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Research Article

**A NOVEL METHOD DEVELOPMENT AND VALIDATION OF
DECITABINE AND CEDAZURIDINE USING RP-HPLC
METHOD****Shaik Ejas*, Y. Rajendra, Khaja Zeeyauddin**
MAK College of Pharmacy, Moinabad, Hyderabad.**Article Received:** May 2022**Accepted:** May 2022**Published:** June 2022**Abstract:**

The objective of the current work is to develop a simple, efficient, economical and compatible RP-HPLC method for the analysis of Decidabine and cidazuridine in bulk, dosage forms. Samples were separated on Phenomenex luna C18, 150 mm X 4.6 mm, 5m column with mobile phase composed of Methanol: Buffer (pH 3.75 with Potassium dihydrogen ortho Phosphate in isocratic mode. The detection wavelength was fixed at 225nm. The retention time of Decidabine and cidazuridine was 4.1 min and the method showed a good linearity in the concentration range of 25-150µg/mL with a correlation coefficient of 0.999. The method was validated for specificity, linearity, limit of detection, limit of quantification, precision, robustness and stability. All the validation parameters were within the compendia requirements. The proposed method was successfully adopted for the analysis in bulk, pharmaceutical dosage forms.

Keywords: Decidabine and cidazuridine , Phenomenex luna Column, PDA Detection, RP HPLC

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INTRODUCTION:

HPLC is a condensing for High Performance Liquid Chromatography (It has similarly been depicted as High-Pressure LC). HPLC has been around for concerning 35 years as well just like the greatest separating strategy utilized.

HPLC is a partition technique that involves:

The infusion of a minuscule volume of liquid model squarely into a cylinder stacked with small amounts (3 to 5 micron (μm) in size called the decent stage).

Where individual pieces of the example are dropped down the stuffed cylinder (section) with a fluid (portable stage) expected through the segment by high strain conveyed by a siphon.

These parts are separated from one another by the section pressing that incorporates different synthetic or potentially actual associations between their atoms as well as the pressing pieces.

These separated components are identified at the leave of this cylinder (segment) by a course through gadget (indicator) that decides their amount. A result from this locator is known as a "fluid chromatogram In concept, LC and HPLC work similarly except the speed, effectiveness, sensitivity and simplicity of operation of HPLC is significantly exceptional.

HPLC COMPONENTS**1. Pump:**

The function of the heart is to require a fluid (called the moveable phase) with the runny chromatograph at a exact flow degree, spoken in mils per minutes (mL/min). Regular flow rates in HPLC remain in the 1-to 2-mL/min variety. Characteristic hearts can reach stress in the series of 6000-9000 psi (400-to 600-bar). During the chromatographic experiment, a pump can supply a consistent mobile phase structure (isocratic) or an enhancing mobile stage composition (slope).

2. Injector:

The injector helps to present the liquid example into the circulation watercourse of the moveable stage. Common example volumes are 5-to 20-microliters (μL). The injector necessity also be able to by attitude the tall weights of the fluid system. A car sampler is the automatic variation for when the customer has several examples to assess or when hands-on shot is not sensible.

3. Column:

Considered the "heart of the chromatograph" the column's stationary phase separates the example elements of interest utilizing various physical as well as chemical criteria. The little bits inside the column are what trigger the high back pressure at typical flow prices. The pump should press tough to relocate the moveable stage finished the column as well as this resistance triggers a high pressure within the chromatograph.

Sorts of columns

Analytical [inner size (i.d.) 1.0 -4.6- mm; sizes 15-- 250 mm] Preparative (i.d. > 4.6 mm; sizes 50-- 250 mm).

Capillary (i.d. 0.1 -1.0 mm; different sizes).

Nano (i.d.< 0.1 mm, or in some cases stated as < 100 μm).

4. Detector:

The identifier can see (recognize) the singular particles that come out (elute) from the segment. An indicator effectively gauges how much those atoms by the goalmouth that the scientist can quantitatively investigate the example parts. The indicator gives a result to a recorder or PC those outcomes in the fluid chromatogram (i.e., the diagram of the identifier reaction).

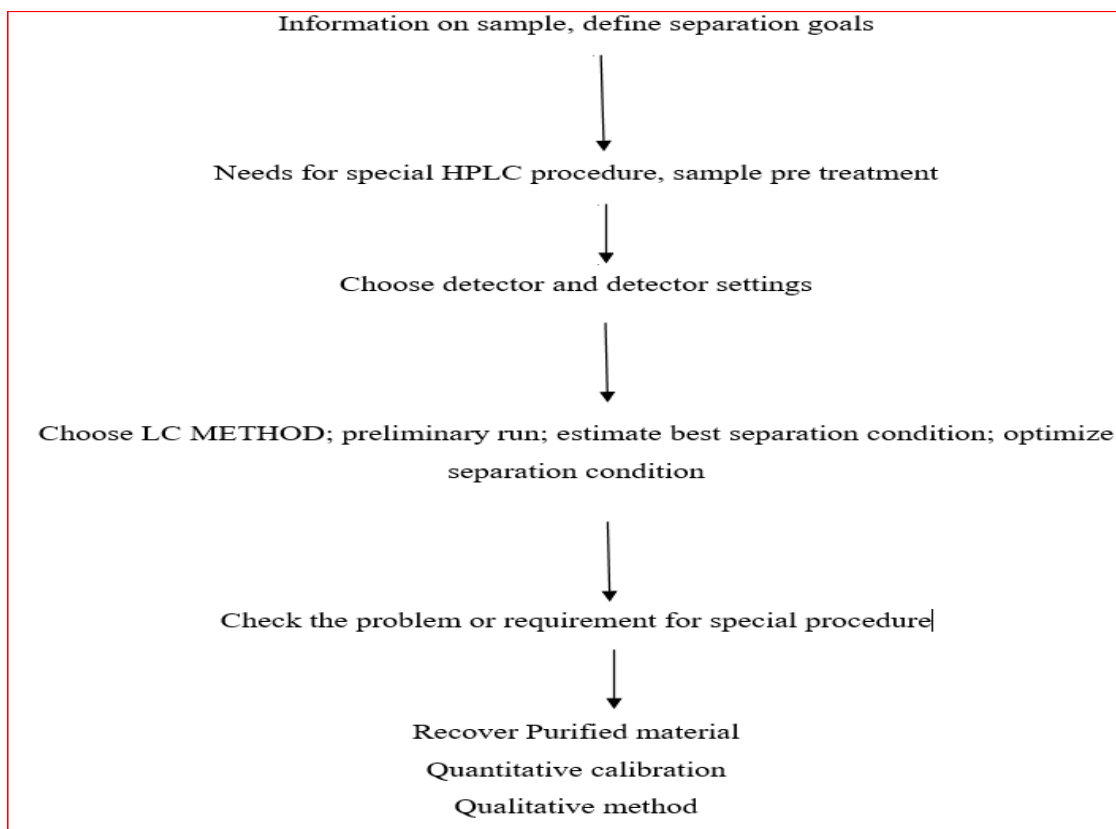
5. Computer:

Often called the information framework, the PC not just controls each ace of the modules of the HPLC instrument yet it takes the sign from the locator and utilizations it to decide the hour of elution (maintenance season) of the example parts (subjective investigation) and how much example (quantitative examination).

Advantages of HPLC

- It gives explicit, delicate, and exact strategy for examination of various muddled examples.
- There is simplicity of test planning and test presentation.
- Speed of investigation
- Investigation by HPLC is explicit, exact and exact.
- Proposals benefit over gas chromatography in investigation of numerous glacial, ionic materials, metabolic items and thermolabile as well as non-unpredictable substances.

METHOD DEVELOPMENT



Method validation is the interaction used to affirm that the scientific strategy utilized for a particular test is reasonable for its expected use. Results from technique approval can be utilized to pass judgment on the quality, dependability, and consistency of logical outcomes; it is a rudimentary part of any great insightful practice.

As indicated by ICH Guidelines Authentication of a Logical system is to exhibit that it is reasonable for its expected reason.

Endorsement of logical techniques is facilitated to the four most ordinary sorts of legitimate system:

- Recognizing evidence tests;
- Quantitative tests for contaminations' substance;
- Limit tests for the control of contaminations;
- Quantitative preliminary of the powerful moiety in instances of prescription substance or drug thing or other picked component(s) in the medicine thing.

Normal approval attributes which ought to be careful are recorded beneath:

- Accuracy

- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Accuracy

The accuracy of an intelligent procedure imparts the closeness of course of action between the value which is recognized either as a standard certifiable worth or a recognized reference regard and the value found. This is to a great extent named validity.

Repeatability

Repeatability conveys the exactness under comparable working conditions all through a short stretch of time. Repeatability is moreover named intra-test precision.

Reproducibility

Reproducibility imparts the exactness between research focuses (agreeable examinations, ordinarily applied to standardization of approach).

