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RP-HPLC Method for Determination of Imeglimin Hydrochloride in Bulk and Tablet Formulation

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A B S T R A C T

Imeglimin hydrochloride (IMEG.HCl) is a new tetrahydro triazine-containing class of oral antidiabeticagents referred to as 'glimins', is used to treat type 2 diabetes (T2D). It is the firstanti-diabetic drug of this type to receive approval. It is an inhibitor of oxidative phosphorrylation that also works to improve muscle glucose absorption and restore regular insulin secretion[1]. A reverse phase HPLC method was developed and validated for quantitative determination of Imeglininhydrochlorideusing a BRISA LC[2] C18 (25 mm x 0.46 mm, 5µm) Column, isocratic mobile phase of Methanol: Phosphate buffer (10mM) pH6.0, at a flow rate of 1ml/min. The analyte was monitored at 243nm and retention time was found to be 3.097min.The peak obtained was symmetrical with tailing factor less than 2 and theoretical plates more than 2000. The developed HPLC method showed good linearity (R^2=1), the intra- and inter-day precision was less than 2%, LOD and LOQ were 0.137 and 1.417 µg/ml andaccuracy for three different levels was found to be 101.23%w/w-100.83%w/w respectively.The method was validated in accordance with ICH guidelines Q2 (R1).[2] and was found to be Specific, Accurate, Precise, Robust and can be successfully applied for routine analysis of Imeglininhydrochloridein bulk and in pharmaceutical dosage form.

Key Words: RP-HPLC, Methanol (MeOH), Imeglimin hydrochloride (IMEG.HCl)

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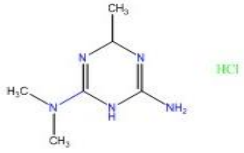
INTRODUCTION

Imeglimin is a new tetrahydro triazine-containing class of oral antidiabetic agents referred to as 'glimins', is used to treat type 2 diabetes (T2D). It is an inhibitor of oxidative ephosphorylation that also works to improve muscle glucose absorption and restore regular insulin secretion[1]. Imeglimin hydrochloride (IMEG.HCL) is white crystalline powder, chemically(6R)-(+)-4-dimethylamino-2-imino-6-methyl-1,2,5,6-tetrahydro-1,3,5-triazinehydrochloride with Molecular Formula of $C_6H_{14}ClN_5$, Molecular Weight of 191.66G/Mol, Melting Point of 223-225°C, with pKaValue of 10.21.Imeglimin hydrochlorideis soluble inorganic solvent such as ethanol, DMSO and methanol. Freely soluble in acetone, sparingly soluble in ethyl acetate.Literature survey cites only one RP-HPLC method for estimation of Imeglimin hydrochloride, in which high concentrationand linearity range has been observed. This work describes the development and validation of new RP-HPLC method for estimation of Imeglimin hydrochloride in bulk and tablets using different validation parameters as per ICH guidelines Q2(R1)[2].



Imeglimin Hydrochloride

MATERIALS AND METHOD

High Performance Liquid Chromatograph 10 AT SHIMADZU- SPD20A Detector System, injection is Rheodyne 20 µl andLC-solutions software.

UV-Visible Spectrophotometer Shimadzu-1900i. Software Version Lab SolutionUV-Vis was used for data processing.

CHEMICALS AND REAGENTS

IMEG.HCl API was obtained by Metrochem API Private Ltd. Hyderabad.Methanol HPLC Grade (FINAR), Water (FINAR), Phosphate buffer.

Preparation of Phosphate buffer

10 mM of Phosphate buffer was prepared by dissolving 680.4 mg of potassium dihydrogen orthophosphate in 500 mL of water and adjusting the pH to 6.0 with 0.1M sodium hydroxide (NaOH).

Preparation of Mobile Phase:

The working mobile phase was prepared in the ratio of 80:20 (Methanol: Phosphate buffer) filtered, degassed and sonicated for 10 min.

Preparation of Standard Imeglimin Hydrochloridesolution

Accurately weighed 10mg of Imeglimin Hydrochloride standard was transferred into 10mL volumetric flask, 3-5mL of Mobile phase was added and sonicated for 5 min to dissolve it completely and the volume was made up to 10ml with Mobile phase to get 1000µg/mL of standard Imeglimin Hydrochloride solution and labelled as **STD STOCK.**

Preparation of Sample Imeglimin Hydrochloride solution.

