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Analytical Method Development and Validation of RP-HPLC Method for Estimation of Imeglimin in Bulk and Pharmaceutical Dosage Form

Aniket. P. Khaire[*1], Vijaya. U. Barge[2]

[1,2]Student, Guide, Department of Pharmaceutical Quality Assurance, Pune District Education Association's, Shankarrao Ursal College of Pharmaceutical Science and Research Centre Kharadi, Pune -14.

[*]**Corresponding author:**

Aniket. P. Khaire

Pharmaceutical Quality Assurance Department, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Savitribai Phule Pune University, Kharadi, Pune 411014, Maharashtra, India.

Email ID: aniketkhaire9272@gmail.com

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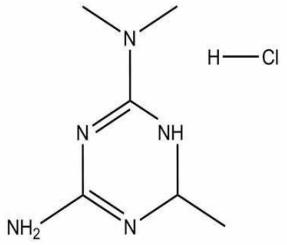
ABSTRACT

A new technique for evaluating the oral antidiabetic medication Imiglimin hydrochloride has been created and verified using High-Performance Layer Chromatography (HPLC) for both tablet and bulk forms. Acetone is used as a mobile phase in the procedure. Formic acid, toluene, and methanol in particular ratios. Drug absorbance is substantial at 240 nm, where chromatographic separation was accomplished on silica gel TLC plates using densitometry scanning. The findings of the validation, which adhered to ICH Q2R1 recommendations, were good in terms of linearity, accuracy, precision (both intra- and inter-day) and robustness. The new class of oral antidiabetic drugs known as "glimins," which contain tetrahydrotriazine, is called Imeglimin hydrochloride (IMEG. HCL) and is used to treat type 2 diabetes (T2D). It is the first of its kind to be approved as an anti-diabetic medication. In addition to improving muscle glucose uptake and restoring regular insulin secretion, it is an inhibitor of oxidative phosphorylation1. Using a Hypersil ODS C18 (150 x 4.6mm 5µ) Column and an isocratic mobile phase of Buffer pH 3.5 and Methanol (80:20)) pH6.0, a reverse phase HPLC technique was created and verified for the quantitative measurement of Imeglimin hydrochloride at a flow rate of 1 ml/min. The procedure complied with ICH recommendations Q2 (R1) for validation. and was discovered to be robust, precise, accurate, and specific. It may be used effectively for routine analysis of Imeglimin hydrochloride in pharmaceutical dosage forms as well as in bulk.

Keywords: *Imeglimin HCL, HPLC, New method development, Validation, ICH guidelines.*

1. INTRODUCTION

Drugs that assist reduce elevated blood sugar, a defining feature of diabetes mellitus, are known as antidiabetic drugs. Diabetes is brought on by the body's incapacity to make or use insulin, a hormone that is essential for regulating blood sugar levels. The immune system attacks and destroys pancreatic beta cells in type 1 diabetes, which accounts for 5–10% of cases and needs to be managed with insulin therapy [1]. Although it can strike at any age, type 2 diabetes, which accounts for 85–90% of cases, usually affects adults. The pancreas generates insulin in type 2 diabetes, but the body's cells develop resistant to its effects, which raises blood sugar levels [2]. Imeglimin hydrochloride (IMGH) is an oral antidiabetic medication under investigation. IMGH is being developed to treat diabetes mellitus type 2. IMGH's mode of action differs from that of other antidiabetic medications. It targets the cell's mitochondria in an effort to increase insulin sensitivity and secretion. The pharmaceutical business uses a chemical molecule called IMGH. It is an experimental medication specifically designed to treat type 2 diabetes mellitus [3-4]. The pathogenesis of type 2 diabetes has three components, which are evidently unaddressed by anti-diabetic drugs now available on the market: increased glucose production, insulin resistance, and pancreatic b-cell dysfunction. [6-8]. The "Glimins" family includes the recently approved oral anti-diabetic medication Imiglimin hydrochloride. Its molecular formula is C6H14ClN5, and its weight is 191.66 g/mol. Its chemical structure is (R)-6-imino-N, N,4-trimethyl-1,4,5,6-tetrahydro-1,3,5-triazin-2-amine hydrochloride [9-10].



