

## A new spectrophotometric method for the determination of cardiovascular drugs in dosage forms



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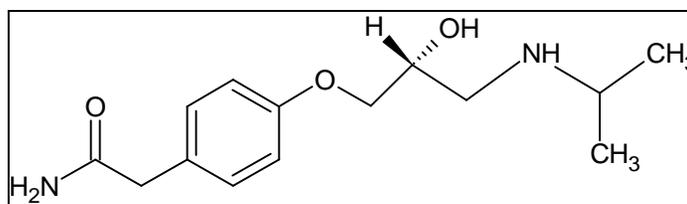
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**Abstract.** A simple, accurate, and precise spectrophotometric method has been proposed for the determination of three cardiovascular drugs, namely: Atenolol (ATE), Doxazosin mesylate (DOX) and Lisinopril dihydrate (LID) in pharmaceutical formulations. Proposed method is based on the derivatization of drugs with 1,2-naphthoquinone-4-sulfonic (NQS). The optimum experimental conditions have been studied. Beer's law is obeyed over the concentration of 0.5–3, 0.4–8, and 5–50  $\mu\text{g/mL}$  for ATE, DOX, and LID, respectively. The detection limits were 0.11, 0.12, and 1.16  $\mu\text{g/mL}$  for ATE, DOX, and LID, respectively, with a linear regression correlation coefficient of 0.9993, 0.9998, and 0.9997 and recovery in range from 98.25–102.57, 97.20–100.57, and 97.83–101.80 for ATE, DOX, and LID, respectively. Effects of pH, temperature, reaction time, and NQS concentration on the determination of ATE, DOX, and LID, have been examined. This method is simple and can be applied for the determination of ATE, DOX, and LID in pharmaceutical formulations in quality control laboratories.

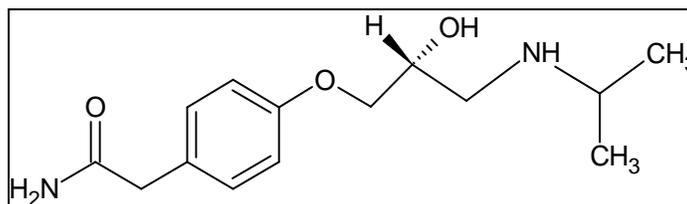
**Keywords:** Spectrophotometric, cardiovascular drugs, dosage forms, sodium 1,2-naphthoquinone-4-sulfonic (NQS)

### 1 INTRODUCTION

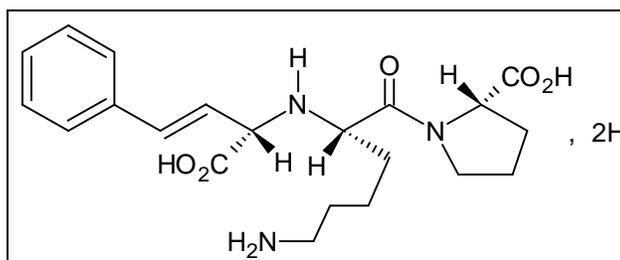
There are several cardiovascular diseases and multiple cardiovascular agents for their treatment. The most common cardiovascular disease is hypertension. Hypertension increases the risk of stroke and other heart diseases. Cardiovascular drugs are used to control this disease, with no cure being currently available. Diuretics,  $\alpha$ -adrenergic antagonists,  $\beta$ -receptor antagonist, angiotensin-converting enzyme inhibitors (ACE) and calcium channel blockers are the primary routes of treatment for hypertension (Nhung et al., 1996). Atenolol (Fig.1.(i)) is an antihypertensive, antianginal, and antiarrhythmic drug. Chemically, it is [(RS)-2-{4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl} acetamide] is a selective  $\beta_1$ -receptor antagonist, a drug belonging to the group of beta blocker. Doxazosin mesylate Fig.1.(ii) is a quinazoline compound that is selective inhibitor of the  $\alpha_1$  subtype of  $\alpha$ -adrenergic receptors. Its chemical name is [1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-4-[(2RS)-2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl]Piperazine methanesulphonate. Lisinopril (Fig.1.(iii)), [(2S)-1-[(2S)-6-Amino-2-[[[(1S)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate], is an angiotensin converting enzyme (ACE) inhibitor.



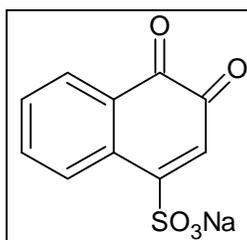
(i) ATE



(ii) DOX



(iii) LID



(iv) NQS

Fig.1. Chemical structures of the (i) ATE, (ii) DOX, (iii) LID and (iv) NQS.

