Original Article

A Systematic Analytical Quality By Design Approach For Estimation Of Decitabine And Cedazuridine Combined Pharmaceutical Formulations Using RP-HPLC

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Abstract

The present research work emphasises systematic development of a simple, fast, sensitive, reproducible and cost-effective reversed-phase high-performance liquid chromatographic (RP-HPLC) method, applying the principles of Quality-by-Design (QbD) for analysis of Decitabine (DCT) and Cedazuridine (CDZ) in combined oral pharmaceutical formulation. Preliminary risk assessment was performed and then screening studies were carried out using a four-factor-two-run fractional factorial design (FFD). Eventually, optimization studies were executed out employing a central composite design (CCD) with flow rate and mobile phase ratio as the critical method parameters (CMPs), and tailing factor (TF), % assay and theoretical plate count (TPC) as the critical analytical attributes (CAAs). The optimized RP-HPLC method for determination of DCT and CDZ used X-Terra C18 column (250 X 4.6mm, 5µm particle size) and mobile phase consist of 0.1%OPA: Methanol, 45:55 v/v. The retention time for DCT and CDZ was found to be 2.923min and 3.948min. The method was linear in the range of 10–200 µg/ml with 0.992 correlation coefficient. The % RSD for repeatability, intraday, and inter day precision was found to be less than 2% indicating the optimized method was precise. The LOD and LOQ were 0.22µg/ml, 0.24µg/ml and 0.67 µg/ml,0.72µg/ml, respectively. According to the ICH guidelines, the percent recovery of spiked samples ranged between 99.57 1.47 and 100.79 1.73. The developed method was validated in accordance with the ICH Q2 (R1) guidelines, as well as the principles and science of analytical quality by design (AQbD), for estimating DCT and CDZ in bulk drugs and marketed formulations with a high degree of linearity, accuracy, precision, sensitivity, and robustness.

Keywords — Quality-by-design, Decitabine, Cedazuridine, chromatogram, Repeatability .

INTRODUCTION

Analytical QbD is defined as a science and risk based model for developing analytical methods, with the goal of comprehending the established objectives for controlling the important method variables that affect the critical method attributes in order to obtain better process performance, high robustness, ruggedness, and flexibility for continuous improvement[1,2]. Analytic QbD, like process QbD, produces a well-known, fit-for-purpose, and robust method which thus consistently delivers the desired throughput over its life cycle[3,4]. Robustness and ruggedness should be tested earlier in the development stage for QbD, HPLC method to ensure the method's efficiency over the product's lifetime[5]. Otherwise, if a non-rugged or non-robust system is used, it can take a significant amount of time and energy to re-develop, re-validate, and re-transfer analytical methods. The primary goal of AQbD is to identify failure modes, create robust method operable design regions or design space within meaningful system suitability criteria, and manage the system's continuous life cycle. A review of the literature reveals that QbD approaches for the HPLC method have been reported[6-8].

Genes that are essential for the regulation of cellular differentiation and proliferation may regain their normal function when decitabine causes hypomethylation in cancer cells. The development of covalent adducts between decitabine integrated into DNA and DNA methyltransferase may also be responsible for decitabine's cytotoxicity in rapidly proliferating cells. Decitabine

has a low level of sensitivity in non-proliferating cells. Decitabine is a chemical compound with the molecular formula $C_8H_{12}N_4O_4$ and a mass of 228.21 daltons. In accordance with the International Union of Pure and Applied Chemistry (IUPAC), its molecular name is 4-amino-1-[(2R,4S,5R) the compound [4-hydroxy-5-(hydroxymethyl)oxolan-2-yl] -1,3,5-triazin-2(1H)-one [9]. Cytidinedeaminase (CDA), an enzyme that catalyses the breakdown of cytidine, especially the cytidine analogue decitabine, is inhibited by cedazuridine. A CDA inhibitor is cedazuridine with molecular weight of 268.21 daltons and the chemical formula $C_9H_{14}F_2N_2O_5$. The IUPAC name for it is (4R) -1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl] One -4-hydroxy-1,3-diazinan [10]. Decitabine's systemic exposure is increased when cedazuridine is also administered.

The literature lacks information on quantitative analysis and characterisation despite several articles reporting the synthesis, effectiveness, and pharmacokinetics of DCT and CDZ. There have been other HPLC methods of related nucleoside analogues published in the literature, including decitabine [11,12], zebularine[11], fluorodeoxycytidine[13], 5-iodo-2-pyrimidinone-2'-deoxyribose [14], bromodeoxyuridine[15], and azacytidine[16]. These nucleosides were evaluated using C18 columns eluted with aqueous buffers containing methanol or acetonitrile as organic modifiers because the majority of them have moderate to high polarity. There are no known AQbD strategies for the two medications used together. This study's aims to develop and validate an RP-HPLC method for the simultaneous determination of DCT and CDZ in bulk and tablets in conformity with ICH Q2 (R1), ICH Q8 Pharmaceutical Development, and ICH Q10 Pharmaceutical Quality System guidelines, as well as to assess the critical quality analytical attributes for the developed method in order to establish a robust, optimised method that meets system suitability requirements.

