Validated RP-HPLC Method for the Determination of Nelaribine in Bulk and Tablet Dosage Form

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ABSTRACT

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Nelaribine in bulk and tablet dosage form with greater precision and accuracy. Separation was achieved on Cosmiscil C18 column (150X4.6mm i.d.,5μm) in isocratic mode using Triflouro acetic acid PH-3.6 buffer and Acetonitrile in the ratio of 90:10(v/v) as mobile phase, pumped in to the column at flow rate of 1.0 mL min−1 and the detection of eluent from the column was carried out using variable wavelength UV detector at 248 nm. The total run time was 15 min and the column was maintained at ambient temperature. The retention time of Nelaribine was 4.003 min. The standard curves were linear over the concentration range of 25-150 μg/ml with R^2 0.999 and the LOD and LOQ values for Nelaribine were 0.04 μg/ml and 0.12 μg/ml, respectively. The percentage recovery was found to be 101.76 – 98.72 %, the % RSD was found to be 0.43. The percentage amount of a marketed tablet formulation of Nelaribine was found to be 101.2 %. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. Hence the proposed method can be applied for the routine quality control analysis of Nelaribine in bulk and tablet dosage forms.

KEYWORDS: Nelaribine, RP-HPLC, Method Development, Validation, ICH guidelines

1. INTRODUCTION

NELARABINE is a purine nucleoside analog converted to its corresponding arbinosylguanine nucleoside tri phosphate results inhibition of DNA synthesis and cytotoxicity. The drug is ultimately metabolized into the active 51-tri phosphate ara –GTP which disrupts the DNA synthesis and induces apoptosis. Nelarabine is used to treat T-cell acute Lymphoblastic leukemia and T-cell lymphoblastic lymphoma. The Chemical name of Nelaribine (2R,3S,4S,5R)-2-(2-amino-6-methoxy-9H-purin-9-yl)-5 -(hydroxymethyl) oxolane-3,4-diol and chemical formula is $C_{10}H_{11}ClFN_5O_3$ and molecular weight is 297.27 g/mol.

MATERIALS AND METHODS

Chemicals and solvents

The reference sample of NELARABINE was supplied by M/s Natco laboratories,Hyd. The Branded formulations of NELARABINE (Arranon Vials; Glaxosmithkline) were purchased from the local market. Ortho phosphoric acid, Acetonitrile, and water used are of HPLC grade.

Instrumentation

A Aliance waters (2487) HPLC instrument, equipped with a Cosmicsil Adza C-18 (150x4.6mm), 5μm, an LC 20 AD pump and a SPD 20AD UV-Visible detector was employed in this study. Chromatographic analysis and data acquisition was monitored by using empower-2software. A 20 μL Hamilton injection syringe was employed for sample injection. Degassing of the mobile phase was done by using a SV scientific ultrasonic sonicator. A Mettle Toledo AL 104 electronic balance was used for weighing the materials. Class 'A' Borosil glass ware (Borosil, India) was employed for volumetric and general purpose in the entire study.

Chromatographic Conditions

The mobile phase consists of PH 3.6 buffer : Acetonitrile in the ratio of 90:10 v/v%. The mobile phase was pumped from solvent reservoir in the ratio of 90:10 %v/v to the column in the flow rate of 1.0 ml/min whereas run time set was 15 min. The separation was performed on Cosmicsil C18 column

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and the column was maintained the temperature of 25°C and the volume of each injection was 10 μL. Prior to injection, the column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluents were monitored at 248 nm.

Preparation of buffer solution: (PH: 3.6):

1ml of Trifloroacetic acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then pH adjusted to 3.6with dil. Trifloroacetic acid solution.

Preparation of mobile phase:

Buffer and Acetonitrile are in the ratio 90:10 %v/v, filtered through 0.45 μ filter under vacuum.

Preparation of standard solution: (100μg/ml):

Accurately Weigh and transfer accurately 31.25 mg of Nelarabine working Standard into a 50 ml clean dry volumetric flask, and add about 30 ml of diluents, dissolve and dilute to volume with diluents. Transfer 2.0 ml above solution into 200 ml volumetric flask and dilute to volume with diluent(Milli-Q water as diluent).

Preparation of sample solution:

Accurately pipette out 2.0 ml of sample solution in to 200 mL volumetric flask without any loss of solution. Add 120 ml 0f diluent, shake well to dissolve and dilute to volume with diluents and mix well. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Label Claim: 37.5 mg of Nelaribine

Validation of Proposed method:

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision, Specificity, Forced degradation studies, Linearity, Accuracy, Limit of detection and Limit of quantification.

