IJPHT Journal
Papers Having Good Impact

International Journal of Pharmacy and Herbal Technology (Online)

Home Page: https://www.ijprdjournal.com/

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF BULK DRUG IN PHARMACEUTICAL DOSAGE FORMS

Shubhada S. Pawar, Sanjay K. Bais and Vighnesh Nagnath Putta*
Fabtech College of Pharmacy, Sangola
Tal-Sangola, Dist.-Solapur
Maharashtra -413307

ABSTRACT

This study used UV spectrophotometry to determine the active pharmaceutical ingredient (API) in the dosage form from the AUC. Analyte concentration for AUC method, was measured using which combines absorbance over a predetermined wavelength range.[1] This work describes the design, optimization, and validation of the AUC method, including selecting the appropriate wavelength range, generating the curve, and measuring stability and accuracy on track. The precision, accuracy and LOD & LOQ method for routine analysis in pharmaceutical laboratories have been demonstrated by verifying its performance according to International Committee for Harmonization (ICH) guideline.[2] The AUC method provides a reliable and convenient method when measuring API in pharmaceutical forms using UV spectrophotometry. The Mobile phase used throughout the analysis of Cilnidipine is Methanol of HPLC Grade.

***Keywords**: Cilnidipine, Area Under Curve Method, Validation.*

*Corresponding Author Email: puttavighnesh@gmail.com
Received on 02 July, 2024, Accepted 10 July, 2024

Please cite this article as: Putta Vighnesh *et al*., Development and Validation of UV Spectrophotometric Methods for Determination of Bulk Drug in Pharmaceutical Dosage Forms
International Journal of Pharmacy And Herbal Technology 2024.

INTRODUCTION

The accurate and reliable determination of pharmaceutical compounds in dosage forms is essential for ensuring the efficacy, safety, and quality of medicinal products. Among various analytical techniques, UV spectrophotometry is a widely used method due to its simplicity, cost-effectiveness, and versatility. In recent years, the Area Under Curve (AUC) method has gained prominence as a valuable approach for quantitative analysis in UV spectrophotometry, offering enhanced sensitivity and specificity compared to traditional peak-based methods.[1]

This research aims to explore the development and validation of UV spectrophotometric methods utilizing the AUC approach to determine pharmaceutical compounds in tablets. The adoption of the AUC allows for a comprehensive analysis of the entire UV spectrum, capturing all relevant absorbance information and minimizing potential interference from impurities or excipients present in the sample matrix.[2]

Through systematic method development and validation procedures, including linearity, accuracy, precision, and LOD & LOQ assessments, suitability and reliability of the AUC method for pharmaceutical analysis will be thoroughly evaluated.[3]

Cilnidipine is a dual L/N-type calcium channel blocker used in the treatment of hypertension. It exerts its antihypertensive effect by blocking both L-type calcium channels, resulting in vasodilation and subsequent reduction in blood pressure. This unique mechanism of action distinguishes it from other calcium channel blockers and contributes to its efficacy in managing hypertension.[4]

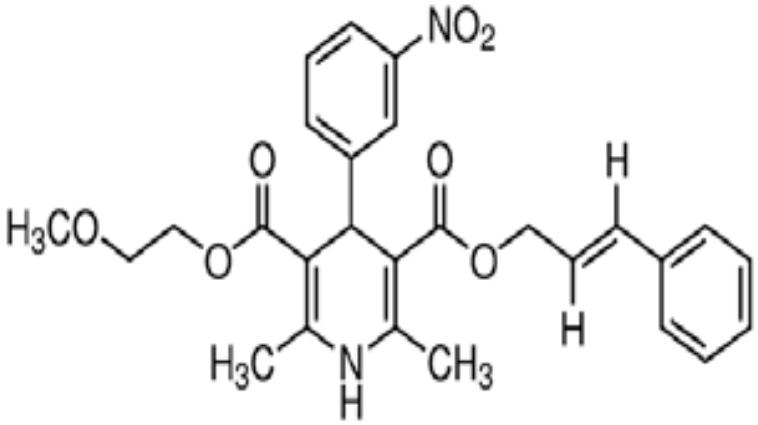

Fig. No.1: Molecular Structure of Cilnidipine

Molecular formula of Cilnidipine: $C_{27}H_{28}N_2O_7$
Molecular weight of Cilnidipine: 492.5 g/mol

MATERIAL AND METHODS:

Chemical and reagents:

Mumbai-based J.B. Chemical and Pharmaceutical Pvt. Ltd. provided Cilnidipine. Methanol of (HPLC) grade used throughout the analysis.

