STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR IMEGLIMIN HCL IN PHARMACEUTICAL DOSAGE FORM

ABSTRACT:

Background: Imeglimin HCL is a medication used in pharmaceutical products. To ensure its quality and effectiveness, it's crucial to have a reliable method to measure its concentration in these products. High-Performance Liquid Chromatography (HPLC) is a common technique for this purpose.

Objective: This study aimed to develop and validate a precise and accurate stability indicating HPLC method for measuring Imeglimin HCL in pharmaceutical formulations.

Methods: The method optimization process included selecting a suitable chromatographic column and determining the optimal mobile phase composition. A Credchrom C18 column (250mm x 4.6 mm x 5µm) was chosen, and a mobile phase consisting of Phosphate Buffer and acetonitrile (80:20, v/v) was identified as optimal. The selected conditions provided satisfactory resolution and a retention time of 2.5 minutes for Imeglimin HCL, with detection achieved at a wavelength of 241nm. Validation parameters, including linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), were thoroughly evaluated. Precision was assessed through %RSD values for repeatability and intra-day and inter-day precision. Accuracy was determined by % recovery, with values ranging from 98.00% to 102%. Subsequently, force degradation studies were conducted to further assess the method's stability.

Results: The developed RP-HPLC method exhibited excellent linearity (R^2 close to 1) and sensitivity, with LOD and LOQ values of 0.577722 µg/ml and 1.750673 µg/ml, respectively. Precision, as indicated by %RSD values, ranged from 0.994733 for repeatability to 0.988377–0.7480963 for intra-day precision and 0.988377–10.9883477 for inter-day precision. Accuracy, reflected by % recovery, was within acceptable limits (98.62–100.34%). These results, along with successful force degradation studies, confirmed the method's reliability and stability.

Conclusions: In conclusion, the developed RP-HPLC method offers a sensitive, precise, and accurate means for the estimation of Imeglimin HCL in pharmaceutical formulations. Its robustness and stability make it suitable for routine analysis in pharmaceutical laboratories, ensuring the quality and efficacy of Imeglimin-containing products.

Keywords: Imeglimin HCL, RP-HPLC, Method Development, Method Validation, Force Degradation Studies, ICH guidelines.

INTRODUCTION:

An Antidiabetic drug is any medication that helps lower high blood sugar levels, a hallmark of diabetes mellitus. Diabetes arises from the body's inability to produce or effectively use insulin, a hormone vital for controlling blood glucose levels.

There are two main types:

- Type 1 Diabetes.
- Type 2 Diabetes.

Type 1 diabetes happens when your body doesn't make insulin, a hormone that helps your cells use sugar for energy. It usually starts in childhood or young adulthood, and people with Type 1 diabetes need to take insulin every day to stay healthy. Type 1 diabetes, constituting 5-10% of cases, results from the immune system attacking and destroying pancreatic beta cells, requiring insulin therapy for management [1].

Type 2 diabetes is more common and often develops later in life. With Type 2, your body either doesn't make enough insulin or can't use it effectively. This can be managed with lifestyle changes like healthy eating, exercise, and sometimes medication. Type 2 diabetes, comprising 85-90% of cases, typically affects adults but can occur at any age. In type 2 diabetes, the pancreas produces insulin, but the body's cells become resistant to its effects, leading to elevated blood sugar levels [2]. This resistance causes insulin to be less effective in regulating glucose, necessitating various treatment approaches such as lifestyle changes, oral medications, and sometimes insulin therapy.

Imeglimin Hydrochloride, a newly approved oral anti-diabetic drug, falls under the "Glimins" class. Its chemical structure is (R)-6-imino-N, N,4-trimethyl-1,4,5,6-tetrahydro-1,3,5-triazin-2-amine hydrochloride, with a molecular formula of C6H14ClN5 and a weight of 191.66 g/mol. Imeglimin primarily acts on mitochondria, improving their energy production and protecting pancreatic β-cells. It reduces liver glucose production, boosts insulin secretion from pancreatic cells, and enhances muscle glucose uptake. By targeting defective cellular energy metabolism, Imeglimin addresses a core issue in type 2 diabetes mellitus [3].

