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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF IMEGLIMIN HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATION BY UV-SPECTROPHOTOMETER

Uttam Prasad Panigrahy[1]*, Anupam Roy[2], Shayed Anowar Hussain[3], Kabya Pratim Das[4], Ananya Deka[5]

[1]*Associate Professor, Faculty of Pharmaceutical Science, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwahati, Assam, India
[2,3,4,5]Research Scholar, Faculty of Pharmaceutical Science, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwahati, Assam, India
*Corresponding Author E-mail: uttampanigrahy@gmail.com

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ABSTRACT:

Imeglimin Hydrochloride is an anti diabetic drug which blocks oxidative phosphorylation and inhibits hepatic gluconeogenesis, increase muscle glucose uptake, and restore normal insulin secretion. A rapid, simple, specific, and economic UV-spectrophotometric method has been developed to determine the stability indicating method for Imeglimin using distilled water as a solvent. The absorption spectrum of Imeglimin Hydrochloride showed maximum absorbance at 240nm and obeyed beer's law in concentration range 1-16µg/ml. Linear regression of absorbance on concentration gave the equation y=0.059x+0.003 with correlation coefficient 0.999. The method developed is validated for parameters like specificity, linearity, range, accuracy, precision, LOD, LOQ, robustness as per Q2A specifications of the ICH guidelines. The drug sample was exposed to acid, alkali, and oxidation by hydrogen peroxide, thermal and photolytic conditions to study the forced degradation of the drug. The forced degradation studies, it was found that the drug Imeglimin Hydrochloride in the presence of degradation products. By the forced degradation studies, it was found that the drug Imeglimin Hydrochloride is maximum degraded in the presence of acid and peroxide degradation, moderately degraded in alkali and thermal degradation and mild degradation in photolytic degradation as per the studies performed. So, it was concluded that the drug should be stored in cool and dark place, away from acid, alkali and peroxide environment. The obtained results proved that this method can be employed for the routine analysis of Imeglimin Hydrochloride in bulk as well as in the commercial formulations.

KEY WORDS: Imeglimin Hydrochloride, UV-Spectrophotometry, Maximum absorbance, Correlation coefficient, ICH guidelines.

1. INTRODUCTION:

Imeglimin HCl is a novel oral agent for the treatment of type 2 diabetes (T2D). Imeglimin's mechanism of action involves dual effects: (a) amplification of glucose-stimulated insulin secretion (GSIS) and preservation of β-cell mass; and (b) enhanced insulin action, including the potential for inhibition of hepatic glucose output and improvement in insulin signalling in both liver and skeletal muscle (Hallakou-Bozec et al., 2021). Imeglimin HCl is chemically known as (R)-6-imino-N, N, 4-trimethyl-1, 4, 5, 6-tetrahydro-1, 3, 5-triazin-2-amine hydrochloride was shown in Figure 1. Literature review tells that very few analytical methods

have been reported for the determination of Imeglimin HCl which includes development and Validation of RP-HPLC Method for Estimation of Anti-Diabetic Drug in Bulk and Tablet Dosage Form (Salvi et. al., 2023). Isocratic RP-UHPLC methodology for the quantitative measurement of Imeglimin Hydrochloride was developed (Jain et. al., 2023). A sensitive chiral liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique to estimate the (+) and (−) enantiomers of imeglimin in its formulation (Ramalingam et al., 2023). Pharmacokinetics of Imeglimin in Caucasian and Japanese Healthy Subjects (Fouqueray et al., 2022). A Review on Imeglimin Hydrochloride Immediate Release Tablet (Rautela et al., 2023). Imeglimin population pharmacokinetics and dose adjustment predictions for renal impairment in Japanese and Western patients with type 2 diabetes (Tomita et al., 2022). Imeglimin Hydrochloride: A Novel Approach to Type 2 Diabetes Management (Sharma et al., 2024). The present study was aimed to develop a novel, simple, economic and validated RP-HPLC method for the estimation of Imeglimin HCl to ICH guidelines (Shabir, 2003).

