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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ESTIMATION OF DECITABINE BY UV SPECTROSCOPIC METHOD

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ABSTRACT

A new simple, accurate, rapid, precise and reproducible spectrophotometric method for the quantitative estimation Decitabine in bulk form. The developed visible spectrophotometric method for the quantitative estimation of Decitabine is based on measurement of absorption at maximum wavelength 254 nm using with Methanol**:** water (30**:**70) as a solvent. The stock solution of Decitabine was prepared, and subsequent suitable dilution was prepared in diluent to obtained standard curve. The standard solution of Decitabine shows absorption maxima at 254 nm. The drug obeyed beer lambert's law in the concentration range of 5 - 25 µg/ml with regression 0.9991 at 254 nm. The overall % recovery was found to be 99.22% which reflects that the method was free from the interference of the impurities and other excipients used in the bulk form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be 0.81103 and 0.9856, respectively which is<2% hence proved that method is precise.

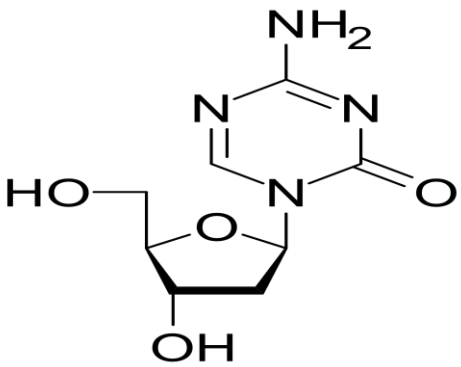
The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Decitabine in bulk form.

KEYWORDS: Decitabine, UV Visible Spectrophotometry, Method development, Validation, ICH guidelines, Accuracy, Precision.

DRUG PROFILE

Name : Decitabine

Description : Decitabine is used to treat myelodysplastic syndrome Decitabine is in a class of medications called hypomethylation agents.



IUPAC Name : 4-Amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one

Chemical formula : C8H12N4O4

Molecular mass : 228.21g/mol

Category : hypomethylation agents.

Mechanism of action: Myelodysplastic syndromes (MDS) are a group of hematopoietic neoplasms that manifest in peripheral cytopenia's and may eventually progress to secondary acute myeloid leukemia (sAML). Included in the over 45 genes commonly mutated in MDS patients are those involved in DNA methylation and histone modification, and it is well-established that alteration of the epigenetic landscape is a feature of myeloid leukemia's.

Decitabine is considered a prodrug, as it requires transport into cells and subsequent phosphorylation by distinct kinases to generate the active molecule 5-aza-2'-deoxycytidine-triphosphate, which is incorporated by DNA polymerase during DNA replication. Once incorporated into DNA, decitabine is recognized as a substrate by DNA methyltransferase

enzymes (DNMTs), specifically DNMT1, but due to the presence of an N5 rather than C5 atom, traps the DNMT through the irreversible formation of a covalent bond. At low concentrations, this mode of action depletes DNMTs and results in global DNA hypomethylation while at high concentrations, it additionally results in double-strand breaks and cell death.

Pharmacodynamics

Decitabine phosphorylated intracellularly, is incorporated into DNA and exerts numerous effects on gene expression. The use of decitabine is associated with neutropenia and thrombocytopenia. In addition, decitabine can cause fetal harm in pregnant women; effective contraception and avoidance of pregnancy are recommended during treatment with decitabine.

Volume of distribution	:	4.59_+ 1.42 L/kg
Protein binding	:	Decitabine exhibits negligible[<1%]
Metabolism	:	It is rapidly metabolized in the liver by cytidine deaminase, which explains its short half-life of 8–30 minutes.
Route of elimination	:	Decitabine can be inactivated through its major elimination pathway involving deamination by cytidine deaminase found principally in the liver, but also in granulocytes, intestinal epithelium, and plasma. Urinary clearance of intact drug accounted for 29% of plasma clearance in mice.
Half-life	:	The terminal elimination half-life ($t_{1/2}$) of decitabine is 37–47 minutes.
Clearance	:	Decitabine has a clearance of 125 L/hr/m^2 (53% CV) when administered intravenously at 15 mg/m^2 for three hours every eight hours over three days, and a clearance of 210 L/ hr /m^2 (47% CV) at 20 mg/m^2 for one hour once daily over five days.
Brand Name	:	Dacogen, Dcitas 30mg , Mylodec ,Visvin, D-NIB, Deczuba
Toxicity	:	The most common toxicity is myelosuppression, mainly displaying as neutropenia and thrombocytopenia.
Affected organisms	:	Humans and alternative mammals.

