https://doi.org/10.48047/AFJBS.6.2.2024.2600-2611

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

A rapid stability-indicating HPLC method for determination of imeglimin hydrochloride in pure and dosage forms

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Article History

Volume 6, Issue 2, Apr-Aug 2024

Received: 10 August 2024

Accepted: 30 August 2024

Published: 30 August 2024

doi: 10.48047/AFJBS.6.2.2024.2600-2611

Abstract: A new, rapid, simple, precise and accurate stability-indicating reversed phase-HPLC method has been developed and validated for quantitative determination of imeglimin hydrochloride (IMGH) in pure form and tablets. An isocratic HPLC method, using Thermo hypersile BDS C_{18} reversed phase column (150 mm × 4.6 mm i.d., particle size 5 µm) with isocratic mobile phase consisting of ((Methanol:(0.05 M phosphate buffer pH 3.0)) (200:800 V:V:V), was investigated to separate (IMGH) from its stress degradation products. The flow rate was 1.5 mL min^{-1} at temperature 30 ± 2ºC and UV detector was used at 240 nm for detection. The elution time of IMGH was found to be 2.915± 0.015 minutes. The developed method was validated for system suitability, linearity, accuracy, precision, limits of detection and quantitation, specificity, stability, robustness and for system suitability parameters as per ICH guidelines. The calibration curve was found to be linear with the equation y=298310.419x + 21402.624, with a correlation coefficient of (R^2=0.99984) over a concentration range of 1.0–20 µg mL^{-1}. The limits of detection and quantification were 0.299 and 0.907 µg mL^{-1}, respectively. The recovery value of this method is 100.21% and the reproducibility is within 0.90%. Stability tests were done through exposure of the analyte solution for five different stress conditions: Reflux with 1.0 mol L^{-1} hydrochloric acid (HCl), reflux with 1.0 mol L^{-1} sodium hydroxide (NaOH), reflux with 30% hydrogen peroxide (H_2O_2), exposure to ultraviolet radiation (UV) radiation and thermal conditions. The proposed method could be used for routine analysis of IMGH in tablets

Keywords: *Imeglimin Hydrochloride; Stability indicating HPLC-method; Method validation; Stress degradation; Tablets.*

Introduction

Imeglimin hydrochloride (IMGH) is an investigational oral antidiabetic agent. IMGH is being developed for the treatment of type 2 diabetes mellitus. It is intended to improve glycemic control by targeting mitochondrial bioenergetics. IMGH is unique in its mechanism of action compared to other antidiabetic drugs. It works by targeting the mitochondria in cells, aiming to improve both insulin secretion and sensitivity. IMGH is a chemical compound used in the pharmaceutical industry. Specifically, it is an investigational drug developed for the treatment of type 2 diabetes mellitus. The hydrochloride form is a salt of IMGH, which is the active pharmaceutical ingredient (API)[1]. IMGH is chemically designated as (R)-6-imino-N,N,4-trimethyl-1,4,5,6-

tetrahydro-1,3,5-triazin-2-amine hydrochloride (Figure 1)[1]. IMGH belongs to the class of dihydro-1, 3, 5-triazine derivatives. As a first-in-class treatment for type-2 diabetes (T2D), IMGH is a novel oral agent that is currently being studied T2D [1]

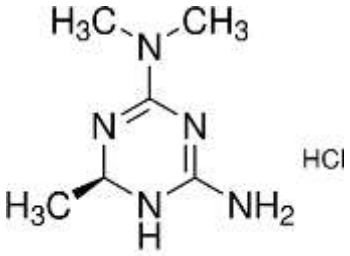


Figure 1. The chemical structure of Imeglimin Hydrochloride (IMGH).

Literature survey reveals that there is few methods have been reported for the assay of IMGH in pharmaceutical dosage forms, including spectrophotometry [2-4], upper high-performance chromatography (UHPC) [5-8], LC–MS/MS method [9].

There is only few methods dealing with stability indicating methods for determination of IMGH [5-8] but this method includes some drawbacks such as too long separation time and lower sensitivity. Also, there is no official pharmacoepeial methods are available for the determination of IMGH in drug substance and drug product. Therefore the aim of the proposed study is to find an inexpensive, new, sensitive, simple, accurate, precise, rubst and rapid stability indicating RP-HPLC method applying isocratic mode for determination of IMGH in bulk powder and tablets.

