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Two Modified Spectrophotometric Methods for The Estimation of Indomethacin in The Pharmaceutical Formulations and Biological Fluids Using Bromate-Bromide Reagent, Indigo Carmine and Methyl Violet 2B Dyes

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Abstract

This research presents the development of two sensitive indirect spectrophotometric methods for the quantitative determination of indomethacin, applicable to its pure form, pharmaceutical formulations, and biological samples. The two methods A and B were developed based on the reaction of the drug indomethacin with bromine, which was generated in situ using an excess of the friendly environmental materials bromate-bromide mixture in an acidic medium through the principle of green chemistry. The residual amount of bromine reagent was then quantified by reacting it with either indigo carmine (method A) or methyl violet 2B (method B) dyes, and the absorbance of the remaining two dyes, with the absorbance of the remaining dyes measured at wavelengths of 610 nm and 582 nm, respectively. Both methods A and B follow Beer's law within the concentration ranges 1-22 and 0.4-18 µg/mL with an excellent molar absorptivity value 2.51×10^4 and 3.37×10^4 L/mol.cm, correspondingly. The accuracy values for both methods were calculated to be between 99.90% to 100.18%, and 99.97% to 100.12%, while a relative standard deviation (RSD%) in the ranges 0.1536 to 0.1959 and 0.0540 to 0.1293 for methods A and B, respectively. The evaluation of results of the two proposed methods was a achieved by applying t and F tests. The results showed that there is no significant difference between the proposed and reference methods. Both recommended methods A and B were used successfully to analyse indomethacin in its pharmaceutical forms (capsules, suppositories and spray), in the serum and urine, with no interference detected from excipients additives present in the commercial pharmaceutical preparations.

Keywords: Bromate-bromide mixture, Indigo carmine and Methyl violet 2B, Indomethacin, Spectrophotometry.

تطوير طريقتين طيفيتين لتقدير الاندوميثاسين في المستحضرات الصيدلانية والسوائل البيولوجية باستخدام مزيج البرومات–البروميد وصبغتي الانديكو كارمن والمثيل البنفسجي 2B

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الخلاصة

تقدم هذه الدراسة تطوير طريقتين طيفيتين حساستين وغير مباشرتين لتقدير الإندوميتاسين ويمكن تطبيقهما على شكله النقي، ومستحضراته الصيدلانية، والعينات البيولوجية. اذ تستند كلا الطريقتين المطورتين A وB على مفاعلة عقار الاندوميتاسين في وسط حامضي مع البروم المتولد انيا في موقع التفاعل باستخدام فائض من خليط المواد الصديقة للبيئة محلول البرومات – البروميد في وسط حامضي من خلال مبدا الكيمياء الخضراء . بعدها يتم تقدير الكمية المتبقية من كاشف البروم وذلك بمفاعلته مع صبغتي الانديكو كارمن (الطريقة A) والميثيل البنفسجي 2B (الطريقة B), ثم يقاس الامتصاص لمحلول الصبغتين المتبقيتين عند الطولين الموجيين 610 و582 نانومتر، على التوالي. كانت كلتا الطريقتين A وB تتبعان قانون بير ضمن مدى التراكيز 1–22 و0.4–18 ميكروغرام/مل مع قيم للامتصاص المولاري بمقدار 2.51×10^4 و3.37×10^4 لتر/مول.سم على التوالي, تم حساب قيم الدقة لكلا الطريقتين لتكون بين 99.90% إلى 100.18%، و 99.97% إلى 100.12%، في حين أن الانحراف المعياري النسبي (RSD%) في النطاقات 0.1536 إلى 0.1959 ومن 0.0540 إلى 0.1293 للطريقتين A وB على التوالي. ، وتم تحقيق تقييم نتائج الطريقتين المقترحتين من خلال تطبيق اختباري t و F. أظهرت النتائج أنه لا يوجد فرق كبير بين الطريقتين المقترحتين والطرق المرجعية. تم استخدام الطريقتين الموصى بهما A وB بنجاح لتحليل الإندوميثاسين في أشكاله الصيدلانية (كبسولات وتحاميل ورذاذ)، في المصل والبول، دون الكشف عن أي تداخل من المواد المضافة الموجودة في المستحضرات الصيدلانية التجارية.

1. Introduction

Indomethacin (INDm) is a white crystalline powder, chemically identified as [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetic acid, (Figure 1). It is classified as a nonsteroidal anti-inflammatory drug that belongs to a group of acetic acid derivatives [1]. INDm is commonly used to treat conditions such as arthritis, tendonitis, and relieve pain, and reduce fever. INDm is also used to relieve pain after dental or orthopaedic surgery. It has side effects, especially in high doses and its symptoms include vomiting, dizziness and gastrointestinal bleeding [2]. Furthermore, INDm has therapeutic connections with other drugs used for treating Covid-19 [3]. INDm in certain circumstances has more effective than aspirin [4].

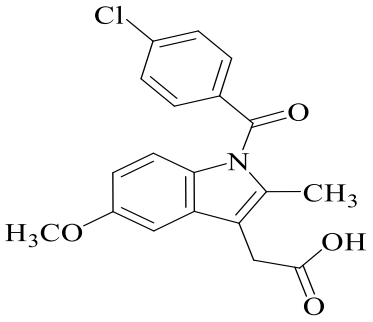


Figure 1: Chemical structure of INDm

The estimation of INDm is crucial for quality control in the pharmaceuticals industries, bioavailability studies, and monitoring drug therapy. A range of analytical methods have been developed for quantifying indomethacin in pharmaceutical formulations, as well as in biological samples such as human serum and urine, rabbit blood plasma, and in environmental matrices like wastewater [5-7]. These methods include flow injection analysis with phosphotungstic acid [8], chemiluminescence based on sulfur and nitrogen co-doped carbon quantum dot-$KMnO_4$ reaction [9], gas chromatography-mass spectrometry (GC-MS) [10], RP-HPLC [11], HPLC using equal BDS C_{18} column and UV detection at 278 nm [12]. Additionally, liquid chromatography tandem mass spectrometry [13], micellar electrokinetic

chromatography (MEKC) with UV detection [14], voltametric sensor using multi-walled carbon nanotube composite modified carbon-ceramic electrode [15], homemade ISNAG-fluorimeter using potassium hexacyanoferrate (III) [16], electrochemical method using a cadmium sulfide nanoparticles/multi-walled carbon nanotubes modified gold electrode [17] and voltametric using TiO_2 nanoparticle modified carbon ionic liquid electrode [18].
UV-Vis spectroscopy is one of the simplest methods for estimating INDm, notable for its straightforward preparation, relatively not time consuming for analysis and low cost. Various spectrophotometric methods have been developed for the estimation of INDm in its medicines. In UV- spectroscopic methods, potassium hydroxide is utilized to decompose of INDm into p-chlorobenzoic acid. This method allows for the direct estimation of p-chlorobenzoic acid at 228nm and indirectly to estimate the INDm content in pharmaceutical preparations [19]. Additionally, other methods employing the mixed solvency concept [20], ethanol [21] and mix hydrotropic solutions (sodium acetate, sodium citrate, and urea) [22] were used as solvents for solubilizing INDm and estimating it at 320nm, 266nm and 320nm, respectively.