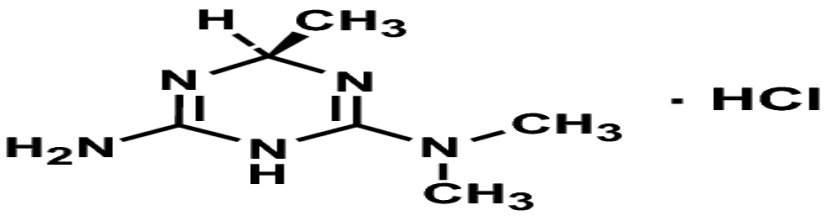

Figure 1: Chemical structure of Imeglimin HCl

2. MATERIALS AND METHODS:

Chemicals and Reagents:

Imeglimin HCl bulk drug were kindly provided as gift sample by Metrochem API Private Limited, Hyderabad, India. Water (Merck Chemical Company, HPLC-Grade) were used in the study. Imeglyn® tablet contain 500mg is obtained from a local pharmacy manufactured by Zydus Healthcare Ltd., India.

Instrumentation

The UV-Spectroscopic studies were performed on Shimadzu UV-1900 UV-Visible Spectrophotometer system, equipped with UV Probe software. All weights were taken on electronic balance (Model: CY64, Make: Citizen Scale) and Sonicator (Model: LMUC-3, Make: Enertech) were used in the study.

Preparation of Standard stock solution of Imeglimin Hydrochloride

A precisely measured quantity of Imeglimin Hydrochloride, 100mg, was transferred to distilled water into a volumetric flask of 100 ml. The volume was made using distilled water to produce a standard solution with a concentration of 1000 µg/mL. From the above solution 10ml was pipetted out and transferred into a volumetric flask of 100 ml. The final volume was made using distilled water to produce a standard solution with a concentration of 100µg/mL. Further dilutions were prepared using these stock solutions.

Preparation of working standard solutions

By pipetting 0.1, 0.2, 0.4, 0.8 and 1.6 mL of stock solution into 10 mL volumetric flasks, six working standard solutions of Imeglimin Hydrochloride were created. The volume was then make up to the proper level with distilled water to create concentrations of 1, 2, 4, 8 and 16 µg/mL respectively.

Selection of Analytical wavelength

The working standard solution of 8 µg/mL was scanned in a UV spectrophotometer from a range of 200-400nm against distilled water as a blank solution in order to determine the

analytical wavelength. The maximum absorbance at a given wavelength (max) was discovered to be at 240 nm which was shown in figure 2.

Preparation of sample solution

Tablets containing 500 mg of Imeglimin Hydrochloride having brand names Imeglyn® was procured. Five Imeglimin Hydrochloride tablets were broken into a fine powder using a triturator. The powder containing 100 mg of Imeglimin Hydrochloride equivalent weight was then precisely weighed and put into a 100 ml standard volumetric flask. Distilled water was used to dissolve the ingredients, and it was then ultra sonicated for 15 minutes. The entire solution was filtered using Whatmann filter paper (No. 41), and distilled water was then added upto the mark to create the 1000 µg/ml solution and again 10ml was pippetted out and transferred in a volumetric flask with a volume of 100 ml and distilled water was then added upto the mark to create the 100 µg/ml solution and at 240 nm, the sample solution's absorbance was measured in comparison to a distilled water blank solution.

Estimation of drug content in tablet formulation

Assay procedure

The absorbance of a series of sample solutions, each containing 8 µg/mL of Imeglimin Hydrochloride, was measured. Through fitting the responses into the regression equations of the calibration curve, the amount of Imeglimin Hydrochloride in tablet dosage form was established, and the outcomes were consistent with the relevant label claim to obtain % recoveries was shown in Table 1.

Validation of Proposed Method

The method's linearity, precision, accuracy, ruggedness, and robustness were all evaluated in accordance with ICH recommendations during validation.

Specificity

The analyte was assessed in the presence of the components and it was found that there was no interaction with the analyte.

Linearity

When measuring at different analyte concentrations, different amounts of stock solution were diluted with distilled water to produce Imeglimin Hydrochloride concentrations of 1, 2, 4, 8 and 16µg/mL. Plotting absorbance along concentration (µg/ml), the calibration curve was created.

