Spectroscopic study on Indacaterol maleate: Analytical applications for quality control of capsules

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Three simple and sensitive spectrophotometric methods (A, B and C) have been developed for the quantitative estimation of Indacaterol maleate (IND) in bulk drug and capsules. Method A is based on the measurement of the difference absorption spectra of IND in acidic and basic media. The difference spectrum exhibits maxima and minima at 274.6 and 257.8 nm, respectively. Method B is based on the oxidative coupling reaction with an acidic solution of the chromogenic agent 3-methylbenzothiazoline-2-one hydrazone (MBTH) and the drug upon treatment with ceric ammonium sulfate (CAS) produces an orange color peaking at 545 nm. Method C is based on the reaction with 4-aminoantipyrine (4-AAP) in presence of alkaline oxidizing agent; potassium hexacyano ferrate and diluted ammonia (K_3 [Fe (CN) $_6$] / NH_3) and measuring the produced red color at 510 nm. Beer's law is obeyed in the concentration range of 1.0-16.0 µg mL^{-1}, 2.0 – 20.0 µg mL^{-1} and 3.0 – 30.0 µg mL^{-1} for Methods A, B and C, respectively. The proposed methods were validated according to ICH guidelines and they were successfully applied for the determination of IND in its capsule dosage forms without interference from excipients.

Keywords: Indacaterol Maleate (IND), Spectrophotometric, difference Spectrophotometry, 3-methylbenzothiazoline-2-one hydrazone (MBTH), 4-aminoantipyrine (4-AAP), pure form, Capsules.

Introduction:

Indacaterol (IND) is chemically named as 5-{(1R)-2-[(5,6-diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl}-8-hydroxy-2(1H)-quinolinone maleate[1] (Fig. 1). It is a new, long-acting β_2-agonist bronchodilator used for maintenance treatment of airflow problems in patients with chronic obstructive pulmonary disease[2] (COPD). Two HPLC/MS method were developed for its determination in pure form and capsule dosage forms[3,4]. To the best of our knowledge no spectrophotometric methods have been reported up till now for determination of IND either in pure form or in capsules. This prompted us to develop new simple, sensitive and rapid spectrophotometric methods for the assay of IND in pure form and capsules. The first method is based on measurement of difference absorbance of IND in 0.1N NaOH solution and 0.1 N HCl where the drug undergoes bathochromic, hyperchromic shifts exhibiting a maximum absorbance at 274.6 nm in basic medium and hypsochromic, hyporchromic shifts exhibiting a maximum absorbance at 257.8 nm. The second and third methods are based on the oxidation followed by coupling reaction of Indacaterol maleate with MBTH in presence of ceric ammonium sulphate and 4-AAP in the presence of alkaline oxidizing agent for methods B and C, respectively. The proposed spectrophotometric methods have the advantages of being simple, rapid, not expensive and convenient suggesting the applicability in quality control laboratories.

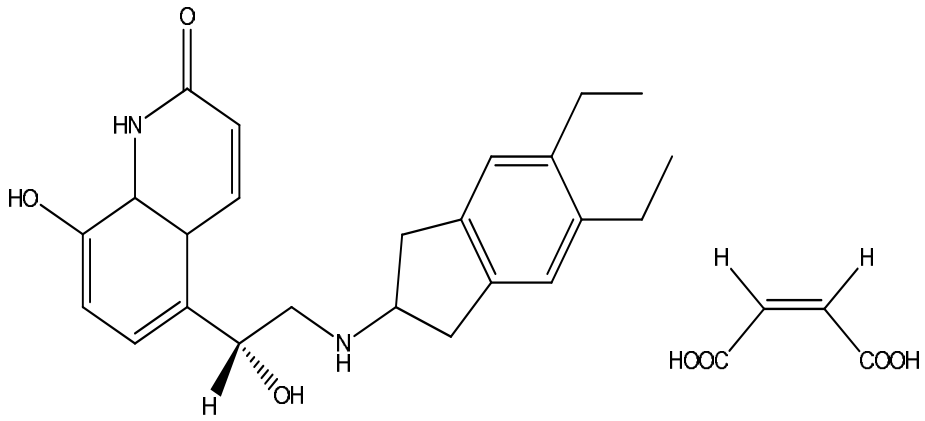


Fig. (1): Chemical structure of Indacaterol maleate (IND)

Experimental

Apparatus

- Spectrophotometric analyses were carried out on a Shimadzu (Kyoto, Japan) UV-1601 PC, UV-Visible double-beam spectrophotometer with matched 1 cm path-length quartz cells (method A)

-Spectrophotometric analyses were carried out on JENWAY UV/ Visible Scanning Spectrophotometers 6315a United Kingdom with matched 1 cm path-length quartz cells. Absorption spectrum of the reaction product was recorded on a fast scan speed between 400-710 nm setting slit width to be 1 nm and sampling interval to be auto (method B and C).

Materials and pharmaceutical formulations

1-Indacaterol maleate (IND) was kindly provided by Wuhan Vanz Pharm Inc. High Technology Industry Park, Wuhan Economic & Technology Development Zone, Wuhan, 430056, China (Purity 99 %.).

2-Onbrez® capsules contain 150 µg Indacaterol Maleate (IND), manufactured by Novartis Pharma Inc., East Hanover, USA. Batch no. 143984 were obtained from commercial source.

Chemicals and reagents

All chemicals were of analytical reagents grade and double distilled water was used throughout the study.

-Acetonitrile, methanol, ethanol and sulphuric acid (98%) all of HPLC grades (Sigma, St. Louis, MO, USA).

- Additive substances including surfactants like cetrimide, Brij, cyclodextrin, sodium dioctylsulfosuccinate (SDOSS) and sodium dodecyl sulphate (SDS) were obtained from Sigma, St. Louis, MO, USA.

-Sodium hydroxide and hydrochloric acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were prepared as 0.1 N aqueous solutions.

- 3-Methylbenzothiazoline-2-one hydrazone (MBTH) (Sigma, St. Louis, MO, USA) 0.3% w/v acidic solution was prepared in 0.2 M HCl (Merck, Darmstadt, Germany); this solution should be freshly prepared.

- Ceric ammonium sulphate (CAS) (Merck, Darmstadt, Germany) 0.5% w/v acidic solution was prepared in 5% H_2SO_4 solution.

- 4-Aminoantipyrine (4-AAP) (Sigma-Aldrich Co Ltd., UK) 0.2% w/v aqueous solution.

- Ammonium hydroxide 25% (El-Nasr Pharmaceutical Chemicals (ADWIC), Egypt) was used as 0.1 M aqueous solution and potassium hexacyano ferrate (HCF) (Winlab, UK) 0.8% w/v aqueous solution.

Preparation of stock and standard solutions

A stock solution (100 µg mL^{-1}) was prepared by dissolving 10.0 mg of IND in 100 mL methanol. Serial dilutions with the same solvent were prepared to obtain the appropriate concentration range. The standard solutions were stable for at least two weeks when kept in the refrigerator.

