ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF IMEGLIMIN IN BULK AND MARKETED DOSAGE FORM BY RP-HPLC

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ABSTRACT

A rapid, simple, economical, accurate, and precise RP-HPLC method was developed and validated for the estimation of Imeglimin in bulk and marketed dosage forms. Chromatographic separation was achieved under isocratic conditions using a Kinetex® C18 100A column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of 0.1% orthophosphoric acid (pH adjusted to 3.0 with triethanolamine) and methanol in a 20:80 (v/v) ratio, delivered at a flow rate of 1.0 mL/min. Detection was carried out at 237 nm. The retention time of Imeglimin was found to be 5.731 minutes. The calibration curve exhibited linearity over the concentration range of 2–12 µg/mL with a correlation coefficient (r^2) of 0.998. The method was validated as per ICH guidelines and found to be sensitive, precise, and robust, with %RSD less than 2. This method can be effectively applied for routine quality control analysis of Imeglimin in pharmaceutical formulations.

Keywords: Estimation, Imeglimin, RP-HPLC, Validation.

INTRODUCTION:

Imeglimin[1] is the first of the "glimins,"[2] a new class of glucose-lowering[3] drug developed for the treatment of type 2 diabetes mellitus (T2DM) [2]. Imeglimin, a dihydro-1, 3, 5-triazine that has been studied as a potential novel anti-diabetic medication is a cyclic Metformin derivative [4]. Correcting abnormalities in both insulin secretion and insulin sensitivity [5] is a requirement for achieving optimal glucose management in type 2 diabetes [6]. Imeglimin has a special method of action that specifically targets the three pathophysiologic elements of type 2 diabetes: decreased muscle glucose uptake, excessive hepatic gluconeogenesis, and increased apoptosis in beta cells. It is an oxidative phosphorylation inhibitor that reduces hepatic gluconeogenesis, boosts muscular glucose uptake, and returns insulin levels to normal. Recent phase II and phase III studies have demonstrated the efficacy of Imeglimin alone and in combination with other hypoglycemic agents, in improving glycated hemoglobin (HbA1c) and fasting plasma glucose (FPG) levels [7]. Therefore, it may be suitable for safe and effective combination with other drugs commonly used to treat type 2 diabetes and its common complications.

This is a crystalline white powder, very soluble in water **[8]**, methanol, and ethanol. Chemically, it is (6R)-(+)-4-dimethylamino-2-imino-6-methyl-1,2,5,6-tetrahydro-1,3,5-triazine**[9]** **Fig. 1,** with a molecular weight of 191.6 and a molecular formula of $C_6H_{14}ClN_5$.

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MATERIALS AND METHODS

Chemicals and Reagents:

The Imeglimin reference samples have been obtained from Dr. Reddys Labs, Hyderabad, India as gift samples. Imextor tablets (Label claim: Imeglimin 500 mg) was procured from the local drug store, Tarnaka, Hyderabad. HPLC grade Methanol has been attained from Avanto Performance Materials, Maharashtra, India, OPA was obtained from SD Fine Chem Limited, Mumbai, India, and Mili Q water.

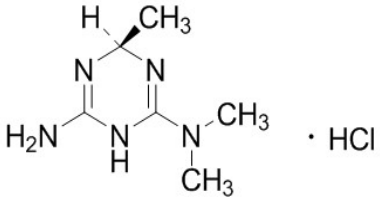

Figure 1: Structure of Imeglimin

Instrumentation and chromatographic conditions:

The Proposed work has been carried out by utilizing HPLC (Waters), Pump (Binary), Manual with UV-Visible detector (UV-Detector), Column: C18 Kinetex 5µ column, data was recorded using Breeze software. Various combinations of mobile phases were screened and finally, the isocratic elution has been carried out with a mobile phase consisted of 0.1% orthophosphoric acid (pH adjusted to 3.0 with triethanolamine) and methanol in a 20:80 (v/v) ratio, delivered at a flow rate of 1.0 mL/min and the retention time of Imeglimin was thoroughly investigated. The 0.1% orthophosphoric acid Buffer the ratio of 20:80v/v with Methanol was tuned to produce a symmetric peak with a brief duration. **Fig 2** illustrates the parameters of the experiment: the UV detection wavelength was set to 237 nm, the flow rate has been set to 1mL/min, the injection volume was 20µL, the runtime was set to 10 min, and the retention time has been estimated to be 5.622.