MATERIALS AND METHODS

Chemicals and Reagents: Water and Methanol.

Instrumentation

The Spectroscopic analysis was carried out using Double beam PG Instruments recording UV-Visible Spectrophotometer (SHIMADZU UV-1601**)** with 1mm path length matched quartz cells was used for analytical purpose.

Diluent preparation: in a 100ml volumetric flask take 30:70 water and Methanol.

Preparation of standard Stock Solution of Decitabine

Accurately weighed 100mg of Decitabine was weighed accurately and transferred into 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Decitabine Working standard solution of Decitabine containing 10 µg /ml for method. Finally add those above solutions and prepare the final solution is about 10µg/ml.

5.5-METHOD OPTIMIZATION

Optimization of selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, Acetone, etc. First optimize the different solvents. From that solvents Water and methanol combination satisfied the all the optimized conditions.

Wavelength Selection: The standard solutions are preparing by transferring the standard drug in a selected solvent or mobile phase and finally diluting with the same solvent or diluent. That prepared solution is scanned in the visible wavelength range of 200-400nm.This has been performed to know the maxima of Decitabine. While scanning the Decitabine solution we observed the maxima at 254 nm. The visible spectrum has been recorded on (SHIMADZU UV-1601) make UV – Vis spectrophotometer model UV-1601.The scanned visible spectrum is attached in the following page. The λ_{max} of the Decitabine was found to be 254 nm in diluents as solvent system.

Preparation of calibration curve for Decitabine: Standard solutions of Decitabine in the concentration range of 5 µg/ml to 25µg/ml. were obtained by transferring (5, 10, 15, 20 and 25 µg/ml. Decitabine stock solution (100 ppm) to the series of clean and dry 10 ml volumetric

flasks. The volumes in each volumetric flask were made up with the solvent system and mixed. The absorbencies of the solutions were measured at 254 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 5µg/ml at 254 nm for Decitabine. . The results are shown in Table no. 9

METHOD VALIDATION

1. Accuracy

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (75%, 100%, and 125%) of pure drug of Decitabine were taken and added to the pre- analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. Results in the table no 11.

2. Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Decitabine (API) the percent relative standard deviations were calculated for Decitabine is presented in the table no 12.

Intermediate Precision

Intra-assay & inter-assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Decitabine revealed that the proposed method is precise. The results in the table no 13.

3. Linearity & Range

The calibration curve showed good linearity in the range of 5-25µg/ml, for Decitabine (API) with correlation coefficient (r^2) of 0.9999. A typical calibration curve has the regression equation of y = 0.0787x + 0.0107 for Decitabine.

Standard solutions of Decitabine in the concentration range of 5 µg/ml to 25 µg/ml were obtained by transferring (5,10,15,20 and 25 ml) of Decitabine stock solution (100ppm) to the

series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 254 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 5 to 25 µg/ml at 254 nm for Decitabine. The result in the table no 9.

4. Method Robustness

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e., at 252 nm, and 256 nm. The respective absorbances of 10µg/ml were noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Decitabine (API). The results were shown in Table 14

5. LOD & LOQ

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.38039154 & 1.1572327 µg/ml respectively.

RESULTS AND DISCUSSION

The standard solutions of Decitabine with Water (10µg/ml) Acetonitrile (10µg/ml) methanol (10µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Decitabine was found to be at 254 nm. Therefore, 254 nm was selected as λ_{max} of Decitabine for the present study. The calibration curve of Decitabine was found to be linear in the range of 5-25 µg/ml at 254 nm. Therefore, it was clear that Decitabine can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 75%, 100% and 125% of Decitabine standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly

developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and rug.

