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Spectrophotometric determination of secnidazole in pure form and pharmaceutical formulation

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ABSTRACT

Four simple and sensitive spectrophotometric methods were proposed for determination of secnidazole in pure and in tablet forms. The methods (I, II, III) depends on the reduction of secnidazole molecule with zinc dust and hydrochloric acid), forward by coupling reaction between the drug and vanillin reagent in acidic condition (method I), the red colored product was measured at λ_{max} 557 nm. Beer's law was obeyed in the range 5-55 µg/ml. Method II, 1, 2-Naphthoquinone-4-sulphonate sodium react in alkaline media through nucleophilic substitution reaction producing an orange-brown colored product showing maximum absorption at 480 nm. Beer's law was obeyed in the range 0.2-2.2 µg/ml. Method III, charge transfer complex was formed with tetracyanoethylene with maximum absorbance at 397 nm. Beer's law was obeyed in the range 0.15-1.2 µg/ml.

Method IV based on bromination-oxidation reaction using bromate-bromide mixture with methyl orange as reagent and measuring the absorbance of the unbleached dye at 510 nm. Beer's law was obeyed in the range 3-8 µg/ml.

Under optimized conditions, the methods were applied successfully to the tablets containing secnidazole. The results obtained are in good agreement with those obtained using official and reference methods.

INTRODUCTION

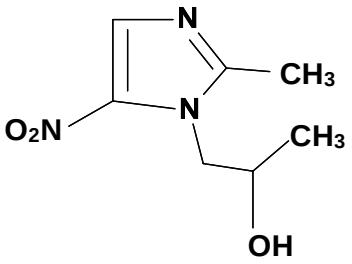


Figure (1): structure of secnidazole

Secnidazole (SEC) is chemically known as 1-(2-Methyl-5-nitroimidazol-1-yl) propan-2-ol. Secnidazole is not official in any pharmacopoeia.

A nitroimidazole anti-infective are effective in the treatment of dientamoebiasis (Girginkardeşler et al., 2003) .It has also been tested against Atopobium vaginae (De Backer et al. 2009).

Secnidazole is structurally related to the commonly used 5-nitroimidazoles metronidazole and tinidazole. These drugs share a common spectrum of activity against anaerobic micro-organisms and they appear particularly effective in the treatment of amoebiasis, giardiasis, trichomoniasis and bacterial vaginosis (Pavan et al., 2013).

It is used to eradicate *Helicobacter pylori* in peptic ulcer disease with other antimicrobials and proton pump inhibitor (Silva et al., 2002; Ahuja et al., 1998).

A literature survey revealed that secnidazole has been estimated in pharmaceuticals by UV, spectrophotometry (Bansode et al., 2013; Sonpetkar et al., 2012; Saffaj et al., 2004; Khalile et al., 2011; Abul Khier et al., 2008; Krishnan et al., 2013; Dawish et al., 2012; Saffaj et al., 2007), high performance liquid chromatography (Momtovani et al., 2009; Farooqui et al., 2010; Alhalabi et al., 2012; Lv et al., 2013), and voltammetry (El Sayed et al., 2010).

The aim of the present work is to develop simple, sensitive and cost-effective spectrophotometric method for the determination of SEC in its pure form and tablet forms.

Apparatus:

Labomed® Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1cm quartz cells connected to

Windows compatible computer using UV Win. 5 Software v 5.0.5.

Spectronic Genesys® UV-VIS Spectrophotometer connected to an IBM PC computer load with FLWINLAB software

MATERIALS and METHODS

All chemicals used are of analytical reagent grade.

Secnidazole (Amoun Company, Egypt. 99% Purity).

Fladazole® tablets labeled to contain 500 mg of secnidazole. Batch No.134631 (Amoun Pharmaceutical).

Vanillin (EL Nasr Pharmaceutical Chemicals Company, Batch No.2011/1)

The solution was prepared by dissolving 4 gm. of vanillin in 100 ml absolute methanol (99.8%)

Sulphuric acid (97-99%, El Nasr pharmaceutical chemicals Company, Batch No.2012/3)

1,2-Naphthoquinon-4-sulphonate (NQS)0.5% (w/v). 0.5g of NQS was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 20 ml distilled water, and make up the volume up to the mark with bidistilled water to obtain a solution of 0.5 % (w/v).The solution was freshly prepared and protected from light during the use.

Sodium hydroxide solution (7×10^{-2}M)

0.28 g of sodium hydroxide is accurately weighed and transferred into 100 ml volumetric flask and made up to the mark with distilled water.

Tetracyanoethylene (98%, B.No: 138050100 ACROS Chemicals company). Solution of 5×10^{-3} M dissolved in 100 ml acetonitrile.

Sodium bicarbonate (98%, B.No: 34090 El Nasr pharmaceutical chemicals company). Solution of 1×10^{-3} M dissolved in 100 ml water was used.

Methyl Orange 60 µg/ml (Universal Fine Chemicals, India) was dissolved in 20 ml methanol then completed to 100 ml with bidistilled water (stable for 2 weeks at least)

5 M HCl (El-Nasr Chemicals, Egypt) was prepared by diluting 225 ml of concentrated HCl (34%) to 500 ml.

Bromate / Bromide stock solution was prepared by dissolving 0.1 gm of potassium bromate (Winlab, England) and 1.0 gm of potassium bromide (Winlab, England) in 100 ml bidistilled water (stable for 10 days at least).

