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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DECITABINE AND CEDAZURIDINE USING RP-HPLC

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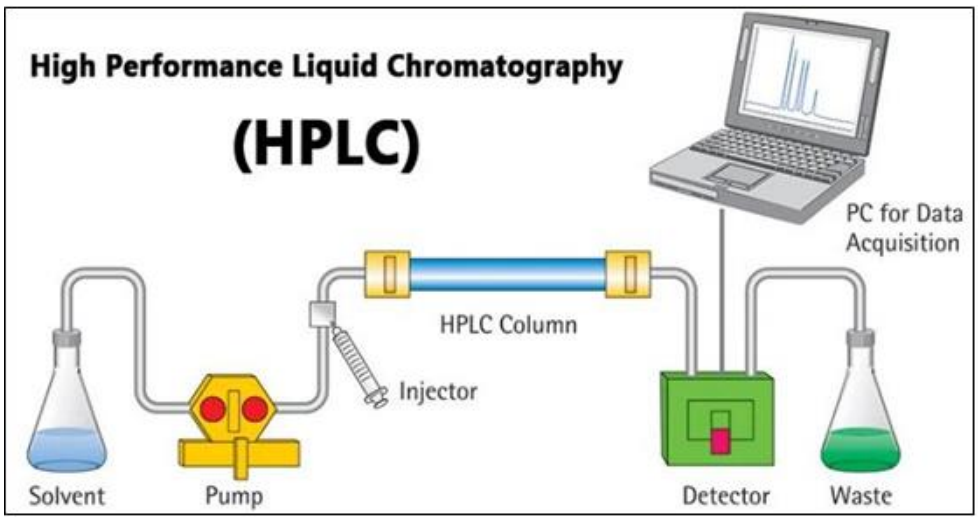
ABSTRACT

A simple, rapid, accurate and precise isocratic reversed phase high performance liquid chromatographic method has been developed and validated for simultaneous estimation of Decitabine and Cedazuridine in tablet dosage form. The chromatographic separation was carried out on Zorbax C18 column (150 mm x 4.6 mm I.D., 5µm particle size) with a mixture of 0.01N potassium dihydrogen phosphate buffer and acetonitrile in the ratio of 65:35% v/v as a mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 245nm. The retention times were 2.263 minutes and 3.001 minutes for Decitabine and Cedazuridine respectively. Calibration plots were linear (r2=0.999 for both Decitabine and Cedazuridine respectively) over the concentration range of 8.75-52.5 µg/mL for Decitabine and 25-150µg/mL for Cedazuridine. The method was validated for linearity, precision, accuracy, ruggedness and robustness. The proposed method was successfully used for simultaneous estimation of Decitabine and Cedazuridine in tablet dosage form. Validation studies revealed that the proposed method is specific, rapid, reliable and reproducible. The high % recovery and low % RSD confirms the suitability of the proposed method for routine quality control analysis of Decitabine and Cedazuridine in bulk and tablet dosage form.

KEYWORDS: Decitabine, Cedazuridine, Validation, HPLC.

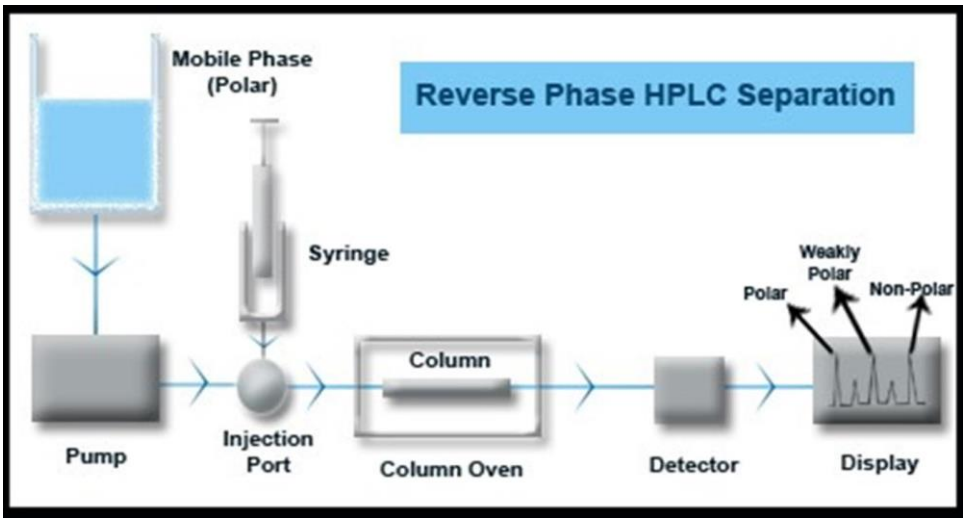
INTRODUCTION

High-performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture. The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy. In the 1960s, the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns. HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.



Reverse phase
The column packing is non-polar (e.g C18), the mobile phase is water+ miscible solvent (e.g methanol).

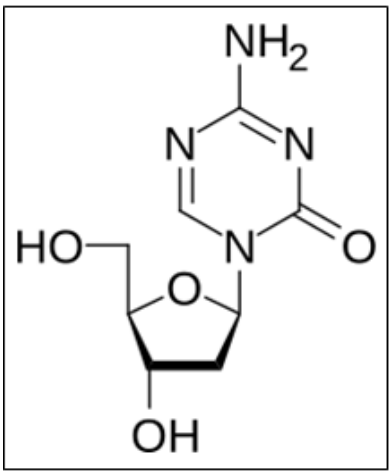
It can be used for polar, non- polar, ionizable, and ionic samples.



DRUG PROFILE
1.0 Decitabine
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. 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. It is also available as an oral combination product together with the cytidine deaminase inhibitor cedazuridine..

Structure



Structure of Decitabine
IUPAC Name: 4-Amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H)-one

Chemical Formula: $C_8H_{12}N_4O_4$
Mol. wt: Average: 228.203 g/mol Monoisotopic: 228.08 g/mol
pKa: 3.79 (Strongest Acidic)
5.48 (Strongest Basic)

Mechanism of Action
Myelodysplastic syndromes (MDS) are a group of hematopoietic neoplasms that manifest in peripheral cytopenias and may eventually progress to secondary acute myeloid leukemia (sAML). Included in the over 45 genes commonly mutated in MDS patients are those involved in DNA methylation and histone modification, and it is well-established that alteration of the epigenetic landscape is a feature of myeloid leukemias.

Decitabine is considered a prodrug, as it requires transport into cells and subsequent phosphorylation by distinct kinases to generate the active molecule 5-aza-2'-deoxycytidine- triphosphate, which is incorporated by DNA polymerase during DNA replication. Once incorporated into DNA, decitabine is recognized as a substrate by DNA methyltransferase enzymes (DNMTs), specifically DNMT1, but due to the presence of an N5 rather than C5 atom, traps the DNMT through the irreversible formation of a covalent bond. At low concentrations, this mode of action depletes DNMTs and results in global DNA hypomethylation while at high concentrations, it additionally results in double-strand breaks and cell death.

The general hypothesis regarding decitabine's therapeutic efficacy is that the global hypomethylation it induces

results in the expression of previously silent tumour suppressor genes. However, there are other putative mechanisms also related to this change in DNA methylation, including indirect alteration of transcription through effects on transcription factors, indirectly altering histone modifications and chromatin structure, and activating pathways involved in DNA damage response. The overall effect of decitabine is a decrease in neoplastic cell proliferation and an increase in the expression of tumour suppressor genes.

Pharmacokinetics
Decitabine is a prodrug analogue of the natural nucleotide 2'-deoxycytidine, which, upon being phosphorylated intracellularly, is incorporated into DNA and exerts numerous effects on gene expression.3,4,5,8,11 The use of decitabine is associated with neutropenia and thrombocytopenia. In addition, decitabine can cause fetal harm in pregnant women; effective contraception and avoidance of pregnancy are recommended during treatment with decitabine.

Absorption
Decitabine administered intravenously at 15 mg/m2 for three hours every eight hours over three days resulted in a Cmax of 73.8 ng/mL (66% coefficient of variation, CV), an AUC0-∞ of 163 ng*h/mL (62% CV), and a cumulative AUC of 1332 ng*h/mL (95% CI of 1010-1730).

Similarly, decitabine at 20 mg/m2 for one hour once daily over five days resulted in a Cmax of 147 ng/mL (49% CV), an AUC0-∞ of 115 ng*h/mL (43% CV), and a cumulative AUC of 570 ng*h/mL (95% CI of 470-700).

Volume of distribution: Decitabine as an apparent volume of distribution of 4.59 ± 1.42 L/kg.4

Protein binding: Decitabine exhibits negligible (< 1%) plasma protein binding.

Metabolism
Decitabine is phosphorylated inside cells by the sequential action of deoxycytidine kinase, nucleotide monophosphate kinase, and nucleotide diphosphate kinase, prior to being incorporated into newly synthesized DNA by DNA polymerase. Decitabine not incorporated into cellular DNA undergoes deamination by cytidine deaminase followed by additional degradation prior to excretion.

Route of elimination: Less than 1% of administered decitabine is excreted in the urine.

Half-life
Decitabine has a half-life of 0.62 hours (49% CV) when administered intravenously at 15 mg/m2 for three hours every eight hours over three days, and a half-life of 0.54 hours (43% CV) at 20 mg/m2 for one hour once daily over five days.

Clearance
Decitabine has a clearance of 125 L/hr/m2 (53% CV) when administered intravenously at 15 mg/m2 for three hours every eight hours over three days, and a clearance of 210 L/hr/m2 (47% CV) at 20 mg/m2 for one hour once daily over five days.

Toxicity
Decitabine has demonstrated mutagenic potential in L5178Y mouse lymphoma cells and an *Escherichia coil lac-I* transgene within the colonic DNA of mice. Decitabine treatment increased chromosomal rearrangements in fruit fly larvae. In mouse models, decitabine exposure *in utero* (approximately 7% of the recommended daily dose) resulted in decreased weight and decreased male fertility. Adult male mice administered with between 0.3 and 1% of the recommended daily dose of decitabine three times a week for seven weeks had smaller testes with abnormal histology, decreased sperm count, and decreased fertility.

There is no known antidote for decitabine overdose. Patients experiencing an overdose are at an increased risk of severe adverse effects such as myelosuppression, including prolonged and severe neutropenia and thrombocytopenia. Symptomatic and supportive measures are recommended

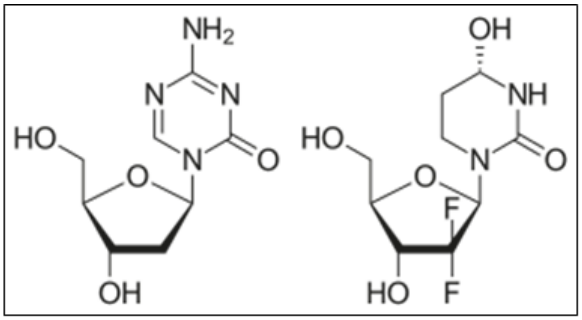
Side effects: Cough, fatigue, weakness/asthama, headache, mood or sleep disturbances

2.0 Cedazuridine
Cedazuridine is a small molecule inhibitor of cytidine deaminase that is used as a pharmacoenhancer of decitabine to increase oral bioavailability of this DNA methylase inhibitor treatment of myelodysplastic syndromes. The combination of oral decitabine and cedazuridine is associated with a low rate of minor serum enzyme elevations during therapy that is usually attributed to decitabine. The oral combination has not been linked to cases of clinically apparent liver injury.

