ISSN 2581 – 4346

Available Online at www.ijms.co.in
Innovative Journal of Medical Sciences 2021; 5(3):19-23

RESEARCH ARTICLE

RP-HPLC method development and validation for simultaneous determination of decitabine and cedazuridine in pure and tablet dosage form

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Received on: 10 July 2021; Revised on: 15 Aug 2021; Accepted on: 01 Sep 2021

ABSTRACT

Introduction: An attempt has been made to develop a validated stability indicating RP-HPLC method for the estimation of decitabine and cedazuridine. Literature survey revealed that many analytical methods have been reported individually or in combination with other drugs. Retention times were decreased and that run time was decreased, so the method developed was simple and economical. **Materials and Methods:** It includes the general information on RP-HPLC and method development, general information on forced degradation studies and stress conditions like acid, base, peroxide, thermal, photolytic and neutral. Discussion: The article discusses about drug profiles and official status of selected drugs i.e., decitabine and cedazuridine. **Results:** In this article the previous literature available for drugs is used for developed research work which Include stability indicating RP-HPLC method development and validation for simultaneous estimation of decitabine and cedazuridine in bulk and their pharmaceutical dosage form. Using Waters HPLC 2695 system, quaternary gradient pump equipped with auto sampler injector with 20 µL is injected eluted with the mobile phase containing 65% 0.01 N KH2 PO4: 35% acetonitrile which is pumped at a flow rate of 1 mL/min and detected by PDA detector at 245 nm. The peak of decitabine and cedazuridine was eluted at retention times of 2.263 min and 3.001 min, respectively. **Conclusion:** In this paper, HPLC method for the selected drugs showed good linearity.

Keywords: Decitabine, Cedazuridine, Tablet

INTRODUCTION

A drug may be defined as a substance meant for diagnosis, cure mitigation, prevention or treatment of diseases in human beings or animals or for altering any structure or any function of the body. Drugs play a key role in the progress of human civilization by curing diseases. Today majority of the drug used are of synthetic origin. These are produced in the bulk and used for their therapeutic effects in pharmaceutical formulations. There are biologically active chemical substances generally formulated into convenient dosage forms such as tablets, capsules, ointments, and injectable, these formulations deliver the drug substance in stable, non-toxic, and acceptable form, ensuring its bioavailability and therapeutic activity.[1-10]

Based on modes of chromatography

Normal-phase high-performance liquid chromatography (NP-HPLC)

Normal-phase liquid-liquid chromatography uses a polar stationary phase and less polar mobile phase. To select an optimum mobile phase, it is best to start with a pure hydrocarbon mobile phase such as heptane's.

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If the sample is strongly retained, the polarity of the mobile phase should be increased, perhaps by adding small amounts of methanol or dioxin.

In the normal phase mode, separations of oil-soluble vitamins, essential oils, nitro phenols, or more polar homologous series have been performed using alcohol/heptane as the mobile phase. Column used in normal-phase chromatography for chiral separation: Chiracel OJ and Chiracel OD [Table 1].[11-20]

Table 1: Comparison of normal-phase and reverse-phase HPLC

Properties	Normal phase	Reversed phase
Polarity of stationary phase	High	Low
Polarity of mobile phase	Low to medium	Low to high
Sample elution order	Least polar First	Most polar first
Retention will increase by	Increasing surface of stationary phase	Increasing surface of stationary phase.

Reverse-phase high-performance liquid chromatography (RP-HPLC)

Reverse-phase chromatography uses hydrophobic bonded packing, usually with an octadecyl or octyl functional group and a polar mobile phase, often a partially or fully aqueous mobile phase.

Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. In general, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of compounds in table is reversed (thus the name reverse-phase chromatography).[21-23]

Drug profiles

Decitabine [Figure 1 and Table 2]

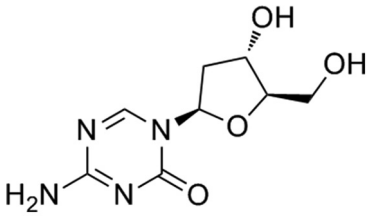

Figure 1: Chemical structure of decitabine

Table 2: Drug profile of decitabine

IUPAC name[21]	4-Amino-1-[(2R,4S,5R) -4-hydroxy-5-(hydroxymethyl) oxolan-2-yl]-1,2-dihydro-1,3,5-triazin-2-one
Molecular formula	$C_8H_{12}N_4O_4$
Molecular weight	228.2053 g/mol
Description[22]	Decitabine is indicated for the treatment of patients with myelodysplastic syndromes (MDS) including refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia.
Solubility	Soluble in dimethyl sulfoxide (DMSO) and sparingly soluble in water.
Therapeutic category[23]	Nucleoside metabolic inhibitor
Storage	Store at−20°C
Melting point	193–196°C

Cedazuridine [Figure 2 and Table 3]

