Development and Validation of Stability-indicating HPTLC for Determination of Imeglimin Hydrochloride as Bulk Drug and in Tablet Formulation

Padmanabh B. Deshpande*, Ankit Rathod, Nikita Bhadane, Shreyas Raut
All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Pharmaceutical Quality Assurance, Kennedy Road, Near RTO, Pune-411001

***Abstract*—A simple, precise, and sensitive stability-indicating High-Performance Thin Layer Chromatography (HPTLC) method was developed and validated for quantitative estimation of Imeglimin hydrochloride as bulk drug and in tablet formulation. The separation was achieved on precoated silica gel 60 F_{254} TLC plates using Chloroform: Ethyl acetate: Methanol (6: 1.5: 2.5, v/v/v) as the mobile phase. Densitometric scanning was performed at 241 nm. Forced degradation studies revealed the susceptibility of Imeglimin under all analyzed stress conditions. The method that was optimized has been inspected for linearity, accuracy, precision, limit of detection, limit of quantification, and robustness according to ICH guidelines. The developed method exhibited good linearity over a concentration range of 200-1200 ng band^{-1} with correlation coefficient (R^2) of 0.996. Limit of detection and quantitation were found to be 14.54 ng band^{-1} and 44.07 ng band^{-1}, respectively. The % drug content in commercial tablets was determined with satisfactory recovery values and %RSD <2.0, fulfilling ICH guidelines. This validated method is suitable for routine quality control analysis of Imeglimin hydrochloride.**

***Index Terms*- Imeglimin hydrochloride, HPTLC, Stability Studies, Validation**

I. INTRODUCTION

Imeglimin Hydrochloride, chemically named (4R)-6-N, 6-N, 4-trimethyl-1, 4-dihydro-1, 3, 5-triazine-2, 6-diamine hydrochloride, is a first-in-class oral antidiabetic agent belonging to the 'glimins', a novel class of compounds containing a tetrahydrotriazine ring [1]. Imeglimin has been developed to target multiple pathophysiological mechanisms underlying Type 2 Diabetes Mellitus (T2DM), including mitochondrial bioenergetics, insulin resistance, β-cell dysfunction, and hepatic glucose output [2]. A thorough study of the literature showed that analytical methods such as Spectrophotometry [3-5], High Performance Liquid Chromatography (HPLC) [6-8], Ultra Performance Liquid Chromatography [9] has been reported for the determination of Imeglimin in pharmaceutical dosage form. One HPTLC method for Imeglimin has been claimed by Suresh Kumar K et al. [10] involving only method optimization and validation. The above study was not targeted for stability study. The less amount of literature provides the need for developing a new suitable stability indicating densitometric method for determination of Imeglimin. Based on this fact, an attempt was made in this study to develop and validate a sensitive stability-indicating HPTLC method for estimation of Imeglimin in bulk and pharmaceutical formulation with acceptable degradation in accordance with International Conference on Harmonization Guidelines.

II. MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standard Efonidipine hydrochloride ethanolate and Metoprolol was obtained as gift sample from Zuventus Healthcare Ltd. (Mumai, India). The pharmaceutical dosage form used in this study was Efnocar-MX tablets labelled to contain 25 mg of Efonidipine hydrochloride ethanolate and 40 mg of Metoprolol were procured from the local market. Ethyl acetate, Toluene, Methanol, Chloroform (All AR grade) were obtained from Thomas Baker Pvt Ltd (Mumbai, India). Ethyl acetate was obtained from Loba Chemie Pvt Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

The drug was separated chromatographically on Merck TLC plates that were precoated with silica gel 60 F_{254} plates (10 cm × 10 cm with 250 µm layerthickness) from E. MERCK, (Darmstadt, Germany) using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag

twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Chloroform: Ethyl acetate: Methanol (6: 1.5: 2.5, v/v/v) as mobile phase. The mobile phase was saturated in the chamber for 20 min. After development, TLC plates were dried in a current of air with the help of a hair drier. The CAMAG thin layer chromatography scanner III was used to scan for all developments using WinCATS software version 1.4.2 and was scanned at 241 nm. A deuterium lamp was the source of radiation that emitted UV spectrum between 200 and 400 nm.

Preparation of standard stock solution

Standard stock solution of Imeglimin was prepared by dissolving 10 mg of drug in 10 mL of methanol to get concentration of 1000 µg mL^{-1} from which 1 mL was further diluted with methanol to achieve final concentration of 100 ng μL^{-1} for the drug.