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.

Cedazuridine was first reported in 2014,4 and was subsequently approved by the FDA on July 7, 2020, in combination with decitabine for sale by Astex Pharmaceuticals Inc under the name INQOVI®.

Structure



Structure of cedazuridine

IUPAC Name: (4R)-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl) oxolan-2-yl] -4-hydroxy-1,3-diazinan-2-one **Chemical Formula:** $C_9H_{14}F_2N_2O_5$ **Mol. wt:** 567.05 g/mol

pKa: 19.12 (Strongest Acidic)
9.45 (Strongest Basic)

Mechanism of Action

Myelodysplastic syndromes (MDS) represent a heterogeneous group of hematopoietic neoplasms arising from a variety of underlying mutations that manifest in peripheral cytopenias and may eventually progress to secondary acute myeloid leukemia (sAML). There are over 45 genes commonly mutated in MDS patients, including those involved in DNA methylation and repair, histone modification, RNA splicing, transcription, signal transduction, and cellular adhesion. It is hypothesized that initial clonal founder mutations give rise to progressive acquisition of secondary mutations and facilitate disease progression to sAML.

Hypomethylating agents such as decitabine are metabolized into triphosphate derivatives that are subsequently incorporated into DNA. Once incorporated, these agents inhibit the activity of DNA methylases such as DNMT1, leading to progressive DNA hypomethylation and eventual activation of tumour suppression genes and apoptotic pathways. However, hypomethylating agents given orally are vulnerable to first-pass metabolism by cytidine deaminase, and hence typically have to be administered through intramuscular or intravenous routes. Co-administration with cedazuridine, which is an efficient inhibitor of cytidine deaminase, drastically increases the oral bioavailability of decitabine, allowing for combination oral therapy.

Pharmacodynamics

Cedazuridine is a cytidine deaminase inhibitor that is co-administered with hypomethylating agents such as decitabine in order to increase their oral bioavailability. In combination with hypomethylating agents, cedazuridine may cause myelosuppression and embryo-fetal toxicity and should be administered with appropriate monitoring.

Absorption

Cedazuridine (100 mg) taken orally with decitabine (35 mg) once daily for five days resulted in a day 1 AUC and steady-state AUC (coefficient of variation) of 103 (55%) and 178 (53%) ng*hr/mL for decitabine and 2950 (49%) and 3291 (45%) ng*hr/mL for cedazuridine, respectively. Overall, the 5-day cumulative AUC for decitabine was 851 (50%). Similarly, the Cmax for decitabine and cedazuridine was 145 (55%) and 371 (52%) ng/mL, respectively. The median Tmax for decitabine was 1 hr (range 0.3 to 3.0 hrs) and for cedazuridine was 3 hrs (range 1.5 to 6.1 hrs).

The bioavailability of decitabine, as assessed by comparing the AUC of oral decitabine co- administered with cedazuridine to intravenous decitabine alone, was 60% on day 1 (90% CI of 55-65%). The corresponding values on day 5 and considering the cumulative day 5 dose were 106% (90% CI: 98, 114) and 99% (90% CI: 93, 106). Hence, the oral bioavailability of decitabine approaches 100% over the 5-day treatment cycle.

Volume of distribution: The apparent volume of distribution (and coefficient of variation) of decitabine and cedazuridine at steady state was 417 (54%) and 296 (51%), respectively.

Protein binding: Neither decitabine nor cedazuridine display extensive plasma protein binding. The bound fraction of decitabine between doses of 17 and 342 ng/mL was between 4 and 6%, while that of cedazuridine for doses between 1000 ng/mL and 50000 ng/mL was between 34 and 38%.

Metabolism

The metabolism of cedazuridine is not well-established. Cedazuridine is known to be converted to an epimer that is roughly 10-fold less effective in inhibiting cytidine deaminase and is subsequently degraded through unknown pathways.

Route of elimination: Roughly 46% of cedazuridine is found in urine, 21% of which is unchanged, and 51% is found in feces, 27% of which is unchanged.

Half-life: Cedazuridine has a steady-state half-life of 6.7 hours, with a coefficient of variation of 19%.

Clearance: Cedazuridine has an apparent steady-state clearance of 30.3 L/hours, with a coefficient of variation of 46%.

Toxicity

Cedazuridine administered orally to mice in 7 days on/21 days off cycles for a total of 91 days in doses of 100, 300, or 1000 mg/kg produced abnormal effects only at the 1000 mg/kg dose, which is roughly 108 times the

recommended dose in humans. These effects included abnormal histology of the testes, epididymis, and ovaries, as well as decreased sperm count; these effects were reversible following cedazuridine removal.

Side effects

The most common side effects of decitabine/cedazuridine include fatigue, constipation, hemorrhage, muscle pain (myalgia), mucositis (mouth sores), arthralgia (joint pain), nausea, dyspnea, diarrhea, rash, dizziness, fever with low white blood cell count (febrile neutropenia), edema, headache, cough, decreased appetite, upper respiratory tract infection, pneumonia, and transaminase increased. The combination can cause fetal harm. It is taken by mouth.

Literature Survey

Journal: Asian Journal of Pharmaceutics

Author: G.Dharmamoorthy, M Anupama (2022)

Past Work: 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 2 PO 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.

Journal: Innovative Journal of Medical Sciences

Author: Mungara Meghana, (2020).

Past Work: Introduction: An attempt has been made to develop a validated stability indicating RP-HPLC method for the estimation of decitabine and cedazuridine. Literature survey revealed that many analytical methods have been reported individually or in combination with other drugs. Retention times were decreased and that run time was decreased, so the method developed was simple and economical. Materials and Methods: It includes the general information on RP-HPLC and method development, general information on forced degradation studies and stress conditions like acid, base, peroxide, thermal, photolytic and neutral. Discussion: The article discusses about drug profiles and official status of selected drugs i.e., decitabine and cedazuridine. Results: In this article the previous literature available for drugs is used for developed research work which Include stability indicating RP-HPLC method development and validation for simultaneous estimation of decitabine and cedazuridine in bulk and their pharmaceutical dosage form. Using Waters HPLC 2695 system, quaternary gradient pump equipped with auto sampler injector with 20 Î¼L is injected eluted with the mobile phase containing 65% 0.01 N KH2 PO4: 35% acetonitrile which is pumped at a flow rate of 1 mL/min and detected by PDA detector at 245 nm. The peak of decitabine and cedazuridine was eluted at retention times of 2.263 min and 3.001 min, respectively. Conclusion: In this paper, HPLC method for the selected drugs showed good linearity.

Journal: Current Trends in Biotechnology and Pharmacy

Author: Alimunnisa and Lakshmana Rao A (2022).

Past Work: A simple, rapid, accurate and precise isocratic reversed phase high performance liquid chromatographic method has been developed and validated for simultaneous estimation of Decitabine and Cedazuridine in tablet dosage form. The chromatographic separation was carried out on Zorbax C18 column (150 mm x 4.6 mm I.D., 5 µm particle size) with a mixture of 0.01N potassium dihydrogen phosphate buffer and acetonitrile in the ratio of 65:35% v/v as a mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 245 nm. The retention times were 2.263 minutes and 3.001 minutes for Decitabine and Cedazuridine respectively. Calibration plots were linear (r2=0.999 for both Decitabine and Cedazuridine respectively) over the concentration range of 8.75-52.5 µg/mL for Decitabine and 25-150 µg/mL for Cedazuridine. The method was validated for linearity, precision, accuracy, ruggedness and robustness. The proposed method was successfully used for simultaneous estimation of Decitabine and Cedazuridine in tablet dosage form. Validation studies revealed that the proposed method is specific, rapid, reliable and reproducible. The high % recovery and low % RSD confirms the suitability of the proposed method for routine quality control analysis of Decitabine and Cedazuridine in bulk and tablet dosage form.

Journal: Journal of Pharmaceutical Research International

Author: B. Mohammed Ishaq.; L. Siva Sanker Reddy; M. Sreenivasulu.; (2020)

Past Work: Aim: The aim of our present work was to develop and validate a reverse phase high-performance liquid chromatography (RP-HPLC) method for the

simultanious determination of Decitabine (DEC) and Cedazuridine (CED).

Methodology: The developed method was further applied to observe the degradation of analytes under the influence of different forced degradation conditions. Analytes were resolved on C18, 250 x 4.6 mm, particle size 5 µm Xterra column, using a mobile phase combination of 0.1% Ortho Phospharic Acid buffer pH 6.5: Methanol (40:60v/v) with flow rate of 1mL/min and injection volume of 10 µL. Quantification was carried out with PDA detector at an isosbestic point of 220 nm with a linear calibration curve in the concentration range of 35-175 µg/mL for DEC and 100-500 µg/mL for CED.

Results: Validation of the developed method was performed as per ICH guidelines viz., linearity, accuracy, precision, and robustness. The limits of detection (LOD) and the limits of quantification (LOQ) for CED were found to be 2.69 µg/mL and 8.15 µg/mL respectively. LOD and LOQ for DEC 1.55 µg/mL and 4.68 µg/mL respectively. Moreover, validated method was applied to study the degradation profile of analytes under various stress degradation conditions.

Conclusion: The proposed method was found to be sensitive, specific and was successfully applied for the simultaneous estimation of Decitabine (DEC) and Cedazuridine (CED) in bulk drug, and tablets..

Journal: Chemistry World Conference
Author: Tarekegn Tadesse Unade (2025)

Past Work: In the last few decades, several anticancer drugs have been introduced to the market. The introduction of a large number of new drugs and their formulations may lead to the possibility of the distribution of substandard drug products to the consumers. So, stringent quality control of pharmaceuticals is essential for ensuring the availability of quality products to the consumers. The stringent quality control of pharmaceuticals mainly requires the availability of fast, cost-effective, and sensitive modern analytical methods. A combination of Decitabine and Cedazuridine in tablet form is among a recently approved oral anticancer agent. No stability indicating official analytical method was reported for quality control of this recently approved anticancer preparation. Thus, there is a need for the development and validation of a new method to be applicable for routine quality control of the combination in pharmaceutical industries and regulatory authorities. Method validation and forced degradation studies were performed as per the International Conference on Harmonization Guidelines. Separation was conducted on Agilent XDB -C18 (150 mm x 4.6 mm, 3.5µ) column using a mobile phase consisting of mixture of 0.1 % formic acid buffer and acetonitrile (40: 60, v/v), at a flow rate of 1.0 mL min-1. The developed method was linear for both drugs with a coefficient of determination (R2) > 0.999. The limit of detection (LOD) of the method was 1.05 µgmL- 1 and 3.0 µgmL-1 for Decitabine and Cedazuridine respectively. The recovery results for the accuracy study were ranged from 98.5% - 101.7 %, and the % RSD for the precision results was less than 1.26. A new stability-indicating HPLC method was successfully developed and validated for the assay of both drugs simultaneously, and thus it can be employed for the routine quality control in pharmaceutical industries and regulatory authorities.

