ORIGINAL ARTICLE

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

G. Dharmamoorthy[1], M. Anupama[1], M. Sai Nandhini[1], C. Pavithra[1], Y. Kesava Reddy[1], Anna Balaji[1]

[1]Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy, Sree Sainath Nagar, Rangampeta, Tirupathi, Andhra Pradesh, India

Abstract

Objective: A new sensitive accurate and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of cedazuridine and decitabine in bulk and pharmaceutical formulation. **Materials and Methods:** Chromatographic separation was achieved through Altima C18 column (4.6*150 nm, 5 µm) using 0.01 Kh_2PO_4:acetonitrile (60:40 v/v) mixture used as the mobile phase. The Waters 2695, Reciprocating Water-510 pump system with PDA detector, and EMPOWERPRO software were monitored at detection wavelength 257 nm on flow rate 1 mL/min and the method was validated as per ICH guidelines (ICH,Q2 [R1]). **Results and Discussion:** Cedazuridine and decitabine were eluted at 2.248 min and 2.956 min, respectively, with good resolution. Plate count and tailing factor were very satisfactory, so this method was optimized and to be validated. **Conclusion:** This RP-HPLC method was successfully applied for the simultaneous determination of cedazuridine and decitabine in their pharmaceutical formulation and, hence, can be used for the routine analysis of these drugs in combined dosage form.

Key words: Cedazuridine, decitabine reverse-phase high-performance liquid chromatography, ICH guidelines, validation

INTRODUCTION

Cedazuridine chemically described as (4R)-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-4-hydroxy-1,3-diazinan-2-one.[1,2] Its empirical formula is $C_9H_{14}F_2N_2O_5$ and its molecular weight 268.217. Chemical structure of Cedzuridine shown in Figure 1 Cedazuridine is a cytidine deaminase inhibitor coadministered with the hypomethylating agent decitabine for the treatment of variable forms of myelodysplastic syndrome [3]

Decitabine chemically described as 41-(2-Deoxy-α-D-ribofuranosyl)-5-azacytosine; NSC 127717; 4-Amino-1-(2-deoxy-α-D-erythro-pentofuranosyl)-s-triazin-2(1H)-one. Its empirical formula is $C_8H_{12}N_4O_4$ and its molecular weight 228.21. Chemical structure of Decitabine is shown in the Figure 2. A pyrimidine nucleoside analog is used mainly in the treatment of leukemia, especially acute non-lymphoblastic leukemia.[4,5] Decitabine is an antimetabolite antineoplastic agent that inhibits the synthesis of DNA. Its actions are specific for the S phase of the cell cycle. It also has antiviral and immunosuppressant properties. Decitabine is a cytidine antimetabolite analog with potential antineoplastic activity. Decitabine incorporates into DNA and inhibits DNA methyltransferase, resulting in hypomethylation of DNA and intra-S-phase arrest of DNA replication. [6-8]

Address for correspondence:
Dr. G. Dharmamoorthy, Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy, Sree Sainath Nagar, Rangampeta, Tirupathi - 517 102, Andhra Pradesh, India. Mobile: +91 9603774847.
E-mail: dharmamoorthy111@gmail.com

Received: 29-12-2022
Revised: 02-07-2022
Accepted: 28-07-2022

MATERIALS AND METHODS

Materials

- Cedazuridine and decitabine pure drugs (API) received from spectrum laboratories.
- Combination of cedazuridine and decitabine injections (VYXEOS), received from local market.
- Distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen orthophosphate buffer, and orthophosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments

- Electronics Balance-Denver
- p^H meter-BVK enterprises, India
- Ultrasonicator-BVK enterprises

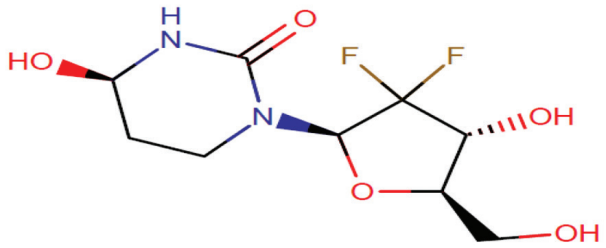

Figure 1: Chemical structure of cedazuridine

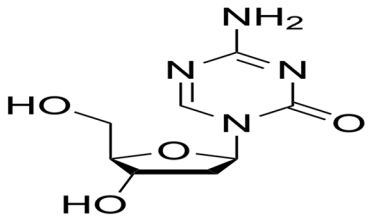

Figure 2: Chemical structure of decitabine

- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, photo diode array detector, and auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of cedazuridine and decitabine solutions.

Methods

Diluent

Based on the solubility of the drugs, diluent was selected, acetonitrile and water taken in the ratio of 50:50.

Preparation of standard stock solutions

Accurately weighed 50 mg of cedazuridine, 17.5 mg of decitabine and transferred to 50 mL volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 min. Flask was made up with diluents and labeled as standard stock solution (1000 µg/mL of cedazuridine and 350 µg/mL decitabine).

Preparation of standard working solutions (100% solution)

A 1 mL from each stock solution was pipetted out and taken into a 10 mL volumetric flask and made up with diluent (100 µg/mL of cedazuridine and 35 µg/mL of decitabine).

Preparation of sample stock solutions

Accurately taken one tablet equivalent to dose of 100 mg of cedazuridine and 35 mg of decitabine and that was transferred into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 min, further, the volume was made up with diluent and filtered by HPLC filters (1000 µg/mL of cedazuridine and 350 µg/mL of decitabine).

