Original Article

# Spectrophotometric Determination Of Anti-Obesity Drug (Lorcaserin) By Azo Coupling Reaction With Brady's Reagent

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## Abstract

The dedication of lorcaserin in its pure form and pharmaceutical preparations, with a simple, exact, precision, accurate and managed spectrophotometric technique is studied. The azo coupling reaction of dinitrophenylhydrazine (2,4DNPH) reagent oxidized by potassium iodate with lorcaserin in alkaline medium. The optimum conditions for the formation of the azo coupling dye were studied from the impact of the reagent's extent and the volume of concentrated $H_2SO_4$, concentration and volume of oxidizing agent $KIO_3$, volume of NaOH, the quick response of time and the temperature effect. This method obeys Beer's law with concentrations ranging from (25- 500) µg. $mL^{-1}$ at max wavelength 590 nm. The Calculated values from the current study were LOD = 0.523 µg. $mL^{-1}$, LOQ = 1.743 µg. $mL^{-1}$, linearity coefficient = 0.9990, sandals sensitivity =0. 294µg.$cm^{-2}$ and stabilization constant is 5. $1\times10^8$.The proposed method has been successfully applied for the dedication of lorcaserin in its pharmaceutical preparations.

## 1. INTRODUCTION:

Pharmaceutical analysis has traditionally been defined as analytical chemistry that deals with drugs as both bulk drug substances and pharmaceutical products (formulation) [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].

Lorcaserin is a medication used to treat obesity. Lorcaserin 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 - 24.8 percent lost at least 10% of their body fat [7]. After discontinuing lorcaserin, a significant number of subjects gained weight—up to 35% of the weight they had lost [8,9]. It reduces the incidence of type II diabetes in obese people by roughly the same amount that lifestyle changes do [10,11]. Figure (1) shows the chemical structure of lorcaserin.

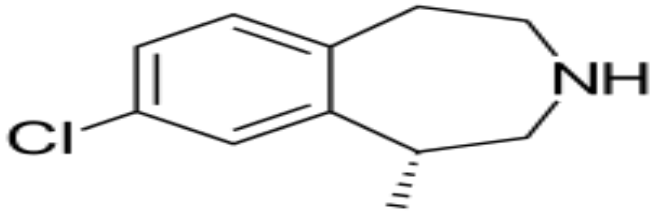



**Figure (1):**The chemical structure of lorcaserin [12]

The organic compound $C_6H_3$ $(NO_2)_2NHNH_2$ is 2,4Dinitrophenylhydrazine (2,4-DNPH or DNPH). DNPH is a reagent used in qualitative organic analysis instructional laboratories. Brady's reagent, also known as Borche's reagent [13], is made by dissolving DNPH in a solution of methanol and concentrated sulfuric acid. This solution detects ketones and aldehydes. The formation of a yellow, orange, or red precipitate of dinitrophenylhydrazone indicates a positive test [13, 14]. Aromatic carbonyls produce red precipitates of 2, 4 DNPH [16].

$RR'C=O + C_{11}H_{14}CLN \rightarrow C11\ H_{12}\ CLN=CRR' + H_2O$

Figure (2) show the chemical structure 2, 4 DNPH

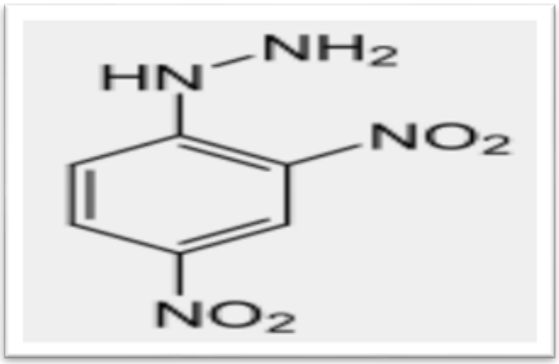



**Figure (2)** the chemical structure of (2,4DNPH) reagent [17]

They reported to estimate the lorcaserin drug in pure form and pharmaceutical formulation by chromatographic methods [18-20].

## 2.EXPERIMENTAL PART:

All the chemicals were had high purity and provided form different world company such Lorcserin / chine, Bradys reagent (2,4DNPH) / India, sulfuric acid from BDH, Potassium iodate from UK, Sodium hydroxide /India.

