



DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR CLOMIPHENE CITRATE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, accurate, precise, and cost-effective UV spectrophotometric method was developed and validated for the estimation of Clomiphene Citrate in tablet dosage form. Clomiphene Citrate exhibited maximum absorbance (λ max) at 244 nm using Methanol: Water (50:50 v/v) as the solvent system. The developed method obeyed Beer–Lambert’s law in the concentration range of 10–50 μ g/mL with good linearity. The calibration curve showed a regression equation of $y = 0.001x + 0.0432$ with a correlation coefficient (R^2) of 0.9972. The developed analytical method was validated according to ICH guidelines with respect to parameters such as linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). The %RSD values obtained for intra-day and inter-day precision studies were found to be less than 5%, indicating good precision of the method. Accuracy studies were carried out by recovery studies at 50%, 100%, and 150% levels, and the average recovery was found to be 100.78%, confirming the accuracy of the method. The LOD and LOQ values were found to be 3.18 μ g/mL and 9.66 μ g/mL, respectively. The developed method was found to be simple, sensitive, reliable, and suitable for routine quality control analysis of Clomiphene Citrate in pharmaceutical tablet formulations.

KEYWORDS: Clomiphene Citrate, UV Spectrophotometry, Method Validation, Tablet Dosage Form, ICH Guidelines.

1. INTRODUCTION

Clomiphene Citrate is a non-steroidal synthetic drug belonging to the class of Selective Estrogen Receptor Modulators (SERMs). Chemically, it is designated as 2-[4-(2-chloro-1,2-diphenylvinyl) phenoxy]-N, N-diethyl ethanamine citrate with a molecular formula of $C_{22}H_{26}ClNO_8$ and molecular weight of 598.09 g/mol. It appears as a white to pale yellow crystalline powder and is freely soluble in methanol and ethanol.^[1]

Clomiphene Citrate is widely used in the treatment of female infertility associated with anovulation and irregular ovulation. The drug stimulates the release of gonadotropins such as Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), thereby

promoting ovulation. It is extensively prescribed in conditions such as Polycystic Ovary Syndrome (PCOS), ovulatory dysfunction, and unexplained infertility.^[2]

Due to the presence of aromatic rings and conjugated double bonds in its chemical structure, Clomiphene Citrate exhibits significant absorption in the ultraviolet region, making it suitable for UV spectrophotometric analysis. The chemical structure of Clomiphene Citrate is shown in Fig. 1.

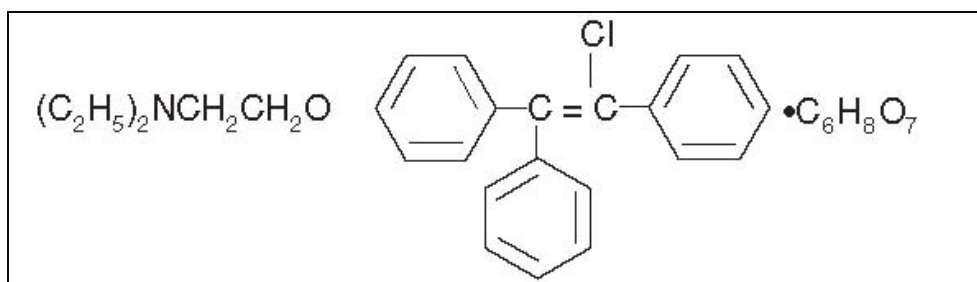


Fig. 1: Chemical structure of Clomiphene Citrate.

A detailed literature survey revealed that very limited analytical methods have been reported for the estimation of Clomiphene Citrate by UV spectrophotometry in pharmaceutical dosage forms. Therefore, the present work was undertaken to develop and validate a simple, precise, accurate, and economical UV spectrophotometric method for the estimation of Clomiphene Citrate in marketed tablet formulations according to ICH guidelines.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Clomiphene Citrate was used as the working standard for the study. Methanol was procured from Loba Chemie Pvt. Ltd., Mumbai, India, and distilled water was used

throughout the analysis. All chemicals and reagents used in the study were of analytical grade.

2.2 Instruments Used

The instruments and apparatus used in the present work included a UV–Visible Spectrophotometer with matched quartz cells of 1 cm path length, analytical balance, volumetric flasks, pipettes, sonicator, and Whatman filter paper. All instruments were calibrated before use.

2.3 Selection of Wavelength

A standard solution of Clomiphene Citrate was scanned in the UV region between 200–400 nm using Methanol: Water (50:50 v/v) as solvent system. The drug showed maximum absorbance (λ_{max}) at 244 nm, which was selected for further analytical studies.

