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SIMPLE AND RAPID SPECTROPHOTOMETRIC DETERMINATION OF ATENOLOL AND ESMOLOL β-BLOCKERS IN PHARMACEUTICAL FORMULATIONS AND SPIKED WATER SAMPLES

D. K. Sharma and Pushap Raj*

Department of Chemistry, Himachal Pradesh University, Shimla - 171005, Himachal Pradesh, India.

Keywords:

Atenolol, Esmolol, Spectrophotometry, Photometric titrations, Formulation and spiked water analysis

Correspondence to Author:
Pushap Raj

Department of Chemistry, Himachal Pradesh University, Shimla - 171005, Himachal Pradesh, India.

E-mail: pushap.hp@gmail.com

ABSTRACT: Atenolol and esmolol drugs belonging to a β–blocker group are frequently used for the treatment of cardiovascular diseases. These drugs are also charged as water pollutants. A new simple and rapid spectrophotometric method has been developed for their determination in pharmaceutical formulations and in spiked water samples. The proposed method is based on the derivatization of the amino function present in these drugs to the corresponding yellow copper (I) drug dithiocarbamate derivative through reaction with carbon disulphide, pyridine and copper (I) perchlorate in aqueous acetonitrile. The analysis is accomplished for their determination in pharmaceutical formulations by measuring absorbance at 425 nm for atenolol and 420 nm for esmolol. For the determination of these drugs in spiked water samples, the coloured complex is extracted into chloroform and the absorbance of the chloroform extracts is measured at 415 nm and 410 nm for atenolol and esmolol respectively. Linear correlation is obtained in the range 1.00 - 22.00 and 1.50 - 25.00 µg mL^{-1} with detection limits of 0.10 and 0.15µg mL^{-1} for atenolol and esmolol respectively. The proposed methods show good recoveries of atenolol and esmolol from pharmaceutical formulations and spiked water samples and are in the ranges from 99.00 - 100.40% and 98.20 - 100.80% respectively. The 1:1 stoichiometry of the colour reaction between drug as dithiocarbamate derivative and copper (I) perchlorate has been established by photometric titrations. The latter also provides an alternative method for the determination of above drugs in pharmaceutical formulations.

INTRODUCTION: Beta-blockers also called beta-adrenergic antagonists constitute one of the most frequently prescribed drugs to treat cardiovascular diseases like heart rhythm disorders, angina pectoris, hypertension, tachycardia or acute myocardial infarction [1, 2].

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Among various β–blockers, atenolol and esmolol are recommended as the first-line treatment for cardiovascular diseases.

Atenolol (**Fig. 1A**) is chemically 2-[4-{(2RS)-2-hydroxy-3-[(1-methylethyl) amino] propoxy} phenyl] acetamide. It is a β-selective adrenergic blocking agent, which is used concurrently with diuretics to reduce heart muscle contraction and lowers blood pressure by blocking the action of the nervous system on the heart. Esmolol hydrochloride, methyl 3- {4- [2- hydroxy- 3- (isopropylamino) propoxy] phenyl} propionate hydrochloride (**Fig. 1B**), is an ultra-short acting

adrenergic receptor antagonist used for the rapid control of heart rate in patients with atrial fibrillation or atrial flutter.

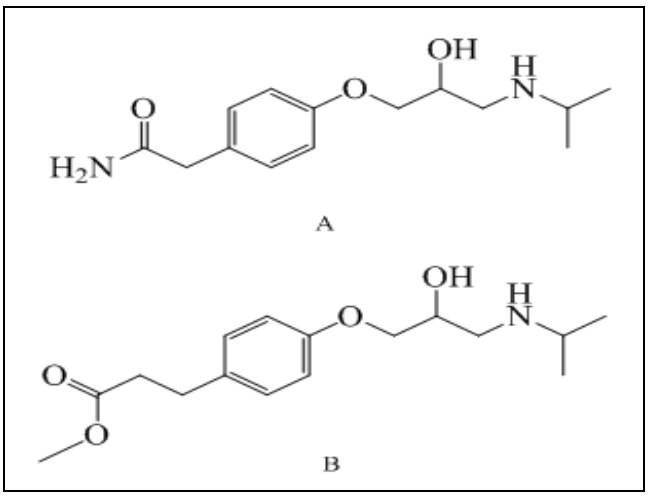

FIG. 1: CHEMICAL STRUCTURES OF DRUGS: A ATENOLOL, B ESMOLOL

Due to the wide therapeutic range of these drugs, their periodic monitoring in pharmaceutical formulations is required for ensuring the quality of marketed drugs and consequently for adjusting the corresponding dose. In the last few years, water scarcity and water quality have become a worldwide concern. Everyday large amount of water is contaminated by different pollutants coming from domestic or industrial uses. Among those pollutants found in water, there is also a mention of β-blockers including atenolol and esmolol. Detection of these drugs in water samples have also been reported by some workers [3, 4]. The drugs enter water when people excrete them and simply flush out the outdated and unused drugs. In view of the above, there is a dire need to determine these drugs in commercial pharmaceutical formulations (for the purpose of quality control and to maintain uniform therapeutic standards) and in water samples (for monitoring water pollution).