### 2.1 Instruments

- A Shimadzu UV-Vis 1800 Spectrometer (Japan)
- Sensitive Balance Electronic Balance type ABS 120 – 4 KERN.
- Water Bath Korea VISON.
- Electrical Balance.

### 2.2 Preparation of standard solutions.

**1. Solution of Lorcaserin:** The preparation of solution of lorcaserin (1000 µg. $mL^{-1}$) was by solubility the appropriate weight 0.1g of lorcaserin in volumetric flask 100 mL of deionized water.
**2. Reagent Solution:**2,4-dinitrophenyl hydrazine (2,4-DNPH) [0.01M]: dissolving 0.05 g in 0.625 mL of concentrated sulfuric acid and diluting to 25 mL with deionized water.
**3. Sodium hydroxide [10M]:** dissolving 4g NaOH in 10 mL deionized water.
**4. Potassium iodates [0.007 M]:**0.015 g of $KIO_3$ dissolving in10 mL of deionized water.

## 3. RESULT AND DISCUSSION

### 3.1 General procedure

Add 1.5 mL of 2,4DNPH reagent [0.01M] to 1.5mL of $KIO_3$[0.007 M] after that 2mL of standard solution of lorcaserin drug (1000 µg.$mL^{-1}$) ,finally 0.5 mL of NaOH [10M] and diluted to 10 mL with deionized water at room temperature and the absorbance was measured against blank solution(prepared from the same solutions except the lorcaserin .The reading of the absorbance measured at the max wavelength ,the figures (3) show the max wave length for lorcaserin at 222nm , the max wave length for 2,4DNPH was 359nm and the max wavelength for the color product was 590nm

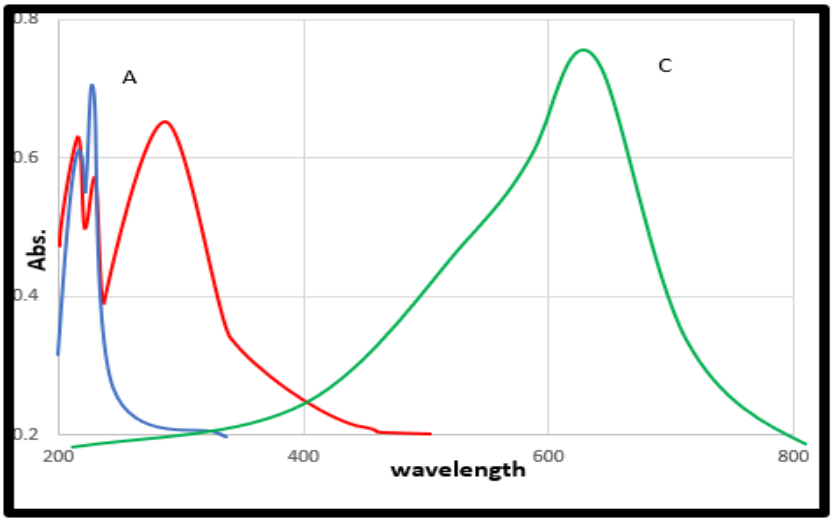



**Figure (3)** Abs. spectra for (A) lorcaserin, (B) 2.4 dinitrophenyl hydrazine (C) Color product for reaction of

### 3.2 Optimizing conditions for reaction

#### 1. Effect of reagent concentration:

The effect of reagent concentration 2,4DNPH range from (0.002 -0.02 M) was studied and showed that the reaction was dependent on a reagent concentration, where the highest absorption intensity was achieved at a concentration of the reagent of 0. 01M.It has the highest absorbance was at 0.618, As showing in figure (4)