Chemical structure of Imeglimin Experimental work

2. MATERIALS AND METHOD

Instrumentation and Chromatographic Conditions

The Agilent 1260 Infinity II High-Performance Liquid Chromatography (HPLC) system you mentioned is a highly acclaimed type that is renowned for its effectiveness and dependability in compound separation. The Openlab EZ Chrome software and UV detector aid in accurate data processing and analysis. The Zodiac C8 column (150 x 4.6 mm, 5µ) combined with a mobile phase of water: methanol (80:20) at a flow rate of 1 mL/min strengthens this analytical setup even more. With a 50 µL injection volume and a 240 nm detection wavelength, this system allows for highly precise target compound separation and quantification. Another effective analytical equipment is the double beam UV-visible spectrophotometer (Jasco UV 550) that you mentioned. It is particularly useful for classifying and quantifying compounds based on their absorption spectra. Accurate measurements are ensured by the matching quartz cells.

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND OPTIMIZATION

The standard solution of Imeglimin HCL was used for method development trials to optimize the method for determination of Imeglimin HCL 1000 mg. Teat samples were prepared by optimizing sample preparation which was used for method development trials to optimize the method as a Specific, Accurate, Precise and robust.

3. PREPARATION OF SOLUTION

Preparation of Buffer solution:

Weigh and transfer 2.38 g of sodium dihydrogen phosphate in 1000 mL volumetric flask. Add 700 mL water, sonicate to dissolve and dilute up to the mark with water. mix well and adjust pH 3.5 ± 0.05 with Phosphoric acid. Filter through 0.45µ nylon membrane disc filter.

Preparation of Mobile phase:

A mixture buffer pH 3.5 and methanol in the ratio 80:20 *v/v*, was prepared, mixed and sonicated to degas used as mobile phase.

Preparation of diluent:

0.1 % OPA in water and methanol (70:30%*v/v*) was used as diluent.

Preparation of Blank:

Use diluent as blank.

Preparation of Standard solution:

Weighed and transferred accurately about 40 mg of Imeglimin HCL working standard into 100 mL clean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 30 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 10.0 mL of this stock solution to 50 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Imeglimin HCL standard solution: 80 ppm)

Preparation of Sample solution:

Weighed and transferred 2 Imeglimin HCL tablets in to 250 mL clean and dry volumetric flask. Added about 200 mL of diluent, sonicate for 60 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Further diluted above stock solution 2.0 mL of this sample stock solution to 200 mL volumetric flask make

up with Diluent and mixed well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Sample Solution: 80 ppm)

4. RESULTS AND DISCUSSION

Table.No.1: Final reversed phase High performance liquid chromatographic condition.

Column	Hypersil ODS C18 (150 x 4.6mm) 5µ
Mobile Phase	Buffer pH 3.5 and Methanol (80:20)
Flow Rate	1 mL/min
Injection Volume	10 µL
Wavelength	240 nm
Column Temp.	25°C
Auto sampler Temp.	25°C

Observation: Imeglimin analyte eluted at 3.60 minutes with acceptable chromatography (Asymmetry: 1.12 and Theoretical plates 5516)

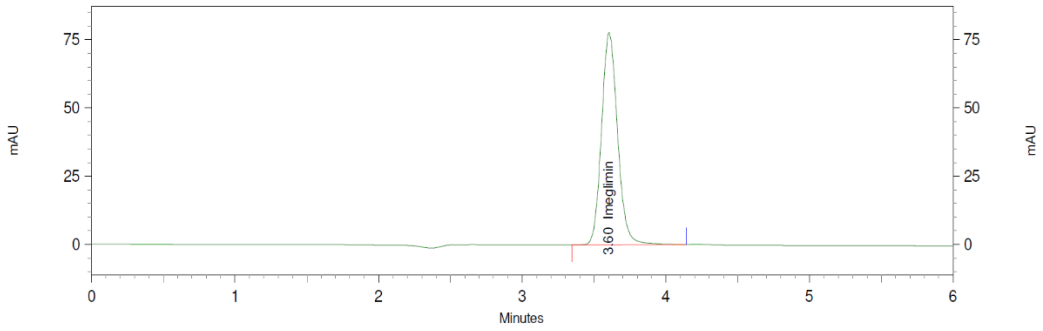


Fig. 1: Typical chromatogram for Imeglimin

Method Development:

The standard solution of Imeglimin HCL was used for method development trials to optimize the method for determination of Imeglimin HCL 1000 mg. Teat samples were prepared by optimizing sample preparation which was used for method development trials to optimize the method as a Specific, Accurate, Precise and robust.