Literature survey revealed that several methods have been reported for the analysis of these drugs in pharmaceutical formulations and / or in biological fluids but less work has been directed for their determination in water samples. These methods include: spectrophotometry [5-8], NMR spectroscopy [9], fluorimetry [10], potentiometry [11], GC [12-13], HPLC [14-18] and capillary electrophoresis [19-21]. Despite the precision and accuracy of chromatographic and capillary electrophoresis methods, these are time-consuming and expensive due to high analytical cost, limiting the operation of these instruments to highly qualified laboratory persons. Spectrophotometry is considered the most convenient analytical technique because of its inherent simplicity, low cost, and wide availability in most laboratories and consequently methods based on this technique continues to flourish.

In this piece of work, a simple, rapid and sensitive spectrophotometric method is proposed for the determination of the studied drug compounds. The method is based on the reaction of either of the drug compound with carbon disulphide in the presence of pyridine (base) and copper (I) perchlorate in aqueous acetonitrile (8:2 v/v) to form intensively yellow copper (I) drug dithiocarbamate complex showing maximum absorbance at 425 nm for atenolol and 420 nm for esmolol. The analysis has been accomplished both by direct colorimetric as well as photometric titrimetric procedures. The latter procedure is rapid and precise as no calibration graph is required to be prepared, but the former is more sensitive. Optimizations of various experimental parameters, stoichiometry and proposed mechanism of colour reaction have been studied. The method has successfully been applied to the analysis of pharmaceutical formulations of these drugs. The method has also been validated to determine these drugs in water samples. In this context, the coloured complex formed from each drug is extracted with chloroform and absorbance of chloroform extract is measured at 415 nm for atenolol and 410 nm for esmolol.

MATERIAL AND METHODS:

Instrumentation: All the absorbance measurements have been carried out using Varian-cary 100 Bio UV-Visible Spectrophotometer and Bousch and Lomb spectrophotometer (spectronic 20 D+) with one cm matched glass cells. Microwave oven, (Samsung make) was used for heating purposes.

Pure Samples: The analytical standards of atenolol and esmolol both containing 98% of the active ingredients were procured from Sigma Aldrich Bangalore India.

Pharmaceutical Formulations: The following pharmaceutical formulations of these drugs were

purchased from local authorised dealers: ATEN-50 tablets (Zydus Cadila Healthcare Ltd. India) labelled to contain 50mg of atenolol per tablet, Tenolol-50 tablets (IPCA Laboratories Ltd. India) labelled to contain 50mg of atenolol, Neotach vials (Neon Laboratories Ltd. India) labelled to contain 10 mg of esmolol hydrochloride per millilitre of aqueous solution.

Reagents: Copper(I) perchlorate was prepared by a method reported by Hathaway *et al.,* [22], and its standard solution was prepared by dissolving more than the calculated amount in acetonitrile and standardized by titrating it against standard ammonium hexanitrocerate (IV) solution using ferroin as indicator [23]. Carbon disulphide (Merck, AR), pyridine (Merck, GS) were used as received. Commercial chloroform was purified by allowing it to stand over anhydrous potassium carbonate (AR) overnight and then distilled. The fractional distilling at 55 °C was collected.

Procedures:

Direct Colorimetric Procedure:

Preparation of Calibration Graph for Atenolol and Esmolol: The stock standard solutions (500 µg mL^{-1}) of atenolol and esmolol were prepared by dissolving precisely weighed 50mg each of the pure drug compound in 100mL of distilled water. The working concentrations were prepared by diluting 1mL of standard stock solution to 10mL with water to form a standard solution having concentration 50µg mL^{-1} in each case. Aliquots (0.10-2.20 and 0.15-2.50mL of atenolol and esmolol solutions respectively) of the standard drug solutions (50µg mL^{-1}) were taken in 5mL measuring flasks and volume made to 2mL with water.

Each solution was mixed with 1mL of pyridine and one drop (~ 50µL) of carbon disulphide and the contents were kept in a microwave for 30 s for the completion of the reaction. Each solution was then mixed with copper (I) perchlorate (1 mL, 10^{-3} M in acetonitrile) and volume made upto the mark with acetonitrile. The yellow colour which developed immediately was measured at 425 nm for atenolol and 420 nm for esmolol against a reagent blank. Calibration graphs were constructed for each drug compound by plotting absorbance corresponding to different concentrations of drug.

