International Journal of
Medicinal Chemistry & Analysis

www.ijmca.com

e ISSN 2249 - 7587
Print ISSN 2249 - 7595

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DECITABINE AND CEDAZURIDINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

Yamini S*[1], Rajitha S[2]

[1]Student, Department of Pharmaceutical Analysis, KLR Pharmacy College, Palwancha, Telangana 507115, India.
[2]Assistant Professor, Department of Pharmaceutical Analysis, KLR Pharmacy College, Palwancha, Telangana 507115, India

ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Decitabine and Cedazuridine was done by RP-HPLC. The Phosphate buffer was pH 4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of PH 4.5(20:80 v/v). Kromosil C18 Column (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1 ml min-1. The linearity range of Decitabine and Cedazuridine were found to be from 100-500 mg/ml of Decitabine and 1-5mg/ml of Cedazuridine. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. Decitabine %RSD 0.2 and Cedazuridine %RSD 0.6. Intermediate precision for Decitabine %RSD 0.2 and Cedazuridine %RSD 0.1 The percentage recovery varies from 98-102% of Decitabine and Cedazuridine. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Kromosil C18, Decitabine and Cedazuridine, RP-HPLC

INTRODUCTION

The acronym HPLC, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called high pressure liquid chromatography, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

The components of a basic High-Performance Liquid Chromatography [HPLC] system are shown in the simple diagram in figure 5. A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute. An injector is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column.

Reversed phase HPLC (RP-HPLC) consists of a

Corresponding Author: - **Yamini S,** Email: yamin335186030@gmail.com

non-polar stationary phase and an aqueous, moderately polar mobile phase.

One common stationary phase is silica which has been treated with RMe2SiCl, where R is a straight chain alkyl group such as C18H37 or C8H17. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention Time (Rt) is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. The energy released in this process is proportional to the surface tension of the eluent (water: 73 erg/cm², methanol: 22 erg/cm²) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding less-polar solvent (MeOH, ACN) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically changing the polarity of the mobile phase during the course of the analysis.

Myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic neoplasms with variable underlying etiology and presentation, including neutropenia and thrombocytopenia. Further mutations leading to increased proliferation of cancerous cells can eventually lead to secondary acute myeloid leukemia, which has a poor prognosis.9,10 Among treatment options, nucleoside analogues such as decitabine and azacitidine integrate into cellular DNA and inhibit the action of DNA methyltransferases, leading to global hypomethylation and related downstream therapeutic benefits. Decitabine was developed by MGI Pharma/SuperGen Inc. and was approved by the FDA for the treatment of MDS on February 5, 2006. It was first marketed under the name Dacogen.11 It is also available as an oral combination product together with the cytidine deaminase inhibitor.

Myelodysplastic syndromes (MDS) are a group of hematopoietic neoplasms that give rise to variable cytopenias progressing to secondary acute myeloid leukemia (sAML), which is invariably fatal if untreated. Hypomethylating agents such as decitabine and azacitidine are used to treat MDS through inducing DNA hypomethylation and apoptosis of cancerous cells. Although effective, these compounds are rapidly metabolized by cytidine deaminase (CDA) prior to reaching systemic circulation when administered orally, necessitating intramuscular or intravenous administration routes. Cedazuridine is a fluorinated tetrahydrouridine derivative specifically designed to inhibit CDA and facilitate oral administration of hypomethylating agents.

MATERIALS AND METHODS

Table 1: Instruments used

Sl. No	Instrument	Model
1	HPLC	Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

SL. No	Chemical	Brand
1	DECITABINE	Mavyret
2	CEDAZURIDINE	Mavyret
3	KH_2PO_4	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization:Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate

with buffer (pH 4.5), Methanol in proportion 20: 80 v/v respectively.

Wave length selection:UV spectrum of 10 µg / ml Decitabine and Cedazuridine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 254. At this wavelength both the drugs show good absorbance.