Working solution was freshly prepared daily by diluting 2.5 ml of stock solution to 100 ml with bidistilled water to give final concentration 25 µg/ml.

Principle:

Preparation of standard drug solutions:

Standard reduced form of secnidazole solution for method I, II and III:

This solution was prepared by dissolving 50 mg, 20 mg and 15 mg of secnidazole in 15 ml methanol in a beaker. The methanolic solution of secnidazole was treated with 2 ml of 34% concentrated HCl and 0.4 g of zinc powder were added then the reaction mixture was stirred for 5 min. and heated for 10 min. The solution was filtered through whatman filter paper (90 mm) to remove insoluble matter. The residue was washed with 10 ml portions of methanol three times then collected and completed with methanol to 100 ml in a volumetric flask for method I, method II and method III respectively.

The final working standard solution of reduced secnidazole containing 20 µg/ml and 15 µg/ml for method II and method III respectively was prepared by further dilution. This solution was stable for at least one week.

Preparation of standard drug solutions for method IV:

Stock solution of secnidazole was prepared by dissolving 50 mg of the pure drug in 20 ml in methanol and diluting to 100 ml in calibrated flask.

Working solution of lower concentration (50µg/ml) was prepared by further dilution of stock solution with methanol.

Procedures:

Method I (vanillin):

To a series of 10 ml calibrated flasks, an increasing the concentration range (5-55) µg/ml of reduced form of secnidazole solution volume covering were transferred, followed by addition of 1.5 ml of 4% vanillin and 2 ml of concentrated H_2SO_4 with occasionally shaked and heated on a water bath at 50°C for 15 min and cooled to room temp, finally the volume was brought up to the mark with

absolute methanol. The absorbance was measured at 557 nm versus reagent blank. A calibration graph was prepared by plotting the measured absorbance versus concentration. The concentration of the unknown sample was read from the calibration graph or computed from the regression equation derived using the Beer's law data.

Method II

(1, 2-Naphthoquinon -4-sulphonate):

To a series of 10 ml calibrated flasks, an increasing volume covering the concentration range (0.2-2.2) µg/ml reduced form of secnidazole solution were transferred, followed by addition of 1ml of 0.5 % NQS and 1ml of 7×10^{-2} M NaOH with occasional shaking and the solution left 10 min at room temperature, finally the volume was brought up to the mark with bidistilled water. The absorbance was measured at 480 nm versus reagent blank. A calibration graph was prepared by plotting the measured absorbance versus concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the Beers law data.

Method III (Tetracyanoethylene):

To a series of 10 ml calibrated flasks, an increasing volume covering the concentration range (0.15-1.2) µg/ml reduced form of secnidazole solution were transferred, followed by addition of 1ml of 5×10^{-3} M tetracyanoethylene and 1ml of NaHCO3 with occasional shaking and diluted to mark with bidistilled water. The solution was then left for 10 min in ice bath. The absorbance was measured at 397 nm versus reagent blank. A calibration graph was prepared by plotting the measured absorbance versus concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the Beers law data.

Method IV (Methyl orange):

To 1ml bromate - bromide working solution in 10 ml volumetric flasks, add (3-8) µg/ml of a secnidazole drug solution then acidify using 0.4 ml 5 M HCl, close flasks and stand for 15 minutes, add 1 ml dye working solution then stand for another 2 minutes and complete to mark with bidistilled water then measure absorbance against reagent blank at 510 nm.

Pharmaceutical preparation:

For fladazole ®Tablets:

Methods (I, II and III)

Twenty tablets of fladazole® tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg, 20 mg and 15 mg of secnidazole in method (I,II and III) were dissolved in 30 ml of methanol, stirred for about 5-10 min, filtered through whatman filter paper to remove the insoluble matter .The methanolic solution of secnidazole was treated with 2 ml of concentrated HCl and 0.4 g zinc powder was added, then the reaction mixture was stirred for 5 min. and heated on a water bath (50°C) for 10 min. the solution is filtered to remove insoluble matter. The residue was washed with 10 ml portions of methanol three times, collected and completed with methanol to 100 ml in a volumetric flask. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