Method Validation

1.System suitability: System suitability test was carried out in validation and routine use to verify that the analytical system is working properly to give accurate and precise results for further analysis. Standard solution was injected six times and the chromatograms were recorded.

Parameter	**Imeglimin**	**Acceptance Criteria**
Relative Standard Deviation (RSD)	**0.1**	**< 2.0**
Tailing Factor	**1.14**	**< 2.0**
Theoretical Plates	**5514**	**> 2000**
Retention Time		**+10**

Table.No.2: System suitability data for Imeglimin

2.Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table

Solution	**Specificity data**		
	Retention time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	3.60	Purity angle	Purity threshold
		1.47	2.33
Sample solution	3.60	1.25	2.14

Table.No.3: Specificity (Identification and Interference)

Sample Name: BLANK

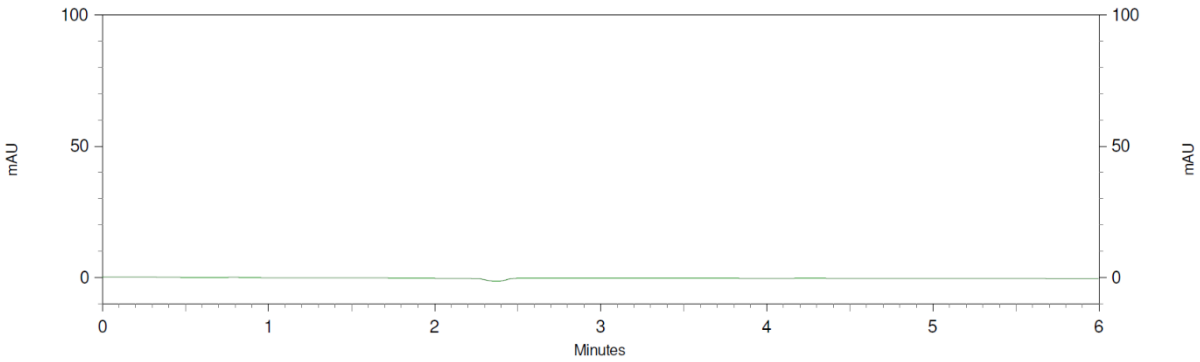


Fig.2: Chromatogram of Blank