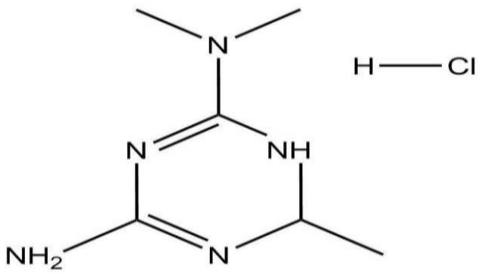

Fig 1 - Chemical Structure of Imeglimin HCL

MATERIAL AND METHOD –

Pharmaceutically active ingredient (Imeglimin HCL) was kindly obtained as a gift from Zydus pharmaceutical pvt. Ltd. Acetonitrile (HPLC grade) was purchased from SD fine-chem ltd. Chemicals and solvents, (analytical grade) and HPLC water were purchased from Loba Chemie. All solvents and solutions were filtered through a Millipore 0.45 µm filter and sonicated.

METHOD OPTIMIZATION –

The objective of this study was to develop a sensitive, precise, and accurate method RP-HPLC (Shimadzu HPLC) with photodiode array detector method for analyzing drugs in pharmaceutical dosage forms. To achieve this, various tests were conducted to optimize the method under isocratic conditions. This involved experimenting with different mobile phase compositions and C18 columns of varying lengths to achieve satisfactory resolution of Imeglimin HCL. Ultimately, the Credchrom C18 column (250mm x 4.6 mm x 5µm) was selected for providing both satisfactory resolution and runtime. A series of aqueous mobile phases containing phosphate buffer and acetonitrile in a ratio of 80:20 % v/v were tested. Additionally, the analysis of the drugs was performed at different wavelengths (220, 225, 241, and 256 nm), and 241nm is selected as the appropriate wavelength for estimation of Imeglimin HCL was determined by overlaying the UV spectra of the drugs. Following optimization of the mobile phase and wavelength, the flow rate was set at 1 ml/min, and the retention time for Imeglimin HCL was determined to be 2.5 minutes.

1. **Preparation of mobile phase:**

   Prepare a mobile phase consisting of phosphate buffer adjusted to ph. 4.2 and acetonitrile in a ratio of 80:20 (v/v). Transfer the prepared phosphate buffer and acetonitrile separately into containers. Subject both solutions to sonication for 15 minutes before use in HPLC analysis.

2. **Preparation of phosphate buffer:**

   Dissolve monosodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4) in distilled water to achieve desired concentrations for the final volume. Mix the solutions appropriately, adjusting the ph. with a ph. meter up to ph. 4.2.

3. **Preparation of standard stock solution 1 of Imeglimin HCL (1000ppm):**

   Accurately weigh 50 mg of Imeglimin HCL API working standard and transfer it to a 50 ml volumetric flask. Add 75% of the diluent (HPLC water) and dissolve thoroughly. Adjust the volume up to 50 ml with diluent and sonicate the solution for 15 minutes.

4. **preparation of standard stock solution 2 of Imeglimin HCL (100ppm):**

   measure accurately 1 ml of standard stock solution 1 of Imeglimin HCL and transfer it to a 10 ml volumetric flask. Add 75% of the diluent and dissolve properly. Adjust the volume up to 10 ml with diluent and sonicate the solution for 15 minutes.