Only a limited number of visible spectrophotometric methods have been developed for the assay of INDm in both pure form and its drug preparations. Two notable methods involve the diazo coupling reaction of INDm either with diazotized 4-carboxyl-2,6-dinitrobenzene [23] or with p-phenylenediamine dihydrochloride (PPDD) in sulphuric acid medium [24]. INDm was reacted with cuprous ions producing colour cuprous complex [25], Others based on the reduction of INDm with ammonium oxalate to form violet colour product showed maximum absorption at 578 nm [26] and based on the oxidation of INDm with an excess of bromosuccinamide and the residual NBS was estimated by bleaching the color of methyl orange dye in an acidic medium [27]. However, some of these methods face challenges such as low sensitivity, low accuracy due to the matrix interferences in uncontrolled conditions. Others required temperature control. The current investigation presents two newly developed indirect spectrophotometric methods using friendly environmental materials bromate-bromide mixture reagent [28-29] and two organic dyes for estimating INDm in the bulk, in the pharmaceutical forms and biological fluids.

2. Experimental

2.1. Scientific equipment

All spectrophotometric measurements and absorption spectra were carried out and recorded via a double-beam spectrophotometer (UV-1900i) using 1.0-cm matched fused silica cells. While a professional Benchtop pH meter Model (BP3001) was used for measuring the pH values.

2.2. Chemical reagents and standard solutions

The highest purity of chemicals was adopted in this work.
Standard INDm solution (100 µg/mL): A 0.010 g of pure INDm (Sammara-Iraq (SDI)), was weighed and dissolved in 20 mL of ethanol, and the volume was brought to 100 mL with distilled water (Dw) using a calibrated flask [30].
$KBrO_3$-KBr mixture solution (200 µg/mL): A 0.020 g of potassium bromate ($KBrO_3$) and 0.200 g of potassium bromide (KBr) were weighed and dissolved in 100 mL of Dw using a calibrated flask [31].
Indigo carmine dye solution, Idc (2.5×10^{-3} M): It was prepared by dissolving 0.1166 g of Idc dye (Fluka) in a 100-mL of Dw using a calibrated flask.[32]

Methyl violet 2B solution, MV2B (2×10^{-3} M): It was prepared by weighing 0.0717 g of MV2B dye and dissolved in small portion of Dw and the volume of solution was diluted with the same solvent to 100-mL in a calibrated flask.[33]
Hydrochloric acid solutions (1M and 5M). Two hydrochloric acid solutions (1M and 5M) were prepared by diluting appropriate amounts of the concentrated hydrochloric acid to the mark with Dw using 100-mL calibrated flasks.

2.3. Essential procedure

2.3.1. Method A

In a series of 25 mL conical flasks, varying volumes of INDm standard solution covering the concentration range of 0.2 to 3.5 µg/mL were added, along with 1.0 mL of 1M hydrochloric acid solution and 2 mL of a 200 µg/mL $KBrO_3$-KBr mixture solution. The solutions were left at room temperature for 10 minutes to complete the reaction, and a 1.7 mL of 2.5×10^{-3} M Idc dye was added. The solutions were then shaken thoroughly and kept constant for 3 minutes. Finally, the volumes were adjusted up to the marks with Dw and their absorbance were measured at 610 nm against corresponding reagent blank.

2.3.2. Method B

In this method, 0.7 mL of 5M hydrochloric acid solution and 3.0 mL of $KBrO_3$ - KBr mixture solution (200µg/mL) were added to a series of 25 mL conical flasks containing an increasing quantity 0.1 - 4.5 mL of INDm standard solution (100 µg/mL). The flacks were mixed thoroughly and left for 20 minutes at room temperature. Following this, 1.5 mL of 2×10^{-3} MV2B dye was then added and mixed well. After a waiting period of 5 minutes, the solutions were diluted to the marks with Dw and the absorbance of each flask was recorded at 582 nm against reagent blank solution.

2.4. Essential procedure for INDm analysis in their drugs

2.4.1. INDm capsules solution (100 µg/mL)

The contents of ten INDm capsules, each contains 25mg of INDm, were thoroughly mixed and homogenised well. An accurate weight equivalent to 0.01g of pure INDm was dissolved in 20 mL of ethanol [34]. The solution was then transferred to 100 mL calibrated flask and the volume was completed to the mark with Dw. Subsequently, both recommended procedures A and B were then followed for the estimation of INDm by taking an aliquot of diluted solutions of the drug.

2.4.2. INDm suppositories solution (100 µg/mL)

Five of suppositories, each containing INDm, were weighed, melted, and thoroughly mixed. An equivalent weight to 0.01 g of pure INDm was dissolved in ethanol. The solution was filtered three times using Whatman 42 filter paper. The filtrate solution was then transferred to a 100-mL calibrated flask and the final volume was brought to the mark with Dw. An aliquot of the filtrate solution was then used for analysis of INDm by designated procedures in methods A and B [34].

2.4.3. INDm spray solution (100 µg/mL)

Three containers of Elmetacin spray, each containing 8 mg of INDm per mL, were thoroughly mixed. A volume of 1.25 mL of the spray solution was pipetted into a 100-mL calibrated flask containing 20 mL of ethanol. The solution was shaken and completed to the mark with Dw. A suitable volume of the final solution of the spray was then analysed using the procedures designated in methods A and B.

2.5. Essential Procedure for assaying INDm in the spiked biological fluids

A 5 mL of acetonitrile was added to a 1mL of one serum sample obtained from healthy individuals and mixed well. The sample solution was then introduced in a centrifuge for 5 minutes at 2500 rpm. The supernatant was used for investigating the INDm recovery %. For urine samples, a 1 mL of one spiked urine sample was 50-fold diluted with Dw [35-36].
An appropriate amount of the standard INDm solution (100 µg/mL) were added to (0.5 and 3 mL) and (1 and 3 mL) of the two treated urine and serum samples, respectively. The amount of INDm was then determined following the two proposed procedures A and B.

3. Results and Discussion

3.1. Optimization of the reaction conditions

All various parameters that influence the sensitivity of the colour product development were evaluated using 4 µg/mL of INDm in a final volume 25 mL in both methods A and B.

