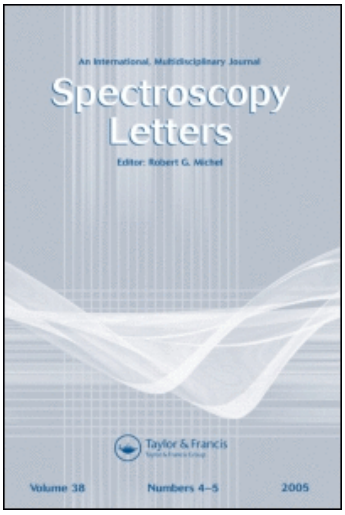

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Stability-Indicating Assay of Secnidazole in the Presence of Its Degradation Products

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To cite this Article Moustafa, Azza A. and Bibawy, Lories I.(1999) 'Stability-Indicating Assay of Secnidazole in the Presence of Its Degradation Products', Spectroscopy Letters, 32: 6, 1073 — 1098
To link to this Article: DOI: 10.1080/00387019909350051
URL: http://dx.doi.org/10.1080/00387019909350051

SPECTROSCOPY LETTERS, 32(6), 1073–1098 (1999)

STABILITY - INDICATING ASSAY OF SECNIDAZOLE IN THE PRESENCE OF ITS DEGRADATION PRODUCTS .

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Keywords : Secnidazole, derivative spectrophotometry , RP-HPLC , TLC- densitometry, colorimetry, pharmaceutical formulations .

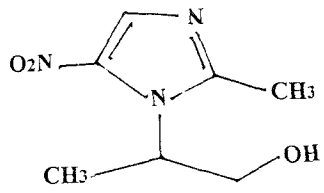
ABSTRACT

Four new selective, precise, accurate and rapid first derivative spectrophotometric, RP-HPLC, TLC densitometric and colorimetric methods are described for the determination of secnidazole in the presence of its degradation products, 2 - methyl - 5- nitroimidazole and hydroxy

propanol.(Method A) is applying the first derivative spectrophotometry at 296 nm . (Method B) is RP- HPLC based on using 30% methanol as a mobile phase at a flow rate of 1.0 ml / min. and 5 μ Bondapak C_{18} column (5 micron, 150 X 4.6 mm) as a stationary phase. Detection was carried out using a diode detector at 319 nm. (Method C) is densitometry using ethyl acetate as mobile phase and the spot was scanned with a spectrodensitometry at 311 nm. (Method D) is colorimetry based on diazotization of sulfanilamide with the nitrite ions liberated by alkaline hydrolysis of secnidazole and subsequent coupling of the diazonium salt with N-1-(naphthyl)- ethylenediamine dihydrochloride. Regression analysis of a Beer's plot showed good correlation in the concentration 4 - 30 μ g ml^{-1} , 2-20 μg. ml^{-1} , 4-18 μg, 0.8-6.0 μg.ml^{-1} with mean percentage recoveries: 99.87 ± 0.56 % , 100.12 ± 0.80 % , 99.33 ± 0.50 % and 99.68 ± 0.39 % for methods A,B, C and D , respectively. These methods are suitable for stability testing of secnidazole in bulk powder retaining their accuracy in the presence of up to 70 % for method A and up to 90 % for method B , C and D . The proposed methods were applied for the analysis of pharmaceutical formulations and the recoveries were 99.40 -100.52 % . The results obtained compared statistically with those obtained with the reported method .

INTRODUCTION

Secnidazole is 1- (2 - hydroxy propyl) - 2-methyl-5- nitroimidazole and are used as antiprotozoals [1]. The structural formula is as follows:



Several methods have been reported for the determination of secnidazole including spectrophotometry [2 , 3] , polarography [4] , differential pulse polarography [5], GLC [6] and HPLC [7-9].

The main task of this work is to establish simple, fast and accurate stability - indicating methods for the determination of secnidazole in bulk powder and in pharmaceutical preparations which can be used in quality control labs.

EXPERIMENTAL

Instruments

*UV/VIS spectrophotometer (SHIMADZU) UV- 1601 PC .The derivative curves of the spectra of solutions were obtained with the

following instrumental parameters : Scan speed 120 nm/min. : Chart speed 60 nm/min. : Response time 10 s.