Precision

When a method is applied repeatedly to numerous samplings of homogenous samples, the precision is the degree of agreement among individual test findings. It was expressed as a coefficient of variation and gives an indicator of the outcomes of random mistake.

a) Repeatability

Repeatability was determined by preparing six replicates of 8 µg/ml of sample and standard separately to determine the system precision and method precision respectively and the absorbance was measured at 240nm.

b) Intermediate/ruggedness precision

The typical variations that were examined on various days (inter-day & intra-day) by various analysts (analyst 1 & analyst 2) led to the determination of intermediate precision.

In order to conduct the intermediate/ruggedness precision, solution containing 8 µg/ml of Imeglimin Hydrochloride was prepared and the outcomes were given as %RSD and the precision result was good, with a percent relative standard deviation less than 2.

Accuracy

Recovery studies were used to gauge the suggested method's accuracy. The pre-analyzed formulation was used for the recovery trials, and various concentrations (50%, 100%, and 150% of the pure drug) were added. The solutions were made in three copies, and the percent recovery was computed.

Limit of Detection and Limit of Quantitation

Based on the response and slope of the regression equation, the parameters LOD and LOQ were established. The International Conference on Harmonization (ICH) recommended the following equations to calculate the signal-to-noise ratio (S/N), i.e., 3.3 for LOD and 10 for LOQ, from which the limit of detection (LOD) and limit of quantitation (LOQ) of the drug were determined.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Robustness Studies

By scanning the samples kept at ±2 ºC and ±5nm while making small variations to the experimental parameters, such as the temperature and wavelength, the robustness of the approach was assessed.

Forced Degradation Study

Drug samples were exposed to heat deterioration, peroxide oxidation using 0.3% H_2O_2, alkaline hydrolysis using 0.1N NaOH, acid hydrolysis using 0.1N HCl, and photo degradation under UV light. The spectra of the treated samples were recorded, together with the changes in absorbance value, after the samples had been scanned and treated.

3. RESULTS AND DISCUSSION:

Wavelength Selection

The detection of wavelength at 240nm was selected as the drug showed optimal absorbance at that wavelength was shown in figure 2.

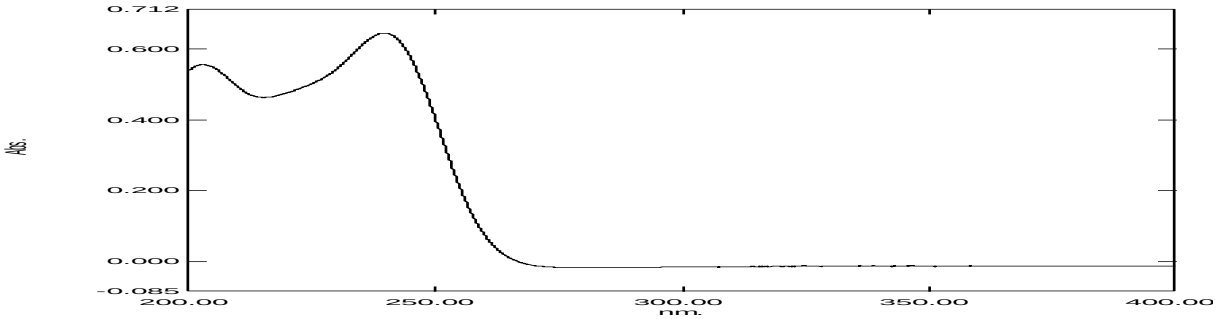

Figure 2: Wavelength selection at 240nm

Assay

SL. No.	Formulation	Label claim (mg)	Amount found (mg) ±SD	% Recovery±SD
1	**Imeglyn®**	500	495.93±3.586	99.19±0.72

Table 1: Assay of commercial formulations

Specificity

The analyte was assessed in the presence of the components and it was found that there was no interaction with the analyte was shown in figure 3.

