Spectrophotometric study for the determination of trace amount of an important medication used in the cardiovascular diseases

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Abstract:

In this work, a simple, fast, inexpensive and an accurate two methods was suggested for the spectrophotometric estimation of medication used for cardiovascular diseases such as atenolol. The first method was depend on oxidizing-reducing process which was happened between that’s medication and an excess quantity of chromium as an oxidizing agent in acidic medium of 2N sulphuric acid. The residual chromium was then reacted with 1,5-diphenylcarbazide to form a reddish-violet soluble complex measured at 543 nm. while the second method depends on charge transfer reaction between atenolol and alizarin reagent to produce a wine-red water soluble and very stable product measured at 523 nm. Beer's law was obeyed from 1.2 to 40 ppm and 2 to 30 ppm for first and second method respectively. While the sensitivity of the present work was measured using molar absorptivity 0.4661×10^4 and 0.8656×10^4 μg.mol⁻¹.cm⁻¹ of first and second method respectively. Sandell's sensitivity was 0.0571 μg.cm⁻² of first method and 0.0307 μg.cm⁻² for second method. This method was successfully applied to determine atenolol in pharmaceutical preparation.

Keywords: Atenolol, Chromium (VI), Alizarine, 1,5-diphenylcarbazide, Pharmaceutical preparation.

Introduction:

The chemical name of atenolol (ATN) is2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide, chemical formula is C_{14}H_{22}N_{2}O_{3} and the molar mass is 266.431 g/mole (Fig. 1). ATN is a medication of the beta blockers group, primarily ATN have been used in cardiovascular diseases. In 1976 was introduced and developed as a replacement for propranolol for the hypertension treatment, ATN works to slow down the heart and reduced its workload. The most important effective of ATN was reducing blood pressure.[1,2]
Fig. 1: Chemical formula of ATN [1]

Spectrophotometric methods were used in literatures for determination of ATN such as: determination of ATN and amloidipinedrugs in tablet dosage forms and bulk drug using Vierodt’s method[3]. Or using bromination of ATN with bromate-bromide mixture in acid medium [4,5], also, ceric ions (IV) have been used as an oxidizing agent in acidic medium to assay ATN[6,7]. Moreover, titration of ATN in acetic acid with acetous perchloric acid in presence of crystal violet as an indicator[8]. Charge transfer reaction was used for estimated ATN with phenol red reagent at pH 3[9], 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 2,4-dinitrophenol (DNP) and 2,4,6-trinitrophenolreagents [10].Oxidation –reduction reaction also was used to determine ATN with various oxidizing agent such as:potassium permanganate [11] and ferric ions [12].Complex formation reaction have been reported as a reaction leads to form a color products between ATN and Fe(III) chloride and Cr(III) sulphate[13], phosphomolybidic acid[14] Chloramine-T[15]. Titrimetric method combined with spectrophotometric method was used to assay ATN in tablets[16]. ATN was determined in basic medium with sodium nitroprusside[17], pyridine, carbondisulphide and copper(I) in acetonitrile medium[18]. Derivative spectrophotometric method have been reported in literatures to estimate ATN and dual wavelength[19-21]. Finally, simultaneous determination of ATN and ivabradine in synthetic mixture[22]

Alizarin (Alz)and 1,5-Diphenylcarbazide (DPC) (Fig.2)have been used in this work for estimating ATN, DPC is an organic compoundused for colorimetric measurements and considered as an artificial donor used in photochemical and photosynthesis of electron transport. The chromate-1,5-diphenylcarbazide chelate was in fact shows an intense pinkish-violet color at the pH equal to (0.2), depending on this fact ATN was reduced chromate ions to chromium III, then the unreacted chromate was then formed with DPC pinkish-violet complex measured at 546 nm.[23-25]. In the other hand,Alz (Fig.2) also named as mordant or Turkey red was chemically known as 1,2-dihydroxyanthraquinone, Alz is an orange-red crystals or powder, found in the roots madder genus plants. The most commercially an important used of Alz as a red textile dye. Alz was used in this paper as a reagent reacted with ATN through charge transfer reaction to form a red, soluble and a very stable complex measured at 525 nm.[26,27].