Imeglimin Hydrochloride 10 tablets (IMEXTOR 500) were accurately weighed and their average weight was calculated. The tablets were finely triturated and accurately weighed a quantity of powder containing 50mg of Imeglimin Hydrochloride and transferred to 50ml volumetric flask, solubilized in 25ml of mobile phase and sonicated for 15mins. Then, the volume was made up to the 50ml mark with the mobile phase to obtain final concentration of 1000µg/mL of Imeglimin Hydrochloride and was labelled as **'SMP STOCK'.**

Standardsolution (10µg/ml)in Methanol and Phosphate buffer (pH 6) in ratio of 80:20 was scanned in UV region of 200-400nm by using UV-Visible Spectrophotometer. The Spectra obtained is presented in **Fig 1.**

Then, the above std sol^{n}was filtered through 0.45µm nylon membrane filter and 20µL was injected into the HPLC system under standardized chromatographic conditions to get a stable baseline and to observe for peak of Imeglimin Hydrochloride. The chromatograms obtained is presented in **Fig 2**.

VALIDATION OF HPLC METHOD

Specificity

Solutions of standard and sample were prepared and 20µL was injected into HPLC. It was observed that other substances present in the formulation did not interfere with the peak of Imeglimin Hydrochloride and thus the method was specific. The peak purity of Imeglimin Hydrochloride was checked by comparing the spectra at different level viz. peak start, peak apex and peak end position of the spot.

Linearity

Suitable quantity of standard solution was transferred into a series of 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the concentration of 0.1,0.2,1, 2,5, 10, 30µg/mL of Imeglimin Hydrochloride. Peak areas and R_f valueswere calculated and the graph was plotted against concentration. The correlation coefficient (R^2) of least square linear regression of IMEG was calculated.

Accuracy

Recovery studies were determinedby adding known amounts of Std IMEG to pre-analysed samples at three different concentration levels i.e., 80 %, 100 %, 120%of assay concentration. The percentage recovery, standard deviation and% RSD was calculated.

Precision

Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Limit of detection and Limit of quantification

Detection limit was determined based on the standard deviation of peak area and was calculated by formula

$$LOD = 3.3 \times \frac{\text{Standard Deviation of Y-intercept}}{\text{Average slope of six calibration curves}}$$

Quantification limit was determined based on the standard deviation of peak arca and was calculated by formula

$$LOQ = 10 \times \frac{\text{Standard Deviation of Y-intercept}}{\text{Average slope of six calibration curves}}$$

Robustness

The robustness was carried out by deliberately varying some parameters by changing wavelength, change in flow rateby ±2% and mean, S.D and % RSD were calculated.

The data obtained for all validation parameters is presented in **Table-2.**

RESULTS AND DISCUSSION

The standard solutions of Imeglimin Hydrochloride (10µg/mL) in mobile phase Methanol: Phosphate Buffer (10mM) pH 6.0 (80:20v/v) was scanned in the UV region of 200 to 400 nm using Shimadzu 1900i UV-Visible Spectrophotometer and the spectra obtained is presented in **Fig 1.**

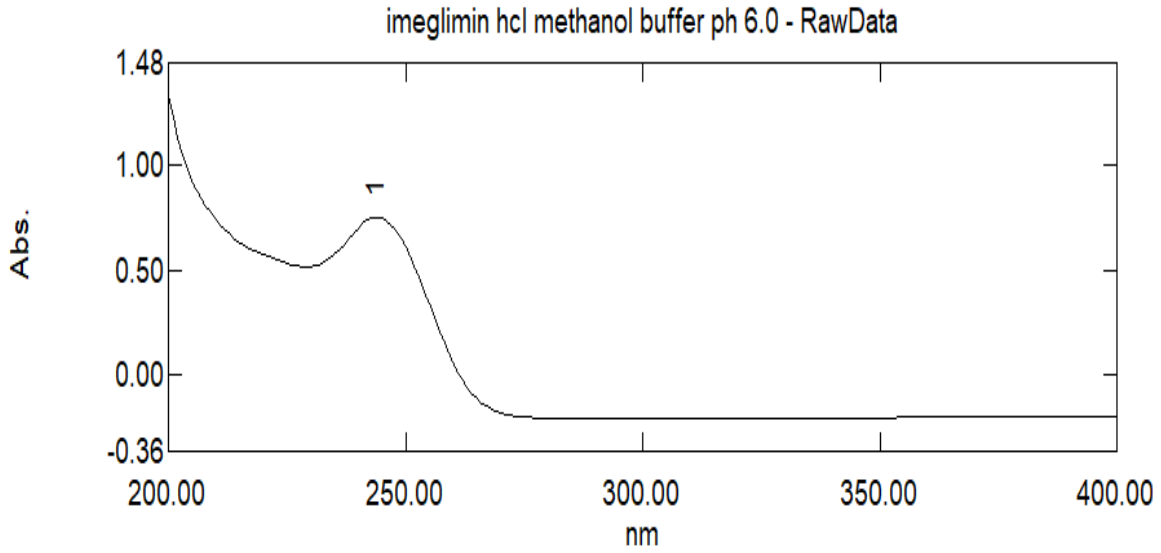


Figure 1: UV Spectra of Imeglimin Hydrochloride (10µg/mL)

From the UV Spectra, the λmax was found to be 243nm.

