INDIRECT SPECTROPHOTOMETRIC DETERMINATION OF THE ATENOLOL BY USING TOLUIDINE BLUE DYE

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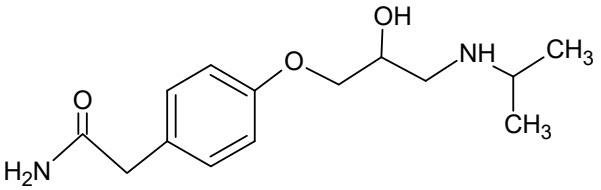
Abstract: *An efficient and eco-friendly indirect spectrophotometric method has been devised to determine the amount of atenolol in both pure form and in tablet formulations. This method relies on the oxidation of atenolol using an excess of standard potassium permanganate in an acidic medium. The remaining oxidant is determined by measuring the decrease in absorbance of toluidine blue dye at a suitable wavelength of 600 nm. The method followed Beer's law across concentrations in the ranges of 0.5-14 µg/mL for atenolol with molar absorptivity of 1.6×10^4 $L\cdot mol^{-1}\cdot cm^{-1}$. The method proved effective in the estimation of atenolol in tablets, and the results were compared favorably with the standard addition method.*
Keywords: *atenolol, determination, potassium permanganate, spectrophotometric, toluidine blue.*

1. Introduction

Atenolol (ATN) is known chemically as 2-[4-[(2RS)-2-Hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]acetamide (Scheme 1). ATN functions as cardio-selective β-blockers, which oppose the effect of adrenaline on the heart and arteries, which reduces the heart rate and contraction of the heart muscle, leading to a decrease in blood pressure and improved heart perfusion. Therefore, it is used in the treatment of hypertension, angina, myocardial infarction, and irregular heartbeat [1-2].



Scheme 1. Atenolol $C_{14}H_{22}N_2O_3$, molar mass: 266.3gm/mole

A review of literature reveals that there are various methods available for the determination of ATN, which include spectrophotometry [3-9], spectrofluorimetry [10-11], HPLC methods [12-13], and electrochemical methods [14-15]. UV-Vis molecular absorption spectrometry is regarded as an easy and practical analytical method for the characterization and analysis of chemical compounds in different fields, including chemistry [16-17] and pharmacy [18]. It does not require expensive instruments or toxic organic solvents. Therefore, the simple and rapid estimation of drugs is still considered important. *The aim of this study* is to validate and develop analytical methods for the determination of ATN of drugs in pharmaceutical formulations so that they are simpler, more sensitive, and more eco-friendly.

2. Experimental part

2.1. Apparatus. The measurements were made using a Shimadzu UV-Visible 1650 PC Double-beam spectrophotometer with a glass cell (1cm). The dissolution process was carried out using an Ultrasonic Cleaner Power Sonic 405 equipped by Tech-Korea.

2.2. Reagents and solutions. The measurements were made using a Shimadzu UV-Visible 1650 PC Double-beam spectrophotometer with a glass cell (1cm). The dissolution process was carried out using an Ultrasonic Cleaner Power Sonic 405 equipped by Tech-Korea.

ATN (100 µg/mL) was prepared by dissolving 0.010 gram in 2 mL of ethanol and completing the volume to 100 mL in a volumetric bottle using distilled water.

Toluidine blue dye solution (200 µg/mL) was created by dissolving 0.020 gram of the dye in 100 mL of distilled water in a volumetric bottle.

Hydrochloric acid (3M), which was prepared by diluting 25 mL of strong acid with distilled water up to 100 mL in a volumetric bottle.

To create a stock solution of $KMnO_4$ at a concentration of 5×10^{-3} M, 0.079 grams of the compound was dissolved in 100 mL of distilled water. To ensure thorough dissolution, the solution was subjected to ultrasonic vibration for 5 minutes in a volumetric flask. The solution was then calibrated using sodium oxalate before being stored in the dark bottle. A solution with a concentration of 2×10^{-3} M was prepared by diluting the original stock solution.

2.3. ATN tablets solution (100 µg/mL). Five tablets of each preparation (each tablet containing 100 mg of atenolol) were crushed and mixed well, and the exact weight of the powder (equivalent to 10 mg) was dissolved in a sufficient amount of distilled water with 2 ml of absolute ethanol and then transferred to a 100 ml volumetric flask, and the volume was made up to the mark with distilled water. The solution was subjected to ultrasonic shaking for 5 minutes until complete dissolution, followed by filtration and determination as described below.