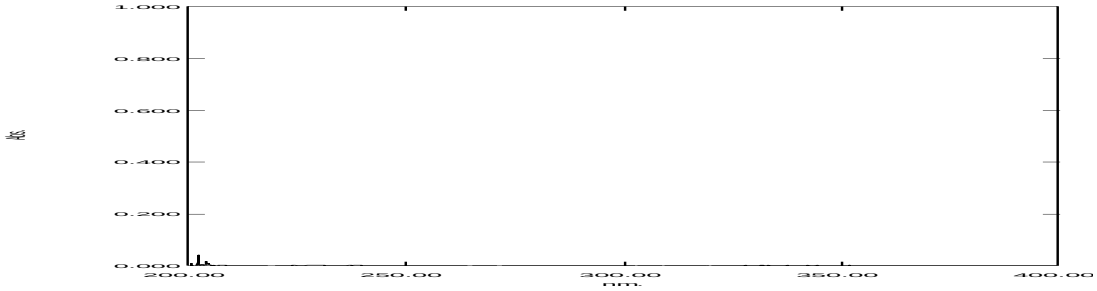

Figure 3: Specificity

Linearity

Various standards in the range 1 to 16 µg/ml of Imeglimin Hydrochloride were observed into UV system. A graph of absorbance (on Y-axis) versus concentration (on X-axis) is plotted and the correlation coefficient was calculated was shown in Table 2 and 3 and figure 4, 5 and 6.

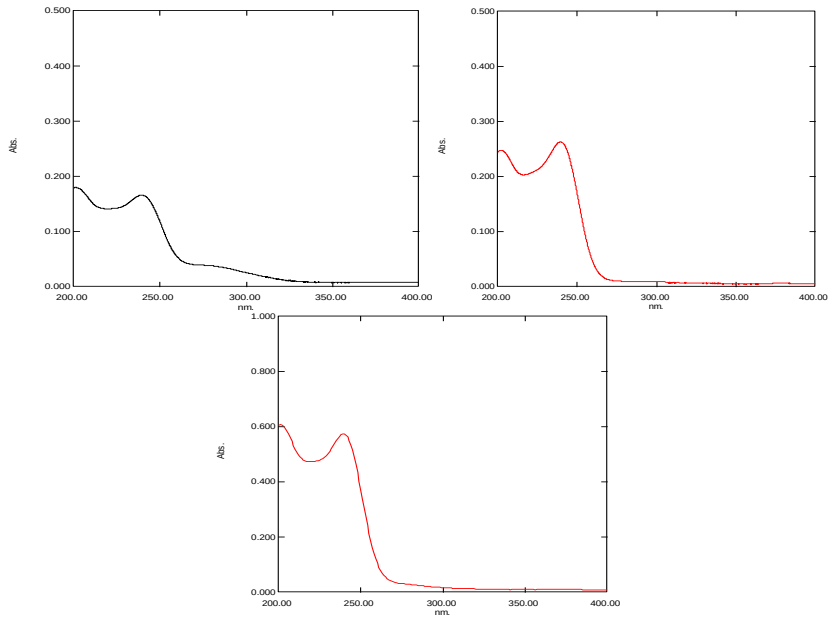

Figure 4: Linearity Spectra of Imeglimin Hydrochloride

(Set-1)

Conc. (µg/ml)	Abs
1	0.056
2	0.129
4	0.242
8	0.484
16	0.947

Table 2: Linearity of Imeglimin Hydrochloride

(Set-1)

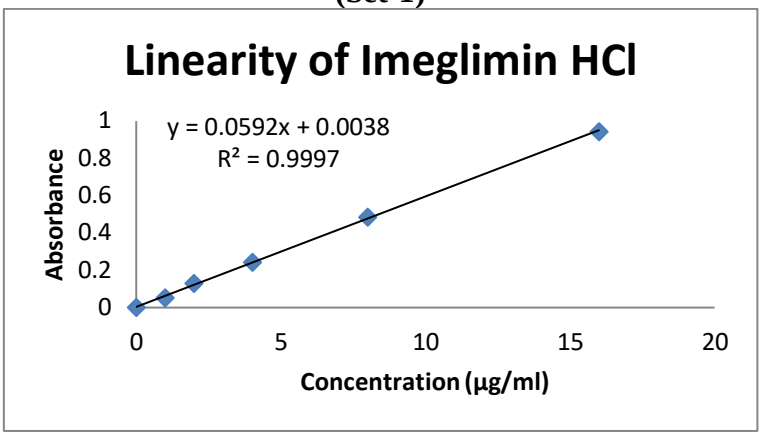

Figure 5: Linearity of Imeglimin Hydrochloride

(Set-2)