METHOD VALIDATION –

Method validation in Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) serves to ascertain the appropriateness of an analytical method for its intended application, thereby ensuring the attainment of requisite levels of accuracy, precision, and reliability. This process

encompasses method development, optimization, and validation adhering to International Conference on Harmonisation (ICH) guidelines. Method development entails the systematic selection of the mobile phase, chromatographic column, and detector based on the physicochemical properties of the analyte, such as polarity and functional groups. Validation stages encompass the comprehensive assessment of key parameters including accuracy, specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ). RP-HPLC is widely employed in pharmaceutical analysis, facilitating the detection, separation, and quantification of chemical compounds. Method validation plays a pivotal role in ensuring the quality, safety, and efficacy of pharmaceutical products, while also enabling the evaluation of clinical responses. [4].

1. **Linearity –**

   Prepare an aliquot of 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 ml were pipette out from the above stock solutions and transferred into a 10 ml volumetric flask and volume was make up with diluents 05, 10, 15, 20, 25, 30, 40, and 50 µg/ml of Imeglimin HCL respectively [5].

2. **Specificity –**

   Weigh accurately about 50mg of Imeglimin HCL working standard to a 50ml volumetric flask. Dissolve it in 75% of diluent and sonicate it. Make up to 50 ml mobile phase.

3. **Limit of detection (LOD) –**

   The limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected, though not necessarily quantitated. It is a limit test that specifies whether or not an analyte is above or below a certain value [6].

4. **Limit of quantitation (LOQ) –**

   The limit of quantitation (LOQ) is defined as the lowest concentration of the analyte in a sample that can be determined *with acceptable* precision and accuracy under the stated operational conditions of the method.

5. **Precision –**

   The precision of this method was evaluated in terms of repeatability, intraday precision (within a day), and interday precision (across three consecutive days) [7].

6. **Accuracy –**

   To get a concentration of 80%, 100%, 120% of drug, pipette out 2ml and 3ml, of mixed standard stock solution into separate 10 ml volumetric flask and volume is made with mobile phase. Further dilute 3ml this solution to 10ml with mobile phase [8].

7. **Robustness –**

   Robustness assesses the method's reliability when small variations are introduced intentionally in parameters such as ph, temperature, and mobile phase composition [9]. A robust method remains effective despite minor changes. The impact of changes in detection wavelength, within ±5 nm, was specifically studied [10].

8. **Ruggedness –**

   To determine the degree of reproducibility of the results by this method involved the studies of the analyst to analyst and day to day; that is to carry out precision study in six

replicates of an assay of a single batch sample by two different analysts on two different days [11].

9. Forced degradation studies

Forced degradation studies, also known as stress degradation studies, were conducted to assess the stability of Imeglimin HCL according to ich guidelines. The drug was subjected to various stress conditions including acidic, alkali, oxidative, thermal, and photolytic conditions [12].

- **Acidic degradation –**

Untreated Imeglimin HCL solution was withdrawn from a stock solution of 1000 µg/ml and diluted to 100 µg/ml in a 10 ml volumetric flask. 0.1n HCL was added, and the solution was refluxed for 1 hour at 80°c. After cooling, the solution was neutralized with 0.1N NAOH and diluted to volume with diluent [13].

- **Alkali degradation –**

Untreated Imeglimin HCL solution was withdrawn from a stock solution of 1000 µg/ml and diluted to 100 µg/ml in a 10 ml volumetric flask 0.1n NaOH was added, and neutralization was done with 0.1n HCL and the volume made up to 10ml [12,13].

- **Oxidative degradation** –

  1 ml of stock solution was mixed with 1 ml of 3% h_2o_2 and allowed to react for 2 hours at 80°c. The volume was then adjusted to 10 ml with diluent [13].

- **Photolytic degradation –**

  Imeglimin HCL powder was exposed to sunlight for 2 days, after which a 10 µg/ml sample solution was prepared and injected into the HPLC system [14].

- **Thermal degradation –**

  Imeglimin HCL powder was exposed to 80ºc for 48 hours. A diluted sample was then injected into the HPLC system to measure peak height, area, and retention time [14,15].

RESULT AND DISCUSSION

- **Spectrum of Imeglimin HCL –**

  The standard solution was scanned from 200-400nm on UV spectrophotometer and from spectrum the 241 nm as maximum wavelength was selected for study.