The literature reveals various methods for the determination of the mentioned drugs in pharmaceutical preparations. Among these methods are UV spectroscopy (Kasture & Ramteke 2006; Vetuschi & Ragno 1990; Vrushali et al., 2010; Vishnu et al., 2010), spectrofluorometry (Damiani P.C. 2011), reversed phase HPLC (Kavitha & Muralidharan 2010; Kumar et al., 2010) and HPTLC (Sathe & Bari 2007) for ATE, Several analytical methods that have been reported for the estimation of DOX in biological fluids or pharmaceutical formulations which includes RP-HPLC (Chokchai et al., 2007; Kulsum et al.,

2011; Dhanya et al., 2011), HPLC with mass spectrometry (HPLC/MS) (Ning et al., 2007), UV-Spectrophotometry (Padma & Vidya 2011; Aydomu & Barla 2008; Bebawy et al., 2002) and for LID is potentiometry (British Pharmacopoeia 2004), RP-HPLC (Naidu et al., 2005), HPTLC (Argekar & Powar 2000), HPLC (Beasley et al., 2005), UV-Spectrophotometric (Prasad et al., 1999; Priyanka et al., 2009; Rahman et al., 2005; Stanisiz B 2004; Paraskevas et al., 2002), spectrofluorometric (El-Gindy et al., 2001). Sodium 1,2-naphthoquinone-4-sulfonic (NQS) Fig.1.(iv) has been used for the determination of many compounds. It is a popular spectrophotometric reagent due to its efficient reactivity with both primary and secondary amines, and high reaction rate (Wang et al., 2004; Darwish et al., 2009; Quan-M & Zhan-Jun.Y.2007; Li Zhang et al., 2008; Ebraheem et al., 2011; Ahmed et al., 2011; Elbashir et al., 2011; Ahmed & Elbashir 2012). NQS proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group. The applications of NQS for determination of pharmaceutical bearing amine group have recently been reviewed by Elbashir *et al.* (Elbashir et al., 2011). The uses of (NQS) for spectrophotometric determination of three drugs under study have not been reported yet. Therefore in this work a rapid spectrophotometric method for determination of the content of ATE, DOX, and LID in pharmaceutical formulations which is based on the reaction of NQS with amino group of ATE, DOX, and LID molecules to form orange and greenish blue compounds, at 454, 460, and 481 nm for ATE, DOX, and LID, respectively.

## 2 EXPERIMENTAL

### 2.1 Instrumentation

All of the spectrophotometric measurements were made with a Double beam UV1800 ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells (SHIMADZU Japan) also temperature controller was used for the spectrophotometer measurements. pH meter model pH 211 (HANNA Italy) was used for adjusting pH.

### 2.2 Chemicals and materials

All chemicals used were of analytical reagent grade. Sodium 1,2-naphthoquinone-4-sulphonate (NQS) (Aldrich Chemical Co., St. Louis, USA); Double distilled water was used to prepare all solutions.

The following available commercial preparations were analyzed; ATE tablets (AMIPHARMA laboratories, Sudan), labeled to contain 50 mg ATE per tablet; DOX tablets (Pharmasyr Co. Syria) labeled to contain 4 mg DOX per tablet; LID tablets (Pharma International Co. Amman-Jordan), labeled to contain 5 mg LID per tablet.

### 2.3 Preparation of standard and sample solutions

#### 2.3.1. Stock standard solution of ATE, DOX and LID (1000 $\mu$ g/mL)

An accurately weighed 0.1000g standard sample of the three drugs was dissolved in double distilled water for ATE, DOX and LID, transferred into a 100 mL standard flask and diluted to the mark with double distilled water for ATE, DOX and LID mixed well. This stock solution was further diluted to obtain working solutions in the ranges of 0.5–3, 0.4– 8 and 5–50 $\mu$ g/ mL for ATE, DOX and LID, respectively.

#### 2.3.2 Sodium 1, 2-naphthoquinone-4-sulfonic solution (0.3%, 0.5% w/v)

An accurately weighed 0.3000g and 0.5000g of NQS was dissolved in double distilled water, transferred into a 100 ml standard flask and diluted to the mark with double distilled water and mixed well to prepare (0.3% ,0.5% w/v), respectively. The solution was freshly prepared and protected from light during use.

### 2.3.3 Buffer solutions

Buffer solution of pH 12.0 was prepared by mixing 50 mL of 0.2 M KCl with 24 mL of 0.2 M NaOH, and buffer of pH 13.0 was prepared by mixing 25 mL of 0.20 M KCl solution with 65 mL of 0.20 M NaOH solution, in 100 mL volumetric flask and adjusted by a pH meter. Buffer solutions of different pH value were also prepared according to literature method (Robinson & Stokes1955).

