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

***Ms. Pooja S. Kasurde, Dr. Pravinkumar N. Sabale, Mr.Sarang D. Kulkarni, Ms. Sharda S. Kulkarni, Ms. Rutuja S. Kulkarni**

India.

***Corresponding Author: Ms. Pooja S. Kasurde**

India.

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1. ABSTRACT

Imeglimin Hydrochloride is a novel anti-diabetic drug used in the management of Type 2 Diabetes Mellitus. The present study focuses on the development and validation of a simple, precise, accurate, and economical Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the determination of Imeglimin Hydrochloride in bulk drug and pharmaceutical dosage form. The chromatographic separation was achieved using a suitable C18 column with an optimized mobile phase consisting of appropriate solvent systems under isocratic conditions. Detection was carried out using a UV detector at a selected wavelength for obtaining optimum sensitivity and peak resolution. The eveloped analytical method was validated according to International Council for Harmonisation (ICH) guidelines for various validation parameters including linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), and system suitability. The developed RP-HPLC method demonstrated excellent linearity over the selected concentration range with acceptable correlation coefficient values. Accuracy studies showed satisfactory percentage recovery, while precision studies indicated low %RSD values, confirming reproducibility of the method. The robustness study demonstrated that minor variations in chromatographic conditions did not significantly affect the analytical performance. The developed and validated RP-HPLC method was found to be simple, rapid, sensitive, accurate, and reliable for routine quantitative estimation of Imeglimin Hydrochloride in bulk drug and pharmaceutical dosage forms. Therefore, the proposed method can be successfully applied for quality control analysis in pharmaceutical industries and research laboratories.

KEYWORDS: Imeglimin Hydrochloride, Anti-diabetic Drug, RP-HPLC, Analytical Method Development, Method Validation, Pharmaceutical Analysis, Quantitative Estimation, Bulk Drug Analysis, Dosage Form Analysis, ICH Guidelines.

INTRODUCTION

Imeglimin Hydrochloride is a novel oral anti-diabetic drug used in the treatment of Type 2 Diabetes Mellitus. It belongs to a new class of tetrahydrotriazine-containing compounds and acts by improving mitochondrial bioenergetics. Imeglimin enhances insulin secretion, improves insulin sensitivity, and reduces hepatic glucose production, thereby helping to control blood glucose levels effectively. Due to its unique mechanism of action, imeglimin has emerged as an important therapeutic agent in the management of diabetes mellitus.

Analytical method development and validation play a significant role in pharmaceutical analysis for the qualitative and quantitative determination of drugs in bulk and pharmaceutical dosage forms. The development of reliable analytical methods is essential for ensuring the quality, safety, and efficacy of pharmaceutical products.

Among various analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is widely used due to its high accuracy, sensitivity, precision, reproducibility, and rapid analysis. RP-HPLC

is one of the most commonly employed chromatographic techniques for the estimation of pharmaceutical compounds because of its ability to separate, identify, and quantify components in complex mixtures. The technique offers advantages such as excellent resolution, shorter analysis time, high sensitivity, and suitability for routine quality control analysis.

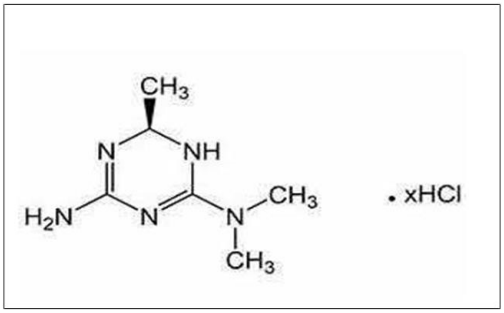
Method development involves optimization of chromatographic conditions such as mobile phase composition, stationary phase, flow rate, wavelength selection, and retention time to achieve proper separation and accurate estimation of the analyte. Method validation is an important process carried out according to International Council for Harmonisation (ICH) guidelines to confirm that the developed analytical method is suitable for its intended purpose. Validation parameters such as accuracy, precision, linearity, robustness, specificity, limit of detection (LOD), limit of quantification (LOQ), and system suitability are evaluated to establish the reliability and consistency of the method.

Several analytical methods including HPLC, UHPLC, UV spectrophotometry, LC-MS, and stability-indicating methods have been reported for the estimation of Imeglimin Hydrochloride in pharmaceutical dosage forms. However, there is still a need for a simple, accurate, precise, and cost-effective RP-HPLC method for routine pharmaceutical analysis.

