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Stability indicating RP-HPLC Method Devlopment and Validation of Decitabine Drug in Formulation

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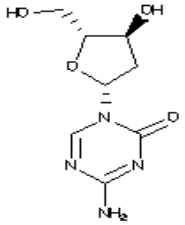
Abstract: A simple, sensitive, precise and stability indicating Reverse phase high performance liquid chromatographic method has been developed for the quantitative analysis of Decitabine drug present in tablet formulation and bulk drug. The HPLC separation was achieved on Zorbax bonus C_{18} Column (250mm x 4.6 mm, i.d, 5 µm particle size) with the mobile phase and detection at 254nm.The proposed method provided linear responses within the concentration range 400-1200 µg mL^{-1} for Decitabine and its related compounds. LOD and LOQ values for the active substance were 0.26 and 0.8 µg mL^{-1}, respectively. Correlation coefficients (*r*) of the regression equations for the impurities were greater than 0.999 in all cases. The precision of the method was demonstrated using intra- day assay RSD% values which were less than 1% in all instances. No interference from any components of pharmaceutical dosage forms or degradation products was observed.

Key words: HPLC, Decitabine, stability indicating, method development and validation.

Introduction:

Chemically Decitabine has found to be 4-amino-1-(2-deoxy-b-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one ($C_8H_{12}N_4O_4$) with the molecular weight of 228.08 gms. Decitabine is pyrimidine analogue of the nucleoside 2'-deoxycytidine[1, 2, 3] . It is believed to exert its antineoplastic effects after phosphorylation by triphospate metabolite of the drug and direct incorporation into DNA and inhibition of DNA methyltransferase, causing hypomethylation of DNA and cellular differentiation or apoptosis in rapidly dividing cells. Non-proliferating cells are relatively insensitive to Decitabine. DNA hypomethylation is achieved at concentrations below those required to significantly inhibit DNA synthesis, which may promote restoration of function to genes associated with control of cellular differentiation and proliferation [4, 5, 6] . Decitabine is specifically indicated for the treatment of multiple types of myelodysplastic



DECITABINE 1

syndromes, including previously treated and untreated, de novo and secondary myelodysplastic syndromes (MDS) of all French-American-British (FAB) subtypes (refractory anemia, refractory anemia with ringed side oblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia) and intermediate-1, intermediate-2, and

high-risk International Prognostic Scoring System (IPSS) groups.

A reverse-phase high-performance liquid chromatography method with electro spray ionization and detection by tandem mass spectrometry is described for the simultaneous quantitative determination of Decitabine was available and other bioanalytical methods are also. But till the date there is no avaibalblity Stability indicating HPLC method for Decitabine [7, 8, 9, 10] . The Developed method for Decitabine is simple, accurate, précised, economic method for routine analysis [11, 12, 13]

Experimental:

Materials: Decitabine working standard having purity of 99.75% .ammonium acetate and Dimethyl sulfoxide purchased from standard chemicals .Commercial Decitabine formulation (Dacogen 5mg powder for injection) from pharmacy shops in Hyderabad.

Shimadzu HPLC equipped with photodiode array was employed with empower software was used for the instrument control, data acquisition and data analysis. Zorbax bonus C_{18} column with 4.6 mm i.d and length 250 mm. The pH value of running buffer solution determined by using polmon 5 points P^H meter to within +0.05. The electrolyte solution was prepared and filtered daily.

Method: Column was run with mobile phase for 1 hr .Between injections, the column conditioned with the mobile phase for 1 min.

Preparation of standard solutions and samples:

The stock solution of Decitabine (4 mg/ml) were prepared by dissolving 100 mg in 25 ml volumetric flask and completing the volume properly.sperate the aliquots (1, 1.5, 2, 2.5 and 3ml) o f stock solution were transferred to a 10 ml volumetric flask and diluted with diluent to make concentration of 0.4, 0.6, 0.8, 1 and 1.2 mg /ml ,respectively .

Formulation containing 5 mg in vial .were accurately weighed and crushed to fine powder. An appropriate amount of the crushed equivalent powder to transferred in volumetric flask to volume with diluent to produce final concentration of 0.8 mg/ml of Decitabine. The solution was filtered through 0.45 µm membrane filters and an appropriate portion was transferred into vial.

Linearity range, accuracy, precision and sensitivity

The peak area for Decitabine was plotted against concentration to calibration curve (5 concentration points).the method of least square was employed to examine linearity of the curve.

