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# Spectrophotometric determination of propranolol in formulations via oxidative coupling with 3-methylbenzothiazoline-2-one hydrazone

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**Abstract**

A simple spectrophotometric method has been developed for the determination of propranolol hydrochloride in pure as well as in dosage forms. The method is based on the oxidative coupling reaction with 3-methylbenzothiazoline-2-one hydrazone. A mixture of an acidic solution of the chromogenic agent and the drug upon treatment with ceric ammonium sulfate produces an orange color peaking at 496 nm. The absorbance–calibration plot was linear over the range 1–10 μg/ml with minimum detectability ($S/N = 2$) of 0.1 μg/ml ($3.38 \times 10^{-7}$ M). The molar absorbitivity was $3.195 \times 10^3$ l/mol/cm with correlation coefficient ($n = 10$) of 0.9999. The different experimental parameters affecting the development and stability of the color were carefully studied and optimized. The proposed method was applied successfully to the determination of propranolol in its dosage forms. A proposal of the reaction pathway was presented.


*Keywords:* Propranolol; MBTH; Pharmaceutical preparations; Spectroscopy; Pharmaceutical analysis

## 1. Introduction

Propranolol hydrochloride (±)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride, is a prototype of Beta-blockers. Antagonism of β-adrenergic receptors affects the regulation of circulation through a number of mechanisms, including a reduction in myocardial contractility and cardiac out put, reduction in the secretion of rennin with a resulting fall in the levels of angiotensin II which contributes importantly to the antihypertensive action of this class of drugs [1].

Literature survey reveals a lot of analytical methods for the determination of propranolol in pharmaceuticals and in biological fluids. The more recent articles concerned with the analysis of propranolol include different techniques, viz UV derivative spectroscopy [2], colorimetry [3–7], synchronous fluorimetry [8], thin layer chromatography for enantiomer separation [9,10], HPLC for enantiomers separation [11–15], HPLC in biological fluids [16–20], cation exchange HPLC in spiked plasma [21], micellar liquid chromatography in urine samples [22], capillary electrophoresis [23–27] and electrochemical methods [28,29]. The assay procedures of propranolol in pure form and in pharmaceutical preparations listed in USP 24 NF 19 (2000) [30] and BP [31] described potentiometric titration, spectrophotometric and chromatographic methods. Propranolol, like other β-blockers exhibits absorption in the UV region but with low sensitivity [31], that is achieved by direct measurement of the absorbance at 290 nm. This has led us to develop a simple and sensitive spectrophotometric method for the determination of propra-

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nolol HCl, based on its reaction with the oxidized MBTH in acidic medium.

## 2. Experimental

### 2.1. Apparatus

Shimadzu UV-1601 PC, UV–Vis spectrophotometer, Kyoto, Japan with 1 cm quartz cells.

### 2.2. Materials and reagents

Propranolol hydrochloride pure sample was kindly provided by Kahira Pharmaceutical Company, Cairo, Egypt. Tablets containing propranolol HCl: Inderal 10 and 20 labeled to contain 10 mg and 20 mg of propranolol hydrochloride per tablet, respectively. Ampoules containing propranolol HCl: Inderal ampoules, labeled to contain 1mg of propranolol HCl per ampoule. Tablets and ampoules were obtained from commercial sources.

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

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

Hydrochloric acid (Merck, Darmstadt, Germany) 0.2 M aqueous solution.

Sulphuric acid (Merck, Darmstadt, Germany) 5% aqueous solution.

Standard solution of propranolol HCL was prepared by dissolving 100.0 mg in 100 ml of distilled water and further diluted with the same solvent as appropriate. This solution is stable for 3 days if kept in refrigerator.

### 2.3. Procedures

#### 2.3.1. Recommended procedure and calibration curve

Transfer accurately measured aliquots of the stock solution into a series of 10-ml volumetric flasks so that the final concentration is in the range of 1–10 μg $ml^{-1}$. Add 1 ml of MBTH solution and 2.5 ml of ceric(IV) ammonium nitrate. Mix well and allow the reaction mixture to stand for 30 minutes at room temperature, then complete to the volume with acetonitrile. Measure the absorbance of the solution at 496 nm against a reagent blank. Simultaneously construct the calibration curve. Alternatively, derive the regression equation.