*A liquid chromatographic system consisted of PERKIN ELMER Isocratic LC pump 25O , with a 7125 Rheodyne valve injector, 20 microlitre fixed loop, equipped with a PERKIN ELMER LC diiode array detector controlled by PE Nelson injector, μ Bondapak C_{18} (5 micron) column (SHIMADZU) was used as stationary phase.

* SHIMADZU - DUAL wavelength flying CS-9301.
* U.V. lamp with short wavelength 254 nm.
* TLC plates (20 x 20 cm) silica gel 60 F_{254} (E. Merck) .

MATERIALS

A. Pure Sample :

Secnidazole , working standard, kindly supplied by Alexandria Co., Cairo, Egypt. The purity of the sample was found to be 99.36 ± 0.32% according to the reported method [3] .

B.Market Samples:

1. Flagentyl tablets (Alexandria Co.), batch No.6900008, labeled to contain 500 mg/tablet.
2. Flagentyl granules (Alexandria Co.), batch No.8314000, labeled to contain 750mg / 7.5 g powder

Reagents

Chemicals used were of analytical grade and the solvents used were of spectroscopic grade .

1. Sulfuric acid, 0.1 N aqueous solution .
2. Deionized water.
3. Methanol HPLC grade (E.Merck).
4. Ethyl acetate (E.Merck).
5. Sodium hydroxide , 2M aqueous solution .
6. Sulfanilamide - (Sigma chemical Co., St. Louis, MO). 0.35% in 2.5M HCL.
7. N-1- (Naphthyl) - ethylenediamine dihydrochloride.- (Riedel) 0.1% aqueous solution .

Preparartion of the degradation products

Weigh about 100 mg. of secnidazole bulk powder , transfer into 100 ml. volumetric flask , dissolve in methanol and subject to artificial sunlight for 24 hrs. The methanol solution was concentrated to a few milliliters then applied in a band form to TLC plates . 20 µl of standard solution in methanol (1 mg. ml^{-1}) was also spotted . The plates were placed in chromatographic tanks previously saturated for 30 min. with the

developing system and then air dried . The spot correponding to the degredation products were visualized under UV light at 254 nm. scraped, extracted with 3x20 ml. methanol , filtered and evaporated just to dryness on a boiling water bath . The residue left after evaporation was used for the preparation of laboratory prepared mixtures .

Standard Stock Solutions :

1. Secnidazole , 1 mg. ml^{-1} in methanol then dilute 2 ml. of this solution to 10 ml with 0.1 N sulfuric acid for (Method A) (200µg.ml^{-1}).
2. Secnidazole , 100 µg .ml^{-1} in 30% methanol for (Method B).
3. Secnidazole , 1 mg.ml^{-1} in methanol for (Method C).
4. Secnidazole , 40 µg.ml^{-1} in water for (Method D).

The degradation products were prepared at the same concentrations and solvents for each of the corresponding methods .

Laboratory prepared mixtures :

1. For derivative spectrophotometric method :

Transfer accurately aliquot portions equivalent to 40 - 300 µg. of secnidazole from its stock solution (200 µg.ml^{-1}) into a series of 10- ml. volumetric flasks , add aliquot portions equivalent to 10 - 90 % of degradation products from its stock solution (200 µg.ml^{-1}) to the same flasks and complete to the mark with 0.1 N. sulfuric acid .

2.For RP-HPLC.

Prepare mixtures of secnidazole and its degradation products containing 20 - 200 μg. of the first and 10 - 90 % of the latter .

3.For the densitometric method

Transfer accurately aliquot portions 1 - 4.5 mg. of secnidazole from its stock solution (1 mg. ml^{-1}) into a series of 5 - ml volumetric flasks . Add from 10 - 90 % of the degradation products using the prepared stock solution (1 mg. ml^{-1}).

For colorimetric method :

Prepare mixtures of secnidazole and its degradation products containing 8 - 60 μg of the first and 10 - 90 % of the latter .

PROCEDURES

1. Method A, derivative spectrophotometric method:

For bulk powder .

a. **Construction of calibration curves .**

Transfer accurately aliquot portions equivalent to 40 - 300 μg. of secnidazole from its stock solution (200 $\mu g.ml^{-1}$) into a series of 10- ml volumetric flasks and complete to the mark with 0.1 N sulfuric acid. Record the first derivative curves of each solution against 0.1 N. sulfuric

acid as a blank . Measure D_1 values at 296 nm. and plot the calibration curve representing the relationship between the measured D_1 values and the corresponding concentration .

b.**Assay of prepared mixtures**

Record the first derivative spectrum of laboratory prepared mixtures containing different ratios of secnidazole and its degradation products . Measure the D_1 values at the previously chosen wavelength . Calculate the concentration of secnidazole from the regression equation . Results obtained are shown in Table 1 .

