Natural Dye of Beetroot: An Agent for Spectrophotometric Determination of Atenolol in the Pharmaceutical Formulations

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***Abstract*—In this study, a simple and indirect spectrophotometric method for the quantification of atenolol in pharmaceutical formulations, utilizing a natural food dye extracted from red beet root, is presented. The process involves the oxidation of atenolol in a 1 mol/L HCl acidic medium, using an excess of potassium persulfate. Subsequently, the resulting tablet solution is employed to fade the red beetroot dye, and the solution is measured spectrophotometrically. The optimized reaction conditions consist of a 16 μg/mL atenolol solution, 2.1 mL (100 μg/mL) of potassium persulfate, and 5 mL (100 μg/mL) of red beetroot dye. Spectrophotometric measurements were performed at 535 nm, and the linear range for quantification was found to be 4–22 μg/mL (R^2 = 0.9987). The method exhibited a limit of detection of 0.01 μg/mL. Notably, the proposed method was successfully applied to analyze various commercial brands of pharmaceutical formulations; yielding results consistent with those obtained using the pharmacopeia method. This research offers a valuable and accessible technique for atenolol quantification, demonstrating potential significance in pharmaceutical analysis and quality control processes.**

***Index Terms*—Atenolol, Beetroot dye, Indirect measurement, Redox reaction, Spectrophotometric method.**

I. Introduction

The analytical procedures that are comprised less harmful, non-toxic reagents used, and the wastes produced are important analytical features. This concept is one of the 12 principles of green chemistry regulations and rules (Vidotti, et al., 2006, Anastas, 1999). Consequently, this urges the claim to use ecofriendly reagents and instruments that are less convoluted in relation to consumption of solvents and the possibility to achieve swift and accurate measurements. Red beetroot natural dye is an organic pigment that absorbs selective wavelengths in the range of purple to red color and contains a high concentration of betaines compound which oversees the red color (Nisa, et al., 2021). The red natural pigment of beetroot is extracted in the highest yield when 5 g of the beetroot sample is heated to 100°C in 10 ml distilled water for 10 min at pH 4 (Hussin, 2022). The organic dye that is friendly to the environment has different application in painting, optical, and industrial fields (Thankappan, Thomas, and Nampoori, 2012, Abdelrahman, Abdelrahman, and Elbadawy, 2013).

Hypertension is a disease of the century. Pharmaceutical industries are competing to manufacture drugs for all diseases particularly chronic diseases such as hypertension. A major issue worldwide is the falsification and tampering of medications that are consumed by the populace. The utilization of these pharmaceuticals presents a risk to individuals' well-being. In Brazil, this concern came to light in 1998, when a range of medications including birth control pills, antibiotics, anticancer drugs, and fever reducers was counterfeited with severe outcomes (Pastore and Capriglione, 1998). Therefore, the examination of pharmaceutical compositions aims not only to ensure the quality control in the manufacturing process but also to demonstrate the appropriateness of the product (Pezza, et al., 2000). Furthermore, within the framework of green chemistry, there is a clear need for effective approaches to regulate the quantity of medication present in pharmaceutical formulations. Atenolol, a β1-selective (cardioselective) adrenoreceptor antagonist, is a medication frequently employed in the treatment of hypertension. It is also utilized for the prevention of heart conditions such as angina pectoris and the regulation of certain types of cardiac arrhythmias (Hoffman, et al., 1987). Atenolol is chemically identified as 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy) benzeneacetamide.

Several analytical methods have been recorded to detect the existence of atenolol in pharmaceutical products.

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Various techniques have been reported in scientific literature for quantifying atenolol in pharmaceutical formulations, such as high-performance liquid chromatography (Weich, et al., 2007), high-performance thin-layer chromatography (Argekar and Powar, 2000), potentiometry (Nikolelis, Petropoulou, and Mitrokotsa, 2002, Saad, et al., 2003, Shamsipur and Jalali, 2005), capillary electrophoresis (Bonato and Briguenti, 2005), and voltammetry (Goyal, et al., 2006, Goyal and Singh, 2006). However, many of these methods are lengthy, requiring significant amounts of organic solvents, or necessitate costly and advanced equipment.

