Original Article

Novel Validated UV Spectroscopic Method for Routine Analysis of Decitabine Drug Substance

Ankit Awasthi, Dheeraj HM, Muddukrishna Badamane Sathyanarayana*

Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, INDIA.

ABSTRACT

Objectives: In the proposed study, a basic, novel, protected economic, delicate and cost-effective UV-Spectrophotometric technique for the analysis of Decitabine which is antineoplastic drug. **Methods:** The created method was approved according to ICH rules. The decitabine indicated maximum absorption at 221 nm. This strategy can successfully apply for estimation of Decitabine in active pharmaceutical ingredient (API) form for routine examination with UV identification at 221 nm. A Shimadzu UV-Visible spectrophotometer 1800 with 1 cm quartz cells and Acetonitrile (ACN) as diluent was utilized in this technique. **Results:** The Developed technique obeyed Beer's-Lambert's law in the conc. of 5.0-15.0 ug/ml, having R^2 of 0.9993 and 99.82% ± 0.75 recovery of decitabine was found. Different approval parameters like linearity, accuracy, precision and robustness were performed and were found to be within the limits as per the guidelines of ICH.

Key words: Decitabine, UV-spectroscopy, Validated method, ICH guidelines, Beer's Lambert's law, Analysis.

INTRODUCTION

Decitabine chemically is 4-amino-1-(2-deoxy-b-D-erythro-pentofuranosyl)-1, 3,5-triazin-2(1*H*)-one having molecular weight of 228.08. Decitabine was found to be pyrimidine simple of the nucleoside 2'- deoxycytidine (Figure 1).[1-3] Mechanism of decitabine shows it has antineoplastic impacts after phosphorylation and direct fuse into DNA and hindrance of DNA methyltransferase, causing hypo methylation of DNA and cellular differentiation or apoptosis.[4-6] It is designed for the treatment of myelodysplastic (MDS) syndrome.[7] It was found that decitabine having oral bioavailability (3.9-14%) which is very low and it has safety profile to the oraldose of 30-240 mg with that of 20 mg/m^2 IV administration compared in MDS patients[8] it was reported that It is unstable in acidic and alkaline condition and metabolized by the enzyme cytidine deaminase which is present in the liver. In impartial pH it is steady for 7 days at 4°C, 96 hr at 20°C and 21 hr at 37°C.[9,10] In basic arrangement, it experiences fast and reversible opening of the 5-azacytosine ring and pursued by irreversible disintegration though, in acidic condition, the glycosidic obligation of aza-nucleoside is separated, which is one of the real foundations for the low oral bioavailability of the decitabine.[10] Decitabine is somewhat dissolvable in ethanol/water (50/50), methanol/water (50/50) and methanol; sparingly solvent in water and dissolvable in dimethylsulfoxide (DMSO)[11] literature surveydemonstrates that there are numerous strategies like RP-HPLC[12,13] and LC–MS[14,15] for assurance of Decitabine. The Stability demonstrating technique for the estimation of Decitabine is additionally accessible[16] however from the broad review of the literatures, there is no official method for the determination of the decitabine by UV Spectroscopy has been

Submission Date: 08-04-2020;
Revision Date: 17-07-2020;
Accepted Date: 07-09-2020

DOI: 10.5530/ijper.54.3s.166

Correspondence:
Dr. Muddukrishna Badamane Sathyanarayana
Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka-576104, INDIA.
Phone: +91 9483932250
E-mail: krishna.mbs@manipal.edu

www.ijper.org

found. According to ICH rules. As UV spectroscopic determination is always first step in any drug analysis so it winds up important to build up an easy, simple, touchy, exact, reproducible method for the estimation of decitabine. Ourprimary aim is to development a method and validation of UV spectrophotometric method for Decitabine bulk as per ICH guideline.[17,18]

MATERIALS AND METHODS

Instrumentation

Shimadzu UV1800 UV-Visible double beam spectrophotometer and one cm matched quartz cells are used in determination. The glass wares are washed with methanol and water and dried in hot air oven prior to use. The absorption spectra of active pharmaceutical ingredients were carried out in a one cm quartz cell in the range of 200-400 nm.

