International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)
A Bridge Between Laboratory and Reader

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MULTIVARIATE ANALYSIS FOR SPECTROPHOTOMETRIC ESTIMATION OF IMEGLIMIN HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The primary aim of this study is to develop and verify a simple, reliable, precise, and convenient UV-visible spectroscopic method for determining the quantity of Imeglimin hydrochloride, following the recommendations outlined in ICH Q2 (R1). The multivariate calibration uses linear regression analysis to establish the association between absorbance and concentration at five specific wavelengths that are evenly distributed. Using water as the solvent, Imeglimin hydrochloride displayed λ_{max} at 240 nm. A linear plot was obtained with a regression coefficient of 0.999 for the concentrations between 5-15 µg mL^{-1}. The % RSD for intra-day and inter-day precision were found to be 0.197 and 0.151, respectively. The assay was determined and found to be 100.11 % w/w.

Keywords: Imeglimin hydrochloride, UV-visible spectrophotometry, Multivariate calibration, ICH guidelines

INTRODUCTION

Imeglimin Hydrochloride, chemically known as (4R)-6-N,6-N,4-trimethyl-1,4-dihydro-1,3,5-triazine-2,6-diamine;hydrochloride. The molecular formula and molecular weight were found to be $C_6H_{14}ClN_5$ and 191.66 g mol^{-1} respectively [1]. Imeglimin Hydrochloride (Figure 1) is an oxidative phosphorylation

inhibitor acts as Anti-diabetic medication. They increase muscle glucose absorption, inhibits hepatic glucogenogenesis, and restore insulin secretion to normal. They enhance insulin action and reverse pancreatic β-cell dysfunction hence acts potent antihyperglycemic effects [2].

Literature surveys demonstrate various techniques for estimating Imeglimin hydrochloride, like UV-Visible Spectroscopy (UV) [2, 3], High Performance Liquid Chromatography (HPLC) [4, 5], Mass Spectroscopy [6].
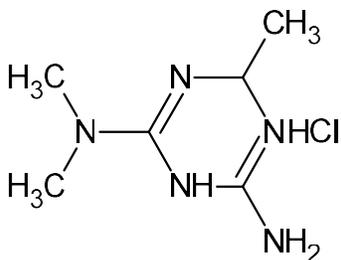
Figure 1: Chemical structure of Imeglimin hydrochloride

The multivariate technique provides clear accuracy in results, so the results have shown increased accurateness and precision than a conventional UV methods. This method simplifies and converts the result to "m" value as a reliant variable. The absorbance of an analyte (X), i.e., Imeglimin hydrochloride, is scanned at 5 diverse wavelengths surrounding its absorbance maxima (λ = 234, 237, 240, 243, 246 nm). The following formula can be expressed by

$$"A_{\lambda 234} = a \, X \, C_x + \; k_1 \text{ -------------------------- (1)}$$

$$A_{\lambda 237} = b \, X \, C_x + \; k_2 \text{ -------------------------- (2)}$$

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

$$A_{\lambda 243} = d \, X \, C_x + \; k_4 \text{------------------------- (4)}$$

$$A_{\lambda 246} = e \, X \, C_x + \; k_5 \text{------------------------- (5)”}$$

Equation system from (1-5) represent the analyte's absorbance at specific wavelengths i.e., 234, 237, 240, 243, 246 nm, linear regression slopes at (a, b, c, d, e), intercepts at (k_1, k_2, k_3, k_4, k_5), and concentration (C_x) respectively. A_T and K_T are the combination of absorbance from the regression equations at five selected wavelengths [7-14].

$$A_T = a \, X \, C_x + \; b \, X \, C_x + \; c \, X \, C_x + \; d \, X \, C_x + \; e \, X \, C_x + \; K_T \text{----------- (6)}$$

The above equation can be further condensed to

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

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

MATERIALS AND METHODS

Materials and reagents required

- Distilled water
- Imeglimin was exgratis by Nuray Chemicals Pvt. Ltd, Thiruvallur.
- The dosage form Lupimeg 500 mg tablets manufactured by LUPIN LTD, acquired from a local medical shop.

Instrumentation

- UV-Visible double beam spectrophotometer [LAB INDIA 3092]
- Analytical balance
- Micropipette

Analytical method development

Solvent selection

Imeglimin Hydrochloride shows high solubility in distilled water. Further dilutions of the standard and sample solutions were made by using water as a solvent.

