DEVELOPMENT AND VALIDATION OF STABILITY INDICATINGULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUSDETERMINATION OFDECITABINE AND CEDAZURIDINEIN PHARMACEUTICAL DOSAGE FORM

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Abstract

The aim of this study is to create and validate a fast, easy-to-use, affordable, sensitive, and accurate method for measuring Decitabine and Cedazuridine in bulk and pharmaceutical products by using Reversed-Phase Ultra-Performance Liquid Chromatography (RP-UPLC). Phenyl column was used with a mobile phase composed of 0.1% trifluoroacetic acid: acetonitrile (40:60 v/v) at a flow rate of 0.5 mL/min. UV detection was used at a wavelength of 220 nm. Cedazuridine and Decitabine correlation coefficients were found to be 0.999 over a concentration range of 25-150 µg/mL and 8.75-52.5 µg/mL, respectively. Cedazuridine and Decitabine have respective retention times of 1.225 and 1.875 minutes. The run time for separating the Cedazuridine and Decitabine peaks was within 3 minutes only. The validation results agreed with what's acceptable and had good limits as per ICH. This method, proposed as a regular analysis and quality control tool for medications that contain these active drugs either individually or in combination, was evident to be a suitable one.

Keywords: *UPLC, Cedazuridine, Decitabine,Method Development, Validation.*

1. Introduction

Decitabine, sold under the brand name Dacogen among others, acts as a nucleic acid synthesis inhibitor. It is a medication for the treatment of myelodysplastic syndromes[1-2], a class of conditions where certain blood cells are dysfunctional, and for acute myeloid leukemia (AML)[3-4]. Chemically, it is a cytidine analog. Decitabine is used to treat myelodysplastic syndromes (MDS) including previously treated and untreated, de novo and secondary MDS of all French-American-British subtypes (refractory anemia, refractory anemia with ringed sideroblasts[5], refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia[6]) and Intermediate-1, Intermediate-2, and High-Risk

International Prognostic Scoring System groups. In patients with chronic kidney disease, Batty and colleagues reported the first case series on the feasibility of therapy with hypomethylatingagents[7]in patients with chronic kidney disease[8].

Decitabine/cedazuridine, sold under the brand name Inqovi, is a fixed-dose combination medication for the treatment of adults with myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML). It is a combination of decitabine, a nucleoside metabolic inhibitor, and cedazuridine, a cytidine deaminase inhibitor[9-10]. The most common side effects of decitabine/cedazuridine include fatigue, constipation, hemorrhage[11], muscle pain (myalgia), mucositis (mouth sores)[12], arthralgia (joint pain), nausea, dyspnea[13-14], diarrhea, rash, dizziness[15], fever with low white blood cell count (febrile neutropenia)[16], edema, headache, cough, decreased appetite, upper respiratory tract infection, pneumonia, and transaminase[17-18] increased. The combination can cause fetal harm.

Till now, there have no UPLC and HPLC reports available for the estimation of Cedazuridine and only one report available for Decitabine. There were no methods available in combination. Hence, we employed UPLC method to determine both drugs.

2. Experimental Study

Solutions and Reagents

The pure Cedazuridine and Decitabine used in this study was provided by Glenmark Pharmaceutical Private Ltd., located in Andheri (E), Mumbai, India (99.8 and 99.8 percent purity). Other reagents, including acetonitrile, trifluoroacetic acid, and water, were procured from Merck (India) Ltd. in Worli, Mumbai, India, and were of HPLC grade.

Collection of instruments

Waters Acquity UPLC system with a quaternary pump and PDA detector (D2 lamp). Processing data was completed using Empower 2.0.

Buffers are chosen

An easy, inexpensive, and appropriate acidic buffer was chosen, such as 0.1 percent trifluoroacetic acid based on product pka values.

Step of mobility

For Standard review, the mobile step was 0.1% TFA buffer in a 40:60 (v/v) acetonitrile mixture and was degassed beforehand. A mobile phase chosen to produce well-defined peaks with a lower tailing factor (2.0) and a plate count of over 2000 was selected.

Prepare the diluent

Mobile phase was used as diluent.