MATERIALS AND METHODS

Materials

Decatabine and Cedazuridine were obtained from Intas Pharmaceutical Pvt. Ltd. in Gujarat as gift samples. The HPLC grade solvents were employed, while all other chemicals and reagents of analytical grade. The pills with the combination formulation were purchased under the trade name INQOVI (Decitabine35 mg and Cedazuridine 100 mg).

Instrumentation

The X-Terra C18 column (250mm 4.6 mm 5 m particle size) and the HPLC WATERS-2695 with Detector UV-VIS Dual Absorbance Detector WATERS-2487 were used at room temperature.

Chromatographic conditions

The isocratic elution of X-TerraC-18 column (250 mm 4.6 mm, 5.0 m particle size) was employed. The mobile phase utilised was a mixture of methanol and ortho phosphoric acid buffer (OPA) at a ratio of 45:55 %v/v. The column was kept at room temperature, and the flow rate was kept constant at 1 ml/min. Eluents were monitored with a PDA detector operating at 229 nm. With the aforementioned chromatographic conditions, an acceptable separation and peak symmetry for the medication were achieved. Using a factorial design, the HPLC technique for DCT and CDZ was adjusted for a number of factors, including mobile phase and pH as two variables at varying values.

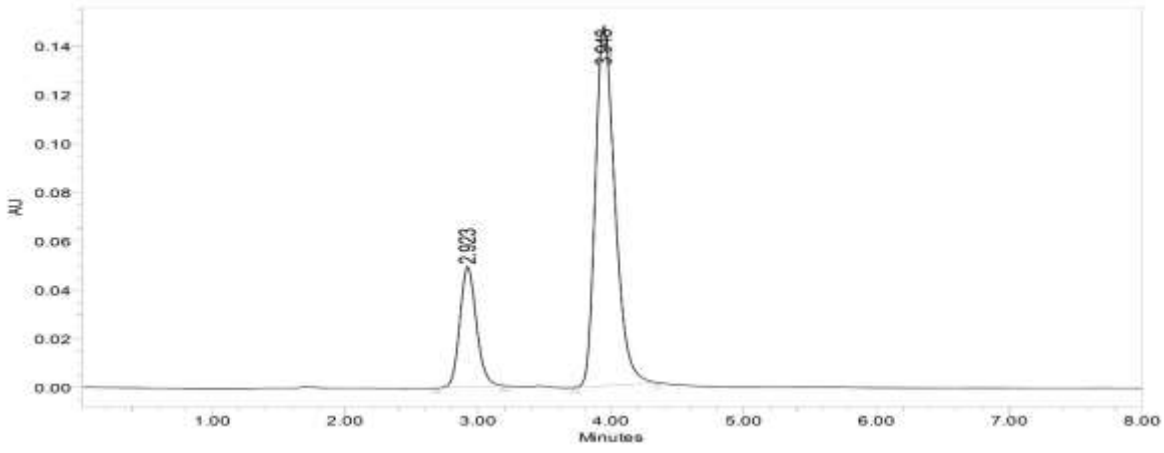


Figure 1: Optimized chromatogram of DCT and CDZ

Preparation of 0.1% ortho phosphoric acid Buffer:

1 ml of orthophosphoric acid was pipetted into 1000 ml of HPLC water.

Mobile Phase Preparation:
In an ultrasonic water bath, combine 450 mL (45%) of the aforementioned buffer with 550 mL (55%) of Methanol HPLC degas for five minutes. Filter through a 0.45 filter under vacuum filtration.

Diluent Preparation: As a diluent, use the mobile phase.

Preparation of the Decitabine&Cedazuridine Standard

Preparation of Standard Solution:
Weigh and transfer the 35 mg and 100 mg decitabine and cedazuridine working standards into a 10 mL clean, dry volumetric flask. Add the diluent and sonicate to completely dissolve it, then add more to the desired volume using the same diluent. (Stock solution)

Pipette 0.1 ml of the aforesaid stock solution of decitabine and cedazuridine into a 10 ml volumetric flask and diluent until the desired concentration is reached (35 ppm Decitabine and 100 ppm of Cedazuridine).

Selection of detection wavelength
Different CDZ and DCT concentrations were scanned in the 200-400 nm range, and a maximum wavelength of 229 nm was accepted as the detection wavelength.

QbD Method Development for RP-HPLC

Quality target product profile selection
The QTPP is necessary for determining the variables that influence the QTPP parameters. The retention time, theoretical plates, and peak asymmetry were identified as QTPP for the proposed RP-HPLC method [17, 18].