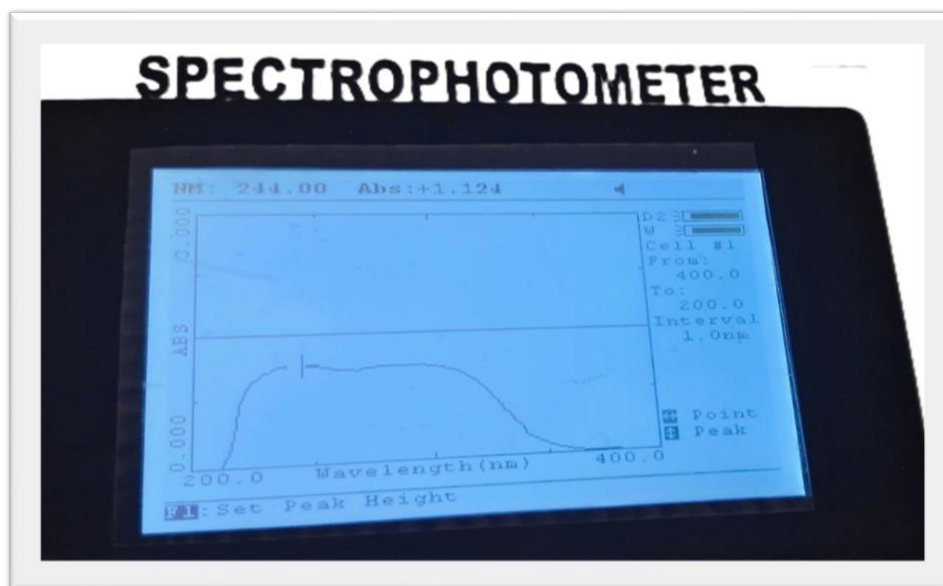


Fig. 2: UV spectrum of Clomiphene Citrate.

2.4 Preparation of Solvent System

The solvent system was prepared by mixing methanol and distilled water in the ratio of 50:50 v/v. The prepared solution was sonicated for proper mixing and used throughout the study.

2.5 Preparation of Standard Stock Solution

An accurately weighed quantity of 50 mg of Clomiphene Citrate was transferred into a 50 mL volumetric flask and dissolved in the prepared solvent system. The volume was made up to the mark with the same solvent to obtain

a stock solution containing 1000 µg/mL of Clomiphene Citrate.

From this stock solution, suitable aliquots were withdrawn and diluted with solvent system to obtain working standard solutions in the concentration range of 10–50 µg/mL.

2.6 Analysis of Marketed Formulation

Twenty tablets of Clofert-50 containing Clomiphene Citrate were accurately weighed and finely powdered.

Tablet powder equivalent to 50 mg of Clomiphene Citrate was transferred into a 50 mL volumetric flask containing solvent system. The solution was sonicated for 10 minutes to ensure complete extraction of the drug and filtered through Whatman filter paper.

The filtrate was suitably diluted with solvent system to obtain a final concentration of 10 µg/mL, and absorbance was measured at 244 nm.

3. METHOD VALIDATION

The developed UV spectrophotometric method was validated according to ICH Q2(R1) guidelines for analytical validation parameters such as linearity, precision, accuracy, robustness, limit of detection, and limit of quantification.

3.1 Linearity

The linearity of Clomiphene Citrate was evaluated by preparing standard solutions in the concentration range

of 10–50 µg/mL. The absorbance of each solution was measured at 244 nm against reagent blank.

The calibration curve was plotted between concentration and absorbance. The method obeyed Beer–Lambert's law within the selected concentration range with good linearity.

Table 1: Linearity study of Clomiphene Citrate.

Sr. No.	Concentration (µg/mL)	Absorbance
1	10	0.053
2	20	0.064
3	30	0.072
4	40	0.084
5	50	0.093

