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Spectrophotometric Methods for Quantitative Determination of Imeglimin HCl in Pure and Dosage Forms

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ABSTRACT: ***Simple, precise, reproducible, and validated spectrophotometric methods have been developed for the determination of imeglimin hydrochloride (IMGH) in pure and dosage forms. The methods are based on the formation of a yellow-colored ion-pair complex between IMGH and three reagents, namely, alizarine red s (ARS), bromophenol blue (BPB), and methyl orange (MO), in an acidic buffer solution with absorption maxima at 410, 420, and 420 nm, respectively. Several parameters, such as pH, buffer type and volume, reagent volume, sequence of addition, and effect of extracting solvent, were optimized. Under the optimum experimental conditions, Beer's law is obeyed over the concentration ranges of 1.0–20 µg/ml for the three reagents, with good correlation coefficients (0.99985-0.99988). The apparent molar absorptivity's and Sandell's sensitivity values are reported for all methods. The limit of detection (LOD) and the limit of quantification (LOQ) values are found to be 0.28, 0.30, and 0.31 µg/ml and 0.86, 0.93, and 0.97 µg/ml for ARS, BPB, and MO, respectively. The stoichiometric ratio of the formed ion-pair complexes was found to be 1:1 (drug: reagent) for all methods, as deduced by Job's method of continuous variation. The proposed methods were successfully applied for the determination of IMGH in dosage forms with good accuracy and precision. A statistical comparison of the results was performed using the Student's t-test and variance ratio F-test at the 95% confidence level, and there was no significant difference between the reported and proposed methods regarding accuracy and precision. Further, the validity of the proposed methods was confirmed by recovery studies via standard addition techniques in accordance with ICH guidelines.***

Keywords: ***Imeglimin hydrochloride, Ion-pair complex, Spectrophotometry, Method validation, Dosage forms.***

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I. 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)[2].

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 [2]

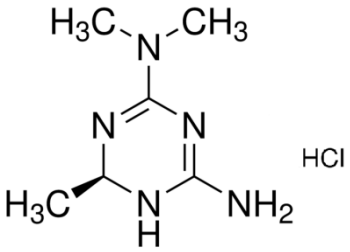


Figure 1. The chemical structure of IMGH.

The literature survey reveals that very few methods were reported for the estimation IMGH in dosage forms which include spectrophotometry [1] and chromatography [3-5]. These reported methods were either not appropriately sensitive or tedious and utilized expensive instruments that are not available in most quality control laboratories. For these reasons, it was worthwhile to develop a new, simple, cost effective spectrophotometric methods for the determination of IMGH in its dosage forms.

The aim of the present work is to develop simple, sensitive, accurate, precise, low-cost and validated extractive spectrophotometric methods for the determination of IMGH in pure and dosage forms. The proposed methods are based on the ability of IMGH to form stable ion-pair complexes with alizarine red s (ARS), bromophenol blue (BPB), and methyl orange (MO) in acidic buffer solution. No interference was observed in the assay of IMGH from common excipients in levels found in dosage forms. These methods are validated by statistical data.

II. Materials and Methods

2.1. Instrumentation:

All absorption spectra were made using a Shimadzu UV-1800 UV/visible double beam spectrophotometer (Sweden) equipped with 10 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm. The pH values of different buffer solutions were checked using a Janeway 3501 A/digital analyzer pH meter equipped with a combined glass-calomel electrode.

2.2. Chemicals and reagents

All reagents, chemicals and solvents used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Pure sample of IMGH was kindly supplied by Al-Esraa pharmaceutical, Egypt with purity (99.9%, Ami life sciences PVT. LTD, India). Twymeeg tablets were obtained from Sumitomo Pharma Co., Ltd., Japan, labeled to contain 500 mg IMGH per tablet; Imeglibright tablets were obtained from Al-Esraa pharmaceutical company, Egypt labeled to contain 500 mg IMGH were purchased from local pharmacies.

