Bioanalytical Method Development and Validation of Antidiabetic Drug: Imeglimin Hydrochloride

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***Abstract*- A simple, rapid, sensitive, and cost-effective isocratic reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the bioanalytical estimation of Imeglimin Hydrochloride in human plasma. Imeglimin, a novel oral antidiabetic agent, belongs to the "glimins" class and exhibits a unique dual mechanism of action by enhancing insulin secretion and sensitivity. At a flow rate of 1.0 mL/min, the technique used a C18 column (Hypersil BDS, 250 × 4.6 mm, 5 µm) with a mobile phase made of methanol and ammonium formate buffer (80:20 v/v) with UV detection at 238 nm. The retention time of Imeglimin Hydrochloride was observed to be approximately 3.57 minutes, indicating a relatively short analysis time. The method was validated as per USFDA guidelines, demonstrating excellent specificity with no interference from blank plasma. Across a concentration range of 12–38 µg/mL, linearity was verified with a correlation coefficient (R2) of 0.9998. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.51 µg/mL and 1.53 µg/mL, respectively. Accuracy studies showed mean recoveries between 98.09% and 99.37%, while precision (intra- and inter-day) results displayed %RSD well within 2%, indicating reproducibility. The method was further proven robust and rugged through deliberate variations in analytical conditions such as flow rate, wavelength, operators, and reagent sources, with acceptable %RSD values confirming method reliability. Protein precipitation using methanol was employed for plasma sample preparation, ensuring efficient extraction. Due to its simplicity, low retention time, economic mobile phase, and high resolution, the developed RP-HPLC method is highly suitable for routine analysis and pharmacokinetic studies of Imeglimin Hydrochloride in biological matrices.**

***Keywords*- Imeglimin Hydrochloride, RP-HPLC, Bioanalytical, Development, Validation, Type 2 diabetes (T2D), % RSD.**

I. INTRODUCTION

A collection of metabolic diseases known as diabetes mellitus (DM) are typified by persistently high blood sugar levels brought on by deficiencies in either insulin secretion, insulin action, or both[1]. The most prevalent form is type 2 diabetes mellitus (T2DM), which accounts for more than 90% of all diabetes cases worldwide[2]. T2DM results from a combination of insulin resistance and progressive β-cell dysfunction[3]. According to the International Diabetes Federation, in 2021, approximately 537 million adults were living with diabetes, and the number is expected to rise to 643 million by 2030[4].

Long-standing hyperglycemia in diabetes is associated with various complications, including cardiovascular disease, nephropathy, retinopathy, and neuropathy[5]. These complications significantly affect the quality of life and increase mortality in diabetic patients. Therefore, managing blood glucose levels through pharmacologic and non-pharmacologic means is essential to prevent or delay the progression of complications[6].

Current pharmacological treatments for T2DM include metformin, sulfonylureas, DPP-4 inhibitors, GLP-1 receptor agonists, and SGLT2 inhibitors[7]. However, many patients fail to achieve adequate glycemic control despite combination therapies, highlighting the need for novel antidiabetic agents with different mechanisms of action. Moreover, existing treatments may be limited by side effects such as hypoglycemia, weight gain, gastrointestinal disturbances, and cardiovascular risks[8].

Imeglimin Hydrochloride: A New Antidiabetic Agent

Imeglimin Hydrochloride is chemically known as (6R)-(+)-4- dimethylamino-2- imino-6-methyl-1, 2, 5, 6 tetrahydro-1, 3, 5-triazine hydrochloride[8]. Imeglimin is an oral anti-diabetic drug that is marketed under the Twymeeg brand. Imeglimin Hydrochloride is the first drug from a new class of oral hypoglycemic agents known as “glimins.” It was approved in Japan in 2021 for the treatment of T2DM. 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[9].

Imeglimin has a unique dual mechanism of action: it improves both insulin secretion from pancreatic β-cells and insulin sensitivity in peripheral tissues[10]. The drug exerts its action at the mitochondrial level by modulating oxidative phosphorylation and reducing reactive oxygen species (ROS), which are implicated in the pathogenesis of insulin resistance and β-cell dysfunction. By targeting mitochondrial dysfunction, it acts on a root cause of T2DM rather than just controlling symptoms[11-12]. Several clinical trials have confirmed the efficacy and safety of Imeglimin Hydrochloride in patients with T2DM[13]. Interestingly, current research has demonstrated that it 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[11,12,14,15].

The literature survey revealed that various UV[16,17,18] and HPLC[19,20,21,22] methods had been reported for the estimation of Imeglimin Hydrochloride either individually or in combination with other drugs. Therefore, an economical RP-HPLC system was used to develop a simple bioanalytical method for estimation of Imeglimin Hydrochloride and validate as per USFDA guideline.

