Journal of Pharmaceutical Research International

32(32): 10-16, 2020; Article no.JPRI.63354
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

RP-HPLC-PDA Method Development, Validation and Stability Studies of the Novel Antineoplastic Drug Combination - Decitabine and Cedazuridine

B. Mohammed Ishaq[1], L. Siva Sanker Reddy[1*], S. Venu[1], M. Sreenivasulu[1] and K. Vanitha Prakash[2]

[1]Department of Pharmaceutical Analysis and Chemistry, Santhiram College of Pharmacy, Nandyal, Kurnool Dist., A.P., India.
[2]SSJ College of Pharmacy, Hyderabad, Telangana, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors BMI and LSSR performed the experimental work and wrote the first draft copy of the manuscript. Author SV has proof read the article and author MS performed statistical and mathematical analysis. All the authors has read and approved the final copy of the manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3230928
Editor(s):
(1) Dr. Rafik Karaman, Al-Quds University, Palestine.
Reviewers:
(1) Paras Nath Yadav, Tribhuvan University, Nepal.
(2) Thamer Mutlag Jasim, Al Hadi University College, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63354

Original Research Article

Received 15 September 2020
Accepted 21 November 2020
Published 01 December 2020

ABSTRACT

Aim: The aim of our present work was to develop and validate a reverse phase high-performance liquid chromatography (RP-HPLC) method for the simultanious determination of Decitabine (DEC) and Cedazuridine (CED).
Methodology: The developed method was further applied to observe the degradation of analytes under the influence of different forced degradation conditions. Analytes were resolved on C18, 250 x 4.6 mm, particle size 5 µm Xterra column, using a mobile phase combination of 0.1% Ortho Phospharic Acid buffer pH 6.5: Methanol (40:60v/v) with flow rate of 1mL/min and injection volume of 10 µL. Quantification was carried out with PDA detector at an isosbestic point of 220 nm with a linear calibration curve in the concentration range of 35-175 µg/mL for DEC and 100-500 µg/mL for CED.
Results: Validation of the developed method was performed as per ICH guidelines viz., linearity,

**Corresponding author: E-mail: shiva_s_rl@yahoo.co.in;*

accuracy, precision, and robustness. The limits of detection (LOD) and the limits of quantification (LOQ) for CED were found to be 2.69 µg/mL and 8.15 µg/mL respectively. LOD and LOQ for DEC 1.55 µg/mL and 4.68 µg/mL respectively. Moreover, validated method was applied to study the degradation profile of analytes under various stress degradation conditions.

Conclusion: The proposed method was found to be sensitive, specific and was successfully applied for the simultaneous estimation of Decitabine (DEC) and Cedazuridine (CED) in bulk drug, and tablets.

Keywords: Decitabine; degradation; Cedazuridine; Xterra; methanol.

1. INTRODUCTION

The DNA methyltransferase (DNMT) inhibitors decitabine and azacitidine are standard drugs used in therapies for myelodysplastic syndromes and chronic myelomonocytic leukemia [1,2]. These cytidine nucleoside analogues are incorporated into DNA during the S phase of the cell cycle and bind covalently to DNMT [1]. resulting in reduced methylation of Cytosine followed by Guanine residues, [CpG] in genomic DNA, altered epigenetic pattern and modified gene expression. This process, described as DNMT inhibition or DNA hypomethylation, is believed to be part of the clinical activity of DNMT inhibitors [3].

For the treatment of myelodysplastic syndromes and chronic myelomonocytic leukemia, decitabine and azacitidine are administered parenterally for 5-7 days per treatment cycle [4,5]. More courses of treatment are required to obtain a response; Discontinuation of therapy in responders leads to disease progression. An orally bioavailable DNMT inhibitor would reduce the burden of monthly intravenous or subcutaneous infusions, lasting several days, which could remain on treatment for several months or even years. However, both decitabine and azacitidine are not readily bioavailable when administered orally due to rapid inactivation by cytidine deaminase (CDA), which is highly expressed in the gut and liver [6].

Inhibition of CDA with tetrahydrouridine has been shown to improve the oral bioavailability of decitabine. Cedazuridine (E7727, Astex Pharmaceuticals, Pleasanton, CA, USA), a new CDA inhibitor, has been designed to overcome the instability of tetrahydrouridine. It has been shown to be orally bioavailable safely in preclinical studies [5].

