



Research article

Multivariate UV spectrophotometric quantification of Cilnidipine in bulk drug and pharmaceutical formulations

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ABSTRACT

The aim of this research work was to develop a simple, accurate, sensitive and validated Ultra Violet (UV) spectrophotometric assay using the multivariate regression method for the analysis of Cilnidipine. This multivariate calibration technique was based on equations constructed using linear regression analysis using the correlation between absorbance and concentration at five selected equidistant wavelengths. Cilnidipine had a maximum absorbance at 240 nm. The findings were statistically analyzed for significance. A linear plot in the concentration range of 3-9 µg/mL, with a regression coefficient of 0.999 was obtained. The % RSD for intra-day and Inter-day precision were 0.4558 and 0.6099, respectively. The assay was determined and found to be 99.1% - 101.67% w/w.

Keywords: Cilnidipine, Antihypertensive agent, UV spectrophotometry, Multivariate calibration, Assay, ICH guidelines.

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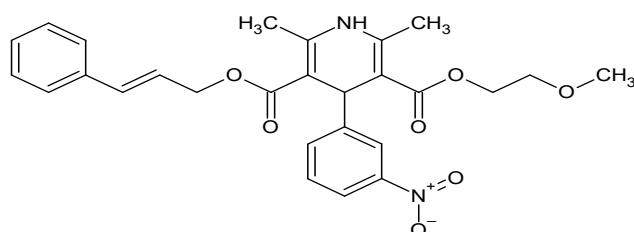
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INTRODUCTION

Cilnidipine is a majorly L-type calcium channel blocker that is used in the ailment of hypertension and other heart related conditions such as stroke and angina. It is often recommended for diabetic patients with these conditions [1]. Clinical and animal tests have proven its efficacy as a Reno protective, cardioprotective and neuroprotective agent. It demonstrates both L-type and N-type Ca²⁺ channel blocking activity and is hence a dual activity drug in antihypertensive pharmacotherapy. Cilnidipine suppresses the cardiovascular neurohumoral regulation, sympathetic nervous system as well as the renin-angiotensin-aldosterone system [2]. Cilnidipine (Figure 1) is a dihydropyridine compound with an IUPAC nomenclature of 3-O-(2-methoxyethyl)-5-O-[(E)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, a molecular formula of C₂₇H₂₈N₂O₇ and a molecular weight of 492.5 g/mol [3]. The drug is official in Indian Pharmacopoeia [4]. Literature review showed findings of various UV-Vis Spectrophotometry [5-17] available for its estimation.

Figure 1: Chemical structure of Cilnidipine



The technique being suggested provides a higher confidence in results as it directly evaluates Cilnidipine and has been attested with greater accuracy and precision than a classical UV-Visible assay. This technique is also more cost effective, direct and rapid than other methods, and can be used for bulk drugs as well as various dosage forms. This multivariate standardization method simplifies the individual result and converts it into a "m" value as a reliant variable [18-20].

Within optimized conditions, this analytical technique would provide excellent sensitivity, resolving power, expeditiousness and cost effectiveness for a validated quantification of Cilnidipine. The absorbance of an analyte (X) i.e. Cilnidipine, is scanned at 5 different wavelengths ($\lambda = 236, 238, 240, 242$ and 244 nm), the following formula can then be applied for any preferred wavelength.

$$A_{\lambda 236} = a X C_x + k_1 \text{----- (1)}$$

$$A_{\lambda 238} = b X C_x + k_2 \text{----- (2)}$$

$$A_{\lambda 240} = c X C_x + k_3 \text{----- (3)}$$

$$A_{\lambda 242} = d X C_x + k_4 \text{----- (4)}$$

$$A_{\lambda 254} = e X C_x + k_5 \text{----- (5)}$$

Where A_λ is the analyte's absorbance, a, b, c, d, and e being slopes of the analyte's linear regression functions; intercepts are denoted as k_1, k_2, k_3, k_4, k_5 at the five specified wavelengths, and C_x is the

analyte's concentration. The selected five equation systems (1–5) listed above can be summarised as follows:

$$A_T = aX C_x + bX C_x + cX C_x + dX C_x + eX C_x + K_T \text{-----} (6)$$

The above equation can be further condensed to

$$A_T = C_x (a + b + c + d + e) + K_T \text{-----} (7)$$

Where A_T and K_T is the summation of the absorbance acquired cum totality of intercepts of regression equations at selected five wavelengths respectively. The concentration of the analyte X is computed by following formula.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

