IJBPAS, October, 2025, 14(10): 5478-5492

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ECO-FRIENDLY FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR THE QUANTIFICATION OF IMEGLIMIN HYDROCHLORIDE EMPLOYING MULTIVARIATE CALIBRATION TECHNIQUE

ILAKIYA M, SANGAVI G, AKSHAYA DEVI R, PREMALATHA T K AND KOKILAMBIGAI K. S*

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Chengalpattu District, Tamil Nadu, India

***Corresponding Author: Dr. Kokilambigai K S: E Mail: kokilams@srmist.edu.in; kokilampharm@gmail.com**

Received 28th July 2024; Revised 10th Sept. 2024; Accepted 3rd Oct. 2024; Available online 1st Oct. 2025

https://doi.org/10.31032/IJBPAS/2025/14.10.9478

ABSTRACT

The objective of this research is to develop a multivariate calibration technique-based UV spectrophotometric method in first derivative method for Imeglimin hydrochloride in bulk and in its pharmaceutical form. The multivariate calibration technique associates the relationship between concentration and amplitude at 5 different wavelengths, hence utilizing the linear regression equations. As a result of this, the instrumental variations are minimized and the correlation is enhanced. When distilled water used as the solvent, Imeglimin hydrochloride indicated a maximum amplitude at 232 nm and linearity between 5 and 15 µg/mL. The developed approach was found to be simple, linear, accurate, and precise, and it was approved in compliance with ICH requirements.

Keywords: Imeglimin Hydrochloride, First order derivative, Multivariate calibration method, Pharmaceutical form, ICH guidelines, Validation

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]**.

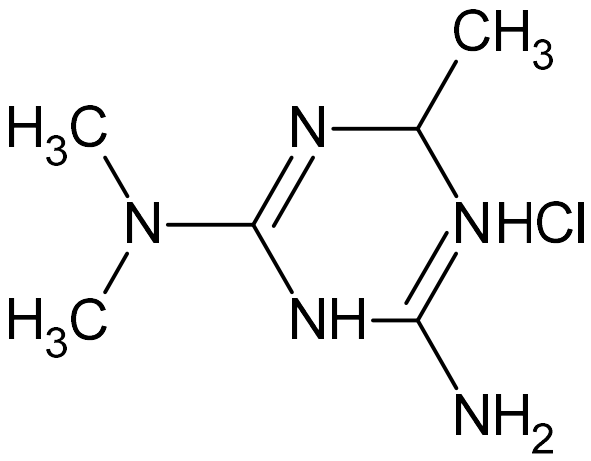

Figure 1: Structure of Imeglimin hydrochloride

Spectrophotometric methods are used as the most effective approach in laboratories because of its intrinsic simplicity, rapid procedure, and cost effective. This method depends on evaluation of Imeglimin Hydrochloride with a high level of precision and accuracy in first order derivative mode. This is an easy method with low cost and can be applied to large amount of drug formulation. This paper demonstrates the evaluation of Imeglimin Hydrochloride in pharmaceutical dosage form having a simple mathematical content with a UV spectral multivariate calibration analysis technique.

Multivariate analysis means the method of converting one dependent variable from usual single species examination to 'm' dependent variables. e.g. wavelengths, are the calibration mode which can included simultaneously **[7-14]**.

The resolving power, sensitivity, rapidity and affordable for the quantitative analysis, quality control and regular analysis of the investing compounds are applied in statistical method under optimized conditions.

If the amplitude of an analyte (X), here Imeglimin Hydrochloride is measured at 5 wavelengths set (λ = 226, 229, 232, 235 and 238 nm), then for each selected wavelength we can write the following equation.

$A_{\lambda 226} = f\ X\ Cx + k_1$ ..... (1)

$A_{\lambda 229} = g\ X\ C_x + k_2$ ..... (2)

$A_{\lambda 232} = h\ X\ C_x + k_3$ ..... (3)

$A_{\lambda 235} = i\ X\ Cx + k_4$ ..... (4)

$A_{\lambda 238} = j\ X\ C_x + k_5$ ..... (5)

Where A represents the amplitudes of the analyte; f, g, h, i, j are the slopes of the linear regression functions of the analyte; k_1, k_2, k_3, k_4, k_5 are the intercepts of the linear regression function at the 5 selected wavelengths and C_x represents the concentration of the analyte. The above 5 equation system (1-5) can be summarised as

$A_T = f \times C_x + g \times C_x + h \times C_x + i \times C_x + j \times C_x + K_{AT}$……. (6)

This can be further simplified to

$A_T = C_x (f+g+h+i+j) + K_{AT}$ …… (7),

Where A_T and K_{AT} represents the aggregate of the amplitude obtained and intercepts of regression equations at 5 wavelength set respectively. The concentration of analyte in the solution can be calculated by using the equation

$$C_x = \frac{A_T - K_T}{(f+g+h+i+j)} \quad \text{..... (8)}$$

GREENNESS EVALUATION TECHNIQUES

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) established a set of pictograms with related signal words, and the analytical eco scale **[15]** is predicated on assigning penalty points relying over both quantity and number. The analytical eco scale approach considers each reagent, including its kind and quantity, potential occupational exposure, energy depletion, and waste. Penalty points are eliminated from a 100 point base score.