Sample Name: STANDARD SOLUTION_1

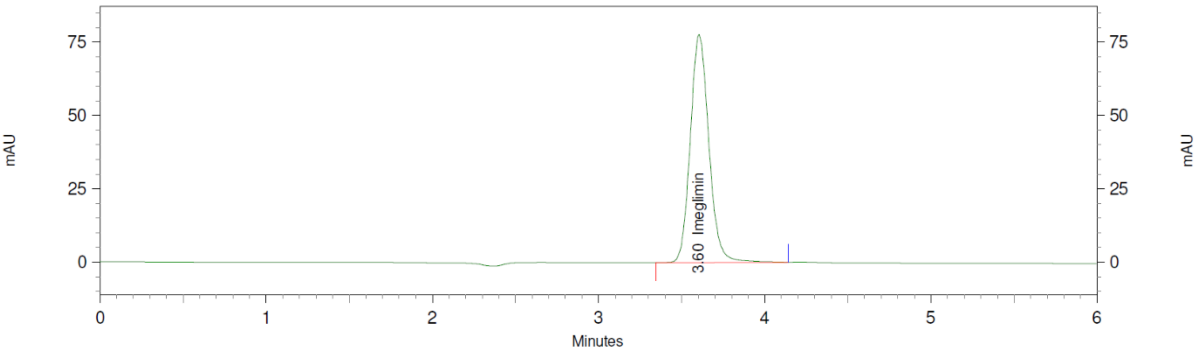


Fig.3: Chromatogram of Standard

Sample Name: SPL

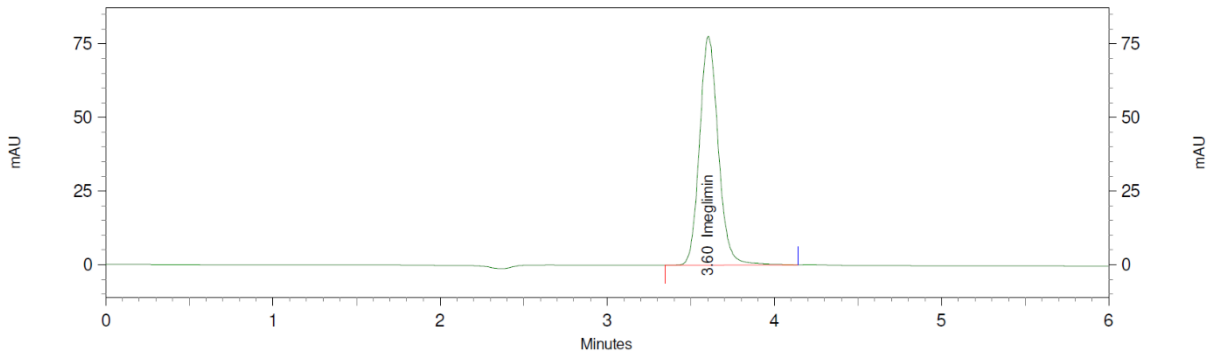


Fig.4: Chromatogram of Sample

Sample Name: PLACEBO

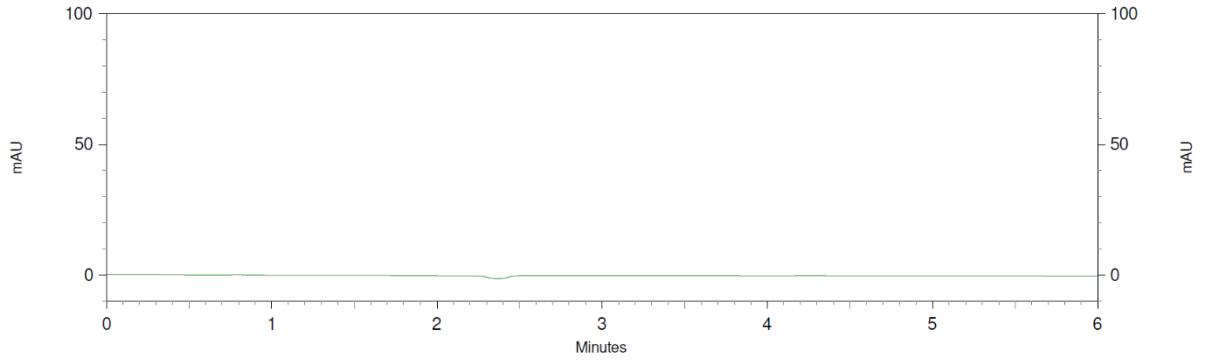


Fig.5: Chromatogram of Placeb

3.Linearity:

- Linearity was evaluated in the range of 50 % to 150 % of Apremilast for working concentration. The working concentration of Imeglimin HCL in solution is 80 µg/mL. The data summarized in Table.
- The data shows that the response is found to be linear.
- Co-relation coefficient (r^2 was found 0.9997.

Level	Conc. (µg/mL)	Area	Mean
50%	40	5216204	5212240
		5223659	
		5196857	
75%	60	7986524	7986165
		7989551	
		7982419	
100%	80	10536521	10547370

		10542594	
		10562996	
125%	100	13129561	13130541
		13132504	
		13129558	
150%	120	15940215	15933737
		15932506	
		15928491	
Corr. Coeff			0.9997
Intercept			85391
Slope			130306
% Y-intercept			0.81

