

UV-Visible Spectrophotometric Method Development and Validation for the Estimation of Treosulfan in Bulk and Pharmaceutical Dosage Forms

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Abstract

Treosulfan is an alkylating anticancer drug widely used in chemotherapy and conditioning regimens before hematopoietic stem cell transplantation. The present study aims to develop and validate a simple, accurate, and cost-effective UV-Visible spectrophotometric method for the estimation of Treosulfan in

bulk and pharmaceutical dosage forms. Treosulfan shows maximum absorbance ($\lambda_{\max}$) at 258 nm using acetonitrile as the solvent. The method was validated according to ICH guidelines for parameters such as linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The calibration curve showed good linearity in the concentration range of 2–12 $\mu\text{g/mL}$ with a correlation coefficient close to 0.999. The developed method was found to be simple, precise, and suitable for routine analysis of Treosulfan in bulk drug and pharmaceutical formulations.

Keywords: Treosulfan, UV-Visible Spectroscopy, Analytical Method Validation, Bulk Drug, Pharmaceutical Dosage Form.

1. Introduction

Treosulfan is a bifunctional alkylating agent used mainly in the treatment of ovarian cancer and as a conditioning agent prior to stem cell transplantation. Chemically, Treosulfan is known as dihydroxybusulfan. It produces cytotoxic effects by alkylating DNA, thereby inhibiting cell division and causing apoptosis in cancer cells. Reliable analytical methods are required to determine the purity, potency, and quality of pharmaceutical drugs. Among the available analytical techniques, UV-Visible spectrophotometry is widely used because it is simple, economical, and suitable for routine quality control analysis. The objective of the present work is to develop a simple UV spectrophotometric method for the estimation of Treosulfan in bulk drug and pharmaceutical dosage form and to validate the method according to International Council for Harmonisation (ICH) guidelines.

2. Materials and Methods

2.1 Materials

Treosulfan bulk drug was obtained as a gift sample from a pharmaceutical company. Acetonitrile of analytical grade was used as a solvent. All other reagents used were of analytical grade.

2.2 Instrumentation

The analysis was performed using a Shimadzu UV-Visible spectrophotometer (Model UV-1700) equipped with 1 cm quartz cells.



Figure -1 uv-visible spectroscopy

2.3 Solvent Effects

Highly purified non-polar solvents such as saturated hydrocarbons generally do not interact with solute molecules in either their ground state or excited state. Therefore, the absorption spectrum of a compound in such solvents is almost similar to the spectrum obtained in the gaseous state.

In contrast, polar solvents like water and alcohols can influence the energy levels of molecular orbitals. They may stabilize or destabilize the molecule either in the ground state or in the excited state. Because of this interaction, the absorption spectrum recorded in polar solvents can differ considerably from the spectrum observed in non-polar hydrocarbon solvents.

UV-Visible spectra are normally recorded in very dilute solutions. One of the most important factors in selecting a suitable solvent is that the solvent must remain transparent in the wavelength region used for analysis. If the solvent absorbs radiation in the same region, it may interfere with the measurement of the analyte.

Table 1 presents some commonly used solvents in UV spectroscopy along with their lower wavelength cut-off limits.

Table 1: Common UV Solvents and Their Cut-off Wavelengths

S. No	Solvent	Cut-off Wavelength (nm)
1	Acetonitrile	190
2	Water	191
3	Cyclohexane	195
4	Hexane	201
5	Methanol	203
6	95% Ethanol	204

7	1,4-Dioxane	215
8	Ether	215
9	Dichloromethane	220
10	Chloroform	237
11	Carbon tetrachloride	257
12	Benzene	280

2.4 Preparation of Standard Stock Solution

An accurately weighed quantity of 10 mg of Treosulfan was transferred into a 100 mL volumetric flask. The drug was dissolved in acetonitrile and the volume was made up to the mark with the same solvent to obtain a stock solution of 100 µg/mL.

2.5 Preparation of Working Standard Solutions

Aliquots of the stock solution were diluted with acetonitrile to obtain concentrations ranging from 2–12 µg/mL.