Analytical eco-scale = 100 - total penalty points

The Green Analytical Procedure Index (GAPI) is a visual depiction made up of five pentagons with distinctive colour coding. The colour coding in the pictogram corresponds to three levels of evaluation at each stage of an analytical technique. The colour coding used by GAPI to determine greenness spans from green to yellow to red, denoting the low, medium, and high environmental impacts connected with the analytical technique, respectively. J. Potka Wasylka provided a succinct overview of GAPI in the year 2018 [16]. The third assessment methodology makes use of AGREE metrics **[17]** special software for assessing the greenness profile. The result of the software is a circle with numbers around the edges that range from 1 to 12 and are oriented clockwise. These figures represent the 12 green analytical chemistry philosophies. Based on the inputs and their weight, the outputs of each of these 12 principles are rated from 0 to 1. This aggregate scale uses the colours red, yellow, and green

to show different numbers. Red means zero, dark green means one or close to one, and yellow means a number between red and dark green. A score that represents the level of greenness is produced by adding the 12 principles and the core.

MATERIALS AND METHODS

Reagents

- 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.

Solubility

- Freely soluble in Distilled water

Instrumentation

- UV- Visible double beam spectrophotometer (Perkin-Elmer Lambda25)
- Electronic weighing balance

Method development

Solubility studies

Imeglimin Hydrochloride was soluble freely in distilled water. Hence distilled water was used as the diluent throughout the study.

Preparation of reference 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 Amplitude maxima

The stock solution of Imeglimin Hydrochloride was diluted with the diluent to obtain a concentration of 10 µg/mL solution. The first order derivative spectra were recorded between 200-400 nm and the amplitude maximum was found to be 232 nm (Figure 2). In order to improve the correlation and to minimize the instrumental fluctuations, amplitude of these solutions were estimated over a range encompassing the amplitude maximum 232 nm i.e., 226, 229, 232, 235, 238 nm respectively.

Preparation of sample solution

About ten tablets of Imeglimin Hydrochloride was weighed and transferred to a mortar, powdered and mixed well. From the tablet powder, a quantity equivalent to 500 mg of Imeglimin Hydrochloride was weighed accurately and dissolved in 100 mL distilled water, filtered and diluted for further analysis.

Method validation

The proposed method was authenticated as per ICH guidelines for linearity, accuracy, precision **[18]**.

Linearity

The Imeglimin Hydrochloride stock solution was mixed with the diluent to achieve concentrations ranging from 5-15 µg/mL. In

order to enhance correlation and reduce instrumental fluctuations, the amplitudes of these solutions were measured around wavelength of 232 nm. i.e., 226, 229, 232, 235, 238 nm in the derivative mode.

The overlay spectra for the linearity in first derivative mode were shown in **Figure 3**. The amplitude of the corresponding concentrations was recorded and represented in **Table 1**. The regression analysis for the respective wavelengths is presented in **Table 2**. **Figures 4-8** displayed the calibration graphs, whereas **Figures 9-13** showed the corresponding residual plots at the five individual wavelengths.

Table 1: UV calibration data at five selected wavelengths for First derivative mode

Concentration (µg/mL)	Amplitude				
	226 nm	229 nm	232 nm	235 nm	238 nm
5	3.7	6	6.8	5.1	0.9
7.5	6	9.1	10.4	7.9	2.9
10	8.2	11.9	13.8	10.9	4.7
12.5	10.6	15.14	17.9	14.5	6.5
15	12.9	18.3	21.6	17.2	8.5

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

Precision

A 10 µg mL^{-1} solution was subjected to UV scanning in the range of 200 to 400 nm. This process was repeated six times within a short time frame for intraday analysis, and on six different days for interday analysis, in order to assess precision. The amplitude values recorded at the selected wavelength sets are displayed in **Figures 14 and 15** for intraday precision and interday precision, respectively.

Accuracy (Recovery studies)

The accuracy of the established method was assessed using standard addition technique at 80 %, 100 %, and 120 %. 0.5 mL of the standard solution and 0.3 mL, 0.5 mL, and 0.7 mL of the sample solution were added to separate standard flasks. These solutions were then diluted with solvent to reach the desired volume. The aliquots after scanning in the first derivative mode, the % recovery were calculated. The accuracy studies results are shown in **Figure 16** and provided in **Table 3**.

Assay

The sample solution's amplitude was measured at a wavelength of 232 nm. An estimation was made of the quantity of drug contained in the formulation and is given in **Table 4**.

RESULTS AND DISCUSSION

The maximum amplitude was recorded at 232 nm using distilled water as the diluent **(Figure 2)**.

Linearity

The linear nature of the collected data was assessed for values ranging from 5 to 15 µg mL^{-1} at 226, 229, 232, 235, 238 nm as shown in Figure 3. The results presented in **Table 1**. All calibration curves within the prescribed concentration range exhibited linearity. The **Figures 4-13** display the calibration graphs and accompanying residual plots, while **Table 2** presents the results of the regression analysis. The linear analytical data of the produced calibration plots demonstrates a strong linear relationship, as indicated by a correlation coefficient of around 0.9973.

