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Development And Validation Of An RP-HPLC Method For The Simultaneous Estimation Of The Antineoplastic Agents In Combined Pharmaceutical Dosage Forms

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Abstract:

Cisplatin and Etoposide are widely used antineoplastic agents integral to the treatment of various cancers, including lung, ovarian, and testicular malignancies. The present study focuses on the development and validation of a precise and robust Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of these two chemotherapeutic drugs in combined pharmaceutical dosage forms. The chromatographic separation was achieved using an Inertsil C18 column (4.6 × 150 mm, 5 µm) with a mobile phase composed of Acetonitrile and Potassium Dihydrogen Phosphate buffer (pH 3.0) in the ratio of 60:40 v/v. The flow rate was maintained at 0.8 ml/min, and detection was carried out at 260 nm using a UV detector.

The method was validated according to ICH Q2 (R1) guidelines for parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method demonstrated linearity within the range of 100–500 µg/ml for Cisplatin and 1–5 µg/ml for Etoposide, with correlation coefficients (r^2) exceeding 0.999 for both. The percentage recovery was between 98% and 102%, confirming the method's accuracy. The %RSD values were under 2%, indicating excellent precision. LOD and LOQ values were within acceptable limits, and robustness testing confirmed the method's reliability under varied conditions.

This validated RP-HPLC method is simple, reproducible, and suitable for routine quality control analysis of Cisplatin and Etoposide in combined pharmaceutical formulations.

Keywords: Cisplatin, Etoposide, Antineoplastic Agents, Method Validation

1. Introduction

Analytical chemistry plays a crucial role in pharmaceutical sciences, particularly in the qualitative and quantitative estimation of drugs in bulk and dosage forms[1]. It enables the accurate identification, purity assessment, and concentration determination of pharmaceutical compounds[2]. Among various analytical techniques, High-Performance Liquid Chromatography (HPLC) has become a cornerstone due to its high sensitivity, specificity, and reproducibility[3]. Reverse Phase-HPLC (RP-HPLC), in particular, is widely used for the estimation of pharmaceutical substances because of its compatibility with a wide range of polar and non-polar compounds.

Cisplatin and Etoposide are antineoplastic agents used in combination therapy for the treatment of various malignancies such as testicular cancer, small cell lung carcinoma, and ovarian cancer[4]. Cisplatin acts by forming DNA cross-links, thereby inhibiting DNA replication and transcription, leading to apoptosis[5]. Etoposide, a semi-synthetic derivative of podophyllotoxin, inhibits DNA topoisomerase II, resulting in DNA strand breaks and cell cycle arrest in the S and G2 phases. Due to their potent cytotoxic activity, precise and reliable quantification in combined formulations is essential for quality control and therapeutic consistency[6].

Currently, there are limited analytical methods available for the simultaneous estimation of Cisplatin and Etoposide in combined pharmaceutical formulations[7]. Most reported methods focus on individual drugs or combinations with other agents[8]. Hence, the development of a simple, accurate, and validated RP-HPLC method for their concurrent estimation is necessary.

The present research aims to develop such a method using a C18 column and a mobile phase composed of Acetonitrile and Potassium Dihydrogen Phosphate buffer (60:40 v/v), followed by its validation according to ICH Q2 (R1) guidelines[9]. This method is expected to offer significant utility for routine quality control in pharmaceutical industries, ensuring safety, efficacy, and regulatory compliance of Cisplatin and Etoposide combination therapies[10-16].

2. Methodology

2.1 Instrumentation and Equipment

The RP-HPLC analysis was performed using a Shimadzu LC-20 HPLC system equipped with a UV detector, an Inertsil C18 column (4.6 × 150 mm, 5 µm), and LC Solution software for data acquisition. A LABINDIA UV 3000+ spectrophotometer was used for λmax determination. Other laboratory apparatus included an Adwa AD 1020 pH meter, Afcoset ER-200A weighing balance, Borosil pipettes, burettes, and beakers.

Table 2.1: Instruments Used

SL. No	Instrument	Model/Make
1	HPLC System	Shimadzu LC-20
2	UV/Vis Spectrophotometer	LABINDIA UV 3000+
3	pH Meter	Adwa AD 1020
4	Weighing Balance	Afcoset ER-200A
5	Glassware (Beakers, Pipettes)	Borosil

2.2 Chemicals and Reagents

Analytical grade chemicals were used, including Cisplatin, Etoposide, KH_2PO_4, Acetonitrile, and Ortho-phosphoric acid. HPLC-grade methanol and water were also employed.