Material

Active pharmaceutical ingredients

Decitabine was procured as gift sample by sShilpa Medicare Limited, Raichur.

Reagents and Chemicals

Acetonitrile HPLC Grade (Finer, Ahmedabad), Methanol (Finer, Ahmedabad), Ammonium Acetate (Rankem), Ortho phosphoric acid (Rankem) and Ultra clear water (Siemens, USA).

Methodology

Ammonium Acetate buffer

Weigh Accurately about 1.925gm. Ammonium Acetate and dissolved in 500 mL of water. Ortho phosphoric acid is used for maintaining the pH to 4.5. Filtration of buffer is carried out in 0.45µm filter.

Diluent

Mixture ofbuffer and acetonitrile in the ratio of (95:5), sonicate for 5 min used as a diluent. Afterthat filtration of diluent was carried out through 0.45µm filter.

λ_{max} Determination

Standard solution

Weigh accurately About 5.0mg of decitabine into a 10.0 mL volumetric flask, add 5.0 mL of diluent into volumetric flask and then sonicate after that volume was made up to mark with the diluent.

Dilutions

Acetonitrile was used as diluent for necessary dilution from standard stock solutions to get the concentration of 20 µg/mL of drug sample.

λ_{max} of the drug sample were scanned with help of UV-Visible spectrophotometer over the wavelength region of 200–400 nm against acetonitrile as blank. The absorption curve shows characteristic λ_{max} at 221 nm for decitabine (Figure 2).

Calibration Curve

Acetonitrile as a solvent for the preparation of calibration curve at λ_{max} 221 using Shimadzu 1800UV–Visible spectrophotometer.

Standard stock solution

Preparation of standard stock sample is done by weighing accurately 5.0mg of decitabine into 10 ml volumetric flask and dissolve in 3.0ml mixture of diluent. And volume is made up to the mark with same diluent. Ultra sonication was done to get a clear solution. Then 5.0, 7.5, 10.0, 12.5, 15.0, µg/ml were prepared with acetonitrile through serial dilution and absorbance were taken at λ_{max} 221nm against the Acetonitrile as blank. The data obtain from concentration v/s absorbance gives standard calibration curve data reported in (Table 1: Concentration and Absorbance of Standard Solution, Figure 3).

Method Validation

Linearity and Range

The linearity was obtained at 5.0 to 15.0 µg/ml concentrations. As per the data obtain plotting the absorbance versus the concentration the standard calibration curve was obtain by linear regression analysis (Table 2). The slope for Decitabine obtained was y = 0.146x + 0.1474, the calibration curve was found to be linear within this concentration (The correlation coefficient (R^2) was 0.9993).

Precision

Precision is evaluated by carrying out five independent sample of 10 ug/ml. If %RSD is less than 2% then that method is precise.

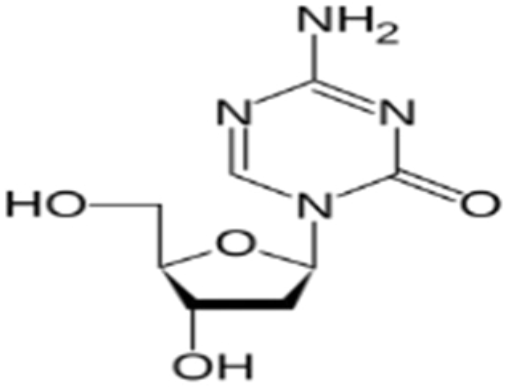


Figure 1: Chemical structure of decitabine.