Photometric Titration Procedure: Aliquots of standard solutions ($2x10^{-4}$ M) of atenolol and esmololin distilled water were taken in colorimetric tubes and the volume made to 2mL with water. Each solution then mixed with 1 ml of pyridine and 1 drop of carbon disulphide and kept in microwave for 30 s. Each solution was then titrated photometricallyat room temperature (~24 °C) with standard copper (I) perchlorate ($2x10^{-4}$M) by adding reagent in small instalments, stirring the solution magnetically (with a specially designed stirring disk) each time and measuring the absorbance at their respective wavelengths *i.e.* 425 nm for atenolol and 420 nm for esmolol against a reagent blank.

Absorbance values were corrected to the initial volume of the solution by multiplying the absorbance readings by a factor V+ v/v, where V is the initial volume and v is the volume of standard copper (I) perchlorate added for a particular absorbance reading being measured. A plot of absorbance versus mL of titrant was then made and the best straight line drawn between the points taken well before and after the equivalence point. An inverted L-shaped titration curve was obtained in each case and the intersection of linear segments was taken as the end point.

Analysis of Pharmaceutical Formulations: Two formulations of atenolol *viz.* ATEN-50 and Tenolol-50 containing 50mg of atenolol per tablet and one formulation of esmolol *viz.* Neotach containing 10mg mL^{-1} of injection were used. A known number of tablets (20 tablets) of each formulation of atenolol were weighed and crushed into powder. Stock solution was prepared by dissolving accurately weighed amount equivalent to 50mg of active ingredient in distilled water and sonicated for 10 min. The solution was filtered and residue was washed 2-3 times with distilled water. The filtrate and washings were diluted to 100mL with distilled water. The stock solution of esmolol from its injection formulation was prepared by dissolving accurate volume of injection to a known volume of water. Suitable aliquots of the extracts were taken for analysis. The experimental details of direct colorimetric and photometric titrimetric determinations were the same as described above for pure compounds.

Analysis of Spiked Water Samples: For the determination of above drugs in spiked water samples, the above direct colorimetric procedure has been modified as follows. 10mL of distilled water taken in different flasks were mixed with aliquots of the standard solution (50µg mL^{-1}) of each drug. Each solution was mixed with 1mL of pyridine and one drop (~ 50µL) of carbon disulphide. The contents were kept in a microwave for 30 s for the completion of the reaction. Each solution was then mixed with copper (I) perchlorate (1mL, 10^{-3} M in acetonitrile). Each yellow coloured solution was equilibrated with 5mL of chloroform for five minutes. The yellow chloroform layer was separated and dried by shaking with anhydrous sodium sulphate (2g) and the absorbance of the resulting yellow colour was measured at 415 nm and 410 nm for atenolol and esmolol respectively. The calibration graphs were constructed by plotting absorbance against concentration of the drug in the usual way. Atenolol and esmolol in the ranges 1.00-22.00 and 1.50-25.00µg mL^{-1} can be determined by this procedure.

RESULTS AND DISCUSSION: The reactions of primary and secondary amines with carbon disulphide in the presence of a base to form corresponding dithiocarbamates and the reaction of latter with some metal ions like copper (I), copper (II), cobalt (II) and nickel (II) to form respective coloured metal dithiocarbamates have been studied in our laboratory. Based on these reactions various spectrophotometric methods for the determination of amines and carbon disulphide have also been developed [24]. The advantages of these colour reactions have now been extended to the determination of atenolol and esmolol containing secondary amino function. Of the above metal ions, copper (I) has been preferred in the present work as it not only forms colourless solution in acetonitrile but the colour intensity of metal drug dithiocarbamates is maximum.

The amino function of each drug is transformed into corresponding copper (I) drug dithiocarbamate complexes showing maximum absorbance at 425 and 420 nm respectively and form the basis of the proposed method for the determination of atenolol and esmolol in pharmaceutical formulations. For the adaptation of this method for the determination of atenolol and esmolol in spiked water samples, the copper (I) drug dithiocarbamate is selectively extracted into chloroform and absorbance of the chloroform extract is measured at 415 and 410 nm respectively. During the investigation on the development and stability of yellow colour, it was observed that colour development with drug is slow and colour intensity attains stable values after 15 min.

With a view to speed up the colour development, the reaction was carried out in a microwave oven. Microwave assisted reactions accelerate the reaction rate and is an effective alternative to conventional procedure as there is no loss of analyte and contamination from atmosphere [25]. The absorption spectra of copper (I) drug dithiocarbamates without and after extraction with chloroform are shown in **Fig. 2** and **3** respectively. The plausible reaction mechanism is depicted in **Fig. 4**.