Method IV

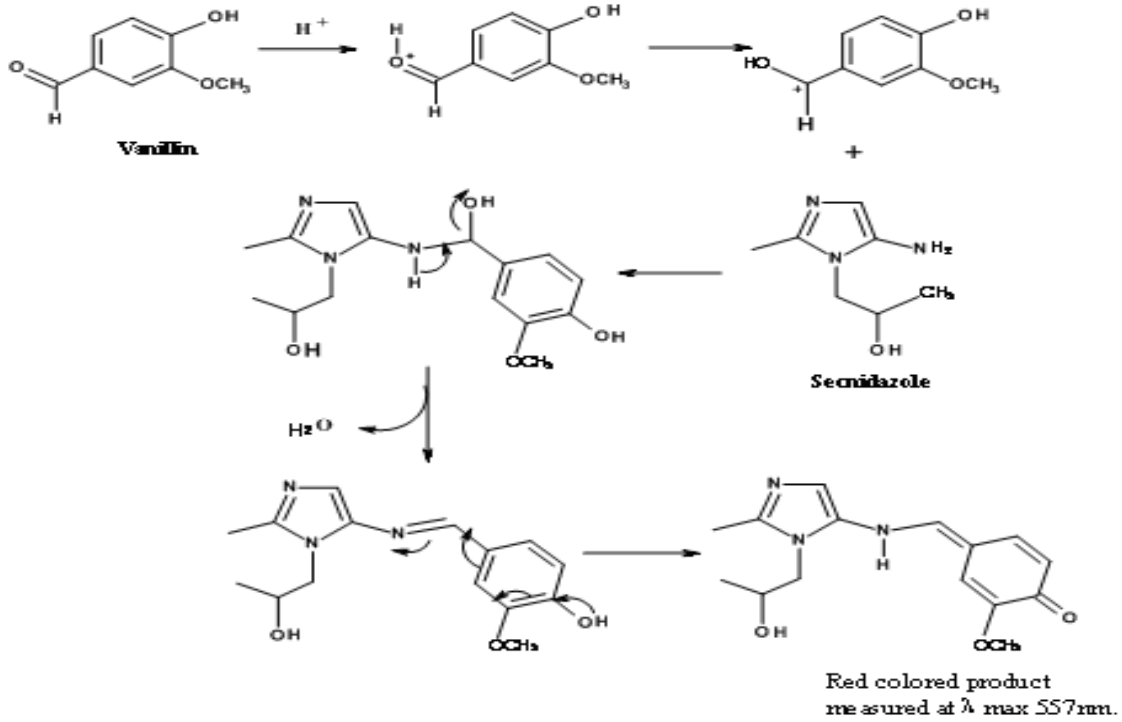
Twenty tablets of fladazole® tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg of secnidazole was dissolved in 30 ml methanol and stirred for about 5-10 min, filtered to remove the insoluble matter. The residue was washed with 10 ml portions of methanol three times, collected and completed with methanol to 100 ml in a volumetric flask Aliquots from this solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

RESULTS and DISCUSSION:

Method I:

Enamine is formed by a condensation reaction of primary amine and an aldehyde or ketone in the presence of an acid catalyst (Youngman et al., 2001). The formation of enamine forms the basis for the spectrophotometric determination of compound of pharmaceutical significance. Vanillin, an aromatic aldehyde, has been applied to quantification of drug

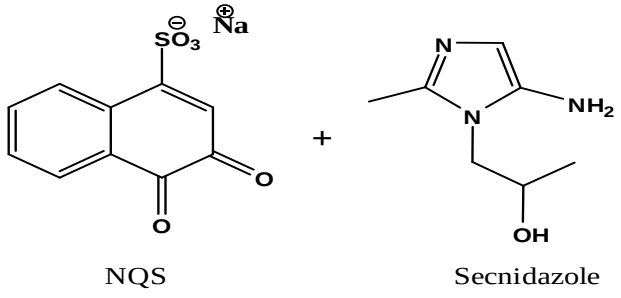
with primary amine in acidic medium. The proposed method is based on the formation of chromogenic enamine between the primary amine group of reduced form secnidazole and aldehyde group of vanillin. The most probable condensation step for the formation of enamine between drugs and vanillin is presented in scheme (1). The absorption spectrum of the chromogen formed between secnidazole and vanillin was recorded between 450 nm to 650 nm against respective reagent blank are shown in figure (2). The red-colored enamine exhibits λ_{max} at 557 nm for reduced secnidazole .The reagent blank showed negligible absorbance at 557 nm.



Scheme (1): Proposed reaction mechanism between reduced form secnidazole and vanillin (Prashanth et al., 2013).

Method II:

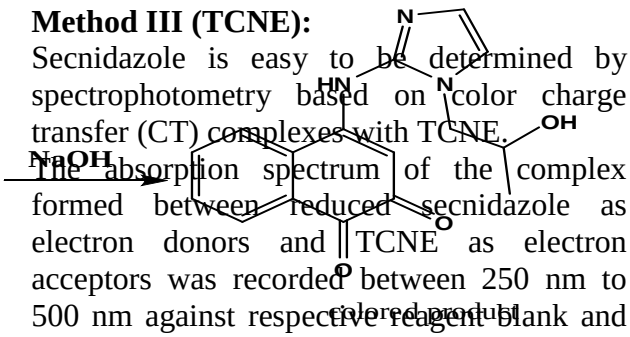
The reduced form of secnidazole containing of primary amino groups which can react with NQS in alkaline media through nucleophilic substitution reaction producing an orange-brown colored product showing maximum absorption at λ_{max} 480 nm against respective reagent blank are shown in figure (3).



Scheme (2): Proposed reaction mechanism between reduced form secnidazole and NQS (Ribeiro et al., 2007).

Method III (TCNE):

Secnidazole is easy to be determined by spectrophotometry based on color charge transfer (CT) complexes with TCNE. The absorption spectrum of the complex formed between reduced secnidazole as electron donors and TCNE as electron acceptors was recorded between 250 nm to 500 nm against respective reagent blank and shown in figure (4). The yellow-colored exhibits λ_{max} at 397 nm of secnidazole. The reagent blank showed absorbance at 330 nm.