Analysis of marketed formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of tablet powder equivalent to 10 mg of Imeglimin was weighed and transferred to 10 mL volumetric flask containing 7 mL of methanol. The contents were sonicated for 20 min, filtered and volume was made with methanol. From this stock solution, 1 mL was further diluted to 10 mL with methanol. 4 µL volume of this solution was applied on TLC plate to obtain final concentration of 400 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 241 nm. The amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drug

The stability studies were performed by subjecting the standard solution of bulk drug to the physical stress (hydrolysis, peroxide, heat and light) and stability was accessed. The stress degradation studies were carried out at initial drug concentration of 1000 ng μL^{-1}. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 100 ng $band^{-1}$. The oxidative degradation was carried out in 30 % H_2O_2 and the sample was diluted with methanol to obtain solution having concentration 100 ng $band^{-1}$. Thermal stress degradation was performed by keeping the solid drugs individually in oven at 80ºC for a period of 4 h. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h $square\ meter^{-1}$. Thermal and photolytic samples were diluted with methanol to get the concentration of 100 ng $band^{-1}$ of the drug.

100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in

the mobile phase comprising Chloroform: Ethyl acetate: Methanol (6: 1.5: 2.5, v/v/v) was selected as optimum to attain well-defined and resolved peak for the drug. The retention factor was found to be 0.37 ± 0.05.

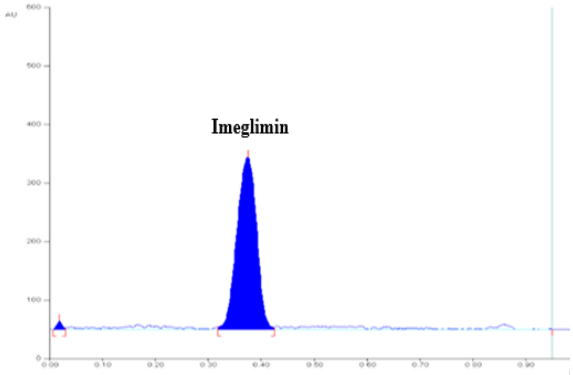

Fig 1: Densitogram of standard solution of Imeglimin (800 ng $band^{-1}$, Rf= 0.37 ± 0.05)

Forced degradation studies

The stress degradation study of Imeglimin hydrochloride demonstrated its susceptibility to hydrolytic, oxidative, and thermal as well as photolytic stress conditions. Significant degradation was evident under alkaline and thermal stress, as indicated by a noticeable reduction in peak area. Peak purity values greater than 995 confirmed that the Imeglimin hydrochloride peaks remained spectrally homogeneous under all stress conditions, affirming the stability-indicating capability of the developed analytical method. The findings of degradation studies are represented in Table 1.

Table 1: Forced degradation studies

Stress conditions/ duration	% degradation
Acid / 0.1N HCl Kept at RT for 2 h	09.71
Alkali/0.05 N NaOH Kept at RT for 2 h	22.29
Oxidative /30 % H_2O_2 Kept at RT for 30 min	11.25
Dry heat/ 80ºC/ 4 h	28.42
Photolysis UV light	10.13
Fluorescence light	09.88

Method Validation

The optimized method was validated in accordance with ICH guidelines with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.

III. RESULTS AND DISCUSSION

The main aim in developing this stability indicating HPTLC method is to achieve the satisfactory resolution of drug and also from its degradation products. To obtain better separation, a number of method trials were conducted initially using different mobile phases. Finally + 1260.1. The 3D Densitogram demonstrating linearity is shown in Fig. 2. The calibration curve, obtained by plotting the amount of drug spotted (ng band^{-1}) versus peak area, is displayed in Fig. 3.

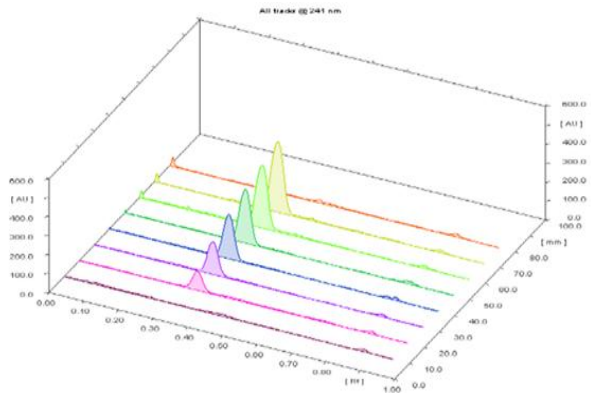

Fig.2: 3D spectra of linearity in concentration range (200-1200 ng band^{-1})

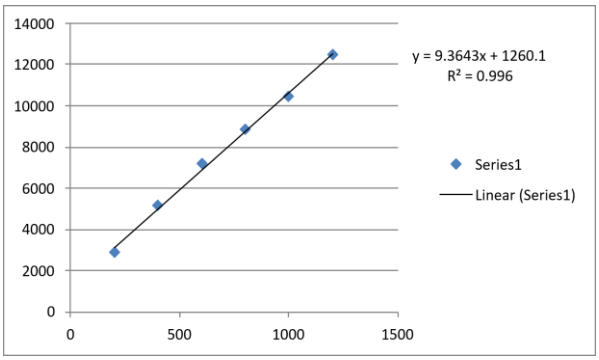

Fig.3: Calibration curve of Imeglimin

Precision

Set of three different concentrations in three replicates of standard solutions of Imeglimin were prepared. All the solutions were analyzed on the same day and on three consecutive days in order to record intra-day and inter-day variations in the results. The % R.S.D. values for intra-day and inter-day precision were found to be in the range of 1.08 to 1.33 and 1.59 to 1.89, respectively. The smaller values of % R.S.D. obtained indicate that the developed method is precise.