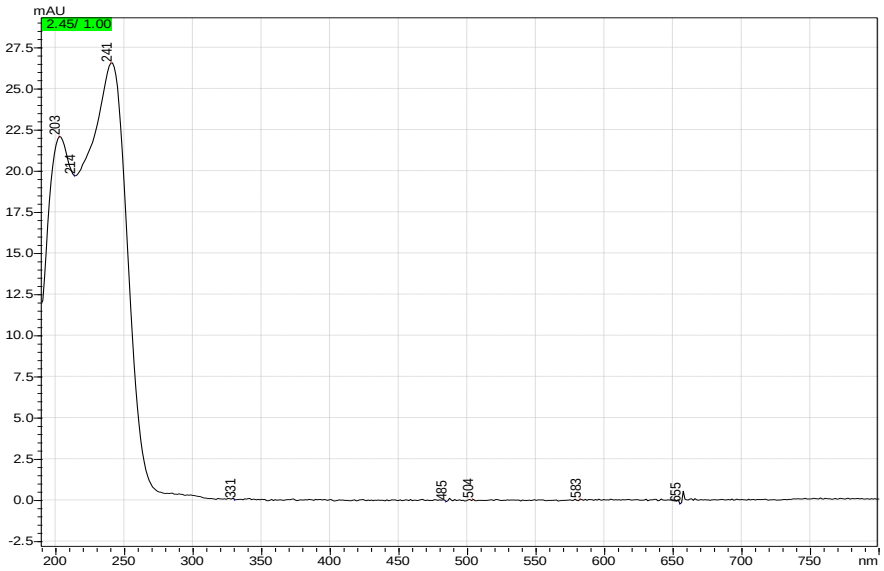

Fig 2 – spectrum of Imeglimin HCL

- **Linearity –**

Eight standard solutions from 05 to 50 µg/ml strength (05, 10, 15, 20, 25, 30, 40, and 50) were analyzed for linearity in HPLC. Standard stock solution is 0.1 mg/ml concentration, R^2 is 0.9991 indicates strong association and RSD% is 2% for peak regions ensures consistency (Fig 3,4) (Table 1).

