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Research Paper

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Ecofriendly spectrophotometric techniques for the determination of imeglimin hydrochloride in pharmaceutical formulations

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ABSTRACT: ***Accurate, precise, eco-friendly, sensitive, and validated spectrophotometric methods were described for determination of*** *imeglimin hydrochloride (IMG)* ***in pure form and pharmaceutical formulations. These methods utilize N-bromosuccinimide (NBS) as an environmentally friendly oxidizing agent alongside three specific dyes: indigocarmine (IC), rhodamine B (RB), and orange G (OG). Under acidic conditions, excess NBS is used to oxidize*** *IMG,* ***and the unreacted NBS is subsequently quantified through its reaction with predetermined amounts of the dyes. Absorption measurements were recorded at 610 nm for IC, 550 nm for RB, and 478 nm for OG. The analytical technique was implemented and validated by carefully examining and optimizing various factors that could potentially affect the reaction. Strong linear correlations, with correlation coefficients ranging from 0.9990 to 0.9994, were established under optimal conditions. These methods were effective within concentration ranges of 1.0–14, 1.0–12, and 1.0–16 µg/ml, respectively. The limit of detection (LOD) was determined to be 0.29 µg/ml for IC, while RB and OG exhibited an LOD of 0.30 µg/ml. The accuracy and precision of these methods were assessed through both intra-day and inter-day measurements. No significant interference was detected from common tablet excipients.***

Keywords: Imeglimin hydrochloride; N-bromosuccinimide; Spectrophotometry; Method validation; Tablets.

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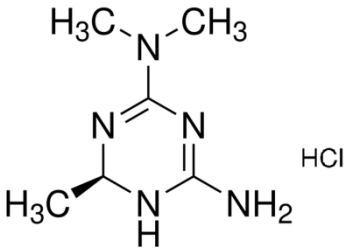
1. Introduction

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]



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 new, simple, cost effective spectrophotometric methods for the determination of IMGH in its dosage forms.

N-bromosuccinimide (NBS) is an environmentally friendly reagent widely used as an efficient oxidizing and brominating agent. Spectrophotometric determinations using NBS can be performed either by directly measuring the chromogenic derivative of the drug or indirectly by assessing the residual NBS with color-producing reagents [6-11].

Currently, numerous concerns are being expressed regarding the health risks associated with the implementation of analytical methods. Green analytical chemistry (GAC) techniques were established as an option that reduces environmental impact while maintaining procedural efficiency.

This study aims to develop innovative spectrophotometric methods that are simple, highly sensitive, accurate, and cost-effective for quantifying IMGH in its pure form and pharmaceutical dosage forms. The proposed methods utilize NBS as an eco-friendly reagent in combination with indigocarmine (IC), rhodamine B (RB), and orange G (OG) dyes. The presence of commonly used pharmaceutical excipients at typical concentrations did not interfere with the determination of IMGH. The methods were statistically validated for accuracy, precision, sensitivity, selectivity, robustness, and ruggedness following the guidelines established by the International Council for Harmonisation (ICH) [12].

2. EXERIMENTAL

2.1. Equipment

A Shimadzu UV-1601 UV/Vis spectrophotometer (Sweden) equipped with a 10 mm glass cell was used for absorbance measurements. This device offers exceptional wavelength precision, with an accuracy of ±0.2 nm. It operates at a scanning speed of 200 nm/min and a bandwidth of 2.0 nm, covering a wavelength range of 200 to 900 nm.

2.2. Chemicals and reagents

All chemicals, reagents, and solvents used in this study were of high-purity analytical grade. Additionally, all solutions were freshly prepared at regular intervals. Double-distilled water was used throughout the experiment.

2.2.1. Pure IMG and pharmaceutical formulations

Pure-grade IMG was kindly provided by Al-Esraa Pharmaceutical Company, Badr City, Cairo, Egypt, with a potency of 99.90 ± 0.80% as determined by the reported method [1]. The commercial pharmaceutical formulation, Twymeeg film-coated tablets contain 500 mg IMG per tablet and were produced by Sumitomo Pharmaceutical Co. Ltd., Japan, and purchased from the local market.