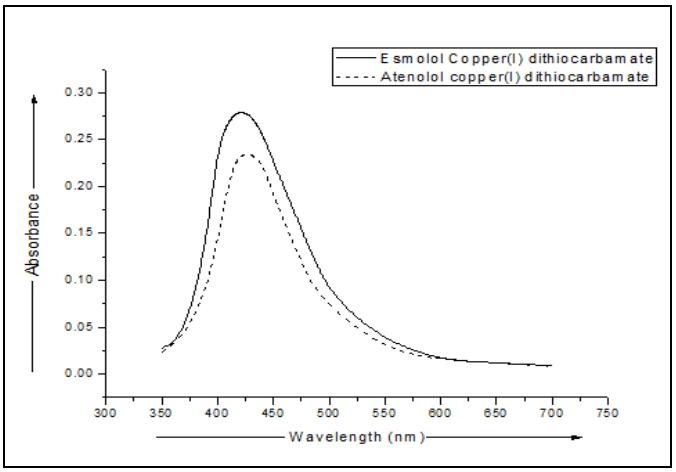

FIG. 2: ABSORPTION SPECTRA OF DRUG AS COPPER (I) DRUG DITHIOCARBAMATE COMPLEX

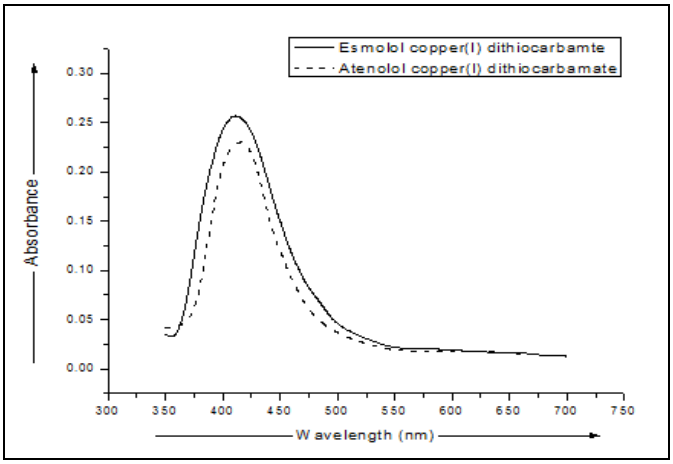

FIG. 3: ABSORPTION SPECTRA OF DRUG AS CHLOROFORM EXTRACTABLE COPPER (I) DRUG DITHIOCARBAMATE COMPLEX

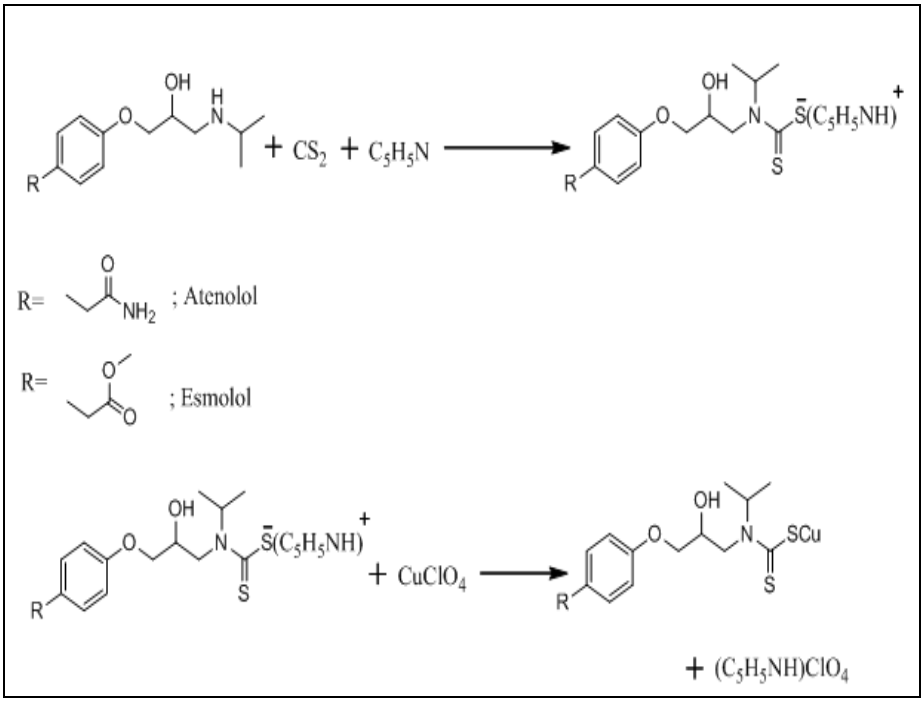

FIG. 4: PLAUSIBLE REACTION MECHANISM FOR THE PROPOSED METHOD

Optimization of Experimental Parameters: The various experimental parameters affecting the colour development and its stability were carefully studied and optimized in order to achieve the maximum sensitivity. The parameters optimized include heating time, effect of reagent volume, effect of extracting solvents and stability of colour.