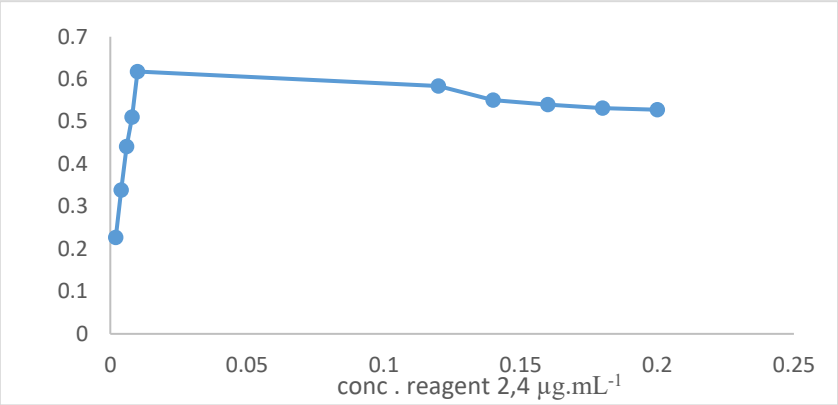



**Figure 4.** Effect concentration of reagent 2, 4 DNPH on reaction with lorcaserin drug

#### 2. Effect the volume of 2, 4 DNPH reagent

After the best concentration of the reagent 2,4DNPH in the current studied, which was recorded the effect of the volume of the reagent on the intensity of absorption resulting from taking different volumes of reagent (0.25 – 3.5) mL, where it was found that the highest absorbance of 2, 4 DNPH reagent at 1.5 ml where the absorbance value was 0.626 as shown in the figure 5.

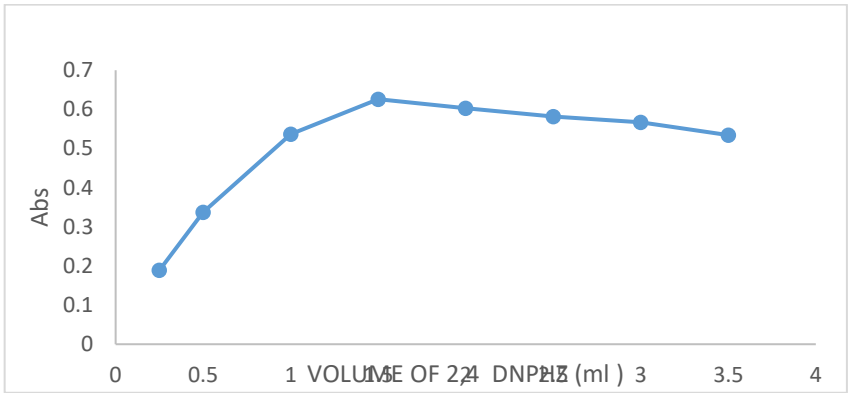



**Figure 5.** Effect volume of reagent 2,4DNPH on reaction with lorcaserin drug

#### 3. Effect the volume of sulfuric acid:

Different volume of concentrated sulfuric acid used arrange from (0.1-0.9) mL to prepare a reagent We obtained the highest absorptive density for the effect of acid volume used to prepare the reagent for lorcaserin give highest absorbance value is 0.645 at a volume of 0.625 mL of acid as shown in the figure6.

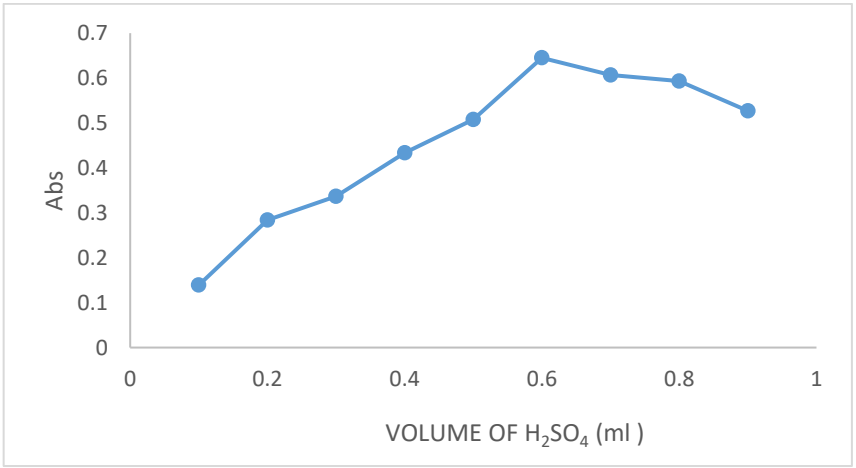



**Figure 6.** Effect volume of $H_2SO_4$ on reaction of 2,4 DNPH with lorcaserin drug

### 4. Effect Concentration of $KIO_3$:

That effect of $KIO_3$weight was recorded at different weight starting from (0.004 to 0.03) g, we were found that the best absorbance for the oxidizing agent was at 0.015g dissolved in 10ml deionized water; concentration is 0.007 M. The highest absorbance reached the peak at 0.015g of $KIO_3$ then it began to decrease, where a difference in the concentration of the oxidizing factor showed a significant effect on the absorbance, as it reached a peak with absorbance is 0.653 as shown in the figure 7.