General procedures

Construction of the Calibration Curves: (Method A)

Accurately measured aliquots equivalent to 1.0-16.0 µg IND was quantitatively transferred from the stock solution into two separate sets of 10 mL volumetric flasks. The set of the drug consists of two series. The first series of flasks were made up to the volume with 0.1 N NaOH and the absorption spectra were recorded against blank solution of 0.1 N NaOH at 274.6 nm. The flasks of the second series were made up to the volume with 0.1 N HCl and the absorption spectra were recorded against blank solution of 0.1 N HCl at 257.8 nm. The absorbance difference (δ A) between acidic solution and equimolar basic solution was measured by subtracting the spectra of the second series (in 0.1 N HCl) from the spectra of the first one (in 0.1 N NaOH) for each concentration of IND drug and the absorbance was taken immediately. The (δA) values of the difference absorption spectra were plotted vs. concentration of IND (µg mL^{-1}) to get the calibration graphs. Alternatively, the corresponding regression equation was derived.

Construction of the Calibration Curves (Method B)

Accurately measured aliquots of the stock solution were transferred into a series of 10-mL volumetric flasks to obtain the final concentration is in the range of 2.0 – 20.0 µg mL^{-1}. 1 mL of MBTH solution and 2.5 mL of ceric (IV) ammonium sulphate were added to each flask. The reaction mixture was mixed well and allowed to stand for 25 minutes at room temperature, then the flasks were made up to the volume with acetonitrile. The absorbance of the solution was measured at 545 nm against a reagent blank. Simultaneously, the calibration curve was constructed by plotting the values of the absorption spectra vs. concentration of IND (µg mL^{-1}). Alternatively, the corresponding regression equation was derived.

Construction of the Calibration Curves (Method C)

Aliquot volumes of IND standard solution were transferred into a series of 10- mL volumetric flasks to get final concentration within the range of 3.0- 30.0 µg mL^{-1}. Then 0.6 mL of ammonia solution, 2.0 mL of 4-

aminoantipyrine solution, and 1.0 mL of potassium hexacyano ferrate solution were added to each flask. The reaction mixture was mixed well and allowed to stand for 15 minutes at thermostatic waterbath adjusted to 70°C then diluted to volume with distilled water. The absorbance values at 510 nm were measured against a reagent blank and then plotted against the final concentration to get the calibration graph. Alternatively, the corresponding regression equation was derived.

Applications

Procedure for Capsules :(Method A, B, C)

The contents of ten capsules were weighed and mixed well. A weighed quantity of the powder equivalent to 1.5 mg of IND was transferred into a small conical flask and extracted three successive times each with 30 mL of methanol. The extracts were collected, filtered and transferred quantitively into a 100 mL volumetric flask and completed to the volume with the same solvent. Three different concentrations covering the working concentration range for each method were transferred into three sets of 10 mL volumetric flasks and the procedures for the calibration graph for each method were followed, the analysis was performed as described under **"Construction of calibration curves (Method A, B, C)"**. The nominal content of the capsules was determined using the corresponding regression equation.

Results and Discussion:

Method A

Difference spectrophotometry has proved particular usefulness in the determination of drugs by eliminating specific interference from degradation products and additives of the formulation matrix. The method is based on measurement of direct absorbance of IND using equimolar solutions of weak acid (0.1 N HCl) and weak base (0.1 N NaOH) (Fig. 2.a), respectively then measurement of the absorbance difference (δA) between two solutions. A marked bathochromic shift to longer wavelengths in alkaline solution is observed for most of the phenolic drugs[5]. IND showed two characteristic peaks at 257.8 and 274.6 nm with negative and positive absorbance, respectively (Fig. 2.a.).

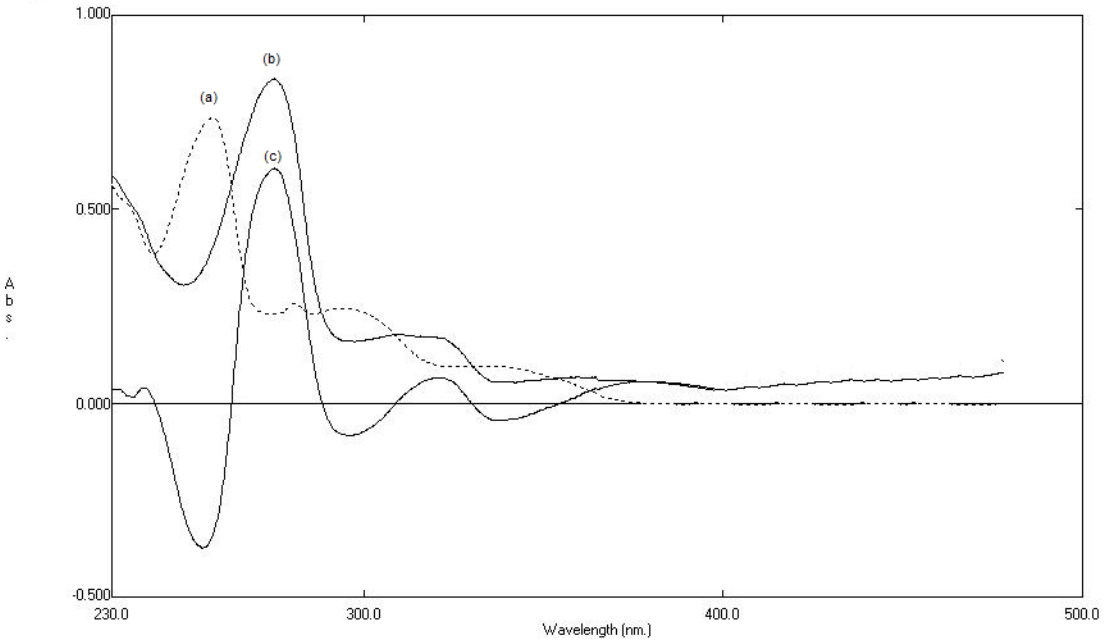


Fig. (2.a.): Absorption spectra of IND (12.0 μg mL^{-1}) in 0.1 N HCl, 0.1 N NaOH and difference absorption spectra of IND.

Method B:

This method is based on the oxidation followed by coupling reaction of Indacaterol maleate with MBTH in presence of ceric ammonium sulphate to produce an orange colored species peaking at 545 nm (Fig. 2.b). MBTH has been frequently utilized for the spectrophotometric determination of several pharmaceutical compounds fenofibrate[6], lipoic acid[7], tolterodine tartrate [8], pregapaline[9], isoxuprine hydrochloride [10], ethamsylate [11], propranolol [12] and sulphonamides[13].