Recognition LIMIT

The ID farthest reaches of an individual logical strategy is the most insignificant proportion of analyte in a model which can be perceived anyway not actually quantitated as an exact worth.

QUANTITATION LIMIT

The quantitation farthest reaches of an individual legitimate technique is minimal proportion of analyte in a model which can in any case hanging out there with proper exactness and accuracy. Quite far is a limit of quantitative analyzes for low levels of blends in model systems, and is used particularly for the confirmation of contaminations and also corruption things.

LINEARITY

The linearity of a quick system is its ability (inside a given reach) to get test results which are clearly relating to the obsession (proportion) of analyte in the model.

RANGE

The extent of a smart procedure is the stretch between the upper and lower center (proportions) of analyte in the model (counting these obsessions) for which it has been displayed that the logical strategy has a healthy level of precision, precision and linearity.

2. REVIEW OF LITERATURE

1. **B. Mohammed Ishaq** et., alia developed technique was more put on observe the destruction of analytes drunk of various forced deterioration conditions. Analytes were fixed on C18, 250 x 4.6 mm, fragment dimension 5 μm Xterra column, using a mobile stage combination of 0.1% Ortho Phosphoric Acid barrier pH 6.5: Methanol (40:60 v/v) with circulation rate of 1mL/min and shot volume of 10 μL . Metrology was accomplished with personal organizer detector at an isosbestic point of 220 nm with a straight calibration contour in the concentration series of 35-175 $\mu\text{g}/\text{mL}$ for DEC and also 100-500 $\mu\text{g}/\text{mL}$ for CED. precision, precision, as well as toughness. The limits of discovery (LOD) and the limits of quantification (LOQ) for CED were discovered to be 2.69 $\mu\text{g}/\text{mL}$ and also 8.15 $\mu\text{g}/\text{mL}$ respectively. LOD as well as LOQ for DEC 1.55 $\mu\text{g}/\text{mL}$ and 4.68 $\mu\text{g}/\text{mL}$ respectively. Additionally, verified approach was put on examine the degradation profile of analytes under various stress and anxiety destruction conditions. Verdict: The suggested approach was located to be sensitive, details and also was efficiently looked for the synchronised estimation of Decitabine (DEC) as well as Cedazuridine (CED) wholesale drug, and also tablet computers.

2. **PRABAHARAN.P** et al for the decision of Decitabine was chosen at 244 nm. Different hearings remained performed with different mobile stages in various ratios, but Ammonium Acetate barrier pH 4.5: ACN was picked as excellent peak symmetry. The Holding period of decitabine was discovered to be 3.786 min. The different analytical performance parameters such as linearity, accuracy, accuracy, and uniqueness, LOD, LOQ were figured out according to International Meeting on Harmonization ICH Q2B guidelines. The calibration curves were gotten by plotting peak location versus the focus over the variety of 50-150 $\mu\text{g}/\text{mL}$. From linearity the correlation coefficient R2 value was discovered to be 0.998. The future HPLC technique was additionally confirmed for system suitability, system precision and technique precision. The % RSD in the peak location of medication was located to be less than 2%. The number of academic plates was discovered to be greater than 2000, which indicates reliable efficiency of the column. The LOD for this technique was discovered to be 0.0003 $\mu\text{g}/\text{mL}$. The LOQ for this approach was located to be 0.0009 $\mu\text{g}/\text{mL}$, shows the level of sensitivity of the technique. The percentage of recuperation of was discovered to be 99.77 programs that the suggested method is extremely accurate

3.PLAN OF WORK

Weight problems has become one of the life-threatening illnesses in both created as well as in some parts of developing nations of the globe for which proper treatment needs to be required to protect against the threat of some of the harmful illness like atherosclerosis etc. So in our project we are trying to approximate Decitabine and also Cedazuridine (An anti-weight problems medication) wholesale as well as pharmaceutical dose types by RP-HPLC which is more easy quick accurate precise approach.

IN OUR APPROACH OF 'EVALUATION OF DECITABINE AS WELL AS CEDAZURIDINE, WE HAVE ACTUALLY WORKED ON THE FOLLOWING CRITERIA.

1. Precision
2. Accuracy
3. L.O.D.
4. L.O.Q.
5. Linearity.
6. Array.
7. Effectiveness.

STAGES OF INTENDED WORK.

1. To assess literature.

- To pick correct solvent system for evaluation.
- To establish chromatographic methods for single element formulation.
- To verify the established methods based on the ICH standards.


4. INTRO TO DRUG ACCOUNT.

Decitabine and also Cedazuridine is a novel artificial statin,

Mechanism of action.

Decitabine as well as Cedazuridine competitively hinders HMG-CoA reductase, which is a rate-determining enzyme included with biosynthesis of cholesterol, in a manner of competition with the substrate so that it inhibits cholesterol synthesis in the liver. Because of this, the expression of LDL-receptors complied with by the uptake of LDL from blood to liver is accelerated and afterward that the plasma TC lowers. Better, the continual inhibition of cholesterol synthesis cutting-edge the liver reduces levels of extremely reduced density lipoproteins.

Table 1.7 Structural features of Decitabine and Cedazuridine

| Official Name | Chemical Name | Structure |
|---------------|---|---|
| Decitabine | (3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3,5-dihydroxyhept-6-enoic acid. |  |
| Cedazuridine | (4R)-2'-deoxy-2',2'-difluoro-3,4,5,6-tetrahydropyrimidin-5(1H)-one, (4R) | |

Characteristic profile of Decitabine and Cedazuridine

| Drug | Decitabine and Cedazuridine |
|----------------------|--|
| Molecular formula | C ₈ H ₁₂ N ₄ O ₄ , C ₉ H ₁₄ F ₂ N ₂ O ₅ |
| Molecular weight | 228,268 |
| CAS number | 2353-33-5,1141397-80-9 |
| Colour | white crystalline powder |
| Odour | Odorless |
| Taste | Bitter |
| Appearance | Amorphous Hygroscopic powder |
| Melting range | 200°C,162-165°C. |
| Solubility | freely soluble in pyridine, chloroform, dilute hydrochloric acid, and tetrahydrofuran |
| Therapeutic category | Used to treat chronic myelomonocytic leukemia. |

Marketed formulations of Decitabine and Cedazuridine (Tablet formulations)

1. Dacogen 1 mg,35mg

2. INQOVI 1 mg,35 mg,100 mg decitabine and also cedazuridine Each film-coated tablet of INQOVI contains 1.045 mg, 2.09 mg, or 4.18 mg of Decitabine and also Cedazuridine calcium, which is equivalent to 1 mg, 2 mg, or 4 mg, respectively of free base and the

adhering to non-active ingredients: lactose monohydrate, reduced substituted hydroxy propyl cellulose, hypromellose, magnesium luminometer silicate, magnesium stearate, and also movie finishing having the following inactive components: hypromellose, titanium dioxide, triethyl citrate, and colloidal anhydrous silica

Reported methods for Decitabine and Cedazuridine.

| S. No | Type of method | Matrix | Solvent suitability | Detector wavelength(nm) |
|-------|-------------------|--------------------|--|-------------------------|
| 1. | UPLC | Tablet | Methonal:Acetonitrile (20:80) | 246 |
| 2. | LC-TMS | Human plasma-urine | Methonal-0.2%aceticacid in water(70:30v/v) | |
| 3 | Spectrophotometry | Tablet | Acidic potassium permanganate | 551 |
| 4 | HPTLC | Tablet | Toluene: methonal:glacial acetic acid(7.6:2.36:0.04v/v) | 239 |
| 5 | HPLC-ESI-MS/MS | Human plasma | | |
| 6 | HPTLC | Tablet | Ethylacetate-methonol-ammonia-1 drop of formic acid (7:2:0.8) | 246 |
| 7 | HPLC | Tablet | 0.5%aceticacid: acetonitrile(35:65v/v) | 246 |
| 8 | Spectrophotometry | Tablet | Ferric chloride in presence of o-phenanthroline or 2,2'bipyridyl or potassium ferricyanide | 511,531,756 |
| 9 | LC | Tablet | Orthophosphoric acid: ACN: triethylamine(19.8:80:0.2v/v)Ph 3 | 236 |

5. EXPERIMENTAL SECTION: OPTIMIZED METHOD

Buffer:

Transfer 1.36 gr of potassium di hydrogen ortho Phosphate in to a 1000ml beaker. Add regarding 800ml of milli-Q water and sonicate to degas and make up to final quantity and pH adapted to 3.75 with water down orthophosphoric acid solution.

Mobile phase:

Buffer and Acetonitrile are taken in 20:80% v/v, sonicated to degas.