MATERIALS AND METHODS

Chemicals and reagents

Methanol (Gradient grade, Finar Chemicals), Cilnidipine was obtained as a gift sample from Ideal Analytical and Research Institute, Pondicherry. The marketed tablet formulation used was Cilaheart-10, Mankind Pharma, India, (Label claim – 10 milligram Cilnidipine), acquired from a local market.

Instrumentation

LAB INDIA 3092 UV-Visible double beam spectrophotometer, Ultra Sonicator Bath, Analytical balance, Micropipette

Analytical method development

Choice of the solvent

In Methanol, Cilnidipine was found to be freely soluble. Hence, it was used for further dilutions of both standard and sample drug.

Standard stock solution

Cilnidipine standard stock solution was prepared by the dissolution 10 mg of the standard drug in 5 mL of Methanol and then making up to the mark in a 10 mL standard flask with the same solvent. 1mL of this solution was transferred to another 10mL volumetric flask and made up to 10mL with diluent to obtain a 100 µg/mL concentration. From this standard stock solution, several concentrations (3-9 µg/mL) of solution were prepared.

Determination of $\lambda_{\max}$

The standard stock solution was diluted in methanol to obtain 6 µg/mL. This solution was measured in the Ultra-Violet region from 200 - 400 nm. The $\lambda_{\max}$ was obtained as 240 nm (Figure 2). The linear curve was obtained with a graph plotting the absorbance against the concentration (Table 1). The solutions were scanned across the range surrounding 240 nm i.e., 236, 238, 240, 242, 244 nm to better enhance the correlation and to diminish instrumental oscillations.

Preparation of sample solution

20 tablets of Cilnidipine were accurately weighed and powdered. A weight corresponding to 10 mg was measured into a 10

ml volumetric flask, dissolved and made up to the mark with methanol to obtain 1 mg/mL. This solution was then filtered and used for further analysis ^[21-23].

Method Validation

According to ICH Q2B guidelines this method was validated for sensitivity, precision, accuracy, and linearity.

Linearity

The different concentrations over the range of 3-9µg/mL was prepared from the standard stock solution of Cilnidipine. In order to minimize instrumental fluctuations and to better the correlation, these solutions were scanned overrange of wavelength surrounding its absorbance maxima at 236, 238, 240, 242, 244 nm respectively. The absorbances were recorded and the standardizations were obtained by plotting a concentration vs absorbance graph. (Figure 3, Table 1).

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg/mL)	Absorbance [#]				
	236 nm	238 nm	240 nm	242 nm	244 nm
3	0.239	0.246	0.252	0.249	0.246
4.5	0.361	0.372	0.382	0.372	0.370
6	0.475	0.499	0.505	0.492	0.485
7.5	0.594	0.621	0.627	0.620	0.611
9	0.719	0.741	0.761	0.743	0.737

[#]Average of 5 determinations; UV= Ultra violet

By calculating the detection limit and quantification limit using the below formula, the sensitivity of the method was determined.

$$\text{LOD} = 3.3 \sigma / S \text{-----} (8)$$

$$\text{LOQ} = 10 \sigma / S \text{-----} (9)$$

Here, σ is the standard deviation (SD) of the lowermost concentration and

S is the slope of the standard curve.

Precision

To assess the intra-day and inter-day precision, 6 µg/mL solution was scanned six times in a short interval of time in one day for intraday precision and on six different days for inter-day precision.