EXEPRIMENTAL

Instrumentation

Shimadzu HPLC series LC-2030C Plus (Shimadzu, , japan) consists of solvent pump, autosampler, column compartment and UV-DAD detector. The pH measurements were made on compact ino Lab pH Level 1 precision pH meter (Janeway pH-meter instrument) (pH 3501) (England)

Chemicals and reagents

HPLC grade methanol were purchased from Merck, (Scharlab S .L, Spain). NaOH, HCl and 30% H_2O_2 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Potassium dihydrogen orthophosphate obtained from Supelco, USA. IMGH raw material was obtained from Al-Esraa pharmaceutical Co. (Badr City, Cairo, Egypt). Bi-distilled water was used throughout the work.

Pharmaceutical dosage forms

Twymeeg film-coated tablets contain 500 mg IMGH per tablet and were produced by Pharmaceutical Co. (Sumitomo Pharma Co., Ltd., Japan).

Imeglibright tablets 500 mg tablets contain Imeglimin Hydrochloride IMGH were produced by Al-Esraa pharmaceutical company, (Badr City, Cairo, Egypt).

Chromatographic conditions

The chromatographic separation was performed using BDS- Thermohyersil C18 (150 mm × 4.6 mm), 5.0 µm particle size column; the column temperature was maintained at 30±2 ºC. The Autosampler utilized Methanol : Water as a rinse solution, the total run time was 5.0 minutes. The elution quaternary pump ran an isocratic flow using mobile phase consisting of a mixture of methanol : phosphate buffer (pH 3.0) (20:80 V:V) at a flow rate of 1.5 mL min^{-1}. The eluate was monitored at 240 nm using UV DAD-detector. The retention time of the drug was found to be 2.835± 0.015 min. The injection volume was 50 µL.

Preparation of stock and standard working solutions

A stock solution of IMGH (100 µg mL^{-1}) was prepared by dissolving 10 mg of IMGH in mobile phase in 100 mL volumetric flask, then shake and sonicate for 5 min till completely dissolved and then, complete the volume to 100 mL with the same solvent. The working standard solutions were prepared by diluting aliquots of stock solution with mobile phase to obtain final concentrations ranging from 1.0 to 20 µg mL^{-1}. Working solution of the drug was stable for one week.

Construction of calibration curve

Aliquots of standard solution, ranging from 1.0 to 20 µg mL^{-1} were prepared in a series of 100 mL volumetric flasks. 50 µL was injected into the instrument. Detection was performed at wavelength 240 nm. The calibration graph was constructed by plotting the peak areas obtained at the wavelength 240 nm versus the corresponding injected concentrations.

Procedure for dosage forms

Ten tablets (Twymeeg tablets contain 500 mg IMGH per tablet) were weighed, finely powdered and an accurately weighed amount of the powdered tablets equivalent to 10 mg of IMGH was dissolved in 50 mL of the mobile phase in 100 mL volumetric flask. The flask sonicated for 10 min and the solution was filtered through a 0.45 µm membrane filter and then the final solution was completed to the mark with the mobile phase, then diluting aliquots of stock solution with mobile phase to obtain final concentration 10 µg mL^{-1}. The procedure was then completed as mentioned above under the general procedure.

Stability tests

Forced degradation studies were performed to provide an indication of the stability-indicating properties and specificity of the method. Intentional degradation was attempted using acid hydrolysis, base hydrolysis, hydrogen peroxide oxidative degradation, thermal degradation and UV-radiation degradation. A degradation sample was prepared by dissolving 10 mg of IMGH in 100 mL water through shaking and sonication. Then 10 mL of this solution was taken in each of three 50 mL round bottomed flasks to perform the first three degradation tests. To the first flask, 10 mL of 1.0 mol L^{-1} HCl was added for acidic degradation. To the second flask, 10 mL of 1.0 mol L^{-1} NaOH was added for basic degradation. To the third flask, and 10 mL of 30 %(v/v) H_2O_2 was added for oxidative degradation. All the three flasks were refluxed for about 1.0 h at 60 °C. After completing degradation treatments, samples were allowed to cool to room temperature and treated as follows: The pH values of the first and second flasks were neutralized with 1.0 mol L^{-1} NaOH and 1.0 mol L^{-1} HCl, respectively. To the third flask 1.0 N sodium bisulfite solution was added to destroy H_2O_2. The volume of all the three flasks was adjusted to 50 mL with the mobil phase. Suitable aliquots of resultant degradation samples were taken and subjected to analysis after suitable dilutions with the mobil phase against the control samples (which lacked the degradation treatment) and 50 µL solution was injected into the system and the chromatogram was recorded to assess the stability of samples.