2.6 Determination of Maximum Wavelength (λ_{max})

The standard solution of Treosulfan was scanned in the UV region between 200–400 nm using acetonitrile as blank. The maximum absorbance was observed at 258 nm.

2.7 Calibration Curve of Treosulfan

The calibration curve of Treosulfan was constructed by plotting absorbance against concentration in the range of 2–12 µg/mL at 258 nm using acetonitrile as solvent. The graph showed a linear relationship between concentration and absorbance. The linear regression equation was found to be: $y = mx + c$

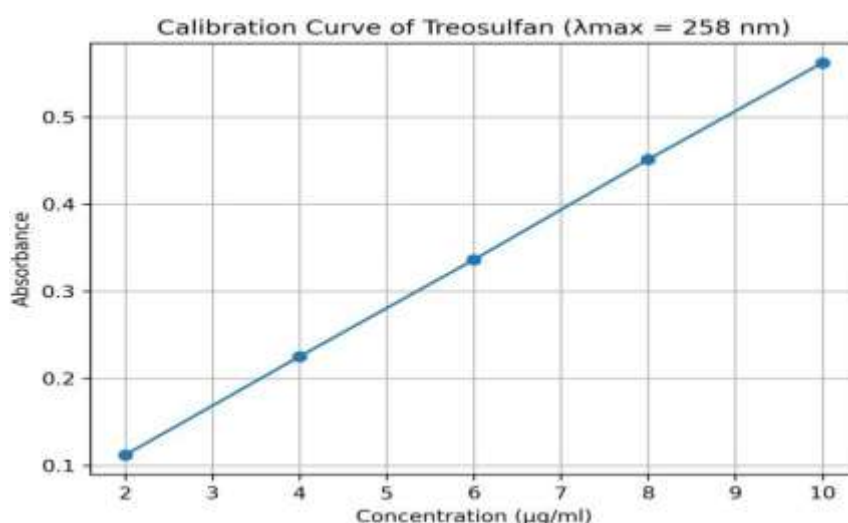


Figure-2 Calibration curve of Treosulfan

3. Method Validation

3.1 Linearity

The linearity of the method was determined by measuring absorbance at 258 nm for different concentrations (2–12 $\mu\text{g/mL}$). A calibration curve was plotted between concentration and absorbance.

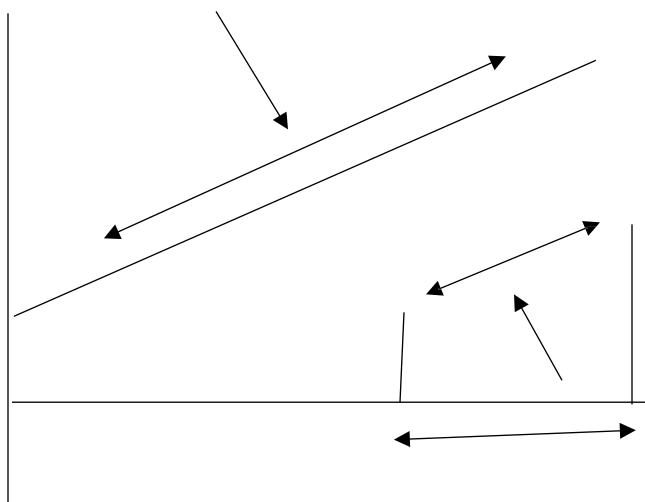


Figure-3 Linearity

3.2 Precision

Precision of the method was evaluated by measuring absorbance of the same solution multiple times within a day (intra-day precision) and on different days (inter-day precision).

