

Spectrophotometric Determination of Nitrofurantoin Drug in its Pharmaceutical Formulations Using MBTH as a Coupling Reagent

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Abstract

A direct, sensitive and efficient spectrophotometric method for the determination of nitrofurantoin drug (NIT) in pure as well as in dosage form (capsules) was described. The suggested method was based on reduction NIT drug using Zn/HCl and then coupling with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of ammonium ceric sulfate. Spectrophotometric measurement was established by recording the absorbance of the green colored product at 610 nm. Using the optimized reaction conditions, beer's law was obeyed in the range of 0.5-30 µg/mL, with good correlation coefficient of 0.9998 and limits of detection and quantitation of 0.163 and 0.544 µg/mL, respectively. The accuracy and precision of the proposed method represented by recovery and relative standard deviation were satisfactory; about 99.33% and 1.16%, respectively. The proposed method was applied for determination of NIT in its pharmaceutical forms and the results compared successfully with those obtained by standard method (British pharmacopeia method).

Keywords: Nitrofurantoin , MBTH , Oxidative coupling reaction.

التقدير الطيفي لدواء النيتروفورانتوين في المستحضرات الصيدلانية باستخدام MBTH ككاشف ازدواج

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الخلاصة

تم وصف طريقة طيفية مباشرة حساسة وفعالة لتقدير دواء النيتروفورانتوين (NIT) بشكله النقي وكذلك في المستحضرات الصيدلانية (كبسولات). تستند الطريقة المقترحة على اختزال دواء NIT بواسطة الزنك في الوسط الحامضي ثم بعد ذلك الاقتران مع 3-مثيل-2-بنزوثيازولينون هيدرازون هيدروكلوريد (MBTH) وبوجود كبريتات الامونيوم . حيث قدرت القياسات الطيفية بواسطة تسجيل الامتصاصية للنتائج الاخضر اللون عند 610 نانوميتر. باستخدام ظروف التفاعل المثلى كان حدود قانون بير 0.3-5.4 مايكروغرام/مل مع معامل ارتباط جيد بحدود 0.9998. وبلغ حد الكشف وحد الكمية 0.163 و 0.544 مايكروغرام/مل على التوالي. تم حساب الدقة والمصادقية للطريقة المقترحة عن طريق نسبة الاسترجاع والانحراف القياسي المعياري وكانت مقبولة بحدود 99.33% و 1.16% على التوالي. طبقت الطريقة بنجاح في تقدير NIT في المستحضرات الصيدلانية وتم مقارنة النتائج مع الطريقة القياسية (طريقة الدستور الدوائي البريطاني).

الكلمات المفتاحية: نيتروفورانتوين، MBTH، تفاعلات ازدواج تاكسدي .

Introduction

Nitrofurantoin (Figure 1), is 1-(5-nitro-2-furfurylidene)-1-amino hydration ⁽¹⁾ with a molecular weight of 238.16 g/mol. It is a lemon-yellow, odorless fine powder, very slightly soluble in water and alcohol, but it is soluble in dimethyl formamide⁽²⁾.

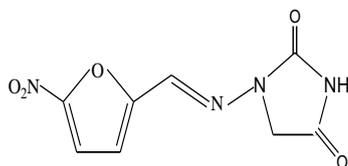


Figure (1):- Structure of Nitrofurantoin

Nitrofurantoin , a nitro furan derivative, is an antibiotic, that is preferable choice of oral use for wide spectrum of infections especially for urinary tract infections ⁽³⁾. The mechanism of action in killing the germs is not known accurately, however, it is well absorbed in the intestines and up to very high concentrations in the urine⁽⁴⁾. A survey of the literature exposed that numerous techniques have been reported for analysis of NIT in both body fluids and pharmaceuticals, among them a spectrophotometric ⁽⁵⁾, HPLC⁽⁶⁻⁸⁾, liquid chrom tandem mass spectrometry ⁽⁹⁾, square wave voltametric ^(10, 11), and flow injection chemiluminescence methods ⁽¹²⁾.

