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DEVELOPMENT AND VERIFICATION OF RP-HPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF DECITABINE IN TABLET DOSAGE FORMULATION

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ABSTRACT

Decitabine is an anti-cancer chemotherapy drug. This article describes method development and method verification of Assay of Decitabine in tablet formulation. A new, precise, rapid, accurate RP-HPLC method has been developed for the estimation of Decitabine in pharmaceutical tablets dosage form. After optimization the good chromatographic separation was achieved by using YMC Triart C18, 250 x 4.6mm, 5µm (Part No. TA12S05-2546WT) column. The mobile phase consists of gradient elution using of mixture of 0.02M Potassium dihydrogen phosphate buffer and Methanol in the ratio of 97:3 as Mobile phase A and Water and Acetonitrile in the ratio of (40:60) as Mobile phase B . The flow rate is 1.5 mL/min, injection volume is 5 µL, the column temperature is 30°C and the detection is by UV at 242 nm. The retention time of decitabine was found about 6.6 minutes. The linearity of this method was found in the concentration range of 50-150 µg/mL. The correlation coefficient R^2 value is found to be 1.000. This method was found to be good percentage recovery about 100.5 % indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of formulation. The method was extensively verified according to ICH guidelines for Linearity, Range, Accuracy, Precision, specificity and Robustness.

KEYWORDS: Decitabine tablets, Development, Verification, HPLC, ICH, Antineoplastic.

INTRODUCTION

Decitabine is chemically 4-amino-1-(2-deoxy-β-D-ERYTHRO-PENTOFURANOSYL)-1, 3, 5-triazin-2(1H)-one and molecular formula is $C_{18}H_{12}N_4O_4$ and molecular weight is 228.21 g/mol. Decitabine a hypomethylating agent. Decitabine is believed to exert its antineoplastic[2] effects after phosphorylation and direct incorporation into DNA and inhibition of DNA methyltransferase, causing hypomethylation of DNA and cellular differentiation or apoptosis. It hypomethylates DNA by inhibiting the methyl transferase enzyme. It is useful for the treatment of patients with myelodysplastic syndrome (MDS). The chemical structure of Decitabine as shown in Fig.1.From the literature survey, it was found that Decitabine was estimated by analytical methods such few UV-Visible methods and high performance liquid chromatography (HPLC) Method for injectable formulation. To the best of our knowledge, there is no reported HPLC method for estimation of decitabine assay in tablet dosage forms. The aim and objective of this study was to develop and verify the assay of Decitabine in Decitabine tablet dosage form.

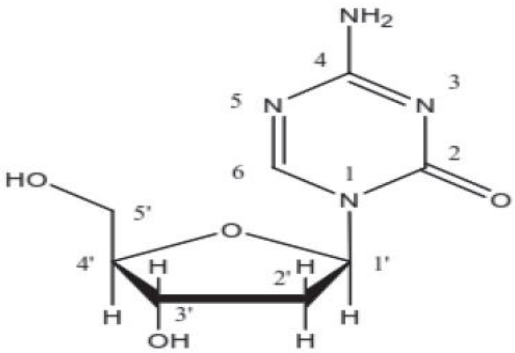

Fig. 1: Structural formulation of decitabine.

EXPERIMENTAL

MATERIALS AND METHODS

Instruments and Chromatographic condition

All chemicals used in the investigation were of HPLC grade. Chromatographic analysis for detection of Decitabine was performed using the RP-HPLC (Waters and Agilent) equipped with Empower software, waters 2695 UV and Agilent 1260 VWD detector by using YMC Triart C18, 250 x 4.6mm, 5µm analytical column. The mobile phase consists of gradient elution using of mixture of 0.02M Potassium dihydrogen phosphate buffer and Methanol in the ratio of 97:3 as Mobile phase A and Water and Acetonitrile in the ratio of (40:60) as

Mobile phase B . Gradient elution mobile phase-B, 0.0min-0, 10.0 min-0, 10.5-100, 14.5 min-100, 15.0 min-0, 20.0 min -0, at The flow rate is 1.5 mL/min, injection volume is 5 µL, the column temperature is 30°C and the detection is by UV at 242 nm. The Diluent is DMSO.

Selection of wavelength

Decitabine has wavelength maxima at about 201 nm and 244 nm. Wavelength of 242 nm has been finalized as Decitabine peak has optimum response and proper baseline was observed at this wavelength.

