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Indirect Spectrophotometric Determination of Niclosamide in Pharmaceutical Preparations

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الخلاصة

يتضمن البحث تطوير طريقة طيفية غير مباشرة بسيطة وحساسة لتقدير النيكلوساميد في المستحضرات الصيدلانية . تعتمد الطريقة على أكسدة الصيغة المختزلة للنيكلوساميد بوساطة ايون الحديد الثلاثي في الوسط الحامضي ومفاعلة الحديد الثنائي الناتج مع الكاشف 1،10– فينانثرولين لينتج معقد الفيروين والذي يكون ذائبا في الماء ومستقرا (تم الحصول على استقرارية أفضل بإضافة محلول EDTA) ويعطي أعلى امتصاص عند الطول الموجي510 نانوميتر مقابل المحلول الصوري، وكانت قيمة الامتصاصية المولارية 2.19 ×10^4 لتر.مول$^{-1}$. سم$^{-1}$. وينطبق قانون بير في مدى التركيز من 2.5 إلى225 مايكروغرام /10 مل. وقد تراوح الخطأ النسبي بين– 1.80 و+1.50% والانحراف القياسي النسبي بين ± 0.8 و ± 2.07 % اعتمادا على تركيز النيكلوساميد. وقد تم تطبيق الطريقة بنجاح لتقدير النيكلوساميد في المستحضرات الدوائية .

الكلمات الدالة : النيكلوساميد ،الأكسدة والاختزال ,طريقة طيفية ، 1, 10– فينانثرولين

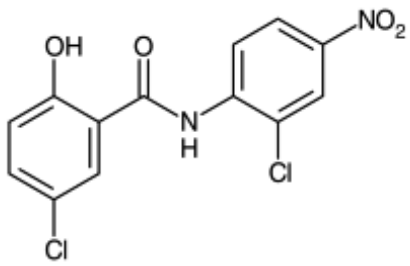
Absrract

A simple and sensitive indirect spectrophotometric method is developed for the determination of niclosamide in pharmaceutical preparations. The method is based on the oxidation of reduced form of niclosamide with iron (III) in acidic medium, then the subsequent reaction of iron (II) with 1,10-phenanthroline to produce a ferroin complex which is water - soluble , stable (using EDTA solution improve the stability of the complex) , and has a maximum absorption at 510 nm against the reagent blank with a molar absorptivity of 2.19×10^4 $l.mol^{-1}.cm^{-1}$. Beer's law is valid over the concentration range of 2.5 to 225 µg/10 ml. The relative error is in between - 1.80 and +1.50 % and the relative standard deviation from ± 0.89 to ± 2.07 % depending on the concentration of niclosamide . The proposed method has been applied successfully to determine niclosamide in pharmaceutical preparations.

Keywords : *niclosamide , oxidation - reduction , spectrophotometry, 1,10-phenanathroline.*

Introduction

Niclosamide (NIC) is an anthelmintic, BCS class II drug of low solubility and high permeability[1]. It is the first choice in the treatment of beef tapeworm (*Taenia saginata)*, fish tapeworm (*Diphyllobothrium latum)* and pork tapeworm (*Taenia solium).* It inhibits phosphorylation in the mitochondria of Cestodes. Both in vitro and in vivo, the scolex and proximal segments are killed on contact with the drug [2]. NIC is used also as a molluscicide for the treatment of water in Schistosomiasis control programmes[3] NIC and its two novel synthesized derivatives constructed to float on the water surface were able to kill cercariae[4,5], also possessed promising activity in vitro against an apicomplexan parasite *Toxoplasma gondii*[6]. NIC chemically is 5 – chloro - N - (2- chloro – 4 – nitrophenyle) - 2 –hydroxybenzamide. NIC is a yellowish – white or yellowish , fine crystals , practically insoluble in water , slightly soluble in ethanol[7]. Niclosamide has different proprietary names such as : Yomesan; Niclocide ; Cestocide[8], and has the following structure:



M.Wt. =327.1 g/mol

$C_{13}H_8Cl_2N_2O_4$

Chemical structure of niclosamide

Several methods have been reported for the determination of NIC, these methods include derivative spectrophotometric method[9]. Other spectrophotometric methods depend on reduction of NIC with zinc powder in presence of hydrochloric acid followed by reaction with different reagents such as metol and potassium dichromate to give a colored product[10] and p-benzoquinone to form a pink product which absorbs at 506 nm [11]. NIC was also determined in tablets by dissolution of the tablets in 0.1 N sodium hydroxide solution followed by measurement of the absorbance at 375 nm[12]. Also other techniques have been utilized for the determination of NIC such as liquid chromatography[13], gas - liquid chromatography[14] , cyclic voltametry[15] and fluorometry[16]. The standard method for determination of NIC includes potentiometric titration[7].

The present work aims mainly to develop a simple and sensitive spectrophotometric method for the determination of NIC in pharmaceutical preparations basded on the oxidation of the reduced form of NIC with iron (III) and the liberated iron (II) reacts with 1,10- phenanthroline in aqueous solution to form a colored complex that has been proved successfully for the determination of niclosamide in both pure form and in its pharmaceutical preparation .

Experimental

Apparatus

All spectrophotometricmeasurements were carried out on a double beam spectrophotometer type CECIL CE 7200 and Shimadzu UV-160A UV-visible recording spectrophotometer with 1.0 cm matched quartz cells , pH measurements were performed by pH meter type TRANS BP 3001.

Thermostatic controlled water-bath type memmert was used for heating process .

Reagents

All chemicals used are of analytical reagents grade.

Standard solution of niclosamide (10000 µg/ml).

This solution was prepared by dissolving 0.5 g of pure NIC (Sigma-Italy) in 50 ml of 1 : 1 ethanol-acetone mixture in a volumetric flask.

Standard reduced form solution of niclosamide(500 µg / ml)

A 5.0 ml of NIC (10000 µg / ml) solution was transferred to dry beaker, add 0.1 g of zinc powder, 5ml of 1M HCl and heat the mixture in boiling water - bath for 15 min., cool and filter the solution into a 100 ml volumetric flask, and then the volume was completed to mark with distilled water [7].

Reduced working niclosamide (RNIC) solution (50 µg / ml)

A 10 ml of 500 µg / ml is diluted with distilled water to the mark in a 100 ml volumetric flask .

Ferric sulphate (3.74×10^{-3}M)

This solution was prepared by dissolving 0.1499 of ferric sulphate (BDH) in distilled water in the presence of 3ml of 0.25M sulphuric acid and the volume was completed to 100 ml in a volumetric flask with distilled water.

1,10- phenanthroline monohydrate solution (1.68×10^{-2} M)

This solution was prepared by dissolving 0.3348g of the 1,10-phenanthroline monohydrate (Fluka) in 5 ml of ethanol and the solution was made up to 100 ml in a volumetric flask with distilled water.

Ethylenediaminetetraacetic acid (EDTA) Solution(1%)

This solution was prepared by dissolving 1 g of disodium salt of the EDTA (Fluka) in 100 ml distilled water.

Tablets (Yomesan) solution (10000µg / ml)

Ten tablets of yomesan (each tablet contain 500 mg of niclosamide) are finely powdered , an accurately weighed of the powder equivalent to 0.5 g of NIC is dissolved in 20 ml of ethanol – acetone 1:1 mixture and the residue is filtered into 100 ml calibrated flask and then the volume was completed to the mark by repeated washing with the same solvent . This solution was treated in the same way as the standard niclosamide to prepare the reduced and the working solutions of niclosamide drug .