Conditions of Chromatography

For the UPLC experiments, phenylcolumn (100 mm x 2.6 mm, 1.6 µm) was used. The elution was conducted with isocratic conditions using acetonitrile: TFA (0.1% volume) (60:40 by volume) at a flow rate of 0.5 mL/min. The injection volume was 5 µL, and the run time was 3 minutes, with the column temperature set to room temperature and

the absorbance measured at 220 nm (Because maximum absorbance was observed at this wavelength for both molecules. So, this was selected as wavelength).

Standard Solution Preparation

To dilute 100 mg of Cedazuridine and 35 mg of Decitabine, measure out the drug and transfer it to a 100 mL volumetric flask, then add approximately 70 mL of a diluent and sonicate for 30 minutes to dissolve it, after which you must add more diluent to the total amount. Further dilute 5 mL of the above solution to 50 mL with diluents.

Sample Solution Preparation

Accurately weigh and transfer sample, weight equivalent to100 mg of Cedazuridine and 35 mg of Decitabinein to a 100 mL volumetric flask, then add approximately 70 mL of a diluent and sonicate for 30 minutes to dissolve it, and add more diluent to the total amount. Further dilute 5 mL of the above solution to 50 mL with diluents.

Validation Process

System Precision

The system's performance has been validated through assessment of device suitability parameters. Limits were found to be met for a variety of parameters, including plate count, tailing, and RSD percentage.

Specificity

Being able to identify and test a given analyte in the presence of other elements required to be combined in the Standard and the standard solution is known as specificity. Blank Standards and those with Cedazuridine and Decitabine will be tested using chromatograms.

Accuracy

Being close to the real meaning of the technique is what defines accuracy. Three concentrations will be used to test the recovery trials. The drug's quantity, percentage of recovery, and standard deviations were calculated after every injection at each level.

Precision

It is the level of agreement between the various test results that determines the precision of the analytical methodology. Researchers examined the effects of sampling a homogeneous population more than once. The current process was evaluated in terms of its ability to provide repeatable, intraday, and inter-day results. It was examined by sampling the materials on the same day and over the course of different days.

Linearity

Linearity is the feature of analytical process which allows for a direct proportion of analytical results in response to a certain concentration of the analyte in the Standard. A total of seven series of standard solutions were selected for the assessment of the linearity curve. The calibration curve was drawn by comparing regular solution concentration with peak area. Using the least square method, the slope, intercept, and coefficient of correlation were calculated.

Forced Degradation

The peaks in the chromatogram should agree. ICH guidance Q1(A) R2 was performed in conjunction with stress degradation experiments. The peaks of degradation should be well distanced and at least 1.0 resolution between peaks. For the largest peaks to go over, a separation must occur. A degradation of around 20 percent has been attained via

several various stress conditions like acid, alkali, peroxide, reduction, thermal and photo in what is known as a forced degradation experiment.

Robustness

Robustness refers to a procedure's resistance to small process parameter changes, as well as its reliability in normal operation. An organic solution was introduced into the UPLC system for a robustness analysis, and the chromatographic settings (such as flow rate and mobile-phase organic content) were modified. The separation factor, retention time, and peak asymmetry were determined by evaluating the effects of altered parameters.

3. Results and Discussion

The aim of this study is to establish a single isocratic UPLC method for the simultaneous quantification of Cedazuridine and Decitabine in bulk and pharmaceutical dosage forms that is reliable, precise, and cost effective. According to the UV spectra of these compounds, an appropriate wavelength for simultaneous estimation of two drugs was chosen.

Optimization of the method

Using buffers (0.1% orthophosphoric acid, 0.1% formic acid, 0.1% triethylamine) and acetonitrile as mobile phase different trials were conducted in isocratic and gradient modes. Various stationary phases including phenyl and C18 were used to test the system. The resolution and retention times were improved by changing the mobile step composition at each trial. In the end, the separation was achieved using a Phenyl column (100mm x 2.6mm, 1.6µm) and a mobile phase of 0.1% TFA: acetonitrile (40:60 v/v) with a flow rate of 0.5 mL/min and UV detection at a wavelength of 220 nm. The entire performance lasted threeminutes only. Conditions for optimized chromatography are provided in table 1.

System Suitability

To attain results, the following device suitability parameters were established after six consecutive injections of normal solution: theoretical plate number, time, peak area, tailing factor, and resolution.