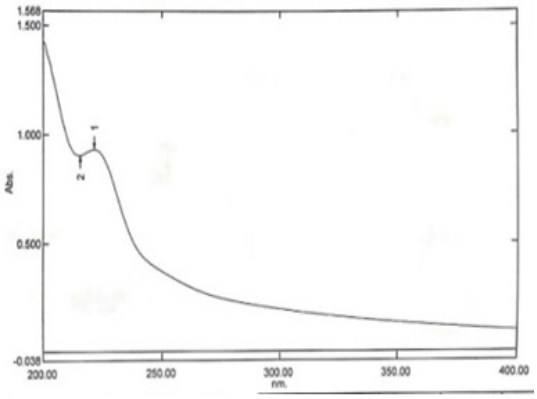


Figure 2: Determination of (λ_{max}).

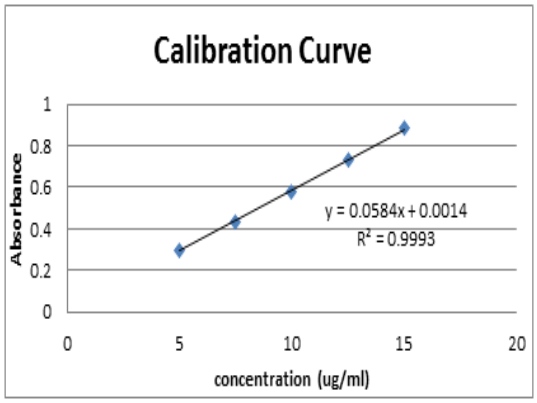


Figure 3: Standard calibration curve.

Table 1: Concentration and Absorbance of Standard Solution.	
conc.	**Abs.**
5.0ug/ml	0.3011
7.5ug/ml	0.432
10.0ug/ml	0.5819
12.5ug/ml	0.7307
15.0ug/ml	0.8819

Table 2: Repeatability of 10 ug/ml drug sample.	
No. of injections	**Abs.**
01	0.5887
02	0.5871
03	0.5868
04	0.5875
05	0.5865
Average	0.58732
Std. dev	0.00086
%RSD	0.14567

Table 3: Recovery studies.			
	Conc.	**Abs.**	**%Recovery**
75%	7.5 ug/ml	0.432	98.99
100%	10.0ug/ml	0.5819	100.00
125%	12.5 ug/ml	0.7307	100.46
		Average	99.82
		Std Dev.	0.75
		%RSD	0.75

Table 4: Robustness results.						
Deliberate Changes	**Intra-day**	**Inter-day**	**221nm**	**225nm**	**Analyst 1**	**Analyst 2**
Results %RSD	0.673	0.689	0.689	0.671	0.689	0.685

Accuracy

Accuracy of the method can be assessed by recovery studies it is performed in 3 times by standard addition method at 75%, 100% and 125% concentration levels. 99.82% ±0.75average recovery was found which shows that method is satisfactory in nature (Table 3).

Robustness

The analysis was done to check the robustness of method by small change in the method and conditions (Table 4).

RESULTS AND DISCUSSION

In the present work we have developed and validated an UV spectroscopic technique. The methods were approved according to ICH guidelines. The linearity was 5.0-15.0µg/ml for UV spectroscopic technique individually, demonstrating the R^2 of 0.9993. The UV spectroscopic method was approved for linearity, precision, reproducibility and robustness. Furthermore, 99.82% ±0.75 recovery of decitabine was found. All the outcomes were well within the specification according to ICH rules and thus the proposed method can be

effectively utilized for the determination of decitabine drug substances for daily and routine analysis.

ACKNOWLEDGEMENT

The author would like to thank Manipal College of Pharmaceutical Science, MAHE for providing the materials and instruments to conduct this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

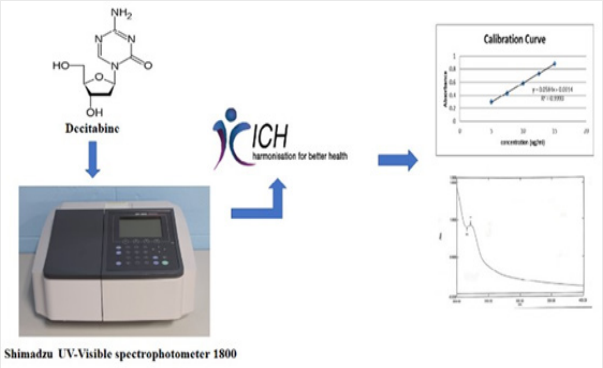
ABBREVIATIONS

ICH: International Conference for Harmonization; **UV:** Ultra Violate; **HPLC:** High Performance Liquid Chromatography; **RSD:** Relative Standard Deviation.