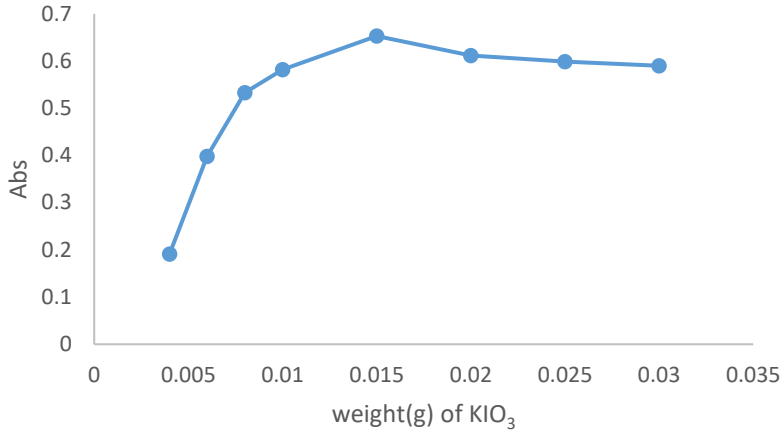



**Figure 7.** Effect the concentration of $KIO_3$ on the reaction of 2,4DNPHwith lorcaserin drug

### 5. Effect volume of $KIO_3$:

The effect of adding different volumes of the oxidizing agent, arranged from (0.25-3.5) mL of potassium iodate to obtaining the best absorbance for this oxidizing agent was at 1.5 mL, where it was recorded The highest absorbance is 0.678 after that we noticed a sharp decrease in the absorbance as it is shows in figure 8.

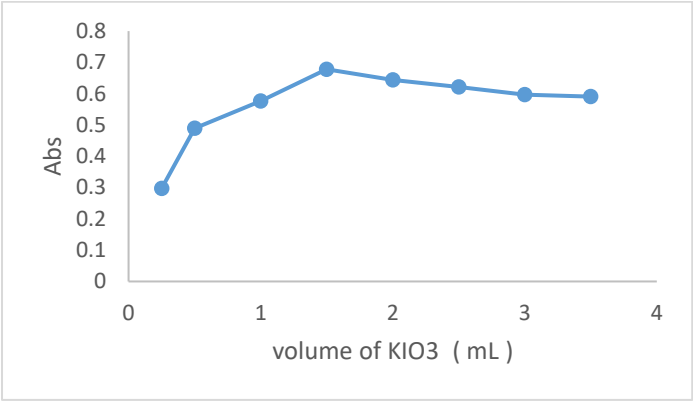



**Figure 8.** Effect the volume $KIO_3$ on the reaction of 2,4DNPHwith lorcaserin drug

### 6. Effect of NaOH volume:

Eight samples of different volume were taken from the base(NaOH) arranged from (0.25-3.5) mL the results of this study showed that adding of 0.5 mL of NaOH with concentration (10 M) is the best volume that helps the drug to give azo coupling dye at highest absorbance's 0.701 as shown in figure 9.

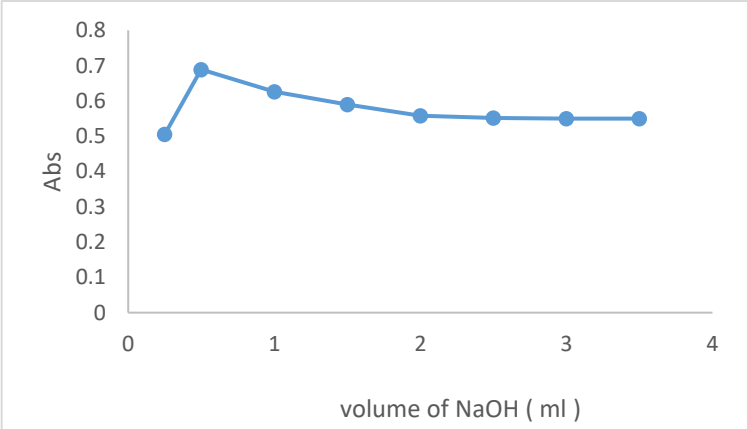



**Figure9.** Effect volume of NaOH on the reaction of 2,4DNPHwith lorcaserin drug

### 7. Effect of reaction time:

We studying the effect of time on the stability for the reaction of lorcaserin drug with a reagent 2,4DNPH in the presence of $KIO_3$ and NaOH the absorption of the reaction product was determined at different times from (5 - 90) min. The highest absorbance was measured is 0.702 at time 10 min, and it was measured under optimal conditions. After that, we noted gradually it began to stable no effect of time; this study was made clear through the readings, after leaving the solution for 10 min, as in the figure10.