3.1.1. Effect of dye amount

The effect of different quantities 0.1-2.0 mL of 2.5×10^{-3} M Idc in method A and 2×10^{-3} M in method B on absorbance in presence of acidic medium of 0.5 mL (method A) and 1.0 mL (method B) of 2M hydrochloric acid solutions was investigated. The solutions were shaken and diluted to the mark with Dw. The absorbance was then measured at the selected wavelengths 610 and 582 nm against their reagent blanks foe method A and B, respectively. The results in Figure 2 show that 1.7 mL of Idc and1.5 mL of MV2B solutions are the optimum volumes for the reaction because they gave the best linearity with an excellent coefficient (R^2=0.9994) (method A) and (R^2=0.9995) (method B). Based on these results, volumes of 1.7 mL and 1.5 mL were selected for use in subsequent experiments

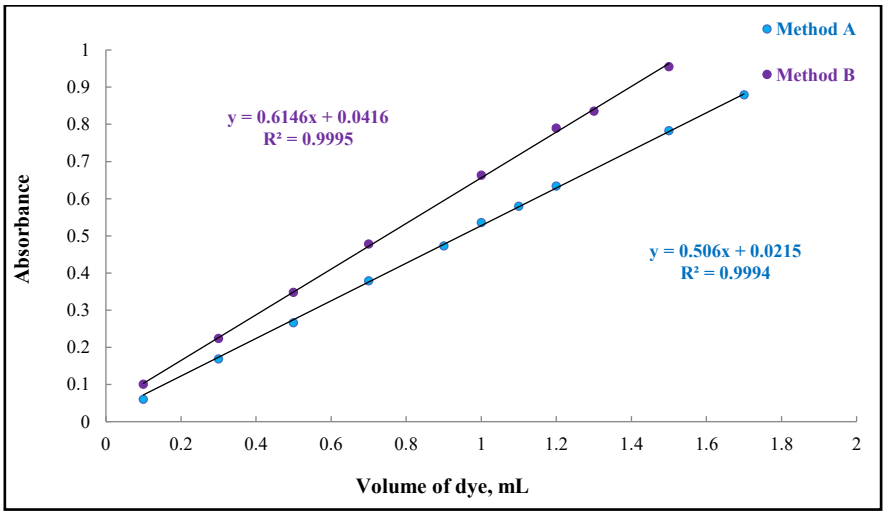


Figure 2: The optimum amounts of Idc (2.5×10^{-3} M) and MV2B (2×10^{-3} M) dyes for the reaction

3.1.1. Influence of $KBrO_3$ - KBr mixture solution

In acidic medium, the influence of diverse quantities 0.5-4.0 mL of $KBrO_3$ - KBr mixture solution in presence of 1.7 and 1.5 mL of Idc and MV2B dye solutions on the absorbance was studied in method A and method B, respectively. The solutions were mixed well, diluted to the mark with Dw and their absorbance were measured at 610 (method A) and 582 nm (method B) against the reagent blank solution. The experimental results presented in Figure 3 demonstrate that the volumes of 2.0 and 3.0 mL of $KBrO_3$ - KBr mixture solution are sufficient to obtain the optimal bleaching for both dyes in methods A and B, respectively.

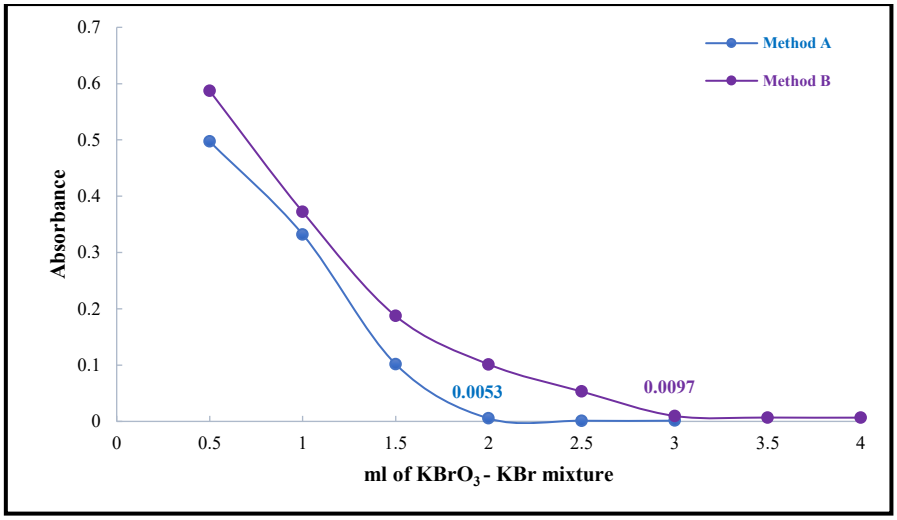


Figure 3: Effect of the amount of $KBrO_3$ - KBr mixture on absorbance for methods A and B

3.1.2. Effect of acid type and its concentration

The influence of various strong and weak acids (HCl, H_2SO_4, HNO_3, H_3PO_4, and CH_3COOH) (2M) on the absorbance of the remaining Idc and MV2B dyes was examined in both methods A and B. The data in Figure 4 indicate that the HCl solution exhibits the highest sensitivity compared to the other acids, likely attributed to pH-dependent effects. Therefore, 2M of HCl solution was selected for the subsequent experiments in both methods.

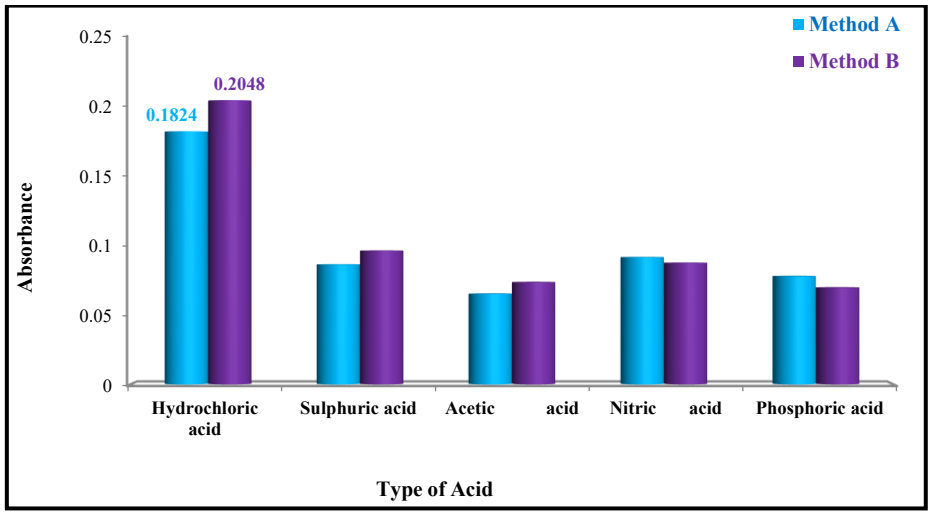


Figure 4: Effect of acid type on absorbance in both methods A and B

The effect of 0.5 (method A) and 1.0 mL (method B) of different concentrations 0.5 - 6.0 M of HCl solutions was also investigated. The results in Figure 5 illustrate that the concentrations of 1M HCl and 5M of HCl were the optimal in the method A and method B, respectively, due to the highest absorbance values, therefore 0.5 mL of 1M and 1.0 mL of 5M concentrations of HCl was selected for the next experiments of A and B methods.

