ICH and USP requirements. It is inferred that the method was found to be simple, specific, precise and linear. The method was found to have suitable applications in routine laboratory analysis with high degree of accuracy and precision.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding

According to authors, the research described in this paper did not receive any financial support.

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