Various cost-effective, precise, and resource-efficient methods have been observed for the analysis of atenolol, such as diffuse reflectance spectroscopy (Gotardo, et al., 2008), visible spectrophotometry (Gülcü, Yücesoy and Serin, 2004, Salem, 2002, Al-Ghannam and Belal, 2002, Amin, Ragab and Saleh, 2002, Al-Ghannam, 2006), and UV derivative spectrophotometry (Ferraro, Castellano and Kaufman, 2004). Among these, the UV-visible spectrophotometer remains the most widely used technique globally due to its simplicity, rapidity, accuracy, sensitivity, and suitability for routine pharmaceutical quality control (Wati, Chandra, and Rivai, 2020).

To adhere to the principles of green chemistry, this study employed a spectrophotometer to analyze the presence of atenolol in a pharmaceutical formulation utilizing beetroot natural dye.

II. Materials and Methods

A. Instruments

The measurement of absorbance was conducted using the Agilent Technologies Cary Series UV-Visible double beam Spectrophotometer, which was equipped with a glass cell having an optic path length of 1.0 cm.

B. Materials, Chemicals, and Solutions

A stock solution of atenolol, with a purity of 99.9%, was obtained from Awamedica Pharmaceutical Company in the Kurdistan Region of Iraq and used as received. To prepare a solution of 1000 μg/mL, 0.1 g of atenolol was dissolved in 20 mL of distilled water and warmed for approximately 10 min. The resulting solution was then diluted to 100 mL with distilled water in a volumetric flask. Daily working standard solutions were prepared by diluting the stock solution appropriately with distilled water. A solution of potassium persulfate (100 μg/mL) was prepared by dissolving 0.1 g of solid potassium persulfate (Fluka) in a small amount of warm water and then diluting it to 1000 mL with distilled water. A solution of hydrochloric acid (SCP) with an approximate concentration of 1.0 M was created by diluting 8.3 mL of concentrated HCl in a 100 ml volumetric flask using distilled water. To produce a dye solution of red beetroot with a concentration of 100 μg/mL, 0.1 g of fresh red beetroot was dissolved in 10 ml of distilled water, heated to 100°C for 10 min at pH 4, and then further diluted to 1000 mL using distilled water.

C. Interfering Solutions

Individual solutions of glucose (SCP), lactose (BDH), and starch (Difco) with concentrations of 1000 μg/mL were prepared by dissolving 0.1 g of each respective solid compound in 100 ml of distilled water.

D. Pharmaceutical Formulations

Pharmaceutical tablets from two distinct commercial brands, specifically Novaten (50 mg) and Vascoten (100 mg), were subjected to analysis. These formulations were obtained from local drug stores and underwent testing before their designated expiration dates.

For each pharmaceutical company, 28 tablets of Novaten and 20 tablets of Vascoten were weighed, crushed, and the resulting sample powder from each company was carefully measured and placed into separate 50 mL beakers. Subsequently, the powder was dissolved in 20 mL of distilled water, heated, and continuously stirred for 10 min to improve solubility. Any insoluble excipients were then removed through filtration using Whatman No. 41 membrane filter paper. The filtered solution was further diluted to a final volume of 100 mL in a volumetric flask, using the same solvent.

E. Method

An indirect spectrophotometric method was employed to determine the content of pharmaceutical tablets (Kokhasmail, Tahir, and Azeez, 2020) and atenolol in the previously reported method (Basima, Afyaa, and Najih, 2022). The determination of atenolol involved two steps. In the first step, atenolol was oxidized using an excess of potassium persulfate reagent as the oxidizing agent, under acidic conditions with HCl as illustrated in Fig 1 (Shadjou, et al., 2011). The second step involved the determination of the excess potassium persulfate reagent by measuring the bleaching reaction of the red beetroot food dye in an acidic medium.

F. Analytical Procedure

In a 25.0 mL volumetric flask, either a standard solution or a sample solution containing atenolol (100 μg/mL) was mixed with varying concentrations ranging from 4 to 22 μg/mL, along with 2.1 mL of potassium persulfate solution (100 μg/mL) and 1.0 mL of HCl (1 mol/L). The mixture was vigorously shaken and left to stand for 10 min at a controlled temperature of 40 ± 2°C. Subsequently, 5 mL of red beetroot solution (100 μg/mL) was added, and the mixture was further diluted to the mark with distilled water. The absorbance of the resulting solution was measured at 535 nm, relative to a reagent blank prepared under similar conditions but without the presence of atenolol. This process allows for precise analysis and quantification of atenolol using spectrophotometric measurements.