Table 3: Linearity of Imeglimin Hydrochloride

Conc. (µg/ml)	**Abs**
1	0.067
2	0.132
4	0.267
8	0.491
16	0.988

(Set-2)

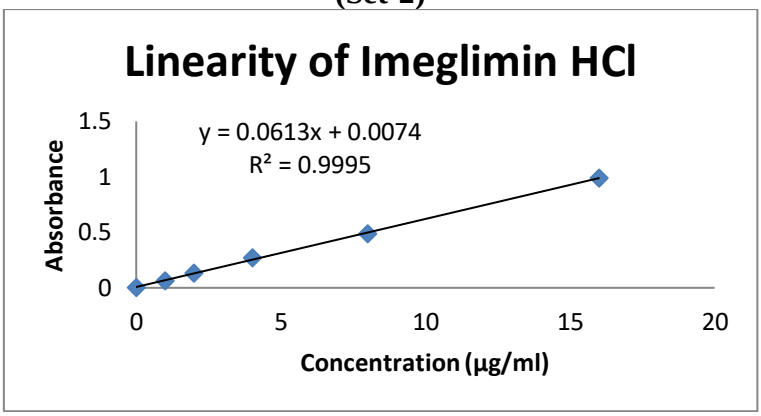

Figure 6: Linearity of Imeglimin Hydrochloride

LOD and LOQ

Calculation of S.D and Slope

Set-1 $y = 0.059x + 0.003$, $R^2 = 0.999$

Set-2 $y = 0.061x + 0.007$, $R^2 = 0.999$

Average of m = 0.06(slope)

S.D of c = 0.0028 (S.D)

LOD = 3.3×S.D /Slope

= 3.3×0.0028/0.06

= 0.16 µg/ml

LOQ = 10×S.D/Slope

= 10×0.0028/0.06

= 0.47 µg/ml

Range

Lowest concentration to highest concentration is 1 µg/ml to 16 µg/ml.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation was shown in Table 4 and 5 and figure 7 and 8.

a) Repeatability

a. System precision

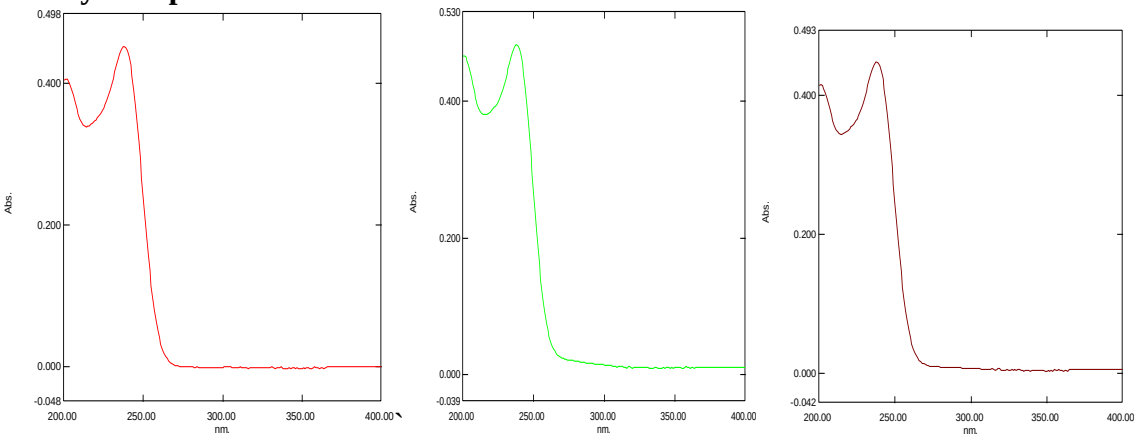

Figure 7: Spectra of system precision

Conc. (µg/ml)	Abs.	Avg.	S. D	%R.S.D
8	0.451	0.455	0.0057	1.3
8	0.459			
8	0.462			
8	0.453			
8	0.447			
8	0.459			