2.2.2. Standard solutions preparation

Stock standard solutions of IMG equivalent to 100 µg/mL were prepared by dissolving an exact weight of pure IMG in bidistilled water in a 100 mL measuring flask and completed to the mark with bidistilled water. The standard solutions were found stable for at least one week without alteration when kept in an amber coloured bottle and stored in a refrigerator when not in use.

A stock solution of NBS (200 µg/mL) was prepared using Sigma-Aldrich NBS by dissolving 0.02 g in the smallest possible volume of bidistilled water in a 100 mL volumetric flask, followed by dilution with distilled water to the desired concentration and subsequent calibration [13].

A 1.0% (w/v) potassium bromide (KBr) solution was prepared by dissolving 1.0 g of KBr in 100 mL of bidistilled water.

A 5.0 mol/L HCl solution (Merck, Darmstadt, Germany, Sp. gr. 1.18, 37%) was prepared by diluting 43 mL of concentrated HCl to 100 mL with bidistilled water. The solution was standardized following the recommended procedure [14] before use. All prepared standard solutions were stored in a refrigerator when not in use.

Stock solutions of IC, RB, and OG dyes (Sigma-Aldrich, 90% dye concentration) were prepared by dissolving 112 mg of each dye in bidistilled water to obtain a 1000 µg/mL concentration. These solutions were further diluted to 200 µg/mL using a 100 mL calibrated flask.

2.3. Recommended procedures

Different aliquots (0.1-1.2 mL), (0.1-1.4 mL), and (0.1-1.0 mL) of a standard 100 µg/mL IMG solution using IC, RB, and OG methods, respectively, were transferred into a series of 10 mL calibrated flasks by means of a micro burette. To each flask 2.0 mL each of 2.0 mol/L HCl; 2.0 mL of NBS solution (200 µg/mL) and 1.0 mL of 1.0% (w/v) KBr were added successively. The flasks were stoppered, content mixed, and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 1.0 mL of (200 µg/mL) IC, RB, or OG solution were added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The absorbance of each solution was measured at 520, 663 and 610 nm for IC, RB, and OG methods, respectively, after 3.0 min against a reagent blank. In all methods, a standard graph was prepared by plotting the absorbance versus the concentrations of IMG. The concentration of the unknown analyte was read from the calibration graph or computed from the regression equation derived using Beer's law data.

2.3. Assay procedure for dosage forms

Twenty Twymeeg tablets were accurately weighed and finely ground into a powder. An amount equivalent to 10 mg of IMG was dissolved in bidistilled water in a 100 mL volumetric flask. The mixture was stirred for 5.0 minutes and then filtered through Whatman No. 42 filter paper. The filtrate was diluted to 100 mL with bidistilled water to prepare a stock solution of IMG (100 µg/mL). The spectrophotometric methods were applied to analyze this solution. A suitable aliquot was then examined using the previously described methodologies. The exact concentration of the drug in the dosage form was determined using the corresponding regression equation.

3. RESULTS AND DISCUSSION

3.1. Absorption spectra

In an acidic environment, oxidizing agents permanently transform many colored compounds into colorless ones [15]. The proposed methods are based on the reaction between IMG and an excess amount of NBS, followed by the quantification of the remaining NBS through its interaction with a predetermined amount of IC, RB, and OG dye. Absorbance is then measured at 610 nm, 550 nm, or 478 nm, depending on the dye used (Figure 2). These methods utilize the bleaching effect of NBS on the dyes, where oxidative degradation causes the dyes to lose their color. As the concentration of IMG increases, it reacts with more NBS, leading to a depletion of available NBS. This reduction in NBS concentration results in a greater retention of the dye's original color when a fixed amount of dye is introduced. Consequently, a direct correlation is observed between IMG concentration and the increase in absorbance at the respective λmax, enabling accurate quantification of IMG.