Preparation of stock standard solution

Stock standard solutions (100 µg/ml) of IMGH were prepared by dissolving 10 mg of pure IMGH in bidistilled water and diluted to the mark in a 100 ml volumetric flask. The standard solutions were stable for at least 7.0 days when kept in the refrigerator. Serial dilution with the same solvent was performed to obtain the appropriate concentration range

Reagents

ARS, BPB, and MO (Merck) and used without further purification. Stock solutions (1.0×10^{-3} mol/l) of reagents were prepared by dissolving the appropriate weight 34.23 mg, 67.00 mg and 32.73 mg of ARS, BPB and MO respectively in100 ml of bidistilled water. These solutions were kept in the refrigerator.

The pH profile of the universal buffer was prepared [6,7]. by mixing different ratios of 0.2 M Boric Acid + 0.05 M Citric Acid and 0.1 M Na_3PO_4 to give a buffer range of pH 2 to 12. The pH of each solution was adjusted to an appropriate value by the addition of 0.2 mol/l hydrochloric acid or sodium hydroxide with the help of the pH meter. Freshly prepared solutions were always employed. Chloroform, methylene chloride, and carbon tetrachloride were obtained from (BDH Chemicals Ltd., Poole, England) and anhydrous sodium sulfate was obtained from (Prolabo).

2.3. General recommended procedure

Accurately measured aliquots (0.1–2.0 ml) of standard IMGH solution (100 µg/ml) was transferred into 10 ml measuring flasks. 2.0 ml buffer at the optimum pH 4.0, 4.5 and 4.5 using ARS, BPB and MO, respectively were added. Then, 2.0 ml of each reagent (1.0×10^{-3} mol/l) was added and the volume was completed to 10 ml with bidistilled water. The formed ion associate complexes were extracted with 10 ml chloroform. The solution was shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the chloroform layer was passed through anhydrous sodium sulfate. The absorbance of the yellow colored ion-pair complexes was measured at 410, 420, and 420 nm, using ARS, BPB and MO, respectively against corresponding reagent blank similarly prepared. All measurements were made at room temperature. In the three proposed methods, a standard curve was prepared by plotting the absorbance values versus concentrations of IMGH to calculate the amount of drug in unknown analyte samples.

2.4. Applications for dosage forms

Ten tablets containing IMGH were finely crushed, powdered, and weighed. An accurate weighed quantity of the powdered tablets equivalent to 10 mg of IMGH was transferred into a 100 ml volumetric flask, about 20 ml of bidistilled water was added and the flask was sonicated for 30 min. The volume was completed to the mark with bidistilled water, mixed well and filtered through a Whatman No.1 filter paper into 100 ml volumetric flask, discarding the first 10 ml, then the conical flask was washed with bidistilled water. The wash was added to the same volumetric flask, and then the flask was made up to volume with bidistilled water. Aliquots containing IMGH in the final concentration ranges were analyzed as described under “General recommended procedure”. The concentration of IMGH was determined either from the calibration curve or using the corresponding regression equation. The method of standard addition was used for the accurate determination of IMGH content.

2.5. Stoichiometric relationship

The stoichiometric ratios of the ion-associates formed between IMGH and the reagents were determined by applying the continuous variation[8] and the molar ratio[9] methods at the optimum wavelengths. In continuous variation method, equimolar solutions were employed: a 1.0×10^{-3} mol/l standard solution of IMGH and 1.0×10^{-3} mol/l solution of dye were used. A series of solutions was prepared in which the total volume of IMGH and the dye was kept at 2.0 ml. The drug and reagent were mixed in various complementary proportions (0.2:1.8, 0.4:1.6, 0.6:1.4, 0.8:1.2, 1.0:1.0, 1.2:0.8, 1.4:0.6, 1.6:0.4, 1.8:0.2) and completed to volume in a 10 mL calibrated flask with the appropriate

solvent for extraction following the above mentioned procedure. In the molar ratio method, the concentration of IMGH is kept constant to 1.0 ml of (1.0 $x10^{-3}$ mol/l) while that of dye (1.0 $x10^{-3}$ mol/l) is regularly varied (0.2–2.0 ml). The absorbance of the prepared solutions was measured at optimum condition and at the optimum wavelength for each complex.

III. RESLUTS AND DISCUSSIONS

3.1. Absorption spectra

To the best of our knowledge, spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure form, pharmaceutical formulations and biological samples, due to its simplicity, less expensive, less time consuming and reasonable sensitivity with significant economic advantages [10-14].