Standard stock solution

Solubilizing 500 milligrams of the active pharmaceutical ingredient in distilled water to obtain 5000 μg mL^{-1} serves as the standard stock solution of Imeglimin hydrochloride. This standard stock solution was used to make an aliquot of solutions with concentrations ranging from 5-15 μg mL^{-1}.

Determination of Absorption maxima

From the standard stock solution, 10 μg mL^{-1} was prepared and scanned in UV spectrophotometry in the region from 200 to 400 nm, to identify the maximum absorbance. The λ_{max} was at 240 nm and is presented in Figure 2. The linearity was acquired in concentration limits of 5-15 μg mL^{-1}. The solutions were scanned over various wavelengths about 240 nm in order to reduce the oscillations of the instrument and improve the correlation, wavelength including 234, 237, 240, 243, 246 nm, respectively.

Preparation of sample solution

Ten Imeglimin hydrochloride tablets were precisely measured and pulverized. In order to obtain 5000 μg mL^{-1}, a weight identical to 500 mg has been transferred to a 100 ml standard flask, and it was then further dissolved, diluted, and made up to the mark with water. The resulting filtrate is used for further analysis after filtering.

Method Validation

The above method has been evaluated in accordance with ICH Q2 (R1) guidelines for precision, linearity, sensitivity, and accuracy [15].

Linearity

The standard stock solution of Imeglimin hydrochloride was used to prepare different concentrations ranging from 5-15 μg mL^{-1}. To eliminate instrumental errors and enhance correlation prepared solutions were scanned over the wavelength range around

their respective maximum absorbance at 234, 237, 240, 243, 246 nm. The absorbances were recorded, and a concentration versus absorbance graph was used to obtain the results. The limit of detection and quantification were calculated using the formula below to determine the method's sensitivity **(Figure 3, Table 1)**.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg mL^{-1})	Absorbance				
	234 nm	237 nm	240 nm	243 nm	246 nm
5	0.466	0.481	0.495	0.461	0.412
7.5	0.632	0.657	0.664	0.637	0.554
10	0.794	0.819	0.839	0.794	0.697
12.5	0.970	1.027	1.034	0.970	0.859
15	1.139	1.198	1.225	1.129	0.990

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

$$LOD = 3.3\ \sigma/S \ \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \ (8)$$

$$LOQ = 10\ \sigma\ /S \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \ (9)$$

Where, S stands for standard curve slope, and σ for the standard deviation (SD) of the lowest concentration.

Precision

10 µg mL^{-1} solution was scanned in the UV region from 200 to 400 nm six times in a short period of time on one day for intraday and six different days for interday to evaluate the precision studies.

Accuracy

Assessing the recovery study at 80%, 100%, and 120% was done using the conventional addition technique. To three 10 ml standard flasks, 0.5 ml of the reference solution was pipetted. Sample solutions of 0.3, 0.5, and 0.7 ml were added to the same flasks and made up to the mark. After UV scanning these solutions, the recovery percentage was computed.

Assay

By measuring the extracted tablet solution's absorbance at 240 nm, the amount of Imeglimin hydrochloride that is present in the tablet dosage form has been determined.

RESULTS AND DISCUSSION

Using distilled water as a solvent, the λ_{max} of Imeglimin Hydrochloride was found to be 240 nm **(Figure 2)**.

This approach is linear within the applied concentration range from 5 to 15 µg mL^{-1}. This linear regression analysis demonstrates a strong linear relationship with $R^2 = 0.999$-0.9998. The % RSD values were 0.197 and 0.151 for intra-day and inter-day precision. The LOD and LOQ values were 0.4679 and 1.4179 g mL^{-1}, respectively. Hence, the obtained values were within the ICH validation parameter limits.

Linearity

Linearity at 234, 237, 240, 243, 246 nm was recorded with concentration range from 5-15 µg mL^{-1} **(Figure 3)**, with low relative standard deviation values demonstrates the method accuracy and precision. LOD and

LOQ were calculated. The calibration plots were shown in **Figures 4 to 8** and data is presented in **Table 2**.

Precision

The technique's specificity is demonstrated by the low standard deviation (SD) readings; the percentage RSD for intra-day and inter-day precision was determined to be 0.197 and 0.151, respectively. At each wavelength, it varies by less than 2 %. The low percentage of relative standard deviation suggests that this approach is precise and accurate **(Figure 9, 10)**.

Recovery

The percentage recovery of Imeglimin Hydrochloride was from 98.75 % to 101.90 % w/w, as per ICH guidelines **(Figure 11, Table 3)**.

