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# Spectrophotometric Determination Of Orlistat In Pharmaceutical Formulation And Some Body Fluid By Folin's Reagent

**Athraa Aqeel Ali[1], Muthana Saleh Mashkour[1], Hazim Y. Saeed[2]**

*1- Department of Chemistry, Faculty of Science, Kufa University.*
*2- Department of Radiology Techniques, College of Medical Technology, The Islamic University, Najaf, Iraq*

## Abstract

The purpose of current study is to estimation the orlistat in pure form and pharmaceutical formulations by a simple, exact, precision, accurate and sensitive spectrophotometric technique via the use of the organic reagent NQS (1,2-Naphthoquinone-4-sulfonic acid sodium salt) In an alkaline medium. The maximum absorption of pure drug (Orlistat) was recorded at the wavelength (λmax) 212nm and the wavelength of the reagent NQS at 365nm. The wavelength of the products was 469 nm. The optimum conditions for this reaction were studied, where included the best reagent concentration (NQS), the optimum volume of the reagent, Acidity function has an effective role in increasing the absorbance the of color product, best time for the stability of the colored, and the effect of different temperature was also studied.

The ideal addition of drug, reagent and base solution recorded an increase in absorbance, as the order of addition was( Drug +NQS + NaOH ). After completing the study of the optimal conditions and obtaining the best results, a calibration curve was built for orlistat, which was found to comply the Beer-Lambert's law obeyed with range of concentrations (20 - 400) µg. $mL^{-1}$and the Linearity correlation was 0.9992 and the value of the molar absorptive constant was 1735.06 $L.mol^{-1}.cm^{-1}$ and Sandal's sensitivity is 0.0122 µg.cm-² and its stability constant was $5.43\times10^{5}$. This study showed that the effect of some additives in the pharmaceutical compounds had no effect on the product reaction. Determination of Orlistat in the pharmaceutical formulation and some body fluid by NQS reagent was very successfully.

**Key Words:** Orlistat, NQS, Pharmaceutical formulation, Drug analysis and body fluid

## 1. Introduction:

Pharmaceutical analysis has traditionally been defined as analytical chemistry that deals with drugs as both bulk drug substances and pharmaceutical products (formulations)[1,2]. However, other branches of analytical chemistry, such as bioanalytical chemistry, drug metabolism studies, and analytical biotechnology are also involved in academia and the pharmaceutical industry[3,4].Drug development in the pharmaceutical industry is a lengthy process that frequently takes more than a decade from the start of a research project to the appearance of a drug on the market [5,6].

Orlistat is a medication used to treat obesity. Orlistat causes varying degrees of weight loss. In one-year clinical trials, between 35.5 percent and 54.8 percent of subjects lost 5% or more of their body weight, though not all of this weight was necessarily fat. Between 16.4 percent and 24.8 percent lost at least 10% of their body fat [7,8]. After discontinuing orlistat, a significant number of subjects gained

weight—up to 35% of the weight they had lost[9,10].It reduces the incidence of type II diabetes in obese people by roughly the same amount that lifestyle changes do[11,12]. Figure 1 show the chemical structure of orlistat drug.

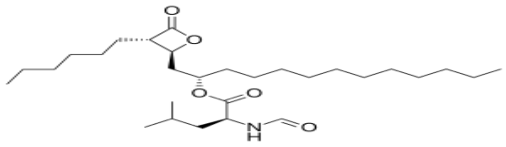

**Figure (1) the chemical structure Orlistat [13]**

Folin's reagent, also known as sodium 1,2-naphthoquinone-4-sulfonate, is a chemical reagent used to derivative amino acids and amines to detect their concentrations[14,15].In an alkaline solution, the reagent combines with them to create a luminous substance [16-18]. Figure (2) show the chemical structure of NQS reagent.

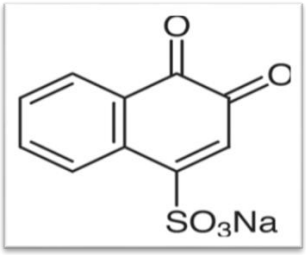


Figure (2) the chemical structure of NQS reagent [19]

They reported various methods used to approach the orlistat drug in pure form, pharmaceutical formulation and some body fluid such as chromatographic methods [20], UV-Visible spectrophotometric [21] and electrochemical method such as cyclic voltammetry [22].

## 2. Experimental part:

All the chemicals were had high purity and provided form different world company such Orlistat / chine, Folin's reagent (NQS) / India and Sodium hydroxide / BDH, Ethanol / UK.

### 2.1 Instruments

A Shimadzu UV-Vis 1650 Spectrometer double beam (Japan)

Sensitive electronic balance Type ABS 120 – 4 KERN.

Circular water bath Korea VISON .