Chromatographic conditions:

| | | |
|-----------------------------|---|--|
| Flow rate | : | 1.2 mL/min |
| Column | : | Phenomenex luna C18, 150 x 4.6 mm, 5m. |
| Detector wave length | : | 248 nm |
| Column temperature | : | 30°C |
| Injection volume | : | 10mL |
| Run time | : | 10 min |
| Diluent | : | Methanol |

EXPERIMENTAL

Decitabine as well as Cedazuridine API was acquired as present sample from Range pharma study services. Potassium dihydrogen Orthophosphate and also Orthophosphoric acid were of analytical quality supplied by Rankem, Mumbai. Acetonitrile and water made use of were of HPLC quality. Readily available Decitabine as well as Cedazuridine tablet computers were procured from regional Drug store.

Preparation of Characteristic standard facility

A common supply remedy of Decitabine and also Cedazuridine was ready by dissolving 10 mg each of Decitabine and also Cedazuridine in a 10ml clean completely dry volumetric flask, 7mL of Methanol was added by way of healthy by way of sonicated for around 10min and after that made up to 10mL with Methanol to obtain a 1 µg/ mL conventional stock remedy.

Calibration contour were prepared by dilution of above supply solution in the variety of 25 µg/ mL- 150 µg/ mL.

Table 2.0 Preparation of Standard stock solution

| S.No | Pipetted from stock (mL) | Volume of flask (mL) | Concentration in ppm |
|------|--------------------------|----------------------|----------------------|
| 1 | 0.25 | 10 | 25 |
| 2 | 0.5 | 10 | 50 |
| 3 | 0.75 | 10 | 75 |
| 4 | 1.0 | 10 | 100 |
| 5 | 1.25 | 10 | 125 |
| 6 | 1.50 | 10 | 150 |

Preparation of Sample solution:

Tag Insurance Claim: 1mg of Decitabine and also Cedazuridine Five tablet computers having 1 mg of Decitabine and Cedazuridine were evaluated and after that Powdered. A quantity of powder matching to 1 mg of Decitabine as well as Cedazuridine and was moved in a 100 mL volumetric flask, with 70mL of methanol and also sonicated for 25min, to make certain complete solubility of the medicine, and quantity made up with the diluent (Methanol) and also filtered through 0.45 µm membrane filter. From this 1ml was pipetted out and moved to 10 mL volumetric flask and made up to 10mL by Methanol to get the focus of 1 µg/ mL of Decitabine as well as Cedazuridine.

Trail 1: Chromatographic conditions

| | |
|--------------------|--|
| Column | Phenomenex luna C18, (150 x 4.6 mm, 5mm) |
| Flow rate | 1.2 mL/min |
| Wavelength | 220 nm |
| Column temperature | 30°C |
| Injection volume | 10mL |
| Run time | 10 min |
| Diluent | Methanol |
| Mobile phase | Water and methanol 50:50v/v |
| Retention time | 3.55 min |

Trail 2: Chromatographic conditions

| | |
|--------------------|---|
| Column | Phenomenex luna C18, (150 x 4.6 mm, 5mm) |
| Flow rate | 1.2 mL/min |
| Wavelength | 220 nm |
| Column temperature | 30°C |
| Injection volume | 10mL |
| Run time | 10 min |
| Diluent | Methanol |
| Mobile phase | Buffer and Acetnitrile are taken in 50:50% v/v, sonicated to degas. |
| Retention time | 3.748 |

| | |
|--------------------|---|
| Column | Phenomenex luna C18, (150 x 4.6 mm, 5mm) |
| Flow rate | 1.2 mL/min |
| Wavelength | 248 nm |
| Column temperature | 30°C |
| Injection volume | 10mL |
| Run time | 10 min |
| Diluent | Methanol |
| Mobile phase | Buffer and Acetnitrile are taken in 20:80% v/v, sonicated to degas. |
| Retention time | 4.1 min |

6. OUTCOMES AS WELL AS CONVERSATION

METHOD RECOGNITION

The technique was validated for the Parameters like linearity, precision, restriction of discovery (LOD), restriction of quantification (LOQ), Accuracy, Specificity, toughness based upon ICH/CPMP guidelines (14-16).

PRECISION

Precision was determined in terms of repeatability of application and measurement. Repeatability of conventional application remained approved out using six reproduces of very same typical concentration. Repeatability of example measurement remained approved out in six different sample preparations from same homogenous blend of significant sample. The results of Precision research studies are revealed in Table 2.1

Table 2.1: Method precision of Decitabine and Cedazuridine

| Sample preparation | % Assay Decitabine and Cedazuridine |
|--------------------|-------------------------------------|
| 1 | 100.72 |
| 2 | 100.81 |
| 3 | 100.41 |
| 4 | 100.19 |
| 5 | 101.20 |
| 6 | 103.20 |
| Mean | 101.08 |
| ± SD | 0.995987 |
| % RSD | 0.89 |

The % RSD for repeatability of sample preparation is 0.89%. This shows that exactness of the technique is satisfactory as % relative standard deviation is not more than $\pm 2.0\%$ ⁽¹⁷⁾

chromatograms of method precision

| | Top Designation | RT | Part | USP Bowl Count | USP Tailing |
|---------|-----------------------------|-------|---------|----------------|-------------|
| 1 | Decitabine and Cedazuridine | 4.061 | 2687218 | 7756 | 1.07 |
| 2 | Decitabine and Cedazuridine | 4.127 | 2689612 | 7755 | 1.06 |
| 3 | Decitabine and Cedazuridine | 4.144 | 2678915 | 7745 | 1.06 |
| 4 | Decitabine and Cedazuridine | 4.145 | 2673009 | 7691 | 1.06 |
| 5 | Decitabine and Cedazuridine | 4.145 | 2699978 | 7684 | 1.06 |
| 6 | Decitabine and Cedazuridine | 4.152 | 2755090 | 7657 | 1.07 |
| Mean | | | 2697304 | | |
| Std.Dev | | | 29785.0 | | |
| %RSD | | | 1.1 | | |

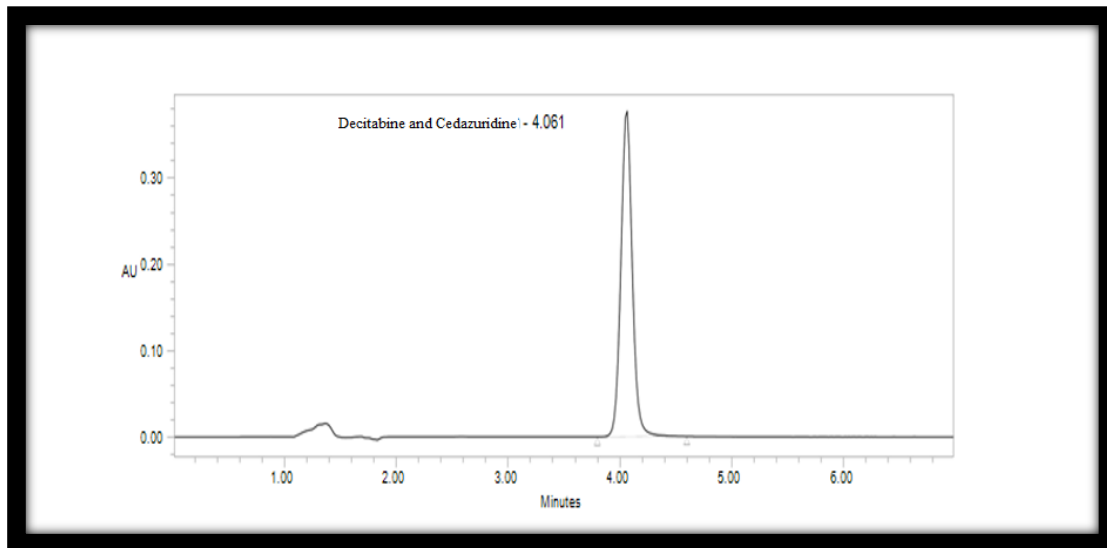


Table 2.2: Precision results

Accuracy

Precision of the approach was determined by evaluation of requirement at 3 various degrees. Values were located to be within the limit given up. The mean recuperation remained in the series of 99.43-100.54% which reveals there is no disturbance from excipients.

$$\% \text{ Recovery} = b-a/c \times 100.$$

Where,.

- a = The amount of medication located prior to the addition of basic drug.
- b = The quantity of medication discovered after the enhancement of typical medicine.
- c = The amount of basic medication added.

