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# Spectrophotometric Determination of Nitrofurantoin in its Bulk and Pharmaceutical Formulations

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## ABSTRACT
Nitrofurantoin is an antibacterial agent works against gram positive and gram-negative bacteria. The main purpose of this work was to determine nitrofurantoin concentration in pure form and marketed pharmaceutical preparations. For this assay two, rapid, accurate and precise spectrophotometric methods were proposed. The methods are based on the reduction of nitrofurantoin by zinc powder in hydrochloric acid medium followed by diazotization of reduced nitrofurantoin and coupling with 8-hydroxy quinolin to give a colored product which is stable, water-soluble and has a maximum absorption at 365 nm (method A). In the second method (method B), the reduced product reacts immediately with p-benzoquinone to give a color product, which has maximum absorbance at 400 nm. The calibration graphs were linear over the concentration range of 0.5-25 and 1-35 µgml$^{-1}$ for both methods A and B respectively. With molar absorption coefficient of 1.782×10$^4$ and 0.897×10$^4$ Lmole$^{-1}$cm$^{-1}$ for both methods A and B respectively. The proposed methods were applied successfully for commercially available nitrofurantoin dosage forms and the results were statistically compared with those obtained by reference method. Additionally, the results showed no interference of the excipients in the assay process.

**Keywords:** Nitrofurantoin, p-benzoquinone, Coupling reaction, Azo dye, spectrophotometric determination

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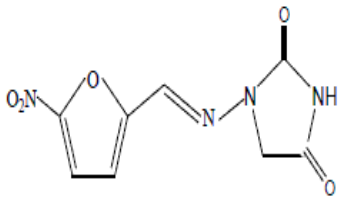
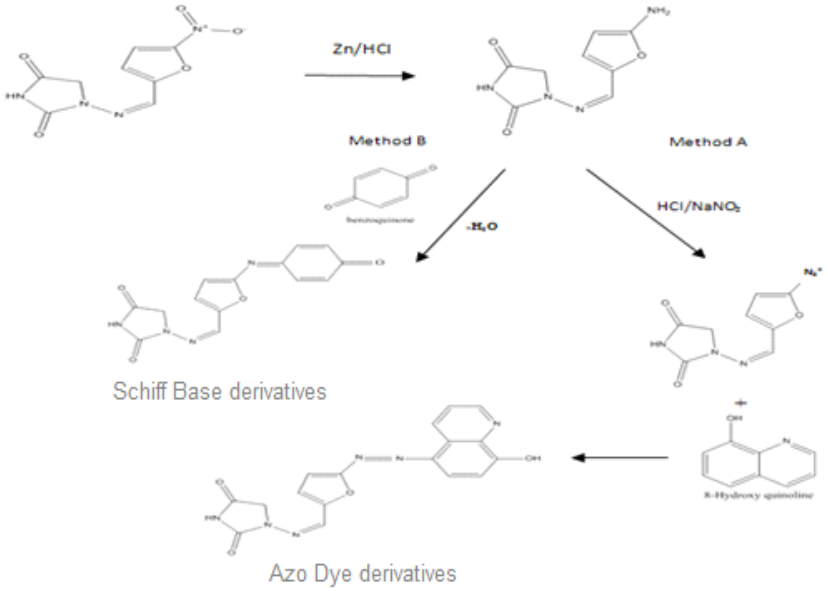
## INTRODUCTION
Nitrofurantoin (NUF) is an antimicrobial substance works effectively against both gram-positive and gram-negative bacteria (1). It is affective against Citrobacter, Enterobacter, Corynebacterium, Escherichiacoli, Salmonella, Enterococcus faecalis and Staphylococcusaureus (2,3).