Accuracy

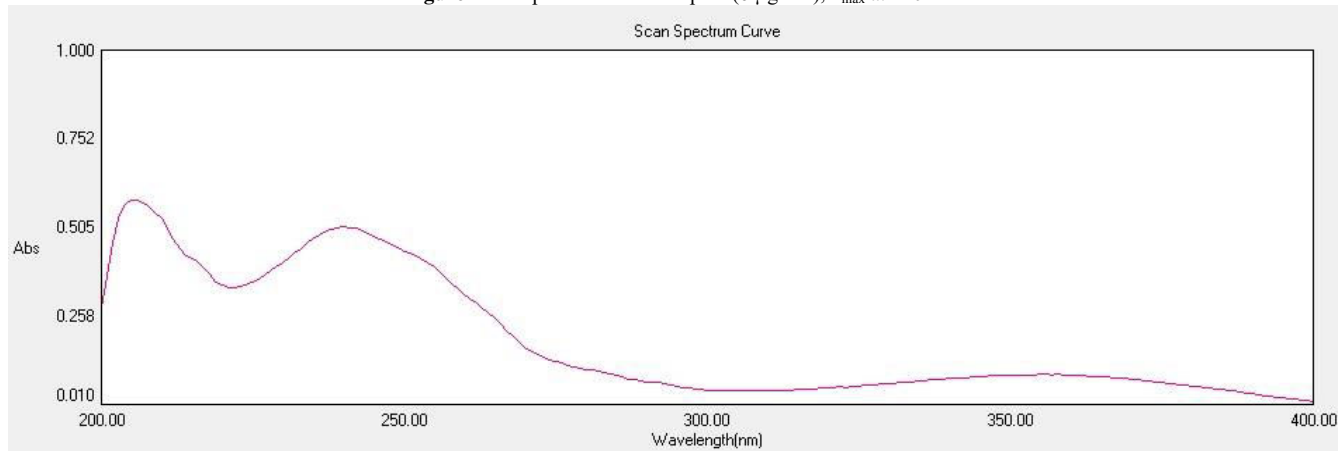
Using the standard addition technique, the recovery study for the suggested technique was resolved at 80%, 100%, and 120%. The standard and sample stock solutions were prepared. 0.3 mL of standard was pipetted out into a three standard 10mL volumetric flasks and to it 0.48, 0.3, 0.72 mL of sample solution were added respectively, making up to a capacity of 10 mL with Methanol. These solutions were measured with a UV spectrophotometer, and the percentage recovery was calculated.

Assay

The amount of Cilnidipine present in the tablet formulation was calculated by measuring the absorbance of the extracted tablet solution at 240 nm.

RESULTS AND DISCUSSION

The $\lambda_{\max}$ of Cilnidipine was found to be 240 nm with Methanol as the solvent as shown in Figure 2.

Figure 2-UV spectrum of Cilnidipine (6 µg/mL), λ_{max} at 240 nm

The technique is linear within the assigned concentration range of 3-9 µg/mL. The linear regression analysis shows good linear relationship with $R^2=0.9997 - 0.9999$ for all the calibration plots. For precision, the % relative standard deviation was found to be 0.4558 and 0.6099. The LOD and LOQ obtained are 0.1436 µg/mL and 0.4352 µg/mL respectively. Therefore, the values were found to fall according to ICH guideline limits of validation parameters.

Linearity

The linearity was recorded at 236, 238, 240, 242 and 244 nm in the concentration range of 3-9 µg/mL and depicted in Figure 3 and corresponding calibration curves and residual plots are presented in Figures 4 to 8 & 9-13 respectively. For each of the wavelengths, the low values of % relative standard deviation show that the technique is accurate and precise. The LOD and LOQ were calculated and reported in Table 2.