#### 2.3.2. Procedure for the tablets

Weigh and pulverize ten tablets. Transfer a portion of the powder equivalent to 10.0 or 20.0 mg of propranolol hydrochloride into a small flask. Shake with 2 × 30 ml of acetone for 10 min, then filter into a conical flask wash the beaker and filter with few ml of acetone and pass the washings to the same flask. Evaporate acetone using a rotatory evaporator till dryness. Dissolve the residue in 3 × 30 ml of water and filter, if necessary, into 100 ml volumetric flask. Complete to the mark with distilled water. Proceed as described under Section 2.3.1. Determination of the nominal content of the tablets was achieved using the standard addition method.

#### 2.3.3. Procedure for ampoules

Mix the contents of 5 ampoules, transfer an accurately measured volume of the solution equivalent to 1.0 mg into a 10 ml calibrated flask. Complete to the mark with distilled water. Proceed as described under Section 2.3.1. Determination of the nominal content of the ampoules was achieved either from the calibration curve or using the regression equation.

## 3. Results and discussion

Propranolol was found to react with MBTH in the presence of Ce(IV) to produce an orange colored species peaking at 496 nm (Fig. 1). MBTH has been frequently utilized for the spectrophotometric determination of several pharmaceutical compounds, viz 4-quinolone antibacterials [32], acetaminophen and phenobarbital [33], ritodrine and amoxycillin [34] and sulphonamides [35].

### 3.1. Study of the experimental conditions

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. The effect of MBTH concentration was studied by adding various volumes of its solution to a fixed concentration of propranolol, 0.75 ml of 0.3% MBTH in 5% sulfuric acid was enough to develop the color in its full intensity (Fig. 2). To ensure the presence of an excess concentration of MBTH, 1 ± 0.2 ml of 0.3% MBTH solution was used for all experiments within the working concentration range.

It was found that the use of 0.2 M HCl as a solvent for MBTH gave the maximum stability of the color produced. Fig. 2 shows that 2 ml of 0.5% (w/v) ceric(IV) ammonium nitrate solution in 5% sulfuric acid gave the maximum color intensity, so to ensure enough concentration of the oxidizing agent, 2.5 ml of its solution was used. Other oxidizing agents such as iron ammonium sulfate, ceric ammonium sulphate and ferric chloride were attempted, all gave characteristic colors.

Fig. 3 shows that the color intensity reached a maximum after the drug solution had been reacted with MBTH and ceric(IV) ammonium nitrate for 25 min. Therefore, a 30 min development time was

suggested as the optimum reaction time. The order of addition of the reagents is an essential part of the experiment, addition of MBTH after ceric(IV) ammonium nitrate produced the lowest absorbance reading. The formed chromophore was found to be stable for at least 3 h.

The nature of the diluting solvent is of significant importance. Different 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, meanwhile neither water nor dilute sulfuric acid can be used, this is attributed to the observed insolubility of the formed chromophore in water.

Different sensitizers (quinine, fluorescein and rhodamine B), at concentration of 5 μg/ml were tested by adding to the reaction mixture, it was found that there was neither inhibitory nor enhancing effect on the intensity of the color produced.

In the same manner, the effect of different surfactants (cetrimide, gelatin and sodium dodecylsulphate) on the color intensity at three different concentrations of 2.5, 7.5 and 15 μg/ml was studied. All of the tested surfactants have a little diminishing effect on the absorbance (Table 1). This may be attributed to the partial consumption of ceric ammonium nitrate by the surfactants, being oxidized to oxidation products other than the active species.

The effect of foreign substances on the color intensity was studied by adding three different concentrations, 1.0, 5.0, 20.0 μg/ml of each of the following substances: fructose, glucose, lactose, sucrose and starch (Table 2). It was found that sucrose completely vanished the color and greatly interfered with the product formation even in the lowest concentration, this may be attributed to the complete consumption of the oxidizing agent, while glucose, fructose and lactose have a little diminishing effect on the intensity of the color produced even at the highest concentration.