Optimization of Column:The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5mm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow

Standard Solution 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. (Stock solution) Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

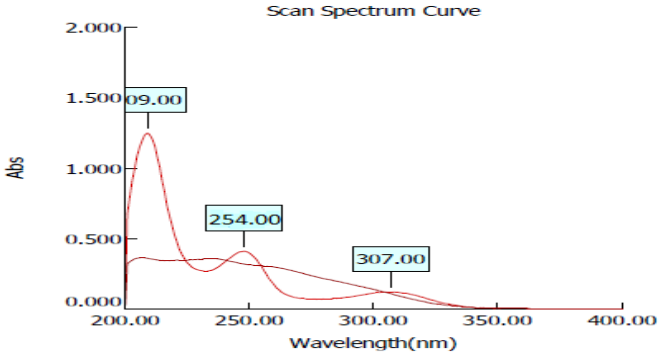
Sample Solution Preparation: Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Decitabine and Cedazuridine (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 3 ml of Decitabine e and Cedazuridine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Inject 20 □L of the standard, sample into the chromatographic system and measure the areas for Decitabine and Cedazuridine peaks and calculate the %Assay by using the formulae. Tailing factor for the peaks due to Decitabine and Cedazuridine in Standard solution Should not be more than 2.0. Theoretical plates for the Decitabine and Cedazuridine peaks in Standard solution should not be less than 2000.

RESULTS AND DISCUSSIONS

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Decitabine and Cedazuridine was obtained and the isobestic point of Decitabine and Cedazuridine showed absorbance's maxima at 254 nm. Chromatogram for Decitabine and Cedazuridine sample Preparation.From the above chromatogram it was observed that the Decitabine and Cedazuridine peaks are well separated. Retention time of Decitabine – 2.669min.Retention time of Cedazuridine - 3.855 min. Chromatogram for Decitabine and Cedazuridine Standard Preparation. Retention time of Decitabine – 2.569 min. Retention time of Cedazuridine - 3.842 min.

Figure 1: Overlay spectrum of Decitabine and Cedazuridine



Optimized Chromatogram Is Obtained By Following Conditions

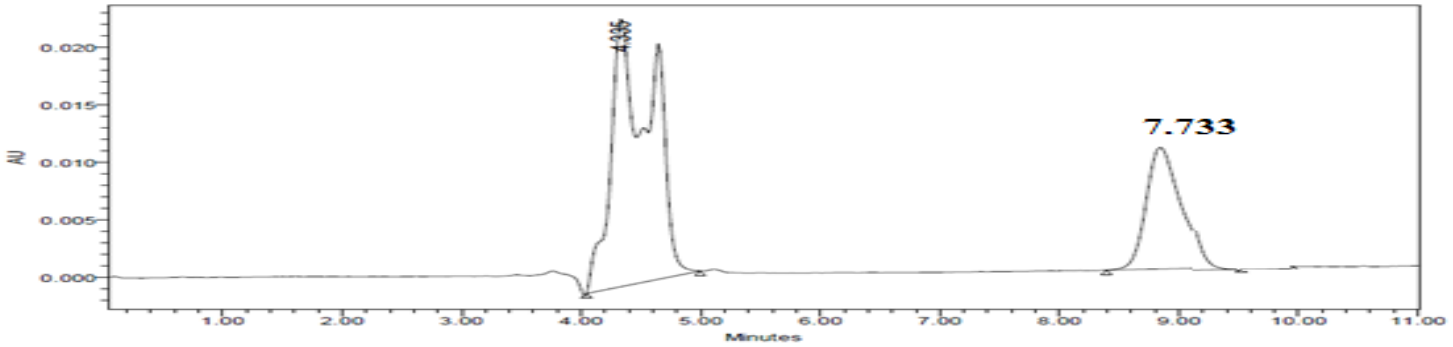
Trial-1

Table 3: Chromatographic condition

Parameters	Description
Flow rate	1ml min^{-1}
Column	Chromosil C_{18} Column(250mm x 4.6mm)5µ
Mobile Phase	ACN: water (30:70 v/v)
Detector	PDA
Column temperature	Ambient
Wavelength	254 nm
Type of elution	Isocratic
Injection volume	20µl