AIM AND OBJECTIVE

Aim

Method Development and Validation for the Simultaneous Estimation of Decitabine and Cedazuridine using RP-HPLC.

Objectives

- To developed sensitive, rapid, accurate, precise and desirable method for estimation of drug by RP-HPLC method.
- To develop RP-HPLC method for Decitabine and Cedazuridine.
- To validate Novel Qualitative and Quantitative Analytical method development for estimation of Decitabine and Cedazuridine in bulk and pharmaceutical formulation as per ICH guidelines.
- Statistical analysis of the recovery data obtained from different techniques for Decitabine and Cedazuridine.

MATERIAL AND METHOD

Chemicals and reagents

Acetonitrile (HPLC Grade), Orthophosphoric acid (AR Grade), Potassium dihydrogen orthophosphate (Merck, AR Grade), water (HPLC Grade). Generic Healthcare Pvt. Ltd., Pune, provided standard medicine samples of Decitabine and Cedazuridine besylate, as well as tablets available in a 35:100 ratio including INQOVI (Decitabine & Cedazuridine) 35mg & 100mg Tablet, Decitabine 10mg and Cedazuridine 10mg Standard.

Instrumentation

The work was carried out on chromatographic system Shimadzu, series LC 2030, which processes pump Quaternary system. All weighing was done Mettler Toledo Semi Micro Balances. A Manti Lab Solutions ultrasonicator cleaner (India) was used for degassing the mobile phase

Chromatographic condition

Compounds were separated using Kromasil C8 (4.6mm x 250mm, 5µ particle size) column at 40°C temperature, flow rate 1.00mL/min with an isocratic mobile phase consisting of Buffer and Acetonitrile of ratio 50:50 and pH maintained till 5.0 using orthophosphoric acid. Decitabine & Cedazuridine were evaluated by UV detection at 245nm with the injection volume of 10µL and the run time 5 min.

Table: Optimized Chromatographic Condition.

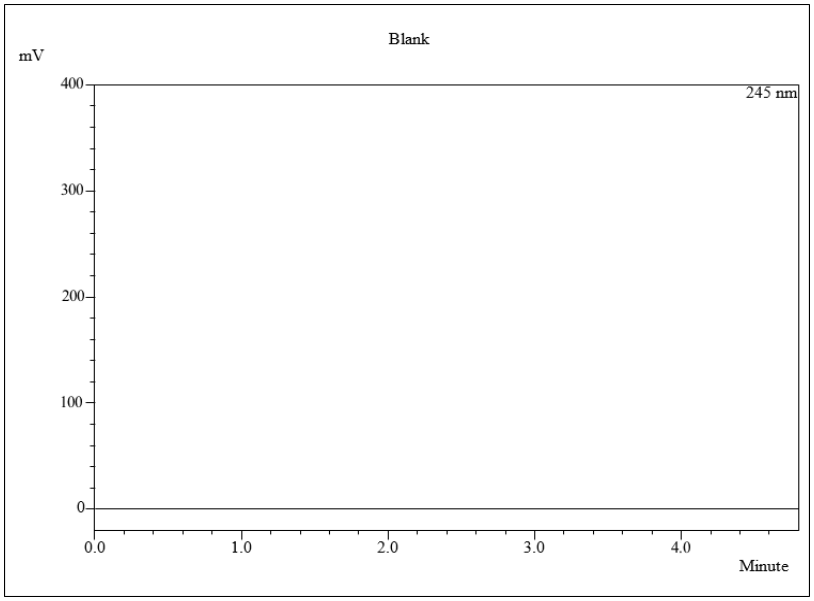
Optimized Chromatographic Condition		
Mobile Phase (V/V)	Acetonitrile	Buffer
	50	50
Acetonitrile : Potassium Phosphate Buffer		
pH of Aqueous Phase	5.00	
Flow Rate (mL/min)	1.00	
Detection Wavelength (nm)	245.00	

6.4 Preparation of buffer solution for mobile phase

Roughly 6.8gm of potassium dihydrogen orthophosphate were accurately weighed and added to 500 mL of HPLC grade water, mixed well and sonicated for proper dissolution and lastly the solution volume was made 1000 mL with HPLC grade water.

6.5 Preparation of mobile phase

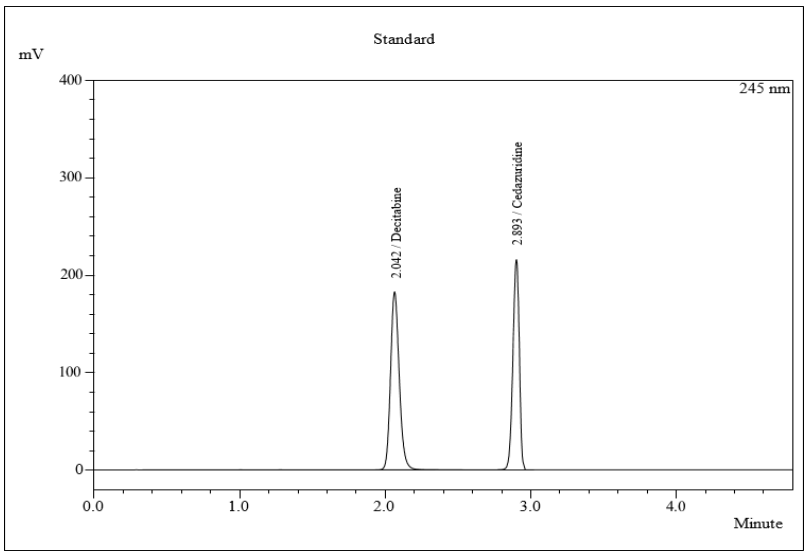
Mixed 50 ml of buffer solution and 50 ml acetonitrile. Sonicated with stirring, maintaining the pH to 5.0 using orthophosphoric acid. Degas and filter through a 0.20-micron membrane filter.



Typical chromatogram of Blank

6.6 Preparation of standard solution

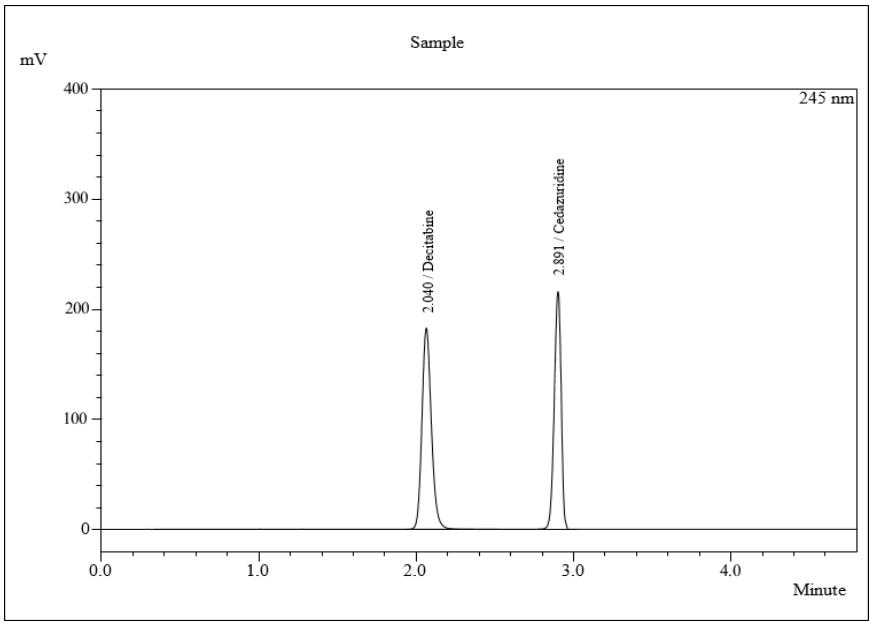
Decitabine 10 mg, Cedazuridine 10 mg, weighed and added in a 50 mL volumetric flask, where they were mixed in 30 mL of mobile phase, sonicated and diluted predetermined volume using mobile phase. Dilute 5 ml of this solution to 100 ml with mobile phase and mix. Further dilute 5 ml of this solution to 10 ml with mobile phase to get 10.0 mg/mL of Decitabine 10.0 mg/mL of Cedazuridine. A figure 3.3 signifies the typical chromatogram of standards of Decitabine and Cedazuridine.



Typical chromatogram of Standards

6.8 Preparation of sample solution

Accurately weighed about 10 mg of tablet powder (Decitabine and Cedazuridine) was transferred to 100 ml of volumetric flask containing 60ml of Diluent. The sample was dissolved by sonication for 15 mins and volume was made up to the mark with Diluent. The resulting solution was filtered using membrane filter 0.22µ. The filtrate was further diluted appropriately with diluent to get 10 µg/ml of Decitabine and 10.0 µg/ml of Cedazuridine. This diluted sample was then analyzed by HPLC.



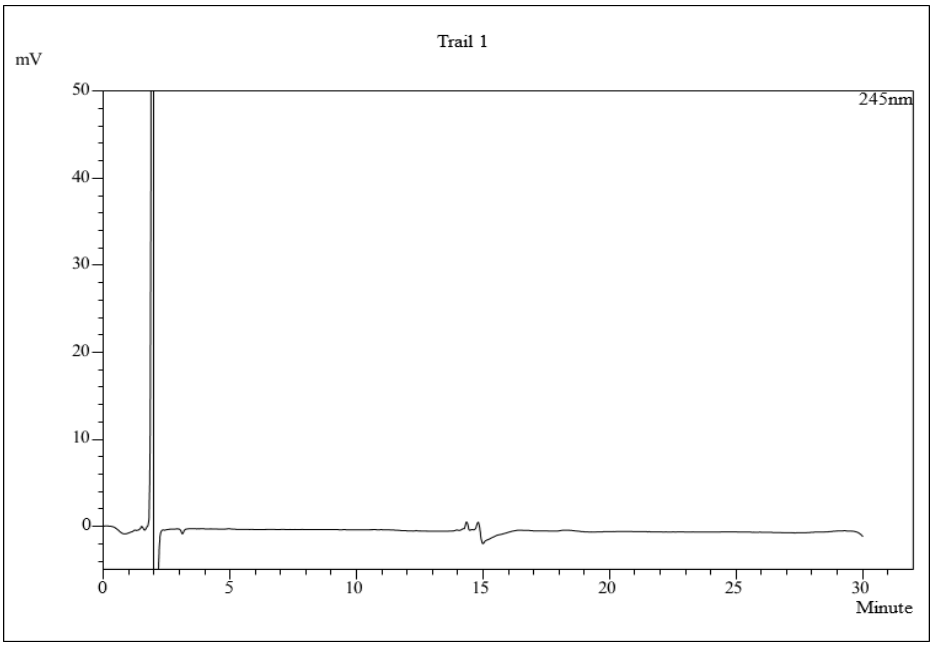
Typical chromatogram of Samples

Results and Trials

Trial 1

- Mobile phase- 50 ml of Water solution and 50 ml Methanol. pH to 4.5 using orthophosphoric acid
- Column: Kromasil C18 (100mm x 2.1mm ID, Particle size: 1.7 µm)
- Flow rate- 1.00 ml/min
- Detection wavelength (λmax)- 245nm
- Injection Volume: 10microliter
- Column temperature: Room temperature
- Retention time: Peak not Observed
- Runtime: 30.000 Minutes
- Observation and Conclusion:

Peak not observed, Peak properties not match in this chromatographic condition hence peak is rejected.