Therefore, the present study focuses on the development and validation of an RP-HPLC method for the determination of Imeglimin Hydrochloride in bulk drug and pharmaceutical dosage form. The developed method aims to provide reliable analytical performance for routine quality control and pharmaceutical analysis. The validated RP-HPLC method may be useful in pharmaceutical industries and research laboratories for the accurate determination of Imeglimin Hydrochloride in bulk and marketed formulations.

COMPOUND SUMMARY OF IMMEGLIMIN

Structure



Other Names of Imeglimin: Imeglimin, Imeglimin Hydrochloride

Molecular Formula

$C_6 H_{14} ClN_5$

Molecular Weight

191.66 g/mol

Molecular Mass

191.66 g/mol

Route of Administration

Orally

Pharmacokinetics Data

- **Protein Binding:** 1.2%–6.4%
- **Metabolism:** Poorly metabolized; largely excreted unchanged
- **Elimination Half-life:** Approximately 13 hours
- **Excretion:** Primarily through kidney (urine)

METHODS AND MATERIALS

A simple, accurate, and precise RP-HPLC method was developed for the estimation of Imeglimin HCL in bulk drug and pharmaceutical dosage forms. Different mobile phase compositions were tested to achieve optimal peak shape, retention time, and resolution.

The optimized chromatographic conditions used an Agilent C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of phosphate buffer and acetonitrile in a suitable ratio with a flow rate of 1.0 mL/min. Detection was performed using a UV detector at 241 nm.

Standard and sample solutions were prepared in methanol and filtered through Whatman filter paper before analysis. The solutions were injected into the HPLC system, and chromatograms were recorded. The method was validated according to ICH guidelines for parameters including accuracy, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

Method Objective

To develop and validate an RP-HPLC method for the quantitative estimation of Imeglimin HCL in bulk drug and pharmaceutical dosage forms.

Materials Required

- Imeglimin HCL
- Methanol (HPLC grade)
- Acetonitrile (HPLC grade)
- Phosphate Buffer
- Water (HPLC grade)
- HPLC Instrument
- UV Detector
- Analytical Balance
- Sonicator
- Whatman Filter Paper

Instruments Required

- Agilent HPLC System
- Agilent C18 Column (250 mm × 4.6 mm, 5 µm)
- UV Spectrophotometer
- pH Meter
- Electronic Balance

- Sonicator

Method of Analysis by RP-HPLC

Imeglimin HCL standard was accurately weighed and transferred into a clean volumetric flask. Methanol was added as solvent, and the solution was sonicated for 10–15 minutes to obtain a clear homogeneous solution. The solution was then filtered through Whatman filter paper.

Chromatographic separation was carried out using an Agilent C18 column (250 mm × 4.6 mm, 5 µm). The optimized mobile phase consisted of phosphate buffer and acetonitrile in a suitable ratio. The mobile phase was filtered and degassed before use.

The flow rate was maintained at 1.0 mL/min, and detection was carried out at 241 nm using a UV detector. Standard and sample solutions were injected into the HPLC system, and chromatograms were recorded for analysis.

CHARACTERIZATION / ANALYSIS OF IMEGLIMIN HCL

UV–Visible Spectrophotometric Analysis of Imeglimin HCL

Beer-Lambert Law Used in UV Spectroscopy

The concentration determination in UV spectroscopy is based on the equation:

$$A=\varepsilon cl$$

Where:

- (\varepsilon) = molar absorptivity
- (c) = concentration
- (l) = path length

Determination of λmax

Procedure

- Prepare standard stock solution of Imeglimin HCL (100 µg/mL)
- Dilute to obtain 10 µg/mL solution
- Scan between 200–400 nm
- Record wavelength showing maximum absorbance

Observation

- λmax observed = 241 nm

Preparation of Standard Stock Solution

- Weigh accurately 10 mg of Imeglimin HCL
- Transfer into 100 mL volumetric flask
- Dissolve in methanol and make volume up to 100 mL

Concentration obtained

10 mg100 mL=100µg/mL

List Of Chemical Reagents

Sr No	Name of Chemicals	Manufacture
1.	Acetonitrile(HPLCgrade)	Merck Ltd.,India
2.	Methanol(HPLCgrade	Merck Ltd.,India
3.	0.1%formic Acid (HPLCgrade)	Merck Ltd.,India
4.	water(HPLCgrade)	Merck Ltd.,India

Selection Formulation

Sr No	Brand Name	Formulation	Strength	Manufacturer
1.	Imeglyn Tablet	Imeglimin HCl Tablet	500 mg	Zyduscadila

IMEGLIMIN HCL ANALYSIS: RP-HPLC & UV-VIS.