Procedure and calibration graph

Accurately measured volumes containing 2.5-225 µg of of RNIC were transferred into a series of 10 ml calibrated flask . To each of these flasks 0.6 ml of 3.74×10^{-3} M ferric sulphate and 2.5 ml of 1.68×10^{-2} M 1, 10 - phenanthroline monohydrate solutions were added , then the flasks left to stand for 30 minutes at room temperature,followed by addition of 1ml of 1% EDTA solution and diluted to the mark with distilled water , the absorbance of the red colored product was then measured at 510 nm a gainst the reagent blank solution.A linear calibration graph is obtained over the concentration range of 2.5 to 225 µg niclosamide/10ml (i.e 0.25 to 22.5µg / ml) (Fig 2). The sensitivity of the method , expressed as the molar absorptivity has been found to be 2.19×10^{4} $l.mol^{-1}.cm^{-1}$.

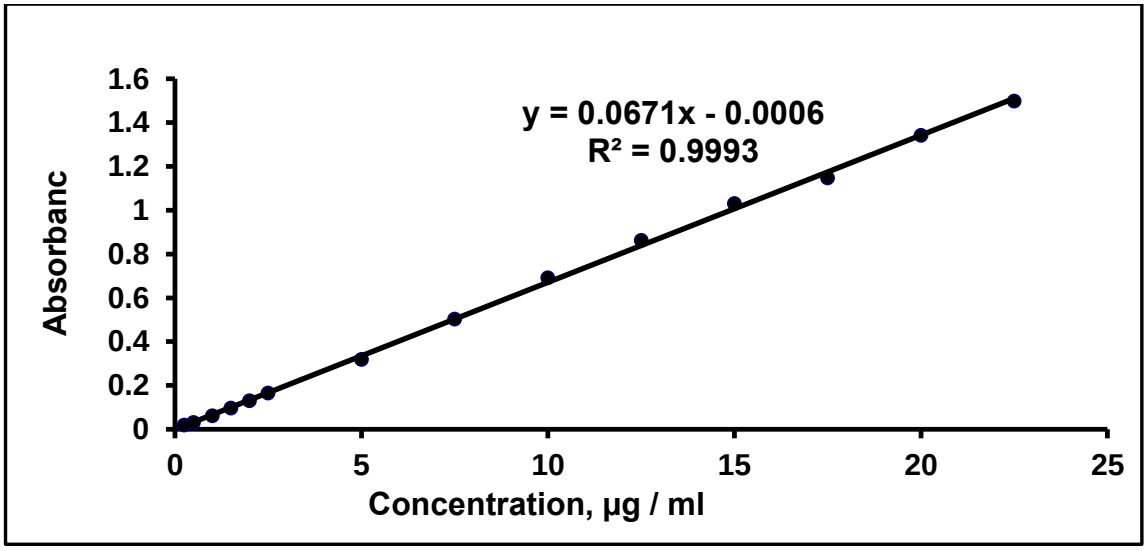
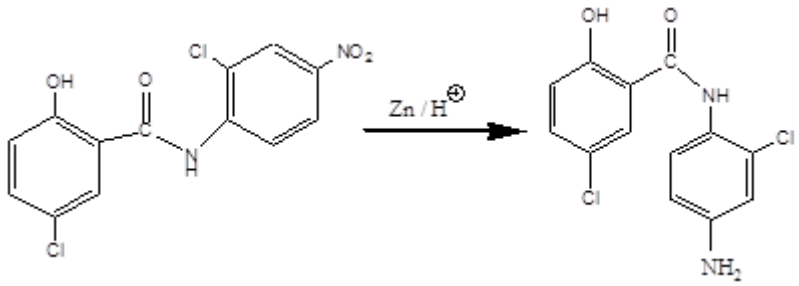


Fig.2. Calibration graph of NIC determination using the proposed method

Results and Discussion

Principle of the method

The method involves the reduction of nitro-group in niclosamide by zinc and HCl to produce the corresponding amine as shown in the following equation:



Then the corresponding amine of NIC undergoes oxidation - reduction reaction with Fe(III) to produce Fe(II) which subsequently reacts with 1,10- phenanthroline to produce a red colored complex of ferroin having an absorption maximum at 510 nm as shown in the following reaction :

Fe^{+2} + 1,10- phenanphthroline ⟶ Red complex

Optimum reaction conditions

The effect of various parameters on the intensity of the colored product has been studied and optimum conditions have been selected **.**

Effect of ferric sulphate solution

The effect of different amounts of (oxidizing agent) ferric sulphate solution on the absorbance has been investigated (Table 1).