Centrifuge Germany Hettich

### 2.2 Preparation of standard solutions

#### 1. Solution of Orlistat

The preparation of solution of Orlistat (1000 µg/ml) was by solubility of appropriate weight 0.1 g from drug in volumetric flask 100 ml with absolute ethanol.

#### 2. Solution of 1, 2 napthoquine-4-sulfonate-sodium (Folin's reagent)

By dissolving of 0.07 g of NQS (Folin's reagent) in 10 ml of deionized water in volumetric flask with a 10 ml capacity as a percent W/V%. Dark flask used to prepare this solution.

#### 3. Solution of NaOH

Dissolving 0.02 g of sodium hydroxide base in 50 ml of deionized water and transferring the mixture to a volumetric flask with a capacity of 50 ml, a solution with a concentration of 0.01 mol. $L^{-1}$ was prepared.

#### 4. Preparation of solutions of interferences

1 g of each interfering substance dissolving it in 100 ml of solvents. Such as the magnesium citrate, povidone, titanium dioxide, Gelatin and others these substances were dissolved and filtrated and

taken the filter to investigate the interfering inside the medication using NQS reagent

## 3. Results and discussion

### 3.1 General procedure:

An aliquot of standard stock solution was transferred into a 10 mL volumetric flask contain 1.5 mL of orlistat, 1 mL of reagent NQS, and 1 mL of NaOH. The mixture was mixed well and diluted to 10 mL with deionized water at room temperature. The absorbance was measured at absorption maximum 469 nm against a blank**.** Figure (3) show the absorption spectra of orlistat drug, NQS reagent and the color product

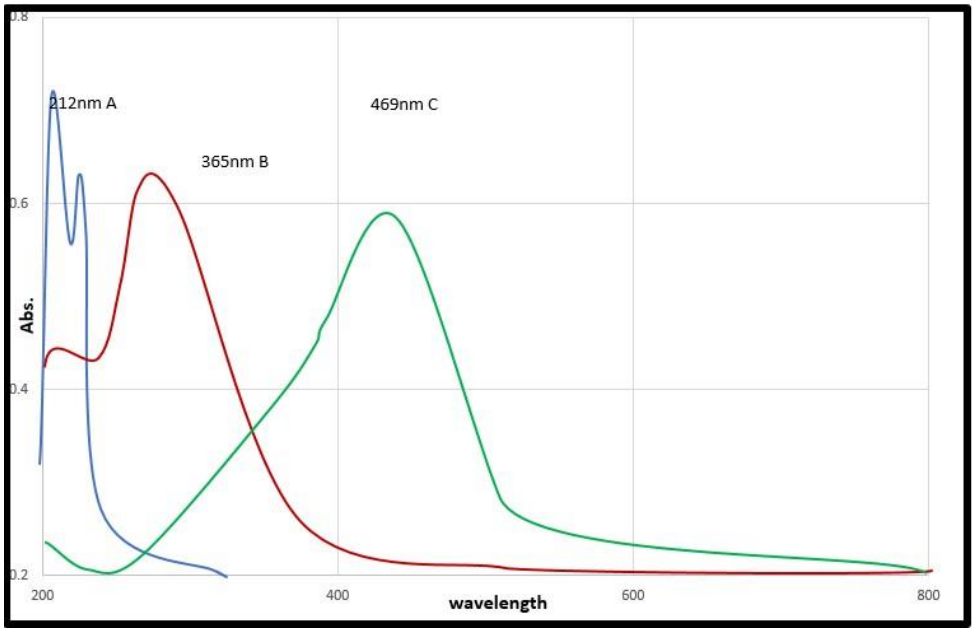


**Figure 3 Absorption spectra for A. Orlistat, B. NQS reagent and C. Color product for reaction of A and B**

### 3.2 Optimizing reaction condition:

#### 1. Effect of NQS concentration

The reagent was weighed out in increments of (0.1-1) g, and the varying weights were thoroughly dissolved in 10 ml of deionized water into volumetric flask. A blank was having all component except drug. 1.5 ml of orlistat with 1mL of reagent (different concentrations) and the volume of NaOH was 1 ml to forming the product. Than dilute solution to 10 ml by deionized water. The absorbance was measured at the maximum wavelength 469 nm. Where the highest absorbance was recorder at a concentration of 0.7% (w/v) % it was 0.584 as show in figure 4.