3. Results and discussion

Toluidine blue dye has a blue color in its aqueous solution, which appears at 600 nm. Experimentally, a quantitative oxidation of toluidine blue was noticed in the presence of the oxidizing agent KMnO4. The absorbance of the dye decreased by increasing the concentration of the oxidizing agent by bleaching its color. Based on this characteristic, it makes it possible to indirectly determine the spectrophotometric determination of the ATN drug. The increasing concentration of ATN leads to a decrease in the concentration of KMnO4 for bleaching toluidine blue color. It also leads to an increase of the absorbance (600 nm), which is proportional to the drug concentration (Fig. 1). However, the optimum conditions for the quantitative determination of the above drug have been considered.

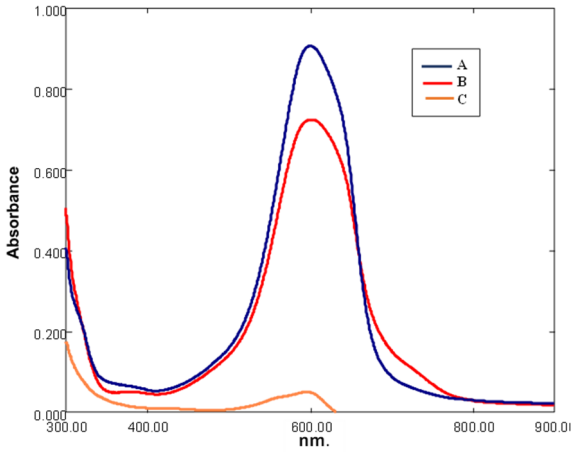


Fig.1. Final absorption spectra of 20 µg/mL of toluidine blue dye in hydrochloric acid medium (A) without and (B)with the presence of $KMnO_4$ (1 mL of 2×10^{-3} M) and ATN (10 µg/mL) (C) reagent blank

3.1. Optimum reaction conditions. *Amount of toluidine blue dye:* In order to calculate the optimal amount of toluidine blue dye needed for estimating the drug compound under study, which

follows Beer's law, increasing amounts of the dye (200 µg/mL) were transferred to 10-mL volumetric flasks, each containing 1 mL of (1M) HCl. The volume was supplemented with distilled water to the mark, followed by measurement of absorbance at a wavelength of 600 nm. According to the standard curve depicted in Fig. 2, the concentration was proven to be 20 µg/mL.

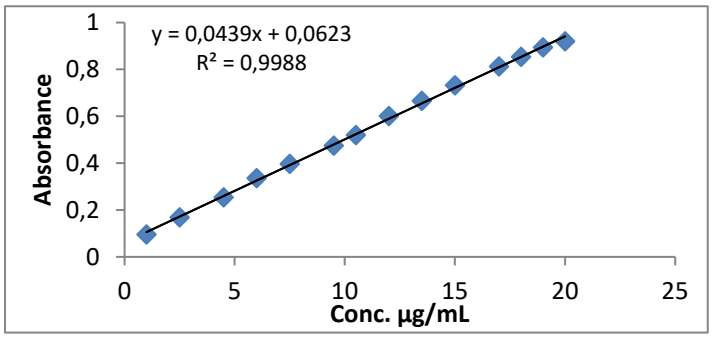


Fig. 2. Standard curve for toluidine blue dye

Selection of oxidant agent: the study involved adding 1 mL of various oxidizing agents with a concentration of 2×10^{-3} M each into a 10 mL volumetric flask already containing 1 mL of 1 M HCl and 1 mL of 200 µg/mL toluidine blue dye. The flasks were filled up to the indicated level with distilled water and left for 10 minutes at room temperature before measuring the absorption at 600 nm. Fig. 3A indicates that potassium permanganate is the most effective oxidizing agent.

Different volumes of potassium permanganate: the effect of the volumes of oxidizing agent $KMnO_4$ (2×10^{-3}M) was studied, different volumes of (0.1-0.9 mL) were used, and it was found that 0.7 mL of $KMnO_4$ can completely bleach toluidine blue dye (Fig. 3B).