Precision were determined employing solutions prepared by using formulation. The final filtrate were properly diluted to produce the 0.8 mg/ml for reproducibility and the method also employed with the analyst for intermediate precision..Six separate solution were prepared formulation samples and chromatograms obtained within the same day to assess the intraday precision Detection limit and quantification limit were taken at which the peak response of blank and minimum concentration levels of their signal to noise ratio comparisons .

Forced degradation studies of Decitabine

Forced degradation studies were performed to provide an indication of stability of the drug and specificity of the proposed method. Acid hydrolysis was performed in 0.1N HCl at 60 °C for 8 h. The study in alkaline condition was carried out in 0.1N NaOH at 60 °C for 8 h. These were repeated at lower temperature of 40 °C keeping all the other conditions constant. Oxidative studies were carried out at room temperature in 6 and 20% hydrogen peroxide for 24 h. additionally, the drug powder was exposed to dry heat at 50 °C for 45 d and at 60 °C for 7 d. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution.

Results and discussion

Degradation behavior

HPLC studies on DTB under different stress conditions suggested the following degradation behavior:

Acidic condition

The drug gradually decreased with time on heating at 60 °C in 0.1 N HCl, forming degradation products at RRT 0.33 and 1.20, intially. The rate of hydrolysis in acid was slower as compared to that of alkali. Acid degradation obtained for Decitabine was within the acceptance criteria. From the above table and its chromatogram it is clear that the assay preparation Single Point Threshold was less than that of Purity Index of Decitabine. The Detail of acid degradation mention in table no.1 (figure no.4)

Table 1: Summary of acid degradation

Time	DTB Area %	Impurity Areas	Impurity Area%	RRT	Name of the impurity	DTB Peak Purity Index	DTB Single Point Threshold
Initial	96.4	705550 632857	1.91 1.71	0.33 1.20	UK Hydrolyte	1.000	0.99995
1Hr at 60^0C	59.4	11071417 1009582 25080 51136 127746 20116	36.5 3.33 0.08 0.17 0.42 0.07	0.33 0.64 0.75 0.80 1.19 2.02	UK UK UK UK Hydrolyte UK	1.000	0.99986

Table 2: Summary of alkali degradation

Time	DTB Area %	Impurity Areas	Impurity Area%	RRT	Name of the impurity	DTB Peak Purity Index	DTB Single Point Threshold
Initial	64.8	17893752 34623	35.1 0.07	1.20 2.03	Hydrolyte UK	1.0000	0.99994

Table 3: Summary of Oxidative degradation

Time	DTB Area %	Impurity Areas	Impurity Area%	RRT	Name of the impurity	DTB Peak Purity Index	DTB Single Point Threshold
Initial	99.95	20189	0.05	1.20	Hydrolyte	1.0000	0.99995
1Hr at 60^0C	99.0	303823 68198	0.82 0.18	1.20 2.03	Hydrolyte UK	1.0000	0.99995
3Hr at 60^0C	98.4	459061 164556	1.23 0.44	1.21 2.03	Hydrolyte UK	1.0000	0.99995
6Hr at 60^0C	97.2	28778 706943 321644	0.08 1.89 0.86	0.81 1.20 2.03	Uk Hydrolyte UK	1.0000	0.99995
22Hrs at 60^0C	89.1	48353 276949 241211 45322 2384928 1202139	0.13 0.73 0.63 0.12 6.24 3.15	0.71 0.75 0.81 0.92 1.20 2.03	UK UK UK UK Hydrolyte UKs	1.0000	0.99995

Degradation in alkali

The drug was found to be highly labile to alkaline hydrolysis. The reaction in 0.1N NaOH at 60 °C was so fast and the degrade products formed with the 1.20 and 2.20 RRT, intially. The Base degradation of Decitabine values obtained were within the acceptance criteria i.e., the assay preparation Single point Threshold was less than the Purity Index of the Decitabine. The details of alkali degration studies were mentioned in table no.2 (figure no.5)

Oxidative conditions

The drug was stable to hydrogen peroxide (6%) at room temperature. The reaction in the presence of 6% H_2O_2 at 60 °C was so fast and the degrade products formed with the 1.20 RRT, initially. The details of oxidative degradation studies were mentioned in table no.3 (figure no.6)

Solid-state study

The solid-state studies showed that DTB was stable to the effect of temperature. When the drug powder was

exposed to dry heat at 50 °C for 45 d and at 60 °C for 7 d, no decomposition of the drug was seen.