Run time	10min

Figure 2: Chromatogram of Trial-1

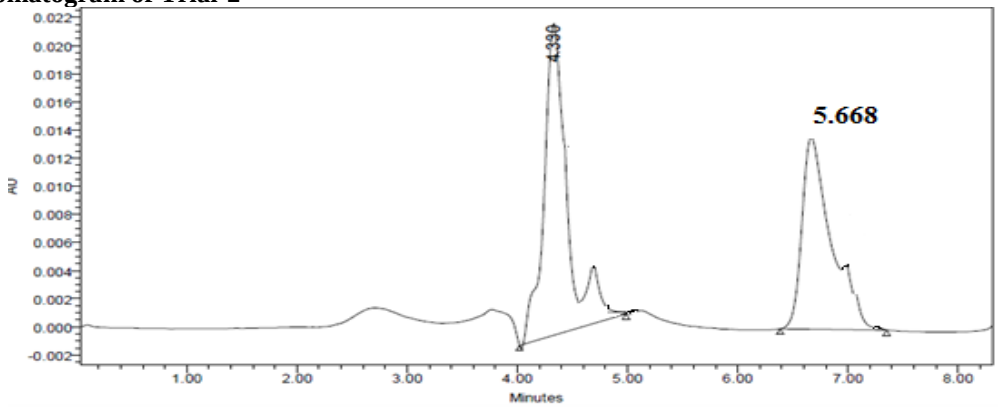


Trial-2

Table 4: Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Chromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	water: Methanol P^H 2.5 (30:70 v/v)
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254nm
Injection volume	20µl
Run time	10min

Figure 3: Chromatogram of Trial-2



Assay Results:

Weight of 10 tablets : 1.25 grams

Average Weight : 0.125grams

$$= \frac{125260}{124581} \times \frac{10}{100} \times \frac{0.3}{10} \times \frac{10}{25} \times \frac{10}{3} \times \frac{99.7}{100} \times \frac{125}{0.5} \times 100 = 100.24\%$$

Table 5: Results of system suitability parameters for Decitabine and Cedazuridine

S. No	**Name**	**Retention time(min)**	**Area (µV sec)**	**Height (µV)**	**USP resolution**	**USP tailing**	**USP plate count**
1	Decitabine	2.669	124505	223532	**1.2**	1.2	4523.3
2	Decitabine	2.5264	123442	134544	1.2	1.2	5020.2
3	Decitabine	2.5265	123431	124386	1.2	1.2	4061.2
4	Decitabine	2.5266	125432	134568	1.2	1.2	5032.4

5	Decitabine	2.5267	122434	146852	1.2	1.2	5076.4
6	Decitabine	2.5268	124438	145782	1.2	1.2	6024.8
7	Cedazuridine	3.855	1308495	154566	1.3	1.3	6090.3
8	Cedazuridine	3.902	1309496	156428	1.3	1.3	5023.2
9	Cedazuridine	3.903	1306498	152634	1.3	1.3	8060.7
10	Cedazuridine	3.904	1342499	158426	1.3	1.3	7080.1
11	Cedazuridine	3.905	1343451	158484	1.3	1.3	6054.4
12	Cedazuridine	3.906	1346455	158423	1.3	1.3	7080.6

VALIDATION PARAMETERS:

PRECISION:

Precision of the method was carried out for standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Figure 4: (a) chromatogram for standard injection -1

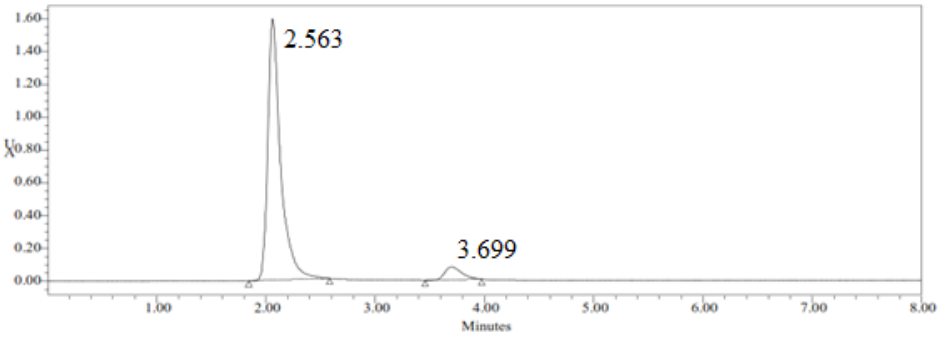
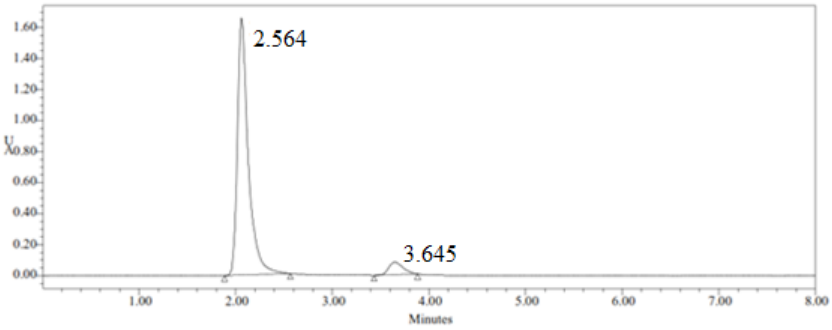