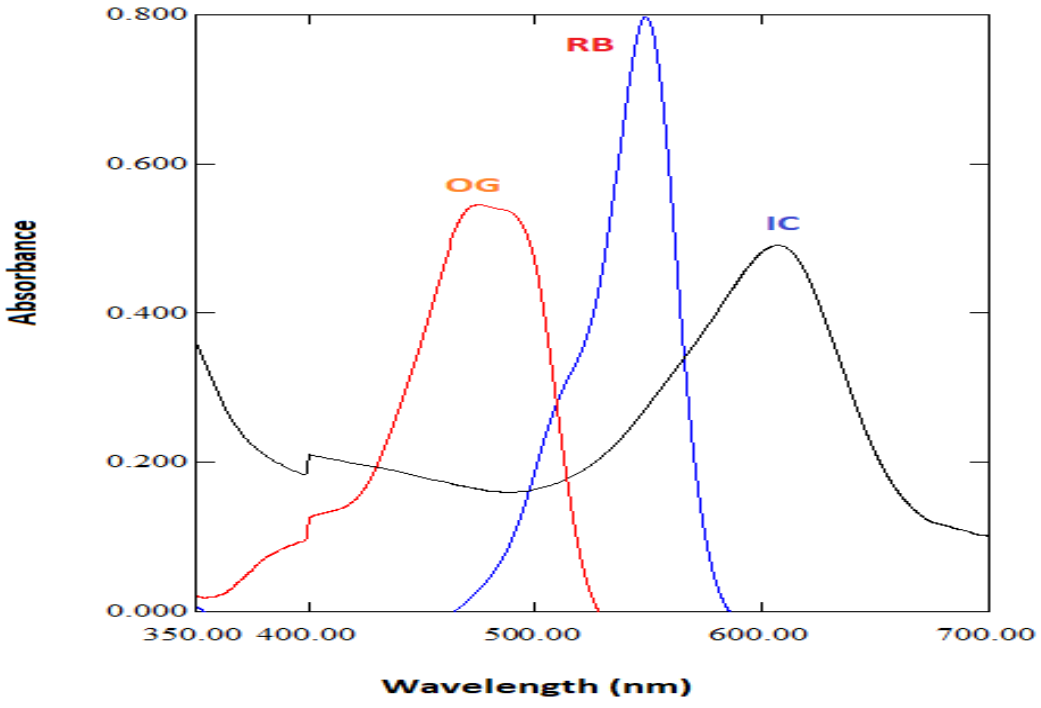
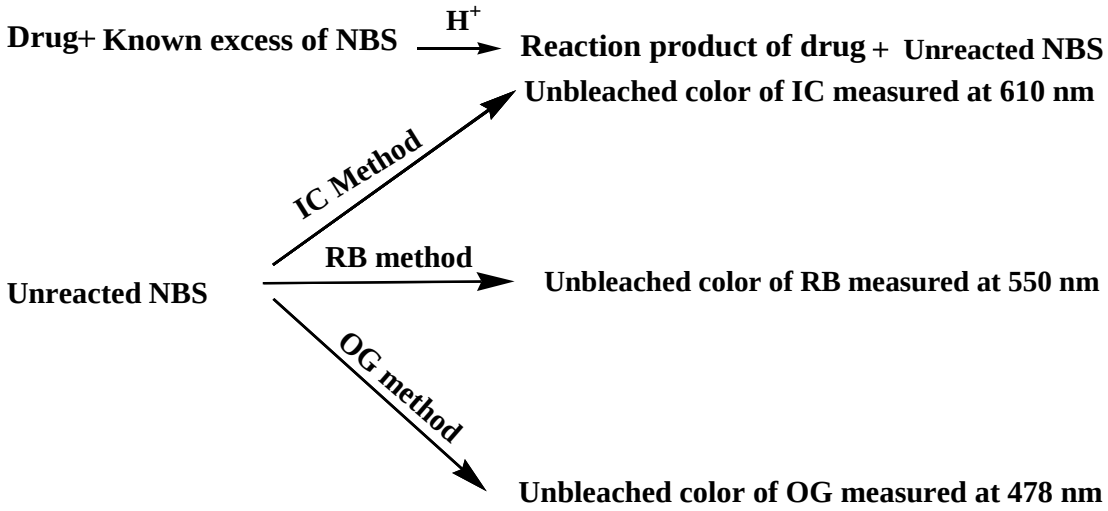


Figure 2. The absorption spectra for the unbleached IC, RB, and OG dyes.