### 2.3.4 Sample Solutions

The contents of 20 tablets were evacuated and well mixed. Then an accurately weighed amount equivalent to 100 mg was transferred into a 100mL calibrated flask, and dissolved in about 40mL in double distilled water for ATE, DOX and LID. After shaking for 10 min, the contents were made up to volume with water, filtered rejecting the first portion of the filtrate. The prepared solution was diluted quantitatively with double distilled water for ATE, DOX and LID to obtain a suitable concentration for the analysis.

## 2.4 General recommended procedure

About 1.00 mL of (0.5–3,0.4–8 and5–50 $\mu$ g/mL) for ATE, DOX and LID, respectively, were transfer in to 10mL volumetric flask subsequently, 1.5mL of pH 12.0 for LID, 2 and 1.5mL of pH 13.0 for ATE and DOX respectively were added and 1 mL of 0.5% NQS were added for ATE and DOX and 1 mL of 0.3% (NQS) solution was added for LID, the solution was heated in a thermostat at 70oC for 10 minutes for ATE , and 5 and 30 min at room temperature for LID and DOX, the mixture was diluted with double distilled water for all drugs. The absorbance of the solution was measured at 454, 460, 481 nm for ATE, DOX and LID respectively against a reagent blanks treated similarly.

## 2.5 Determination of the stoichiometric ratio of the reaction (Job's method)

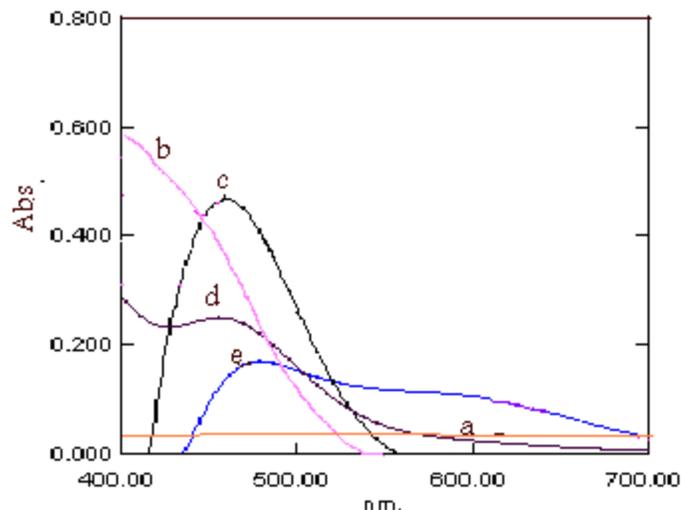
The Job's method (Oliner & Boyd 1964) of continuous variation was employed. Equimolar ( $2.5 \times 10^{-3}$ M) aqueous solutions of ATE, DOX and LID and (NQS) were prepared. Series of 10-mL portions of the master solutions of ATE, DOX and LID and NQS were made up comprising different complementary proportions ( 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0 ), The solution was further treated as described under the general recommended procedures.

## 3 RESULT AND DISCUSSION

### 3.1 Absorption spectra

According to the procedure the absorption spectrum of products produced by the reaction of ATE, DOX and LID with (NQS) are recorded in (Fig.2). as can be shown in (Fig.2) the maximum absorption wave length peak ( $\lambda_{max}$ ) at 454, 460,and 481nm for ATE, DOX and

LID, respectively and the  $\lambda_{\text{max}}$  of NQS was 360 nm (the determination of products against the reagent blank). Obviously ATE, DOX and LID has no absorption in the range 400-800 nm, in order to eliminate interference.



**Fig 2.** Absorption spectra of (a) ATE, DOX and LID (1, 5 and 10 $\mu\text{g}/\text{mL}$  respectively) (b) Absorption spectra of NQS (0.5%) (c) Absorption spectra of ATE (2 $\mu\text{g}/\text{mL}$ ) with NQS 0.5% (d) Absorption spectra of DOX (5 $\mu\text{g}/\text{mL}$ ) with NQS 0.5% (e) Absorption spectra of LID (10 $\mu\text{g}/\text{mL}$ ) with NQS 0.3%.

### 3.2 Stoichiometry of Derivatization Reaction

Under the optimum conditions Table.1. the stoichiometry of the reaction between ATE and DOX with NQS was investigated by Job's method and were found to be 1:1 and LID was found to be 1:2 because DOX and ATE molecules contains only one center (primary or secondary amino group respectively) Fig.3. While LID molecule contain primary and secondary amino group. Based on this ratio, the reaction pathway was postulated to be proceeded as shown in Scheme 1.

