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Analytical Method Development and Validation of Stability Indicating RP-HPLC Method For Imeglimin Hydrochloride

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ABSTRACT

The present study describes the development and subsequent validation of Reverse phase HPLC (RP-HPLC) method for the analysis of Imeglimin hydrochloride. A novel economic, simple, rapid, accurate, reproducible, and precise Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for Imeglimin hydrochloride. The method was performed on a YOUNG LIN-HPLC system-ACME9000. The method developed for Imeglimin hydrochloride was quantitatively measured using an isocratic RP-HPLC methodology. The chromatographic separation of Imeglimin hydrochloride was achieved on RP-HPLC equipped with Hypersil BDS C18 (250mm x 4.6mm, 5 um) column using isocratic elution with a mobile phase consisting of MeOH: Buffer in a ratio of (70:30% v/v) at a flow rate of 1.0ml/min with an injection volume of 20µl, where detection was carried out by UV- 730D detector at 239nm The retention time for Imeglimin hydrochloride was found to be 3.47 min. The developed method was successfully with results falling within acceptable criteria validated for different validation parameters as per (ICH-Q2 (R1)) guidelines. The linear regression equation was found to be y = 27.83x - 8.512 with a correlation coefficient (R2) > 0.999 which shows excellent linear correlation. Accuracy, precision, specificity, system suitability, robustness, linearity, LOD and LOQ were determined for method validation. The results were found to be well within recommended limits as per ICH guidelines. Stability studies of Imeglimin hydrochloride were carried out under acidic, basic, peroxide, photolytic and thermal conditions. Degradation was observed in acidic, basic, and oxidative conditions, but not in photolytic and thermal conditions.

Keywords: Imeglimin hydrochloride, RP-HPLC, Development, Validation, Stability studies, Type 2 diabetes (T2D).

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INTRODUCTION

Diabetes continues to be one of the primary causes of illness and mortality, resulting in tremendous pressures on healthcare systems globally. The worldwide prevalence of type 2 diabetes (T2D) has increased in the recent years.[1] From the findings of a recent study, the rise in T2D prevalence is primarily due to urbanization and more advanced socioeconomic position.[2] Although public health measures that involve education, awareness among the populace, and encouraging the adoption of healthy lifestyle changes are critical for reducing this global healthcare challenge, there is still a need for the development of new therapies for the treatment of T2D with improved management of blood glucose levels and minimal adverse reactions.[3] Furthermore, emerging approaches are focusing on overcoming the obesity barrier in treating T2D. As a result, discovering innovative medicines that can control blood glucose levels and boost cellular energetics is of the highest importance.[4] The prevalence of T2DM, which presently affects more than 380 million individuals globally, is anticipated to climb to more than 592 million people by 2035.[5-6] Several promising therapeutic drugs for the management of T2D have been developed in recent years, including imeglimin.[3]

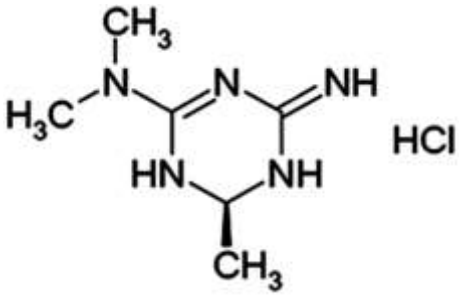

Figure 1: Structure of Imeglimin hydrochloride [7]

Imeglimin is an oral anti-diabetic medication sold under the brand name Twymeeg. In Japan, it received approval in June 2021. It inhibits oxidative phosphorylation while simultaneously improving muscle glucose uptake and restoring normal insulin secretion. It is the first anti-diabetic medicine of this type to be approved. It is a tetrahydrotriazene-containing tiny molecule that belongs to an entirely novel category of oral anti-diabetics known as glimins.[8] Imeglimin hydrochloride is chemically known as (6R)-(+)-4- dimethylamino-2- imino-6-methyl-1, 2, 5, 6-tetrahydro-1, 3, 5-triazine hydrochloride.[9] Imeglimin was developed with the goal of providing type 2 diabetes patients with a safe, well-tolerated medicine that can effectively address the underlying metabolic imbalances in these individuals.[10] Imeglimin was approved for usage in T2D patients in Japan, and multiple developing research studies demonstrated its efficacy as a monotherapy or in combination with insulin in T2D patients. Imeglimin promotes its glucose-

lowering impact through multiple pathways, including boosting pancreatic beta-cell function and preserving its integrity, improving insulin sensitivity in the liver and skeletal muscles and decreasing liver gluconeogenesis.[11] Imeglimin's underlying molecular mechanism of action primarily involves enhancing mitochondrial function and reducing mitochondrial-derived free radicals.[11-12] Interestingly, current research has demonstrated that Imeglimin has protective properties beyond its glucose-lowering impact across a variety of cell types and tissues, as proven in multiple experimental disease models, including cardiovascular and neurodegenerative.[12-15] Additionally, these findings may expand the future usage of Imeglimin beyond its current use in T2D patients.

MATERIALS AND METHOD

Chemicals and Reagents:

A gift sample of Imeglimin hydrochloride was obtained from Ami Life sciences Pvt. Ltd. A marketed preparation of Imeglimin hydrochloride tablets were bought from a nearby pharmacy. Sodium acetate anhydrous (Finar), Ammonium formate, HPLC Grade water, HPLC Grade methanol (Thermo Fisher Scientific, India), Hydrogen peroxide, Sodium hydroxide, HCL, Acetonitrile, Acetic acid (Merk Life Science Pvt.Ltd.) are used in the study.