Table 1. Method suitability conditions

Parameter	Suitable conditions
Column	Phenyl (100mm x 2.6 mm, 2.6 µ)
Moving Phase	0.1% Tri fluoro acetic acid: Acetonitrile (40:60 v/v)
Volume of injection	5 µL
Stream rate	0.5 mL/min
Temperature of column	25°C
Wavelength	220 nm
Time duration	3 minutes
Retention time of Cedazuridine	1.225 min
Retention time of Decitabine	1.875 min

Table 2. Results of system suitability

Parameter	Cedazuridine	Decitabine	Acceptance criteria
Number of theoretical plates	4653	3289	Not less than 2000
Tailing	1.02	1.09	Not more than 2.0
Resolution	-	3.52	Not less than 2.0
Peak elution time (min)	1.225	1.875	--
% RSD	0.56	0.74	Not more than 2.0

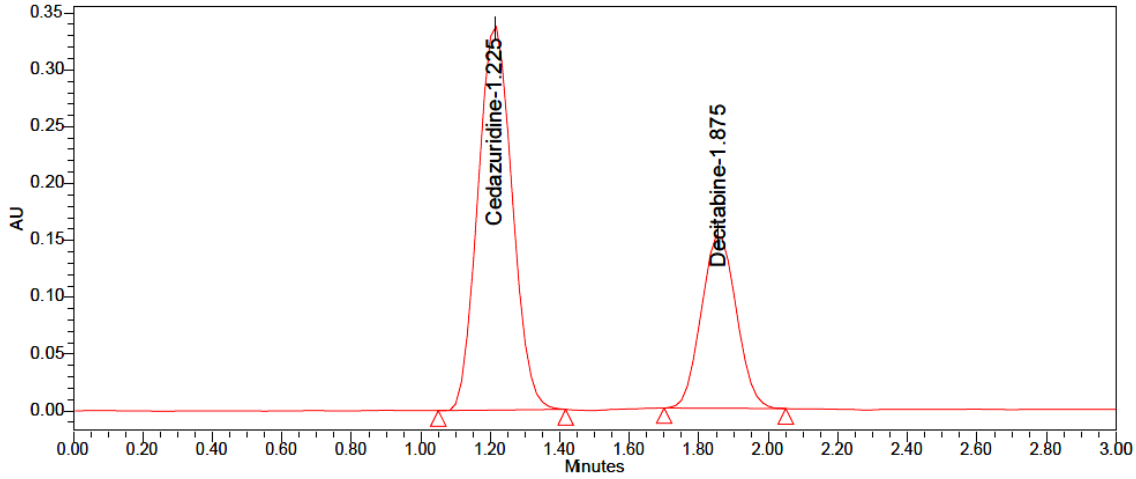

Figure 1. Chromatogram of standard

Specificity

There was no carryover from Cedazuridine and Decitabine at the elution time. As seen in Figure 2, the blank chromatogram is present.

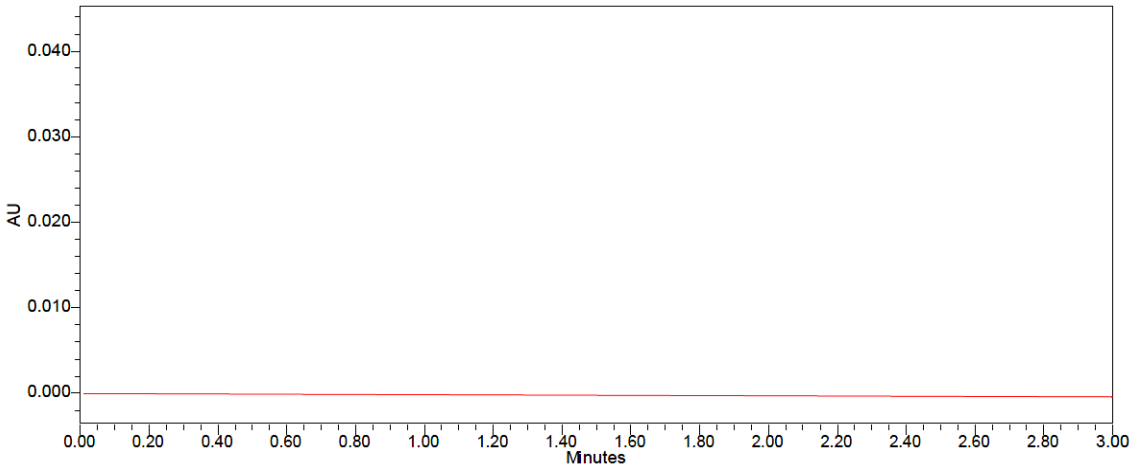

Figure 2. Chromatogram of blank

Linearity

By using a calibration curve to determine the linearity of the area of peak, its corresponding concentration was discovered. From this graph, it appears that the range of 25-150 µg/mL of Cedazuridine and 8.75-52.5 µg/mL of Decitabine had a straight line. Linearity results were demonstrated in table 3.