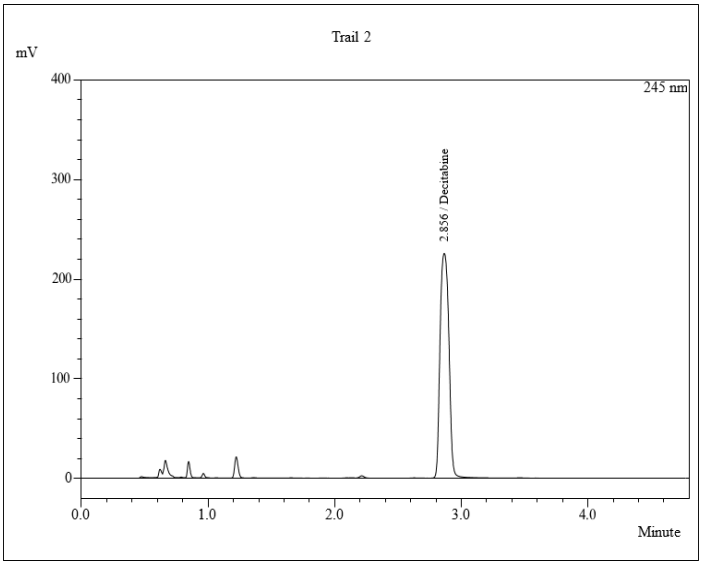


Typical Chromatogram of Trial 1

Trial 2

- Mobile phase- 50 ml of Buffer and 50 ml Acetonitrile.(50:50 v/v)
- Column: Kromasil C18 (100mm x 2.1mm ID, Particle size: 1.7 µm)
- Flow rate- 1.00 ml/min
- Detection wavelength (λmax)- 245nm
- Injection Volume: 10microliter
- Column temperature: Room temperature
- Runtime: 5.00 Minutes
- Retention time (RT): Decitabine 2.856 Minutes
- Observation and Conclusion:

Decitabine peak observed However poor peak properties of Decitabine this chromatographic condition, Poor peak shape and minimum theoretical plates. Poor baseline, Peak properties not match in this chromatographic condition hence peak is rejected.

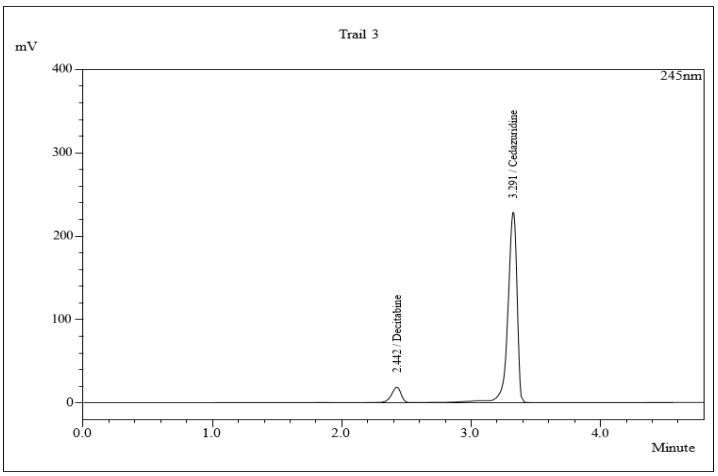


Typical Chromatogram of Trial 2

Trial 3

- Mobile phase- 50 ml of Buffer solution and 50 ml Methanol:Acetonitrile (50:50 v/v) pH was adjusted 4.0.
- Column: Kromasil C18 (100mm x 2.1mm ID, Particle size: 1.7 µm)
- Flow rate- 1.00 ml/min
- Detection wavelength (λmax)- 245nm
- Injection Volume: 10 microliter
- Column temperature: Room temperature
- Runtime: 5.000 Minutes
- Retention time (RT): Decitabine- 2.442 Minutes Cedazuridine- 3.291 Minutes
- Observation and Conclusion:

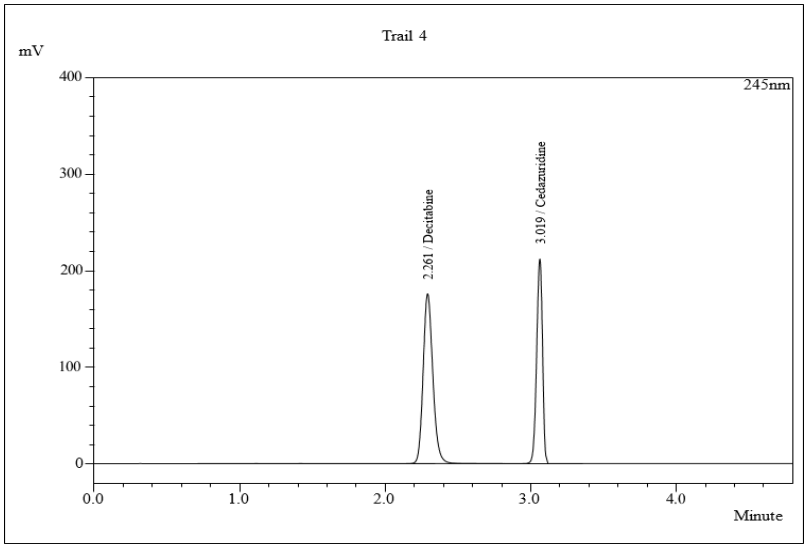
Both peak observed, Decitabine and Cedazuridine peaks eluted this chromatographic condition, Poor peak shape and minimum theoretical plates, more retention time as expected, peak tailing and Peak splitting observed. Peak properties not match in this chromatographic condition hence peak is rejected.



Typical Chromatogram of Trial 3

Trial 4

- Mobile phase- 40 ml of buffer solution and 60 ml Acetonitrile. Sonicated with stirring, maintaining the pH to 5.5 using orthophosphoric acid
- Column: Phenomenex C18 (100mm x 2.1mm ID, Particle size: 1.7 µm)
- Flow rate- 1.00 ml/min
- Detection wavelength (λmax)- 245nm
- Injection Volume: 10microliter
- Column temperature: Room temperature
- Runtime: 5.000 Minutes
- Retention time (RT): Decitabine- 2.261 Minutes Cedazuridine- 3.019 Minutes
- Observation and Conclusion:
  Both peak eluted this mobile phase, Poor peak shape and peak properties and minimum theoretical plates. More retention time as expected Peak properties not match in this chromatographic condition hence peak is rejected.

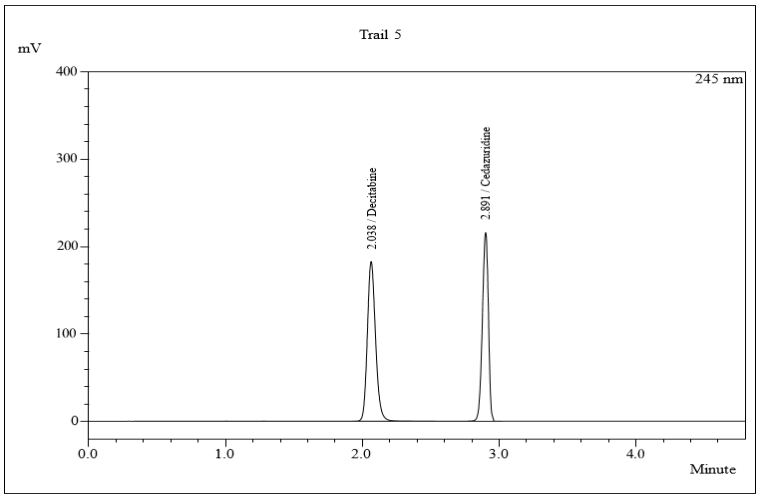


Typical Chromatogram of Trial 4

Trial 5 (Optimized Chromatographic Condition)

- Mobile phase- 50 ml of buffer solution and 50 ml acetonitrile. Sonicated with stirring, maintaining the pH to 5.0 using orthophosphoric acid
- Column: Phenomenex C18 (100mm x 2.1mm ID, Particle size: 1.7 µm)
- Flow rate- 1.00 ml/min
- Detection wavelength (λmax)- 245nm
- Injection Volume: 10microliter
- Column temperature: Room temperature
- Runtime: 10.0 Minutes
- Retention time (RT): Decitabine- 2.038 Minutes Cedazuridine 2.891 Minutes
- Observation and Conclusion:

Both Peak observed/peak eluted this mobile phase, Poor peak shape and appropriate theoretical plates. Peak properties match with this chromatographic condition hence peak is accepted. Optimized chromatographic condition.



Typical Chromatogram of Trial

METHOD VALIDATION

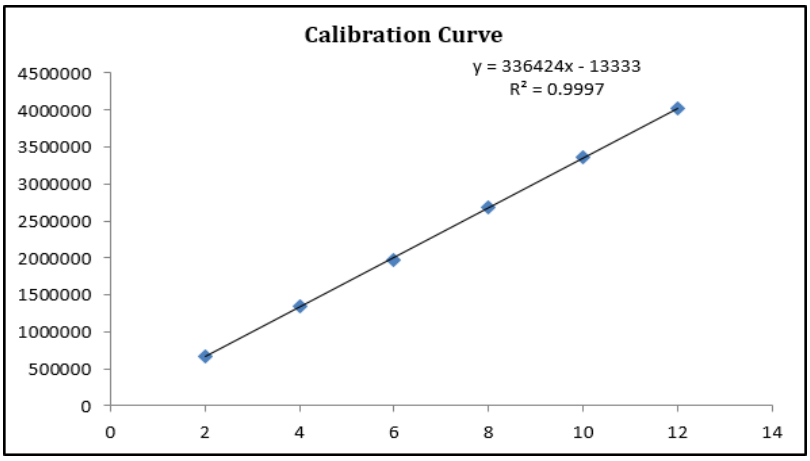
Method Validation

The proposed RP-HPLC method was validated as per ICH guidelines.