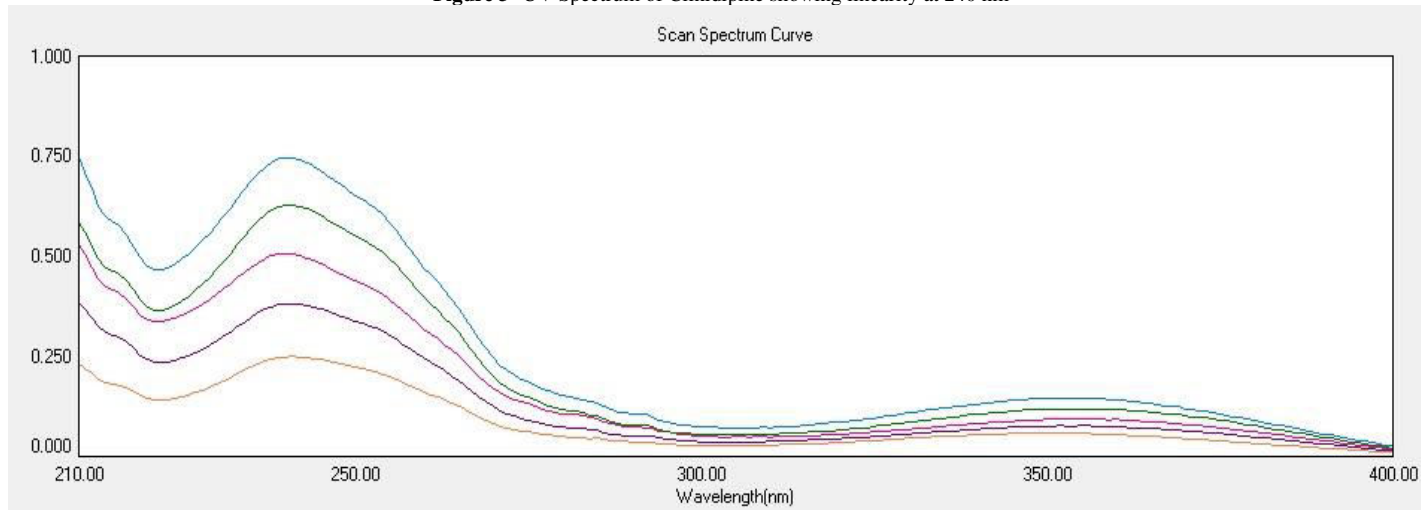
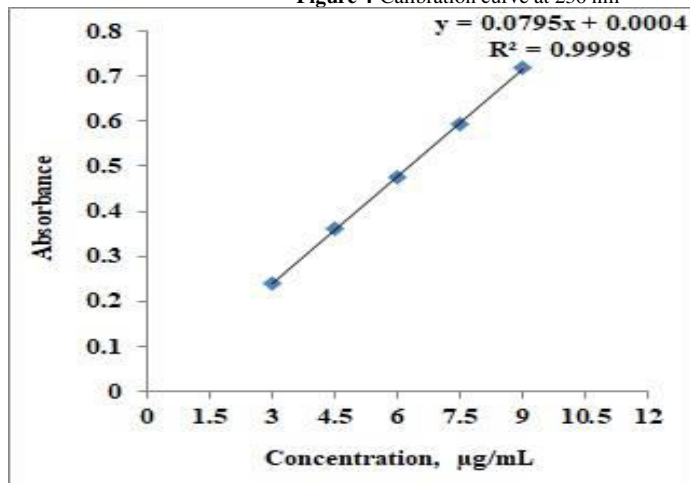
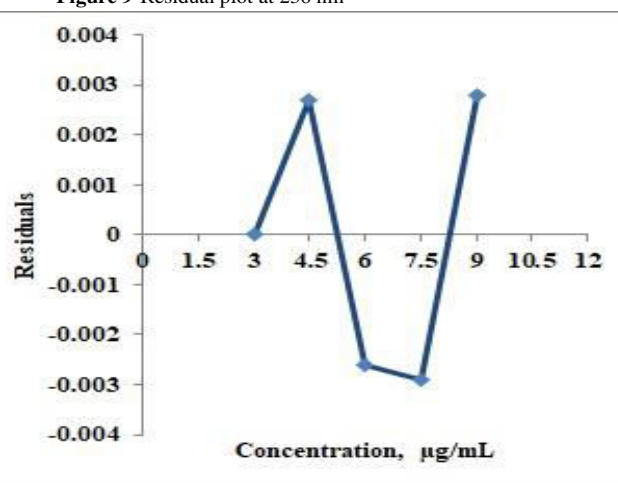
Figure 3- UV Spectrum of Cilnidipine showing linearity at 240 nm**Figure 4-**Calibration curve at 236 nm**Figure 9-**Residual plot at 236 nm