Factors for method I (vanillin)

i- Effect of the reagent concentration:

Vanillin is slightly soluble in water but soluble in absolute methanol. The effect of

vanillin on the sensitivity of the reaction was studied. It was observed that when 0.5-3 ml of 4% (w/v) was examined, 1.5 ml vanillin give maximum chromogen red colored product figure (6).

ii- Effect of acid volume :

The reaction was very slow in dilute acid medium, thus concentrated sulphuric acid was used .The intensity of red colored product was found to be maximum on using 2 ml of sulphuric acid figure (7).

iii- Effect of time:

The color development slowly at room temperature, on heating the reaction mixture on thermostatically controlled water-bath an increase in the intensity of the produced color was observed, the absorbance reached maximum after heating in a water bath at (55 ± 5°**C**) for 15 min and remained stable for at least 30 min .

Factors for method II (NQS):

i- Effect of the reagent concentration:

NQS is soluble in water. The effect of changing the concentration on the absorbance of solution containing a fixed amount of drugs was studied. It is evident that the absorbance increases with increasing NQS concentration and reached maximum on using 1ml of 0.5 % (w/v) NQS which achieves a suitable volume for maximum color intensity figure (8).

ii- Effect of alkalinity:

Different bases of different molarity such as sodium hydroxide, potassium hydroxide of 7×10^{-2} M (0.28 g) concentration were examined in order to obtain high sensitivity. It was found that 1.0 ml of sodium hydroxide gives maximum color intensity of secnidazole and beyond these amounts, the absorbance would be decreased. Therefore, 1 ml of 7×10^{-2}M was chosen as the optimum concentration of sodium hydroxide figure (9).

iii- Effects of temperature and reaction time:

The reaction time was determined by following the color development at room temperature and thermostatically controlled water-bath at different temperatures. It was observed that the absorbance reached maximum after leaving the solution 10 min at room temperature. This temperature and reaction time were chosen for color development .It was found that the absorbance of the chromogen remained stable for at least 30 minutes.

Factors for method III (TCNE):

i- Effect of solvent

Different solvents were investigated in order to select the suitable for TCNE method. These solvents included acetonitrile, absolute ethanol and methanol .It was found that acetonitrile is considered to be an ideal solvent for this experiment because it has a suitable solvating power for TCNE as well as producing more stable and reproducible absorbance.

ii- Effect of reagent volumes

The effect of changing the TCNE concentration on the absorbance of solution containing a fixed amount of drug was studied. It is evident that the absorbance increases with increasing TCNE concentration and reached maximum on using 1ml of 0.064 % (w/v) 5×10^{-3} M TCNE achieves a suitable volume for maximum color intensity figure (10).

iii- Effect of base

Different bases such as sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate of 1×10^{-3} M concentration were examined in order to obtain high sensitivity. It was found that 1.0 ml of sodium bicarbonate gave maximum color intensity and beyond these amounts, the absorbance would be decreased. Therefore, 1ml of 1×10^{-3} M was chosen as the optimum concentration of sodium bicarbonate figure (11).

iv- Effect of time and temperature

The reaction time was determined by following the color development in ice-bath, at room temperature and thermostatically controlled water-bath at different temperatures. It was observed that the absorbance reached maximum after 10 min in ice-bath and remained stable for at least 30min. This temperature and reaction time were chosen for color development.

Study of the experimental parameters for method IV:

i- Effect of 5M HCl volume:

5 M HCl was used throughout experiment and it was found that 0.4 ml HCl with methyl orange is the appropriate acid volume and increasing HCl volume results in a decrease in absorption figure (12).

ii- Effect of volume of 2 5 µg ml^{-1} bromate-bromide:

Bromate - bromide volume was studied by varying the reagent volume while other factors were held constant. It was found that of 25 µg/ml of 1 ml of bromate-bromide is sufficient for its bleaching action of methyl orange using these stated concentrations figure (13).

iii- Effect of the reaction time:

Time required to brominates and oxidize the drug before addition of dye and time required to irreversibly oxidize dye after its addition was studied. The bromination reaction was found to be complete in 15 minutes while contact times up to 25 minutes had been examined and no further bromination was detected. A contact time of 2 minutes was necessary for the bleaching of the dye color by the residual bromine and the color of the dye remains stable for at least two hours after mixing with the reaction mixture.

Validation of the proposed methods

The validity of the proposed methods was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision, robustness and specificity according to ICH recommendations (ICH, 2005).

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance versus the final concentrations (µg/ml) were found to be rectilinear over the concentration ranges cited in the table (1)

The calibration graph was described by the equation:

$$Y = a + bX$$

(Where Y=absorbance, a=intercept, b=slope, X=concentration in µg/ml).

Correlation coefficient, intercept and slope for the calibration data are summarized in table (1).

Limits of detection and limits of quantification

Limits of detection (LOD) were determined by evaluating the lowest concentrations of the analyte that can be detected according to the following equation:

$$LOD = 3.3\ S/K$$

Limits of quantification (LOQ) were determined also by establishing the lowest concentrations that can be detected according to the following equation:

$$LOQ = 10\ S/K$$

Where S is the standard deviation of the three replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph. The results are summarized in table (2).

Accuracy and precision

Accuracy was evaluated as percentage relative error between the measured concentrations for secnidazole. The accuracy of the proposed methods was checked by performing recovery experiments of the dosage forms through standard addition technique. The results are shown in tables (3 and 4) are compiled and show that the accuracy is good. The precision of the method was calculated in term of intermediate precision (intraday and interday). Two different concentrations were repeated five times of secnidazole and analyzed during the same day (intra-day precision) and five consecutive days (inter-day precision). The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable. The results are summarized in table (6).