2 . **Method B, RP HPLC :**

a . **Construction of calibration curves** :

Transfer aliquot portions equivalent to 20 - 200 μg of standard stock solution (100 μg . ml^{-1}) into six separate 10 - ml volumetric flasks and dilute up to volume with 30 % v/v methanol. Inject 20 μl of the solution from each of the above flasks and record the chromatograms maintaining the flow rate at 1.0 ml /min., monitor the effluent at 319 nm . Construct the calibration curve by plotting the concentration of the cited drug in μg .ml^{-1} against the peak area .

b. **Assay of the prepared mixtures** :

Inject 20μl of each solution of laboratory prepared mixtures

Table 1 : Comparison between the proposed methods and reported one for the determination of secnidazole in the presence of its degradation products .

Sample No.	% of Degradation Product	First Derivative Found*,%	HPLC Found*,%	Densitometric Method Found*,%	Colorimetric Method Found*,%	Report Method Found*,%
1	10	100.31	99.22	99.14	98.45	109.21
2	20	99.64	99.69	99.92	100.56	121.41
3	30	99.79	98.75	99.82	99.24	127.56
4	50	100.56	100.21	98.59	99.67	150.26
5	60	99.24	99.77	100.66	100.52	159.24
6	70	101.05	99.77	99.76	98.97	147.52
7	90	107.14**	99.52	100.04	99.57	200.14
	Mean C.V.	100.1 0.66%	99.49 0.47%	99.7 0.67%	99.57 0.78%	

* Found % of pure sample

** % Rejected

containing different ratios of secnidazole and its degradation products and record the chromatogram. Measure the peak area and calculate the concentration of secnidazole from the regression equation. Results obtained are presented in Table 1 .

3 . Method C, densitometric method :

a . **Construction of calibration curves :**

Transfer accurately aliquot portions equivalent to 1 - 4.5 mg of standard stock solution (1 mg .ml $^{-1}$) into separate 5 - ml volumetric flasks and complete to volume with methanol . Perform TLC analysis on 20 X20 cm plates coated with a 0.250 mm layer of silica gel 60 F_{254} .Before use , wash the plates with methanol ,dry in air and activate at 120^0 C for 30 min. Apply 20 µl of each solution on the plate , place in chromatographic tank previously saturated for one hour with the developing mobile phase , ethyl acetate . Develop the plate by ascending chromatography for a distance of 16 cm ,then remove and dry in air . Detect the spots under UV lamp and scan the plate at 311 nm. (Photo mode : reflection and scan mode zigzag). Construct the calibration curve as in Method B .

b. **Assay of prepared mixtures** :

Apply 20 µl of different samples of laboratory prepared mixtures to TLC plates and proceed as mentioned under 3,a Construction of

calibration curves starting from, "place in chromatographic tank ....." and calculate the concentration of secnidazole from the regression equation. Results obtained are presented in Table 1.

4. **Method D, colorimetric method** .

a . **Construction of calibration curves :**

Transfer aliquot portions equivalent to 8 - 60 μg of standard stock solution (40 μg .ml^{-1}) into separate glass stoppered tubes , complete the volume of each tube to 1.5 ml with water and add 0.5 ml 2M sodium hydroxide . Immerse the tubes in boiling water bath for 10 min ., then remove and cool in ice water . Transfer the contents of each tube quantitatively into 10 - ml volumetric flasks by the aid of 2 ml water , add 4 ml of sulfanilamide reagent and mix well . Finally , add 1 ml of N -1 - (naphthyl) - ethylenediamine dihydrochloride reagent , mix thoroughly and let stand for 5 min. to develop the color. Complete the volume with water and measure the absorbance at 536 nm. against reagent blank prepared similarly . Construct the calibration curve by plotting the concentration of the drug in μg . ml^{-1} against the absorbance .

a . **Assay of prepared mixtures** :

Transfer accurately aliquot portions of the laboratory prepared mixtures containing different ratios of secnidazole and its degradation

products into a separate glass stoppered tubes . Proceed as mentioned under Method D. a .Construction of calibration curves starting with the word, " complete the volume of each tube to 1.5 ml with water....." . Calculate the concentration from the regression equation . Results obtained are shown in Table 1 .

