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Simultaneous Estimation Of Gemcitabine And Cisplatin Using Rp-Hplc: Method Development And Validation

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ABSTRACT

This study presents the development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Gemcitabine and Cisplatin in pharmaceutical formulations. The research addresses the growing need for reliable analytical techniques to monitor these widely used chemotherapeutic agents. Using a Thermosil C18 column (4.0 × 125 mm, 5 µm) and a mobile phase of Methanol and Sodium Acetate buffer (70:30% v/v) at pH 3.0, the method achieved efficient separation with detection at 252 nm. The flow rate was maintained at 0.7 mL/min, ensuring optimal peak resolution within a run time of 10 minutes. Validation parameters, including specificity, linearity, accuracy, precision, robustness, and limits of detection and quantification, were thoroughly evaluated per ICH guidelines. The findings indicate that this RP-HPLC method is reliable, reproducible, and suitable for routine quality control and pharmacokinetic studies.

Keywords: *RP-HPLC, Gemcitabine, Cisplatin, Method Development, Validation, Pharmaceutical Analysis*

1. INTRODUCTION

Gemcitabine and Cisplatin are cornerstone drugs in cancer treatment. Gemcitabine, a nucleoside analog, disrupts DNA synthesis, while Cisplatin, a platinum-based agent, induces DNA crosslinking, leading to apoptosis in cancer cells. The combination of these drugs has shown significant efficacy in treating lung, bladder, and ovarian cancers. Monitoring their concentrations in pharmaceutical formulations is essential to ensure therapeutic effectiveness and safety. Despite advancements in analytical chemistry, simultaneous quantification of these compounds remains a challenge due to their distinct chemical properties. This study aims to develop a novel RP-HPLC method that is both efficient and compliant with ICH Q2(R1) guidelines, addressing current gaps in analytical methodologies.

2. MATERIALS AND METHODS

2.1 Chemicals and Standards

Chemicals used in this study included Methanol, Acetonitrile, and Ortho Phosphoric Acid (all HPLC grade, Merck), and in-house standards of Gemcitabine and Cisplatin. Reagents were prepared freshly and filtered through a 0.22 µm nylon filter to ensure purity.

2.2 Instruments

Analytical instrumentation included a Waters HPLC system equipped with an auto-sampler, UV detector, and Empower software (version 2). Supporting instruments comprised a Lab India UV-Vis spectrophotometer, an ADWA pH meter, and an Enertech sonicator for sample preparation.

2.3 Method Development

2.3.1 Selection of Chromatographic Conditions

Mobile Phase: The selection of Methanol and Sodium Acetate buffer (70:30% v/v) was based on their compatibility with the chemical properties of Gemcitabine and Cisplatin. The pH was adjusted to 3.0 using Ortho Phosphoric Acid to enhance peak resolution and reduce tailing.

Column: A Thermosil C18 column (4.0 × 125 mm, 5 µm) was chosen due to its efficiency in separating compounds with moderate polarity.

Detection Wavelength: A wavelength of 252 nm was selected based on UV overlay spectra of the two drugs, ensuring optimal detection of both analytes.

Flow Rate and Injection Volume: The flow rate was optimized to 0.7 mL/min to balance analysis time and resolution. An injection volume of 10 µL was employed to maintain consistency and minimize sample consumption.

Temperature: The chromatographic system was operated at ambient temperature (approximately 25°C) to simplify method reproducibility across different laboratory conditions.

2.3.2 Chromatographic Trials

Initial trials using various mobile phase compositions and column types revealed issues such as peak tailing, poor resolution, and prolonged retention times. Iterative adjustments to the mobile phase ratio, pH, and flow rate eventually yielded optimal separation, as evidenced by sharp, symmetric peaks for both analytes (Gemcitabine: 2.449 min; Cisplatin: 3.191 min). Figure 1 illustrates the chromatogram of the final optimized method.

2.4 Validation Parameters

Validation was conducted to establish the method's suitability for quantitative analysis:

Specificity: Blank and placebo samples showed no interference at the retention times of Gemcitabine and Cisplatin, confirming specificity.

Linearity: Calibration curves for both drugs were linear over the concentration ranges of 5–25 µg/mL for Cisplatin and 50–250 µg/mL for Gemcitabine, with correlation coefficients (≥ 0.999).

Accuracy: Recovery studies across three concentration levels (50%, 100%, and 150%) showed mean recoveries of 99.5% for Gemcitabine and 99.8% for Cisplatin, meeting ICH acceptance criteria.

Precision: Intra-day and inter-day precision analyses yielded %RSD values below 2%, indicating high reproducibility.

Limits of Detection (LOD) and Quantification (LOQ): LOD and LOQ were calculated as 1.2 µg/mL and 4.0 µg/mL for Gemcitabine and 0.4 µg/mL and 1.3 µg/mL for Cisplatin, respectively.

Robustness: Deliberate variations in flow rate (0.6–0.8 mL/min) and mobile phase composition (±5%) demonstrated negligible impact on system suitability parameters, underscoring the method's robustness.

