International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Development and Validation of a Stability Indicating Reverse Phase: High Performance Liquid Chromatography Method for Estimation of Imeglimin

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Abstract

The aim of present research work is to ‘ DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING REVERSE PHASE – HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ESTIMATION OF IMEGLIMIN’ the RP- HPLC is analytical techniques in which separation of active constituent from in a mixture .The high-performance liquid chromatography (HPLC) of Shimadzu SCL-10AVP inbuilt with binary pump (LC-10ATVP), UV detector (SPD-10AVP), Rheodyne 20µl loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC-Solution software was used to interpret the HPLC reports. Zodiac C18, 5µm; 150 x 4.6 mm ID., column purchased from UltraChrom Innovatives Pvt. Ltd was used throughout the analysis. Digital weighing balance (ME-204) purchased from Mettler-Toledo (USA), ultra-sonicator Labman® purchased from UltraChrom Ltd, India. Digital pH meter from MettlerToledo was purchased from (Mumbai-India). 50 µ micro-syringe was purchased from Hamilton USA. 0.20µ and 0.45µ nylon membrane filters were purchased from Phenomenex® Mumbai, India the developed analytical method for the estimation of imeglimin in both bulk and tablet formulation has obliged the ICH guidelines. As per the ICH guidelines, the developed method has complied the linearity range (calibration data), accuracy/drug recovery studies (%), repeatability, precision studies (intraday and interday/intermediate), and robustness. Moreover, as per the ICH guidelines, the system suitability test performed for imeglimin has achieved all guidelines; including, tailing factor (T), separation factors (α), theoretical plates (N), capacity factor (k’), resolution ® and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained few degradation products for imeglimin.

Hence, this developed and validated method for investigation by reverse phase high performance liquid chromatography (RP-HPLC) can be used for routine analysis of estimation of imeglimin from marketed formulation.

Introduction

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today. The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid

(mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase.

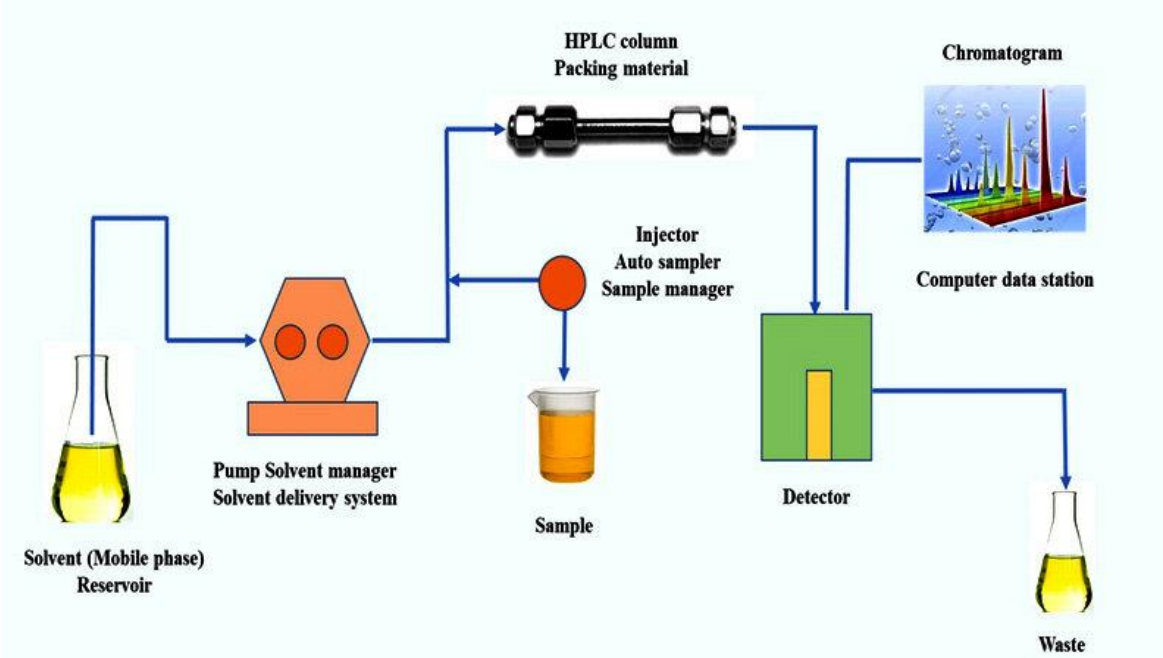


Fig NO.1 : schematic representation of RP- HPLC

Instrumentation

The high-performance liquid chromatography (HPLC) of Shimadzu SCL-10AVP inbuilt with binary pump (LC-10ATVP), UV detector (SPD-10AVP), Rheodyne 20µl loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC-Solution software was used to interpret the HPLC reports. Zodiac C18, 5µm; 150 x 4.6 mm ID., column purchased from UltraChrom Innovatives Pvt. Ltd was used throughout the analysis. Digital weighing balance (ME-204) purchased from Mettler-Toledo (USA), ultra-sonicator Labman® purchased from UltraChrom Ltd, India. Digital pH meter from Mettler-Toledo was purchased from (Mumbai-India). 50 µ micro-syringe was purchased from Hamilton USA. 0.20µ and 0.45µ nylon membrane filters were purchased from Phenomenex® Mumbai, India.

Method development

Analytical method development and validation studies play an important role in discovery, development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency, & performance of the pharmaceutical drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physicochemical properties (pKa, log P, solubility) and determining which mode of detection would be suitable for analysis (i.e., suitable wavelength in case of UV detection)

The majority of the analytical development effort goes into validating a stability indicating HPLC–method. The goal of the HPLC-method is to try & separate quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants. Steps involve in method development are:

1. Understand the physicochemical properties of drug molecule.
2. Set up HPLC conditions.
3. Preparation of sample solution for method development.
4. Method optimization.
5. Validation of method

Method Validation studies

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use. The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures. All analytical methods that are intended to be used for analyzing any clinical samples will need to be validated. The validation of analytical methods is done as per ICH guidelines. Components of method validation studies which have to be evaluated during methods validation are as follows

System Suitability test

- Repeatability
- Precision studies (Intraday and Interday/intermediate)
- Linearity/Calibration studies
- Detection limit (LOD)
- Quantitation limit (LOQ)
- Robustness
- Accuracy/Drug recovery studies
- Forced degradation/Stability indicating studies
- Peak retention time,
- Peak tailing,
- Capacity factor (k´),

1. Repeatability

Repeatability is the variation experienced by a single analyst on a single instrument. It does not distinguish between variation from the instrument or system alone and from the sample preparation process. During validation, repeatability is performed by analyzing multiple replicates of an assay composite sample by using the analytical method [63,65]. The recovery value is calculated. Intermediate precision is the variation within a laboratory such as different days, with different instruments, and by different analysts. The precision is then expressed as the relative standard deviation. 2. Precision studies The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method (53). It consists of two components: repeatability and intermediate precision or system alone and from the sample preparation process. During validation, repeatability is performed by analyzing multiple replicates of an assay composite sample by using the analytical method. The recovery value is calculated.

2. Linearity

Linearity is the ability of analytical procedure to obtain a response that is directly proportional to the co-

ncentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of analyte in samples within a given range. Linearity is usually expressed as the confidence limit around the slope of the regression line. Range is defined as the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

3. Detection limit (LOD)

The detection limit (DL) or limit of detection (LOD) of an individual procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value ( ). In analytical procedures that exhibit baseline noise, the LOD can be based on a signal-to-noise (S/N) ratio (3:1), which is usually expressed as the concentration of analyte in the sample.(book) The signal-to-noise ratio is determined by: s = H/h Where H = height of the peak corresponding to the component. H = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.

4. Limit of Quantification (LOQ)

The limit of Quantitation (LOQ) or Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy [54]. For analytical procedures such as HPLC that exhibit baseline noise, the LOQ is generally estimated from a determination of S/N ratio (10:1) and is usually confirmed by injecting standards which give this S/N ratio and have an acceptable percent relative standard deviation as well.

5. Robustness studies of HPLC

Robustness is defined as the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters (e.g. pH, mobile phase composition, temperature and instrumental settings) and provides an indication of its reliability during normal usage. Determination of robustness is a systematic process of varying a parameter and measuring the effect on the method by monitoring system suitability and/or the analysis of samples.

6. Accuracy or Drug recovery studies

Accuracy is the nearness of a measured value to the true or accepted value. Accuracy indicates the deviation between the mean value found and the true value (43). Lt is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. It may often be expressed as the recovery by the assay of known, added amounts of analyte.

7. Forced degradation and stability indicating studies

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The FDA and ICH guidance state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors.