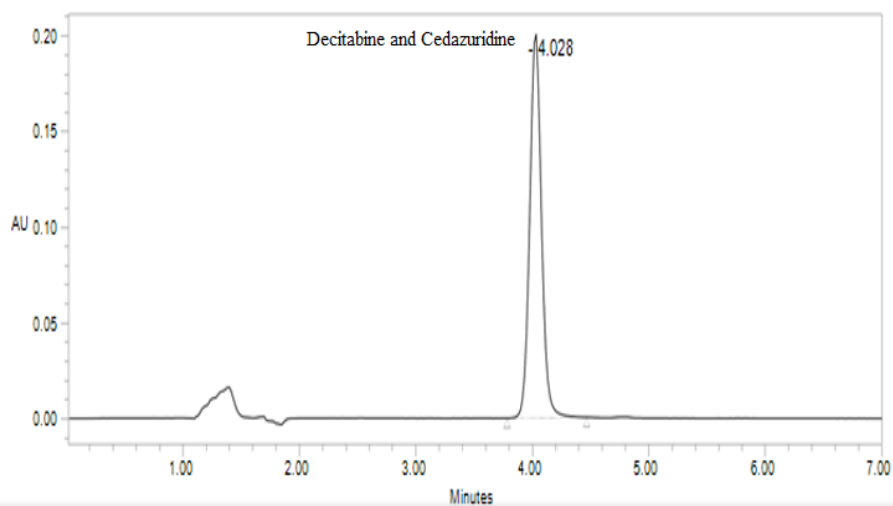
Table2.3: Recovery studies of Decitabine and Cedazuridine

| concentration | Sample preparation | % Recovery of Decitabine and Cedazuridine | AVG | ±SD | %RSD |
|---------------|--------------------|---|--------|-------|-------|
| | 1 | 99.54 | | | |
| 50% | 2 | 99.15 | 99.43 | 0.245 | 0.246 |
| | 3 | 99.61 | | | |
| | 1 | 99.17 | | | |
| 100% | 2 | 100.84 | 100.06 | 0.836 | 0.835 |
| | 3 | 100.16 | | | |
| | 1 | 100.98 | | | |
| 150% | 2 | 99.88 | 100.54 | 0.58 | 0.57 |
| | 3 | 100.76 | | | |

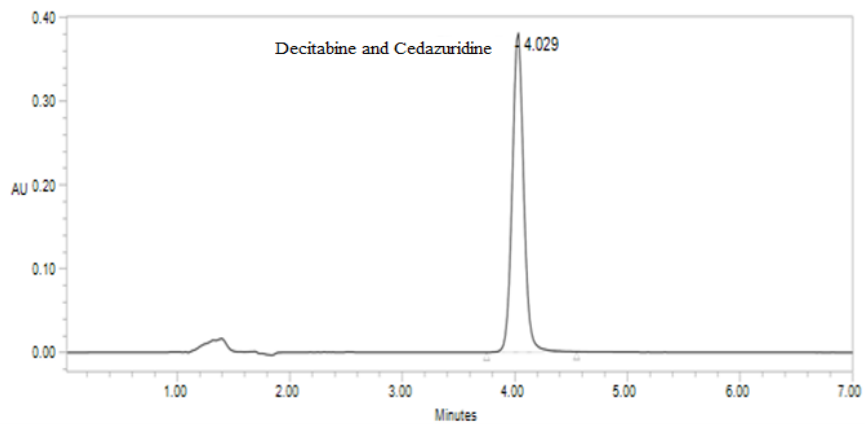
| | Highest Designation | RT | Part | USP Count | Bowl | USP Tailing |
|---|-----------------------------|-------|---------|-----------|------|-------------|
| 1 | Decitabine and Cedazuridine | 4.029 | 1327757 | 7817 | | 1.08 |
| 2 | Decitabine and Cedazuridine | 4.036 | 1327284 | 7894 | | 1.08 |
| 3 | Decitabine and Cedazuridine | 4.045 | 1328785 | 7857 | | 1.08 |

Figure 2.9: chromatograms of Accuracy

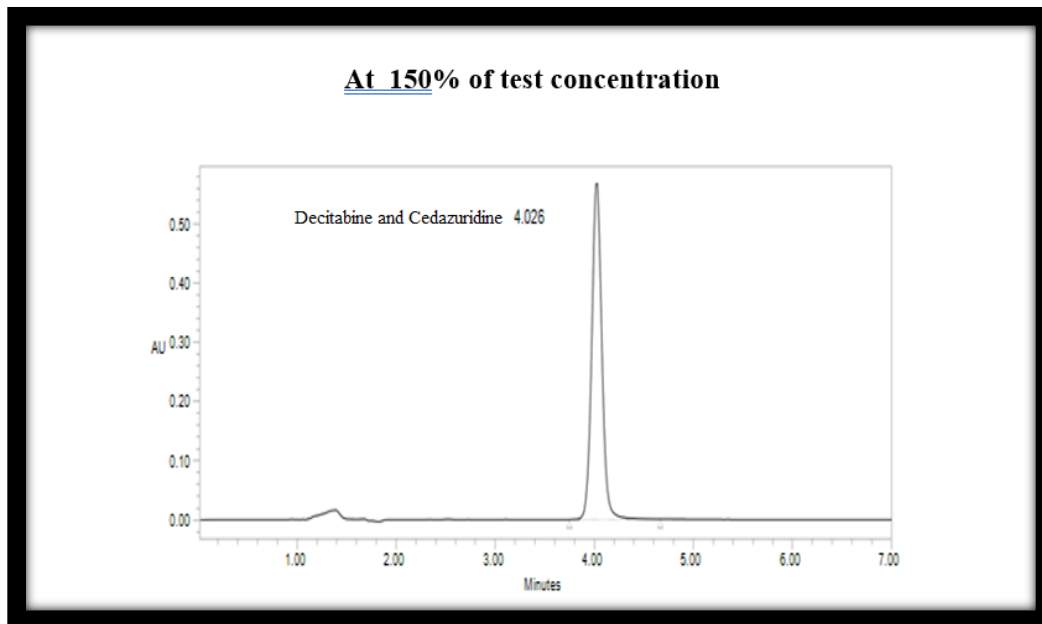
At 50% of test concentration



At 100% of test concentration



| | Top Designation | RT | Part | USP Count | Bowl | USP Tailing |
|---|-----------------------------|-------|---------|-----------|------|-------------|
| 1 | Decitabine and Cedazuridine | 4.022 | 2645822 | 7831 | | 1.08 |
| 2 | Decitabine and Cedazuridine | 4.039 | 2690199 | 7743 | | 1.07 |
| 3 | Decitabine and Cedazuridine | 4.037 | 2672138 | 7933 | | 1.07 |



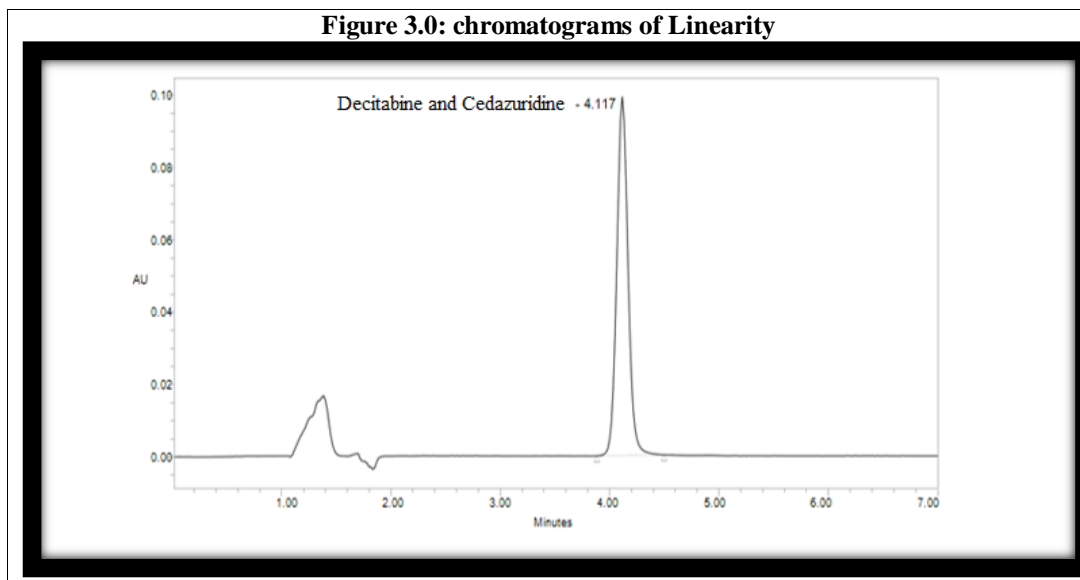
| | Top Designation | RT | Part | USP Count | Bowl | USP Tailing |
|---|-----------------------------|-------|---------|-----------|------|-------------|
| 1 | Decitabine and Cedazuridine | 4.027 | 4041058 | 7767 | | 1.06 |
| 2 | Decitabine and Cedazuridine | 4.038 | 3997273 | 7871 | | 1.07 |
| 3 | Decitabine and Cedazuridine | 4.047 | 403237 | 7774 | | 1.07 |

Linearity

Aliquots of typical Decitabine and also Cedazuridine supply solution remained occupied in dissimilar 10ml volumetric flasks as well as weakened approximately the mark with the diluents such that the final focus of Decitabine and also Cedazuridine are in the variety of 25-150 $\mu\text{g}/\text{mL}$. Each of these drug solutions (10 μL) was injected in to the column, and the peak location and retention time were tape-recorded. Assessment was done with PDA detector at 248nm and a Correction graph remained got by plotting peak location versus attentiveness of Decitabine as well as Cedazuridine. The story of Peak location of each example versus respective focus of Decitabine and Cedazuridine was found linear in the variety of 25-150 $\mu\text{g}/\text{mL}$ with correlation coefficient of 0.999. Direct reversion least square fit data learnt from the capacities are given in table3. The own linear regression formula being $Y = 26504x + 152930$. The regression qualities were determined for this approach as well as given up.

