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Spectrophotometric Determination of Atenolol Using Indigo Carmine Dye

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Abstract

A simple, rapid, accurate and precise spectrophotometric method is proposed for the determination of atenolol in both pure form and in its pharmaceutical formulation. The method is based on the oxidation of atenolol with chromate in acidic medium of 1N H_2SO_4, the residual amount of oxidizing agent is then reacted with indigo carmine dye in the presence of oxalate as a catalyst, the increase in the absorbance of the dye, which is proportional to the amount of the determinants atenolol is then measured at 610 nm. Beer's law is obeyed in the concentration range of 30-1500 µg/25 ml with a molar absorptivity of 0.73×10^4 $l.mol^{-1}.cm^{-1}$, Sandell's sensitivity index of 0.364 $\mu g.cm^{-2}$, and a relative standard deviation of ± 0.263 to ± 0.376% depending on the concentration level. The limit of detection (LOD) and limit of quantification (LOQ) are 0.3259 and 1.0863 µg ml^{-1}, respectively. The proposed method has been applied successfully to the determination of atenolol in pure and pharmaceutical preparation.

Keywords: Atenolol, Indigo carmine, oxalate, Sulphuric acid.

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التقدير الطيفي للاتينولول باستخدام صبغة الانديكوكارمين

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الملخص

اقترحت طريقة طيفية بسيطة وسريعة ودقيقة لتقدير الاتينولول في شكله النقي وفي احد مستحضراته الصيدلانية. تعتمد الطريقة على أكسدة الاتينولول مع الكرومات في الوسط الحامضي من حامض الكبريتيك (1عياري)، ثم تتفاعل الكمية المتبقية من العامل المؤكسد مع صبغة الانديكوكارمين وبوجود الاوكزالات كمحفز، ان الزيادة في الامتصاصية المقاسة للصبغة والتي تتناسب مع كمية الاتينولول تقاس عند 610 nm. ينطبق قانون بير في مدى التركيز من 30 µg/25 ml إلى 1500 وامتصاصية مولارية 0.73×10^{4} $mol^{-1}.cm^{-1}$، ودلالة ساندل للحساسية هي 0.364 $\mu g.cm^{-2}$، وانحراف قياسي نسبي 0.263± إلى 0.376± تبعاً لمستوى التركيز. وبلغت قيمة حد الكشف (LOD) وقيمة حد التقدير الكمي (LOQ) للطريقة 0.3259 µg ml^{-1} و1.0863 على التوالي. تم تطبيق الطريقة المقترحة بنجاح لتقدير الاتينولول في شكله النقي وفي احد مستحضراته الصيدلانية.

الكلمات الدالة: الاتينولول، صبغة الانديكوكارمين، الاوكزالات، حامض الكبريتيك.

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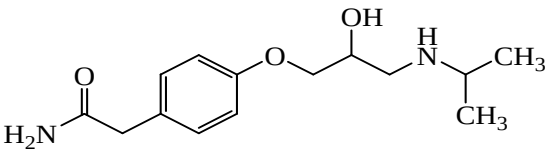
1. Introduction:

Atenolol, ATN (Scheme 1) is a cardioselective β-blocker antagonist, chemically known as 2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide [1]. ATN is used to treat chest pain (angina) and high blood pressure (hypertension). hypertension is also used to decrease the severity of heart attacks and control of some forms of cardiac arrhythmia and for the management of hypertension, prevention of chest pain. It may be used alone or concomitantly with other antihypertensive agents including thiazide-type diuretics, hydralazine, prazosin and α-methyldopa. [2-4].



Atenolol: $C_{14}H_{22}N_2O_3$
M.wt : 266.431 g/mol

Scheme 1: Chemical structur of atenolol

It is necessary to develop analytical methods for the determination of drug in commercial dosage forms because of the great use of drug. Several analytical methods have been reported which are based on the bromination reaction of ATN with a known excess of bromate-bromide mixture in acid medium [5,6] or based on the oxidation of the ATN by a known excess of cerric (IV) in acid medium [7], or titration of the drug in glacial acetic acid with acetous perchloric acid to the visual end point using crystal violet as indicator [8]. UV spectrophotometric methods with sensitive titrimetric used for determination of ATN in tablet formulations [9].