III. Results and Discussion

A. Absorption Spectra

The molecular absorption spectrum of the red beetroot solution (5.0 mL, 100 μg/mL) exhibited its maximum

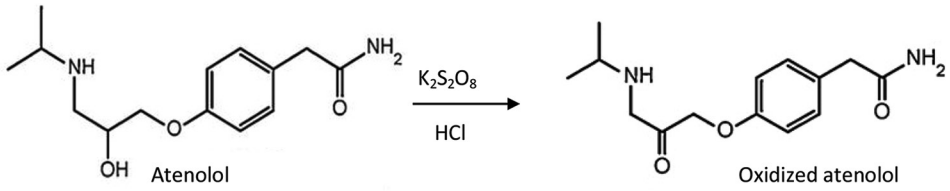

Fig. 1. Oxidation reaction mechanism of atenolol by oxidizing agent potassium persulfate in HCl acidic medium.

absorbance at 535 nm when compared against the blank solution of distilled water (Fig. 2a). On the other hand, a remarkable decrease in absorbance was observed for the resulting bleaching solution containing 10 μg/mL of atenolol at the same wavelength (Fig. 2b). Conversely, the blank solution comprising 2.1 mL (100 μg/mL) of potassium persulfate and red beetroot dye displayed no significant absorbance (Fig. 2c). These observations illustrate the distinctive spectral responses and indicate the effectiveness of the method in detecting and quantifying atenolol in the presence of the specific reagents.

B. Different Proton Providers

The reaction of 16 μg/mL atenolol with 2.1 mL (100 μg/mL) of potassium persulfate and the 5.0 mL (100 μg/mL) solution of red beetroot dye was investigated using different acidic solutions, namely, 1 mol/L of HCl, HNO_3, H_2SO_4, and CH_3COOH, individually (Fig. 3a). Among these acids, Muriatic acid (HCl), being the strongest acid, proved to be the most effective in achieving sufficient oxidation of atenolol and bleaching of the red beetroot dye. The optimal volume of 1.0 mL (1 mol/L) HCl solution was identified and used for subsequent studies (Fig. 3b). These findings highlight the significance of using HCl as the acidic medium to attain the desired reactions for the analysis of atenolol and red beetroot dye in the method.

C. Sequence of Addition

The effect of different sequences of addition for the components, namely, 2.1 mL (100 μg/mL) potassium persulfate, 16 μg/mL atenolol solution, 5.0 mL (100 μg/mL) red beetroot dye, and 1.0 mL (1 mol/L) HCl, was investigated by measuring the absorbance at 535 nm (Fig. 4). The optimal absorbance was achieved when the sequence of addition was as follows: Atenolol, potassium persulfate, HCl, and finally red beetroot dye. These results validate the mechanism of the redox reaction between atenolol and potassium persulfate, followed by the bleaching of the red beetroot dye by the resulting solution from the drug reaction in the acidic medium. The preferred order of addition ensures the most efficient and accurate determination of atenolol content in the presence of red beetroot dye, allowing for reliable analysis using the described method.

D. Bleacher and Dye Concentrations

Elementary experiments were conducted to determine the optimal amount of red beetroot dye to be added, and it was found that adding 5 mL of 100 μg/mL red beetroot dye

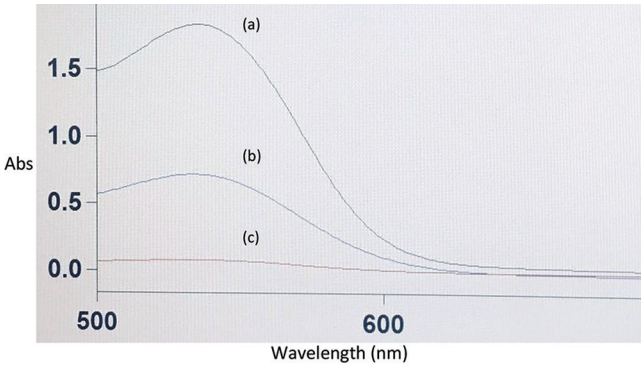

Fig. 2. Absorption spectra of (a) 5.0 mL of (100 μg/mL) red beetroot, (b) 10 μg/mL atenolol, 2.1 mL (100 μg/mL) of potassium persulfate, and 5.0 mL red beetroot dye, and (c) blank reagent of 2.1 mL (100 μg/mL) of potassium persulfate and red beetroot solution against distilled water.

yielded stable and intense absorbance, making it a reasonable concentration for the procedure.