Mobile Phase consists of Methanol: Phosphate Buffer (10mM) pH 6.0 (80:20v/v) at a flow rate of 1 mL/min, at 243 nm shows good resolution of Imeglimin Hydrochloride peak, hence it was standardized and selected for the project work.

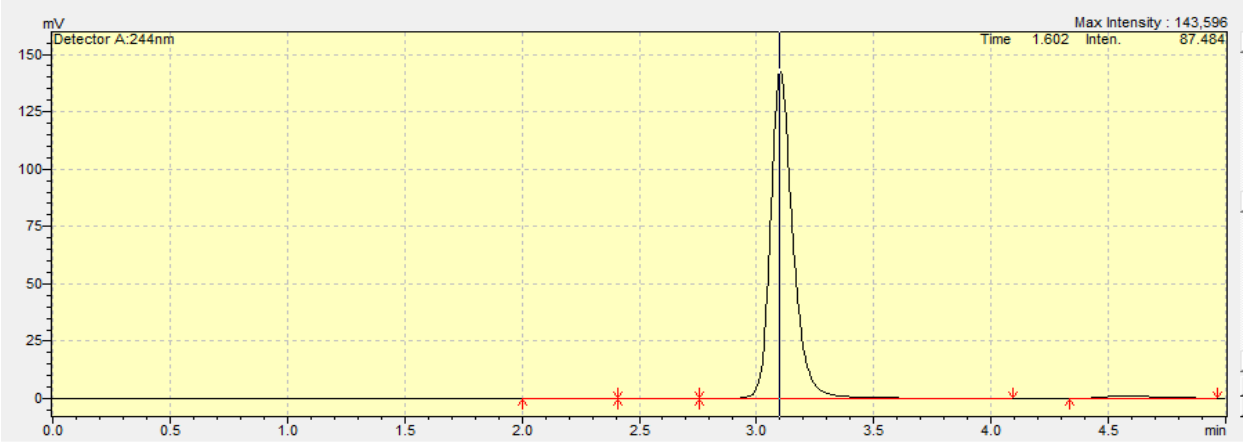


Figure 2: Chromatogram for Imeglimin hydrochloride (10µg/ml)in MPcontaining Methanol: Phosphate buffer 10Mm pH 6.0 (243nm) 80:20

Linearity

The linearity of an analytical procedure specifies the results which are directly proportional to the concentration of analyte in the sample. The linearity and range were determined from coefficient of correlation (R^2) obtained by plotting AUC vs. CONCENTRATION at 243 nm. The linearity was observed in concentration range of 0.1-30 µg/ml and Calibration graph is presented in **Fig 3.**

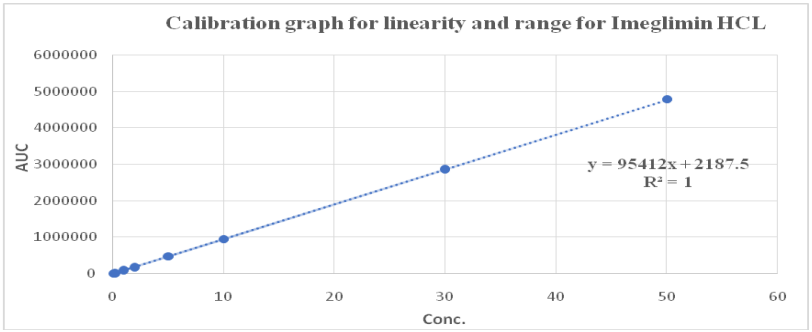


Figure 3: Calibration graph for linearity and range for Imeglimin Hydrochloride

Precision

The standard solution of 10 µg/mL were selected for Interday and intraday. the %RSD for Intraday and Inter- day studies for Imeglimin Hydrochloride was within the acceptance criteria of less than 2%.

Precision (%)	%RSD
Inter-day	1.878
Intra-day	1.009

Limits of detection and limit of quantification

The **LOD** and **LOQ** for Imeglimin Hydrochloridewas found to be **0.137 and 0.417 µg/ml** respectively.