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes present mainly in anionic form at pH ≥ 4.0. So, when treated with an acid dye at acidic pH using buffer solutions, a yellow ion-pair complex which is extracted with chloroform is formed. The absorption spectra of the ion-pair complexes, which were formed between IMGH and reagents were measured in the range 350–550 nm against the blank solution and the maximum absorbance's were measured at wavelengths 410, 420, and 420 nm, using ARS, BPB and MO, respectively.

3.2. Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve the complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH of buffer, the type of organic solvent, volumes of the dye, reaction time and temperature for the extraction of ion-pair complexes.

3.2.1. Effects of pH

It was observed that the effective extraction of the complex depends on the buffer pH value. The effect of pH was studied by extracting the colored complexes in the presence of universal buffer. It is evident that the maximum absorbance's of the ion pair complexes were obtained at pH 4.0, 4.5, and 4.5 using ARS, BPB and MO, respectively (Figure 2). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-5.0 ml). The higher absorbance value and reproducible results were obtained by using 2.0 ml of buffer solution.

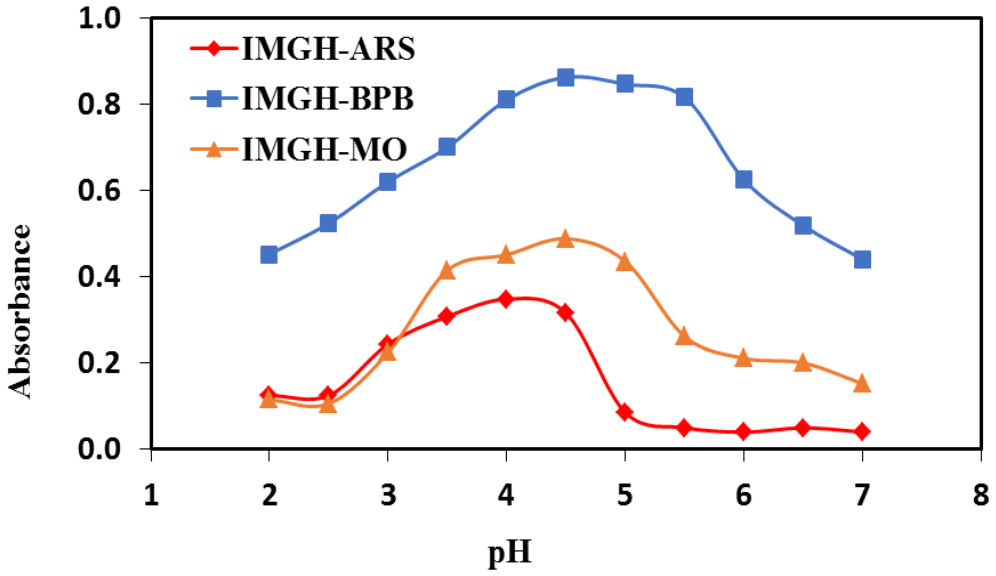


Figure 2. Effect of pH on the absorbance of 10 µg/mL IMGH complexed with ARS, BPB, and MO.

3.2.2. Effect of reagent concentration

The effect of the reagent was studied by measuring the absorbance's of solutions containing a fixed concentration of IMGH (10 µg/mL) and various volumes of the ARS, BPB and MO (1.0×10^{-3} M) reagents in the range of (0.5–5.0 ml). The results showed that the absorbance of the extracted ion-pair increased by increasing the reagent volume till 2.0 ml. So, the maximum color intensity of the complex was achieved with 2.0 ml of (1.0×10^{-3} M) of each reagent solution. Although a larger volume of the reagent had no pronounced effect on the absorbance's of the formed ion-pair complexes (Figure 3).