Instrumentation:

The Younglin-HPLC system was utilized which featured a UV detector (730D). Analytical Column: C18 (Hypersil BDS) (250mm x 4.6mm, 5 um), Autochrom 3000 software, and M Lab pH meter. Manual sample injection was performed, and the Shimadzu Model-ATX224 was used for the analytical balance. UV-spectrum of Imeglimin hydrochloride was detecting by Shimadzu UV1800 Spectrophotometer Japan Corporation.

Chromatographic Conditions:

Chromatographic separation was achieved on Younglin-HPLC system using C18 (Hypersil BDS) (250mm x 4.6mm, 5 um) stationary phase and the isocratic mobile phase composition. UV detection at 239 nm at ambient temperature with a 20µl injection volume. Pump used SP930 D and flow rate of the detection of analyte was 1.0 ml/minutes. About 20 µl of injection volume of each sample injects into the system and employed at an ambient column temperature. By using above condition, we get retention time of 3.47 minute.

Preparation Mobile Phase:

Buffer:

Dissolve 1.74gm sodium acetate in 900ml of HPLC grade water, and adjust the pH to 3.0 using dilute acetic acid solution. Make the final volume to 1000ml with HPLC grade water.

Mobile Phase:

Prepare a mixture of methanol and buffer in the ratio 70:30 and filter it via a 0.45µm membrane filter, then degas it by using an ultrasonic bath.

Preparation of Standard Stock Solution:

Accurately weighed quantity 50mg of Imeglimin hydrochloride was dissolved in a mixture of methanol and water in the ratio 50:50 volume and filled with 100ml mark to obtain a stock solution of 500µg/ml. Pipette out 2ml of standard stock solution and further dilute it with 20ml of mobile phase to obtain the working solution of 50µg/ml. The resulting solution was filtered through a 0.45µm membrane filter and ultra-sonicated for 10 minutes with intermittent shaking. The resultant solution was used for further validation using RP-HPLC.

METHOD DEVELOPMENT:

After the finishing of six experiments with variations in run time, column and mobile phase the drug observed to be in good peak shape at the sixth trial. The % RSD, Tailing Factor and Theoretical plate show that drug is within the acceptance standards. The approach was found to be satisfactory. The chromatogram is shown in (figure 2)

Table 1: Optimized chromatographic conditions

Parameters	Results
Mobile phase	MeOH: Buffer (70:30)
Column	Hypersil BDS C18 (250mm x 4.6mm, 5 um)
Flow rate	1.0ml/minute.
Injection volume	20µl.
Temperature	Ambient
Wavelength	239nm
Run time	7 minutes
Elution mode	Isocratic
Diluent	Mobile phase

METHOD VALIDATION:

The analytical method was validated for the following parameters using ICH guidelines:

Specificity:

The specificity of the procedure was determined by comparing the chromatograms of the blank, standard, and sample. The retention time found was tabulated in Table 2. There is no interference from blank at the retention time of Imeglimin hydrochloride. Retention time of Imeglimin hydrochloride in test solution and standard solution are matching with each other hence, specificity is justified.

System Suitability:

It is an essential component of chromatographic technique. It tests system performance before and during analysis, including equipment, electronics, samples, and analytical operations. The developed method was found to be appropriate for use because the tailing factor and peak resolution for Imeglimin hydrochloride were within acceptable limits. Table 3 tabulates data on system suitability.

Linearity:

The linearity concentrations were used to calculate the slope, intercept values, and R2 value via the regression equation (Y = mx + C). Linearity refers to the ability of a method to produce test findings that are proportional to the concentration of the analyte in the sample. Imeglimin hydrochloride solutions with concentrations ranging from 25-75µg/ml were produced and 20µl of each solution was injected into an RP-HPLC instrument, and peak areas were recorded. The calibration curve is plotted against the dilution concentrations on the x-axis and the corresponding peak areas on the y-axis. This will result in the slope and intercept values of the regression line. Linearity detected between 25-75µg/ml. The regression equation is y = 27.83x - 8.512, with a correlation coefficient (R2) = 0.999, which shows excellent linear correlation. Table 4 tabulates data on Linearity. In Figure 3 the standard calibration curve was displayed.

LOD and LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following formulas: - LOD = 3.3σ/s and LOQ = 10σ/s. The standard deviation of the response ('σ') and the slope of the calibration curve ('s') are used in this calculation. The developed method's sensitivity is determined by its limit of detection and limit of quantification. The values calculated for LOD and LOQ were 1.73µg/ml and 5.26µg/ml, respectively. Table 5 shows the LOD & LOQ data.

Accuracy:

Accuracy is defined as the proximity of the obtained value to the true value. It was calculated as the percentage recovery of the standard API to the blank. The method's recovery was determined by adding a known concentration of the drug standard. The recovery was carried out at three levels: 80, 100, and 120% of the Imeglimin hydrochloride standard concentration. The three samples were prepared for each recovery level, and the recoveries were calculated. Table 6 shows the accuracy data. In Figure 4, 5, 6 the chromatograms were displayed.

Precision:

It is the degree of agreement between individual test results when the procedure is repeated for numerous samplings. It was determined by analyzing the method's repeatability, intra-day and

inter-day precision. The results were calculated as %RSD and shown in Table 7 & 8.

Robustness:

It is the method's ability to remain unaffected by few but intentional modifications in method parameters. The wavelength, mobile phase composition, and flow rate all changed slightly during the analysis. The results were calculated as %RSD and are shown in Table 9.

