

**FULL PAPER**

# Highly development and validation of a spectrophotometric method for Mogadon drug in pharmaceutical tablets by diazotization reaction

Alaa A. Omran<sup>a,\*</sup> | Hanan Hassan Ahmed<sup>b</sup> | Wathiq Kh. Mohammed<sup>c</sup> | Reem Mohsin Khalaf<sup>d</sup> |  
Khulood Majid Alsaraf<sup>e</sup> | Shamam Kareem Oudah<sup>f</sup>

<sup>a</sup>Department of Engineering, AL-Nisour University College, Baghdad, Iraq

<sup>b</sup>Department of Pharmacy, Al-Noor University College, Nineveh, Iraq

<sup>c</sup>Department of Pharmacy, Al-Hadi University College, Baghdad, 10011, Iraq

<sup>d</sup>Department of Pharmacy, Mazaya university college Iraq

<sup>e</sup>Department of Pharmacy, Al-Esraa University College, Baghdad, Iraq

<sup>f</sup>Department of Pharmaceuticals, College of Pharmacy, National University of Science and Technology, Dhi Qar, Iraq

This method was based on the use of the diazotization method, as a simple, sensitive method, and easy spectrophotometric way, for the determination of Mogadon drug or called Nitrazepam (NZZP) in pharmaceutical tablets. This way depends upon the reduction of the nitro to amino group; reacts with reagent catechol to form a color complex with the best maximum absorption at 463 nm. The optimal conditions were studied for an experiment such as effect of base volume, effect of kind and acid volume, contact time, and temperature. The spectrophotometric way has been successfully useful to determine NZZP in pharmaceutical tablets. The best absorbance as optimal volume NaOH at 1 mL and 1 mL HCl. Optimal time required to complete the azo coupling reaction was found to be 3 min for NZZP drug. Where the range concentration 1-20 mg/10 mL, it obeys Lambert Beer Law Correlation coefficient ( $R^2 = 0.9982$ ). The selectivity of the suggested was reveals the influence of number of some materials foreign (like lactose, starch, glucose, dextrose). The data show that the examined interferences non-overlap with suggested method. The recovery of NZZP drug in tablets was in the range of (96% -100.1 %). The statistical result compared with these found by a way reported in literature. This simple, sensitive, and selective, method can be utilized to do control analysis for drug determination.

**\*Corresponding Author:**

Alaa A. Omran

E-mail: [aishamohaisha7@gmail.com](mailto:aishamohaisha7@gmail.com)

Tel.: +964 780 074 4220

**KEYWORDS**

Spectrophotometric; pharmaceutical; diazotization method; nitrazepam; catechol.

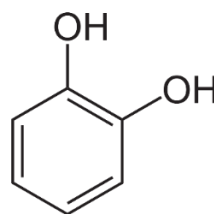
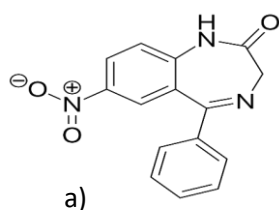
**Introduction**

Spectrophotometry is a one branch of electromagnetic spectroscopy, which deals with the measurement of the interaction of light with materials [1-6]. It considers a global and inexpensive technique in which the incident light passed through the sample solution undergoes absorption or transmission by the chemicals inside the solution. Spectrophotometry is applied most

commonly to ultraviolet, visible, and infrared radiation [7-11].