List of Instruments

Sr. No.	Name of Instrument	Name Of Company
1	HPLC Instrument	Agilent Tech. Gradient System with Auto injector
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column (C18)	Agilent C18 (250 mm × 4.6 mm, 5µm)
5	Balance	WENSAR High Resolution Balance
6	Sonicator	Ultrasonics Electronic Instrument

Selection of Analytical Technique

Among various analytical techniques such as UV Spectrophotometry, HPLC, HPTLC, and LC-MS, **Reverse Phase High Performance Liquid Chromatography (RP-HPLC)** was selected for the estimation of Imeglimin HCl in bulk drug and pharmaceutical dosage form.

RP-HPLC was selected because of the following advantages:

- High accuracy
- High precision
- Good sensitivity
- Better peak resolution
- Short analysis time
- Suitable for quantitative estimation
- Reliable and reproducible results

The analysis was carried out using Agilent Gradient HPLC system equipped with Auto Injector, DAD detector and C18 column.

Analytical Technique Selection

Sample → Solubility Study → UV Analysis → Method Development → RP-HPLC Analysis → Validation

Selection of Formulation

List of brand names of formulation of Trelagliptin Succinate

Sr No	Brand Name	Formulation	Strength	Manufacturer
1.	Trelaglip	Tablet	100 mg	Zuventus Healthcare

HPLC Analysis

The analysis of the drug was carried out on **Agilent Tech. Gradient System with Auto Injector, DAD and Gradient Detector**. The system was equipped with **Reverse Phase (Agilent) C18 column (4.6 mm × 250 mm, 5 µm)** and operated using **Chemstation 10.1 software**.

Stock Preparations

Stock I: Standard Sample Preparation

Standard Trelagliptin Succinate **5 mg** was dissolved in **10 mL methanol**.

Concentration = 500 µg/mL

Stock II: Tablet Solution Preparation

Accurately weighed **29.37 mg** tablet powder was transferred into **10 mL methanol**.

Concentration = 500 µg/mL

Accuracy Solution Preparations

Take **10 µg/mL tablet solution** for accuracy study.

80% Level

- Take **0.1 mL tablet solution**
- Add **4 µg/mL standard TLG**
- Make up volume to **10 mL with mobile phase**

100% Level

- Take **0.1 mL tablet solution**
- Add **5 µg/mL standard TLG**
- Make up volume to **10 mL with mobile phase**

120% Level

- Take **0.1 mL tablet solution**
- Add **6 µg/mL standard TLG**
- Make up volume to **10 mL with mobile phase**

HPLC Method Development

Selection of Analytical Technique

HPLC was selected as the analytical technique for estimation of Imeglimin HCL in bulk drug and pharmaceutical dosage form due to its **high sensitivity, precision, and accuracy**.

Instruments

The analysis was carried out using

- Agilent Tech Gradient System with Auto Injector
- DAD Detector
- Reverse Phase C18 Column (250 mm × 4.6 mm, 5 µm)
- UV detector
- Chemstation 10.1 software

Selection of Stationary Phase

The column selected for the method was:

- Agilent C18 Column
- Dimension: 250 mm × 4.6 mm
- Particle size: 5 µm

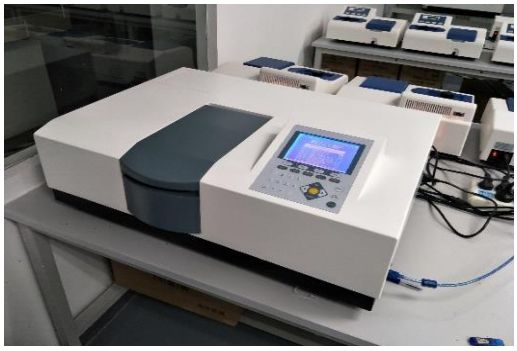
C18 column was selected because it provides:

- Good retention
- Symmetrical peak shape
- High reproducibility
- Stable chromatographic performance

Solubility Studies
Solubility studies were performed to identify a suitable solvent.

RESULTS
- Freely soluble in Methanol
- Poorly soluble in Water
- Soluble in pH-adjusted 0.1% Orthophosphoric Acid buffer (pH 3)

UV Spectrophotometric Analysis
UV-Visible Spectrophotometer was used to determine λmax of Imeglimin HCL in the range of 200–400 nm.