Table 1 : Effect of ferric sulphate amount on absorbance

Fe^{+3}(ml) (3.74×10^{-3} M)	Absorbance / µg of RNIC					R^2
	25	50	75	100	125	
0.1	0.162	0.277	0.398	0.470	0.513	0.9650
0.2	0.185	0.298	0.437	0.558	0.642	0.9941
0.4	0.180	0.314	0.487	0.595	0.655	0.9730
0.6	0.173	0.328	0.493	0.620	0.717	0.9901
0.8	0.181	0.329	0.483	0.568	0.668	0.9845
1.0	0.173	0.319	0.439	0.482	0.583	0.9680

The results illustrated in Table 1 indicate that 0.6 ml of 3.74×10^{-3} M ferric sulphate solution gives the highest absorbance over a range of determined concentration of 25 to 125µg RNIC / 10 ml , therefore it is recommended for the subsequent experiments.

Effect of 1,10- phenanthroline monohydrate reagent amount

The effect of different amounts of 1,10-phenanthroline monohydrate reagent on the absorbance of solutions containing different amounts of RNIC is studied. The results indicated that the absorbance increases with increasing reagent concentration and reached maximum on using a volume of 2.5 ml of 1.68×10^{-2} M 1,10- phenanthroline monohydrate which also gives the highest value of determination coefficient (Table 2).

Table 2: The effect of reagent amount on absorbance

Amount of 1.68×10^{-2} M 1,10-phenanthroline solution(ml)	Absorbance / µg of RNIC					R^2
	25	50	75	100	125	
0.5	0.160	0.232	0.268	0.291	0.322	0.9459
1.0	0.169	0.304	0.441	0.518	0.589	0.9761
1.5	0.170	0.327	0.485	0.635	0.759	0.9979
2.0	0.171	0.313	0.479	0.654	0.788	0.9982
2. 5	0.168	0.325	0.480	0.658	0.817	0.9992
3.0	0.171	0.320	0.454	0.622	0.750	0.9987
3.5	0.174	0.320	0.470	0.577	0.682	0.9925

Effect of pH

The effect of pH on color intensity is examined since the extent of complex formation and hence, the absorbance of the final solution is often a function of hydrogen ion concentration. Different volumes of different acid and base solutions have been used and the absorbances and pH values were measured as in Tables (3,4).

Table 3: Effect of different acids on absorbanc

Acid used (1M)	Absorbance / ml of acid				pH-range
	0.1	0.3	0.5	0.7	
HCl	0.326	0.272	0.248	0.242	2.36-1.61
HNO_3	0.325	0.292	0.250	0.231	2.34-1.63
H_2SO_4	0.297	0.223	0.217	0.202	1.98-1.59
CH_3COOH	0.313	0.317	0.307	0.314	4.33-3.98

Table 4: Effect of different bases on absorbance.

Base used (0.1 ml of 1 M)	A	pH
NaOH	Turbid	11.65
Na_2CO_3	Turbid	10.40
$NaHCO_3$	0.236	9.68

- **Absorbance without addition of acid or base = 0.328**
- **pH without addition of acid or base = 4.42**

The results shown in Tables (3) and (4) indicate that there is no improvement in absorbance on the addition of acids or bases, so they are not recommended for subsequent experiments.

Effect of buffer solution

The effect of buffer solution on the absorbance of produced complex was studied by addition 3 ml of three buffer solutions of pH 4.4 with different compositions [17] as in Table 5.