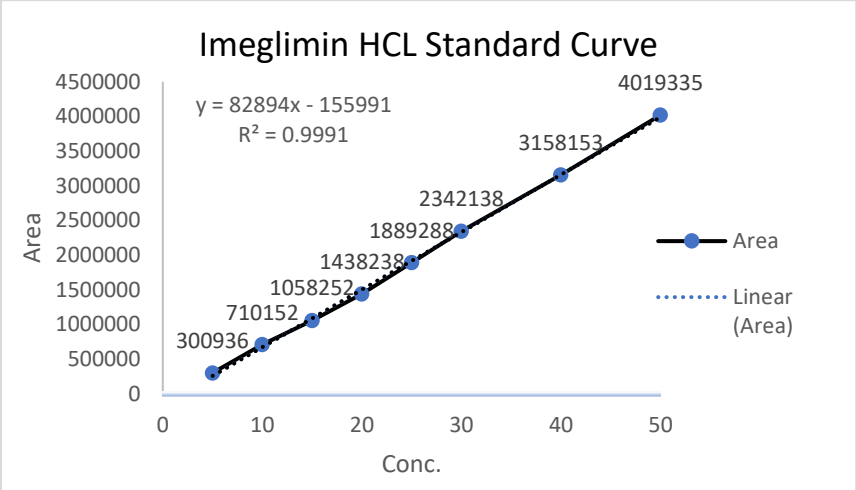

Fig 3 – Calibration Curve of Imeglimin HCL

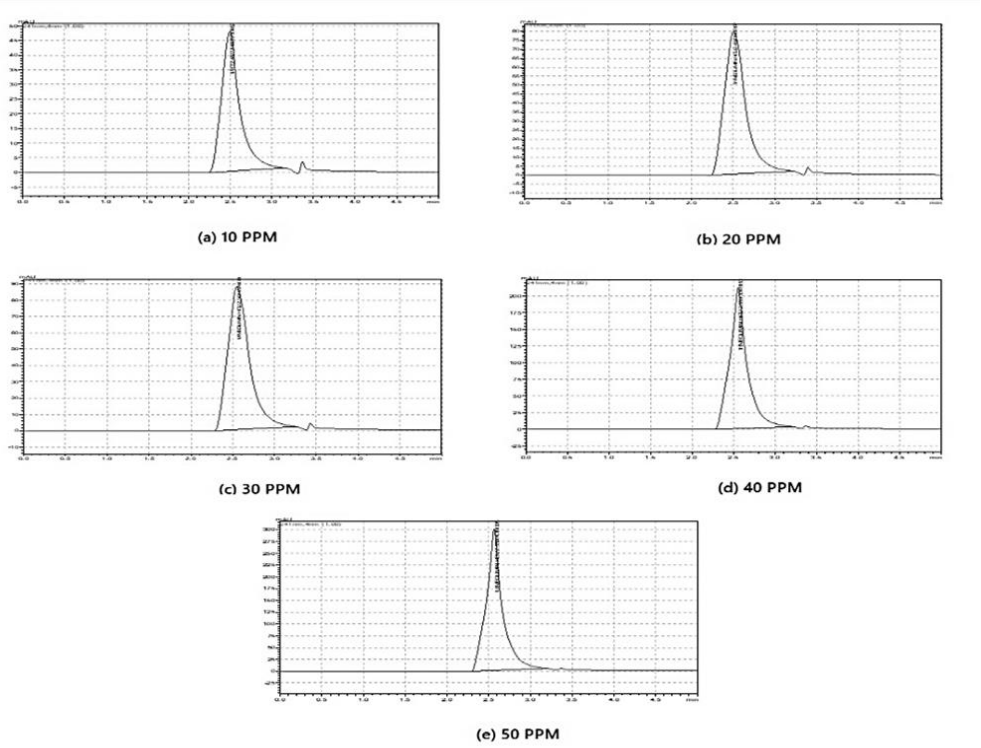

Fig 4 – linearity area of Imeglimin HCL

Sr No.	Conc. (µg/ml)	Area
1	05	300936
2	10	710152
3	15	1058252
4	20	1438238
5	25	1889288
6	30	2342138
7	40	3158153
8	50	4019335

Table 1 – linearity area of Imeglimin HCL

- **Accuracy –**

To check how accurate our proposed method is, we tested it by adding different amounts of a standard drug to the product at 80%, 100%, and 120% of its concentration.

Then, we estimated the contents using an assay method [14]. The percentage of recovery for Imeglimin HCL falls within a specific range (Table 2).

We got the accuracy in range in between 98% to 101%

Conc. (µg/ml)	Spiked	Total	Amount Recovery	% Recovery
20	16	36	35.78	99.38
20	16	36	36.24	100.66
20	16	36	35.58	98.83
20	20	40	39.45	98.62
20	20	40	39.85	99.62
20	20	40	40.02	100.05
20	24	44	44.15	100.34
20	24	44	43.74	99.40
20	24	44	43.59	99.06

Table 2 – Accuracy of Imeglimin HCL

- **LOD and LOQ –**

The LOD shows the lowest analyte concentration reliably detectable with confidence in an analytical method.

The LOQ establish the lowest concentration of the analyte that can be accurately quantified with sufficient precision and accuracy (Table 3).

DRUG	LOD	LOQ
Imeglimin HCL	0.577722 µg/ml	1.750673 µg/ml

Table 3 – LOD AND LOQ of Imeglimin HCL

- **Precision –**

In the precision validation method, we conducted repeatability, intra-day, and inter-day precision tests. These analyses revealed slight variations in the results, which nonetheless met the acceptance criteria. This demonstrates the method's capability to produce consistent and reliable results, confirming its suitability for pharmaceutical laboratories (table 4,5,6).