II. MATERIALS AND METHOD

Chemicals and Reagent

Ami Life Sciences Pvt. Ltd. provided a complimentary sample of Imeglimin Hydrochloride. HPLC Grade Water (Thermo Fisher Scientific India Pvt. Ltd.), Ammonium formate (Thermo Fisher Scientific India Pvt. Ltd.), Acetic acid (Thermo Fisher Scientific India Pvt. Ltd.), Hydrogen peroxide, Sodium hydroxide, Hydrochloric acid, Acetonitrile, Sodium acetate anhydrous (Finar), and Formic acid, Methanol (Gradient grade) (Thermo Fisher Scientific India Pvt. Ltd.).

Instrumentation

Chromatographic separation of the drug was performed on Agilent Technologies- HPLC system (1100) equipped with EZ Chrom Elite software with UV detector (730D) and Digisun pH meter. Separation was attained using C18 (Hypersil BDS) (4.6 mm × 250mm, 5µm) analytical column. Shimadzu Model-ATX224 electronic balance was used for weighing. UV detection at 238 nm at ambient temperature with a 20µl injection volume and flow rate of the detection of analyte was 1.0 ml/minutes.

Preparation of Mobile Phase

Buffer: Accurately weighed 0.63 gm of ammonium formate was dissolved in 900 ml of HPLC grade water and pH was adjusted to 3.5 using formic acid. After that, HPLC-grade water was added to get the volume up to 1000 ml.

Mobile Phase: A homogenous mixture of methanol:buffer in the ratio 80:20 was prepared and shaken well. The solution was then filtered using a 0.2 µm membrane filter and sonicated for 5 min.

Preparation of Stock Solution

Imeglimin Hydrochloride 25 mg API powder was accurately weighed and transferred in 100 ml volumetric flask. The drug was dissolved and diluted up to mark with mobile phase to obtain a standard stock solution of 250 µg/ml.

Extraction of Plasma and Sample Preparation

Frozen human plasma was thawed to ambient temperature. Aliquots of 200 µl plasma were taken into eppendorf tube and 1800 µl of stock solution was added and the plasma proteins were precipitated by using methanol. After one minute of vortexing the tube, the solution was centrifuged for ten minutes at 6000 rpm at 5 °C. After being extracted, the supernatant was sent to HPLC vials. The clear supernatant was filtered using 0.42 membrane filter paper prior to being fed into the HPLC apparatus.

Method Development

After finishing four experimental trials with variations in run time, column and mobile phase the drug observed to be in good peak shape at fourth trial. The % RSD, Tailing Factor and Theoretical plate shows that the drug is within the acceptance criteria. The approach was found to be satisfactory. The chromatogram is shown in figure 2,

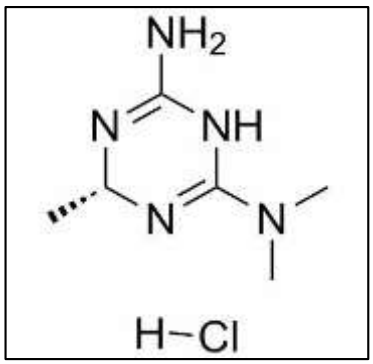

Fig. 1: Imeglimin Hydrochloride

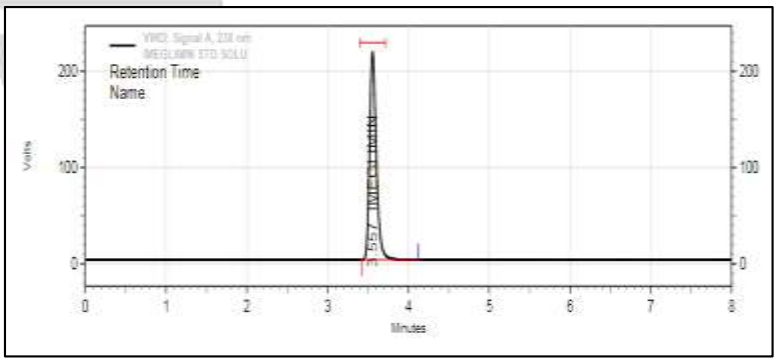

Fig. 2: Chromatogram of optimized method for Imeglimin Hydrochloride

Table 1: Optimized chromatographic conditions

Parameters	Result
Mobile phase	Buffer:MeOH (20:80)
Column	Hypersil, C18 (250 mm x 4.6 ID, Particle size: 5 um)
Flow rate	1.0 ml/min
Injection volume	20 µL
Temperature	Ambient
Wavelength	238 nm
Run time	8 minutes
Elution mode	Isocratic
Diluent	Mobile phase

III. RESULT AND DISCUSSION

Specificity

The ability to detect the target analyte precisely and particularly in the presence of other elements that might be anticipated to be present in the sample matrix is known as specificity. Specificity was evaluated by comparing the chromatogram of blank, plasma blank solution, standard solution and test solution.