3.2. Chemistry of the reaction

NBS is a highly effective reagent capable of oxidizing or brominating various compounds. It is extensively utilized in the analysis of pharmaceutical drugs and is considered the primary bromine-containing organic compound for this purpose [16-20]. Additionally, NBS is specifically employed to introduce bromine atoms at the allylic position of alkenes [21]. The reaction process occurs in two sequential stages. In the first stage, IMG undergoes bromination using an excess amount of NBS in an HCl solution. In the second stage, the remaining unreacted NBS is quantified by allowing it to react with a predetermined amount of IC, RB, and OG dyes, followed by absorbance measurement at their respective λmax. The proposed reaction mechanism for the spectrophotometric methods is depicted in Scheme 1. A direct correlation was observed between the absorbance and IMG concentration in all methods. These techniques leverage the bleaching properties of NBS, where oxidative degradation leads to color fading of the dyes, enabling the quantification of IMG based on the resulting absorbance changes.



Scheme 1. The recommended chemical route for the proposed spectrophotometric approaches involves the utilization of NBS and dyes.

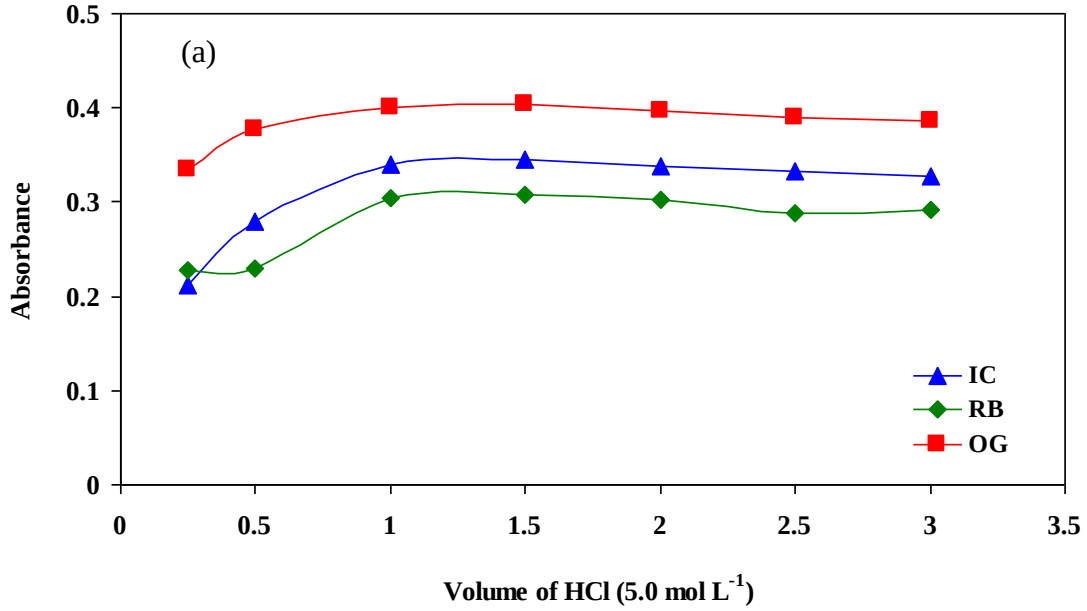
3.3. Analytical parameters optimization

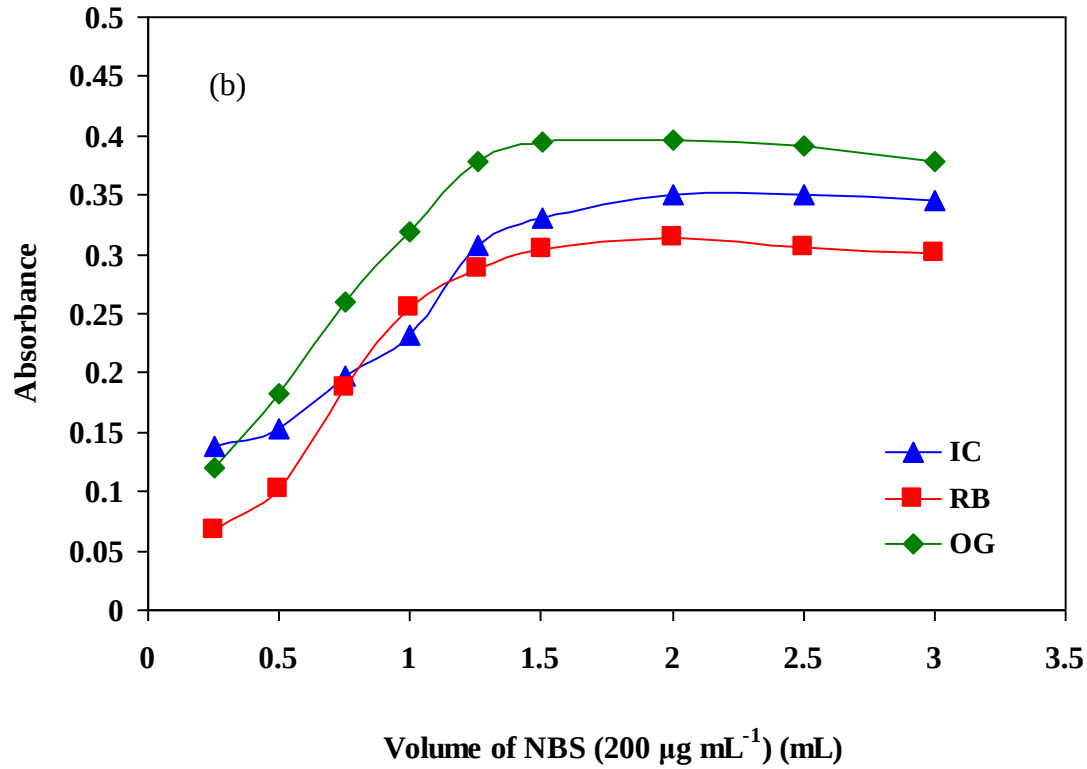
