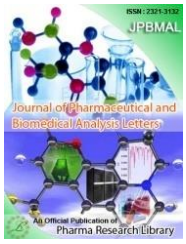

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RS Method for Quantification of Imeglimin HCl in Its Pure and Tablet Dosage form by using RP-HPLC Technology

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A B S T R A C T

This study focused on developing and characterizing floating microspheres of Propranolol HCl, aiming to enhance gastric retention and sustained drug release. A UV-Visible spectrophotometric method was validated for drug quantification in 0.1N HCl at 256 nm, exhibiting strong linearity over the concentration range of 2 to 10 µg/ml. Compatibility studies using FTIR spectroscopy confirmed no chemical interactions between the drug and polymers, ensuring formulation stability. The prepared microspheres showed high percentage yields between 81.58% and 95.50%, with drug entrapment efficiency improving as polymer concentration increased due to higher solution viscosity reducing drug diffusion during formation. Further evaluations revealed that the microspheres exhibited satisfactory swelling behavior and mucoadhesive properties essential for prolonged gastric residence. In vitro release experiments demonstrated efficient and controlled drug liberation over 12 hours, with release kinetics fitting diffusion-controlled models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas. Optimized formulations showed promising potential for sustained release and enhanced gastroretention, which could improve therapeutic effectiveness and patient adherence.

Keywords: Propranolol HCl, floating microspheres, UV-Visible spectrophotometry, sustained release, mucoadhesion, drug release kinetics, FTIR compatibility.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

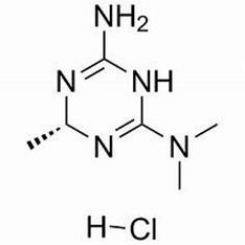

Fig.1: Imeglimin HCL

Table 1: Imeglimin HCL drug profile

Molecular Formula	$C_6H_{13}N_5$
Molecular Weight	155.205 g/mol
IUPAC Name	(2S)-N^6,N^6,2-Trimethyl-1,2-dihydro-1,3,5-triazine-4,6-diamine
Chem Spider ID	26232690
Density	**1.29 ± 0.1 g/cm³,**
Boiling Point	239.9 ± 23.0 °C

Vapour Pressure	Data not available
Flash Point	Not applicable
Refractive Index	43.29 $m^3 \cdot mol^{-1}$
Polar Surface Area	66.01 $Å^2$
LogP	-0.92 (Octanol/Water)
Generic Name	Imeglimin
Brand Names	Twymeeg
Drug category	Antidiabetic agent, Oral hypoglycemic agent, Gluconeogenesis inhibitor
Potency	Not specified in the available data
Adverse Effects	Not specified in the available data
Mechanism of Action	Mechanism of Action: Imeglimin is the first approved drug in a novel class of anti-diabetic medications. It functions by inhibiting oxidative phosphorylation, which leads to: • Reduced hepatic gluconeogenesis • Enhanced glucose uptake in muscles • Restoration of normal insulin secretion

2. Materials and Methods

Table 2: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 3: Chemicals used

S.No	Chemical	Brand
1	Imeglimin	Supplied by Pharmatrain
2	Ortho phosphoric acid	FINAR chemical LTD
3	Water and Methanol for HPLC	Standard solutions Ltd
4	Acetonitrile for HPLC	Standard solutions Ltd
5	HCl, H_2O_2, NaOH	MERCK

Wave length selection:
UV spectrum of 10 µg / ml Imeglimin in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 245. At this wavelength both the drugs show good absorbance.

Optimization of Column:
Spursil ODS (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.5 ml/min flow.

Optimized Chromatographic Conditions:
Instrument used : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector
Temperature : Ambient
Column : Sprusil ODS C_{18} (4.6 x 250mm, 5.0µm)
Buffer : 0.1% Ortho phosphoric acid buffer
Mobile phase : 20ml 0.1% OPA: 80ml Methanol
Flow rate : 1 ml per min
Wavelength : 238 nm
Injection volume : 10 µl
Run time : 15min.