Linearity

Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ml were taken from stock solution of concentration 100 μg/ml

of Nelaribine and then diluted up to mark with diluent. Such that the final concentrations were in the range 25-150 ppm of Nelaribine. Volume of 10μl of each sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. A linear relationship between peakarea vs. concentration was observed in the range of study. The observations and calibration curve were shown in figure.2.

Linearity and Construction of calibration curve

The quantitative determination of the drug was accomplished by the external standard method. The mobile phase was filtered through a 0.45μ membrane filter before use. The flow rate of the mobile phase was adjusted to 1.0 mL/min. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. The column temperature is maintained at 25±10 C throughout the study. Linearity of the peak area response was determined by taking six replicate measurements at seven concentration points. Working dilutions of NELARABINE in the range of 25-150 μg/mL were prepared by taking suitable dilutions of the standard solutions in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase. Twenty microlitres of the dilutions was injected each time into the column at flow rate of 1.0 mL/min. Each dilution was injected six times into the column. The drug in the eluents was monitored at 248 nm and the corresponding chromatograms were obtained. From the chromatograms, the mean peak areas were noted and a plot of concentrations over the peak areas was constructed. The regression of the plot was computed by least squares method. The linear relationship was found to be in the range of 25-150 μg / mL between the concentration of NELARABINE and peak area response. This regression equation was later used to estimate the amount of NELARABINE in pharmaceutical dosage forms. The linearity plot was shown in the Fig.2 and the linearity data and the statistical parameters for the linearity plot are reported in Table 3 & 4.

VALIDATION OF THE PROPOSED METHOD:

The method was validated in compliance with ICH guidelines. The following parameters were determined for validation.

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Specificity

The method specificity was assessed by comparing the chromatograms obtained from the drug with the most commonly used excipient mixture with those obtained from the blank solution. The blank solution was prepared by mixing the excipients in the mobile phase without the drug. The drug to excipient ratio used was similar to that in the commercial formulations. The commonly used excipients in formulations like lactose, starch, microcrystalline cellulose, ethyl cellulose, hydroxyl propyl methylcellulose, magnesium stearate and colloidal silicon dioxide were used for the study. The mixtures were filtered through 0.45µ membrane filter before injection. An observation of the chromatograms indicates absence of excipient peaks near the drug peak in the study runtime. This indicates that the method is specific.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the method was studied in terms of repeatability (intra-day assay) and intermediate precision (inter-day assay). Method repeatability was studied by repeating the assay three times in the same day for intra-day precision and intermediate precision was studied by repeating the assay on three different days, three times on each day (inter-day precision). The intra-day and inter-day variation for determination of NELARABINE was carried out at three different concentration levels 25, 50, 75 µg/mL level. The coefficient of variation values as presented in the Table.5 shows that the method provides acceptable (<2) intraday and inter-day variation.

Accuracy

Accuracy of the method was evaluated by standard addition method. An amount of the pure drug at three different concentration levels in its solution has been added to the pre analysed working standard solution of the drug. The sample solutions were analysed in triplicate at each level as per the proposed method. The percent individual recovery and %RSD for recovery at each level are calculated. The results are tabulated (Table.6). A recovery ranged from 98.72 – 102 % has been obtained by the method indicates its accuracy.

Robustness

A study was conducted to determine the effect of deliberate variations in the optimized chromatographic conditions like composition of the mobile phase, flow rate, and mobile phase pH. The effect of these changes on the system suitability parameters like tailing factor and number of theoretical plates and on assay was studied. A single condition was varied at a time keeping all other parameters constant. The results were found to be within the allowed limits which indicate that the method is specific.

Variations in flow rate of the mobile phase:

Demonstrated robustness of method by changing the flow rate for 0.9 ml/min and 1.1 ml/min instead of specified flow rate (1.0 ml/min). By injecting the replicate injections of standard in 0.9 ml/min and 1.1 ml/min flow rate and found that system suitability parameters were passed. The %RSD of peak area, tailing factor and theoretical plates of Nelarabine standard was found within the limits.