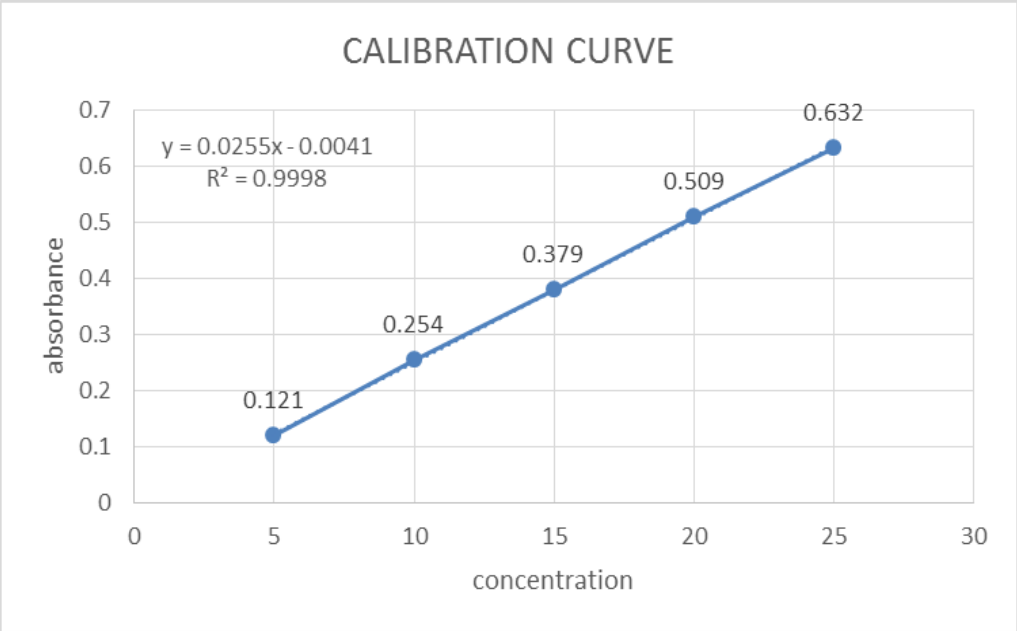


Fig. 2: Calibration curve of Decitabine API).

Table 1: Results of accuracy.

Level of Recovery	Sample Conc. (µg/ml)	Recorded conc. (µg/ml)	Absorbance	% Recovery	Mean % Recovery
75%	7.5	7.38	0.191	98.45	98.45
75%	7.5	7.38	0.189	97.42	
75%	7.5	7.38	0.193	99.48	
100%	10	9.96	0.258	99.61	98.96
100%	10	9.96	0.255	98.45	
100%	10	9.96	0.256	98.84	
125%	12.5	12.43	0.322	99.46	99.09
125%	12.5	12.43	0.320	99.07	
125%	12.5	12.43	0.319	98.76	

Acceptance criteria: correlation coefficient should not be less than 0.999

Repeatability

Table 2: Results of Repeatability.

Sr. No.	Conc. (µg /ml)	Wavelength (nm)	Absorbance
1	10	254	0.256
2	10	254	0.254
3	10	254	0.251
4	10	254	0.256
5	10	254	0.259
6	10	254	0.252
Mean □ S.D.			0.254666
Standard Deviation			0.002944
% RSD			1.15599%

Table 3: Results of intra-Day & inter-Day.

Conc. taken (µg/mL)	Observed Conc. Of Decitabine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	Statistical Analysis	Con. found (µg/mL)	Statistical Analysis
10	0.255	Mean = 0.256 SD = 0.002082 %RSD = 0.8110	0.258	Mean = 0.255 SD = 0.002517 %RSD =0.9856
10	0.259		0.253	
10	0.256		0.255	

Table 4: Results of Linearity.

Concentration(µg /ml)	Absorbance(n=6)
5	**0.121**
10	**0.254**
15	**0.379**
20	**0.509**
25	**0.632**

Acceptance criteria: correlation coefficient should not be less than 0.990

Table 5: Result of Method Robustness Test.

Concentration (µg/ml)	Wavelength	Absorbance	Statistical Analysis
10	252	0.257	Mean = 0.255 SD = 0.002898 % RSD = 1.136579
10		0.254	
10		0.251	
10	256	0.253	
10		0.259	
10		0.256	

CONCLUSION

- A sensitive & selective UV method has been developed & validated for the analysis of Decitabine API.

- Further the proposed UV method has excellent sensitivity, precision and reproducibility.
- The result shows the developed method is yet another suitable method for purity which can help in the analysis of Decitabine in different formulations.

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