Assay of Pharmaceutical Formulations :

1.For tablets :

Weigh 10 tablets and finely powder . Transfer accurately an amounts of the powder each equivalent to 50 mg of secnidazole into three separate 50 - ml volumetric flasks , dissolve in the same solvent as mentioned under " D- Standard stock solutions ." Filter the solution into separate 50- ml volumetric flasks, wash each with its proper solvent and complete to volume. Dilute the solutions to the same concentrations of standard stock solutions and proceed according to construction of calibration curves for each method .

2. Granules for oral suspensions :

Finely powder the granules and proceed as under tablets, starting with the word, "Transfer accurately an amount of the powder each equivalent to 50 mg of secnidazole....." .

RESULTS AND DISCUSSION

1.Derivative spectrophotometric method :

Derivative spectrophotometric method is used for the determination of secnidazole in the presence of its degradation products . The zero order absorption spectra of secnidazole and its degradation products (2-methyl-5-nitroimidazole and hydroxy propanol) in 0.1 N sulfuric acid show an overlapping which interferes with the direct determination of pure secnidazole, Figure 1.. The present work is devoted to the application of first derivative technique to resolve such spectral overlapping for the determination of intact secnidazole in the presence of its degradation products , Figure 2.

By applying the first derivative technique a linear correlation was obtained between the D_1 values and the concentration range over 4 - 30 $\mu g.ml^{-1}$ for pure secnidazole from which the regression equation was calculated :

$$D_1 = 1.4 \times 10^{-5} + 7.39 \times 10^{-4}\ C \qquad r = 0.9998$$

Where D_1 is first derivative value and C is the concentration in $\mu g.ml^{-1}$ and r is the correlation coefficient .