Sr. No	Peak Area	Mean	SD	%RSD
1	1388138			
2	1406157			

3	1415411	1412930.5	14054.89	0.994733
4	1418313			
5	1422425			
6	1427139			

Table 4 – Repeatability

Sr. No.	Peak Area (20 µg/ml)	Peak Area (30 µg/ml)	Peak Area (40 µg/ml)
1	1388138	2342138	3158153
2	1406157	2366459	3169456
3	1415411	2376498	3188145
Mean	1403235.3	2361698.3	3171918
SD	13869.255	17667.777	15146.818
%RSD	0.988377	0.7480963	0.4775287

Table 5 – Intra- Day Precision

Sr. No.	Peak Area (Day 1)	Peak Area (Day 2)	Peak Area (Day 3)
1	1388138	1388132	1388128
2	1406157	1406148	1406142
3	1415411	1415406	1415401
Mean	1403235.3	1403228.7	1403223.7
SD	13869.255	13869.378	13868.729
%RSD	0.988377	0.9883904	0.9883477

Table 6 – Inter - Day Precision

- **Robustness**

  The Robustness of method validated for the slight change in flow rate, slight change in mobile phase composition and change in wavelength. From the observations it was found that method is robust with slight change in chromatographic parameter as shown in [Table 7].

Change in Flow Rate						
Sr. No	**Flow Rate (ml/min)**	**Retention Factor**	**Peak Area**	**Mean**	**SD**	**%RSD**
1	0.8	2.564	1439946	1438536	1287.14	0.089476
2	1	2.486	1438238			
3	1.2	2.438	1437424			
Change in Mobile Phase						
Sr. No	**Ratio**	**Retention**	**Peak**	**Mean**	**SD**	**%RSD**

	(V/V%)	Factor	Area			
1	80:20	2.482	1434652	1435538	821.429	0.057221
2	75:25	2.647	1435689			
3	60:40	2.846	1436274			
Change in Wavelength						
Sr. No	**Wavelength (Nm)**	**Retention Factor**	**Peak Area**	**Mean**	**SD**	**%RSD**
1	236	2.491	1404648	1426711	10962.3	0.76836
2	241	2.491	1418959			
3	246	2.491	1434462			

Table 7 – Robustness

- **Ruggedness**

  In experimental conditions such as changes in equipment, analysts, reagents, and environmental factors [15]. When establishing acceptance criteria for ruggedness in method validation, it's essential to consider the potential sources of variability that could affect the performance of the method [16].

- **Forced Degradation Studies**

  In the force degradation studies, it was found that acidic degradation resulted in 6.19%, alkali degradation resulted in 24.93%, oxidation degradation resulted in 17.50%, light degradation resulted in 10.01%, and thermal degradation showed 0% degradation. Our peak was not affected by any of the degradant parameters, and all were well resolved (Fig 5) (Table 8).