A new spectrophotometric method is depend on the charge transfer reaction of ATN with phenol red at pH 3.0 [10], or 2,3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ), 2, 4-dinitrophenol (DNP) and 2, 4, 6-trinitrophenol (picric acid; PA) as pi-acceptors to give highly colored radical anion species [11], or redox reaction between ATN and $KMnO_4$ in alkaline medium [12], or based on the formation of a colored complex between the drug and metal ions like Fe(III) chloride and Cr(III) sulphate. The maximum absorption wavelength is 454 nm for Fe(III)-ATN complex and 594 nm for Cr(III)-ATN complex [13] or losartan poatassium and ATN with ferroin reagent and methyl orange respectively at pH 7.0 [14]. Also ATN can be

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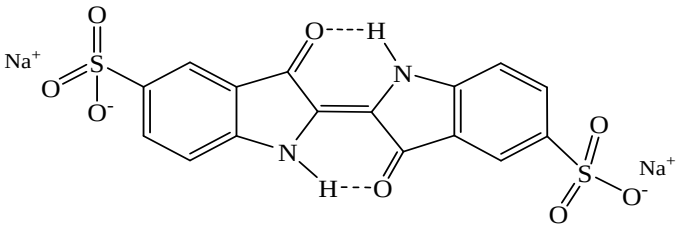
deamined in basic medium, followed by addition of sodium nitroprusside to generate a coloured complex that absorbs at 495 nm [15].

Other spectrophotometric method was based upon the formation of white precipitate for the ion pair compound by phosphomolybidic acid with ATN in aqueous medium [16]. Chloramine-T, metanil yellow and indigo carmine have been used for the determination of ATN spectrophotometrically [17]. Also spectrophotometric method based on the derivatization of the amino function present in ATN to the corresponding yellow copper (I) drug dithiocarbamate derivative through reaction with carbon disulphide, pyridine and copper (I) perchlorate in aqueous acetonitrile [18]. Q-absorbance ratio spectrophotometric method used for simultaneous determination of atenolol and ivabradine hydrochloride in synthetic mixture [19].

A spectrophotometric method has been proposed for the determination of ATN using cerium (IV) in perchloric acid medium. The method is based on measuring the decrease in absorbance of cerium(IV) [20], or based on ratio derivative and dual wavelength [21], also dual wavelength used for estimation of ATN and indapamide in their combined dosage form [22]. Finally, zero-, first-, second- and third-order derivative spectrophotometric methods were developed for quantitave determination of ATN in pharmaceutical preparation [23].

Indigo carmine, I.C. (Scheme 2), 5,5′-indigodisulfonic acid sodium salt. It is a redox and pH indicator, an organic salt derived from indigo by sulfonation and it is soluble in water. Furthermore, I.C. used as a food colorant and also as a dye in the manufacturing of capsules[24].



Indigo Carmine:$C_{16}H_8N_2Na_2O_8S_2$
M.wt : 466.36 g/mol

Scheme 2: Chemical structur of Indigo carmine

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2. Experimental:

2.1 Apparatus:

Spectral and absorbance measurements are carried out using JASCO V – 630 UV-Visible computerized double-beam spectrophotometer. In all measurements, matched cells of 1 cm are used. The pH measurements are carried out using HANA pH meter.

2.2 Reagents And Materials:

All chemicals used are of analytical reagent grade.

2.2.1 Atenolol (1000 µg/ ml) solution:

This solution was prepared by dissolving 0. 1000 g of atenolol in distilled water and the volume was completed to 100 ml with distilled water in a volumetric flask. The solution was then transferred to a dark bottle in order to be stable for at least 2 days. Working solution of 100 µg/ml atenolol solution was prepared by appropriate dilution of the stock solution with distilled water.

2.2.2 Chromate solution, (8.6×10^{-4} M) solution:

This solution is prepared by dissolving 0.0167 g of potassium chromate (Fluka) in 100 ml distilled water in a volumetric flask. The solution was transferred to a dark bottle and it is stable for at least one month.

2.2.3 Indigo Carmine, (1×10^{-3} M) solution:

This solution is prepared by dissolving 0.1165 g of indigo carmine (BDH) indistilled water, then the volume is completed to 250 ml with distilled water in a volumetric flask. This solution is stable for at least 3 days.

2.2.4 Sodium oxalate solution, 0. 1 M:

This solution is prepared by dissolving 1.34 g of sodium oxalate (Fluka) in distilled water then is the volume completed to the mark with distilled water in a 100-ml volumetric flask.