UV Spectrophotometr
- Analytical Technologies Limited UV-Visible Spectrophotometer
- Double beam system
- Quartz cuvette (1 cm path length)
- Deuterium lamp

Optimized Chromatographic Conditions

Parameter	Condition
Instrument	Agilent HPLC
Software	Chemstation 10.1
Column	Agilent C18 (250 × 4.6 mm)
Particle Size	5 µm
Mobile Phase	Methanol : Water (0.1% Formic Acid)
Ratio	55 : 45
Detection Wavelength	249 nm
Flow Rate	0.7 mL/min
Temperature	Ambient
Injection Volume	20 µL
pH	3
Run Time	15 min
Filter	0.45 µm

preparation of Standard Stock Solution
- Accurately weighed 10 mg of Imeglimin HCL and transferred into 100 mL volumetric flask.
- Dissolved in Methanol
- Volume made up to 100 mL
- Final concentration = 100 µg/mL
- Solution was sonicated for 15 min for complete dissolution.

Study of Chromatographic Conditions
Prepared 10 µg/mL solution and subjected to chromatographic analysis using different mobile phases.

Trial Mobile Phases
- Methanol + 0.1% OPA Water (90:10)
- Methanol + 0.1% OPA Water (70:30)
- Methanol + 0.1% OPA Water (50:50)
- Methanol + 0.1% OPA Water (40:60)
- Methanol + 0.1% OPA Water (20:80)
- Methanol + 0.1% OPA Water (55:45)

Final optimized mobile phase
Methanol : Water (0.1% Formic Acid) = 55 : 45 v/v

Method Development Summary
The RP-HPLC method for estimation of Imeglimin HCL was optimized using:
- C18 Column
- Methanol : Water (55:45) mobile phase
- Flow rate 0.7 mL/min
- Detection at 249 nm

The developed method showed
- Good peak symmetry
- Sharp peak
- Suitable retention time
- Accurate quantification

Parameters Evaluated
Standard drug analysis was carried out using:
- Melting point
- Solubility
- UV Spectrum and λmax
- HPLC Chromatogram
- Retention Time

EXPERIMENTAL WORK SUMMARY

- **Analytical Technique:** HPLC chosen for estimating Imeglimin HCL due to its high sensitivity, precision, and accuracy.
- **Instruments Used:** Agilent Tech Gradient System with Auto Injector, DAD Detector, Reverse Phase C18 Column (250 × 4.6 mm, 5 µm), UV detector, Chemstation 10.1 software.
- **Stationary Phase:** Agilent C18 column (250 × 4.6 mm, 5 µm) selected for good retention, symmetrical peaks, reproducibility, and stable performance.
- **Solubility:** Freely soluble in Methanol, poorly soluble in Water, soluble in pH 3 (0.1% Orthophosphoric Acid buffer).

- **Optimized HPLC Conditions**

Mobile Phase: Methanol : Water (0.1% Formic Acid) 55:45 v/v
Flow Rate: 0.7 mL/min
Detection Wavelength: 249 nm
Injection Volume: 20 µL
Column: Agilent C18 (250 × 4.6 mm, 5 µm)
Run Time: 15 min, pH 3, ambient temperature.

- **UV Analysis:** λmax determined using UV-Visible Spectrophotometer (200–400 nm range).
- **Standard Stock Solution:** 100 µg/mL prepared by dissolving 10 mg Imeglimin HCL in 100 mL Methanol, sonicated for 15 min.

- **Method Summary**
  RP-HPLC method optimized for sharp, symmetric peaks, suitable retention time, and accurate quantification.

- **Parameters Evaluated:** Melting point, solubility, UV λmax, HPLC chromatogram, retention time.

Study of Chromatographic Conditions of Imeglimin HCL

Preparation of Standard Solution

- Accurately weighed **10 mg of Imeglimin HCL**.
- Transferred into **100 mL volumetric flask**.
- Dissolved in **Methanol**.
- Volume made up to mark with Methanol to get **100 µg/mL stock solution**.
- Sonicated for **15 minutes** for complete dissolution.
- From this stock solution, **0.1 mL** was transferred into **10 mL volumetric flask**.
- Volume made up to mark with mobile phase.
- Final concentration obtained = **10 µg/mL**.

Chromatographic Conditions

- **Analytical Column:** Agilent C18 Column (250 mm × 4.6 mm, 5 µm)
- **Injection Volume:** 20 µL
- **Flow Rate:** 0.7 mL/min
- **Detection Wavelength:** 249 nm
- **Run Time:** 10 min

Method Development of HPLC

Mobile Phases Tried

Sr. No.	Mobile Phase
1	Methanol : 0.1% OPA Water (90:10)
2	Methanol : 0.1% OPA Water (70:30)
3	Methanol : 0.1% OPA Water (50:50)
4	Methanol : 0.1% OPA Water (40:60)
5	Methanol : 0.1% OPA Water (20:80)
6	Methanol : 0.1% OPA Water (20:80)
7	Methanol : 0.1% OPA Water (50:50)
8	Methanol : 0.1% OPA Water (55:45)