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 0.1555 and 0.1549, respectively. At each wavelength, it varies by less than 2 %. The low percentage of relative standard deviation suggests that this approach is precise and perfect **(Figure 14-15)**.

Recovery

The percentage of the medication that was recovered from the synthetic mixture was found to be between 98.60 - 101.50 %w/w, which was well within the acceptable range of 97 - 103 % w/w as per the ICH guidelines and the results of the accuracy studies were shown in **Figure 16 and Table 3**.

Assay

The amplitude of the tablet solution was measured at a wavelength of 232 nm. The percentage of Imeglimin Hydrochloride (Lupimeg tablets) was determined to be 100.02% w/w. The measured quantity of the medication in the commercially available product was determined to be 500.08 mg, with a relative standard deviation (RSD) value of less than 2% as indicated in **Table 4**.

Evaluation of Greenness Profile

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale is shown in **Table 5**, while the results agree metrics and GAPI is depicted in **Figure 17, 18**.

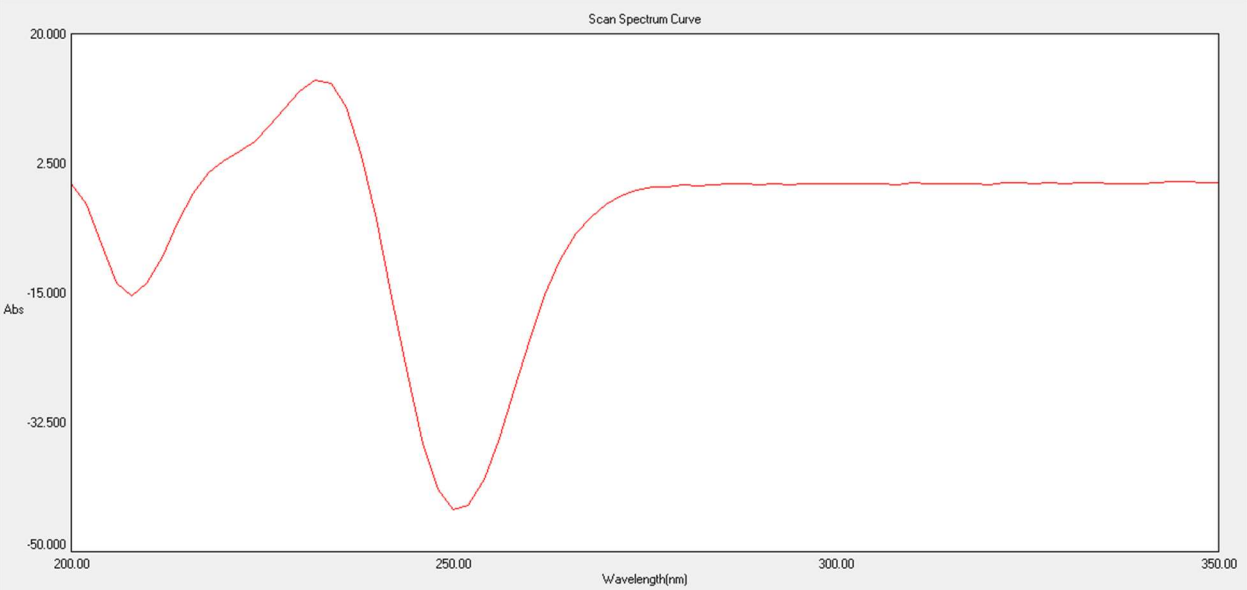

Figure 2: UV spectrum of Imeglimin Hydrochloride, amplitude maximum at 232 nm

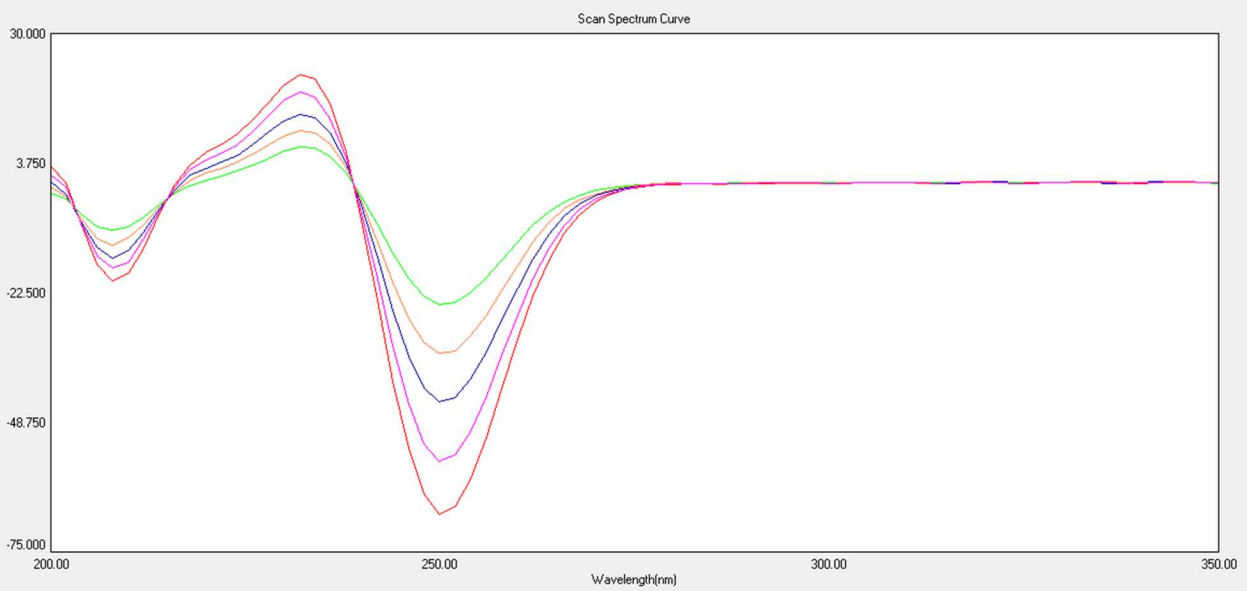

Figure 3: Overlay Spectrum of Imeglimin Hydrochloride in first derivative mode

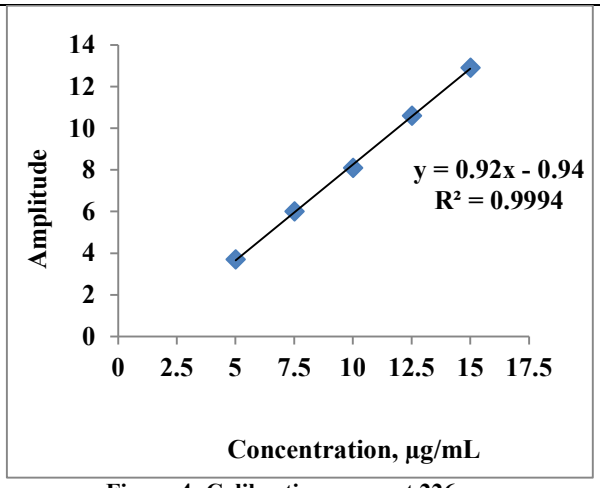

Figure 4: Calibration curve at 226 nm

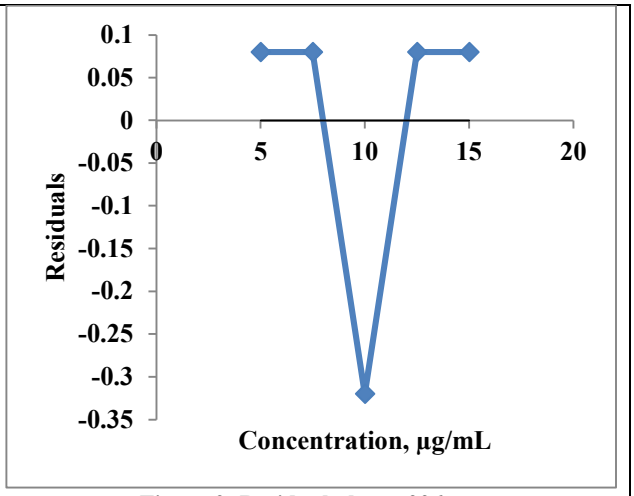

Figure 9: Residual plot at 226 nm

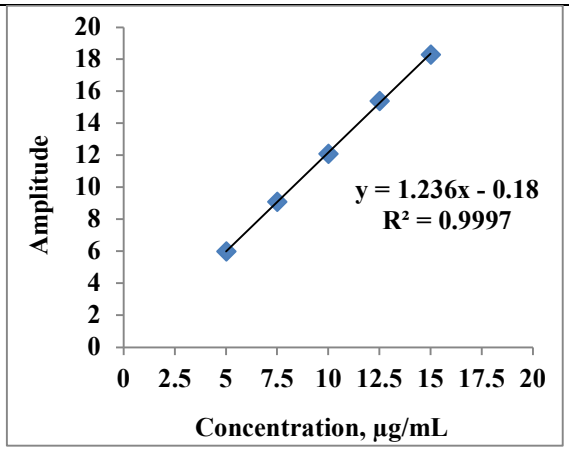

Figure 5: Calibration curve at 229 nm

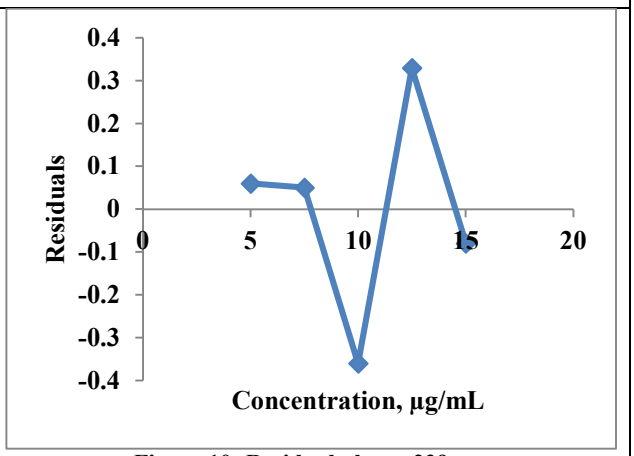

Figure 10: Residual plot at 229 nm

