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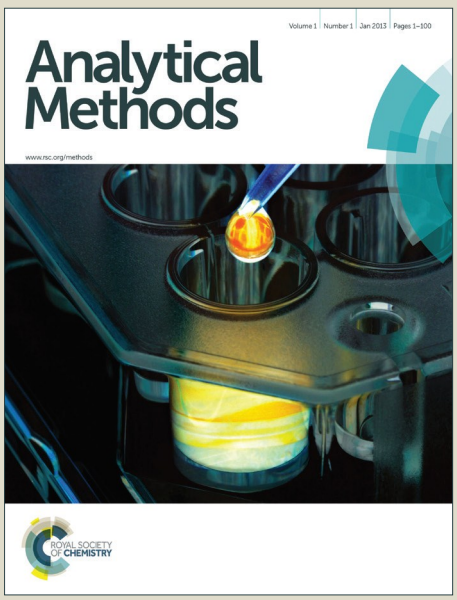

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Colorimetric estimation of alfuzosin hydrochloride in pharmaceutical preparation based on computational study

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Abstract

Computational and theoretical studies were done electronically and geometrically to find suitable, selective and sensitive coupling agent applicable for diazocoupling estimation of alfuzosin hydrochloride (ALF). These studies revealed that 8-hydroxyquinoline (8-HQ) fits better with ALF than other coupling agents based on its higher calculated interaction energy. The proposed method is based on the formation of red colored chromogen through the reaction of AFZ with sodium nitrite in acid medium to form diazonium ion, which was coupled with 8-HQ. Different variables affecting the reactions are optimized. Beer's law is obeyed over the concentration range of 1-12 µg/ml. The Job's plot analysis was applied and the stoichiometric ratio of ALF: 8-HQ was found to be 1:1. The method was successfully applied to the determination of ALF in pharmaceutical formulation with good accuracy and precision.

Keywords

Alfuzosin hydrochloride; 8-hydroxyquinoline; Computational studies; Diazotization technique.

1. Introduction

Alfuzosin hydrochloride (ALF), N-{3-[4-amino-6,7-dimethoxyquinazolin-2-yl) (methyl) amino] propyl} tetrahydro-2-furancarboxamide hydrochloride, **Figure (1)** belongs to the class of second generation alpha1-adrenoceptor antagonists. This group of drugs is used for the treatment of benign prostatic hyperplasia (BPH). The pharmaco-therapeutic effects achieved by blockade of the alpha1-adrenergic receptors which causes relaxation of the smooth muscles in the bladder neck, allowing urine to flow through the prostate, and decreasing the symptoms of BPH. [1] Several analytical methods were reported for the estimation of ALF either individually or in its combination with other drugs including spectrophotometry [2-10], spectrofluorimetry [9,11], HPLC [12-18], HPTLC[14,16,19], LC-MS [20,21] and electrochemically. [22, 23]

Figure (1)

The analysis of pharmaceutical compounds through structural behavior represents a very important part of science. Virtually all drug molecules possess one or more functional groups that can be analyzed in some fashion. [24, 25] However, although the last decade's analytical chemists have developed a variety of visible spectrophotometric techniques for the estimation of pharmaceutical products, evidently this field has become very sophisticated and a lot of reagents have been synthesized either by analytical chemists or organic chemists. Extremely

little attention has been paid to application of computational modeling and theoretical studies for the choice of the most selective and optimum reagent of different reactions applicable for visible spectrophotometric estimation of drugs. Among different visible spectrophotometric techniques, diazotization employing coupling reactions are generally used methods for assay of drugs containing a free aromatic amino group through coupling the resulting diazonium salt with various phenolic reagents. [26, 27]And so, it is clear that the choice of the optimum coupling agent will led to higher selectivitity and sensitivity, the ab initio quantum-mechanical (QM) calculations and molecular modelling tools may help to drastically reduce the number of possible candidates for coupling agents for a given analyte. [28]

In the present work, computational studies were done to find suitable selective and sensitive coupling agent for alfuzosin hydrochloride. Then the best coupling agent, based on computational calculations, was used for estimation of alfuzosin hydrochloride in pharmaceutical preparation. The method is based on the formation of red colored chromogen up on the reaction of AFZ with sodium nitrite in acid medium to form diazonium ion, which was coupled with the best phenolic reagent. The reaction was employed as a basis for spectrophotometric determination of ALF in its tablets.

2. Experimental

2.1. Apparatus

Shimadzu UV visible 1650-PC spectrophotometer (Tokyo, Japan), equipped with 10 mm matched quartz cells.

2.2. Reagents

All reagents used were of analytical grade and solvents were of spectroscopic grades, distilled water was used throughout the procedure. Hydrochloric acid, sodium hydroxide and ethanol (El-Nasr Company, Egypt). Sodium nitrite (Winlab, UK) solution was freshly prepared as 4% (w/v) aqueous solution. 8-hydroxyquinoline (Koch–Light Laboratories Ltd., England),0.35% (w/v) solution was freshly prepared in 1% w/v sodium hydroxide solution in 50% (v/v) aqueous ethanol. 10% (v/v) aqueous solution of hydrochloric acid. 30% (w/v) aqueous solution of sodium hydroxide.

2.3. Materials

2.3.1. Reference sample

Pure alfuzosin hydrochloride (99.25%) was kindly supplied by Eva pharma Company, Cairo, Egypt**.**

2.3.2. Pharmaceutical formulation

Prostetrol® tablets: each tablet is claimed to contain 10 mg alfuzosin hydrochloride (B.No. 501178, manufactured by Eva pharma Company), purchased from local market.

2.4. Computational optimization and energy calculations

In order to avoid time-consuming and experimental trials to select the best reagent, computational approach has been applied as reported elsewhere. [29] In the computational system, the structure of the ALF, reagent and all ALF–reagent complexes were drawn in the Gauss-view software. The conformation of ALF and the reagents were optimized and their energy in the optimized conformations was calculated. In this study, all calculations were carried out using Gaussian 03 software based on the application of density functional theory (DFT) method at the level B3LYP/6-31G (d) basis set. [30, 31] Finally, the binding energy of ALF–reagent complexes, ΔE, was calculated via the following equation:

$$\Delta E = E_{A-B} - E_A - n E_B \quad [1]$$

where A is the drug and B is the reagent. The binding energy (ΔE) between a drug and reagent is calculated as a measure of their interaction, which facilitates the selection of the reagent used for complex synthesis, reagent of a higher ΔE with the drug would be more suitable for complex preparation, compared to those of lower ΔE. [32]

The polarizable continuum model was applied to calculate the energy of complex, where the effect of solvent should be considered during energy calculations as it leads to changes in stability and energy of the drug–reagent complexes in solvent phase compared to gaseous phase. [33]

2.5. Standard solutions

Solution of alfuzosin (0.1 mg/ ml) was prepared by dissolving 10mg of alfuzosin in 100 ml distilled water; the working solution was prepared as required by suitable dilution.