3.3.1. Identification and quantification of acid type and concentration

The reaction between IMG and N-bromosuccinimide (NBS) was evaluated in various acidic media, including HCl, H_2SO_4, HNO_3, and CH_3COOH. Among these, HCl provided the most favorable results. To investigate the effect of HCl concentration, its volume was varied between 0.25–3.0 ml of 5.0 mol/l HCl, while maintaining constant concentrations of NBS and IMG. The results showed that using 1.0–3.0 ml of HCl (5.0 mol/l) resulted in nearly identical absorbance values in the presence of IMG. However, when the HCl volume was below 1.0 ml, the reaction proceeded more slowly and did not reach completion. Therefore, 1.0 ml of 5.0 mol/l HCl was chosen as the optimal concentration for the reaction, as illustrated in Figure 3(a).

3.3.2. Effect of NBS

To determine the optimal concentration of NBS, varying volumes of NBS (200 µg/ml) ranging from 0.25 to 3.0 ml were reacted with a fixed amount of dye in an HCl solution. The results indicated that the highest absorbance value was obtained when 2.0 ml of NBS (200 µg/ml) was used, as illustrated in Figure 3(b).


(a)
Absorbance
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
2.5
3
3.5
IC
RB
OG
Volume of HCl (5.0 mol L-1)


(b)
Absorbance
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.5
1
1.5
2
2.5
3
3.5
IC
RB
OG
Volume of NBS (200 µg mL-1) (mL)