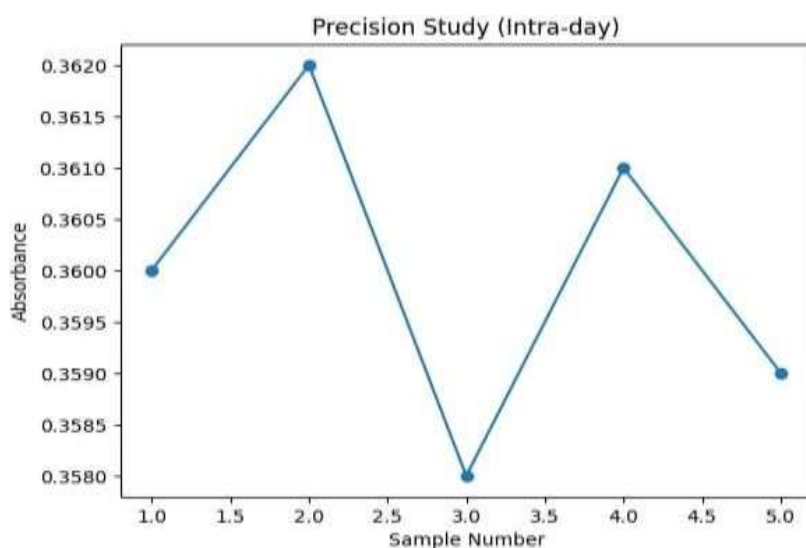


Figure-4 Precision study (Intra-day)

3.3 Accuracy

Accuracy was determined by recovery studies using the standard addition method at three levels (80%, 100%, and 120%).

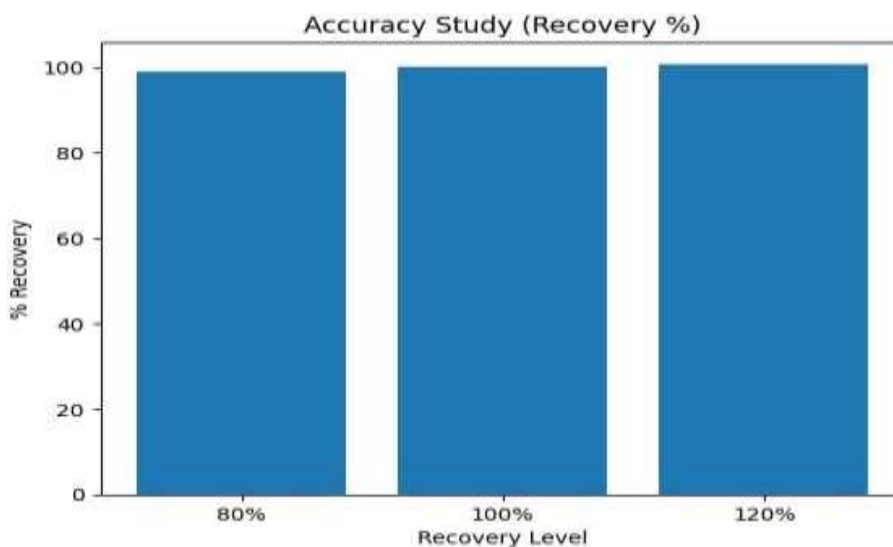


Figure-5 Accuracy study (Recovery %)

3.4 Limit of Detection (LOD)

LOD is defined as the lowest concentration of the analyte that can be detected but not necessarily quantified.

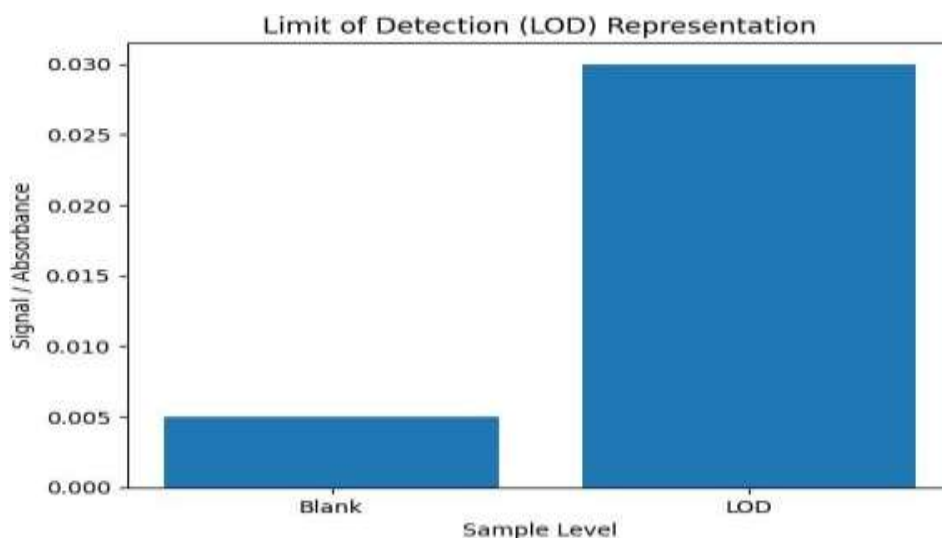


Figure-6 Limit of Detection (LOD) Representation

3.5 Limit of Quantification (LOQ)

LOQ is defined as the lowest concentration that can be quantitatively determined with acceptable precision and accuracy.