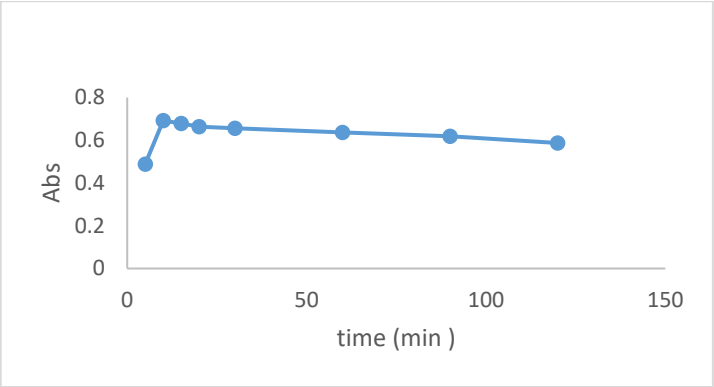



**Figure 10.** Effect the time on the product reaction of 2,4DNPHwith lorcaserin drug

### 8. Temperature effect:

The effect of temperature ranged from (5 – 50) °C in the current study was studied at the optimal conditions for the reaction of anti- obesity drug (lorcserin). The best temperature was 20°C after that began to decline sharply, where the highest absorbance is 0.702, as in the figure11.

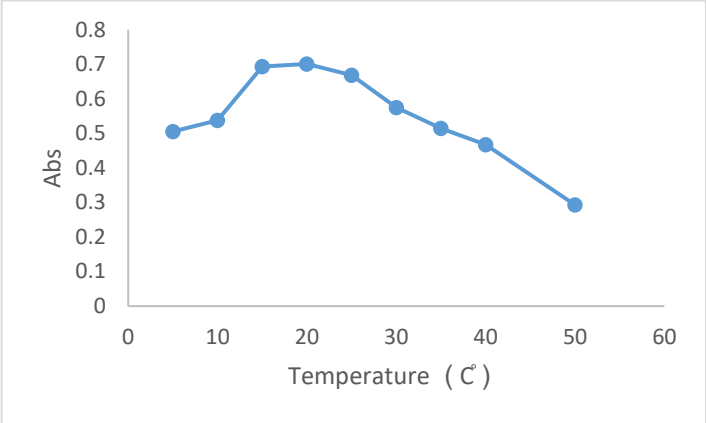



**Figure 11.** Temperature effect on the reaction of 2,4DNPHwith lorcaserin drug

### 9. The effect of addition order:

Where the arrangement of the addition order on the absorption of the reaction was studied based on four procedures to monitor the added ordered substances, which shows the highest absorbance due to the formation of a chemical compound as in the table 1.

**Table 1.** The effect for order of addition

| No. | Order of addition | Abs. |
|---|---|---|
| 1 | Reagent +$KIO_3$ + Drug +NaOH | 0.702 |
| 2 | Reagent + Drug + NaOH + $KIO_3$ | 0.454 |
| 3 | Drug + Reagent + $KIO_3$+ NaOH | 0.611 |
| 4 | Drug + $KIO_3$ + Reagent + NaOH | 0.488 |

### 3.3 Calibration curve:

The figure (12) shows the calibration curve for the estimation of lorcaserin, as it turned out that it is subject to Beer's law between the range( 25-500)µg.mL$^{-1}$ at the optimum conditions for the maximum wavelength 590 nm with correlation linearity ( $R^2$ = 0.9990), so it was the value of the molar absorption constant (Ɛ=6653.46 L.mol$^{-1}$.cm$^{-1}$) ,Sandel's sensitivity also calculated was 0.294 µg.cm$^{-2}$.The high value of the molar absorbance and the sensitivity of Sandal's proved this method of analysis was preferred for determination of lorcaserin in bulk form and pharmaceutical formulation.