Critical quality attributes
The critical quality attributes are method parameters that have a direct impact on the QTPP. The mobile phase composition and buffer pH were two critical method parameters that needed to be controlled in order to maintain QTPP's acceptable response range [19].

Factorial-Design
Following the definition of the QTPP and Critical quality attributes, the factorial design was used to optimise and select the key components of the HPLC method: mobile phase, flow rate, and pH. The various interaction effects and quadratic effects of mobile phase composition, flow rate, and pH of buffer solution on retention time, theoretical plates, and peak asymmetry were investigated using response surface design.

The best suited response for factorial response surfaces was created using Design Expert® (Version 11.0, Stat-Ease Inc., and M M)[20].

Experiment results evaluation and final method condition screening
Design of Experiments (DoE) has been widely used to understand the effects of multidimensional and interaction of input factors on analytical method output responses. The Design Expert tools will be used to optimise the best chromatographic conditions.

Risk assessment
The final optimised method is chosen based on the method's characteristics, such as the fact that the developed method is efficient and will remain operational throughout the life of the product. To assess the method's robustness and ruggedness, a risk-based approach based on the QbD principles outlined in the ICH Q8 and ICH Q9 guidelines was used[21]. The parameters of the method or its performance were evaluated under various conditions for robustness and ruggedness studies, such as different laboratories, chemicals, analysts, instruments, reagents, and days[22].

Control strategy
The analytical control strategy is a predetermined set of controls derived from a thorough understanding of the various parameters, which include fitness for purpose, analytical procedure, and risk management. All of these parameters ensure that

the method's output performance and quality are within the analytical target profile. An analytical control strategy was developed for sample preparation, measurement, and replicate control operations[23].

Analytical method validation

Method validation is documented evidence that provides a high level of assurance that the process used to confirm the analytical process is appropriate for its intended use for a specific method. The developed HPLC method for calculating DCT and CDZ was validated using the ICH Q2 (R1) guidelines [24,25].

Linearity

The linearity of DCT and CDZ was assessed by analysing five independent concentration levels ranging from 10-200 g/ml. Peak area on the y axis was plotted against concentration on the x axis to create the calibration curve. The regression line equation and correlation coefficient were calculated.

Precision

The repeatability was determined by measuring six samples of 100 µg/ml DCT and CDZ. The intraday and interday precision were determined by testing three different concentrations (100, 150 and 200µg/ml) of DCT and CDZ at three different times, on the same day at two-hour intervals, and on three different days. The acceptance limit for RSD was less than 2.

Accuracy

The method's accuracy was determined by calculating recovery rates from marketed formulations at three levels of 80 percent, 100 percent, and 120 percent of standard addition. The percentage of sample recovery was calculated. According to ICH guidelines, the acceptance limit for percent recovery was 98-102 percent of standard addition.

Ruggedness

The ruggedness studies were determined by changing the analyst as an extraneous influencing factor. The calculated percent RSD of the acceptance limit for the peak area was less than 2.

Assay

A total of twenty tablets were powdered and weighed. Transfer 100 mL of volumetric flask to an accurately weighed powder containing 100 mg CDZ and 35mg DCT. Sonicate for 15 minutes, or until the powder dissolves, with 25 mL of methanol. Then, using mobile phase, increase the volume to the desired level. Filter the resulting solution through 0.42 Whatman filter paper. To achieve a concentration of 100 g/ml, dilute 0.5 ml to 10 ml of the filtrate. The solution was analysed using HPLC under the same chromatographic conditions as linearity. The calculation used the mean of three different assays.

RESULTS

Initially, 0.1% TFA: Acetonitrile (50:50), 0.1% TFA: Methanol (30:70), 0.1% TFA: Methanol (25:75), NaH_2PO_4: Methanol (10:90), and 0.1% OPA: Methanol (30:70) mobile phases were tried, and the peak was observed at far retention time. With the mobile phase acetonitrile to water, 80:20 v/v, no single peak was observed. The following mobile phase was tried: 0.1% OPA: Methanol, 40:60 v/v. The peak shape and symmetry were improved by adjusting the buffer pH. The optimised chromatographic conditions satisfied the system suitability test parameters. The optimised mobile phase is a 45:55 v/v mixture of 0.1% OPA and methanol. Following that, the factorial design was used to optimise various parameters within the design space.

QbD method for developing HPLC methods[26]

Profile of a high-quality target product

The QTPPs chosen for optimization of HPLC chromatographic conditions were retention time, theoretical plates, and peak asymmetry.

Critical quality attributes

The mobile phase composition was 0.1% OPA: Methanol, 45:55 v/v, with good peak shape and resolution.