Instrumentation:

For all spectral measurements, a Systronics 1800UV/VIS double beam spectrophotometer with quartz cells that were matched by 1 cm was used.

METHODOLOGY:

Procedure of Standard stock solution:

Weigh out 100 mg of cilnidipine. Then, dissolve it in an adequate amount of mobile phase and transfer it to a 100 ml volumetric flask. Correct the volume to achieve a concentration of 1000µg/ml. Pipetting out 10 ml of the stock solution into a 100 ml volumetric flask allowed for the concentration to be adjusted to 100µg/ml. [5] A working standard solution containing 10µg/ml of Cilnidipine in the mobile phase was prepared by pipetting one millilitre from the standard stock solution mentioned previously.[6]

Selection of Wavelength range and Solvent:

A range of operational parameters were established for concentrations of 2, 4, 6, 8, and 10 µg/m2. The final wavelength range of 200–400 nm was selected after a variety of wavelengths were tested. This was done because the corresponding concentration and area had a linear relationship.[7]

Selection of Wavelength Range:

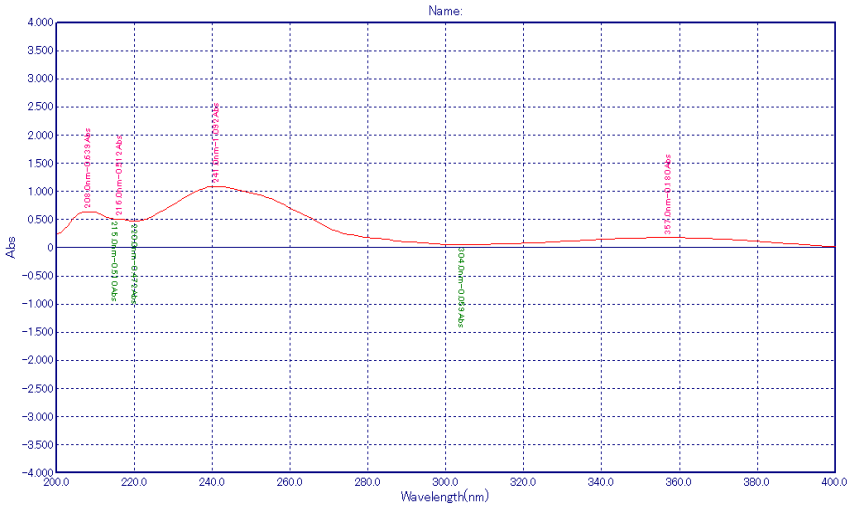

Fig. No.2: Selection of Wavelength Range

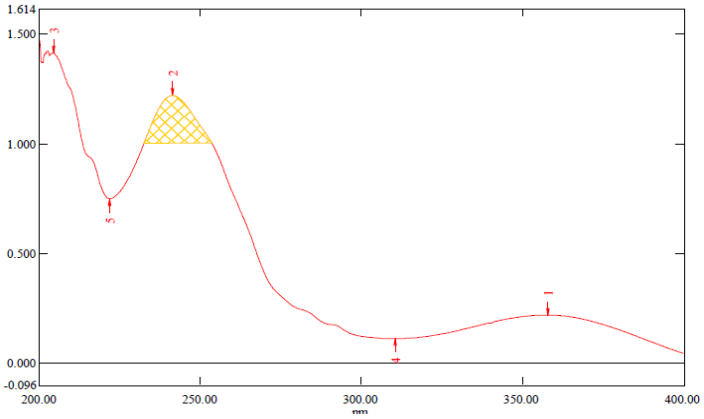

Fig. No.3: Area Under Curve Spectra of Cilnidipine

AUC Method:

When it comes to UV spectrophotometry, the Area Under Curve method is a useful tool for analysing and determining the concentration of substances in a solution by using their absorbance characteristics over a range of wavelengths.[8]

AUC Area Calculation:

λ1 and λ2, which stand for the start and end points of the curve region, the integrated absorbance with respect to wavelength is calculated using the area under the curve method. Computing the area under curve between λ1 and λ2 was done using UV probe software. wavelength ranges from 200 to 400 nm were integrated in this research area.[9]

Calculating area: $(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where,

λ1 and λ2 = wavelength range start and end points of the curve region

α = area of portion bounded by curve data and a straight line connecting the start and end point

β = area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis.[10]

Assay of Tablet (Marketed Formulation):

Twenty tablets containing 10 mg of cilnidipine were weighed, the average weight was calculated, and the weight corresponding to 10 mg of cilnidipine was then transferred to a 10 ml volumetric flask containing the suggested mobile phase. Following a 15-minute sonication, the mixture was filtered through Whatman filter paper no. 42 to create a 1000 µg/ml stock solution. Solvent was then added to the final volume. Following the preparation of a 10µg/ml dilution of Cilnidipine using the appropriate stock solution, the absorbance was assessed.[11]

Sr. No.	Cilnidipine		
	Area	Amount Recovered (µg/ml)	% Recovery
1	3.287	10.029	101.90
2	3.310	10.097	101.82
3	3.291	10.050	102.07
4	3.310	10.118	102.45
5	3.280	9.999	101.90
6	3.275	9.987	101.44
Mean	3.288	10.10	100.45
% RSD	0.381	0.529	0.529

Table No. 1: Assay of Tablet (Marketed Formulation)

RESULT

Validation Method:

Validation parameters such as linearity, precision, and accuracy, along with the LOD and LOQ, were used to validate by ICH guidelines.

Linearity:

Analytical methods such as linearity can be measured by examining various standard solution concentrations. To achieve concentrations of 2, 4, 6, 8 and 10µg/ml, dilutions were made using the Standard Stock solution. These solutions area under curve (AUC) values were integrated between 220 and 260 nm after being scanned from 400 to 200 nm.[12] For Cilnidipine, a calibration curve was constructed by graphing the peak areas on the y-axis against the concentration on the x-axis. For Cilnidipine, a linear relationship was found in graph at concentrations between 2 and 10 µg/ml. [13]

Parameter	Cilnidipine
Range	2-10 µg/ml
Slope	0.1103
Intercept	0.0941
Correlation coefficient	0.9996

Table No. 2: Linearity Values of Cilnidipine

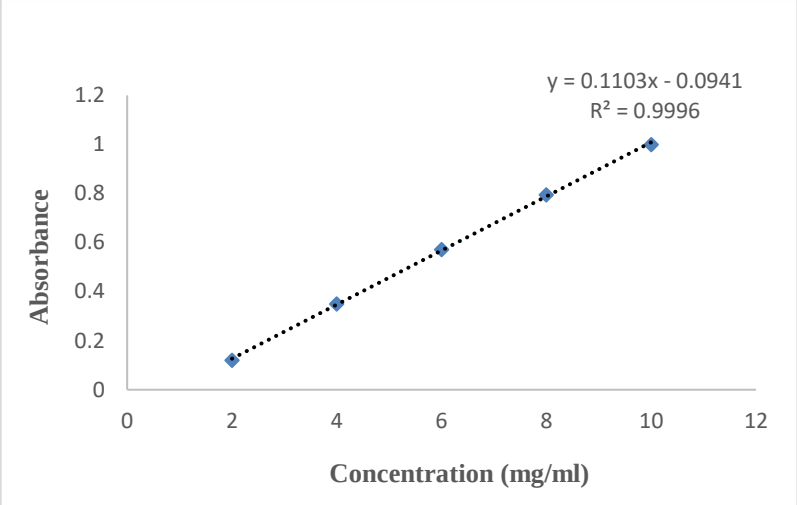

Fig. No.4: Calibration Curve of Cilnidipine

Precision:

The precision of AUC method in UV spectrophotometry refers to its ability to provide consistent and accurate measurements of analyte concentration. Precision is typically assessed through the calculation of standard deviation and relative standard deviation of replicate measurements.[14]

