

Visible Spectrophotometry Method for Quantification of Atenolol Using Cerium IV-Rhodamin 6G Complex

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ABSTRACT

Atenolol is a very important drug used to treat chest pain (angina) and high blood pressure. Due to this, medical importance, a spectrophotometric method is proposed to determine Atenolol in its pure form and pharmaceutical preparations. The proposed method relies on two important steps, the first is the oxidation-reduction reaction between Atenolol and an excess amount of cerium (IV) as an oxidizing agent in the presence of acidic medium, then the second step occurs between unreacted cerium (IV) that was decreased rhodamine 6G absorption intensity. This is an indirect method for estimating Atenolol as it relies on the decreased color intensity of a dye Rhodamine 6G, which is proportional to the increase in the amount of the cerium (IV) in the acid medium at the wavelength of 525 nm. The proposed method follows Beer's law within the range (50–800) $\mu\text{g}/25\text{ mL}$, with good sensitivity relative to the molar absorption coefficient value $2.53 \times 10^4\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ and the Sandell value equal to $0.0105\text{ }\mu\text{g}\cdot\text{cm}^{-2}$. The proposed method has been successfully applied to quantify Atenolol in pure form and its pharmaceutical preparations.

Keywords: Atenolol, Cerium Ion, Pharmaceutical Preparations, Rhodamine 6G.

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INTRODUCTION

The chemical and commercial name of medication are 2-[4-[2-hydroxy-3-(propan-2-yl-amino)propoxy]phenyl] acetamide and atenolol, respectively, atenolol important uses was to treat chest pain, high blood pressure, as well as control of cardiac arrhythmia, and decreasing severity of heart attack.¹⁻³

Through a literary survey, ATL was spectrophotometrically estimated in various reactions such as: oxidation-reduction reaction,^{4,5} charge-transfer complex formation reaction,^{6,7} and other methods have been used non-aqueous acetous perchlorate,⁸ or ratio derivative and dual wavelength.^{9,10} Also spectrophotometric method was based on the reaction of atenolol with reagents like phenol red,¹¹ Methyl orange,¹² crystal violet,¹³ Atenolol was also measured in 0.1 N of hydrochloric acid,¹⁴ perchloric acid,¹⁵ methanol medium,¹⁶ Sodium nitroprusside.¹⁷ Other spectrophotometric methods have estimated this drug in various sample such as: pharmaceutical formulations.¹⁸⁻²¹ In the other hand, methods with various techniques were used to estimate

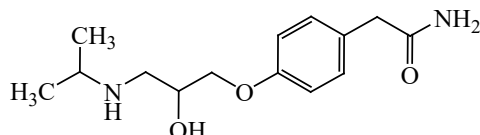


Figure 1: Atenolol structure

ATL such as: flow injection,²³ surface methodology,²² voltametric,²³⁻²⁵ high performance liquid chromatography (HPLC),²⁶ continuous flow membrane coupled with HPLC,²⁷ and HPLC coupled with photo diode array detection,²⁸ and an oxidimetric treatment of atenolol and propranolol with potassium permanganate at $\text{pH} \geq 9$ was carried out spectrophotometrically.²⁹

The organic fluorescent reagent used in this work was Rhodamine 6G (Figure 2), with the chemical name 9-[2-(Ethoxycarbonyl)phenyl]-N-ethyl-6-(ethylamino)-2,7-dimethyl-3H-xanthen-3-iminium. Rhodamine 6G was often used as a trace dye within water to determine the direction and rate of transportation and flow. Rhodamine 6G dye is used widely in the applications of biotechnology like: fluorescence, ELISA, fluorescence microscopy, and correlation spectroscopy.³⁰

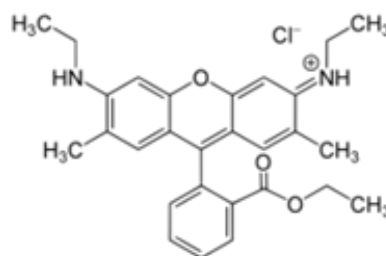


Figure 2: Chemical structure of Rhodamine 6G

The amount of cerium (IV) is proportional inversely to the rhodamine 6G amount; therefore, the increase of cerium (IV) decreased the absorption of rhodamine 6G. Depending on this principle, The first step of the proposed method include the addition of an excess amount of cerium (IV) to the ATL solution in the presence of acidic medium. Then, the remaining amount of cerium (IV) reduces the absorption of rhodamine 6G dye which was measured at 525 nm.