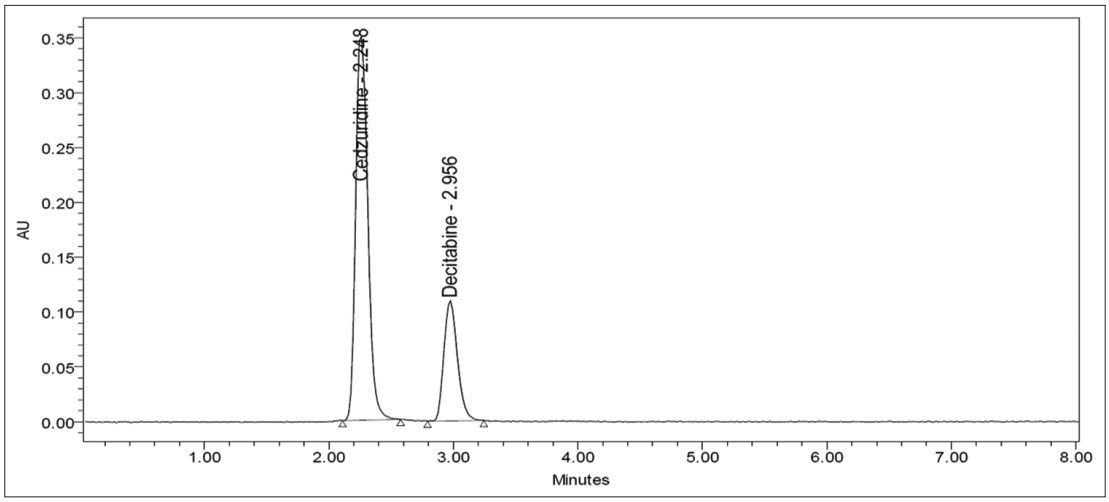

Figure 3: Optimized chromatogram

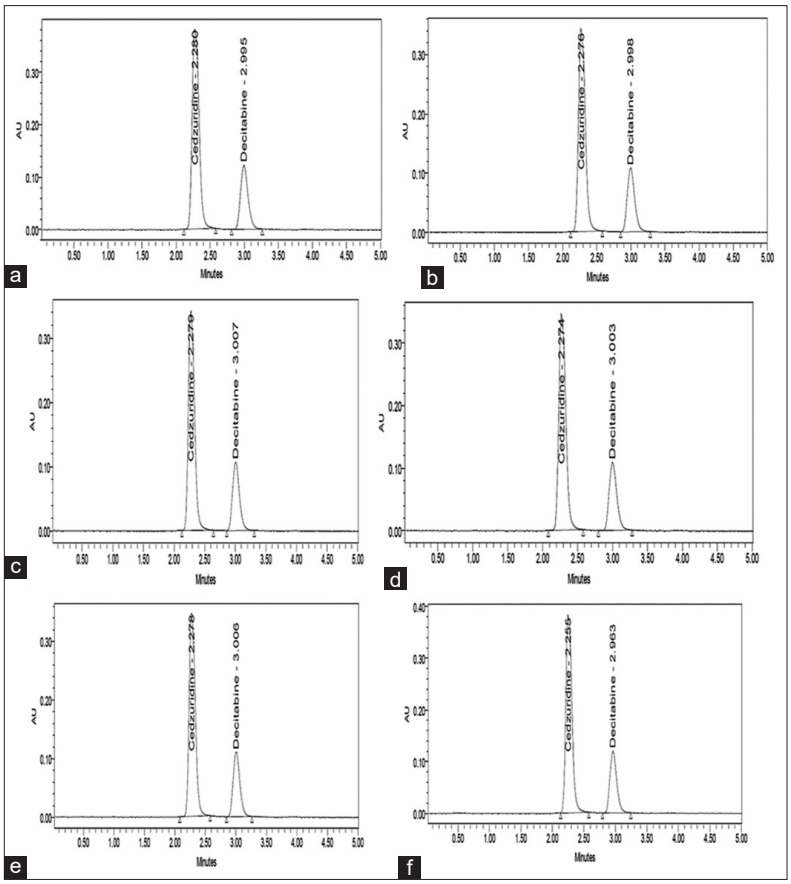

Figure 4: (a-f) System suitability chromatogram

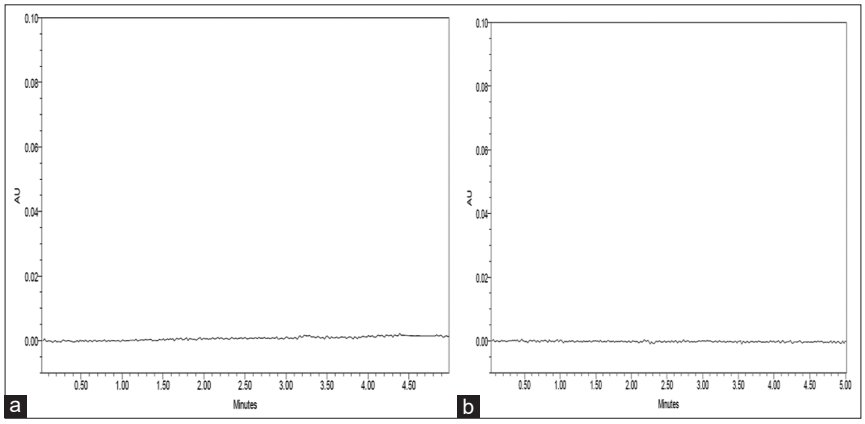

Figure 5: (a) Chromatogram of blank. (b) Chromatogram of placebo

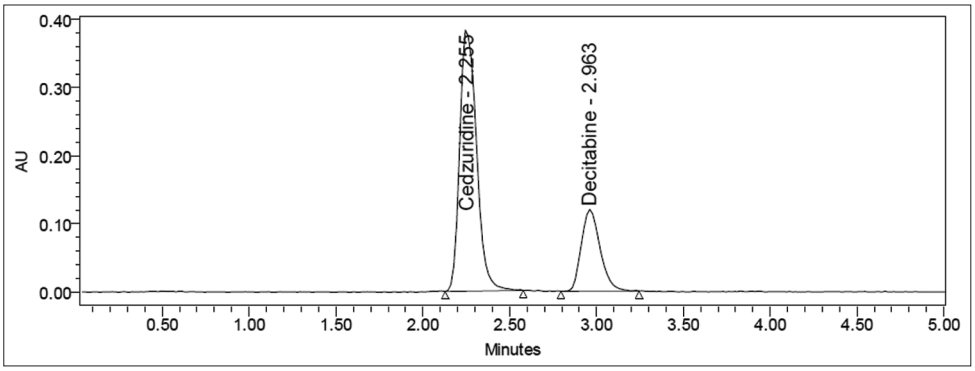

Figure 6: Typical chromatogram

Preparation of sample working solutions (100% solution)

A 1.0 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent (100 µg/mL of cedazuridine and 35 µg/mL of decitabine).

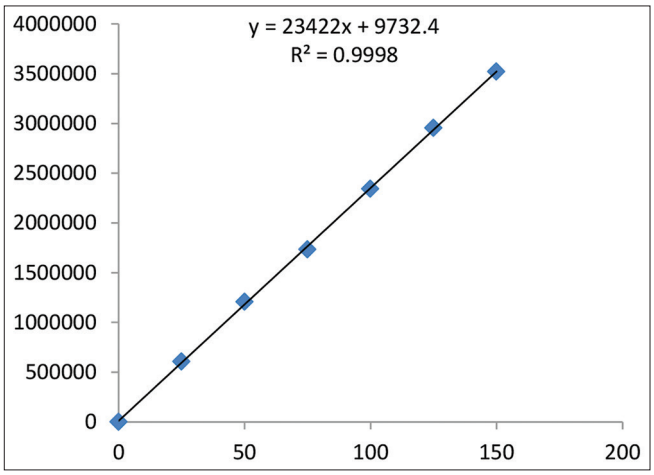

Figure 7: Calibration curve of cedazuridine

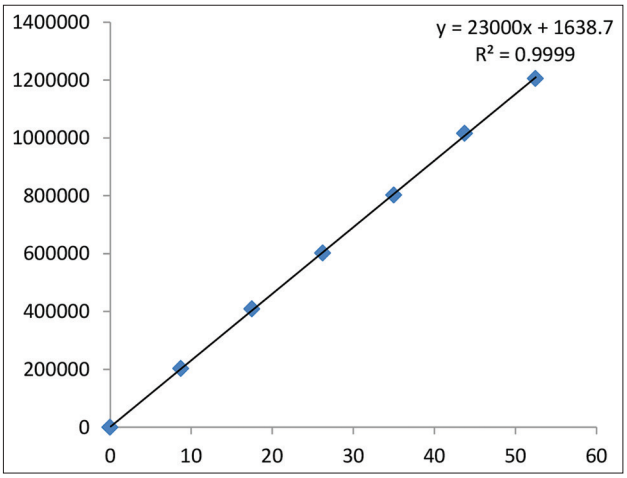

Figure 8: Calibration curve of decitabine

Preparation of buffer

0.1% OPA buffer: 1 mL of orthophosphoric acid was diluted to 1000 mL with HPLC grade water.

Buffer: 0.1 N potassium dihydrogen orthophosphate

Accurately weighed 1.36 g of potassium dihydrogen orthophosphate in a 1000 mL of volumetric flask add about 900 mL of Milli-Q water added and degas to sonicate and finally make up the volume with water then added 1 mL of triethylamine then pH adjusted to 3.8 with dil. orthophosphoric acid solution.

Optimized method

Chromatographic conditions

Mobile phase	0.01N Kh_2Po_4:acetonitrile (60:40)
Flow rate	1 mL/min
Column	Altima C18 (4.6×150 mm, 5 µm)
Detector wave length	257 nm
Column temperature	30°C
Injection volume	10 µL
Run time	10 min
Diluent	Water and acetonitrile in the ratio 50:50
Results	Both peaks have good resolution, tailing factor, theoretical plate count, and resolution.

Decitabine and cedazuridine were eluted at 2.248 min and 2.956 min, respectively, with good resolution. Plate count and tailing factor were very satisfactory, so this method was optimized ,optimized chromatogram shown in Figure 3 and developed method was validated as per ICH guidelines.[8,9]

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines and results are shown in Table 1and chromatograms are shown in the Figure 4