Linear regression data for calibration curves

| | |
|-------------------------|------------------------------------|
| Drug | Decitabine and Cedazuridine |
| Linearity Range | 25 -150 µg/mL |
| Slope(m) | 265040 |
| Y Intercept(b) | 152930 |
| Correlation coefficient | 0.9998 |

**Linearity 25%**

| | Peak Name | | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-----|-----------|-------------|--------------|------------------------|--------------------|
| 1 | Decitabine and Cedazuridine | and | 4.118 | 706789 | 100.00 | 7923 | 1.08 |

Linearity 50%

| | Peak Name | | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-----|-----------|-------------|--------------|------------------------|--------------------|
| 1 | Decitabine and Cedazuridine | and | 4.118 | 706789 | 100.00 | 7923 | 1.08 |

Linearity 75%

| | Peak Name | | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-----|-----------|-------------|--------------|------------------------|--------------------|
| 1 | Decitabine and Cedazuridine | and | 4.032 | 1969939 | 100.00 | 7959 | 1.08 |

Linearity 100%

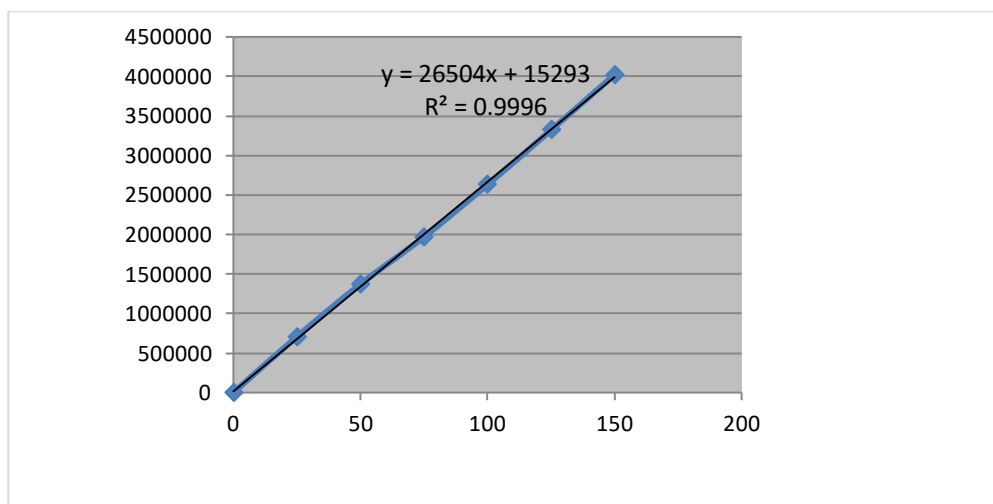
| | Peak Name | | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-----|-----------|-------------|--------------|------------------------|--------------------|
| 1 | Decitabine and Cedazuridine | and | 4.042 | 2631647 | 100.00 | 7731 | 1.08 |

Linearity 125%

| | Peak Name | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-------|---------|--------|-----------------|-------------|
| 1 | Decitabine and Cedazuridine | 4.048 | 3320339 | 100.00 | 7847 | 1.08 |

Linearity 150%

| | Peak Name | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-------|---------|--------|-----------------|-------------|
| 1 | Decitabine and Cedazuridine | 4.062 | 4021882 | 100.00 | 7829 | 1.07 |

Fig 3.1: Calibration curve of Decitabine and Cedazuridine by HPLC**Limit of Discovery as well as Restriction of Metrology**

The level of sensitivity of measurement of Decitabine and Cedazuridine by use of proposed approach was estimated in footings of the limit of metrology (LOQ) by way of healthy by way of the most affordable concentration identified under the chromatographic problem as the limit of discovery (LOD). The LOQ as well as LOD were calculated by the use of formulas $LOD = 3 \times N/B$ as well as $LOQ = 10 \times N/B$ where N is the normal deviation of the peak location of the medication by way of healthy by way of B is the slope of equivalent calibration story. (LOD) restriction of discovery and also (LOQ) restriction of quantification was discovered to be 1.9 $\mu\text{g}/\text{mL}$ and also 5.7 $\mu\text{g}/\text{mL}$ respectively.

Specificity

The mobile phase developed for the technique dealt with the drug very efficiently, as is displayed in (Figure 1.3). The holding time of Decitabine and also Cedazuridine was 4.1 min. The wavelength 248nm was selected for the detection due to the detail that it

resulted in better discovery level of sensitivity for the medicine.

Robustness

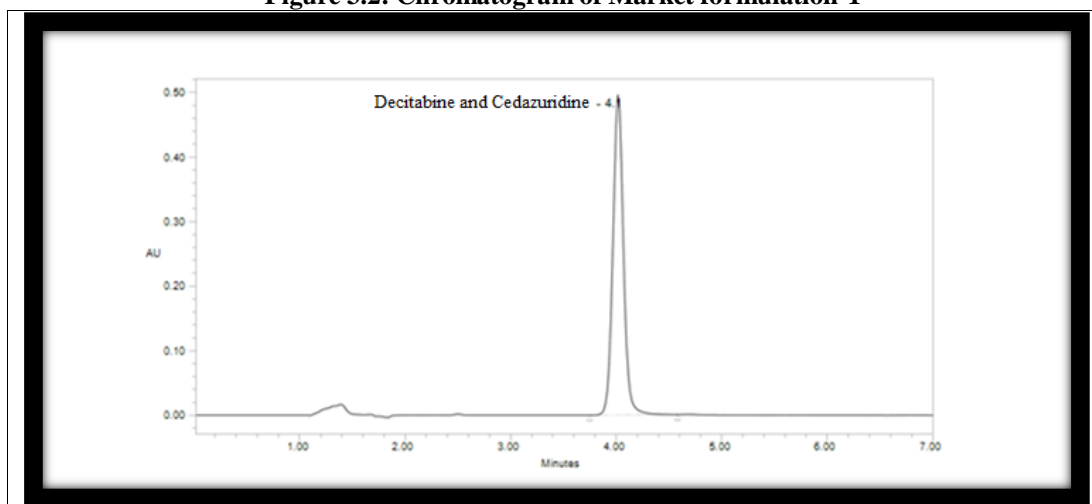
Toughness of the approach was established by making mild changes in the chromatographic problems, such as modification in mobile stage, flow price and also column temperature. It remained experiential that there were no significant modifications in the chromatograms, which showed that the RP-HPLC approach developed is Robust. The robustness restriction for mobile stage difference are well within the limit, which reveals that the technique is consuming decent system appropriateness and accuracy below provided collection of conditions as well as were within the acceptance criteria of not greater than 2%.

System Viability Tests.

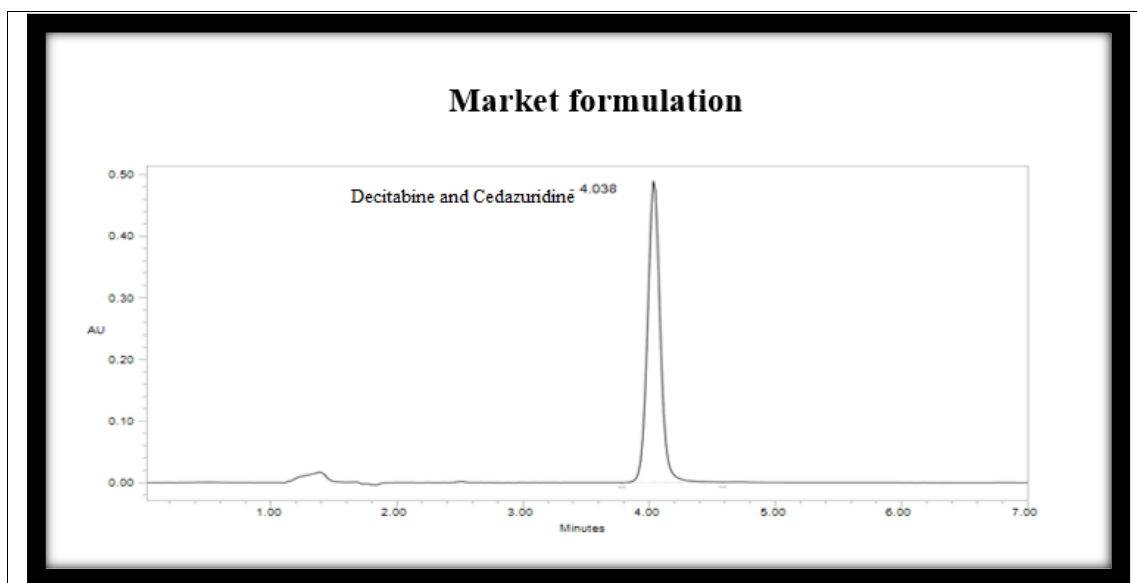
The system suitability examinations were performed on freshly prepared common supply solution of Decitabine and also Cedazuridine. Criteria that were studied to review the suitability of the system are given up Table 2.5.