### 3.2. Analytical performance

A typical spectrum for propranolol reaction product is shown in Fig. 1 from which a calibration graph of propranolol concentration versus absorbance was extracted. The absorbance–concentration plot was found to be linear over the concentration range 1–10 μg/ml. Linear regression analysis of the data ($n = 10$) gave the following equation:

$$A = 6.67\times10^{-3} + 0.1065C \qquad r = 0.9999$$

where $A$ is the absorbance in 1-cm cell, $C$ is the concentration of the drug in μg/ml with a correlation coefficient of 0.9999.

The apparent molar absorpitivity was found to be $3.195 \times 10^3$ mol/l/cm and $A$ 1%, 1 cm was about 108.

Statistical evaluation [36] of the regression line gave the following values: standard deviation of the residuals ($S_{y/x}$) is $4.05 \times 10^{-3}$; standard deviation of the intercept ($S_a$) is $2.77 \times 10^{-3}$; standard deviation of the slope ($S_b$) is $4.46 \times 10^{-4}$ while the percentage error is 0.19%. These small figures point out to the high precision of the proposed method. The limit of detection (LOD) is 0.1 μg/ml ($3.38 \times 10^{-7}$ M).

The proposed method was applied to the determination of pure sample of propranolol hydrochloride. The results obtained by the proposed method were compared with those obtained by the reference method [35]. Statistical analysis of the results obtained by both methods using the Student's $t$-test and the variance ratio $F$-test, revealed no significant difference in the performance of the two methods regarding accuracy and precision, respectively (Table 3).

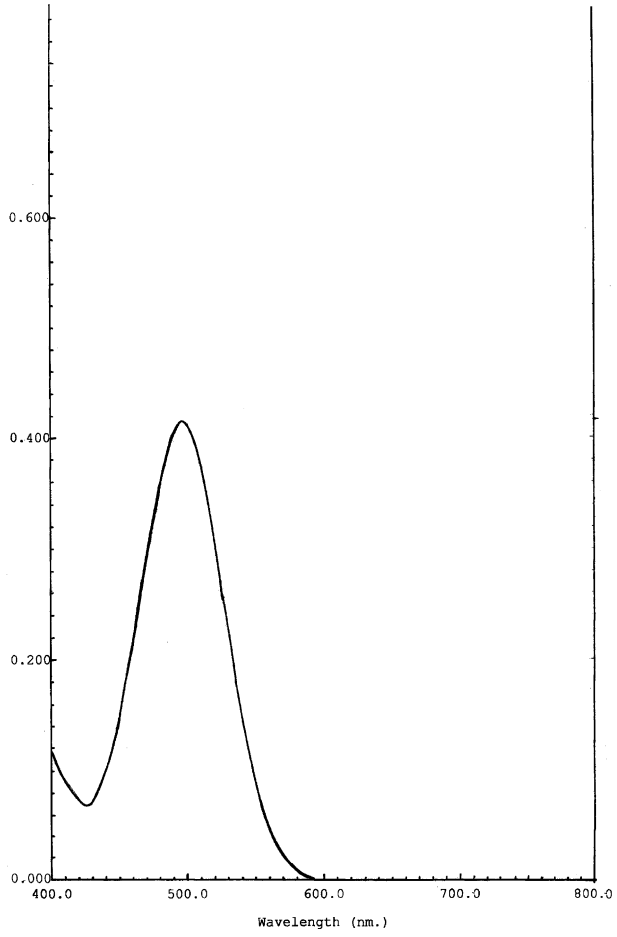



Fig. 1. Absorption spectrum of the reaction product of propranolol hydrochloride (4 μg/ml) with MBTH–ceric ammonium nitrate system.