Robustness and Ruggedness

Robustness of the method was examined by small changes in the method variables such as change in the volume of the reagent (±0.05ml), change in bromated- bromine mixture and dye (±0.05ml), change in volume of base (±0.05ml) , change in reaction time (±2min) and change in the volume of the acid (±0.05 ml). The results are listed in table (7).

The ruggedness was analysis by two different analysts and on two different instruments by same analyst. The intermediate precision, expressed as % RSD, which is a measure of ruggedness was within the acceptable limits as shown in the table (8).

The minor changes that may take place during the experiment didn't affect the absorbance of the reaction products.

Analysis of pharmaceutical preparations:

The proposed methods were applied to the analysis of the drug in dosage forms and the results were statistically compared with

reference method (Krishnan et al., 2013) by calculating Student's *t*- test and F-values. The evaluated *t*- and F-values were less than the tabulated values at the 95% confidence level. The results are listed in table (5).

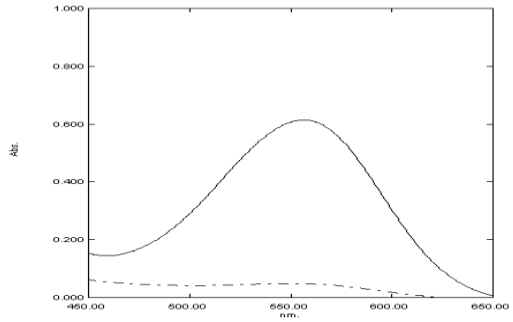


Figure (2): Absorption spectra of (method I) and Secnidazole (45 µg/ml) showing λ_{max} at 557 nm against reagent blank.

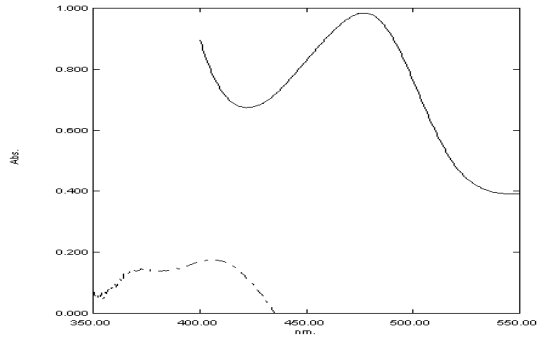


Figure (3): Absorption spectra of (method II) for the reaction between NQS and Secnidazole(2.2 µg/ml) showing λ_{max} at 480 nm against reagent blank.

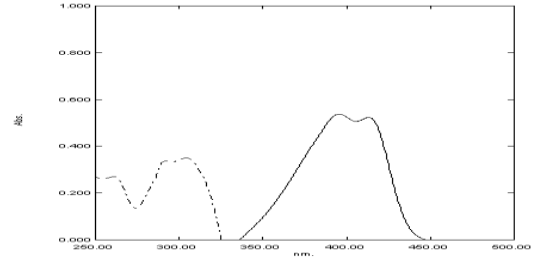


Figure (4): Absorption spectra of (method III) for the reaction betweenTCNE and Secnidazole (0.75 µg/ml) showing λ_{max} at 397 nm against reagent blank.

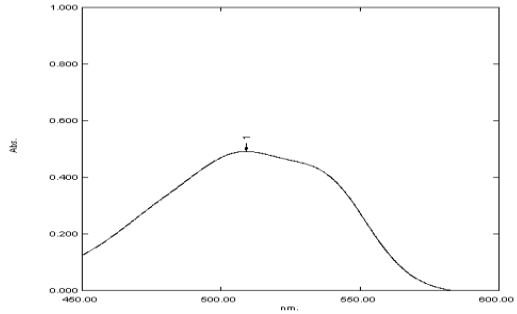


Figure (5): Absorption spectra for reaction between (7) µg/ml Secnidazole with Methyl orange showing λ_{max} at 510 nm.

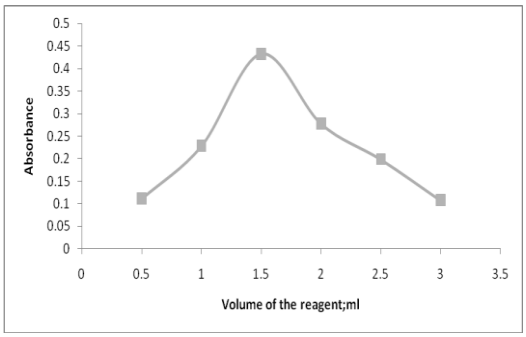


Figure (6): The effect of Vanillin volume on the absorbance of (35 µg/ml) Secnidazole.