Preparation of Buffer for mobile phase (0.1% OPA):

0.1ml of Orthophosporic acid was added then diluted with Mili Q water to make 100 ml in a volumetric flask. After passing Orthophosphoric acid through a 0.2µm membrane filter and degassing, the pH has been adjusted to 3 with Triethanol amine.

Preparation of Mobile Phase:

Mixed A mixture of).1% OPA buffer 200ml (20percent) and 800ml of Methanol HPLC grade (80percent) was degassed in the ultrasonic water bath until it was completely dissolved and filtered by a 0.2µm membrane filter.

Diluent Preparation: The mobile phase is being utilized as diluent.

Preparation of working standard solution of Imeglimin

Because the drug was found to be water soluble. The standard stock solution was made by dissolving 25 mg of Imeglimin in a small amount of water, then filling the volume to 25 ml with distilled water to achieve a concentration of 1000 10 µg/ml and sonicating for 10 minutes to ensure complete solubilization. By diluting the stock solution with Milli Q water, a working standard solution of 10 µg/ml was prepared. A membrane filter having a pore size of 0.2µm has been utilized to filter the final solution.

Sample preparation

20 tablets are weighed and powdered; weight equivalent to 25 mg of Imeglimin is taken in a 25 ml volumetric flask dissolved in few ml of distilled water, then filling the volume to 25 ml with distilled water to achieve a concentration of 1000 10 µg/ml and sonicating for 10 minutes to ensure complete solubilization. By diluting the stock solution with Milli Q water, a working standard solution of 10 µg/ml was prepared. A membrane filter having a pore size of 0.2µm has been utilized to filter the final solution

Method Validation:

The method was validated for precision, linearity, robustness, specificity, sensitivity, accuracy, and parameters of system suitability by the following procedures by using the mentioned optimized chromatographic conditions as per ICH guidelines.

Selectivity:

The impact of excipients on the assay outcome is ascertained by the selectivity test. First, a standard sample of Imeglimin was injected to ascertain the method's selectivity. Next, the device was used to run blank solutions and commercial items one after the other. The test results demonstrated that at the Imeglimin retention time, the components other than medication did not provide a detectable signal.

Accuracy:

Recovery studies were conducted in triplicates at three various concentrations of 50%, 100%, and 150% for Imeglimin, respectively, to assess the accuracy of the approach. The sample was

mixed with known concentrations of standard drugs, and the peak area has been ascertained. The average recovery % values are displayed in **(Table 2).**

Linearity:

Using the mobile phase, multiple aliquots of the standard stock solutions of Imeglimin was taken and diluted to the desired final concentrations of 2-12 µg/mL for Imeglinin, in separate 10 ml volumetric flasks. Plotting the peak area vs. analyte concentration created calibration curves, as illustrated in **(Table 3)**.

Precision:

Repeatability

Six measurements of the same solution are the basis for the results about the repeatability of peak area measurement for Imeglimin. The % RSD for Imeglimin is displayed in **(Table 4)**.

LOD and LOQ:

This method's LOD and LOQ for Imeglimin were assessed using the signal-to-noise ratio (SNR) approach outlined in ICH recommendations. In general, it is thought that a SNR of 3:1 to 2:1 is suitable for determining the detection limit. LOQ typically requires a SNR of 10:1.

System suitability:

Six replicates of samples containing Imeglimin were given to assess equipment, analytical operations, electronics, and sample suitability. The % RSD of the retention time along with area, the theoretical plate number, and the Resolution were the parameters used to calculate the system suitability.

Robustness:

The degree of method robustness was assessed by purposefully and gradually adjusting chromatographic parameters, like column, wavelength, and flow rate ratio as in **Table 7**.