Mogadon drug or called Nitrazepam, is a 1,4-benzodiazepine that is 1,3-dihydro-2H-1,4-benzodiazepine-2-one which is substituted at positions 5 and 7 by phenyl and nitro groups, in that order. Also known by the trade name Mogadon, is a benzodiazepine hypnotic drug used to treat insomnia and severe anxiety [1,11]. It has sedative, analgesic, and skeletal muscle relaxant

properties. Its formula is  $C_{15}H_{11}N_3O_3$ , its molar mass is 281.3 g/mol, with the chemical structure which is displayed in Figure 1a. Several methods have been utilized for the estimation of NPZ drug in pharmaceutical products, having spectrophotometric, TGA analysis coupled to FTIR, HPLC, flow injection, capillary electrophoresis, chemiluminescence, and fluorimeter. The utmost the spectrophotometric way reported suffer from the disadvantages the same as the utilization of non-aqueous solvent, large of time to reaction complete, and the stability of color compound. The best idea of this method is to provide sensitive simple and fast spectrophotometric estimation of PNZ drug in pharmaceutical and these ways is eco-friendly, and not have any solvent organic [7,13-15].



**FIGURE 1** The chemical structure of a) Nitrazepam, b) catechol

## Experimental

### Freshly prepared drugs solutions:

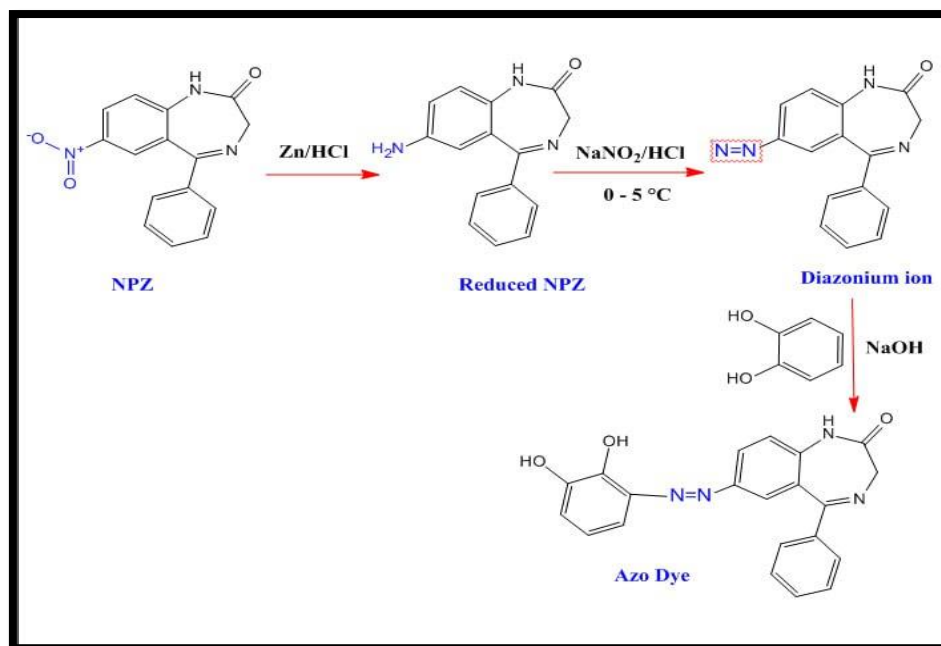
All chemicals applied were of high analytical degree. Solution of the Nitrazepam (NZZ) (100 mg/L) prepare freshly via dissolving of NZZ 0.1 g with a small amount of methanol and in distilled water in a 100 mL elementary flask. Catechol solution (10 mg/L) was prepared *via* 0.01 g in 100 mL of distilled water. The solution of sodium nitrite is prepared *via* 0.1 g in 100 ml DW. Sodium hydroxide 0.1N solution: accurate weight 0.4 g of (NaOH) was dissolved in 100 mL of DW. Sodium bicarbonate 0.1 N solution: accurate weight 0.8 g of ( $NaHCO_3$ ) was dissolved in 100 mL of DW in a volumetric flask of 100 mL. Sodium

