Spectrophotometric determination of atenolol via oxidation and bleaching color reaction for methyl red dye

S A Zakaria¹, R A Zakaria² and N S Othman¹³

¹Chemistry Department /College of Science / University of Mosul / Mosul, Iraq
²Chemistry Department / College of Education for Pure Science /University of Mosul /Mosul, Iraq
³Corresponding author: Email: nsn20002004@uomosul.edu.iq

Abstract. A selective and sensitive spectrophotometric method has been suggested for the quantitative assay of atenolol (ATNL) as pure and in its manufactural formulation (Tablet). The suggested procedure included oxidation of ATNL with an excess quantity of the oxidant N-bromosuccinimide (NBS), and then the excess of NBS was occupied in bleaching the color of methyl red dye(MRD), then measuring the absorbance of remaining MRD at 518 nm. The absorbance of the unbleached color of MRD corresponds to the ATNL concentration in the sample solution. Beer's law was followed in the range of 0.1-2.0 μg.ml⁻¹ with molar absorptivity value equal to 8.8864x10⁴ L.mol⁻¹ .cm⁻¹. The suggested method was applied to the assay of ATNL in commercial tablets, with satisfactory results.

1. Introduction

Atenolol (ATNL), chemically identified as 2-[4-[(2RS)-2-hydroxy-3-[(1-methylethyl) amino] propoxy]phenyl] acetamide (Figur1). It is a white powder, it is soluble in ethanol but water is sparingly soluble. {[1] ATNL is a selective β1 receptor antagonist, ATNL accumulated evidence suggests that ATNL is not sufficiently effective as a primary tool to treat hypertension[2] and for reducing morbidity and mortality in patients with heart failure [3].

Figure1. Chemical structure of ATNL.

Literature survey exposes that several techniques have been reported for the assay of ATNL, or in combination with other drugs in formulations using flow injection analysis[4-7], HPLC[8-11], spectrofluorometric method[12-14], derivative spectroscopy[15].Various spectrophotometric methods have been reported for determination of ATNL such as: complexation with Fe(III) and Cr(III) ions [16],formation a colored product with bromothymol blue [17] or phenol red[18], condensation reaction with vanillin[19], Schiff's base formation by coupling ATNL with salicylaldehyde[20], ion-pair formation via reaction with bromocresol green in 1, 2-dichloroethane medium[21],oxidative derivatization with KMnO₄ in alkaline medium[22], or in acidic medium[23], gas chromatography-mass spectrometry [24], electrochemical[25] and voltammetric determination[26].
The objective of the investigation reported in this new work is to evaluate a sensitive and accurate method for the determination of ATNL in an aqueous medium. The proposed method relied on the use of spectroscopy measurements, due to the simplicity and accuracy of spectrophotometric methods, and the spectrometer device is cheap and available in most laboratories, in addition to the fact that the proposed method was distinguished by its simplicity and availability of the required chemical materials in addition to that it has high sensitivity compared to some of the spectral methods mentioned in the literature [18-20,22]. The method based on the oxidation of ATNL in an acidic medium by N-bromosuccinimide, then bleaching the color of methyl red dye by the unreacted N-bromosuccinimide which its intensity proportional to the amount of ATNL.

2. Experimental

2.1. Instruments

The absorbance and the spectrums were achieved by using Jasco UV Spectrophotometric (JascoV-630, Japan) using two silica cells, the pH of solutions estimated by using Philips PW 9420 pH meter with a combined glass electrode.

2.2. Reagents

All chemicals used are pure as possible for analytical studies.

2.3. Preparation of solutions

Working ATNL solution, 20 µg/ml
A 0.0100 g of ATNL (provided from general establishment for medical appliance and drugs / SDI – Samaraa / Iraq) was dissolved in 5 ml of absolute ethanol, then diluted to 100 ml with distilled water in the calibrated flask, 20 ml of the above solution was diluted to 100 ml with distilled water in a calibrated flask.