Mobile phase optimization

Optimization of the cell in chromatography is essential to achieve the best separation and analysis; different solvents such as Acetonitrile, methanol, and HPLC grade water, and buffers were used. Chromatographic Conditions was optimized using Phenomenex C18 Luna, measuring 250 mm by 4.6 mm by 5 µm with mobile phase: 0.1% OPA (pH adjusted to 3 with Triethanol amine) and Methanol in the ratio of (20:80) in an isocratic elution mode with 1 mL/min of flow rate. The detection wavelength was 237 nm

Symmetry is good and there are no significant effects.

After injection of 20mL of each test and Sample (formulation), chromatograms were recorded for 10 minutes. The Peak was found to be accurate and unaffected by excipients in the tablet formulation. The optimized chromatogram is shown in Figure 2.

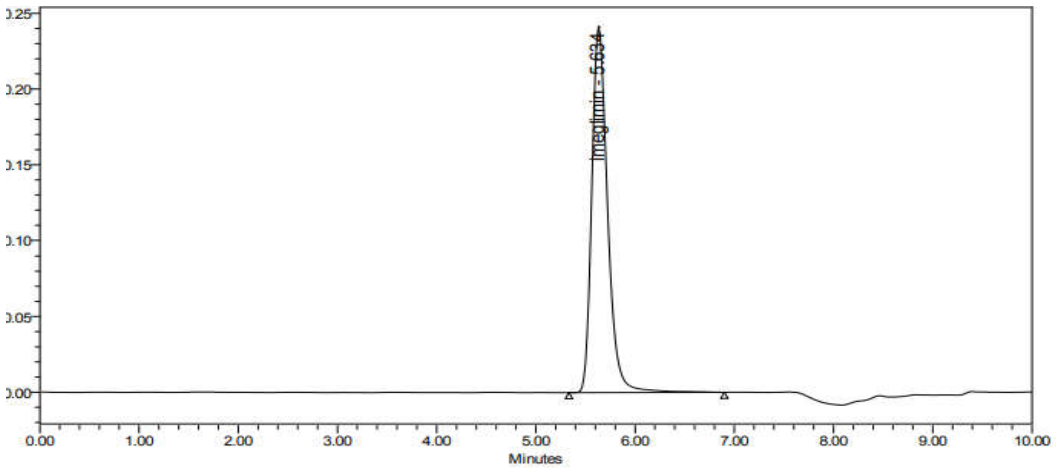

Figure 2: Chromatogram of conditions optimized with Imeglimin

RESULT AND DISCUSSION

Method development:

An isocratic, rapid, and simple RP-HPLC approach was for the instantaneous estimation of Imeglimin. The Proposed work has been carried out by utilizing HPLC (Waters), Pump (Binary), Manual with UV-Visible detector (UV-Detector), Column: C18 Kinetex 5µ column, data was recorded using Breeze software. Various combinations of mobile phases were screened and finally, the isocratic elution has been carried out with a mobile phase consisted of 0.1% orthophosphoric acid (pH adjusted to 3.0 with triethanolamine) and methanol in a 20:80 (v/v) ratio, delivered at a flow rate of 1.0 mL/min and the retention time of Imeglimin was thoroughly investigated. The 0.1% orthophosphoric acid Buffer the ratio of 20:80v/v with Methanol was tuned to produce a symmetric peak with a brief duration. The temperature within the column compartment was kept at 25°C. According to **(Table 1)** & **(Figure 2)** the observed retention times for Imeglimin was 5.654 minutes, respectively.

OPTIMIZED METHOD

The conditions of the method are given in **Table 1** and further tests were carried out with the same chromatographic conditions.

Table 1: Showing chromatographic conditions of the optimized parameters

Parameters	Optimized condition
Stationary Phase	Kinetex C18 (250 mm x 4.6 mm x 5 µm) column
Mobile Phase(v/v)	0.1% Orthophosphoric acid (using Triethanol amine to bring pH down to 3): Methanol (V/V: 20:80)
Flow rate(mL/min)	1 mL/min
Detection Wavelength(nm)	237nm
Temperature	Ambient
Injection Volume(µL)	20 µL
Run time(minute)	10 minutes
Retention Time(minute)	Imeglimin (5.646min)

Selectivity:

The chromatogram of Imeglimin the mobile phase is displayed in Fig 3. The Imeglimin retention time did not exhibit any interfering peaks.