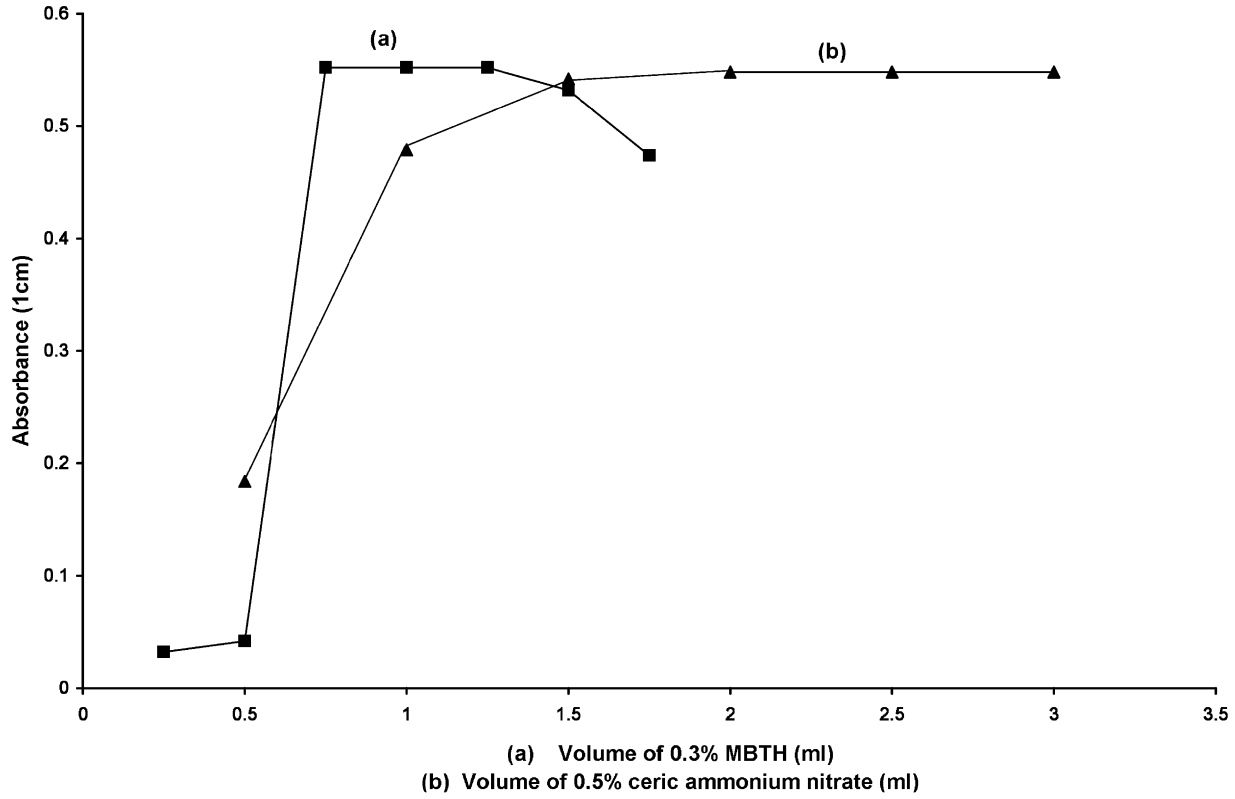



Fig. 2. Effect of volume of 0.3% MBTH (a) and 0.5% ceric(IV) ammonium nitrate (b) on the development of the reaction product of propranolol (5 μg/ml).