Chemical Structure of NUF

NUF is useful for treatment and prophylaxis of the urinary tract infections (UTI). It exerts its action by inhibiting the bacterial growth (4,5). Nowadays, there is increased attention in the use of NUF due to the concept of bacterial resistance facing the commonly used antimicrobials such as quinolones or trimethoprim/sulfamethoxazole. Few analytical methods are registered for the assessment of NUF in pharmaceutical preparations and as pure drugs. Those include determination using HPLC (6), spectrophotometric analysis (7), electroanalytical method (8), Voltammetric assay (9) and Liquid Chromatographic methods also have been applied for determination NUF in pharmaceutical preparations [10-13]. The nitro group of the NUF possibly, used as a tool for the quantitative evaluation of this compound. It could be reduced into an amino group, followed by conjugation with a variety of color producing moieties. The obtained amino group could be diazzotized and coupled to 8-hydroxy quinolin to give the azo dye derivatives. On the other hand, the p-benzoquinone is a well-known sensitive reagent commonly used for spectrophotometric determination of amino group containing compounds (14). The amino group has the ability to react with the p-benzoquinone to produce the imine (Schiff base) moiety, which are commonly colored chromospheres.

The aim of the present study is to develop new and accurate methods for determination and estimation NUF in its pure form and in the traditional pharmaceutical preparations. The first step of the process was reduction of the drug by zinc dust to convert the nitro group in the NUF to amino group in acid medium. The method A including formation of colored product from the diazotization of the reduced NUF and coupling with 8-hydroxy quinolin to form azo dye, whereas method B including the reaction of the reduced product with p-benzoquinone to form colored imine product.




Scheme 1. Expected mechanism for method A and B

## EXPRIMENTAL

### Apparatus

All absorbance estimations were performed by a UV-Visible spectrophotometer (Jena Model 1100, Germany) in department of Pharmaceutical Chemistry, Basrah Pharmacy college, Basra, Iraq. The UV-Visible spectrophotometer was outfitted with a quartz cell with a 10 mm way length. Startureus electrical balance was used for sample weighing.

### Materials and Reagents

All reagents utilized in this study were of analytical grade with high degree of purity.

### Reduction of NUF (Stock Solution)

The reduced solution of NUF was set up by dissolving 0.05 g of NUF in 50mL of ethanol. This arrangement was moved into 125 ml measuring flask and to which, 20 ml of refined water, 20 ml of concentrated hydrochloric acid HCL, and 3.0 g of zinc powder were included. So as to finish the reduction procedure the flask was permitted to stand for 15 min at room temperature (25°C), at that point the solution had been filtered, and the volume was completed to the 100 ml with refined water to get 500 µg $mL^{-1}$ of reduced NUF solution, at that point it was moved into a dark colored jug. This working solution was daily prepared (15).

### Calibration Curve of the Azo dye (Method A)

To a number of 25 ml beakers, specific volumes from the stock solution had been added and diluted to get solutions at concentration range of (0.5-25) $\mu g.ml^{-1}$.

To these solutions, 1N hydrochloric acid (1 ml) with 0.5% sodium nitrite solution (1ml) were added. Then, it was shacked frequently for 3 min, to get the diazonium salt.

Then 2.5 ml of 0.1% 8-hydeoxy quinolin reagent and 2 ml 1N sodium hydroxide solution had been added, and the volumes were completed with distilled water. The absorbance belongs to the formed colored azo dye then, measured at 365 nm, at 5 min interval against the blank reagent (that prepared similarly, but without the drug addiction).

### Calibration Curve of the Schiff Base

Into a number of 25 ml flasks containing aliquots of the reduced NUF stock solution required to get a concentration range of 1.0-35 µg $ml^{-1}$, 1 ml of (100 mg $ml^{-1}$) p-Benzoquinone solution were added to give Schiff base derivatives. Then, the volume diluted to 25 ml with ethanol and measures the absorbance at 400 nm compared to a reagent blank solution. The calibration curve was drawn by plotting the concentration of the NUF in µg $ml^{-1}$ versus the absorbance (method B).

### Solutions of pharmaceutical preparations

The pharmaceutical products used to fulfill this research were purchased from the local Iraqi market. Capsule of Uvamin retard, 100 mg (Acino, Switzerland), and Furantil tab, 50mg (Bio Active, UK) were used in this experiment.