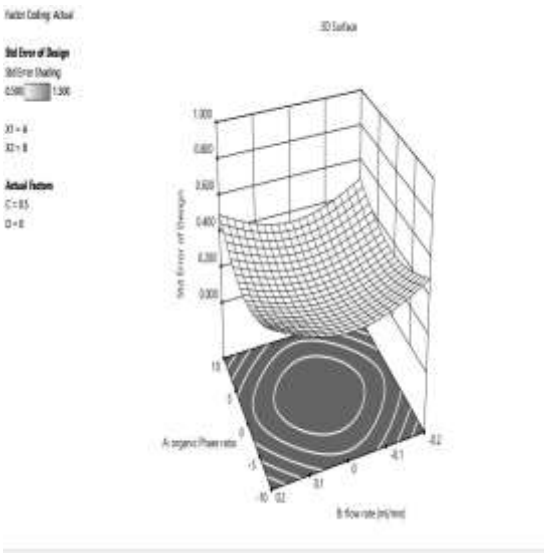
Factorial design [27]

For the proposed HPLC method development, the box plot factorial design was chosen. Table 1 depicts the optimization of various parameters.

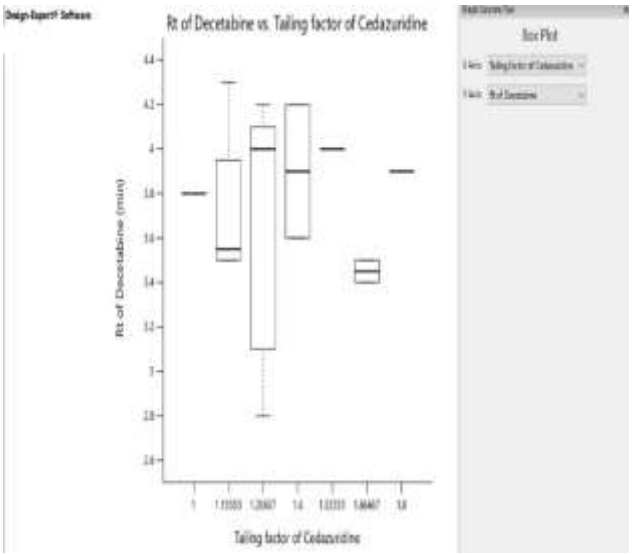
Table 1: Factors

Factor	Name	Units	Type	SubType	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	organic Phase ratio	ml/min	Numeric	Continuous	-10.00	10.00	-1 ↔ -10.00	+1 ↔ 10.00	0.0000	10.33
B	flow rate				-0.2000	0.2000	-1 ↔ -0.20	+1 ↔ 0.20	0.0000	0.2066
C	Buffer pH				-3.00	4.00	-1 ↔ -3.00	+1 ↔ 4.00	0.5000	3.61
D	Aqueous phase				-10.00	10.00	-1 ↔ -10.00	+1 ↔ 10.00	0.0000	10.33

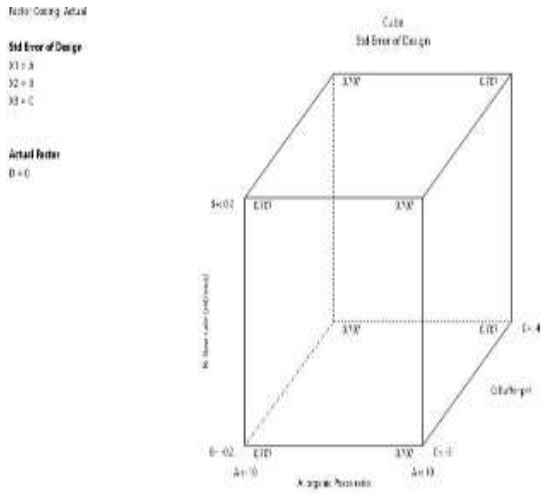
The different design of experiments (DOE) carried out under factorial design space is enclosed with following factor screening studies.



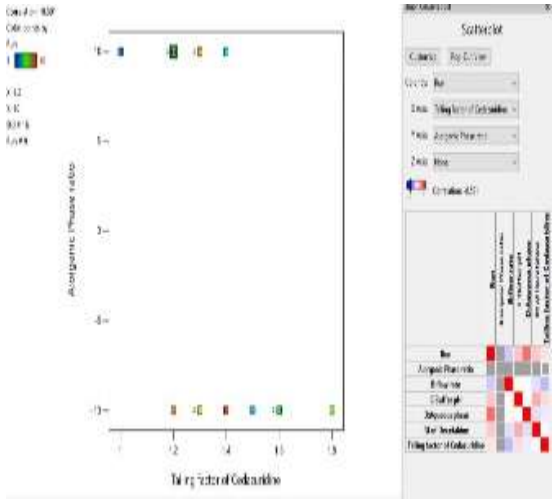
a) 3D SURFACE PLOT



b) BOX PLOT



c) CUBE PLOT



d) SCATTER PLOT

Figure 2: [a] 3D surface of factor coding actual, [b]Box plot of Rt of DecetabineVsTailing factor of Cedazuridine, [c]Cube plot of factor coding actual, [d]Scatter plot.