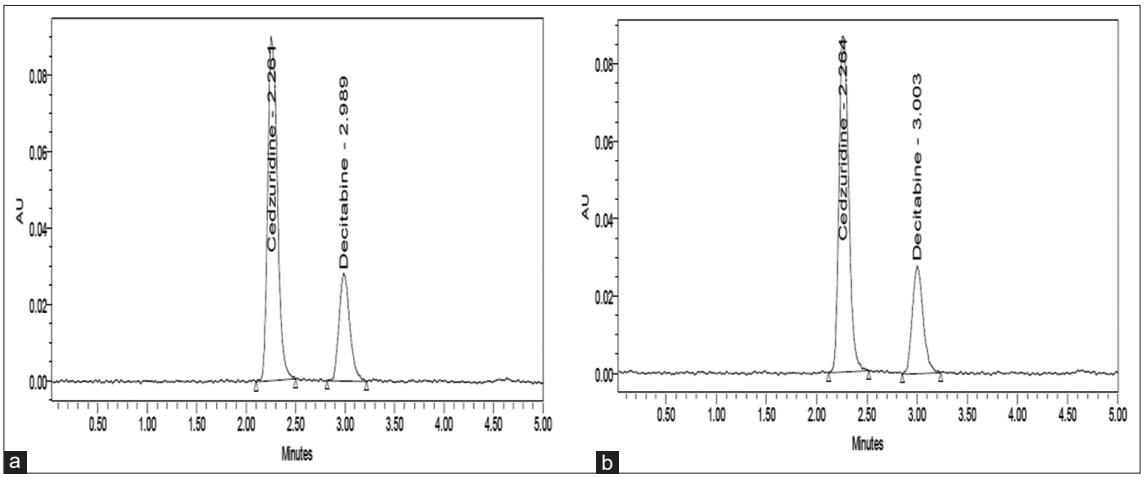

Figure 9: (a and b) Linearity 25% chromatogram of cedazuridine and decitabine

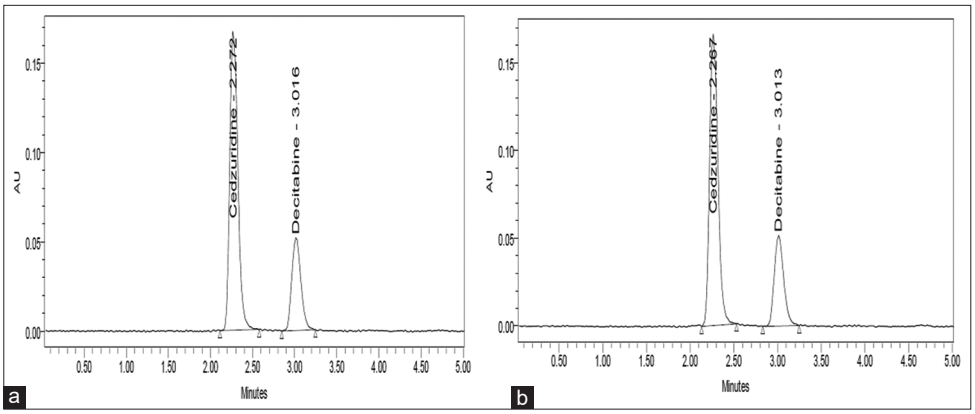

Figure 10: (a and b) Linearity 50% chromatogram of cedazuridine and decitabine

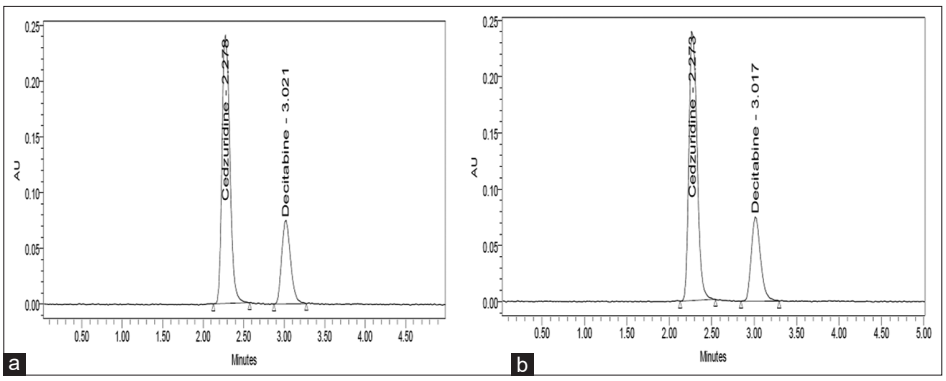

Figure 11: (a and b) Linearity 75% chromatogram of cedazuridine and decitabine

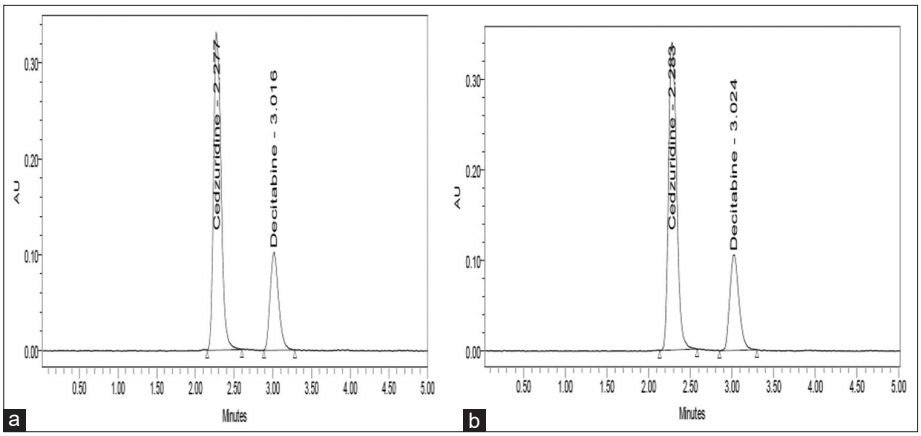

Figure 12: (a and b) Linearity 100% chromatogram of cedazuridine and decitabine

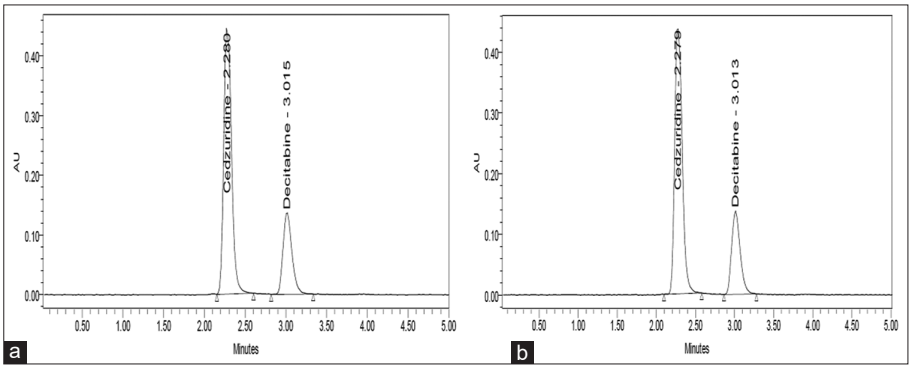

Figure 13: (a and b) Linearity 125% chromatogram of cedazuridine and decitabine

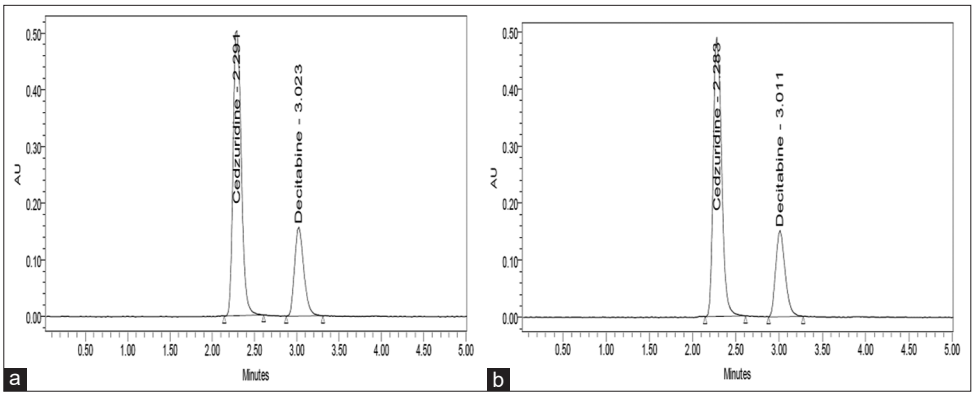

Figure 14: (a and b) Linearity 150% chromatogram of cedazuridine and decitabine

Table 1: System suitability parameters for decitabine and cedazuridine

S. No.	Decitabine			Cedazuridine			
Inj.	RT (min)	USP plate count	Tailing	RT (min)	USP plate count	Tailing	RS
1	2.255	2810	1.25	2.963	3419	1.24	3.8
2	2.274	2570	1.26	2.995	3452	1.23	3.8
3	2.276	2634	1.25	2.998	3425	1.22	3.8
4	2.278	2646	1.22	3.003	3447	1.22	3.8
5	2.279	2743	1.23	3.006	3545	1.21	3.8
6	2.280	2749	1.22	3.007	3330	1.23	3.8

Table 2: Linearity table for cedazuridine and decitabine

Cedazuridine		Decitabine	
Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
0	0	0	0
25	605,933	8.75	202,932
50	1,207,420	17.5	408,904
75	1,733,745	26.25	602,047
100	2,341,936	35	802,829
125	2,953,881	43.75	1,015,969
150	3,521,583	52.5	1,204,968

Table 3: System precision table of cedazuridine and decitabine

S. No.	Area of cedazuridine	Area of decitabine
1.	2,362,271	802,666
2.	2,362,585	812,403
3.	2,376,806	800,378
4.	2,380,599	792,146
5.	2,362,401	799,147
6.	2,359,228	797,382
Mean	2,367,315	800,687
S.D	8987.8	6745.7
%RSD	0.4	0.8

SD: Standard deviation, RSD: Relative standard deviation

Table 4: Repeatability table of cedazuridine and decitabine

S. No.	Area of cedazuridine	Area of decitabine
1.	2,360,336	808,277
2.	2,363,658	792,765
3.	2,362,250	805,286
4.	2,350,655	805,538
5.	2,369,852	794,302
6.	2,384,599	806,891
Mean	2,365,225	802,177
S.D	11,351.2	6797.0
%RSD	0.5	0.8

SD: Standard deviation, RSD: Relative standard deviation

Discussion: According to ICH guidelines, plate count should be more than 2000, tailing factor should be <2, and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation

Specificity

Discussion: Retention times of decitabine and cedazuridine were 2.255 min and 2.963 min, respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific and chromatograms are shown in the Figures 5-7.

Table 5: Intermediate precision table of cedazuridine and decitabine

S. No.	Area of cedazuridine	Area of decitabine
1.	2,339,906	796,050
2.	2,331,953	792,540
3.	2,299,174	796,299
4.	2,300,820	795,997
5.	2,366,903	797,939
6.	2,361,216	799,979
Mean	2,333,329	796,467
SD	28,891.0	2463.5
%RSD	1.2	0.3

SD: Standard deviation, RSD: Relative standard deviation

Table 6: Accuracy table of cedazuridine

% level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% recovery	Mean % recovery
50%	50	49.78	99.57	99.74%
	50	49.87	99.73	
	50	49.94	99.88	
100%	100	99.07	99.07	
	100	99.52	99.52	
	100	99.69	99.69	
150%	150	150.0	100.0	
	150	149.1	99.4	
	150	151.2	100.8	

Table 7: Accuracy table of decitabine

% level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% recovery	Mean % recovery
50%	17.5	17.71	101.22	99.93%
	17.5	17.63	100.74	
	17.5	17.39	99.35	
100%	35	35.04	100.11	
	35	34.87	99.63	
	35	34.90	99.72	
150%	52.5	52.61	100.22	
	52.5	52.18	99.38	
	52.5	51.98	99.01	