2. HPLC method :

Various systems were evaluated as mobile phase for the separation

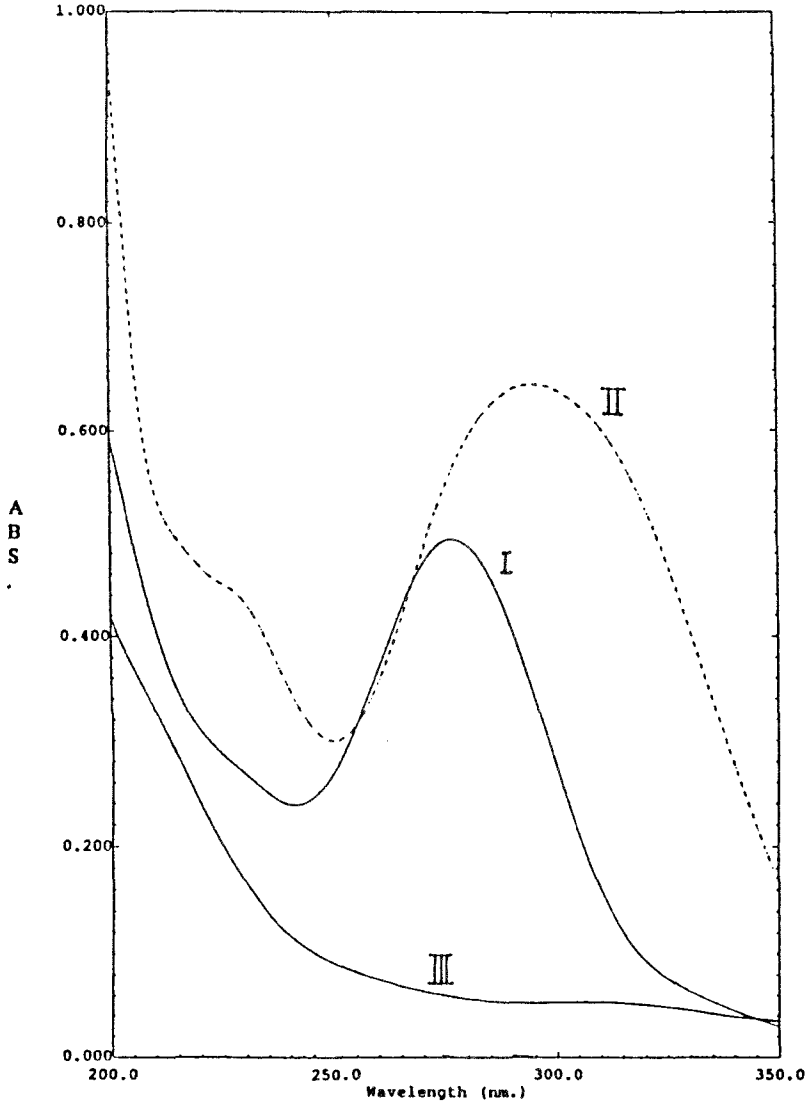


Figure 1: Zero order absorption spectra of secnidazole I, 2-methyl-5-nitroimidazole II and hydroxy propanol III each 20 $\mu g.ml^{-1}$ in 0.1N sulphuric acid.

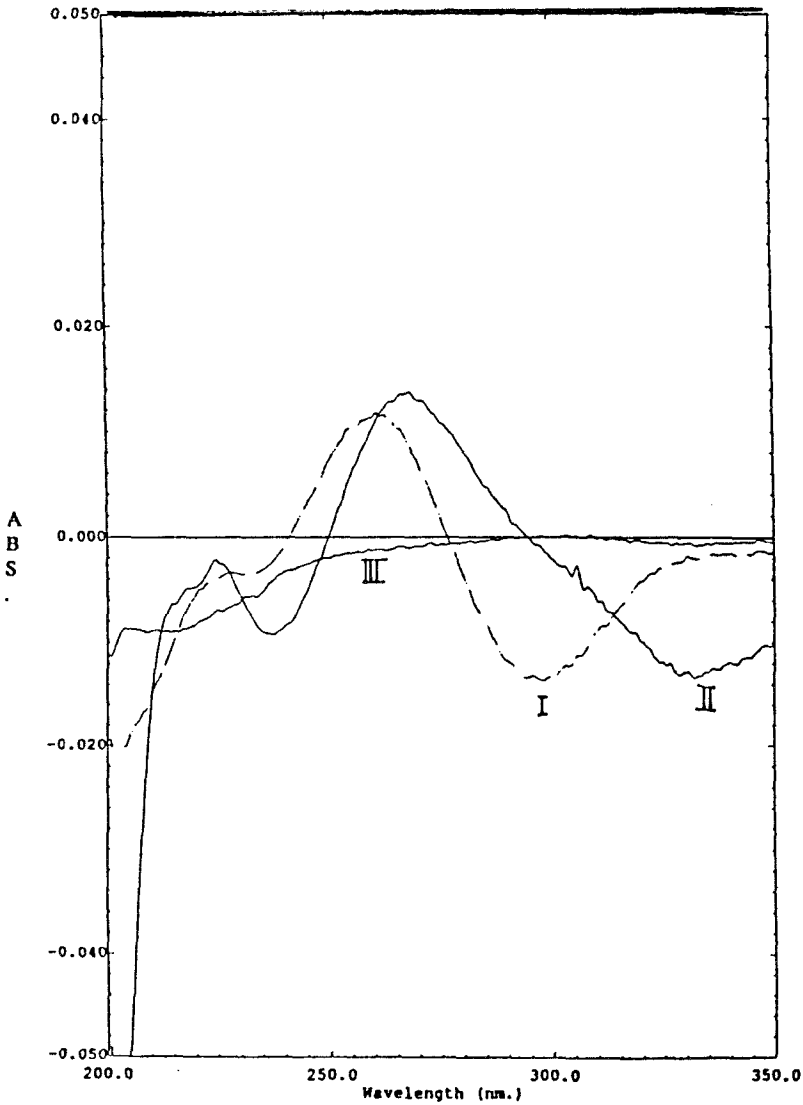


Figure 2 : First derivative spectra of secnidazole I, 2-methyl-5-nitroimidazole II and hydroxy propanol III each 20 $\mu g.ml^{-1}$ in 0.1N sulphuric acid.

of secnidazole and its degradation products, 30% methanol gave better resolution and sensitivity of secnidazole. The mobile phase composition was optimized. Under the described conditions the analyte peak was well defined, resolved and free from tailing. The retention time was (t_r = 3.91 min) at a flow rate of 1.0ml/min as shown in Figure 3 . The optimum wavelength for detection was 319 nm at which good detector response was obtained for secnidazole . Linearity was obtained in the concentration range 2-20 $\mu g.ml^{-1}$. The calibration curve could be represented by the following regression equation:

$$A = 1.4785\ C - 0.0022 \qquad r = 0.9999$$

where A is the area under the peak, C is the concentration in $\mu g\ .ml^{-1}$ and r is the correlation coefficient.