STABILITY STUDY:

The degradation tests for Imeglimin hydrochloride were carried out in accordance with ICH guidelines. Imeglimin hydrochloride was tested for stability using acid, alkali, hydrogen peroxide, photolysis, and thermal degradation. The percentages of degradation were determined and reported in Table 10. The Figures 7-11 shows chromatograms.

Acid Degradation:

Take 10ml of stock solution into a 100ml volumetric flask. Dilute it with 10ml of 0.1M HCL solution. After that, heat the aforesaid solution on water bath at 50°C for 3 hours. Then the solution was cooled to room temperature, neutralized with 0.1M NaOH, and the volume was made to 100ml with mobile phase. This solution was injected into the system and the chromatogram was recorded. Then the % of degradation was calculated.

Base Degradation:

Take 10ml of stock solution into a 100ml volumetric flask. Dilute it with 10ml of 0.1M NaOH solution. After that, heat the aforesaid solution on water bath at 50°C for 3 hours. Then the solution was cooled to room temperature, neutralized with 0.1M HCL, and the volume was made to 100ml with mobile phase. This solution was injected into the system and the chromatogram was recorded. Then the % of degradation was calculated.

Peroxide Degradation:

Take 10ml of stock solution into a 100ml volumetric flask. Dilute it with 10ml of 3% H_2O_2 solution. After that, heat the aforesaid solution on water bath at 50°C for 3 hours. Then the solution was cooled to room temperature and the volume was made to 100ml with mobile phase. This solution was injected into the system and the chromatogram was recorded. Then the % of degradation was calculated.

Photolytic Degradation:

Place the drug in a Petri plate and expose it to ultraviolet light for 12 hours. Then take the drug out after 12 hours of exposure. Then take 10ml of stock solution into a 100ml volumetric flask and the volume was made to 100ml with mobile phase. This solution was injected into the system and the chromatogram was recorded. Then the % of degradation was calculated.

Thermal Degradation:

Place the drug in a Petri plate and put it into oven at 50°C for 4 hours. Then take 10ml of stock solution into a 100ml volumetric flask and the volume was made to 100ml with mobile phase. This solution was injected into the system and the chromatogram was recorded. Then the % of degradation was calculated.

MARKETED SAMPLE ANALYSIS:

Accurately weighed 10 tablets of Imeglimin hydrochloride, calculating the average weight and comparing the individual tablet weights to the average. Average weight was found 555.74. Then finely powder it and measure quantity of powder equivalent to 55-57mg was measured and transferred to 100ml volumetric flask. This test compares the label claim to the actual drug contained in the tablet. The results were calculated for marketed samples and are shown in Table 11. In Figure 12 & 13 the chromatograms were displayed.

RESULTS AND DISCUSSION:

METHOD DEVELOPMENT:

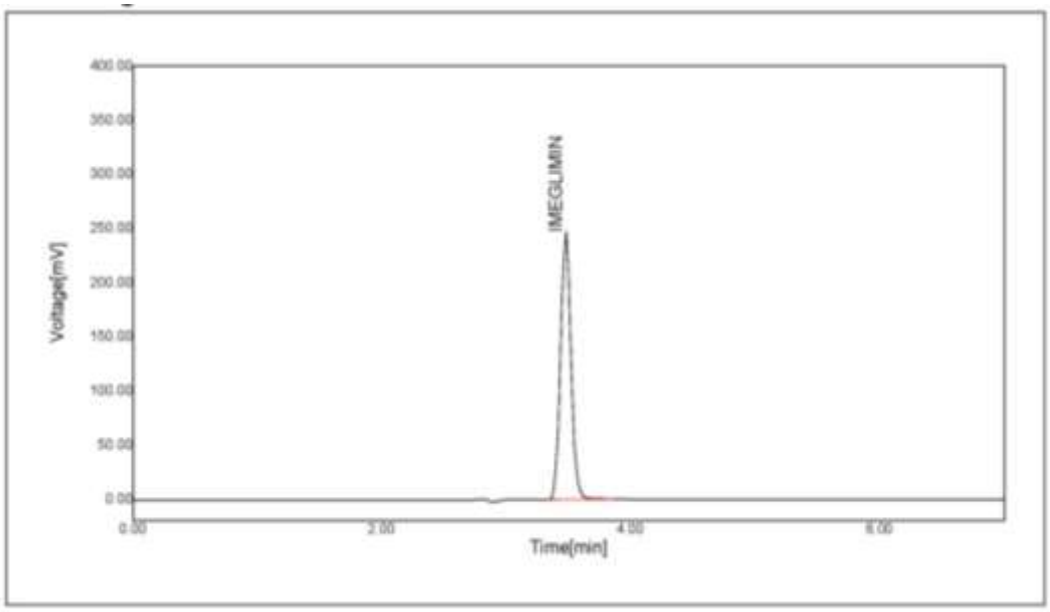

Figure 2: Chromatogram for Optimized method of Imeglimin hydrochloride

METHOD VALIDATION:

Specificity:

Table 2: Specificity of Imeglimin hydrochloride

Sr. No.	Solution	Retention time
1.	Blank solution	0.00
2.	Imeglimin hydrochloride standard	3.47
3.	Imeglimin hydrochloride sample	3.47

Inference:

Retention time of Imeglimin hydrochloride in test solution and standard solution are matching with each other.Hence specificity is justified.