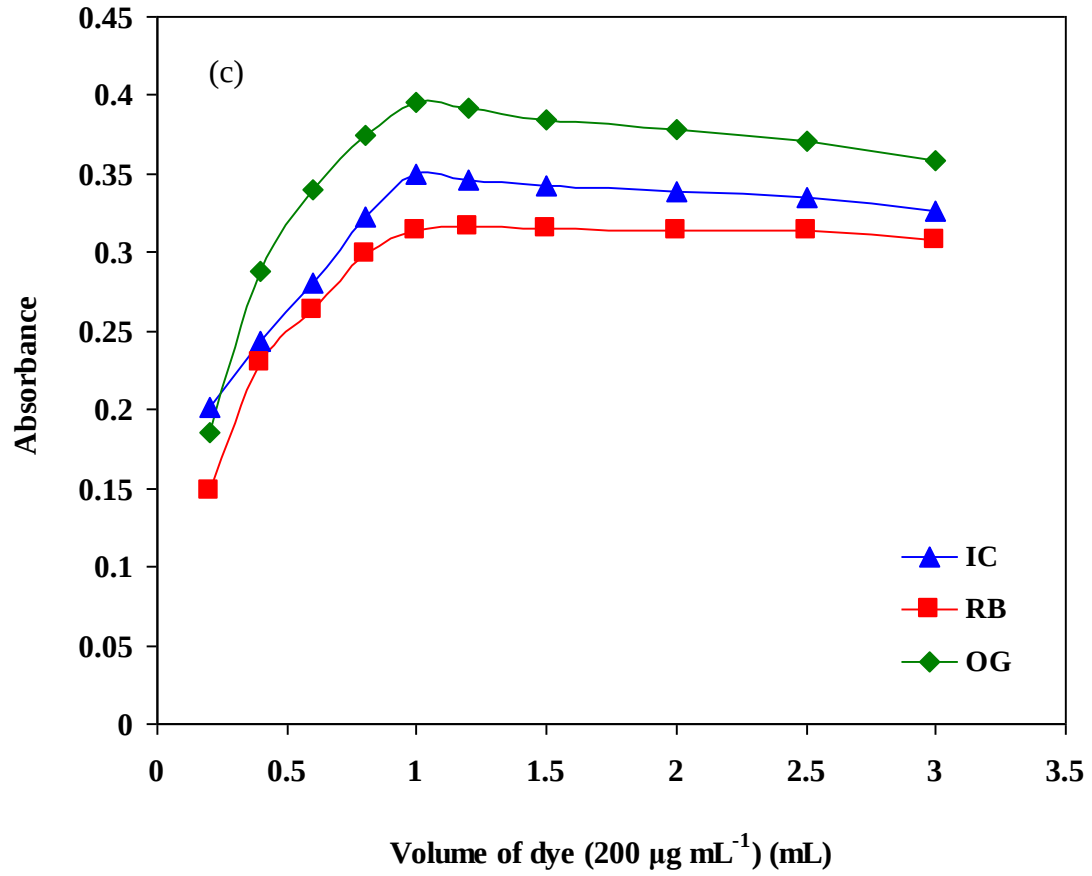


Figure 3. The impact of (a) HCl volume (5.0 mol/l), (b) NBS volume (200 µg/ml) and (c) dye volume (200 µg/ml) on the oxidation product of IMG.

3.3.3. Effect of KBr

The influence of potassium bromide (KBr) volume on the reaction was evaluated within the range of 0.5–3.0 ml. The results indicated that 1.0 mL of KBr (1.0% w/v) was the optimal amount, as it effectively accelerated the oxidation process.

3.3.4. Effect of dye

To determine the ideal volume of IC, RB, and OG dyes at a concentration of 200 µg/ml, a study was conducted by varying the dye volume between 0.25 and 3.0 mL. The findings revealed that the oxidation products achieved the highest color intensity when 1.0 mL of dye solution was used (Figure 3(c)).

3.3.5. Effects of temperature and duration of mixing

The impact of temperature on both sample and blank solutions was investigated by immersing them in a water bath at varying temperatures (25–50°C). The highest color intensity was observed at 25 ± 2°C, and increasing the temperature did not yield consistent results. Additionally, the effect of reaction time on the oxidation process was examined over a range of 2.0–20 minutes. The study showed that a 5.0-minute reaction time at 25 ± 2°C consistently produced stable and reproducible absorbance readings. Furthermore, a standing period of 3.0 minutes was necessary for the complete decolorization of the dye by the remaining NBS. The unreacted dye absorbance remained stable for at least 10 hours after this period.

3.3.6. Impact of the sequence of addition

The optimal sequence for reagent addition was determined as follows: IMG-HCl-NBS-KBr-Dye. Alternative sequences resulted in lower absorbance values under the same experimental conditions.

3.4. Method validation

3.4.1. Linearity and sensitivity

A clear correlation was observed between absorbance at the maximum wavelength (λmax) and IMG concentration under optimized conditions. The linear concentration ranges for IMG were 1.0–12 µg/ml using IC, 1.0–14 µg/ml using RB, and 1.0–10 µg/ml using OG. For enhanced accuracy, the Ringbom concentration range [22] was established. The limits of detection (LOD) and limits of quantitation (LOQ) for the proposed methods were determined using the formulas:

LOD = (3s / b) and LOQ = (10s / b)

Where **s** is the standard deviation of 10 repeated measurements of the reagent blank, and b represents sensitivity, defined as the slope of the calibration curve. The calculated values were: LOD: 0.30 µg/mL (IC or RB), 0.29 µg/ml (OG). LOQ: 1.0 µg/ml (IC or RB), 0.97 µg/mL (OG).