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Unfortunately all the mentioned techniques are expensive and complicated. The sensitive UV-visible spectrophotometric methods for the determination of the drug are limited and literature contains only few methods for the determination of NIT in pure and dosage forms. This work describes the development of simple and rapid method based on colorimetric reaction for the quantitative determination of NIT in pure and pharmaceutical forms. The proposed method carried out at room temperature is based on the reduction of the nitro group of NIT, followed by coupling with MBTH in the presence of ammonium ceric sulphate as oxidizing agent.

Experimental

Apparatus

A digital double beam Shimadzu UV-VIS 260 spectrophotometer (Shimadzu, Kyoto, Japan) was used for all measurements (spectral and absorbance). The absorbance measurements were carried out using matched 1cm quartz cells.

Reagents and standards

- All the chemicals and reagents were of analytical grade and the freshly prepared solutions were always used throughout this work.
- **Nitrofurantoin (NIT) solution(500 µg/mL)**⁽¹³⁾.

The reduction solution of NIT was prepared by dissolving 0.0500 g of NIT in 50mL of ethanol. This solution was transferred into 125 mL beaker and 20 mL of distilled water, 20 mL of concentrated hydrochloric acid, and 3.0 g of zinc powder were added. In order to complete the reduction process the beaker was allowed to stand for 15 min at room temperature (25°C), then the solution was filtered into 100 mL volumetric flask, and the volume was diluted to the mark with distilled water to obtain 500 µg mL⁻¹ of NIT reduction solution then transferred in a brown bottle. Working solution was prepared daily by appropriate dilution using distilled water.

- **Methylbenzothiazoline- 2- one hydrazone hydrochloride (0.3%w/v)**⁽¹⁴⁾, **Sigma-Aldrich**

The acidic solution of this reagent was prepared by dissolving 0.3000g of the reagent in 100 mL of 0.2 M HCl. This solution should be freshly prepared. Working solution was prepared daily by appropriate dilution using the same solvent.

- **Ammonium ceric sulphate, ACS (0.03M, BDH)**

This acidic solution was prepared by dissolving 1.9789 g of the oxidant in 100mL of 1% H₂SO₄ solution. Working solution was prepared daily by appropriate dilution using the same solvent.

- **Hydrochloric acid (Merck, Germany)** 0.2M aqueous solution.
- **Sulphuric acid (Merck, Germany)** 1% v/v aqueous solution.
- **Pharmaceutical preparations**

The different pharmaceutical preparations were purchased from the commercial source in the local market.

1. Nitrofurantoin capsules, 100 mg (Uvamin retard, Switzerland).
2. Furantil capsules, 50mg (Bio Active T-UK).

Solutions of pharmaceutical preparations

Ten and/or fifteen capsules of commercial NIT (labeled to contain 100 and/or 50mg of nitrofurantoin per capsule) were accurately weighted and finely powdered. An amount of the powder equivalent to 50 mg of NIT was dissolved in 30 mL of ethanol, then the solution was filtered into a 50 mL volumetric flask. And the volume was diluted to the marked with the same solvent to obtain 1000 µg mL⁻¹ of NIT. This solution was transferred into 125 mL beaker and was reduced as previously described. Further appropriate solutions of pharmaceutical capsules were prepared using distilled water. For the proposed method, the content of a tablet was calculated using the corresponding regression equation of the appropriate calibration graph.

General procedure (constructing the calibration curve)

An increasing volumes of standard drug solution in the range (0.01-0.6) mL of 500µg/mL reduce NIT were transferred into a series of 10 mL volumetric flasks to obtain the concentration range of 0.5-30 µg/mL. To each flask, 0.5 mL of MBTH reagent (0.3% w/v) and 0.5 mL of ammonium ceric sulfate (0.03M) were added. After 5 min the contents were diluted to the mark with distilled water and mixed well. The absorbance of the colored product was measured after 15 min at 610 nm against the corresponding reagent blank prepared similarly omitting the drug content.

Stoichiometry of the coupling reaction using Job's method and mole ratio method

⁽¹⁵⁾

The stoichiometry of oxidative coupling reaction was calculated using equimolar of reduced NIT and MBTH (2mM) at constant oxidant concentration adopting Job's method of continuous variation, and mole ratio methods. Job's method of continuous variation of equimolar solutions was employed: a 2mM of standard solution of reduced NIT and MBTH were used. A series of solutions was prepared in which the total volume of the NIT and MBTH was kept at 10 mL. The drug and reagent were mixed in varying complementary

proportions (0:5, 1:4, 2:3....5:0, inclusive) and completed as directed under the recommended procedure. The absorbance of the resultant product was measured at optimum wavelength.