Validation of developed stability-indicating method

Specificity:

The peak purity of DTB was assessed by comparing the retention time of standard DTB sample good correlation was obtained between the retention time of standard and sample. Placebo and blank was injected and there were no peaks. There are no interferences hence method is specific (figure no.2 and 3)

Linearity

The response for the drug was strictly linear in the concentration range between 400 and 1200 µg ml^{-1}.(figure no.6).

The regression equation for y = 5E+06x+55963 with correlation coefficient (R^2) 0.9998 (table.4, figure no.1)

Table 4: Summary of linearity

Level	Peak Area
50	2092536
75	3142970
100	4221257
150	6275361
200	8281369

Figure 1: Linearity graph

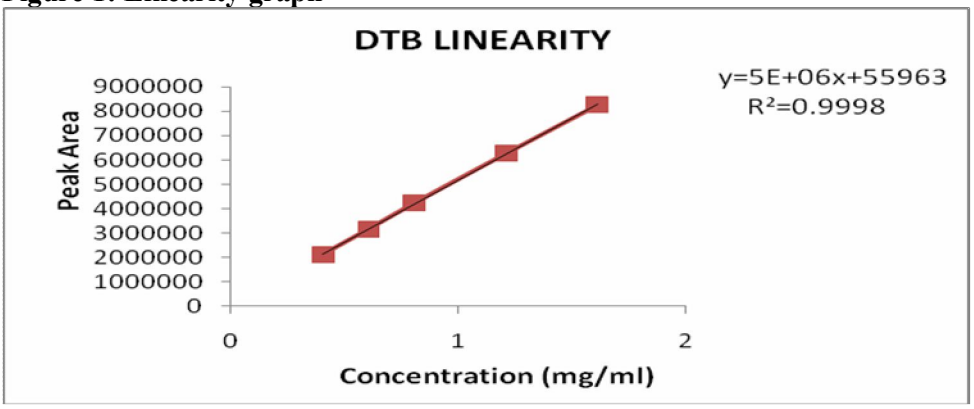


Figure 2: Standard Chromatogram

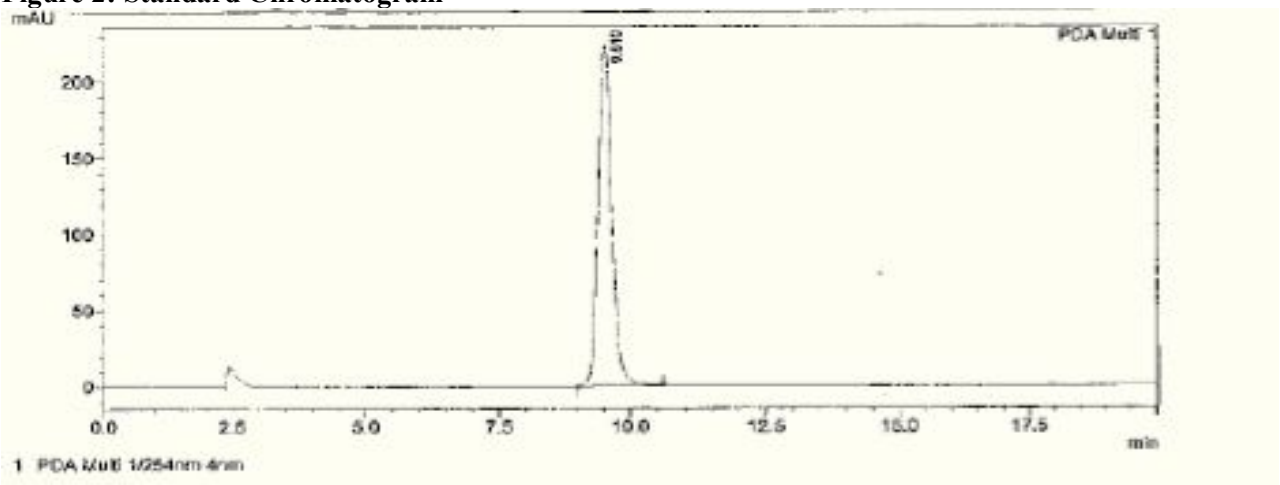


Figure 3: Sample Chromatogram

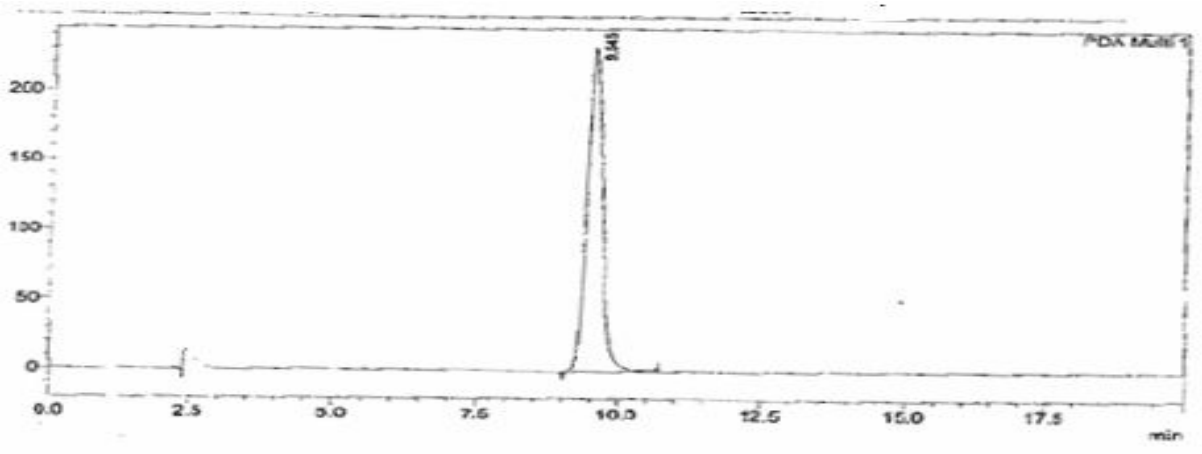


Figure 4: Acid degradation

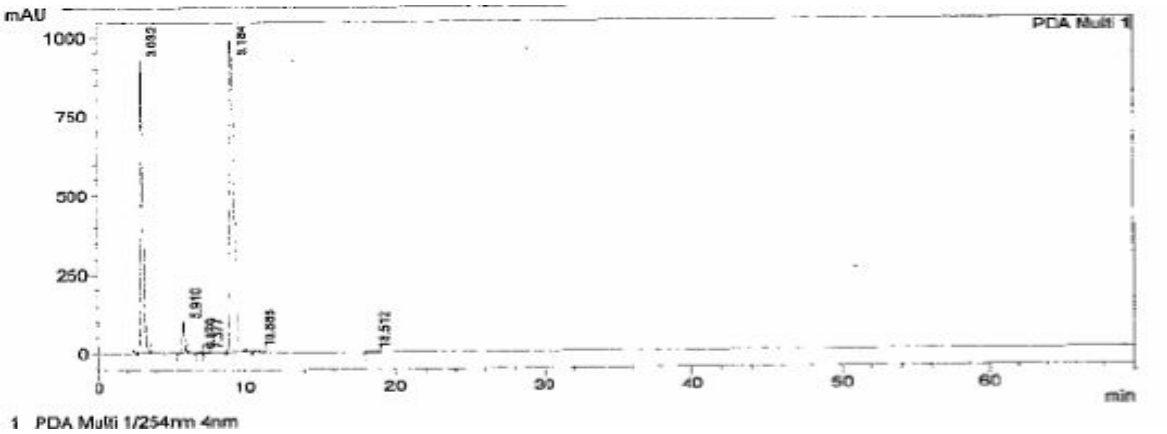


Figure 5: Base degradation

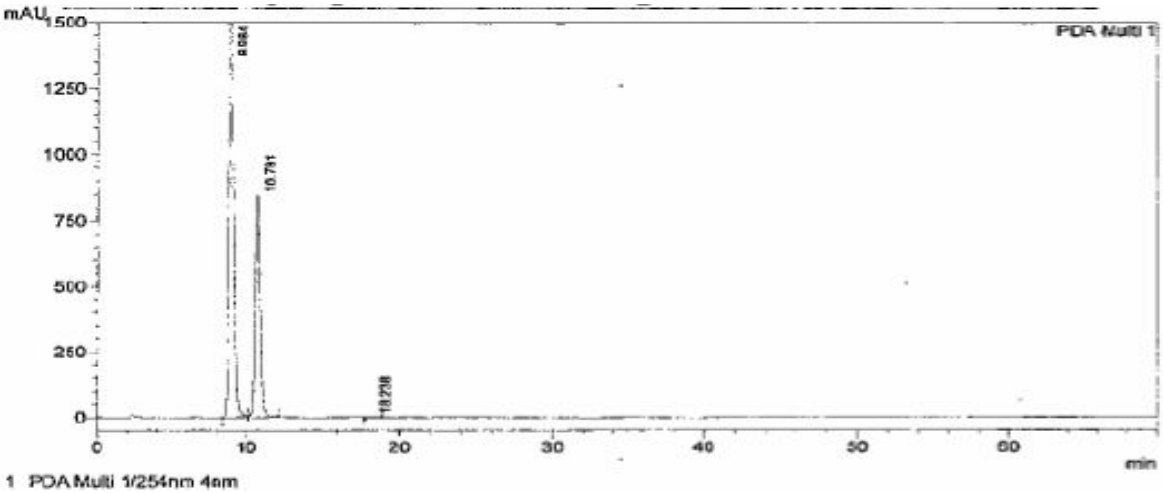


Figure 6: Oxidative degradation