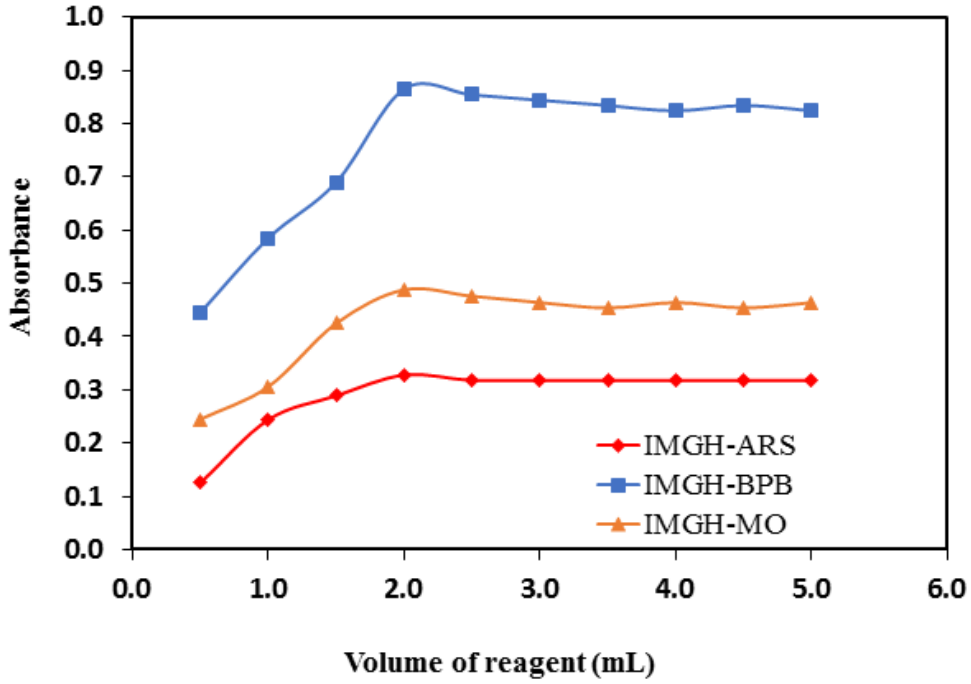


Figure 3. Effect of volume of (1.0×10^{-3} M) reagent on the absorbance of 10 µg/mL IMGH complexed with ARS, BPB, and MO

3.2.3. Effect of extracting solvent

The effect of several organic solvents viz., chloroform, carbon tetrachloride, dichloromethane, methanol, ethanol, acetonitrile, toluene, and chlorobenzene were studied for effective extraction of the colored species from the aqueous phase. Chloroform was found to be the most suitable solvent for extraction of colored ion pair complexes for all reagents quantitatively. Experimental results indicated that double extraction with total volume 10 ml chloroform, yielding maximum absorbance intensity, stable absorbance and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

3.2.4. Effect of shaking time and temperature

The optimum shaking time was investigated by shaking from 1-5.0 min at ambient temperature (25 ± 2°C). Maximum and constant absorbance value were obtained when extracted after 2.0 min of shaking for all complexes. Therefore, shaking time of 2.0 min was maintained throughout the experiment (Figure 4). The effect of temperature on colored complexes was studied by measuring the absorbance values over the temperature range 25-50°C. It was found that the absorbance of the colored ion pair complex was constantly up to 30°C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of dichloromethane. Therefore, the temperature

chosen was room temperature (25 ± 2°C) as the best temperature for determination of IMGH in bulk and pharmaceutical formulations (Figure 5).