Table 3. Results of linearity

S. No	Cedazuridine		Decitabine	
	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
1	25.00	785496	8.75	310165
2	50.00	1542305	17.50	655947
3	75.00	2263512	26.25	953245
4	100.00	3125478	35.00	1256348
5	125.00	3896524	43.75	1502369
6	150.00	4602157	52.50	1845796

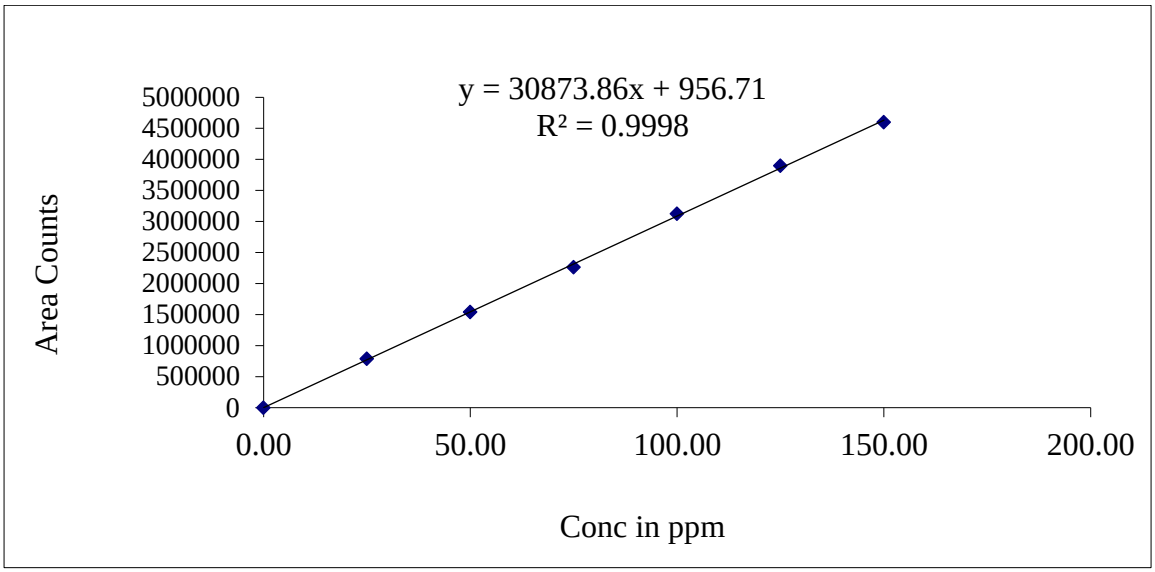

Figure 3. Calibration plot of Cedazuridine

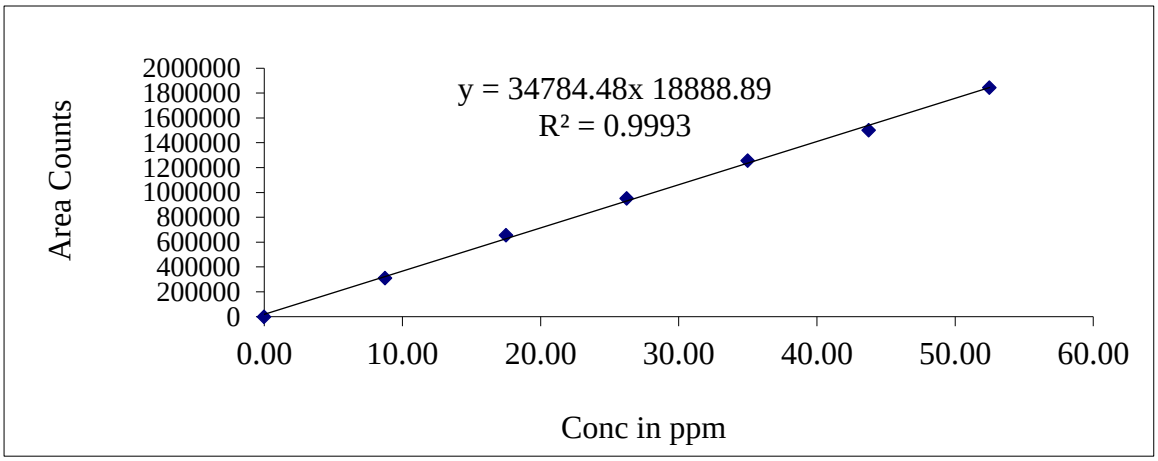

Figure 4. Calibration plot of Decitabine

Precision

Intraday and intermediate precision variances were assessed in relation to the procedure's accuracy. The Standards were examined six times on the same day to

obtain intraday results for Cedazuridine and Decitabine. The system's intermediate precision was explored by analyzing data in the same laboratory using a variety of examiners and tools. It is very accurate, with an RSD percentage of less than 2%. The process was precise, yielding the best drug recoveries at each additional concentration. Table 4 shows the method precision results.

Table 4. Outcomes of method precision

S. No.	Cedazuridine		Decitabine	
	Area	% Assay	Area	% Assay
1	3152468	99.5	1254785	101.1
2	3142517	99.2	1232654	99.3
3	3162543	99.8	1228958	99
4	3185642	100.5	1246523	100.4
5	3132568	98.8	1222857	98.5
6	3165294	99.9	1262539	101.7
Mean	3156839	99.6	1241386	100