Table 2.5: VALIDATION SUMMARY

| Validation Parameter | Results |
|------------------------|---------------|
| Calibration range | 25 -151 µg/mL |
| Theoretical Plates (N) | 7715 |
| Tailing factor | 1.07 |
| LOD (mcg/ml) | 1.954 |
| LOQ (mcg/ml) | 5.78 |

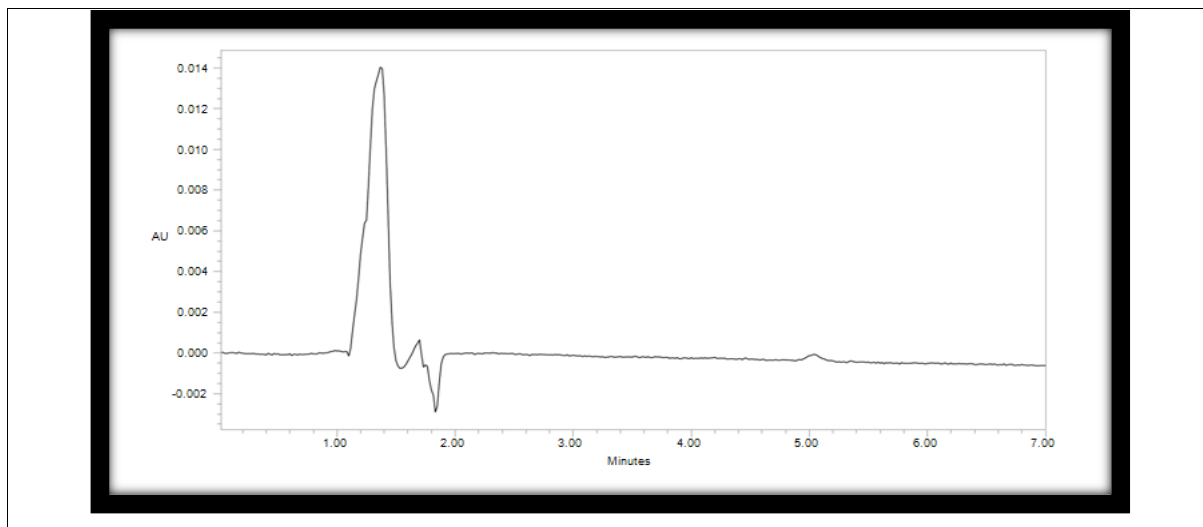
Figure 3.2: Chromatogram of Market formulation-1

| | Peak Name | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-------|---------|--------|-----------------|-------------|
| 1 | Decitabine and Cedazuridine | 4.029 | 3453689 | 100.00 | 8011 | 1.07 |



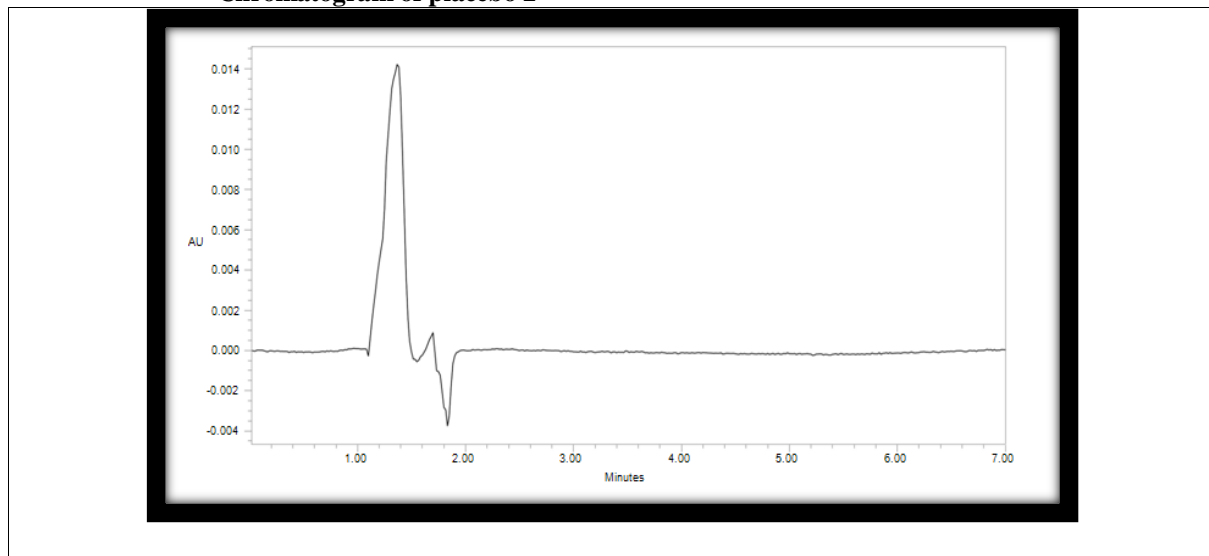
| | Peak Name | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-------|---------|--------|-----------------|-------------|
| 1 | Decitabine and Cedazuridine | 4.039 | 3426269 | 100.00 | 7937 | 1.07 |

Figure 3.3 Chromatogram of placebo 1

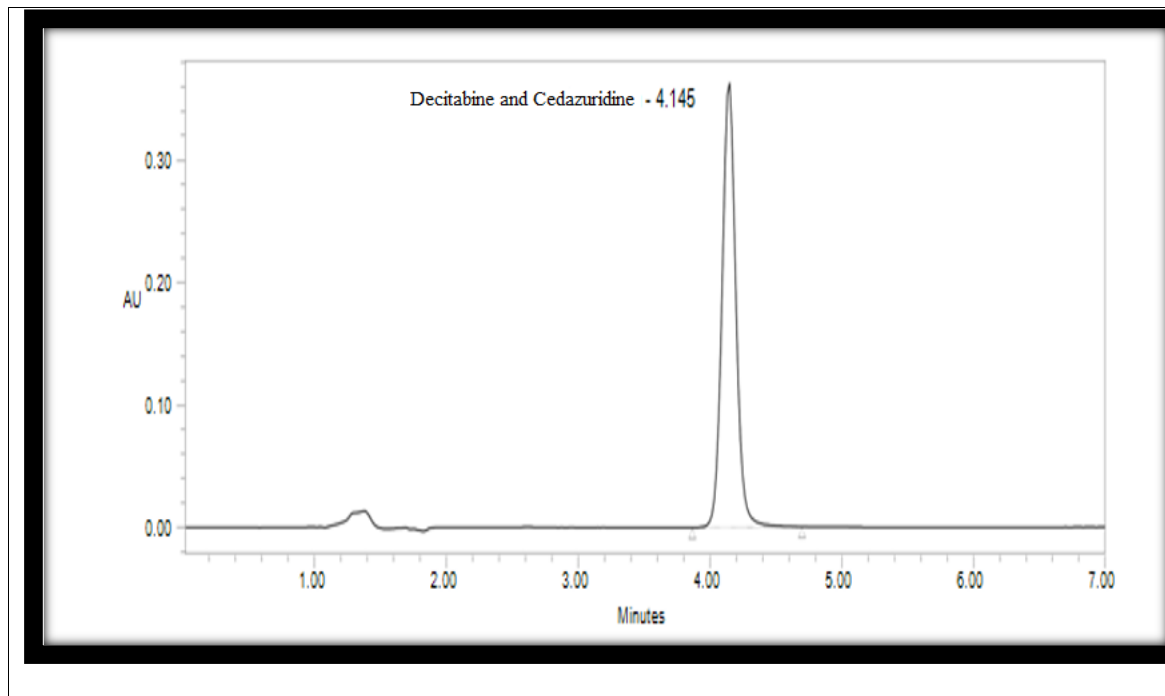
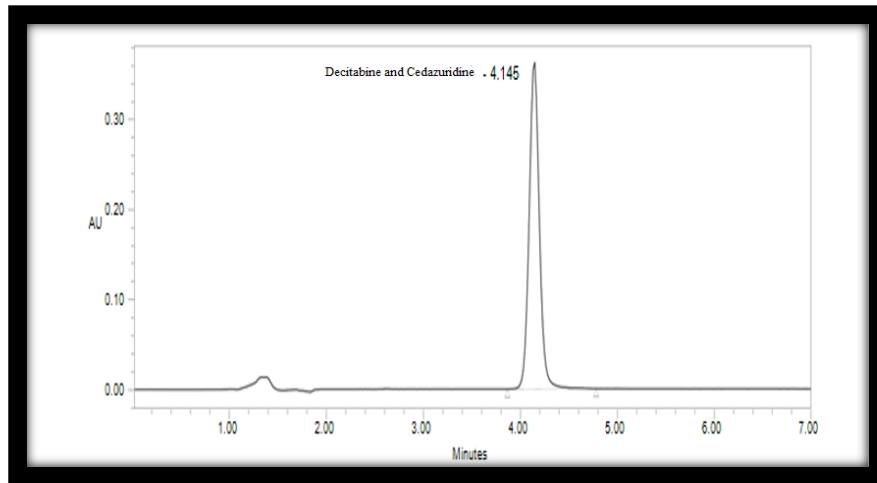