8. Tailing factor

Our treatment of chromatography in this section assumes that a solute elutes as a symmetrical Gaussian peak, such as that shown in Figure 1 as dotted line. This ideal behaviour occurs when the solute's partition coefficient, KD is the same for all concentrations of solute

KD = [Ss] / [Sm]

[S]s= concentration of solute in the stationary phase,

[S]m = concentration of in the mobile phase,

Material and method

1. Reagents

HPLC grade acetonitrile and deionised water were purchased from Merck (Mumbai, India). 0.20µ and 0.45µ nylon membrane filters were used and purchased from UltraChrom Innovatives Pvt. Ltd. (India). All other chemicals and reagents were used of analytical grade.

2. Standard stock solutions

Standard stock solution of marketed formulation was prepared by dissolving 10 mg of the drug in 10 ml of acetonitrile-methanol-water (4:4:2 v/v) in a 20 mL volumetric flask. Furthermore, freshly prepared sample solution was sonicated for 10-20 minutes and filtered through 0.20µ nylon filters. Required serial dilution was made for evaluating the validation studies

3. Chromatographic conditions

Chromatographic separation was achieved on Acclaimed mix-mode HILIC-1 (150 mm × 4.6 mm, 5µm) applying an isocratic elution based on water- acetonitrile (40:60, v/v) as a mobile phase. The ultraviolet detector was operated at 230 nm. The buffer solution was filtered through 0.2 µm nylon membrane filter and degassed for 10-20 min in an ultrasonic bath prior to its use. The mobile phase was pumped through the column at a flow rate of 1.1 mL min-1. The column temperature was adjusted to 28◦C and the injection volume was 20 µL.

4. Sample preparation for drug recovery studies

Exactly 10-20 tablets of marketed formulation was containing 500 mg of were weighed separately and then crushed to the fine powder. An accurately weighed 10 mg amount of the finely powder was transferred to 25 mL volumetric flask. It was then mixed with 10 mL of equal volume of acetonitrile-methanol-water (4:4:2, v/v) and sonicated for 20 mins. Furthermore, the solution was filtered through 0.20µ filter and then analysed with HPLC technique.

5. Sample preparation for Linearity/Calibration studies

Accurately measured aliquots of stock solutions equivalent to 32.15-500 µg, of REM, respectively were transferred separately into a series of 10 mL volumetric flasks. The final volume was adjusted with same mobile phase, and then 20 µL were injected into HPLC. A calibration curve (linearity graph) was plotted by calculating peak area against concentration.

6. Precision studies of the proposed method

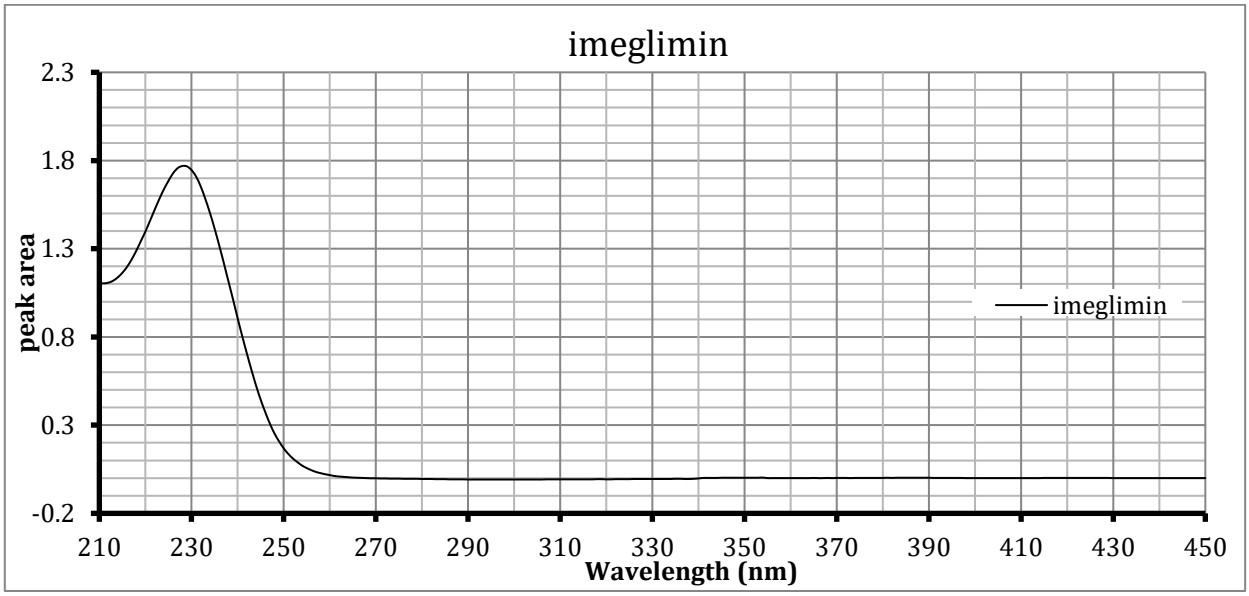
Nine similar concentrations of the marketed formulation (100 ppm) was analyzed within the same day (intraday precision), using the chromatographic condition Similarly, the same concentrations (100 ppm) of marketed formulation were analyzed in 3 successive days using the same chromatographic condition to determine the intermediate precision.

7. Robustness for the chromatographic method

The flow rate of the mobile phase was changed by ±1 decimal from 1 mL/min to 1.1 mL/min and to 0.9 mL/min to evaluate the effect of the flow rate; similarly, the variation of organic modifier used as acetonitrile was changed by ±2% from 70% to 72% and 68% to monitor the peak area and retention time. Finally, the effect of wavelength was monitored by making deliberate variation from 230 to 228 and 232 nm and the differences in system suitability parameters such as retention time, peak tailing, capacity factor, resolution and theoretical plates were tested and evaluate Discussion

Results and discussion

1.1. UV spectral analysis of imeglimin



UV spectra of imeglimin

1.2. Application of new proposed RP-HPLC method

After comprehensive literature survey, there are very few HPLC analysis of imeglimin have been reported. Moreover, the reported article has used the C18 based RP-HPLC for the quantification of imeglimin. However, since the imeglimin is highly polar diabetic drug so it hardly retains in C18 based RP-HPLC. Moreover, it is recently approved in the indian market so very few studies have been done on this particular drug.

Therefore, owing to it polar nature, alternative technique like Mix-Mode chromatography have been attempted. Most particularly, Acclaimed mix mode HILIC-1, 5µ column; 150 x 4.6 mm exhibited the good peak symmetry and height. Moreover, it improved peak sensitivity at 230 nm UV detection since as shown in figure 6.1 its λmax value is almost 230 nm; Importantly, along with method development and validation studies its force degradation studies have also been performed.

1.3 C18 based reverse phase HPLC

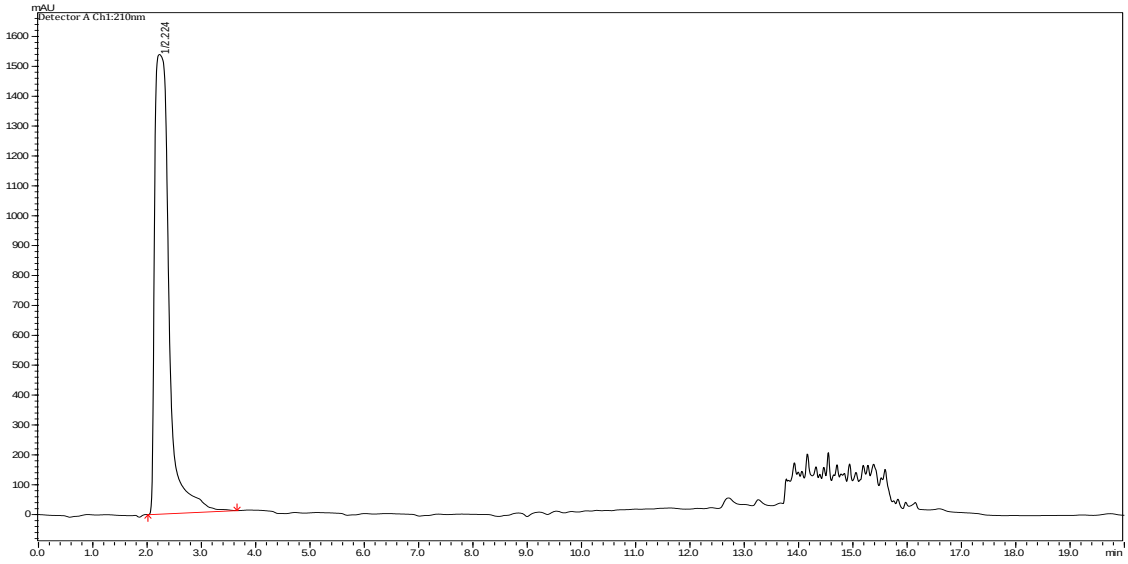


Figure 1.2; 1st trial of HPLC analysis of imeglimin HCl

Table No. 1.2; 1st trial of HPLC analysis of imeglimin HCl

Peak#	Ret. Time	Area	Height	Area %	T. Plate#	Resolution	k'	Tailing F.	Separation
imeglimin	2.224	28411931	1537681	100	556.12	--	0	2.236	0