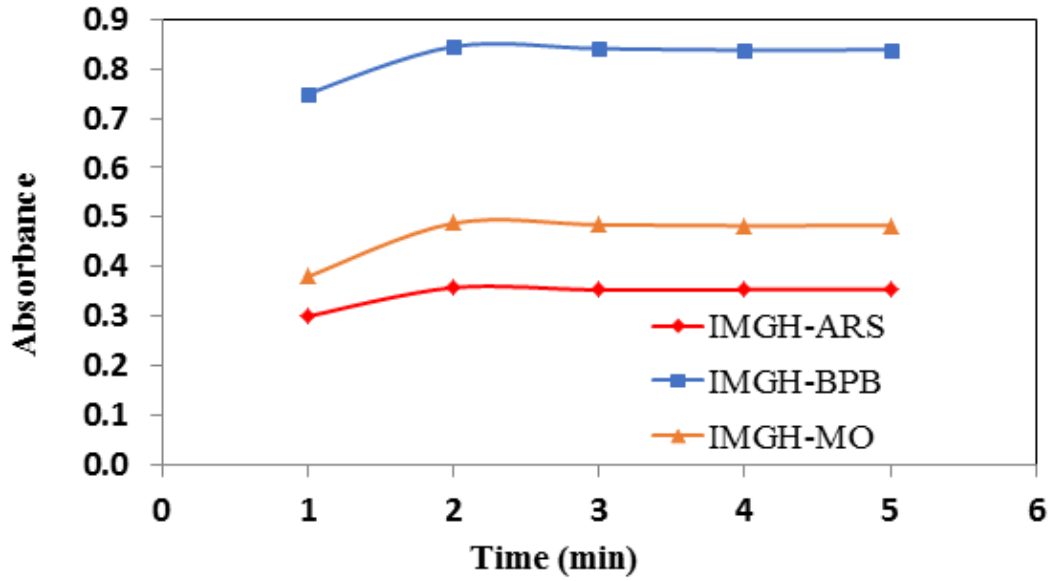
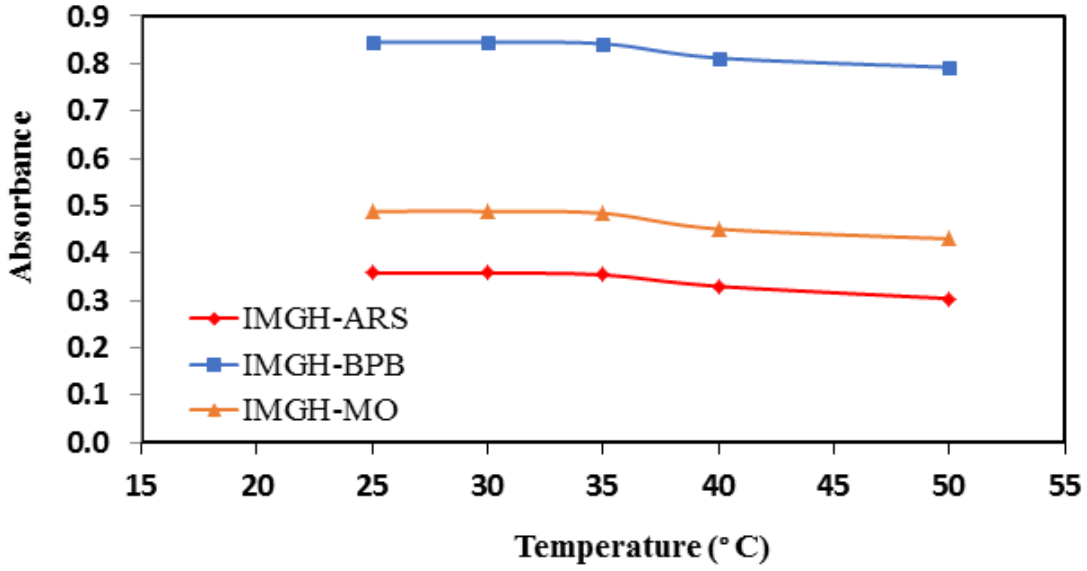


Figure 4. Effect of time reaction on the absorbance of 10 µg/mL complexed IMGH with ARS, BPB, and MO.



Figures 5. Effect of temperature of reaction on the absorbance of 10 µg/mL complexed IMGH with ARS, BPB, and MO.

3.2.5. Stoichiometric ratio

The molar ratio of the ion pair complexes (IMGH: dye) was determined by the continuous variations and molar ratio methods (Figures 6 and 7). The results indicate that the molar ratio of (IMGH: dye) is (1:1) ion-pair complex are formed through the electrostatic attraction between the positive charged $IMGH^+$ and negatively charged dye, (ARS^-, BPB^-, and MO^-). The extraction equilibrium can be represented as follows:

$$IMGH^+_{(aq)} + D^-_{(aq)} \longleftrightarrow IMGH^+D^-_{(aq)} \longleftrightarrow IMGH^+D^-_{(org)}$$

Where $IMGH^+$ and D^- represent the protonated drug and the anion of the dye (ARS^-, BPB^-, and MO^-), respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively.