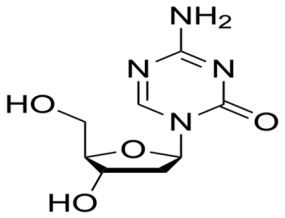

Figure 2: Chemical structure of cedazuridine

Table 3: Drug profile of cedazuridine

IUPAC name	(4R)-1-[(2R,4R,5R)- 3,3-difluoro-4-hydroxy-5-(hydroxymethyl) oxolan-2-yl]-4-hydroxy-1,3-diazinan-2-one
Molecular formula	$C_9H_{14}F_2N_2O_5$
Molecular weight	268.217 kg/molg·mol−1
Description	Cedazuridine is a cytidine deaminase inhibitor co- administered with the hypomethylating agent decitabine for the treatment of variable forms of myelodysplastic syndrome (MDS).
Solubility	Soluble in water.
Therapeutic category	DNA methyltransferase (DNMT) inhibitor which is coadministered with hypomethylating agents like decitabine.
Storage	Store at−20°C
Melting point	162-165°C

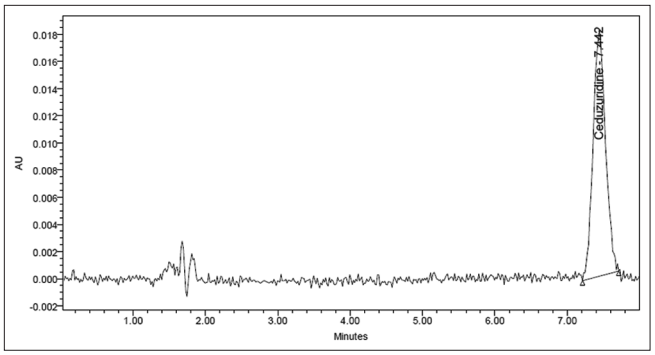

Figure 3: Chromatogram of Trial-1

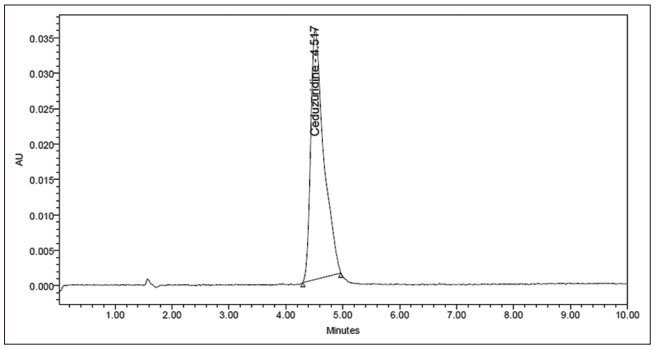

Figure 4: Chromatogram of Trial-2

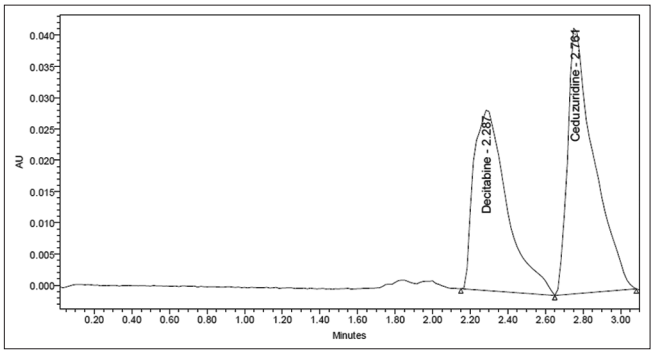

Figure 5: Chromatogram of Trial-3

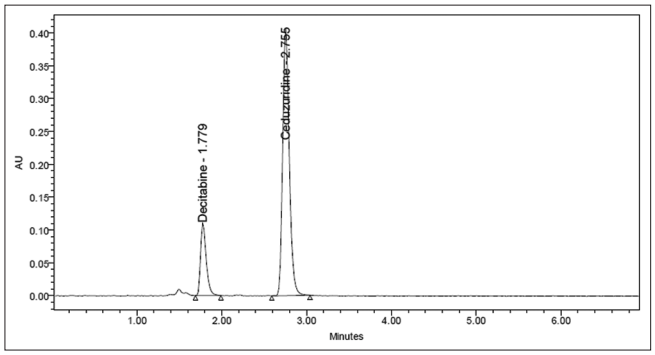

Figure 6: Chromatogram of Trial-4

EXPERIMENTAL WORK

Equipment [Table 4]

Table 4: List of equipment used in HPLC

S. No.	Name	Model	Manufacturer
1.	HPLC	ALLIANCE	Waters HPLC 2695 system
2.	HPLC software	-	Empower software 2.0 version
3.	pH meter	-	Eutech
4.	Weighing balance	-	Sartorius
5.	UV/VIS spectrophotometer	-	PG instrument T60
6.	UV/VIS Spectrophotometer software	-	UVWin 6 software
7.	Pipettes, beakers, and burettes	-	Borosil
8.	Ultrasonicator	UCA701	Unichrome