On the other hand, it was observed that higher concentrations of potassium persulfate, exceeding 100 μg/mL, led to diminish in the intensity of the red beetroot dye's color. To establish the appropriate volume for the procedure, different volumes ranging from 0.5 mL to 2.4 mL of 100 μg/mL potassium persulfate were tested under the experimental conditions, and it was determined that 2.1 mL of the oxidant provided the best results, as shown in Fig. 5.

E. Temperature, Time, and Stability

The absorbance of the redox reactions involving 16 μg/mL of atenolol, 2.1 mL (100 μg/mL) of potassium persulfate solution in 1.0 mL acidic medium of (1 mol/L) HCl, along with 5 mL (100 μg/mL) of red beetroot dye, was examined at various heating temperatures and times, as shown in Table I. It was observed that increasing the heating temperature to 40 ± 2°C for 10 min resulted in the maximum absorbance of the resulting bleaching solution. This is attributed to the fact that at higher temperatures, the electrons possess more energy, allowing them to overcome the activation energy required to break bonds with the current molecule and move to the next. In essence, the temperature represents the average kinetic energy of the particles in the reaction system. Therefore, higher temperatures provide more energy, making it easier for electrons to transition between molecules. In addition, increased temperature enhances diffusion rates, leading to more frequent interactions between molecules that have yet to undergo the reaction, thus facilitating the overall reaction process (Meixner, Rennenberg and Kuhn, 2002).

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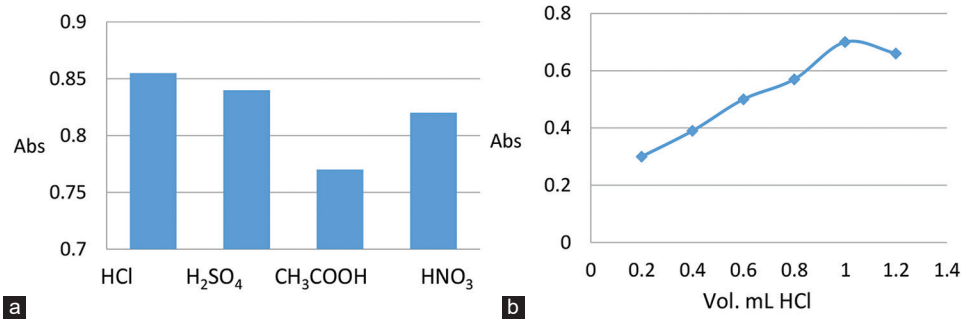

Fig. 3. Absorbance of yielded bleaching solutions of atenolol with (a) 1 mol/L of HCl, H_2SO_4, CH_3COOH, and HNO_3, individually, (b) different volumes (0.2–1.2 mL) of 1 mol/L HCl solution.

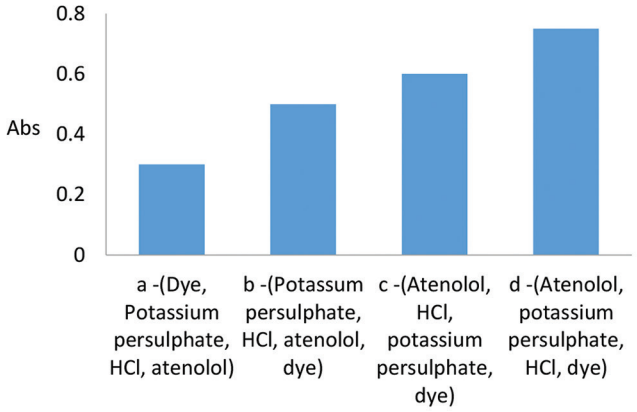

Fig. 4. Absorbance of different sequences of mixing atenolol, potassium persulfate, HCl, and red dye of beetroot.

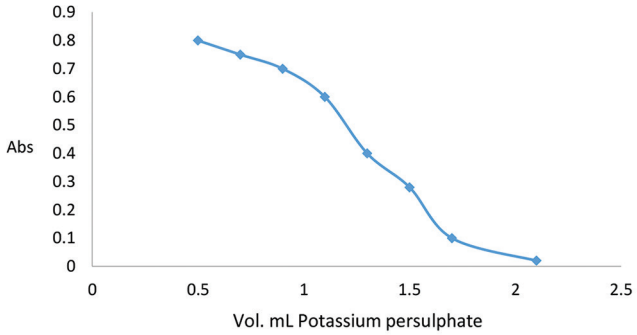

Fig. 5. Amount of bleacher potassium persulfate solution of red beetroot dye in an acidic medium.