Optimized Mobile Phase

- **Methanol : Water (0.1% OPA) = 55 : 45**
- pH adjusted to **3.0**

Analysis of Standard Drug

The standard drug was analyzed by:

- Melting point
- Solubility
- UV spectra and λmax
- HPLC chromatogram
- Retention time

Selection of Wavelength by UV Spectrophotometry

Standard Stock Solution (Stock I)

- Weighed **10 mg of Imeglimin HCL**
- Transferred into **100 mL volumetric flask**
- Dissolved in Methanol
- Volume made up to 100 mL
- Final concentration = **100 µg/mL**

Stock II

- Took **0.2 mL from Stock I**
- Transferred into **10 mL volumetric flask**
- Made volume with mobile phase
- Final solution prepared for analysis

Optimized Chromatographic Conditions

- **Column:** Agilent C18 (250 × 4.6 mm)
- **Particle Size:** 5 µm
- **Injection Volume:** 20 µL
- **Flow Rate:** 0.7 mL/min
- **Detection:** 249 nm
- **Run Time:** 15 min
- **Mobile Phase:** Methanol : Water (0.1% Formic Acid) (55:45)
- **pH:** 3.0

Procedure for Calibration Curve

- Prepared stock solution of **100 µg/mL**.
- Pipetted:
  - 0.2 mL
  - 0.4 mL
  - 0.6 mL
  - 0.8 mL
  - 1.0 mL

- Diluted each to **10 mL** with mobile phase.

Final concentrations obtained
- 2 µg/mL
- 4 µg/mL
- 6 µg/mL
- 8 µg/mL
- 10 µg/mL
- Samples were injected into HPLC.
- Peaks were recorded at **249 nm**.
- Calibration graph plotted between:
  - Concentration
  - Peak Area

System Suitability Parameters
System suitability was performed to check:
- Resolution
- Reproducibility
- Performance of chromatographic system
- Five replicate injections were taken.

Validation of Method
The developed RP-HPLC method was validated according to **ICH guidelines**.
Linearity
Linearity shows direct relationship between:
- Drug concentration
- Peak area

Concentration Range

Sr. No	Concentration (µg/mL)
1	2
2	4
3	6
4	8
5	10

Acceptance Criteria
- Calibration curve should be linear.
- Correlation coefficient should be **not less than 0.999**.

Accuracy
Accuracy indicates closeness of measured value to true value.
% Recovery.

Acceptance Criteria
- Mean recovery should be **98–102%**
- %RSD should be **less than 2.0%**

Trials Performed During Method Development

Trial No.	Mobile Phase	Observation
Trial 1	Methanol : Water (0.1% OPA) = 90:10	Peak not satisfactory / poor separation
Trial 2	Methanol : Water (0.1% OPA) = 70:30	Broad peak observed
Trial 3	Methanol : Water (0.1% OPA) = 50:50	Improved separation
Trial 4	Methanol : Water (0.1% OPA) = 40:60	Peak broad / retention issue
Trial 5	Methanol : Water (0.1% OPA) = 20:80	Poor peak shape
Trial 6	Methanol : Water (0.1% OPA) = 20:80	(Flow 0.7) Unsatisfactory
Trial 7	Methanol : Water (0.1% OPA) = 50:50	Better
Trial 8	Methanol : Water (0.1% OPA) = 55:45	(Flow 0.7 Best peak, sharp, symmetric

RESULT DISCUSSION
Chromatogram of Trial 2.

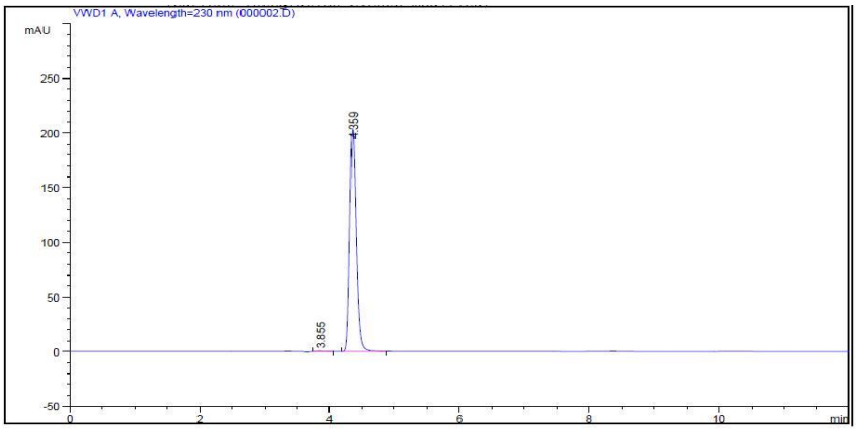


Fig. No. 11: Chromatogram of Trial 2.