Chromatographic Parameters

1. Analytes: Imeglimin (100ppm)
2. Column: Zodiac C18; 5µ, 150 X 4.6 mm. ID.
3. Mobile Phase: 15 mM KH_2PO_4- acetonitrile; 10:80 v/v
4. Flow rate: 1 ml/min
5. Elution mode: Isocratic elution mode
6. Wavelength selected: 230 nm
7. Temperature: Room temperature
8. Run time: 20 mins
9. Retention time: imeglimin (2.22 min)

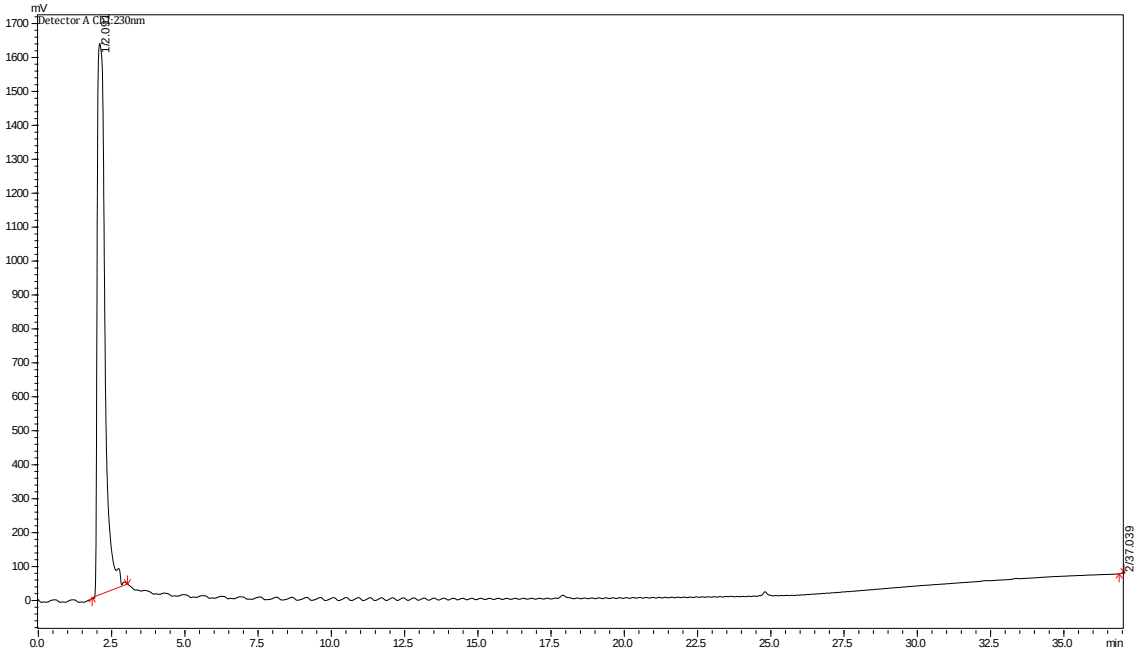


Figure 1.3; 2nd trial of imeglimin (2.09 min) at flow rate 1 mL/min

Table No. 6.3; method development parameters of imeglimin

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.	Separation
imeglimin	2.091	29751864	1624297	99.9883	471.95	--	0	2.263	0

Chromatographic Parameters

1. Analytes: Imeglimin (100ppm)
2. Column: Zodiac C18; 5µ, 150 X 4.6 mm. ID.

3. Mobile Phase: 15 mM formic acid- acetonitrile; 10:80 v/v
4. Flow rate: 1 ml/min
5. Elution mode: Isocratic elution mode
6. Wavelength selected: 230 nm
7. Temperature: Room temperature
8. Run time: 20 mins
9. Retention time: imeglimin (2.09 min)

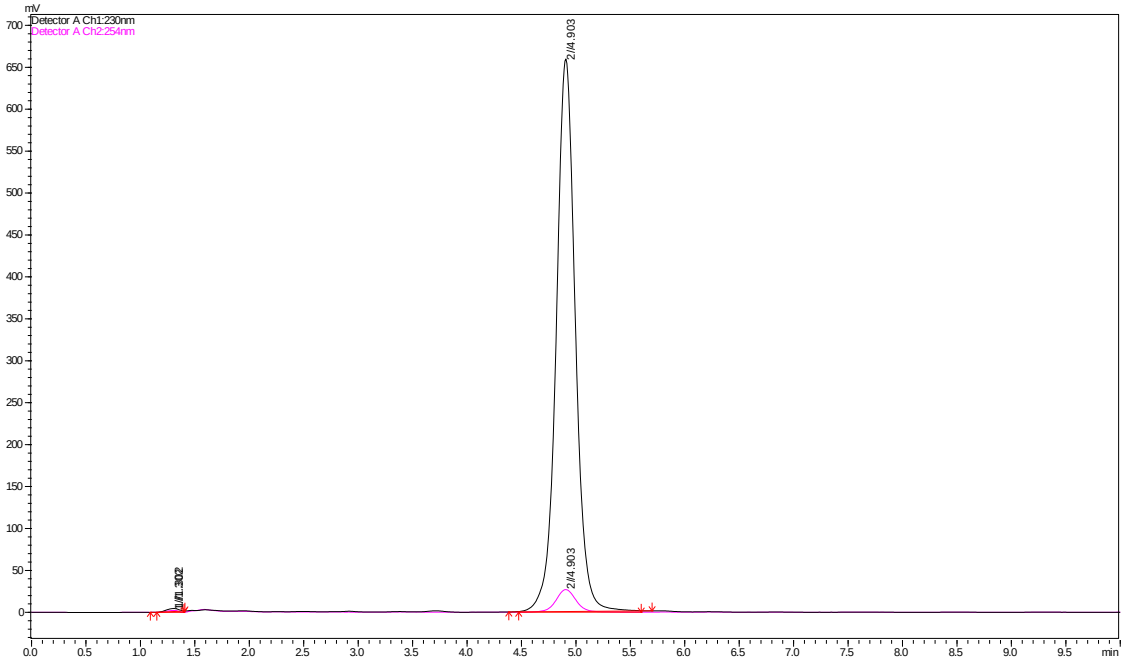


Figure 1.4; Method development of imeglimin (4.44 min) at flow rate 1 mL/min

Table No. 1.4; method development parameters of imeglimin

Peak#	Ret. Time	Area	Height	Area%	T Plate#	Resolution	k'	Tailing F.
1	1.336	48103	3643	0.5599	256.416	--	0	1.507
imeglimin	4.449	8542803	770826	99.4401	3901.801	10.066	2.331	1.054

1.2 Chromatographic Parameters

1. Analytes: Imeglimin (100ppm)
2. Column: Mix-mode HILIC-1; 5µ, 150 X 4.6 mm. ID.
3. Mobile Phase: 10mM ammonium acetate acetonitrile; 70:30 v/v
4. Flow rate: 1 mL/min
5. Elution mode: Isocratic elution mode
6. Wavelength selected: 230 nm
7. Temperature: Room temperature
8. Run time: 10 minutes
9. Retention time: imeglimin (4.44 min)

1.5. System suitability tests for Imeglimin

Table No. 1.6; System suitability data of imeglimin

System suitability parameters	Imeglimin (IMG)	Acceptable Values
Theoretical plates (*N*)	3874	> **2000**
Capacity Factor (*K'*)	2.766	> **1.5 - <10**
Resolution (*R*)	---	≥ **2**
Selectivity/Separation factor (α)	0.00	> ***k'***
Asymmetry/Tailing factor (*T*)	1.03	> **2**
Retention time (t_R)	4.903 min.	> ***k'***
Wavelength of Detection (nm)	230 nm	> **200 nm**
Repeatability (%RSD)	0.20	< **2**
Intra-Day Precision (%RSD)	0.20 – 0.84	< **2**
Inter-Day Precision (%RSD)	0.07 – 0.88	< **2**
Linearity range	15.65 – 100 µg/ml	**NA**
Regression equation	Y= 35958x - 43049	**NA**
SE of intercept (S_e)	92429.58	**NA**
SD of intercept (S_a)	41335.76	**NA**
Correlation Coefficient (r^2)	0.998	**NA**
LOQ (µg/mL)	3.79 µg/ml	**NA**
LOD (µg/mL)	11.49 µg/ml	**NA**

System System system suitability test included the theoretical plate (N), capacity factor (k'), resolution (R), separation factor (α), tailing factor (*T*), Mean±SD and RSD% which should always less than 2% for 6 repeatetive injections of same concentration. Table No. 6.2; displayed the system suitability studies for imeglimin

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1.6. Method validation

The method was validated according to ICH guidelines

1.7. Repeatability

Implementing the procedure mentioned under section ( ), the freshly prepared stock solution of imeglimin of same concentrations (100 µg/mL), were evaluated for six injections within the same day. The % RSD was calculated and found it is less than 2%; shown in (Table 6.2).