Sulphuric acid solution, 1N. This solution is prepared by appropriate dilution of concentrated sulphuric acid solution to the mark with distilled water in a 250-ml volumetric flask.

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3. Results and Discussion:

3.1 Study of optimum conditions:

The effect of various parameters on the oxidation-reduction reaction and the intensity of the coloured complex has been studied and optimum conditions have been selected.

3.2 Effect of sulphuric acid amount

In order to choose the optimum amount of sulphuric acid for the reaction of chromate with atenolol and indigo carmine, different amounts (0-3.5) ml of sulphuric acid solution (1N) are tested. The results are shown in Fig.1 indicate that 1.0 ml of 1N H_2SO_4 is considered optimum (pH =3.8), as it gives the more stable coloured dye. Therefore, it is recommended for subsequent experiments.

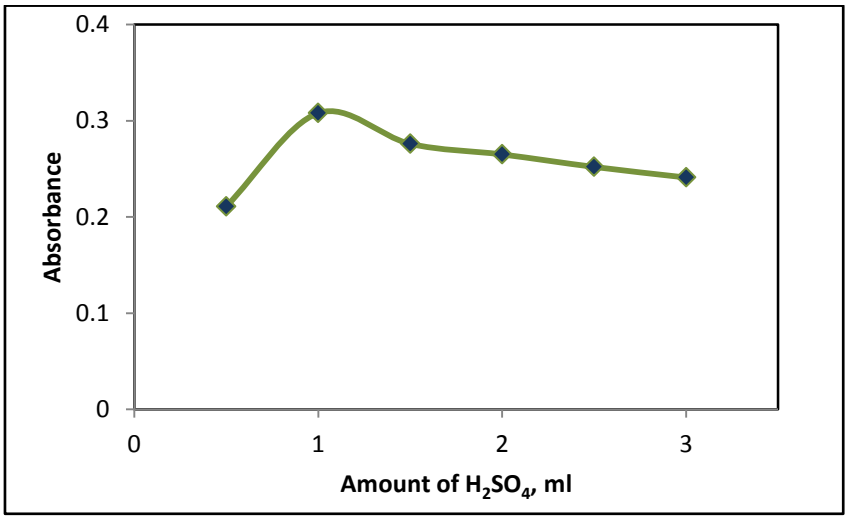

Fig. 1: Effect of sulphuric acid.

3.3 Effect of oxalate ion amount

Oxalate usually served a promoting activator or catalyst for the oxidation system of chromium (VI) [25], therefore, different amounts of oxalate ion solution 0.1 M (0.5-5.0) ml with (100) µg/25ml atenolol are studied and it was found from the experimental results that 2 ml of oxalate ion solution which give best abosorbance was optimum and recommended for the subsequent experiments as shown in Fig. 2.

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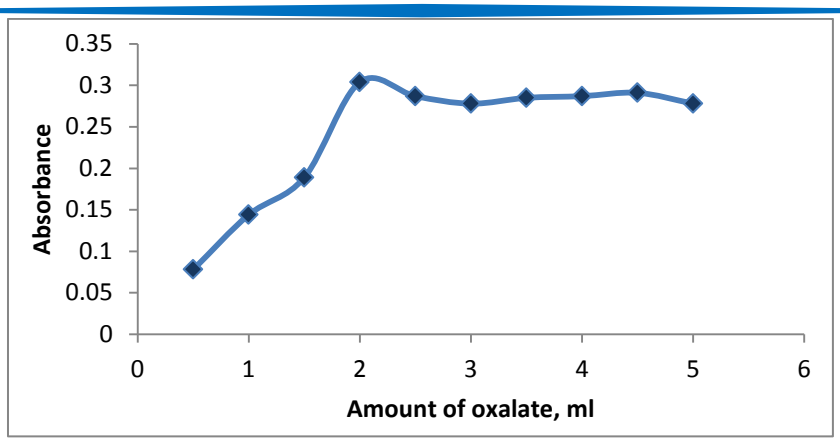

Fig. 2: Effect of oxalate ion.

3.4 Effect of chromate ion amount

Different amounts (1.0-2.0) ml of chromate (VI) ion solution 8.6×10^{-4} M with different amounts (50-1000) µg/25ml of atenolol are studied and it was found from the experimental results that 1.5 ml of chromate solution which gives higher value of determination coefficient (0.997139) was optimum and recommended for the subsequent experiments as shown in Table1.