The degree of agreement (or proximity to agreement) between a set of measurements taken from repeated sampling of the same homogeneous sample carried out under the given conditions is the definition of precision in an analytical procedure. Intraday precision was tested by integrating a 10µg/ml concentration standard solution area at six different independent series in a single day. To conduct inter-day precision studies, a standard solution containing 10µg/ml was integrated over three days. The percentage of RSD was calculated.[15]

Conc. µg/ml	Area			Mean	SD	%RSD
	Trial A	Trial B	Trial C			
6	0.572	0.573	0.574	0.573	0.001	0.17452
8	0.795	0.796	0.797	0.796	0.009	0.125628
10	1.000	1.001	1.002	1.001	0.001	0.0999

Table No.3: Intraday Precision of Cilnidipine

Conc. µg/ml	Area			Mean	SD	%RSD
	Trial A	Trial B	Trial C			
6	0.572	0.575	0.578	0.575	0.003	0.521739
8	0.794	0.796	0.798	0.796	0.002	0.251256
10	1.002	1.005	1.008	1.005	0.003	0.298507

Table No. 4: Inter day Precision of Cilnidipine

Accuracy:

The degree to which the results obtained from the spectrophotometer are near the true value or a recognised standard is known as accuracy, especially when discussing the area under the curve (AUC) method.[16]

At 80%, 100%, and 120% accuracy levels, the analytical method was tested using the 10µg/ml standard solution. The area under the curve (AUC) in the 200–400 nm wavelength range was measured, and the results were then expressed as a percentage of recovery. For each level, three determinations were needed before the percentage RSD could be calculated.[17]

Level	Concentration (µg/ml)		Area	% Recovery	Mean% Recovery
	Sample	Standard			
80%	10	8	4.705	99.13	98.12667
			4.704	98.10	
			4.708	97.15	
100%	10	10	5.923	100.77	100.6233
			5.938	100.50	
			5.935	100.60	
120%	10	12	9.026	99.26	97.00333
			9.030	96.25	
			9.035	95.50	

Table No.5: Accuracy of Cilnidipine

LOD and LOQ :

The smallest analyte concentration at which a measurable response is obtained is known as the Limit of Detection (LOD). The minimum concentration of the analyte at which a response can be accurately determined is known as the Limit of Quantification (LOQ).[18]

The linearity calibration curve was used to calculate the intercept's standard deviation. Using the following mathematical formula, the Limit of Detection and Limit of Quantification were determined:

$$LOD = 3.3 \times \sigma/S$$

Where,

σ is the standard deviation of the Intercept

S is the Mean slope of the calibration curve

$$LOQ = 10 \times \sigma/S$$

Where,

Σ is the standard deviation of the Intercept

S is the Mean slope of the calibration curve [19]

Characteristics	**Cilnidipine**
Sigma (σ)	0.018796
Limit of Detection	0.562341
Limit of Quantification	1.704065

Table No.6: LOD & LOQ of Cilnidipine

CONCLUSION

The developed method can be suitably analysed for routine analysis of Cilnidipine in bulk and tablet dosage form because the area under curve method was novel, inexpensive, dynamic, user-friendly, and economical for simultaneous Cilnidipine estimation.The common excipient found in pharmaceutical formulations does not cause any interference for it.

Key findings of the study include the development of methods with high sensitivity and specificity for detecting APIs, along with comprehensive validation according to ICH guidelines. The validation results confirmed the methods' linearity, accuracy, precision, LOQ & LOD over the tested concentration ranges. Additionally, the validated methods were effectively applied to analyse bulk drug samples and various pharmaceutical formulations, demonstrating their practical utility. In summary, this study provides robust, validated analytical tools that significantly enhance quality control processes for pharmaceutical products, ensuring their safety, efficacy, and quality. The developed UV spectrophotometric methods offer a reliable and efficient solution for the routine analysis of pharmaceutical dosage forms containing the studied API.

The successful application of these methods to bulk drug samples and different pharmaceutical formulations underscores their versatility and practical utility in real-world quality control processes. By providing efficient and validated analytical tools, the study significantly enhances the capability to ensure the safety, efficacy, and quality of pharmaceutical products. In conclusion, the developed UV spectrophotometric methods represent a robust, reliable, and efficient solution for the routine analysis of pharmaceutical formulations. This work supports the ongoing need for effective quality control measures in the pharmaceutical industry, ultimately contributing to better healthcare outcomes.