System Suitability:

Table 3: System Suitability Study for Imeglimin hydrochloride

Name	Area	RT (min)	TP	TF
Standard Injection 01	1415.4122	3.47	13968	1.01
Standard Injection 02	1384.3175	3.47	13968	0.98
Standard Injection 03	1397.1516	3.47	13972	0.98
Standard Injection 04	1377.5016	3.45	13975	0.99
Standard Injection 05	1376.2813	3.47	10646	1.13
Mean	1390.1328	3.47	-	-
SD	16.3825	0.01	-	-
%RSD	1.18	0.26	-	-

Inference: Theoretical plates, tailing factor, % RSD for area and retention time for Imeglimin hydrochloride peak observed within acceptance criteria, hence system is suitable for analysis of Imeglimin hydrochloride and system suitability is justified.

Linearity:

Table 4: Calibration Standards Peak Area

Conc. (ppm or µg/ml)	Area
25.10	681.0613
37.65	1044.0798
50.20	1406.5801
62.75	1723.2285
75.30	2087.8198
Correlation coefficient ®	0.9997
Intercept	-8.5124
Slope	27.8300

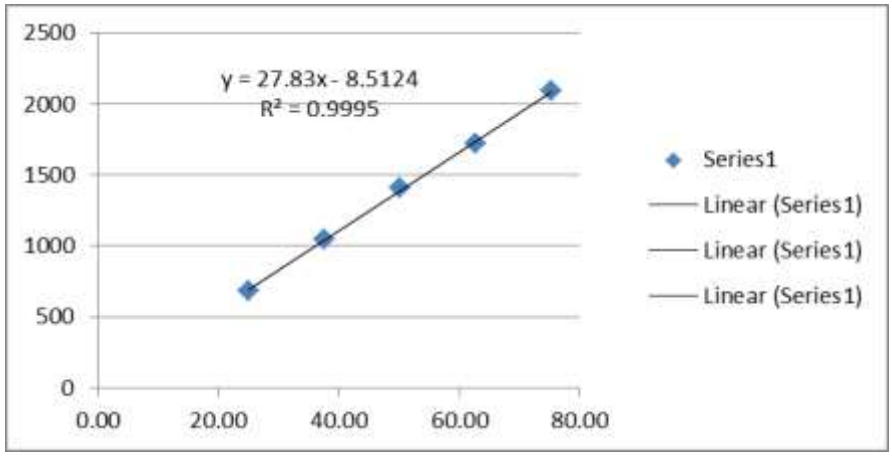

Figure 3: Standard Calibration Curve for Imeglimin hydrochloride

Inference: Correlation coefficient observed within acceptance criteria, hence method is linear and linearity is justified.

LOD and LOQ:

Table 5: LOD & LOQ of Imeglimin hydrochloride

Conc. (ppm or µg/ml)	Area
25.10	681.0613
37.65	1044.0798
50.20	1406.5801
62.75	1723.2285
75.30	2087.8198
Correlation coefficient ®	0.9997
STEYX	14.6247
SLOPE	27.8300
LOD (µg/ml)	1.73
LOQ (µg/ml)	5.26

Inference: LOD & LOQ observed 1.73µg/ml and 5.26µg/ml respectively.

Accuracy:

Table 6: Statistical Validation of Recovery Studies

Accuracy Level	MEAN % Recovery	SD	%RSD
Accuracy at 80 %	99.69	1.4849	1.49
Accuracy at 100 %	98.35	0.2573	0.26
Accuracy at 120 %	100.79	0.4830	0.48