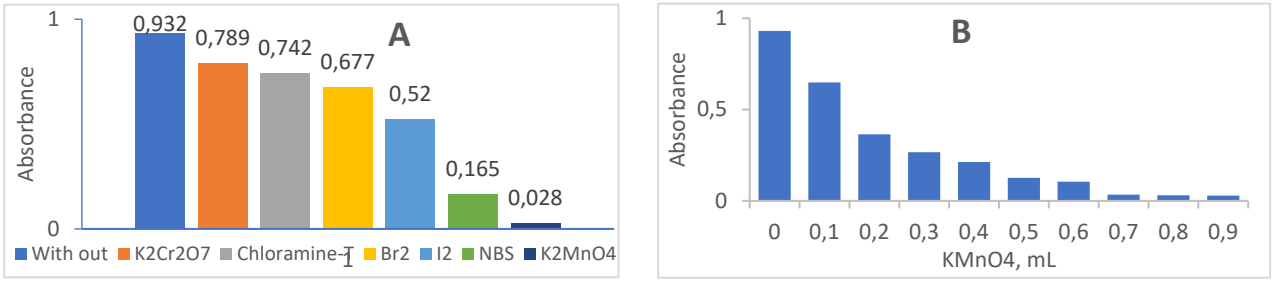


Fig. 3. (A) Effect of various oxidizing agent on dye, (B) Volume of potassium permanganate

Effect of different acid: to identify the optimal acid to analyze the oxidizing processes, 1 ml of different acids listed in Table 1 were tested at a concentration of 1.0 M. They were added to 10 µg/mL of ATN drug with 0.7 ml of oxidizing agent ($KMnO_4$) and left for 20 minutes. 200 µg/mL of toluidine blue dye was added and left for 5 minutes, and then the absorbance was measured at 600 nm. The results appear in Table 1, which shows that hydrochloric acid is the best to give it the highest absorbance.

Table 1. Effect of different acids for the oxidizing processes

Acid (1mL,1M)	HCl	H_2SO_4	HNO_3	H_3PO_4	CH_3COOH
absorbance	0.490	0.412	0.238	0.255	0.034

Effect of the amount of hydrochloric acid: the effect of the amount of HCl required for the ATN oxidation process was investigated. Table 2 demonstrates that 3M of HCl is the most effective concentration, while Fig. 4 indicates that the highest absorption occurs with 1 mL of this concentration.

Table 2. Effect of acid concentration

Molarity of hydrochloric acid	0.5	1	2	3	4	5
absorbance	0.161	0.493	0.518	0.600	0.516	0.524

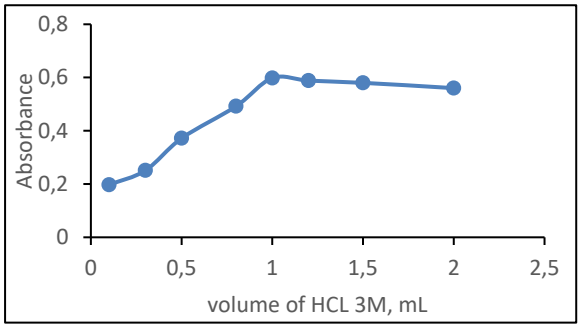


Fig. 4. Effect of the volume of hydrochloric acid

Effect of oxidation time: time is an essential factor in the oxidation reaction. For this reason, the time for the oxidation of ATN was studied. 0.7 mL of $KMnO_4$ (2×10^{-3} M) was mixed with a fixed amount of ATN (10 µg/mL) in 1 mL of 3 M HCl. Then the flasks were shaken and waited for at different times. The 1 mL of 200 µg/mL toluidine blue dye was added and topped up to 10 mL and measured at 600 nm. The results are included in Figure 5A, which indicates that 30 minutes is the optimal time for the oxidation of ATN. Also, 5 minutes was the appropriate time for the oxidation of toluidine blue dye.

Impact of temperature: a study of the reaction's stability across various temperatures, and the results indicate in Figure 5B that the absorbance remains stable for an hour at room temperature (25 ± 2°C).