METHODOLOGY

Apparatus and Chemical Materials

Double beam JASCOV-630UV-visible spectrophotometer with 1-cm matched cells was used for all absorbance measurements. pH measurements have been measured using HANA pH meter.

The chemical solutions were prepared with an analytical reagent grade of chemical materials. Atenolol solution, 100 $\mu\text{g}\cdot\text{mL}^{-1}$ (SDI), was prepared by dissolving 0.01 g of ATL in 100 mL distilled water using a suitable volumetric flask. The reagent Rhodamine 6G solution 0.002% (BDH), was prepared when 0.002 g of Rhodamine 6G was dissolved in distilled water using a 100 mL volumetric flask. The oxidizing agent solution 500 $\mu\text{g}\cdot\text{mL}^{-1}$ was prepared by dissolving 0.2255 g of ammonium ceric sulfate (Fluka) in 5 mL of concentrated sulphuric acid, and completed to the mark with distilled water using a 100 mL volumetric flask. Finally, Sulphuric acid solution, 1%, was prepared with an appropriate dilution of concentrated Sulphuric acid with distilled water in 250 mL volumetric flask.

Preparation of Pharmaceutical Dosages

Ten tablets of 100 mg Vascorel tablet Medochemie LTD-Cyprus have been weighed and ground into a fine powder, dissolved in distilled water, then filtered through Whatman No. 42 filter paper. The filter solution was diluted to obtain 100 $\mu\text{g}/\text{mL}$ as the concentration suitable for analysis

RESULTS AND DISCUSSION

Using the oxidation-reduction reaction to estimate ATL, the optimal quantities of each component of the reaction were

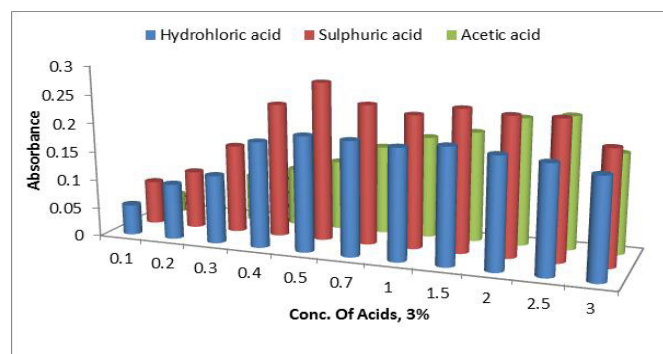


Figure 3: Type and quantity of acids

studied and the optimal amount was selected to obtain a stable color complex using 100 μg of ATL, as follows:

The Optimum Type And Quantity of Acid

Several types of acids were studied, including (acetic acid, hydrochloric acid and sulfuric acid) with a concentration of 3%, where different volumes were added with an amount ranging between (0.1–3.0) mL of these acids as shown in Figure 3.

We conclude from the results shown in Figure 3 that the volume of 0.5 mL of 3% sulfuric acid was chosen as the best depending on the highest absorbance value.

The Optimum Quantity of Cerium (IV)

To estimate the optimal amount of oxidizing agent, ammonium ceric sulphate was prepared at a concentration of 0.7×10^{-2} M, required for ATL oxidation. The various amount between (0.5–3) mL of Ce(IV) were added to volumetric flasks of 25 mL containing (50–500) μg of ATL, then add 0.5 mL of 3% sulphuric acid. Leave this mixture for 20 minutes to complete the oxidation process, and then add the reagent Rhodamine 6G at a concentration of 1.8×10^{-3} M. After diluting all the volumetric flasks to the mark with distilled water, the absorbance intensity was measured at the selected wavelength 525 nm. The experimental results proved that 1-mL of the oxidizing agent Ce(IV) gave the best absorbance value and the value correlation coefficient was 0.97054. Therefore, 1-mL of Ce(IV) was adopted for the subsequent experiments.