Methyl red dye solution, 3 × 10^{-4} M
A 0.0080 g of MRD (Fluka) was dissolved in 50 ml of absolute ethanol and diluted to 100 ml with D.W. in a calibrated flask.

N-Bromosuccinimide solution, 4 × 10^{-4} M
A 0.0071 g of N-bromosuccinimide (Fluka) was dissolved in 100 ml distilled water in a calibrated flask.

The hydrochloric acid solution, 1 M
An 8.3 ml of concentrated hydrochloric acid was diluted to 100 ml with distilled water in a calibrated flask.

The standard solutions of pharmaceutical formulation

Vascoten tablets solution, 100 µg/ml
A weight equivalent to one tablet (100 mg/Tablet) from five of powder (Atenolol tablets) was dissolved in a 100 ml calibrated flask with distilled water. A solution of 100 µg/ml concentration was prepared by taking 10 ml of the above solution and diluted with distilled water in a 100 ml volumetric flask, 20 and 30 µg/ml standard solutions were prepared by appropriate dilution. The same procedure was applied for the preparation of the standard solution of vascoten tablet.

3. General procedure and calibration graph

To a set of 20 ml volumetric flasks, 0.1 to 2.0 ml of ATNL solution (20 µg/ml) were transferred. A 0.7 ml of HCl (1 M) and 1.5 ml of N-bromosuccinimide (0.0004 M) were added, the solutions were raised at room temperature (25°C) for 25 min., lastly 1.6 ml of MRD solution was added, after 5 min. all solutions were diluted to the mark with distilled water. The absorbance was measured at 518 nm versus the blank. Figure 2 indicated the linearity of the method over the range of concentration from 0.1 to 2.0 µg/ml. The value of molar absorptivity is equal to 8.864 × 10^{4} L mol^{-1} cm^{-1} expresses that the method is sensitive.
Figure 2. The calibration curve of ATNL under the suggested method.

4. Results and Discussion

4.1. Principle of method
The method included oxidation of ATNL by adding an excess of NBS.

The unreacted NBS was used in bleaching the colour of MRD [27].
The residual MRD measured spectrophotometric, and the absorbance is measured at 518 nm, which is increased linearly with an increasing amount of ATNL.

4.2. Optimum conditions
The experiments to achieve optimum conditions for the determination of ATNL included:

4.2.1. Amount of MRD
The experimental results in Figure 3 included that 1.6 ml of MRD is considered an optimum amount according to the high intensity and excellent value of the determination coefficient.

![Figure 3. The effect of MRD amount on absorbance.](image)

\[ y = 0.7514x - 0.0006 \]
\[ R^2 = 0.9971 \]

4.2.2. Type of optimum oxidant
NBS from experimental data was found to be a useful agent, compared with other oxidizing agents such as (NCS and Ce\(^{4+}\)) have been also tested, but none offered real advantages over NBS (Figure 4).

![Figure 4. Bleaching the color of MRD: 1- MRD only, 2 - MRD with NCS, 3- MRD with ceric ammonium sulphate, and 4- MRD with NBS.](image)

The NBS was chosen as an oxidizing agent for the subsequent experiments because it gave the largest dye bleaching compared to that of ceric ammonium sulphate and NCS.

4.2.3. Effect of oxidant amount
Various volumes from 0.5 to 2.5 ml of 0.0004M of NBS were studied without ATEL to bleach the color of MRD. Figure 5 shows that 1.5 ml of NBS solution was the optimum volume and it's enough to get maximum bleaching of MRD color's, therefore it was recommended in the subsequent experiments.
4.2.4. Choice of acid and its amount
The primary experiments have shown that ATNL could oxidize in an acidic medium so that different types of acids were examined (table 1).

Table 1. Selection of acid.

| 1ml of 1M acid | Absorbance | pH   |
|---------------|------------|------|
| H₂SO₄         | 0.204      | 3.24 |
| HCl           | 0.394      | 3.34 |
| HNO₃          | 0.202      | 3.32 |
| CH₃COOH       | 0.189      | 4.08 |

The results above in table 1 shows that HCl gave the highest intensity of the residual MRD and is consider as an acid for the present reaction with an optimum amount of 0.7 ml (table 2).