Table No. 11: Trial-2 of chromatogram of Imeglimin HCL.

No.	RT[min]	Area[mV*s]	Area%	TP	TF
1	4.359	1372.2932	100.00	9521	0.83

Observation: Ghost peak was observed, so method was reject
Chromatogram of final Trial 8

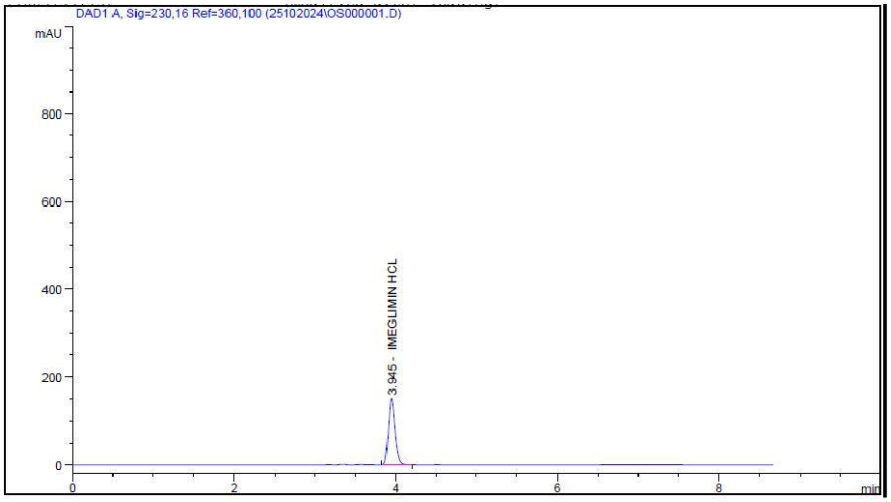


Fig. No. 17: Chromatogram of final Trial 8.

Table No. 17: Final Trial-8 of chromatogram of Imeglimin HCL.

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.945	905.14233	11477	0.79	-

Observation: Sharpe peak were obtained satisfactory result was found, so method was selected
Calibration experiment
RP-HPLC Method

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 2- 10µ g/mL for Imeglimin HCL (**Table No:19)** depict the calibration data of Imeglimin HCL The respective linear equation for Imeglimin HCL was Y= 428.2x+59.02 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Imeglimin HCL is depicted in **(FigNo.20).**

Table No. 19: Linearity data for Imeglimin HCL.

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP- HPLC Method	2	906.1423	905.3432	905.7428	0.57	0.06
	4	1800.2926	1801.027	1800.6603	0.52	0.03
	6	2610.3544	2608.360	2609.3576	1.41	0.05
	8	3474.3530	3476.523	3475.4382	1.53	0.04
	10	4351.7192	4349.407	4350.56	1.63	0.04
	Equation		Y= 428.2x+59.02			
	R2		0.999			

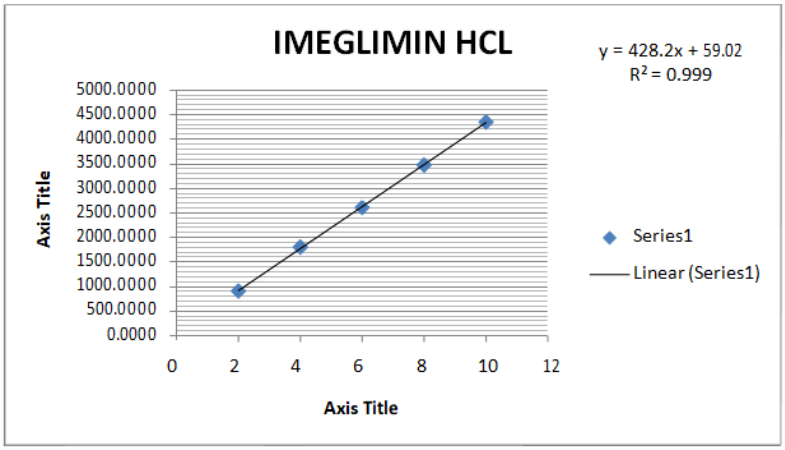


Fig. No. 20: Calibration curve of Imeglimin HCL (HPLC).

8.2. Analytical of Method Validation

1. Linearity

From Imeglimin HCL standard stock solution, different working standard solution (2- 10µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using mixed volume loop injector Chromatograms were recorded. The area for each concentration was recorded.

Table No. 20: Linearity of Imeglimin HCL.