Regression equation: $y = 0.001x + 0.0432$

Correlation coefficient (R^2): 0.9972

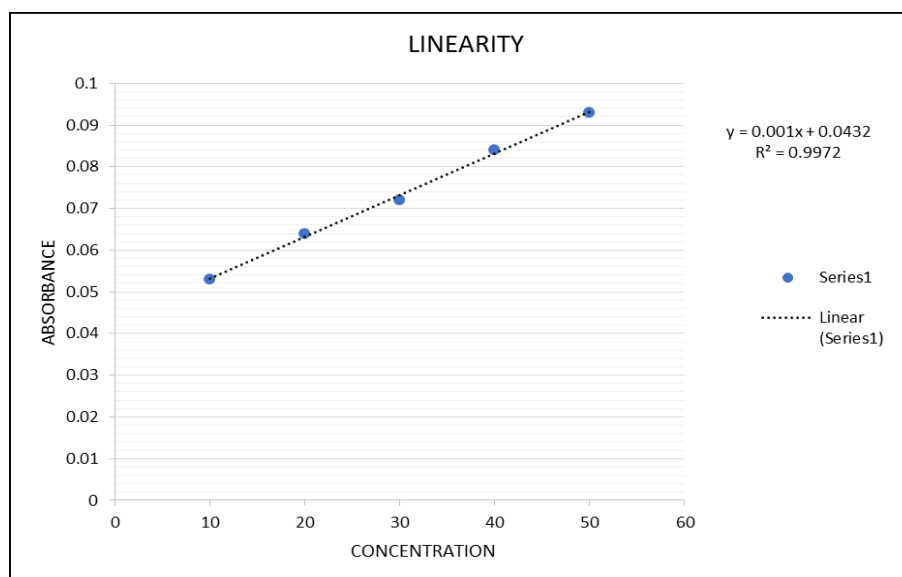


Fig. 3: Calibration curve of Clomiphene Citrate.

3.2 Precision

Precision of the developed method was evaluated in terms of intra-day and inter-day precision studies using

three concentrations of Clomiphene Citrate (10, 20, and 30 µg/mL).

Intra-day Precision

Table 2: Intra-day precision study of Clomiphene Citrate.

Concentration (µg/mL)	Trial 1	Trial 2	Trial 3	Mean	SD	%RSD
10	0.658	0.620	0.659	0.6456	0.0288	4.25
20	0.688	0.687	0.688	0.6876		
30	0.701	0.702	0.700	0.7010		

Inter-day Precision

Table 3: Inter-day precision study of Clomiphene Citrate.

Concentration (µg/mL)	Trial 1	Trial 2	Trial 3	Mean	SD	%RSD
10	0.660	0.665	0.657	0.6606	0.0217	3.17
20	0.698	0.695	0.695	0.6960		
30	0.700	0.701	0.710	0.7003		

The %RSD values obtained for intra-day and inter-day precision studies were found to be less than 5%, indicating good precision and reproducibility of the method.

3.3 Accuracy

Accuracy of the method was determined by recovery studies using standard addition method at 50%, 100%, and 150% levels.

Table 4: Recovery study of Clomiphene Citrate.

Level	Sample Concentration (µg/mL)	Standard Added (µg/mL)	Absorbance	% Recovery
50%	5	10	0.476	101.51
100%	5	20	0.621	99.67
150%	5	30	0.786	101.16

Mean % Recovery = 100.78%

The percentage recovery values indicated good accuracy of the developed analytical method.

3.4 Robustness

Robustness of the developed UV spectrophotometric method was evaluated by introducing slight variations in analytical conditions such as wavelength and solvent composition. The developed method remained unaffected by these small variations, indicating that the method is robust and reliable for routine analysis.

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

The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantification (LOQ) using the following equations:

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

Where,

σ = Standard deviation of intercept

S = Slope of calibration curve

Table 5: Result of LOD and LOQ

Sr. No.	Name of Drug	LOD (µg/mL)	LOQ (µg/mL)
1	Clomiphene Citrate	3.18	9.66

4. RESULTS AND DISCUSSION

The proposed UV spectrophotometric method was successfully developed for the estimation of Clomiphene Citrate in tablet dosage form using Methanol: Water (50:50 v/v) as solvent system. The drug showed maximum absorbance at 244 nm and obeyed Beer–Lambert’s law in the concentration range of 10–50 µg/mL.

The calibration curve demonstrated good linearity with correlation coefficient (R^2) value of 0.9972. The developed method showed satisfactory precision with %RSD values below 5% for both intra-day and inter-day precision studies.

Accuracy studies showed mean percentage recovery of 100.78%, indicating accuracy and reliability of the developed method. The low values of LOD and LOQ

demonstrated good sensitivity of the proposed analytical procedure.

The developed UV spectrophotometric method was found to be simple, rapid, accurate, precise, economical, and suitable for routine quantitative analysis of Clomiphene Citrate in pharmaceutical tablet formulations.

5. CONCLUSION

The developed UV spectrophotometric method for estimation of Clomiphene Citrate in tablet dosage form was found to be simple, rapid, accurate, precise, sensitive, and cost-effective. The method obeyed Beer–Lambert’s law in the concentration range of 10–50 µg/mL and was validated according to ICH guidelines.

The precision and accuracy results confirmed the reliability of the developed analytical method. Low values of LOD and LOQ indicated good sensitivity of the proposed method. Therefore, the developed UV spectrophotometric method can be successfully employed for routine quality control analysis of Clomiphene Citrate in pharmaceutical tablet formulations.

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7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

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