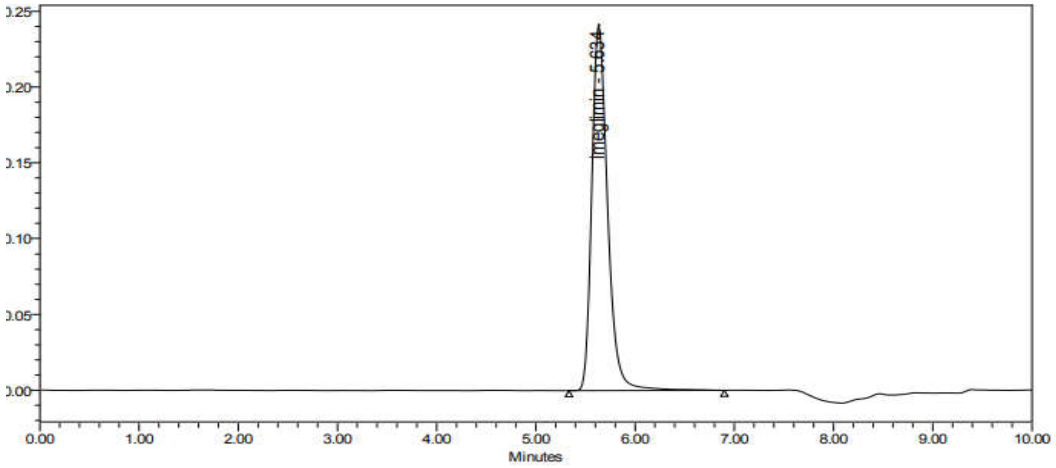

Figure 3: Chromatogram of Imeglimin in mobile phase.

Accuracy:

Table 2. Recovery results for Imeglimin

Concentration	Volume of standard solution taken of conc (10 µg/ml)	Volume of sample solution taken of conc (10 µg/ml)	Final concentration in (µg/ml)	% Recovery ± SD (n=3)	% mean Recovery
50	0.2	0.2	4 µg/ml	98.1 ± 0.1	99.3
100	0.2	0.4	6 µg/ml	100.2 ± 0.6	
150	0.2	0.6	8 µg/ml	99.6 ± 0.10	

From the data given in **Tables 2** the % mean recovery of Imeglimin was found to be 99.3 and the results are found to be within the limits.

Linearity and range

Preparation of working standard solution

The working standard solutions of 2,4,6,8, 10 & 12 **µ**g/mL were prepared from the standard stock solution as given in the **Table 3.**