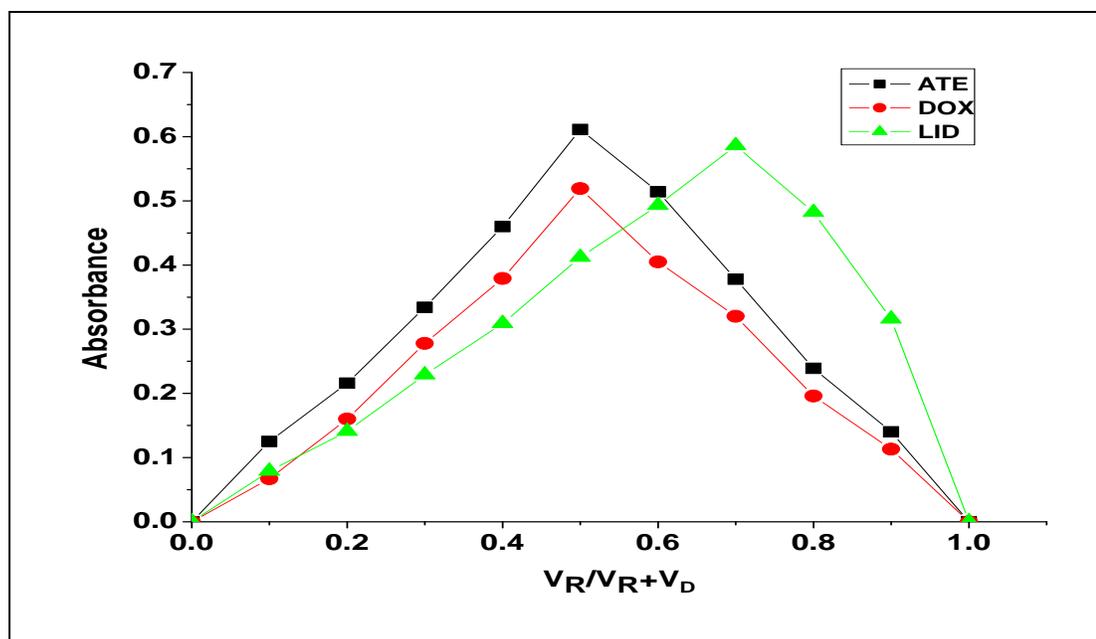
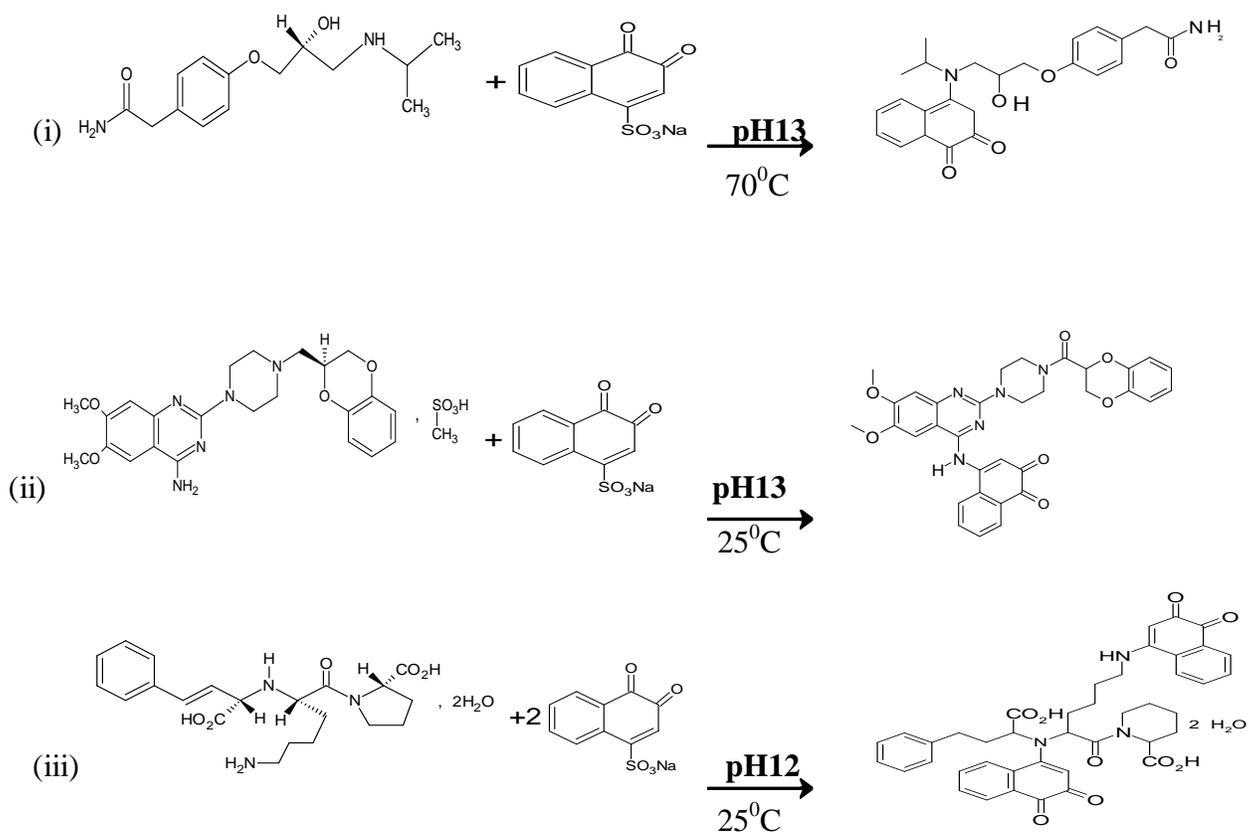


Fig.3. Job's plots of continuous variation of product: ATE, DOX and LID with NQS.