Figure 5: Calibration curve at 238 nm

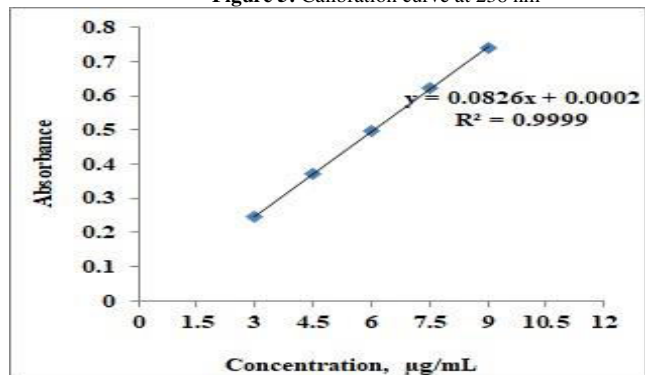


Figure 10: Residual plot at 238 nm

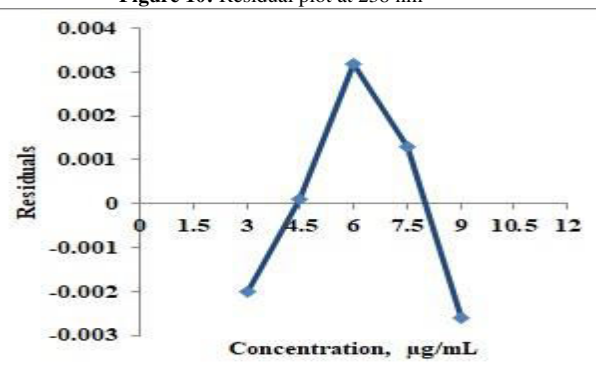


Figure 6: Calibration curve at 240 nm

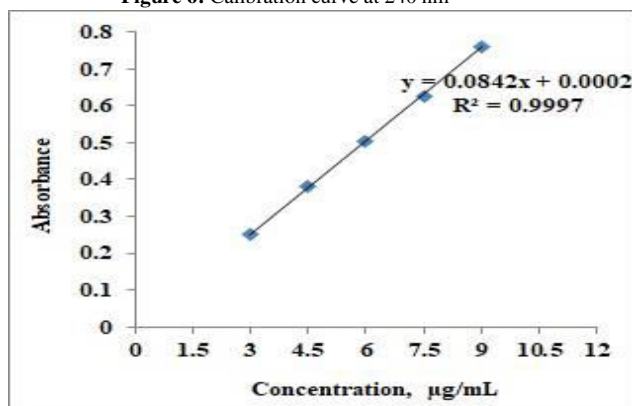


Figure 11: Residual plot at 240 nm

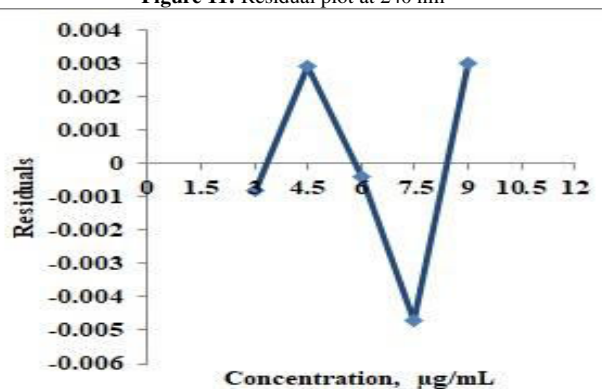


Figure 7-calibration curve at 242 nm

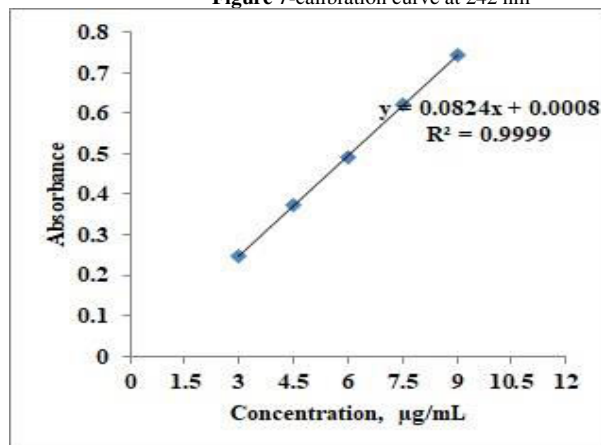


Figure 12-Residual plot at 242 nm

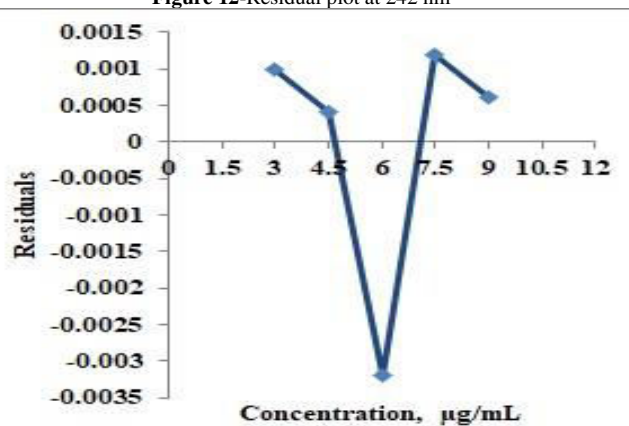


Figure 8: Calibration curve at 244 nm

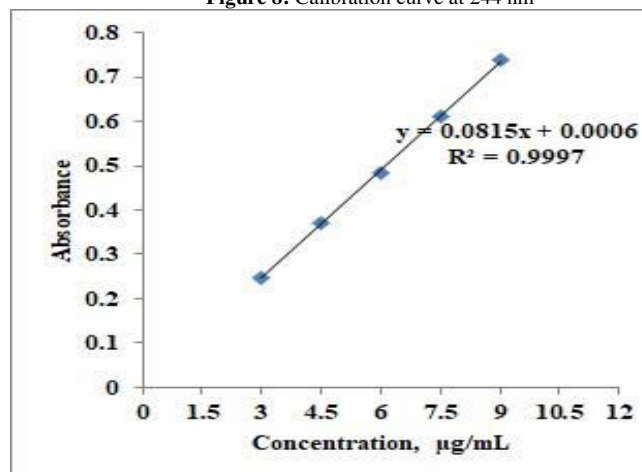


Figure 13: Residual plot at 244 nm