Table 5: Effect of buffer solution

Buffer used (pH 4.4)	Absorbance	pH of final solution
KH- phthalate - NaOH	0.286	4.55
Acetic acid – sod.acetate	0.306	4.48
Formic acid - formate	0.305	4.47
Without	0.328	4.42

The results in Table 5 indicate that using of these buffer solutions decrease the absorbance, therefore, it has been recommended to eliminate the use of these buffer solutions

Effect of temperature and reaction time on absorbance

Table 6: The effect of temperature / room temprature

Temp. C°	Absorbance / Time (min)					
	5	10	15	20	25	30
20*	0.223	0.285	0.301	0.320	0.323	0.326
40	0.330	0.327	0.331	0.352	0.350	0.351
50	0.328	0.339	0.351	0.362	0.361	0.365
60	0.344	0.364	0.367	0.374	0.372	0.378
70	0.352	0.360	0.377	0.386	0.392	0.384
80	0.366	0.368	0.379	0.392	0.421	0.481
90	0.397	0.392	0.444	0.458	0.507	0.514
100	0.405	0.432	0.461	0.601	0.625	0.649

The reaction time was determined by following the color development at different temperatures in thermostatically controlled water-bath. The absorbance was measured at 5 min. intervals against reagent blank treated similarly (Table 6).

The results in Table 6 indicate that the absorbance of the resulting complex increased with increasing of temperature and time reaction, probably due to side reactions at high temperatures. Also, the blank value increase tremendously, so room temperature and 30 min. as a reaction time were selected for subsequent experiments.

Order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the general procedure, otherwise less absorbance intensity was observed .

Effect of surfactants

The effect of different types of surfactants on absorbance of complex has been studied . The results shown in Table 7 confirm that there is no improvement in the absorption, therefore they were excluded .

Table 7 : Effect of surfactant agents

Surfactants	**Absorbance**
Sodium dodecyl sulphate (1×10^{-3} M)	**Turbid**
Cetylepyridinium chloride (1×10^{-3} M)	**0.300**
Triton X-100 1%	**0.203**
Without surfactants	**0.326**

Effect of time on the complex stability

The effect of time on the development and stability of the colored complex for different amounts of RNIC is investigated under the optimum experimental conditions. The absorbance of the complex increased continuously with the time, and it is found that the stability is enhanced in the presence of masking agents such as NaF and EDTA.

Effect of masking agents on stability

The effect of masking agents on the stability and the sensitivity of the colored complex is investigated by carrying out a series of experiments using 0.5,1.0,1.5,2.0 ml of 1% of NaF or EDTA-Na_2 solutions added individually before dilution to the mark.The obtained results in Table 8 indicate that using of 1ml of 1% EDTA-Na_2 gives the highest absorbance and improve the stability of the complex up to 24 hours, so it has been recommended for the subsequent experiments.

Table 8: Effect of masking agents on stability

Time* (min.)	Absorbance / 100 µg of NIC		
	Without	NaF 1%	EDTA 1%
0	0.462	0.626	0.687
5	0.510	0.627	0.688
10	0.560	0.628	0.690
15	0.606	0.628	0.693
20	0.630	0.628	0.693
25	0.642	0.629	0.692
30	0.648	0.629	0.692
35	0.650	0.629	0.692
40	0.652	0.629	0.691
45	0.653	0.629	0.691
50	0.654	0.629	0.691
55	0.655	0.629	0.691
1 hr	0.657	0.629	0.691
2 hr	0.685	0.630	0.691
1 day	0.754	0.621	0.681

* After addition of masking agent solution.

Final absorption spectra

When reduced form of niclosamide is treated according to the recommended procedure, the absorption spectra shows a maximum absorption at 510 nm. characteristic of the ferroin chromophore, in contrast to the reagent blank which shows a low absorbance (0.053) at this wavelength, emphasizing the need for measurements to be performed against the reagent blank (Fig.3).