Fig.2: Chemical formula of DPC and Alz [23, 27]

Experimental section:

Instruments:

All readings of the intensity of absorption for the colored products were carried out using a computerized JASCO V–630 UV-Visible double-beam spectrophotometer and cells with dimensions of 1cm. The pH readings have been measured using HANA pH-meter.

Chemical materials: All the chemicals used were of the highest purity

Atenolol solution (1000 μg.ml⁻¹): 0.1 g of ATN was dissolved with distilled water using 100 ml volumetric flask. Atenolol working solution was then prepared using appropriate dilution with distilled water from atenolol stock solution.
Potassium Chromate solution ($4.3 \times 10^{-4}$ M): 0.0084 g of potassium chromate (Fluka) was dissolved in 100 ml distilled water using a clean and dry volumetric flask.

Alizarin red solution ($1 \times 10^{-3}$ M): 0.024 g of alizarin red reagent (BDH) was dissolved in distilled water, then completed the volume to 100 ml with distilled water in a volumetric flask.

1,5-Diphenylcarbazide solution ($1 \times 10^{-3}$ M): DPC solution was prepared by dissolving 0.0605 g of DPC (BDH) with distilled water, then completed the volume to 2500 ml using distilled water and a suitable volumetric flask.

Sodium oxalate solution ($1 \times 10^{-2}$ M): using 0.134 g of sodium oxalate (Fluka) to prepare 0.01 M solution used as a catalyst of atenolol oxidation process, when that weight was dissolved in distilled water then, completed to 100 ml with distilled water using a suitable volumetric flask.

Sulphuric acid solution 2N: an appropriate dilution of concentrated sulphuric acid with distilled water was used to prepare 2N of sulphuric acid solution in a 250-ml volumetric flask.

Procedure for dosage forms (100 $\mu$g/ml) solution.

10 tablets [100 mg Vasoten tablet] Edochemi LTD-Cyprus were crushed then, a portion equivalent to 0.01 g weighed and dissolved in distilled water, After stirring and shaken well, the mixture was filtered with filter paper, the final volume was complete to 100 ml with distilled water in a volumetric flask.

Results and Discussion:

To study the optimum condition, 100 $\mu$g of ATN was used in a final volume of 25 ml for methods 1 and 2.

Sulphuric acid effect:

In fact, an intense pinkish-violet color was formed at pH 0.2 which was resulted from chromate-1,5-diphenylcarbazide chelate [23-25]. This fact was used for estimating ATN in pharmaceutical preparations when ATN was oxidized with an excess quantity of chromate, then, the unreacted chromate formed a pinkish-violet complex at pH 0.2 using 2N of sulphuric acid, therefore, a various quantity of sulphuric acid (0.5-4.0) ml have been studied. Table 1 indicating that 2 ml of $\text{H}_2\text{SO}_4$ considered as an optimum quantity for the subsequent experiments, while pH for method two is equal to 5.68 without any addition of acids and bases which was not effect on the color intensity, for this reason, this study was only omitted for method two.

| ml of 2N sulphuric acid | Absorbance | Final pH |
|------------------------|------------|----------|
| 0.5                    | 0.557      | 0.93     |
| 1.0                    | 0.579      | 0.71     |
| 1.5                    | 0.589      | 0.48     |
| 2.0                    | 0.621      | 0.23     |
| 2.5                    | 0.614      | 0.18     |
| 3.0                    | 0.618      | 0.20     |
| 4.0                    | 0.616      | 0.16     |

Oxidizing agent amount:
To check the optimum amount of chromate ions which was consumed by ATN through the oxidation reduction reaction, a various amount (0.3-2.0) ml of (4.3×10⁻⁴ M) chromate ions with (50-500) μg of ATN have been studied. The practical results indicated that 1.0 ml of chromate ions was optimum as it gives the best value of Correlation coefficient (0.9972).