Sr. No.	Concentration µg/ml Imeglimin HCL	Area Imeglimin HCL
1	2	905.7428
2	4	1800.6603
3	6	2609.3576
4	8	3475.4382
5	10	4350.5600

2. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%).

Accuracy 120%

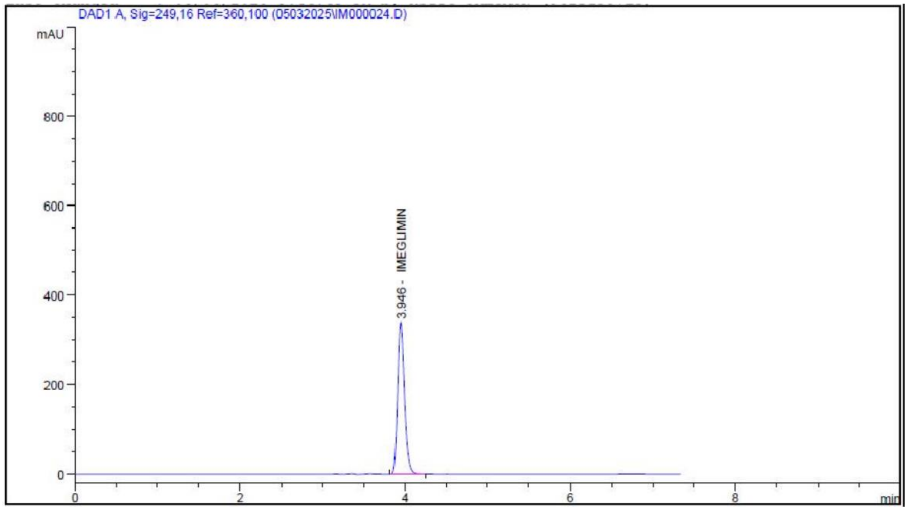


Fig. 35: Chromatogram of Accuracy 120%.

Table 22: Result of Recovery data for Imeglimin HCL.

METH OD	Drug	Level (%)	Amt. taken (µg/m l)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLCMethod	IGM	80%	2	1.6	3.63± 0.012	1.63± 0.012	101.5±0.78
		100%	2	2	4.00±0.004	2.00±0.004	99.77±0.21
		120%	2	2.4	4.39±0.002	2.39±0.002	99.42±0.08

Mean of each 2 reading for RP-HPLC method

Statistical Validation of Recovery Studies Imeglimin HCL

METH OD	Drug	Level (%)	Mean % Recovery	Standard Deviation*	% RSD
RP- HPLC Method	IGM	80%	101.59	0.78	0.77
		100%	99.77	0.21	0.21
		120%	99.42	0.08	0.08

Denotes average of three determinations for RP-HPLC

Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-102% **(Table No. 22, 23)**.

3. System suitability parameters: (Repeatability)

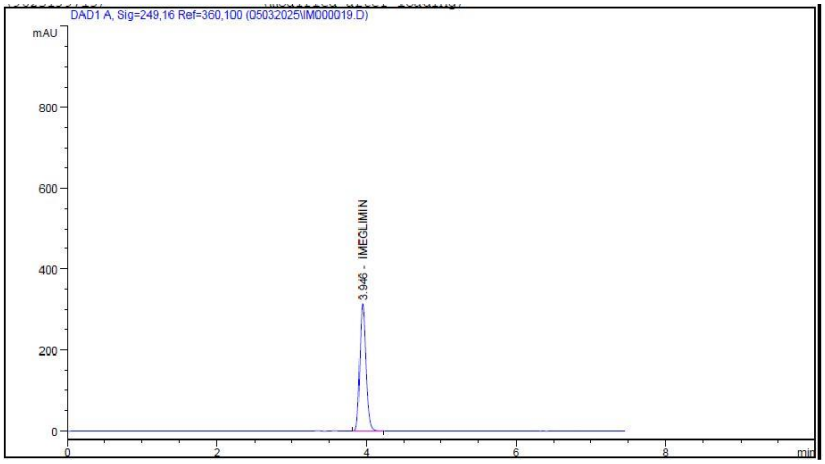
To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Imeglimin HCL system suitability parameters were studied.

4. Precision

The method was established by analyzing various replicates standards of Imeglimin HCL. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded

5. Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied. The mobile phase composition was changed in (± 1 ml/min^{-1}) proportion and the flow rate was varied by (± 1ml/min^{-1}), and wavelength change (± 1 ml/min^{-1}) of optimized chromatographic condition. The results of robustness studies are shown in **(Table No.26**). Robustness parameters were also found satisfactory; hence the analytical method would be concluded.



Chromatogram for Marketed Formulation.