Table 2. Effect of acid amount on absorbance.

| 1M HCl solution(ml) | Absorbance /µg of ATNL |
|---------------------|-------------------------|
|                     | 15          | 20          | 25          | 30          | R²         |
| 0.3                 | 0.292       | 0.322       | 0.333       | 0.404       | 0.9442     |
| 0.5                 | 0.368       | 0.398       | 0.420       | 0.453       | 0.9973     |
| 0.7                 | 0.412       | 0.420       | 0.436       | 0.477       | 0.9412     |
| 1.0                 | 0.388       | 0.391       | 0.425       | 0.437       | 0.9542     |

The presence of the acid is essential for the completion of the oxidation process of ATNL, and a volume of 0.7 ml of 1M HCl solution was chosen to give it the highest absorbance of the remaining MRD, compared with volumes 0.5 and 1.0 ml, which indicates a complete oxidation of ATNL.

4.2.5. The effect of time on oxidation and bleaching the color of MRD
The effect of the time on oxidation of ATNL by NBS and the time needed for optimum bleaching of MRD color were studied (table 3).
Table 3. The effect of time on oxidation and bleaching of the MRD.

| After addition of oxidant, min. | Standing time before dilution, min | 5  | 10  | 15  | 20  | 30  | 40  |
|-------------------------------|-----------------------------------|----|-----|-----|-----|-----|-----|
| 5                            |                                   | 0.415 | 0.421 | 0.421 | 0.426 | 0.422 | 0.425 |
| 10                            |                                   | 0.447 | 0.442 | 0.434 | 0.438 | 0.436 | 0.446 |
| 15                            |                                   | 0.521 | 0.522 | 0.527 | 0.529 | 0.529 | 0.522 |
| 20                            |                                   | 0.590 | 0.585 | 0.585 | 0.571 | 0.567 | 0.565 |
| 25                            |                                   | 0.616 | 0.616 | 0.620 | 0.616 | 0.622 | 0.620 |
| 30                            |                                   | 0.609 | 0.608 | 0.607 | 0.604 | 0.599 | 0.606 |

The results in table 3 show that the standing time of 25 minutes was necessary for the complete oxidation of ATNL and 5 minutes was necessary for bleaching of the color of MRD.

4.2.6. Order addition of components
The order followed as given under general procedure gave high absorbance so that it fixed in the subsequence experiments (table 4).

Table 4. Effect of the order of addition.

| Order number | Order of addition       | Absorbance |
|--------------|-------------------------|------------|
| I            | ATNL+HCl+NBS+MRD        | 0.626      |
| II           | ATNL+NBS+HCl+MRD        | 0.293      |
| III          | ATNL+NBS+MRD+HCl        | 0.146      |
| IV           | ATNL+HCl+MRD+NBS        | 0.034      |

5. Absorption spectrum
When ATN with 20 µg of ATNL was treated according to the recommended procedure, a decrease in the absorbance compared with an absorbance of MRD alone at the maximum absorption of 518 nm (Figure 6).

Figure 6. The absorption spectrum of (A) MRD, (B) residual MRD after oxidation 20 µg of ATNL, and (C) blank against distilled water.
6. Application of the method
To test the applicability of the present method, it has been applied to the determination of ATNL in pharmaceutical formulation (tablet). In table 5 the values of RE% and RSD% make sure that good accuracy and precision were obtained.

| Drug                  | Amount taken | Recovery, % | RE*, % | RSD*, % |
|-----------------------|--------------|-------------|--------|---------|
| Atenolol 100 mg/tablet| 20           | 100.11      | +0.11  | 0.40    |
| Actavis, Jorden       | 30           | 99.59       | -0.41  | 0.14    |
| Vascoten 100 mg/tablet| 20           | 99.75       | -0.25  | 0.49    |
| Limassol-Cyprus       | 30           | 99.59       | -0.41  | 0.11    |

* The average of four determinations.