**Effect of catalyst amount:**

Oxalate ions was usually used as a catalyst for the oxidation system of chromium VI [28], So, various amounts (1.0-3.0) ml of 0.01 M C₂O₄⁻² solution with 100μg/25ml ATN have been studied. The practical results indicated that 2 ml of 0.01 M C₂O₄⁻² solution gives the best absorbance value and recommended for the subsequent experiments as shown in Fig. 3.

**Effect of DPC and Alz amount:**

The optimum amount of DPC and Alz for method one and two was studied, the first method showing that 2 ml of DPC was an optimum amount, when (0.5-40) ml of 1×10⁻³ M DPC was studied experimentally. While the second method studied the effect of Alz quantity by the addition of various amount (1.0-4.0) ml of 1×10⁻³ M Alz reagent to the solution containing (50-500) μg.ml⁻¹ ATN. It was resulted that the higher value of absorbance achieved using 3 ml of 1×10⁻³ M Alz, the correlation coefficient value equal to 0.9989, therefore 2 ml of DPC and 3 ml of Alz was used in the subsequent work for both methods respectively.

**Order of addition:**

Order of addition in the first method was studied, the results was tabulated in Table 2 indicating that the first order of addition was optimum to be selected for the subsequent experiments.

| Reaction components          | Order number | Absorbance |
|------------------------------|--------------|------------|
| At + Cr + Ox + S + DPC       | I            | 0.619      |
| At + Ox + Cr + S + DPC       | II           | 0.643      |
| At + S + Cr + Ox + DPC       | III          | 0.673      |
| At + DPC + Cr + S + Ox       | IV           | 0.648      |
| At + Cr + DPC + Ox + S       | V            | 0.687      |
| At + Cr + S + Ox + DPC       | VI           | 0.662      |
| At + DPC + S + Ox + Cr       | VII          | 0.671      |

At=atenolol, Cr=Chromate, S=Sulphuric acid, DPC=1,5-Diphenylcarbazide, OX=Oxalate.
Effect of surfactants

A various kind of surfactants species have been prepared and its effect on the intensity of absorbance have been studied for methods 1 and 2, for this purpose, anionic surfactant (sodium dodecyl sulphate), cationic surfactant (cetyltrimethylammonium bromide and cetylpyridinium chloride), and non-ionic surfactant (Triton X-100, Tween 20 and Tween 80) have been selected. Table 3 showing that all kinds of surfactants do not lead to any important effect for both methods. Therefore, this study was omitted.

| Surfactant                     | Absorbance / ml of surfactant added | Without surfactant |
|-------------------------------|-------------------------------------|--------------------|
|                               | 2.0       | 4.0       | 6.0       | λ_max |                      |
| Cetyltrimethylammoniumbromide | 0.633     | 0.637     | 0.635     | 543   | 0.623                |
| Sodium dodecylsulphate        | 0.628     | 0.626     | 0.612     | 544   |                      |
| Cetyl pyridinium chloride     | 0.639     | 0.630     | 0.627     | 542   |                      |
| Triton X-100                  | 0.643     | 0.637     | 0.631     | 545   |                      |
| Tween 20                      | 0.645     | 0.641     | 0.640     | 543   |                      |
| Tween 80                      | 0.630     | 0.626     | 0.621     | 541   |                      |
| Cetyltrimethylammoniumbromide | 0.201     | 0.203     | 0.204     | 523   | 0.209                |
| Sodium dodecylsulphate        | 0.214     | 0.213     | 0.210     | 523   |                      |
| Cetyl pyridinium chloride     | 0.209     | 0.193     | 0.190     | 521   |                      |
| Triton X-100                  | 0.219     | 0.214     | 0.211     | 524   |                      |
| Tween 20                      | 0.208     | 0.206     | 0.202     | 525   |                      |
| Tween 80                      | 0.197     | 0.204     | 0.207     | 523   |                      |

Stability of the resulted colored complex in both methods:

The stability of the resulted colored complex for method 1 and 2 was studied under the optimum conditions, the effect of time on the absorbance at the selected wavelength at 543 and 523 nm for methods 1 and 2 respectively was shown in Fig. 4, which was showed that the colored complex formed immediately and become stable after 5 and 2 minutes for method 1 and 2 respectively and the absorbance was remained nearly constant more than 2 hours.