Table 2: Specificity of Imeglimin Hydrochloride

Sr. No.	Solution	Retention time
1	Blank	0.00
2	Plasma Blank	0.00
3	Imeglimin Hydrochloride Standard Sample	3.57
4	Imeglimin Hydrochloride Test Sample	3.57

There is no interference found from the blank and plasma 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

To determine whether the chromatographic system's resolution and repeatability are sufficient for analysis, the system suitability test is utilized. Data was gathered from five replicate injections of the standard solution in order to conduct the test.

Table 3: System Suitability Study for Imeglimin Hydrochloride

Name	Area	RT (min)	TP (NLT 2000)	TF (NMT 2)
Standard_Inj_01	21059818	3.581	9191	1.34
Standard_Inj_02	20966855	3.576	9267	1.35
Standard_Inj_03	20987110	3.581	9258	1.36
Standard_Inj_04	21048038	3.576	9165	1.36
Standard_Inj_05	21036354	3.576	9321	1.34
Mean	21019635	3.578		
SD	40449.1436	0.0027		
%RSD (NMT 2)	**0.19**	**0.08**		

Theoretical plates and Tailing factor observed within acceptance criteria, also % RSD of replicate injections for area and retention time observed within acceptance criteria, here system is suitable for analysis of Imeglimin Hydrochloride.

Linearity

An analytical process is said to be linear if it can produce test findings that are exactly proportionate to the analyte concentration within a specified range. The linearity concentrations were used to calculate the slope, intercept values, and R2 value via the

regression equation (Y = mx + C). The regression equation is y = 819286x + 384831, with a correlation coefficient (R2) = 0.9998, which shows excellent linear correlation.

Table 4: Calibration Standards Peak Area

Conc. (ppm or ug/ml)	Area
12.50	10650978
18.75	15723405
25.00	20764509
31.25	26161244
37.50	31034739
Correlation coefficient (NLT 0.995)	0.9998
Intercept	384831
Slope	819286

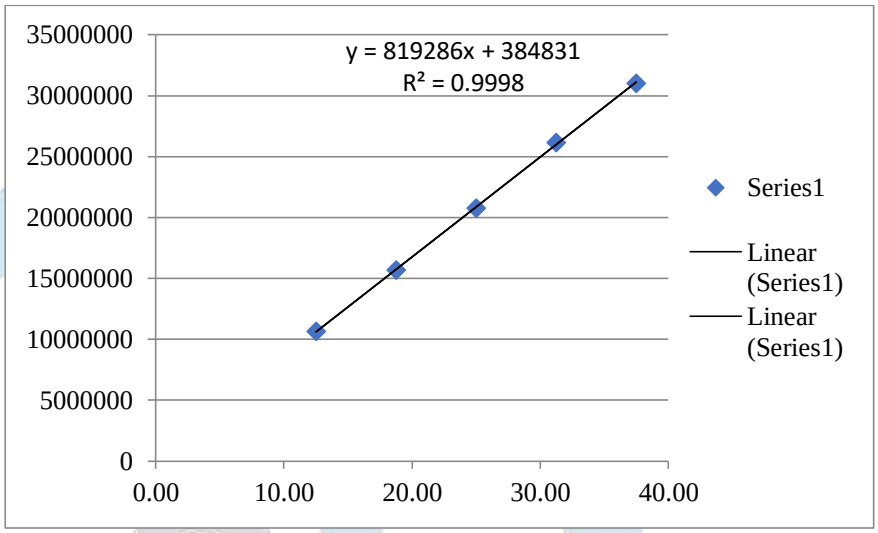

Fig. 3: Standard Calibration Curve for Imeglimin Hydrochloride

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentration of Imeglimin Hydrochloride in plasma in the range 12-38 µg/ml. Correlation coefficient was observed within acceptance criteria. Thus, the developed method is linear and linearity is justified.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD is the lowest concentration of the analyte that can be detected & LOQ is the lowest concentration that can be quantitatively measured based on the steyx and the slope. The LOD and LOQ were calculated using the following formulas: LOD = 3.3σ/s and LOQ = 10σ/s.

Table 5: LOD & LOQ of Imeglimin Hydrochloride

Conc. (ppm or ug/ml)	Area
12.50	10650978
18.75	15723405
25.00	20764509
31.25	26161244
37.50	31034739
Correlation coefficient ® (NLT 0.995)	0.9998
STEYX	125454
SLOPE	819286
LOD (ug/ml)	**0.51**
LOQ (ug/ml)	**1.53**

Accuracy

Accuracy is defined as the proximity of the obtained value to the true value. In the present study, the accuracy was checked by recovery studies, by addition of standard drug solution to pre-analyzed sample solution at three different concentrations at 80%, 100%, 120% level. The three samples were prepared for each recovery level, and the recoveries were calculated.