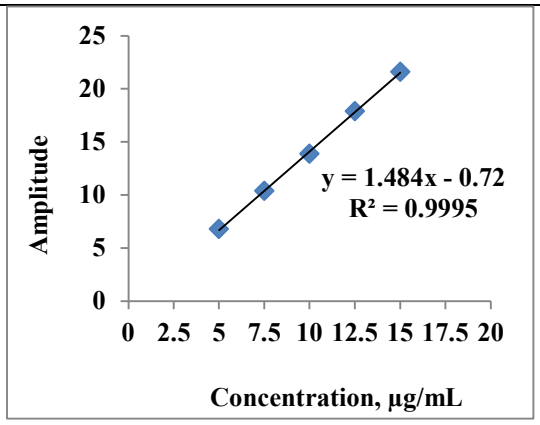

Figure 6: Calibration curve at 232 nm

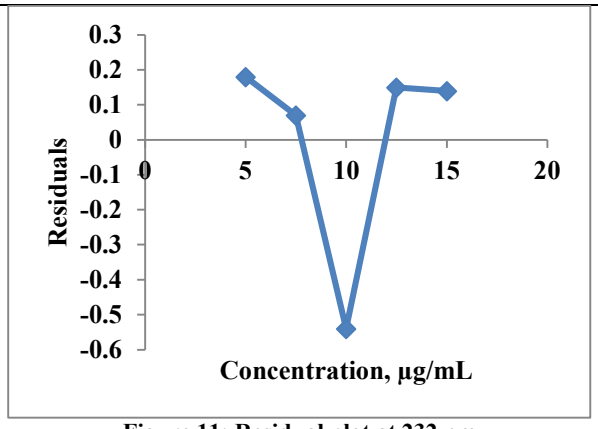

Figure 11: Residual plot at 232 nm

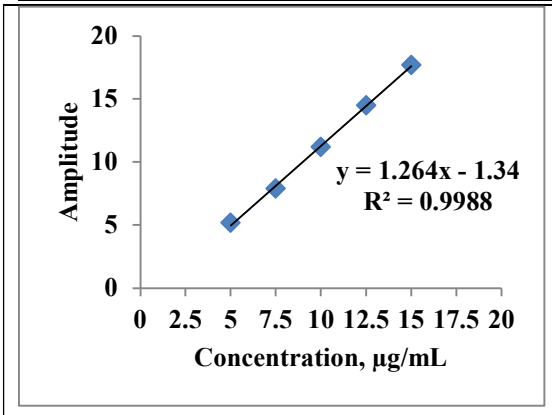

Figure 7: Calibration curve at 235 nm

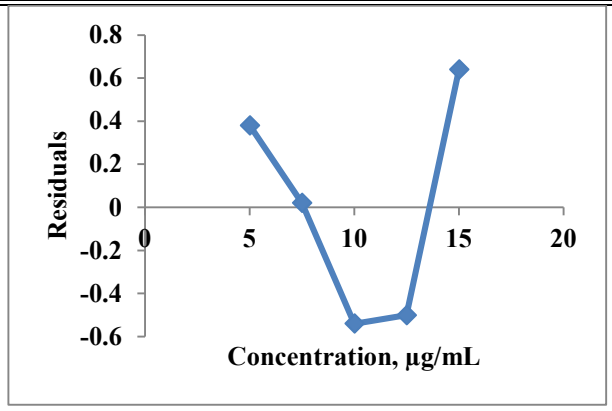

Figure 12: Residual plot at 235 nm

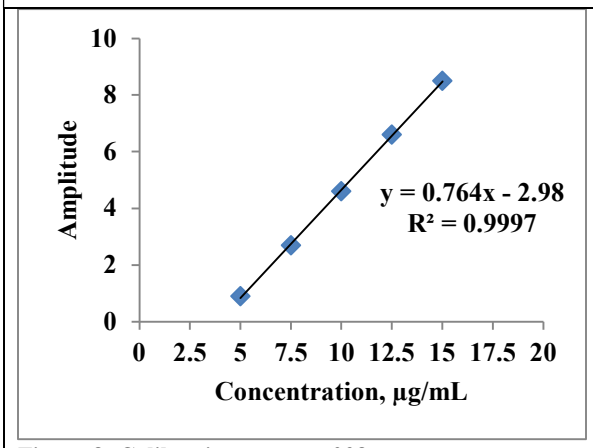

Figure 8: Calibration curve at 238 nm

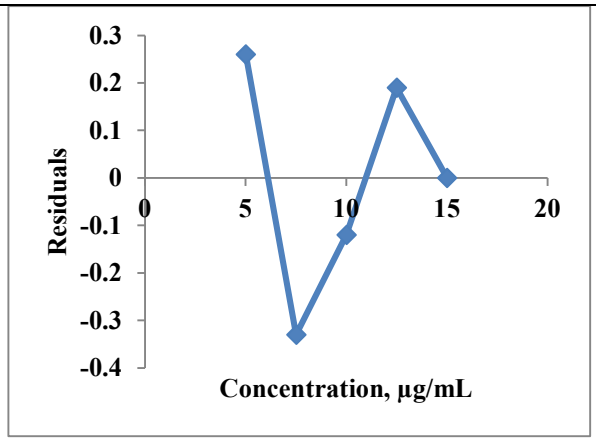

Figure 13: Residual plot at 22638 nm

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

Wave length (nm)	Regression equation	Slope	Intercept	R^2	LOD (µg/mL)	LOQ (µg/mL)
226	Y = 0.92x - 0.98	0.92	0.98	0.9994	0.3704	1.1226
229	Y=1.236x - 0.18	1.236	0.18	0.9997	0.2532	0.7675
232	Y=1.484x - 0.72	1.484	0.72	0.9995	0.3323	1.0071
235	Y=1.264x - 1.34	1.264	1.34	0.9988	0.5307	1.6084
238	Y=0.764x - 2.98	0.764	2.98	0.9997	0.2615	0.7925