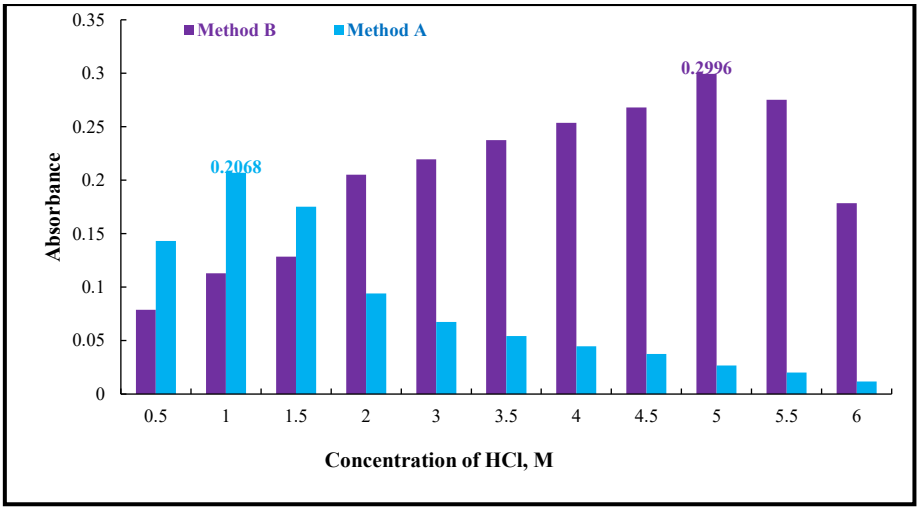


Figure 5: Effect of hydrochloric acid concentrations on absorbance for methods A and B

The influence of several quantities from 0.3 to 2.0 mL of 1M HCl (method A) and 5M HCl (method B) on absorbance was also investigated. The results in Figure 6 indicate that the volumes 1.0 mL of HCl (1M) and 0.7 mL of HCl (5M) were selected as the optimum amounts for both methods A and B, respectively.

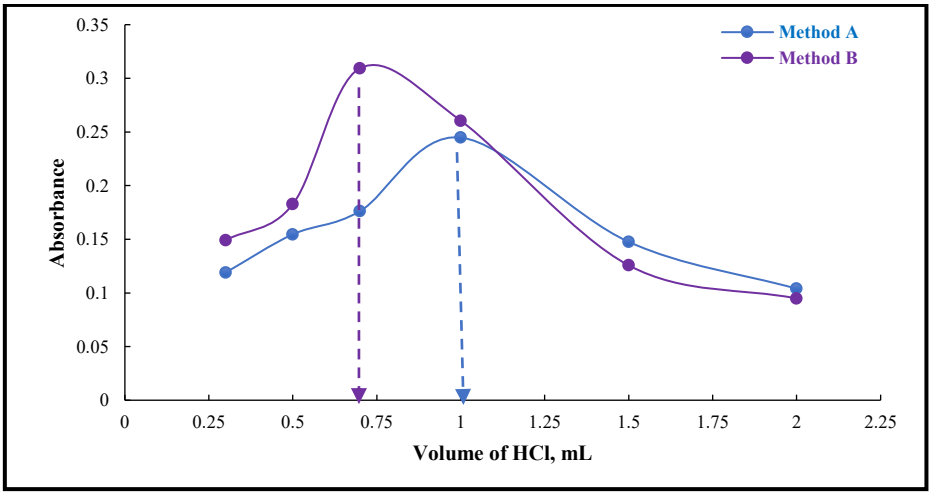


Figure 6: Influence of HCl amounts on absorbance in the methods A and B

3.1.3. Reaction time effect of INDm bromination

The impact of varying time periods from 5-30 minutes on the bromination of INDm in acidic medium for both methods was examined. The results, summarized in Figure 7 indicated that the reaction of INDm with liberated bromine needs at least 10 minutes (in method A) and 20 minutes (in method B) to complete.

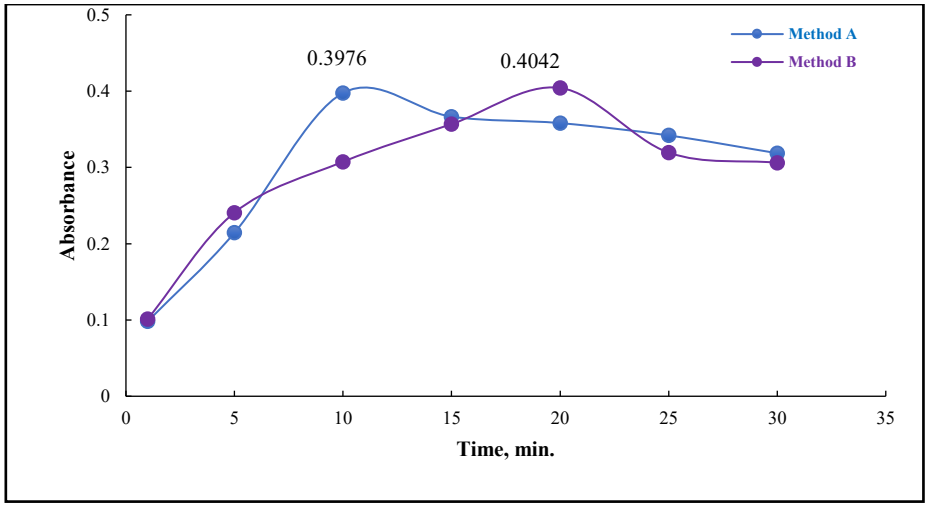


Figure 7: Effect of time of INDm bromination on absorbance in the methods A and B

3.1.4. Bleaching time effect

It is necessary to estimate the time that is required for the bleaching of Idc and MV2B dyes by the unreacted bromine reagent in the methods A and B, correspondingly. The results which are listed in Table 1. indicated that the bleaching time of Idc requires 3 minutes (in method A), whereas, the bleaching time of MV2B dye requires 5-10 minutes. Therefore, 3 and 5 minutes were chosen in the subsequent experiments as bleaching time for Idc and MV2B dyes in both methods A and B, respectively.

Table 1: Effect of bleaching time of Idc and MV2B dyes on absorbance in method A and B.

Method	Absorbance/ time needed for bleaching dyes				
	Immediately	**3**	**5**	**10**	**15**
A	0.3971	**0.4363**	0.4092	0.4011	0.3805
B	0.4073	0.4622	**0.5084**	0.5020	0.4719

3.1.5. Effect of temperature

The effect of different temperatures 25, 40, 50 and 60 °C (25°C = room temperature) on the absorbance by using a water bath with thermostatic control was also carried out for both methods A and B. The experimental results reveal that the bromination of INDm and the bleaching of Idc and MV2B were optimum at room temperature for both methods A and B.

3.1.6. Effect of sequence additions

The influence of different sequence additions of the reactants on absorbance of the remaining Idc and MV2B dyes was examined. The experimental results revealed that the ideal sequence of additions is in the following arrangement [INDm + HCl + ($KBrO_3$ – KBr) mixture + dye] for both methods A and B.

3.1.7. Time effect on the colour stability of the dye

The effect of time on the color stability of the remaining Idc and MV2B dyes after bleaching them with the excess of bromine in the both methods A and B was investigated. The results in Figure 8 show that the color of the bleaching Idc and MV2B dyes stays stable for about 2.5 hours at room temperature and there is no noticeable change in color and absorbance during this period.