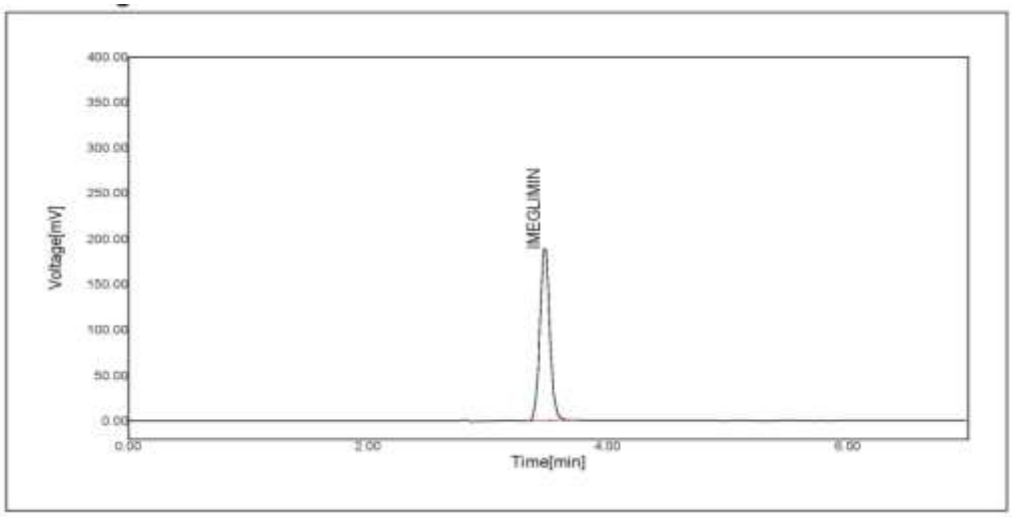

Figure 4: Chromatogram of Accuracy at 80%

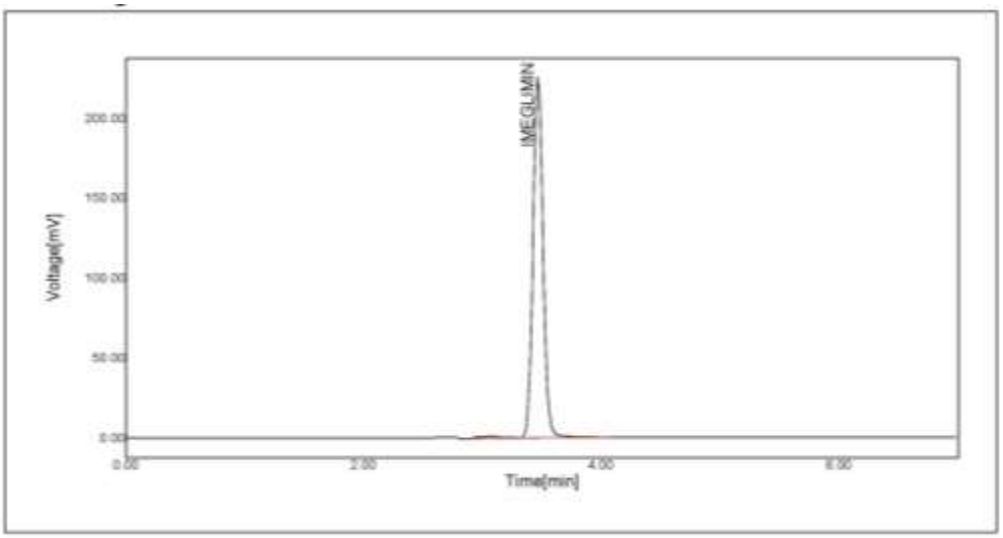

Figure 5: Chromatogram of Accuracy at 100%

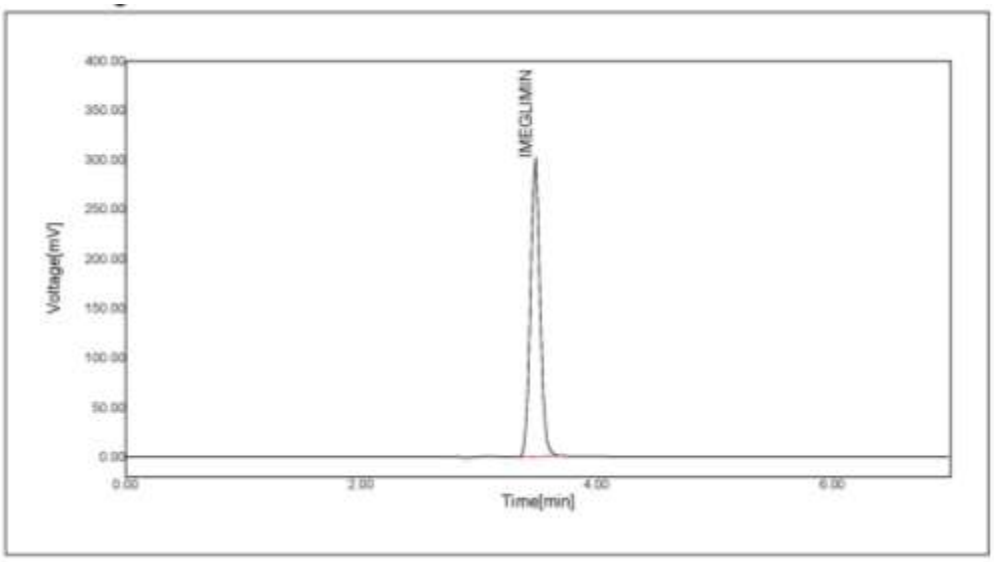

Figure 6: Chromatogram of Accuracy at 120%

Inference: % Mean recovery observed within acceptance criteria, also % RSD of recovery observed within acceptance criteria, hence accuracy is justified.

Precision:

Intraday Precision:

Table 7: Result and Statistical Data for Intraday Precision

Name	Preparations	% Assay
Set-1	prep-01	99.18
	prep-02	100.85
Set-2	prep-01	99.69
	prep-02	99.32
Mean		99.76
SD		0.7578
% RSD		0.76

Inference: Overall % RSD for results of set-1 and set-2 performed in single day observed within acceptance criteria so method is precise in terms of repeated analysis in single day, hence intraday precision is justified.

Interday Precision:

Table 8: Result and Statistical Data for Interday Precision

Name	Preparations	% Assay
Day-1	prep-01	99.18
	prep-02	100.85
Day-2	prep-01	97.96
	prep-02	97.16
Mean		98.7875
SD		1.6064
% RSD		1.63

Inference: Overall % RSD for results of Day-1 and Day-2 analysis performed that observed within acceptance criteria so method is precise in terms of repeated analysis in different days, hence interday precision is justified.

Robustness:

Table 9: Robustness of Imeglimin hydrochloride

Name	Preparations	%Assay
Robustness change in method parameters		
Original method parameters	Test prep-1	99.18
Original method parameters	Test prep-2	100.85
Flow rate 0.90 ml/min	Test prep	100.10
Flow rate 1.10 ml/min	Test prep	100.49
Wavelength 237 nm	Test prep	101.19
Wavelength 241 nm	Test prep	101.70
MeOH: Buffer, 65:35	Test prep	97.55
MeOH: Buffer, 75:25	Test prep	101.92
Mean		100.37
SD		1.4407
%RSD (NMT 2)		1.44

Inference: Overall % RSD of results with change in flow rate, wavelength and mobile phase composition observed within acceptance criteria so method is robust in terms of slight change in internal method parameters, hence Robustness is justified.

