Development and Validation of a Novel RP-HPLC Method for the Simultaneous Estimation of Antineoplastic Agents in Pharmaceutical Dosage Forms

Thunga Anitha*[1], S. Lakshmi Naga Aparna[2], U. Mohan Kumar[3], Meruva Jyothi[4], P. Rangaswamy[5]
[1,2,3,4,5]Department of Pharmaceutical Analysis, Nirmala College of Pharmacy, Ukkayapalli, Kadapa, Y.S.R District, Andhra Pradesh -516002

Abstract: High-Performance Liquid Chromatography (HPLC) is a vital technique for the qualitative and quantitative analysis of pharmaceutical substances. The present study describes the development and validation of a novel Reverse-Phase HPLC (RP-HPLC) method for the simultaneous estimation of selected antineoplastic agents. Chromatographic separation was achieved using a Kromosil C_{18} column (250 mm × 4.6 mm, 5 µm) with a mobile phase composed of Methanol and Phosphate buffer (pH 4.5) mixed in a 30:70 v/v ratio. The mobile phase was delivered at a constant flow rate of 1.0 mL/min and detection was carried out at 254 nm using a UV detector.

The method exhibited excellent linearity over a wide concentration range, with correlation coefficients (r^2) greater than 0.999 for both analytes. Validation was performed according to ICH guidelines, evaluating parameters such as specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), and robustness. The percentage recoveries were within the range of 98–102%, and precision studies showed %RSD values below 2%, confirming the method's accuracy and reproducibility. LOD and LOQ values were satisfactory, indicating the sensitivity of the method. Robustness testing confirmed that minor variations in method parameters did not significantly affect system performance.

The developed RP-HPLC method is thus simple, precise, accurate, and robust, and can be effectively applied for routine quality control and stability assessment of antineoplastic agents in pharmaceutical dosage forms.

Keywords: Method development, Validation, ICH guidelines, Pharmaceutical analysis, Quality control

1. INTRODUCTION

High-Performance Liquid Chromatography (HPLC) has emerged as one of the most essential and reliable techniques in pharmaceutical analysis, particularly for the separation and quantification of active pharmaceutical ingredients. Reverse-Phase HPLC (RP-HPLC) is especially advantageous due to its simplicity, reproducibility, sensitivity, and versatility, making it highly suitable for the simultaneous estimation of multiple analytes even in complex mixtures.

Antineoplastic agents play a critical role in the treatment of various malignancies by inhibiting or preventing the proliferation of cancerous cells. Given the therapeutic importance of these agents, precise and accurate analytical methods are required to ensure their quality, efficacy, and safety. Developing validated analytical methods is crucial to comply with regulatory standards and to facilitate routine quality control during pharmaceutical manufacturing.

The present study focuses on the development and validation of a novel RP-HPLC method for the simultaneous estimation of selected Decitabine and Cedazuridine in pharmaceutical formulations. A mobile phase consisting of Methanol and Phosphate buffer (pH 4.5) in a 30:70 v/v ratio was employed to achieve efficient separation. Detection was carried out using a UV detector at 254 nm, and chromatographic conditions were optimized to obtain sharp, symmetrical peaks with acceptable retention times.

The developed method was validated according to ICH Q2(R1) guidelines, evaluating parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). The aim was to establish a simple, accurate, precise, and robust method that could be routinely employed for the simultaneous estimation of antineoplastic agents in quality control laboratories.

2. METHODOLOGY

2.1. Materials and Reagents

All chemicals used were of analytical grade. Methanol (HPLC grade), potassium dihydrogen phosphate, and orthophosphoric acid were procured from certified suppliers. Double-distilled water was used throughout the study. Standard samples of antineoplastic agents were obtained with proven purity for method development and validation.

2.2. Instrumentation

The chromatographic analysis was performed using a High-Performance Liquid Chromatography (HPLC) system equipped with a UV detector. The system included a quaternary pump, manual injector with a 20 µL fixed loop, and a data acquisition system for analysis and interpretation of chromatograms.