Variations in the pH of the mobile phase:

The effect of variation in the pH of the mobile phase was evaluated at ± 0.2 levels. The system suitability results were found to be within the limits as shown in the following table-

Variations in the temperature of column:

Robustness of the method by changing the column temperature for 28°C and 31°C instead of specified column temperature (30°C) are shown. By injecting the replicate injections of standard in 28°C column and 31°C column temperature and found that system suitability parameters were passed. The %RSD of peak area, tailing factor and theoretical plates of Nelarabine standard was found within the limits.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a measurable response. LOD is determined based on signal to noise ratio (S/N) of three times typically for HPLC methods. The limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. It is the lowest concentration at which the

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precision expressed by a RSD of less than 2%. In this study the analyte response is 10 times greater than the noise response. For this study six replicates of the analyte at lowest concentration in the calibration range were measured and quantified.

Average Baseline Noise obtained from Blank = 98.1936 µV

Signal Obtained from LOD solution = 7985 µV

LOD = 3.3×σ/s

$\quad$ **= 3.3×98.1936/7985**

$\quad$ **= 0.0405**

LOQ = 10×σ/s

$\quad$ **= 10×98.1936/7985**

$\quad$ **= 0.1229**

System precision and System suitability

System precision and system suitability studies were carried out by injecting six replicates of the working standard solution. The % RSD for the peak areas obtained was calculated. The data presented in Table 2.3.9 (% RSD<1) establishes reproducible performance of the instrument. The system suitability parameters are given in Table 2.3.10.

Assay calculation:

$\%Assay = \frac{TA}{SA} \times \frac{SW}{100} \times \frac{5}{50} \times \frac{100}{2} \times \frac{P}{100} \times \frac{100}{L.A}$

Where,
TA = peak area response due to Nelarabine from sample
SA = peak area response due to Nelarabine from standard

SW = Weight of Nelarabine working standard taken in mg

P $\quad$ = purity of Nelarabine working standard taken on as is basis

L.A = Labeled amount of Nelarabine in mg

%Assay $\qquad\qquad$ = 862111/845634x31.25x2/25x200/2x99.2/100x100/5

= 100.192%

CONCLUSION

The present study was aimed at developing a sensitive, precise and accurate HPLC method for the analysis of NELARABINE in bulk drug and in pharmaceutical dosage forms. using C18 column, flow rate was 1.0ml/min, mobile Phase: Trifluoroacetic acid buffer solution pH 3.6 ±0.05 and solvent mixture (Acetonitrile) in (90:10 v/v). Detection wave length was 248nm.The instrument used was WATERS HPLC auto sampler. The retention times were found to be 4.003 mins. The analytical method was validated according to ICH guidelines (ICH Q2b). The correlation coefficient (r2) was found to be 0.998, % recovery was 101.76-98.72% and %RSD for precision on replicate injection was 0.43 and intermediate precision for intraday precision at condition- I, II and III was 0.7, 0.2 and 0.3 interday precision at condition-I,II and III was 1.2,0.3 and 0.3 respectively. The precision study was precise, robust, and repeatable. LOD value was 0.0405 and LOQ value was 0.1229.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust. The lowest values of LOD and LOQ as obtained by the proposed method indicate the method is sensitive. The standard solution of the drug was stable up to 24 hours as the difference in percent assay is within limit.

System suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria.

The tailing factor, the number theoretical plates and HETP are in the acceptable limits. Hence the author concludes that the proposed HPLC method is sensitive and reproducible for the analysis of NELARABINE in pharmaceutical dosage forms with short analysis time.

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Figures:

Chemical Structure:
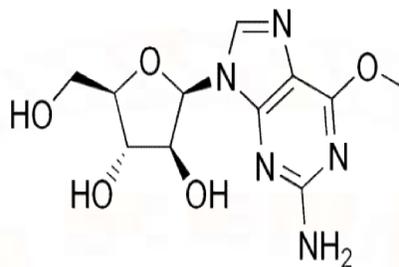
Figure.1
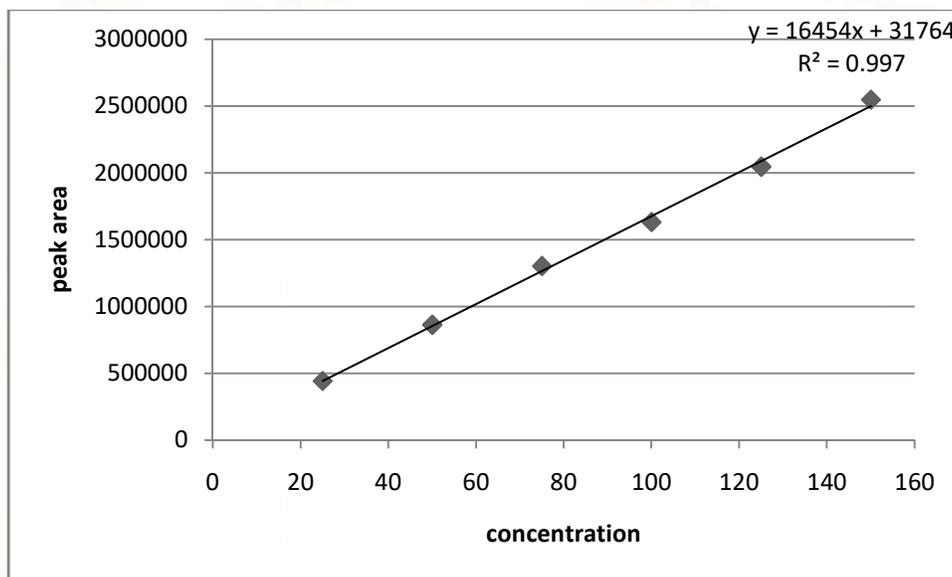
Fig.2 Linearity Plot for the proposed method