2.6. General analytical procedure

Transfer aliquots of drug solution of (0.1 mg/ ml) containing (0.01 - 0.12 mg) into a series of 10 ml volumetric flasks, add 0.5 ml of 10% hydrochloric acid and 0.5 ml of 4% sodium nitrite. Then the solutions were shaken thoroughly for 5 minutes to allow the diazotization reaction to go to completion. Add 1 ml of 0.35% 8-HQ, stir the solutions for 3 minutes. Then 0.5 ml of 30% sodium hydroxide was added to each flask and the mixtures were left to stand for 5 minutes. Finally, complete the volume with ethanol to the mark and measure the absorbance of the formed red color azodye at 515 nm against a reagent blank treated similarly. Constructing calibration curve relating absorbance to concentration in the range of (1–12 µg /ml).

2.7. Determination of molar ratio

The Job's method of continuous variation [34] was employed. In a series of 10 ml volumetric flasks (0.5, 1, 1.5….., 2.5 ml) of ($7.52x10^{-5}$M) ALF solution were mixed with 0.5 ml of 10 % hydrochloric acid, 0.5 ml of 4% sodium nitrite, (2.5, 2, 1.5……., 0.5 ml) of 8-HQ ($7.52x10^{-5}$M) were added to each flask then add 0.5 ml of 30 % sodium hydroxide drop wise to each flask, complete the volumes to the mark with ethanol and measure the absorbance of the formed azo dye complex at 515 nm against a reagent blank treated similarly.

2.8. Application to pharmaceutical preparation

Ten tablets were weighed and finely powdered. Appropriate weight of powder equivalent to the 10 mg of the drug was accurately weighed, transferred to 100 ml volumetric flask and the volume was made up to 75 ml with water. The solution was shaken vigorously for 15 min, then sonicated for 30 min and filtered through Whatman filter paper no 41. The volume was completed to 100 ml with the same solvent. Necessary dilutions of the filtrate were made with water to obtain different concentrations of ALF. Samples were analyzed using the procedures stated under general analytical procedure. To assess the accuracy of the proposed methods, standard addition technique was applied.

3. Results and discussion

Diazotization employing coupling reaction is a generally used method for assay of drugs containing a free aromatic amino group. The presence of amino group in ALF hydrochloride enabled the use of diazotization of the drug with nitrous acid and coupling the resulting diazonium salt with optimum phenolic reagent.

3.1. Computational studies

Experimentally testing the many variables involved in any reaction mechanisms is both time-consuming and expensive. This provides enough motivation for the optimization of experimental conditions using computer-based methods. [35, 36] In order to obtain a clue about the tendency of ALF for different coupling agents, 8-HQ, α-naphthol, β-naphthol, resorcinol and phloroglucinol, to select the most suitable reagent for diazocoupling reaction, some ab initio quantum-mechanical calculations were carried out. The calculations were performed with DFT method at the level B3LYP/6-31G (d) basis set, which derives properties of the molecule based on a determination of the electron density of the molecule. Unlike the wave function, which is not a physical reality but a mathematical construct, electron density is a physical characteristic of all molecules. It was believed that if we can determine the electron density of a molecule, we can say numerous things about the molecule, and this

forms the basis for DFT. There are several major advantages of this approach. The first is that the method is based on a property that exists in real molecules, not a purely mathematical invention. The second is that the wavefunction becomes more complicated mathematically as the number of electrons increases. In DFT, the density depends only on the x-y-z coordinates of the individual electron. In practical terms, DFT can be said to scale three-dimensionally, or as N_3, where N is the number of basis functions. Ab initio methods, on the other hand, scale as N_4, and as a result DFT calculations are faster with better accuracy. [37-39] The use of such level of calculation is fully justified by the fact that B3LYP/6-31G (d) is considered to be standard model chemistry for many applications, particular good for organic molecules and clearly provide better results for reaction chemistry calculations. [40] To obtain good estimates of the interaction energy, basis sets with polarization functions on all the atoms must be used. [41] Given the size of our systems, the computer needs (i.e., CPU, memory and disk space) for such calculations rapidly increase with the size of the basis set. For this reason, the medium size 6-31G basis sets are most frequently used with biomolecules. [42]

Geometry optimization is the most important step for the calculation of the interaction energy. [43] Optimization of the structures of the ALF, reagent and all ALF–reagent complexes via calculation of the minimized

energies of their respective structures was carried out. Optimized molecular structures of the studied molecules are given in Figure (2).

Table (1) summarizes the calculated interaction energies for complexes formed between ALF and coupling phenolic agents in the gaseous as well as solvent (ethanol) phase. From the data given in Table 1 it is obvious that the interaction energy calculated decreases in the order: ALF (8-HQ) > ALF (β-naphthol) > ALF (α-naphthol) > ALF (resorcinol) > ALF (phloroglucinol). The highest stability was obtained for ALF-8-HQ complex. Moreover, in solution phase, the order of interaction energy is quite similar to that obtained in gaseous phase, which suggests that binding between ALF and coupling agent is strong enough to persist also in solution phase. [35]

Thus, based on the above calculation results, 8-HQ could possibly be used as a suitable, sensitive, selective coupling agent and expected to yield the highest efficiency for diazocoupling reaction of ALF hydrochloride.

Table (1) **Figure (2)**.

3.2. Spectral characteristics of the reaction

The absorption spectrum of ALF solution ($1.88x10^{-5}$M), in distilled water was recorded Figure 3 (a). The spectrum shows two maximum

(λ_{max}) absorption peaks at 246 and 330 nm. The treatment of ALF with nitrous acid (generated in situ from sodium nitrite and a strong acid, such as hydrochloric acid) leads to formation of diazonium intermediate. This diazonium intermediate is unstable, fairly strong electrophile and can undergo coupling reaction with 8-HQ ($7.51x10^{-5}$M) to form azodye prouct. The product will absorb longer wavelengths of light than the reactants because of increased conjugation. The absorbance of the formed red color azodye was recorded at 515 nm against a reagent blank treated similarly Figure 3 (b).The absorption intensity of this new absorption maximum increased with increase in ALF concentrations in the interaction solution Figure 3 (C). This increase in the new absorption band with graual increase of ALF concentrations was indicative for the quantitative estimation of ALF. The proposed scheme for the diazotization reaction pathway of ALF with 8-HQ can be represented as Figure (4).

Figure 3 (a) **Figure 3 (b)** **Figure 3 (C)**

Figure (4)

3.3. Optimum reaction conditions

3.3.1. Effect of acid on diazotization

The diazotization reaction must be carried out under condition of fairly high acidity to provide powerful nitrosating agent because primary

aromatic amines are not powerful nucleophiles. [44] It was found that the absorbance was increased with increasing acid volume till 0.5 ml as shown in Figure (5). Above this range a decrease in absorbance was observed. Thus, 0.5 mL of 10% HCl was used.

Figure (5)

3.3.2. Effect of sodium nitrite volume

Sodium nitrite has a main role for conversion of amines into diazo compounds.[45] It was found that 0.5 ml of 4% $NaNO_2$ solution sufficient enough to give maximum absorbance as shown in **Figure (6)**.