The used pharmaceutical samples were exactly weighted and finely powdered. A quantity of the powder containing 50 mg of NUF dissolved ethanol (30 ml), followed by filtration of the solution to exclude the insoluble additives. Then, the volume diluted by ethanol to get a concentration of 1000 µg $ml^{1}$. This samples was moved into a 125 ml glass beaker and it's reduced as earlier mentioned. Then, it was coupled with the colouring chromophore as mentioned earlier. The absorbencies were measured and the concentrations were calculated from the calibration curves.

## RESULTS AND DISCUSSION

Drugs with nitro group ($NO_2$) are extremely important kinds of drugs. But its spectrophotometric determination (especially NUT) is not simple because of the weak affinity of this group to conjugate directly with other reagents. So, to increase this reactivity, an attempt to change the nitro group ($NO_2$) into a more reactive group amino group ($NH_2$) was performed. This amino group, then, either undergo diazotization reaction to give the azo dye derivatives (method A) or reacted with a carbonyl compounds to give Schiff base derivatives (method B).

The spectra of absorption of the resulted colored complex formed by the reaction of reduced NUF and 8-hydroxy quinolin in acidic environment versus its corresponding blank reveals a maximum absorbance at 365 nm (method A) Fig. 1.

The reaction produces the colored complex involves two steps. At the first one, reduced NUF was reacted with sodium nitrite solution in presence hydrochloric acid HCl, that led to diazotization reaction and formation of diazonium ion. In the next step, formed diazonium ion was attached with the 8-hydroxy quinolin, to give orange-red complex of azo-dye in a basic medium. The second method (method B) involves a single step conjugation between the reduced NUT and the carbonyl group of the p-benzoquinone. The colored complex shows maximum absorption at 400 nm (method B) as shown in Fig. 2.

The planned methods had been adjusted to get completed reaction, maximum sensitivity and high absorbance. The experimental variables were optimized by using of 15 µg $ml^{-1}$ NUF for method A and 20 µg $ml^{-1}$ NUF for method B.

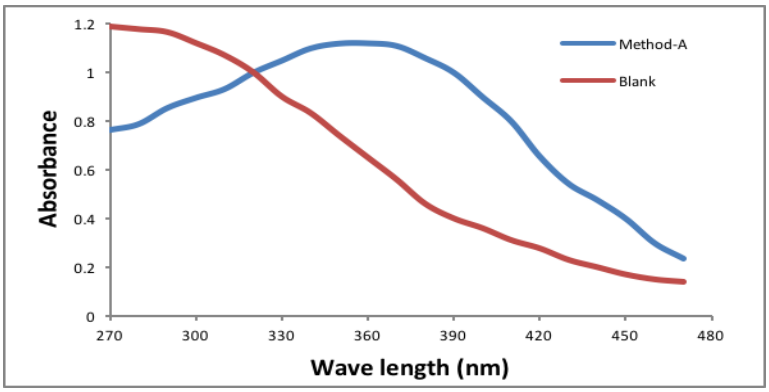



Fig 1. Absorption spectra for reduction nitrofurantoin (15 µg $ml^{-1}$) and 8-hyaroxy quinolin (0.1%) method A against reagent blank

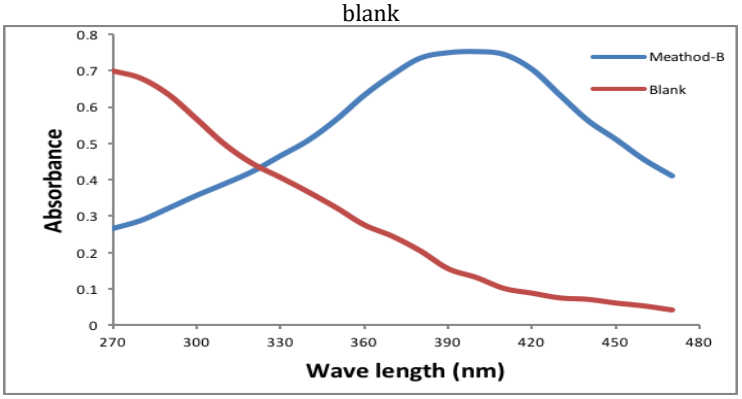



Fig 2. Absorption spectra for reduction nitrofurantoin (20 µg $ml^{-1}$) and p-Benzoquinone method B against reagent blank.