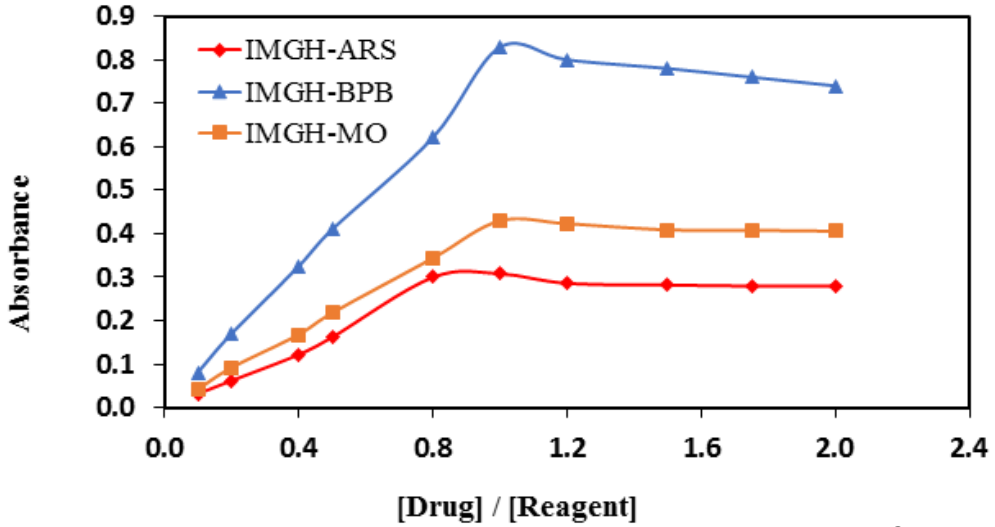


Figure 6. Mole ratio plots for the ion-association complexes of IMGH (1.0 x 10^{-3} M) with various volumes of reagent solution (1.0 x 10^{-3} M) at the optimum conditions.

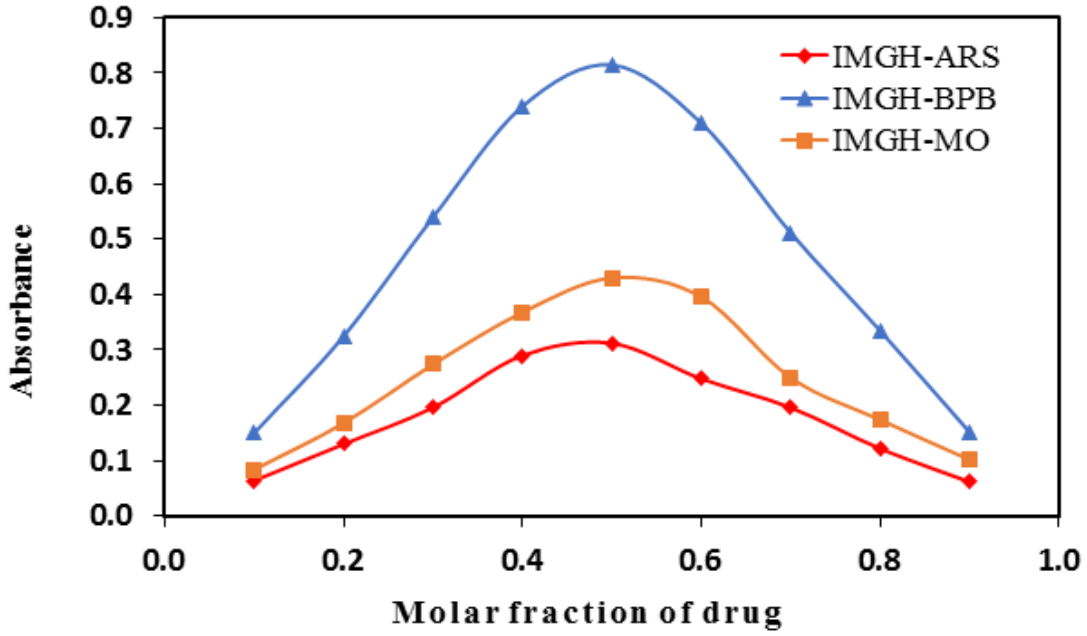


Figure 7. Job's method of continuous variation graph for the reaction of IMGH with ARS, BPB, and MO, [drug] = [dye] = (1.0×10^{-3} M) (N=3.0).