System Suitability

System Suitability was performed to confirm that the system was appropriate for the analysis to be performed.

Table 1: Validation parameters for Imeglimin HydrochloridebyHPLC method

Parameters	Data for Imeglimin Hydrochloride
Regression Equation	95412x + 2187.5
Regression coefficient(r^2)	1.000
Limit of detection (µg/ml)	0.137
Limit of Quantification (µg/ml)	0.417
Precision (% RSD) Inter-day Intra-day	 1.878 1.009
Assay(%w/w)	101.57-102%w/w

Accuracy

The percentage recovery of Imeglimin Hydrochloride at three different levels (40%,50%,60%) was found to be from 101.23%w/w-100.83%w/w for Imeglimin Hydrochloride which is well within the acceptance criterialimits (95-105%w/w) and the overlain chromatogram is presentedin **Fig 4.**

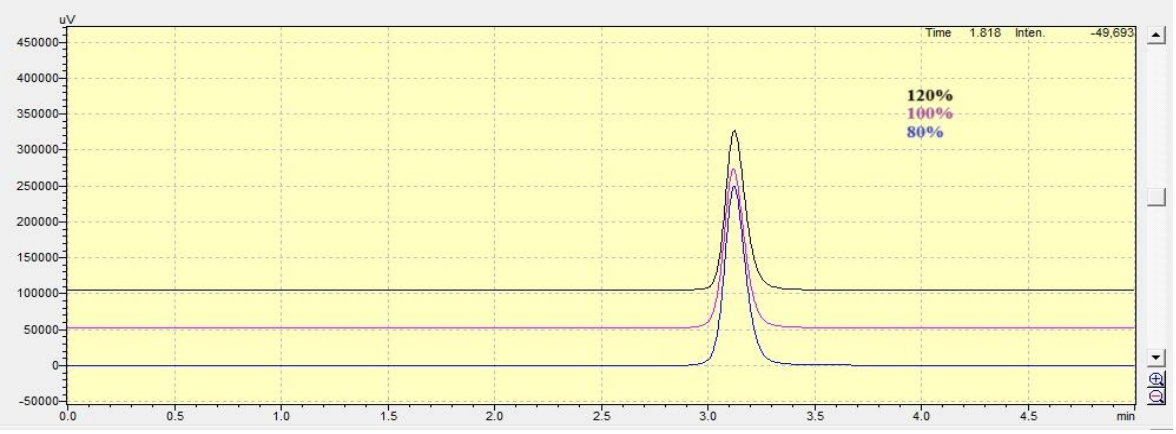


Figure 4: Overlain Chromatogram for Accuracy studies of Imeglimin Hydrochloride at three differentlevels.

Table 2: Percentage Recovery data for accuracy studies at three different levels.

Drug	**Conc.of STD (µg/ml)**	**Conc of Sample(µg/ml)**	**Total Conc (A+B) (µg/ml)**	**Peak Area*for Mixture STD+Sample (µg/ml)**	**Total amount (A+B) from graph (µg/ml)**	**Recovery of Std (µg/ml)**	**%Recovery of Std (%w/w)**
Imeglimin HCL	8	10	18	1704787	18.099	8.099	101.23%w/w
	10	10	20	1908097	20.03	10.03	100.3%w/w
	12	10	22	2104708	22.1	12.1	100.83%w/w
	8	10	18	1704787	18.099	8.099	101.23%w/w

***Average of three readings**

CONCLUSION

The retention time for Imeglimin HClby RP-HPLC method was found to be 3.097min. The developed method was validated as per ICH Q2 (R1) guidelines[10]. The linearity and range,was performed on series of concentrationand was found to be linear in the concentration ranges of 0.1-30µg/ml for IMEG.HCl with correlation coefficients of 1.000(Fig 2). LOD and LOQ of IMEG were found to be 0.137 and 0.417µg/ml. The %RSD for precision of proposed method was found to be less than 2% indicating that the method was stable during Inter and Intraday studies. Accuracy was determined by standard addition method at three different levels. (80%, 100% and 120%) and the mean % recovery at three different levels was found to be100.3-101.23**%**w/w for IMEG.HCl which was well within the range of 95-105%w/w, hence the method was found to be accurate. The percentage assay for IMEG.HCl was found to be 101.57-102%w/wrespectively. Hence the developed and validated method was found to be specific, accurate, precise, linear and robust and thus can be routinely applied for determination of Imeglimin Hydrochloride in bulk and pharmaceutical dosage form.

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