Table 4: Summary of system precision

b. Method precision

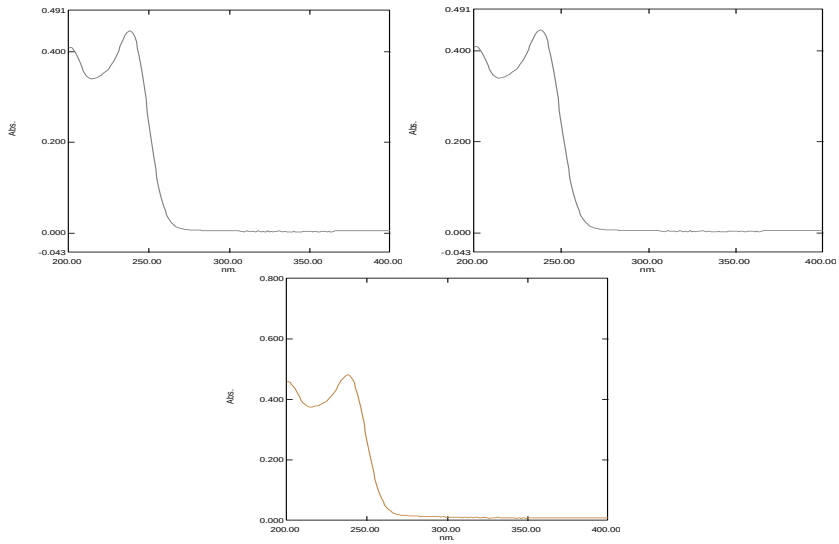

Figure 8: Spectra of method precision

Conc. (µg/ml)	Abs.	%Assay	Avg.	S. D	%R.S.D
8	0.486	99.59	99.975	0.429	0.4
8	0.481	98.57			
8	0.483	98.98			
8	0.485	99.39			
8	0.481	98.57			
8	0.482	98.77			

Table 5: Summary of method precision

b) Ruggedness (Intermediate Precision)

Intermediate precision was determined by the typical variations to be studied on different days (inter-day & intra-day) by different analysts (analyst 1 & analyst 2). The tests were carried out by preparing drug solution of concentration 8µg/ml of Imeglimin Hydrochloride and analyzing. The results were reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2 was shown in Table 6.

Inter Day/ANALYST-1		Intra Day/ANALYST-2	
Conc. (µg/ml)	Abs.	Conc. (µg/ml)	Abs.
8	0.486	8	0.451
8	0.488	8	0.469
8	0.485	8	0.460
8	0.484	8	0.455
8	0.481	8	0.467
8	0.484	8	0.468
Avg.	0.485	Avg.	0.462
S.D	0.0023	S.D	0.0075
%R.S.D	0.5	%R.S.D	1.6

Table 6: Summary of intermediate precision

Accuracy Studies

The accuracy of the method, recovery studies were carried out by adding different amounts (50%, 100% and 150%) of bulk samples of Imeglimin Hydrochloride within the linearity range were taken and added to the pre-analyzed formulation of concentration 8µg/ml. From that percentage recovery values were calculated. The results were within the range and were found to be highly accurate was shown in Table 7.

Level	Amount Added (µg/ml)	Amount Found (µg/ml)	%Recovery	Average % Recovery	% SD	% RSD
50%	4	4.02	100.59	99.54	0.98	1.0
50%	4	3.94	98.62			
50%	4	3.98	99.41			
100%	8	8.01	100.10	99.62	0.42	0.4
100%	8	7.96	99.49			
100%	8	7.94	99.28			
150%	12	11.92	99.31	99.26	0.21	0.2
150%	12	11.88	99.03			
150%	12	11.93	99.45			

Table 7: Summary of Accuracy studies

Robustness

The Robustness of the method was determined by making slight changes in the experimental conditions such as the temperature ±2°C and wavelength ± 5nm was shown in Table 8, 9, 10 and 11.