Time of Oxidation Process

The time required to complete the oxidation process between each of ATL and the oxidizing agent Ce(IV) ions was studied as shown in Table 1.

The Optimum Amount of Rhodamine 6G

Volumes ranging from (0.5–4) milliliters of reagent R6G at a concentration of 1.8×10^{-3} M were added to volumetric bottles of 25 mL containing different quantities ranging from (50–500) μg of Atenolol. Then the optimal quantities of sulfuric acid, oxidizing agent, cerium ion were added. Waiting for 20 minutes to complete the oxidation process, the absorption intensity was measured at the selected wavelength of 525 nm, where the practical results showed that a volume of 2 mL of the R6G reagent at a concentration of 1.8×10^{-3} M gave the best values for the absorbance and the correlation coefficient. Therefore, this volume was adopted in the subsequent experiments.

The Effect of Surfactants

Several types of different surface tension factors (positive, negative and neutral) have been studied, where sodium dodecyl sulfate (SDS) was used as an example of the negative type. In contrast, cetylpyridinium chloride (CPC) was used as an example of the positive type, and Triton X-100 was used as an example of the neutral type, and through the laboratory results, it was noted that the use of surface tension factors of

Table 1: Effect of time on oxidation process

Oxidation time	5	10	15	20	25	30
Absorbance	0.122	0.167	0.211	0.256	0.251	0.248

all kinds, it harmed the nature of the reaction, so this study was neglected from later experiments.

Order of Addition

The additive sequences were studied using the redox reaction to quantify ATL, and sequence no. I was considered the optimum as it gives the highest value of absorbance, as shown in Table 2.

Stability of the Resulted Color

The effect of time on the color intensity and absorbance intensity was measured at the selected wavelength 525 nm. Under the optimum conditions, the absorbance was recorded at various intervals of time, which indicated that the resulted colored product remained constant after 10 minutes or more than an hour, as shown in Figure 4.

Beer's Law and Final Spectrum

The standard curve of ATL and the final absorption spectrum were studied after fixing the optimal conditions for the determination of ATL. Quantities ranging between 50 to 800 µg of ATL were added to 25 mL volumetric flasks, then an excess amount (1 mL) of the oxidizing agent cerium ion Ce(IV) 0.7×10⁻² M, followed by the addition of 0.5 mL of 3% sulphuric acid, have been added. After a waiting period of 20 minutes to complete the oxidation process, Rhodamine 6G was then added