3.3. Method of Validation

3.3.1. Linearity, detection, and quantification limits

At described experimental conditions for IMGH determination, standard calibration curves with reagents were constructed by plotting absorbance vs. concentration of IMGH (Figure 8). The statistical parameters were given in the regression equations calculated from the calibration graphs A = aC + b, where A is the absorbance and C is the concentration in µg/ml. The linearity of calibration graphs was proved by the high values of the correlation coefficient (r^2) and the small values of the *y*-intercepts of the regression equations. The apparent molar absorptivity of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed

spectrophotometric method were also calculated and recorded in Table 1. The molar absorptivity of BPB > MO > ARS ion-pair complexes.

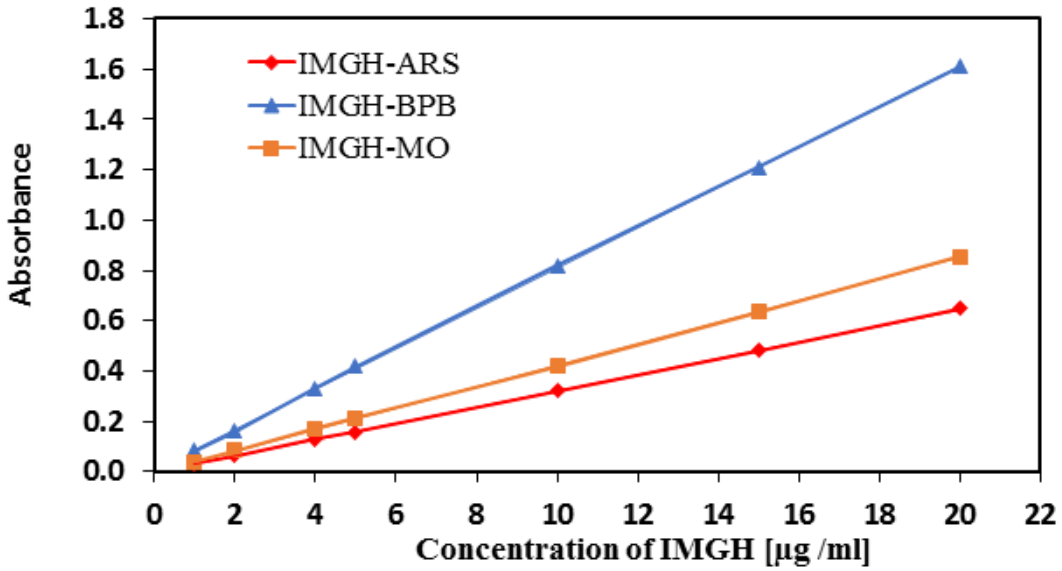


Figure 8. Calibration curves of IMGH complexed with ARS, BPB, and MO dyes.

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following equation [15, 16]:

$$LOD = 3s / k \text{ and } LOQ = 10\, s / k$$

Where s is the standard deviation of ten replicate determinations values of the reagent blank and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, LOD and LOQ were found to be be 0.28, 0.30, and 0.31 µg/ml and 0.86, 0.93, and 0.97 µg/ml for ARS, BPB, and MO, respectively.

Table 1. Statistical analysis of calibration graphs and analytical data in the determination of IMGH using the proposed methods.

Parameter	ARS	BPB	MO
Wavelengths λmax (nm)	410	420	420
pH	4.0	4.5	4.5
Beer's law limits (µg/mL)	1-20	1-20	1-20
Molar absorptivity ε ($L/mol^{-1}.cm^{-1}$) × 10^4	5.01416	12.45393	6.61753
Regression equation [a]			
Slope (b)	0.03231	0.08024	0.04264
Intercept (a)	-0.00193	0.00901	-0.00220
Standard error	0.00278	0.00743	0.00412
Correlation coefficient (R^2)	0.99988	0.99986	0.99985
Mean(n=6)	99.17	98.23	99.14
RSD % [b]	0.65	0.47	0.87
LOD (µg/mL) [b]	0.28401	0.30536	0.31875
LOQ (µg/mL) [b]	0.86063	0.92532	0.96589

[a] A = a + bC, where C is the concentration in µg/ml, A is the absorbance units.
[b] LOD, limit of detection; LOQ, limit of quantification; SD , standard deviation; RSD%, relative standard deviation.