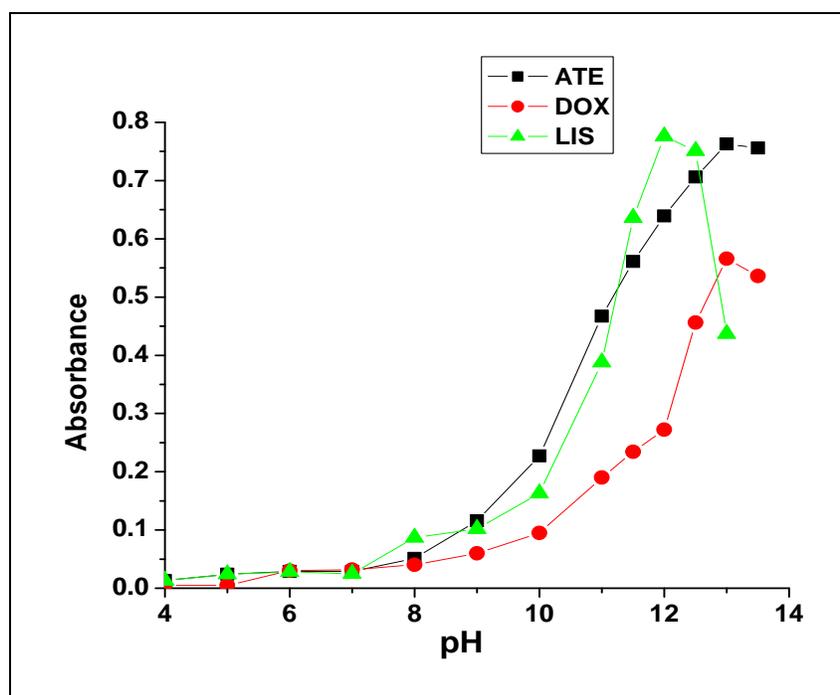


**Scheme 1.** Reaction pathway of (i) ATE ;(ii) DOX and (iii) LID with NQS

### 3.3 Optimization of Derivatization reaction and Spectrophotometric procedure

#### 3.3.1 Effect of pH

The effects of pH on the reaction of ATE, DOX and LID with (NQS) were examined by varying the pH from 4.0 to 13.5. The results revealed that ATE, DOX and LID have difficulty to react with (NQS) in acidic media (Fig.4). This was possibly due to the existence of the amino group of ATE, DOX and LID in the form of hydrochloride salt, thus it loses its nucleophilic substitution capability. As the pH increased, the readings increased rapidly, as the amino group of ATE, DOX and LID (in the hydrochloride salt) turns into the free amino group, thus facilitating the nucleophilic substitution. The maximum readings were attained at pH values of 12.0 for LID and 13.0 for ATE and DOX. At pH values more than 12.0 for LID and more than 13.0 for ATE, DOX a decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction of ATE, DOX and LID with (NQS).



**Fig.4.** Effect of pH on absorbance of product ATE, DOX and LID with NQS

### 3.3.2 Effect of reaction temperature and time

The effect of temperature on the reaction was also studied by varying the temperature from 25 °C to 90 °C for ATE. The reaction does not go in room temperature and the highest absorbance is obtained at 70 °C for 10 minutes. However, for DOX and LID it were found that the reaction with NQS were not affected by increasing the temperature, and the reaction at room temperature ( $25 \pm 5$  °C) went to completion in 30minutes for DOX and 10 minutes for LID. (Fig.5 and Fig.6).