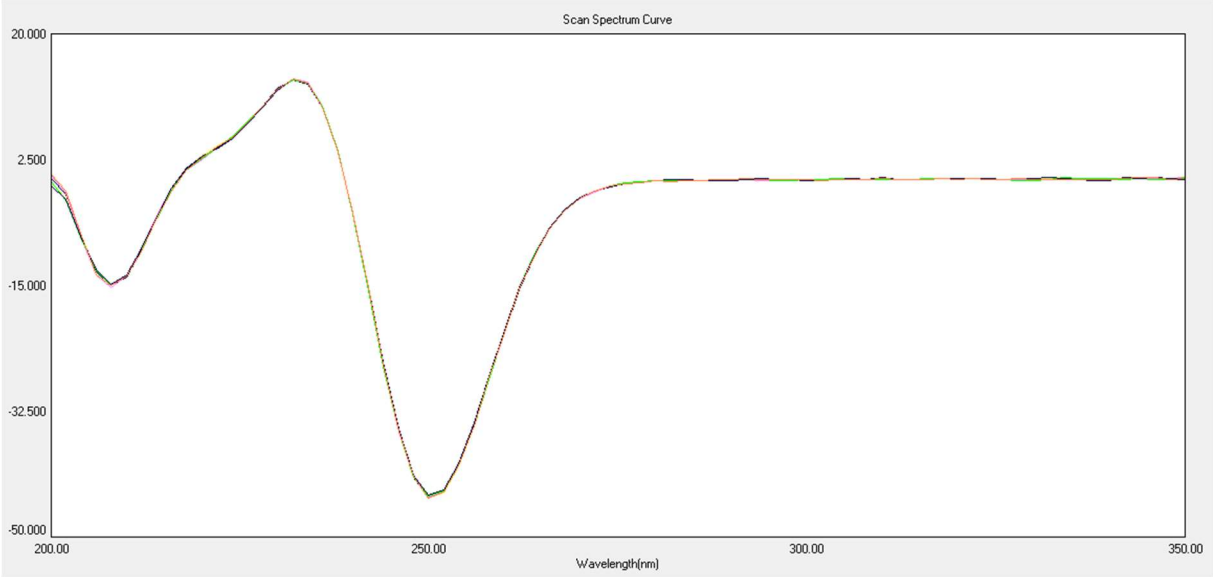

Figure 14: Overlay UV spectrum showing Intra-day precision

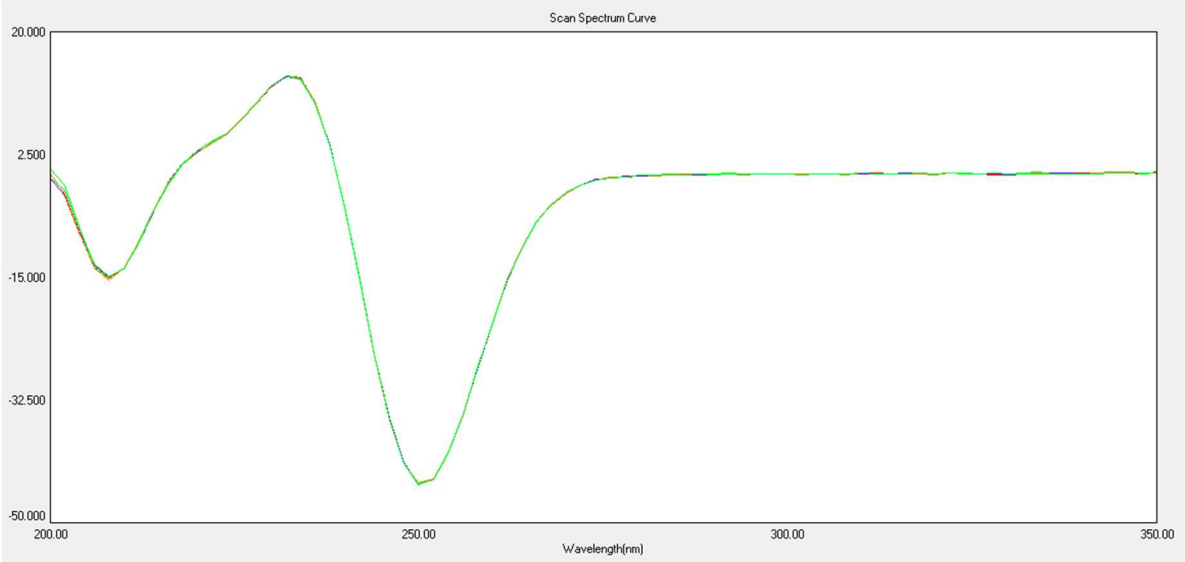

Figure 15: Overlay UV spectrum showing Inter-day precision

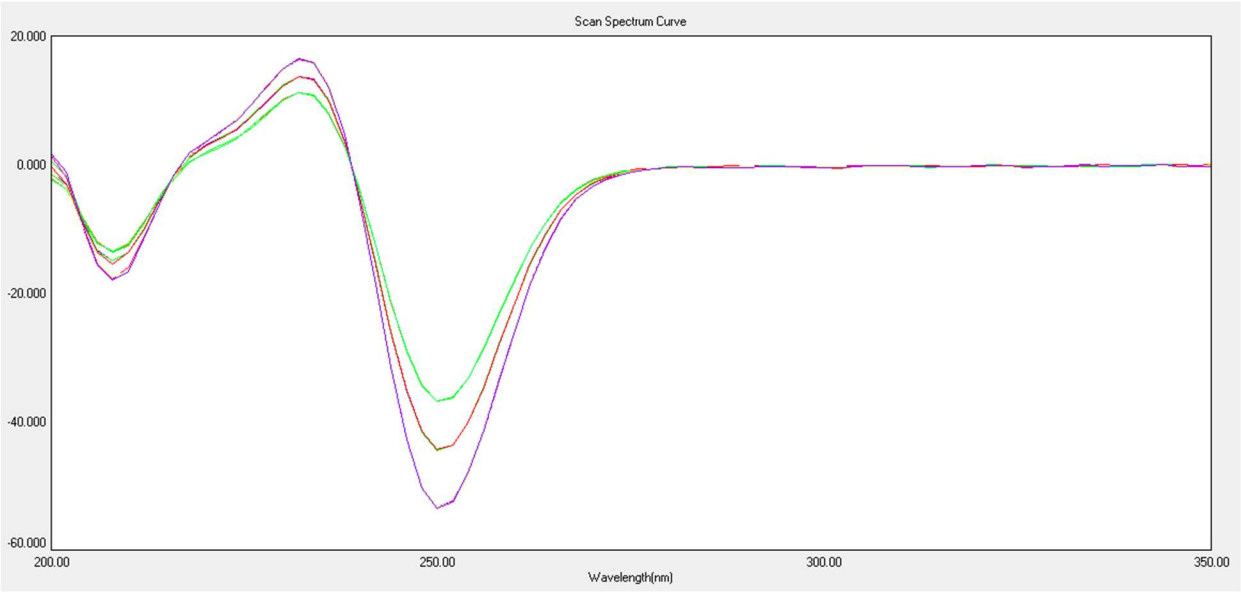

Figure 16: UV spectrum showing accuracy in first derivative mode

Table 3: Recovery studies for first derivative mode

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Amount Recovered (µg/mL)	% Recovery
226	5	3	8.12	101.50
		5	9.86	98.60
		7	11.98	99.83
229	5	3	7.89	98.63
		5	10.15	101.50
		7	12.11	100.92
232	5	3	8.01	100.13
		5	10.05	100.50
		7	11.96	99.67
235	5	3	7.99	99.88
		5	9.89	98.90
		7	12.05	100.42
238	5	3	8.02	100.25
		5	10.12	101.20
		7	11.92	99.33

Table 4: Assay of Imeglimin Hydrochloride for first derivative mode

Label claim (mg)	Amount obtained (mg)	% Assay
500	501.29	100.26
500	496.91	99.38
500	502.05	100.41
Average		100.02
SD		0.5549
% RSD		0.5548

Table 17: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total penalty Points	Score