Table 8: Sensitivity table of cedazuridine and decitabine

Molecule	LoD	LoQ
Cedazuridine	0.51	0.18
Decitabine	1.53	0.54

LoD: Limit of detection, LoQ: Limit of quantitation

Table 9: Robustness data for cedazuridine and decitabine

S. No.	Condition	%RSD of cedazuridine	%RSD of decitabine
1	Flow rate (-) 0.9 mL/min	0.7	0.7
2	Flow rate (+) 1.1 mL/min	0.3	1.1
3	Mobile phase (-) 55B: 45A	0.5	0.7
4	Mobile phase (+) 45B: 55A	0.2	0.5
5	Temperature (-) 25°C	0.4	0.3
6	Temperature (+) 35°C	0.2	0.7

RSD: Relative standard deviation

Table 10: Assay data of cedazuridine

S. No.	Standard area	Sample area	% assay
1	2,362,271	2,360,336	99.31
2	2,362,585	2,363,658	99.45
3	2,376,806	2,362,250	99.39
4	2,380,599	2,350,655	98.90
5	2,362,401	2,369,852	99.71
6	2,359,228	2,384,599	100.33
Average	2,367,315	2,365,225	99.51
SD	8987.8	11,351.2	0.48
%RSD	0.4	0.5	0.5

SD: Standard deviation, RSD: Relative standard deviation

Table 11: Assay data of decitabine

S. No.	Standard area	Sample area	% assay
1	802,666	808,277	100.75
2	812,403	792,765	98.81
3	800,378	805,286	100.37
4	792,146	805,538	100.40
5	799,147	794,302	99.00
6	797,382	806,891	100.57
Average	800,687	802,177	99.99
SD	6745.7	6797.0	0.8
%RSD	0.8	0.8	0.8

SD: Standard deviation, RSD: Relative standard deviation

Linearity

Discussion: Six linear concentrations of cedazuridine (25–150 µg/mL) and decitabine (8.75–52.5 µg/mL) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for cedazuridine were y = 23,422x + 9732 and of decitabine were y = 23,000x + 1638. Correlation coefficient obtained was 0.999 for the two drugs. Linerity data

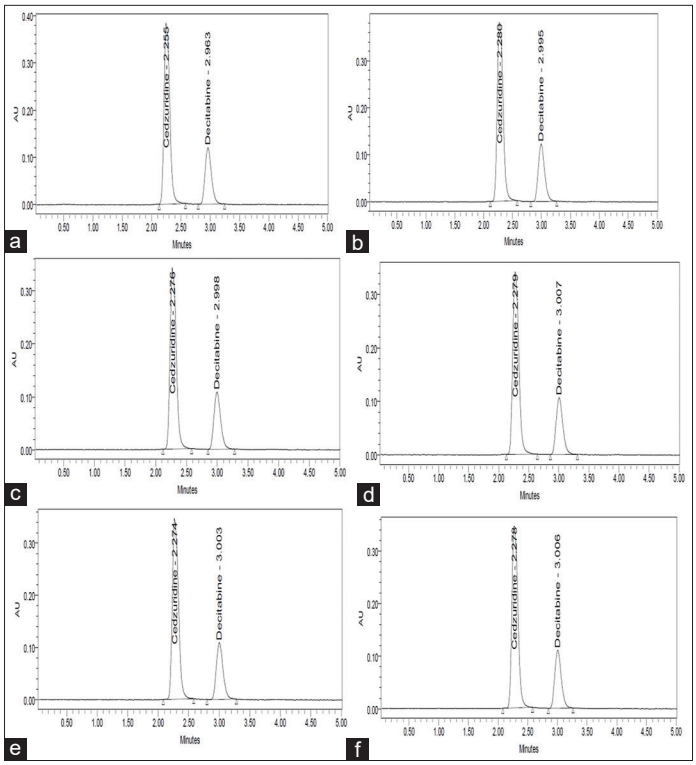

Figure 15: (a-f) System precision chromatogram

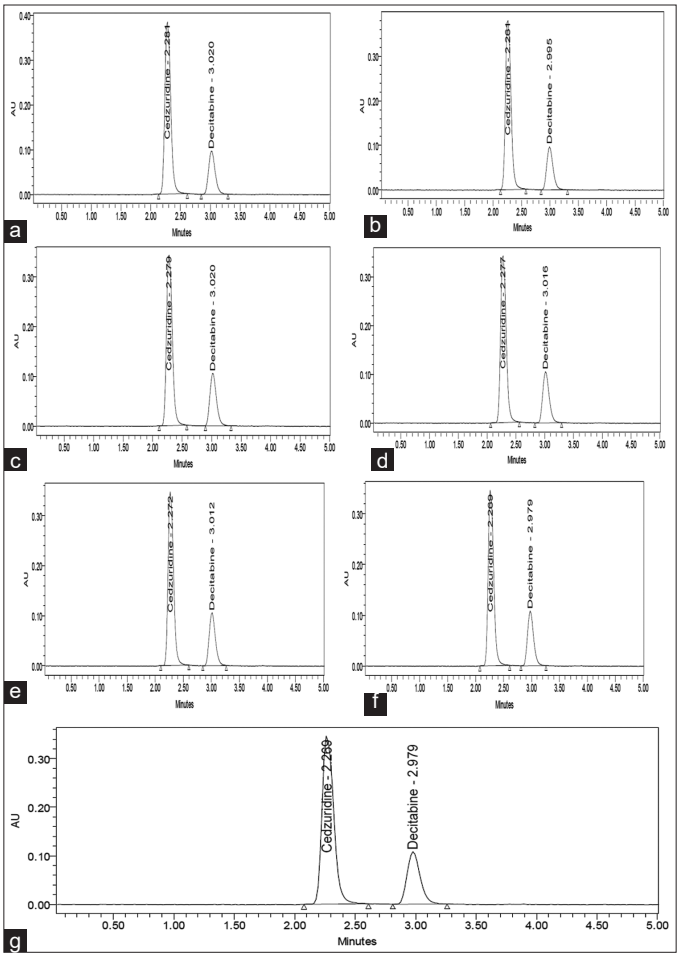

Figure 16: (a-g) Repeatability chromatogram

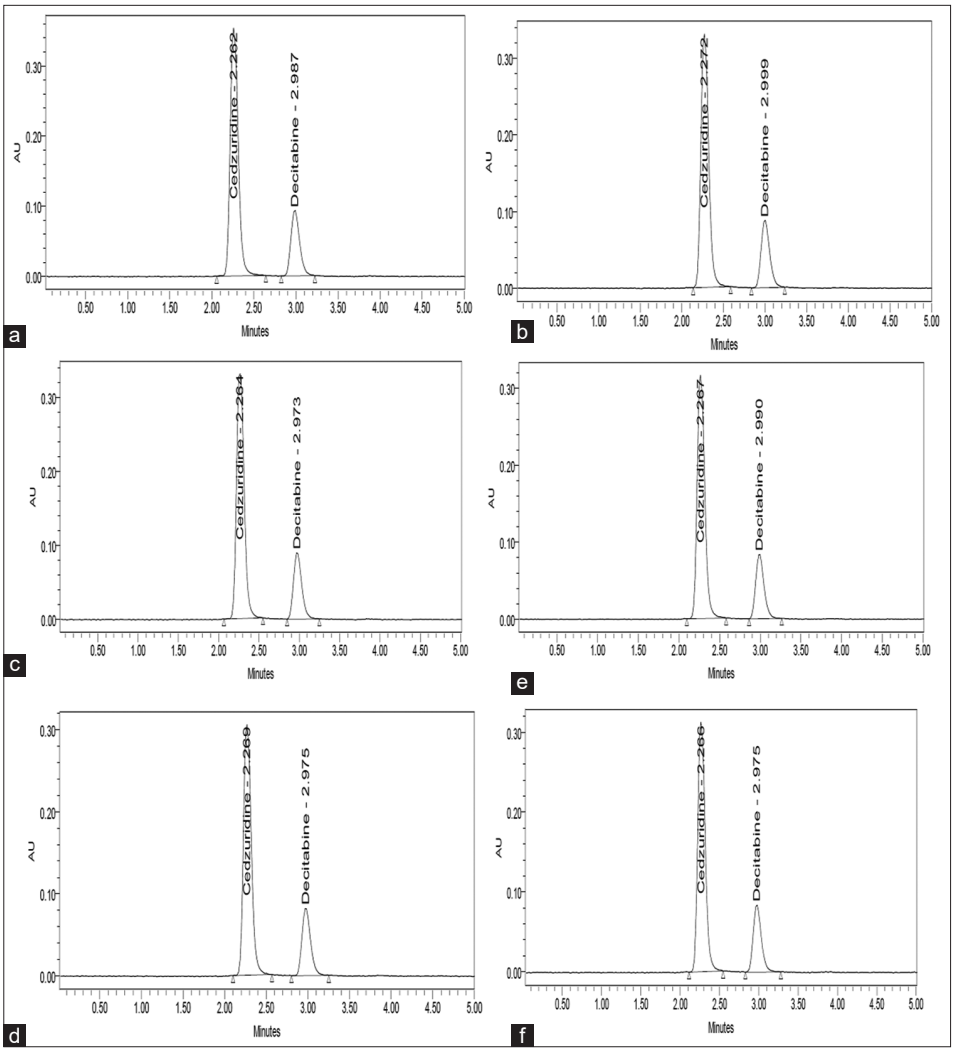

Figure 17: (a-f) Interday precision chromatogram

Table 12: Degradation data of cedazuridine

S. No.	Degradation condition	% drug ungraded	% drug degraded
1	Acid	95.93	4.07
2	Alkali	92.42	7.58
3	Oxidation	92.42	7.58
4	Thermal	97.60	2.40
5	UV	97.43	2.57
6	Water	97.43	2.57

Table 13: Degradation data of decitabine

S. No.	Degradation condition	% drug ungraded	% drug degraded
1	Acid	95.61	4.39
2	Alkali	91.88	8.12
3	Oxidation	96.01	3.99
4	Thermal	96.74	3.26
5	UV	97.30	2.70
6	Water	99.11	0.89

of Citzuridine and Dicitabine shown in the Table 2,Calibration curves of Citzuridine and Dicitabine shown in the Figures 8 and 9 Linearity Chromatograms are shown in the Figures 10-15.