The proposed methods were validated through statistical analysis, comparing the obtained results with those of a previously reported method [1]. Based on the Student's t-test and variance ratio (F-test) (Table 1), no statistically significant difference was found between the proposed methods and the reference method [1] in terms of accuracy and precision.

Table 1. Analytical and regression parameters of the developed approaches for determining IMG.

Parameters	**IC**	**RB**	**OG**
Beer's law limits, µg/ml	1.0-12	1.0-14	1.0-10
Ringboom limits, µg/ml	3.0-10	3.0-2	3.0-8.0
Molar absorptivity, x 10^4 L/mol.cm	0.7013	0.5871	0.9779
Sandell sensitivity, ng/cm^2	27.33	32.65	19.60
Regression equation[a]			
Intercept (a)	0.0059	0.0089	0.0139
Standard deviation of intercept (S_a)	0.018	0.032	0.04
Slope (b)	0. 0.0314	0.0216	0.0422
Standard deviation of slope (S_b)	0.016	0.028	0.022
Correlation coefficient, (r)	0.9998	0.9997	0.9994
Mean ± SD	99.80±0.75	٩٩.75±0.90	99.60±0.68
RSD%	0.75	0.90	0.68
RE%	0.79	0.95	0.71
Limit of detection, µg/ml	0.30	0.30	0.29
Limit of quantification, µg/ml	1.0	1.0	0.97
Calculated *t*-value [b]	0.20	0.28	0.64
Calculated *F*-value [b]	1.14	1.27	1.38

[a] $A = a + bC$, where C is the concentration in µg/ml, A is the absorbance units, a is the intercept, b is the slope.

[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).

3.4.2. Evaluation of Accuracy and Precision

To assess the accuracy and precision of the proposed methodologies, solutions containing three different IMG concentrations were prepared and analyzed. The study was conducted on six replicate samples, and the results are summarized in Table 2. The accuracy of the methods was evaluated based on the percentage relative error (RE%), while precision was assessed using the relative standard deviation (RSD%). Lower values of RE% and RSD% indicate higher accuracy and precision. Both intra-day and inter-day precision were examined, and the results demonstrated that the proposed methods exhibit high accuracy and precision, ensuring excellent repeatability and reproducibility.

Table 2. Intra-day and inter-day accuracy and precision of the developed approaches.

Method	Taken (µg/ml)	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
	Intra-day				
IC	4.0	99.30	0.48	-0.70	3.972 ± 0.020
	8.0	98.90	1.10	-1.10	7.912 ± 0.091
	12	100.30	0.89	0.30	12.036 ± 0.112
RB	4.0	99.80	0.78	-0.20	3.992±0.033
	8.0	99.30	0.74	-0.70	7.944 ± 0.062
	12	100.10	0.98	0.10	12.012 ± 0.124
OG	3.0	99.10	1.10	-0.90	2.973±0.034
	6.0	99.50	1.17	-0.50	5.970±0.073
	9.0	100.10	1.40	0.10	9.009 ± 0.132
	Inter-day				
IC	4.0	100.30	0.56	0.30	4.012±0.024
	8.0	99.80	0.82	-0.20	7.984 ± 0.069
	12	100.40	0.92	0.40	12.048 ± 0.116
RB	4.0	99.40	0.68	-0.60	3.976 ± 0.028
	8.0	99.40	0.96	-0.60	7.952 ± 0.080
	12	99.60	1.15	-0.40	11.952 ± 0.144
OG	3.0	100.10	0.78	0.10	3.003 ± 0.025
	6.0	99.30	0.84	-0.70	5.958 ± 0.053
	9.0	99.80	1.93	-0.20	8.982 ± 0.182

[a] RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Mean ± standard error, confidence limit at 95% and five degrees of freedom (t = 2.571).