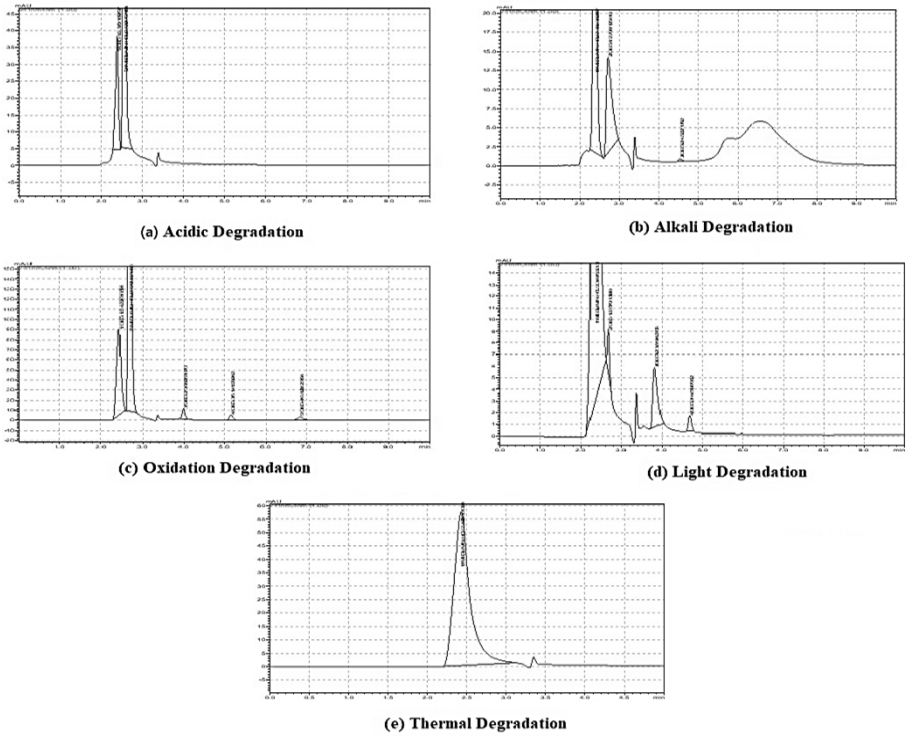

Fig 5 – Forced Degradation Studies of Imeglimin HCL in (a) Acidic degradation, (b) Alkali Degradation, (c) Oxidation Degradation, (d) Light Degradation and (e) Thermal degradation

Sr. No.	Degradation Conditions	%Drug Undegraded	%Drug Degraded
1	Acidic	93.81	6.19
2	Alkali	75.07	24.93
3	Oxidation	82.50	17.50
4	Light	89.99	10.01
5	Thermal	100	0

Table 8 - Forced Degradation Studies of Imeglimin HCL

ASSAY –

An analytical method was developed to determine the content of Imeglimin in Imeglyn -500 mg tablets by Zydus pharmaceutical Pvt. Ltd. Twenty tablets were weighed and triturated to obtain a fine powder. A portion equivalent to 50 mg Imeglimin was dissolved in 25 ml of diluent in a 50 ml volumetric flask, which was then ultrasonicated for 20 minutes and made up to volume with diluent. The resulting solution was filtered and diluted further to obtain a concentration of 100µg/ml imeglimin. This solution was filtered again and injected into an RP-HPLC system for analysis. Assaying six samples of the tablets yielded an average value of 504 mg (100.8%) with a standard deviation of 0.584 and a % relative standard deviation of 0.58. The assay values fell within the acceptable range of 98-102% against the claimed amount in the tablets.

Sr. No.	Amount present in (µg)	Amount found in (µg)	% label claim
1	20	19.83	99.15
2	20	19.86	99.3
3	20	19.92	99.6
4	20	19.98	99.9
5	20	20.01	100.05

Table 9 – analysis of Imeglimin HCL marketed formulation

CONCLUSION –

In conclusion, the study successfully developed a sensitive, precise, and accurate RP-HPLC method for estimating Imeglimin HCL in pharmaceutical dosage forms. Optimization of mobile phase composition and wavelength selection enabled satisfactory resolution and runtime using a Credchrom C18 column (250mm x 4.6 mm x 5µm). Method validation demonstrated excellent linearity, sensitivity (with LOD and LOQ values of 0.577722 µg/ml and 1.750673 µg/ml, respectively), precision (%RSD values ranging from 0.4775287 to 10.988377), and accuracy (% recovery ranging from 98.62% to 100.34%). These results underscore the method's robustness and reliability, making it suitable for routine analysis in pharmaceutical laboratories. Overall, this research contributes significantly to advancing analytical techniques for drug analysis, particularly in the context of Imeglimin HCL pharmaceutical formulations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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