RP-HPLC-Based Quantitative Analysis of Multi-Component Antineoplastic Dosage Forms: Method Development and Validation

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***Abstract*—A simple, precise, accurate, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Cisplatin and Etoposide in combined antineoplastic dosage forms. Chromatographic separation was achieved using an Inertsil C18 column with a mobile phase consisting of methanol and phosphate buffer (pH 3.0) in the ratio of 70:30 v/v. Detection was carried out at 260 nm with a flow rate of 0.8 mL/min. The retention times for Cisplatin and Etoposide were found to be approximately 2.57 and 3.84 min, respectively. The developed method was validated according to ICH Q2(R1) guidelines for specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). The correlation coefficients were found to be greater than 0.999 for both analytes. Percentage recovery ranged from 98–102%, indicating excellent accuracy. The developed method was found to be suitable for routine quality-control analysis of Cisplatin and Etoposide in pharmaceutical dosage forms. The method demonstrated excellent reproducibility, sensitivity, and reliability for simultaneous estimation of both drugs. The study confirms the applicability of the developed RP-HPLC method for regular analytical and industrial quality assurance purposes.**

***Index Terms*—RP-HPLC, Cisplatin, Etoposide, Method Validation, Antineoplastic Agents, ICH Guidelines.**

I. INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, necessitating the development of effective therapeutic agents and reliable analytical methods for their quality assessment[1]. Combination chemotherapy involving Cisplatin and Etoposide has been widely employed in the treatment of various malignancies, including lung cancer, ovarian cancer, testicular cancer, and lymphomas[2]. Accurate quantification of these drugs in pharmaceutical formulations is essential to ensure therapeutic efficacy and patient safety[3].

High-performance liquid chromatography (HPLC) is among the most powerful analytical techniques used in pharmaceutical analysis due to its high sensitivity, selectivity, precision, and reproducibility[4]. RP-HPLC has become the preferred analytical method for the simultaneous determination of multiple drug components because of its ability to separate compounds with different physicochemical properties within a short analysis time[5].

Cisplatin is a platinum-containing antineoplastic agent that exerts its therapeutic action by forming DNA cross-links, thereby inhibiting DNA replication and transcription. Etoposide is a semisynthetic derivative of podophyllotoxin that acts by inhibiting topoisomerase II, leading to DNA strand breakage and apoptosis[6]. The simultaneous estimation of these drugs requires a robust chromatographic method capable of providing accurate quantification without interference from excipients or degradation products[7]. The objective of the present study was to develop and validate a simple RP-HPLC method for simultaneous estimation of Cisplatin and Etoposide in pharmaceutical dosage forms according to ICH guidelines[8-12].

II. MATERIALS AND METHODS

Chemicals and Reagents

Reference standards of Cisplatin and Etoposide were obtained from certified pharmaceutical sources. HPLC-grade methanol, analytical-grade potassium dihydrogen phosphate, orthophosphoric acid, and purified water were used throughout the study.

Instrumentation

Chromatographic analysis was performed using an HPLC system equipped with a UV detector, autosampler, solvent delivery system, and data acquisition software. Separation was achieved using an Inertsil C18 column (4.6 × 150 mm, 5 µm particle size).

Chromatographic Conditions

Table 1: Optimized Chromatographic Conditions

Parameter	Condition
Column	Inertsil C18 (4.6 × 150 mm, 5 µm)
Mobile Phase	Methanol : Phosphate Buffer (70:30 v/v)
pH	3.0
Flow Rate	0.8 mL/min
Detection Wavelength	260 nm
Injection Volume	10 µL
Run Time	10 min
Temperature	Ambient

The mobile phase was filtered through a 0.45 µm membrane filter and degassed before use.

Preparation of Standard Solution

Accurately weighed quantities of Cisplatin and Etoposide were transferred into volumetric flasks and dissolved in the mobile phase to obtain standard stock solutions. Appropriate dilutions were made to prepare working standards for calibration studies.

Preparation of Sample Solution

Commercial dosage forms containing Cisplatin and Etoposide were accurately weighed and processed to obtain sample solutions equivalent to the required concentrations. The solutions were filtered before chromatographic analysis.

III. METHOD DEVELOPMENT

Several chromatographic trials were conducted using different columns, mobile phase compositions, flow rates, and pH conditions. The optimized chromatographic conditions provided symmetrical peaks, adequate resolution, and acceptable system suitability parameters.