**Effect of the reagent volume**

The volumes used in the concentrations of 0.5-4 ml from 8-hydroxy quinolin (0.1%) were investigated. It was noticed that 2.5 ml showed the maximum absorbance, and it was utilized in later experiments (Method-A). On other method, the volumes in the range of 0.25-3 ml of p-Benzoquinone with concentration of 100 µg $ml^{-1}$ p-Benzoquinone had been used. The results proved that 1 ml is the ideal volume required to have the highest absorbance. Therefore, it was used in the subsequent experiments (Method-B) (Fig.3).

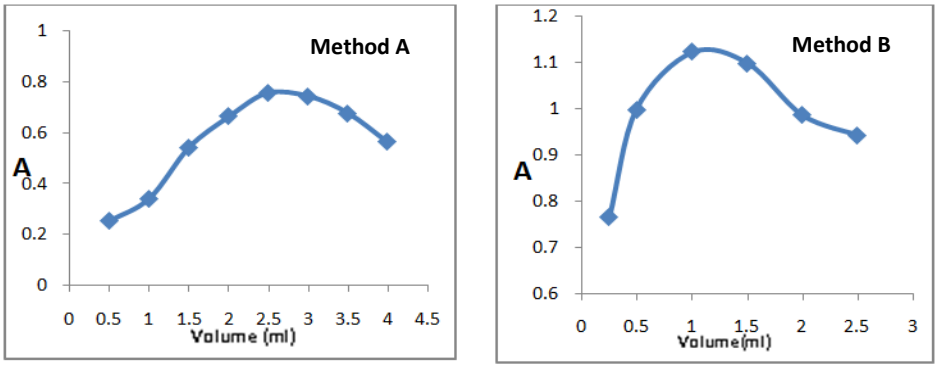



Fig.3.The effect of reagent volume on the absorbance for both methods A and B.

**The effect of the volume of nitrite ($NaNO_2$) solution**

The influence of $NaNO_2$ solution volume on the intensity of absorption was also investigated. Volumes with concentration of 0.1% $NaNO_2$ around 0.5 - 3 ml were used. It was noticed that 1 ml of nitrite is the best possible volume for greatest absorption (Fig.4).

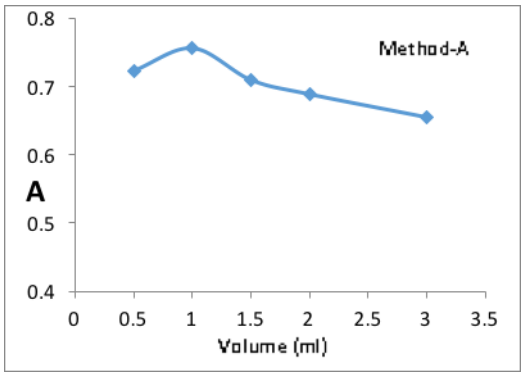



Fig 4. Effect of sodium nitrite volume on absorption intensity

**Effect of acid type used**

A variety of acids had been used in this experiment. Acids like HCl, $H_2SO_4$, $HNO_3$ and $CH_3COOH$, with 1 M concentration were investigated. The results revealed that HCl leads to get the highest absorption for the colored product. HCl, 0.25-3 ml of 1M were examined. It was observed that 1 ml leads the greatest absorption and it was used in the subsequent experiments (method A).