Catechol, known as 1,2-dihydroxybenzene or pyrocatechol, is a toxic chemical organic colorless compound, the molecular formula  $C_6H_4(OH)_2$ . It is the ortho isomer of the three isomeric benzene diols [16,17]. the chemical structure as shown in Figure 1(b). Several methods have been reported to determine this drug in biological and pharmaceutical samples, such as flow injection voltammetry, micellar liquid chromatographic, micellar electro kinetic capillary chromatographic, thin layer chromatographic, thin layer chromatographic-densitometry, high performance liquid chromatographic, and reverse phase-high performance liquid chromatographic, spectrophotometric [12,18-23].

carbonate 0.1N solution with accurate weight 0.5 g of ( $Na_2CO_3$ ) was dissolved in 100 mL of DW in a volumetric flask of 100 mL. Hydrochloric acid solution (0.1 N): accurate volume (0.813 mL) of HCl has a specific gravity 1.18  $g \cdot mL^{-1}$  and 37.0% was diluted to 100 mL with distilled water.

### Preparation of azo dye

Under optimal conditions, NZZ drug was reacted with  $NaNO_2$  solution in acidic medium (HCl), to form a Diaz onium salt by a reaction named a dia-zotization reaction. After the formation Diaz onium salt, it is combined with catechol as a coupling agent in NaOH, which results resulting color from the formation of a color azo dye as appear in the Scheme 1:

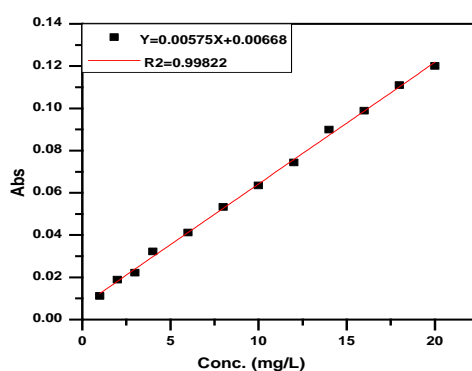


**SCHEME 1** Preparation of color azo dye by starting of NPZ

#### Calibration Curve

The absorbance of NPZ drug increases linearly as the concentration of NPZ drug rises, calibration curve was gained from the series of stock solution for drug the linearity, regression equation, determination of ( $R^2$ ),

intercept, and slope, and also the linear concentration of the calibration graph ranged from 1-20 mg/L of NPZ drug. Furthermore, several factors of the analytical achievement of the proposed way are briefly depicted in Figure 2.



**FIGURE 2** Calibration curve of drug NPZ at the optimum condition

#### Optimization conditions of the reaction

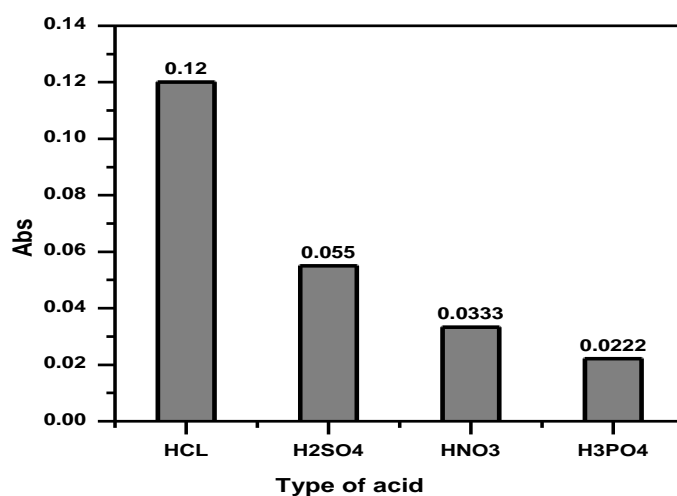
##### Influence of acid concentration

The diazotization reaction of NPZ drug was carried out in acidic medium. Thus, the influence of different amounts of acids was