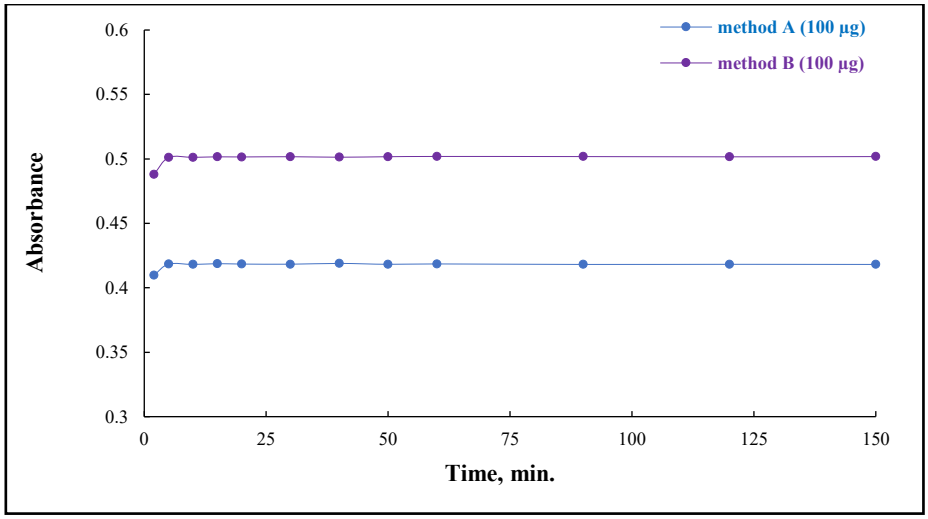


Figure 8: Effect of time on the color stability of the remaining dyes in both methods A and B.

3.1.8. Summary of optimal conditions and spectral characteristics

Under the optimal reaction conditions which were established for assaying INDm in methods A and B and confirmed in Table 2. The final absorption spectra were recorded by adding an excess of $KBrO_3$ - KBr mixture solution to 25 mL calibrated flasks containing 4 and 8 µg/mL of standard INDm in an acidic medium of HCl solution followed by the addition of Idc (method A) or MV2B (method B). The resulting bleaching dyes of Idc and MV2B exhibited maximum absorption peaks at 610 and 582 nm, respectively against their blank solutions. The final absorption spectra for the estimation of INDm in both methods are presented in in Figures 9 and 10.

Table 2: Summary of the optimum conditions for assaying INDm in methods A and B

Parameter	Results	
	Method A	**Method B**
$KBrO_3$ - KBr mixture solution (200µg/mL)	2 mL	3 mL
Volume and concentration of HCl	1 mL of 1M	0.7 mL of 5M
Type of dye	Idc	MV2b
Volume and concentration of the dye	1.7 mL of 2.5×10^{-3} M	1.5 mL of 2.0×10^{-3} M
Temperature, ºC	RT	RT
Standing bromination time of INDm, min.	10	20
Standing bleaching time, min.	3	5
$\lambda_{max,}$ nm	610	582
Stability, min.	150	150

Therefore, the final absorption spectrum formed for methods A and B is represented by Figures 10 and 11 under the optimum conditions determined previously.

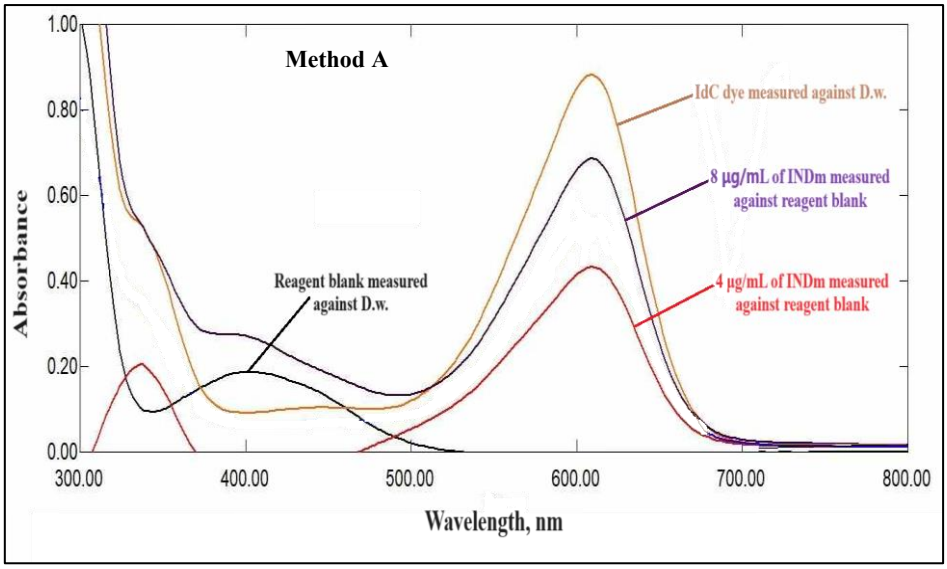


Figure 9: Final absorption spectra for the determination of INDm by using Idc dye according to methods A

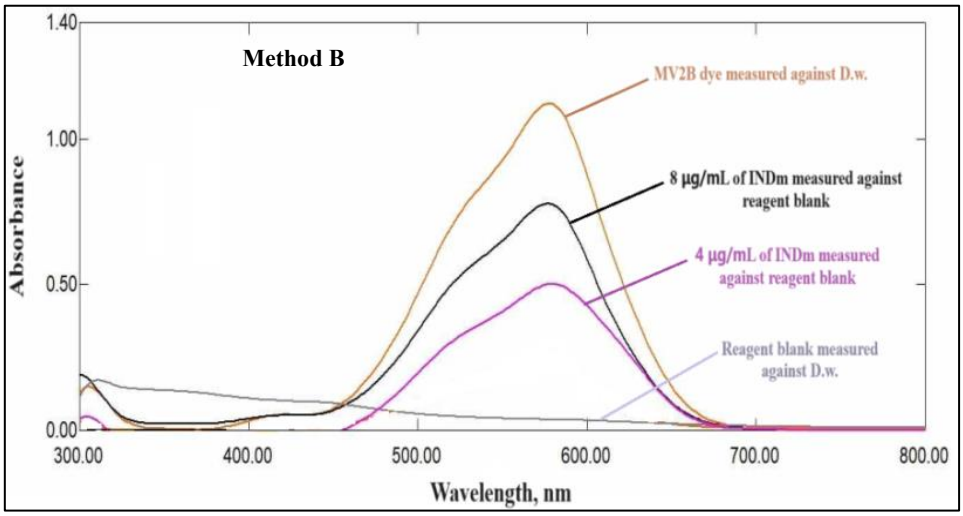


Figure 10: Final absorption spectra for the determination of INDm by using MV2B dye according to method B

3.1.9. Reproducibility and Validity of Beer's law

By applying the optimal of the two recommended methods at [λ_{max}= 610 nm in method A] and [λ_{max} =582 nm in method B], a linear calibration curves (Figure 11) were constructed and followed Beer's law within the concentration ranges 1-22 and 0.4-18 µg/mL of INDm with determination coefficients (R^2) 0.9996 and 0.9995 and molar absorptivity values 2.51×10^4 and 3.37×10^4 l/mol.cm for methods A and B, correspondingly. The LOD and LOQ values were evaluated and found to be 0.0205 and 0.0683 µg/mL (in method A), and 0.0028 and 0.0093 µg/mL (in method B), respectively. Additionally, accuracy, precision (RSD%) and Sandal's sensitivity for both procedures A and B were evaluated and validated. The results are summarized in Table 3.