For thermal degradation, IMGH powder was dispersed onto a Petri-dish and left in an oven at 60 °C for 24 h then the solution was prepared from it in a concentration of 100 µg mL^{-1} using the mobile phase as solvent.

For degradation through UV-radiation 2.0 mL of the sample was retained in the UV radiation from 5.0 to 60 minutes and then the radiated solution diluted with mobile phase to 10 mL, then finally injected into the LC and compared with the control sample.

Method validation

The methods were validated according to the International Conference on Harmonization Guidelines [10] for validation of analytical procedures.

Linearity

Linearity of the method was tested for different concentrations in a range from 1.0 to 20 µg mL^{-1}. A graph was plotted between the peak areas versus concentrations to obtain the calibration curve. The seven concentrations of active drug component were subjected to regression analysis by least-squares method to calculate

correlation co-efficient and calibration equation. The method of linear regression was used for the data evaluation.

LOD& LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) were determined by injecting a series of samples of low concentration and from the calibration curve the LOD and LOQ were estimated as per ICH guidelines.

Precision

Precision is a measure of the reproducibility of the whole analytical method under normal operating conditions. The precision was expressed as the relative standard deviation (RSD).

% RSD = (Standard deviation/ average) x 100

Precision was done for three level of concentration, each concentration repeated three times in the same day for intraday precision and then the procedure repeated in another day for inter day precision.

Accuracy

Accuracy or trueness was determined by applying the method to samples in which known amounts of analyte have been added. These should be analyzed against standard and blank solutions to ensure that the sample solution accuracy results are comparable. Accuracy of the method was tested by % recovery of IMGH on three concentrations; each concentration was repeated three times. Known amounts of IMGH were added to a synthetic mixture of the drug product components (placebo) and subjected to analysis procedure. The % recovery was calculated for the drug added. The mean percentage recovery was calculated.

Ruggedness

Ruggedness was tested for different analytical conditions like different day and analyst to measure the capability of the method to remain unaffected by these variations that expected during method usage.

Robustness

Robustness of the method indicates the reliability of an analysis to assess the system suitability parameters under the influence of small but deliberate variations in method parameters. Robustness was examined by small change in the temperature (±2.0 °C), flow rate (±0.1 mL/min), percentage of methanol solvent (±1%) , wavelength of detection (±2.0 nm), and injection volume (±1.0 µL).

Solution stability

Sample solution and the standard solutions containing IMGH were prepared as per the test procedure. All these solutions were divided into two portions. One portion was stored at room temperature and the other portion was stored in the refrigerator at 2-5°C. Freshly prepared solutions and the solutions which were stored at room temperature and refrigerated condition (2-5°C) up to 24 hours were injected at different time intervals. Percent assay obtained at initial was compared with the % assay obtained at different time intervals.

RESULTS AND DISCUSSION

System suitability

The conditions affecting the chromatographic performance of IMGH were carefully studied in order to recognize the most suitable chromatographic system. So, the optimum chromatographic performances were achieved via using isocratic mobile phase composed of methanol, acetonitrile and phosphate buffer (pH 3.0) (30:30:40, V:V:V), injection volume 20 µL, column temperature 25°C, detection wavelength 305 nm and flow rate 1.5 mL min^{-1}. The results of three runs indicate high system suitability (Table 1).The retention time (t_R) value of IMGH was 2.961±0.015 minutes. The RSD of peak area was 0.90%.