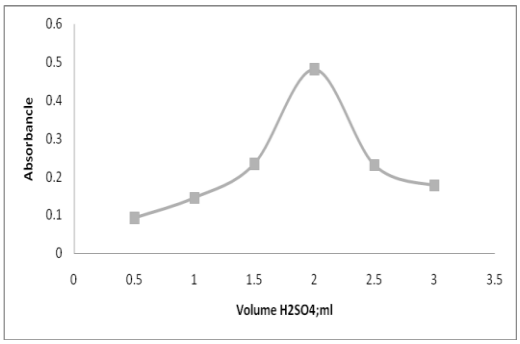


Figure (7): The effect of acid volume on the absorbance of (35 µg/ml) Secnidazole

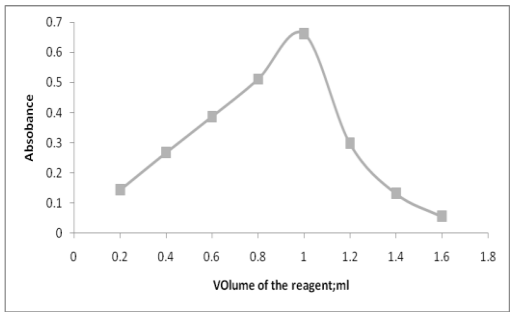


Figure (8): The effect of NQS volume on the absorbance of (1.4 µg/ml) Secnidazole

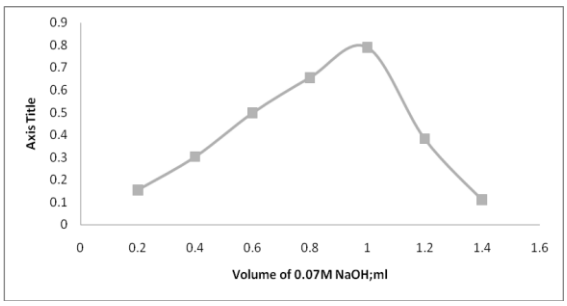


Figure (9): The effect of base volume on the reaction of NQS with(1.8 µg/ml) Secnidazole

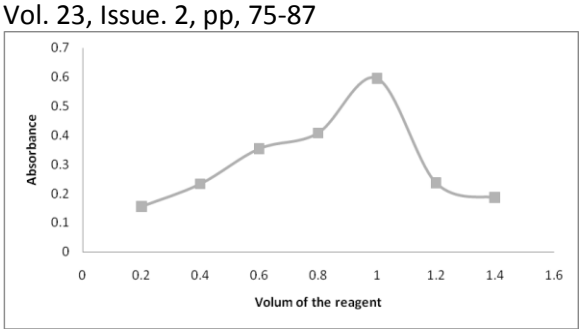


Figure (10): The effect of TCNE volume on the absorbance of (0.75 µg/ml) Secnidazole

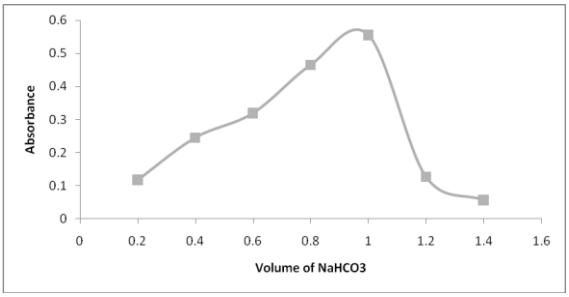


Figure (11): The effect of base volume on the reaction of TCNE with (0.75 µg/ml) Secnidazole

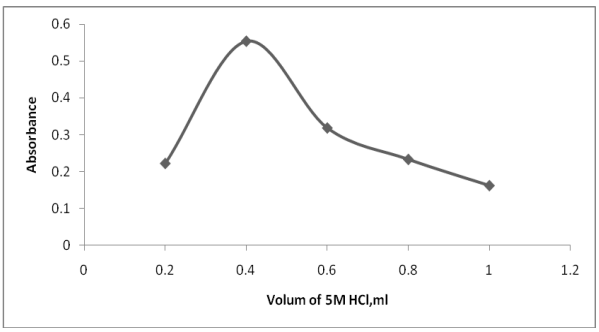


Figure (12): Effect of volume 5M of HCl on absorbance of Methyl orange (60µg/ml) in presence of (7 µg/ml) Secnidazolee at 510nm.

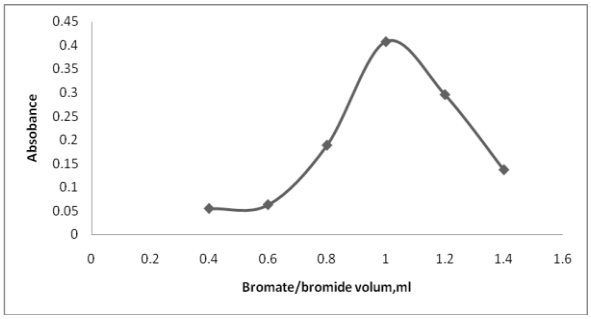


Figure (13): Effect of volume Bromate-Bromide mixture (25µg/ml) on absorbance of Methyl orange (60µg/ml) in presence of (6µg/ml) Secnidazole at 510 nm.