![Fig. 4: Stability of the colored complex for Methods 1 and 2](image-url)

Calibration curve:

Calibration curve for determination of ATN in the first method was carried out with a 25 series of flasks contained of a various amounts (0.3-10) ml of 100 μg/ml ATN solution,
2ml of 2N sulphuric acid, 1 ml of 4.3×10-4 M potassium chromate solution, 2 ml of 0.01 M sodium oxalate solution, and 2 ml of 1,5-Diphenylcarbazide solution, after 5 minutes, all the flasks were measured against the reagent blank at the selective wavelength 543 nm. Also (0.5-7.5) ml of ATN solution were used to study the calibration curve in the second method after the addition of 2 ml of 1×10⁻³ M Alz reagent, then the intensity of absorbance was measured at 523 nm against the reagent blank after standing all the calibrated flasks for 2 minutes.

Fig. 5 showing that methods 1 and 2 were followed beer's law in the range of (30-1000) μg/25 ml and (50-750) respectively. While the values of molar absorptivity and sandal's sensitivity were equal to for method 1 and 2 respectively.

Absorption spectrum:

After study the optimum conditions for methods 1 and 2, the final spectra of ATN was showed in figures 6 and 7 below:

![Absorption Spectrum](image_url)
Fig. 7: Absorption spectrum of 100 μg/25 ml ATN for method 2 measured against a: reagent blank, b: reagent blank against distilled water

**Accuracy and precision:**

Two concentrations of ATN were used to check the accuracy and precision values of the suggested methods, the results in Table 4 show that the accuracy and precision were almost reliable.

| Amount of ATN taken, μg/25ml | Recovery*, % | RSD*, % |
|-----------------------------|--------------|--------|
| Method 1                    |              |        |
| 100                         | 100.16 ± 0.0011 |        |
| 200                         | 100.03 ± 0.0014 |        |
| 500                         | 100.05 ± 0.0021 |        |
| Method 2                    |              |        |
| 100                         | 100.09 ± 0.0062 |        |
| 200                         | 100.27 ± 0.0028 |        |
| 500                         | 99.891 ± 0.0021 |        |

* Average of five determinations

**Nature of the reactions:**

The nature of the reaction can be studied in the first and second method by using the continuous variations method (Job’s method). In the first method, the ratio of atenolol to chromate and atenolol to alizarin were 1:1, the same ratio was founded between atenolol and alizarin for second method as shown in Fig.8.

The suggested[28] equations below:
The resulted colored complex in the first method may have the following structure [23,25]:

Also, the probable reaction between atenolol and alizarin reagent is:

Effect of foreign chemicals:
To study the efficiency of the proposed method, several types of chemicals are added to the atenolol in the drug manufactured as colored or flavorful materials for the taste of the drug. The following table shows the results obtained for the first and second methods.

| Drugs | µg Tet. present/25ml | µg Tet. measured/25ml | R*, % | R.E*, % |
|-------|---------------------|-----------------------|-------|---------|
| Method 1 | [100 mg Vascoten tablet) Edochemi LTD-Cyprus] | | | |
| 50  | 50.08 | 100.15 | ± 0.38 |
| 100 | 100.32 | 100.31 | ± 0.31 |
| 300 | 299.38 | 100.18 | ± 0.26 |
| Method 2 | | | | |
| 50  | 49.92 | 99.83 | ± 0.42 |
| 100 | 99.82 | 99.64 | ± 0.35 |
| 300 | 298.79 | 99.41 | ± 0.28 |