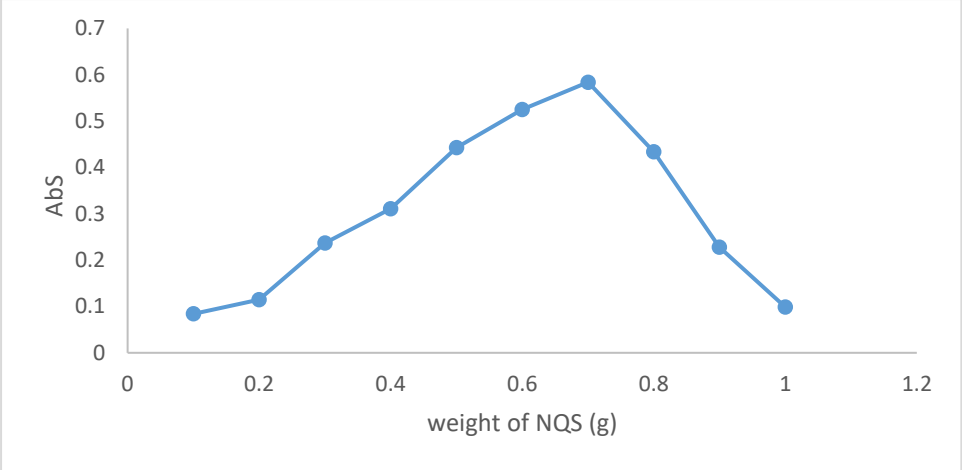


**Figure 4. Effect the weight of NQS on reaction with orlistat in alkaline medium**

## 2. Effect of NQS volume

The change in the volume of the reagent by taking different volumes ranging from (0.25-3ml) at concentration 0.7% (w/v) % was studied. 1.5ml of orlistat drug different volume of reagent and 1ml of base NaOH with a concentration 0. 01mol.$L^{-1}$ was added and the solution diluted in a volumetric flask 10mL capacity with ionized water. Blank for each volume of the reagent without adding the drug. The best absorption was recorded, which is 0.587 at a volume of 1 ml of the reagent at the greatest wavelength 469nm, as shown in figure 5.

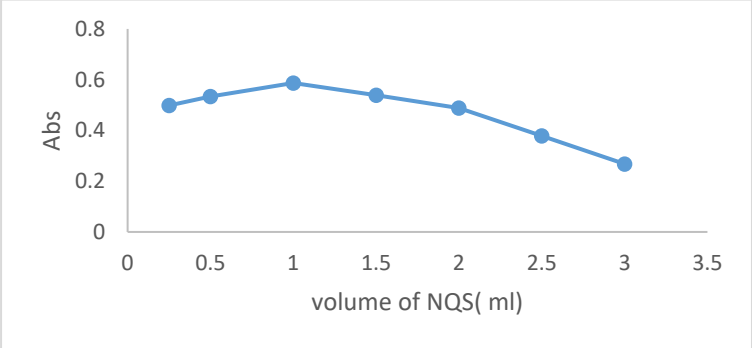


**Figure 5. Effect volume of NQS on reaction with orlistat in alkaline medium**

## 3. Effect of NaOH concertation

Different concentrations of the base were prepared for studying the effect changing the concentration of the base NaOH. The weights of base ranged between (0.005 and 0.25) g. we're dissolving in 100 ml of deionized water, and 1 ml was withdrawn from it and added to 1.5ml orlistat and 1 ml of NQS reagent, after that the volume completed to 10 ml by deionized water. Blank's solution for each of these concentrations was used, the highest absorbance was recorded it was 0.589 at concentration of 0.05 g. As shown in Figure 6.

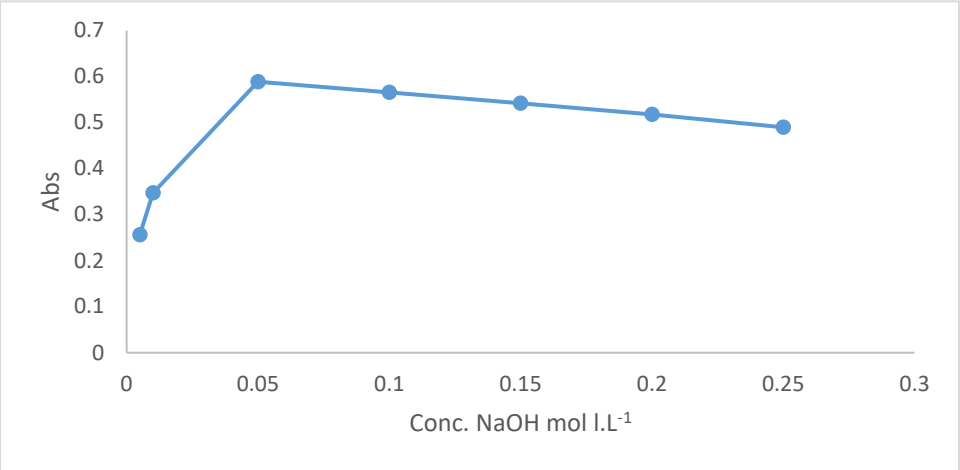


**Figure 6. Effect the concentration of NaOH (mol. $L^{-1}$) on the reaction of orlistat with NQS reagent**

## 4. Effect of NaOH volume:

The effect of changing the volume of the base NaOH was conducted, when the base concentration was 0.01 mole. $L^{-1}$. Different volumes of the base were ranging from (0.25 to 3) mL, were added to 1.5 ml of orlistat and 1 ml of NQS reagent, then volume of the solution completed to 10 ml by deionized water. Blank's solutions were prepared for each volume, the highest absorbance was recorded it was 0.594 at the ideal base volume 1 mL with the greatest wavelength 46nm, as shown in Figure 7.