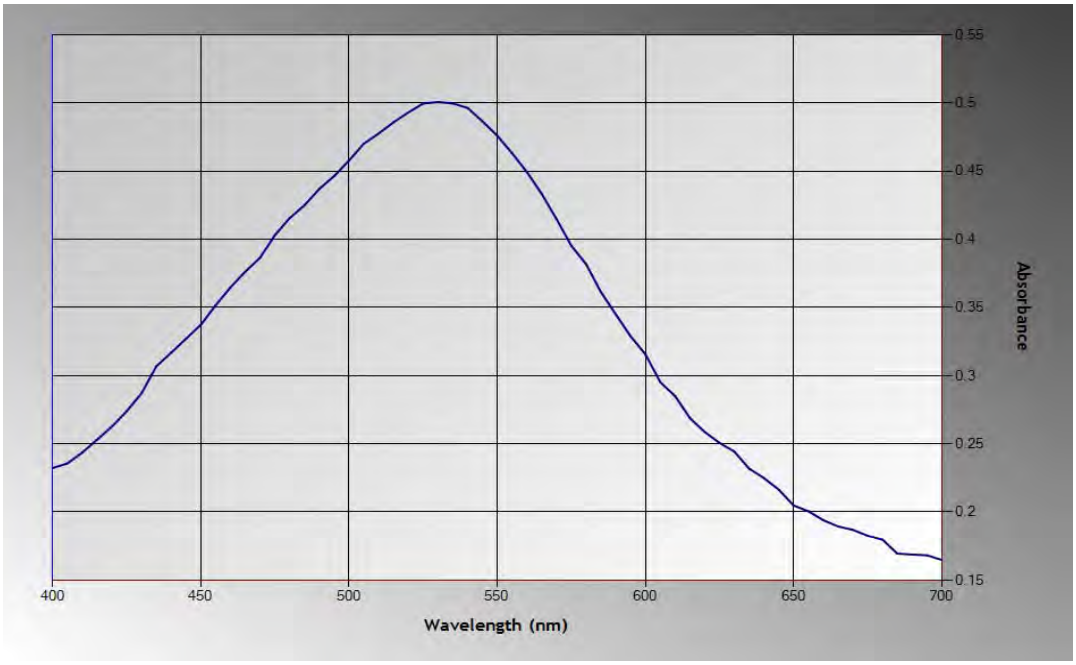


Fig. (2.b): Absorption spectrum of the reaction product of Indacaterol maleate (12.0 µg mL^{-1}) with MBTH-ceric ammonium sulphate system.

Method C:

The phenolic hydroxyl group of IND it can be oxidatively coupled with 4-aminoantipyrine in presence of alkaline oxidizing agent to produce an intensely colored stable product peaking at 510 nm. Typical absorption spectrum of the oxidative coupling reaction product is shown in Fig. 2.c.

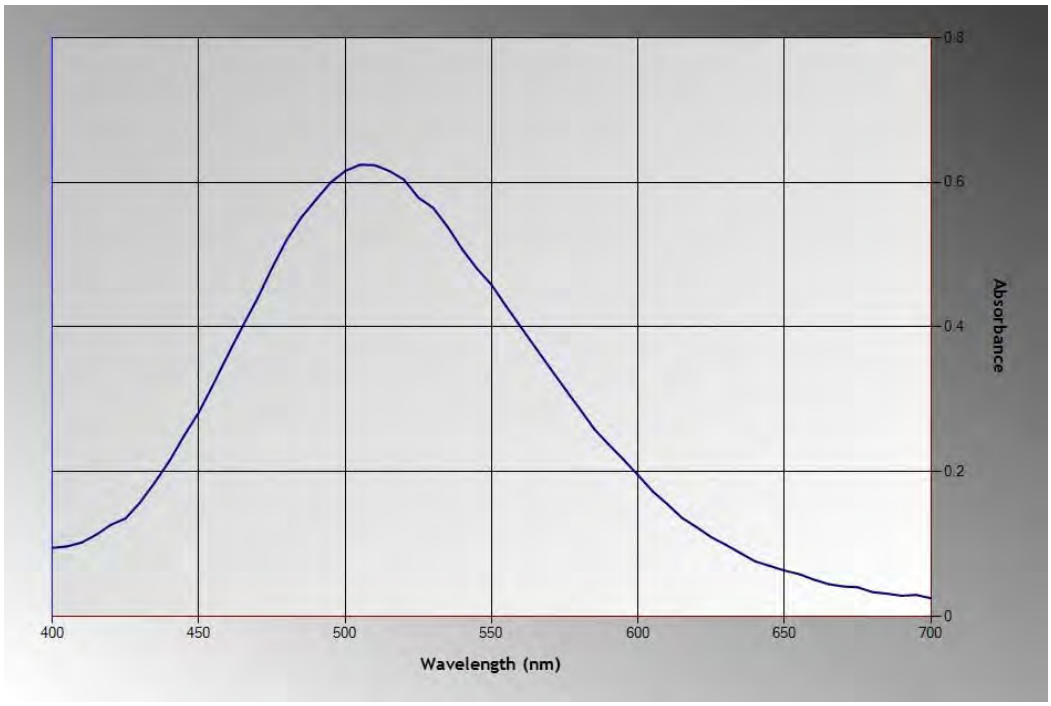


Fig. (2.C): Absorption spectrum of the reaction product of Indacaterol maleate (18.0 µg mL^{-1}) with 4-aminoantipyrene (4-AAP).

Optimization of experimental parameters

Method A

Effect of molar concentration of NaOH:

The influence of the concentration of NaOH on the absorption intensity was investigated over the range of 0.05 to 0.5 N. It was found that increasing in the concentration of NaOH produces a corresponding increase in the absorption value up to a concentration of 0.1N after which the absorbance remained constant. So that, 0.1 N was chosen as the optimum concentration of the NaOH in the present study.

Effect of time:

Study of the time effect on the difference absorbance of IND was performed in 0.1 N NaOH vs. 0.1 N HCl. It was found that the difference absorbance readings of IND are constant for at least two hours.

Method B

Effect of different diluting solvents:

Different diluting solvents including; water, dilute sulphuric acid, ethanol, methanol and acetonitrile were investigated. It was found that each of acetonitrile, methanol and ethanol can be used as diluting solvent but acetonitrile gave the maximum color intensity.

Effect of order of addition of the reagents:

The order of addition of the reagents is an essential part of the experiment, the highest absorbance reading produced in case of addition of ceric (IV) ammoniumsulphate after MBTH.

Effect of volume of MBTH solution:

Different volumes of MBTH 0.3% solution ranging from 0.5-2.0 mL were added to a fixed concentration of IND. It was found that 1.0 ± 0.25 mL was ideal to develop the maximum color intensity (Fig. 3.a). It was found that the maximum stability of the color produced using 0.2M HCl as a solvent for MBTH.

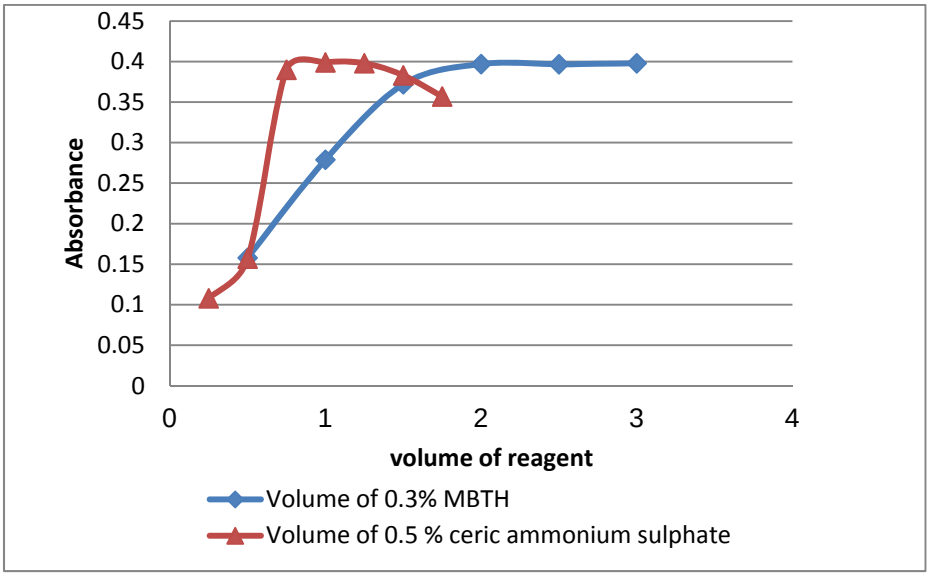


Fig.(3.a): Effect of volume of 0.3% MBTH (a) and 0.5% ceric (IV) ammonium Sulphate (b) on the development of the reaction product of Indacaterol maleate (9.0 µg mL^{-1}).