Chemically Decitabine (molecular formula: $C_8H_{12}N_4O_4$; Mol Wt.: 228.21 g/mol) is a 4-amino-1- [(2R,4S,5R)-4-hydroxy-5-(hydroxyl methyl) oxolan-2-yl]- 1,2-dihydro-1,3,5-triazin-2-one. Decitabine is physically fine white crystalline powder [7]. Cedazuridine (molecular formula: $C_9H_{14}F_2N_2O_5$; Mol Wt.: 268.21 g/mol) is (4*R*)-1-[(2*R*,4*R*,5*R*)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-4-hydroxy-1,3-diazinan-2-one [8]. Fig. 1 (a & b) shows the chemical structures of DEC and CED.

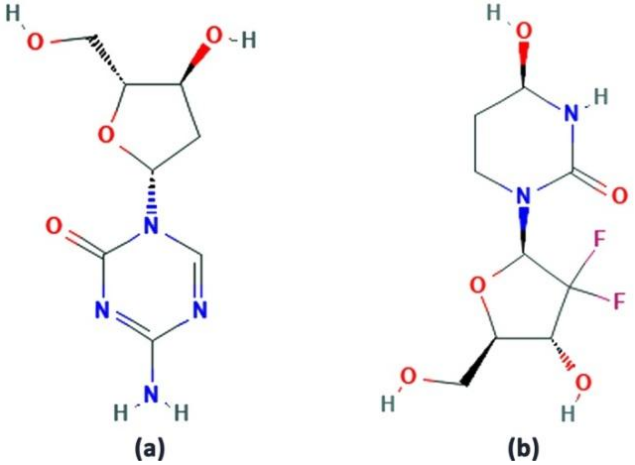

Fig. 1. Chemical structures of a) Decitabine; b) Cedazuridine

In spite of a number of publications describing the synthesis, efficacy, and pharmacokinetics of DEC and CED, information on quantitative analysis and characterization is lacking in the literature. Several HPLC methods of similar nucleoside analogs, including decitabine, [9,10] zebularine [11], fluorodeoxycytidine [12], 5-iodo-2-pyrimidinone-2'-deoxyribose [13], bromodeoxyuridine [14] and azacitidine [15], were reported in the literature. Most of these nucleosides have moderate to high polarity and were tested with C18 columns eluted with aqueous buffers containing methanol or acetonitrile as organic modifiers. Many of the methods reported have been developed primarily for bioassays with LC/MS detection and are not suitable for the determination of impurities and degradation products in the analysis of pharmaceutical substances and products. The objective of this study was to develop and validate a Stability Indicator HPLC method for the simultaneous determination of DEC and CED in bulk and tablets according to ICH Q2 (R1) guidelines and evaluate the nature of the stability indicator of the developed method.

2. METHODOLOGY

2.1 Chemicals and Solvents

The pure DEC and CED were procured as a gift sample from Zydus Pharma, India. Tablets of Inqovi® (35 mg decitabine and 100 mg Cedazuridine) were purchased at Mukesh pharmacy, Hyderabad, India. HPLC grade Acetonitrile, Methanol, Di Potassium Hydrogen Ortho phosphate, Ortho phosphoric acid and water were obtained from SD Fine Chem, Mumbai, India. All other chemicals used were of AR grade.

2.2 HPLC–PDA Instrumentation and Chromatographic Conditions

The HPLC system was a Waters LC (Waters, Milford, MA, USA) consisting of a quaternary gradient system (controller 600), an inline degasser (Waters, model AF), a photodiode array detector (Water, model 2998) and an autosampler (Waters, model 717 plus). The data was processed using the Empower Pro software (Waters, Milford, MA, USA). Chromatographic separation assay was performed with a Xterra C-18 analytical column (250 mm × 4.6 mm inner diameter, 5 µm particle size) maintained at ambient temperature. The mobile phase consists of 0.1% Ortho Phosphoric Acid buffer pH 6.5: Methanol (40:60v/v). The mobile phase was pumped at a flow rate of 1.0 mL min^{-1}. The detection wavelength was 220 nm. Mobile phase was used as diluent for the preparation of working standards of analytes.

2.3 Preparation of Standard Solutions

A Mixed standard stock solution of DEC (105 µg/ml) and CED (300 µg/ml) was prepared by accurately weighing 35 mg of DEC and 100 mg of CED and dissolved in 10 ml volumetric flasks containing 5 ml of methanol and flask was sonicated to dissolve the contents and made up to the mark with the same. 0.3 mL of these samples was transferred into 10 mL volumetric flask containing 5 ml of diluent (mobile pahse), sonicated for 5 minutes and the remaining volume was made upto the mark with diluent to get final concentration of 105 µg/ml and 300 µg/ml for DEC and CED respectively.