Table 3: Linearity data of Imeglimin

S. N0	Concentration in µg/mL	Absorbance
1	2	0.045
2	4	0.08
3	6	0.12
4	8	0.16
5	10	0.201
6	12	0.243

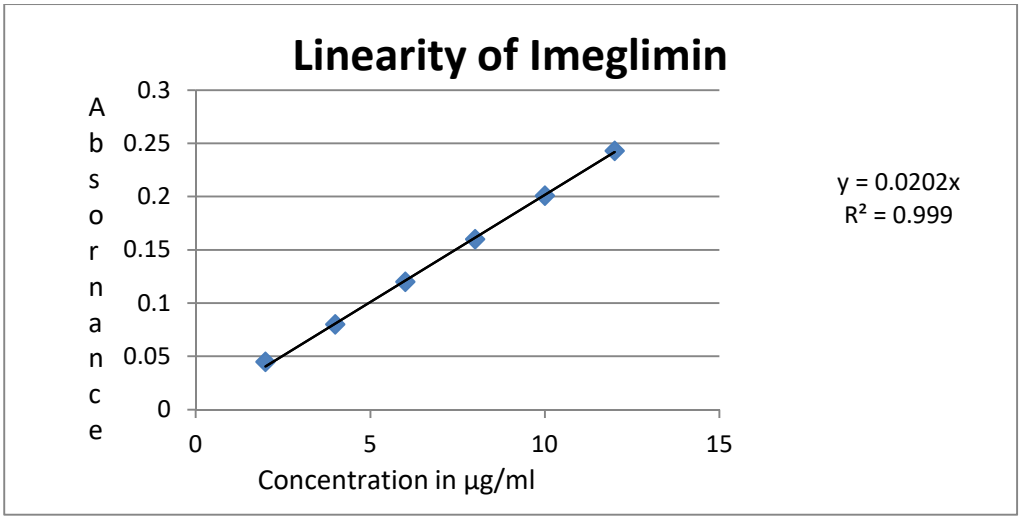

Figure 4: Graph for linearity data of Imeglimin

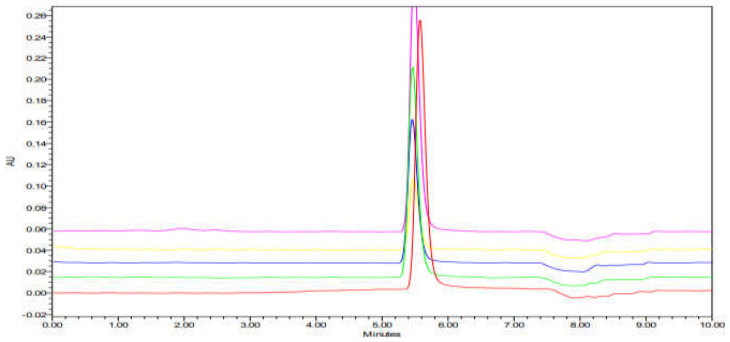

Figure 5: Overlay of linearity

Specificity

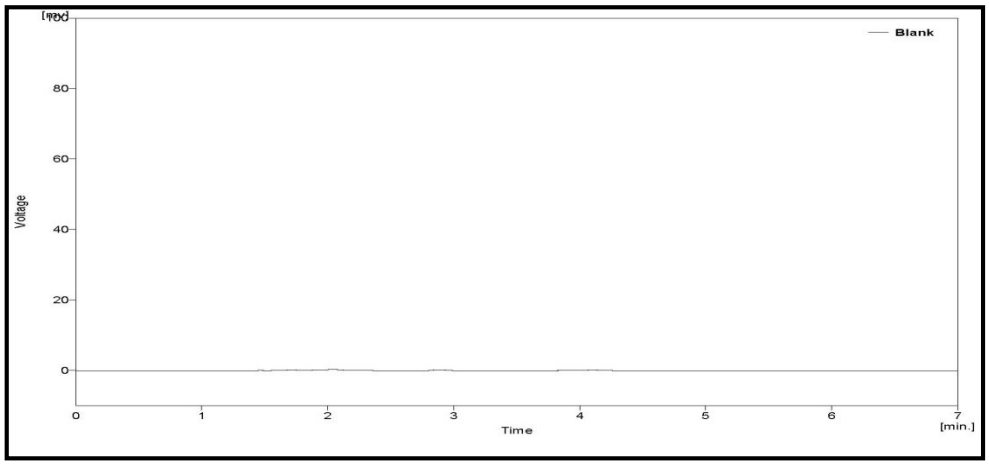

Figure 6: Chromatogram of blank.

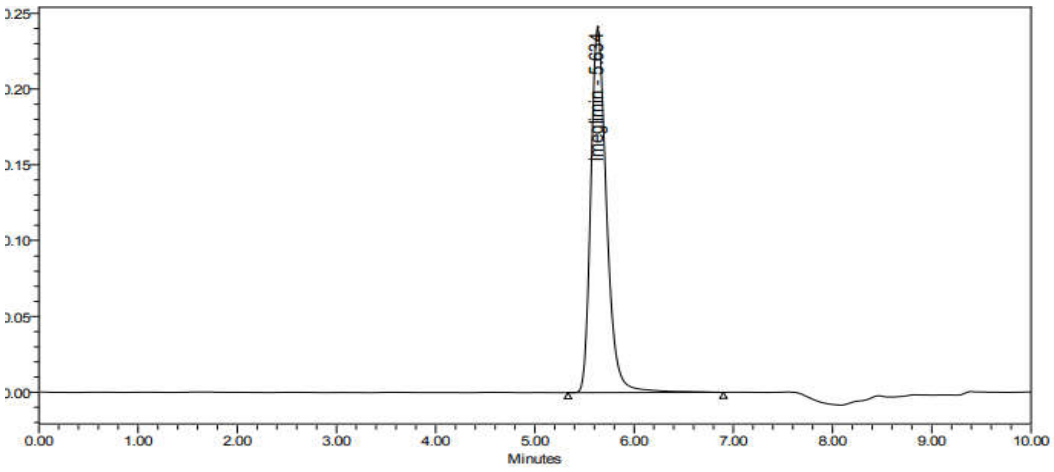

Figure 7: Chromatogram of standard.