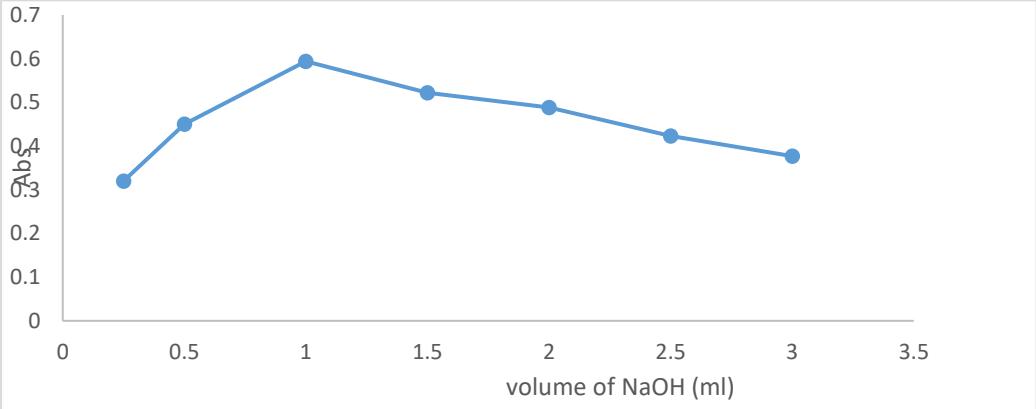


**Figure 7. Effect the volume of NaOH on reaction of orlistat with NQS reagent**

## 5. Effect of Orlistat volume:

The effect of changing the volume of the drug Orlistat whose concentration is (1000µg.$mL^{-1}$) was studied by taking different volume ranging from (0.25 to 4) mL added to 1 ml of the reagent NQS and 1 mL of the base NaOH and complete to 10 ml with ionized water, its highest absorption was 0.612 at the best volume of the drug, it was 1.5 ml at the greatest wavelength 469 nm. As shown in Figure 8.

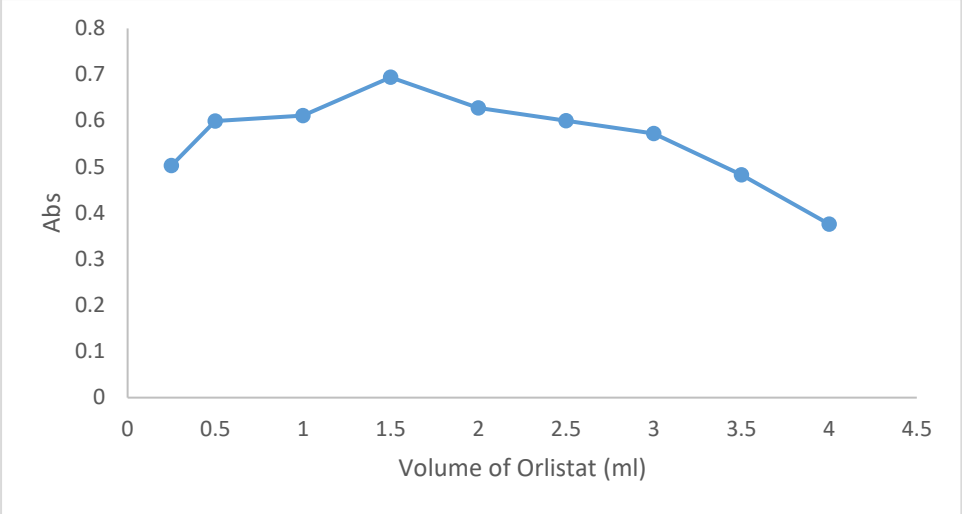


**Figure 8. Effect volume of Orlistat on the reaction with NQS in alkaline medium**

## 6. Effect of the time:

Studying the effect of time on the stability of the color product was achieved. The reaction of 1.5 ml from Orlistat at concentration 150µg.mL$^{-1}$ added to 1 mL of the NQS reagent at concentration 0.7 % (w/v %), then 1 ml of the NaOH at concentration of 0.01 mol. L$^{-1}$ added to them. The color product was measured at different time ranged from (5 -120) min. The best time was recorded at 15 min, which gave the highest absorption it was 0.622 and it was fixed as the best time for this study. As shown in Figure 9.