Preparation of Buffer And Mobile Phase:
Preparation of 0.1% Ortho phosphoric acid buffer:
Pipetted 1 ml of ortho phosphoric acid in 1000 ml HPLC water.
Preparation of mobile phase:
Mix a mixture of above buffer 200ml (20%), and 800ml Methonol HPLC (80%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.
Diluent Preparation:
Use the Mobile phase as Diluents.
Validation parameters:
1. ASSAY:
Preparation of standard solution:
Standard Solution Preparation:
Accurately weigh and transfer 10 mg working standard into a 10 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.
Impurity Solution Preparation:
Accurately weigh and transfer the equivalent weight of 1 mg of Impurity - D6 HCL into a 10 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.
Procedure:
Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for peaks and identify the impurities known and unknown.
System suitability:
Tailing factor for the peaks in Standard solution should not be more than 2.0
Theoretical plates for the peaks in Standard solution should not be less than 2000.
Resolution for the peaks in standard solution should not be less than 2.
Validation parameters:
Linearity:
Preparation of stock solution:
Accurately weigh and transfer 10 mg of Imeglimin and 1mg of impurity D6 HCL working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to

dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Imeglimin, 1ppm Imp-D6 HCL):

0.1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (20ppm of Imeglimin, 2ppm Imp- D6 HCL):

0.2 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (30ppm of Imeglimin, 3 ppm Imp- D6 HCL):

0.3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (40ppm of Imeglimin, 4ppm Imp- D6 HCL):

0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (50ppm of Imeglimin,5ppm Imp- D6 HCL):

0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark withDiluents.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION:

Preparation of stock Solution:

Accurately weigh and transfer 10mg of **Imeglimin** working standard, 1mg of impurity D6 HCL into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specifiedlimits.

Intermediate precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 10 mg of **Imeglimin** working standard, 1mg of impurity D6 HCL into a 10 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 10 mg of **Imeglimin** working standard, 1mg of impurity D6 HCL into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 5mg of Imeglimin working standard, 0.5mg of impurity D6 HCL into a 10 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of **Imeglimin** working standard, 1mg of impurity D6 HCL into a 10 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution).Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 15mg of **Imeglimin** working standard, 1.5mg of impurity D6 HCL into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for **Imeglimin** and calculate the individual recovery and mean recovery values.

Limit of detection:

Preparation of Imeglimin solution:

Preparation of 0.02µg/ml solution: Accurately weigh and transfer 20mg of **Imeglimin** working standard, into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.8 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of IMP- D6 HCL solution:

Preparation of 0.07µg/ml solution: Accurately weigh and transfer 2 mg of IMP- D6 HCL into a 20 ml clean dry

volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 2.4ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent

Limit of quantification:

Preparation of Imeglimin solution:

Preparation of 0.06µg/ml solution:

Accurately weigh and transfer 20 mg of **Imeglimin** working standard, into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of IMP- D6 HCL solution:

Preparation of 0.24 µg/ml solution:

Accurately weigh and transfer 2mg of IMP-A into a 20 ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 6ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1.36ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.8 ml/min to 1.2 ml/min

Standard solution 30 µg/ml of **Imeglimin,** 3 µg/ml of **IMP-** D6 HCL prepared and analyzed using the varied flowrates along with method flow rate.

The Organic composition in the Mobile phase was varied from 72% to 88%: Standard solution 30 µg/ml of Imeglimin, 3 µg/ml of IMP- D6 HCL prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

Forced Degradation Studies

Accurately weigh and transfer 10mg of Imeglimin working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Acid degradation:

Pipette 3 ml of Imeglimin of the above stock solution into a 10ml volumetric flask added about 3 ml of 0.1N Hcl and sonicated for 10minutes and kept it in darkness for 24 hours then refluxed under heat at 60 degrees in a heating mantle for 1 hours. Neutralized the sample solution using 0.1N NaOH and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Base Degradation:

Pipette 3 ml of Imeglimin of the above stock solution into a 10ml volumetric flask added about 3 ml of 0.1N NAOH and sonicated for 10minutes and kept it in darkness for 24 hours then refluxed under heat at 60 degrees in a heating mantle for 1 hours. Neutralized the sample solution using 0.1N HCL and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Thermal Degradation:

Pipette 3 ml of Imeglimin of the above stock solution into a 10ml volumetric flask and kept in oven under heat at 105 degrees for 24 hours and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Peroxide Degradation:

Pipette 3 ml of Imeglimin of the above stock solution into a 10ml volumetric flask added about 3ml of 3% Hydrogen Peroxide (H_2O_2) and sonicated for 10 minutes and kept in darkness for 24 hours and refluxed under heat at 60 degrees in a heating mantle for 1 hours. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Photo Degradation:

Pipette 3 ml of Imeglimin of the above stock solution into a 10ml volumetric flask added about and kept in darkness for 24 hours. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system

3. Results and Discussion

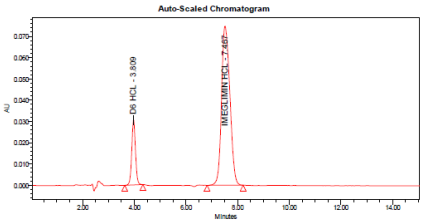

Fig.2: Chromatogram for Sample

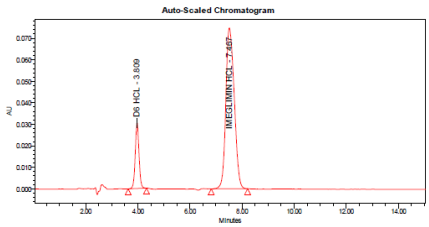

Fig.3: Chromatogram for Standard

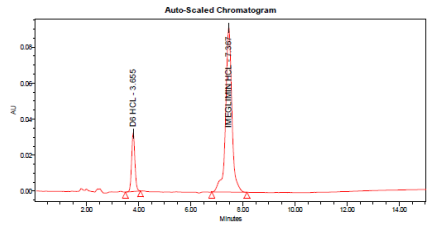

Fig.4: Chromatogram for linearity-5

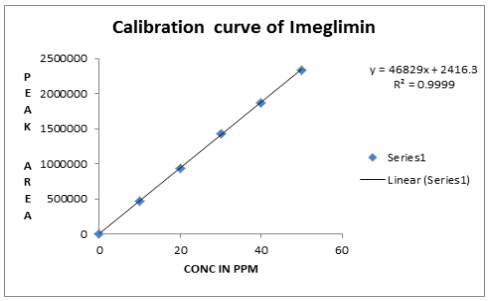

Fig.5: Calibration graph for Imeglimin

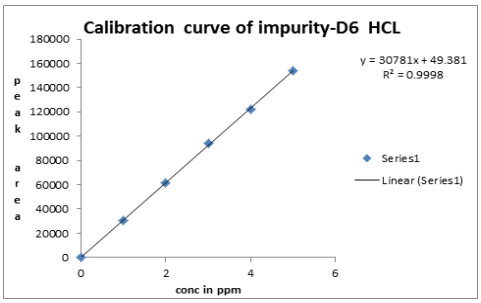

Fig.6: Calibration graph for D6 HCL

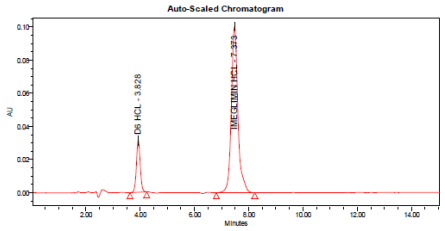

Fig.7: Chromatogram for Precision -6

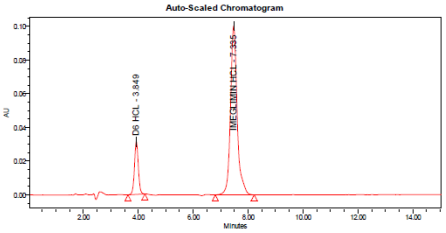

Fig.8: Chromatogram for ID Precision -6

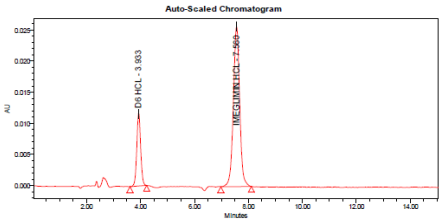

Fig.9: Chromatogram for Accuracy 50%-3

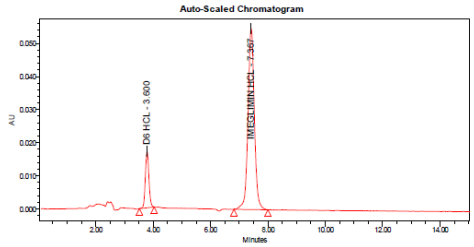

Fig.10: Chromatogram for Accuracy 100%-3

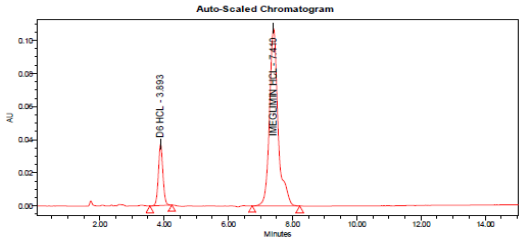

Fig.11: Chromatogram for Accuracy 150%-3

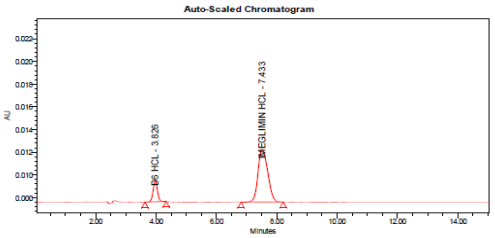

Fig.12: Chromatogram of Imeglimin showing LOD

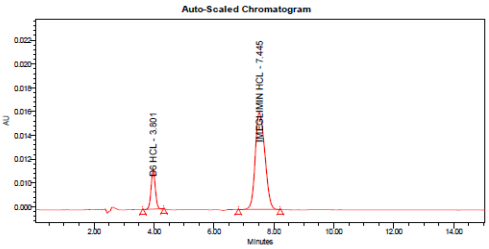

Fig.13: Chromatogram of Imeglimin showing LOQ

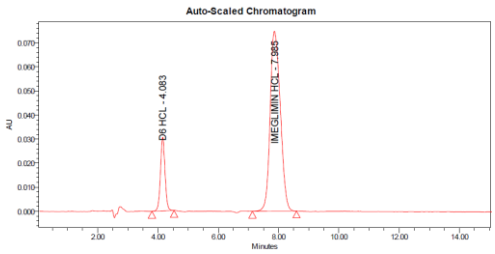

Fig.14: Chromatogram showing less flow

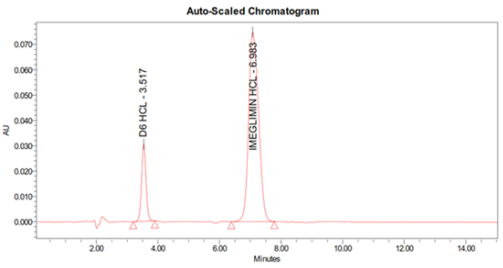

Fig.15: Chromatogram showing more flow

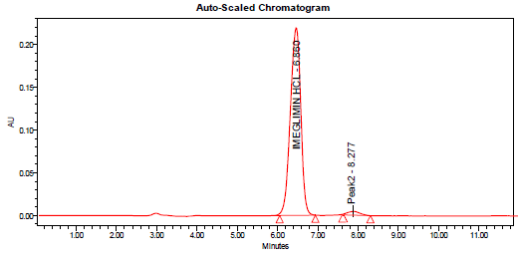

Fig.16: Chromatogram showing Acid

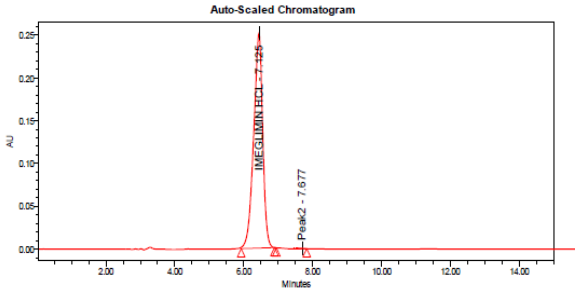

Fig.17: Chromatogram showing Base