In mole ratio method an increasing volume of MBTH (0.5, 1, 1.5, 2.....4mL) was added to 1 mL of reduced NIT at constant oxidant concentration. In varying proportions of both drug and reagent, the solutions were mixed and diluted with distilled water in 10mL volumetric flask, then the absorbance was measured at optimum wavelength and under optimal time and temperature against a reagent blank.

Results and Discussion

Nitro group containing drugs are very important kinds of drugs, but the spectrophotometric determination of these drugs (especially NIT) is not easy because of the poor affinity of these drugs to react directly with other coupling reagents. In order to increase this reactivity an attempt to convert the nitro group to a more reactive group (amino group) was done. The present work depends on a simple oxidative coupling reaction between reduced NIT and MBTH in the presence of an efficient oxidant (ACS). A green colored product was formed and have a maximum absorbance at 610nm. The absorption spectra of the reaction product and the reagent blank are presented in Figure 2, and it was used in all subsequent experiments.

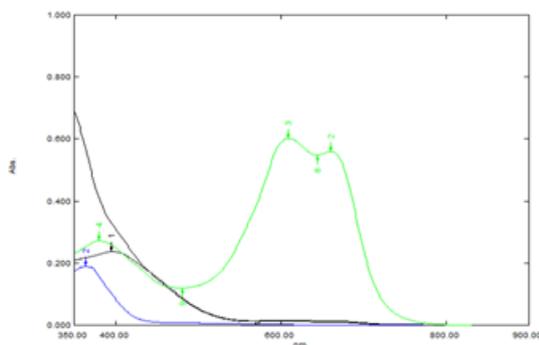


Figure (2) :- Absorption spectra of the product obtained by the reaction of MBTH with 25 µg/mL of reduced NIT in presence of ammonium ceric sulphate versus reagent blank, and the reagent blank versus distilled water.

Optimum reaction conditions

The proposed method was optimized to achieve complete reaction formation, highest sensitivity and maximum absorbance. All the experimental parameters were optimized using 25 µg/mL of NIT.

Effect of reaction time

The color intensity reached a maximum after the reduced drug (25µg/ mL) solution had been reacted after 10 min with MBTH and ACS in aqueous medium and became stable after 15 min and remained stable for at least 50 min (Figure 3). This experiment was repeated after optimized all the reaction parameters and the results were the same.

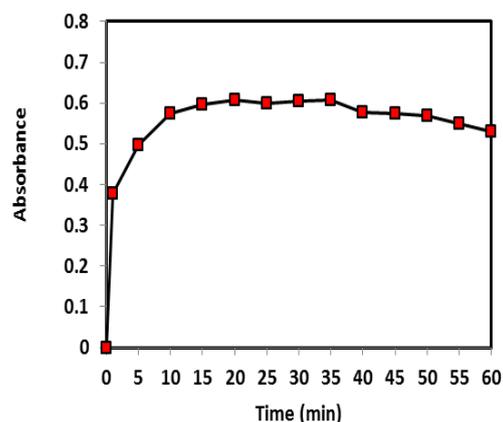


Figure (3):- Stability time

Optimization of the experimental conditions

MBTH is an efficient coupling reagent for many drugs. In order to study the effect of the amount of reagent, different volumes in the range (0.1-3.0)mL of 0.3%w/v MBTH was examined in the presence of 0.5mL of ammonium ceric sulfate (0.03M). The results (Figure 4) show that 0.5 mL of the MBTH solution was enough to obtain a maximum absorbance, and it was used in the subsequent experiments.

Different oxidants were studied to accomplish the coupling reaction such as sodium periodate, ferric chloride, N-Bromo succinimide(NBS), potassium thiosulphate and ACS. The maximum absorbance was obtained only when using ACS as oxidant. The optimum volume of the used oxidant used (ACS) was examined. This study was carried out using different volumes of 0.03M of ACS in the range (0.1-2.0) mL. An increase in absorbance was obtained up to 0.5 mL of ACS as shown in the Figure 5. Beyond this volume the absorbance was decrease with increasing the amount of oxidant because of the increasing the absorbance of blank, therefore 0.5 mL of ACS was chosen, and was used in the subsequent experiments.