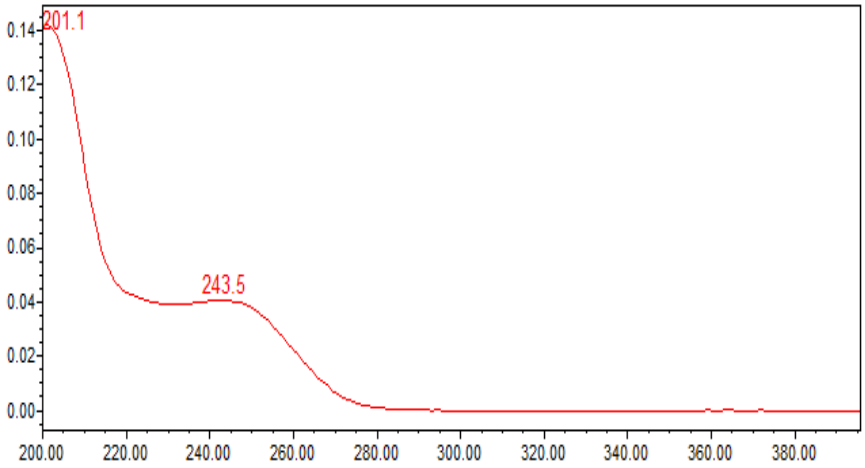

Fig. 2: UV spectrum of decitabine.

Selection of diluent

Decitabine is slightly soluble in methanol and sparingly soluble in DMSO and water in the pH range of 2 to 11. Experiment was conducted by preparing about 50 µg/mL of Decitabine using API in DMSO and pH 2.5 Sodium Hydrogen Sulfite buffer solution as a diluent and injected into HPLC. Peak response was found to be good in both injected solutions. Based on the Decitabine molecule nature, DMSO has been finalized as diluent in order to avoid degradation of Decitabine during sample preparation.

Selection of Standard and Sample concentration

Decitabine peak response and percent relative standard deviation (RSD) for area response was found to be satisfactory for standard solution of 100 µg/mL. Accordingly selected the standard and sample concentration as 100 µg/mL.

Selection of injection volume

During method development experiments different injection volumes were explored. An injection volume of 5µL has shown optimum peak response and symmetrical peak shapes for Decitabine, hence the same has been finalized.

Preparation of pH 6.8 Buffer: Dissolved 2.72g of Potassium Dihydrogen Phosphate into 1000ml of HPLC grade water, adjusted the pH to 6.8 with diluted sodium hydroxide, filter ed through 0.45µ membrane filter and degassed in sonicator for 10 minutes.

Mobile phase A: Mixed the pH 6.8 Buffer solution and Methanol in the ratio of 97:3 v/v%. Degassed the mobile phase by sonication for 10 minutes.

Mobile Phase B: Mixed the Water and Acetonitrile in the ratio of 40:60 v/v%. Degassed the mobile phase by sonication for 10 minutes.

Standard solution preparation: Weighed accurately and transferred about 50 mg of Decitabine standard into a clean and dry 100 mL volumetric flask. Added 70 mL of diluent, sonicated to dissolve. Diluted to volume with diluent and mixed well. Further Transfer 5.0 mL standard stock solution into 25 mL volumetric flask, diluted to volume with diluent and mixed well.

Sample solution preparation

Taken 25 mg equivalent of decitabine tablets and transferred into a clean and dry 50 mL volumetric flask. Added 30 mL of the diluent and sonicated for 30 minutes with intermittent shaking to dissolved and make up the volume with diluent and mixed well. Centrifuged a portion of the sample solution at 3000 rpm for 5 minutes. Pipetted 5.0 mL of supernatant solution and transferred into a clean and dry 25 mL volumetric flask, diluted to volume with diluent and mixed well.

Placebo solution preparation

Accurately weighed and transferred 25 mg equivalent placebo into 50 mL volumetric flask, added 30 mL of diluent, mixed well and sonicated for 30 min with intermittent shaking to dissolved and make up the volume with diluent and mixed well. Centrifuged a portion of the sample solution at 3000 rpm for 5 minutes.

Method verification

System suitability

System suitability was performed based on the above finalized conditions. Injected diluent as blank and standard in five replicate injections into the chromatograph. Results summarized table 1.

Acceptance criteria

The results of the system suitability parameters should be within the specified limits as mentioned in the table 1.

Specificity

Placebo interference

To establish non-interference, placebo solutions were prepared in duplicate and injected into HPLC.