Distilled water	0	0	100
Instrument	0		
Occupational hazard	0		
Waste	0		

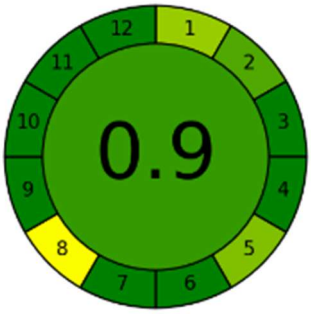

Figure 17: Agree metrics output for the proposed method

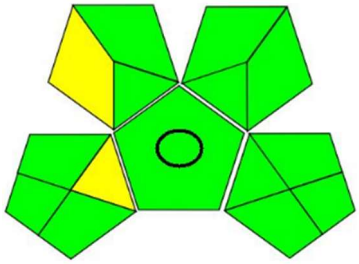

Figure 18: GAPI Pictogram for the proposed method

CONCLUSION

The newly developed spectrophotometric multivariate analysis technique for the evaluation of Imeglimin Hydrochloride was validated as per ICH guidelines by evaluating different validation parameters and was found to be within the acceptable limits. The proposed approach demonstrated high accuracy, precision, sensitivity, and reproducibility in evaluating Imeglimin Hydrochloride in its Pharmaceutical form. The established method, which involves basic mathematical principles, is more valuable and more consistent compared to existing UV spectrophotometric methods. Therefore, we strongly recommend employing this method for routine examination of Imeglimin Hydrochloride in pharmaceutical form.