Std. dev	18691.94	0.591	15667.79	1.265
% RSD	0.592	0.59	1.262	1.27

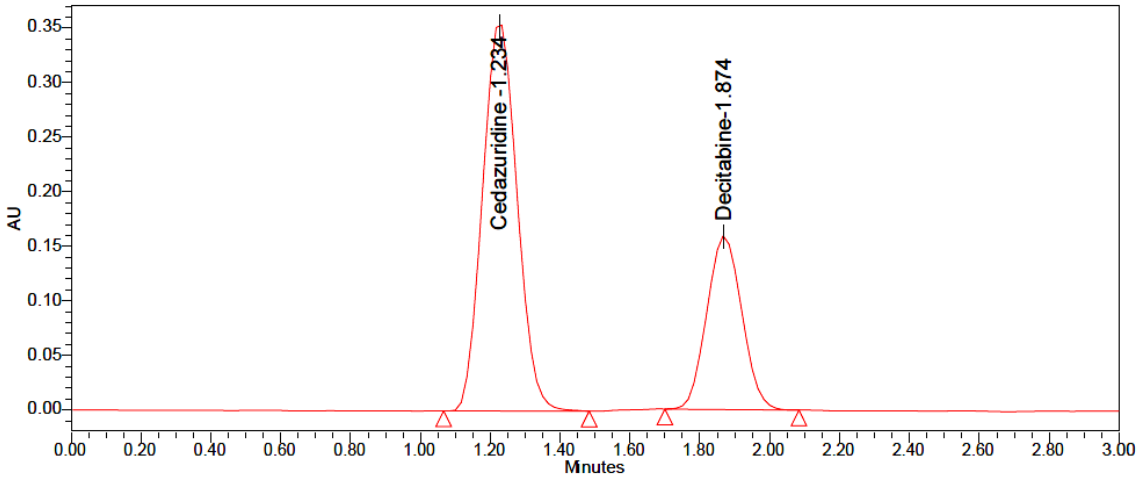

Figure 5. Chromatogram of method precision

Intermediate Precision (Ruggedness)

Intermediate precision results were shown in table 5.

Table 5. Results of intermediate precision

S.No.	Cedazuridine		Decitabine	
	Area	% Assay	Area	% Assay
1	3147598	99.3	1265845	101.8
2	3131256	98.8	1248758	100.5
3	3145268	99.2	1239568	99.7
4	3168594	100.0	1222547	98.4
5	3145785	99.3	1258475	101.2
6	3195684	100.8	1235683	99.4
Mean	3155698	99.6	1245146	100.2
Std dev	22957.62	0.717	15814.77	1.247
% RSD	0.727	0.72	1.27	1.24

Accuracy

By measuring the recovery experiments at three stages, the method's precision was reached (50 percent, 100 percent, and 150 percent). APIs were made with concentrations of Cedazuridine of 50, 100, and 150 micrograms/mL and Decitabine of 17.5, 35 and 52.5 micrograms/mL. For each stage of the spike, the test solution was injected three times, and the assay was performed in accordance with the test process. In addition to being able to determine the percentage of recovered data, the mean and relative standard deviations have also been found. The strategy was effective because the recovery values fell within the target range. Table 6 presents the accuracy results.