Acceptance criteria

No peak shall be detected in the placebo chromatogram at the retention time of Decitabine.

Forced Degradation Studies / Stress Study

To establish non-interference from the degradants, stressed samples and placebo solutions were prepared along with the unstressed placebos and samples and injected into HPLC. Results summarized table 2&3.

Acceptance criteria

Peak purity shall pass for Decitabine in the stressed samples. Purity angle shall be less than Purity Threshold.

Linearity

A series of solutions of Decitabine were prepared in the concentration of ranging from 50.6 µg/mL to 151.8 µg/mL (50 to 150% level of the test concentration) and analyzed as per test method.

A graph was plotted using concentration in µg/mL on X-axis versus peak area on Y-axis. Calculated the correlation coefficient and Y-intercept value at 100 % response and found within the limits. Intercept, Slope value was calculated and reported. Results summarized table 4 and Fig 3.

Acceptance criteria

The correlation coefficient should be not less than 0.999.

Y-Intercept should not be more than ±2% of the response at 100% level.

Report the Intercept, Slope and Residual sum of squares value.

Accuracy

Recovery study was performed at 150% levels by spiking the placebo with the drug substance. Samples were spiked in triplicates. Spiked samples were extracted and analyzed. Percent recovery and its mean recovery were calculated. All the results were found to be within the limits. Results summarized table 5.

Acceptance criteria

The individual % recovery of Decitabine shall be between 97.0 and 103.0.

Method precision

Prepared three sample solutions as per test method by using the drug product for assay. The percent assay of each replicate, average of three replicates and % RSD were calculated. Results summarized table 6.

Acceptance criteria

The relative standard deviation of three replicate assay results shall be not more than 2.0%.

Ruggedness

Solutions Stability at Room Temperature (RT)

Stability of standard and sample preparations were established at room temperature (RT). Sample solutions prepared in precision sample were considered for solution stability study. The final dilutions of the standard and sample solutions were then stored on the bench top at controlled room temperature in tightly closed HPLC vials and analyzed. Standard solutions were analyzed after 18, 24 and 32 hours, similarly sample solutions were analyzed after 12 & 24 hours. For sample solutions, the assay and difference in the percent assay between initial and next interval was calculated and for standard solutions similarity factor was calculated. Results summarized table 7&8.

Acceptance criteria

For sample solution, the difference in % Assay results from initial to next time interval shall not be more than ± 2.0. For Standard similarity factor shall be 0.98 to 1.02

RESULTS AND DISCUSSION

System suitability

Table 1: System suitability results.

Parameter	ProposedAcceptance Criteria	Observed
Blank Interference	No peak > 0.1% shall be observed in the blank chromatogram at the retention time of Decitabine	Nil
Tailing Factor	NMT 2.0	1.09
% RSD from replicate injections of standard solution	NMT 2.0%	0.74

Specificity

No peak was detected in the Blank & placebo chromatogram at the retention time of Decitabine peak when compared to the standard chromatogram.

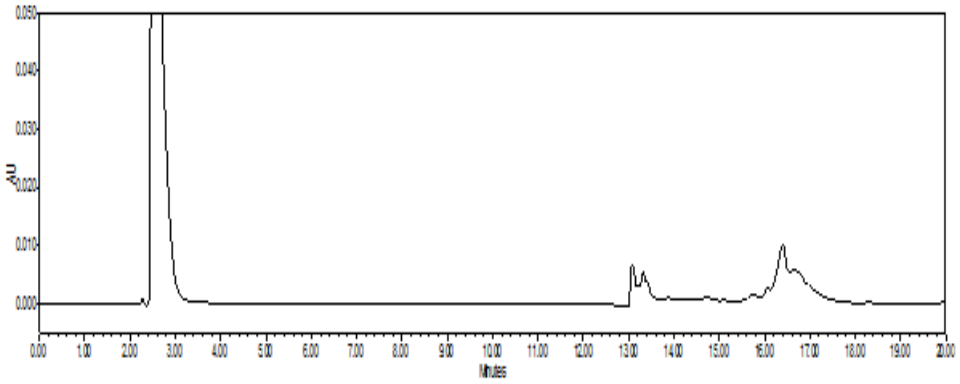

Fig: 2. Typical chromatogram of diluent (Blank).