The results listed in Table 5 demonstrate the success of the proposed method for estimating ATNL in pharmaceutical formulation (tablet) for two different companies.

7. Estimation of t-test and F-distribution
The suggested method is positively applied for the determination of ATNL in its pharmaceutical formulation (Vascoten tablet). The performance of the suggested method is judged by the application of a t-test at the 95% confidence level for 4 degrees of freedom and F-distribution in comparison with the standard method[1]. The results have been showing that the calculated values for t-exp. and F are less than the tabular values and equal to 1.199 and 7.39 respectively indicating that there is no significant difference between the suggested and standard method for the determination of ATNL.

8. Conclusion
The suggested method described a simple, sensitive and accurate spectrophotometric method for the estimation of ATNL using N-bromosuccinimide as an oxidant agent and the unreacted NBS bleached the MRD color. The ATNL in pharmaceutical preparation has been determined with satisfactory results.

References
[1] British Pharmacopoeia Commission, Great Britain. Medicines Commission, General Medical Council (Great Britain) British Pharmacopoeia (2000).
[2] H. Tomiyama and A. Yamashina. Beta-blockers in the management of hypertension and/or chronic kidney disease. Inter. J. Hyper. 2014,1-7 (2014).
[3] J. Nicolantonio, H. Fares, A. Niazi, S. Chatterjee, F. D'Ascenzo, E. Cerrato, G. Biondi-Zoccai, C. Lavie, D. Bell and J. O'Keefe. β-Blockers in hypertension, diabetes, heart failure, and acute myocardial infarction: a review of the literature. Open Heart 2(1), e000230 (2015).
[4] N. Al-Awadie and A. Khudhair. Determination of atenolol in pharmaceutical formulations by continuous flow injection analysis via turbidimetric (T180°) and scattered light effect at two
opposite position (2N90°) using Ayah 4SW-3D-T180-2N90-Solar-CFI Anal. Iraqi J. Sci. 55(1), 12-26 (2014).

[5] A. Khataee, R. Lotfi, A. Hasanzadeh, M. Iranifam and S. Joo. Flow-injection chemiluminescence analysis for sensitive determination of atenolol using cadmium sulfide quantum dots. Spectrochim. Acta Part A: Mol. and Biomol. Spectr. 157, 88-95 (2016).

[6] N. Turkey and E. Mezaal. Assessment of long-distance chasing photometer (NAG-ADF-300-2) by estimating the drug atenolol with ammonium molybdate via continuous flow injection analysis. Bagh. Sci. J. 17(1), 78-92 (2020).

[7] E. Mezaal and N. Turkey. Assessment of long distance chasing photometer (NAG-ADF-300-2) by estimating the drug atenolol with povidone-iodine via CFIA. Ibn AL-Haitham Journal For Pure and Appl. Sci. 33(1), 65-83 (2020).

[8] H. Hashem, I. Ehab and E. Magda. A novel stability indicating HPLC-method for simultaneous determination of atenolol and nifedipine in presence of atenolol pharmacopeial impurities. J. Appl. Pharma. Sci. 5(08), 017-25 (2015).

[9] A. Raoufi, M. Ebrahimi and M. Bozorgmehr. Application of response surface modelling and chemometrics methods for the determination of atenolol, metoprolol and propranolol in blood sample using dispersive liquid–liquid microextraction combined with HPLC-DAD. J.Chroma. B 1132, 121823 (2019).

[10] W. El-Alfy, O. Ismaiel, M. El-Mammli and A. Shalaby. Determination of atenolol and trimetazidine in pharmaceutical tablets and human urine using a high performance liquid chromatography-photo diode array detection method. Inter. J. Anal. Chem. 20191-8 (2019).