2.4 Analysis of Formulation

Twenty tablets were weighed accurately and ground into fine powder in a mortar. A quantity equivalent to 35 mg of DEC and 100 mg of CED was transferred in a volumetric flask 5ml of diluent was added and sonicated to ensure the solubility. Finally, the volume was made up to 10 ml to achieve the primarily stock solution of the tablets. An aliquot of 0.3 ml was withdrawn and transferred into 10 ml volumetric flask. Volume was made with the mobile phase to achieve 105 µg/ml and 300 µg/ml for DEC and CED respectively. The resulting solution was filtered through a 0.45 µm Millipore nylon filter paper if required.

2.5 Validation of Chromatographic Method

The developed method was validated as per the guidelines of ICH (ICH Guidelines, Q2 (R1), 2005) [16].

2.5.1 System suitability

System suitability parameters were measured to verify system performance. The precision of the system was determined in six repeated injections of standard preparations. All important characteristics were measured, including the peak area, the resolution of the peaks and the theoretical plate number.

2.5.2 Specificity

To assess the strategy specificity, working placebo solution (blank) in the absence of the

DEC and CED and standard solution having a concentration of 105 µg/ml and 300 µg/ml for DEC and CED respectively, as well as formulations were introduced into the HPLC system and analyzed chromatograms.

2.5.3 Accuracy (recovery)

Recovery studies were performed to determine Accuracy. In this process, it was tested at three different levels that were 50, 100 and 150% and analyzed chromatogram.

2.5.4 Precision

Precision (Intraday and Interday) of the analytical technique was proven by using optimized concentration of DEC and CED by six replicate injections. Average and % Relative Standard Deviation (RSD) of peak area and Assay were determined from chromatograms.

2.5.5 Linearity

Linearity was confirmed by preparing and analyzing the pure analytical standards at five different concentrations. The developed method shows ideal linearity over a range of 35 to 175 µg/ml and 100 to 500 µg/ml for DEC and CED respectively.

2.5.6 Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ of DEC and CED were determined by using the standard deviation (SD) of the response and the slope. LOD = 3.3*SD/Slope and LOQ = 10*SD/Slope.

2.5.7 Robustness

Robustness as a measure of the method's ability not to be affected by small but deliberate changes in chromatographic conditions was investigated by testing the influence of small changes in mobile phase flow rate (± 0.2 units), change in column temperature (± 2°C) and wavelength variation (± 2%).

3. RESULTS AND DISCUSSION

3.1 System Suitability Study

To ensure the validity of the analytical procedure, a system suitability test was established. The following parameters like theoretical plat number (N), Resolution, Retention time (Rts) and tailing factor were analyzed by using working standard solution containing DEC (105 µg/mL) and CED (300 µg/mL) injecting six times into HPLC system. The results are presented in Table 1.

3.2 Specificity

To assess the strategy specificity, blank solution, DEC and CED standard having a concentration of 105 and 300 µg/mL respectively as well as formulations were injected into the HPLC system. No peaks were found in blank; the Rts reported in standard and samples were similar. Fig. 2(a-c) shows the representative chromatograms of blank, Standard and sample.

3.3 Accuracy (Recovery)

Accuracy was determined at three different levels that were 50, 100 and 150%. The results are shown in Table 2. Mean % Recoveries at 50, 100 and 150% for DEC were found to be 99.85, 100.21 and 100.95% respectively. Similarly for CED 99.38, 99.78 and 100.88% at 50, 100 and 150% respectively.

3.4 Precision

Precision of the analytical method were established for both intra and interday by using concentration of 105 and 300 µg/mL of DEC and CED six replicate injections. The results are shown in Table 3.

3.5 Linearity

Linearity curves were constructed by plotting peak areas on X axis versus drug concentration along Y axis and the regression equations were computed. The curves were plotted over the concentration ranges of 35-175 µg/mL and 100-500 µg/mL for DEC and CED Respectively. Fig. 3a and 3b shows the linearity curves of DEC and CED.

3.6 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ values of DEC were found to be 1.55 and 4.68 µg/mL respectively. For CED LOD and LOQ were found to be 2.69 µg/mL and 8.15 µg/mL respectively.

3.7 Robustness

The study reveals that there was no much deviation in the robust chromatograms in assessment with optimized one. The results were shown in Table 4.