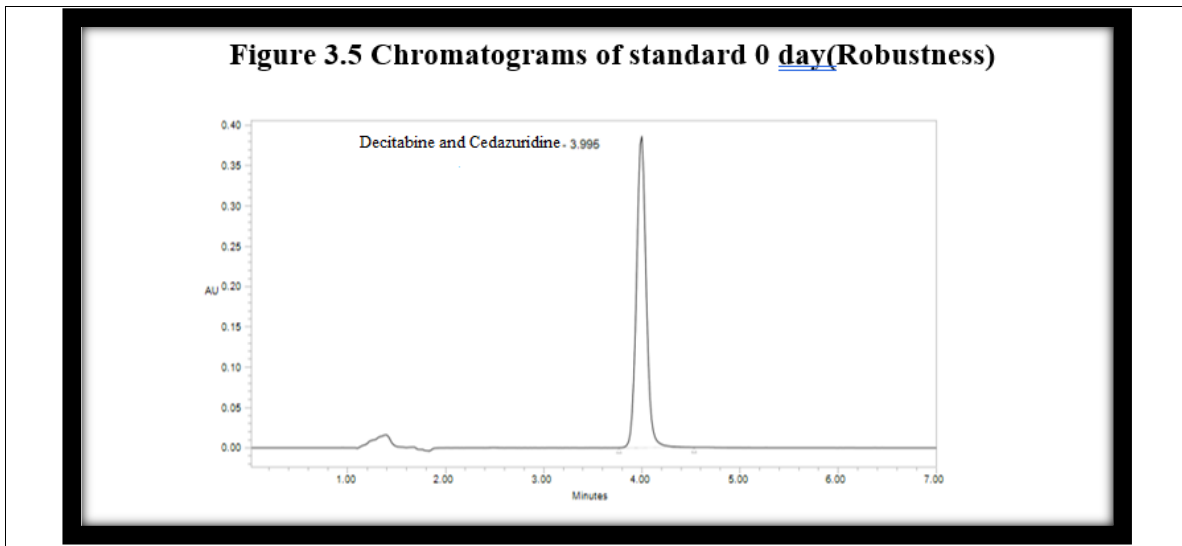
| | Peak Name | RT |
|---|-----------------------------|-------|
| 1 | Decitabine and Cedazuridine | 4.146 |

Chromatogram of placebo 2



| | Peak Name | RT |
|---|-----------------------------|-------|
| 1 | Decitabine and Cedazuridine | 4.147 |

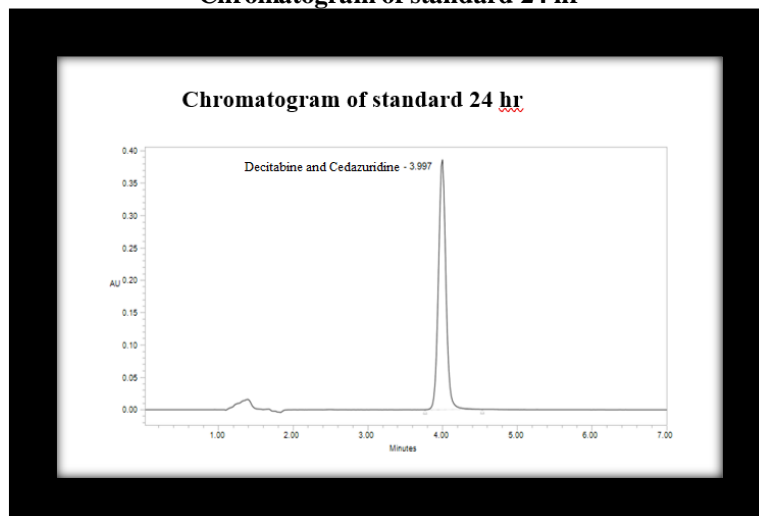
Figure 3.4 Chromatograms of standard



| | Peak Name | RT | Area | %Area | USP Plate Count | USP Tailing |
|---|-----------------------------|-------|---------|--------|-----------------|-------------|
| 1 | Decitabine and Cedazuridine | 3.996 | 2674235 | 100.00 | 7911 | 1.07 |

| | Top Designation | RT | Part | USP Count | Bowl | USP Tailing |
|---------|-----------------------------|-------|---------|-----------|------|-------------|
| 1 | Decitabine and Cedazuridine | 4.146 | 2668166 | 7652 | | 1.07 |
| 2 | Decitabine and Cedazuridine | 4.146 | 2659191 | 7664 | | 1.07 |
| 3 | Decitabine and Cedazuridine | 4.159 | 2657405 | 7704 | | 1.08 |
| 4 | Decitabine and Cedazuridine | 4.169 | 2661142 | 7683 | | 1.08 |
| 5 | Decitabine and Cedazuridine | 4.177 | 2679895 | 7763 | | 1.08 |
| 6 | Decitabine and Cedazuridine | 4.192 | 2664853 | 7813 | | 1.08 |
| Mean | | | 2665109 | | | |
| Std.Dev | | | 8223.6 | | | |
| %RSD | | | 0.4 | | | |