Table (1): Analytical parameters for spectrophotometric determination of Secnidazole through the proposed methods

Parameters		Method I (Vanillin)	Method II (NQS)	Method III (TCN)	Method IV (Methyl orange)
λmax, nm		557	480	397	510
Volume of H_2SO4, ml		2	.-	-	-
Volume of 1×10^{-3} M$NaHCO_3$(ml)		-	-	1	-
Volume of 5M HCl (ml)		-	-	-	0.4
Volume of 25 µg/ml bromate-bromide mixture (ml)		-	-	-	1
Volume of 0.07M NaOH (ml)		-	1	-	-
Reagent Conc.		4%*w/v*	0.5%*w/v*	0.064%*w/v*	60 µg/ml
Time required to oxidize the drug before dye addition (min.)		-	-	-	15
Time required to irreversibly oxidize the dye (min.)		-	-	-	2
Reagent volume (ml)		1.5	1	1	1
Temperature		55±5°C	25±5°C	Ice bath	-
Reaction time (minutes)		15	10	10	-
Diluting solvent		Methanol	bidistilled water	bidistilled water	bidistilled water
Beer's law limits (µg/ml)		5-55	0.2-2.2	0.15-1.2	3-8
Regression equation*	**Slope (b)**	0.0132	0.3805	0.7124	0.1558
	Intercept (a)	0.0414	0.0396	0.0264	-0.2257
Correlation coefficient		0.9999	0.9999	0.9998	0.9999

*A = a + bC where A is absorbance, C is the concentration of the drug in$\mu g ml^{-1}$

Table (2): Statistical data for the determination of Secnidazole with Vanillin, NQS, TCNE and Methyl orange:

parameters	Method I (Vanillin)			Method II (NQS)			Method III (TCNE)			Method IV (Methyl orange)		
	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
	5	5.0455	100.91	0.2	0.20078	100.39	0.15	0.15104	100.69	3	3.006	100.20
	15	14.8182	98.79	0.6	0.59238	98.73	0.3	0.30545	101.82	4	4.0409	101.02
	25	25.2727	101.09	1	1.0076	100.76	0.45	0.45003	100.006	5	5.0408	100.81
	35	34.9697	99.91	1.4	1.39921	99.94	0.6	0.59321	98.87	6	6.03217	100.53
	45	45.0455	100.10	1.8	1.80394	100.22	0.75	0.74340	99.12	7	7.08434	101.20
	55	55.0455	100.08	2.2	2.1955	99.79	0.9	0.89921	99.91	8	8.03217	100.40
							1.05	1.0578	100.74			
							1.2	1.19961	99.97			
Mean			100.15			99.97			100.14			100.69
N			6			6			8			6
±SD			0.822			0.698			0.9439			0.3833
±RSD			0.821			0.698			0.9426			0.3807
±SE			0.336			0.285			0.334			0.1565
Variance			0.676			0.488			0.891			0.1469
L.D.			1.506			0.0575			0.0416			0.79925
L.Q.			4.299			0.1916			0.1386			2.6641
Sandell's sensitivity(µgml^{-1} per 0.001A)			0.0519			0.0032			0.000895			0.02525
Apparent Molar absorbitivity LMol^{-1}cm^{-1}**			2927.96			81920.51			143180.7			12960.84

*Mean of three different experiments. **Calculated in the basis of molecular weight of the drug.

Table (3): Application of standard addition technique for the determination of Fladazole® in tablets using Vanillin and NQS method

Items	Method I Vanillin				Method II NQS			
	Added pure drug (µg/ml)	Fladazole tablet (µg/ml)	Conc. found (µg/ml)	Recovery* %	Added pure drug (µg/ml)	Fladazoletablet(µg/ml)	Conc. found (µg/ml)	Recovery* %
	5	0	4.9230	98.46	0.2	0	0.1987	99.35
	5	5	10.0385	100.39	0.2	0.2	0.3947	98.68
	5	10	15	100	0.2	0.4	0.6026	100.43
	5	15	20.0385	100.19	0.2	0.6	0.7987	99.83
	5	20	24.9230	99.69	0.2	0.8	1.0013	100.13
Mean*				99.74				99.68
N				5				5
S.D.				0.7623				0.6909
R.S.D.				0.7642				0.6930
V				0.5811				0.4773
S.E.				0.3409				0.3090

*Mean of three different experiments.

Table (4): Application of standard addition technique for the determination of Fladazole® tablets using TCNE and Methyl orange methods

Items	Method III TCNE				Method IV Methyl orange			
	Added pure drug (µg/ml)	Fladazoletablet (µg/ml)	Conc. found (µg/ml)	Recovery* %	Added pure drug (µg/ml)	Fladazoletablet(µg/ml)	Conc. found (µg/ml)	Recovery* %
	0.15	0	0.14836	98.91	3	0	2.9639	98.78
	0.15	0.15	0.30528	101.76	3	1	4.0090	100.225
	0.15	0.3	0.45221	100.49	3	2	5.06363	101.27
	0.15	0.45	0.59772	99.62	3	3	6.0166	100.28
	0.15	0.6	0.75036	100.05	3	4	7.01385	100.20
Mean*				100.16				100.03
N				5				5
S.D.				1.0658				1.237
R.S.D.				1.0640				1.236
V				1.1360				1.530
S.E.				0.4767				0.555

Table (5): Statistical data for the determination of pharmaceutical tablets of Fladazole through the proposed methods compared with the reference method (Krishnan et al., 2013).