Linearity: Several aliquots of standard Decitabine and Cedazuridine solutions were placed in various 10 ml volumetric flasks and the capacity was filled with mobile phase to achieve a final concentration of Decitabine and Cedazuridine of 2-12 µg/ml and 5-30 µg/ml, respectively. The UV-Vis detector at 245 nm was used for the evaluation, and the peak area for each peak was recorded. The calibration curve was drawn as a plot of concentration versus peak area. The calibration curve slope and intercept values were y = 336424x + 13333 (R2 = 0.9997) for Decitabine and y = 214915x - 13333 (R2 = 0.9999) for Cedazuridine.

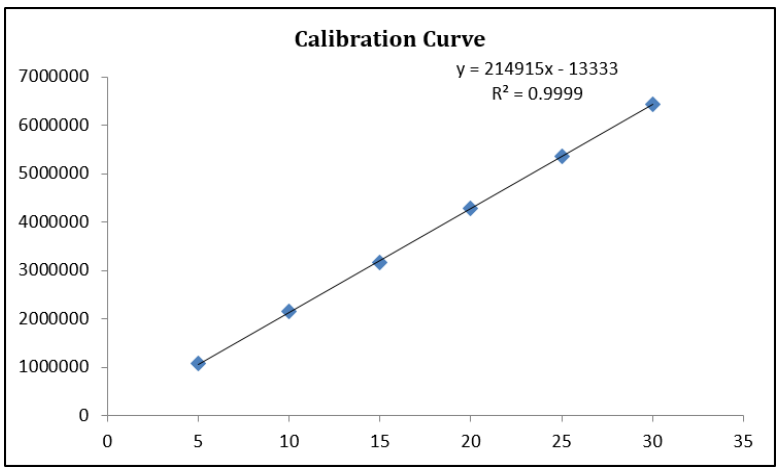
Linearity Data of Decitabine

Linearity		
Sr. No	**Concentration (µg/mL)**	**Peak Area**
1	2	671420
2	4	1342840
3	6	1964260
4	8	2685680
5	10	3357100
6	12	4028520
Slope		336424.29
Standard Error		22625.31

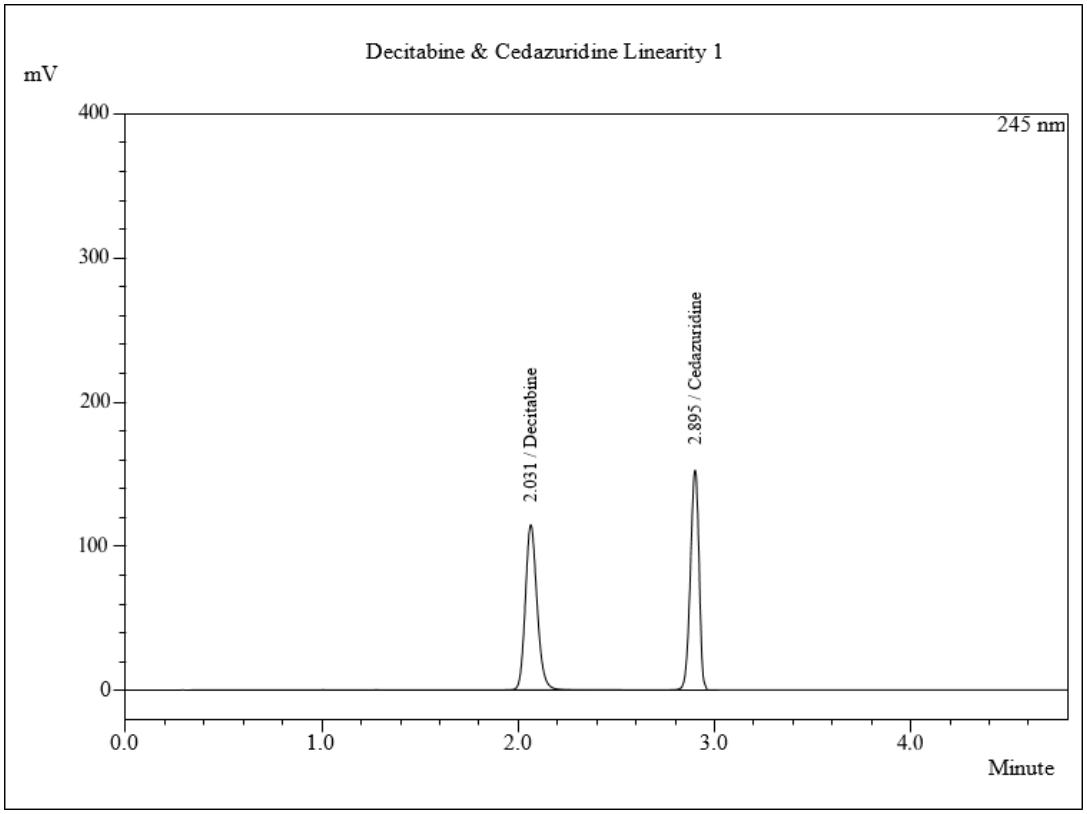


Calibration curve of Decitabine Linearity Data of Cedazuridine

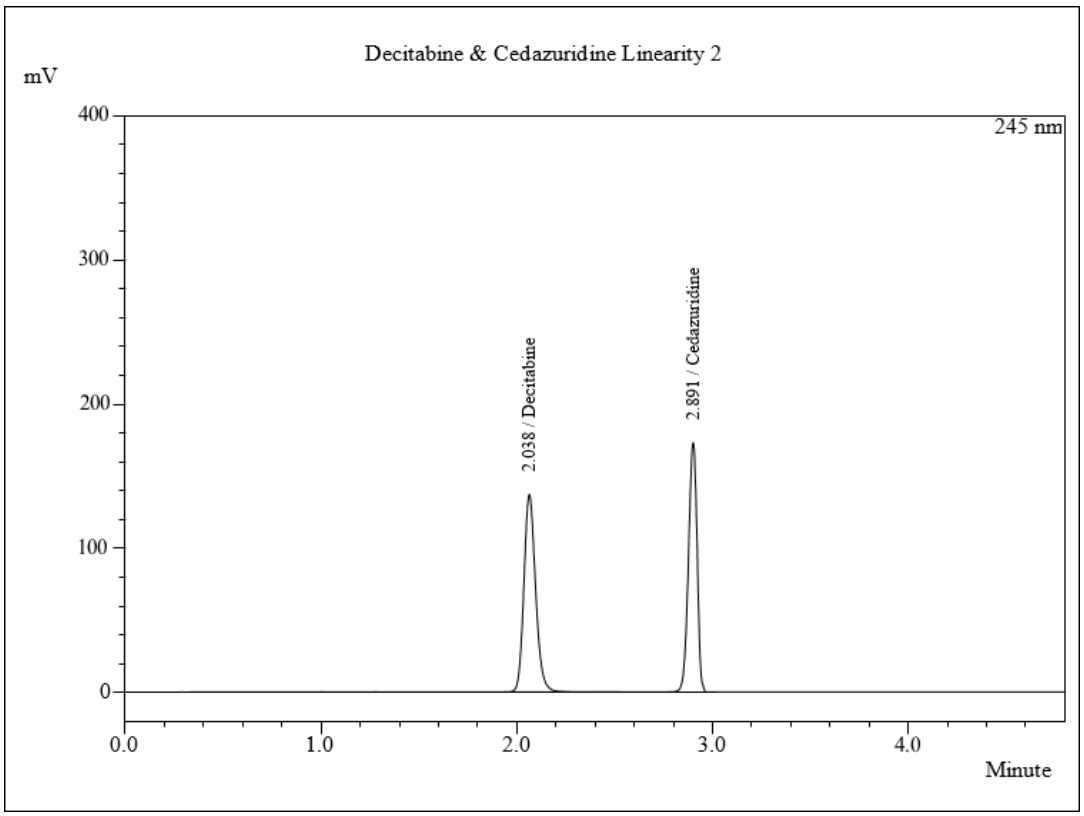
Sr. No	Concentration (µg/mL)	Peak Area
1	5	1073145
2	10	2146290
3	15	3169435
4	20	4292580
5	25	5365725
6	30	6438870
Slope		214914.71
Standard Error		22625.31