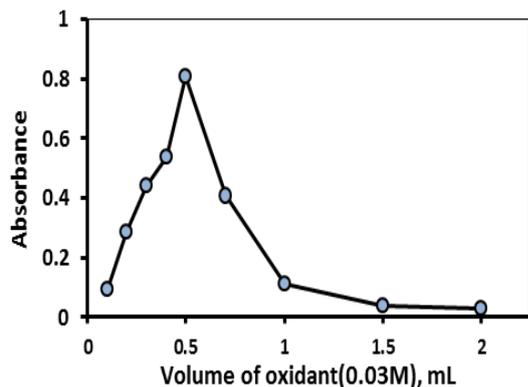


Figure (4):- Effect of the reagent

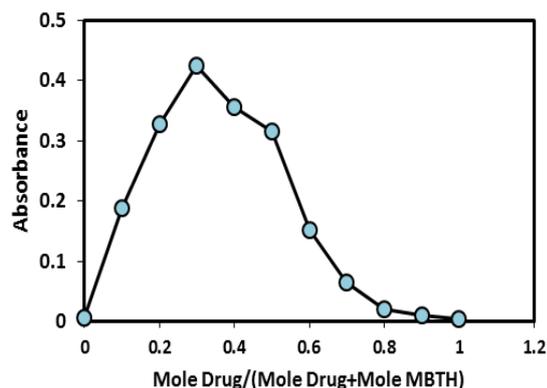


Figure (5):- Effect of the oxidant

The effect of order of addition is also studied under the obtained optimum results. According to the results, it was found that the order of addition of reagents (Drug + MBTH + ACS) as shown in Figure 6, gave the maximum absorbance and stability in measurement.

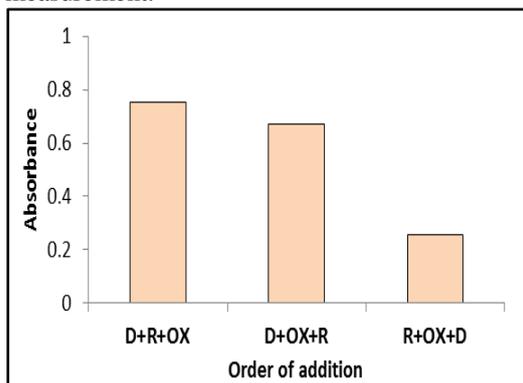


Figure (6):- The effect of the order of addition (D=Drug (NIT), OX= oxidant (ACS), R=reagent(MBTH))

The effect of temperature on the oxidative coupling reaction was also studied using three different temperatures (5, 25, 65)^oc. Figure 7 shows that a maximum absorbance and a good stability were obtained when the formed product developed at ambient temperature (25^oc).