3.3.2. Accuracy and precision

In order to evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of IMGH were prepared and the assay procedure was analyzed in six replicates, and percentage relative standard deviation (RSD%) values were obtained within the same day to evaluate the repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision). The percentage relative error (RE%) was calculated using the following equation:

$$\textbf{RE \% = [(Founded – Added) / Added] x 100}$$

The analytical results of intra-day and inter-day precision (RSD%) and accuracy (RE%) were summarized in Tables 2. These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

Table 2. Intra-day and inter-day precision and accuracy data for IMGH obtained by the proposed methods.

Method	Added concentration (µg/mL)	Intra-day			Inter-day		
		Recovery %	Precision RSD% [a]	Accuracy RE%	Recovery %	Precision RSD% [a]	Accuracy RE%
ARS	5	98.99	0.50	-1.01	99.42	1.04	-0.58
	10	100.52	1.12	0.52	98.84	0.55	-1.16
	20	98.57	0.72	-1.43	99.42	1.09	-0.58
BPB	5	100.13	1.01	0.13	101.07	1.65	1.07
	10	99.87	1.08	-0.13	100.13	1.00	0.13
	20	100.29	0.75	0.29	100.39	0.73	0.39
MO	5	98.48	0.82	-1.52	98.95	0.65	-1.05
	10	100.33	0.89	0.33	98.88	0.52	-1.12
	20	98.72	0.337	-1.28	101.93	0.92	1.93

[a] Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.

3.3.3. Ruggedness and robustness

For the evaluation of the method robustness, some parameters were interchanged; pH and dye volume. The capacity remains unaffected by small deliberate variations and RSD% values ranged from 0.62 to 2.05%. Method ruggedness was expressed as RSD% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts (RSD% range 0.70-1.82%) and instruments (RSD% range 0.84-1.95%), suggesting that the developed methods were robust and rugged.

3.3.4. Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany IMGH in its dosage forms (hydroxypropyl cellulose, Avicel, croscarmellose sodium, magnesium stearate, talc and areosel) was studied. The results indicated that there is no interference from excipients and additives, indicating a high selectivity for determining IMGH in its dosage forms.

3.4. Applications to dosage forms

The proposed methods have been successfully applied to the determination of IMGH in dosage forms (Twymeeg and Imeglibright tablets, labeled to contain 500 mg IMGH per tablet). Six replicates determinations were made. Moreover, to check the validity of the proposed methods, dosage forms were tested for possible interference with standard addition method (Table 3). Therefore, it is concluded that the excipients in dosage forms of IMGH did not cause any interference in the analysis of IMGH. A statistical comparison of the results for determination of IMGH in tablet

dosage forms using the proposed and reported methods[3] is shown in Table 5. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed and reported methods at the 95 % confidence level with respect to accuracy and precision[16] (Table 3).

Table 3. Application of the proposed methods for the determination of IMGH in dosage forms (tablets) and statistical comparison with the reported method [3].

Samples	Recovery [a] ± RSD			
	Proposed Methods			Reported Method [3]
	ARS	*BPB*	*MO*	
Twymeeg tablets	99.84±0.74	99.58±0.89	99.68±0.52	100.36±0.49
t-value [b]	1.25	1.70	2.13	
F-value [b]	2.28	3.30	1.13	
Imeglibright tablets	99.95±0.65	100.93±0.92	99.63±0.95	
t-value [b]	1.12	1.22	1.53	
F-value [b]	1.76	3.53	3.76	

[a] Average of six determinations.

[b] The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

IV. Conclusion

The proposed methods describe the application of extractive ion–pair complex formation reaction with dyes for the quantification of IMGH in pure and dosage forms. The proposed methods are simple, rapid, cost-effective, and more sensitive for determining IMGH in pure and dosage forms. The most attractive feature of these methods is its relative freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal high precision and accuracy of the proposed methods besides being robust and rugged. Therefore, the validated method could be useful for routine quality control assay of IMGH in pure and dosage forms.

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