RP-HPLC Method Development and Validation for the Simultaneous Estimation of Cedazuridine and Decitabine in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of analysis. The main aim of the present work is to develop a simple, precise, Valid, speedy and decisive chromatographic strategy for the estimation of Cedazuridine and Decitabine quantitatively in fixed dosage form. Effective Chromatographic separation was achieved by using Hypersil C_{18} Column (250 mm X 4.6 mm internal diameter, 5 µm particle size) using mobile phase composed of Methanol and Buffer (pH 4.0) in the proportion of 60:40(v/v) under controlled temperature. The Mobile phase was siphoned using a gradient HPLC system at a flow rate of 1.0 ml/min and quantification was based on peak area measurements at 254 nm. RT (Retention Time) for Decitabine and Cedazuridine was found to be 2.569 min and 3.842 min. The dimensionality of both the drugs found to be linear with a statistic value of 0.999. The acceptance criteria of precision was Relative variance should be less than 2.0 which indicates that the method can be performed repeatedly. Reliability of the proposed method was assessed by evaluation of validation parameters like linearity, precision, specificity, accuracy, LOD, LOQ values as per ICH guidelines. The results obtained on the validation parameters met ICH and

USP requirements. The proposed method of chromatography has been applied to dosage form without additives interference and is specific for the estimation of Decitabine and Cedazuridine

Key Words: Decitabine, Cedazuridine, Specificity, Accuracy, Precision

INTRODUCTION

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify and quantify the compounds that are present in any sample[1]. Decitabine a nucleoside metabolic inhibitor[2] chemically 4-amino-1-[(2R,4S,5R)-4-hydroxy-5(hydroxymethyl)oxolan-2-yl]-1,2-dihydro-1,3,5-triazin-2-one used for the treatment of Myelodysplastic syndromes (MDS) having molecular formula $C_8H_{12}N_4O_4$ and molecular weight 228.208 g/mol[3]. It is being used in the treatment of cancer, myelodysplastic syndrome showing activity towards hematopoietic tumors[4]. It mainly works by integrating cellular DNA and inhibiting the action of DNA methyl transferases, leading to global hypomethylation and related downstream therapeutic benefits[5]. The chemical structure of Decitabine is exhibited in Figure 1. Cedazuridine a cytidine deaminase inhibitor chemically (4R)-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5 (hydroxymethyl)oxolan-2-yl]-4-hydroxy-1,3-diazinan-2-one having molecular formula $C_9H_{14}F_2N_2O_5$ and molecular Weight 268.21 g/mol[6]. It is being used in the treatment of cancer, myelodysplastic syndrome, showing activity towards hematopoietic tumors. It is a fluorinated tetrahydrouridine derivative specifically designed to inhibit CDA and facilitate oral administration of hypomethylating agents[7]. The chemical structure of Cedazuridine is exhibited in Figure 2.

The present strategy mainly focuses on developing and validating a novel reversed-phase chromatographic method for the estimation of Decitabine and Cedazuridine in bulk and pharmaceutical dosage forms. After performing extensive literature Survey[8-12] on chromatographic analysis of several dosage forms an attempt was made to develop a new rapid, valid, speedy and accurate method for the estimation of Decitabine and Cedazuridine quantitatively.

MATERIALS AND METHODS

Chemicals and Reagents

Decitabine and Cedazuridine were kindly gifted by Nutech Biosciences Pvt Ltd, Hyderabad having certified purity limits and were used without any chemical treatment. For separation of drugs solvents of HPLC Grade was used in analysis. Decitabine and Cedazuridine combined marketed formulation purchased from local pharmacy was utilized for analysis.

Instrument

Liquid Chromatography system used consists of Waters HPLC having Empower Software with 2695 separation module having PDA detector with universal loop injector of injection capacity 20 µL. The column used was Agilent C_{18} Column, 5µ (250× 4.6 mm) at surrounding temperature. Several mobile phases were tested in order to search out the suitable conditions for separating the drugs.

Optimized Chromatographic conditions

The mobile phase having Methanol and Phosphate buffer having pH 4.0 in proportion of 60:40 by volume was preferred because it ideally resolves the height with Retention Time (RT) of 2.569 min and 3.842 min for Decitabine and Cedazuridine respectively. Standard drug were scanned over a large range of wavelength ranging from 200 nm to 390 nm and wavelength was selected at 254 nm because of showing reasonably good response with characteristic UV Spectrum exhibited in Figure 3

Preparation of Buffer

Accurately weighed and transferred 3.464 g of Potassium Dihydrogen Phosphate into 1000 ml clean dry volumetric flask. To this add 500 ml of HPLC water and sonicated for five minutes to dissolve it completely and make up the volume to mark with HPLC water and pH was adjusted to 4.0 by addition of few drops of Orthophosphoric acid.

Preparation of Mobile Phase

Accurately measured 600 milliliter of Methanol (60%) and 400 milliliter of phosphate buffer (40%) were mixed and kept for sonication in inaudible water tub for 10 minutes and after

sonication filter the above solution using 0.45 µ membrane filter under vacuum prior its use and used as diluent

Standard Stock Preparation

Accurately weigh and transfer 10 mg of Decitabine and Cedazuridine 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. The Chromatogram was exhibited in Figure 4.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Decitabine and Cedazuridine (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and Sonicate to dissolve it completely and make volume up to the mark with the same solvent. The Chromatogram was exhibited in Figure 5.