[11] Y. Bilal and A. Sakir. Determination of atenolol in human urine by using HPLC. Sep. Sci. Plus. 1(1), 4-10 (2018).

[12] A. Tabrizi, F. Bahrami and H. Badrouj. A very simple and sensitive spectrofluorometric method based on the oxidation with cerium (IV) for the determination of four different drugs in their pharmaceutical formulations. Pharma. Sci. 23(1),50-58 (2017).

[13] N. Safwat, M. Abdel-Ghany and M. Ayad. Sensitive derivative synchronous and micellar enhanced ecofriendly spectrofluorimetric methods for the determination of atenolol, diclofenac and triclosan in drinking tap water. J.AOAC Inter. 104(1), 103–112 (2020).

[14] E. Bakir, M. Gouda, A. Alnajar and W. Boraie. Spectrofluorometric method for atenolol determination based on gold nanoparticles. Acta Pharma. 68(2), 243-50 (2018).

[15] M. Mohammad, M. Abdullah and S. Sabir. Simultaneous determination of atenolol and amlodipine using second derivative spectroscopy. Polytechnic J. 9(2), 25-9 (2019).

[16] K. Divya and B. Narayana. New visible spectrophotometric methods for the determination of atenolol in pure and dosage forms via complex formation. Indo Amer. J. of Pharma. Res. (IAJ PR) 4(1), 194-203 (2014).

[17] Y. Zhuk and S. Vasyuk. Quantitative determination of atenolol in tablets. Inter. J. of Advances in Pharma. Biol. and Chem. 5(3),350-354 (2017).

[18] I. Elgailani and T. Alghamd. Development of spectrophotometric method for the determination of atenolol in Normoten drug. Int. J. Chem.9 58-64, (2017).

[19] M. Mohamed, E. Mohamed, M. Samy and A. Mai. Spectrophotometric determination of irbesartan,isosartan,atenolol and hydrochlorothiazide in bulk and dosage forms. Asian J. of Pharma. Anal. and Med. Chem. 4(2), 88-106 (2016).

[20] A. Raghad. Spectrophotometric determination atenolol in pharmaceutical preparation by Schiff’s base formation. Kirkuk Univ. J. /Sci. Stud. (KUJSS) 13(2),253-265 (2018).

[21] S. Antakli, L. Nejem and M. Jounaa. Determination of atenolol in tablet formulation by analytical spectrophotometry. Res. J. Pharma. and Technol. 13(2), 609-14 (2020).

[22] E. Vaikosen, J. Bioghele, R. Worlu and B. Ebeshi. Spectroscopic determination of two beta-blockers–atenolol and propanolol by oxidative derivatization using potassium permanganate in alkaline medium. Rev. Anal. Chem. 39(1),56-64 (2020).
[23] H. Sayyed, R. Katapalle, S. Salim and F. Mazhar. Permanganate study of oxidation of atenolol in acidic medium. World J. Pharma. and Pharma. Sci. 6(2),1281-1289 (2017).
[24] Y. Bilal, and A. Sakir. Determination of atenolol in human urine by gas chromatography - mass spectrometry methods. J. of Chromatogr. Sci. 49,365-369 (2011).
[25] M. Shaterian, A. Aghaei, M. Koohi, M. Teymouri and A. Mohammadi-Ganjgah. Synthesis characterization and electrochemical sensing application of CoFe₂O₄/graphene magnetic nanocomposite for analysis of atenolol. Polyhedron. 182, 114479 (2020).
[26] R. Zilberg, V. Maistrenko, L. Kabirova and D. Dubrovsky. Selective voltammetric sensors based on composites of chitosan polyelectrolyte complexes with cyclodextrins for the recognition and determination of atenolol enantiomers. Anal. Meth. 10(16), 1886-94 (2018).
[27] A. Manjunatha, S. Anu and S. Puttaswamy. Oxidative decolorization of methyl red dye with chloramine-T — kinetic and mechanistic chemistry. Indian J. Chem. Tech. 20, 416-422 (2013).