Precision

System precision

Discussion: From a single volumetric flask of working standard solution, six injections were given and the obtained areas were mentioned above. Average area, standard deviation, and % relative standard deviation (RSD) were calculated for two drugs. % RSD obtained as 0.4% and 0.8%, respectively, for cedazuridine and decitabine. As the limit of precision was <"2," the system precision was passed in this method. Results are shown in the Table 3 and chromatograms are shown in Figure 16.

Repeatability

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working

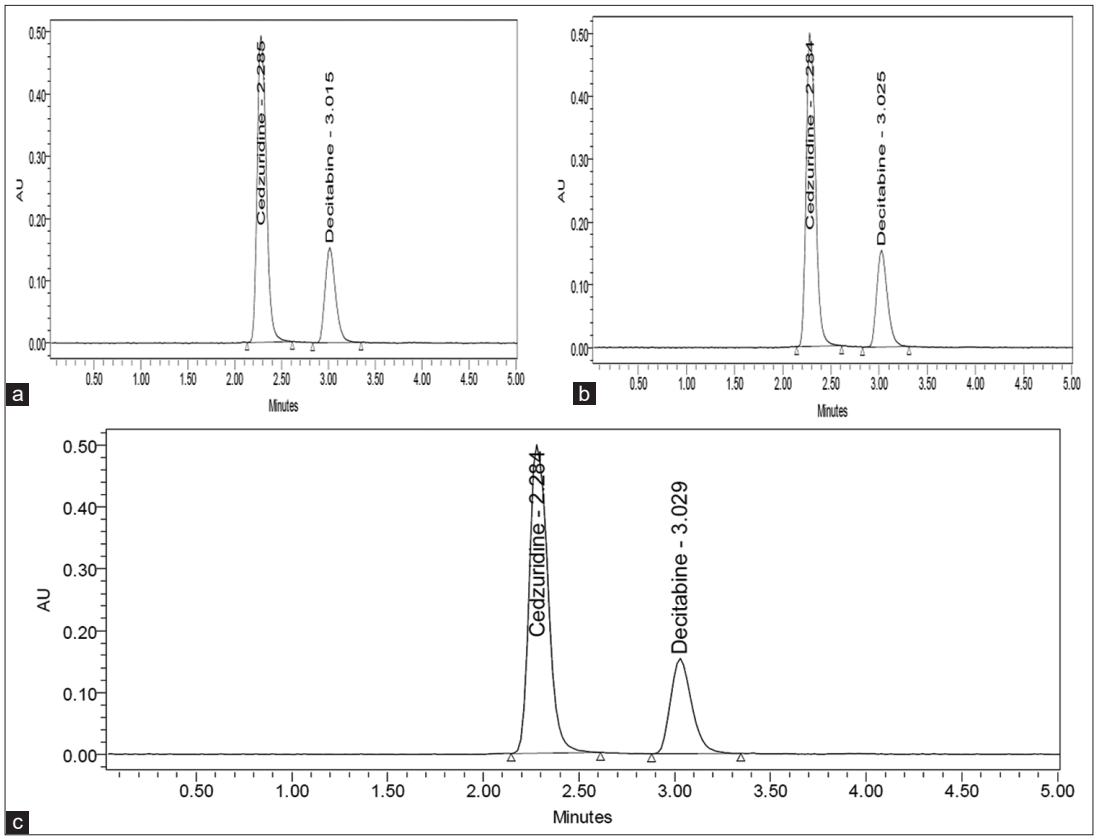

Figure 18: (a-c) Accuracy 50% chromatogram of cedazuridine and decitabine

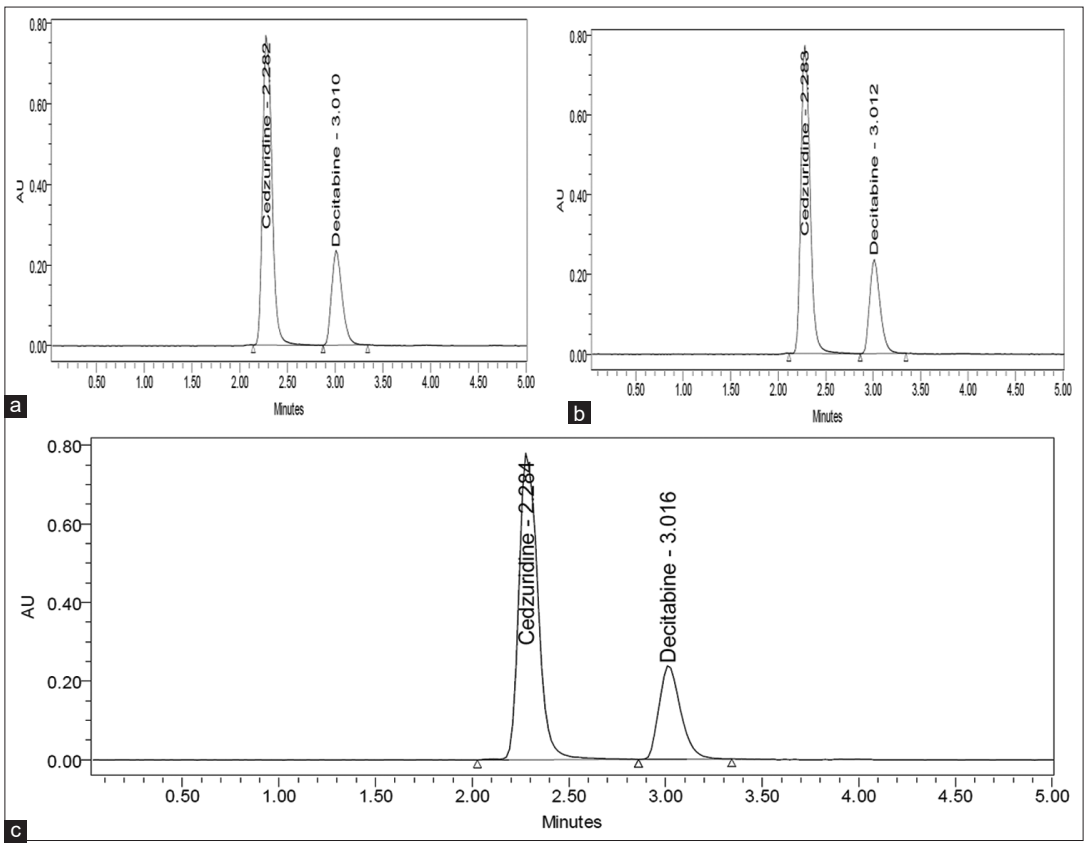

Figure 19: (a-c) Accuracy 100% chromatogram of cedazuridine and decitabine