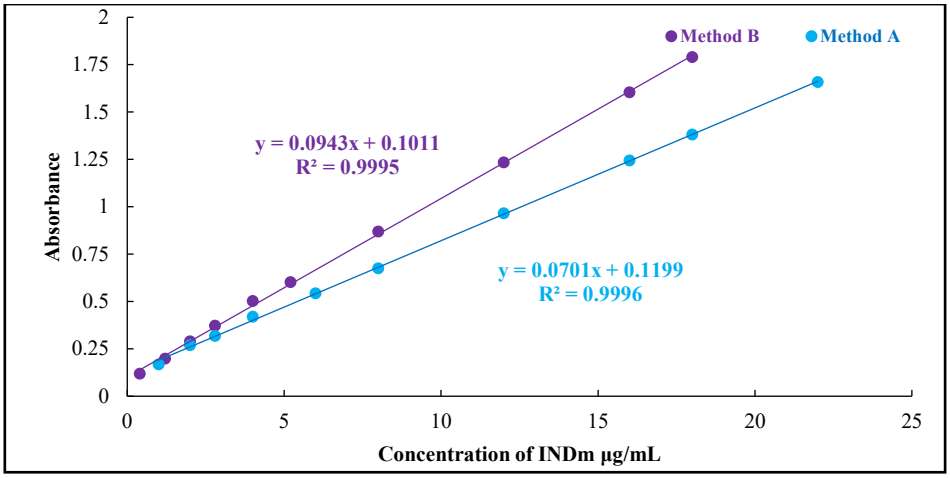


Figure 11: Standard calibration curves for estimating INDm according to the proposed methods A and B

Table 3: Analytical data of the development methods A and B

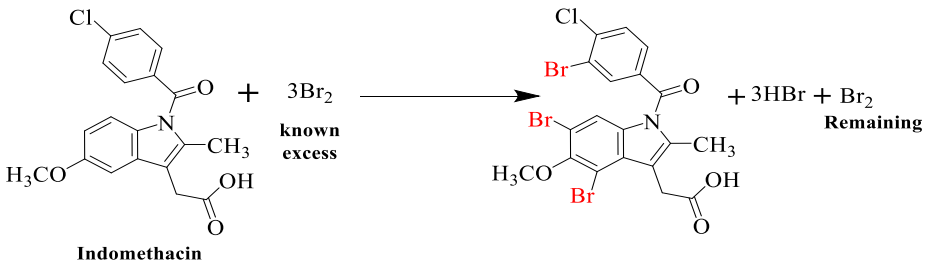
Parameters	Analytical values	
	Method A	Method B
Linearity range, µg /mL	1-22	0.4-18
Molar absorptivity, l/mol.cm	2.51×10^4	3.37×10^4
Sandell's Sensitivity, µg/cm^2	0.0142	0.0106
LOD, µg /mL	0.0205	0.0028
LOQ, µg /mL	0.0683	0.0093
Intercept	0.1199	0.1011
Slope	0.0701	0.0943
Determination coefficient (R^2)	0.9996	0.9995

3.1.10. Reaction mechanism

Based on the principle of the two recommended procedures, the suggested reaction mechanism of the reactants includes three steps [37]. The first step involves generating the bromine site using a $KBrO_3$-KBr mixture solution in an acidic medium as in the following equation:

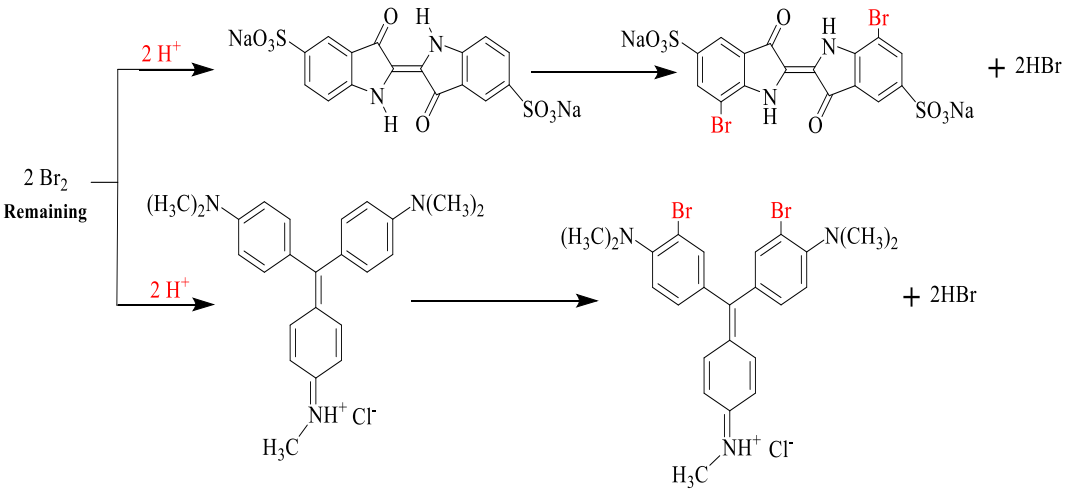
$$BrO_3^- + 5Br^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$

The second step involves the bromination of INDm drug with the generated bromine agent (scheme 1) [38]:



Scheme 1: Bromination of INDm

In step three, the residual amount of bromine reagent reacts with Idc in method A, and with MV2B dyes in method B, as illustrated in Scheme (2) [38]:



Scheme 2: Bleaching reactions of Idc and MV2B reagents with Bromine

3.1.11. Effect of excipients

The impact of various pharmaceutical additives was studied to confirm the selectivity and the applicability of the two proposed methods A and B for determining INDm. The results presented in Table 4 demonstrate that these substances have no significant effect on the recoveries percentage of INDm.

Table 4: Effect of excipients on INDm recovery percentage for methods A and B

Additive	Reco. (%) of 100 µg of INDm / µg of additive added					
	Method A			Method B		
	100	500	1000	100	500	1000
Glucose	97.94	98.12	98.34	97.47	97.88	98.34
Fructose	97.75	98.42	101.03	96.59	98.11	100.03
Sucrose	98.29	98.78	97.85	98.05	98.71	98.46
Isopropyl alcohol	96.62	97.49	97.22	97.22	97.16	96.58
Gelatine	96.57	97.83	96.46	97.60	97.85	98.41
Gum	98.33	97.81	96.24	98.27	98.39	97.62
Starch	99.34	99.18	99.85	99.41	99.38	99.15

3.1.12. Application

The applicability of the two suggested methods A and B have been applied for assaying INDm in the available commercially pharmaceuticals (capsules, suppositories, and spray) at three different quantities 50, 100, and 200 µg of INDm and in the biological fluids (serum and urine), for two different quantities 100 and 200 µg of INDm. The results are summarized in Tables 5 and 6 indicated that the two proposed approaches A and B succeed for assaying INDm in the pharmaceuticals and biological fluids with an acceptable result.