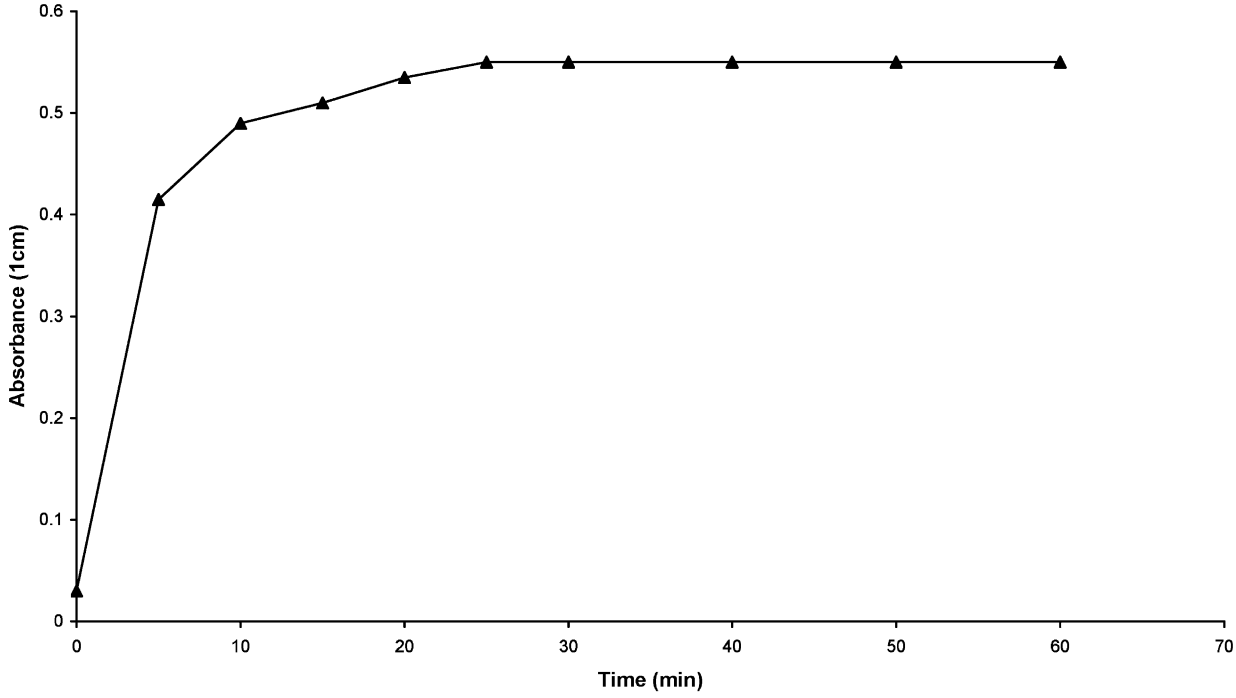



Fig. 3. Effect of time on the development of the reaction product of propranolol (5 μg/ml) with MBTH.

### 3.3. Pharmaceutical applications

The proposed method was applied to the determination of propranolol in its pharmaceutical preparations, tablets and ampoules. It was found that upon application of the method to the determination of propranolol in tablets, an apparent interference from the tablet excipients was noticed. The percentage recovery was low (around 85%), this may be due to the presence of lactose which partially consumes the oxidizing agent. To eliminate this interference the standard addition method was adopted. Table 4 shows the percentage recoveries obtained after applying the standard addition method. Meanwhile, the proposed method was successfully applied to the determination of propranolol in ampoules, the results obtained were satisfactory and there was no interference. Generally the results obtained were compared with those obtained with the official BP method [31]. Statistical analysis of the results using Student's $t$-test and variance ratio $F$-test, revealed no significant difference between the performance of the two methods at the 95% confidence level regarding accuracy and precision, respectively.

Compared with the spectrophotometric method described by Prasad et al. [37] which depends on measuring propranolol at 290 nm, the proposed method is more specific, as it involves the reaction of the intact compound with the reagent and measuring the resulting absorbance at the visible region (496 nm) where no interference may be encountered from impurities (related compounds or degradation products) or from the common excepients which may absorb light at 290 nm.

### 3.4. Mechanism of the reaction

The stoichiometry of the reaction was studied using equimolar concentrations of the drug and MBTH at constant Ce(IV) concentration adopting Job's method of continuous variation [39], a molar ratio of 1:2 (drug:MBTH) respectively, was obtained by the applied method as shown in Fig. 4.

Based on the observed molar reactivity of the reaction, the mechanism of the reaction between propranolol and MBTH in acidic medium in the presence of Ce(IV) to yield an orange color was postulated in Scheme 1. The oxidation of MBTH by Ce(IV) 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 [38]. 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.

The formation constant of the reaction product $K_f$ was calculated adopting the following formula [40]:

Table 1
Effect of different surfactants on the performance of the proposed method for determination of propranolol HCl (5 μg/ml)

| Surfactant concentration (μg/ml) | Surfactant | Absorbance |
|---|---|---|
| | no surfactant | 0.548 |
| 2.5 | cetrimide | 0.545 |
| 2.5 | sodium dodecyl sulphate | 0.447 |
| 2.5 | gelatin | 0.544 |
| 7.5 | cetrimide | 0.543 |
| 7.5 | sodium dodecyl sulphate | 0.424 |
| 7.5 | gelatin | 0.478 |
| 15 | cetrimide | 0.528 |
| 15 | sodium dodecyl sulphate | 0.403 |
| 15 | gelatin | 0.470 |

Table 2
Effect of different surfactants on the performance of the proposed method for determination of propranolol HCl (5 μg/ml)

| Foreign substances | Absorbance | | |
|---|---|---|---|
| | Concentration | | |
| | 20 μg | 5 μg | 1 μg |
| 1. Fructose | 0.438 | 0.481 | 0.535 |
| 2. Glucose | 0.511 | 0.532 | 0.543 |
| 3. Lactose | 0.451 | 0.491 | 0.533 |
| 4. Sucrose | | | |
| 5. Starch | 0.539 | 0.546 | 0.548 |
| No foreign substance | 0.548 | | |