Effect of volume of ceric(IV) ammonium sulphate solution:

Different volumes of CAS 0.5% in 5% sulfuric acid solution ranging from 0.25-3.0 mL were added to a fixed concentration of IND. Fig. 3.a shows that 2.5 ± 0.25 mL gave the maximum color intensity.

Effect of time on the formation and stability of the formed product:

Different heating time ranging from 5- 60 min was investigated. Fig. 3.b reveals that a 25 ± 5.0 min development time was selected as the optimum reaction time to develop the maximum the color intensity. The formed chromophore was found to be stable for at least 2 h.

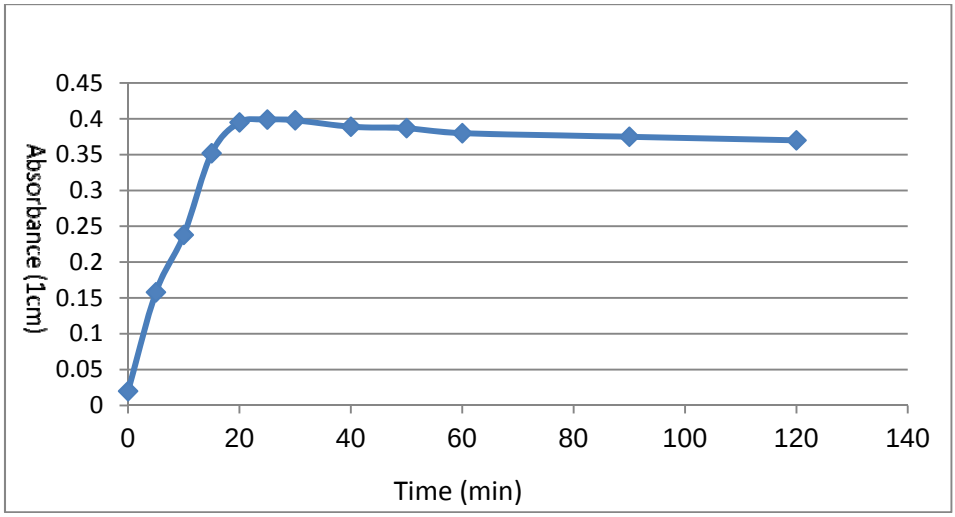


Fig.(3.b): Effect of time on the development of the reaction product of Indacaterol maleate (9 µg mL^{-1}) with MBTH.

(Method C)

Effect of volume of Ammonia solution:

The effect of different volumes of 0.1 M aqueous ammonia solution ranging from 0.2-1.4 mL was studied. As shown in (Fig. 4.a), the optimum volume was found to be 0.6 ± 0.2 mL Moreover, the study revealed that below 0.2 mL of ammonia solution, no reaction product was formed and above 0.8 mL the reaction showed a marked decrease in the absorbance.

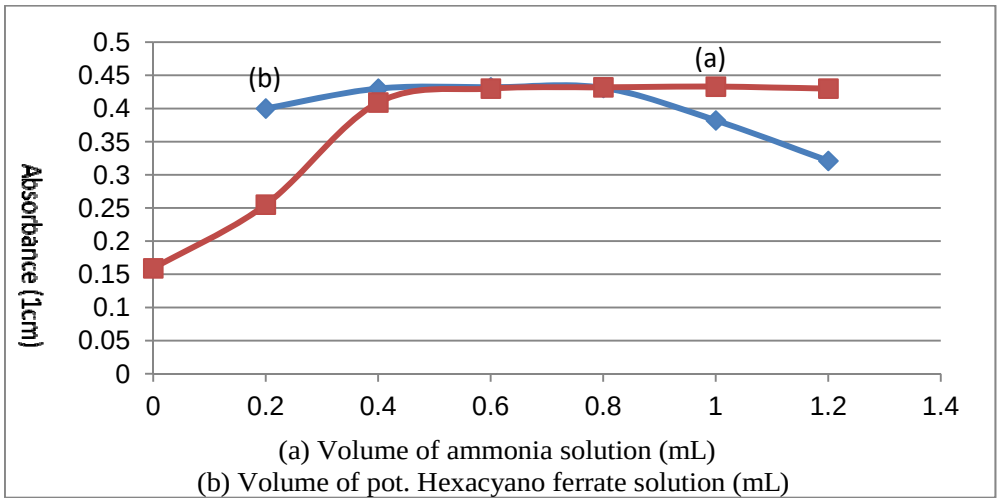


Fig.(4.a) Effect of volume of 0.1M ammonia solution, and 0.8% potassium hexacyano ferrate solution on the development of the reaction product of Indacaterol maleate (12.0 µg mL^{-1}).

Effect of volume of Hexacyano ferrate solution:

The effect of different volumes of 0.8% aqueous HCF ranging from 0.2-1.6 mL on the color intensity of the oxidative coupling reaction of IND with 4-aminoantipyrine is illustrated in (Fig. 4.a). The optimum volume was found to be 1.2 ± 0.2 mL

Effects of volume of 4-aminoantipyrine solution:

Different volumes of 0.2% aqueous 4-aminoantipyrine solution ranging from 0.5- 3.0 mL were attempted and the corresponding absorbance values were measured. It was found that the optimum volume was 2.0 ± 0.5 mL as shown in (Fig. 4.b).

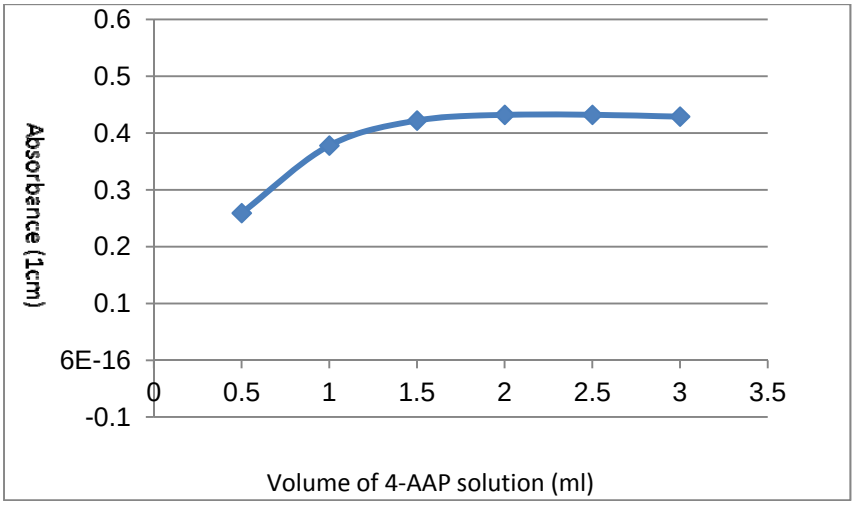


Fig.(4.b) Effect of volume of 4-AAP solution on the development of the reaction product of Indacaterol maleate (12.0 µg mL^{-1}).