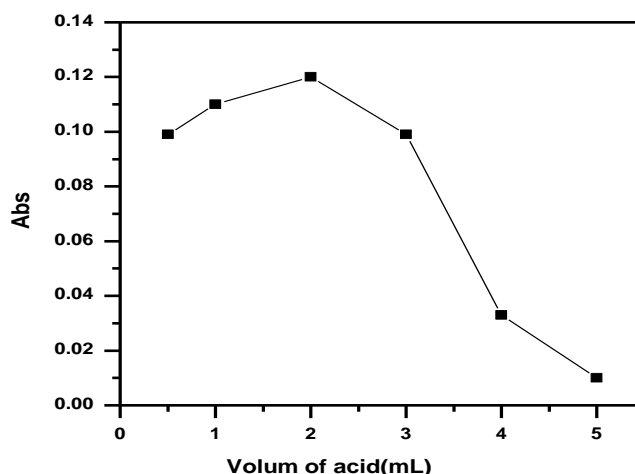
studied using ( $H_2SO_4$ ,  $H_3PO_4$ ,  $HNO_3$ , and  $HCl$ ) (0.1 N) [32]. It was found that  $HCl$  is the utmost excellent acid, to give sensitivity and the best absorption, as shown in (Figure 3). Therefore, different volumes of hydrochloride acid (1- 5 ml) have been utilized for the common assay. Both the selectivity and better

absorbance were obtained. When the HCl volume was used about 2 mL, it was the utmost suitable in acid medium because it

gave high absorbance for the azo dye with corresponding minimum absorbance of reagent blank, as shows in Figure 4 [20,24-25].



**FIGURE 3** Effect of kind of several acids

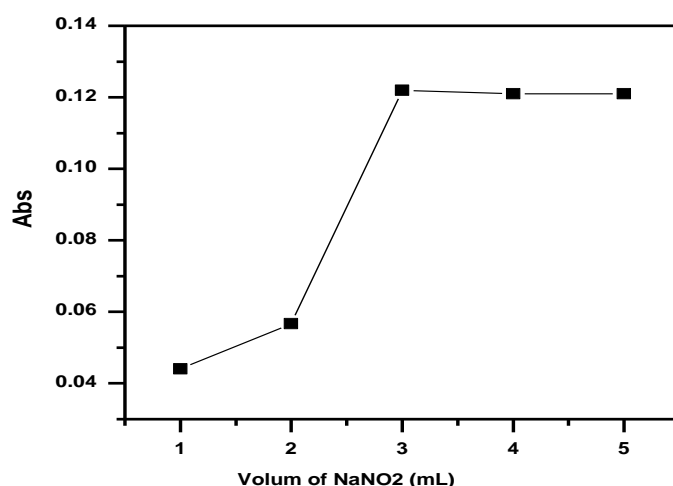


**FIGURE 4** Effect of HCl volume (mL) at the optimum condition

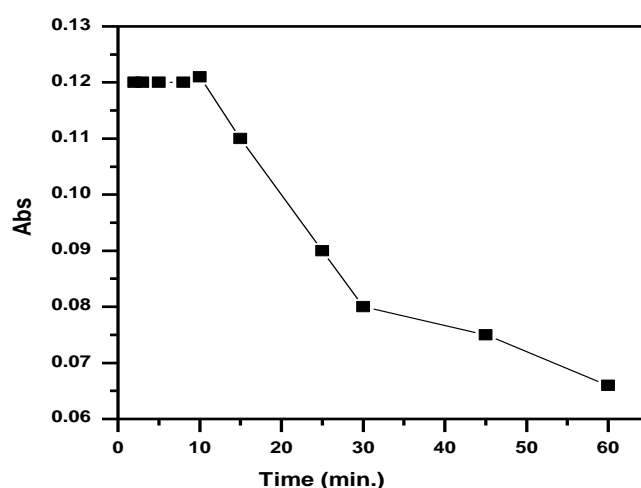
#### *Influence volume of sodium nitrite (NaNO<sub>2</sub>) and time*

The effect of NaNO<sub>2</sub> quantity was studied using different solutions and 0.1 N of NaNO<sub>2</sub> about 0.1-2 mL. The result is demonstrated in Figure 5. Thus, 3 mL solution of sodium nitrite was considered as a proffered volume that required a reaction for 5 minutes for the dia-

zotization method and the best absorbance was obtained after about 5 minutes as a reaction time for NaNO<sub>2</sub> amount. The experimental data showed that the azo dye colored developed directly after mixing for 5 minutes and the absorbance rested most and constant for at least 1 h at 25 °C and using for all experiments [26,27], as illustrated in Figure 6.