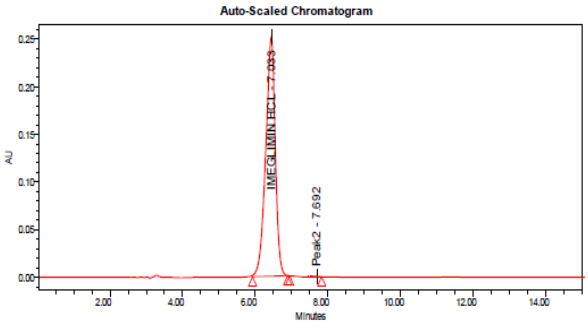

Fig.19: Chromatogram showing peroxide

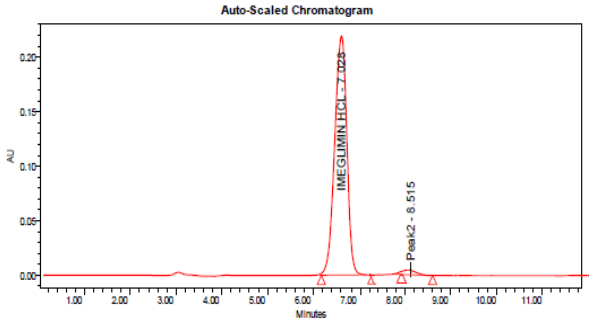

Fig.18: Chromatogram showing Thermal

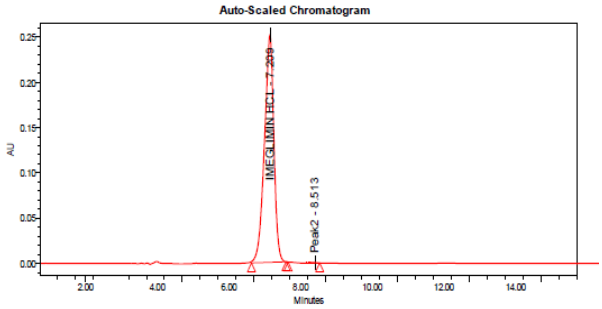

Fig.20: Chromatogram showing Photo

Table 4: Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Imeglimin (Standard)	7.604	1445036	230610	1.03	5546
1.2	D6HCL	2.435	95230	6031	1.09	5547
2	Imeglimin (Sample)	7.602	1442658	24337	1.3	5542

Table 5: Area of different concentration of Imeglimin

S. No	Imeglimin		D6 HCL	
	Concentration (µg/ml)	Areas	Concentration (µg/ml)	Areas
1	10	377390	1	23551
2	20	737622	2	47103
3	30	1159924	3	70655
4	40	1529719	4	93206
5	50	1933145	5	117758

Table 6: Analytical performance parameters of Imeglimin

Parameters	Imeglimin	D6 HCL
Slope (m)	46829	30781
Intercept (c)	2416.3	49.381
Correlation coefficient (R^2)	0.9999	0.9998

Table 7: Results of Precision for Imeglimin

Injection	Area	Area
Injection-1	1438542	94845
Injection-2	1435243	94426
Injection-3	1432415	93103
Injection-4	1436412	94698
Injection-5	1432136	94245
Injection-6	1431458	93394
Average	1434368	94118.5
Standard Deviation	2815.314	711.382
%RSD	0.2	0.8

Table 8: Results for variation in flow for Imeglimin

S. No	Flow Rate (ml/min)	System Suitability Results of Imeglimin		System Suitability results of D6	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.8	5483	1.05	3289	1.01
2	1	5491	1.04	3275	1.04
3	1.2	5462	1.08	3202	1.09

Table 9: Degradation results for Imeglimine HCL

Parameters	Voclosporin	