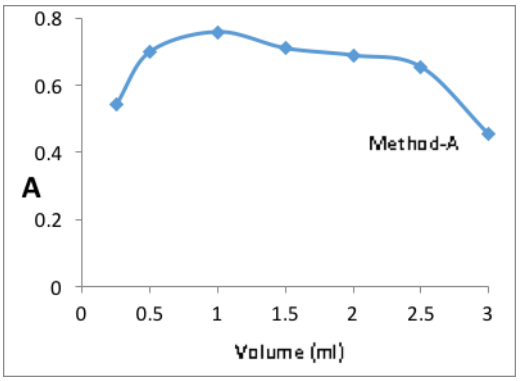



Fig 5. Effect of HCl volume on the absorption intensity

**Effect of the reaction time**

The azo-dye formation was found to end within 10 min and the azo complex was stay stable for more than 24 hour at method-A. Otherwise the colored complex of p-benzoquinon and reduced NUF was found to be ended within 5 min and remain stable for 24 hour.

**Effect of temperature**

The obtained product was tested at various temperatures between 5-45 °C. It's found that the absorbance decline with elevated temperatures, obviously owing to dissociation of the product. The colored chromophore was found to be stable and showing the largest absorbance at temperatures between 20–35°C. The later experiments then, performed at the room temperature for both methods A and B (Fig. 6 and 7).

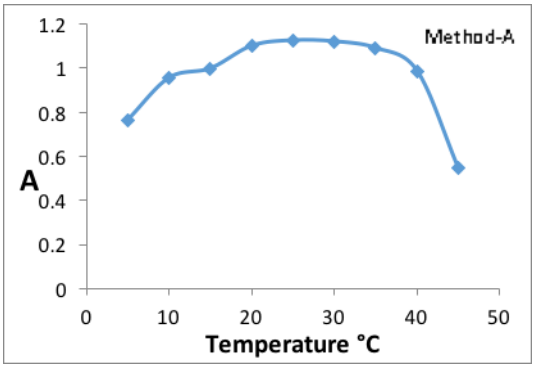



Fig 6. Effect of temperature on the absorption intensity method A

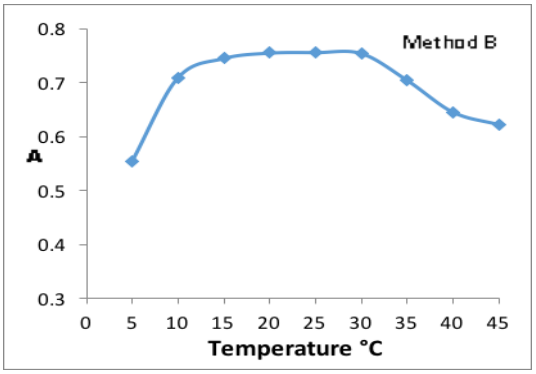



Fig 7. Effect of temperature on the absorption intensity method B

**Effect of Base volume**

The products formed were found to give a colour with highly absorbance. It seems to be more stable and more intense at alkaline conditions. So, various basic solutions like ammonium hydroxide solution, sodium hydroxide, sodium acetate, sodium carbonate and potassium hydroxide had been tested. The higher stability and sensitivity were seen just with sodium hydroxide solution. The NaOH volumes influence was also studied. Volume around (0.5 to 3 ml) sodium hydroxide produced a maximum absorbance (in method A). 1.5 ml was the best volume and used in the subsequent tests Figure (8). Greater concentrations of the base perhaps result in incomplete decolonization of the colored complex.

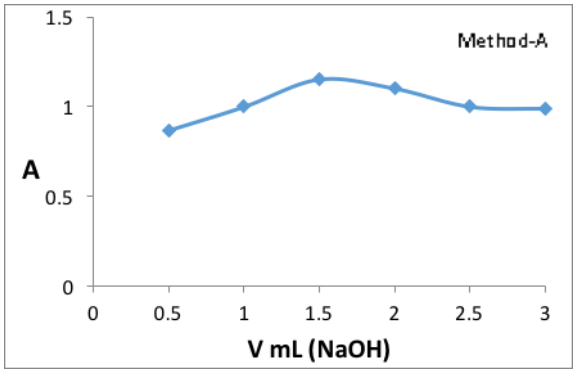



Fig 8. Effect the volume of sodium hydroxide (1N) on the absorption intensity in method A

**Calibration curve**

The constructed calibration curves at the ideal conditions for estimation of NUF in both methods (A and B) were exposed in Figures 9 and 10.