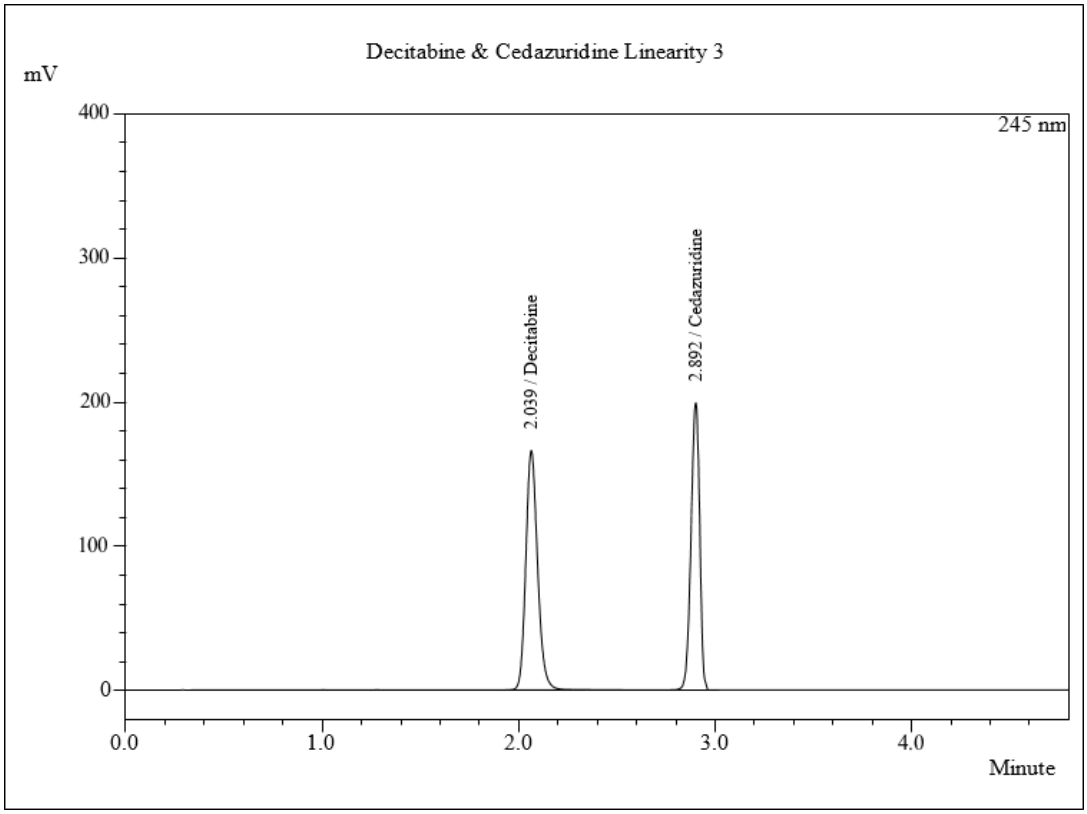
Calibration curve of Cedazuridine



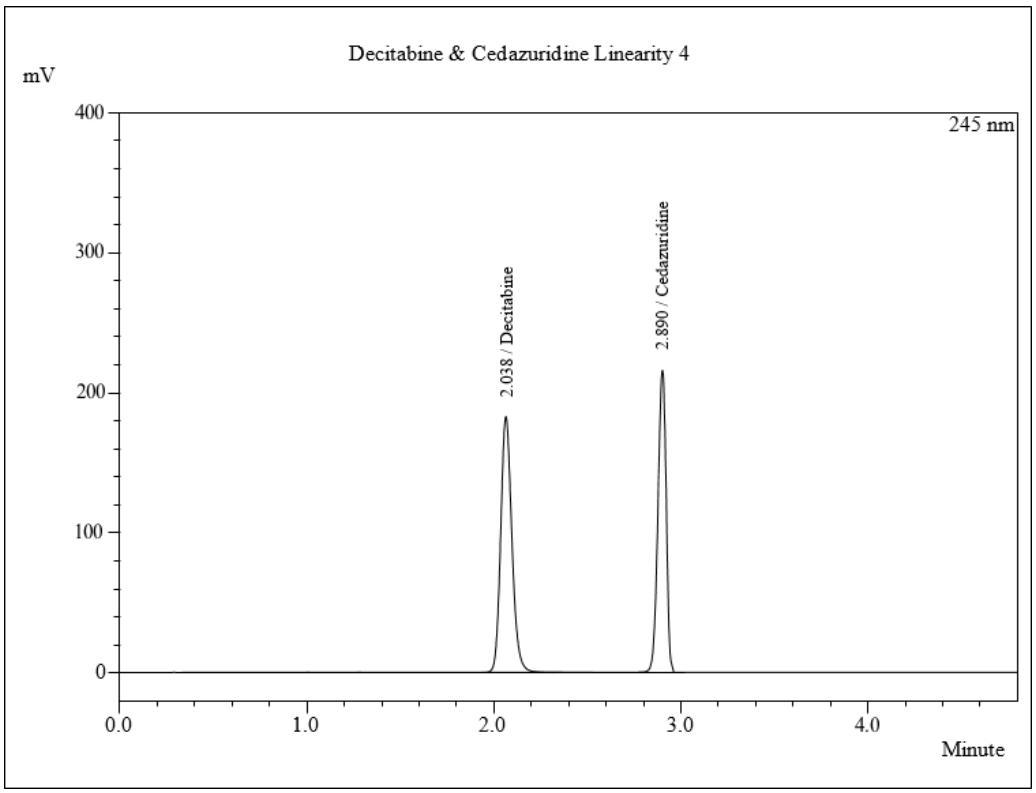
Chromatogram of Injection 1



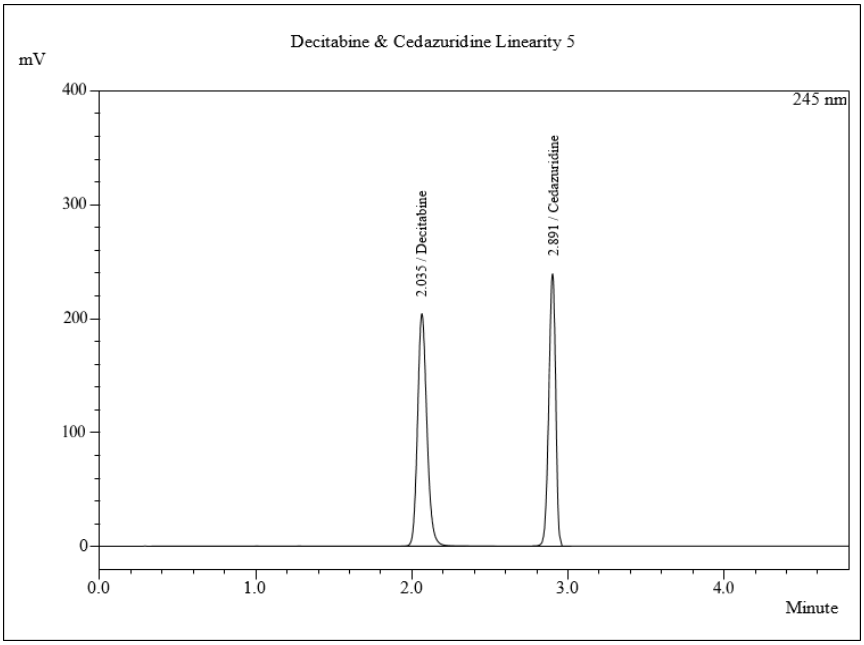
Chromatogram of Injection 2



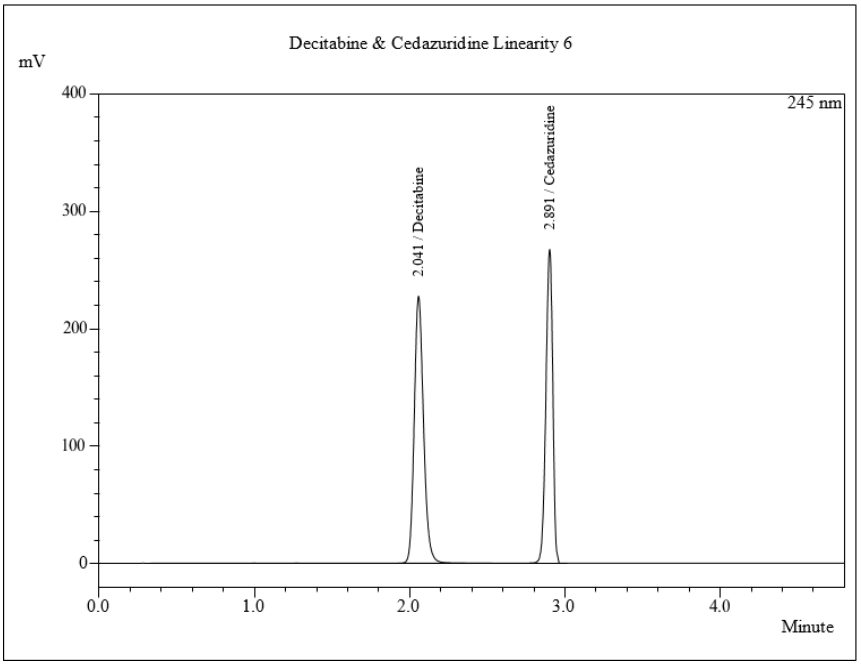
Chromatogram of Injection 3



Chromatogram of Injection 4



Chromatogram of Injection 5



Chromatogram of Injection 6

System Suitability: System suitability study was performed with five replicate analyses of solution of 100 % target concentration of Norfloxacin and Tinidazole. Several chromatographic parameters were determined, including retention duration, peak area, column efficiency, tailing factor, and resolution between the peaks, and the method was evaluated using these data.

Optimized Chromatographic Condition

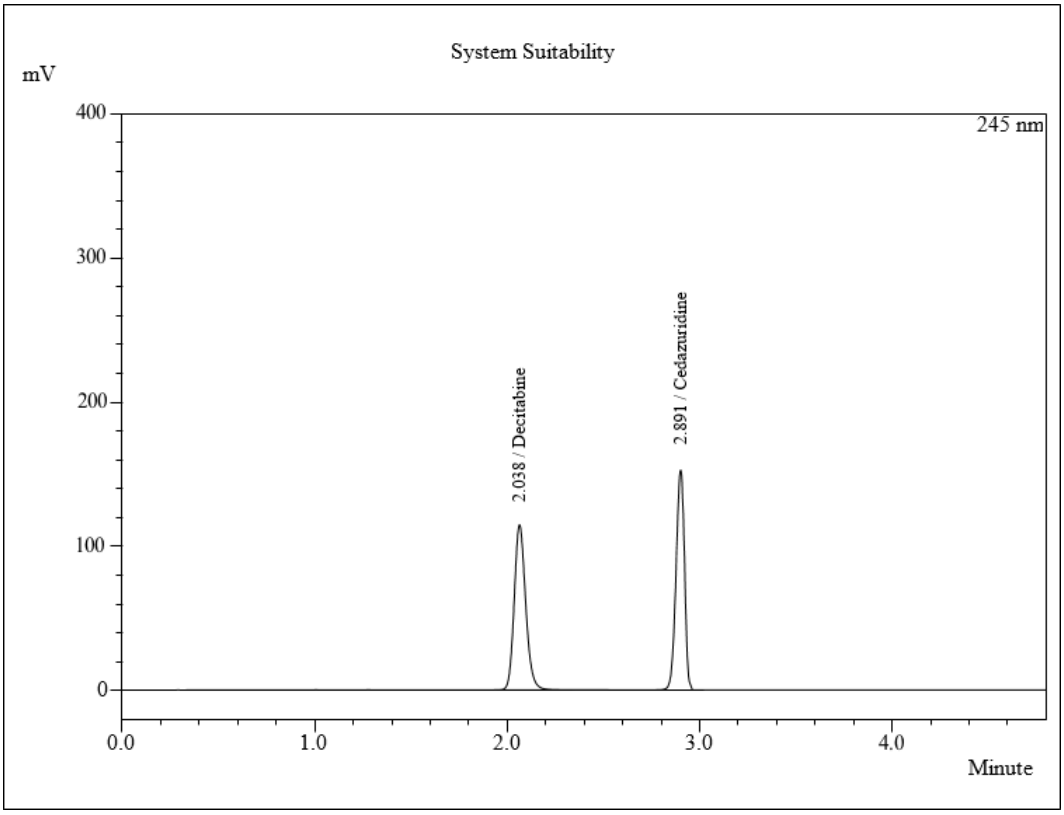
Optimized Chromatographic Condition		
Mobile Phase (V/V)	Acetonitrile	Buffer
	50	50
Acetonitrile : Potassium Phosphate Buffer		
pH of Aqueous Phase	5	
Flow Rate (mL/min)	1	
Detection Wavelength (nm)	245	

System Suitability Parameter of Decitabine

System Suitability Parameter		
Retention time (min)	**Concentration (µg/mL)**	2.041
Peak area	2	671420
Theoretical plates		11209
Asymmetric Factor		1.2

System Suitability Parameter of Cedazuridine

System Suitability Parameter		
Retention time (min)	**Concentration (µg/mL)**	2.889
Peak area	5	1073145
Theoretical plates		13294
Asymmetric Factor		1.0



Chromatogram of System Suitability Injection

Specificity: The RP-HPLC method's specificity was determined by comparing the chromatograms of mixed standards and sample solutions. Retention time (tR), resolution (RS), and tailing factor (Tf) were all computed. There was a strong association between the results of mixed standards and sample solutions.