RESULTS AND DISCUSSION

Preparation of Calibration Curves by HPLC

Serial dilutions of Decitabine and Cedazuridine ranging from 10- 100 µg/ml were made, and their chromatograms were recorded. Height space of Drug was calculated, and also the individual activity curve was planned against quantitative of the area underneath the curve, and their respective concentrations and results are reported.

HPLC Method Validation

The Developed method was validated for Linearity, Accuracy, Specificity, Precision, LOD, LOQ parameters as described in ICH Guidelines.

Linearity and Range

One-dimensionality in a strategy is its ability to induce to take a glance at results and was constructed using the mean areas at their respective concentrations over a given range. Linearity for Decitabine was within the range of 100 µg/ml to 500 µg/ml and Cedazuridine was in the range of 10 to 50 µg/ml respectively. The coefficient of correlation value for calibration plot of Decitabine and Cedazuridine was 0.999 which shows good linearity for the drugs. The Linearity curves were exhibited in figure 6 and 7. Linearity results are tabulated in Table 1 and Table 2

Accuracy

The Certainty of an approach is that the intimacy of the measured worth to actuality worth for the sample. The mean recovery was found to be 99.84% for Decitabine and 100.51% for Cedazuridine respectively. The %RSD of the sample was found to be below 2 and results were tabulated in Table 3 and Table 4

Precision

Precision of the strategy was evaluated by performing repeatability in the same day and inter-day studies. The Percentage Relative Standard Deviation of each study was calculated and was found to be less than 2 showing the strategy was precise and the results were shown in Table 5.

LOD & LOQ

Limit of Detection, Limit of Quantitation values of the method were 0.24 and 0.71 for Decitabine, 0.03 and 0.09 for Cedazuridine respectively. The results obtained are within the limits.

Robustness and Ruggedness

Robustness and Ruggedness studies were carried out by injecting five replicate injections of Decitabine and Cedazuridine on different days and variations was calculated in terms of percentage relative variance which was found to be less than 2% and results were reported in Table 6 and 7. Effect of change in mobile composition results were reported in Table 8 and 9

CONCLUSION

A systematic and practical approach was utilized to develop an efficient and robust RP-HPLC method for the separation of drugs. Different trials were carried out to determine the optimized chromatographic conditions and initial attempt was performed by utilizing low proportion of organic solvents for the elution of compounds by reducing retention time of the compounds. The acceptable results are achieved by the proposed chromatographic conditions. The proposed method is easy, speedy and measurably substantial. During the analysis of drug no interfering peak was found within the chromatogram indicating that there is no excipient interference.

Conflict of Interest

There is no Conflict of Interest among the authors regarding the publication of this paper

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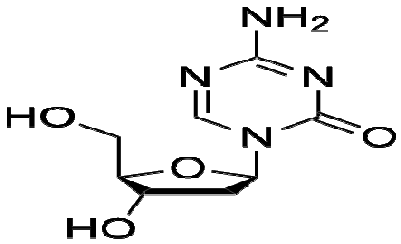