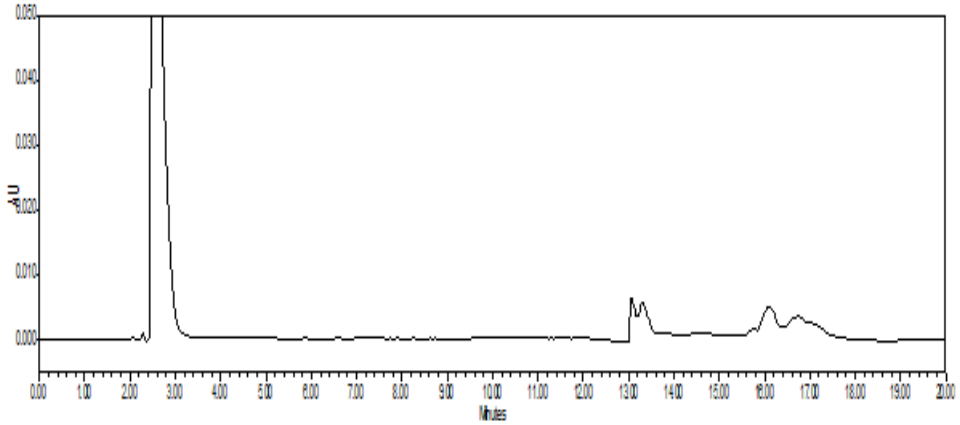

Fig. 3: Typical chromatogram of placebo solution.

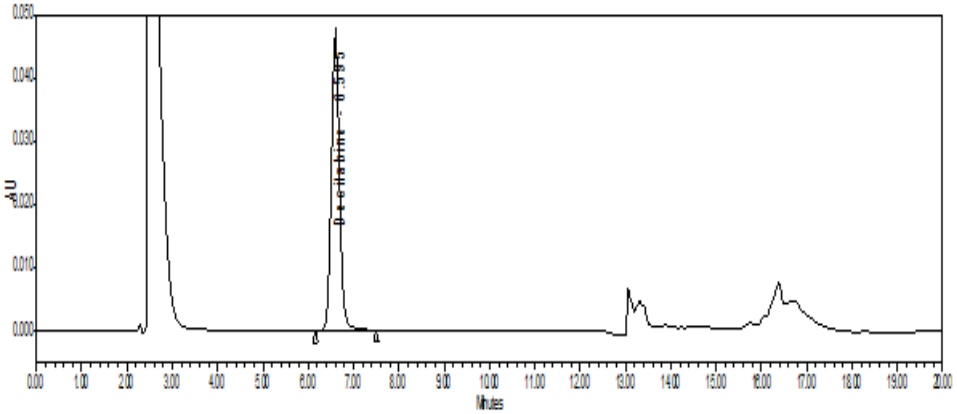

Fig. 4: Typical chromatogram of standard solution.

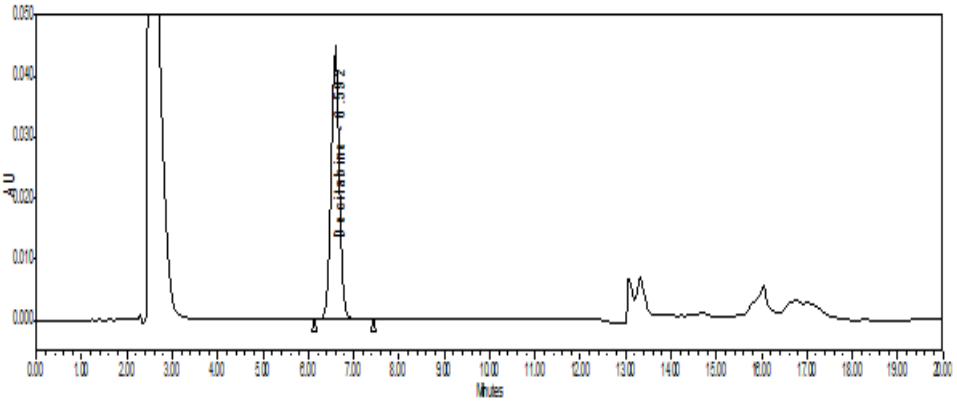

Fig: 5. Typical chromatogram of sample solution.

Conclusion

The method meets the requirements of placebo interference.

Forced Degradation Studies/Stress Study

All the forced degradation samples stressed have resulted in pure analyte peak. The purity angle of the main analyte is less than the purity threshold which happens to the basis for peak purity (based on waters software). The details of the same are summarized in the below Table.

Table 2: Specificity results.