	Area	% Degraded
Standard	1159924	-----
Acid	142258	3.76
Base	142258	5.14
Peroxide	142258	4.24
Thermal	146154	2.39
Photo	146150	5.63

4. Conclusion

A simple, precise, and robust RP-HPLC method was successfully developed and validated for the estimation of Imeglimin HCL in bulk and pharmaceutical dosage forms in line with ICH Q2(R1) guidelines. Chromatographic separation using an Inspire C18 column with acetonitrile and trifluoroacetic acid (35:65v/v) as the mobile phase provided sharp and symmetrical peaks with excellent system suitability parameters. The method showed linearity across the concentration range of 10–50 µg/mL (R^2 = 0.999), with high reproducibility confirmed by precision studies (%RSD < 1%). Accuracy results, with a mean recovery of 98.10%, demonstrated the method's reliability, while sensitivity was confirmed through low LOD (0.57 µg/mL) and LOQ (1.98 µg/mL) values. Robustness studies indicated that small deliberate variations in flow rate and mobile phase composition did not significantly affect chromatographic performance, establishing the method's reliability under varied analytical conditions. Overall, the developed RP-HPLC method is simple, cost-effective, stability-indicating, and compliant with international guidelines. Its accuracy, precision, sensitivity, and robustness make it highly suitable for routine quality control, batch release testing, and regulatory applications of Iptacopan in the pharmaceutical industry.

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