Table 3
Application of the proposed method for the determination of propranolol in pure form

| μg Taken | μg Found | % Found | |
|---|---|---|---|
| | | Proposed method | Reference method [a] [35] |
| 1.00 | 0.99 | 99.00 | |
| 2.00 | 1.98 | 99.00 | |
| 3.00 | 2.99 | 99.67 | |
| 4.00 | 4.00 | 100.00 | |
| 5.00 | 5.01 | 100.20 | |
| 6.00 | 6.03 | 100.50 | |
| 7.00 | 7.04 | 100.57 | |
| 8.00 | 8.06 | 100.75 | |
| 9.00 | 8.95 | 99.44 | |
| 10.00 | 9.95 | 99.50 | |
| $X \pm SD$ | | 99.88 ± 0.60 | 100.25 ± 0.83 |
| $t$ | | 1.45 (2.179) | |
| $F$ | | 1.90 (3.86) | |

Figures in parenthesis are the tabulated values of $t$ and $F$ at $p = 0.05$
[a] Number of experiments = 4.

Table 4
Application of the proposed method to the determination of propranolol in dosage forms

| Preparation | μg Taken | μg Found | % Recovery | Reference method [31] |
|---|---|---|---|---|
| Inderal [a] tablets (10 mg propranolol HCl/tablet) | 2.00 | 2.04 | 102.00 | |
| | 4.00 | 3.99 | 99.75 | |
| | 6.00 | 6.02 | 100.33 | |
| X±SD | | | 100.69±1.12 | 100.46±0.51 |
| *t* | | | 0.31 (2.776) | |
| *F* | | | 6.65 (19.0) | |
| Inderal [a] tablets (20 mg propranolol HCl/tablet) | 2.00 | 2.03 | 101.50 | |
| | 4.00 | 4.03 | 100.75 | |
| | 6.00 | 5.99 | 99.83 | |
| X±SD | | | 100.69±0.84 | 100.09±0.59 |
| *t* | | | 1.01 (2.776) | |
| *F* | | | 2.03 (19.00) | |
| Inderal [b] ampoules (1 mg propranolol HCl/ampoule) | 2.50 | 2.53 | 101.20 | |
| | 5.00 | 5.11 | 102.20 | |
| | 7.50 | 7.57 | 100.93 | |
| X±SD | | | 101.44±0.67 | 101.07±0.65 |
| *t* | | | 0.69 (2.776) | |
| *F* | | | 1.06 (19.00) | |

Number of experiments in reference method = 3, the figure in parenthesis are the tabulated values of $t$ and $F$ at $p = 0.05$.
[a] Product of Kahira Pharmaceutical Company, Cairo, Egypt.
[b] Product of ZENECA limited, Macclesfield Cheshire SK10, UK.

$$K_f = \frac{A/A_m}{\left(\frac{1-A}{A_m}\right)^{n+1} C^n n^n}$$

where $A$ is the maximum absorbance of the continuous variation curve (Fig. 4). $A_m$ is the absorbance corresponding to intersection of the two tangents of the continuous variation curve. $C$ is the molar concentration corresponding to the maximum absorbance. $n$ is the number of molecules of the reagent in the reaction product. $K_f$ was found to be equal to $9.49 \times 10^6$. This high figure obtained indicates a very stable reaction product. The Gibbs free energy of the reaction $\Delta G$ was also calculated adopting the following equation:

$$\Delta G = -2.303 RT \log K_f$$

where, $R$ is the universal gas constant (8.314 J). $T$ is the absolute temperature (273+25 °C) $K_f$ is the formation constant of the reaction. The value of $\Delta G$ was found to be −39810 K J/mole. The negative value of $\Delta G$ points out to the spontaneous nature of the reaction.