**FIGURE 5** Effect volume of NaNO<sub>2</sub>

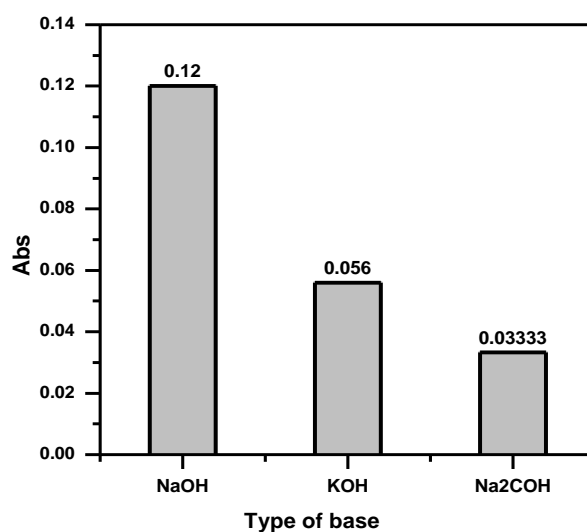


**FIGURE 6** Influence of stability time of diazanuim salt at optimum condition

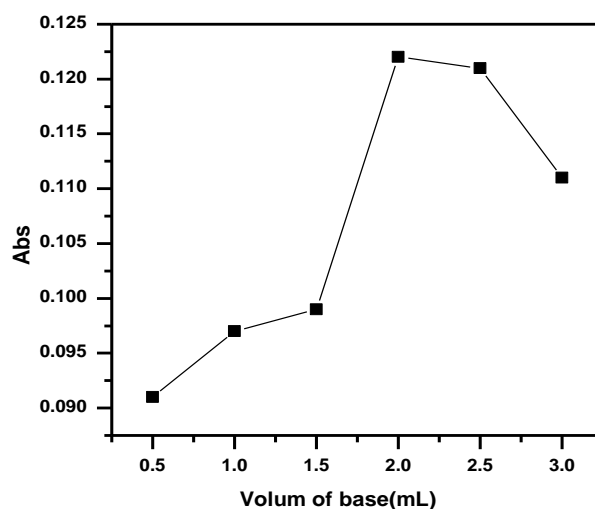
#### *The influence of basic medium*

The alkaline solutions were tested like KOH, NaOH, and Na<sub>2</sub>COH. The result, depicted in Figure 7, revealed that NaOH was the good basic medium for azo reaction diazanuim among the drug utilized in later experiments.

The influence of several volumes of 0.1 N NaOH was studied by changing volume by about (0.5-3 mL) while keeping other factors constant. The result is depicted in Figure 8. The perfect efficiency to produce azo dyewas found when using 2 mL of 0.1 N NaOH [28,29].



**FIGURE 7** Influence of different kinds of alkaline bases



**FIGURE 8** Influence of NaOH volume (mL) at the optimum condition

#### *Influence of solution temperature*

The influence of solution temperature solution onto the maximum absorbance of azo dye color product was also studied. The data are listed in Table 1. The product colored of azo

dye developed directly after mixing and reach the best absorbance about 5 min. The colored of azo dye was stable for 3 h. Therefore, the period of 15 minutes was chosen as the best optimum conditions at 25 °C [30].