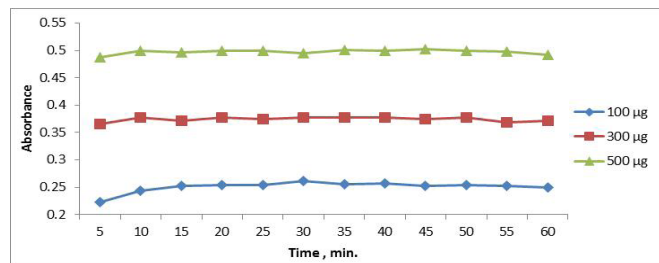


Figure 4: Effect of time

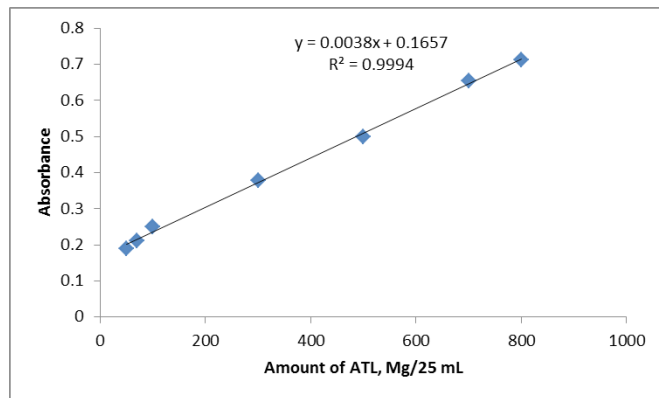


Figure 5: Calibration Curve of Atenolol

Table 2: The sequence of addition

Reaction component	Sequence	Absorbance
ATL+S+Ce+R	I	0.258
ATL+ R+Ce+ S	II	0.041
ATL+S+R+Ce	III	0.029
ATL+ Ce+S+R	IV	0.231

ATL=Atenolol, S=Sulphuric acid, Ce=Cerium ions(IV), R=Rhodamine 6G.

to the reaction components, and the intensity of absorption was measured after diluting the solutions in all volumetric flasks to the mark using distilled water at the wavelength of 525 nm. as shown in (Figures 5 and 6), the proposed method follows Beer's law within the concentration range (50–800) µg. Sandel's significance was within limits 0.0105 µg.cm⁻², and the molar absorption coefficient is within limits 2.53×10⁴ L.mol⁻¹.cm⁻¹ The present method for determining ATL was applied in its pharmaceutical preparations.

Accuracy and Precision:

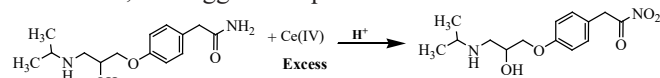
The compatibility of the current calibration curve method for the determination of ATL and for four concentrations has been studied as shown in Table 3.

The results in Table 3 show that the accuracy and precision were reliable.

Nature of the Reactions

The reaction ratio between ATL and Ce(IV) was studied using Job's method (continuous variations method), the obtained results are shown in Figure 7 illustrate that 1:1 was the ratio of ATL to Ce(IV).

So that, the suggested equation was:



Ce(IV) + Rhodamine 6G → Bleaching the color of Rhodamine 6G

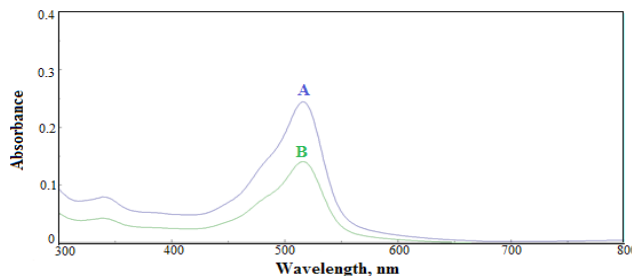


Figure 6: Final absorption spectrum of 100 µg ATL measured against A: reagent blank, B: blank against Distilled water

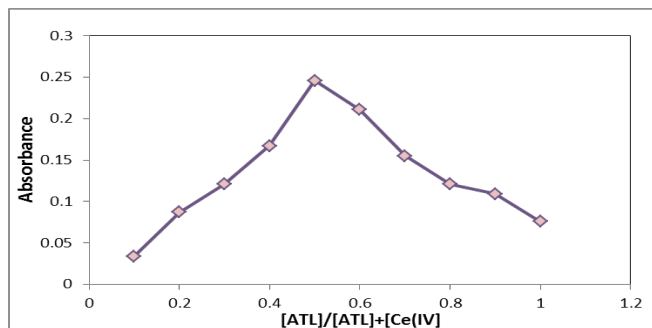


Figure 7: Job's plot for ATL–Ce(IV)

Table 3: Accuracy and precision

Statistical values	Amount of ATL taken, µg/ 25 mL			
	100	300	500	750
Recovery*, %	100.25	99.83	99.74	99.89
RSD*, %	± 0.376	± 0.344	± 0.287	± 0.234

Interferences

During the drug's manufacturing process, there are chemicals added in specific proportions to the medicine to improve the taste, smell, and appearance of the medicine, including for example (gum acacia, glucose, sodium chloride, fructose, starch, and menthol). The effect of these substances on the proposed method for quantifying ATL in its pharmaceutical preparations was studied as shown in Table 4.