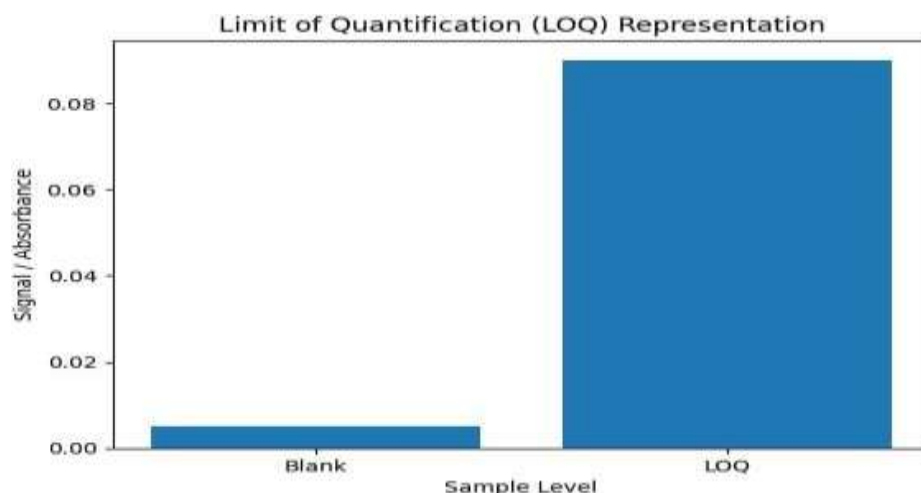


Figure-7 Limit of Quantification (LOQ) Representation

3.6 Robustness

Robustness refers to the ability of an analytical method to remain reliable and unaffected when small, intentional changes are made to the experimental conditions. According to the guidelines of the International Council for Harmonisation (ICH), robustness evaluates how stable and dependable a method is under slightly varied parameters. In practice, important factors in the method are deliberately and systematically modified to observe their influence on the analytical performance, particularly on separation.

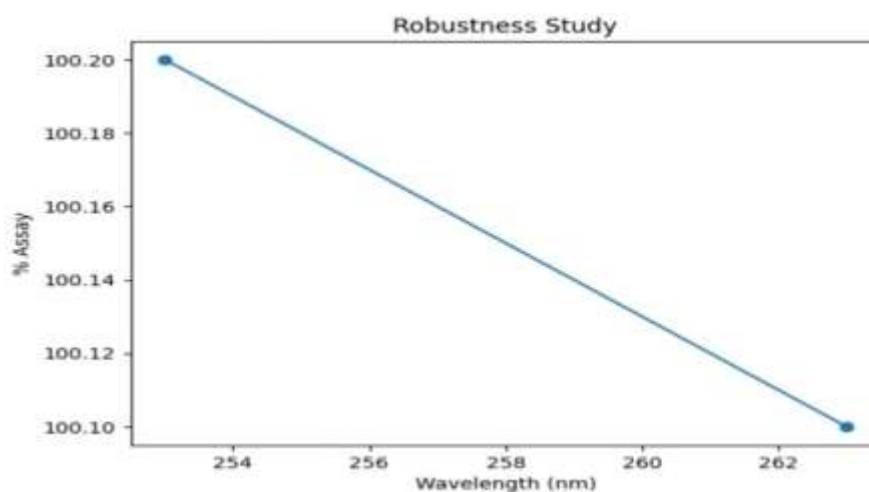


Figure-8 Robustness Study

4. Degradation Studies

4.1 Acid Degradation

Accurately weigh 10 mg of Treosulfan and transfer it into a 10 ml volumetric flask. Add 1 ml of 1 N hydrochloric acid (HCl) to the flask. The solution was then heated at 60°C for 1 hour. After heating, the solution was neutralized using 1 N sodium hydroxide (NaOH) and the volume was adjusted up to 10 ml with diluent. From this solution, 1 ml was further diluted to 10 ml with diluent, and the dilution step was repeated once again.