Assay

The UV absorbance of the selected dosage form was recorded at 240 nm. The amount and assay results were 500.55 mg and 100.11 % w/w, respectively with % RSD values as in **Table 4**.
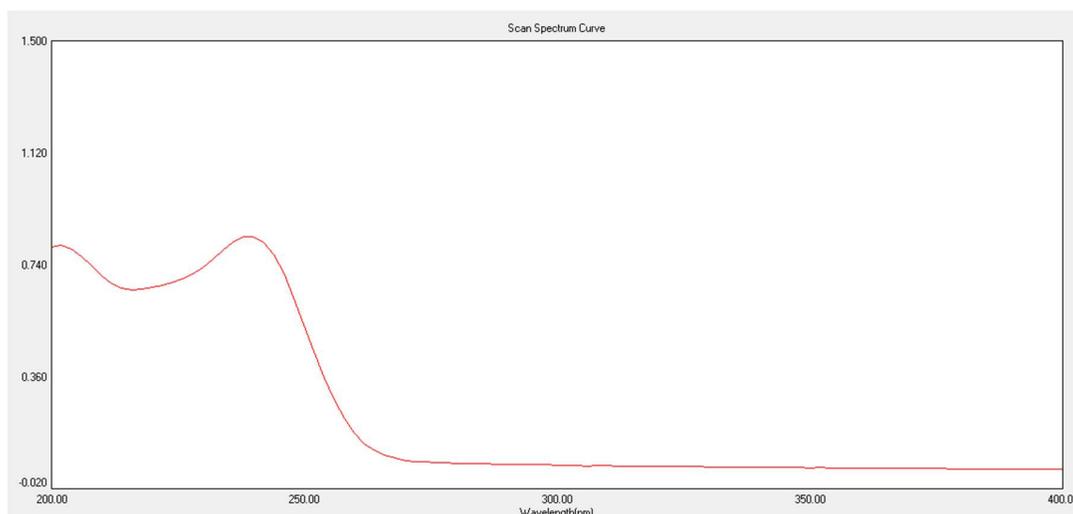

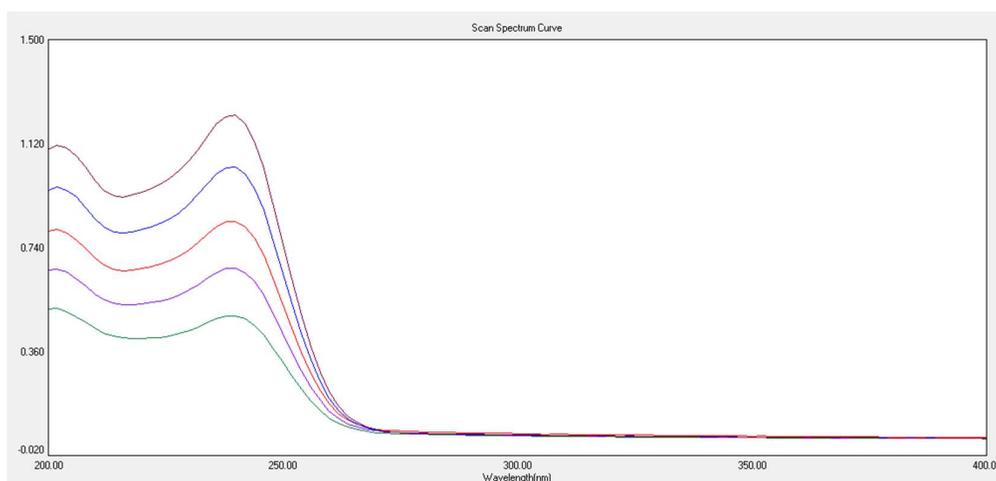**Figure 2: UV spectrum of Imeglimin Hydrochloride (10 µg mL^{-1}), λ$_{max}$ at 240 nm**