Table.No.4: Linearity of Imeglimin HCL

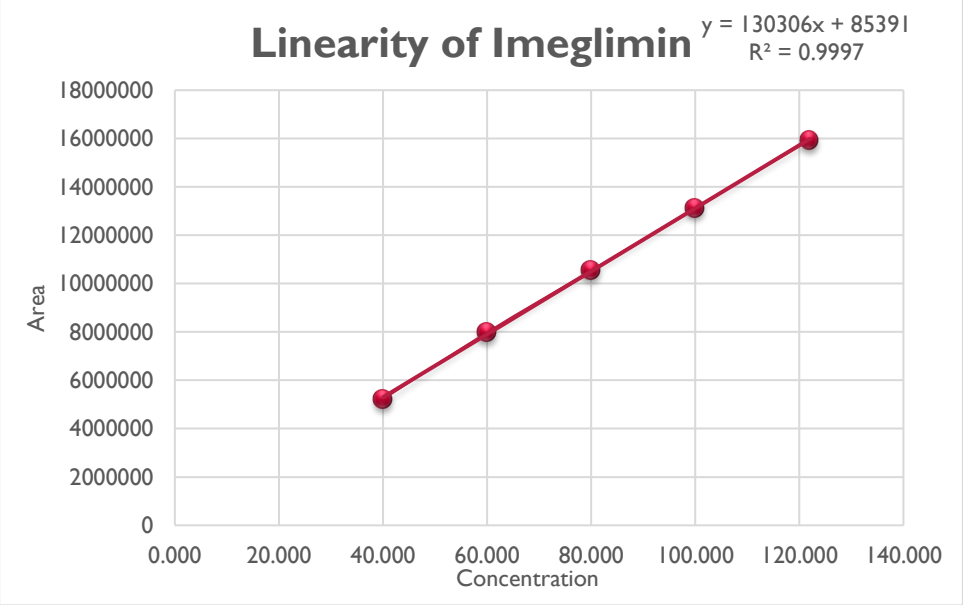


Fig.6: Linearity plot of Imeglimin HCL

4.Accuracy (Recovery):

Evaluated accuracy from 50% to 150% of Imeglimin HCL tablet, working concentration level. Each level prepared in triplicates. The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 95.0% - 105.0%.

Level (%)	Imeglimin HCL Added Conc. (µg/mL)	Imeglimin HCL Recovered Conc.	Area	% Recovery	Mean % Recovery
50	40.02	39.98	5260418	99.26	100.05
	40.05	40.04	5316849	99.73	
	39.99	40.05	5328513	101.15	

100	40.05	80.01	10569524	99.62	100.45
	79.98	80.10	10659856	101.18	
	79.97	88.02	10582103	100.54	
150	120.02	120.12	15959854	100.65	100.08
	119.98	119.99	15820419	100.04	
	120.03	119.96	15793527	99.54	

Table.No.5: % Recovery for Imeglimin HCL

5. PRECISION:

5.1Method Precision:

- Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system. The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

Sample	**Area**	**% Assay**
Sample 1	10253267	96.72
Sample 2	10359142	98.11
Sample 3	10256824	96.29
Sample 4	10429653	98.15
Sample 5	10329567	97.52
Sample 6	10355206	98.31
Mean		**97.51**
STD DEV		**0.8398**
% RSD		**0.861**

Table.No.6: Method precision

5.2 Intermediate Precision:

six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Sample	**Area**	**% Assay**
Sample 1	10450219	98.89
Sample 2	10365109	97.30
Sample 3	10296524	96.51
Sample 4	10356869	97.46
Sample 5	10485623	98.91
Sample 6	10326857	97.33
Mean		**97.73**
STD DEV		**0.9646**
% RSD		**0.987**

Table.No.7: Intermediate Precision

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**AD/HPLC-07**	**AD/HPLC-24**
Column No.	**HPLC-21**	**HPLC-08**
Sample No.	**%Assay**	
1	96.72	98.89
2	98.11	97.30
3	96.29	96.51
4	98.15	97.46
5	97.52	98.91
6	98.31	97.33
Mean	97.51	**97.73**
Mean of Precision % Assay	**97.63**	
Absolute Mean difference % assay	**0.9**	

Table.No.8: Intermediate Precision Pool Data

6. ROBUSTNESS:

- This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.
- System suitability criteria were fulfilled.
- The difference of % assay value in each modified condition is within acceptance criteria.

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	10253267	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.3 ml/min	10352915	1.0
	1.2 ml/min	10182411	-0.7
Change in wavelength (±2 nm)	242 nm	10126527	-1.2
	238 nm	10412320	1.6

TableNo.9: Robustness for Imeglimin HCL

7. CONCLUSION

HPLC's ease of use, sensitivity, specificity, and ability to analyze complicated samples have earned it a valuable place in the analysis area. This method was used in the current study to estimate the formulation of the Imeglimin HCL tablet. The

investigation made use of an HPLC Agilent 1260 Infinity II with a Hypersil ODS C18, 5µ, 4.6 x 150mm column, and a UV/PDA detector as well as Openlab EZ Chrome workstation software. Imeglimin HCL standard and sample solutions were made in diluent. For the chromatogram's development, many pure solvents with varied polarities and in various ratios were tested as the mobile phase. The results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of above-mentioned Imeglimine HCL in their formulation.

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