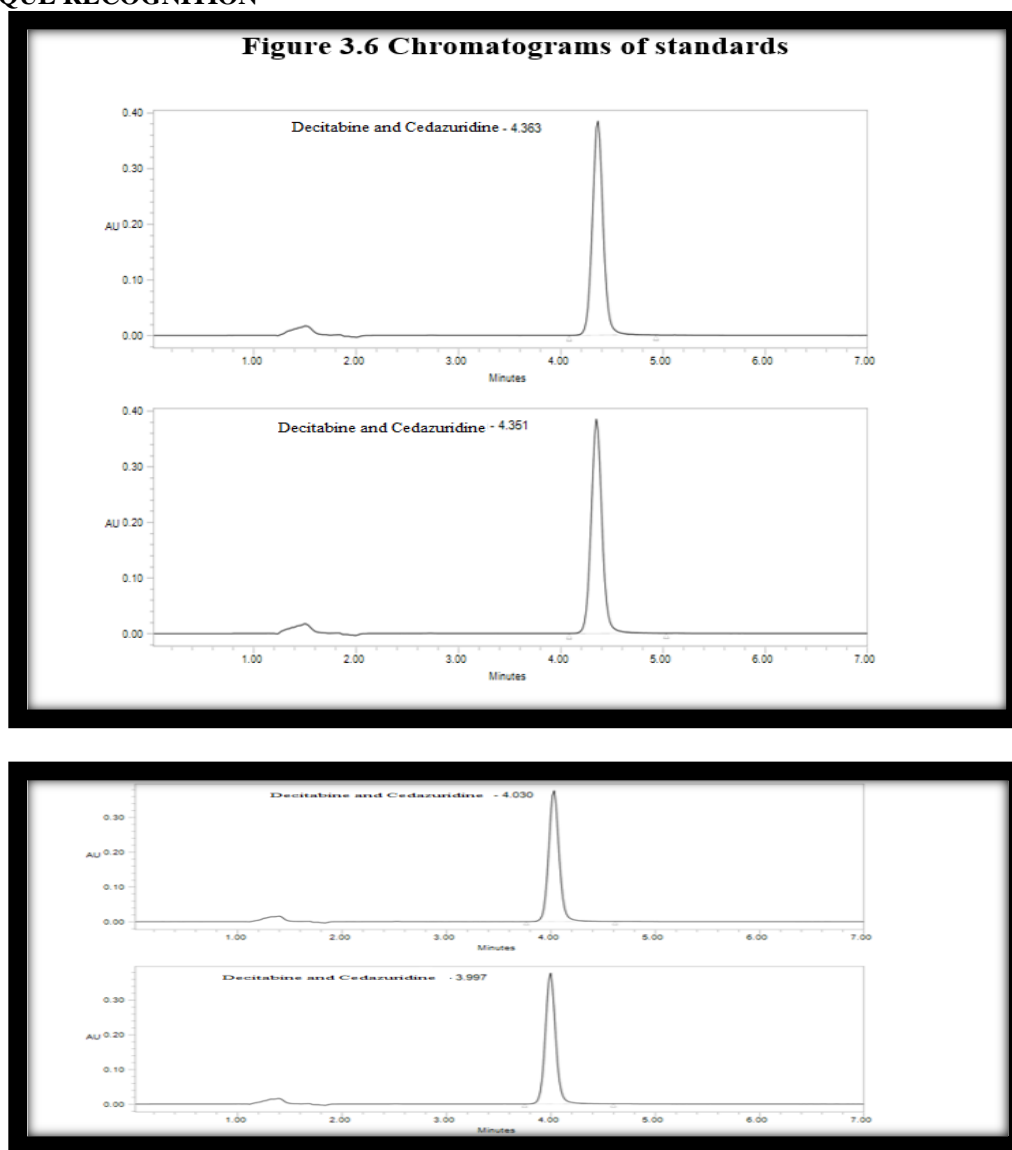
Chromatogram of standard 24 hr



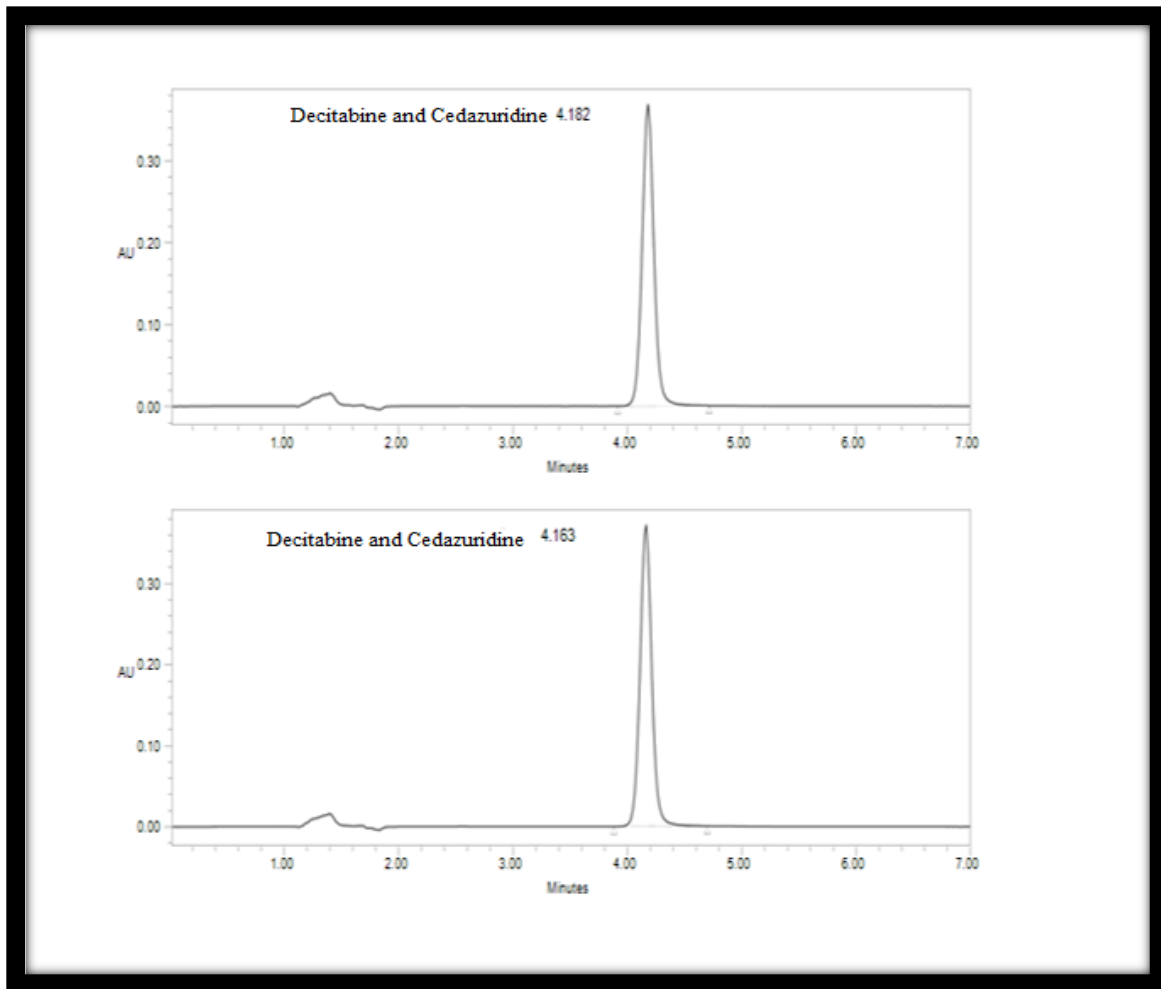
| | Peak Name | RT | Area | USP Count | Plate | USP Tailing |
|---------|-----------------------------|-------|---------|-----------|-------|-------------|
| 1 | Decitabine and Cedazuridine | 4.352 | 2855234 | 8261 | | 1.08 |
| 2 | Decitabine and Cedazuridine | 4.364 | 2849417 | 8306 | | 1.07 |
| Mean | | | 2852327 | | | |
| Std.Dev | | | 41123 | | | |
| %RSD | | | 0.2 | | | |

| | Top Designation | RT | Area | %Area | USP Count | Plate | USP Tailing |
|---|-----------------------------|-------|---------|--------|-----------|-------|-------------|
| 1 | Decitabine and Cedazuridine | 3.998 | 2674235 | 100.00 | 7957 | | 1.07 |

TECHNIQUE RECOGNITION



| | Peak Name | RT | Area | USP Count | Plate | USP Tailing |
|---------|-----------------------------|-------|---------|-----------|-------|-------------|
| 1 | Decitabine and Cedazuridine | 3.998 | 2617407 | 7998 | | 1.07 |
| 2 | Decitabine and Cedazuridine | 4.031 | 2622044 | 8017 | | 1.07 |
| Mean | | | 2619727 | | | |
| Std.Dev | | | 3279.4 | | | |
| %RSD | | | 0.2 | | | |



| | Peak Name | RT | Area | USP Count | Plate | USP Tailing |
|---------|-----------------------------|-------|----------|-----------|-------|-------------|
| 1 | Decitabine and Cedazuridine | 4.164 | 2665205 | 8042 | | 1.07 |
| 2 | Decitabine and Cedazuridine | 4.183 | 26652326 | 8056 | | 1.07 |
| Mean | | | 2658765 | | | |
| Std.Dev | | | 9107.9 | | | |
| %RSD | | | 0.4 | | | |

| | Peak Name | RT | Area | USP Count | Plate | USP Tailing |
|---------|-----------------------------|-------|---------|-----------|-------|-------------|
| 1 | Decitabine and Cedazuridine | 4.112 | 2622159 | 8203 | | 1.06 |
| 2 | Decitabine and Cedazuridine | 4.117 | 2629457 | 8056 | | 1.07 |
| Mean | | | 2625808 | | | |
| Std.Dev | | | 5160.4 | | | |
| %RSD | | | 0.3 | | | |

| | Peak Name | RT | Area | USP Count | Plate | USP Tailing |
|---------|-----------------------------|-------|---------|-----------|-------|-------------|
| 1 | Decitabine and Cedazuridine | 4.146 | 2605918 | 8246 | | 1.05 |
| 2 | Decitabine and Cedazuridine | 4.158 | 2608323 | 8133 | | 1.06 |
| Mean | | | 2607121 | | | |
| Std.Dev | | | 1700.2 | | | |
| %RSD | | | 0.2 | | | |

7. RECAP AND ALSO VERDICT

Reverse phase high performance fluid chromatographic technique was established and also verified for the assessment of Decitabine and also Cedazuridine in Tablet computer dosage kind. A Phenomenex Luna C18, 150 × 4.6 mm i.d, 5µm fragment dimension with mobile phase including buffer 0.01 M potassium dihydrogen ortho phosphate pH (3.75) readjusted with dil orthophosphoric as well as acetonitrile in the proportion of 20:80% v/v was made use of. The circulation price was 1.2 ml/min as well as eluents are monitored at 248nm.

From the normal chromatogram of Decitabine as well as Cedazuridine as, it was discovered that the holding period was 4.1 min. Barrier 0.01 M potassium dihydrogen orthophosphate pH (3.75) was originated to be most appropriate to acquire a height well specified and devoid of tailing. In the present developed HPLC technique, the criterion and also sample prep work obligatory a smaller amount time and also no laborious removal.

An excellent linear connection ($r= 0.999$) was observed between focus series of 25-150 µg/ ml. The assay of Decitabine and also Cedazuridine tablet computers was recouped which indicates high precision of the method. The absence of Additional optimals in the Chromatogram suggests non-interference of the common Expipients utilized in the

tablets. This shows that established HPLC technique is easy, linear, precise, subtle and reproducible.

Hence the established technique can be easily used for the monotonous excellence switch of unpackaged as well as tablet computer dose kind of Decitabine as well as Cedazuridine within a short evaluation is time.

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