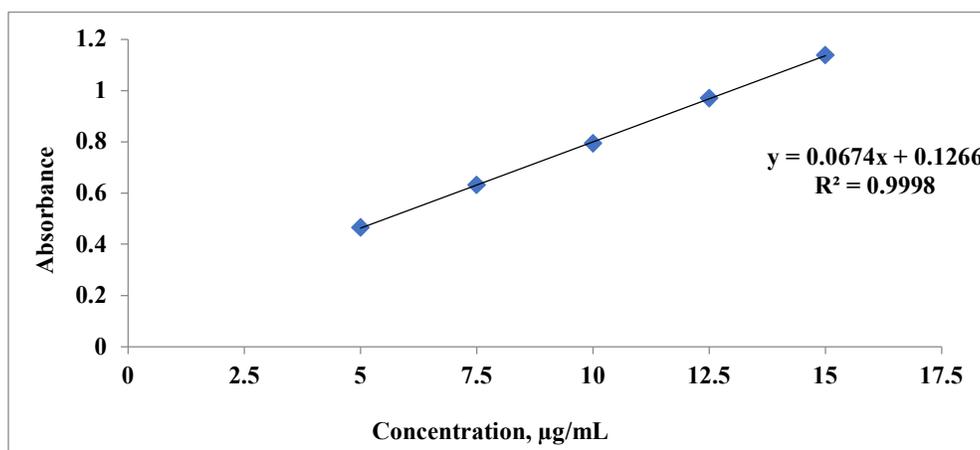**Figure 3: UV Spectrum of Imeglimin Hydrochloride showing linearity at 240 nm**
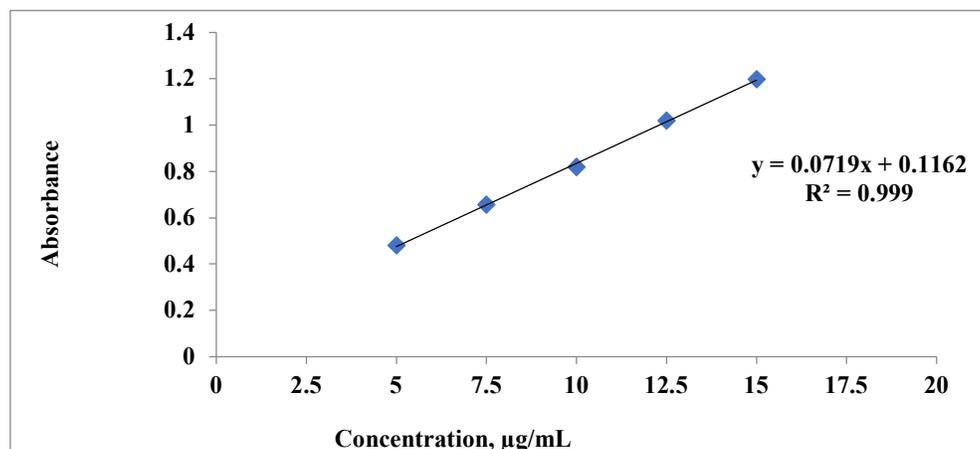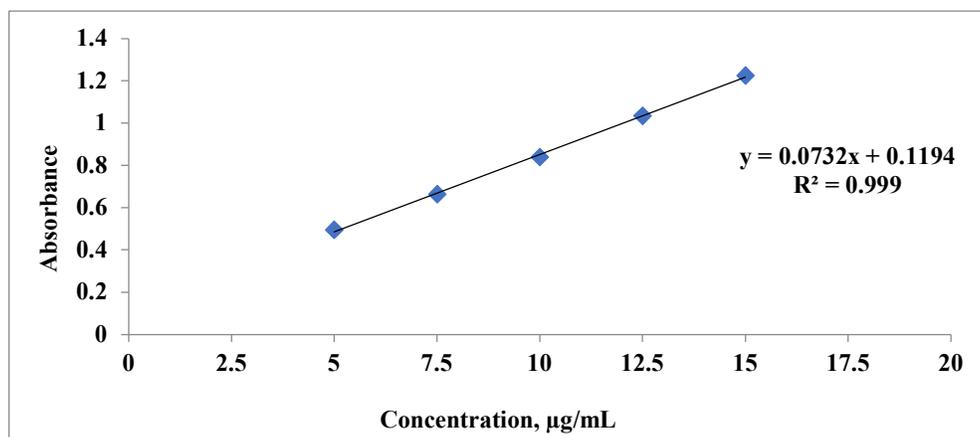
Figure 4: Calibration curve at 234 nm

The equation shown on the chart is $y = 0.0674x + 0.1266$, $R^2 = 0.9998$.

Figure 5: Calibration curve at 237 nm

The equation shown on the chart is $y = 0.0719x + 0.1162$, $R^2 = 0.999$.