Table 1: Effect of chromate (VI) ion.

ml of 8.6×10^{-4} M chromate solution	Absorbance / µg atenolol in 25 ml						r^2
	50	100	250	500	700	1000	
1.0	0.189	0.271	0.361	0.411	0.494	0.544	0.960312
1.5	0.269	0.308	0.332	0.438	0.509	0.609	0.997139
2.0	0.211	0.268	0.301	0.409	0.454	0.592	0.993536

3.5 Effect of Indigo carmine amount

The effect of the amount of indigo carmine dye on the absorbance of the reaction mixture is investigated. It was found from the experimental results that 1.0 ml of indigo carmine reagent 1.0×10^{-3} M was optimum (determination coefficient =0.995441), and recommended for the subsequent experiments as shown in Table 2.

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Table 2: Effect of Indigo carmine amount.

ml of 1.0×10^{-3} M Indigo Carmine solution	Absorbance / µg atenolol in 25 ml						r^2
	50	100	250	500	700	1000	
0.5	0.166	0.253	0.345	0.411	0.473	0.526	0.954183
1.0	0.278	0.302	0.321	0.442	0.512	0.611	0.995441
1.5	0.188	0.222	0.287	0.387	0.422	0.569	0.993950

3.6 Effect of order of addition:

The different orders of addition were studied. The results shown in Table 3 indicate that the first order was optimum because it gives highest absorbance value and best stability (because it is the best order of addition that make Cr(VI) react with At then the residual of it react with I.C), therefore it is selected for the subsequent experiments.

Table 3: Effect of order of addition.

Reaction components	Order number	Absorbance
At + Cr + Ox+ H + I.C	I	0. 309
At + OX + Cr + H + I.C	II	0.282
At + H + Cr + OX + I.C	III	0.273
At + I.C + Cr + H + OX	IV	0.108
At + Cr + I.C + OX + H	V	0.187
At + Cr +H + OX + I.C	VI	0.262
At + I.C +H + OX + Cr	VII	0.111

At= atenolol, Cr=Chromate, H=Sulphuric acid, I.C=Indigo carmine, OX=Oxalate.

3.7 Development time and stability period:

To test the effect of time on the absorbance for different amounts of ATN at the wavelength of maximum absorption at 610 nm, under the optimum experimental conditions, the absorbances were measured at different intervals of time. The experimental results are shown in Fig.3 indicating that maximum absorbance is obtained immediately and remains constant for at least 2 hours.

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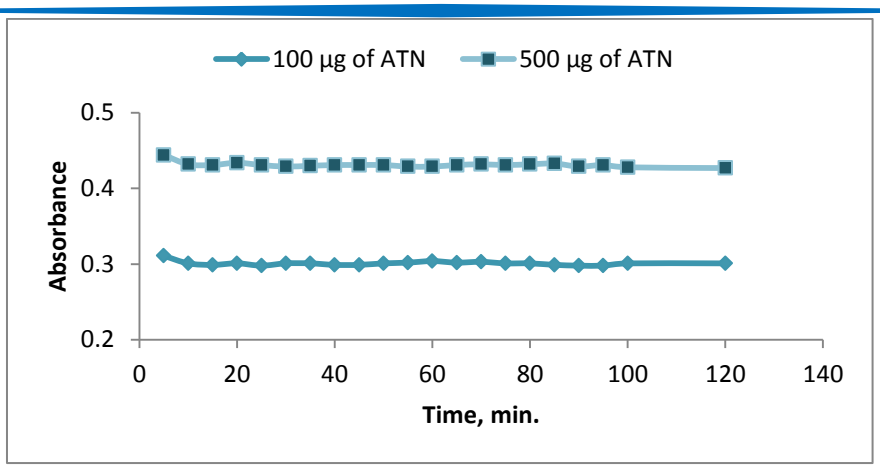

Fig. 3: Effect of time and stability period.