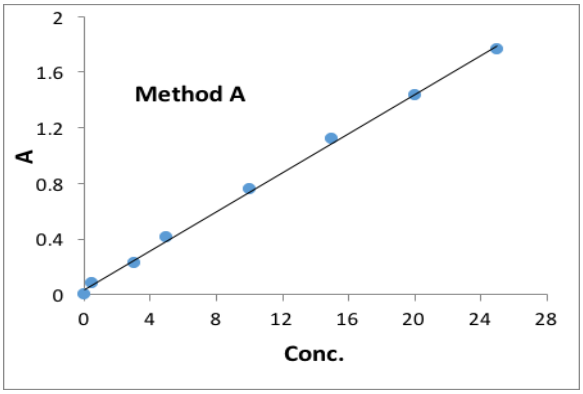



Fig 9. the calibration curve (method A)

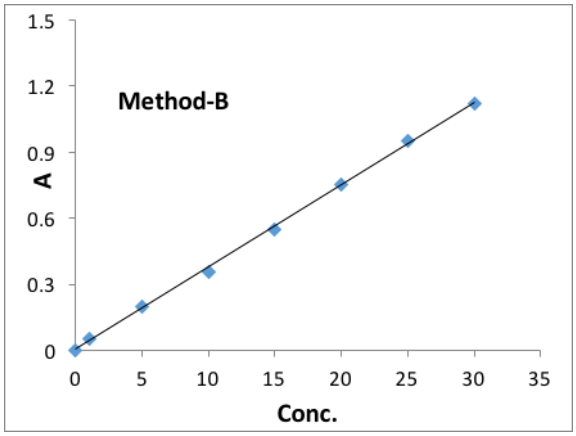



Fig 10. The calibration curve in method B

It was noticed that there's a linear relationship between NUF concentration and its absorbance at a concentration range of 0.5-25 μgml$^{-1}$ and 1.0-30 μgml$^{-1}$, for both methods respectively (16). The molar absorptivity calculated were seen to be 1.782x10$^4$and 0.897x10$^4$ L mol$^{-1}$ cm$^{-1}$, respectively. The Sandell's sensitivity found to be 0.353x10$^{-3}$ and 0.934x10$^{-3}$ μg cm$^{-2}$ for both methods respectively. All the results were listed in Table 1.

Table 1. The Analytical factors for both methods

| Parameters | Method-A | Method-B |
|---|---|---|
| λmax, nm | 365 | 400 |
| Linear range (μg mL$^{-1}$) | 0.5-25 | 1.0-35 |
| The Molar ,(ε)absorptivity | 1.782x10$^4$ | 0.897x10$^4$ |
| Sandell sensitivity | 0.353x10$^{-3}$ | 0.937x10$^{-3}$ |
| Slope (b) | 0.070 | 0.037 |
| Intercept (a) | 0.036 | 0.003 |
| LOD (μg mL$^{-1}$) | 0.256 | 0.750 |
| correlation coefficient ($R^2$) | 0.9989 | 0.9998 |
| LOQ (μg mL$^{-1}$) | 0.436 | 0.993 |

## INTERFERENCES

The interferences impact of the excipients such as lactose, talc, acacia, sucrose, starch, magnesium stearate, benzoic acid, glucose, and aspartate on the various levels for the quantitative assessment of NUF in the two methods were tested. The additives were checked at concentrations which are twenty-times more than NUF concentration depending to the procedure of the calibration curve for the two methods. The interference observed was considered to be tolerable with percent of error less than ± 2%.