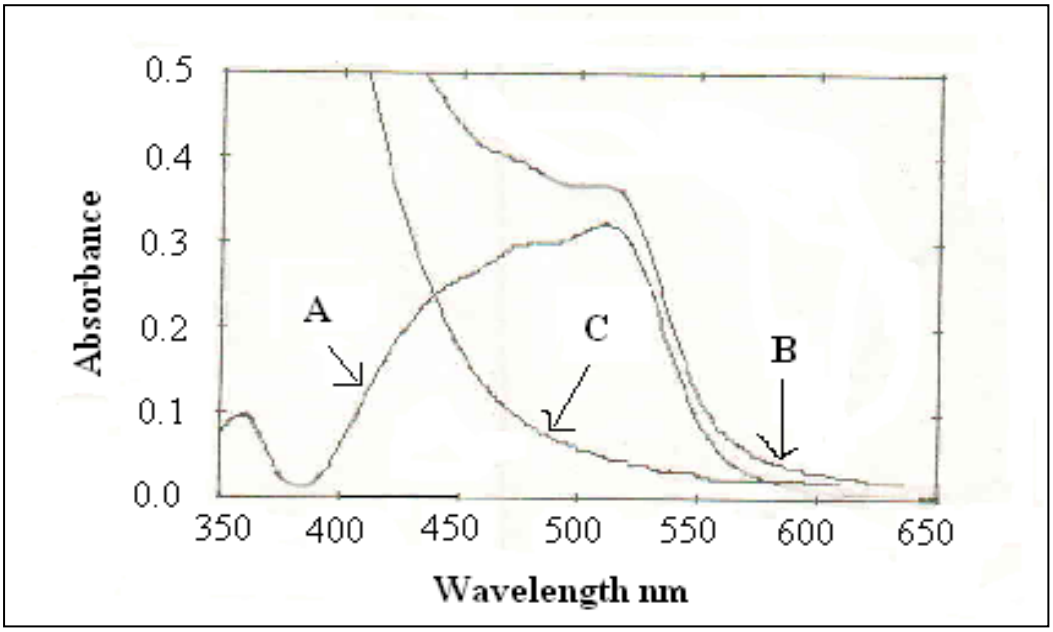
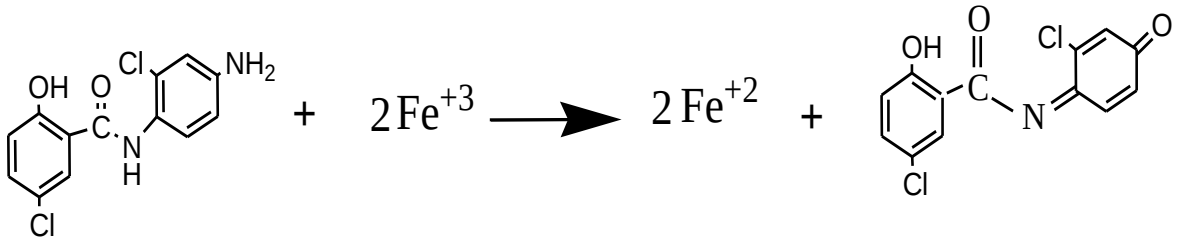


Fig. 3. Absorption spectra of 50µg niclosamide / 10ml treated according to recommended procedure and measured against (A) reagent blank,(B) distilled water and (C) reagent blank measured against distilled water

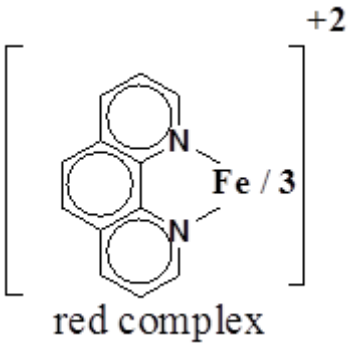
Stoichiometry of the reaction

The stoichiometry of the reactants was investigated by the Job's method[18] .The experimental results indicate that the iron (III) reacts with RNIC in the ratio of 2:1 as the following suggested reaction :



RNIC

The produced ferrous ion is chelated by 1,10-phenanthroline to form the well-known red chelate[19] :



red complex

Effect of Interferences

The influence of excipients compounds (glocose, lactose, starch and arabic gum) which often accompanied pharmaceutical preparations were studied by adding four different amounts (100,250,500 and 1000 µg) to 50 µg of RNIC in a final volume 10 ml . The results in Table (9) indicate that the studied excipients compounds do not interfere in the determination of niclosamide using proposed method.