Table 6.Results of accuracy

Accuracy	Amount of Cedazuridine	% Recovery	Amount of Decitabine	% Recovery
50*	50	100.5	17.5	100.9
100*	100	100.5	35	101.3
150*	150	99.4	52.5	99.9

* Results are mean recovery of three sample preparations

LOD and LOQ

The concentration level at which the analytes are reliably detected and quantified is the limit of detection and quantification. Cedazuridine and Decitabine had a LOD concentrations of 0.3 µg/mL, 0.11 µg/mL and their S/N values of 3, 10. The LOQ concentrations of Cedazuridine and Decitabinewere 1 µg/mL, 0.35 µg/mL, and their S/N values were 25, 22. (S/N is the ratio of signal to noise).

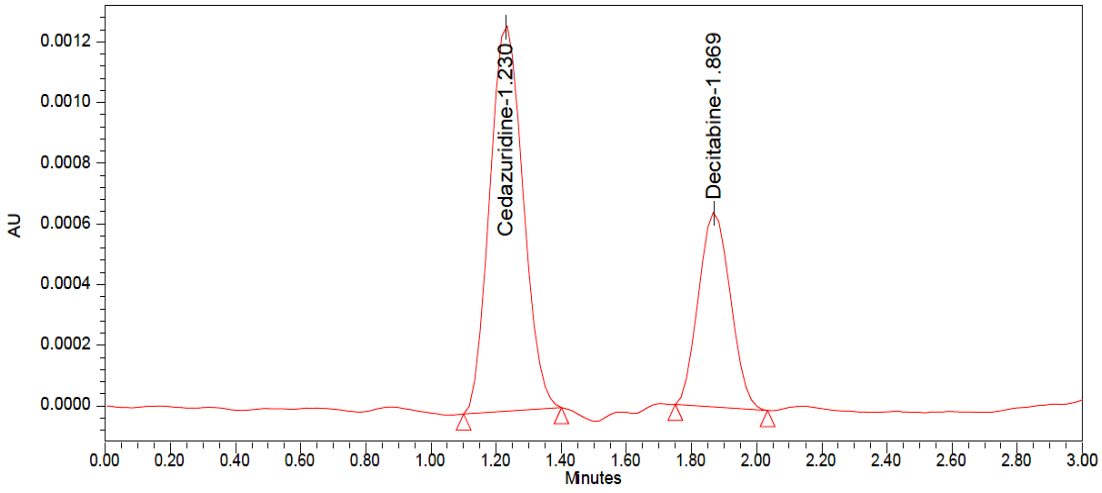

Figure 6. Chromatogram of LOD

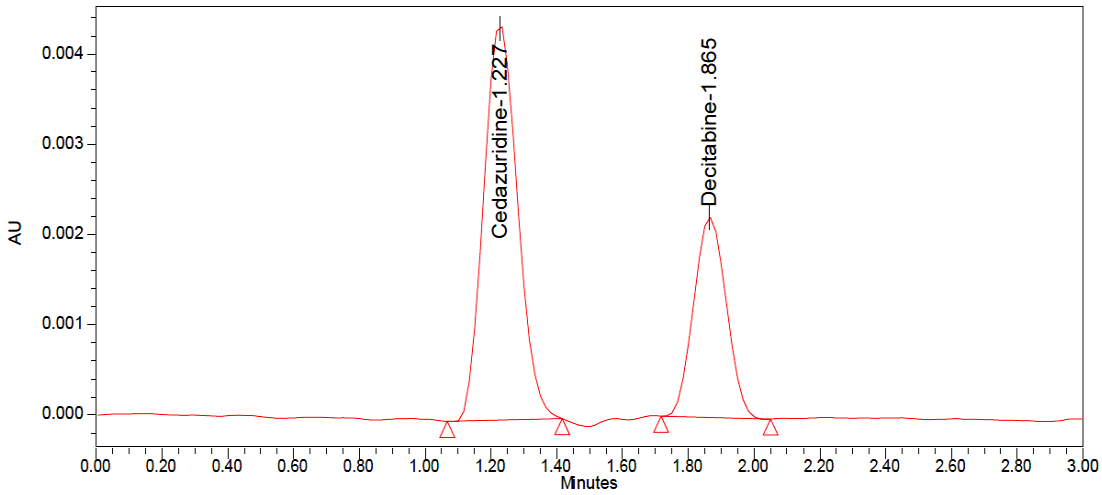

Figure 7. Chromatogram of LOQ

Robustness

To ensure the robustness of the chromatographic technique, the researchers evaluated flow rate and the composition of the mobile phase. By changing the flow rate and mobile phase ratio, the area of drugs changes. So, the percentage of relative standard deviation changes. Here in Table 7 (robustness results) the %RSD values are in within the acceptable limit.

Table 7. Outcomes of robustness

Parameter	% RSD of Cedazuridine	% RSD of Decitabine
Flow Minus (0.45 mL/min)	0.53	0.61
Flow Plus (0.55 mL/min)	0.49	0.89
Organic phase (66:34)	0.74	1.27
Organic phase (54:46)	0.67	0.49

Forced Degradation

The proposed approach can be used for successful evaluations of release and stability tests, and it can be called a stability preferable technique. Acid, Alkali, oxidation, reduction, photo, and thermal degradation are all included in the ICH-required forced degradation analysis. The chromatograms show that the selected drugs remained stable under the stress conditions, despite the presence of degraded peaks. Results of forced degradation were given in table 8.

Acid degradation

A volume of 1 mLsample stock solution was transferred to a volumetric flask with a capacity of 10 mL, to which 1 mL of 1N HCl was added and left to stand for 15 minutes. After 15 min add 1 mL of 1N NaOH and make up to the diluent mark. Filter the solution using syringe filter and injected into UPLC system.

Alkali degradation

A volume of 1 mLsample stock solution was transferred to a volumetric flask with a capacity of 10 ml, 1 mL of 1N NaOH was added, and the mixture was left to stand for 15 minutes. After 15 minutes, add 1 mL of 1N HCl to bring the solution up to the