**Stoichiometry**

The stoichiometry had been investigated for the reaction of NUF and 8-hydroxy quinolin and p-Benzoquinon in both methods A and B by utilization of Job's method and the mole ratio method (17,18). The results revealed that 1:1 complex had been formed at 365 and 400 nm for method A and B respectively (Figures 11 and 12).

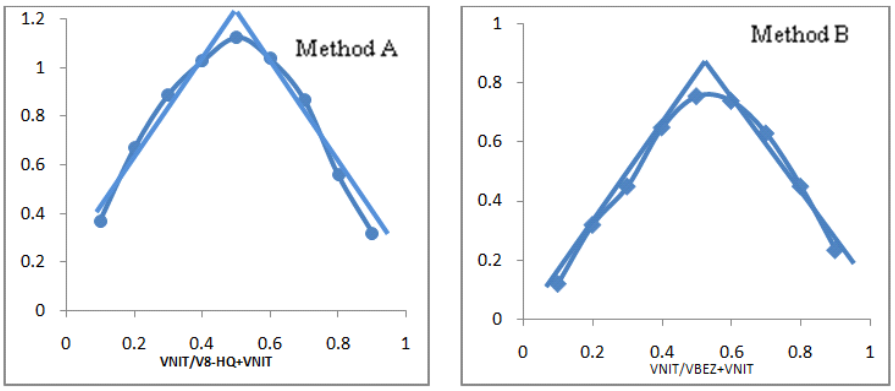



Fig 11. Jobs' method (A and B)

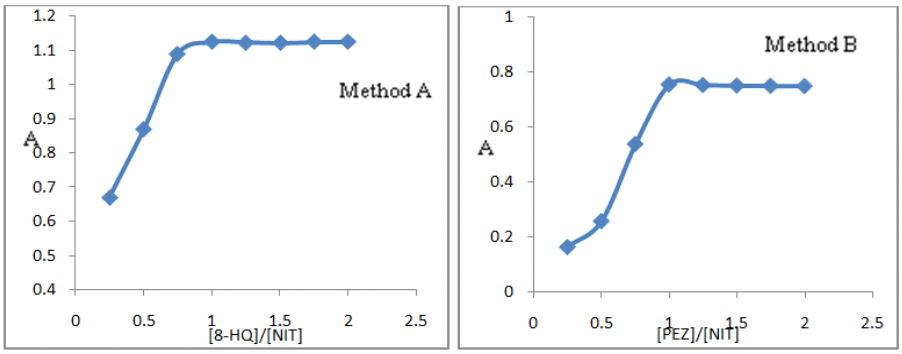



Fig 12. Mole ratio method (A and B)

**Stability constant**

The product structure was adopted depending on the mole ratio and the "continuous variation methods". The continuous variation data can be used to calculate the stability constant of the colored products by using the equation (19):

$$K_f = \frac{A/A_m}{(1 - A/A_m)^{n+1}\, C^n n^n}$$

Where,

$K_f$ =the stability constant, $A$= maximum absorbance of the continuous variation curve, $Am$= absorbance corresponding to junction of the two tangents of the continuous variation curve, $n$= number of molecules of the reagent in the reaction product, $C$= molar concentration of nitrofuration at the maximum absorbance,

The stability constant was found to be equal $3.944 \times 10^{-5}$ and $0.635 \times 10^{-5}$ $L^2$ $mole^{-2}$ for both method A and B as show in Figure 11. That result was preferred to the stability of the colored products and reaction.

The Gibbs free energy ΔG of the reaction was calculated depending on the equation:

$$\Delta G = -2.303 RT \log K_f$$

$R$= universal gas constant, $T$= absolute temperature, $K_f$= formation constant of the reaction.