Table 1. System suitability parameters of DEC and CED

S. No	Analyte	RT(min)	Area	Height (µV)	USP resolution	USP	USP
1	Decetabine	3.097	170698	14786	--	1.12	2137.25
2	Cedazuridine	4.224	346853	26148	3.78	1.14	2657.72

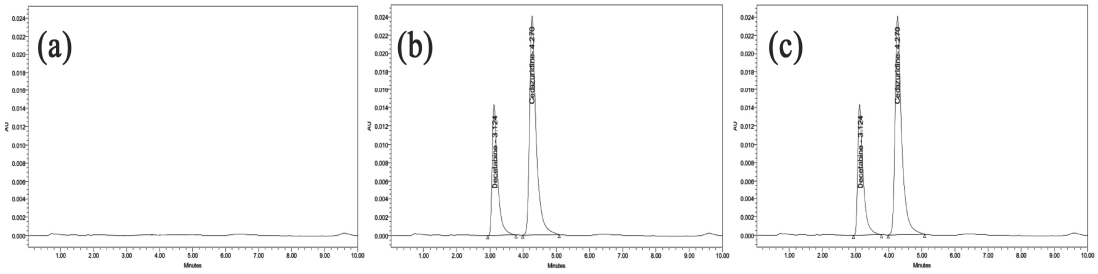

Fig. 2. Representative chromatogram of DEC and CED a) Blank, b) Standard and c) Sample

Table 2. Recovery study

Analyte	Accuracy level*	Peak area*	Amount added (mg)*	Amount found (mg)*	% Recovery*	Mean % Recovery*
DEC	50%	85620	17.5	16.85	99.85	100.34
	100%	171845	35	35.01	100.21	
	150%	259676.0	52.5	52.45	100.95	
CED	50%	172505.0	50	49.97	99.38	100.01
	100%	346412	100	99.98	99.78	
	150%	525309.0	150	150.23	100.88	

**Mean of three determinations at each level*

Table 3. Precision study

Precision	Mean Peak area*		% RSD*		Mean Assay*		% RSD*	
	DEC	CED	DEC	CED	DEC	CED	DEC	CED
Intraday	178302.2	342329.0	0.6	0.3	99.86	99.33	0.25	0.18
Interday	178688.7	348783.8	0.3	0.4	99.92	99.85	1.30	1.22

**Mean of six determinations*

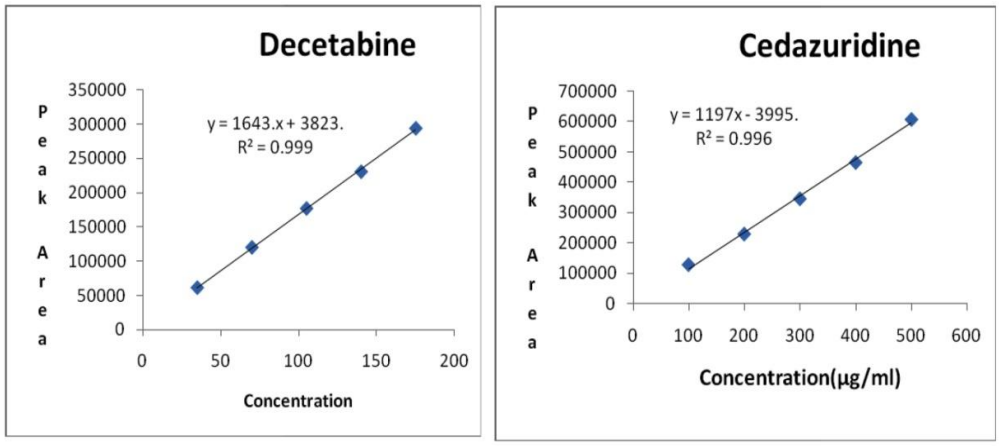

Fig. 3. a) Linearity of DEC and b) Linearity of CED

Table 4. Results of robustness

Parameter	Condition	Decitabine			Cedazuridine		
		RT	Peak Area	% Assay	RT	Peak Area	% Assay
Flow	0.9	3.867	177367.3	98.96	4.567	348578.3	99.67
	1.0	3.097	178302.2	99.86	4.224	348783.8	99.85
	1.1	2.172	178563.9	99.88	3.879	348629.4	99.74
Column	25	2.782	178106.3	99.58	4.182	351673.0	99.98
Temperature	30	3.097	178302.2	99.86	4.224	348783.8	99.85
	35	3.267	178082.8	99.17	4.262	348294.2	98.89
Wave length	218	3.026	178312.7	99.87	4.221	3486325.9	99.79
	220	3.097	178302.2	99.86	4.224	348783.8	99.85
	222	3.106	178320.5	99.96	4.227	348790.3	99.90

3.8 Method Applications

The validated method was applied for the determination of DEC and CED in commercially available tablets. Fig. 2(b-c) shows two typical HPLC chromatograms obtained later the test of the standard DEC and CED reference solution and of the tablets sampling solution, respectively. The results of the trial (n = 6) produced more than 99% (RSD = 0.14%) of the label declaration for DEC and CED in Inqovi® tablets, respectively (Table 3). The average retention time of DEC and CED was found to be 3.097 and 4.242 minutes respectively. The test results indicate that the method is specific for the analysis of DEC and CED without interference from the excipients used to formulate and produce these tablets.