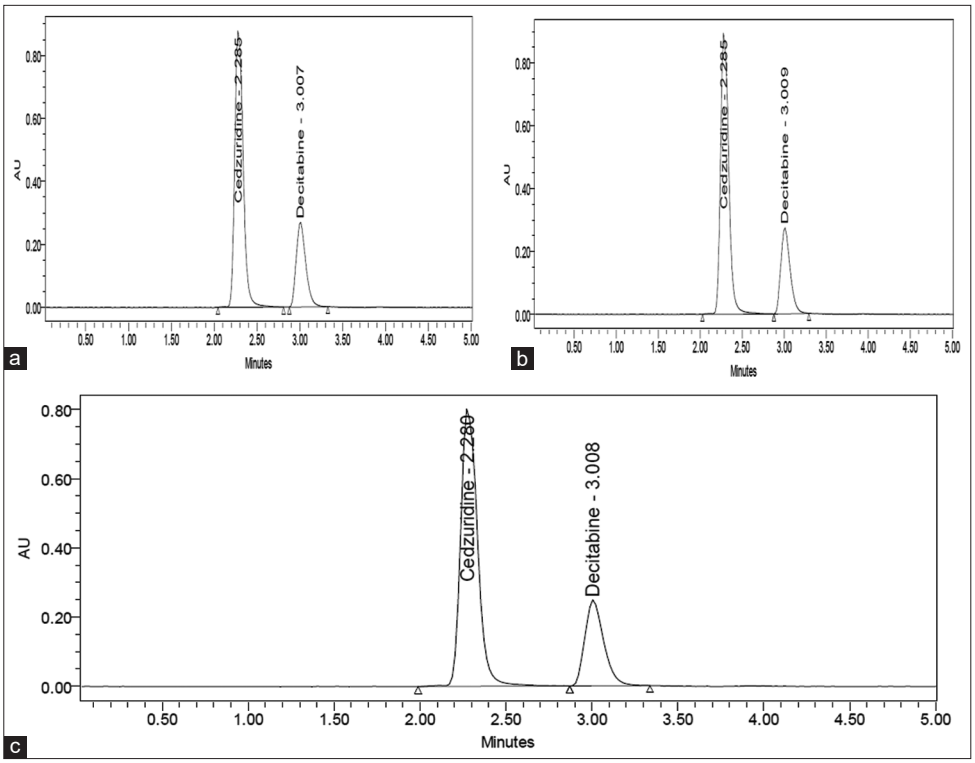

Figure 20: (a-c) Accuracy 150% chromatogram of cedazuridine and decitabine

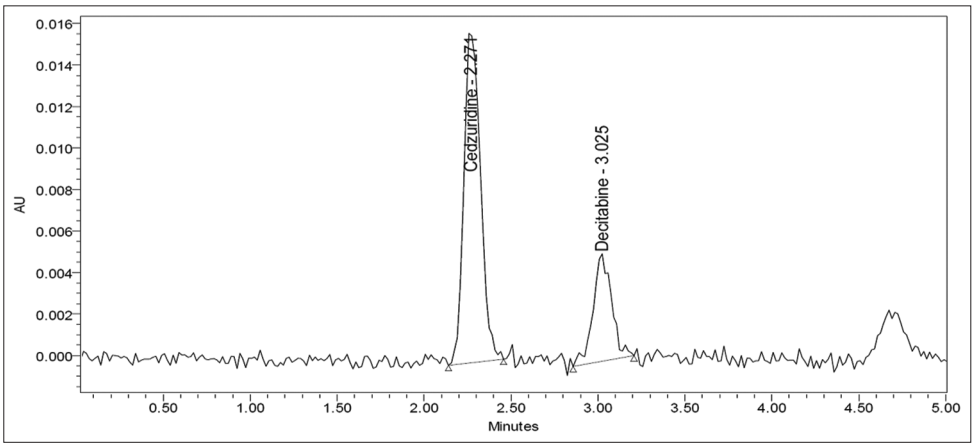

Figure 21: Limit of detection chromatogram of standard

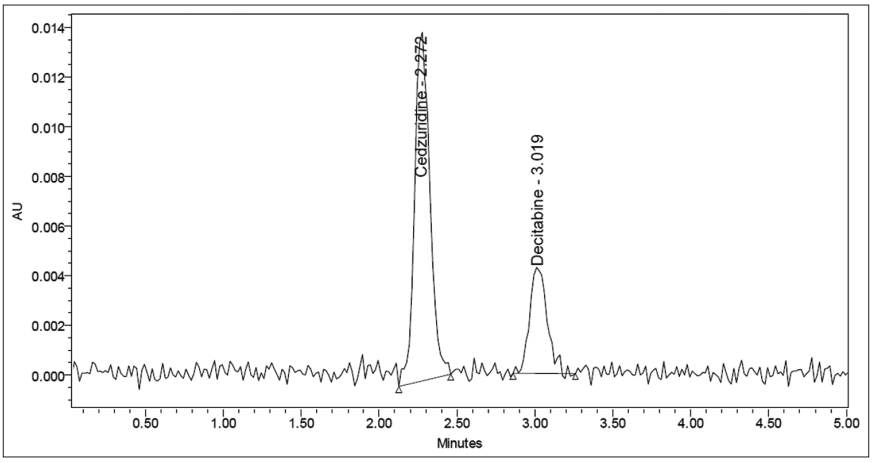

Figure 22: Limit of quantitation chromatogram of standard

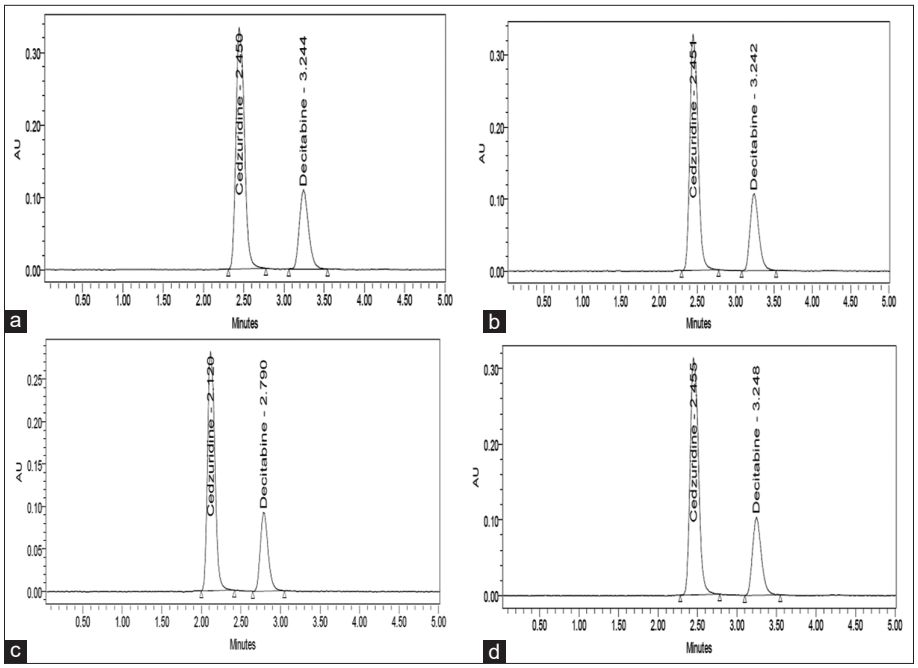

Figure 23: (a-d) Flow minus chromatogram of cedazuridine and decitabine

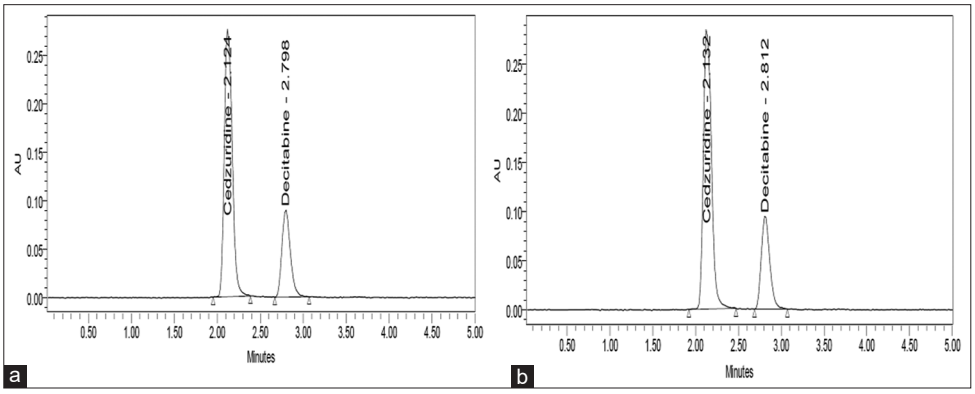

Figure 24: (a and b) Flow plus chromatogram of cedazuridine and decitabine

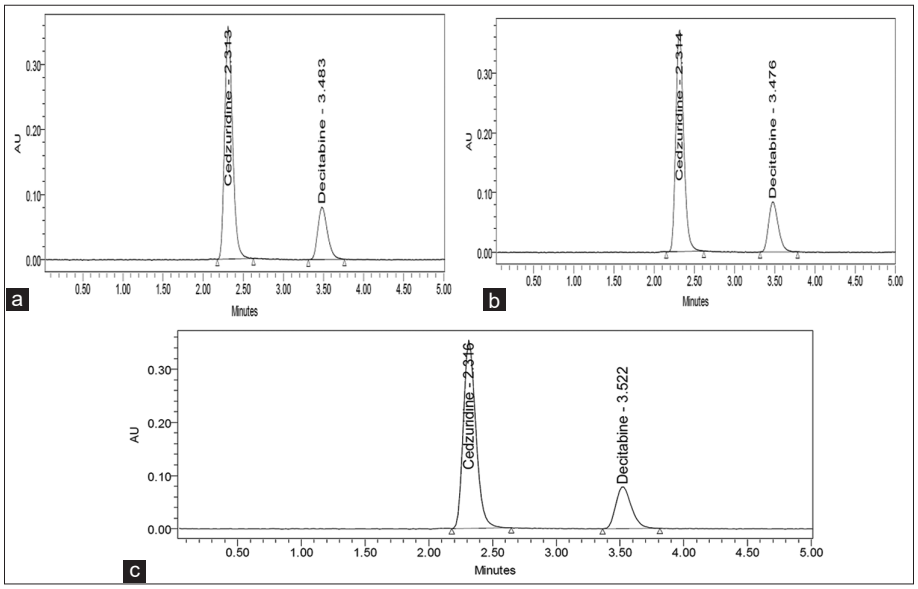

Figure 25: (a-c) Mobile phase minus chromatogram of cedazuridine and decitabine

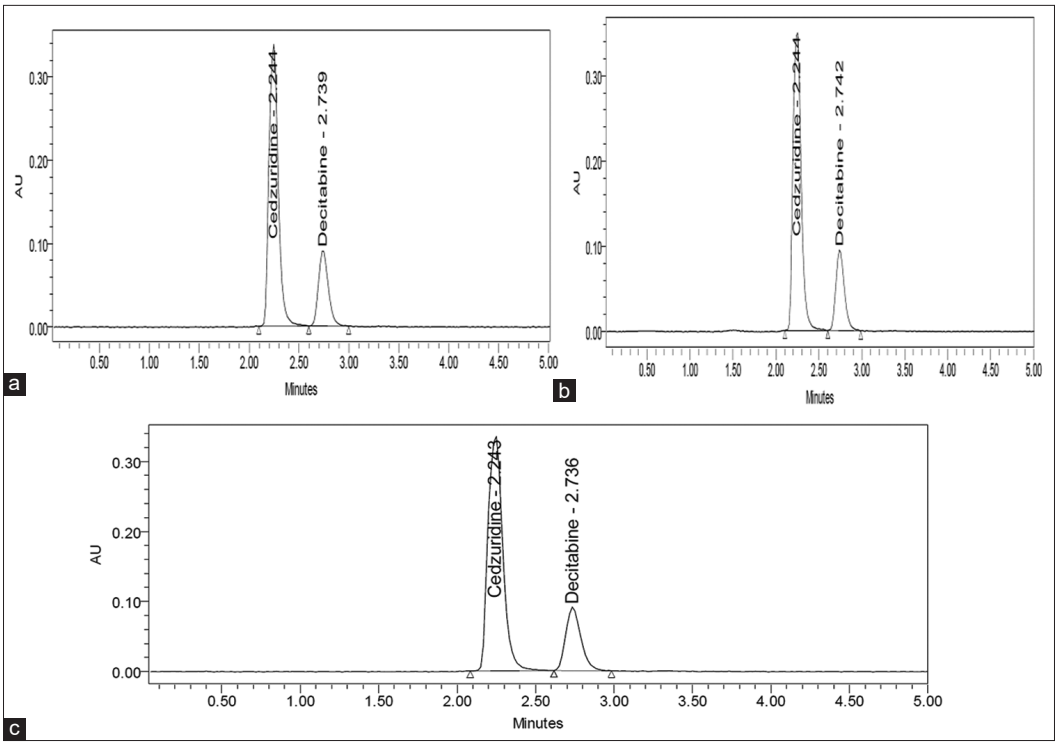

Figure 26: (a-c) Mobile phase plus chromatogram of cedazuridine and decitabine