We concluded from the experimental results shown in Table 3 that the foreign substances studied did not interfere with the current method for the determination of ATL.

Application of Method

The present method was applied to some of the Atenolol preparations shown in Table 5, which shows good recovery rates for Atenolol when applying the currently proposed method. Some well-known Atenolol preparations are included

The present method was applied to some of the Atenolol preparations shown in Table 5, which shows good recovery values for Atenolol when the present proposed method is applied.

The t-test is one of the important statistical values and the values of the t-test³¹ were calculated by comparing the currently proposed method with a modern spectral method proven in the literature³² as shown in Table 6, which indicates that the t-test did not exceed the theoretical values at the level of 95% confidence in eight degrees of freedom ($N_1+N_2-2=8$).

Comparison of the Method

Table 7 compares the current spectral method with two modern spectroscopic methods for estimating ATL proven in the literature³²⁻³⁴ indicating that the proposed method is sensitive and can be successfully applied to identify ATL in its pharmaceutical preparations.

Table 4: Effect of foreign species.

Interferences	Recovery (%) of 100 µg ATL / µg of interference added			
	100	250	500	1000
Menthol	99.41	99.74	99.04	99.76
Glucose	99.57	99.49	99.41	99.82
Starch	99.49	99.46	99.57	99.74
Acacia	100.19	100.19	100.19	99.89
Lactose	100.27	100.27	100.27	99.67

Table 5: Application of method.

Amount of ATL, µg	Pharmaceutical preparation	Recovery(%) of ATL*	µg of ATL measured/25 mL	R.E*, %
100	100 mg Vascoten tablet Medochemie LTD-Cyprus	99.34	99.87	± 0.3291
250		99.21	298.92	± 0.3095
500		99.17	499.14	± 0.2114

* Average of five determinations

Table 6: The t-test calculations

Drug	Pharmaceutical preparation	t-test	Tabulated t-test
100 mg Vascoten tablet	Medochemie LTD-Cyprus	Tablet	1.7357
			2.571

Table 7: Comparison

Analytical parameters	Present method	Literature method		
		[32]	[33]	[34]
Reaction	Oxidation reduction with bleaching	Charge transfer	Complex formation	Oxidation reduction
λ_{\max} (nm)	525	590	594	610
Reagent	Rhodamine 6G	2,3-dichloro-5,6-dicyano-1,4-benzoquinone	Cr(III)	Indigo Carmin
Beer's law range (µg/ml)	2–32	3.0–48.0	10–90	1.2–60
Molar absorptivity ($\text{l.mol}^{-1}.\text{cm}^{-1}$)	2.53×10^4	5.41×10^3	0.4002×10^4	0.73×10^4
RSD*, %	± 0.2114 – ± 0.3291	0.97–1.56	± 0.34 – ± 0.60	± 0.263 – ± 0.376
ell's sensitivity ($\mu\text{g}.\text{cm}^{-2}$)	0.0105	0.0493	6.99×10^{-2}	0.364
Color of the product	Red	Blue	Blue	Blue
Application of the method	Pharmaceutical preparation	Pharmaceutical preparation	Pharmaceutical preparation	Pharmaceutical preparation

CONCLUSION

A spectroscopic method has been proposed to determine ATL in its pure form and its pharmaceutical preparations, as this method, is characterized by the ease, accuracy, and high stability of the formed color complex. The proposed method depends on an oxidation-reduction reaction, where ATL is oxidized using Ce(IV) in the presence of sulphuric acid, which in turn reduces the intensity of the color of the rhodamine 6G dye, the amount of decrease in the intensity of the dye color was measured, which is proportional to the amount of the Ce(IV), which in turn is proportional to the amount of the medicine ATL, the method was applied successfully for the determination of ATL in its pure form and in its pharmaceutical preparations

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