Application of the method:
The best recovery values were obtained from applying the proposed methods 1 and 2 to estimate atenolol in pharmaceutical preparations as shown in Table 6.
Drug Amount of ATN, Recovery(%) of B1*

\[
\begin{array}{ccc}
\text{Drug} & \text{Amount of ATN,} & \text{Recovery(%) of B1*} \\
\text{μg} & \text{Method 1} & \text{Method 2} \\
[100 mg Vascoten tablet) Edochemi LTD-Cyprus] & 50 & 100.39 & 99.97 \\
& 100 & 99.67 & 98.91 \\
& 200 & 99.35 & 98.76 \\
\end{array}
\]

* Average of five determinations.

The suggested methods were compared with the literatures[29, 11] by calculating t-test and compared it with t-tabulated (2.571) for five degrees of freedom at the 95% confidence level [30]. Table 7 indicate that there is no significant difference between the suggested methods and the Literatures.

Table 7: The result of t-test analysis.

| Drug                                      | The value of t-test |
|-------------------------------------------|---------------------|
| [100 mg Vascoten tablet) Edochemi LTD-Cyprus] | ±1.426             |
| Method 1                                  | ±1.903              |

Comparison of the methods:

Table 8 exhibited that the accuracy and sensitivity of the current methods for estimating ATN by measuring some of its analytical variables and comparing it with some recent methods found within the literature.

Table 8: Comparison of the present methods with literatures

| Analytical parameters | Present methods | Literatures method | 29 | 11 |
|-----------------------|-----------------|--------------------|----|----|
| Reaction              | Oxidation       | Ion pair           |    |    |
| Oxidant agent         | reduction       | formation          |    |    |
| Reagent               | 1,5-diphenylcarbazide | Bromocresol       |    |    |
|                       | Alizarin        | green              |    |    |
| Medium and pH         | Acidic          | 1,2-di chloroethane | Basic |
|                       | 0.2             |                    |    |    |
| \(\lambda_{\text{max}}\) (nm) | 543             | 523                 | 414 | 610 |
| Beer’s law range (μg/ml) | 1.2-40          | 2-30                | 2.66-26.63 | 2-14 |
| Molar absorptivity (l.mol⁻¹.cm⁻¹) | 0.4661×10⁴      | 0.8656×10⁴          | ×10⁷ | 2.03×10⁴ |
| Sandell's sensitivity (μg.cm⁻²) | 0.0571          | 0.0307              | ------ | 13.1 |
| Recovery (%)          | 100.03-100.16   | 99.891-100.09       | 97.23-101.53 | 99.95-99.99 |
| Color of the product  | reddish-violet  | wine-red           | yellow | bluish-green |
| Application of the method | Pharmaceutical preparation | Pharmaceutical preparation | Pharmaceutical preparation | Pharmaceutical preparation |

Conclusion:
The proposed methods were simple, direct, sensitive and do not require any extraction process. Thus, this method could be readily applicable for the quality control and routine analysis. The first method was depend on oxidizing-reducing process which was happened between ATN and chromium in of 2N sulphuric acid to form a reddish-violet soluble complex with 1,5-diphenylcarbazide measured at 543 nm. The second method based on charge transfer reaction between ATN and alizarin to form a wine-red water soluble and stable product measured at 523 nm. The linearity of the two methods were obeyed Beer's law from 1.2 to 40 ppm and 2 to 30 ppm for first and second method respectively and molar absorptivity $0.4661 \times 10^4$ and $0.8656 \times 10^4 \frac{\text{µg.mol}^{-1}.\text{cm}^{-1}}{}$ of first and second method respectively. As well as, Sandell's sensitivity was $0.0571 \frac{\text{µg.cm}^{-2}}{}$ for first method and $0.0307 \frac{\text{µg.cm}^{-2}}{}$ for second method. These methods were successfully applied for estimation of ATN in pharmaceutical preparation.

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