Effect of Heating Time: The optimum time required in a microwave oven for completion of colour reaction and consequently to obtain maximum colour intensity and its stability was obtained by varying the heating for the time from 10-80 s. The best results were obtained corresponding to 30 s of heating (**Fig. 5**). Reaction time less than 30 s gives less colour intensity.

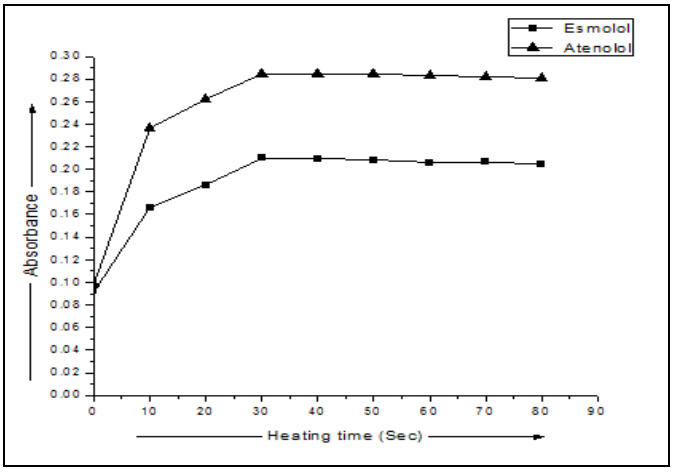

FIG. 5: EFFECT OF HEATING TIME

Stability of Colour: The colour has been found stable for at least 60 min in each case. The kinetic stability of the yellow colour studied with time is shown in **Fig. 6**.

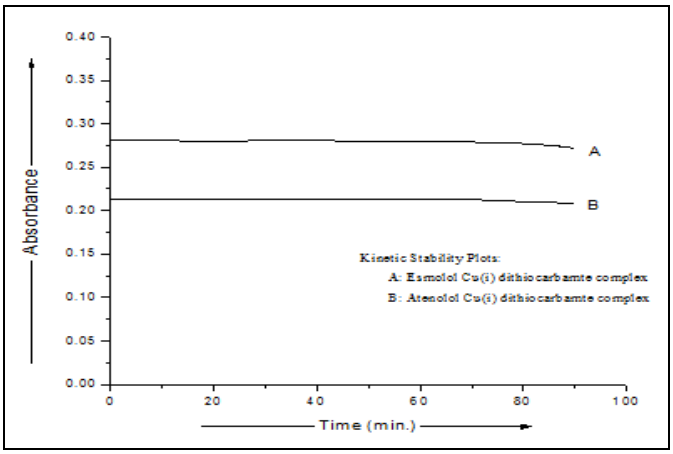

FIG. 6: STABILITY OF COPPER (I) DRUG DITHIOCARBAMATE COMPLEXES

Effect of Reagent Volume and Solvent: The effect of different volumes of particular concentrations of carbon disulphide, pyridine and copper (I) perchlorate on fixed concentration of drugs was investigated. It was found that the reproducible results with highest colour intensity were obtained with 1 drop (~50µL) of carbon disulphide, 1mL of pyridine and 1ml of 10^{-3}M copper (I) perchlorate. Excess addition of reagents causes decrease in the colour intensity.

Both the drugs are more soluble in water than in organic solvents whereas carbon disulphide and copper (I) perchlorate are soluble in organic solvents. Under the situation mixed solvent media for carrying out the reaction was the best choice. Amongst the various organic solvents like methanol, ethanol, DMF, dioxane, acetonitrile and acetone studied, acetonitrile was the best choice owing to its high solvation capacity thus stabilising copper reagent.

Of the various water - acetonitrile systems tested best results in terms of maximum colour and its stability were obtained in 8:2 v/v media.

Effect of Extracting Solvents: Three extracting solvents *viz.* chloroform, methyl isobutyl ketone (MIBK) and ethyl acetate were studied to extract the yellow copper (I) drug dithiocarbamate complex for adaptation to determination of drugs in spiked water samples. Chloroform has been chosen because it extracts the complex almost completely requiring an equilibration time of 5 min and the extract show highest colour intensity and stability (**Fig. 7**).