Effect of temperature and time on the formation and stability of the formed product: The effect of heating on the reaction product was investigated by measuring the color intensity at different temp and heating time ranging from20 -90^{o} C and 5- 40 min, respectively in thermostatic adjusted waterbath. The maximum color intensity was obtained after heating at 70 ± 5.0^{o} C (Fig. 4.c) for 20 ± 5.0 min (Fig. 4.d).The formed product remained stable for more than 2 hours.

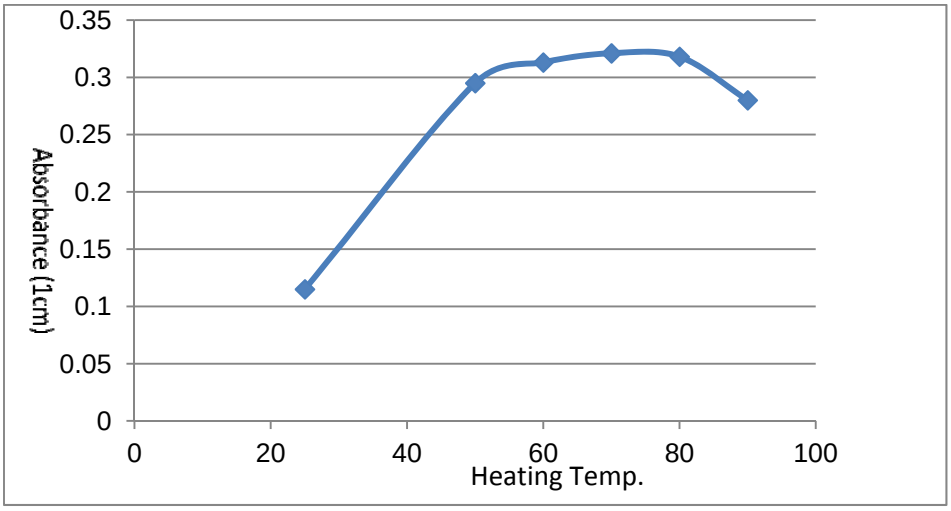


Fig.(4.c): Effect of temperature on the development of the reaction product of Indacaterol maleate (9 µg mL^{-1}) with 4-AAP(b).

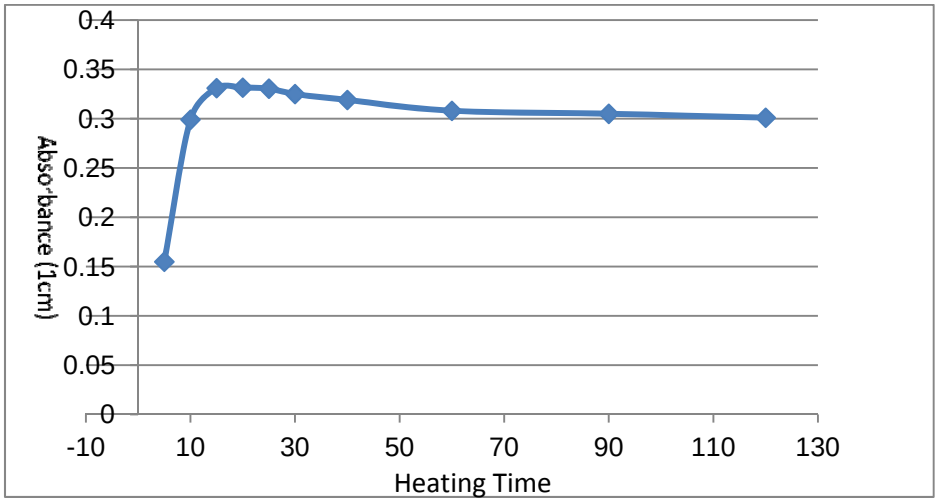


Fig.(4.d): Effect of heating time on the development of the reaction product of Indacaterol maleate (9 µg mL^{-1}) and 4-AAP.

Validation of analytical Performance

The validity of the methods was tested regarding linearity, accuracy, repeatability and precision according to ICH Q2R1 recommendations [14].

Linearity

Linear relationships were established by plotting the values of absorbance against drug concentration and were found to be rectilinear over the concentration ranges cited in Table 1. Statistical analysis of the data gave high values of the correlation coefficient (r) of the regression equations, small values of the standard deviation of residuals (Sy/x), of intercept (Sa), and of slope (Sb), and small values of the percentage relative standard deviation and the percentage relative error (Table 1). The linearity of the proposed methods was proved by the low scattering of the points around the calibration line proving the agreement of the measurements with Beer's law. Method A is considered superior to other methods owing to its simplicity and the highest sensitivity providing high rates of molar absorptivity and specific absorbance values.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantitation (LOQ) was determined according to ICH Q2 (R1) recommendation by establishing the lowest concentration that can be measured below which the calibration graphs are not linear. The limit of detection (LOD) was determined also by evaluating the lowest concentrations of the analytes that can be readily detected. LOQ and LOD were calculated according to the following equations:

LOQ = 10 Sa /*b* and LOD = 3.3 Sa /*b*

Where, Sa = standard deviation of the intercept and b = slope of the calibration curve.

The results of the three proposed methods are summarized in Table 1.

Table1: Performance data of the proposed spectrophotometric methods**:**

Parameter	Method A	Method B	Method C
-Concentration range (µg mL^{-1})	1-16	2- 20	3-30
-LOD (µg mL^{-1})	0.07	0.213	0.125
-LOQ (µg mL^{-1})	0.212	0.651	0.38
-Correlation coefficient (r)	0.9999	0.9999	0.9999
-Slope (a)	0.048	0.042	0.037
-Intercept (b)	0.063	0.021	-0.01
-$S_{y/x}$ (standard deviation of the residuals).	1.6×10^{-3}	3.9×10^{-3}	2.0×10^{-3}
-S_a (standard deviation of the intercept of regression line).	1.0×10^{-3} 1.0×10^{-4}	2.7×10^{-3} 2.0×10^{-4}	1.4×10^{-3} 1.0×10^{-4}
-S_b (standard deviation of the slope of regression line)	0.150	0.211	0.146
-% Error (RSD% / √ n)	0.424	0.559	0.386
-%RSD	99.97	100.04	100.08
-Mean found (%)	0.42	0.56	0.39
± SD	24.271 x	21.270 x	18.709 x
E (Molar absorptivity (l mol^{-1} cm^{-1}))	10^3	10^3	10^3
$A^{1\%}$ (Specific absorbance (dl gm^{-1} cm^{-1}))	477.25	418.25	367.88

N.B. -$S_{y/x}$ =standard deviation of the residuals.

-S_a = standard deviation of the intercept of regression line.

-S_b = standard deviation of the slope of regression line.

-% Error = RSD% / √ n.

- ε =Molar absorptivity (l mol^{-1} cm^{-1})**.**

- $A^{1\%}$ = Specific absorbance (dl gm^{-1} cm^{-1}).