STABILITY STUDY:

Table 10: Stress Degradation Study of Imeglimin hydrochloride

Name	% Assay	% Degradation
Acid Degradation	95.51	4.49
Base Degradation	95.74	4.26
Peroxide Degradation	94.60	5.40

Photolytic Degradation	99.92	0.08
Thermal Degradation	99.97	0.03

Acid Degradation:

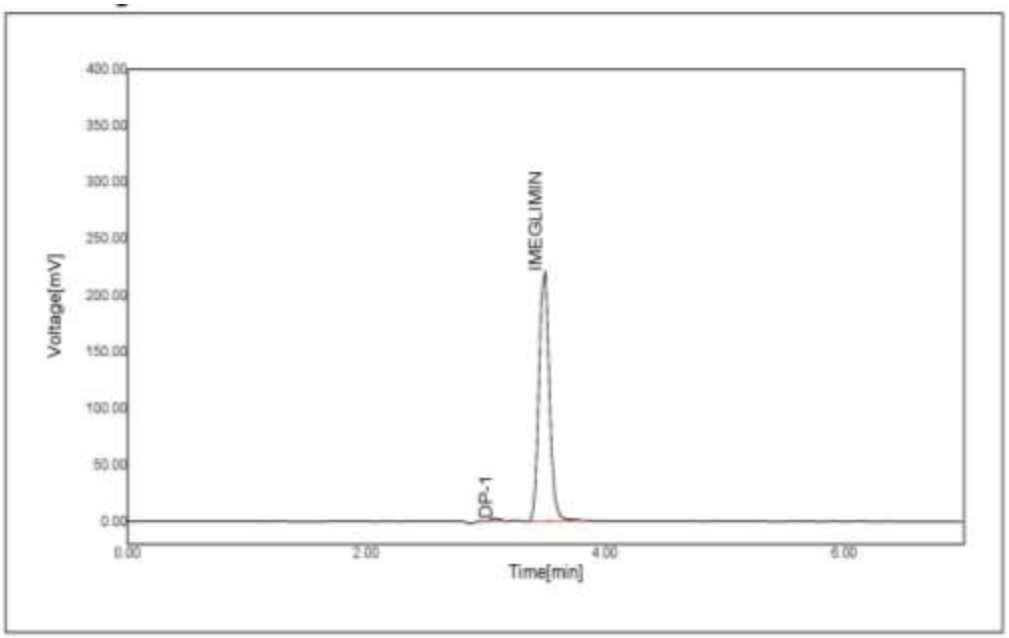

Figure 7: Chromatogram of Acid Degradation

Base Degradation:

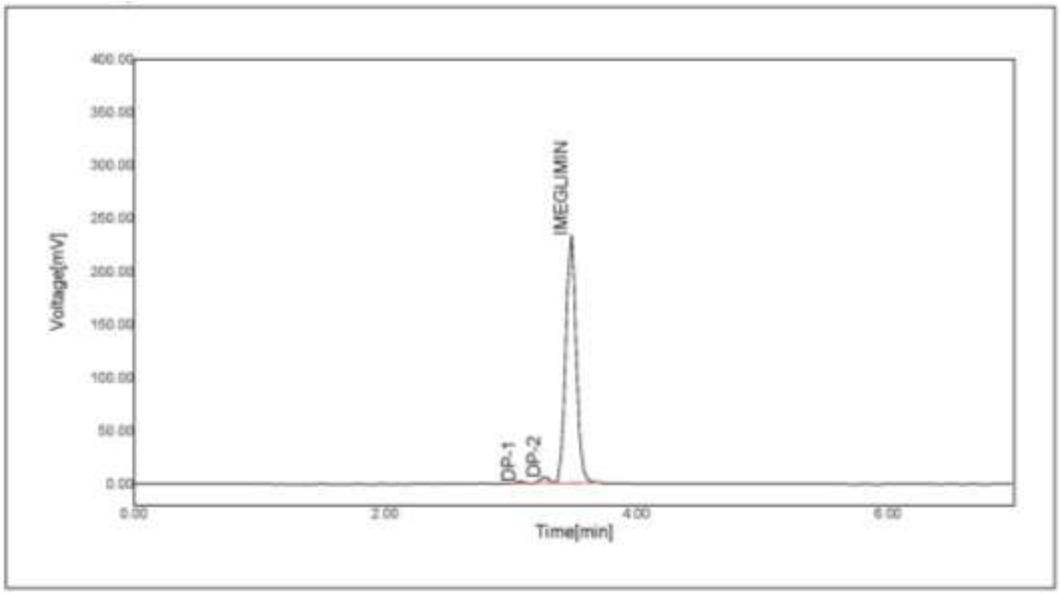

Figure 8: Chromatogram of Base Degradation

Peroxide Degradation:

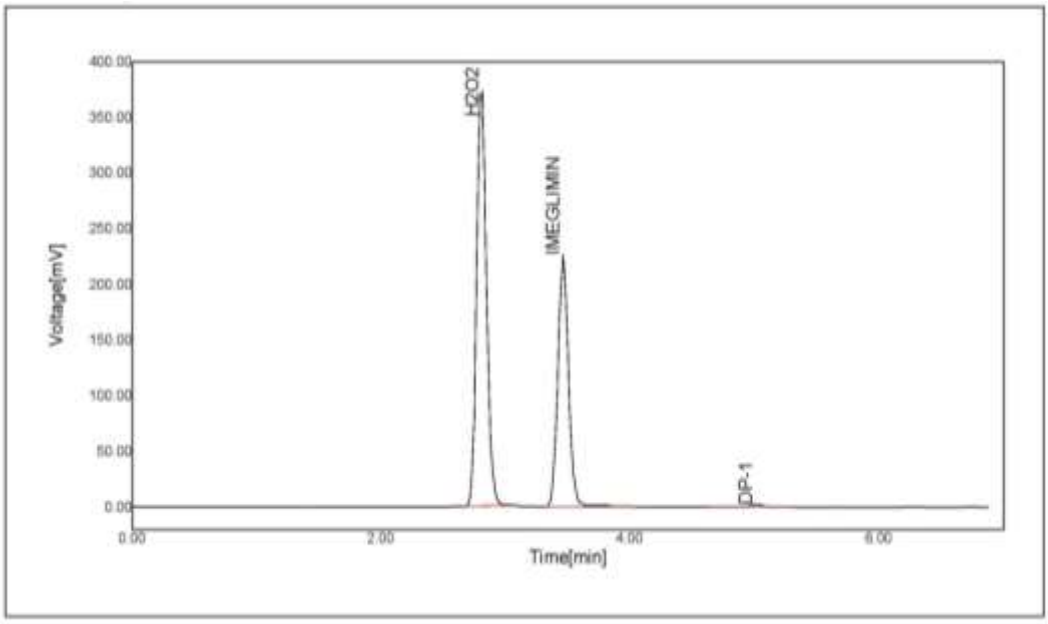

Figure 9: Chromatogram of Peroxide Degradation

Photolytic Degradation:

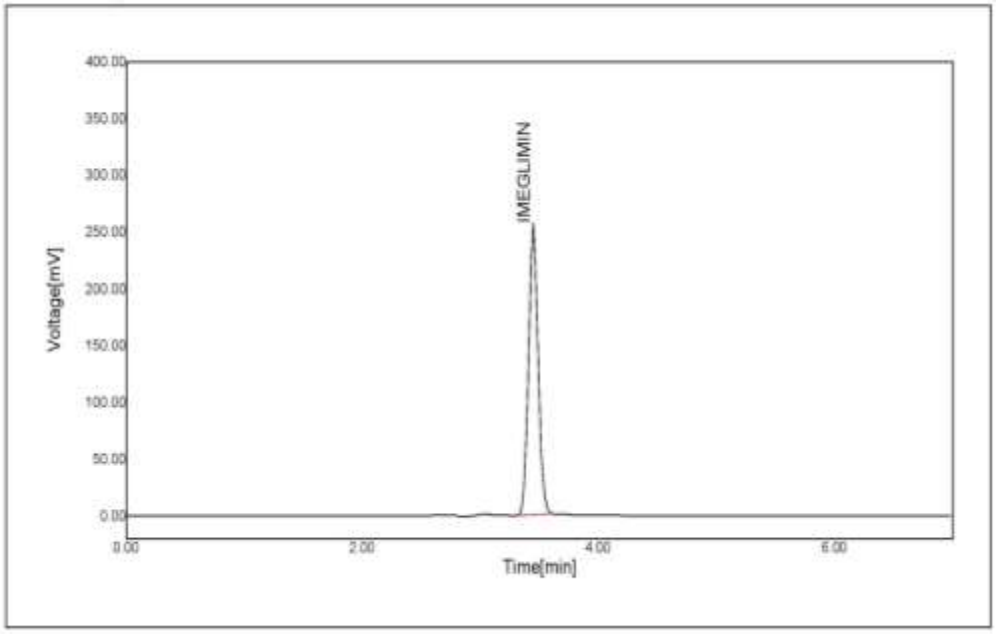

Figure 10: Chromatogram of Photolytic Degradation

Thermal Degradation:

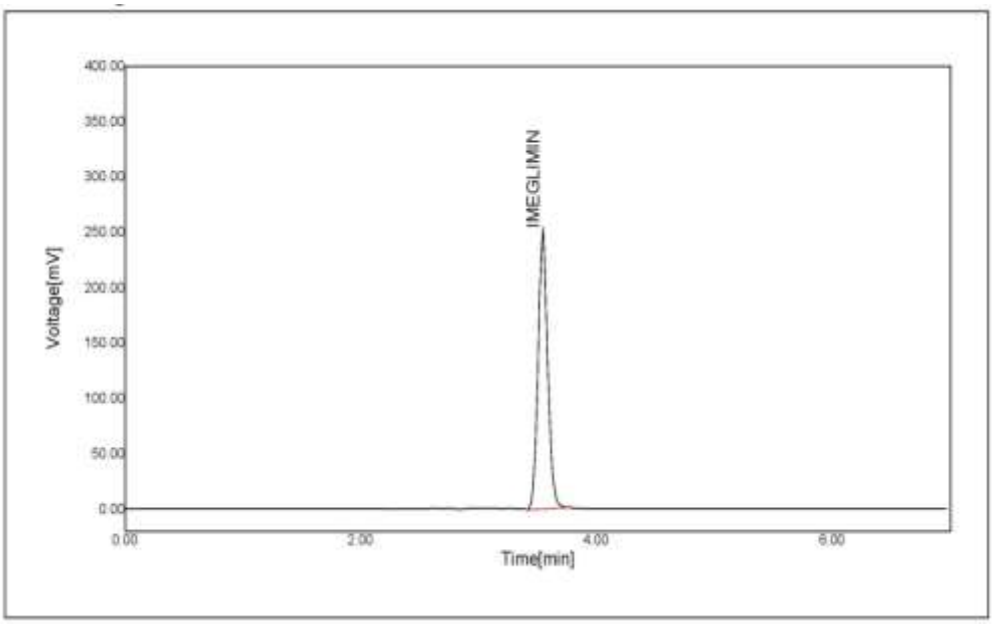

Figure 11: Chromatogram of Thermal Degradation

Inference: There was no degradation observed under photolytic and thermal conditions, however, minor degradation was observed under acidic, basic, and oxidative conditions.