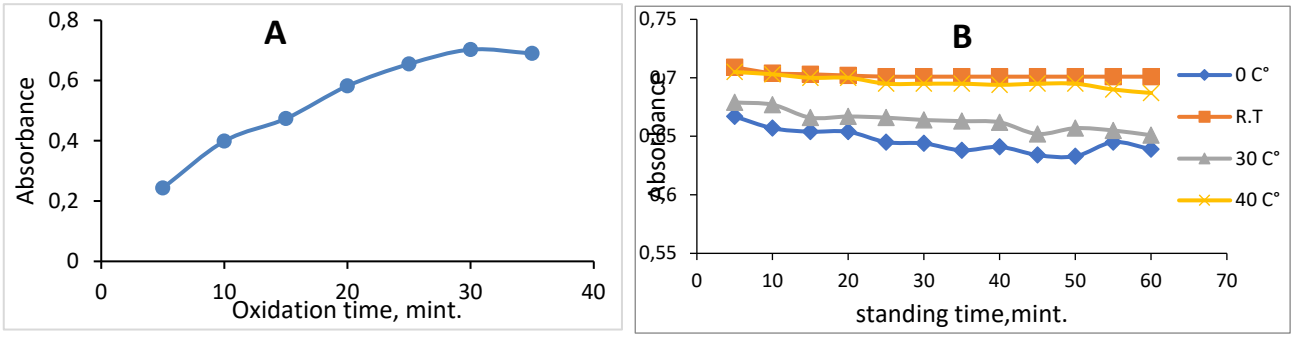


Fig. 5. Effect of oxidation time (A) and temperature (B) on the reaction

3.2. Final calibration curves. Aliquot (1 mL) of 3 M HCl was transferred into 10 mL calibrated flasks containing a standard solution of ATN ranging from (0.5-14) µg/mL. Next, adding 0.7 mL of 2×10^{-3} M solution of $KMnO_4$ and mixed the contents. After allowing 30 minutes to pass, 1 mL of toluidine blue dye was added to each vial. The flasks were shaken and further diluted with distilled water to the mark. The absorbance of each solution was measured against a reagent blank at λ_{max} (600 nm) (Fig. 6). The quantification and detection limits (LOQ and LOD) were determined through replication of blank, as illustrated in Table 3.

Table 3. Analytical parameters

Analytical parameter	**Value**
Beer's low limits (µg/mL)	0.5-14
ε (L/mol.cm)	1.6×10^4
Sandell sensitivity (µg/cm^2)	0.0165
LOQ (µg. mL^{-1})	0.696
LOD (µg. mL-1)	0.2088
R^2	0.9987
Slop	0.0607

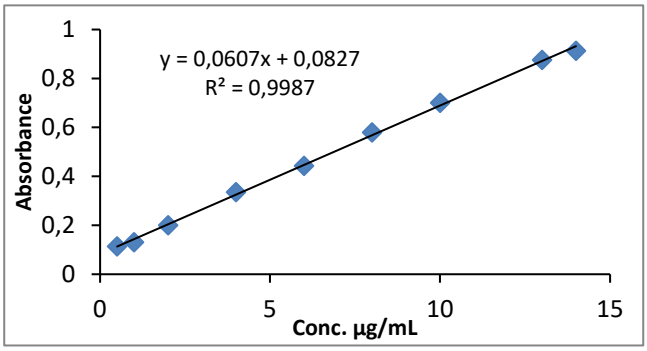


Fig. 6. Calibration curve for estimation of ATN

Accuracy and precision: to evaluate the precision and accuracy of the calibration curve, we prepared solutions with three varying drug concentrations and analyzed them in sets of five replicates. The calculated relative standard deviation (RSD) and recovery percentage are provided in Table 4 and can be deemed satisfactory, particularly for the levels of concentrations studied.

Table 4. Accuracy and precision for proposed method

Conc. of ATN (µg/mL)	RSD* %	Recovery* %	Average recovery %
2	1.880	99.78	99.96
10	0.497	100.50	
14	0.795	99.60	

*Average of five determinations

Suggested reaction mechanism: the suggested mechanism for the reaction between potassium permanganate and ATN or toluidine blue in acidic medium [19] was reported in Fig. 7.