2.3. Chromatographic Conditions

Chromatographic separation was carried out on a Kromosil C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of Methanol and Phosphate buffer (pH 4.5) mixed in the ratio of 30:70 v/v. The mobile phase was filtered through a 0.45 µm membrane filter and degassed before use. The flow rate was maintained at 1.0 mL/min, and the detection wavelength was set at 254 nm. The column temperature was maintained at ambient conditions (~25°C). Injection volume for each run was 20 µL.

The optimized chromatographic conditions are summarized in *Table 1*.

Table 1: Optimized Chromatographic Conditions

Parameter	Specification
Column	Kromosil C18, 250 mm × 4.6 mm, 5 µm
Mobile Phase	Methanol: Phosphate Buffer (pH 4.5) = 30:70 v/v
Flow Rate	1.0 mL/min
Detection Wavelength	254 nm
Injection Volume	20 µL
Column Temperature	Ambient (~25°C)
Run Time	Approximately 10 minutes

2.4. Preparation of Standard Solutions

Accurately weighed quantities of the standard antineoplastic agents were dissolved in methanol to prepare primary stock solutions. Appropriate dilutions were made with the mobile phase to obtain working standard solutions within the linearity range (e.g., 100–500 µg/mL for Analyte A and 1–5 µg/mL for Analyte B).

2.5. Preparation of Sample Solutions

Tablet formulations containing the selected antineoplastic agents were finely powdered. An accurately weighed quantity equivalent to the standard was transferred into a 100 mL volumetric flask containing 70 mL of mobile phase. The mixture was sonicated for 15 minutes and made up to volume with the mobile phase. The resulting solution was filtered through a 0.45 µm membrane filter prior to injection.

2.6. Method Validation

The developed method was validated according to ICH Q2(R1) guidelines. Validation parameters included:

- Specificity: Assessed by injecting blank, standard, and sample solutions to ensure no interference at retention times.
- Linearity: Constructed calibration curves by plotting peak area against concentration for both analytes over the specified range.
- Precision: Evaluated as intra-day and inter-day variations, expressed as %RSD.
- Accuracy: Assessed by recovery studies at three concentration levels (80%, 100%, 120%).
- LOD and LOQ: Calculated based on signal-to-noise ratios (3.3σ/S for LOD and 10σ/S for LOQ).
- Robustness: Studied by making slight variations in flow rate, mobile phase composition, and detection wavelength.

3. RESULTS AND DISCUSSION

3.1 Detection Wavelength Selection

The detection wavelength was selected after scanning standard solutions of Decitabine and Cedazuridine (10 µg/ml) from 200 to 400 nm using a UV spectrophotometer. The overlay spectrum revealed a common absorbance maxima at 254 nm, which was chosen as the detection wavelength due to its optimal sensitivity for both analytes.

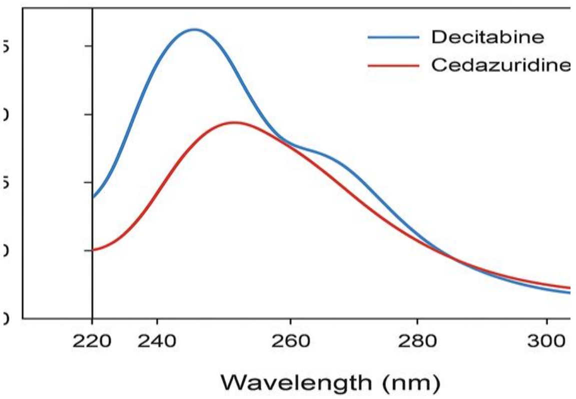

Figure 1: UV Spectrum of Decitabine and Cedazuridine

3.2 System Suitability

System suitability tests were conducted to verify the resolution, column efficiency, and peak shape. The observed parameters met the ICH guidelines.