Table 1.7; Repeatability data of imeglimin

S. No.	**Imeglimin**
	Peak Area; Conc. 100 ppm
1	8054477
2	8022523
3	8056201
4	8066521
5	8052996
Mean	8050543

STD. DEV.	16537.25
RSD (%)	0.20

1.7.1. Intraday precision:

Implementing the procedure mentioned under section (5.5), the freshly prepared stock solution of IMEGLIMIN of three replicates of three same concentrations; 250ppm were tested and evaluated within the same day (intra-day precision). The %RSD was calculated and found less than 2%; shown in (Table 1.4).

Table 1.8; Intraday Precision data of imeglimin

Drug Name: Imeglimin (IMG)				
S. No.	Concentration (ppm)	Area	STD. Deviation	%RSD
1	100 ppm	8071715	21528.49	0.26
	100 ppm	8029231		
	100 ppm	8044410		
2	100 ppm	8151020	69145.16	0.84
	100 ppm	8261006		
	100 ppm	8278611		
3	100 ppm	8164001	16684.59	0.20
	100 ppm	8135719		
	100 ppm	8134523		
Range of %RSD				0.20 – 0.84

1.7.2. Interday (intermediate) precision:

Implementing the procedure mentioned under section (5.5), the stock solution of IMG of three replicates of three different concentrations; 250 ppm, were tested and evaluated in three successive days (interday/intermediate precision). The %RSD was calculated and found less than 2%; shown in (Table 6.5).

Table 1.9; Interday (intermediate) Precision data of imeglimin

Drug Name: Imeglimin (IMG)				
S. No.	Concentration (ppm)	Area	STD. Deviation	%RSD
DAY 1	100 ppm	8194200	15201.58	0.18
	100 ppm	8185512		
	100 ppm	8164624		
DAY 2	100 ppm	8051316	6104.97	0.07
	100 ppm	8039132		
	100 ppm	8045913		
DAY 3	100 ppm	8131021	72354	0.88
	100 ppm	8231003		
	100 ppm	8271612		
Range of %RSD				0.07 – 0.88

The above-mentioned concentrations were analyzed on three successive days using, the procedure mentioned under section (2.7). The % RSD was calculated and the results are shown in (Table 2).

1.8. Linearity

Under linearity or calibration studies, a linear relationship between area under peak values and selected drug concentration was plotted for five chosen concentrations (100, 50, 25, 12.5 and 6.25 µg/ml) of each drug. The regression equations, correlation regression coefficient values (R^2), standard error of intercept (S_e), standard deviation of intercept (S_a), limit of detection (LOD) and limit of quantification (LOQ) were calculated and displayed in table 6.6. Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3.3 and limit of quantification (LOQ) at which S/N is 10 were determined and results are given in (Table 6.10). Low values of LOD and LOQ indicate sensitivity of the applied method for determination of the mentioned drugs in tablets.

Table 1.10; Linearity data of imeglimin

Name of Drug**; Imeglimin (IMG)**			
S. No.	Concentration (µg/mL)	Area	Average (Mean)
1	100 PPM	8962176	8955948
	100 PPM	8949721	
2	50 PPM	4411430	4494852
	50 PPM	4578275	
3	25 PPM	2124505	2092642
	25 PPM	2060779	
4	12.5 PPM	931288	969040
	12.5 PPM	1006792	
5	6.25 PPM	682023	689029
	6.25 PPM	696035	
6	Regression Equation		y=90566x - 93127
7	Correlation coefficient (R^2)		0.9997
8	Std. Error of intercept		60411.15222
9	Std. Dev. of intercept		135083.443
10	LOQ		14.92 µg/ml
11	LOD		4.47 µg/ml

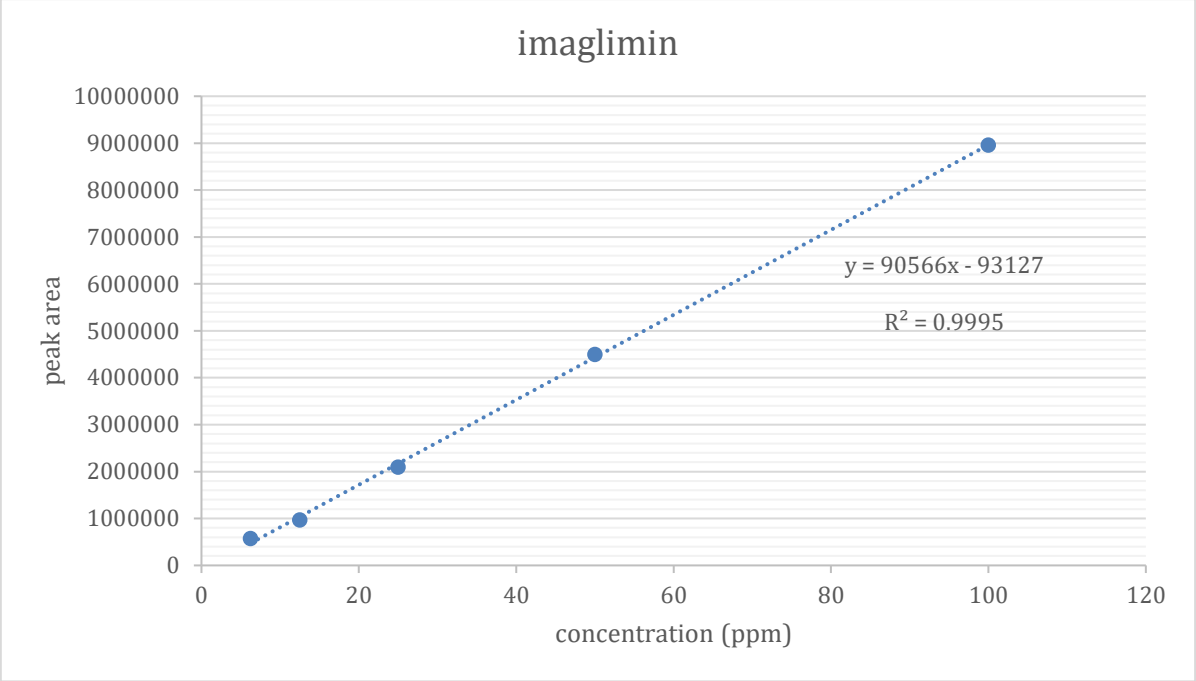

Fig. 1.5; Calibration curve of imeglimin

1.9. Robustness for the chromatographic method

Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in resolution, capacity factor, peak height and peak width (tailing factor). Robustness was studied for IMG, results obtained was displayed in Table 6.11. As resulted, the flow rate of the mobile phase was changed from 1 mL/min to 1.1 mL/min and 0.9 mL/min; results shown in table 6.11. Similarly, the effect of deliberate changes in organic modifier considered as acetonitrile composition (70±2%) evaluated to understand the separation behaviour of imeglimin. Finally, the wavelength was changed by ±2 nm and the effect on its sensitivity was evaluated and reported in Table 6.11.

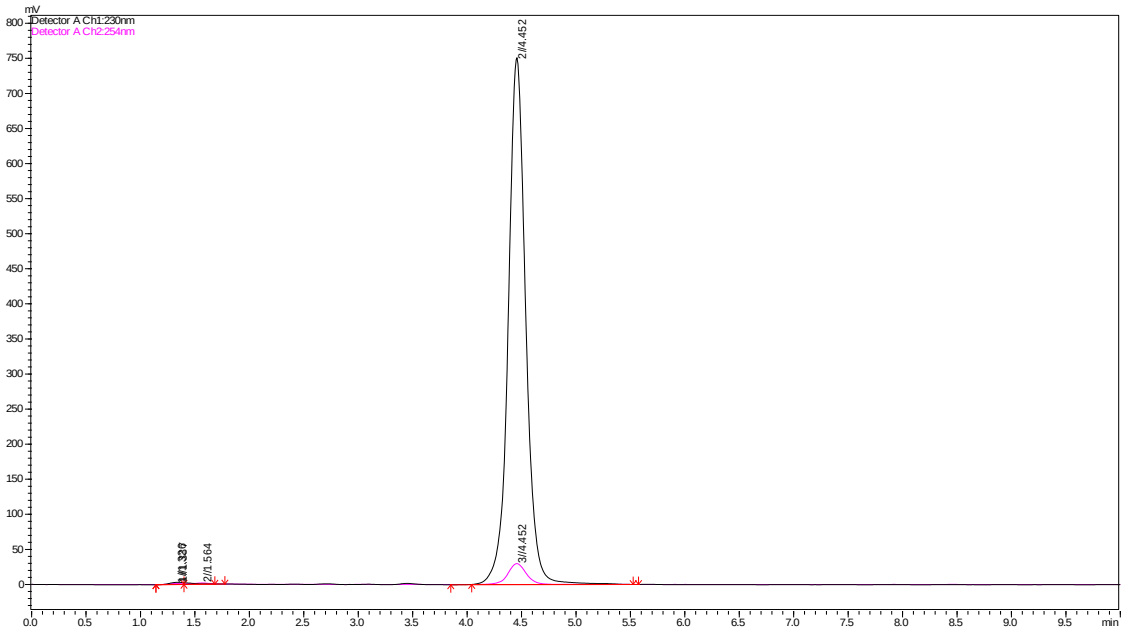

Figure 1.6; Chromatograph of imeglimin at 1.1 ml/min flow rate

Table No. 1.8; effect of flow rate 1.1 ml/min on imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.337	43155	3225	0.5115	208.989	--	0	1.487
imeglimin	4.452	8393031	751322	99.4885	3834.979	9.473	2.329	1.051