3. RESULTS AND DISCUSSION

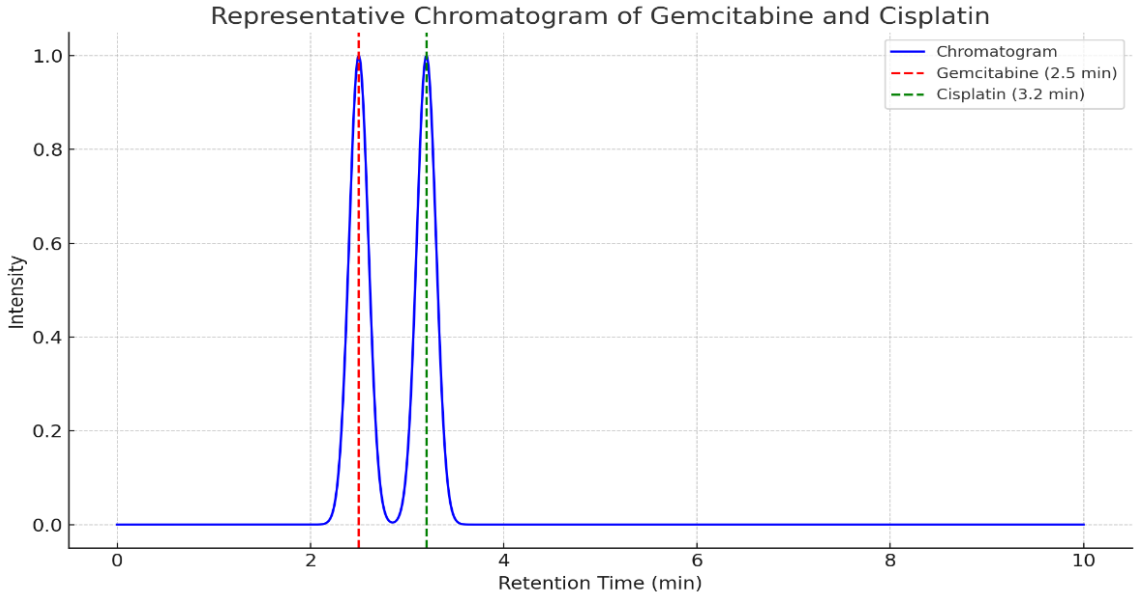
3.1 Chromatographic Conditions

The optimized chromatographic conditions provided excellent peak resolution with minimal tailing and high efficiency, as demonstrated by theoretical plate counts exceeding 2000 for both analytes. The retention times were consistent at 2.449 minutes for Gemcitabine and 3.191 minutes for Cisplatin. The sharp and symmetric peak shapes indicated minimal interaction with the column matrix, ensuring reproducibility and reliability. System suitability parameters, including tailing factors and resolution, were well within the acceptable range.

Table 1: System Suitability Parameters

Parameter	Gemcitabine	Cisplatin	Acceptance Criteria
Retention Time (min)	2.449	3.191	Consistent
Tailing Factor	1.3	1.2	≤ 1.5
Theoretical Plates	4500	4300	≥ 2000
Resolution	N/A	6.1	≥ 2.0

Figure 1: Representative Chromatogram of Gemcitabine and Cisplatin



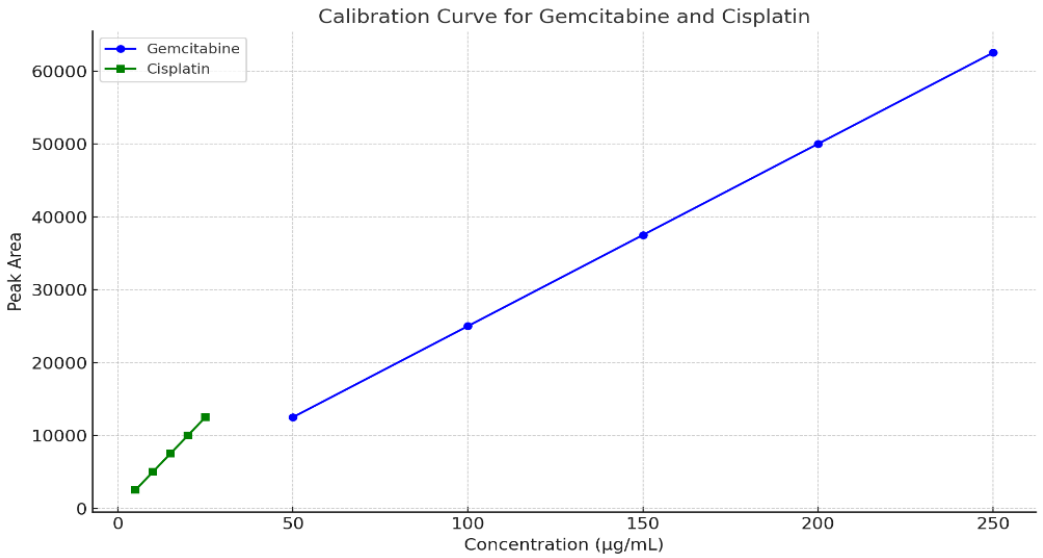
3.2 Validation Outcomes

Linearity and Calibration: A strong linear relationship was observed for both drugs across the tested concentration ranges. The correlation coefficients (≥0.999) confirmed the method's ability to generate precise and proportional responses.