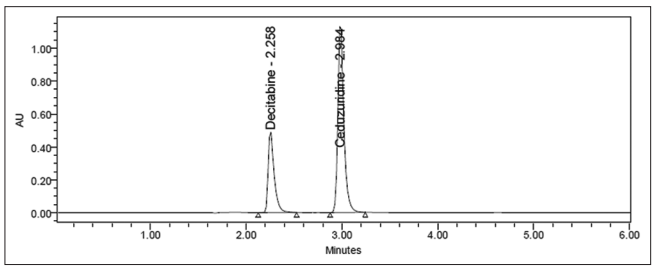

Figure 7: Chromatogram of accuracy 50%

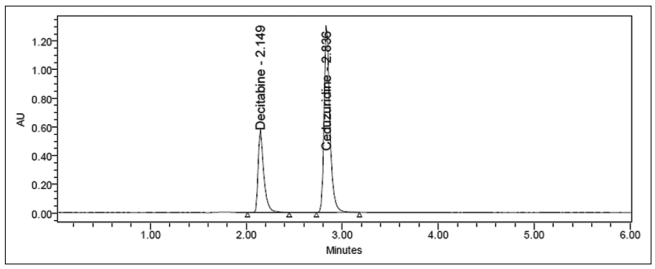

Figure 8: Chromatogram of accuracy 100%

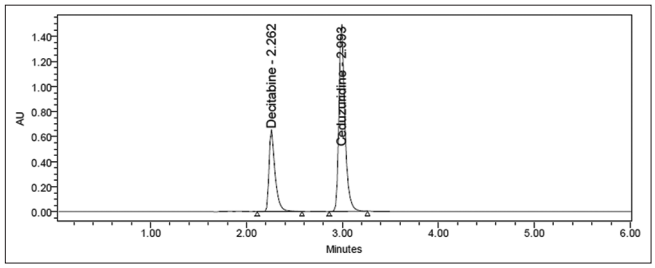

Figure 9: Chromatogram of accuracy 150%

Reagents and chemicals [Table 5]

Table 5: List of chemicals used in HPLC method

S. No.	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Water (Milli-Q)	HPLC	In-house production
3.	Orthophosphoric acid	HPLC	Analytical
4.	Methanol	HPLC	Rankem
5.	Phosphate buffer	HPLC	Rankem
6.	Potassium dihydrogen ortho phosphate buffer	HPLC	Rankem

RESULTS

RP-HPLC method development [Figures 3-6]

Trails in optimization of chromatographic condition [Tables 6-9]:

Trial-1

Table 6: Trial-1 chromatographic conditions

Column	Ascentis 150 C18 (250 mm×4.6 mm, 5 µm)
Mobile phase ratio	Methanol: water (50:50%v/v)
Detection wavelength	265 nm
Flow rate	1 mL/min
Injection volume	10 µL
Run time	10 min
Observation	In this trail only cedazuridine drug peak was eluted, decitabine was not eluted. Hence, further trail is carried out.

Trial-2

Table 7: Trial-2 chromatographic conditions

Column	Ascentis (150 mm×4.6 mm, 5 µm)
Mobile phase ratio	0.1% OPA: methanol (50:50%v/v)
Detection wavelength	265 nm
Flow rate	1 mL/min
Injection volume	10 µL
Runtime	10 min
Observation	In this trail also, only cedazuridine drug peak was eluted, decitabine was not eluted. Hence, further trail is carried out.

Trial-3

Table 8: Trial-3 chromatographic conditions

Column	Altima (150 mm×4.6 mm, 5 µm)
Mobile phase ratio	OPA buffer: acetonitrile (50:50%v/v)
Detection wavelength	265 nm
Flow rate	1 mL/min
Injection volume	10 µL
Runtime	5 min
Observation	In this trail, both drugs were eluted but broad peak shapes were observed for both drugs and less resolution observed. Hence, further trail is carried out.

Trial-4

Table 9: Trial-4 chromatographic conditions

Column	Symmetry (150 mm × 4.6 mm, 5 µm)
Mobile phase ratio	OPA buffer: acetonitrile (50:50 v/v)
Detection wavelength	265 nm
Flow rate	1 mL/min
Injection volume	10 µL
Runtime	10 min
Observation	In this trail, both drugs were eluted but the retention time of decitabine is in voided range (<2 min). Hence, further trail is carried out.

DISCUSSION

Three levels of accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %. Recovery was obtained as 100.01% and 100.29% for decitabine and cedazuridine, respectively [Figures 7-9].

CONCLUSION

The developed RP-HPLC method for the estimation of selected drugs is simple, rapid, accurate, precise, robust, and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive, and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested non-interference of formulation recipients in the

estimation and can be used in laboratories for the routine analysis of selected drugs.

The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence, these can be used for routine analysis of decitabine and cedazuridine.

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