3.4.3. Evaluation of Robustness and ruggedness

To assess the robustness of the proposed method, slight variations were intentionally introduced in the experimental conditions: NBS volume was adjusted within 1.5 ± 0.2 mL and reaction time was varied within 5.0 ± 2.0 minutes. The analysis was conducted using these modified conditions at three different IMG concentrations. The results demonstrated that the method remained unaffected by these changes, as indicated by RSD% values ranging from 0.40% to 2.50%, confirming its robustness.

The ruggedness of the method was evaluated by measuring the RSD% across: three different analysts (intra-analyst variability) and three different instruments (inter-instrument variability). The intra-analyst RSD% ranged from 0.30% to 2.40%, while the inter-instrument RSD% varied from 0.60% to 2.30%, indicating that the proposed methodologies are highly reliable and consistent across different conditions. The detailed results are presented in Table 3.

Table 3. Robustness and ruggedness results of the developed methods (n=3).

Methods	Nominal amount concentration (µg/ml)	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a]			
		NBS volume	Reaction time	Different analysts	Different instruments
IC	4.0	0.80	0.60	0.30	0.60
	8.0	0.90	1.90	1.50	1.75
	12	2.10	2.50	2.10	2.30
RB	4.0	0.54	0.40	1.0	0.75
	8.0	0.60	1.10	1.40	1.10
	12	2.0	1.85	1.90	2.25
OG	3.0	1.10	0.71	0.55	0.84
	6.0	0.78	1.24	1.0	1.50
	9.0	2.40	1.85	2.40	2.20

[a] Volume of (200 µg/ml) NBS is (1.5±0.2 ml) and reaction time is (5.0±2.0 min) were used.

Recovery studies and application

To evaluate the accuracy, reliability, and validity of the proposed methodologies, a recovery experiment was performed using the standard addition method. This involved adding three different concentrations of pure IMG to a fixed amount of IMG from tablet formulations that had already been analyzed. The final concentration was then determined using the proposed methods. Each concentration level was analyzed in triplicate, and the percentage recovery was calculated using the following equation:

$$\textbf{\% Recovery} = \frac{[C_F - C_T]}{C_p} \times 100$$

Where: C_F = Total IMG concentration detected. C_T = IMG concentration in the tablet. C_P = Pure IMG concentration added (spiked).

The results, presented in Table 4, demonstrate that the accuracy of the proposed methods was not affected by the presence of excipients in the pharmaceutical formulations.

A statistical comparison was conducted between the results obtained using the proposed methods and those obtained from a previously reported method [1]. Table 4 presents the Student's t-test and F-test values, calculated at a 95% confidence level with five degrees of freedom [23]. The findings confirm that the obtained results are in good agreement with the labeled claim, indicating no significant difference between the proposed methodologies and the established method. This further validates the accuracy and precision of the suggested approaches.

Table 4. Application of the developed methods for the determining IMG in Twymeeg tablets.

Parameters	Taken (µg/ml)	Added (µg/ml)	Recovery (%)[a]			Reported method [1]
			IC	RB	OG	
	4.0	2.0	101.20	99.60	99.50	
		4.0	100.70	99.30	100.40	
		6.0	99.00	100.60	99.20	
Mean ± SD[a]			100.30±1.15	99.83±0.68	99.٧٠±0.62	99.90±0.90
t-value [b]			0.61	0.14	0.41	
F-value [b]			١.63	1.75	2.11	

[a] Average of six determinations; SD: standard deviation; RSD%: percentage relative standard deviation.

[b] 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).

4. CONCLUSION

For drug analysis, spectrophotometry can be useful in quality control labs and provide optimum sensitivity without the need for expensive equipment. The suggested spectrophotometric techniques are thought to have excellent accuracy, precision, cost-effectiveness, sensitivity, and selectivity. Additionally, they save time by removing the need for time-consuming extraction processes, hazardous solvents, and necessary experimental components. The amount of IMG in both its pure and dose forms was successfully measured using these techniques. The methods used have been extensively verified to meet the requirements of the International Council for Harmonization (ICH) and make use of NBS as an environmentally friendly brominating agent. Consequently, it is evident that these techniques may be regarded as eco-friendly choices for examining genetically modified food in quality control labs.

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