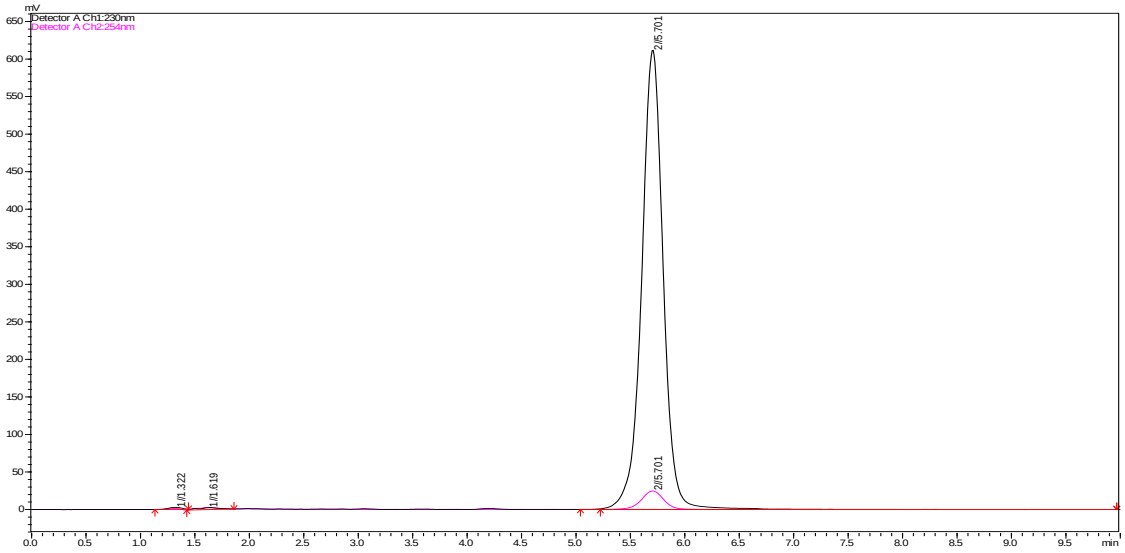

Figure 1.7; Chromatograph of imeglimin at 0.9 ml/min flow rate

Table 1.9; effect of flow rate 0.9 ml/min on Imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.322	22879	2743	0.2711	553.855	--	0	0.852
imeglimin	5.701	8415892	611666	99.7289	4259.499	15.256	3.313	0.975

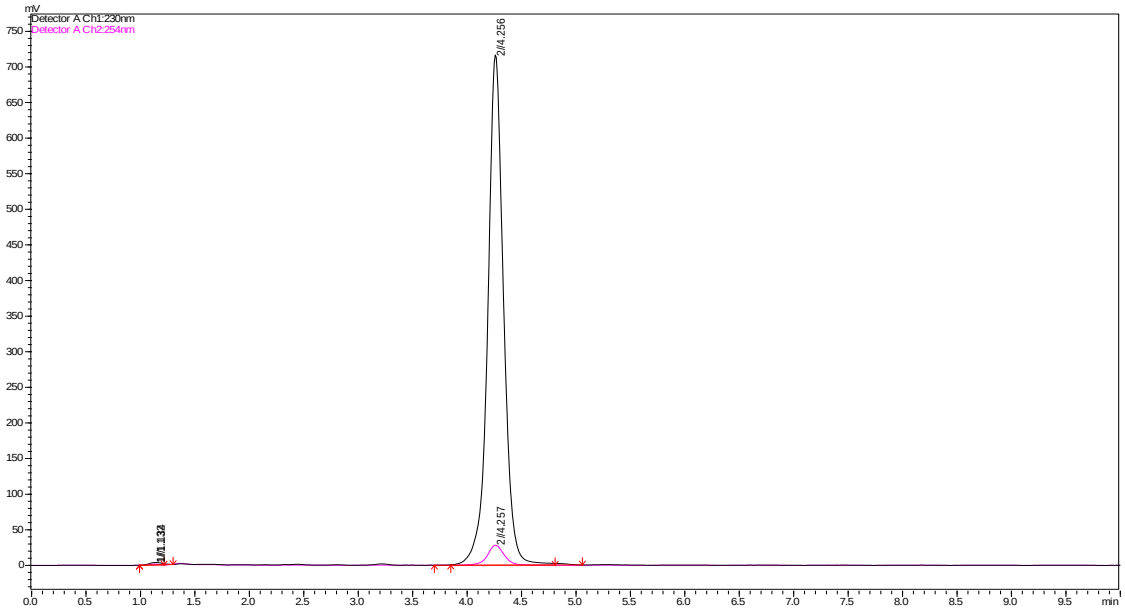

Figure 1.8; robustness studies, effect of acetonitrile 72%

Table No. 1.10; effect of organic modifier (+2%) on Imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.134	28126	3362	0.386	402.753	--	0	1.136
imeglimin	4.256	7257476	716407	99.614	4174.185	12.752	2.752	1.013

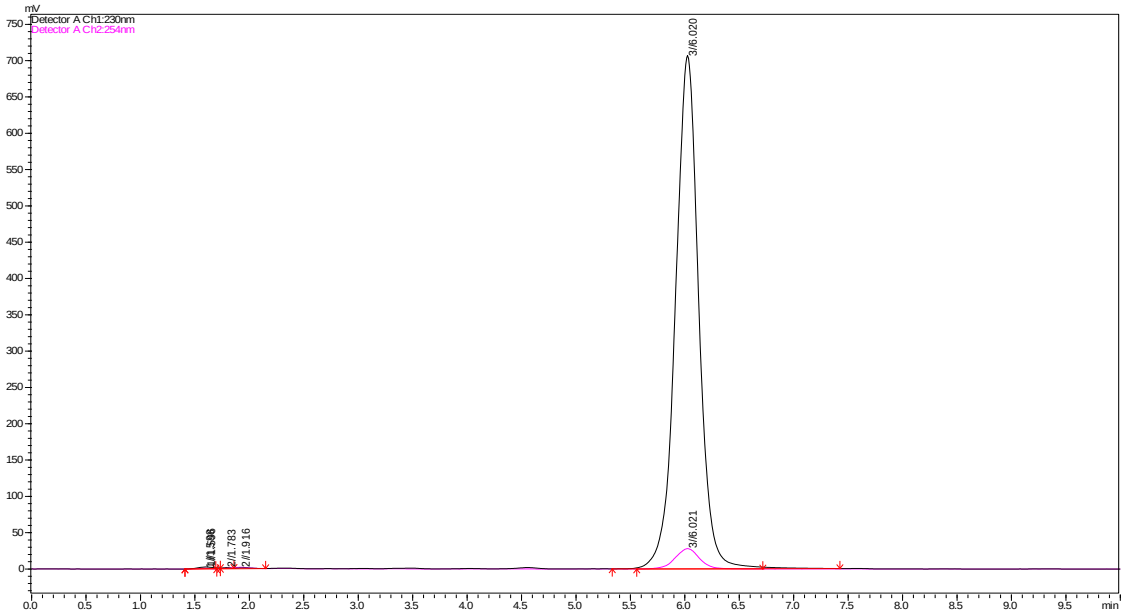

Figure 1.9; robustness studies, effect of acetonitrile 68 %

Table No. 1.11; effect of organic modifier (-2%) on Imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.596	24385	2410	0.2314	479.882	--	0	--
2	1.783	4543	928	0.0431	582.844	0.638	0.117	--
imeglimin	6.02	10507628	706789	99.7255	3835.829	12.384	2.772	0.998