REFERENCES

1. Wijermans P, Lbbert M. Epigenetic therapy with Decitabine for myelodysplasia and leukemia. Future Oncology. 2005;1(5):585-91.
2. Ruter B, Wijermans PW, Lubbert M. Superiority of prolonged low-dose azanucleoside administration Results of 5-aza-2'-deoxycytidineretreatment in high-risk myelodysplasia patients. Cancer. 2006;106(8):1744-50.
3. National cancer institute, cancer topics, Dectiabine, www.cancer.gov/cancertopics/druginfo
4. Schaefer HE, Lubbert M. The hematopathological basis for studying effects of the demethylating agent 5-aza-2'-deoxycytidine (decitabine) in myelodysplasia. Annals of Hematology. 2005;84(1):67-79.
5. Chuang JC, Yoo CB, Kwan JM, Li TW, Liang G, Yang AS, *et al*. Comparison of biological effects of non-nucleoside DNA methylation inhibitors versus 5-aza-2'- deoxycytidine. Molecular Cancer Therapeutics. 2005;4(10):1515-20.
6. www.emedicinehealth.com/drugdecitabine_injection/article_em.html
7. DACOGEN® (decitabine) NDA 021790/S-010 FDA ODAC Briefing Document, Eisai Inc. 2012. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM 290512.pdf
8. Liu D. Recent advances in myelodysplasia: update from 2011 ASH annual meeting. J Hemat Oncolo. 2012;5(S1):A4.
9. Garcia JS, Jain N, Godley AL. An update on the safety and efficacy of decitabine in the treatment of myelodysplastic syndromes. Onco Targets Ther. 2010;3:1-13.
10. Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. Int J Cancer. 2008;123(1):8-13.
11. https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/021790lbl.pdf
12. http://repository-tnmgrmu.ac.in/6827/1/260507917_261330662.pdf
13. Glory H, Teja BB, Ashok KK, Ravindra YR. Stability indicating RP-HPLC Method Development and Validation of Decitabine Drug in Formulation. International Journal of Pharm Tech Research. 2011;3910:237-43.
14. Zhang Y, Sun J, Gao Y, Kong Y, Xu Y, Jia W, *et al*. An HPLC-MS/MS method for simultaneous determination of decitabine and its valyl prodrug valdecitabine in rat plasma. Chromatogr B Analyt Technol Biomed Life Sci. 2013;917-8.
15. Prakash C, Tushar NM. Fast Analysis of Decitabine using a Solid Core HILIC Column themoscientific. 2013;1-3.
16. Stability indicating RP-HPLC method for the estimation of Decitabine in bulk drug and lipid based Nanoparticleshttps://www.ijpsr.info/docs/IJPSR14-05-07-100.pdf
17. ICH Q2A. Guidelines on validation of analytical procedure; Definitions and terminology, Federal Register. 1995;60:11260.
18. ICH Q2B. Guidelines on validation of analytical procedure; Methodology, Federal Register. 1996;60:27464.

PICTORIAL ABSTRACT



SUMMARY

As there is no reported method for investigation accessible for the Analysis of Decitabine by UV spectroscopic technique is accounted for yet. Consequently, the depicted UV spectroscopic method for the determination of Decitabine is fast, explicit, simple, delicate, exact and reproducible for determination of decitabine for routine quality control samples.

About Authors

Muddukrishna Badamane Sathyanarayana: Assistant Professor, Senior scale, Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal.

Cite this article: Awasthi A, Dheeraj HM, Sathyanarayana MB. Novel Validated UV Spectroscopic Method for Routine Analysis of Decitabine Drug Substance. Indian J of Pharmaceutical Education and Research. 2020;54(3s):s667-s670.