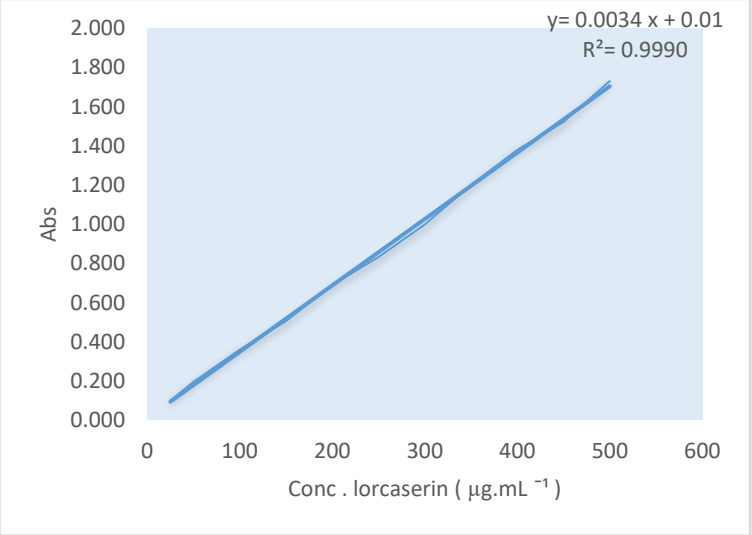



**Figure (12)** Calibration curve of lorcaserin by azo coupling reaction with 2,4DNPH

**Table 2.** Analytical parameter for determining lorcaserin by azo coupling reaction with 2,4DNPH

| Variable | Result |
|---|---|
| The Limits of Beer Law in( µg.mL$^{-1}$ ) | 500 – 25 |
| Absorptivity constant ( L. mol$^{-1}$.cm$^{-1}$) | 6653.46 |
| Liner equation | Y = 0.0034 X + 0.01 |
| Sandells Sensitivity ( µg.cm$^{-2}$) | 0.294 |
| Detection Limits ( LOD ) ( µg.mL$^{-1}$ ) | 0.523 |
| Quantitative Limits ( LOQ ) ( µg.mL$^{-1}$ ) | 1.743 |
| Correlation of Coefficient | 0.9990 |
| R.S.D | 0.174 |
| Erel. % | 0.5 |
| Recovery % | 100.5 |

### 3.4 Estimation of product composition for lorcaserin:

Where this study used the stoichiometric method continuous variance method (Jobs method) to assess the amount of the drug lorcaserin with a reagent DNPH in the alkali medium, the product is formed in the optimal state at the highest wavelength of 590 nm, the ratio of the drug lorcaserin to the reagent 2,4DNPH was (1:1) also the method of mole ratio also showed the same ratio as shown in figures.15-16. [21, 22]