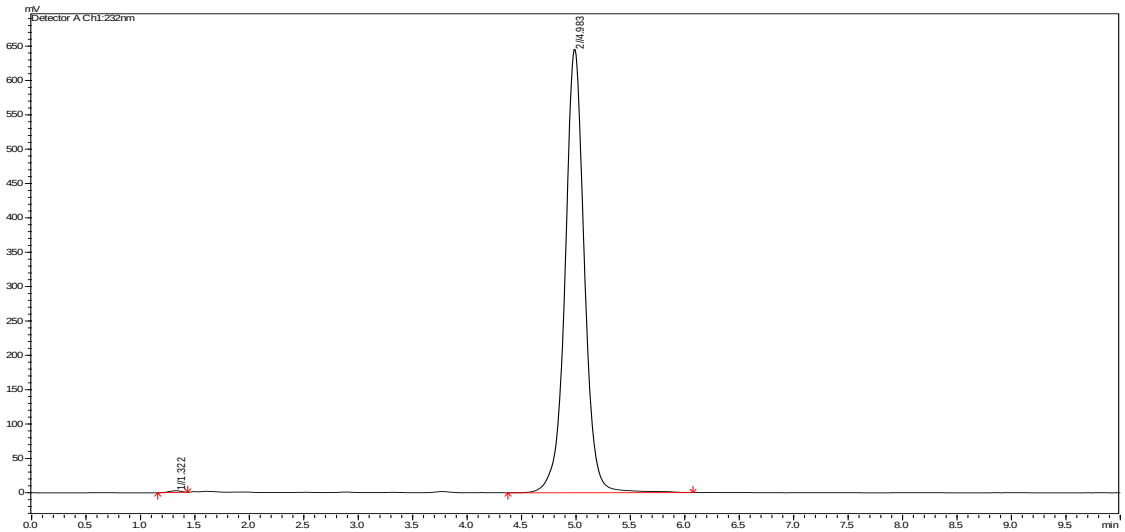

Figure 1.7; robustness studies, effect of wavelength 232 nm

Table No. 1.12; effect of wavelength (+2nm); 230 nm on imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.322	19028	2482	0.2385	606.427	--	0	0.852
imeglimin	4.983	7959143	645225	99.7615	3999.942	13.817	2.769	1.016

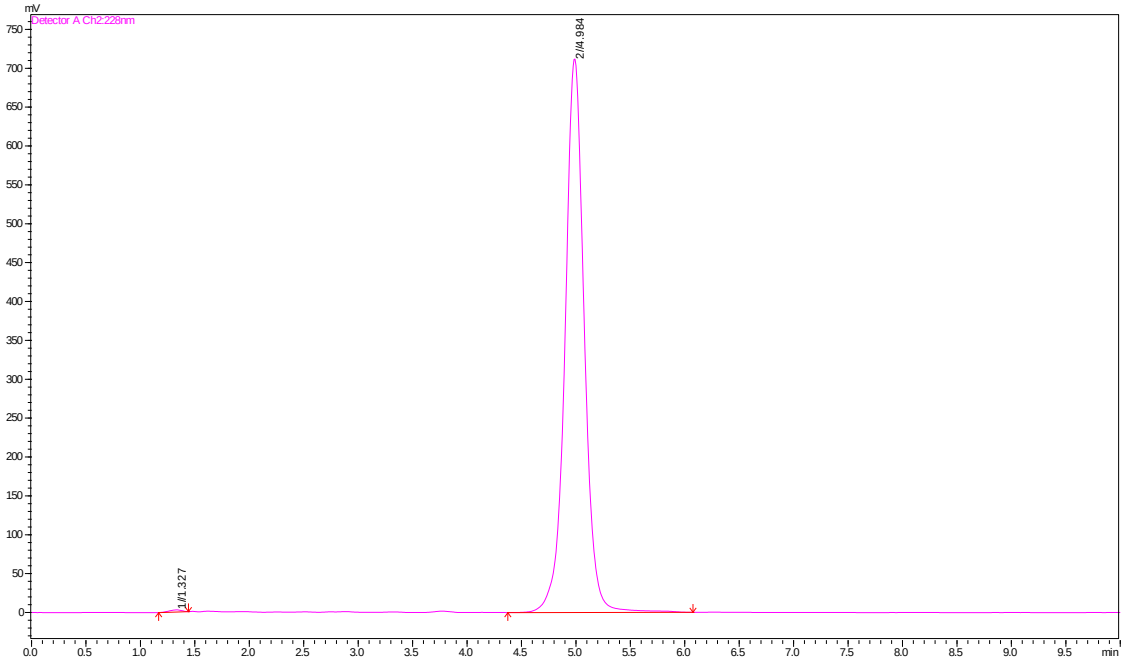

Figure 1.8; robustness studies, effect of wavelength 228 nm

Table No. 1.13; effect of wavelength (-2nm); 228 nm on imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.327	22131	2726	0.2523	545.748	--	0	0.871
imeglimin	4.984	8748478	711879	99.7477	4027.292	13.51	2.756	1.016

Table No. 1.14; Robustness data of Imeglimin

S. No.	F. (-0.2 ml/mL)	F (+0.2 ml/mL)	A (-2 ml)	A (+2 ml)	WL (-2 nm)	WL (+2 nm)
Resolution	----	-----	----	----	----	----
Tailing factor	1.05	0.97	1.01	0.99	1.01	1.01
Capacity factor	2.32	3.31	2.77	2.75	2.76	2.75
Theoretical plates	3834	4259	3835	4174	3999	4027

From all above robustness studies by making deliberated changes in flow rate (± 0.1mL/min), organic modifier used as acetonitrile (±2%) have made some changes in retention time of imeglimin, where reducing both composition and flow rate decrease the retention time and increasing them, increases the

retention time. and wavelength (230 ± 2nm) have not made any significant changes in resolution, capacity factor and tailing factor. Nonetheless, it seems minute changes in robustness studies have not made any significant changes in theoretical plate counts and peak area. The results have been displayed in table No. 6.14.

1.3.6. Accuracy or drug recovery studies

Accuracy of the results was calculated by percentage recovery of 3 different concentrations of each drug. The results including the mean of the recovery and standard deviation as shown in (Table 2).

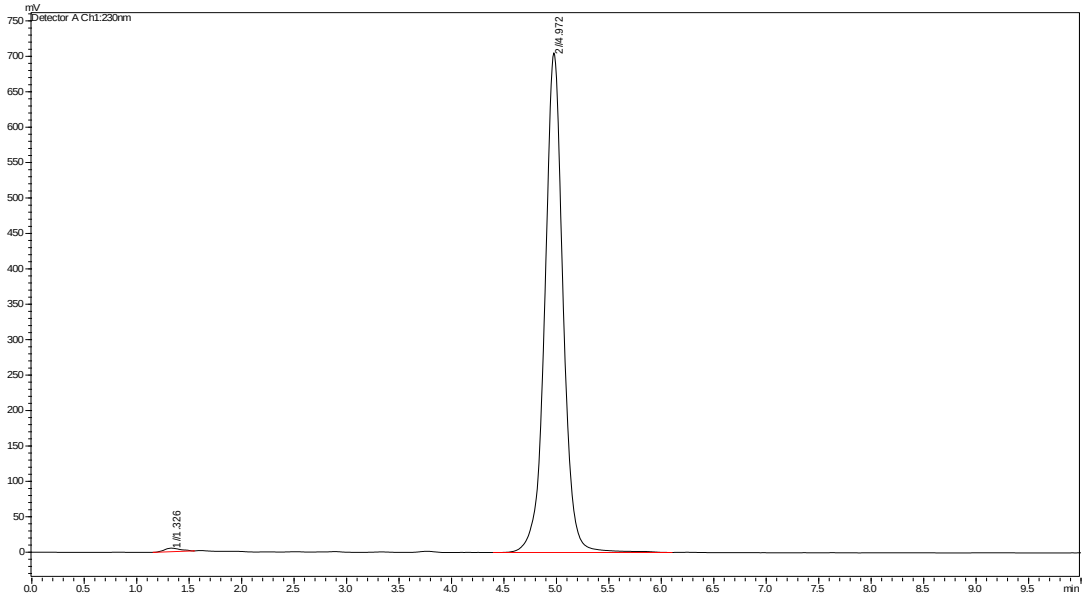

Figure No.1.9; recovery studies of imeglimin at 100%

Table No. 1.15; drug recovery studies of imeglimin at 100%

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.327	22131	2726	0.2523	545.748	--	0	0.871
imeglimin	4.984	8042802	711879	99.7477	4027.292	13.51	2.756	1.016