Design space [28]

With four runs, the response surface study type, box behnken design, and 2-factorial design model were used. The proposed experimental design was used, and the evaluation of mobile phase composition, flow rate, buffer pH, and peak asymmetry was done against the three responses, retention time, theoretical plates, and peak asymmetry, and the results were summarised in figures 2, 3, and 4.

Box-Behnken design are special types of three-level fractionate factorial designs, which allows modeling 1st and 2nd order response surfaces. These designs are more cost-effective than three-level full factorial designs, particularly for large number of input factors.

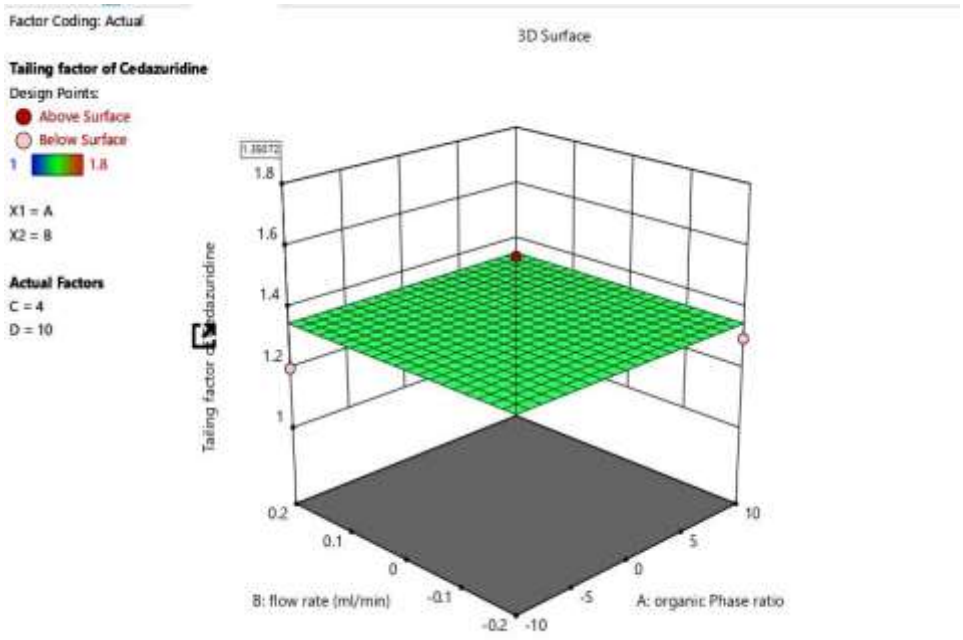


Figure 3: 3D surface plot of DCT and CDZ

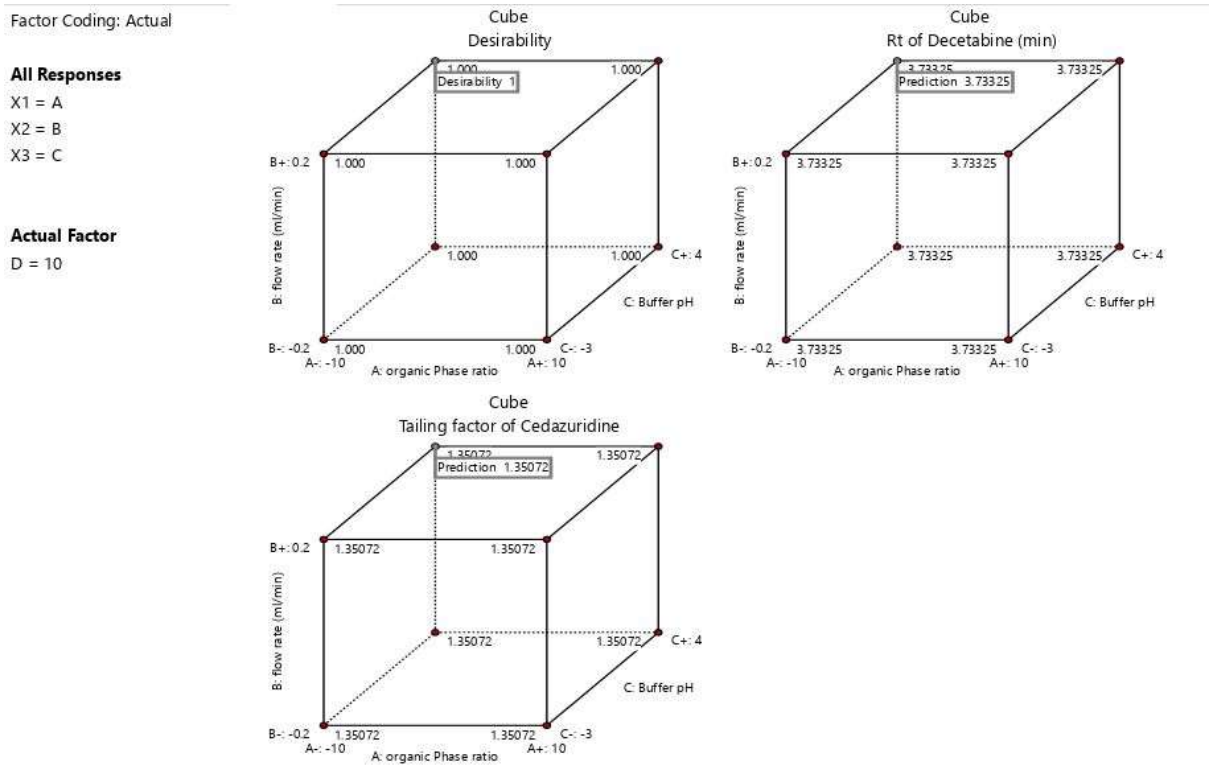