Accuracy & Precision (Method A, B and C)

The intra-day and inter-day accuracy and precisions of the proposed methods were examined by triplicate analysis of IND in pure form, at three different concentrations 3.0, 8.0, and 12.0 µg mL^{-1} for method A, 3.0, 9.0, and 18.0 µg mL^{-1} for method B and 6.0, 9.0, and 18.0 for method C. The analyses were performed for each concentration three different times within 1 day and for 3 consecutive days for intra-day and inter-day, respectively. The analysis was performed as described under **"Construction of calibration curves (Method A, B and C)"**. The results of analyses are summarized in Table 2. The precision of the proposed method were satisfactory, as indicated by good values of percentage recoveries and the low values of SD and RSD.

Table 2: Precision data of the proposed methods for the determination of IND in pure form

Intra-day precision				
Method	**Conc. taken (µg mL^{-1})**	**Mean % found ± SD**	**%RSD**	**%ER**
A	3.0	100.09 ± 0.34	0.34	**0.20**
	8.0	100.93 ± 0.04	0.04	**0.02**
	12.0	99.76 ± 0.48	0.48	**0.28**
B	3.0	99.60 ± 0.60	0.60	**0.35**
	9.0	99.61 ± 0.28	0.28	**0.16**
	18.0	100.69 ± 0.30	0.30	**0.17**
C	6.0	99.71 ± 0.38	0.38	**0.22**
	9.0	100.19 ± 0.32	0.32	**0.19**
	18.0	99.71 ± 0.22	0.22	**0.13**
Inter-day precision				
Method	**Conc. taken (µg mL^{-1})**	**Mean % found ± SD**	**%RSD**	**%ER**
A	3.0	99.41 ± 0.35	0.35	**0.20**
	8.0	100.86 ± 0.09	0.09	**0.05**
	12.0	99.45 ± 0.44	0.44	**0.25**
B	3.0	99.83 ± 0.27	0.27	**0.16**
	9.0	99.62 ± 0.39	0.39	**0.22**
	18.0	100.60 ± 0.38	0.38	**0.22**
C	6.0	99.31 ± 0.26	0.26	**0.15**
	9.0	99.75 ± 0.54	0.55	**0.32**
	18.0	99.15 ± 0.12	0.12	**0.07**

Robustness

The robustness of the proposed methods is demonstrated by the constancy of the absorbance value with the deliberated minor changes in the experimental parameters. For method B changes include: change in reaction time (15 ± 5.0 min), change in the volume of CAS (2.5 ± 0.5mL), and change in the volume of MBTH (1.0 ± 0.25 mL). While in the case of method C, changes include: change in reaction time (20.0 ± 5.0 min), change in heating time (70.0 ± 5.0 ^{O}C) change in the volume of ammonia solution (0.6 ± 0.2 mL), change in the volume of HCF solution (1.2 ± 0.2 mL) and change in the volume of 4-AAP solution (2.0 ± 0.5 mL). These minor changes that may take place during the experimental operation did not affect the absorbance value obtained by the proposed methods.

Pharmaceutical Applications (Method A, B and C)

The proposed methods were applied successfully to the determination of IND in its capsules where no apparent interference from the capsule excipients was observed. Standard addition method was applied to test the validity of the proposed methods. The recovery of IND, was determined by adding a known amount of pure drug at three different concentrations of 2.0, 4.0, and 8.0 µg mL^{-1} for method A, 3.0, 6.0 and 9.0 for method B and 6.0, 9.0, and 12.0 µg mL^{-1} for method C to previously analyzed capsule solution at two different concentrations 3.0 and 6.0 µg mL^{-1} for method A, B and 6.0, and 9.0 µg mL^{-1} for method C. These concentrations of the pure drug were added in separate flasks to each capsule concentration and each solution was reanalyzed for the total drug content. The analysis was carried out in triplicate and was performed as described under **"Construction of calibration curves (Method A, B and C)".** The obtained results are shown in table 3. These control experiments eliminate the interference due to interactions of other constituents encountered in the system or caused by the bulk production. The results obtained were satisfactory, as indicated by the low values of SD.

Table 3: Spectrophotometric methods for the determination of IND by the proposed methods applying the standard addition method:

Method	Capsule* concentration (µg mL^{-1}).	Concentration of added standard (µg mL^{-1}).	Recovery± RSD (%)of added IND**	Mean Recovery ± RSD (%) of added IND
Method A	3.0	2.0	99.37 ± 0.652	100.14 ± 0.80
	3.0	4.0	100.09 ± 0.504	
	3.0	8.0	100.97 ± 0.98	
	6.0	2.0	99.97 ± 0.76	100.00 ± 0.18
	6.0	4.0	99.84 ± 0.48	
	6.0	8.0	100.19 ± 0.34	
Method B	3.0	3.0	99.90 ± 0.70	99.74 ± 0.20
	3.0	6.0	99.80 ± 0.57	
	3.0	9.0	99.51 ± 0.37	
	6.0	3.0	99.72 ± 0.44	99.70 ± 0.19
	6.0	6.0	99.51 ± 0.28	
	6.0	9.0	99.88 ± 0.53	
Method C	6.0	6.0	100.54 ± 0.46	100.00 ± 0.49
	6.0	9.0	99.86 ± 0.31	
	6.0	12.0	99.60 ± 0.62	
	9.0	6.0	100.38 ± 0.47	100.20 ± 0.43
	9.0	9.0	99.71 ± 0.93	
	9.0	12.0	100.52 ± 0.34	

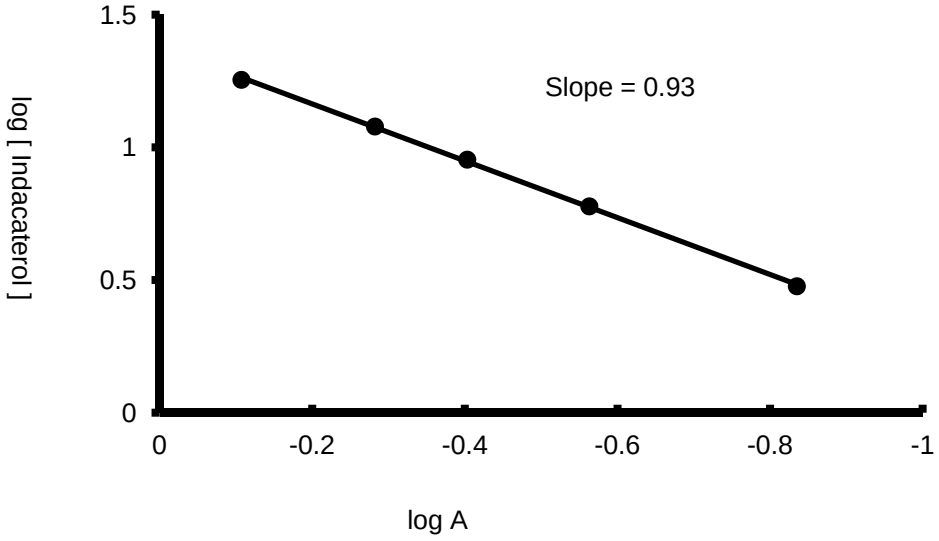
*****Each Onbrez capsule contains 150µg Ind. Mal., Product of Novartis & Chem.Ind. Co., batch no. 143984.
****** Average of 3 determinations.