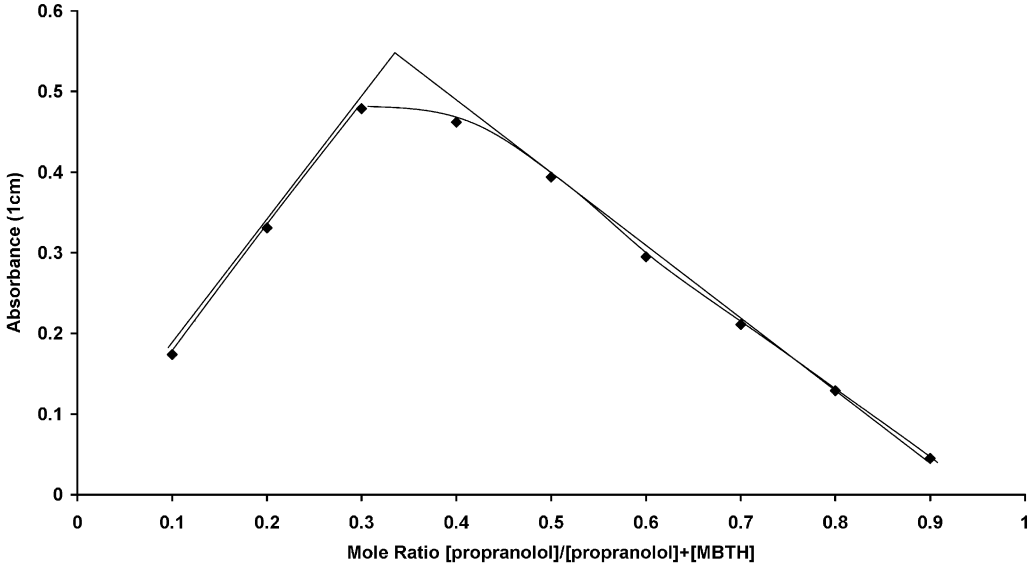
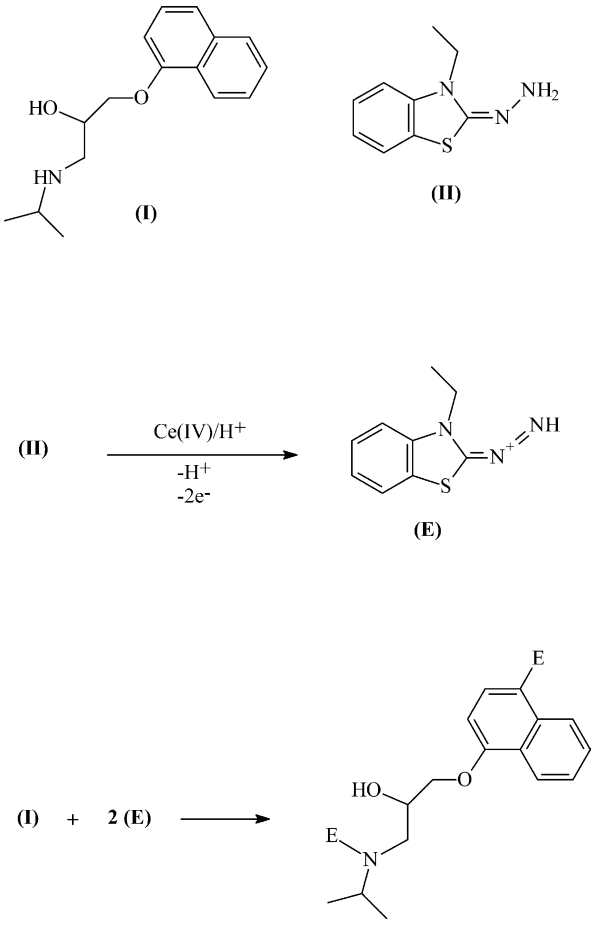



Fig. 4. Determination of molar reactivity of MBTH–propranolol HCl using Job's method of continuous variation (0.005 M) each.




Scheme 1. A proposal of the reaction pathway of the oxidative coupling reaction of propranolol (**I**) and MBTH (**II**) in the presence of ceric(IV) ammonium nitrate

## 4. Conclusion

The proposed method is simple and sensitive compared to other reported spectrometric methods, limit of detection is 0.1 μg/ml ($3.38 \times 10^{-7}$ M) which is comparable to those reported by chromatographic methods. Furthermore, the proposed method does not require elaboration of treatment and procedure, which are usually associated with the chromatographic methods. The proposed method can be used for routine quality control studies.

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