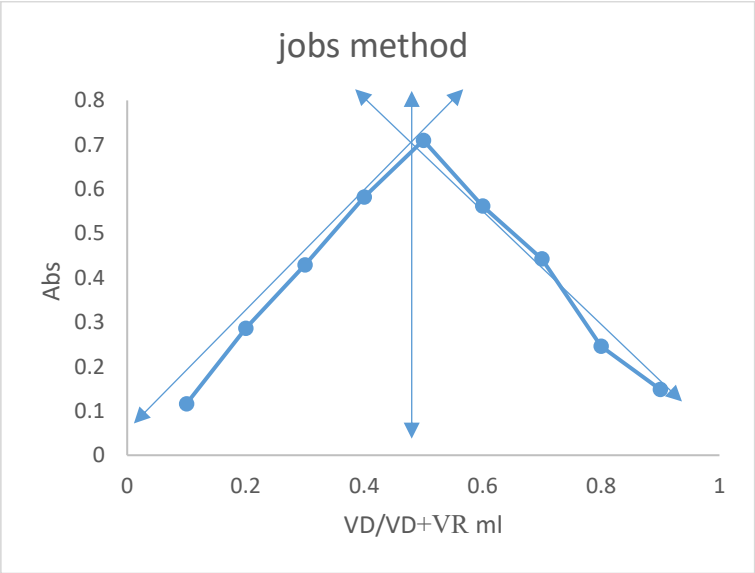



**Figure (13)** Continuous vibration method (jobs method) for lorcaserin product.

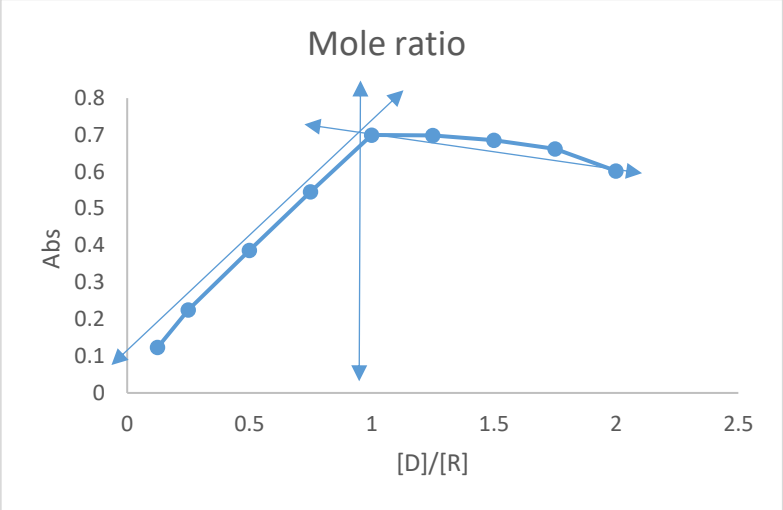



**Figure (14)** Mole ratio for lorcaserin product

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

The degree of stability of the product was determined using the formulas below to calculate the molar ratios for each volume of the property and the detector [23]:

$$C = Am - As/Am$$

Am: the absorption of volume of the product at the greatest absorption
As: the absorption of volume of the product at the point of equivalence

$$K_{Stability} = 1/K_{Instability} \text{ ---------- (1)}$$

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

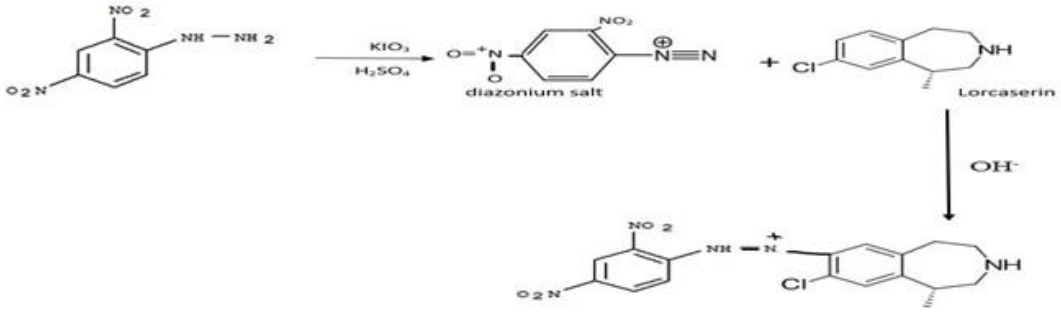
C: concentration of drug in molarity

$$\alpha = \frac{0.700-0.699}{0.700} = 0.0014$$

$$k = \frac{(1-0.0001)}{(0.0014)^2 (0.001)} = 5.1 \times 10^8$$

### 3.6 The suggest mechanism for reaction:

Figure (15) show the suggest mechanism for the azo coupling reaction of 2,4DNPH with lorcaserin drug in the presence of $KIO_3$ as oxidizing agent.




### 3.7Accuracy and precision:

To verify precision and accuracy of the studied method we calculated % R.S. D, %Erel and Recovery by five replicates for the absorbance of concentration 200 µg. $mL^{-1}$ for lorcaserin with 2,4DNPH reagent as shown in Table 3. We noted very precision and accurate method.