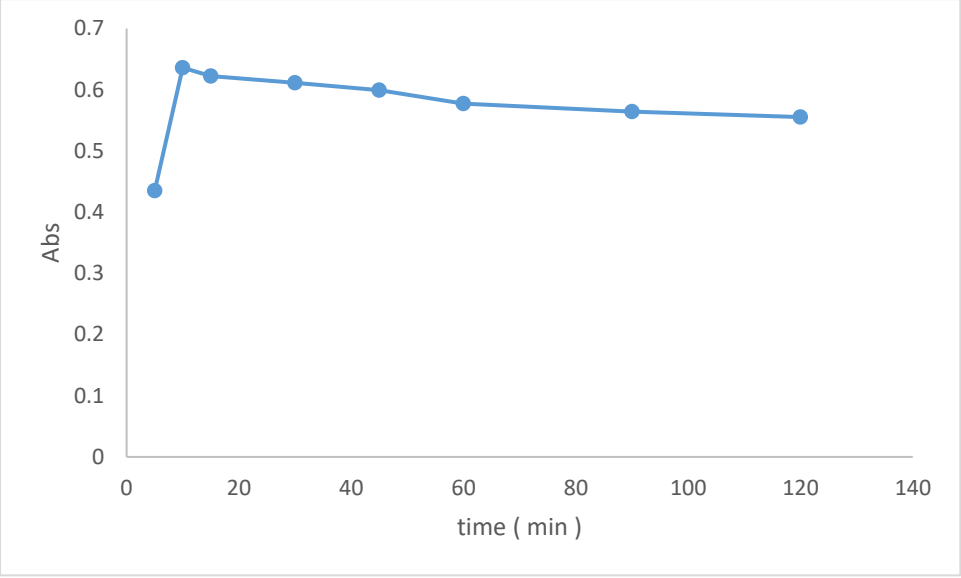


**Figure 9. Effect of time on the stability of the color product**

## 7. Effect of temperature:

The effect of different temperature on the stability of the product was studied, temperatures ranged from (5-50) C°. The best absorbance was 0.675at a temperature of 25 C°. As shown in Figure 10.

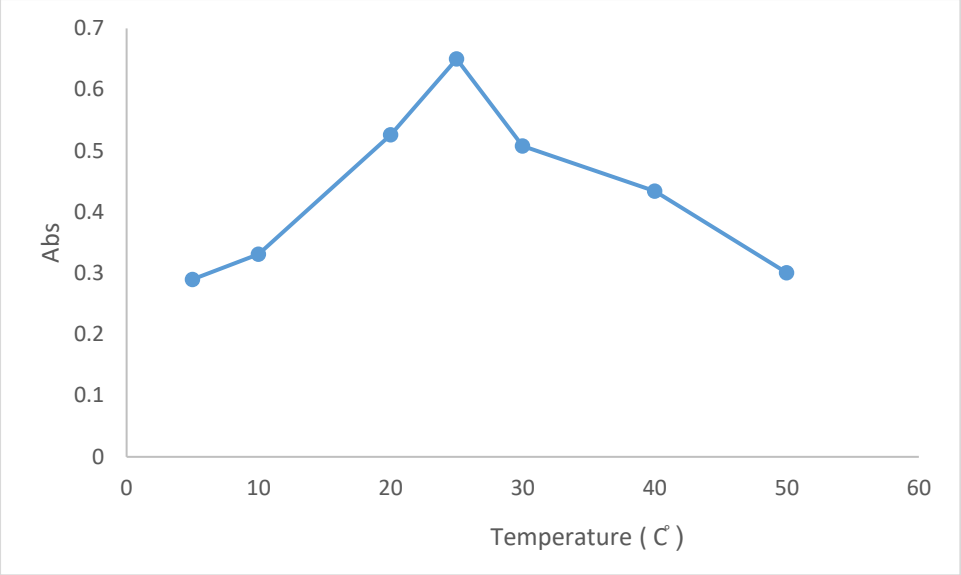


**Figure10. Effect of temperature on the stability of color product**

## 8. Effect of the order of addition:

Based on the solution's best absorbance, the effects of the order of additions for the medication, reagent and base were investigated.