Figure 4: (b) chromatogram for standard injection-2



Intermediate Precession (Ruggedness):

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Figure 4: (c) chromatogram for standard injection-3

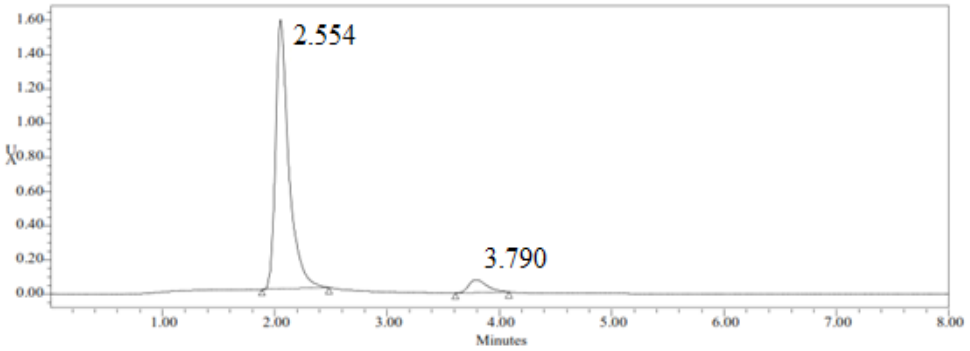


Figure 4: (d) chromatogram for standard injection-4

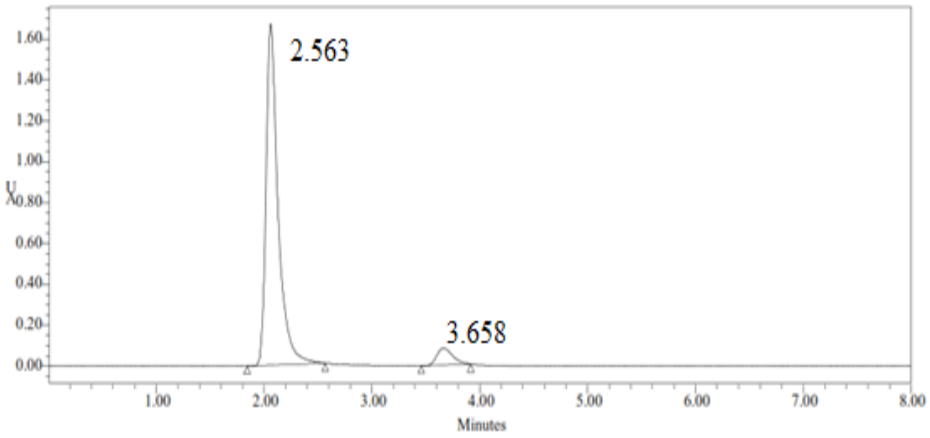


Figure 4: (e) chromatogram for standard injection-5

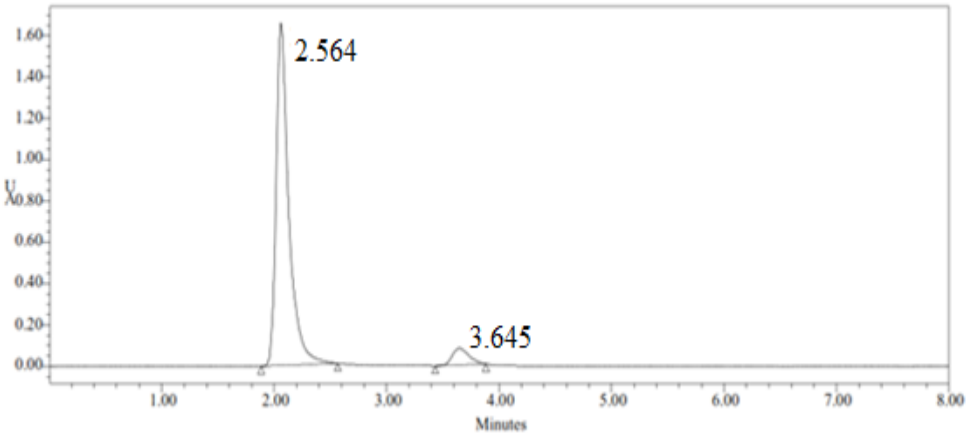


Figure 4: (f) chromatogram for standard injection-6

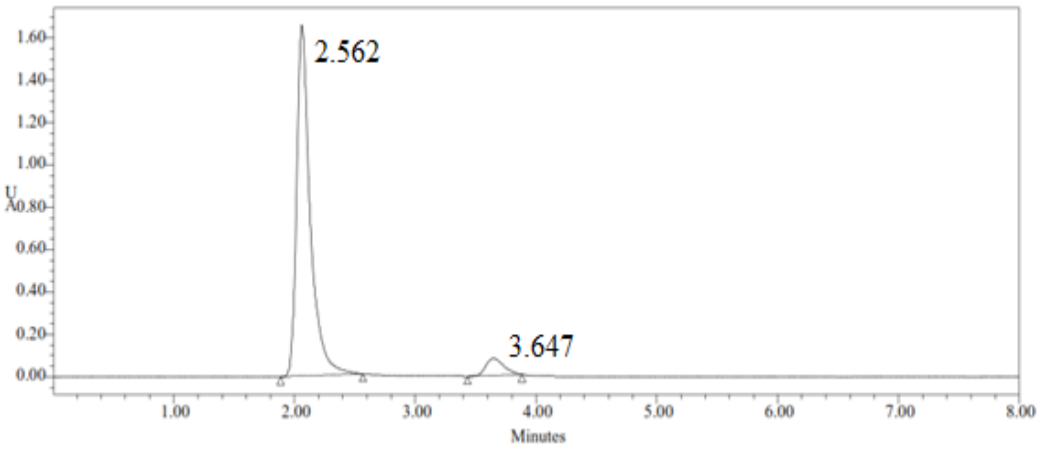


Table 6: Showing % RSD results method precession for Decitabine

Injection	Peak Name	Rt	Area	Height
1	Decitabine	3.699	1302729	341432.2
2	Decitabine	3.790	1302947	523341.4
3	Decitabine	3.663	1303236	374642.4
4	Decitabine	3.658	1303977	327514.3
5	Decitabine	3.647	1309759	374028.1
6.	Decitabine	3.645	1309789	346280.2
mean			1304529.8	
Std.dev			2961.1	
%RSD			0.2	

Table 7: Showing% RSD results method precession for Cedazuridine

Injection	Peak Name	Rt	Area	Height
1	Cedazuridine	3.616	123149	248742.3
2	Cedazuridine	3.634	123766	281441.2
3	Cedazuridine	3.460	124271	271721.2
4	Cedazuridine	3.446	124691	284393.8
5	Cedazuridine	3.437	124956	256318.0
6	Cedazuridine	3.438	125845	226813.0
mean			124162.7	
Std.dev			725.6	
%RSD			0.6	

Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Linearity:

The linearity range was found to lie from 100µg/ml to 500µg/ml of Decitabine, 5µg/ml to 25µg/ml 0f Cedazuridine and chromatograms are shown below.

Limit of Detection for Decitabine and Cedazuridine:

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio

Limit of Quantification (LOQ):

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Figure 5: chromatogram of Decitabine and Cedazuridine showing LOD