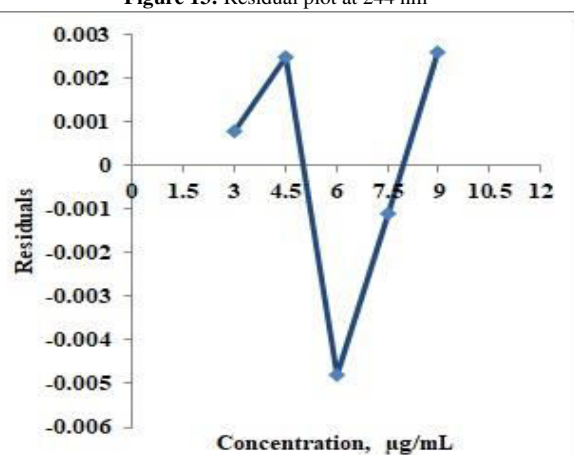


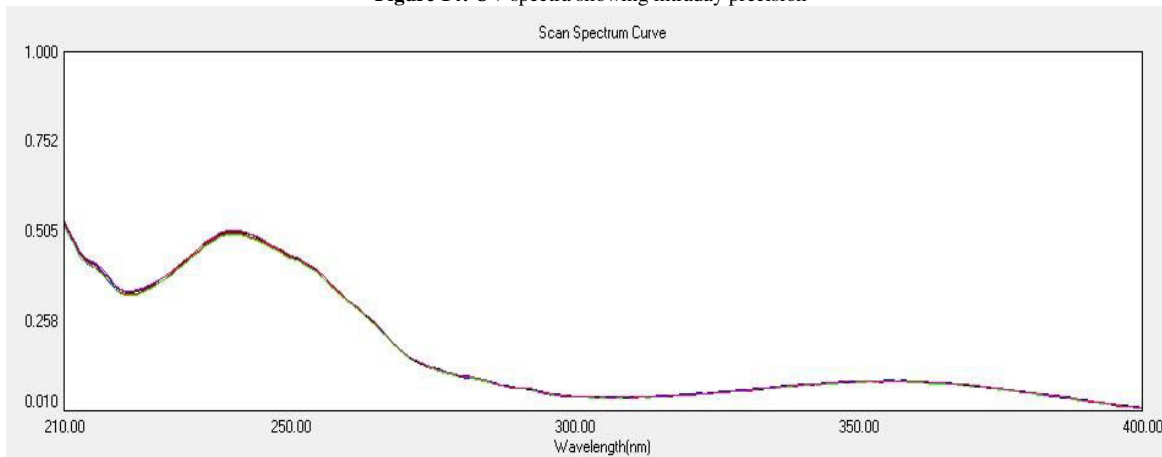
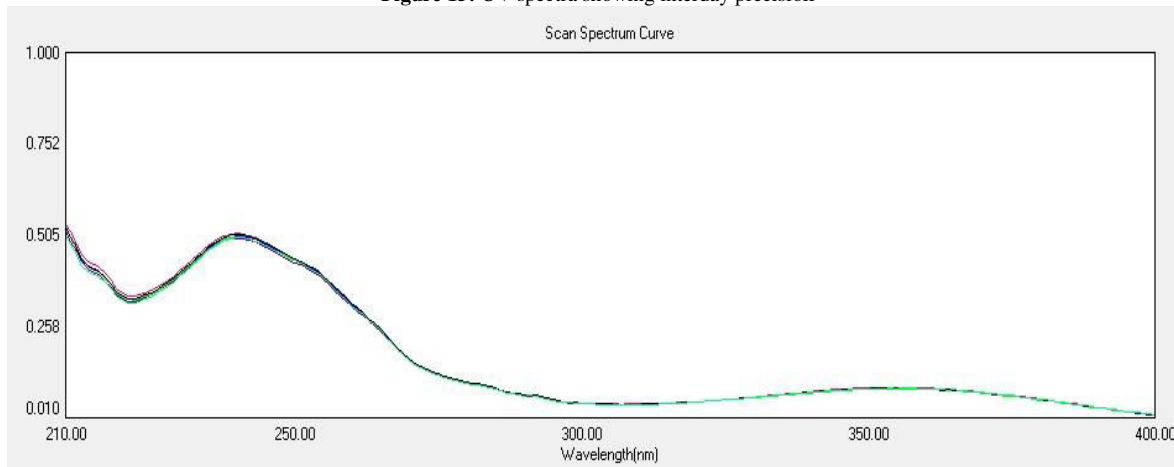
Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	Regression equation	R ²	LOD (µg/mL)	LOQ (µg/mL)	% RSD
236	$y = 0.0795x + 0.0004$	0.9998	0.1319	0.3997	0.6654
238	$y = 0.0926x + 0.0002$	0.9999	0.1098	0.3330	0.5548
240	$y = 0.0842x + 0.0002$	0.9997	0.1436	0.4352	0.7251
242	$y = 0.0824x + 0.0008$	0.9999	0.0840	0.2545	0.4235
244	$y = 0.0815x + 0.0006$	0.9997	0.1439	0.4361	0.7256

* nm = Nanometer; µg/mL = Microgram per millilitre

Precision

The low values of standard deviation indicate that this technique is specific and % RSD for the intra-day and inter-day precision were found to be 0.4558 and 0.6099 respectively. It lies within the limits of less than 2% at each wavelength. The low percentage value of relative standard deviation reveal that the suggested technique is accurate and precise (Figure 14, 15).

Figure 14: UV spectra showing intraday precision**Figure 15:** UV spectra showing interday precision

Recovery

As per ICH guidelines, the % recovery of Cilnidipine was found to be from the range of 99.67% - 101.67% w/w. The recovery

was between the acceptable range of 97 - 103 % w/w (Figure 16, Table 3).