Time plays a crucial role in completing the redox reaction of 16 µg/mL atenolol with 2.1 mL (100 µg/mL) of potassium persulfate solution in a 1.0 mL (1 mol/L) HCl solution, as well as the subsequent bleaching of 5 mL (100 µg/mL) of red beetroot dye. The maximum absorbance was achieved after 10 min at 40 ± 2°C, as indicated in Table I. It is notable that a higher temperature near to 50°C causes decomposition of the oxidizing agent potassium persulfate.

Moreover, the color intensity of the resulting bleached dye solution remained stable for at least 24 h at room temperature (25 ± 3°C) after 40 min of completion. This demonstrates that the reaction is not only efficient but also exhibits good stability over time, providing reliable and consistent results.

TABLE I.
HEATING TEMPERATURES AND TIMES OF YIELDED BLEACHING REACTION OF ATENOLOL DRUG OF THE RED BEETROOT DYE

Temperature °C	Absorbance					
	10 min	20 min	30 min	40 min	50 min	60 min
5±2	0.2055	0.2022	0.2043	0.2173	0.1983	0.2076
10±2	0.5673	0.5723	0.4751	0.4832	0.5394	0.4890
25±2	0.7137	0.7109	0.7097	0.7122	0.7096	0.7105
40±2	0.8102	0.8102	0.8079	0.8089	0.8043	0.8076
45±2	0.8101	0.8102	0.8077	0.8085	0.8043	0.8077

TABLE II
COMMON EXCIPIENTS IN THE DETERMINATION OF ATENOLOL WITH THEIR RELATIVE ERRORS

Interference	Allowance concentration (µg/mL)	E %
Lactose	30	−3.97
Glucose	25	1.38
Starch	20	2.70

TABLE III
ACCURACY AND PRECISION OF THE CALIBRATION CURVE

Concentration of atenolol (µg/mL)	Obtained concentration (µg/mL)	SD	RSD%	E%*
4	3.86	0.0001	0.77	−3.50
16	16.08	0.0001	0.01	0.50
22	21.70	0.0010	0.10	−1.36

*Average of five determinations

F. Interferences

Pharmaceutical tablets were prepared with the addition of common excipients to assess atenolol under optimum conditions. The excipient solutions, namely, lactose, glucose, and starch, were each prepared at a concentration of 1000 µg/mL. These solutions were then mixed individually with 16 µg/mL of pure atenolol to achieve a final volume of 25 mL. The obtained spectra were compared with the spectrum of the 16 µg/mL atenolol standard solution.

In the evaluation, it was found that there were no significant levels of interferences, and the errors observed in the determination of atenolol in the presence of common excipients were lower than 5% (Table II). This indicates that the presence of these common excipients in pharmaceutical tablets does not significantly affect the accuracy and reliability of the determination of atenolol using the proposed method under the specified optimum conditions.

TABLE IV
DETERMINATION OF ATENOLOL IN PHARMACEUTICAL TABLETS

Sample	Label to content (mg/tablet)	Found by proposed[a] method (mg/tablet)	t value (2.78)	F value (19)	Found by reference[a] method (mg/tablet)
A	100	101.8±0.6	0.340	4.69	99.7±0.4
B	50	48.5±0.9	0.254	7.88	49.8±0.5

[a]Average±standard deviation (SD), n=3

TABLE V
COMPARISON OF THE PROPOSED AND THE EXISTING VISIBLE SPECTROPHOTOMETRIC METHODS

Method	Linear range (µg/mL)	Detection limit (µg/mL)	Quantification limit (µg/mL)	References
coupling with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole	5–50	1.3	Non	Al-Ghannam, and Belal, 2002
Complexation reaction	3–48	0.26	0.80	Prashanth and Basavaiah, 2012
Oxidation reaction with chromate, indigo carmine dye	1.2–60	0.3259	1.0863	Saleem, 2019
Complex with bromocresol green	2.66–26.63	0.22	0.66	Antakli, Nejem and Joumaa, 2020
Indirect determination Beet root dye bleaching	4–22	0.01	0.60	Suggested method

G. Calibration Curve and Application

An analytical curve conforming to Beer's law was created by employing atenolol standard solutions across a concentration range of 4–22 µg/mL (Fig. 6). The plotted graph of absorbance versus concentration (µg/mL) exhibited a good correlation coefficient (R^2 = 0.9987), indicating a linear relationship between absorbance and atenolol concentration.