Figure 6: Calibration curve at 240 nm

The equation shown on the chart is $y = 0.0732x + 0.1194$, $R^2 = 0.999$.
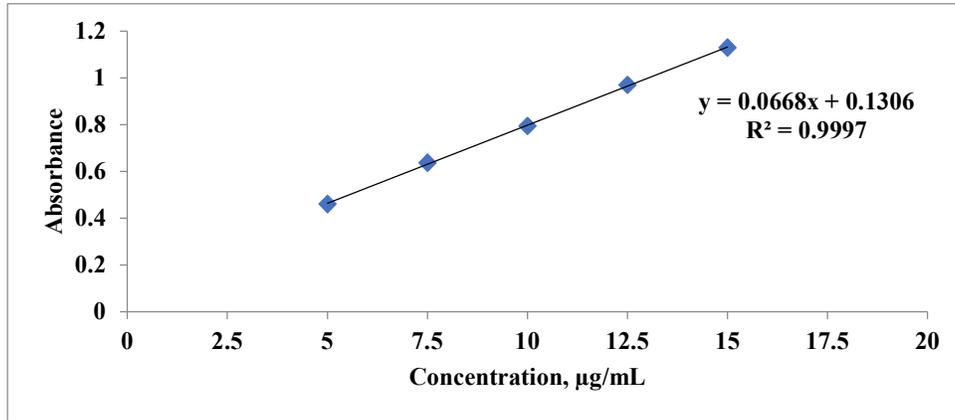
Figure 7: Calibration curve at 243 nm

$y = 0.0668x + 0.1306$
$R^2 = 0.9997$
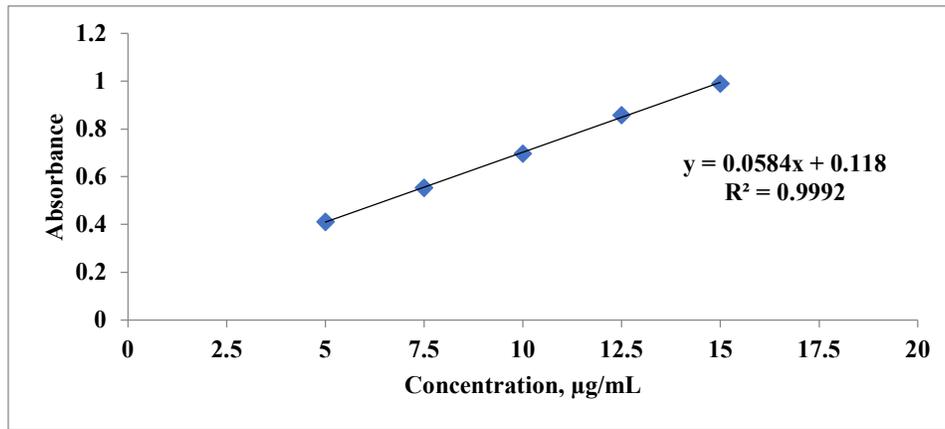
Figure 8: Calibration curve at 246 nm

$y = 0.0584x + 0.118$
$R^2 = 0.9992$

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

Wavelength (nm)	Regression equation	R^2	LOD ($\mu g\ mL^{-1}$)	LOQ ($\mu g\ mL^{-1}$)	% RSD
234	$y = 0.0674x + 0.1266$	0.9998	0.2022	0.6129	0.5162
237	$y = 0.0719x + 0.1162$	0.999	0.4807	1.4567	1.2543
240	$y = 0.0732x + 0.1194$	0.999	0.4679	1.4179	1.2191
243	$y = 0.0668x + 0.1306$	0.9997	0.2775	0.8410	0.7038
246	$y = 0.0584x + 0.118$	0.9992	0.4242	1.2855	1.0688

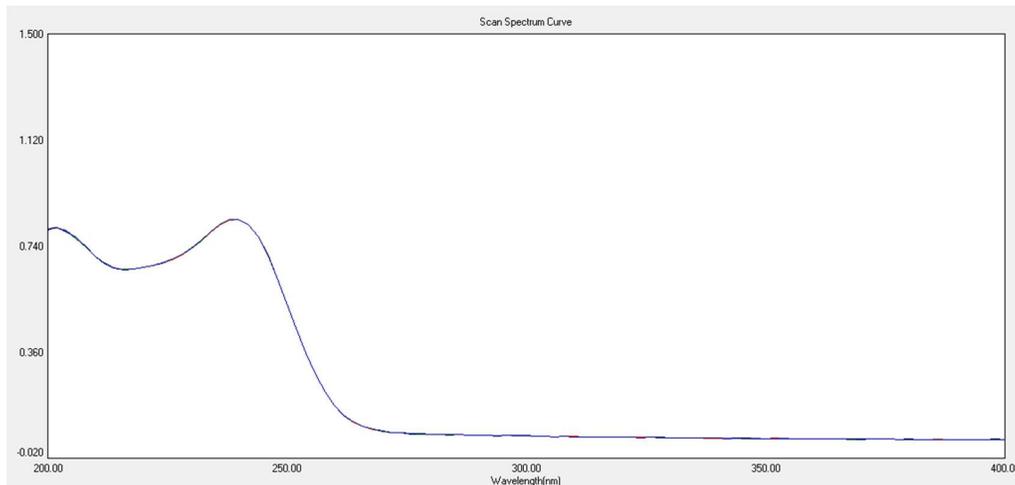*nm = nanometre; $\mu g\ mL^{-1}$ = Microgram per millilitre