Molar ratio and Mechanism of the Reaction (Method B and C)

The stoichiometry of the reactions were studied adopting the limiting logarithmic method [15] using equimolar concentrations between the studied drug and MBTH at constant CAS concentration for method B and 4-AAP at constant concentrations of ammonia and potassium ferricyanide solution for method C. The absorbance of the reaction product was alternatively measured in the presence of either the specified reagents or IND. Plots of log [IND] *vs*. log Absorbance and log [reagent] *vs*. log absorbance gave two straight lines for the both methods, the values of the slopes were 0.93: 1.26 for IND: MBTH (Method B) (Fig. 5) and 1.36: 1.29 for IND: 4-AAP (Method C), respectively (Fig. 6). Hence, it is concluded that, the molar reactivity of the first reaction is 1: 2 drug: reagent (Method B) and the molar reactivity of the second reaction is 1: 1 drug: reagent (Method C) based on the obtained molar ratio, A schematic proposal for the reaction pathway between the studied drug and MBTH in acidic medium in the presence of CAS to yield an orange color was postulated in Scheme 1. The oxidation of MBTH by CAS is accompanied by a simultaneous loss of one proton and two electrons forming an electrophilic intermediate, which has been postulated to be the coupling species. In the second step, an electrophilic reaction between the drug and the electrophilic intermediate takes place, with the formation of the colored product and

elimination of one molecule of water based on the obtained molar ratio[16].c A schematic proposal for the reaction pathway between the studied drug and 4- aminoantipyrine through an oxidative coupling reaction in the presence of alkaline oxidizing agent ($K_3[Fe(CN)_6]/NH_3$) to form an antipyrine dye having the quinonoid structure shown in Scheme 1. It is reported that in this reaction, the para position to the phenolic group should be either free or substituted with a group that can be expelled during the reaction like halogen, carboxyl, sulfonic acid, hydroxyl, and methoxyl groups [17-22]. Thus, the proposed mechanism of the oxidative coupling reaction could be presented as shown in Scheme 2

(A) Log [Indacaterol] *vs* log A



(B) Log [MBTH] *vs* log A.

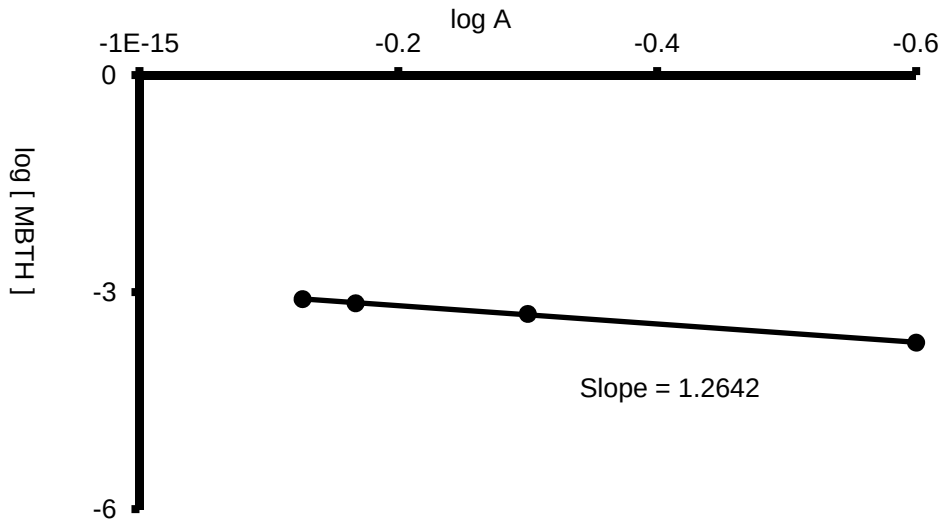
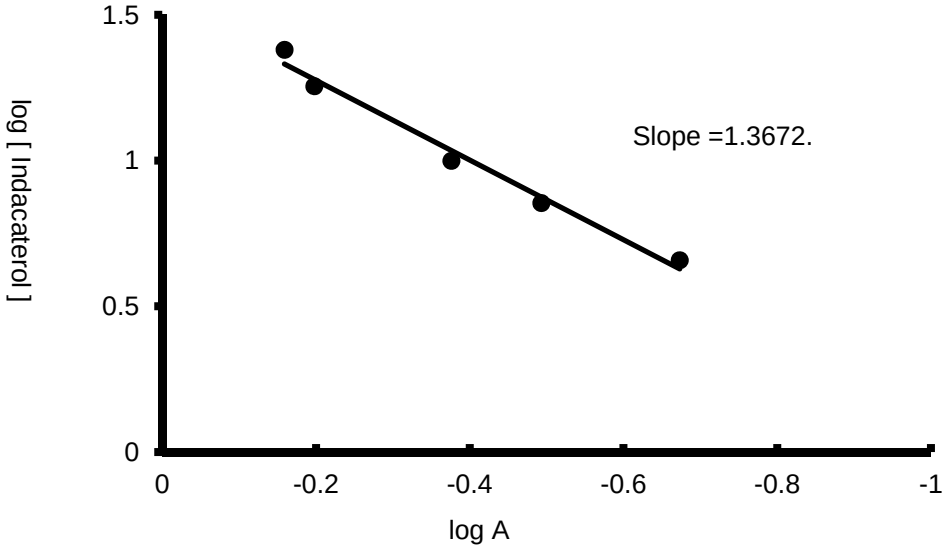


Fig. (5): Stoichiometry of the reaction between drugs and MBTH (0.3 %) adopting limiting logarithmic method.

(A) Log [Indacaterol] *vs* log A



(B) Log [4-AAP] *vs* log A.