Table 5: Determination of INDm in the pharmaceutical preparations by using methods A and B

Pharmaceutical Preparation	Certified Value	Method A			Method B		
		INDm found, µg	Reco. (%) ±RSD (N=5)	Measured value	INDm found, µg	Reco. (%) ±RSD (N=5)	Measured value, mg
Indylon Medochemie Ltd. (Cyprus)	25 mg / Capsule	49.95	99.90±0.072	24.98	49.49	98.98±0.191	24.75
		99.93	99.93±0.085	24.98	100.03	100.03±0.396	25.01
		199.66	99.83±0.063	24.96	200.20	100.10±0.083	25.03
Indosam (SDI-Iraq)	25 mg / Capsule	49.86	99.72±0.126	24.93	49.79	99.58±0.136	24.89
		99.77	99.77±0.111	24.94	99.40	99.40±0.425	24.85
		199.40	99.70±0.186	24.92	197.54	98.77±0.204	24.69
Indomethacine Medico labs Homs (Syria)	100 mg/ Suppositories	48.21	96.42±1.677	96.41	48.19	96.38±0.072	96.38
		97.97	97.97±0.777	97.97	97.11	97.11±1.270	97.11
		196.66	98.33±0.255	98.33	196.86	98.43±0.479	98.43
Elmetacin solution Daiichi-Sankyo (Germany)	8mg/1mL Spray	49.83	99.66±0.134	7.97	49.41	98.82±0.682	7.91
		99.79	99.79±0.088	7.98	99.26	99.26±0.531	7.94
		199.46	99.73±0.036	7.98	199.42	99.71±0.545	7.98

Table 6: Determination of INDm in biological fluids by using methods A and B

Type of sample	Sample, mL	Method A		Method B	
		INDm found, µg	Reco. (%) ±RSD (N=5)	INDm found, µg	Reco. (%) ±RSD (N=5)
Urine	0.5	96.34	96.34±0.073	98.24	98.24±0.873
		196.38	98.19±0.127	192.52	96.26±0.405
	3.0	101.14	101.14±2.091	97.83	97.83±0.887
		202.68	101.34±0.672	203.12	101.56±0.540
Serum	1.0	101.08	101.08±0.958	102.70	102.70±0.958
		201.82	100.91±0.256	201.86	100.93±0.256
	3.0	101.48	101.48±0.684	101.35	101.35±0.596
		202.08	101.04±0.710	201.46	100.73±0.341

3.1.13. Evaluation of the proposed methods

Statistical comparison for evaluation the results for the two proposed approaches A and B to estimate INDm in the pharmaceutical formulations and biological fluids was studied using Student's t-test and F-test at 95% confidence level as shown in Table 7 which indicate that there was no significant difference between the proposed methods and the reference method [30] depending on tabulated values (t=2.306) and (F= 6.39) at the 95% confidence level [39].

Table 7: The calculated results of student's t-test and F-test for the two methods A and B

Method	Sample	INDm Found (µg)*		Reco.* % ± RSD* %		Value of	
		Present method	Literature# method	Present method	Literature# method	t[a]	F[a]
A	Indosam (SDI-Iraq)	98.09	97.67	98.09±0.30	97.67±0.73	1.21	0.17
	Indomethacine Medico labs Homs (Syria)	96.42	96.08	96.42±0.267	96.08±0.438	1.53	0.37
	Elmetacin solution Daiichi-Sankyo (Germany)	98.13	97.74	98.13±0.359	97.74±0.57	1.34	0.40
B	Indosam (SDI-Iraq)	97.80	97.06	97.80±0.45	97.06±0.84	1.78	0.285
	Indomethacine Medico l abs Homs (Syria)	100.32	99.40	100.32±0.36	99.40±0.85	1.91	1.02
	Elmetacin solution Daiichi-Sankyo (Germany)	98.33	97.78	98.33±0.37	97.78±0.72	1.538	0.259

*Average of five estimations, [a] degree of freedom for t-value (N=8) and for F-value (N=4), # [30]

3.1.14. Analysis of INDm by Standard additions method

To verify the efficiency and credibility of the two proposed methods A and B for estimating INDm in its pharmaceutical formulations and to ensure that they were free from the interference of additives, a standard additions method was used [39]. The experimental results shown in Figures 12 and 13, along with those in Table 8 demonstrate a strong correlation between the results of the standard additions method and the results of the two proposed methods [40] for assaying INDm in its medicine (capsule, suppository and spray).

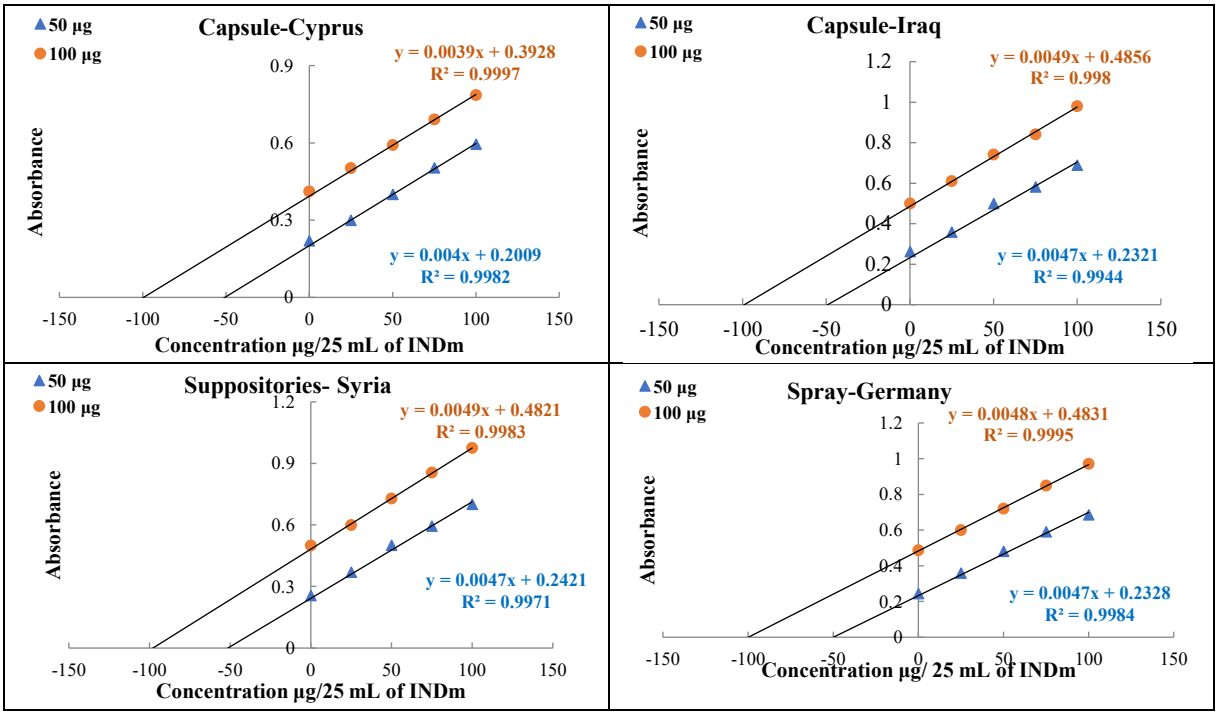


Figure 12: Plots of the standard addition's method for the estimation of INDm in various drugs (method A)