Formulation Details used in Specificity

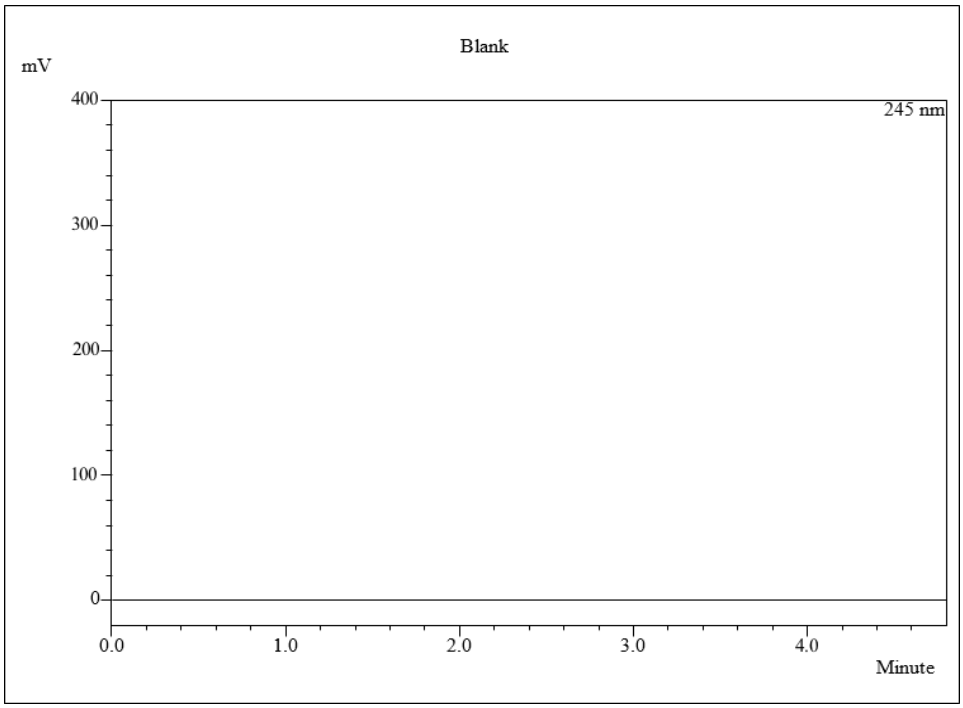
Formulation		
Name of Formulation	INQOVI (Decitabine & Cedazuridine) 35mg & 100mg Tablet	
Type of Formulation	Tablet	Tablet
Concentration (mg)	35	100

Specificity result of Decitabine

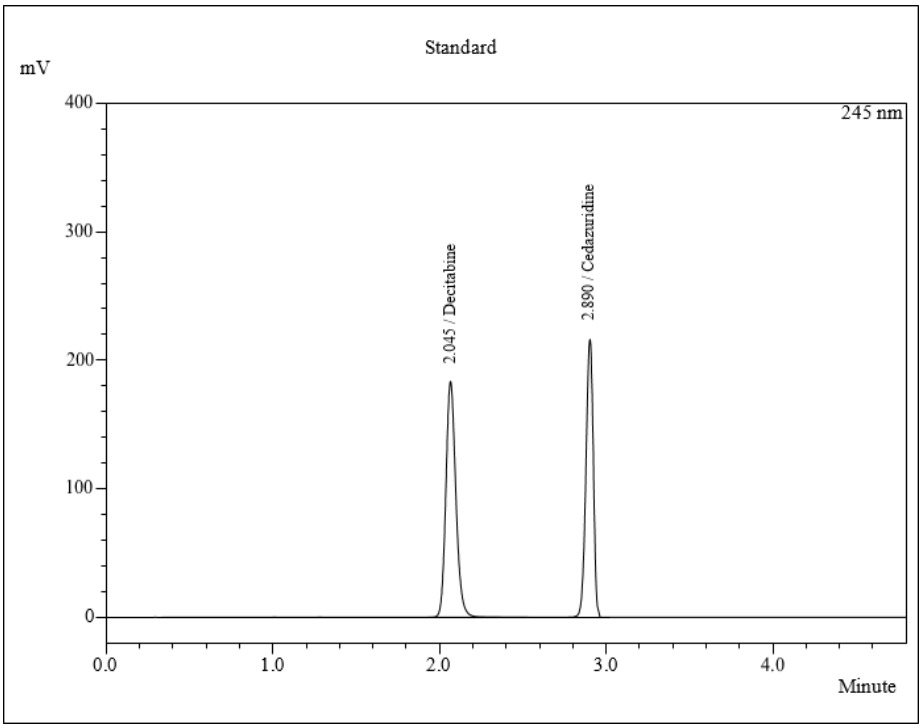
Specificity				
Sample	**Label Claim (mg)**	**Amount Found**	**Recovery**	**Retention Time**
Tablet	35	34.91	99.74286	2.041

Specificity result of Cedazuridine

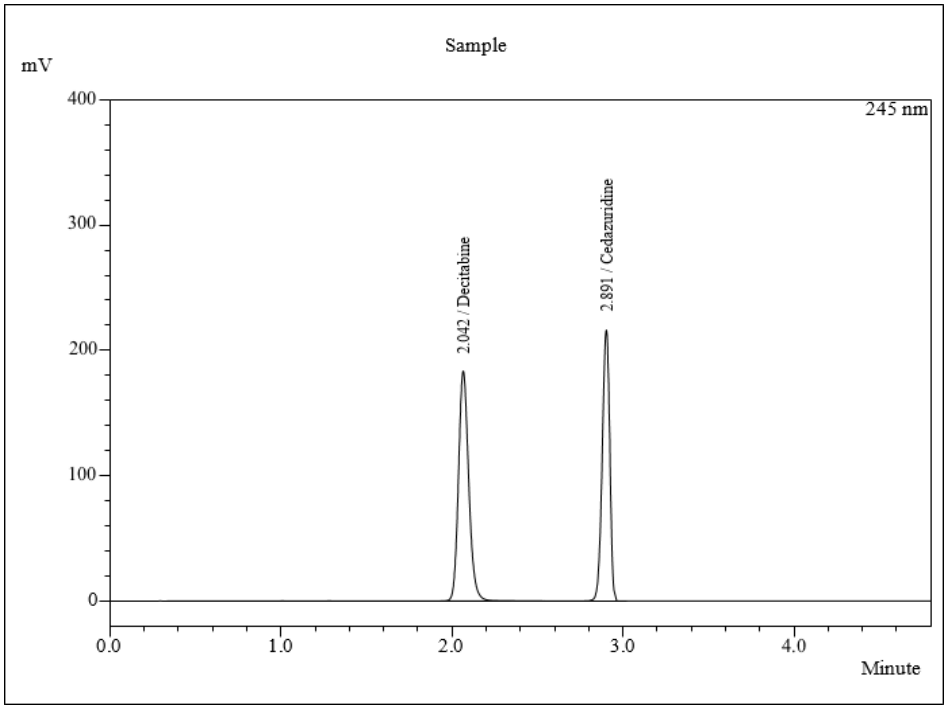
Specificity				
Sample	**Label Claim (mg)**	**Amount Found**	**Recovery**	**Retention Time**
Tablet	100	99.91	99.91	2.889



Chromatogram of Blank



Chromatogram of Standard



Chromatogram of Sample

Precision: Six repetitions of the sample made from commercial tablets were injected to determine method precision, and the assay was calculated to measure the repeatability of retention periods and peak area of standard and sample. The method's precision was validated by utilising a tablet stock solution. The intraday and interday precision tests were conducted by repeating the assay six times on the same day for intraday precision and on different days for interday precision studies. The findings of this study are as follows. The results shown in following figure.

Precision Study of Decitabine

Precision			
Sr. No	**Concentration (µg/mL)**	**Intraday**	**Interday**
1	8	2691185	2741185
2	8	2684036	2751386
3	8	2688715	2812600
4	8	2670008	2828070
5	8	2740927	2837968
6	8	2688668	2847849
Average		2693923.2	2803176
Standard Deviation		22140.80	41712.85
RSD%		0.8219	1.488

Precision Study of Cedazuridine

Precision			
Sr. No	**Concentration (µg/mL)**	**Intraday**	**Interday**
1	20	4298085	4348085
2	20	4290936	4358286
3	20	4295615	4419500
4	20	4276908	4434970
5	20	4347827	4444868
6	20	4295568	4454749
Average		4300823.2	4410076
Standard Deviation		22140.80	41712.85
RSD%		0.5148	0.946

Recovery

The approach's accuracy was determined through recovery trials at three levels (80%, 100%, and 120%) using the standard addition method. The percentage of analyte recovered was used to calculate the accuracy. The proposed method's accuracy was verified in accordance with ICH norms. For Decitabine and Cedazuridine, a tablet powder equivalent to Decitabine 3.5 mg and Cedazuridine 10.0 was placed in three separate 100 ml volumetric flasks separately, and then 8 mg (80%), 10 mg (100%), and 12 mg (120%) of standard Decitabine and Cedazuridine were added to each volumetric flask. The mobile phase phosphate buffer solution: Acetonitrile (50:50 v/v) was then poured to each volumetric flask and sonicated for 5 minutes. The solutions were then filtered, and 1 ml of the filtrate from each was placed in separate 10 ml volumetric flasks and diluted with mobile phase to the desired concentration.

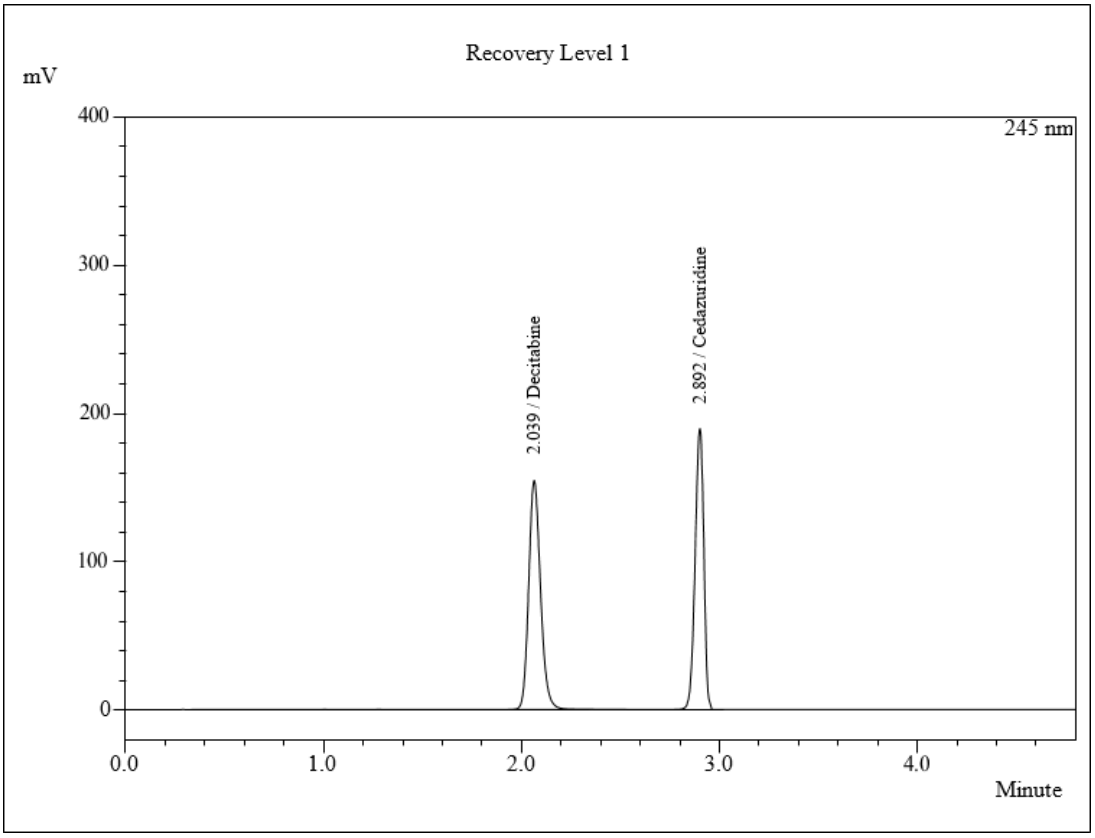
The solutions were injected into the chromatographic apparatus in triplicate, and the peak area was calculated to produce the percent recovery and standard deviation.