Figure 4: Cube plot of DCT and CDZ

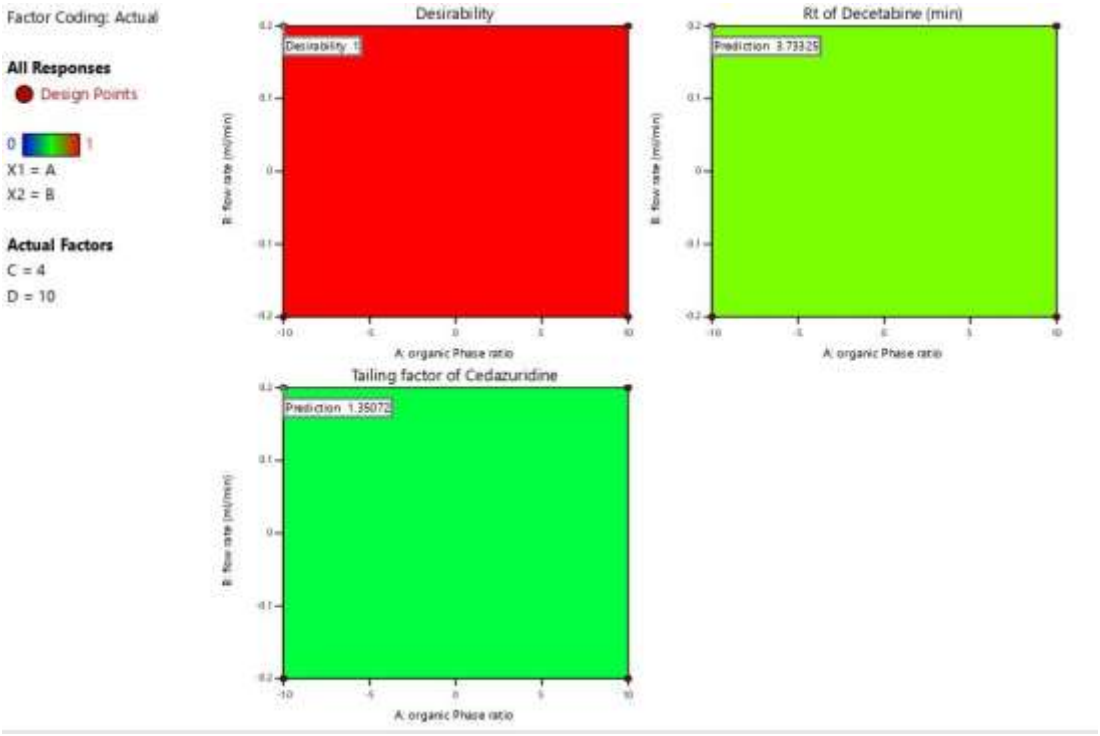


Figure 5: Boxbenhnken plot of DCT and CDZ

Optimized condition obtained

It was obtained by analysing all responses in various experimental conditions with the Design expert 11.0 software, and optimised HPLC conditions and predicted responses are shown in tables 2 and 3.

Table 2: Point Prediction

Two-sided Confidence = 95% Population = 99%

Solution	Predicted Mean	Predicted Median*	StdDev	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Rt of Decetabine†	3.73325	3.71103	0.409083	3.52212	3.95703	2.36069	5.8338
Tailing factor of Cedazuridine†	1.35072	1.33704	0.193725	1.25182	1.45743	0.740543	2.414

- For transformed responses the predicted mean and median may differ on the original scale.

† Standard error (SE) not calculated on original scale.