REFERENCES

1. Singh, S., UV spectrophotometric method development and validation for the estimation of cilnidipine in bulk and tablet dosage form by area under curve method. Asian Journal of Pharmaceutical Analysis, 2018, 8(4), 199-204.
2. Patel, K., Development and validation of UV spectrophotometric method for estimation of cilnidipine in bulk and tablet dosage form by area under curve method. International Journal of Pharma Research & Review, 2020, 9(1), 26-30.
3. Gandhi, R., Comparative study of different analytical methods for estimation of cilnidipine. International Journal of Pharmacy and Pharmaceutical Sciences, 2019, 11(1), 36-42.
4. Jain, P., Application of chemometrics to UV spectrophotometric method development and validation for estimation of cilnidipine in bulk and tablet dosage form by area under curve method. Journal of Pharmaceutical Innovation, 2021, 16(2), 187-195.
5. Sharma, P., & Narasimhan B, Development and validation of UV-spectrophotometric method for estimation of cilnidipine in bulk and tablet dosage form. International Journal of Pharmaceutical Sciences and Research, 2018, 6(3), 1201-1206.
6. Rashmi D. R. Simultaneous Estimation of Cilnidipine and Olmesartan Medoxomil in bulk and combined tablet dosage form by UV-Spectrophotometric methods International Journal of Universal Pharmacy and Bio Sciences, 2014; 3(6): 350-362.
7. Patel H., Damale D. P. Development and Validation of UV Spectrophotometric Method for the Simultaneous Estimation of Cilnidipine and Bisoprolol Fumarate in Tablet Dosage Form World Journal of Pharmaceutical Research, 2018; 7(11): 616-627.
8. Haripriya M. Antony N., Jayasekha. Development and Validation of UV Spectrophotometric method for the Simultaneous Estimation of Cilnidipine and Telmisartan in tablet dosage form utilising simultaneous equation and absorbance ratio method JPBS, 2013; 3(1): 343-348.
9. Safhi M. M. Spectrophotometric Method for the Estimation of Cilnidipine in Bulk and Pharmaceutical Dosage forms. Orient. J. Chem., 2013; 29(1): 131-134.
10. Patel A., Shah B. Development and Validation of Area Under Curve Method for Simultaneous Estimation of Thiocolchicoside and Lornoxicam in Tablet Dosage Form JPSBR, 2014; 4(6): 383-387.
11. ICH Q2A Text on validation of analytical procedure, international conference on harmonization. Tripartite guideline, 1994; 1-5.
12. ICH Q2B Validation of analytical procedure: methodology, international conference of harmonization. Tripartite guideline, 1994; 1-10.
13. S. B. Nagansurkar, S. K. Bais, Priyanka Shinde, Formulation and Standardization of Herbal Cream for Acne Vulgaris, International Journal of Advanced Research in Science Communication and Technology,2023, 3(1), 213.
14. Shirish B Nagansurkar, Sanjay K Bais, Komal Kashinath Maske, Herbal Shampoo for Treatment of Anti Dandruff, International Journal of Advanced Research in Science Communication and Technology,2023, 3 (1), 350.
15. A V Pore, SK Bais, Anjali Bhausaheb Sathe, Review on Commercial Cultivation and Collection Aspects of Medicinal and Aromatic Plants, International Journal of Advanced Research in Science Communication and Technology,2023, 3(1), 550.

16. Amol V Pore, Sanjay Bais, Ganesh Pandurang Shinde, Drug Evaluation Methods International Journal of Creative Research Thoughts, 2023,11 (1), 2023, c199.
17. Sarfaraz M Kazi, Sanjay K Bais, Rohini S Mali, Advance Herbal Technology Journal of Advanced Research in Science Communication and Technology,2023, 3 (1), 249.
18. Vimal Ravi, Kokilambigai KS, Kavitha J, Seetharaman R, Lakshmi KS. "Multivariate UV spectrophotometric quantification of cilnidipine drug and pharmaceutical formulations". Journal of Medical Pharmaceutical and Allied Sciences, 2022; 11(2.2718): 4672-4678.
19. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human use, Validation of Analytical Procedure: Text and Methodology Q2(R1).