Limit of detection (LOD) and Limit of quantitation (LOQ)
LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 400 ng band-1 from tablet solution. The drug concentration was calculated from linearity equation. The mean % recovery was found to be 100.42±1.23 which indicated the accurateness of developed method for estimation in tablet

Linearity

A standard solution of Imeglimin (100 ng µL^{-1}) was applied to the TLC plate in volumes of 2, 4, 6, 8, 10, 12 µL resulting in spotted amounts ranging from 200 to 1200 ng band^{-1}. The correlation coefficient was determined to be 0.996 with the equation y= 9.3643x dosage form.

Robustness

Robustness of the method was determined by making deliberate variations in method parameters. The parameters, such as the wavelength (± 1 nm) and composition of the mobile phase (± 1% methanol), were changed, and the impact on the drug area was observed. The areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

IV. CONCLUSION

Stability indicating high performance thin layer chromatography method developed for the estimation of Imeglimin hydrochloride as bulk drug and in tablet formulation sensitive, precise and accurate. The drug (Imeglimin hydrochloride) was prone to hydrolysis, oxidative, thermal and photolytic stress conditions. The degradation achieved for drug under tested stress conditions was within acceptable limit as proposed by ICH. Validation results confirmed that the values achieved for all parameters were in the limit according to ICH guidelines. Compared with HPTLC method reported [10], the established method is more sensitive as the range for the method developed starts from 200 ng band^{-1} whereas range starts from 1000 ng band^{-1} for reported method. The developed and validated sensitive analytical method will ensure the reliability and accuracy of results, especially in regulated industries like pharmaceuticals, where quality control is paramount. The proposed stability demonstrating

plot. LOD and LOQ were found to be 14.54 ng band^{-1} and 44.07 ng band^{-1}, respectively.

REFERENCE

[1] Fouqueray P, Bolze S, Lubrano-Berthelier C, Hallakou-Bozec S. (2014) Imeglimin–A novel oral antidiabetic that targets the three key defects of type 2diabetes. J Diabetes Complications. 28(4): 434-442.

[2] Hallakou-Bozec S, Bolze S, Fouqueray P. Imeglimin amplifies glucose-stimulated insulin secretion and improves β-cell function in models of Type 2 Diabetes. Cell Rep. 34(5): 108-113.

[3] Vachala SD, Manjunath GV, Pravat Ranjan B, Shashi Prakash R, Srilakshmi KT, Yashodha KJ. (2023) Development and validation of Imeglimin hydrochloride by UV/Visible spectrophotometric method. International Journal of Research and Analytical Reviews. 10(4): 57-67.

[4] Sathawane YJ, Thakare S, Awachat A, Umekar MJ. Development of novel UV-Spectrophotometric method for imeglimin hydrochloride. International Journal of Pharmaceutical Research and Applications. 1637-1644.

[5] Sangavi G, Premalatha TK, Ilakiya M, Akshaya devi R and Kokilambigai KS. (2025) Multivariate analysis for spectrophotometric estimation of imeglimin hydrochloride in bulk drug and pharmaceutical formulations. IJBPAS, 14(3): 1393-1403.

[6] Mubeen G, Sharanabasava N, Lalitha N. (2024) RP-HPLC Method for Determination of Imeglimin Hydrochloride in Bulk and Tablet Formulation. Asian Journal of Pharmaceutical Research and Development. 12. 92-96.

[7] Chikhale H, Ambekar Y, Avhad S, Borse L. (2024) Development and validation of RP-HPLC method for determination of antidiabetic drug (imeglimin HCl) in bulk and its dosage form. Journal of Chemical Health Risks. 14(4): 675-685.

[8] Jahagirdar S, Godge R, Vikhe S, Bornare S. (2024) Estimation of Imeglimin in pharmaceutical tablets by RP-HPLC. International Journal of Drug Delivery Technology. 14(2):724-726.

method may be utilized by pharmaceutical industry for routine analysis and also for checking the stability of drug as bulk and in its formulations.

[9] Jain A, Soni L, Sharma R. (2023) 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. 15(6): 211-217.

[10] Suresh Kumar K, Sajin Arokia Raj M, Arivukkarasu R, Kiruthiga N, Dhinesh Kumar S. (2024) Method development and validation of imeglimin hydrochloride using high-performance thin-layer chromatography in bulk and tablet dosage form. Int. J. of Pharm. Sci. 2(8): 3445-3453.

[11] ICH Guideline Q2A (R1), Validation of analytical procedure, text and methodology Q2 (R1) (2005)

[12] ICH Guideline Q1A (R2), Stability testing of new drug substances and products Q1A (R2) (2003).