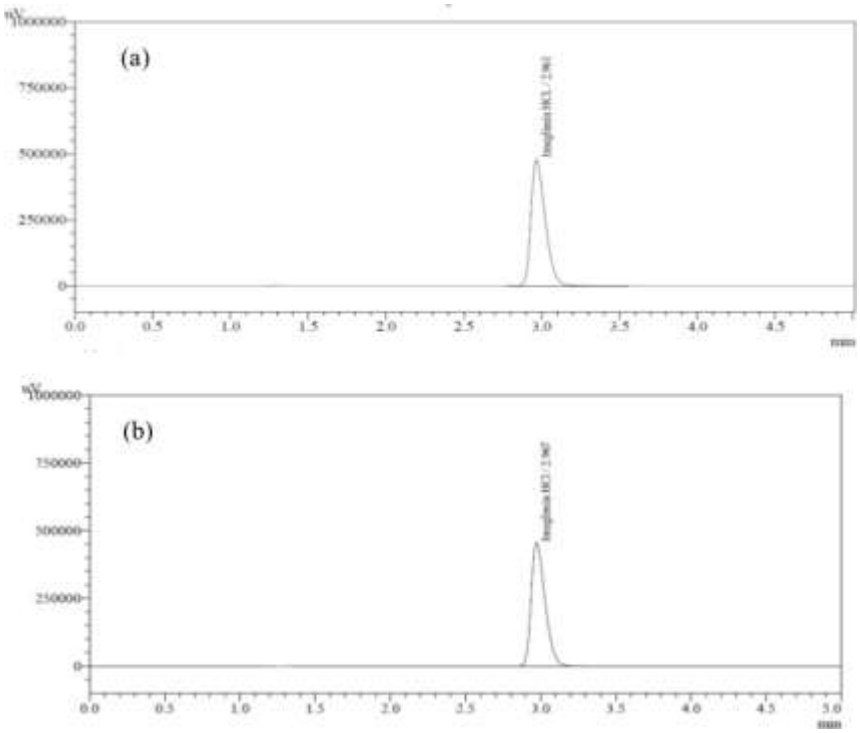


Figure 2. Chromatograms of (10 µgmL^{-1}) IMGH from (a) raw material and (b) tablets.

Table 1. System suitability and regression data.

Parameters	IMGH
System suitability	
t_R± RSD (min)	**2.965 ± 0.015**
N	**3267**
k'	**1.977**
R	
α	
Linearity and regression data	
Linearity range (µg/mL)	**1-20**
Detection limit (µg/mL)	**0.299**
Quantitation limit (µg/mL)	**0.907**
Slope (b)	**298310.419**
Intercept (a)	**21402.624**
SD of y-intercept	**27060.005**
Determination Coefficient (R^2)	**0.99984**

Selectivity, specificity and stability of the method

The resulted peak after tablet analysis is found to be homogeneous and there are no co-eluting peaks indicating specificity of the method. Comparison between the chromatogram of the raw IMGH and that of extracted IMGH from tablets indicates that the excipients in the formulation did not interfere with the determination of (Figure 2).

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradents, matrix (placebo) etc. Specificity was tested by injecting placebo preparation and forced degradation samples.Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Forced degradation was attempted to stress conditions like acid hydrolysis, base hydrolysis, peroxide oxidation, thermal degradation and photolytic degradation. To check and ensure the homogeneity (peak purity) of peak in the stressed sample solutions, photo diode array detector was employed. In forced degradation study it was observed peak purity in all the degradation conditions has been proven for IMGH peak. Results are tabulated in Table 2.

Table 2: Forced Degradation data of IMGH.

Degradation conditions	**%Assay observed**
Acid	97.36
Base	91.23
Oxidative (H_2O_2)	85.17
Thermal	97.35
UV	98.82

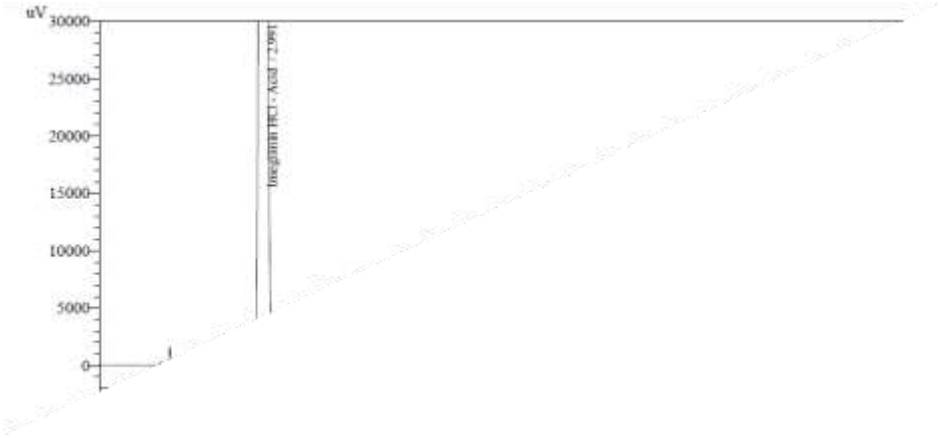


Figure 3. Chromatograms of (10 µgmL^{-1}) IMGH acid degradation.