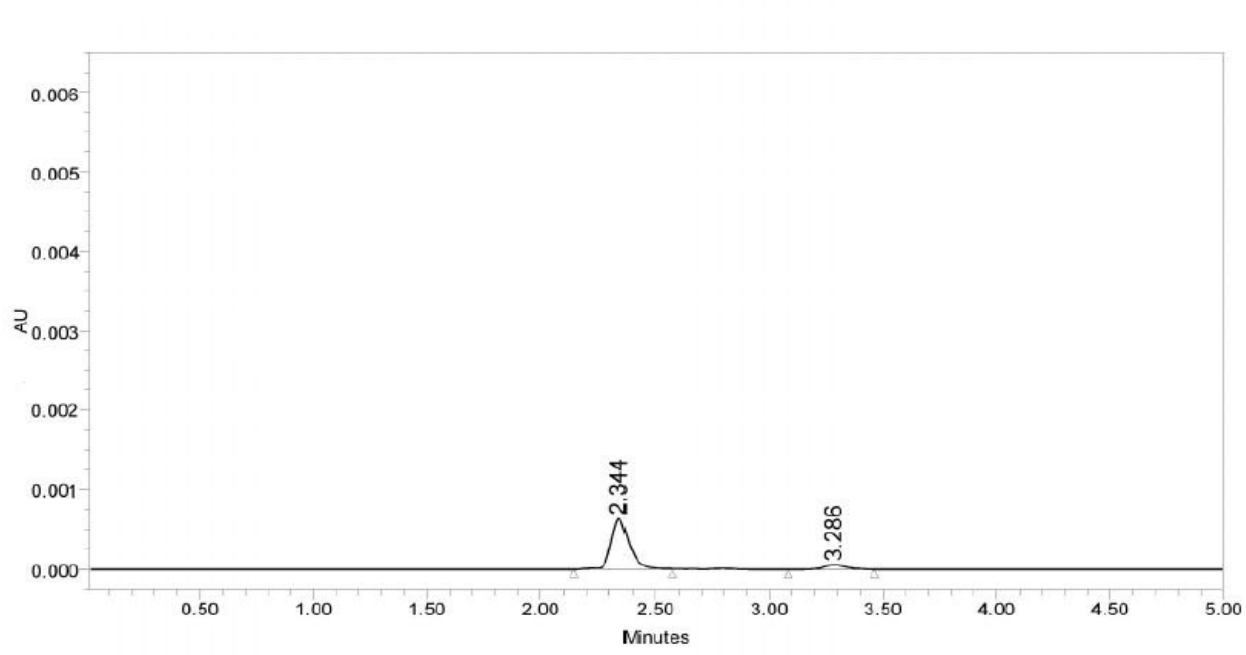


Figure 6: chromatogram of Decitabine and Cedazuridine showing LOQ

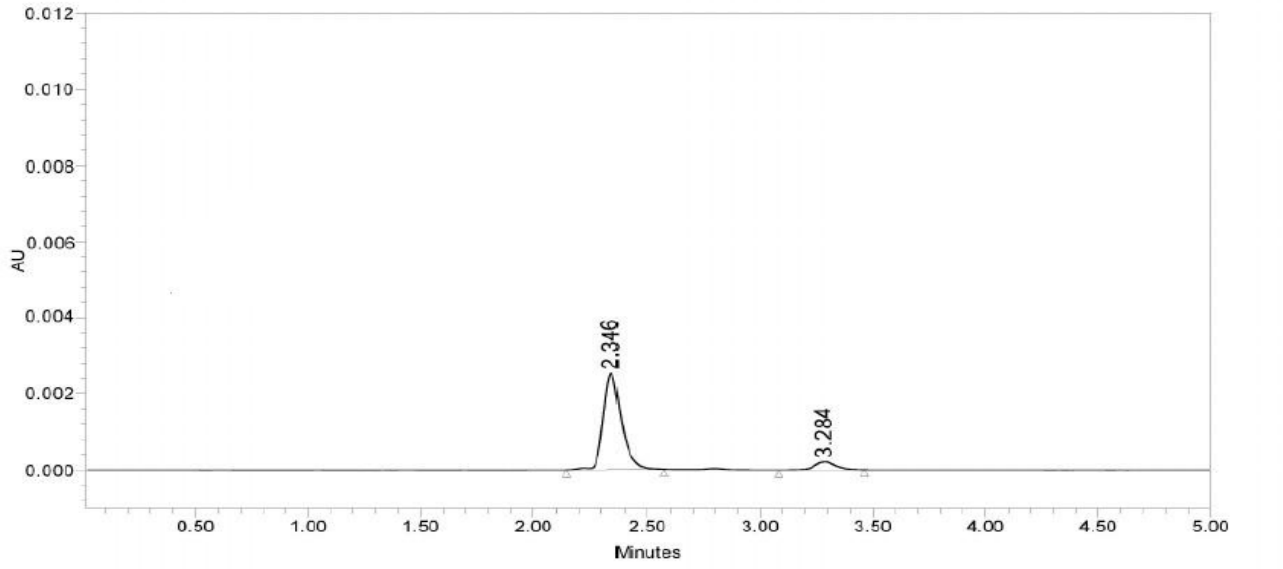


Table 8: Analytical performance parameters of Decitabine and Cedazuridine

Parameters	**Decitabine**	**Cedazuridine**
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Resolution between two drugs must be not less than 2. Theoretical plates must be not less than 2000.Tailing factor must be not less than 0.9 and not more than 2.It was found from above data that all the system suitability parameters for developed method were within the limit. The standard and samples of Decitabine and Cedazuridine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric

factor, and plate count.