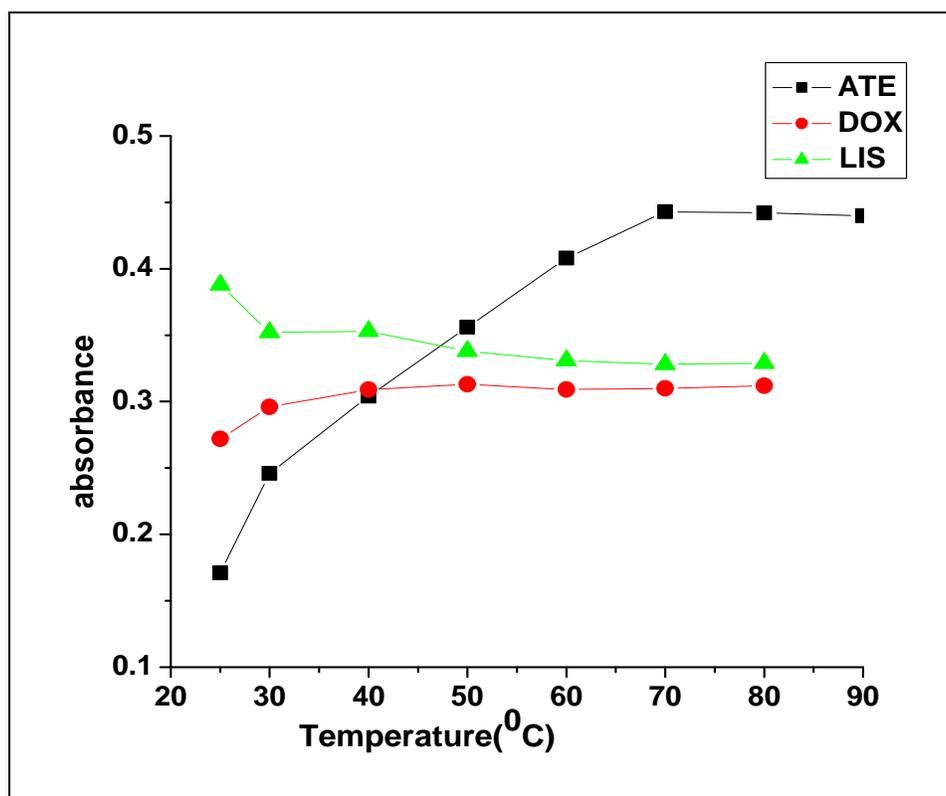


Fig.5.Effect of temperature on absorbance of product ATE, DOX and LID with NQS

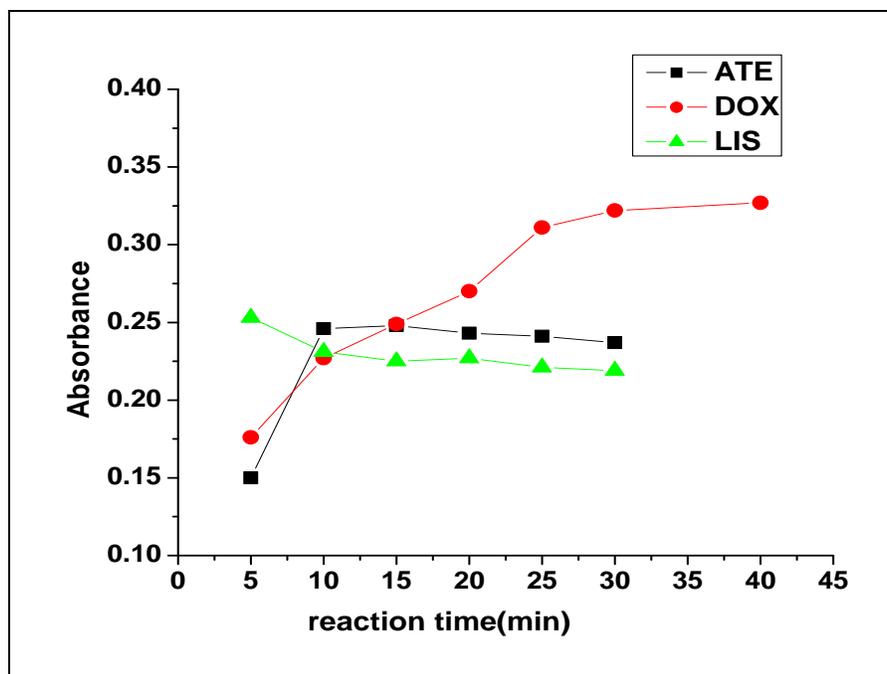


Fig.6. Effect of reaction time on absorbance of product ATE, DOX and LID with NQS

### 3.3.3 Effect of (NQS) concentration

The studying of (NQS) concentrations revealed that the reaction was dependent on NQS reagent. The highest absorption was attained when the concentration of NQS was 0.5% for ATE and DOX and 0.3% for LID. (Fig.7). from the previously described experiments the optimum conditions for the reaction of (NQS) with ATE, DOX and LID were summarized in Table 1.