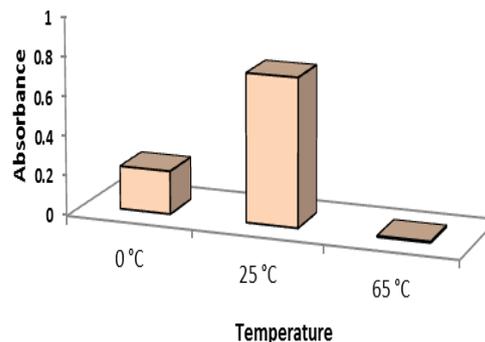
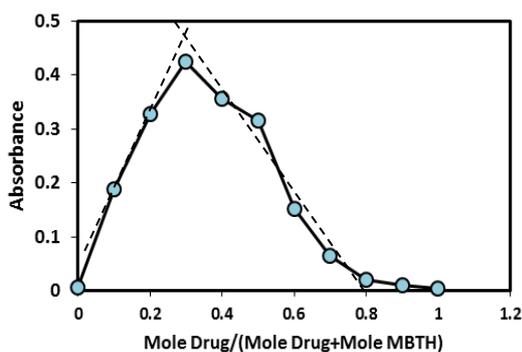
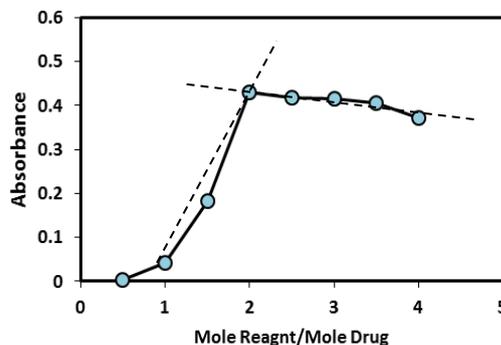
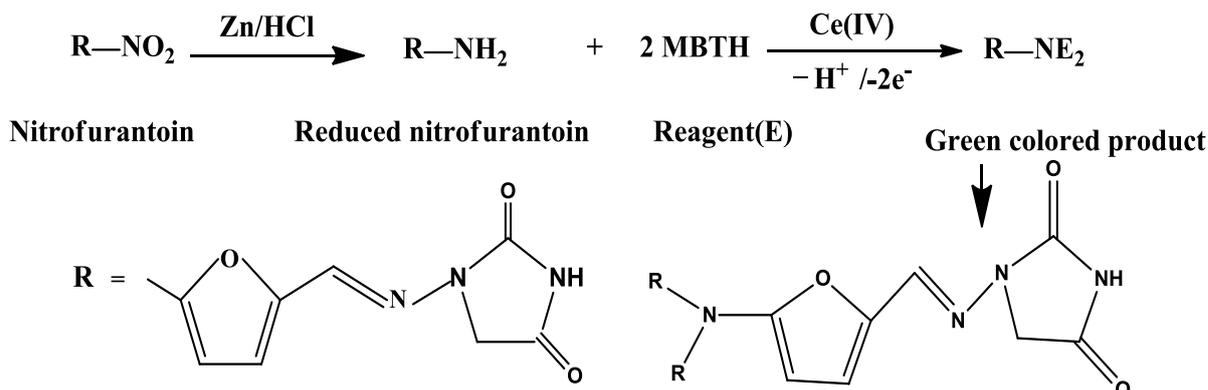


Figure (7):- Effect of temperature

The structure of the product was adopted based on the mole ratio and continuous variation methods ⁽¹⁵⁾, using equimolar solutions (2 mM) of reduced NIT and MBTH reagent. The results obtained in Figures (8 and 9) show that 1:2 ratio reduced NIT to reagent was formed.



when MBTH loses two electrons and one proton under oxidation process, which can be substituted on reduced NIT under the reaction conditions to form a green colored product ⁽¹⁶⁾. Therefore, the mechanism of formation of the product may be suggested according to the following equation:



Conditional stability constant (K_f) of the product and Gibbs free energy of the reaction

The conditional stability constant of the green color product was calculated from the continuous variation data using the following equation⁽¹⁷⁾:

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

Where: A and A_m are the maximum absorbance of the continuous variation curve and the absorbance corresponding to junction of the two tangents of the continuous variation curve respectively (Fig. 8). n is the number of molecules of the reagent in the reaction product (the stoichiometric constant). C is the molar concentration of NIT at the maximum absorbance. K_f was found to be equal to $6.084 \times 10^5 \text{ L}^2 \text{ mole}^{-2}$. This indicates a stable reaction product. The Gibbs free energy of the reaction ΔG was also calculated adopting the following equation:

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

where, R is the universal gas constant ($8.314 \text{ J mole}^{-1} \text{ deg}^{-1}$). T is the absolute temperature ($273 + 25^\circ\text{C}$), K_f is the formation constant of the reaction. The value of ΔG was found to be -33 kJ/mole . The negative value of ΔG refers to the spontaneity of the reaction.

Kinetic of the reaction

The initial rates of the reaction were determined by measuring the slopes of the initial tangents the absorbance time curves for the first 10 min (Fig. 10). Furthermore, logarithmic analysis of the reaction rate (R) was plotted against the logarithm of concentration of the drug (Fig. 1).