**Table 3.** Accuracy and precision of lorcaserin with 2, 4 DNPH

| NO. | Drug Ppm | Abs (xi) | (xi-xv) | $(xi-xv)^2$ |
|---|---|---|---|---|
| 1 | 200 | 0.700 | 0.000 | 0.00 |
| 2 | 200 | 0.701 | 0.001 | 0.000001 |
| 3 | 200 | 0.702 | 0.002 | 0.000004 |
| 4 | 200 | 0.700 | 0.000 | 0.00 |
| 5 | 200 | 0.699 | -0.001 | 0.000001 |
| | | | $\Sigma\ (Xi - XV)^2 = 6 \times 10^{-6}$ | |

$\bar{x} = \frac{\Sigma x}{n} = \frac{3.502}{5} = 0.700$

R. S. D = $\frac{S.D \times 100}{\bar{x}} = \frac{1.22 \times 10^{-3} \times 100}{0.700} = 0.174$

% Erel. = $\frac{201-200 \times 100}{200} = 0.5$

Recovery= 100 + % Erel. = 100 + 0.5 = 100.5

LOD=$\frac{S.D_x 3}{slope} = 0.523$

LOQ =$\frac{S.D_x 10}{slope} = 1.743$

### 3.7 Interferences

This study shows the interferences effect of some substances that are found in pharmaceutical formulation and noted did not have an effect on drug absorption. There is no real effect on the absorption value by contain the functional groups for reagent, the result shown in Table chemical interference because these materials are the ones that do not contain the functional groups for reagent, the result shown in Table 4

**Table.4.** Interferences for lorcaserin pharmaceutical formulation

| **Interferences** | Abs |
|---|---|
| magnesium citrate | 0.00 |
| Starch | 0.00 |
| Polyvinyl alcohol | 0.00 |
| Cellulose | 0.00 |
| Titanium dioxide | 0.00 |

### 3.8 Applications:

Determination of lorcaserin in the pharmaceutical formulation by DNPH Reagent was identified in its capsule within a drug under the trade name Xenical as shown in Table 5. Spectroscopic analysis of the pharmaceutical formulations by the studied method

**Table.Table (5)** Accuracy and precision of lorcaserin pharmaceutical formulation determined by DNPH reagent.

| No. | **Concentration of lorcaserinμg/mL) Presnt** | **Found** | **Relative error percentage** | **%Recoverability** | **%R.S.D** |
|---|---|---|---|---|---|
| 1 | 50 | 49.8 | -0. 4 | 99.6 | 0.099 |
| 2 | 200 | 200.8 | 0.4 - 0 | 100.4 | 0.223 |
| 3 | 500 | 500.4 | 0.08 | 100.08 | 0.182 |

Lorcaserin also was determined in serum and urine. The estimation of serum samples with different amounts of the drug was presented and its absorption was taken for the studied analysis method and the results found as shown in Table (6). To determine the amount of drug in the urine samples were also taken and different amounts of the drug were adding and the absorbance measure the result was shown in Table (7).

**Table (6).** Applications of lorcaserin drug with 2, 4 DNPH in serum

| **NO** | **Conc. present. μg.mL$^{-1}$** | **Conc. found μg.mL$^{-1}$** | **%R.S.D** | **%Error** | **%Recovery** |
|---|---|---|---|---|---|
| 1 | 200 | 200.3 | 0.22 | 0.15 | 100.15 |
| 2 | 300 | 301.0 | 0.17 | 0.30 | 100.3 |
| 3 | 400 | 399.5 | 0.25 | -0.13 | 99.87 |
| 4 | 500 | 499.8 | 0.55 | - 0.04 | 99.96 |

**Table (7).** Applications lorcaserin drug with 2,4DNPH in Urine

| **NO** | **Conc. Present μg.mL$^{-1}$** | **Conc. found μg.mL$^{-1}$** | **%R.S.D** | **%Error** | **%Recovery** |
|---|---|---|---|---|---|
| 1 | 200 | 200.2 | 0.22 | 0.10 | 100.10 |
| 2 | 300 | 300 .3 | 0.17 | 0.10 | 100.01 |
| 3 | 400 | 398.0 | 0.22 | -0.50 | 99.50 |
| 4 | 500 | 499.7 | 0.25 | -0.06 | 99.94 |

## 4. CONCLUSIONS:

The oxidative coupling reaction of 2,4 DNPH after oxidation followed by coupling with lorcaserin in alkaline medium was discovered to be a simple, sensitive, accurate, and cost-effective spectrophotometric method for quantifying lorcaserin in pure form and pharmaceutical preparations. The classical univariate and modified simplex methods were used to optimize the various variables affecting reaction completion. The studied method had a high level of linearity, precision, Accuracy, sensitivity and selectivity for the applications in pharmaceutical formulation and body fluid.

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