Table 3: Confirmation

Two-sided Confidence = 95%

Solution 1 of 17 Response	Predicted Mean	Predicted Median*	StdDev	n	95% PI low	95% PI high
Rt of Decetabine‡	3.73325	3.71103	0.409083	1	2.91911	4.71779
Tailing factor of Cedazuridine‡	1.35072	1.33704	0.193725	1	0.977214	1.82935

For transformed responses the predicted mean and median may differ on the original scale.

† For transformed responses the data mean is calculated on the transformed scale.

‡ Standard error (SE) not calculated on original scale.

System suitability
The system retention time was discovered to be 4 minutes, theoretical plates were 5263, peak asymmetry was 1.35, and the % RSD of a six replicate suitability test was applied to a representative chromatogram to check various parameters such as injections was 0.92.

Linearity
When the graph was plotted with peak area verses concentration, the constructed calibration curve for DCT and CDZ was linear over the concentration range of 10-200 g/ml with a 0.992 correlation coefficient.

Precision
The % RSD for repeatability for DCT and CDZ was less than 0.082 after six measurements of the same concentration (100 g/ml). Precisions were determined for the interday and intraday periods. The % RSD value less than 2 indicated that the developed method was accurate.

Accuracy
The recovery study was used to determine the accuracy. Spiking at three levels, 80%, 100%, and 120%, was used to create sample solutions. The proposed HPLC method's % recovery data is calculated. The % of recovery within 98-102% supports the developed method's accuracy in accordance with the ICH Q2 (R1) guidelines.

Robustness and Ruggedness
DCT and CDZ solutions at 100 g/ml were used for robustness and ruggedness studies. The robustness was investigated by making small but deliberate changes to intrinsic method parameters such as mobile phase pH and flow rate. The ruggedness was investigated as an extraneous influencing factor by a change in analyst. The % RSD for peak area was found to be less than 2 when the pH of the mobile phase, flow rate, and analyst were changed.

LOD and LOQ
Based on the standard deviation of slope and intercept, the LOD and LOQ for DCT and CDZ were determined to be 0.22 g/ml, 0.24 g/ml, and 0.67 g/ml, 0.72 g/ml, respectively.

Assay
When the assay was performed from tablets, the optimised chromatogram of DCT and CDZ showed a resolved peak at retention time 2.923min and 3.948min. For the label claim of DCT and CDZ, the % assay of drug content was calculated. The results of the assay demonstrated the method's ability to measure accurately and precisely in the presence of excipients present in tablet powder.

DISCUSSION
Designing for analytical quality a method for estimating DCT and CDZ in pharmaceutical formulations using HPLC has been developed. For the analysis of DCT and CDZ by HPLC, the analytical target product profile included retention time, theoretical plates, and peak asymmetry. The four variables identified as critical quality attributes that affect the analytical target product profile are the organic phase composition, aqueous phase, flow rate, and pH of buffer solution. The factorial design - Box Behnken design was used with the Design Expert Software Version 11.0 for four factors at three different levels. The risk assessment study determined the critical variables influencing the analytical target profile[27]. In chromatographic separation, column selection, instrument configuration, and injection volume were kept under control, while variables such as mobile phase pH, flow rate, and buffer pH were assigned to a robustness study.

The HPLC method for DCT and CDZ was successfully developed using the quality-by-design approach. The optimised RP-HPLC method for DCT and CDZ determination used an X-Terra C18 column (250 4.6 mm, 5 m particle size) and a mobile phase of 0.1%OPA:Methanol, 45:55 v/v. The retention times for DCT and CDZ were found to be 2.923min and 3.948min, respectively. The method was linear in the 10-200 g/ml range, with a correlation coefficient of 0.992. The % RSD for repeatability, intraday, and interday precision were all less than 2%, indicating that the optimised method was accurate. The LOD and LOQ values were 0.22 g/ml, 0.24 g/ml, and 0.67 g/ml, 0.72 g/ml, respectively. The percentage recovery of spiked samples ranged from 99.57 1.47 to 100.79 1.73. The method was created in accordance with the ICH guidelines.

CONCLUSION
A quality-by-design approach was used to optimise the development of HPLC methods. The method focuses on the target analytical product profile. The experimental design goes into detail about scouting key HPLC method components like mobile phase pH, flow rate, and buffer pH. A multivariant study of several important process parameters, such as the combination of

four factors, namely the organic phase composition, aqueous phase, flow rate, and buffer pH at three different levels, was performed to determine the best performing system and the final design space. A factorial design was used to investigate and optimise their interactions at various levels. This section provides a better understanding of the factors influencing chromatographic separation in terms of the methods' ability to achieve their intended goals. This method provides a practical understanding of knowledge that will aid in the development of a future chromatographic optimization. The validation parameters all met the acceptance criteria. It was discovered that the validated method for determining DCT and CDZ was linear, precise, accurate, specific, robust, and rugged. The QbD method development approach has resulted in a better understanding of method variables, resulting in a lower risk of failure during method validation and transfer. When compared to manual method development, the automated QbD method development approach using the Design Expert software resulted in a more robust, better performing method in less time. The method is repeatable, selective, accurate, and robust, according to data statistical analysis. In the future, this method will be used for routine quality control analysis in the pharmaceutical industry.