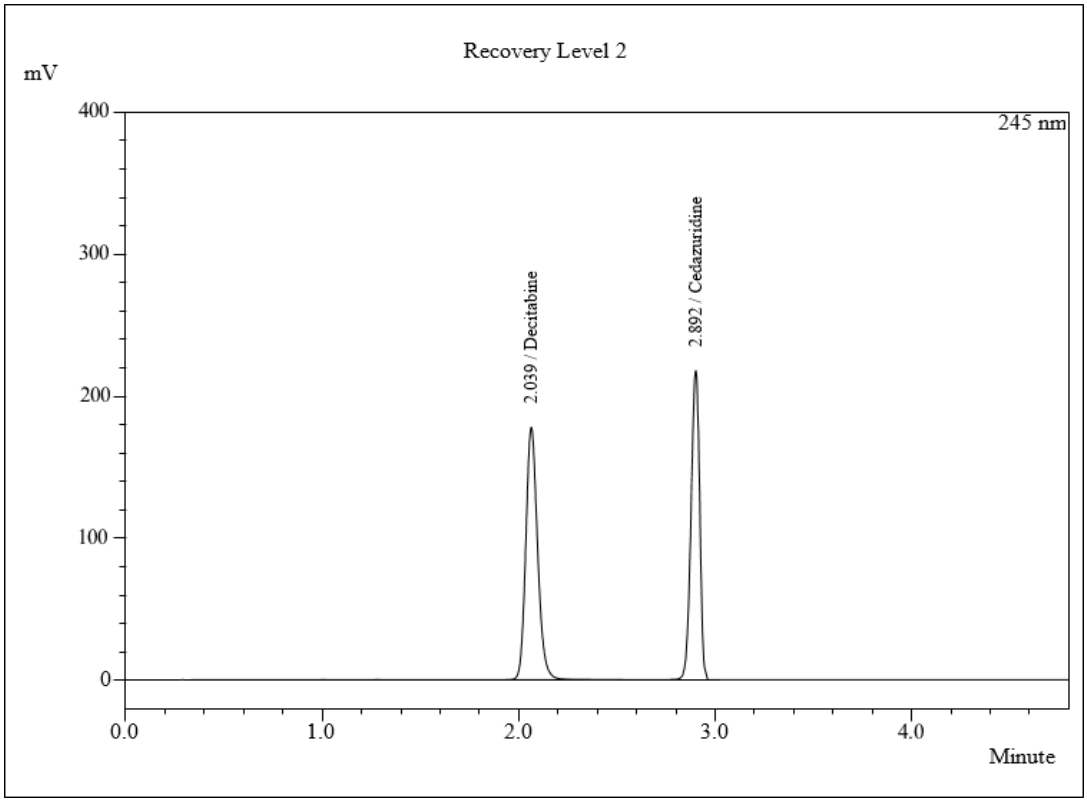
Recovery study of Decitabine

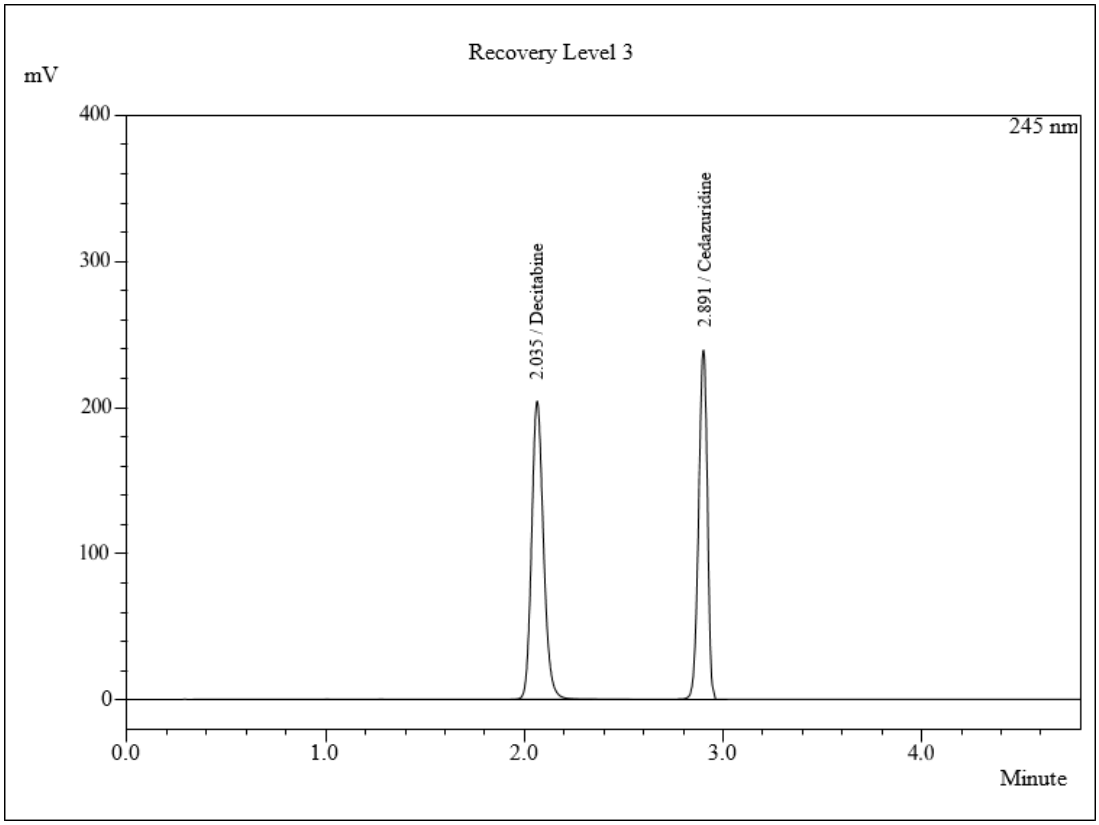
Recovery				
Sr. No	**Amount of Sample** (µg/ml)	**Amount of Drug Added** (µg/ml)	**Amount of Drug Recovered** (µg/ml)	**Recovery %**
1	8	4	3.989	99.73
2	8	8	7.99	99.89
3	8	12	12.01	100.08

Recovery study of Cedazuridine

Recovery				
Sr. No	**Amount of Sample** (µg/ml)	**Amount of Drug Added** (µg/ml)	**Amount of Drug Recovered** (µg/ml)	**Recovery %**
1	20	10	9.989	99.89
2	20	20	19.99	99.96
3	20	30	30.01	100.03







Robustness: The proposed method's robustness was tested by altering the solvent ratio in the mobile phase, flow rate, pH and wavelength range. The sample solutions were introduced into the chromatographic apparatus in 06 increments. Peak area was analysed, as well as its standard deviation and percent RSD.

Robustness study of Decitabine

Robustness					
Sr.No	**Parameter**		**Response**	**Parameter**	**Response**
Acetonitrile : Potassium Phosphate Buffer (V/V)			**Retention Time (min)**	**Detection Wavelength (nm)**	**Peak Area**
1	49	51	1.999	243	2627553
2	50	50	2.041	245	2685868
3	51	49	2.074	247	2719132
Average			2.035	**Average**	2677518
Standard Deviation			0.080	**Standard Deviation**	37850.36
RSD%			0.919	**RSD%**	1.414
Flow Rate (mL/min)			**Retention Time (min)**	**pH of Buffer (mmol/L)**	**Peak Area**
1	0.9		2.172	4.9	2713940
2	1		2.041	5	2686041
3	1.1		2.0369	5.1	2610420
Average			2.083	**Average**	2670134
Standard Deviation			0.0627	**Standard Deviation**	43733.13
RSD%			1.012	**RSD%**	1.6379

Robustness study of Cedazuridine

Robustness					
Sr. No	**Parameter**		**Response**	**Parameter**	**Response**
Acetonitrile : Potassium Phosphate Buffer (V/V)			**Retention Time (min)**	**Detection Wavelength (nm)**	**Peak Area**
1	49	51	2.792	243	4234453
2	50	50	2.889	245	4292768
3	51	49	2.988	247	4326032
Average			2.890	**Average**	4284418
Standard Deviation			0.080	**Standard Deviation**	37850.36
RSD%			1.769	**RSD%**	0.883
Flow Rate (mL/min)			**Retention Time (min)**	**pH of Buffer (mmol/L)**	**Peak Area**
1	0.9		3.001	4.8	4320840
2	1		2.889	5	4292941
3	1.1		2.891	5.2	4217320
Average			1.931	**Average**	4277034
Standard Deviation			0.0627	**Standard Deviation**	43733.13
RSD%			1.140	**RSD%**	1.0225

Limit of detection and Limit of quantification (LOD, LOQ): The suggested method's LOD and LOQ were obtained by gradually injecting lower amounts of the standard solutions under the specified chromatographic conditions. L.O.Q. = 10(SD/S) L.O.D. = 3.3(SD/S) Where SD denotes the standard deviation of the answer and S denotes the slope of the calibration curve. The slope S can be calculated using the analyte calibration curve.

LOD and LOQ Results

Sr. No.	Drug	LOD (µg/mL)	LOQ (µg/mL)
1	Decitabine	0.2219	0.6725
2	Cedazuridine	0.3474	1.0528

CONCLUSION

With a short analytical time, the new approach provides good resolution between Decitabine and Cedazuridine. The approach is simple, accurate, fast, and precise, and it can be used for regular drug analysis without requiring any sophisticated sample preparation.

This is a simple, accurate, and convenient simultaneous estimation of Decitabine and Cedazuridine moreover the novel approach to this simultaneous estimation.

First method developed for simultaneous estimation of Decitabine and Cedazuridine is used. The retention time for Decitabine and Cedazuridine are 2.244 and 2.891

mins simultaneously which are very less as compared to other reported methods.

The analysis get completed only in 5.00 minutes. The proportion of organic phase i.e. methanol required is less than other reported method.

All above factors make the process more efficient, cost effective and less time consuming.

To develop regular method on trial-and-error basis, it require longer time, but approach save time and increases efficiency.

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