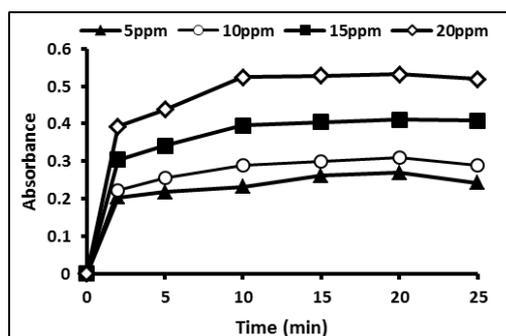


Figure (10):-Absorbance versus time graphs

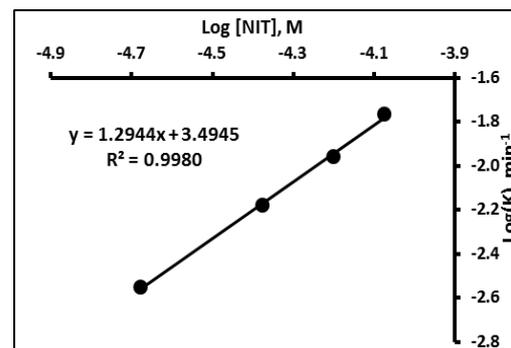


Figure (11):- Log (rate) versus log [NIT] graphs

The rate of reaction was also found to be dependent on NIT concentrations. The rates were followed at room temperature (25°C) with various concentration of NIT in the range of (2.08×10^{-5} to $8.39 \times 10^{-5} \text{ M}$) keeping MBTH and ACS concentrations constant. The reaction rate was found to obey the following equation: $\text{Rate} = k' [\text{NIT}]^n$ where k' is the pseudo-order rate constant and n is the order of the reaction. The rate of the reaction may be estimated by the variable-time method⁽¹⁸⁾ (differential initial rate method) as $\Delta A/\Delta t$, where A is the absorbance and t is the time in minutes. Taking logarithms of rates and concentration, the previous Eq. is transformed into

: $\text{Log (rate)} = \log(\Delta A/\Delta t) = \log k' + n \log [\text{NIT}]$

Regression of $\log(\text{rate})$ versus $\log[\text{NIT}]$ gave the regression equation:

$$\text{Log (rate)} = 3.4945 + 1.2944 \log C \quad (r^2 = 0.9980)$$

Hence $k' = 3122.4 \text{ min}^{-1} = 52 \text{ sec}^{-1}$ and the reaction is first order ($n = 1.2944$) with respect to NIT concentration.

Validation of the proposed method

After optimized all the reaction conditions mentioned above, the calibration graph was plotted between the absorption intensity with the corresponding concentration of NIT.

Regression analysis and the statistical parameters are calculated from the calibration graph using least-square method. The slope, correlation coefficient, intercept for the calibration data and the sensitivity parameters (molar absorptivity and Sandell sensitivity), are summarized in Table 1. A high correlation coefficient ($r=0.9998$) with the small intercept value of the regression equation were confirmed the linearity of calibration curve. The small values of the statistical parameters calculated from regression equation point out to the high precision of the proposed method and low scattering of the points of the calibration curve and high accuracy (Table 1).

Table(1):- Summary of optical characteristics and statistics for the proposed metho

Parameter	Value
λ_{max} (nm)	610
color	Green
Regression equation $y = b x + a$; Y = absorbance, x = concentration($\mu\text{g/mL}$)	$Y=0.0277x+0.0568$
Correlation coefficient, r	0.9998
Linearity percentage, $r^2\%$ ($r^2\% = r^2 \times 100$)	99.9891
Dynamic range ($\mu\text{g/mL}$)	0.5-30
Molar absorptivity, ϵ (L/ mol cm)	6.5970×10^3
Slope, b (mL/ μg)	0.027742
Intercept, a ($a = y - b x$)	0.056758
Standard deviation of the residuals, $S_{y/x}$	0.004377
Standard deviation of the slope, S_b	1.364×10^{-4}
Standard deviation of the intercept, S_a	2.003×10^{-3}
Sandell's sensitivity, S ($\mu\text{g/cm}$)	3.61016×10^{-2}

Sensitivity

Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated according to the $3.3S/k$ and $10S/k$ criterions, respectively⁽¹⁹⁾, where $S(0.001506)$, is the standard deviation of the response of the blank or the standard deviation of intercepts of regression lines and k is the sensitivity, namely the slope of the calibration curve. The LOD and LOQ values were 0.179 and 0.544 $\mu\text{g/mL}$ respectively.