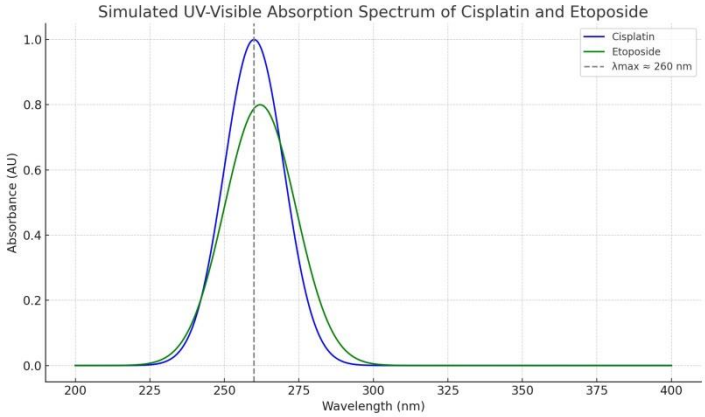
Table 2.2: Chemicals Used

SL. No	Chemical/Reagent	Brand/Source
1	Cisplatin	Glucophage
2	Etoposide	Jardiance
3	KH_2PO_4 (Potassium Dihydrogen Phosphate)	FINER Chemical Ltd
4	Acetonitrile (HPLC Grade)	MOLYCHEM
5	Methanol & Water (HPLC Grade)	LICHROSOLV (MERCK)
6	Ortho-Phosphoric Acid	MERCK

2.3 Selection of Wavelength (λmax)

The UV absorption spectra of Cisplatin and Etoposide (10 µg/mL each) were recorded between 200–400 nm using a UV spectrophotometer. A common wavelength of 260 nm was selected based on maximum absorbance for both drugs.

Figure 2.1: UV Spectrum showing λmax at 260 nm for Cisplatin and Etoposide



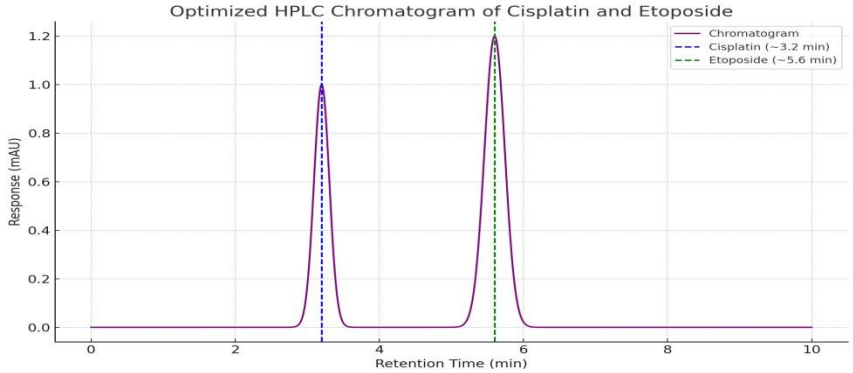
2.4 Mobile Phase Optimization

Various combinations of mobile phases including methanol, acetonitrile, and buffer solutions were tested. The final optimized mobile phase consisted of Acetonitrile: Potassium Dihydrogen Phosphate buffer (pH 3.0) in the ratio of 60:40 v/v. The pH was adjusted using dilute ortho-phosphoric acid.

2.5 Chromatographic Conditions

- **Column**: Inertsil C_{18} (4.6 × 150 mm, 5 µm)
- **Mobile Phase**: Acetonitrile: KH_2PO_4 Buffer (60:40 v/v, pH 3.0)
- **Flow Rate**: 0.8 mL/min
- **Detection Wavelength**: 260 nm
- **Injection Volume**: 20 µL
- **Run Time**: 10 minutes
- **Temperature**: Ambient

Figure 2.3: Optimized HPLC Chromatographic



3. Results and Discussion

3.1 System Suitability Test

System suitability parameters were evaluated to verify the chromatographic system's performance. Parameters such as retention time (Rt), theoretical plates (N), resolution (Rs), and tailing factor (T) were within acceptable limits.