The limit of detection for the method was determined to be 0.01 µg/mL, signifying the lowest concentration of atenolol that can be reliably detected and quantified using this spectrophotometric technique. Overall, the analytical curve's characteristics demonstrate the method's precision and sensitivity in accurately determining the concentration of atenolol in the specified concentration range.

In the proposed procedure, accuracy and precision were achieved by measuring the absorbance of the resulting bleaching solution of the red beetroot dye, which indirectly determined the concentration of atenolol at three different levels (4, 16, and 22 µg/mL). Each concentration was subjected to five replicate measurements (Table III).

The suitability of the suggested procedure was assessed based on two key parameters: The relative standard deviation (RSD %) and the relative error (E %) values. By examining the RSD% and E% values for the three different concentrations of atenolol, the procedure's robustness and accuracy can be ascertained. These parameters help to establish the reliability and practicality of the suggested procedure for the analysis of atenolol in pharmaceutical formulations.

The proposed method was employed to assess commercial atenolol tablets, and the obtained results were statistically compared with the tablet contents determined using the UV spectrophotometry technique which is the procedure of the pharmacopoeia analysis (Table IV). The reference method involved the quantitative determination of atenolol using UV spectrophotometry at 275 nm, which required successive dilutions with methanol and heating at 60°C under shaking for 15 min (Gotardo, et al., 2008, Wati, et al., 2020).

The proposed method was applied to several commercially available pharmaceutical tablets containing atenolol, and the

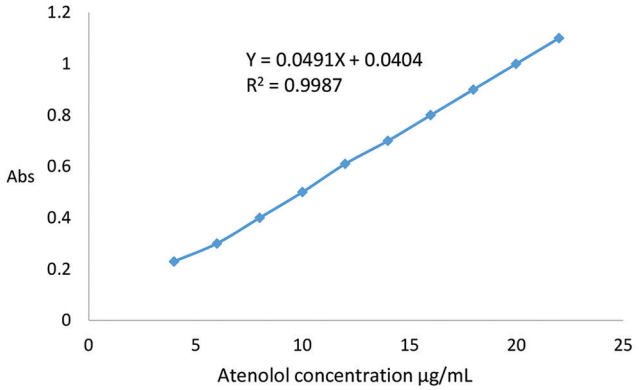

Fig. 6. Calibration curve of indirect spectrophotometric determination of atenolol.

results showed excellent agreement with those obtained using the pharmacopeia method. To further validate the consistency and accuracy of both methods, the results for the formulations assayed by both approaches were compared using the F test and t test at a 95% confidence level.

The calculated F and t values were found to be within the theoretical range, indicating that there is no significant difference between the two methods concerning precision and accuracy. This statistical analysis supports the reliability and suitability of the proposed method for the quantitative determination of atenolol in commercial pharmaceutical tablets and confirms its capability to yield results comparable to those obtained using the established pharmacopeia method.

The results obtained by the recommended method were compared with those in some of the literature corresponding to the linear range of the calibration curve, quantification limit, and detection limit (Table V).

IV. CONCLUSION

The proposed method offers an indirect spectrophotometric approach for accurately quantifying atenolol in tablets. Its notable advantages include its simplicity, utilization of an eco-friendly red beetroot dye, and minimal consumption of reagents and solvents, making it a greener and more cost-

effective option. The method relies on the redox reaction between atenolol and the oxidizing agent potassium persulfate, resulting in the bleaching of the red beetroot dye within the pharmaceutical tablet. The determination of atenolol is accomplished using a spectroscopic technique, which proved successful in yielding reliable results.

By applying the proposed method, atenolol concentrations were determined in tablet samples from various commercial brands. The method exhibited good precision and accuracy, confirming its reliability and suitability for practical use in pharmaceutical analysis.

In summary, the proposed indirect spectrophotometric method presents an efficient and environmentally friendly solution for quantifying atenolol in tablets, making it a valuable tool for pharmaceutical quality control and analysis of different commercial formulations.

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