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CONFLICT OF INTEREST: None.

REFERENCES

1. Sandipan R et al. Quality by design: A holistic concept of building quality in pharmaceuticals. International Journal Pharm Biomed Res. 2021; 3:100-108.
2. The international conference on harmonization ICH technical requirements for registration of pharmaceuticals for human use on pharmaceutical development Q8(R2)(2009).
3. The international conference on harmonization ICH technical requirements for registration of pharmaceuticals for human use on Quality Risk Management Q9(2005).
4. The international conference on harmonization ICH technical requirements for registration of pharmaceuticals for human use on pharmaceutical Quality System Q10(2008).
5. Borman P, Nethercote P, Chatfield M, Thompson D, et al. The application of quality by design to analytical methods. Pharm Tech. 2007; 31:142-152.
6. Schweitzer M, Pohl M, et al. Implications and opportunities of applying QbD principles to analytical measurements. Pharm Tech. 2010; 34:52-59.
7. Galen WE 2004. Analytical Instrumentation handbook 2ndEdn. Marcel Dekker Inc, New York.
8. Sunkara Namratha et al. Analytical method development and validation of valacyclovir in dosage forms by uplc technique. J. Global Trends Pharm Sciences. 2020; 11 (2): 7590 - 7594.
9. Bhatt D, Rane S. QbD approach to analytical RP-HPLC method development and its validation. Int J Pharm Sciences. 2011; 3:79-187.
10. Rajkotwala A, Shaikh S, et.al. QbD approach to analytical method development and validation of piracetam by HPLC. World J Pharm Sciences. 2016; 5:1771-1784.
11. Namratha, S., & A, V. Method development and validation of lopinavir in tablet dosage form using reverse-phase high-performance liquid chromatography. Asian Journal of Pharmaceutical and Clinical Research. 2018; 11(16), 125–128.
12. Prajapati R, Dedania Z et al. QbD approach to HPLC method development and validation for estimation of fluoxetine and olanzapine in pharmaceutical dosage form. J Emerging Tech Innovative Res. 2019; 6:179-195.
13. Dhand V, Dedania Z Nakarani K. QbD approach to method development and validation of Orciprenaline sulphate by HPLC. J Global Trends Pharm Sci. 2020; 11:8634-8640.
14. Krull I, Swartz M, et.al. A quality-by-design methodology for rapid LC method development, part I. Liquid Chroma Gas Chroma N Am. 2008; 26:1190-1197.
15. Myers R, Montgomery D, et.al. Response surface methodology: process and product optimization using designed experiments. 2016; 4thedn, New York: Wiley.
16. Yubing T 2011. Quality by design approaches to analytical methods – FDA perspective. Accessed 15 Dec. 2018.
17. Krull I, Swartz M, Turpin J et.al. A quality-by-design methodology for rapid LC method development part II. Liquid chroma Gas chroma N Am. 2009; 27:48-69.
18. Reid G, Morgado J, et.al. Analytical QbD in pharmaceutical development. Pharma Times. 2013; 46(8):26-33.
19. Molnar RH, Monks K. Aspects of the Design space in high pressure liquid chromatography method development. Journal Chromatogra A. 2010; 1217(19):3193-3200.
20. Monks K, Molnar I, Rieger H, et.al. Quality by design: multidimentional exploration of the design space in high performance liquid chromatography method development for better robustness before validation. J Chromatogra A. 2012; 1232:218-230.
21. Ramalingam P, Kalva B, Reddy Y. Analytical quality by design: a tool for regulatory flexibility and robust analytics. Int. J Ana Chem. 2015; (10):1-9.
22. The International conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human use on Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities) Q11 2012.
23. Orlandini S, Pinzauti S, et.al. Application of quality by design to the development of analytical separation methods. Analytical and Bioanalytical Chemistry. 2013; 405, 443-450.
24. The International conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human use on Validation of analytical procedure: Text and Methodology Q2(R1) 2005.
25. Reid G, Cheng G, et.al. Reversed-phase liquid chromatographic method development in an analytical quality by design framework. Journal Liq Chroma Related Tech. 2013; 36(18):2612-2638.
26. Elder P, Borman P. Improving analytical method reliability across the entire product lifecycle using QbD approaches. Pharmaceutical Outsourcing. 2013; 14:14-19.
27. Schweitzer M, Pohl M, Hanna-Brown M. Implications and opportunities of applying QbD principles to analytical measurements. Pharmaceutical Tech. 2010; 34:52-59.