Figure (6)

3.3.3. Effect of 8-HQ reagent volume

The influence of 8-HQ reagent was investigated in the proposed method by measuring the absorbance at specified wavelengths in the standard procedure for solutions containing a fixed concentration of ALF and varying amounts of coupling reagent. The results indicate that 1 mL of 0.35% 8-HQ is optimum for quantitative estimation of the studied drug as shown in **Figure (7)**. Above this volume a decrease in absorbance was observed. This clearly indicates that all ALF had been reacted by coupling reagent.

Figure (7)

3.3.4. Effect of sodium hydroxide volume

Basically in the end of diazotization reaction sodium hydroxide takes a proton from the benzene ring which has just coupled to restore the aromaticity and stability of that ring. [46] Sodium hydroxide was found to be more suitable for coupling reaction compared to sodium carbonate because the formed dye was stable and more intense in sodium hydroxide medium. The results of study revealed that 0.5 ml of 30% NaOH produces maximum absorbance as shown in **Figure (8)**.

Figure (8)

3.3.5. Effect of time on color development and stability

The effect of the time on the absorption maxima at the suggested wavelength was studied by measuring the absorbances at the relevant maxima at different time intervals (0-60 minutes). The results obtained showed that, full colour development was attained immediately and intensity of the color stayed constant for one hour as shown in figure (9)

Figure (9)

3.4. Molar ratio of the reaction

Determination of the stoichiometry of chemical equilibrium reactions have been performed using several methods such as the Job's method of continuous variation [34] and molar ratio method.[47] In this work we select

Job's method based on simplicity of its theoretical foundation and their straight forward experimental application. It is based on the study of a graphical representation of analytical signals versus ligand mole fraction. [48] The Job's plot was developed where the absorbance peak at 515 nm is plotted as y-coordinate versus the corresponding molar fraction as an x-coordinate as shown in **Figure (10)**. The stoichiometry of the complex formed between ALF and 8-HQ is determined and confirmed to be (1:1).

Figure (10)

3.5. Method validation

3.5.1. Linearity and sensitivity

Under the above mentioned optimum reaction conditions, the calibration curve for the analysis of ALF by the proposed method was obtained by plotting the absorbances as a function of the corresponding ALF concentrations. The regression analysis of the plot using the method of least squares was made to evaluate the intercept (a), slope (b), regression coefficient (r^2) and standard deviations of slope and intercept. Beer's law plot was linear in the range of 1– 12 ug /ml. The limit of detection (LOD) and quantitation (LOQ) were calculated using the formula: LOD or LOQ = kSD_a/b, where k = 3.3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept, and b is the slope. The quantitative parameters of the proposed method are given in **Table (2)**.

Table (2)

3.5.2. Accuracy and precision

Accuracy and precision of the method was evaluated by triplicate analysis of standard drug solution at three different concentration levels lying in linearity range within one day for intra-day and repeating the procedure for three different days for inter-day. Accuracy was expressed as recovery percentage (R%), while precision was expressed as percentage relative standard deviation (RSD%). The results are represented in **Table (3)**.The recovery percentage, standard deviation, and relative standard deviation obtained by proposed method can be considered to be very satisfactory**.** Moreover, the validity of the proposed procedure is further assessed by applying the standard addition technique. The results obtained were shown in **Table (4)**.

Table (3) **Table (4)**

3.6. Application to pharmaceutical formulation

The proposed method was employed for assaying ALF in the pharmaceutical formulation (prostetrol tablets). The results prove the applicability of the method, as demonstrated by the accurate and precise percentage recoveries. The results of assay were statistically compared with another diazocoupling reported method.[3] The results show that the calculated t- and F-values are less than the theoretical values, suggesting that the proposed method is comparable to the reported method with

respect to accuracy and precision**.** The detection sensitivity of the proposed method confirmed the computational calculations when it compared with the reported one. The results are given in **Table (5)**.

Table (5)

Although, the goal of this study is to use the computational investigation in selecting the appropriate reagent for a specific chemical reaction. However, in future work the optimization of this reaction using an experimental design to find out the optimum value of the significant factors will be our goal with computational investigation on different reagents.[49-51] Hence the time and the effort as well as the error will be minimized.

4. Conclusion

In this work, computational and theoretical studies were applied to select most suitable, sensitive and selective coupling agent expected to yield the highest efficiency for diazocoupling reaction of ALF hydrochloride. Results demonstrated that 8-HQ fits better with ALF than other coupling agents based on its higher calculated interaction energy with respect to other coupling agents. Experimental treatment of ALF with nitrous acid to form diazonium intermediate which undergoes cupling with 8-HQ to form azodye prouct. The reaction was employed as a basis for spectrophotometric determination of ALF in its tablets. The

suggested method found to be accurate, selective and equally sensitive with no significant difference of the precision compared with the reference method. They could be applied for routine analysis of pure drug or in its pharmaceutical formulation. In general, application of computational modeling and theoretical studies for choice of the most selective and optimum reagent of different spectrophotometric applications is not limited to the diazotization reactions as it opens a new avenue for optimization variety of visible spectrophotometric techniques.

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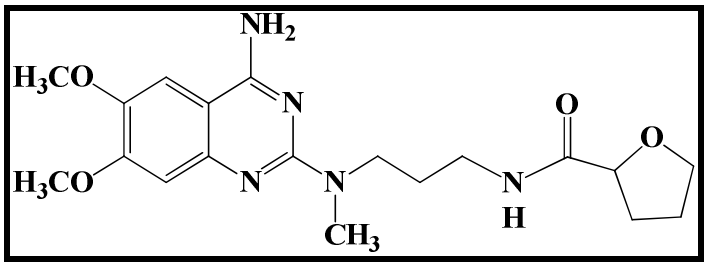