3.8 Absorption spectra and calibration graph:

When atenolol is treated according to the recommended procedure, the absorption spectra and calibration graph are shown in Fig.4 and 5. The sample solution shows maximum absorption at 610 nm. A linear calibration graph is obtained over the range (30-1500)µg / 25 ml with molar absorptivity 0.73×10^4 l.mol^{-1}.cm^{-1} and sandell sensitivity 0.364 µg.cm^{-2}. The limit of detection (LOD) and limit of quantification (LOQ) are 0.3259 and 1.0863 µg ml^{-1}, respectively

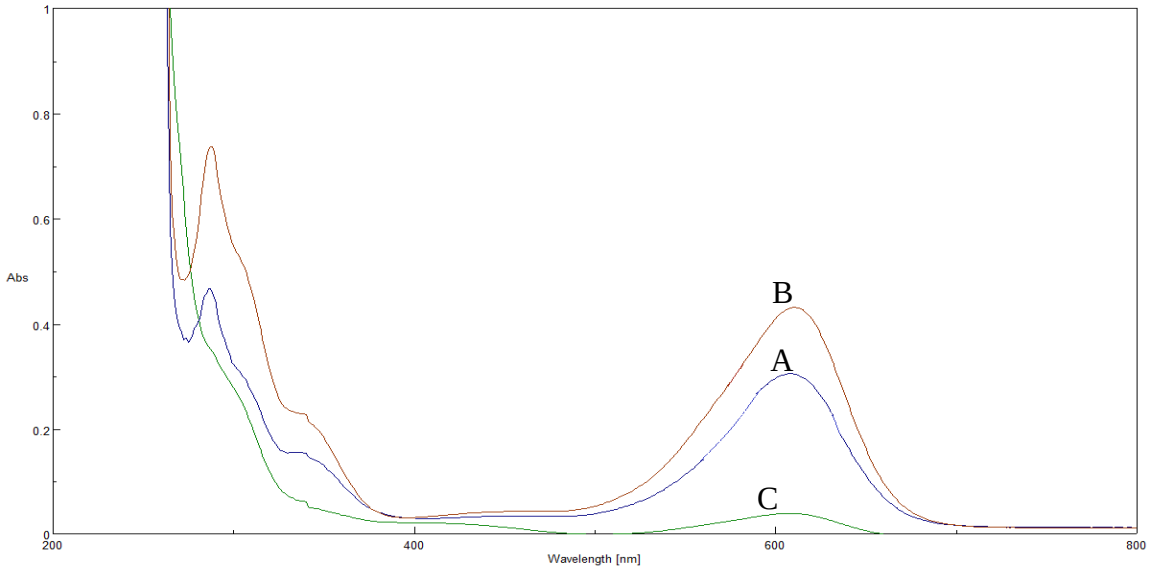

Fig. 4: Absorption spectra of (A) 100 µg of atenolol /25 ml measured against reagent blank, (B) distilled water, (C) blank against distilled water.

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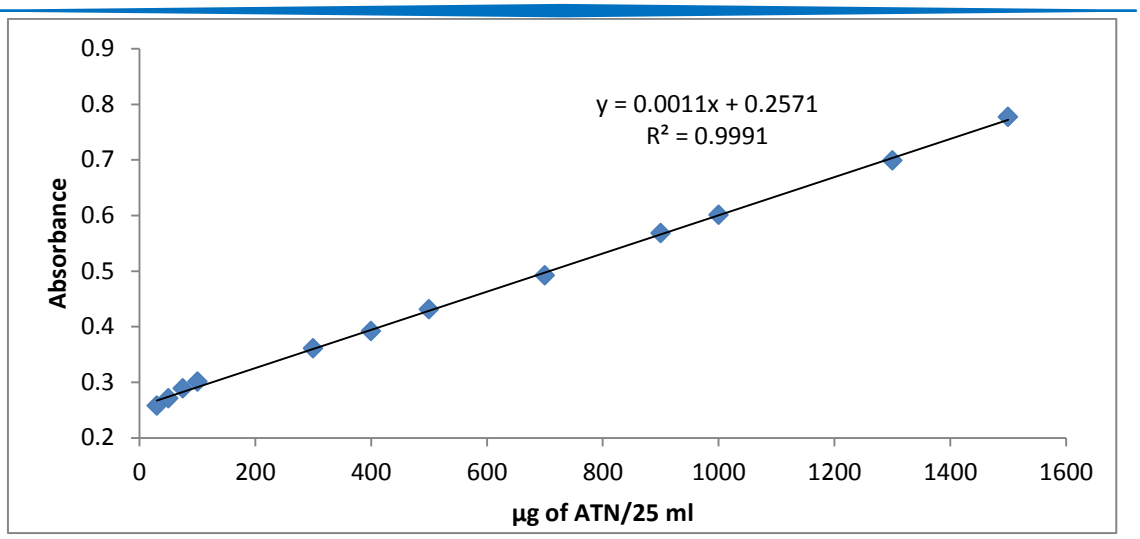

Fig. 5: Calibration graph of ATN.