Table (2) :- Accuracy and precision of the proposed method

Amount of NIT taken, ($\mu\text{g/mL}$)	Found* ($\mu\text{g/mL}$)	%Relative error*	%(Recovery \pm SD)*	%RSD*
6.00	5.95	-0.83	99.17 ± 0.23	1.07
8.00	7.81	-2.38	97.63 ± 0.53	1.96
20.00	20.07	0.35	100.35 ± 0.27	0.45

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

Effect of interferences

In order to examine the usefulness of the method, the studied drug (NIT) was determined in the presence of diluents, excipients and additives which often accompany NIT in its dosage forms such as poly vinyl pyrrolidone, lactose, starch and

magnesium stearate. The experiment accomplished by measuring the absorbance of solution containing 25 $\mu\text{g/mL}$ of NIT in the presence of tenfold of excipient (250 $\mu\text{g/mL}$). The good percentage recoveries were obtained indicating no interference was observed from any of these excipients and additives, and a

high selectivity for determining the NIT in its dosage forms (Table 3).

Table (3):- Analysis of NIT in presence of common interferences by batch method

Excipient (250 µg/mL)	Conc. of NIT, µg/ mL		% (Recovery ± SD)*
	Present	Found	
Poly vinyl pyrrolidone	25.0	25.69	102.76±0.67
lactose		24.50	98.00±0.13
starch		24.19	96.76±0.43
magnesium stearate		24.28	97.12±0.71

*Average of five determinations.

Analytical applications

The proposed method was successfully applied to determine NIT in pharmaceutical preparations by the analysis of three different concentrations of pharmaceutical preparations using the analytical procedure directly and using standard addition methods. The results are given in Table 4 and 5. For evaluating the competence and the success of the proposed method, the results obtained were compared with those obtained by standard BP method⁽¹⁾.

The same pharmaceutical preparations for NIT were analyzed by standard BP method. The results obtained by the two different methods as show in Table 4, were statistically compared using the student t-test and variance ratio F-test at 95% confidence level when degree of freedom(n=3) ⁽¹⁵⁾. In all cases, the calculated values were less than the theoretical one, which indicate that there is no significant difference between either methods in accuracy and precision in the determination of NIT in pharmaceutical preparations.

Table (4):-Application of the proposed method to the determination of NIT in different dosage forms using standard addition method

Dosage form	Taken conc. (µg/mL)	Proposed method		
		Pure drug added conc. (µg/mL)	Total found conc. (µg/mL)	(%Recovery± SD) n=5
Nitrofurantoin (Cap.100 mg), Uvamin retard, Switzerland	5.00	5.00	10.01	100.14±0.010
		10.00	14.81	98.12±0.020
Furantil (Cap.50mg), BioActiveT-UK	5.00	5.00	10.03	100.55±0.003
		10.00	14.44	94.40±0.005

Table (5):- Application of the proposed and official methods to the determination of NIT in different dosage forms directly.

Dosage form	Proposed method				Official method[HPLC]			
	Taken conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%) ^a	RSD (%) ^a	Taken conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%) ^b	RSD (%) ^b
Nitrofurantoin (Cap.100 mg), Uvamin retard, Switzerland	5.00	5.15	102.94	0.97	25.0	25.31	101.22	0.05
	10.00	10.27	102.69	0.73				
	15.00	14.87	99.15	1.34				
Furantil(Cap. 50mg), BioActiveT- UK	5.00	5.01	100.09	1.60	25.0	24.89	99.55	0.03
	10.00	9.85	98.48	1.01				
	15.00	14.74	98.27	0.67				
t (2.776) ^c	0.134							
F (19.000) ^c	1.069							

a, (n=5); b, (n=3); c Theoretical value; Conc., concentration; RSD, relative standard deviation.

Conclusions

The proposed method showed good sensitivity, and low detection limit. In

addition, the data given above reveal that the proposed method was accurate and sensitive with good precision and accuracy; it can be successfully applied to the routine estimation

of NIT in bulk and in pharmaceutical preparations. The values of relative standard deviation were satisfactorily low (less than 2%) with good recoveries which indicate the high reproducibility and accuracy for the proposed method. The reaction was adopted to suggest a new flow injection method for the determination of NIT in a separate work send for publication by the same authors.

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