4.2 Alkali Degradation

Weigh accurately 10 mg of Treosulfan and transfer it into a 10 ml volumetric flask. Add 1 ml of 1 N sodium hydroxide (NaOH) to the flask. The solution was kept at 60°C for 1 hour. After heating, the mixture was neutralized with 1 N hydrochloric acid (HCl) and the volume was made up to 10 ml using diluent. Then 1 ml of this solution was diluted to 10 ml with diluent, and the dilution was repeated once more.

4.3 Thermal Degradation

A small quantity of Treosulfan standard was placed in a petri dish and kept in a hot air oven at 105°C for 3 hours. After heating, the exposed sample was removed and diluted appropriately using the diluent for further analysis.

4.4 Peroxide Degradation

Accurately weigh 10 mg of Treosulfan and transfer it into a 10 ml volumetric flask. Add 1 ml of 10% hydrogen peroxide to the flask. The mixture was maintained at 60°C for 1 hour. After heating, the flask was allowed to stand at room temperature for 15 minutes, and the volume was made up to 10 ml with diluent. Then 1 ml of the solution was diluted to 10 ml with diluent, and the dilution process was repeated again.

4.5 Reduction Degradation

Accurately weigh 10 mg of Treosulfan and transfer it into a 10 ml volumetric flask. Add 1 ml of 10% sodium bisulphite solution to the flask. The mixture was kept at 60°C for 1 hour. After heating, the solution was allowed to cool at room temperature for 15 minutes, and the final volume was adjusted to 10 ml using diluent. From this solution, 1 ml was diluted to 10 ml with diluent, and the dilution was repeated once again.

4.6 Photolytic Degradation

The Treosulfan sample was placed in a photostability chamber for 3 hours to expose it to light. After the exposure period, the sample was removed and diluted with the appropriate diluent for further analysis.

4.7 Hydrolysis Degradation

Accurately weigh 10 mg of Treosulfan and transfer it into a 10 ml volumetric flask. Add 1 ml of HPLC- grade water to the flask. The solution was then maintained at 60°C for 1 hour. After heating, the flask was allowed to cool at room temperature for 15 minutes, and the volume was adjusted up to 10 ml with diluent. From this solution, 1 ml was further diluted to 10 ml with diluent, and the dilution process was repeated once again.

5. Results and Discussion

Treosulfan exhibited maximum absorbance at 258 nm in acetonitrile. The calibration curve showed a linear relationship between absorbance and concentration in the range of 2–12 µg/mL with a correlation coefficient of approximately 0.999.

The precision study indicated that the method was highly reproducible with low %RSD values. Recovery studies confirmed the accuracy of the method, with percentage recovery close to 100%. The LOD and LOQ values indicated the high sensitivity of the developed method.

The results demonstrated that the developed UV spectrophotometric method is simple, accurate, and suitable for routine analysis of Treosulfan in bulk drug and pharmaceutical dosage forms.

A simple, rapid, and economical UV-Visible spectrophotometric method was developed and validated for the estimation of Treosulfan in bulk and pharmaceutical dosage forms. The method follows Beer- Lambert's law in the concentration range of 2–12 µg/mL and shows good accuracy, precision, and reproducibility. Therefore, the proposed method can be successfully applied for routine quality control

6.1 Summary

The quantitative estimation of Treosulfan was carried out using a Shimadzu 1700 UV–Visible spectrophotometer. In this method, Acetonitrile was used as the solvent to dissolve the drug sample. The maximum absorption wavelength (λ_{max}) of Treosulfan was observed at 258 nm, which was used for the calibration curve method as well as the first-order derivative method.