3.9 Accuracy and precision:

To check the accuracy and precision of the calibration curve, ATN is determined at three concentrations. The results shown in Table 4 indicate that these are reliable.

Table 4: Accuracy and precision.

Amount of ATN taken, µg /25ml	Recovery*, %	RSD*, %
100	100.13	± 0.376
300	100.33	± 0.342
500	100.09	± 0.263

* Average of five determinations.

3.10 Nature of the reactions:

Job's method of continuous variations has been used in the determination of the reaction ratio of atenolol with chromate. The obtained results Fig. 6 showed that the ratios of atenolol to chromate is 1:1.

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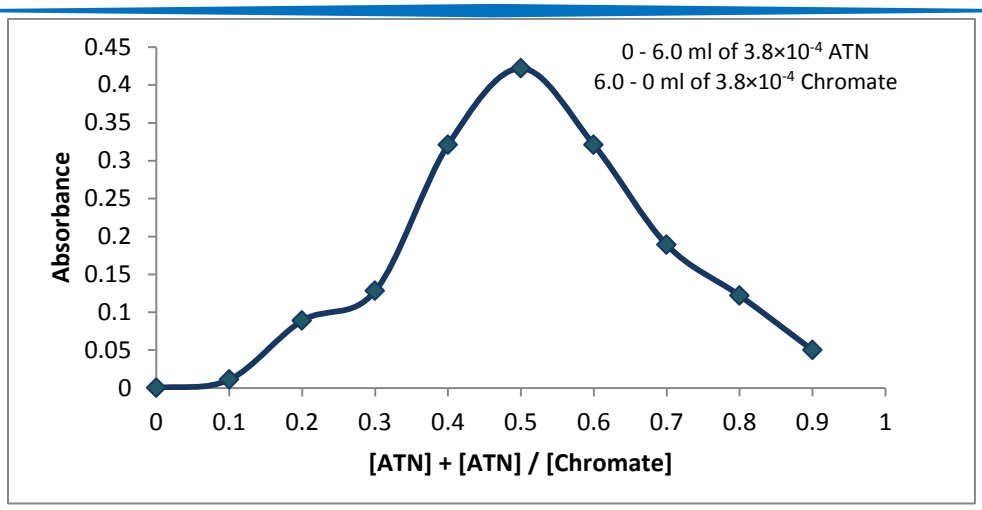
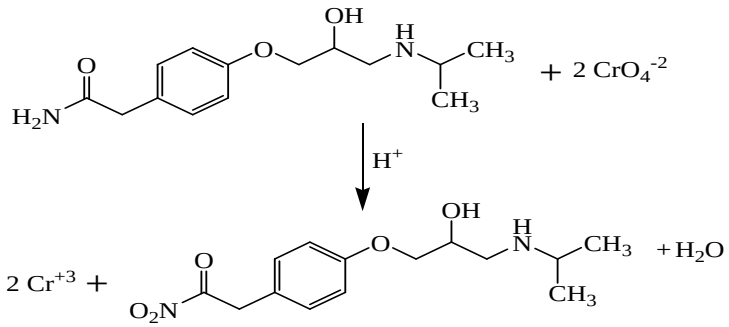
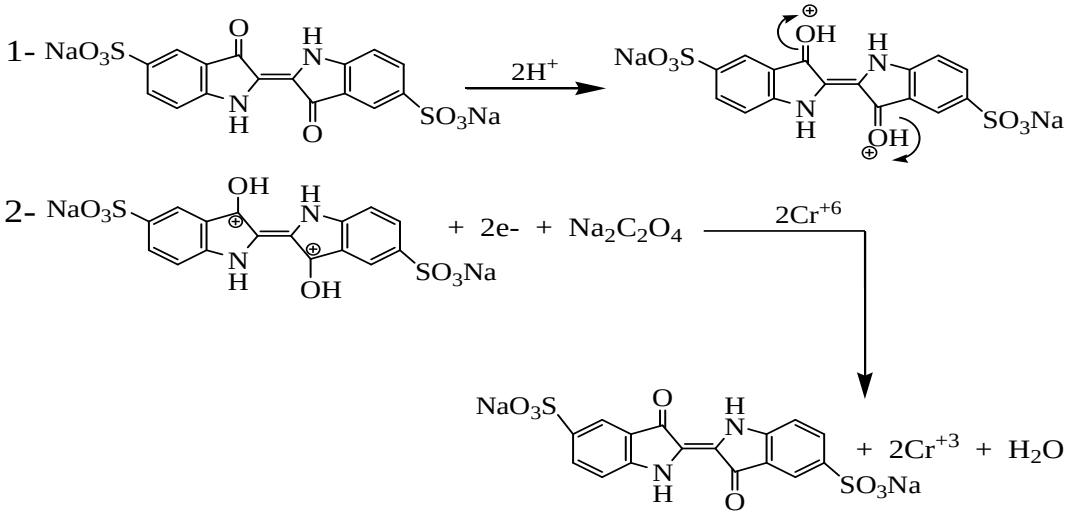