4. CONCLUSION

The developed Rp-HPLC method provides reliable, reproducible, accurate and specific for the quantification of DEC and CED in bulk and their tablets. The newly developed method has been validated as per the regulatory requirements and has shown the acceptable accuracy and precision with adequate sensitivity. This method can be used for the routine analysis of the above titled drugs in Quality control laboratories.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

Authors are thankful to Torrent Pharma, Ahmedabad, India for providing gift sample of APIs and for the management of RGM & Santhiram Group of Institutions for supporting this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Derissen EJ, Beijnen JH, Schellens JH. Concise drug review: Azacitidine and decitabine. The Oncologist. 2013;18(5): 619.
2. Savona MR, Odenike O, Amrein PC, Steensma DP, DeZern AE, Michaelis LC, et al. An oral fixed-dose combination of decitabine and cedazuridine in myelodysplastic syndromes: A multicentre, open-label, dose-escalation, phase 1 study. The Lancet Haematology. 2019; 6(4):194-203.
3. Ngollo M, Dagdemir A, Karsli-Ceppioglu S, Judes G, Pajon A, Penault-Llorca F, et al. Epigenetic modifications in prostate cancer. Epigenomics. 2014;6(4):415-26.

4. Garcia-Manero G, Gore SD, Kambhampati S, Scott B, Tefferi A, Cogle CR, et al. Efficacy and safety of extended dosing schedules of CC-486 (oral azacitidine) in patients with lower-risk myelodysplastic syndromes. Leukemia. 2016;30(4):889-96.
5. Dhillon S. Decitabine/Cedazuridine: First Approval. Drugs. 2020;1-6.
6. Ebrahem Q, Mahfouz R, Ng KP, Saunthararajah Y. High cytidine deaminase expression in the liver provides sanctuary for cancer cells from decitabine treatment effects. Oncotarget. 2012;3(10): 1137.
7. Rogstad DK, Herring JL, Theruvathu JA, Burdzy A, Perry CC, Neidigh JW, et al. Chemical decomposition of 5-aza-2′-deoxycytidine (decitabine): Kinetic analyses and identification of products by NMR, HPLC, and mass spectrometry. Chemical Research in Toxicology. 2009; 22(6):1194-204.
8. Keer H, Azab M, Oganesian A. Inventors; Otsuka Pharmaceutical Co Ltd, assignee. Low dose combination cda substrate drug/cedazuridine with extended administration. United States patent application US 16/574,924; 2020.
9. Hepsiba G, Teja D, Kumar K. Reddy T. Stability indicating RPHPLC method development and validation of decitabine drug in formulation. Int. J. Pharm. Tech. Res. 2011;3:237-43.
10. Das H. Analytical method development and validation of decitabine in API and bulk dosage forms by using rapid and sensitive RP-HPLC. Int. J. Med. Nanotech. 2014;2: 54–63.
11. Beumer JH, Joseph E, Egorin MJ, Covey JM, Eiseman JL. Quantitative determination of zebularine (NSC 309132), a DNA methyltransferase inhibitor, and three metabolites in murine plasma by high-performance liquid chromatography coupled with on-line radioactivity detection. Journal of Chromatography B. 2006; 831(1-2):147-55.
12. Beumer JH, Eiseman JL, Parise RA, Joseph E, Holleran JL, Covey JM, et al. Pharmacokinetics, metabolism, and oral bioavailability of the DNA methyltransferase inhibitor 5-fluoro-2′-deoxycytidine in mice. Clinical cancer research. 2006;12(24):7483-91.
13. Kummar S, Anderson L, Hill K, Majerova E, Allen D, Horneffer Y, et al. First-in-human phase 0 trial of oral 5-iodo-2-pyrimidinone-2′-deoxyribose in patients with advanced malignancies. Clinical Cancer Research. 2013;19(7): 1852-7.
14. Cheung AP, He J, Ha Y. New hydrolytic products detected in aqueous solutions of bromodeoxyuridine. Journal of Chromatography A. 1998;797 (1-2):283-93.
15. Dhawan RK, Ravi R, Subburaju T, Revathi H, Arul C, Rao NG. Development and validation of stability-indicating RP-HPLC assay method for entacapone in entacapone tablets. International Journal of Pharmaceutical Sciences and Research. 2013;4(3):1227.
16. Guideline ICH. Validation of analytical procedures: Text and methodology. Q2 (R1). 2005;1:1-5.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63354