Table 6: Statistical Validation of Recovery Studies

Name	Preparations	Area	Amount added ug/ml	Amount recovered ug/ml	% Recovery (97-103)
Accuracy at 80 %	Prep-1	16541821	19.90	19.67	98.87
Accuracy at 80 %	Prep-2	16648497	20.10	19.80	98.51
Accuracy at 80 %	Prep-3	16578099	20.10	19.72	98.10
Accuracy at 100 %	Prep-1	20701518	25.10	24.62	98.09
Accuracy at 100 %	Prep-2	20679015	25.00	24.59	98.38
Accuracy at 100 %	Prep-3	20694659	25.00	24.61	98.45
Accuracy at 120 %	Prep-1	24982292	29.90	29.71	99.37
Accuracy at 120 %	Prep-2	24858542	30.00	29.57	98.55
Accuracy at 120 %	Prep-3	24816563	30.10	29.52	98.06

Accuracy Level	MEAN %	SD	%RSD (NMT 2)
Accuracy at 80 %	98.49	0.3850	0.39
Accuracy at 100 %	98.31	0.1899	0.19
Accuracy at 120 %	98.66	0.6644	0.67

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

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. It is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements.

Table 7: Result and Statistical Data for Intraday and Interday Precision

Intraday

Name	Preparations	% Assay
Set-1	Prep-1	98.78
	Prep-2	98.94
Set-2	Prep-1	98.23
	Prep-2	98.36
Mean		**98.58**
SD		**0.3369**
% RSD (NMT 2)		**0.34**

Interday

Name	Preparations	% Assay
Day-1	Prep-1	98.78
	Prep-2	98.94
Day-2	Prep-1	98.97
	Prep-2	98.58
Mean		**98.82**
SD		**0.1790**
% RSD (NMT 2)		**0.18**

Overall % RSD for Intraday and Interday results were observed within the acceptance criteria. Thus, the developed method is found to be precise, hence precision is justified.

Robustness

The robustness of analytical methods is a measure of its ability to remain unaffected by small, but deliberate change in method parameters and provides an indication of to remain normal during usage. Robustness tests examine the impact of operational parameters on the analysis results. In the present study robustness was checked by carrying out changes in parameters of analysis such as change in flow rate and change in wavelength.

Table 8: Robustness of Imeglimin Hydrochloride

Robustness changes in method parameters	Preparations	% Assay
Original method parameters	Test prep-1	98.78
Original method parameters	Test prep-2	98.94
Flow rate 0.95 ml/min	Test prep	98.76
Flow rate 1.05 ml/min	Test prep	100.44
Wavelength 236 nm	Test prep	100.04
Wavelength 240 nm	Test prep	98.43
Mean		**99.23**
SD		**0.8084**
%RSD (NMT 2)		**0.81**

Overall % RSD of results with change in flow rate and wavelength were observed to fall within acceptance criteria. Thus, the developed method was found to be robust in terms of slight change in internal method parameters, hence robustness is justified.

Ruggedness

Ruggedness refers to the ability to obtain reproducible results under various conditions that include the use of varied instruments and analysts in real-world situations. Three following conditions were examined. The developed method was assessed for two different operators in the same lab, and changing sources of reagent and solvent.

Table 9: Ruggedness of Imeglimin Hydrochloride

Ruggedness changes in method parameters	Preparations	%Assay
Original method parameters	Test prep-1	98.78
Original method parameters	Test prep-2	98.94
Change in operator	Test prep	98.78
Change in source of reagent	Test prep	99.88
Mean		**99.10**
SD		**0.5287**
%RSD (NMT 2)		**0.53**

Overall % RSD of results with change in source of reagent and change in operator were observed to fall within acceptance criteria. Thus, the developed method was found to be rugged in terms of slight change in external method parameters, hence ruggedness is justified.

IV. CONCLUSION

A simple, selective, stable, and accurate isocratic RP-HPLC bioanalytical method was successfully developed and validated for the quantitative analysis of Imeglimin Hydrochloride. The method demonstrated excellent linearity, precision, specificity, accuracy, and robustness, ruggedness in accordance with ICH guidelines. Its advantages include a shorter retention time, isocratic elution, and the use of a cost-effective and readily available mobile phase, standard UV detection, and high peak resolution. These features make the method highly suitable for routine quality control and stability testing. Therefore, the proposed method offers a reliable, efficient, and practical bioanalytical tool for the routine analysis of Imeglimin Hydrochloride in plasma.

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