**Table1. Effect the order of addition on absorbance for reaction of Orlistat with NQS**

| No. | Order of addition | Abs. |
|---|---|---|
| **1** | **Drug + NQS + NaOH** | **0.675** |
| **2** | **NQS + Drug + NaOH** | **0.599** |
| **3** | **NaOH + Drug + NQS** | **0.482** |

## 3.3 Construction of calibration curve:

Figure (11) shows the calibration curve for the estimation of orlistat with NQS reagent in the alkaline medium, it is subject to Beer's law for the range( 20-400) $\mu g.mL^{-1}$ at the optimum conditions with the maximum wavelength 469 nm the linearity correlation was $R^2 = 0.9992$, the molar absorption constant is ($\varepsilon$=1735.06 $L.mol^{-1}.cm^{-1}$) ,sandals' sensitivity also calculated was 0.285 $\mu g.cm^{-2}$ and its stability constant is $7.4\times10^5$ as show in table 2,The high value of the molar absorbance and the sensitivity of sandals' proved this method of analysis was preferred for determination of orlistat in bulk form and pharmaceutical formulation.

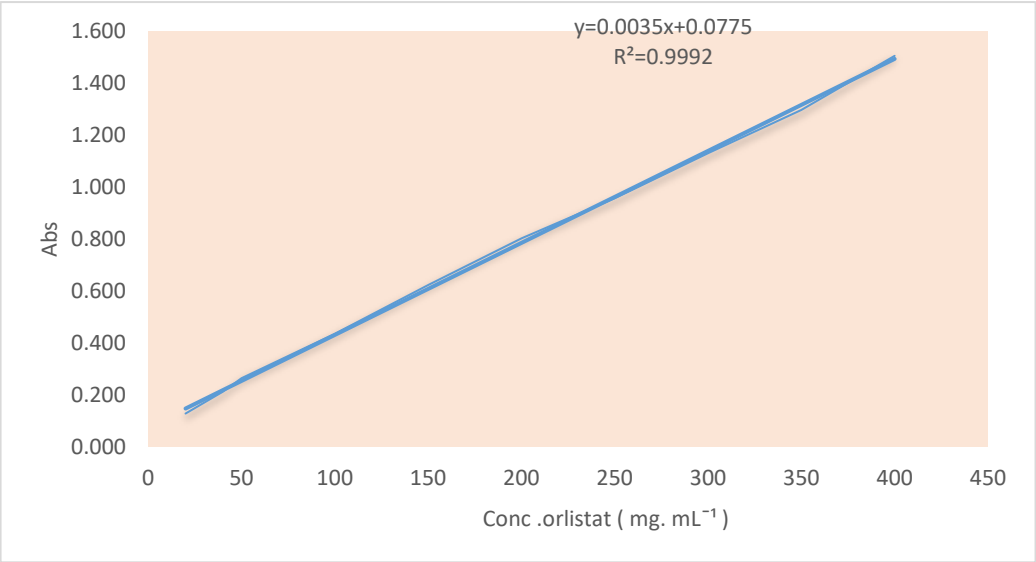


**Figure 11. Calibration curve for Orlistat with NQS reagent**

**Table2. Analytical parameter for determining orlistat with NQS reagent**

| Parameters | Result |
|---|---|
| The Beer Law Limits at( µg.mL$^{-1}$) | 20.0-400.0 |
| Absorptivity constant( L.mol$^{-1}$.cm$^{-1}$) | 1735.06 |
| Liner equation | Y = 0.0035X + 0.0775 |
| Shandell's Sensitivity (µg.cm$^{-2}$) | 0.0122 |
| Detection Limits ( LOD ) (µg.mL$^{-1}$) | 1.1228 |
| Quantitative Limits ( LOQ ) (µg.mL$^{-1}$) | 3.8171 |
| Correlation Coefficient | 0.9992 |
| Stability Constant | 5.43x10$^{5}$ |
| R.S.D | 0.1270 |
| %Erel. | -0.0890 |
| %Recovery | 99.9110 |

### 3.4 Estimation of product composition for orlistat:

Where this study used the stoichiometric method (continuous variance method) (Jobs method) to assess the amount of the drug orlistat with NQS reagent in the alkali medium, the product is formed in the optimal state at the highest wavelength of 469 nm, where the ratio of the drug orlistat to the reagent NQS was (1: 1) also using the molar ratio method showed that the ratio of drug versus reagent was (1: 1) as show in figure 12 and 13[22, 23].