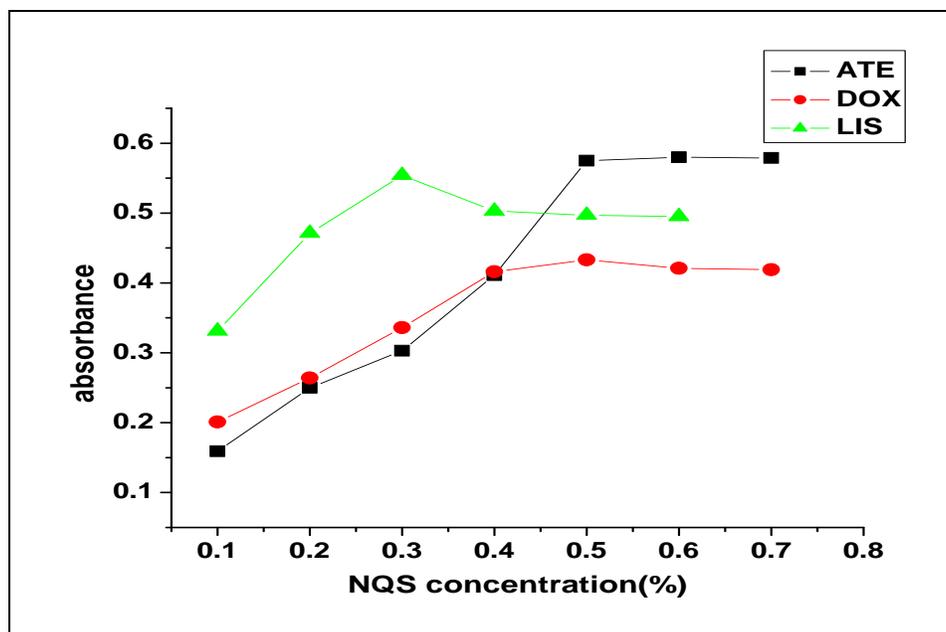


Fig.7. Effect of NQS concentration on absorbance of product ATE, DOX and LIS with NQS

Table 1. Optimum condition for the reaction of ATE, DOX and LIS with NQS

condition	ATE	DOX	LIS
pH	13	13	12
Volume of buffer(ml)	2	1.5	1.5
Temperature	70	25	25
Reaction time (min)	10	30	5
NQS concentration (%)	5	5	3

### 3.4 Validation of the Methods

#### 3.4.1 Linearity and Limits of Detection.

The regression equation for the results was  $Y = 0.0518 + 0.1298X$ ,  $Y = 0.08469 + 0.06546X$ ,  $Y = 0.06839 + 0.0109X$  for ATE, DOX and LIS respectively. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to The International Conference of Harmonization (ICH) guidelines for validation of analytical procedures (ICH 2005). The following formula was used:  $LOD$  or  $LOQ = k S_{Da} / b$ , where  $k = 3.3$  for LOD and 10 for LOQ,  $S_{Da}$  is the standard deviation of the intercept, and  $b$  is the slope. The parameters for the analytical performance of the proposed methods are summarized in Table 2.

Table 2. Parameters for the performance of the proposed method

Parameter	ATE	DOX	LIS
$\lambda_{max}$ , nm	454	460	481
Beer's law limits, $\mu\text{g/ml}$	0.5—3	0.4—8	5—50

Molar absorptivity, l/mol cm	$4.01 \times 10^4$	$4.5 \times 10^4$	$5.4 \times 10^3$
Sandell sensitivity, $\mu\text{g}/\text{cm}^2$	$6.65 \times 10^{-3}$	$1.21 \times 10^{-2}$	$8.17 \times 10^{-2}$
Limit of detection, $\mu\text{g}/\text{ml}$	0.11	0.12	1.16
Limit of quantification, $\mu\text{g}/\text{ml}$	0.34	0.36	3.53
<b>Regression equation, Y* :</b>			
Intercept (a)	0.0518	0.08469	0.0683
Standard deviation of intercept	0.00445	0.00242	0.00385
Slope (b)	0.12983	0.06546	0.0109
Standard deviation of slope	0.00228	$5.3942\text{E}^{-4}$	0.0004
Correlation coefficient ( $r^2$ )	0.9993	0.99986	0.9997
Standard deviation	0.00478	0.00365	0.00479

\* $Y=a+bX$ , where Y is the absorbance, a intercept, b slope and X concentration in  $\mu\text{g}/\text{mL}$ .

### 3.4.2 Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were shown in Table 3.

Table 3. Robustness of the proposed spectrophotometric method

Recommended condition	AT E	Recovery (% $\pm$ SD) <sup>a</sup>	DOX	Recovery (% $\pm$ SD) <sup>a</sup>	LID	Recovery (% $\pm$ SD) <sup>a</sup>
Standard	13.0	98.71 $\pm$ 0.9	13.0	99.79 $\pm$ 0.85	12.0	99.6 $\pm$ 0.59
Ph	12.8	98.34 $\pm$ 0.38	12.8	97.80 $\pm$ 1.23	11.8	100.90 $\pm$ 0.79
	13.2	99.58 $\pm$ 0.56	13.2	99.38 $\pm$ 0.89	12.2	99.58 $\pm$ 0.56
NQS concentration (w/v %)	0.45	99.38 $\pm$ 0.47	0.45	100.60 $\pm$ 1.45	0.25	100.89 $\pm$ 1.23
	0.55	100.65 $\pm$ 0.39	0.55	99.20 $\pm$ 1.25	0.35	101.04 $\pm$ 0.98
Temperature ( $^{\circ}\text{C}$ )	65	100.68 $\pm$ 0.51	23	98.51 $\pm$ 0.67	23	98.72 $\pm$ 1.03
	75	99.35 $\pm$ 0.48	27	99.20 $\pm$ 0.82	27	99.65 $\pm$ 0.88
Reaction time (min)	8.0	97.92 $\pm$ 0.27	28	101.12 $\pm$ 1.05	-	
	12.0	100.16 $\pm$ 0.59	32	99.60 $\pm$ 0.54	-	