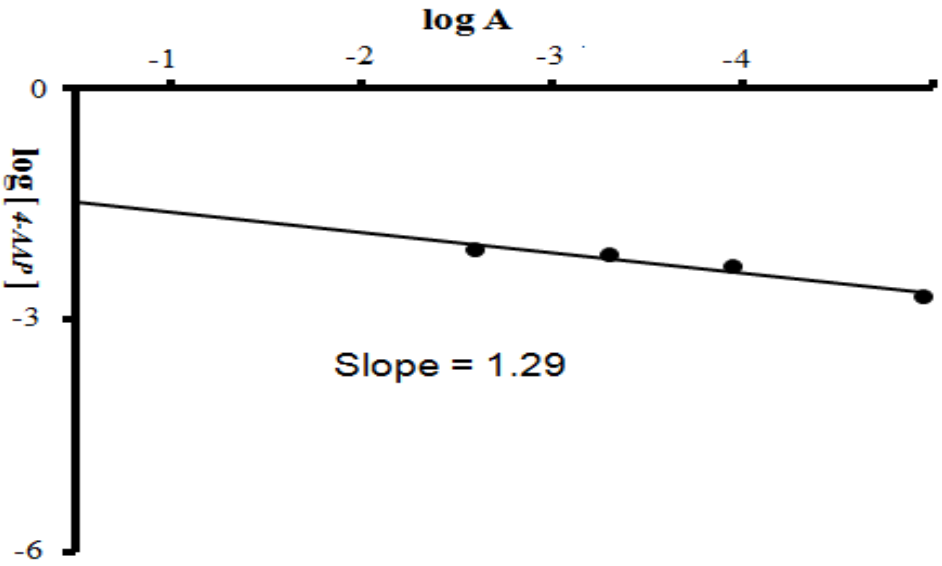
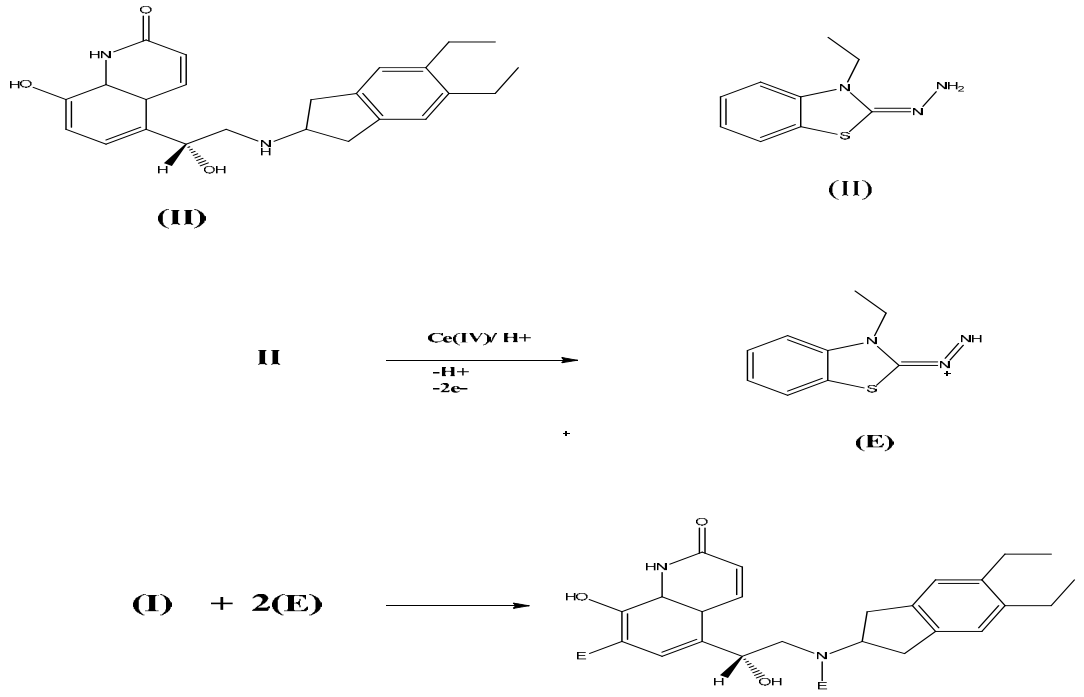
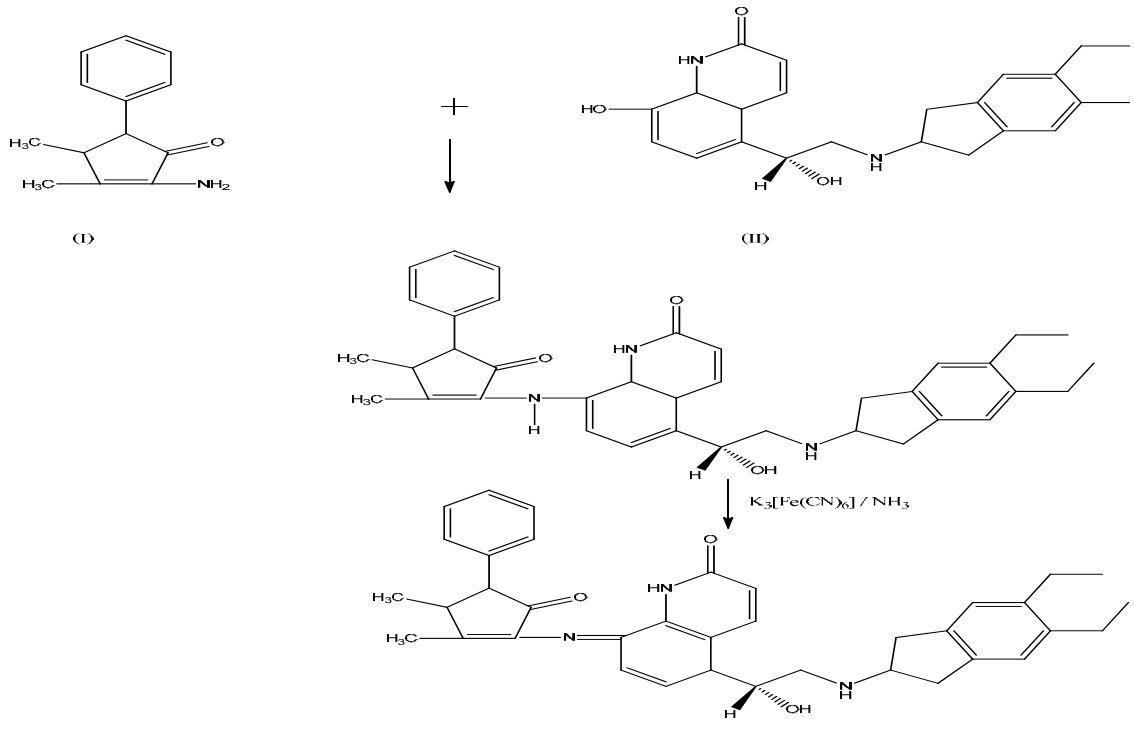


Fig.(6): Stoichiometry of the reaction between drugs and 4-AAP (0.2 %) adopting limiting logarithmic method.



Scheme 1: A proposal of the reaction pathway of the oxidative coupling reaction of Indacaterol maleate and MBTH (II) in the presence of ceric (IV) ammonium sulphate



Scheme 2: A proposal of the reaction pathway of the reaction of Indacaterol maleate and 4-AAP.

Critical comparison of the developed methods

The critical comparison of the two developed spectrophotometric methods for the determination of IND leads to the following advantages/disadvantages:

(1) All methods are sufficiently sensitive and selective for the determination of the analyte in its pharmaceutical formulations.

(2) Methods A, B and C exhibit best linearity (r = 0.9999).

(3) Method A was found to be the most sensitive method providing the highest molar absorptivity and specific absorbance values.

(4) For application in quality control laboratories, method A is considered superior to other methods owing to its rapidness, minimum steps, simplicity and sensitivity providing high rates of sample throughput.

(5) Both methods A and C employ simple diluting solvents (0.1 N HCl, NaOH and distilled water, respectively) providing cost effectiveness. However, distilled water is more eco-friendly.

Other techniques such as HPLC/ MS [3, 4] may also give good results but, because of the low cost and ease of carrying out the spectrophotometric methods, the proposed procedures are likely to be very suitable for the quality control of IND in capsule dosage forms.

Conclusion

The proposed methods are applicable and valid for the assay of Indacaterol maleate. They have the advantages of being less time-consuming and don't require elaborate treatments and expensive solvents required with the chromatographic methods. The increasing order of sensitivity of the propose methods are A > B > C, so the proposed spectrophotometric methods can be used in the determination of Indacaterol in bulk drug and its capsules in quality control laboratories without the interference from common excipients.

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