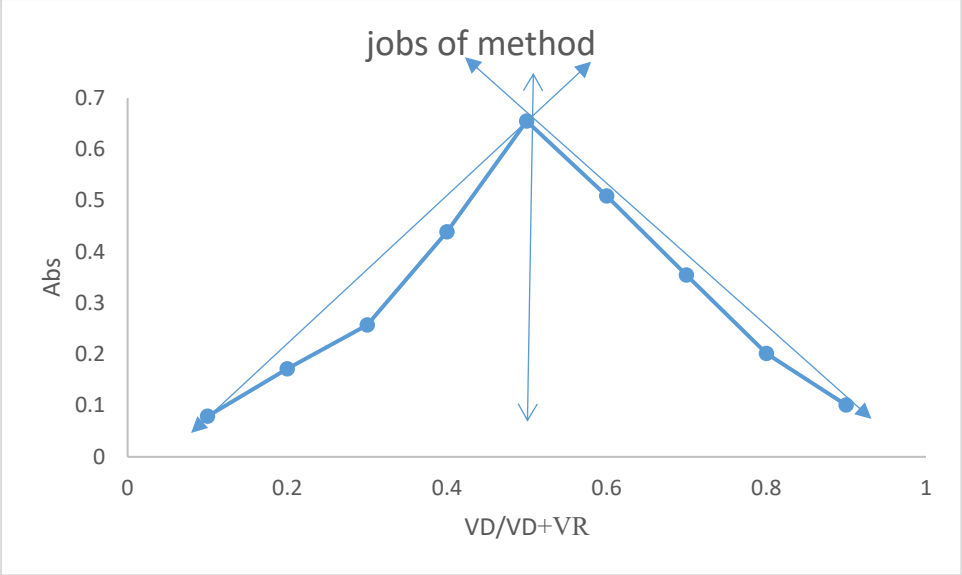


**Figure 12. Job method for the reaction of orlistat with NQS reagent**

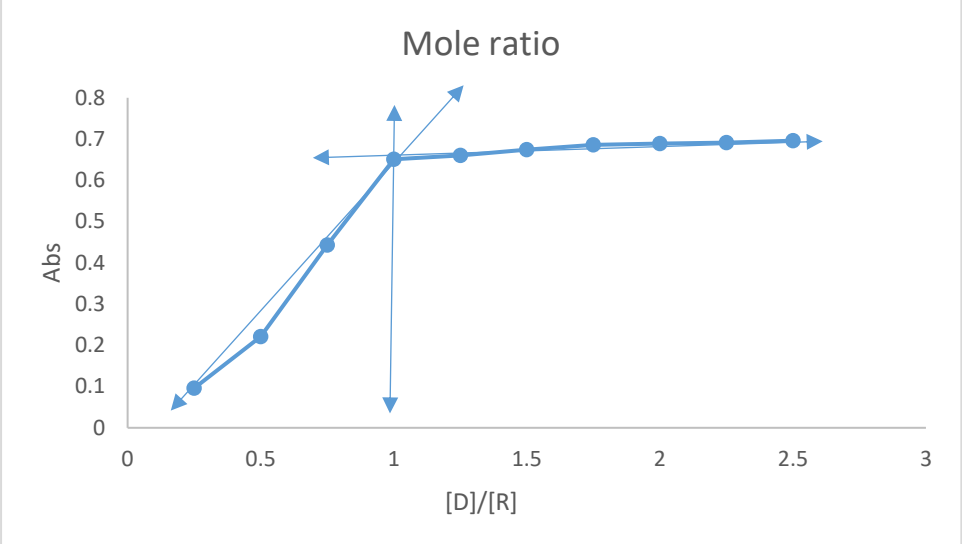


**Figure 13. Mole ratio for the reaction of Orlistat with NQS reagent**

### 3.5 The suggest mechanism for reaction:

Figure 14 show the suggest mechanism for the reaction of orlistat with NQS reagent in the alkaline medium.

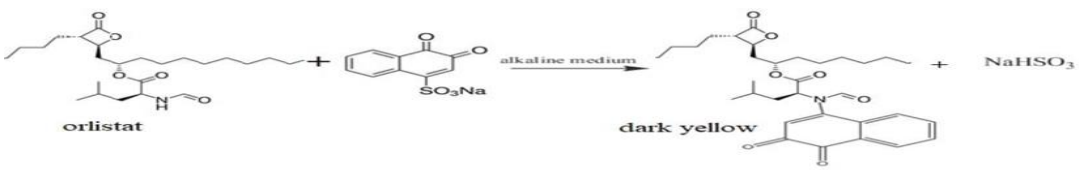


**Figure 14. The suggest mechanism for reaction of orlistat and NQS reagent in alkaline medium**

### 3.6 Precision and accuracy:

To verify precision and accuracy of the studied method, were calculated for orlistat drug using five replicates with a concentration of 150µg.mL$^{-1}$ as shown in Table 3.