Sample Name / Stress Condition	Purity angle	Purity Threshold	Peak Purity
Unstressed Sample	0.11	0.31	Pass
Acid Stressed Sample	0.09	0.27	Pass
Base Stressed Sample	0.09	0.31	Pass
Peroxide stressed Sample	0.12	0.31	Pass

Table 3: Percent Assay for Unstressed and Stressed Samples.

Sample Name	% Assay	% Net Degradation
Unstressed sample	95	NA
Acid stressed sample	85.9	9.1
Base stressed	92.8	2.2
Oxidation stressed	94.3	0.7

Conclusion

Based on the above results the analytical method developed is a specific and stability indicating.

Linearity

A graph was plotted using concentration in µg/mL on X-axis versus peak area on Y-axis. Calculated the correlation coefficient and Y-intercept value at 100 % response and found within the limits. Intercept, Slope value was calculated and reported.

Table 4: Results of linearity of decitabine

% Level	Concentration (µg/mL)	Area
50	50.6	908408
80	81.0	1429860
100	101.2	1803472
120	121.5	2136121
150	151.8	2674643
Coefficient of correlation		1.000
Slope		17448.673
Intercept		24346.145
Bias at 100% response		1.35

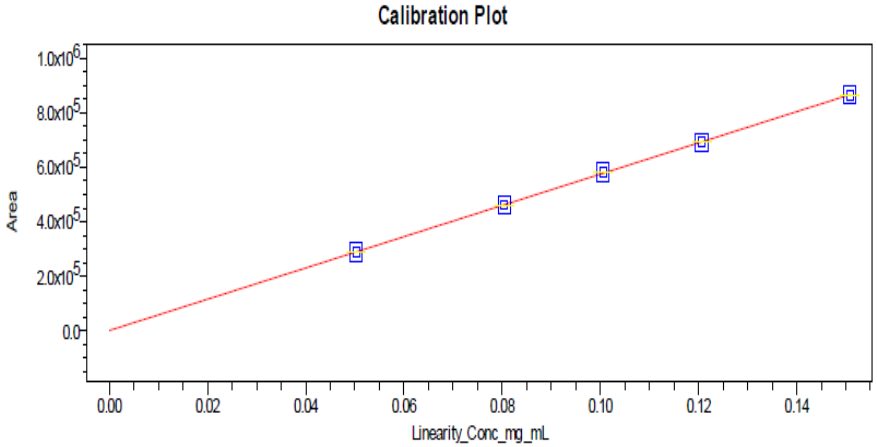

Fig: 6. Linearity plot for decitabine.

Conclusion

Based on the above results the analytical method developed is Linear.

Accuracy

Table 5: Results of accuracy.

Sample No.	Spike level	Percent recovery	Mean percent recovery
1	150%	100.7	100.5
2		99.9	
3		100.8	

Conclusion

Recovery values meet the acceptance criteria. This experiment demonstrates that the diluent and other extraction conditions chosen are adequate to extract up to 150% level of drug loading from the excipients.

Method precision

Table 6: Results of method precision of assay

Sample No.	% Assay
1	96.1
2	96.6
3	95.4
Mean	96.0
%RSD	0.63

Conclusion

The method is precise as the results met the acceptance criteria.

Ruggedness

Solutions Stability at Room Temperature (RT)

Table 7: Standard Solution Stability at Room Temperature

Time in Days	Similarity factor
Initial	NA
18 Hours	0.98
24 Hours	0.99
32 Hours	0.99

Table 8: Sample solution stability at room temperature.

Time in Days	Percent Assay		Percent Difference	
	Test 1	Test 2	Test 1	Test 2
Initial	96.1	96.6	NA	NA
12 Hours	95.5	96.4	0.6	0.2
24 Hours	95.4	96.4	0.7	0.2

Conclusion

Sample solutions were stable for 24 hours when stored at RT condition in tightly closed glass container.

Overall conclusion

Analytical test method for Assay by HPLC for Decitabine in Decitabine tablets was verified for System suitability, Specificity (Placebo interference and Interference from degradation products), Linearity, Method Precision, Accuracy and Ruggedness (Stability of Solutions at Room Temperature). Verification meets all the pre-established acceptance criteria.

The sample recovery in the formulation was in good agreement with their respective label claims. This method was found to be better than previously reported methods. Hence the above method can be used in quality control for routine analysis of tablets formulations.

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Conflict of interest

Declared none.

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