The selected mobile phase consisting of methanol and phosphate buffer (70:30 v/v) at pH 3.0 produced well-resolved peaks with satisfactory peak shapes and minimal tailing. Detection at 260 nm provided sufficient sensitivity for both analytes. Under optimized conditions, Cisplatin and Etoposide exhibited retention times of approximately 2.57 and 3.84 minutes, respectively.

IV. METHOD VALIDATION

Method validation was performed according to ICH Q2(R1) guidelines.

4.1 System Suitability

System suitability studies were performed before analysis to verify chromatographic performance.

Table 2: System Suitability Parameters

Parameter	Cisplatin	Etoposide
Retention Time (min)	2.57	3.84
Tailing Factor	1.20	1.32
Theoretical Plates	4673	6090
Resolution	-	6.02

All system suitability parameters were within acceptable limits.

4.2 Specificity

Specificity was evaluated by injecting blank, standard, and sample solutions. No interfering peaks were observed at the retention times of Cisplatin and Etoposide, demonstrating the specificity of the developed method.

4.3 Precision

Precision was evaluated by six replicate injections of standard solutions.

Table 3: Precision Results

Drug	Mean Area	%RSD
Cisplatin	124505	0.20
Etoposide	1308495	0.62

The %RSD values were below 2%, indicating excellent precision.

4.4 Intermediate Precision

Intermediate precision was evaluated on different days and by different analysts.

Table 4: Intermediate Precision Results

Drug	%RSD
Cisplatin	0.18
Etoposide	0.15

The results demonstrated good reproducibility.

4.5 Accuracy

Accuracy was assessed by recovery studies at three concentration levels.

Table 5: Recovery Studies

Level	Cisplatin Recovery (%)	Etoposide Recovery (%)
50%	100.7	100.8
100%	100.0	100.0
150%	98.8	99.7

Mean recoveries were within the acceptable range of 98–102%.

4.6 Linearity

The linearity ranges were:

- Cisplatin: 100–500 µg/mL
- Etoposide: 5–25 µg/mL

Table 6: Linearity Data

Drug	Range	Correlation Coefficient (R^2)
Cisplatin	100–500 µg/mL	0.9998
Etoposide	5–25 µg/mL	0.9997

Excellent linearity was observed over the studied concentration ranges.

4.7 LOD and LOQ

Table 7: Detection and Quantification Limits

Drug	LOD (µg/mL)	LOQ (µg/mL)
Cisplatin	3.2	9.8
Etoposide	0.15	0.46

The low values indicate good sensitivity of the developed method.

4.8 Robustness

Robustness was evaluated by deliberately varying chromatographic parameters such as:

- Flow rate (±0.2 mL/min)
- Mobile phase composition (±2%)
- Detection wavelength (±2 nm)

No significant changes were observed in chromatographic performance, confirming the robustness of the method.

V. RESULTS AND DISCUSSION

The developed RP-HPLC method successfully separated Cisplatin and Etoposide with good resolution and acceptable chromatographic characteristics. The use of methanol-phosphate buffer as the mobile phase produced sharp, symmetrical peaks and reduced analysis time. Validation results confirmed that the method complied with ICH requirements for analytical method validation.

The high correlation coefficients obtained during linearity studies indicate a direct relationship between concentration and detector response. Recovery studies confirmed the accuracy of the method, while low %RSD values demonstrated excellent precision and reproducibility. Robustness testing revealed that small changes in analytical conditions did not significantly affect the results, highlighting the reliability of the method.

The developed method can therefore be employed for routine quality-control analysis and stability studies of pharmaceutical formulations containing Cisplatin and Etoposide.

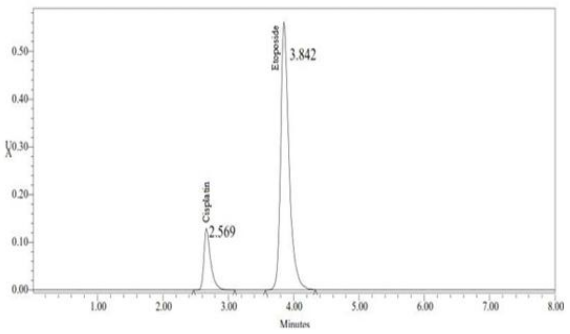

Figure 1: Optimized chromatogram showing separation of Cisplatin and Etoposide mobile phase composition

VI. CONCLUSION

A simple, rapid, precise, accurate, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Cisplatin and Etoposide in pharmaceutical dosage forms. The developed method met all ICH validation criteria and demonstrated excellent specificity, linearity, precision, accuracy, robustness, sensitivity, and reproducibility. Therefore, the method can be effectively used for routine quality-control analysis and pharmaceutical research applications involving Cisplatin and Etoposide formulations.

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