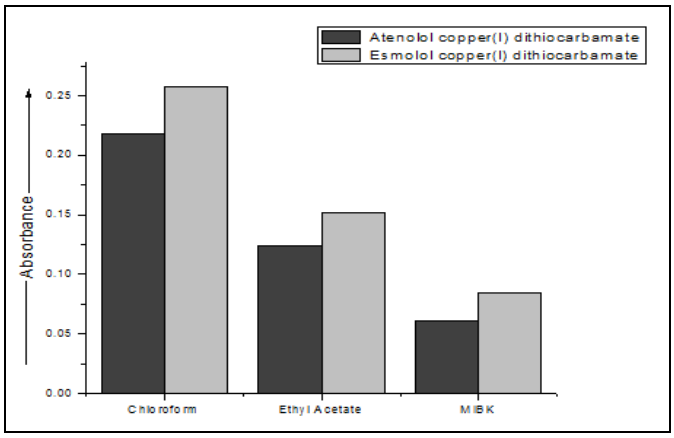

FIG. 7: EFFECT OF EXTRACTING SOLVENTS

Stoichiometry of Colour Reaction: The 1:1 stoichiometry of drug-copper (I) reaction has been established by photometric titrations. The latter is the Job's method of continuous variation. In photometric titrations of each drug (as respective dithiocarbamate derivative) against copper (I) perchlorate at respective wavelengths, absorbance increases till 1:1 drug-copper reagent molar ratio indicating the formation of yellow copper (I) drug dithiocarbamate complex and there after it attains constant values thus establishing the above stoichiometry (**Fig. 8**).

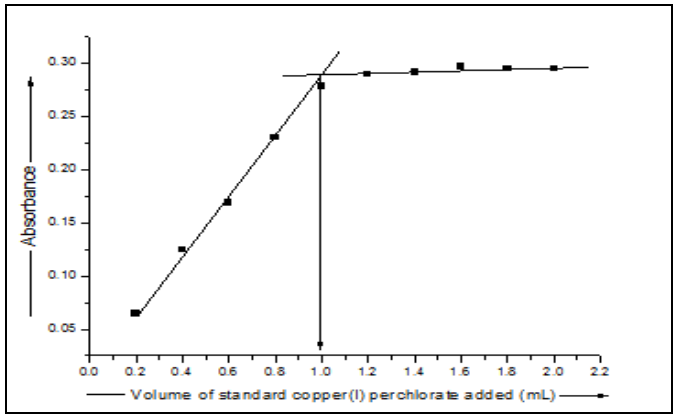

FIG. 8: TYPICAL PHOTOMETRIC TITRATION CURVE OF ESMOLOL (ALSO REPRESENTATIVE OF ATENOLOL) WITH COPPER (I) PERCHLORATE

Validation of Proposed Method: The developed method is validated with respect to linearity, accuracy and precision, limit of detection and quantification according to the guidelines set by International Conference on Harmonization [26]. Under the optimized conditions, the optical and calibration characteristics *viz.* Beer's law range, Sandell's sensitivity, molar absorptivity, slope and intercept values for each drug compound have been calculated and are summarized in **Table 1**.

TABLE 1: SPECTRAL PARAMETERS AND STATISTICAL DATA OF THE REGRESSION EQUATION

Characteristics	Atenolol	Esmolol
λ_{max}(nm)	425	420
Beer's law range (µg mL^{-1})	1.00-22.00	1.50-25.00
Molar absorptivity (ε , L mol^{-1} cm^{-1})	$5.6729x10^3$	$6.6366x10^3$
Stability (min.)	60	60
Slope	0.0177	0.0196
Intercept	0.0340	0.0113
Correlation coefficient (r)	0.9990	0.9987
Sandell sensitivity (µg cm^{-2})	$4.69x10^{-2}$	$5.00x10^{-2}$
Limit of detection (µg mL^{-1})	0.10	0.15
Limit of quantification (µg mL^{-1})	0.31	0.48

Linearity: The proposed method conforms to Beer's law and linear relationship (**Fig. 9** and **10**) in the absorbance-concentration plot obtained in the concentration range of 1.00-22.00µg mL^{-1} and 1.50-25.00µg mL^{-1} for atenolol and esmolol respectively. The regression equations for the plots are as follows. For atenolol Y = 0.0177X + 0.0340, r = 0.9990 and for esmolol Y=0.0196X + 0.0113, r = 0.9987, where Y is the absorbance and X is the concentration of drugs in µg mL^{-1} and r is the correlation coefficient. The results indicate good sensitivity of the proposed method.