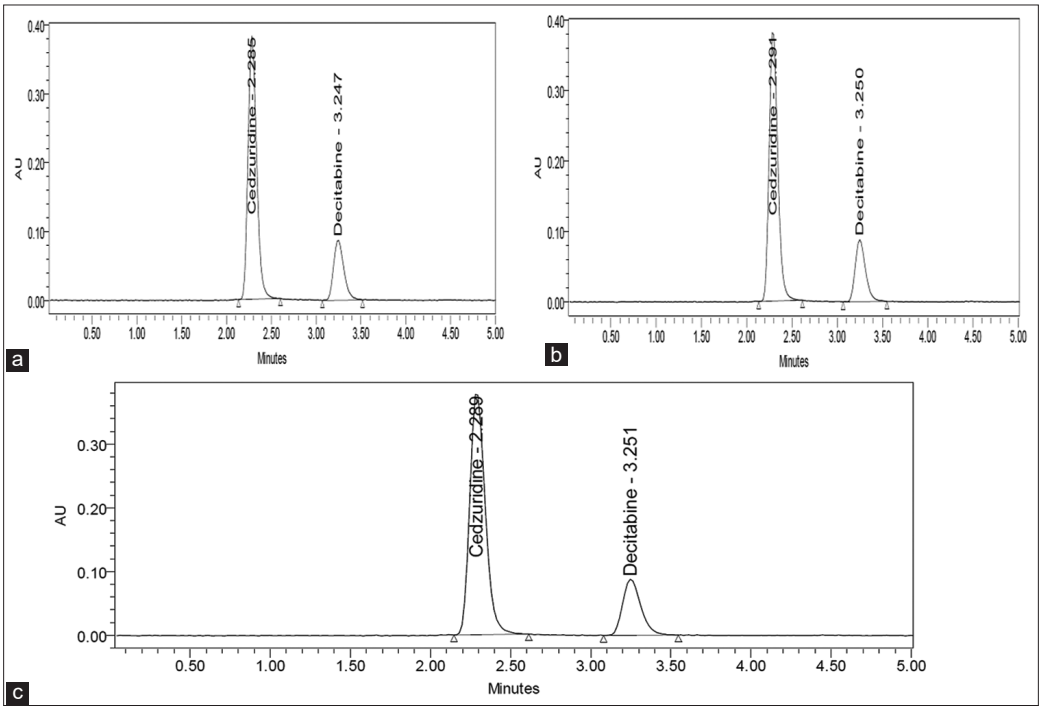

Figure 27: (a-c) Temperature minus chromatogram of cedazuridine and decitabine

sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation, and % RSD were calculated for two drugs and obtained as 0.5% and 0.8%, respectively, for cedazuridine and decitabine. As the limit of precision was < "2," the system precision was passed in this method. Results are shown in the Table 4 and chromatograms are shown in Figure 17.

Intermediate precision (day-day precision)

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area,

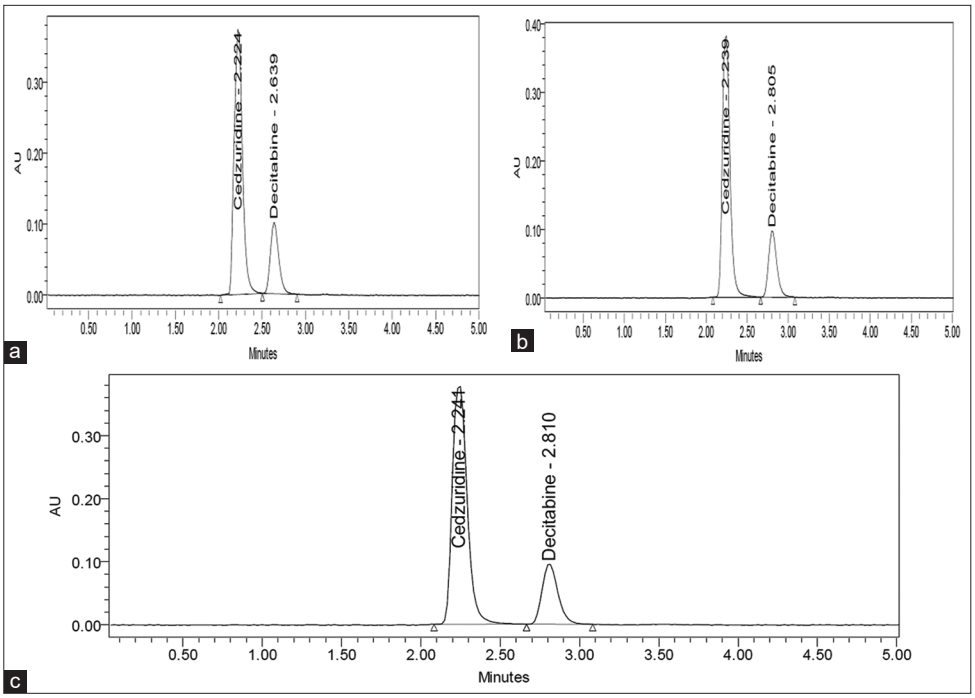

Figure 28: (a-c) Temperature plus chromatogram of cedazuridine and decitabine

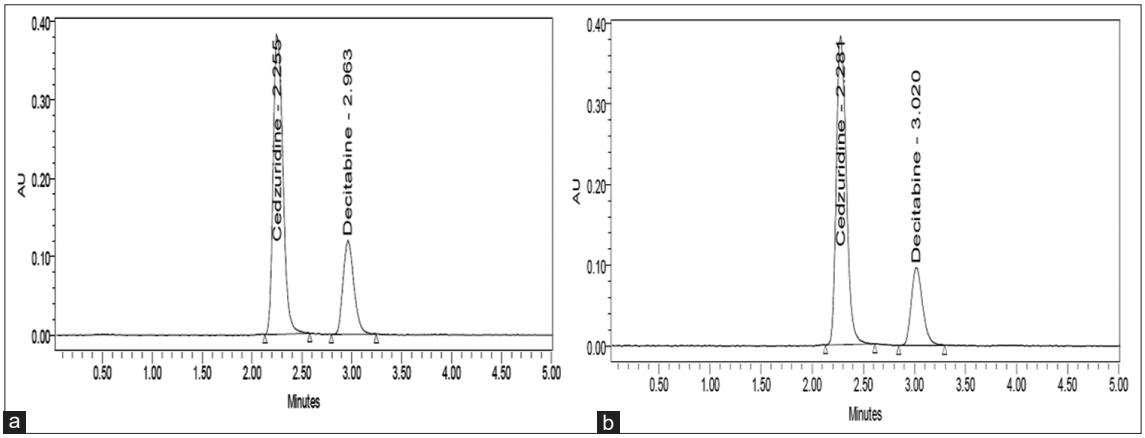

Figure 29: (a) Chromatogram of working standard solution. (b) Chromatogram of working sample solution