(-5nm) i.e. 235nm wavelength				
Conc. (µg/ml)	**Abs.**	**Mean**	**SD**	**%RSD**
8	0.433	0.429	0.003	0.76
8	0.427			
8	0.430			
8	0.433			
8	0.428			
8	0.425			

Table 8: Summary of -5nm wavelength i.e. 235nm

(+5nm) i.e. 245nm wavelength				
Conc. (µg/ml)	**Abs.**	**Mean**	**SD**	**%RSD**
8	0.406	0.404	0.002	0.64
8	0.402			
8	0.405			
8	0.408			
8	0.404			
8	0.401			

Table 9: Summary of +5nm wavelength i.e. 245nm

Conc. (µg/ml)	**Abs.**	**Mean**	**SD**	**%RSD**
8	0.471	0.466	0.003	0.77
8	0.464			
8	0.464			
8	0.462			
8	0.466			
8	0.470			

Table 10: Summary of (-2°C) i.e. 23°C Temperature

Conc. (µg/ml)	**Abs.**	**Mean**	**SD**	**%RSD**
8	0.457	0.462	0.006	1.33
8	0.462			
8	0.469			
8	0.465			
8	0.453			
8	0.467			

Table 11: Summary of (+2°C) i.e. 27°C Temperature

Forced Degradation Study

Drug samples were subjected to alkaline hydrolysis using 0.1N NaOH, Acid hydrolysis using 0.1N HCl, peroxide oxidation using 0.3% H_2O_2, photo degradation by using longer wavelength UV radiation and thermal degradation treated samples were scanned and their respective spectra were recorded and the changes in absorbance value were recorded was shown in Table 12 and figure 9, 10, 11, 12 and 13.

a. Acid Degradation (0.1N HCL)

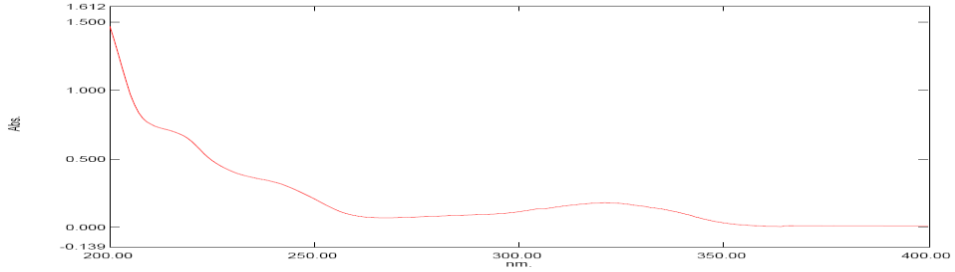

Figure 9: Spectra of acid degradation (0.1N HCl)

b. Alkali Degradation (0.1N NaOH)

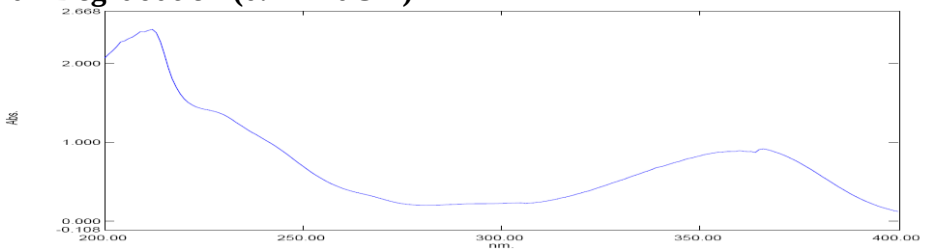

Figure 10: Spectra of alkali degradation (0.1N NaOH)

c. Peroxide Degradation (0.3% H_2O_2)