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Decitabine and Cedazuridine was done by RP-HPLC. The Phosphate buffer was pH 4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of PH 4.5(20:80 v/v). Kromosil C18 Column (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min-1. The linearity range of Decitabine and Cedazuridine were found to be from 100-500 mg/ml of Decitabine and 1-5mg/ml of Cedazuridine. Linear regression coefficient was not more than 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. Decitabine %RSD 0.2 and Cedazuridine %RSD 0.6. Intermediate precision for Decitabine %RSD 0.2 and Cedazuridine %RSD 0.1 The percentage recovery varies from 98-102% of Decitabine and Cedazuridine. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision

REFERENCES

1. Beckett A.H And Stenlake J.B. Text Book Of Pharmaceutical Chemistry 4th Edn,-Part 2 Cbs Publishers And Distriburots, New Delhi,1998, 278-307
2. Douglas Skoog A, James Hollar F. And Timothy Nieman,.A Principles Of Instrumental Analysis. 5thed., Thomson Learning Inc. Singapore,1998, 110-300
3. Sethi P.D. Quantitative Analysis Of Drugs In Pharamceutical Formulation,3rded.,Cbs Publishers And Distributors, 1-29, 1997, 50-64
4. Mendham R.C, Denny J.D, Barnis M. And Thomas, J.K., Vogel"S Text Book Of Quantitative Chemical Analysis, 6thed., Pearson Education, 1, 2003, 676
5. Sharma,B.K., Instrumental Method Of Chemical Analysis, 24th Ed., Goel Publishing House, Meerut, 46, 2005, 68.
6. Chatwal G.R And Anand K.S. Instrumental Methods Of Chemical Analysis,5th Edn Himalaya Publishing House, Mumbai, 2002, 2-149
7. Munson J.W. Modern Methods Of Pharmaceutical Analysis, Medical Book Distributors, Mumbai, 2001,17-54.
8. Willard H.H, Merritt L.L, Dean J.A And Settle F.A, *et al*. Instrumental Methods Of Analysis,7th Edn,Cbs Publishers And Distributors, New Delhi 1988, 436-439.
9. Synder K.L, Kriklad J.J And Glajch J.L, *et al*. Practical Hplc Method Development 2nd Edn,Wiley-Interscience Publication, Usa, 1983, 1-10.
10. Bently And Drivers. Text Book Of Pharmaceutical Chemistry,8th Edn,O'brein,Oxford University Press,1985,1-3.
11. International Conference On Harmonization "Validation Of Analytical Procedures Methodology",14, Federal Register Nov.1996,1-8.
12. Indian Pharmacopeia, 1, 2007, 715.
13. British Pharmacopeia 1, 2007, 136.
14. Martindale The Complete Drug Reference, Thirty Sixth Edition.
15. Merck Index, 12th Edition.
16. B. Mohammed Ishaq. RP-HPLC-PDA Method Development, Validation and Stability Studies of the Novel Antineoplastic Drug Combination - Decitabine and Cedazuridine
17. Sunilkumar Adupa. Development And Validation Method For Decitabine Injection By RP-HPLC. *International Journal Of Pharmaceutical Sciences And Research* 2017
18. Glory hepsiba. Stability indicating RP-HPLC Method Devlopment and Validation of Decitabine Drug in Formulation. *International Journal of PharmTech Research*, 3(1), 2011, 237-243.
19. Yub Raj Neupane. Stability indicating RP-HPLC method for the estimation of Decitabine in bulk drug and lipid based Nanoparticles. *International Journal of Pharma Sciences and Research (IJPSR)* 5(07), 2014
20. Suresh Reddy. Development and Validation of HPLC Method for determination of Decitabine impurity profile in Decitabine for Injection 50mg/vial, 12(4), 2019