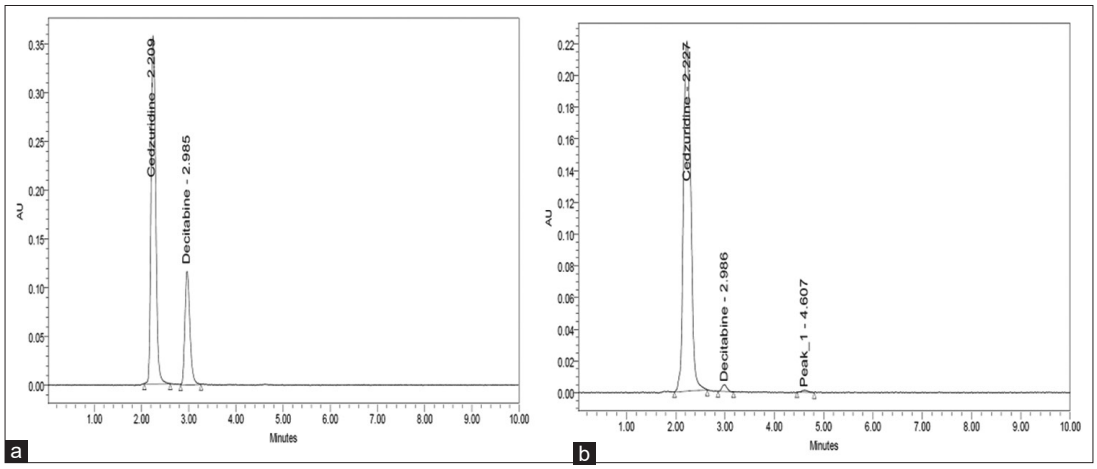

Figure 30: (a) Acid chromatogram of cedazuridine and decitabine. (b) Base chromatogram of cedazuridine and decitabine

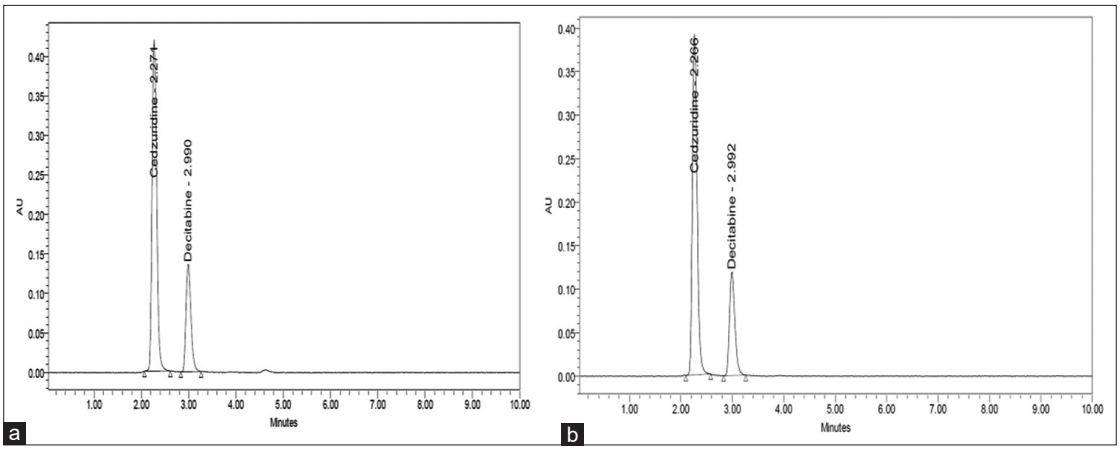

Figure 31: (a) Peroxide chromatogram of cedazuridine and decitabine. (b) Thermal chromatogram of cedazuridine and decitabine

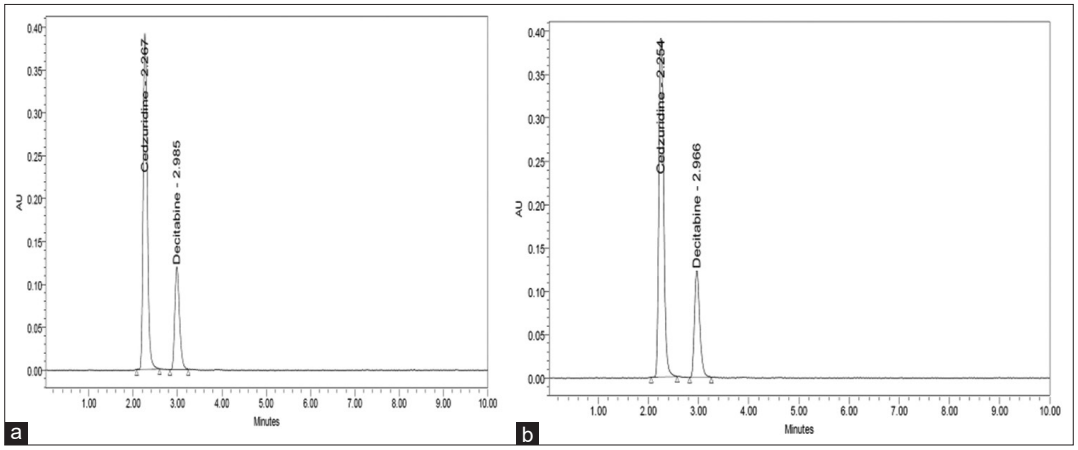

Figure 32: (a) UV chromatogram of cedazuridine and decitabine. (b) Water chromatogram of cedazuridine and decitabine

standard deviation, and % RSD were calculated for two drugs and obtained as 1.2% and 0.3%, respectively, for cedazuridine and decitabine. As the limit of precision was <"2," the system precision was passed in this method. Results are shown in the Table 5 and chromatograms are shown in Figure 18.

Accuracy

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 99.74% and 99.93% for cedazuridine and decitabine, respectively. Accuracy results are shown in the Tables 6 and 7 and chromatograms are shown in Figures 19-21.

Sensitivity

Sensitivity results are shown in the Table 8 and chromatograms are shown in the Figures 22 and 23.

Robustness

Discussion: Robustness conditions such as flow minus (0.9 mL/min), flow plus (1.1 mL/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C), and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Results are shown in the Table 9 and chromatograms are shown in Figures 24-29.

Assay: (INQOVI) Bearing the label claims cedazuridine 100 mg and decitabine 35 mg. Assay was performed with the above formulation. Average % assay for cedazuridine and decitabine obtained was 99.51 % and 99.99%, respectively and and results are shown in the Tables 10 and 11 and chromatograms are in Figures 30 and 31

Degradation

Degradation studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Results are shown in the Tables 12 and 13 and chromatograms are shown in Figures 30-34.

Summary Table

Parameters	Cedazuridine	Decitabine	Limit
Linearity range (µg/mL)	25-150 µg/mL	8.75-52.5 µg/ml	R<1
Regression coefficient	0.999	0.999	
Slope (m)	23,422	23,000	
Intercept (c)	9732	1638	
Regression equation (Y=mx+c)	y=23,422x+9732	y=23,000x+1638	
Assay (% mean assay)	99.51%	99.99%	90-110%
Specificity	Specific	Specific	No interference of any peak
System precision %RSD	0.4	0.8	NMT 2.0%
Method precision %RSD	0.5	0.8	NMT 2.0%
Accuracy %recovery	99.74%	99.93%	98-102%
LOD	0.51	0.18	NMT 3
LOQ	1.53	0.54	NMT 10
Robustness			
FM	0.7	0.7	%RSD NMT 2.0
FP	0.3	1.1	
MM	0.5	0.7	
MP	0.2	0.5	
TM	0.4	0.3	
TP	0.2	0.7	

LoD: Limit of detection, LoQ: Limit of quantitation, RSD: Relative standard deviation

Discussion: Regarding the pH adjustment in mobile phase for the acid and base, degradation studies have movement in retention time of drugs. However, due to neutralized acid sample with 2 N base solution and base sample with 2 N acid solution, there will be no change in retention time**.**

SUMMARY OF METHOD

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the cedazuridine and decitabine. Retention time of cedazuridine and decitabine were found to be 2.248 min and 2.956 min, respectively. %RSD of the cedazuridine and decitabine was found to be 0.5 and 0.8, respectively. % Recovery was obtained as 99.74% and 99.93% for cedazuridine and decitabine, respectively. Limit of detection and limit of quantitation values obtained from regression equations of cedazuridine and decitabine were 0.51, 1.53 and 0.18, 0.54, respectively. % Assay was obtained as 99.51% and 99.99% for cedazuridine and decitabine, respectively. Regression equation of cedazuridine is y = 23,422x + 9732 and y = 23,000x + 1638 of decitabine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in industries.

ACKNOWLEDGMENT

The authors are thankful to spectrum research solution, Hyderabad, for providing gift samples of cedazuridine and decitabine and special thankful to Sree Vidyanikethan College of Pharmacy to provide the facilities to complete this research work.

REFERENCES

1. Sharma BK. Instrumental methods of chemical analysis. In: Introduction to Analytical Chemistry. 23rd edition. Meerut: Goel publication; 2007.
2. Lindholm J. Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis; 2004. p. 13-4.
3. Rashmin. An introduction to analytical method development for pharmaceutical formulations. Indoglobal J Pharm Sci 2012;2:191-96.
4. Malvia R, Bansal V, Pal OP, Sharma PK. A review of high performance liquid chromatography. J Glob Pharma Technol 2010;2:22-6.
5. Skoog DA, Holler FJ, Niemen TA. Principles of Instrumental Analysis. United States: Cengage Learning; 2018; p. 725-60.
6. Ravi Shankar S. Text Book of Pharmaceutical Analysis. 4thed. United Kingdom: Churchill Livingstone;2010; p. 13.1-2.
7. Watson DG. Pharmaceutical Analysis: A Text Book

for Pharmacy Students and Pharmaceutical Chemists. 2nd ed. San Diego, California: Harcourt Publishers Limited; 2006;221-32.
8. Remington JP. Remingtonn's The Sciences and Practise of Pharmacy. 20th ed. United States: Lippincott Williams and Wilkins; 2000.
9. Connors KA. A Textbook of Pharmaceutical Analysis. 3rd ed. Delhi: Wiley Intersciences Inc; 1994. p. 373-421.

Source of Support: Nil. **Conflicts of Interest:** None declared.