- Tailing Factor: 1.3 (Acceptable range: 0.9–2.0)
- Theoretical Plates: 4668.7 (>2000)
- Resolution: >2, indicating good separation of peaks
- Retention Times: Decitabine – 2.669 min; Cedazuridine – 3.855 min

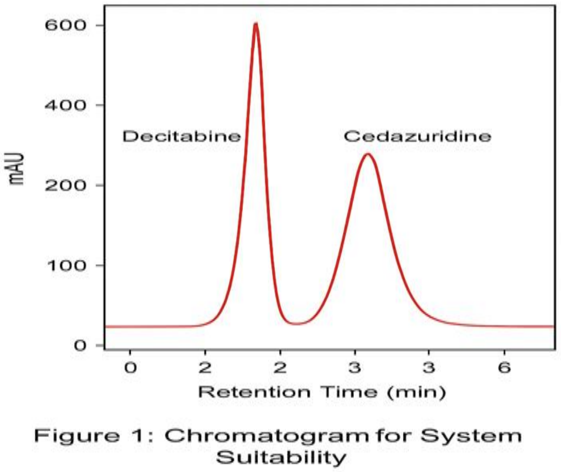

Figure 2: Chromatogram for System Suitability

Table 2. System Suitability Parameters

Drug	Retention Time (min)	Tailing Factor	Theoretical Plates
Decitabine	2.669	1.3	4668.7
Cedazuridine	3.855	1.3	6090.3

3.3 Method Validation

3.3.1 Precision

Precision was evaluated by injecting six replicates of standard solutions. The %RSD values for both drugs were below 2%, confirming the method's repeatability.

Table 3. Method Precision Results

Drug	Mean Area	Standard Deviation	%RSD
Decitabine	13,04,529.80	2,961.10	0.2
Cedazuridine	1,24,162.70	725.6	0.6

3.3.2 Intermediate Precision (Ruggedness)

Assays performed on different days using different systems showed minimal variation. The %RSD values were well within acceptable limits, confirming the ruggedness of the method.

Table 4. Intermediate Precision Results

Drug	Mean Area	Standard Deviation	%RSD
Decitabine	13,05,070.20	3,061.80	0.2
Cedazuridine	1,22,681.80	174.8	0.1

3.3.3 Accuracy (Recovery Studies)

Recovery studies were conducted at 50%, 100%, and 150% concentration levels. The average percentage recovery was between 98–102%, meeting ICH guidelines.

3.3.4 Linearity

Linearity was established for Decitabine (100–500 µg/ml) and Cedazuridine (1–5 µg/ml). The correlation coefficient (R^2) was 0.999 for both drugs.

Table 5. Linearity Results for Decitabine and Cedazuridine

Concentration (µg/ml)	Decitabine Area	Cedazuridine Area
100 / 1	668934	66510
200 / 2	956781	94701
300 / 3	1313873	124802
400 / 4	1563458	152731
500 / 5	1867084	179732

3.3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated based on signal-to-noise (S/N) ratios.

Table 6. LOD and LOQ Values

Drug	LOD (S/N ~3)	LOQ (S/N ~10)
Decitabine	2.9 µg/ml	10.03 µg/ml
Cedazuridine	3.0 µg/ml	10.1 µg/ml

3.3.6 Robustness

To assess robustness, minor changes in flow rate and mobile phase composition were introduced. No significant impact was observed on peak retention, tailing factor, or resolution.

- Flow rate: ±0.2 ml/min
- Mobile phase variation: ±10%
- %RSD remained <1 under all modified conditions.

4. CONCLUSION

The RP-HPLC method developed for the simultaneous estimation of Decitabine and Cedazuridine has been thoroughly validated in accordance with the International Council for Harmonisation (ICH) guidelines, confirming its scientific reliability and practical applicability. The method demonstrated high specificity, ensuring clear separation of both analytes from potential excipients or impurities, and excellent precision, with low %RSD values observed in both intra-day and inter-day analyses.