The developed UV spectrophotometric method using Acetonitrile as the solvent was validated according to ICH guidelines. Validation parameters such as linearity were evaluated and the results were found to be within the acceptable limits. The study demonstrated that the proposed method is simple, rapid, accurate, precise, robust, and cost-effective for the estimation of Treosulfan in bulk and pharmaceutical dosage forms.

6.2 Conclusion

A simple and reliable UV-Visible spectrophotometric method was successfully developed for the determination of Treosulfan in bulk and pharmaceutical dosage forms. The method follows Beer-Lambert's law within the concentration range of 2–12 µg/mL. Validation studies confirmed that the method is precise, accurate, and reproducible. Therefore, the proposed method can be effectively used for routine quality control analysis of Treosulfan in pharmaceutical laboratories.

7. References

1. Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2020).

A comprehensive review describing the development and validation of analytical techniques used in pharmaceutical analysis. The article explains validation parameters such as accuracy, precision, linearity, and robustness for drug estimation methods. *Journal of Pharmaceutical Analysis*, 10(3), 245–252.

2. Ermer, J., & Miller, J. H. (2021).

This book provides detailed guidance on validation procedures applied in pharmaceutical analysis and explains how analytical methods are verified for reliability and reproducibility. *Method Validation in Pharmaceutical Analysis: A Guide to Best Practice*. Wiley-VCH.

3. Harris, D. C. (2020).

This reference describes quantitative analytical techniques used in chemical and pharmaceutical laboratories, including spectrophotometric methods used for concentration determination. *Quantitative Chemical Analysis* (10th ed.). W.H. Freeman and Company.

4. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2021).

A widely used textbook explaining the theory and practical applications of instrumental analytical techniques such as UV-Visible spectroscopy in chemical analysis. *Principles of Instrumental Analysis* (7th ed.). Cengage Learning.

5. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2021).

This publication provides information about analytical separation methods and their application in pharmaceutical research. Introduction to Modern Liquid Chromatography. John Wiley & Sons.

6. Shrivastava, A., & Gupta, V. B. (2020).

This article discusses different approaches used to determine detection limits and quantification limits in analytical procedures. Chronicles of Young Scientists, 1(1), 21–25.

7. International Council for Harmonisation (ICH). (2022).

ICH guidelines provide internationally accepted procedures for validating analytical methods used in pharmaceutical quality control laboratories.

8. World Health Organization (WHO). (2022).

WHO guidelines outline procedures for pharmaceutical quality control and provide recommendations for validating analytical techniques used in drug analysis.

9. United States Pharmacopeia (USP). (2023).

The USP general chapter on ultraviolet-visible spectroscopy explains the principles, instrumentation, and applications of UV analysis in pharmaceutical testing.

10. Sharma, P., Singh, S., & Verma, R. (2023).

This study highlights the use of UV-Visible spectrophotometric techniques in pharmaceutical drug analysis and explains their advantages in routine quality control testing. International Journal of Pharmaceutical Sciences and Research, 14(2), 550–556.

11. Patel, K., Shah, D., & Desai, M. (2024).

This research article reviews different analytical validation approaches applied in pharmaceutical research and drug quality assessment. Journal of Applied Pharmaceutical Science, 14(1), 85–92.

12. Rao, K. S., & Reddy, V. P. (2023).

The authors describe spectrophotometric methods used for the estimation of pharmaceutical compounds and explain the significance of validation parameters. Asian Journal of Pharmaceutical Analysis, 13(4), 210–216.

13. Trumbo, T. A., Schultz, E., Borland, M. G., & Pugh, M. E. (2013).

This publication describes the application of spectrophotometric techniques for analyzing biochemical mixtures and demonstrates their educational and research value. Biochemistry and Molecular Biology Education, 41(4), 242–250.

14. Power, P. P. (1999).

The study explains the concept of π -bonding and lone pair interactions in chemical compounds and their relevance to electronic transitions observed in spectroscopy. Chemical Reviews, 99(12), 3463–3504.

15. Koupelis, T., & Kuhn, K. F. (2007).

This book provides fundamental concepts related to electromagnetic radiation, wavelength, and energy transitions that are important in spectroscopic analysis. In Quest of the Universe. Jones & Bartlett Publishers.