Statistics	Reference method [11]	(Fladazole® tablet)			
		Vanillin	NQS	TCNE	Methyl orange
Mean recovery*± SD	99.79±0.721	99.74±0.762	99.68±0.691	100.16±1.066	100.03±1.237
N	5	5	5	5	5
Variance	0.519	0.5811	0.4779	1.1360	1.530
S.E.	0.323	0.3409	0.3090	0.4767	0.555
t-test**		0.107(2.306)[a]	0.246(2.306)[a]	0.643(2.306)[a]	0.375(2.306)[a]
F-ratio**		1.119(5.05)[b]	1.0890(5.05)[b]	2.185(5.05)[b]	2.944(5.05)[b]

* Average of three experiments.
a and b are Theoretical Student *t-test* and F- ratio at p=0.05.

Table (6): Results of the intraday and interday precision for the determination of Secnidazole with Vanillin, NQS, TCNE and Methyl orange:

Item	conc.ug/ml	Intraday		Interday	
		mean ± SD	RSD	mean± SD	RSD
Vanillin	25 µg/ml	99.97 ± 0.699	0.698	100.0 ± 0.855	**0.855**
	45 µg/ml	100.16±0.856	0.845	100.38±1.043	**1.037**
NQS	0.2µg/ml 1.4	100.14 ± 1.076	1.076	99.75±0.611	**0.612**
	µg/ml 2.2µg/ml	99.98±0.715	0.715	100.18±0.859	**0.858**
		99.95±0.719	0.719	100.07±0.819	**0.818**
TCNE	0.15µg/ml	100. 06± 1.018	0.967	101.15±1.229	**1.129**
	0.6 µg/ml	100.13±0.951	0.949	100.18±0.938	**0.936**
	1.2 µg/ml	100.12±0.958	0.957	100.73±1.035	**1.013**
Methyl orange	5µg/ml	99.93+0.419	0.419	99.92+1.533	**1.535**
	7µg/ml	99.83+0.199	0.206	99.84+0.619	**0.622**

Table (7). Results of the robustness for the determination of Secnidazole with Vanillin, NQS, TCNE and Methyl orange methods

Robustness				
	Recovery % ± SD			
Item	**Vanillin**	**NQS**	**TCNE**	**Methyl orange**
Reagent+0.05ml	99.80 ±0.834	102.07 ±1.090	98.64 ±0.992	**99.96±0.763**
Reagent-0.05 ml	98.29 ± 1.121	99.97± 0.585	99.31± 0.865	**100.52±670**
Br_2+0.05 ml	-	-	-	**100.37±0.922**
Br_2-0.05 ml	-	-	-	**99.87±0.859**
Acid+0.05 ml	99.93±1.116	-	-	**100.47±1.037**
Acid-0.05 ml	98.29± 1.174	-	-	**100.58±1.44**
Base+0.05 ml	-	99.45±1.116	102.143±1.020	-
Base-0.05 ml	-	98.29± 1.174	101.67± 0.925	-
Time+2 min.	100.56 ± 1.228	100.49 ± 0.645	100.01 ± 0.792	**100.47±1.094**
Time-2 min.	99.91 ± 1.170	101.28 ± 836	99.34 ± 0.865	**100.70±1.732**

Table (8): Results of Ruggedness for determination of Secnidazole using Vanillin and TCNE methods

Item		**Recovery % ± SD**
TCNE	0.15 µg/ml	100.69.6±0.944
	0.6 µg/ml	99.10±0.902
Vanillin	25 µg/ml	102.6±1.271
	55 µg/ml	98.98±1.401

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التقدير الطيفى لسكيندازول فى صورته النقيه والصيدليه

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***صيدلانيه بوزارة الصحه، بنغازى ، ليبيا.**

تم فى هذا البحث عمل اربع طرق طيفية دقيقة و حساسة لتقديرالسكنيدازول في صورته النقية والصيدلية وتعتمد الثلاث طرق الأولي على اختزال السكنيدازول بواسطة معدن خارصينِ و حامض هيدروكلورريد.
فى الطريقة الاولى تم تقدير السكنيدازول مع الفانيلين ككاشف وتقدير ناتج التفاعل عند طول موجي ٥٥٧ نانومتر و قد امكن تطبيق قانون بير للتركيز ٥-٥٥مايكرو غرام /مللتر وفى الطريقة الثانية تم تقدير السكنيدازول مع ١و٢ نفثوكينون سلفونات الصوديوم وذلك خلال تفاعل الاستبدال الشغوف بالنواة وتقدير الناتج عند طول موجي ٤٨٠ نانومترو وقد امكن تطبيق قانون بير للتركيز ٢-٢,٢مايكروغرام/مللتر وفى الطريقة الثالثة تم تقدير السكنيدازول عن طريق إنتقال الشحنه مع مع تتراسيانوإيثيلين الذى يعتبر مستقبل للشحنه وقياس المعقد الناتج عند طول موجى ٣٩٧ نانوميترو وقد امكن تطبيق قانون بير للتركيز ٠,١٥-١,٢مايكروغرام/مللتر.
الطريقة الرابعة تعتمد علي الانتاج اللحظى للبرومين كعامل مؤكسد واستخدام ميثيل البرتقال وقد تم قياس ناتج التفاعل عند طول موجى ٥١٠ نانوميترو قد امكن تطبيق قانون بير للتركيز ٣-٨مايكروغرام/مللتر. وقد تم تطبيق الطرق المستحدثه على الأقراص المحتويه للسكيندازول بنجاح وقورنت النتائج مع الطرق المرجعيه
ووجدت أنها مساويه لها فى الدقه والإتقان.