Moreover, accuracy was confirmed through recovery studies, where recoveries ranged within the acceptable limits of 98–102%, establishing the method's ability to quantify the true concentration of the drugs. The method also exhibited outstanding linearity over a wide concentration range for both compounds, with correlation coefficients (R^2) consistently greater than 0.999, indicating a strong direct proportional relationship between concentration and detector response.

Additionally, the method proved to be robust, tolerating small deliberate changes in analytical conditions (such as flow rate and mobile phase composition) without significant impact on system suitability parameters. The determined limits of detection (LOD) and quantification (LOQ) confirm the method's sensitivity and suitability for detecting even low levels of analytes.

Collectively, these validation outcomes demonstrate that this RP-HPLC method is not only scientifically sound but also highly suitable for routine quality control analysis of pharmaceutical dosage forms containing Decitabine and Cedazuridine. Its reliability and reproducibility make it an effective tool for ongoing monitoring in both industrial and regulatory environments.

REFERENCE

[1] Beckett AH, Stenlake JB. *Practical Pharmaceutical Chemistry*. 4th ed. Part 2. New Delhi: CBS Publishers and Distributors; 1998. p. 278, 307.

[2] Skoog DA, Holler FJ, Nieman TA. *Principles of Instrumental Analysis*. 5th ed. Singapore: Thomson Learning Inc.; 1998. p. 110, 300.

[3] Sethi PD. *Quantitative Analysis of Drugs in Pharmaceutical Formulation*. 3rd ed. New Delhi: CBS Publishers and Distributors; 1997. p. 1–29, 50–64.

[4] Mendham J, Denny RC, Barnes JD, Thomas M. *Vogel's Textbook of Quantitative Chemical Analysis*. 6th ed. New Delhi: Pearson Education; 2003. p. 1, 676.

[5] Sharma BK. *Instrumental Methods of Chemical Analysis*. 24th ed. Meerut: Goel Publishing House; 2005. p. 46, 68.

[6] Chatwal GR, Anand KS. *Instrumental Methods of Chemical Analysis*. 5th ed. Mumbai: Himalaya Publishing House; 2002. p. 2–149.

[7] Munson JW. *Modern Methods of Pharmaceutical Analysis*. Mumbai: Medical Book Distributors; 2001. p. 17–54.

[8] Willard HH, Merritt LL, Dean JA, Settle FA. *Instrumental Methods of Analysis*. 7th ed. New Delhi: CBS Publishers and Distributors; 1988. p. 436–439.

[9] Synder KL, Kirkland JJ, Glajch JL. *Practical HPLC Method Development*. 2nd ed. USA: Wiley-Interscience; 1983. p. 1–10.

[10] Bentley R, Driver GC. *Textbook of Pharmaceutical Chemistry*. 8th ed. Oxford: Oxford University Press; 1985. p. 1–3.

[11] International Conference on Harmonisation (ICH). *Validation of Analytical Procedures: Methodology*. ICH Harmonised Tripartite Guideline. Geneva: ICH; 1996.

[12] *Indian Pharmacopoeia*. Vol I. Ghaziabad: Indian Pharmacopoeia Commission; 2007. p. 715.

[13] *British Pharmacopoeia*. Vol I. London: Stationery Office; 2007. p. 136.

[14] Sweetman SC, editor. *Martindale: The Complete Drug Reference*. 36th ed. London: Pharmaceutical Press; 2009.

[15] *The Merck Index*. 12th ed. Whitehouse Station (NJ): Merck & Co., Inc.; 1996.

[16] Ishaq BM, Reddy LSS, Venu S, Sreenivasulu M, Prakash KV. RP-HPLC-PDA method development, validation and stability studies of the novel antineoplastic drug combination – Decitabine and Cedazuridine. *J Pharm Res Int*. 2020;32(32):10–6