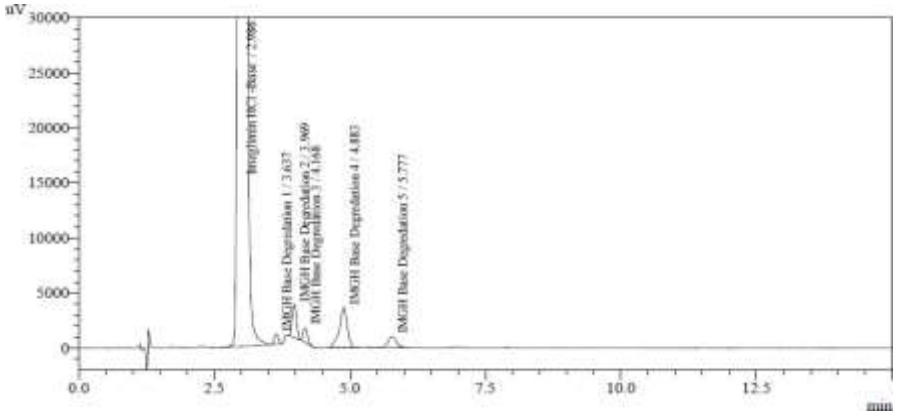


Figure 4. Chromatograms of (10 µgmL^{-1}) IMGH base degradation.