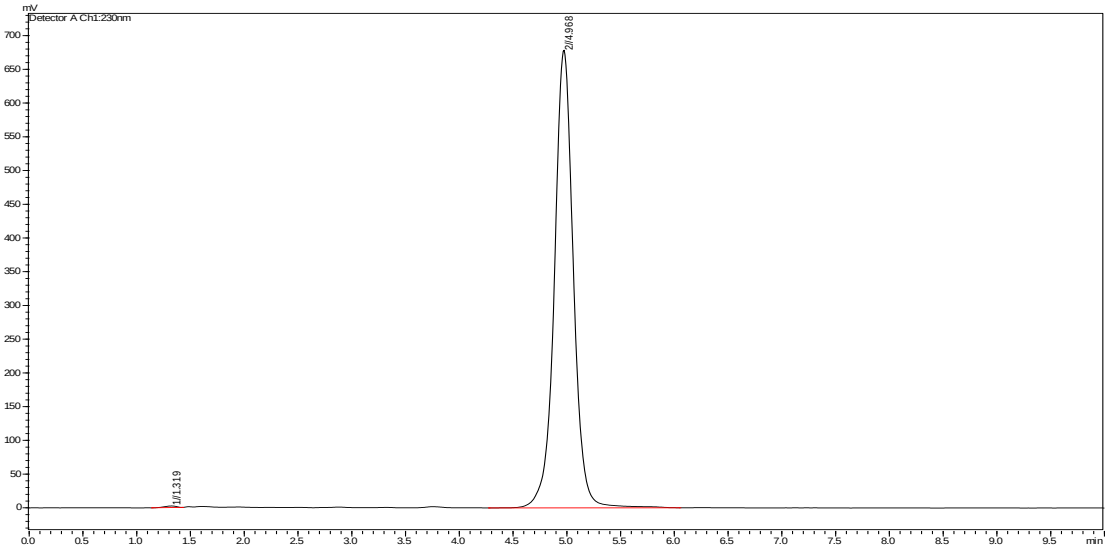

Figure No. 1.10; recovery studies of imeglimin at 120%

Table No. 1.15; drug recovery studies of imeglimin at 120%

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.327	22131	2726	0.2523	545.748	--	0	0.871
imeglimin	4.984	8748478	711879	99.7477	4027.292	13.51	2.756	1.016

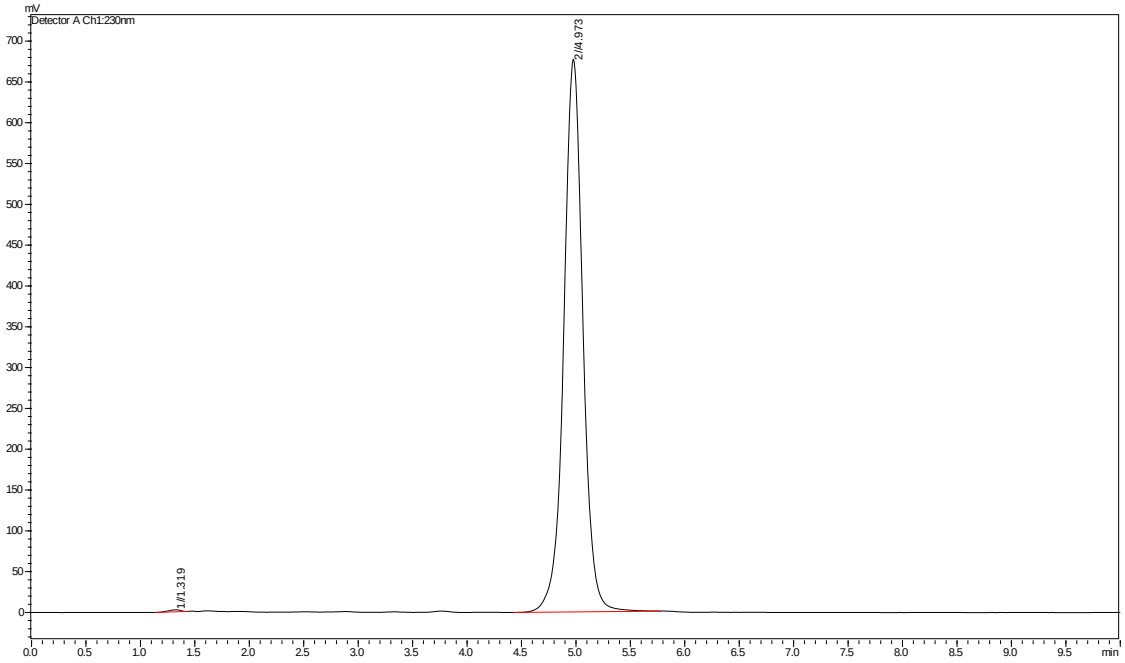

Figure No. 1.10; recovery studies of imeglimin at 100%

Table No. 1.15; drug recovery studies of imeglimin at 100%

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.327	22131	2726	0.2523	545.748	--	0	0.871
imeglimin	4.984	7009040	711879	99.7477	4027.292	13.51	2.756	1.016

Drug Name: imeglimin			Drug content: 500 mg		Marketed formulation: lupimeg		
Std. conc. (%)	Std. (ppm)	Peak area	Drug (%)	Drug (ppm)	Peak area	Avg. peak area	Drug Rec. (%)
100%	100 ppm	8050543	80	80	7009040	7013531	108.90
				80	7018023		
			100	100	8042802	8039514	99.86
				100	8036227		
			120	120	8799478	8785162	90.94

				120	8770847		
			Drug recovery Range (%) as per ICH = 100±10%				100 ± 8.98 %

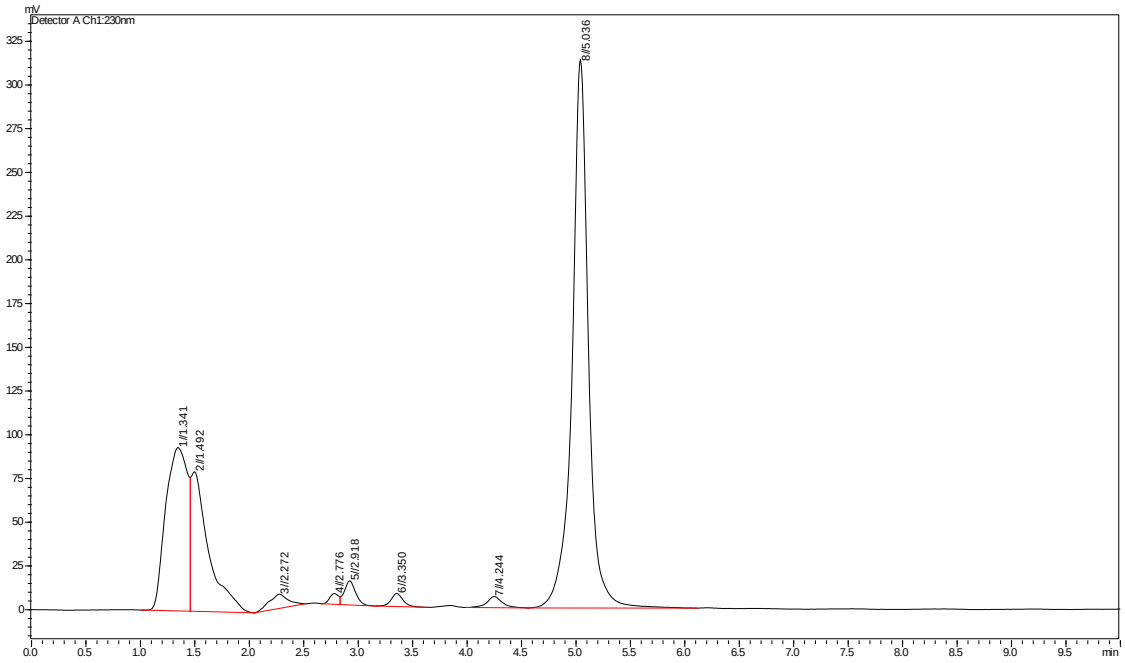

Figure No. 1.11; force degradation studies, effect of 0.1N HCl on imeglimin

Table No. 1.16; effect of 0.1N HCl on imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tail F.
1	1.341	1326425	93377	22.7203	61.979	--	0	--
2	1.492	884969	79777	15.1586	36.453	0.182	0.113	--
3	2.272	100691	8212	1.7247	722.969	1.176	0.695	1.056
4	2.776	35852	6077	0.6141	1726.469	1.664	1.071	--
5	2.918	97904	13690	1.677	3010.313	0.591	1.176	--
6	3.35	57304	7402	0.9816	4156.248	2.055	1.499	1.119
7	4.244	62846	6416	1.0765	4603.007	3.905	2.166	1.065
imeglimin	5.036	3272078	313759	56.0473	6370.578	3.153	2.757	0.974