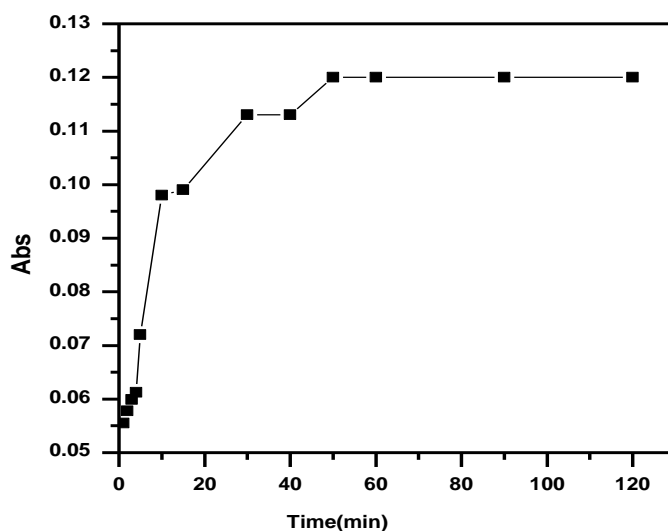
**TABLE 1** Influence of several temperatures on the best absorbance of color dye

| Temperature (°C) | Abs   |
|------------------|-------|
| 15               | 0.066 |
| 25               | 0.12  |
| 30               | 0.11  |
| 40               | 0.08  |

### Effect of time color azo dye

The stability time of the color azo dye was studied using the optimum conditions found

from the preceding result. The color of azo dye was found constant in excess of 2 hours. The data indicate higher absorbance, sensitive, and selectivity [24,31], as shown in Figure 9.



**FIGURE 9** Effect of time color azo dye at the optimum condition

### Interferences

The selectivity of the suggested way, the influence of number of some materials foreign (like lactose, starch, dextrose, and glucose) that generally, existent in dosage forms have

been examined via addition volume (10 mL) of interferences (1000 mg/L) to 2 ml of NPZ (100 mg/L). The data show the examined interferences non-overlap with suggested way in Table 2.

**TABLE 2** NPZ estimation in several excipients utilizing the proposed and official method

| Excipients | Conc. Of NPZ (mg/L) |       | E %    | Rec. % |
|------------|---------------------|-------|--------|--------|
|            | present             | Found |        |        |
| Lactose    | 10                  | 10.1  | 9.909  | 100.1  |
| Glucose    | 10                  | 10.9  | 8.256  | 100.08 |
| Dextrose   | 10                  | 9.87  | -1.317 | 98.68  |
| Starch     | 10                  | 9.12  | -3.092 | 96.90  |

### Conclusion

A sensitive, simple, and perfect spectrophotometric way was used for the estimated of Nitrazepam (NZP) in pharmaceutical tablets. This way did not need control temperature and pH solution. Beer's law conformed over the concentration range of 1-20 mg/L a fair degree of precision and accuracy. That 3 mL of NaNO<sub>2</sub> solution was

selected as the given volume that required a 5-minute reaction for the dia-zotization method. The effect of different base KOH, NaOH, and NH<sub>4</sub>OH was studied. The result revealed that NaOH was the best basic medium for azo dye, and it also showed the perfect efficiency to produce azo dye when using 2 mL of 0.1 N NaOH, and found the azo dye color constant for more than 2 hours with higher absorbance and sensitivity.

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## Conflict of Interest

Authors declare that there is no conflict of interest.

## Orcid:

Alaa A. Omran:

<https://orcid.org/0000-0002-5688-447X>

Hanan Hassan Ahmed:

<https://orcid.org/0000-0001-7198-9811>

Wathiq Kh. Mohammed:

<https://orcid.org/0000-0002-2463-8485>

Reem Mohsin Khalaf:

<https://orcid.org/0000-0002-0656-2931>

Khulood Majid Alsaraf:

<https://orcid.org/0000-0002-2455-2585>

Shamam Kareem Oudah:

<https://orcid.org/0000-0002-3241-9370>

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