Figure 5. Chromatograms of (10 µgmL^{-1}) IMGH Oxidative Degradation

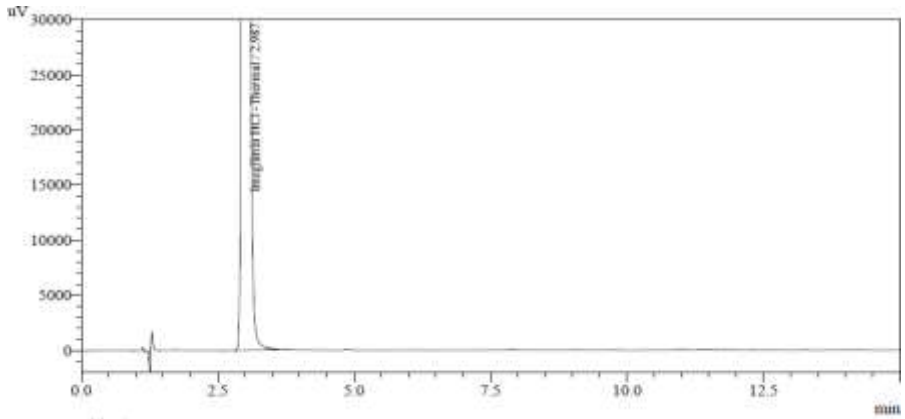


Figure 6. Chromatograms of (10 µgmL^{-1}) IMGH thermal degradation

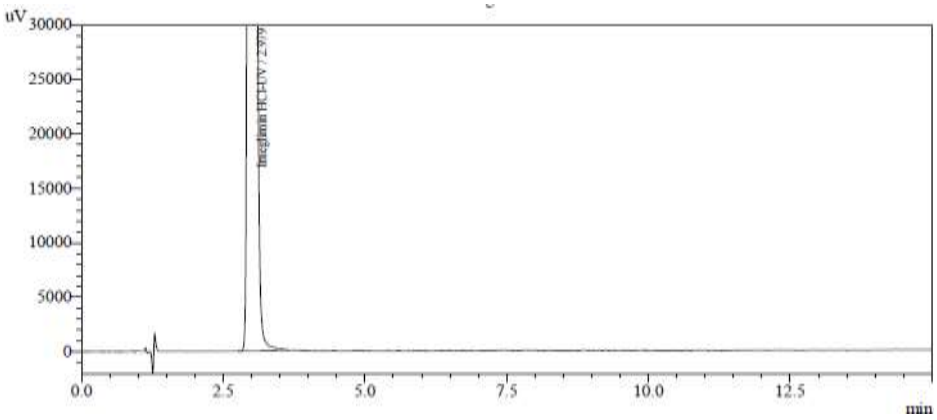
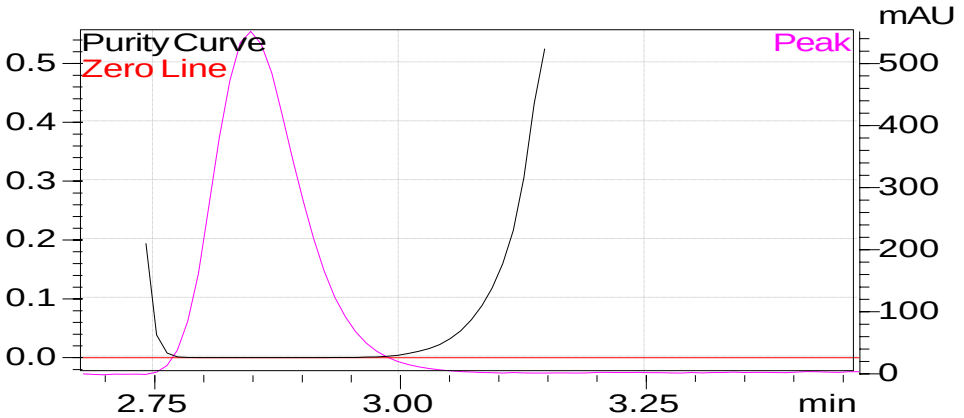


Figure 7. Chromatograms of (10 µgmL^{-1}) IMGH UV degradation.

Figure 8. Chromatograms of Plcebo

Figure 9. Spectrum Of IMGH



(Impurity : Not detected , Peak purity index : 1.00, Single point threshold : 0.999968)

Figure10. Purity index Of IMGH.

Figure 11. Spectrum profile Of IMGH.

Stability of the standard solution was studied by injection of the prepared solution at periodic intervals into the chromatographic system up to about 5.0 days. The results indicate that the RSD of the peak area was within 0.52%.

Linearity, LOD and LOQ

Different concentrations of IMGH solution ranging from 1.0–20 µg mL^{-1} were analyzed. The graph of the peak area against concentration proved linear in the range of 1.0–20 µg $mL^{-1}$and the linearity equation is: y = 298310.419x + 21402.624 and the regression coefficient= 0.99984 (Figure 12). The results have indicated good linearity. The limit of detection (LOD) is defined as the injected quantity giving S/N of 3.0 (in terms of peak height) and was found to be 0.299 µg mL^{-1}. The limit of quantification (LOQ) is defined as the injected quantity giving S/N of 10 (in terms of peak height) and was found to be 0.907 µg mL^{-1} (Table 2).

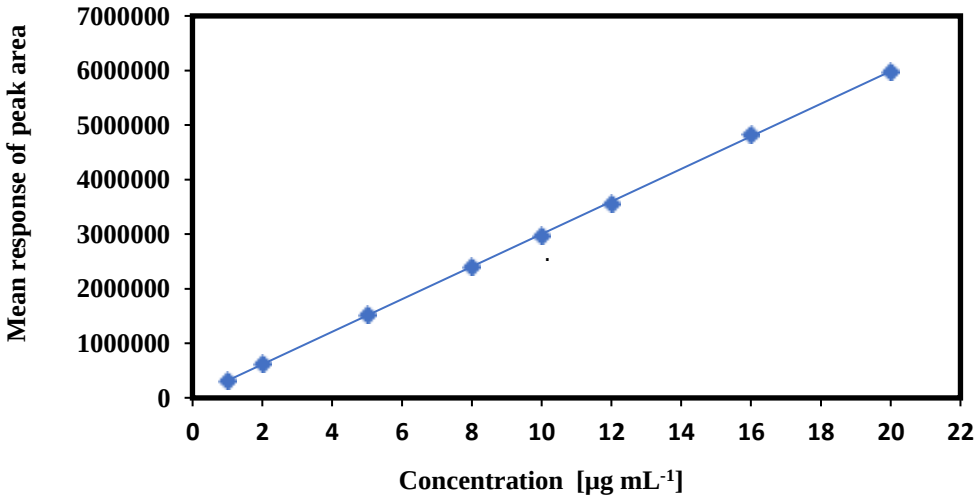


Figure 12. Linearity graph of IMGH.