Table 1 Optimized Chromatographic conditions and system suitability parameters for proposed HPLC method for Nelaribine
Parameter Chromatographic conditions

S.No	Parameter	Value	
1	Column	Cosmicsil Adza C-18 (150x4.6mm), 5µm	
2	Mobile phase	Trifluoro acetic acid pH-3.6 buffer and Acetonitrile in the ratio of 90:10 v/v	
3	Flow rate	1mL/min	
4	Run time	15.0 min	
5	Column temperature	25 ± 1^0 C	

6	Volume of injection	10 µL
7	Detection wave length	248nm
8	Retention time of the drug	4.003 min

Table.2 Accuracy

S.NO	Conc. Of Nelarabine in ppm	Nelarabine peakArea
1	25	442399
2	50	862111
3	75	130223
4	100	1630614
5	125	2045323
6	150	2512346

Table.3 Calibration of the proposed HPLC Method

Concentration of NELARABINE (µg/mL)	Peak Area
25	442399
50	862111
75	1302223
100	1630614
125	2045323
150	2546233

Table.4 Regression characteristics of the linearity plot of NELARABINE

Parameter	Value
Linearity Range (µg)	25-150
Slope (a)	16264
Intercept (b)	30585

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Correlation coefficient	0.998
Regression Equation	Y = 16264X + 30585

Table.5. Precision of the proposed method

Concentration of NELARABINE (µg/mL)	Intra-day Precision			Inter-day Precision		
	Mean	SD	% RSD	Mean amount found (n=9)	SD	% RSD
25	445202	3132.96	0.7%	448172	8624.2	1.2%
50	860569	1947.16	0.2%	858664	2784.8	0.3%
75	1356106	4428.01	0.3%	1304275	4832.2	0.3%

Table.6 Accuracy data (Triplicate values at different concentration levels)

Spike Level	Conc µg/m	Conc µg/ml	Amount found	% Recovery	Mean % Recovery
	Amount added (pure)	Amount added (formulation)			
80%	8	10	7.864	101.84	
	8	10	7.872	101.72	101.76
	8	10	7.863	101.73	
100%	10	10	10.022	99.64	
	10	10	10.030	99.77	99.35
	10	10	10.024	99.72	
120%	12	10	12.156	98.65	
	12	10	12.147	98.81	98.72
	12	10	12.176	98.70	

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Table.7

Variations in the flow rate	Peak area	Average	% RSD
0.9ml/min	864511	866702	0.2%
	867732		
	867865		
1.1ml/min	865567	867083	0.1%
	867783		
	867899		

Table.8

Variations in the P^H	Peak area	Average	% RSD
3.4	865577	867451	0.1%
	867883		
	868894		
3.8	866221	867297	0.1%
	867783		
	867889		

Table.9

Variations in the temperature	Peak area	Average	% RSD
28^0c	588678	588780	0.018%
	588772		
	588892		
31^0c	867745	867509	0.07%
	866785		
	867998		

Table.10 System precision

Injection No.	Peak area
1	845634
2	857096
3	866190
4	853829
5	850789
6	859723

Mean	855543
SD	7138.6
% RSD	0.8%

Table.11 System suitability parameters

Parameters	Values
Theoretical plates (n)	4890
Plates per meter (N)	7509
Tailing factor (T)	1.08
LOD	0.0405
LOQ	0.1229

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