MARKETED SAMPLE ANALYSIS:

Table 11: Marketed Sample Analysis of Imeglimin hydrochloride

Name	Area	RT(min)	Imeglimin in mg	Label claim
Test solution-1	1360.6222	3.48	486.53	500
Test Solution-2	1387.0776	3.53	493.34	500

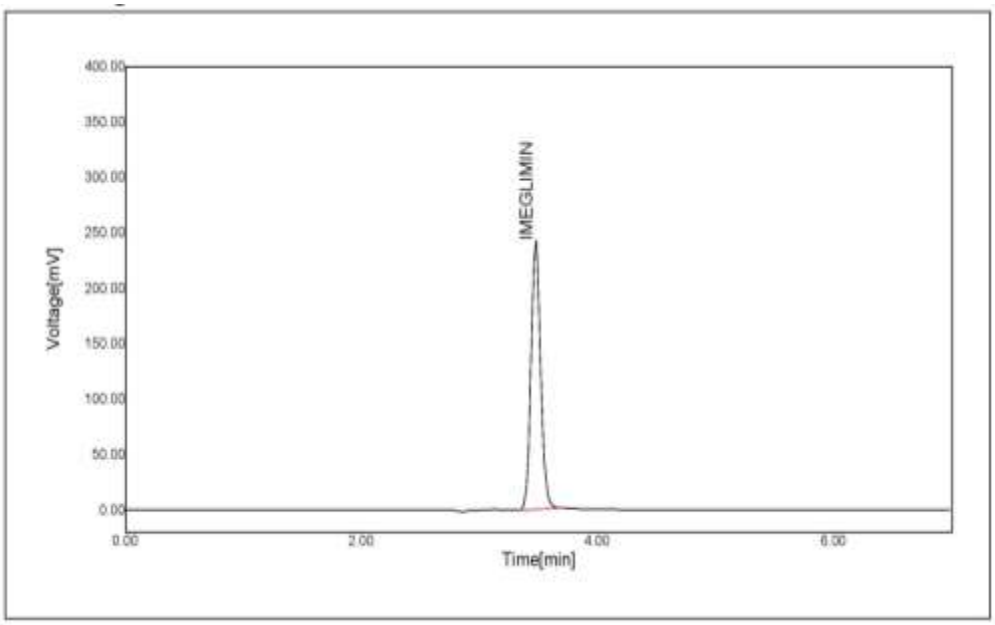

Figure 12: Chromatogram of Marketed Sample Test Solution 1

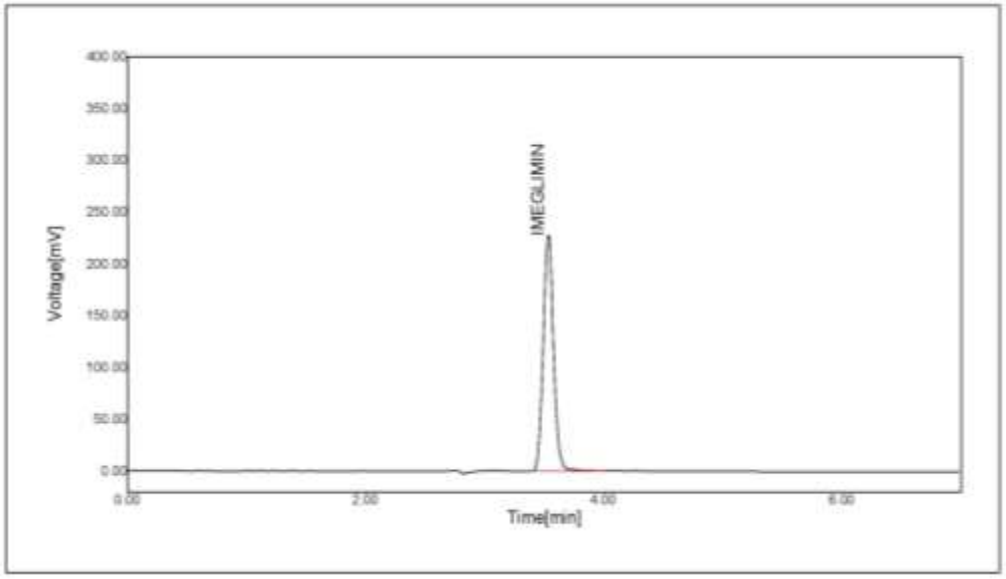

Figure 13: Chromatogram of Marketed Sample Test Solution 2

Inference: The proposed method was applied to the determination of Imeglimin hydrochloride in marketed formulation. The results indicate the developed method was successfully applied for analysis of marketed formulation. All the results found were in good agreement with the label contents of marketed formulation.

CONCLUSION:

In conclusion, a simple, selective, stable, and accurate isocratic stability indicating RP-HPLC method was developed and validated for the analysis of Imeglimin hydrochloride. The developed approach was determined to be appropriate, linear, precise, specific, accurate, and robust in compliance with ICH requirements. The method was superior due to its less retention time, isocratic mode, and use of a relatively inexpensive, easily available mobile phase, column, UV detection, and greater peak resolution. Degradation studies show the drug's stability. This method shows a minor degradation rate under stressful situations. As a result, the proposed method is safe and efficient for routine analysis.

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