Reproducibility, precision and accuracy of the method

Intra-day precision was assessed by injection of the standard solution at three concentrations five times during a day. The same was done for inter-day precision test except that the injection ofthe samples was every day for five days. Results (Table 4) show that there were high intra- and inter-day precisions. The RSD

was calculated for results (%RSD ≤ 0.84%) and it indicates that proposed method has got acceptable level of repeatability.

Accuracy of an analytical method is the closeness of the test results obtained by the method to that of true value. Accuracy of the proposed method was established by recovery experiments. This study was conducted by preparing and anlyzing samples at 20% to 200% of targeted concentration (10 µg mL^{-1}), in triplicate and injected into the chromatographic system. Results obtained from recovery studies are given in Table 4.

Table 4. Reproducibility, precision and accuracy (n=5).

Taken (µg mL^{-1})	Intra-day			Inter-day		
	Found[a] ± S.D.	RSD %	Recovery%	Found[a] ± S.D.	RSD %	Recovery%
2	1.99± 0.008	0.402	99.50	2.01± 0.010	0.498	100.50
4	4.05± 0.009	0.230	101.25	3.95± 0.011	0.278	98.75
8	8.05± 0.015	0.186	100.63	7.98± 0.021	0.267	99.75
10	10.04± 0.028	0.279	100.40	10.13± 0.035	0.346	101.30
15	14.87± 0.035	0.235	99.13	15.05± 0.045	0.299	100.33
20	20.03± 0.027	0.135	100.15	20.14 ± 0.068	0.338	100.70

Application

Analysis of IMGH in Twymeeg 500 mg tablets and Imeglibright 500 mg tablets by the propsed method showed high accuracy with mean recoveries of 99.98±0.35% and 100.21 ±0.46%, respectively (Table 5). The results were compared with a reported method[4]. The calculated values of *f* and *t* indicate that there is no significant difference between both methods.

Table 5. Statistical analysis of results obtained by the proposed method applied on tablets compared with a reported method.

Parameters	Proposed method		Reported methods [3]
	Twymeeg tablets	Imeglibright tablets	
n	5	5	6
Mean recovery %[a]	99.98	100.21	100.36
± SD	0.35	0.462	0.492
± R.S.D%	0.350	0.461	0.490
Variance	0.123	0.213	0.242
S.E	0.157	0.207	0.201
t-value [b]	1.49	0.52	
F-value [b]	1.98	1.13	

[a] *Average of five determinations.*

[b] *Tabulated values for t= 2.447 and F= 4.39at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05)*

Robustness of the method

The robustness of the present method was evaluated within small variation in its parameter and was found to be robust. Robustness was examined by small change in the temperature (±2.0 °C), flow rate (±0.1 mL/min), percentage of methanol solvent (±1%) , wavelength of detection (±2.0 nm), and injection volume (±1.0 µL) (Table 6). The slight variations in the examined factors had no significant effect on the peak areas or retention times (t_R-values).

Table 6. Robustness of the proposed method.

Changes factors	**Temp. (°C)**		**Flow rate (mL/min)**		**Mobile phase (%)**		**Wavelength of detection (nm)**		**Injected volume (µL)**	
Changes	28, 30 and 32		1.40, 1.50 and 1.60		19, 20 and 21		238, 240 and 242		49.0, 50 and 51	
Tested parameter	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R
C.V. (%)	1.27	0.58	1.18	0.45	1.23	0.65	1.08	0.37	1.35	0.5

CONCLUSION

A valid and rapid stability-indicating HPLC-method for the quantification of IMGH in pure form and tablets was established. Compared with the published chromatographic methods, this method represents a strong reduction of the analysis time and it is considered as a stability indicating method. The full run time for separation of the intact IMGH from its degradants is about 5.0 minutes which is very short comparing with the previously published work. With the proposed method a satisfactory separation of IMGH from the degradation products, extended linear range, and rapid analysis time were carried out. Ahigh recovery of IMGH in tablets was achieved. The proposed method ensured a precise and accurate determination of IMGH in tablet formulations and is a stability-indicating method.

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