Figure (1): Chemical structure of Alfuzosin

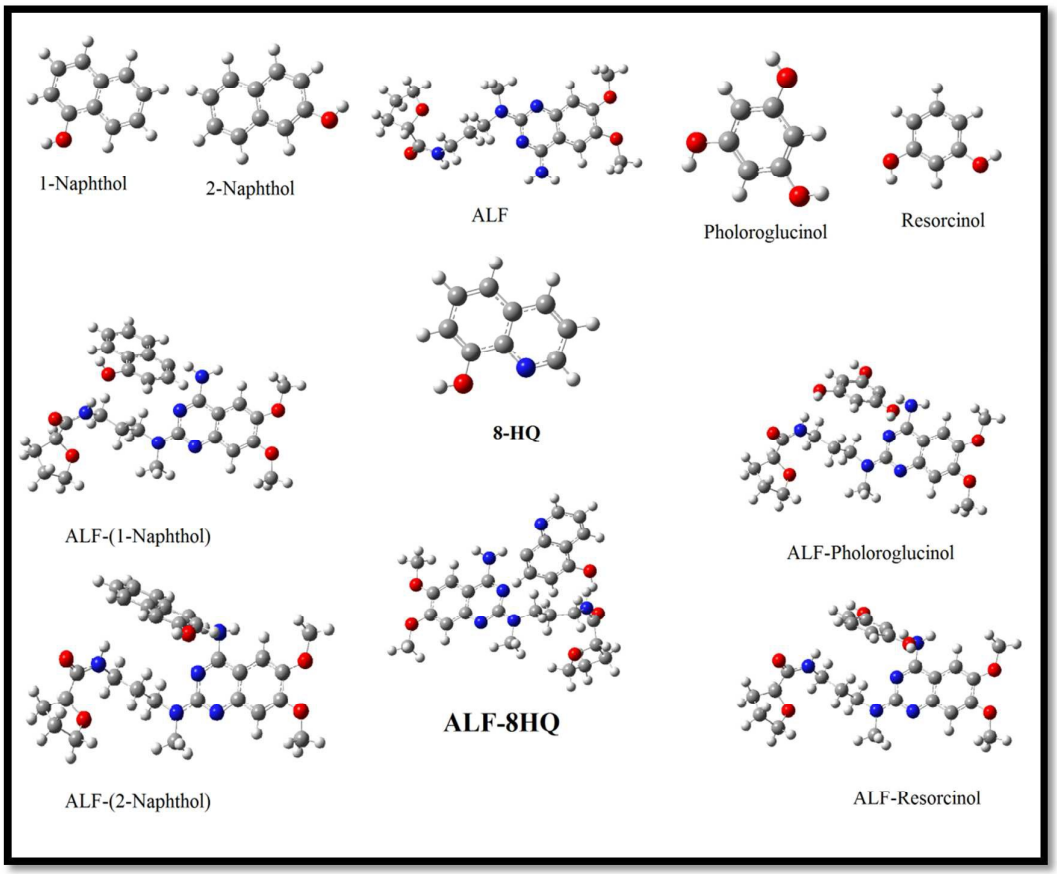

Figure (2): Optimized structures of ALF, phenolic reagents and complexes formed between them.

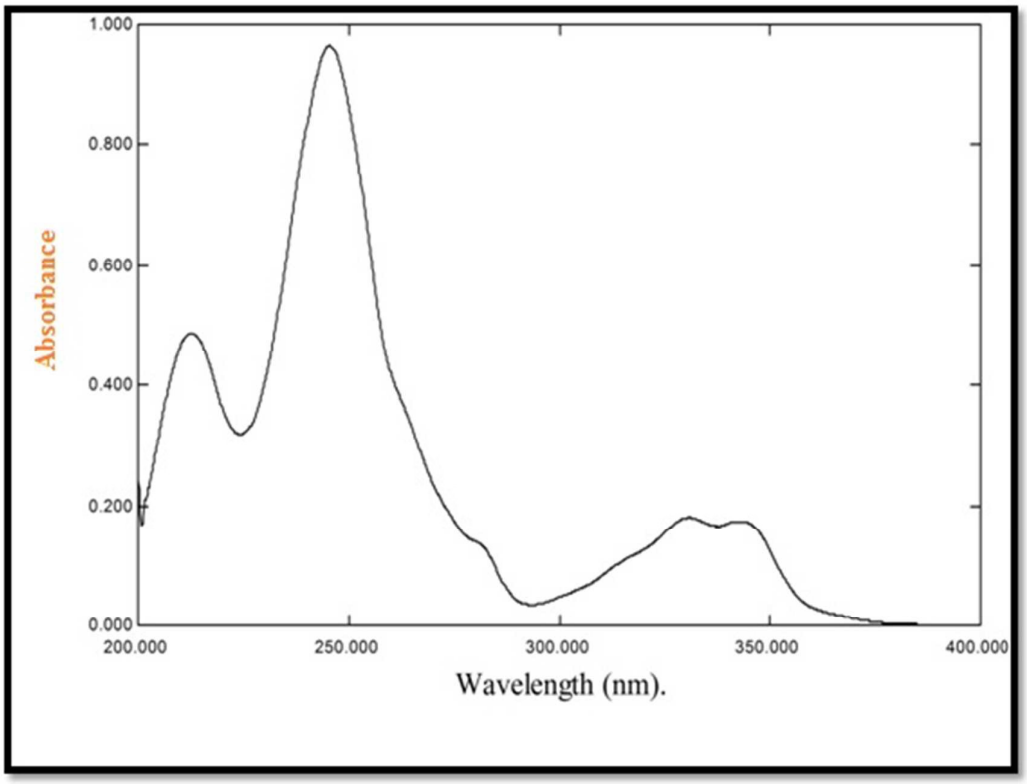

Figure 3 (a): Zero order **spectrum** of (**1.88x10^{-5}**) of ALF.

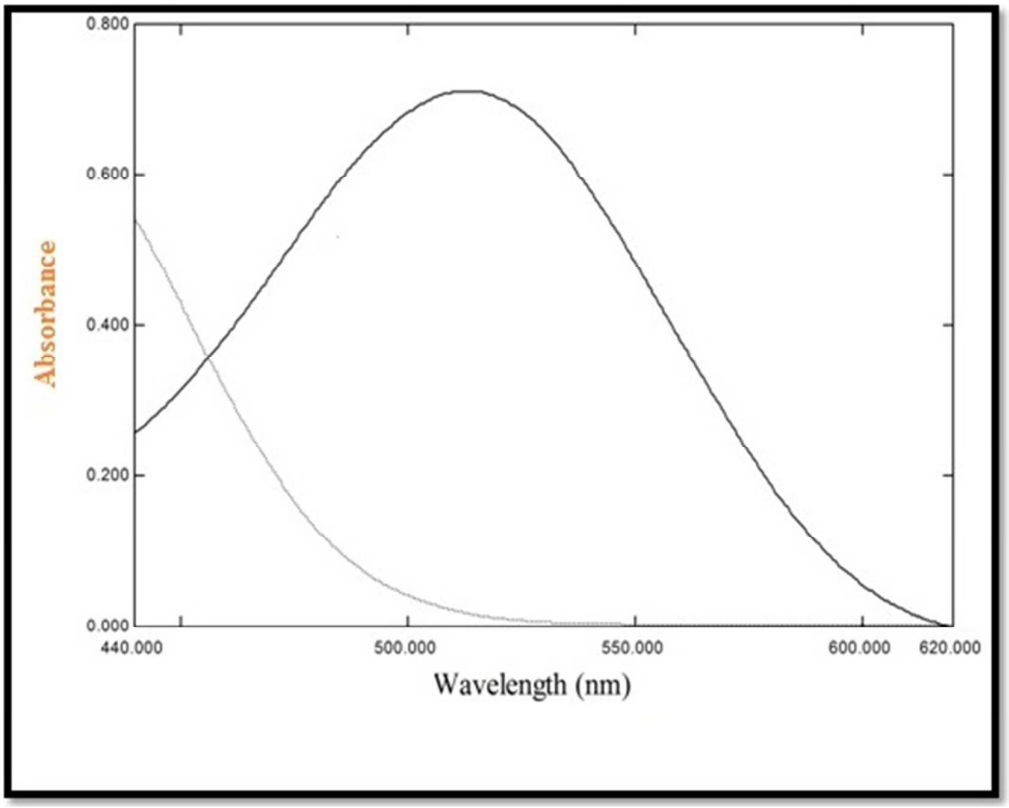

Figure 3 (b): Absorption spectrum of azodye formed between ALF (**8ug/mL**) and 8HQ (—) and reagent only (- - - -) at 515 nm.