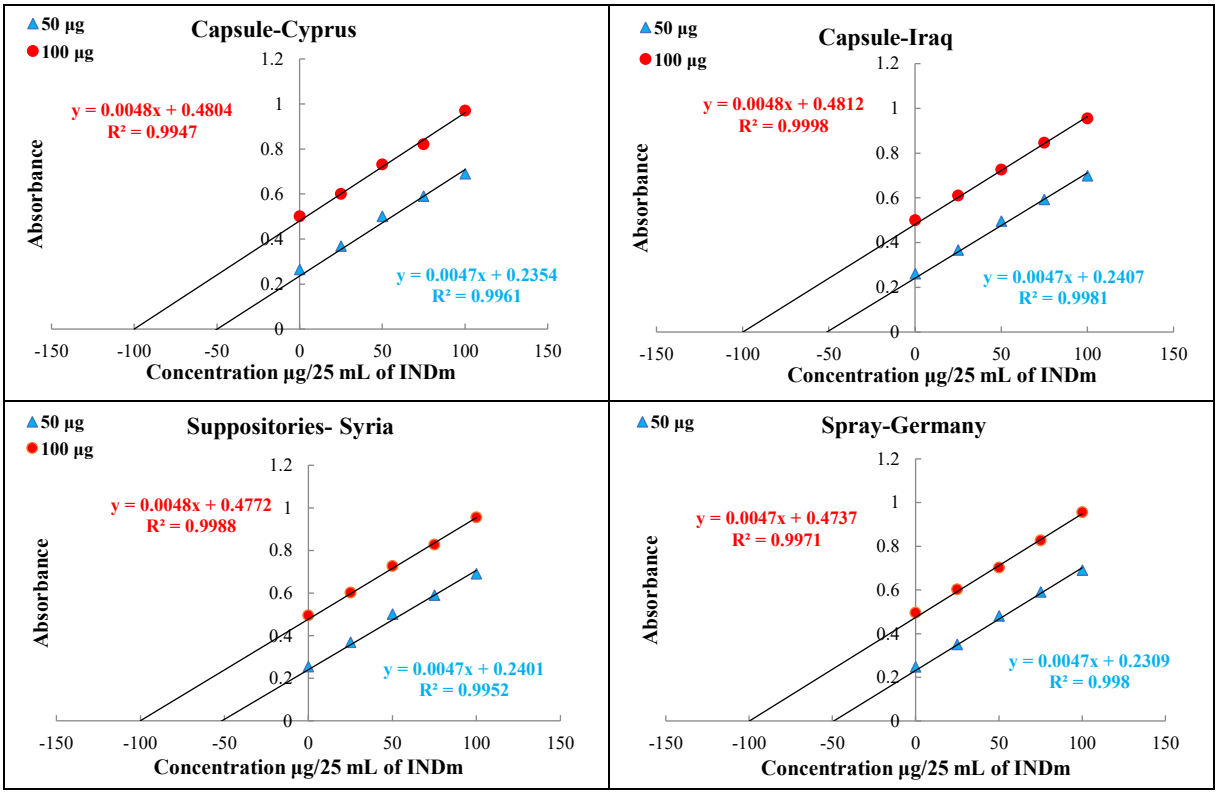


Figure 13: Plots of the standard addition's method for the estimation of INDm in various drugs (method B)

Table 8: The results of standard additions method for assaying INDm in the pharmaceutical formulations

Method	Drug	Certified value	INDm (µg)		Reco. (%)	Measured value, mg	
			Present	Found		Standard addition	Proposed method
A	Indosam (SDI-Iraq)	25 mg/Capsule	50	49.38	98.76	24.69	24.93
			100	99.10	99.10	24.78	24.94
	Indylon Medochemie Ltd.-(Cyprus)	25 mg/Capsule	50	50.23	100.46	25.12	24.98
			100	100.71	100.71	25.18	24.98
	Indomethacine Medico labs Homs-(Syria)	100 mg/ Suppositories	50	51.51	103.02	103.02	96.41
			100	98.39	98.39	98.39	97.97
	Elmetacin solution Daiichi-Sankyo-(Germany)	8mg/1mL Spray	50	49.53	99.06	7.92	7.97
			100	100.65	100.65	8.05	7.98
B	Indosam (SDI-Iraq)	25 mg/Capsule	50	51.21	102.42	25.61	24.89
			100	100.25	100.25	25.06	24.85
	Indylon Medochemie Ltd.-(Cyprus)	25 mg/Capsule	50	50.09	100.18	25.05	24.75
			100	100.08	100.08	25.02	25.01
	Indomethacine Medico labs Homs-(Syria)	100 mg/ Suppositories	50	51.09	102.18	102.18	96.37
			100	99.42	99.42	99.42	97.11
	Elmetacin solution Daiichi-Sankyo-(Germany)	8mg/1mL Spray	50	49.13	98.26	7.86	7.55
			100	100.79	100.79	8.06	7.94

Conclusions

The study included the development of two indirect UV-Vis spectrophotometric methods A and B characterized by simplicity, selectivity and high sensitivity for the determination of INDm in the bulk, pharmaceutical forms and in the biological fluids. Both methods were based on the reaction of INDm in an acidic medium of hydrochloric acid solution with bromine which was generated in situ through environmentally friendly materials $KBrO_3$-KBr mixture. The excess of bromine was then reacted with indigo carmine dye (in method A) and methyl violet 2B dye (in method B). The absorbance of the both residual dyes was measured at wavelengths of 610 and 582 nm in the methods A and B, respectively. Both recommended methods A and B obey Beer's law within the concentration ranges 1-22 and 0.4-18 µg/mL, with molar absorptivity values 2.51 $\times 10^4$ and 3.37×10^4 l/mol.cm, respectively. The two proposed methods, A and B, offer several advantages, including high accuracy, low cost, and sufficient precision, making them suitable alternatives to current spectrophotometric methods. Additionally, they have the benefits of not requiring pre-extraction or temperature control, simplifying the analysis process. The both methods A and B have been successfully applied for the estimation of INDm in the pharmaceutical forms (capsules, suppositories and spray) and in the biological fluids (serum and urine).

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