The result for the applied equation were listed in Table 2 and the negative value for the Gibbs free energy indicated to the spontaneity for the reaction

Table 2. Values of stability constant and Gibbs free energy

| Parameters | Method-A | Method-B |
|---|---|---|
| Stability constant $K_f$ ($L^2$ mole $^2$) | $3.944 \times 10^{-5}$ | $0.635 \times 10^{-5}$ |
| Log Kf | 4.246 | 4.803 |
| Gibbs free energy ΔG (kJ/mole) | -19.349 | -21.887 |

**Accuracy and precision**

To explore the accuracy of the plotted calibration curve, solutions with three concentrations of NUF were utilized in the two methods. The resulted data were summarized in Table 3. The observed results show a good accuracy and precision for both methods.

Table 3. Accuracy and precision of the studied methods

| | Theoretical Amount (µg mL$^{1}$) | Practical Amount* (µg mL$^{1}$) | Relative % of error | Percent of Recovery+SD | %RSD* |
|---|---|---|---|---|---|
| Method A | 5 | 5.06 | 1.20 | 101.21±0.24 | 0.44 |
| | 10 | 10.14 | 1.40 | 101±0.51 | 1.05 |
| | 15 | 15.23 | 1.53 | 101.53±0.33 | 1.47 |
| Method B | 10 | 10.09 | 0.90 | 100.9±0.23 | 0.96 |
| | 15 | 15.08 | 0.533 | 100.53±0.49 | 0.98 |
| | 20 | 19.97 | -0.15 | 0.99.35±0.15 | 1.07 |

*Average values of five determinations, RSD, relative standard deviation.

**Analytical applications**

The developed methods were effectively used for the estimation of NUF in its pharmaceutical forms (capsule or tablet) and the resultant data were expressed in Table 4. The results were statistically compared with a British pharmacopeial standard method (20) by using t-test and F-test with confidence level of 95%. Table 4 revealed that there were no significant differences between the suggested methods and the reference one and with an acceptable precision and accuracy.

Table 4. Application the methods for determination of nitrofurantoin in pharmaceutical preparations

| Drug brand name | **Proposed methods** | | | | | | | | Reference method %)R ± SD( n=5 |
|---|---|---|---|---|---|---|---|---|---|
| | **Method A** | | | | **Method B** | | | | |
| | Taken conc )µg/ml( | Found conc (µg/ml) | R (%) n=3 | RSD (%) n=3 | Taken conc )µg/ml( | Found conc (µg/ml) | R. (%) n=3 | RSD (%) n=3 | |
| NUF | 4 | 4.09 | 102.2 | 0.98 | 4 | 4.15 | 103.7 | 0.95 | 96.55±0.007 |
| | 8 | 8.16 | 100.2 | 0.99 | 8 | 7.97 | 99.64 | 1.05 | 97.99±0.006 |
| | 16 | 15.98 | 99.88 | 1.25 | 16 | 16.33 | 102.1 | 1.16 | 100.09±0.005 |
| t –test<br>F -test | 1.105<br>2.115 | | | | | | | | |
| Furantil | 4 | 4.13 | 103.2 | 0.95 | 4 | 4.08 | 102.0 | 0.99 | 99.32±0.021 |
| | 8 | 8.07 | 100.8 | 1.51 | 8 | 8.05 | 100.6 | 0.88 | 95.50±0.013 |
| | 16 | 15.99 | 99.94 | 1.09 | 16 | 15.87 | 99.19 | 1.32 | 100.09±0.011 |
| t –test<br>F -test | 0.234<br>1.435 | | | | | | | | |

R=Recovery ,SD=Standard deviation, RSD=Relative standard deviation, Theoretical value t=2.776 F=19.01

## CONCLUSION

A sensitive, simple, rapid and precise spectrophotometric methods have been evaluated for the determination of NUF in the bulk and pharmaceutical preparation. The method A depended on the diazotization reaction coupling to formed azo dye with 8-hydroxyquinolin reagent to produced color azo dye absorbed at 365 nm. Method B containing reaction between the NUF drug with p-Benzoquinon to form color product absorbed at 400 nm. The advantage of this procedures was omitting the need to the control the temperature, or solvent extraction with high accuracy and sensitivity.

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