Fig 1: Chemical Structure of Decitabine

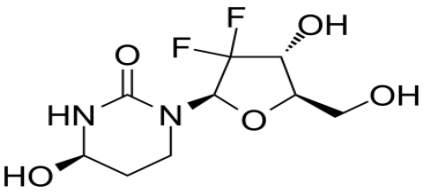

Fig 2: Chemical Structure of Cedazuridine

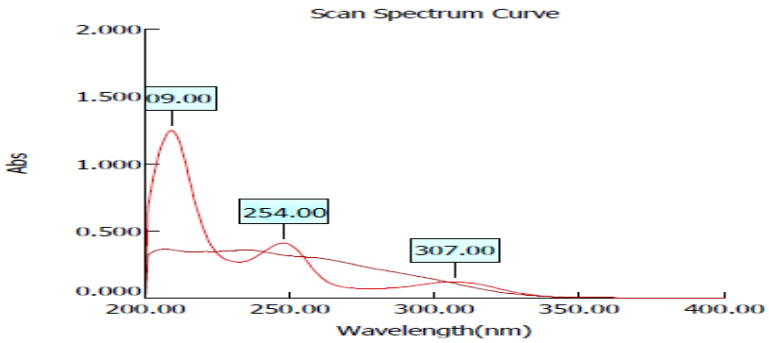

Fig 3: Overlain UV spectrum of Decitabine and Cedazuridine

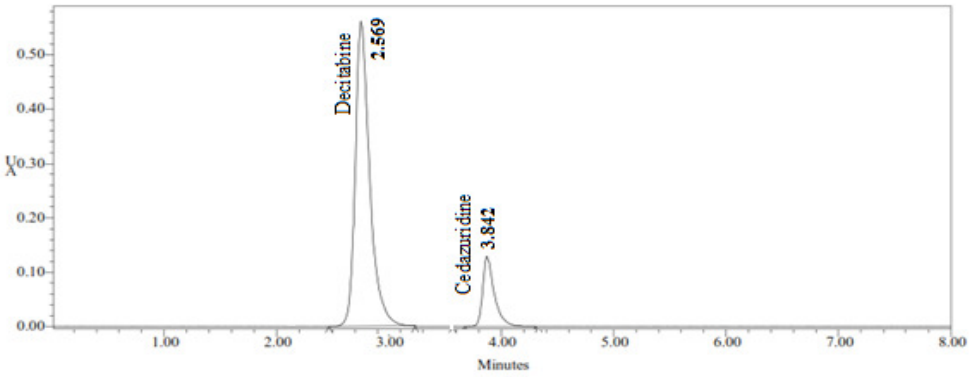

Fig 4: Standard Solution Chromatogram

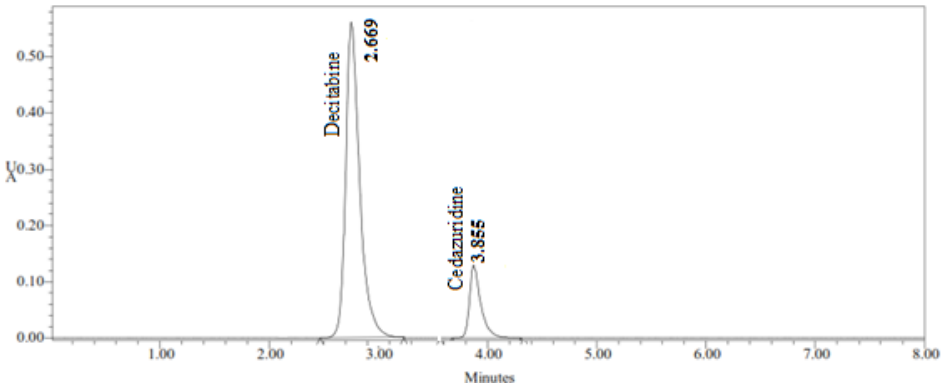

Fig 5: Sample solution Chromatogram

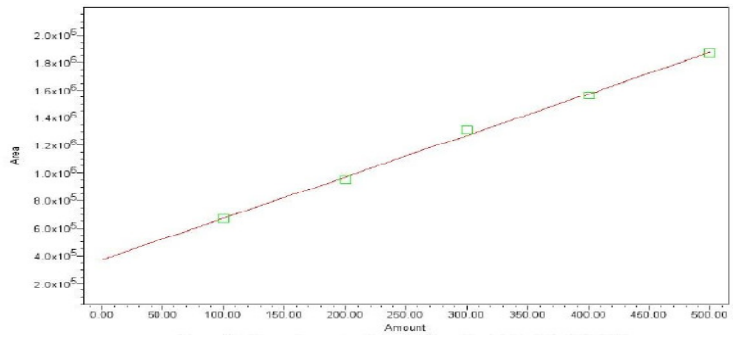

Fig 6: Linearity Curve of Decitabine

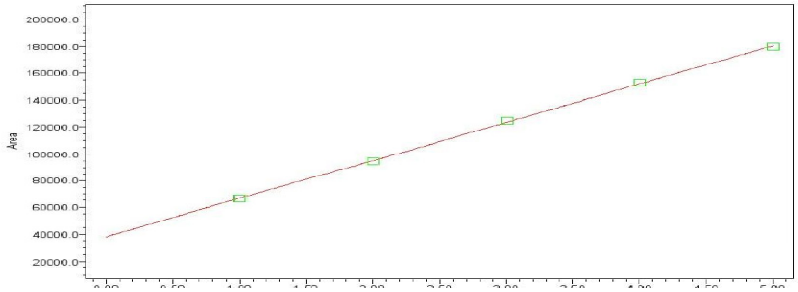

Fig 7: Linearity Curve of Cedazuridine

Table 1: Linearity Data of Decitabine

S.No.	Linearity Level	Concentration(µg/ml)	Area
1	I	100	668934
2	II	200	956781
3	III	300	1313873
4	IV	400	1563458
5	V	500	1867084
Correlation Coefficient			0.999

Table 2: Linearity Data of Cedazuridine

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	10	66510
2	II	20	94701
3	III	30	124802
4	IV	40	152731
5	V	50	179732
Correlation Coefficient			0.999

Table 3: Accuracy Report of Decitabine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.78%	

Table 4: Accuracy Report of Cedazuridine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table 5: Precision Report of Decitabine

S. No	Area of Decitabine	Area of Cedazuridine
1.	1302729	123149
2.	1302947	123766
3.	1303236	124271
4.	1303977	124691
5.	1309759	124956

6.	1309789	125845
Mean	1304529.8	124162.7
S.D	2961.1	725.6
%RSD	0.2	0.6

Table 6: Results of Robustness of Decitabine and Cedazuridine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 7: Results of Robustness of Cedazuridine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 8: Effect of change in mobile phase Composition for Decitabine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing

1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 9: Effect of change in mobile phase Composition for Cedazuridine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2