Fig. 6: Job's plot for ATN – chromate.

As a result the following reaction is suggested:



The probable reaction mechanism between chromate (Cr^{+6}) and indigo carmine dye in the presence of oxalate in acidic medium has been suggested [26] as follows:



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3.11 Effect of interferences:

In order to test the efficiency and selectivity of the proposed method, the effect of some foreign substances (e.g., acacia, glucose, lactose, menthol and starch) that are usually present in dosage forms were studied by adding different amounts of foreign substances to 100 µg atenolol/25 ml. It was observed that the studied foreign species did not interfere in the present method Table 5.

Table 5: Effect of interferences on the determination of 100 µg atenolol.

Interferences	Recovery(%) of 100 µg atenolol / µg of interference added		
	100	500	1000
Acacia	99.67	100.23	99.83
Glucose	100.33	99.76	99.53
Lactose	100.66	99.53	99.50
Menthol	99.34	99.31	99.18
Starch	100.99	100.92	100.83

3.12 Application of the method:

The proposed method was successfully applied to the determination of ATN in its pharmaceutical preparation (tablet). The results which are shown in Table 7 indicate that good recoveries were obtained.

Table 7: Analytical applications.

Atenolol amount, µg	Recovery(%) of ATN * (100 mg Vascoten tablet), Edochemi LTD-Cyprus
100	98.96
300	98.94
500	98.92

* Average of five determinations.

The calculated value of t-test [27], did not exceed the theoretical values at the 95% confidence level for five degrees of freedom when the proposed method has been compared with literature methods [13] as shown in Table 8.

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Table 8: The results of t-test analysis.

Drug	Pharmaceutical preparation	t-test
(100 mg Vascoten tablet) Edochemi LTD-Cyprus	Tablet	0.6086

3.13 Comparison of the methods:

Table 9 shows the comparison between some of analytical variables for the present method with that of other literature spectrophotometric methods.

Table 9: Comparison of the methods.

Analytical parameters	Present method	Literature method	
		[13]	[6]
Reaction	Oxidation reduction	Complex formation	Charge transfer
Reagent	Indigo Carmin	Cr(III)	DDQ
λ_{max} (nm)	610	594	590
Reaction time (min)	5	5	5
Beer's law range (µg/ml)	1.2-60	10-90	3.0-48.0
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	0.73×10^{4}	0.4002×10^{4}	5.41×10^{3}
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.364	6.99×10^{-2}	0.0493
R.S.D. (%)	±0.263 to ±0.376	±0.34 to ±0.60	0.97-1.56
Colour of the product	Blue	Blue	Blue
Application of the method	Pharmaceutical preparation	Pharmaceutical preparation	Pharmaceutical preparation

DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

The results indicate that the proposed method is sensitive and can be applied successfully to the determination of atenolol in pharmaceutical preparation.

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4. Conclusion

A spectrophotometric method for the determination of atenolol has been proposed, which is fairly sensitive, simple, rapid and economical with reasonable precision and accuracy. The optical parameters and statistical comparison justify this method for application in routine atenolol estimation in pure and dosage forms. Also the procedure do not involves any critical reaction conditions or tedious sample preparation steps. The recommended method is well suited for the assay of atenolol in pharmaceutical preparation.

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