<sup>a</sup> Values are mean of 3 determinations

### 3.4.3 Accuracy and precision

The proposed method was applied to some pharmaceutical formulations containing ATE, DOX and LID. The results in Table 4 indicate the high accuracy of the proposed method for the determination of the studied drugs. As can be seen from (Table 6), the proposed method has the advantage of being virtually free from interferences by excipients such as glucose, lactose and starch or from common degradation products. The percentages were 102.10  $\pm$  0.01, 101.03  $\pm$  0.09, 99.58  $\pm$  0.80 for ATE, DOX and LID, respectively.

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80 %, 100 % and 120 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was

3.0 µg/ml of ATE and 8.0 µg/ml of DOX and 50.0 µg/ml of LID. Reparability of the methods was studied by repeating the methods five times. To study intra-day precision, method was repeated 3 times in a day. Similarly the method was repeated on five different days to determine inter-day precision. Table 5.

Table 4. Recovery of the proposed methods

Drug	Sample content (µg/mL)	Added (µg/mL)	Found (µg/mL)	Recovery(%±SD) <sup>a</sup>
ATE	0.3	0.5	0.786	98.25±0.99
	0.3	1.5	1.835	101.90±0.40
	0.3	2.5	2.872	102.57±1.59
DOX	0.4	0.6	0.972	97.20±0.44
	0.4	2.6	2.98	99.60±1.36
	0.4	6.6	7.06	100.57±0.75
LID	5	5	9.78	97.83±0.86
	5	15	19.92	99.69±0.40
	5	40	45.81	101.80±1.10

<sup>a</sup> Recovery was calculated as the amount found/amount taken × 100. Values are mean ± R.S.D. for three determinations.

Table 5. Evaluation of intra-day and inter-day accuracy and precision

Drug	Taken µg/mL	Intra-day			Inter-day		
		Found µg/mL	SD	RSD %	Found, µg/mL	SD	RSD %
ATE	2.4	2.397	0.011	2.90	2.95	0.03	1.44
	3.0	2.985	0.012	3.10	2.961	0.02	0.87
	3.6	3.609	0.005	2.10	3.59	0.008	2.50
DOX	6.4	6.383	0.005	1.50	6.366	0.006	1.04
	8.0	7.988	0.005	2.30	7.963	0.009	1.16
	9.6	9.590	0.006	2.50	9.582	0.011	1.43
LID	40	40.87	0.008	2.40	40.64	0.005	1.49
	50	48.75	0.001	1.44	48.56	0.004	1.42
	60	60.16	0.004	1.41	60.07	0.005	1.48

- SD. Relative error; RSD. Relative standard deviation.
- Mean value of 3 determinations

### 3.5 Applications of the Methods

The proposed method was applied to some pharmaceutical formulations containing ATE, DOX and LID. The results in Table 5 indicate the high accuracy of the proposed method for the determination of the studied drugs. The proposed method has the advantage of being virtually free from interferences by excipients such as glucose, lactose and starch or from common degradation products. The percentages were  $102.10 \pm 0.01$ ,  $101.03 \pm 0.09$ ,  $99.58 \pm 0.80$  for ATE, DOX and LID, respectively Table 6.

Table 6. Determination of the studied drugs in their pharmaceutical dosage forms

Drug	Tablet brand name	Label claim mg/tablet	Proposed method Found (% $\pm$ SD) <sup>a</sup>
ATE	Amitenol	50	$102.10 \pm 0.01$
DOX	Cardosyr	4	$101.03 \pm 0.09$
LID	Lisopril	5	$99.58 \pm 0.80$

<sup>a</sup> Values are mean of five determinations

## 4 CONCLUSIONS

The present study described the evaluation of (NQS) and as analytical reagent in the development of simple, sensitive, and accurate spectrophotometric methods, for the determination of ATE, DOX and LID in pharmaceutical formulations. The described method is superior to the previously reported spectrophotometric methods in terms of the simplicity and sensitivity. The proposed method has comparable analytical performances and devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Therefore, this method can be recommended for the routine analysis of ATE, DOX and LID in quality control laboratories.

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