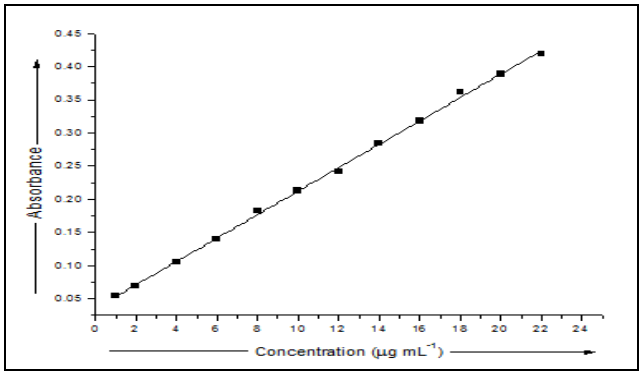

FIG. 9: CALIBRATION GRAPH OF ATENOLOL AS COPPER (I) ATENOLOL DITHIOCARBAMATE COMPLEX

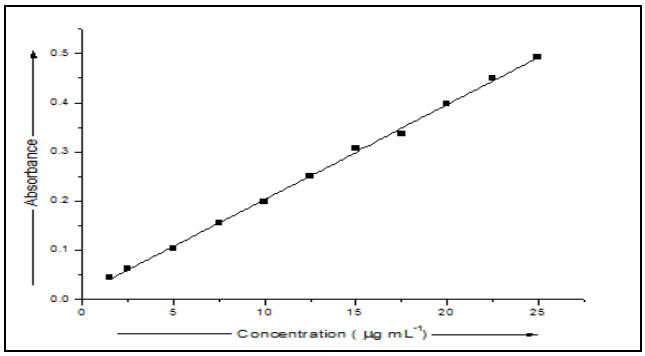

FIG. 10: CALIBRATION GRAPH OF ESMOLOL AS COPPER (I) ESMOLOL DITHIOCARBAMATE COMPLEX

Limit of Detection and Quantification: The limit of detection (LOD) and quantification limit (LOQ) have been determined by evaluating the minimum level at which the active ingredient can be detected and quantified, respectively with reliable accuracy and precision. The following expression was used: LOD = 3.3σ/s and LOQ = 10 σ/s where σ is the standard deviation of the intercept of regression line and s is the slope of calibration curve.

Accuracy and Precision: To test the validity of proposed method, it was applied to the determination of pure sample of atenolol and esmolol over the concentration ranges 2.0 - 18.0 µg $mL^{-1}$with a maximum relative standard deviation (RSD) of 1.20 and 1.01% respectively (**Table 2**). The determination has also been made by photometric titration method in the range 4.2 - 16.8 µgmL^{-1} and 5.2-20.8µgmL^{-1} for atenolol and esmolol with maximum RSD of 0.71 and 0.87% (**Table 3**). The results obtained were in good agreement with those obtained using the comparison methods [6, 7]. The accuracy and reliability of the proposed assay are confirmed by applying the student's t-test.

TABLE 2: APPLICATION OF PROPOSED METHOD TO THE DETERMINATION OF STUDIED DRUGS IN PURE FORMS: DIRECT COLORIMETRIC PROCEDURE

Drugs	Amount taken (µg mL^{-1})	Amount found [a] ± SD (µg mL^{-1})	RE (%)	RSD (%)
Atenolol	2.0	1.99 ± 0.02	0.50	1.00
	6.0	5.98 ± 0.04	0.33	0.67
	10.0	9.92 ± 0.12	0.80	1.20
	14.0	13.87 ± 0.08	0.93	0.58
	18.0	17.95 ± 0.10	0.27	0.56
Esmolol	2.0	1.98 ± 0.02	0.90	1.01
	6.0	5.98 ± 0.05	0.33	0.83
	10.0	9.98 ± 0.05	0.20	0.50
	14.0	13.94 ± 0.10	0.43	0.71
	18.0	17.94 ± 0.04	0.49	0.22

SD standard deviation, RSD relative standard deviation, RE relative error
[a] Mean value of five determinations.

TABLE 3: APPLICATION OF PROPOSED METHOD TO THE DETERMINATION OF STUDIED DRUGS IN PURE FORMS: PHOTOMETRIC TITRATION PROCEDURE

Drugs	Amount taken (µg mL^{-1})	Amount found[a] ± SD (µg mL^{-1})	RE (%)	RSD (%)
Atenolol	4.2	4.18 ± 0.03	0.48	0.71
	8.4	8.32 ± 0.04	0.95	0.48
	12.6	12.64 ± 0.07	0.31	0.55
	16.8	16.74 ± 0.06	0.35	0.36
Esmolol	5.2	5.23 ± 0.04	0.58	0.76
	10.4	10.35 ± 0.08	0.48	0.87
	15.6	15.54 ± 0.09	0.38	0.51
	20.8	20.82 ±0.10	0.09	0.48

[a] Mean value of five determinations

Specificity: The specificity of the proposed method was investigated by observation of any interference encountered from common drug excipients namely lactose, talc, gelatine, gum, acacia, magnesium stearate, maze starch and glucose. The presence of these excipients did not interfere with the proposed method as proved by excellent recoveries obtained.

Application to the Analysis of Drug Formulations: The proposed methods have successfully been applied to the assay of commercial pharmaceutical formulations of atenolol and esmolol (**Table 4** and **5**). The results have been compared by independent methods. The results are in good accordance with maker's specification. The recoveries of active ingredient content are good indicating non-interference of drug excipients in the proposed methods.