Precision:

Precision was determined by analyzing standard preparations of Imeglimin (8 µg/ mL) for six times.

Table 4: Results for Precision.

Injection	Imeglimin	
	RT	Area
1	5.415	2545918
2	5.634	2670031
3	5.634	2670031
4	5.651	2875666
5	5.776	2715662
6	5.714	2915662
Average	5.608	2875886
SD	0.081939	4552.836
%RSD	1.410795	1.381066

From the data given in **Table 4**, the relative standard deviation (% RSD) of 6 determinations of Retention times and peak areas for Imeglimin was found to be within

the limits.

LOD and LOQ:

The calibration curve has been carried through three times, and the intercepts' SD was computed each time. The following was the calculation of LOD & LOQ:

LOQ = 10 * SD/slope of the calibration curve

LOD = 3.3* SD/slope of the calibration curve

Here, SD=Standard deviation of intercepts.

The outcomes have been displayed in (Table 5).

Table 5: Results of LOD & LOQ

Imeglimin	LOD	LOQ
	0.285 µg/mL	0.940 µg/mL

System suitability: Six replicates of a sample containing 2-10 µg/mL for Imeglimin was run and system suitability was tested for %RSD of areas, tailing factor, resolution, and a number of theoretical plates. Within allowable bounds, the outcomes are displayed in Table 6.

Table 6: System suitability results for Imeglimin

S. No.	System suitability parameters	Results	Acceptance criteria
		Imeglimin	
1	%RSD of peak Area (n=6)	0.41	≤2
2	Retention time($R_{t)}$	5.731	>2
3	Theoretical plates(N)	13457	≥2000
4	Tailing factor	1.17866	≤2
5	Resolution (R)	1.1104	>2

Robustness:

The results of the adjustments made to the flow rate and detecting wavelength were compiled in (**Table 7).** % RSD of Imeglimin was found after variation in Flow rate to be 1.1 Imeglimin respectively, whereas and % RSD of Imeglimin after variation in Mobile phase was found to be 1.26 % - 1.41 % respectively and whereas % RSD of Imeglimin after variation in column changes was found to be 1.28 % - 1.48 %.

Table 7: Results of Robustness by variations in flow rate, Mobile phase and Column.

Para-meter	Value	Imeglimin	
		Area ± S.D (n=3)	% RSD
Flow-rate	0.8mL/min	2815397±4460.731	1.1
	1.0mL/min	2680111±887.6917	1.41
	1.2mL/min	2959810±30012.75	1.08
Mobile phase	18:82	2724601 ±4106.57	1.26
	20:80	2670031±963.6917	1.41
	22:78	3276092±60741.34	1.28
Column	1-C18 Luna	2670031±963.6917	1.41
	2-C18 Kinetex	2996661±4552.83	1.38

It was noted from Table 9, robustness results by changes in flow rate, mobile phase, and column that there was little variation in the tailing factor when these changes were made on purpose. Imeglimin tailing factor was discovered to be within acceptable bounds.

Assay

The proposed method was applied for the analysis of Tablet dosage form and the results of the assay were obtained within the specification limit. The % assay of Imeglimin 101.45 % respectively.

Table 8. Assay Data for Imeglimin

Drug	Label claim (mg)	Amount found (mg)	% Assay
Imeglimin	10	10.35	101.45 ± 0.127

CONCLUSION

The proposed HPLC method was found to be economical, simple, sensitive, accurate, precise, specific and robust and can be used for the routine quality control analysis of Imeglimin in Bulk and Marketed dosage forms.

ACKNOWLEDGEMENT:

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CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interests regarding the publication.

Abbreviation Used:

API: Active pharmaceutical ingredient, **RSD**: Relative standard deviation, **SD**: Standard deviations.

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