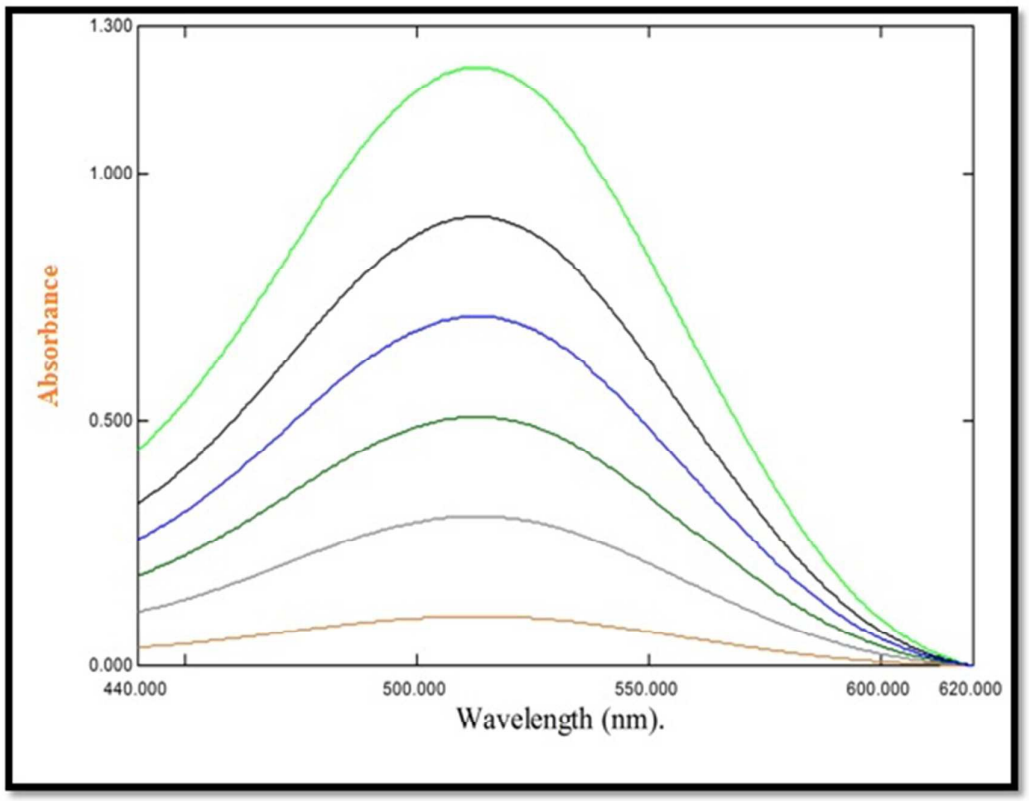

Figure 3 (c): Absorption spectrum of azodye formed between ALF (1-12ug/mL) and 8-HQ reagent.

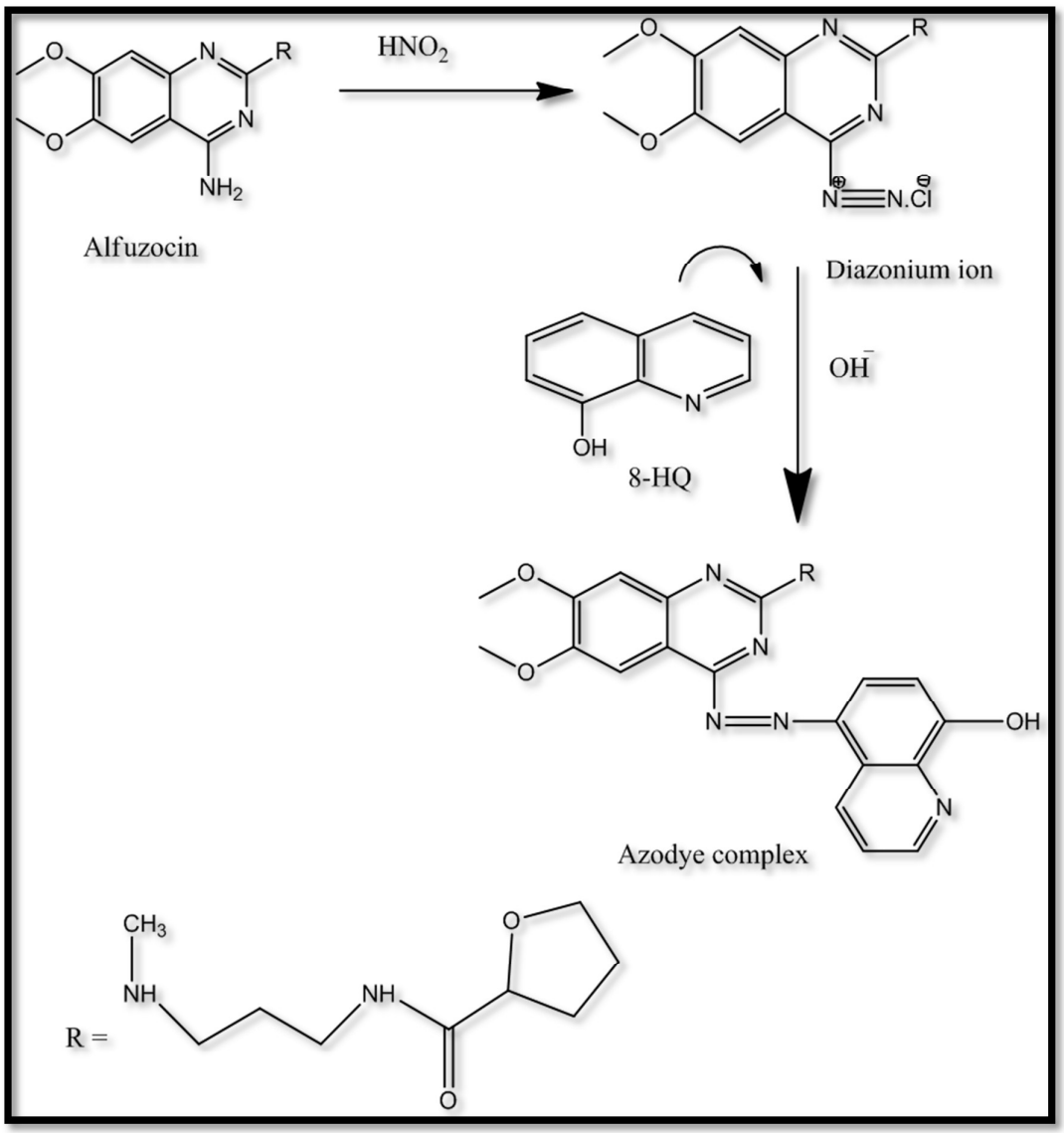

Figure (4): Scheme for the diazotization reaction pathway of ALF with 8-HQ.