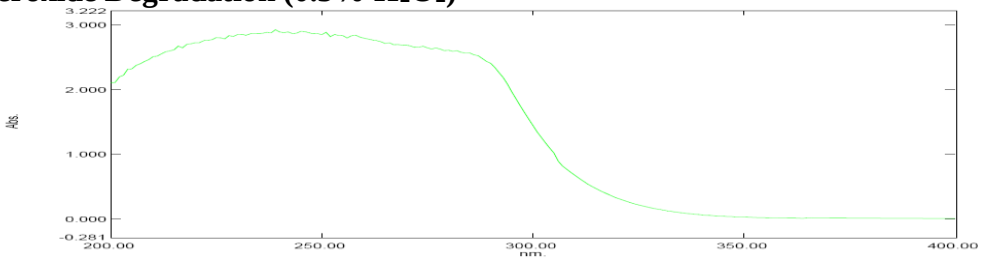

Figure 11: Spectra of peroxide degradation (0.3% H_2O_2)

d. Thermal Degradation

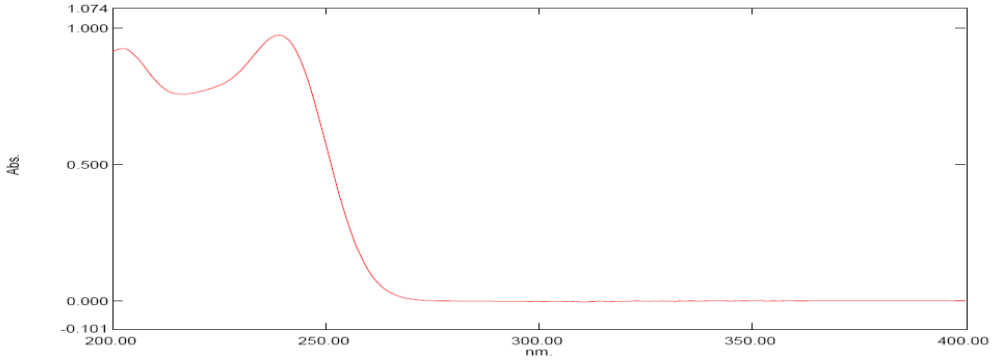

Figure 12: Spectra of thermal degradation

e. Photolytic Degradation

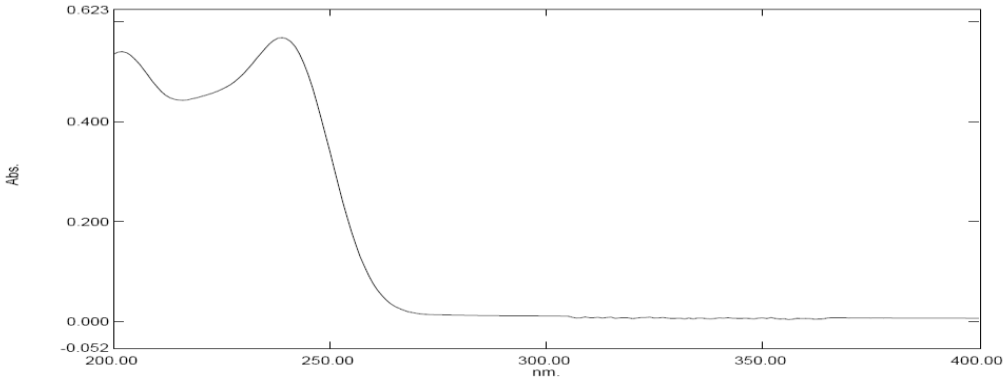

Figure 13: Spectra of photolytic degradation

Degradation Parameter (N=3)	Conc. (µg/ml)	% Degraded	% Recovered
Acid Degradation	8	37.09	62.91
Alkali Degradation	8	11.68	88.32
Peroxide Degradation	8	45.91	54.09
Thermal Degradation	8	11.16	88.84
Photolytic Degradation	8	2.4	97.6

Table 12: Summary of Forced degradation studies

4. CONCLUSION:

A simple method was developed for the determination of Imeglimin Hydrochloride in pure and its pharmaceutical formulations. Imeglimin Hydrochloride exhibited maximum absorption at 240nm in distilled water and obeyed linearity in the concentration range of 1-16µg/ml. The proposed method was statistically validated. This study presents a simple stability-indicating UV-spectroscopic method for estimation of Imeglimin Hydrochloride in the presence of degradation products. By the forced degradation studies, it was found that the drug Imeglimin Hydrochloride is maximum degraded in the presence of acid and peroxide degradation, moderately degraded in alkali and thermal degradation and mild degradation in photolytic degradation as per the studies performed. So, it was concluded that the drug should be stored in cool and dark place, away from acid, alkali and peroxide environment.

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