Table 9 : Effect of excipients for assay of niclosamide

Excipients	Recovery % of 50 µg RNIC per µg excipients added			
	100	**250**	**500**	**1000**
Glocose	99.6	100.0	100.6	101.5
Lactose	99.0	97.6	97.6	97.6
Starch	100.9	99.4	97.9	100.9
Arabic gum	100.6	99.1	98.2	100.6

Application of the method

The proposed method is applied to determine niclosamide in pharmaceutical preparations on appling proposed procedure, good recovery is obtained as shown in Table (10).

Table 10: Analytical applications of the method.

Pharmaceutical Preparation	Amount taken, µg	Amount Measured, µg	Recovery* %
Yomesan 500 mg/ tablet Bayar –Germany	20	20.3	101.5
	50	49.6	99.2
	200	198.0	99.0
Yomesan 500 mg/ tablet Bayar - Turkey	20	20.2	101.0
	50	49.1	98.2
	200	196.4	98.2

***Average of five determinations.**

The performance of the proposed method for the determination of niclosamide in tablets was assessed by calculation of the t-test compared with the standard method[7] at 95% confidence level with eight degrees of freedom. The results in (Table 11) showed that t-value was less than the theoretical value, indicating no significant difference between the proposed method and standard method for the determination of niclosamide in tablets .

Table 11: The results of t-test analysis.

Pharmaceutical Preparation	Recovery, %*		t. exp
	Present method	British pharmacopeia method	
Yomesan 500 mg/ tablet Bayar – Germany	99.2	99.5	0.408
Yomesan 500 mg/ tablet Bayar – Turkey	98.2	98.9	1.076

***Average of five determinations of 50µg .**

Accuracy and precision

To check the accuracy and precision of the method, RNIC is determined at three different concentrations . The results illustrated in Table (12) indicated that the method is satisfactory .

Table 12: Accuracy and precision of the proposed method

Pharmaceutical preparation	Amount taken µg	Relative error, %*	Relative standard diviation , %*
Yomesan 500 mg/ tablet Bayar - Germany	20	+ 1.50	± 1.95
	50	- 0.80	± 1.09
	200	- 1.00	± 0.89
Yomesan 500 mg/ tablet Bayar - Turkey	20	+ 1.00	± 2.07
	50	- 1.80	± 1.26
	200	- 1.74	± 2.03

*** Avarage of five determinations .**

Comparison of the methods

Table (13) shows the comparison between some analytical variables for the present method with that of another literature spectrophotometric method .

Table 13: Comparison of the method.

Analytical parameter	Present method	Literature method[20]
Reagent	1,10-Phenanthroline	p-Dimethylaminobenzaldehyde
Medium of reaction	Aqueous	Non - aqueous
Reaction time (min.)	30	After dilution
pH	4.4	3.0
Temperature (C°)	Room temperature	Room temperature
λ_{max} (nm)	510	454
Beer 's law range (ppm)	0.25-22.5	1-17
Molar absorbtivity (l. mole^{-1} . cm^{-1})	2.19×10^4	3.36×10^4
Color of complex	Red	Yellow
Stability of the color (hr.)	24	3
Application of the method	Tablets	Tablets

Conclusion

The proposed method for the determination of NIC in pharmaceutical formulation is simple, sensitive, accurate and precise. The method is based on oxidation – reduction between RNIC and Fe III, then the subsequent reaction of librated Fe II with 1,10- phenanthroline to produce a ferroin complex which is water - soluble , stable , and has a maximum absorption at 510 nm against the reagent blank. The proposed method has been applied successfully to the determination of the intended compound in its pharmaceutical formulation (tablets).

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