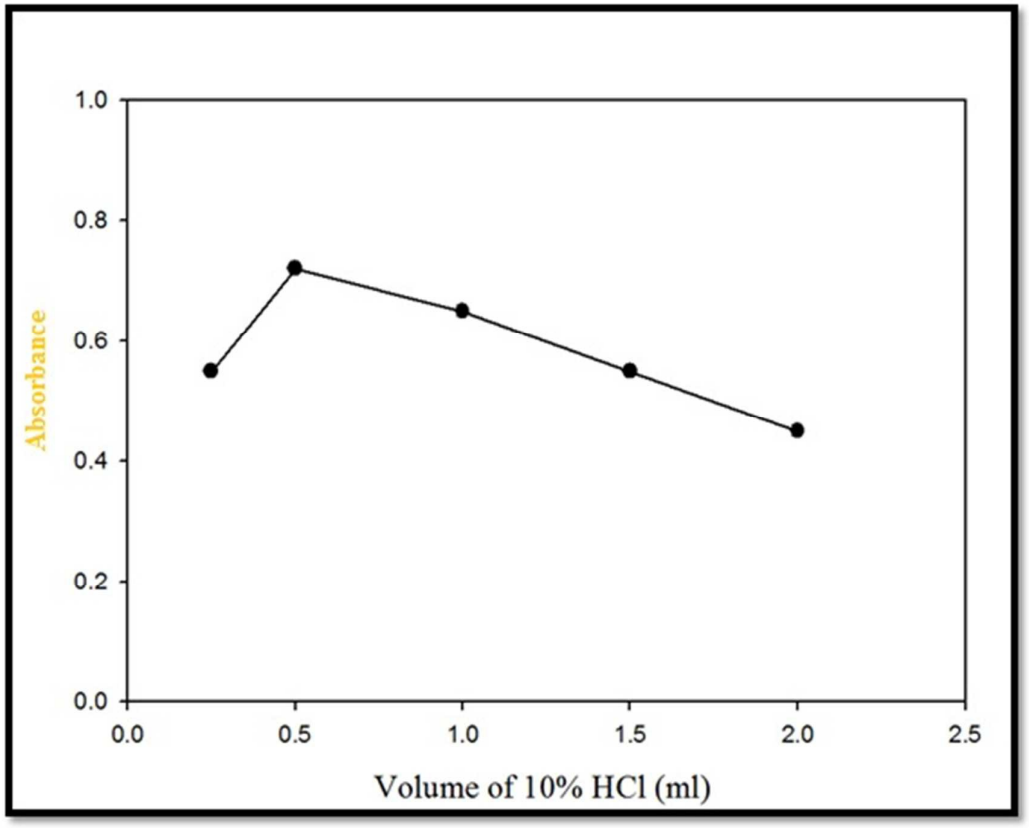

Figure (5): Effect of acidity on the absorbance of the reaction product of ALF (**8ug/mL**) with diazotized 8-HQ at 515 nm.

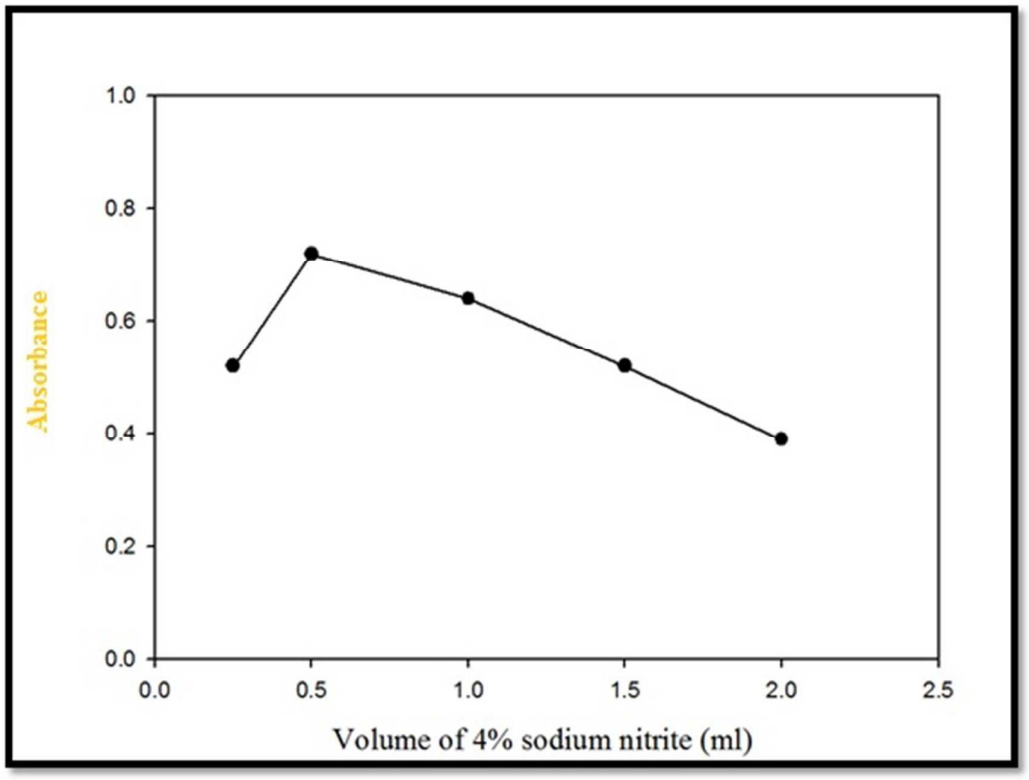

Figure (6): Effect of sodium nitrite volume on the absorbance of the reaction product of ALF (**8ug/mL**) with diazotized 8-HQ at 515 nm.