Analysis of marketed formulation

Assay	Drug	Lable Claimed	Amt. Found	%Lable Claim	SD	%RSD
Rp-HPLC Method	IGM	4	4.05	101.33	0.13	0.414
		4	4.058	101.45	0.42	0.414

Analysis of marketed formulation were also %Lable Claim was found to be 98-101% Satisfactory are conclude.

Summaryo RP-HPLC method

Attempts were made to develop RP-HPLC method for estimation of Imeglimin HCL from t a b l e t . For the RP - HPLC method, Agilent (Autosampler) Gradient System DAD Detector and C_{18} (Agilent) with 250mm x4.6 mm i.d and 5µm particle size Methanol: Water 0.1 % Formic Acid (55:45v/v) pH 3.0 was used as the mobile phase for the method. The detection wavelength was 249 nm and flow rate was 0.7 ml/min. In the developed method, the retention time of Imeglimin HCL were found to be 3.9 min.

The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible.

So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Imeglimin HCL in bulk drug as well as in formulations.

CONCLUSION

Simple, rapid, accurate and precise RP-HPLC have been developed and validated for the routine analysis of Imeglimin HCL in API and tablet dosage forms. Both methods are suitable for the determination of Imeglimin HCL in Single-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

REFFRENCE

1. Sonawane JK, et al. A Review of Stability Indicating Methods and Forced Degradation Studies. *Int J Res Publ Rev.*, 2023; 4(5): 4703-4715. doi:10.55248/gengpi.4.523.42372.

2. Mennickent S, de Diego M. Analytical Method Validation as the First Step in Drug Quality Control. In: *IntechOpen eBooks.* IntechOpen, 2019. doi:10.5772/intechopen.82826.
3. Khaire RD, Pawar PY. A Review on Development of Stability Indicating Analytical Methods for Drugs in Bulk and Pharmaceutical Dosage Form. *Asian J Pharm Anal.* 2022; 261-263. doi:10.52711/2231-5675.2022.00043.
4. Sandhya B. Analytical method development and validation for simultaneous estimation of loratadine and pseudoephedrine in bulk and tablet dosage form by RP-HPLC method. *Int J Allied Med Sci Clin Res.*, 2023; 11(3): 257-265. doi:10.61096/ijamscr.v11.iss3.2023.257-265.
5. Singhvi I, Bhatia NM. Spectrophotometric and HPLC methods for simultaneous estimation of pseudoephedrine hydrochloride and loratadine from tablets. *Indian J Pharm Sci.*, 2006; 68(1): 72-72. doi:10.4103/0250-474X.22968.
6. Indore C. BRNS Sponsored National Symposium: Radiotracer Techniques: Improve Nutritional Status and Health of Animals and Plants. *J Drug Deliv Ther.*, 2020; 10: 1-101. doi:10.22270/jddt.v10i1-a.3885.
7. Kumar D, et al. A Comprehensive Review on Analytical Method Development using RP-HPLC and Recent Advances in Pharmaceutical Applications. *J Res Appl Sci Biotechnol.*, 2023; 2(2): 53-60. doi:10.55544/jrasb.2.2.9.
8. Abu-Lathou A, Hamdan II, Tahraoui A. A New HPLC Approach for the Determination of Hydrophilic and Hydrophobic Components: The Case of Pseudoephedrine Sulfate and Loratadine in Tablets. *Drug Dev Ind Pharm.*, 2005; 31(6): 577-588. doi:10.1080/03639040500214696.
9. Reid IOA. Concurrent chromatographic determination of pseudoephedrine and loratadine from combination syrups and tablets. *Future J Pharm Sci.*, 2021; 7(1). doi:10.1186/s43094-021-00287-3.
10. Anggara L, Satrialdi S, Damayanti S. Ion-pair RP-HPLC strategy for resolving polarity-mismatched APIs in sustained-release capsules: an AQbD-driven mechanistic and statistical optimization. *Pharmacia*, 2025; 72: 1-15. doi:10.3897/pharmacia.72.e168287.
11. Belal F, El-Razeq SA, El-Awady MI, Zayed S, Barghash S. Rapid micellar HPLC analysis of loratadine and its major metabolite desloratadine in nano-concentration range using monolithic column and fluorometric detection: application to pharmaceuticals and biological fluids. *Chem Cent J.*, 2016; 10(1). doi:10.1186/s13065-016-0225-5.
12. Çağlar-Andac S, Toker SE. Simultaneous determination of desloratadine and pseudoephedrine sulfate in tablets by high performance liquid chromatography and derivative spectrophotometry. *Rev Anal Chem.*, 2011; 30: 145-151. doi:10.1515/revac.2011.100.