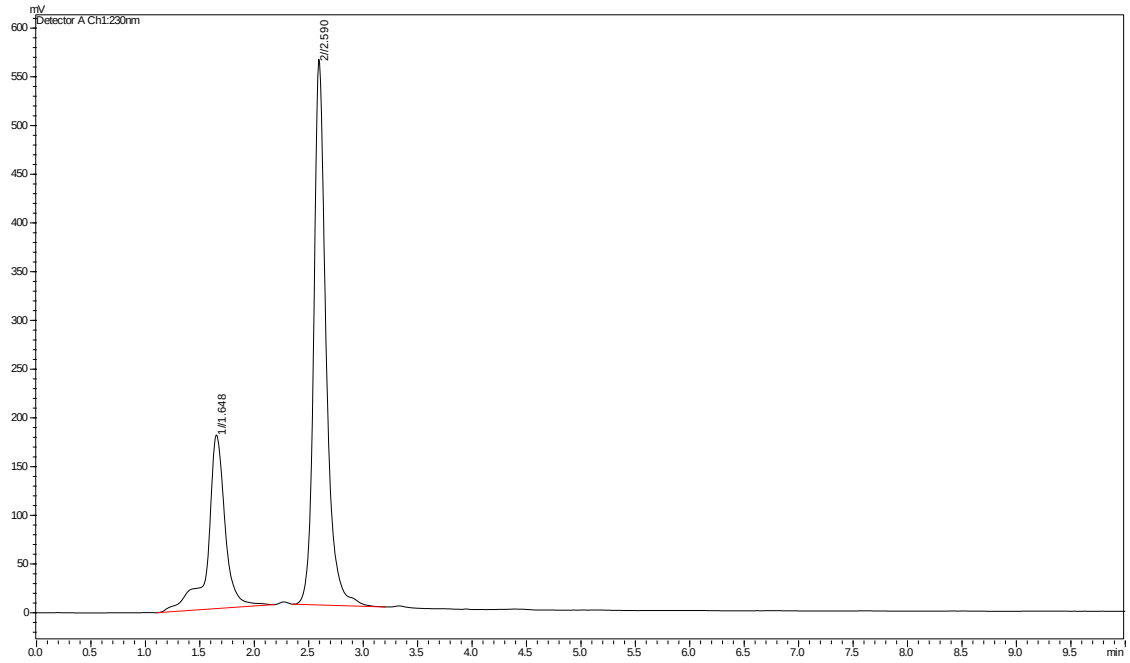

Figure No. 1. 12; force degradation studies, effect of 0.1NaOH on imeglimin

Table No. 1.17; effect of 0.1N NaOH on imeglimin

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tail F.
1	1.648	1976049	178277	30.8481	736.211	--	0	0.834
imeglimin	2.59	4429681	560080	69.1519	2513.81	4.191	0.572	1.331

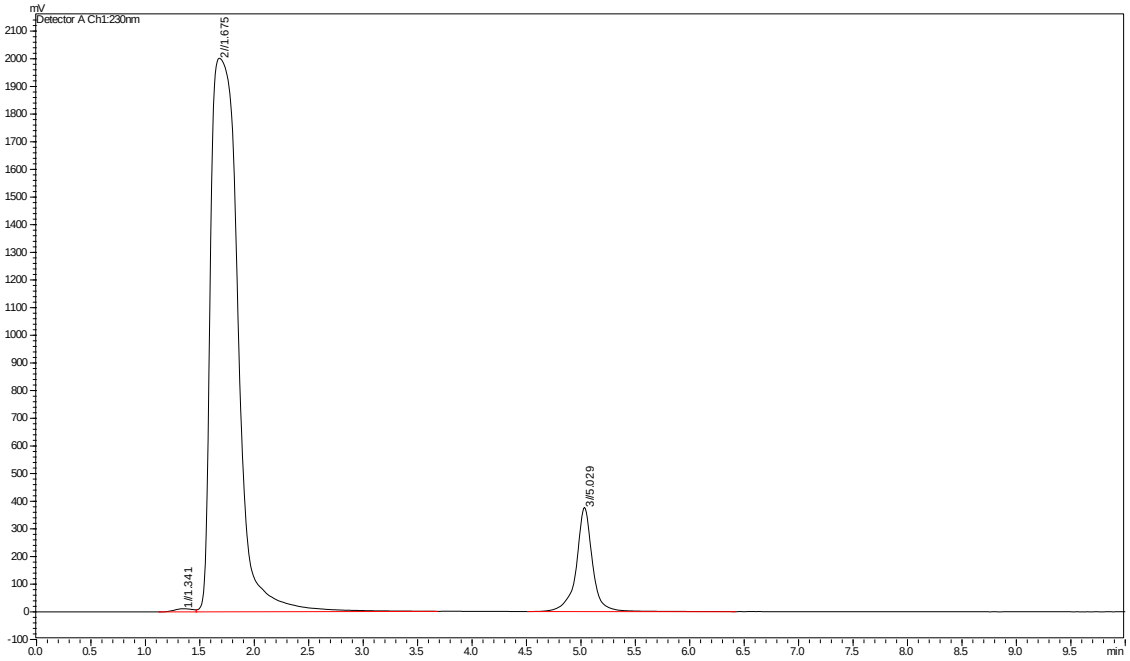

Figure No. 1. 13; force degradation studies, effect of 3% H_2O_2

Table No. 1.18; effect of 3% H_2O_2 on imeglimin

Peak#	Ret. T.	Area	Height	Area%	T.Plate#	Resolution	k'	Tail F.
1	1.341	130104	11246	0.3367	151.939	--	0	--
2	1.675	34631234	2001605	89.6356	317.028	0.822	0.249	1.811
imeglimin	5.029	3874231	376761	10.0276	6445.079	10.701	2.749	0.972

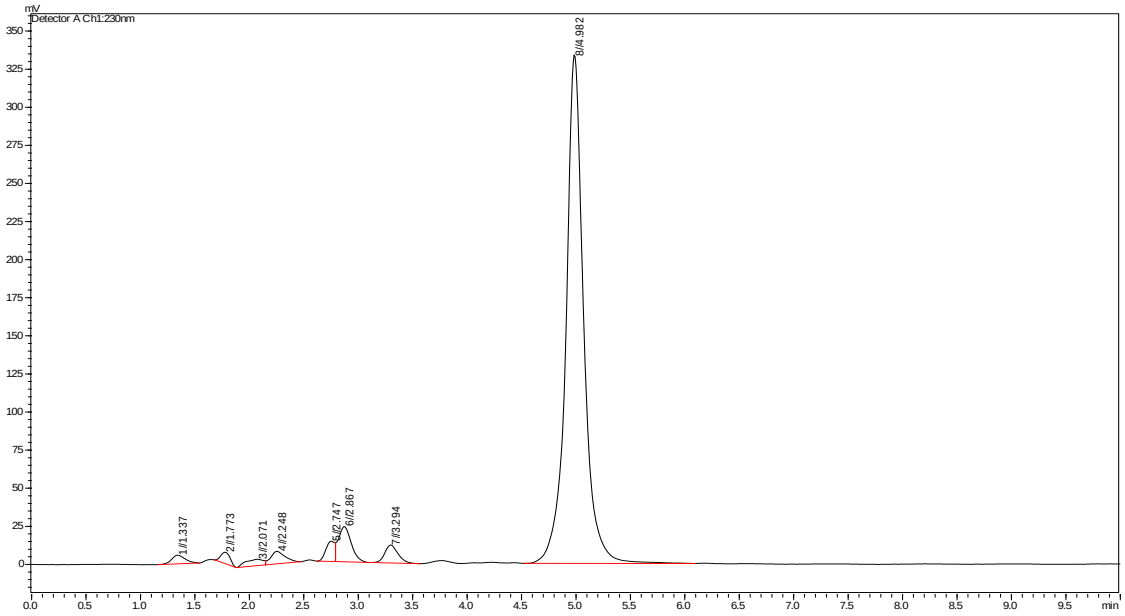

Figure 1.14; force degradation studies, effect of temperature

Table No. 1.19; effect of 45*C temperature

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	k'	Tail F.
1	1.337	49925	5727	1.1462	491.469	0	1.196
2	1.773	42678	7279	0.9798	1802.604	0.326	1.075
3	2.071	49329	4172	1.1325	481.795	0.549	--
4	2.248	74812	8229	1.7176	1197.164	0.681	--
5	2.747	78206	13243	1.7955	323.031	1.055	--
6	2.867	186038	23061	4.2712	1849.769	1.144	--
7	3.294	99450	11757	2.2832	3242.624	1.464	1.22
imeglimin	4.982	3775251	333683	86.674	5165.526	2.725	1.062

	Imeglimin (IMG)		Degradants of IMG
Conditions	% Area Std.	% degradation	No. of degradants
Acid (0.1N/M HCl) + 60°C + 12 Hrs.	56%	44%	7
Base (0.1N/M NaOH) + 60°C + 12 Hrs.	69%	30%	1
Thermal (45°C) + 12 Hrs.	87%	13%	7
Oxidation (3-6% H_2O_2) + Room Temp.	10%	90%	1

Table No. 1.15; effect of force degradation studies on imeglimin From above studies, it was

Conclusion

From all above results and discussion, it has been concluded that the developed analytical method for the estimation of imeglimin in both bulk and tablet formulation has obliged the ICH guidelines. As per the ICH guidelines, the developed method has complied the linearity range (calibration data), accuracy/drug recovery studies (%), repeatability, precision studies (intraday and interday/intermediate), and robustness. Moreover, as per the ICH guidelines, the system suitability test performed for imeglimin has achieved all guidelines; including, tailing factor (*T*), separation factors (α), theoretical plates (*N*), capacity factor (*k'*), resolution (*R*) and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained few degradation products for imeglimin.

Hence, this developed and validated method for investigation by reverse phase high performance liquid chromatography (RP-HPLC) can be used for routine analysis of estimation of imeglimin from marketed formulation.

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