ETHICAL STATEMENT

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

ACKNOWLEDGMENTS

Authors are thankful to The Chancellor, SRM Institute of Science and Technology, and the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur for providing various sources for carrying out this work.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

FUNDING SOURCES

There is no funding to report.

REFERENCES

[1] National Center for Biotechnology Information. PubChem Compound Summary for CID 54763513, Imeglimin hydrochloride. https://pubchem.ncbi.nlm.nih.gov/compound/Imeglimin-hydrochloride.

[2] Tamil Selvan R, Senthilkumar SK, Elakkiya A, Gayathri M, Gokulraj M, Hajima H, Hari Prakash G.A Novel Method Development and Validation of Imeglimin HCl by UV Visible Spectroscopy. Int. J. in Pharm. Sci. 2023; 1(12): 852-9. DOI:10.5281/zenodo.10433883

[3] Yaminee JS, Saurabh T, Abhijit A, Umekar MJ, Lohiya RT. Development of Novel Uv-Spectrophotometric Method for Imeglimin Hydrochloride. International Journal of Pharmaceutical Research and Applications.2023; 8(3): 1637-44

[4] Ajay Sanjay Salvi, Mohini Khamkar S, Lahu Hingane D. Development and Validation of RP-HPLC Method for Estimation of Anti-Diabetic Drug in Bulk and Tablet Dosage Form. Journal of Emerging Technologies and Innovative Research. 2023; 10(5): 366-78.

[5] Ain a, Soni LK, Sharma R. Development and validation of stability indicating RP-UHPLC method for the estimation of Imeglimin hydrochloride used for the treatment of metabolic disorder diabetes mellitus. Int. J. App. Pharm. 2023; 15(6): 211–7. https://doi.org/10.22159/ijap.2023v15i6.49757

[6] Ramalingam S, Subramania MN, Basuvan B, Jaganathan R, Dhavamani AJ, Kandukuri NK, Parimi RV, Bodduna S. A sensitive direct chiral liquid chromatography tandem mass spectrometry method for the enantio—Selective analysis of imeglimin in formulation. J Appl Pharm Sci. 2023; 13(07): 214–9. https://doi.org/10.7324/JAPS.2023.132349.

[7] Kokilambigai KS, Seetharaman R, Kavitha J, Sowndaravel P, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of zaleplon in bulk and pharmaceutical formulations. J Pharm Sci Res. 2017;9(6):824-9.

[8] Sai Susmitha A, Kokilambigai KS, Lakshmi KS, Spectrophotometric Quantification of Telmisartan Employing Multivariate Calibration Technique in Bulk and Pharmaceutical Formulations. Res J. Pharm. and Tech.

2019; 12(4):1799-1805. doi: 10.5958/0974-360X.2019.00300.7

[9] Madhan S, Kavitha J, Lakshmi KS, 2016. Multivariate calibration technique for the spectrophotometric quantification of Ivermectin in pharmaceutical formulation. Asian J. Pharm. Clin. Res. 2019;12(2): 444-51

[10] Durgadevi P, Kokilambigai KS, Lakshmi KS. First order derivative spectrophotometric method for the quantification of Telmisartan employing multivariate calibration technique. Res. J. Pharm. Technol. 2020;13(2):774-80. DOI: 10.5958/0974-360X.2020.00146.8.

[11] Naveena Rani D, Kokilambigai KS, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of Rasagiline in bulk drug and pharmaceutical formulation. Res. J. Pharm. Technol. 2020;13(2):843-9. DOI: 10.5958/0974-360X.2020.00159.6.

[12] Mani Aravinth R, Kokilambigai KS, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi KS. Spectrophotometric quantification of Granisetron in bulk drug and pharmaceutical formulations employing multivariate calibration technique. J. Med. Pharm. Allied Sci. 2021;10(4):3435-9. DOI: 10.22270/Jmpas.V10I4.1442.

[13] Vimal Ravi, Kokilambigai KS, Kavitha J, Seetharaman R, Lakshmi KS. 2022. Multivariate UV spectrophotometric quantification of Cilnidipine in bulk drug and pharmaceutical formulations. J. Med. Pharm. Allied Sci. 2022;11(2):4672- 8

[14] Kokilambigai KS, Logesh M, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi KS. Multivariate calibration technique for spectrophotometric quantification of Ondansetron in bulk drug and pharmaceutical formulations. 2022;11(4):4777-82.

[15] Gałuszka A, Migaszewski ZM, Konieczka P, et al. Analytical EcoScale for assessing the greenness of analytical procedures. TRAC Trends Anal Chem 2012; 37:61–72.

[16] Płotka-Wasylka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta 2018; 181:204–09. "

[17] Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE - Analytical greenness Metric

Approach and Software. Anal Chem 2020; 92(14):10076–82

[18] ICH. Harmonized tripartite guideline, validation of analytical procedures: text and methodology. Vol. Q2(R1). Geneva, Switzerland; 2005.