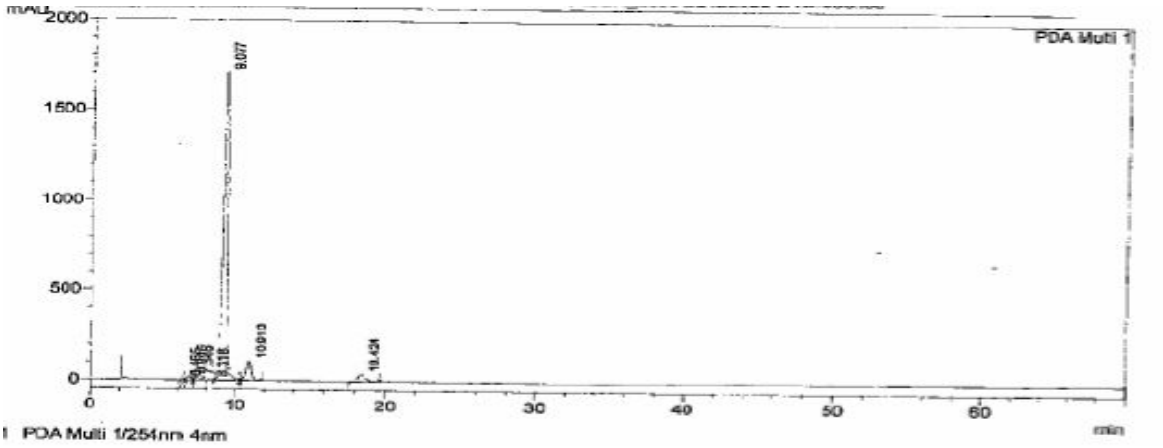


Table 5: Summary of precision

Injection. No	Std Area	Sample area	%assay
1	4302562	4300965	99.85
2	4303215	4298632	98.12
3	4302123	4298923	98.84
4	4302314	4200231	99.42
5	4303212	4300120	99.10
6		4299928	98.90
Mean	4302685		99.02
SD:			0.58
%RSD			0.59

Table 6: Summary of accuracy

Level	%DTB Working Strength	Theoretical Conc (mg/ml)	Peak Area	Measured Conc (mg/ml)	% Recovery
75%	75.71	0.606	3192843	0.600	99.1
	75.71	0.606	3191664	0.600	99.1
	75.71	0.606	3191554	0.600	99.1
100%	100.94	0.808	4305220	0.818	101.3
	100.94	0.808	4303730	0.818	101.3
	100.94	0.808	4304659	0.818	101.3
125%	126.18	1.009	5247926	1.003	99.4
	126.18	1.009	5251976	1.004	99.4
	126.18	1.009	5160274	1.005	99.6
				Mean	99.96
				SD	0.0102
				% of RSD	1.0224

Precision:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (percentage RSD) was found to be less than 2% that proves method is precise (table no.5).

Accuracy (Recovery studies):

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 75%, 100% and 125% concentration levels. Known amounts of standard DTB was added to the pre-analyzed samples and subjected to the proposed HPLC method. Results of recovery studies are shown in table no.6.

Table7: Summary of LOD

Component	Working conc. (mg/ml)	LOD Conc. (mg/ml)	Signal To Noise Ratio
Decitabine	0.8	0.000264	3.3:1

Table 8: Summary of LOQ

Component	Working conc. (mg/ml)	LOD Conc. (mg/ml)	Signal To Noise Ratio
Decitabine	0.8	0.0008	9.1:1

LOD and LOQ:

The LOD concentration obtained is 0.000264mg/ml (or) 0.033% with respect to working concentration of 0.8mg/ml. The LOQ concentration obtained is 0.0008mg/ml (or) 0.1% with respect of working concentration of 0.8mg/ml (table no.7 and 8).

Conclusions:

The proposed method is simple, specific, accurate, precise, Stability indicating and hence can be used in routine for estimation of DTB in formulation and in bulk drug . Statistical analysis of the results has been carried out revealing high accuracy and good precision. The percentages RSD for all parameters was found to be less than two, which indicates the validity of the method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of DTB in formulation.

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