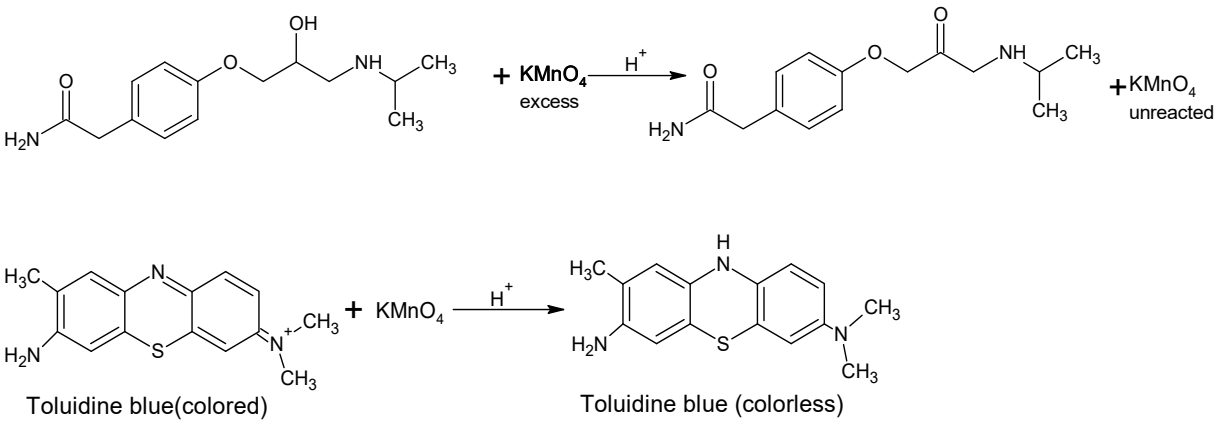


Fig. 7. Suggested reaction mechanism

Quantitative assessment of drug tablets: ewo distinct types of pharmaceutical drug preparations were examined using the recommended procedure. The data presented in Table 5 validated the accuracy and reliability of the present method for the estimation of ATN in tablet formulations. The results show that the recovery percentage using the applying method falls within an acceptable range of 100.98-102.05 for a standard drug sample. The quantity of drugs in tablets was deemed to be within the normal percentage according to the official method. This confirmed the method's validity for analyzing drugs in pharmaceutical preparations.

Table 5. Estimation of ATN in pharmaceutical preparations.

Pharmaceutical formulations	Certified value (mg)	Amount taken (µg⁄mL)	Drug content found (mg)	Recovery (%)	Average recovery (%)
Novaten-100 Ajanta- India	100	1	103.8	103.8	100.98
		2	100.3	100.3	
		5	99.8	99.8	
		10	100.0	100.0	
Atenolol-Bristol Nevada- USA	100	1	103.7	103.7	102.05
		2	101.0	101.0	
		5	103.6	103.6	
		10	99.9	99.9	

Application of standard addition method: The standard addition method involves the addition of different amounts of the studied pure drug to the known amount of pharmaceutical formulation. The results have been documented in Fig. 8 and Table 6. The present method is not likely to interfere with fillers generally formulated with the drugs.

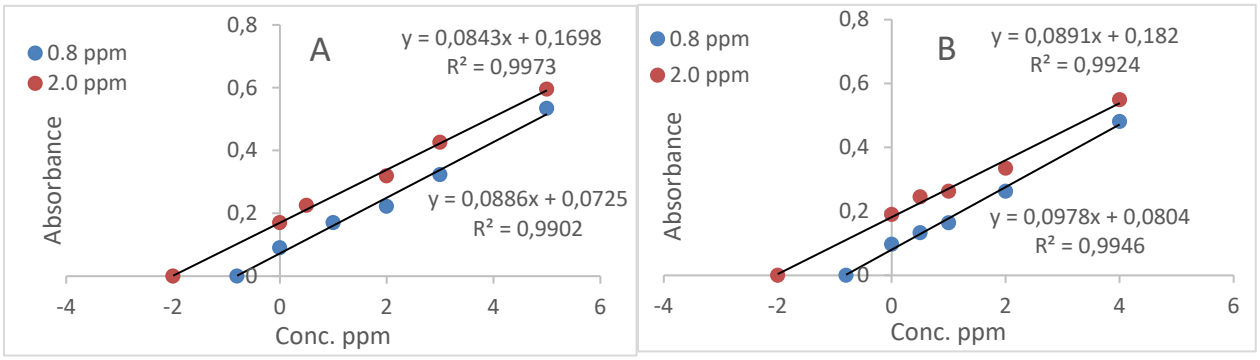


Fig. 8. Standard addition method for determination of ATN in tablets (A) Novaten-100 and (B) Atenolol-Bristol

Table 6. Standard addition method for determination of ATN

Pharmaceutical tablets	Certified Value (mg)	Amount present (µg. mL^{-1})	Drug content (mg)		Recovery of standard addition method (%)
			Present method	Standard addition method	
Novaten-100 Ajanta- India	100	0.8	99.6	102.3	102.3
		2	100.3	100.7	100.7
Atenolol-Bristol Nevada- USA	100	0.8	100.8	102.8	102.8
		2	101.0	102.1	102.1

Conclusion

An eco-friendly method was employed for the estimation of atenolol in tablets. This method is not only simple but also highly accurate and sensitive. It involves a reaction with a non-toxic oxidant and dye without the use of any organic solvents, and the procedure did not require any separation steps.

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Conflicts of Interest: There is no conflict of interest.

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