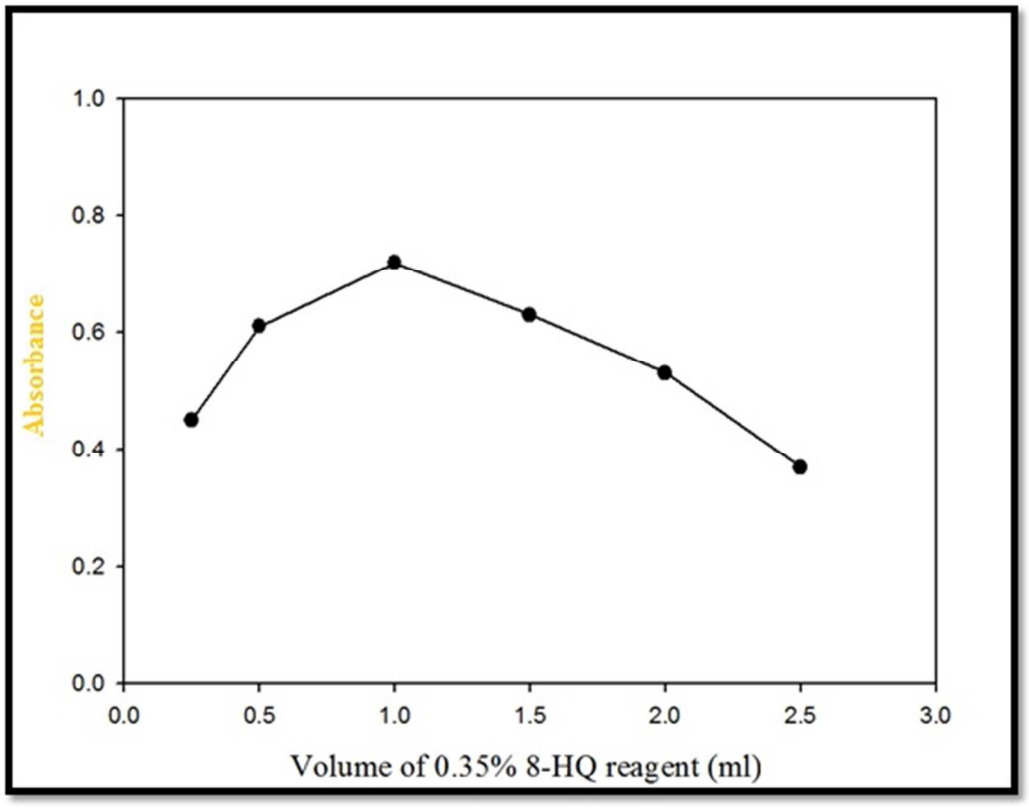

Figure (7): Effect of 8-HQ volume on the absorbance of the reaction product of ALF (**8ug/mL**) with diazotized 8-HQ at 515 nm.

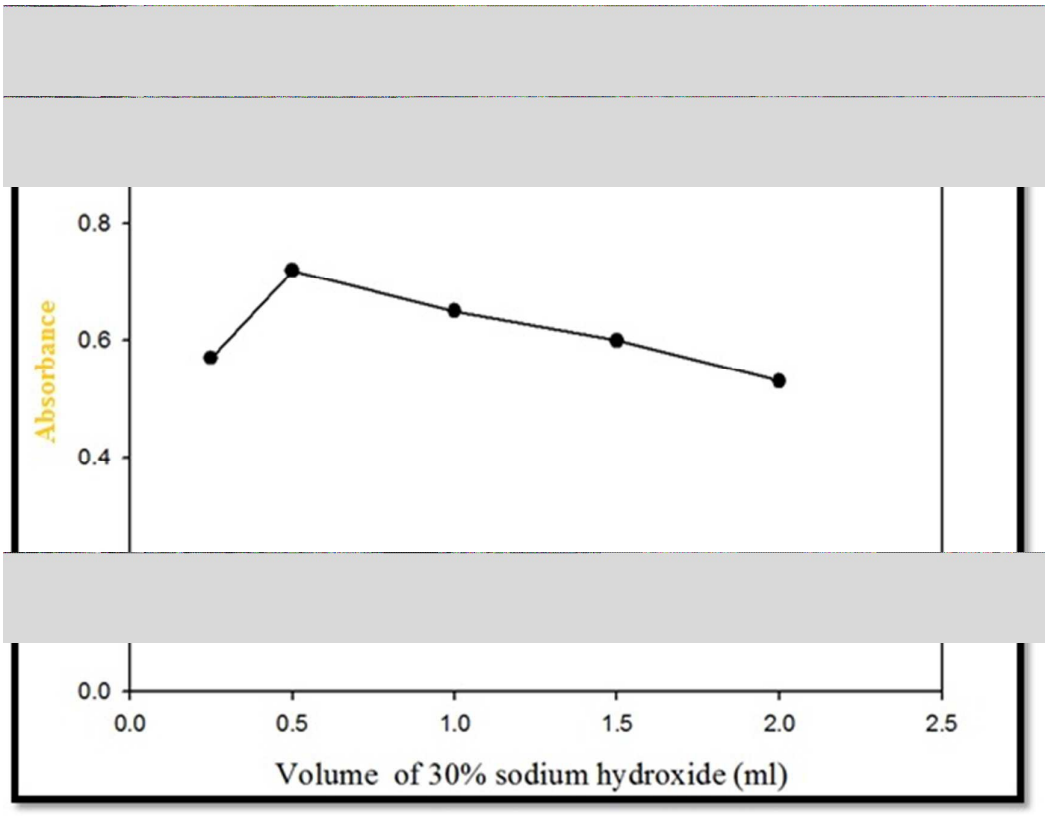

Figure (8): Effect of sodium hydroxide volume on the absorbance of the reaction product of ALF (**8ug/mL**) with diazotized 8-HQ at 515 nm.

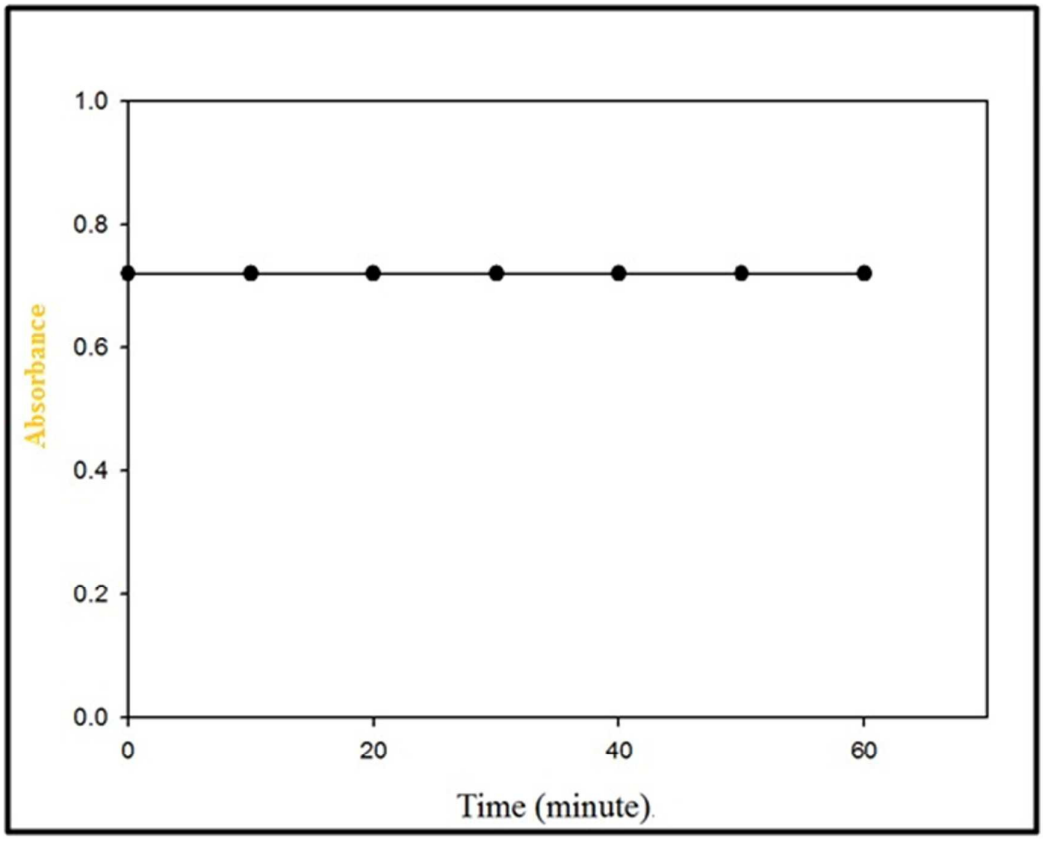

Figure (9): Effect of time on colour stability of reaction poduct of ALF (8ug/mL) with diazotized 8-HQ at 515 nm.