required concentration. Use a syringe filter to filter the solution, which will then be injected into the UPLC system.

Peroxide degradation

A volume of 1 mLsample stock solution was moved to a volumetric flask of 10 mL, add 1 mL of 30% hydrogen peroxide solution and make up to the mark with diluents. Filter the solution using syringe filter and injected into UPLC system.

Reduction degradation

Using a volumetric flask with a capacity of 10 mL, transfer 1 mL of sample stock solution and add 1 mL of 30% sodium bi sulphate solution, then dilute to the required concentration with diluents. Use a syringe filter to filter the solution, which will then be injected into the UPLC system.

Thermal degradation

During the 6-hour baking period, the sample solution was kept at 105°C. The resulting solution was injected into an ultra-performance liquid chromatography system.

Photolytic degradation

A weight of 100mg sample was exposed to sunlight for 6 hrs. and the exposed Standard was analyzed. Prepare the Standard solution by using this Standard and inject into UPLC system.

Table 8. Forced Degradaton results

Stress Parameter	% Degradation	
	Cedazuridine	Decitabine
Acid degradation (1N HCl)	12.7	12.9
Alkali degradation (1N NaOH)	12.5	13.7
Peroxide degradation (30% Peroxide)	14.6	15.7
Reduction degradation (30% sodium bi sulphate)	9.4	10.4
Thermal (sample, 70°C, 6 Hrs)	5.2	2.8
Photo (UV-Vis light- (200 W h/m^2) and fluorescent light (1.2 milliion lux-h)	3.6	4.2

4.Conclusion

In this study, a novel, quick, sensitive, and easy-to-use UPLC method was developed for the simultaneous estimation of Cedazuridine and Decitabine in API and pharmaceutical dosage types. Because there are no UPLC and HPLC methods publishedin combination, this approach is the most practical option. Shorter run time, low cost, and all the other characteristics are benefits. Identifying many Standards necessitates considering these qualities. All the parameters were verified and were found to be within the acceptable range, including linearity, accuracy, specificity, robustness, and process precision. According to our research, the RSD values for all the parameters came in less than 2%, showing that the procedure is accurate and that the results we found are consistent. Therefore, it's possible to use the current approach in QC laboratories for routine study and manufacturing of Cedazuridine and Decitabine pharmaceuticals.

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Conflicts of Interest

None

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