**Table 3. Accuracy and precision for orlistat with NQS reagent**

| NO. | Drug µg.mL$^{-1}$ | Abs (xi) | (xi-xv) | (xi-xv)$^2$ |
|---|---|---|---|---|
| 1 | 150 | 0.673 | -0.0006 | 0.00000036 |
| 2 | 150 | 0.672 | - 0.0016 | 0.00000256 |
| 3 | 150 | 0.674 | 0.0004 | 0.0000016 |
| 4 | 150 | 0.674 | 0.0004 | 0.0000016 |
| 5 | 150 | 0.676 | 0.0024 | 0.00000576 |
| | | | $\Sigma$ (Xi-Xv)$^2$ =11.8 x10$^{-6}$ | |

$$= \frac{\Sigma x}{n} = \frac{3.368}{5} = \mathbf{0.6736}$$

$$\bar{x}$$

$$\mathbf{S.\ D} = \frac{\sqrt{\Sigma (Xi - \bar{x})}}{n-1} = \frac{\sqrt{11.8 \times 10^{-6}}}{5-1} = \mathbf{0.000858}$$

$$\mathbf{R.\ S.\ D} = \frac{S.D}{\bar{x}} \mathbf{x100\%} = \frac{0.0008584}{0.6736} \mathbf{x100\% = 0.127}$$

$$\mathbf{\%\ Erel.} = \frac{0.673 - 0.6736}{0.6735} \mathbf{X\ 100\ \% = -- 0.089}$$

**% Recovery= 100 - % Erel. = 100 -0.0.089 = 99.911**

$$\mathbf{L\ O\ D} = \frac{S.D \times 3}{slope} = \mathbf{1.1228}$$

$$\mathbf{L\ O\ Q} = \frac{S.D \times 10}{solpe} = \mathbf{3.8171}$$

### 3.7 Effect of interferences:

This study showed the effect of the additives in the pharmaceutical formulation had no effect on the measuring their absorbance, where the absorbance values were zero for all of them: magnesium citrate, gelatin, iron oxide, cellulose, sodium lauryl sulfate, povidone and titanium dioxide.

**Table 4: interferences for orlistat with NQS reagent**

| Interferences | Abs. |
|---|---|
| magnesium citrate | 0.00 |
| Gelatin | 0.00 |

| | |
|---|---|
| Iron oxide | 0.00 |
| Cellulose | 0.00 |
| Sodium Lauryl Sulfate | 0.00 |
| Povidone | 0.00 |
| Titanium dioxide | 0.00 |

### 3.8 Calculation of dissociation degree and stability of product:

The degree of stability of the product was determined using the equation as below to calculate the molar concentration for each volume of the property and the detector:

C= Am-As/Am

Am: the absorption of the product at the greatest absorption

As: the absorption of the product at the equivalence point

$K_{stability} = 1/K_{instability}$ ----------1

$K = (1-C)/\alpha^2 C$ ----------2

**C: concentration of drug in molarity**

$$\alpha = \frac{0.696-0.675}{0.696} = 0.030$$

$$k = \frac{(1-0.065)}{(0.030)^2(0.675)} = 5.43 \times \frac{5}{10}$$

### 3.9 Application:

Determination of Orlistat in the pharmaceutical formulation with NQS reagent. Where the drug was identified in its capsule within a drug under the trade name Xenical as shown in Table 5.

**Table 5. Accuracy and precision of Orlistat with NQS reagent**

| No. | Concentration of Orlistat(μg.mL$^{-1}$) -------- present found | | Relative error percentage | %Recoverability | %R.S.D |
|---|---|---|---|---|---|
| 1 | 20.00 | 20.20 | 1.00 | 101.00 | 0.164 |
| 2 | 150.00 | 152.00 | 1.30 | 101.30 | 0.321 |
| 3 | 400.00 | 401.00 | 0.25 | 100.25 | 0.230 |

**Table 6. Applications orlistat drug with NQS reagent in serum**

| No. | Conc. present. μg.mL$^{-1}$ | Conc.found.μg.mL$^{-1}$ | %R.S.D | Relative error percentage | Recovery |
|---|---|---|---|---|---|
| 1 | 150.00 | 150.12 | 0.24 | 0.08 | **100.08** |
| 2 | 250.00 | 250.18 | 0.24 | 0.07 | **100.07** |
| 3 | 350.00 | 350.12 | 0.32 | 0.03 | **100.03** |

**Table 7. Applications orlistat with NQS reagent in Urine**

| No. | Conc,persent. µg.mL$^{-1}$ | Conc.found. µg.mL$^{-1}$ | %R.S.D | % Error | Recovery |
|---|---|---|---|---|---|
| 1 | 150.00 | 150.18 | 0.24 | 0.12 | **100.12** |
| 2 | 250.00 | 250.12 | 0.23 | 0.05 | **100.05** |
| 3 | 350.00 | 349.80 | 0.32 | -0.06 | **99.94** |
| 4 | 450.00 | 449.00 | 0.32 | -0.22 | **99.78** |

## 4. Conclusions:

The oxidative reaction of orlistat by NQS reagent in alkaline medium was discovered to be a simple, sensitive, accurate, and cost - effective spectrophotometric method for quantifying (orlistat) in pure form, pharmaceutical preparations and body fluid. The classical univariate and modified simplex methods were used to optimize the various variables affecting reaction completion. The studied method has a high level of linearity.

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