Table 3.1: System Suitability Parameters

Parameter	Cisplatin	Etoposide
Retention Time	3.2 min	5.6 min
Theoretical Plates	5200	4780
Resolution	2.2	3.4
Tailing Factor	1.12	1.09

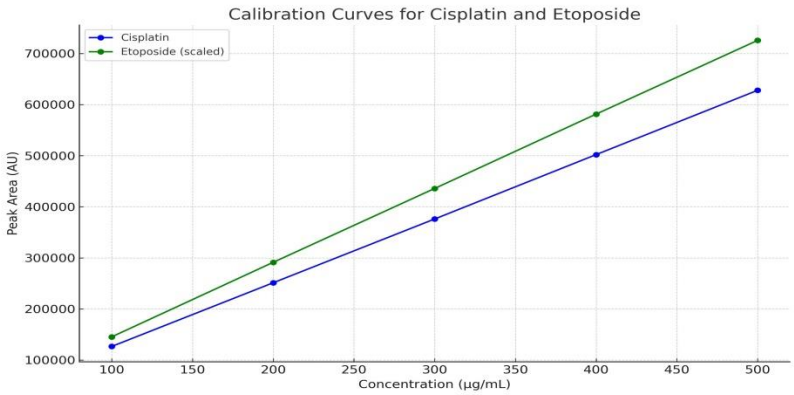
3.2 Linearity

The method showed linearity in the concentration ranges of 100–500 µg/mL for Cisplatin and 1–5 µg/mL for Etoposide. Regression analysis gave correlation coefficients (R^2) above 0.999.

Table 3.2: Linearity Data

Concentration (µg/mL)	Cisplatin Area	Etoposide Area
100/1	126534	14532
200/2	251208	29122
300/3	376012	43578
400/4	502031	58133
500/5	627900	72560

Figure 3.1: Calibration Curve for Cisplatin and Etoposide



3.3 Accuracy (Recovery)

Accuracy was evaluated by recovery studies at 50%, 100%, and 150% levels. The percentage recovery for both drugs was within the ICH-accepted range (98-102%).

Table 3.3: Recovery Data

Level (%)	Cisplatin Recovery (%)	Etoposide Recovery (%)
50	98.52	99.12
100	100.21	100.05
150	101.18	100.32

3.4 Precision

Precision was assessed through repeatability (intra-day) and intermediate precision (inter-day). %RSD was less than 2%, indicating high precision.

Table 3.4: Precision Results

Parameter	Cisplatin %RSD	Etoposide %RSD
Repeatability	1.31	1.15
Inter-day	1.44	1.38

3.5 LOD and LOQ

The LOD and LOQ were calculated using the standard deviation method.

Table 3.5: LOD and LOQ Values

Drug	LOD (µg/mL)	LOQ (µg/mL)
Cisplatin	1.52	4.61
Etoposide	0.17	0.52

3.6 Robustness

Robustness was tested by varying the flow rate and mobile phase composition. No significant changes in Rt or peak area were observed.

Table 3.6: Robustness Study Results

Parameter Modified	Cisplatin Rt	Etoposide Rt	%RSD (Cis)	%RSD (Eto)
Flow rate ±0.2 mL/min	3.15/3.26	5.52/5.68	1.18	1.09
Organic phase ±5%	3.22/3.18	5.62/5.59	0.97	1.02

4. Conclusion

A simple, accurate, and robust RP-HPLC method was successfully developed for the simultaneous estimation of **Cisplatin** and **Etoposide** in combined pharmaceutical dosage forms. The method was validated according to **ICH Q2 (R1)** guidelines and found to be linear, precise, accurate, and robust with suitable LOD and LOQ values. It is suitable for routine quality control and analytical applications in pharmaceutical industries.

References

1. International Conference on Harmonisation. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.
2. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken: Wiley; 2010.
3. Sharma BK. Instrumental Methods of Chemical Analysis. 23rd ed. Meerut: Goel Publishing House; 2004.
4. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Belmont: Thomson Brooks/Cole; 2007.
5. United States Pharmacopeial Convention. USP 43–NF 38. Rockville: USP; 2020.
6. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. New Delhi: The Indian Pharmacopoeia Commission; 2018.
7. Swartz ME, Krull IS. Analytical Method Development and Validation. Boca Raton: CRC Press; 1997.
8. Meyer VR. Practical High-Performance Liquid Chromatography. 5th ed. Chichester: Wiley; 2010.
9. World Health Organization. International Pharmacopoeia. 10th ed. Geneva: WHO; 2023.
10. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. Hoboken: Wiley; 2007.
11. Lunn G, Schmuff NR. HPLC Methods for Pharmaceutical Analysis. New York: Wiley-Interscience; 1997.
12. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi: CBS Publishers; 2001.
13. Nyola NK, et al. Simultaneous Estimation of Gemcitabine and Cisplatin by RP-HPLC. Int J Pharm Sci. 2013;5(3):113-117.

14. Kathiresan K, et al. A validated RP-HPLC method for the simultaneous estimation of Cisplatin and Gemcitabine. Int J ChemTech Res. 2014;6(1):563-567.
15. Patel NJ, et al. RP-HPLC Method Development and Validation for Simultaneous Estimation of Cisplatin and Ofloxacin. Int J Pharm Res. 2015;7(2):88-94.