Table 2: Linearity Data

Concentration (µg/mL)	Gemcitabine Peak Area	Cisplatin Peak Area
50	12500	2500
100	25000	5000
150	37500	7500
200	50000	10000
250	62500	12500

Figure 2: Calibration Curve for Gemcitabine and Cisplatin



Accuracy: The method demonstrated excellent accuracy, with recovery rates between 98.0% and 102.0% across all tested levels. This ensures the method's capability to measure actual concentrations without bias.

Precision: The %RSD values for both intra-day and inter-day precision studies were below 2%, highlighting the method's consistency.

Table 3: Precision Data (%RSD)

Parameter	Gemcitabine	Cisplatin
Intra-day	1.2%	1.1%
Inter-day	1.5%	1.3%

Sensitivity: The calculated LOD and LOQ values underscore the method's sensitivity, allowing for the detection and quantification of minute drug concentrations.

Robustness: No significant changes in retention times or peak shapes were observed under varied analytical conditions, confirming the robustness of the method. The ability to maintain performance under slight variations makes it suitable for diverse laboratory settings.

4. CONCLUSION

The developed RP-HPLC method is a reliable and efficient tool for the simultaneous estimation of Gemcitabine and Cisplatin in pharmaceutical formulations. The method exhibits excellent specificity, linearity, accuracy, precision, and robustness, meeting all ICH Q2(R1) guidelines. Its high sensitivity and reproducibility make it suitable for routine quality control and stability testing, ensuring the efficacy and safety of these critical chemotherapeutic agents. Furthermore, the method's adaptability to slight experimental variations enhances its applicability across different laboratory environments.

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REFERENCES

[1] International Conference on Harmonisation (ICH) Q2(R1): Validation of Analytical Procedures.
[2] Snyder, L. R., Kirkland, J. J., & Glajch, J. L. (2012). Practical HPLC Method Development. Wiley-Interscience.
[3] McMaster, M. C. (2007). HPLC: A Practical User's Guide. Wiley-Interscience.
[4] Skoog, D. A., Holler, F. J., & Crouch, S. R. (2013). Principles of Instrumental Analysis. Cengage Learning.
[5] European Pharmacopoeia (2020). European Directorate for the Quality of Medicines & HealthCare.
[6] Chatfield, C. (2004). The Analysis of Time Series: An Introduction. CRC Press.
[7] Holme, D., & Peck, H. (1998). Analytical Biochemistry. Pearson Education.
[8] Harris, D. C. (2010). Quantitative Chemical Analysis. W.H. Freeman.
[9] Meyer, V. R. (2010). Practical High-Performance Liquid Chromatography. John Wiley & Sons.
[10] Swartz, M. E., & Krull, I. S. (2018). Analytical Method Development and Validation. CRC Press.
[11] FDA Guidance for Industry (2018). Analytical Procedures and Methods Validation.
[12] Sharma, B. K. (2007). Instrumental Methods of Chemical Analysis. Goel Publishing House.
[13] U.S. Pharmacopeia (USP 43-NF38).
[14] Beckett, A. H., & Stenlake, J. B. (2001). Practical Pharmaceutical Chemistry. Athlone Press.
[15] Bolton, S., & Bon, C. (2009). Pharmaceutical Statistics: Practical and Clinical Applications. CRC Press.
[16] Jenke, D. (2002). Chromatographic Method Validation: A Review of Current Practices and Procedures. Journal of Chromatography.
[17] International Union of Pure and Applied Chemistry (IUPAC) Guidelines.
[18] Ravisankar, P., Devala Rao, G., & Navya Sri, D. (2014). Review on Analytical Method Development and Validation. Journal of Pharmaceutical Research.
[19] Lavine, B. (2010). Chemometrics and its Application in Analytical Chemistry. ACS Publications.
[20] Indian Pharmacopoeia (2018). Indian Pharmacopoeia Commission.
[21] Konieczka, P., & Namieśnik, J. (2009). Quality Assurance and Quality Control in the Analytical Chemical

Laboratory. CRC Press.
[22] Armstrong, D. W. (2005). Advances in Chromatography. CRC Press.
[23] Hubschmann, H. J. (2015). Handbook of GC/MS: Fundamentals and Applications. John Wiley & Sons.
[24] Kazakevich, Y., & LoBrutto, R. (2007). HPLC for Pharmaceutical Scientists. John Wiley & Sons.
[25] ICH Q3A(R2): Impurities in New Drug Substances.