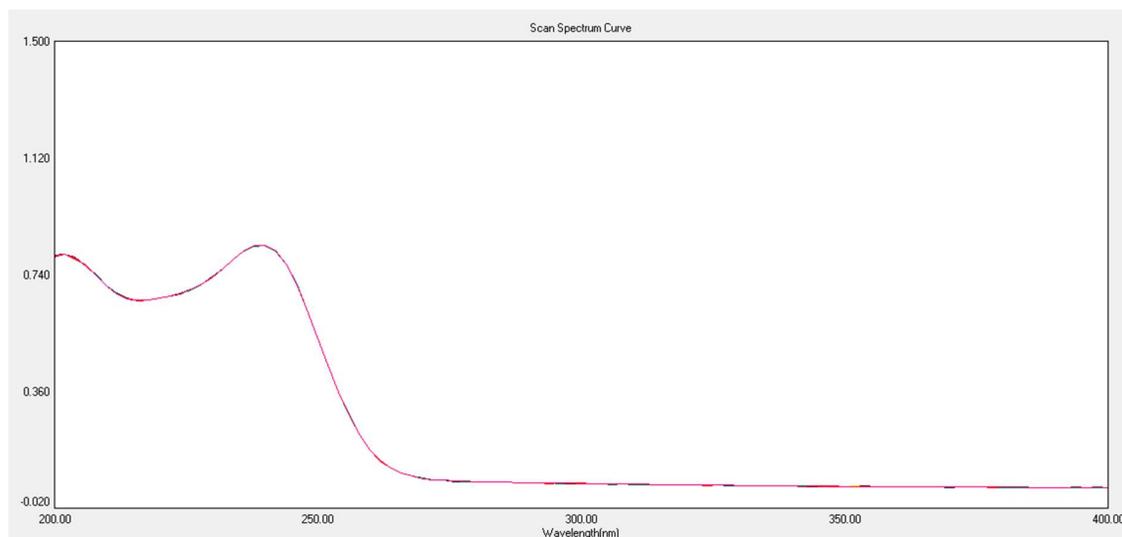**Figure 9: UV spectra showing intraday precision**

Figure 10: UV spectra showing interday precision

Table 3: Recovery Studies

Wavelength (nm)	Amount present ($\mu g\ mL^{-1}$)	Amount added ($\mu g\ mL^{-1}$)	Amount recovered ($\mu g\ mL^{-1}$)	% Recovery
234 nm	5	3	7.98	99.75
		5	10.03	100.30
		7	11.97	99.75
237 nm	5	3	8.03	100.38
		5	10.05	100.50
		7	12.03	100.25
240 nm	5	3	8.11	101.38
		5	9.97	99.70
		7	11.85	98.75
243 nm	5	3	7.95	99.38
		5	10.06	100.60
		7	12.1	100.83
246 nm	5	3	8.09	101.13
		5	10.19	101.90
		7	12.02	100.17

Table 4: Assay of Imeglimin hydrochloride

Label claim (mg)	Amount obtained (mg)	% Assay
500	496.21	99.24
500	502.15	100.43
500	503.28	100.66
Average	500.55	100.11
SD		0.7596
% RSD		0.7588

CONCLUSION

This multivariate analysis is more precise, accurate, sensitive, and economical than a conventional UV-Visible spectrophotometry method for estimating Imeglimin Hydrochloride. It has been revealed that this multilinear regression analysis is useful for testing both the conventional medication and different dosage forms of Imeglimin hydrochloride. This method is validated in accordance with ICH requirements, and the values fall within the validation bounds. This is a basic working process that can be utilised for

routine analysis of Imeglimin hydrochloride in bulk and pharmaceuticals, especially when compared to expensive and advanced techniques like HPLC and HPTLC.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects.

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