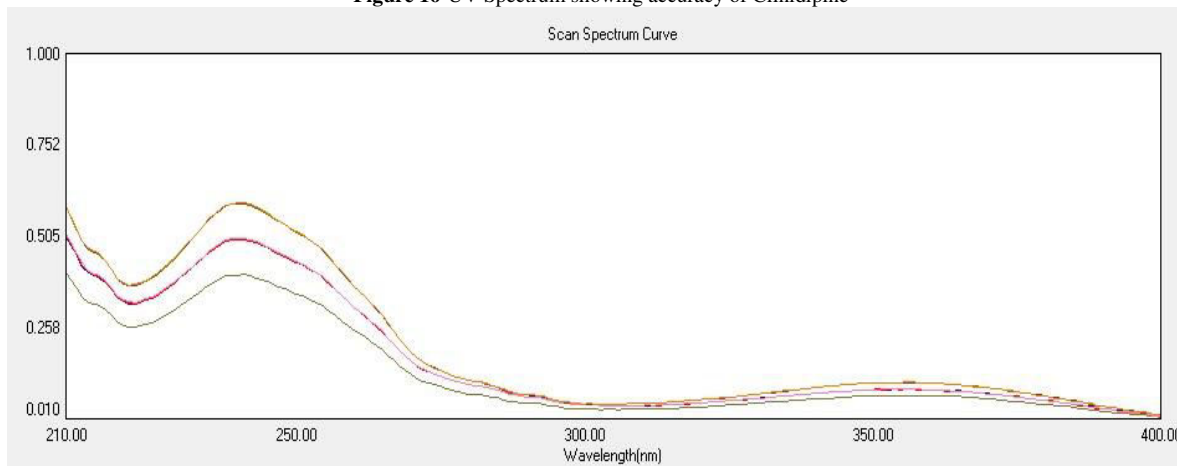
Figure 16-UV Spectrum showing accuracy of Cilnidipine

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
236	3	1.8	0.385	4.79	99.79
		3	0.486	6.01	100.17
		4.2	0.564	7.19	99.86
238	3	1.8	0.396	4.79	99.79
		3	0.499	5.99	99.83
		4.2	0.587	7.18	99.72
240	3	1.8	0.401	4.81	100.21
		3	0.509	6.03	100.50
		4.2	0.592	7.21	100.14
242	3	1.8	0.397	4.79	99.79
		3	0.501	5.98	99.67
		4.2	0.588	7.19	99.86
244	3	1.8	0.386	4.8	100.00
		3	0.493	6.1	101.67
		4.2	0.574	7.22	100.28

Assay

The UV absorbance of the tablet formulation was recorded at 240 nm. The quantity and assay percentage are found to be 9.96 mg and 99.63 % w/w respectively with % RSD values as in Table 4.

Table 4: Assay of Cilnidipine

Label claim (mg)	Amount obtained (mg)	% Assay
10	9.99	99.90
10	10.01	100.10
10	9.89	98.90
Average	9.96	99.63
SD		0.6429
% RSD		0.6453

CONCLUSIONS

This novel multivariate technique is evidently more accurate, precise, reproducible, cost effective and more sensitive than classical UV-Visible Spectrophotometry for Cilnidipine assay. This multilinear regression analysis is proven to be desirable for testing standard drug as well as other dosage forms of Cilnidipine. This method is validated using ICH Quality Guidelines and found to be within the set limits of validation. This is a simple working procedure in comparison to expensive and intricate techniques such as HPLC and HPTLC, and hence can be employed for routine analysis of Cilnidipine formulations in bulk drug and pharmaceuticals.

List of symbols/abbreviations

nm = Nanometer

µg/mL = Microgram per millilitre

g/mol = Gram per Mole

ICH = International Conference on Harmonization

UV = Ultraviolet

HPLC = High Performance Liquid Chromatography

HPTLC = High Performance Thin Layer Chromatography

CONFLICTS OF INTEREST

The authors report no conflict of interest in this study.

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