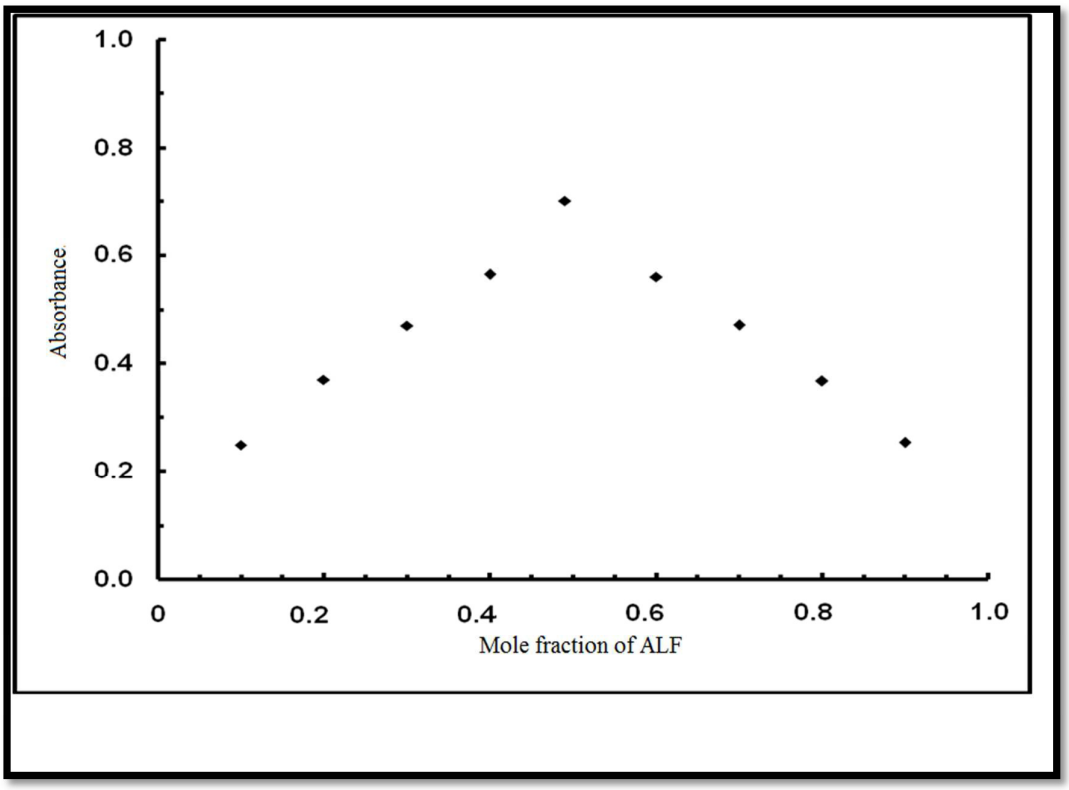

Figure (10): Stoichiometry of the reaction of ALF ($7.52x10^{-5}$M) with 8-HQ by continuous variation (Job's) method.

Table (1): Computational study of interaction energy between various phenolic coupling agents and alfuzocin hydrochloride in gaseous and solvent phase.

Name of complex	**Gaseous phase**		**Solvent phase**	
	ΔE (Hartree)*	ΔE (KJ/mol)	ΔE (Hartree)*	ΔE (KJ/mol)
ALF-8HQ	**-0.0201**	**-52.7725**	**-0.0174**	**-45.6837**
ALF- β-naphthol	-0.0170	-44.6335	-0.0141	-37.0195
ALF- α-naphthol	-0.0131	-34.3940	-0.0099	-25.9924
ALF- resorcinol	-0.0083	-21.7916	-0.0047	-12.3398
ALF-phloroglucinol	-0.0079	-20.7414	-0.0041	-10.7645
* 1Hartree = 2625.5 KJ / mol				

Table (2): Linearity studies and regression equations of the proposed method.

Parameters	Proposed method
λ_{max} (nm)	515
Linearity range (µg/ ml)	**1-12**
LOD* (**µg /ml**)	**0. 2457**
LOQ** (**µg /ml**)	**0. 819**
A (1%,1cm)	883.0833
Response factor ± S.D	0.0883±0.0019
Regression parameters: - Slope - Intercept	0.0895 0.0041
Correlation coefficient (r^2)	0.9999

Table (3): Method validation obtained by applying the proposed method

CONC. (µg/ml)	Intraday			Interday		
	Found Conc.*±SD	**Accuracy (R,%) ±SD**	**Precision (RSD, %)**	**Found Conc.*±SD**	**Accuracy (R,%) ±SD**	**Precision (RSD, %)**
4	**3.97±0.038**	99.41±0.953	0.959	**3.99±0.058**	99.87±1.469	1.47**0**
8	**7.90±0.058**	98.85±0.725	0.733	**8.06±0.080**	100.77±1.011	1.003
12	**11.97±0.105**	99.81±0.875	0.876	**12.07±0.154**	100.59±**1**.28	1.277

* Average of three determinations.

Table (4): Application of standard addition technique to the analysis of prostetrol® tablets by applying the proposed method.

Taken (µg/ ml)	Added standard (µg /ml)	Found Conc.*±SD	**Proposed method (% Recovery)**
4	8	7.89±0.031	**98.73**
	6	6.08±0.107	**101.35**
	4	4.02±0.072	**100.52**
	2	1.97±0.121	**98.68**
Mean ± RSD%			**99.82±1.33**

Table (5): Comparison and Statistical **a**nalysis **o**f **t**he **r**esults **o**btained **b**y **t**he **p**roposed **a**nd **r**eported **p**rocedure **f**or **t**he **d**etermination **o**f **a**lfuzocin **i**n **i**ts **p**harmaceutical **p**reparations.

Parameter	Proposed method	Reported method[a]
λ_{max} (nm)	**515**	**440**
Linearity range (µg/ ml)	**1-12**	**4-20**
Mean	100.132	100.33
S.D.	1.064	**1.399**
N	5	5
Variance	1.1236	**1.959**
t-test	0.249 (2.306)	
F-value	1.729 (6.388)	

The values in the parenthesis are the corresponding theoretical values of t and F at (P = 0.05).

[a] **Reported method: diazo coupling reaction for determination of alfuzosin using phloroglucinol [3].**