TABLE 4: APPLICATION OF PROPOSED METHOD TO THE DETERMINATION OF ATENOLOL IN PHARMACEUTICAL FORMULATIONS

Drug Formulations	Maker's specification[a]	Amount taken (µg)	Direct colorimetric procedure			t-test[c]	Photometric titration procedure			t-test[c]
			Amount found[b] ± SD (µg)	Recovery (%)	RSD (%)		Amount found[b] ± SD (µg)	Recovery (%)	RSD (%)	
Tenolol-50	50 mg per tablet	5.0	4.96 ± 0.07	99.20	1.41	1.28	4.97 ± 0.04	99.40	0.80	1.68
		10.0	10.03 ± 0.09	100.30	0.90	0.74	9.92 ± 0.05	99.20	0.91	1.99
		15.0	14.94 ± 0.12	99.55	0.80	1.11	14.96 ± 0.08	99.70	0.81	0.74
		20.0	20.04 ± 0.16	100.24	0.79	0.55	20.08 ± 0.12	100.40	0.80	1.11
Aten-50	50 mg per tablet	5.0	4.96 ± 0.05	99.12	1.00	1.79	4.98 ± 0.03	99.60	1.21	0.89
		10.0	9.90 ± 0.12	99.00	1.21	1.86	10.06 ± 0.04	100.60	0.80	1.67
		15.0	15.01 ± 0.09	100.08	0.59	0.25	14.95 ± 0.09	99.67	0.60	1.24
		20.0	20.02 ± 0.17	100.10	0.84	0.36	19.94 ± 0.08	99.70	0.85	0.78

[a] Maker's specifications established by an independent method [6].
[b] Mean value of five determinations.
[c] Theoretical t-value at 95% confidence level is 2.78.

TABLE 5: APPLICATION OF PROPOSED METHOD TO THE DETERMINATION OF ESMOLOL IN PHARMACEUTICAL FORMULATIONS

Drug Formulations	Maker's specification[a]	Amount taken (µg)	Direct colorimetric procedure			t-test[c]	Photometric titration procedure			t-test[c]
			Amount found[b] ± SD (µg)	Recovery (%)	RSD (%)		Amount found[b] ± SD (µg)	Recovery (%)	RSD (%)	
Neotach	10 mg per mL	5.0	5.02 ± 0.06	100.40	1.20	0.74	5.02 ± 0.05	100.40	0.99	0.89
		10.0	9.94 ± 0.08	99.40	0.80	1.68	9.94 ± 0.07	99.40	0.70	1.92
		15.0	14.93 ± 0.09	99.53	0.60	1.74	15.08 ± 0.09	100.53	0.60	1.98
		20.0	19.89 ± 0.14	99.45	0.70	1.76	19.93 ± 0.12	99.65	0.85	0.92

[a] Maker's specifications established by an independent method[7].
[b] Mean value of five determinations.
[c] Theoretical t-value at 95% confidence level is 2.78.

Application to the Recovery of Drugs from Spiked Water Samples: The proposed method modified by extracting the colour complex into chloroform has also been applied to the determination of above drugs from spiked water samples. The high recoveries of drugs indicate good accuracy and precision of the method (**Table 6**).

TABLE 6: APPLICATION OF PROPOSED METHOD TO THE DETERMINATION OF DRUGS IN SPIKED WATER SAMPLES

Drug	Amount taken (µg)	Amount found[a] (µg)	Recovery (%)	RSD (%)
Atenolol	5.0	4.92 ± 0.04	98.40	0.81
	10.0	9.88 ± 0.07	98.80	0.71
	15.0	14.92 ± 0.04	99.47	0.25
	20.0	20.04 ± 0.12	100.20	0.60
Esmolol	5.0	4.91 ± 0.04	98.20	0.81
	10.0	10.08± 0.06	100.80	0.60
	15.0	15.06± 0.11	100.40	0.73
	20.0	20.10± 0.14	100.50	0.70

[a] Mean value of five determinations

CONCLUSION: The proposed spectro-photometric method is simple, rapid and sensitive and analysis can be performed by the laboratories of limited means. The method offers the determination of drugs both by direct colorimetric procedure as well as photometric titration procedure. The high accuracy and precision obtained in photometric titrations which are marked by a well-defined intersection at drug to reagent molar ratio of 1:1 also lend support to the stoichiometry of the colour reaction. The photometric titration procedure is more precise and rapid as no calibration curve is required to be prepared but direct colorimetric procedure is more sensitive. The high recoveries of drugs from their commercial pharmaceutical formulations and spiked water samples indicate good accuracy and precision of the method. The instantaneous development of colour and its stability, well established stoichiometry of the reaction and above simplicity and rapidity of procedures are some special attributes of the proposed methods.

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