3. Densitometric method :

The present work is concerned with the application of a densitometric technique for determination of secnidazole. The best separation of the studied drug was obtained using ethyl acetate as developing mobile phase and the Rf value is 0.23. Quantitatively the chromatogram was scanned at 311 nm. By applying this technique a linear correlation was obtained between the area under the peak and the

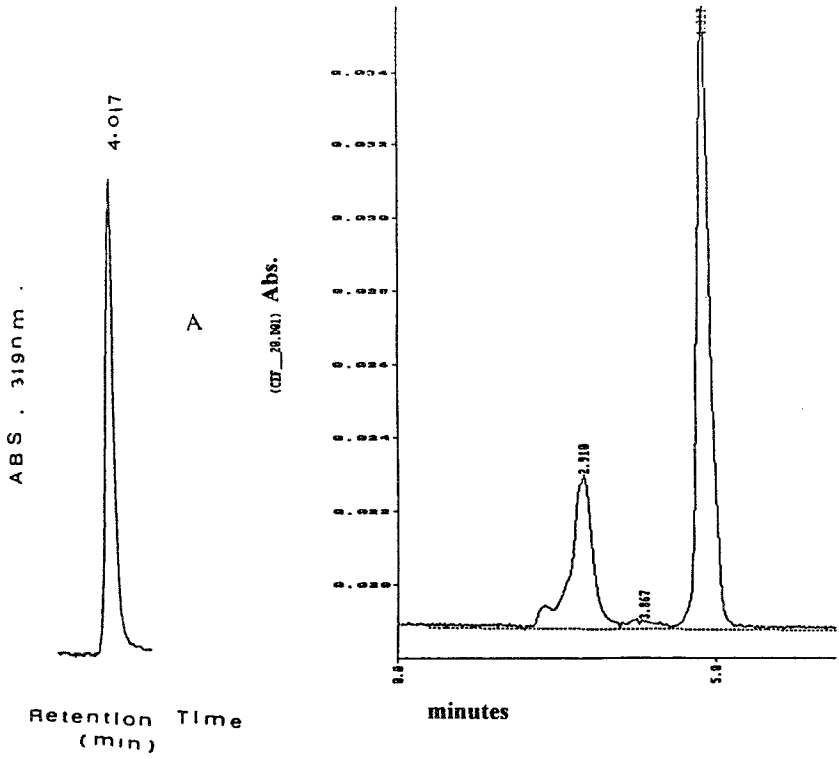


Figure 3 : HPLC chromatograms of pure secnidazole A ; Photodegradation B ; The peak with a retention time of 4.017 min. is secnidazole , the other peaks are 2 - amino - 5 - methyl imidazole at 2.910 and hydroxy propanol at 3.867 .

concentration 4-18 μg of secnidazole from which the linear regression equation was calculated:

$$A = 2.24C - 0.91 \qquad r = 0.9999$$

where A is the area under the peak, C is the concentration in μg and r is the correlation coefficient.

4. Colorimetric method:

Assay of nitroimidazle depend on the basis of estimation of nitrite ions liberated during their alkaline hydrolysis, reported by Lau et al. [10] . The nitrite ion liberated was diazotized with sulfanilamide and coupled with N-1-(naphthyl)- ethylenediamine dihydrochloride. The color formed is measured at λ max at 536nm.

The optimum conditions for the reaction were carefully studied. The reaction kinetics curve for secnidazole hydrolysis shows that the reaction reaches equilibrium within 10 min, in a boiling water bath as shown in Figure 4 , when the alkali concentration is 2M which is the best concentration to enhance the hydrolysis. The effect of the amount of sulfanilamide and the reagent were studied and it was found that 4 ml and 1 ml were used respectively for the maximum color formation. Under the conditions standardized in the proposed method, Beer's law was obeyed within a concentration range of 0.8-6.0 $\mu g.ml^{-1}$ from which the linear regression equation was calculated.

$$A = 0.002 + 0.158C \qquad r = 0.9969$$

Where A is the absorbance, C is the concentration in $\mu g.ml^{-1}$ and r is the correlation coefficient.

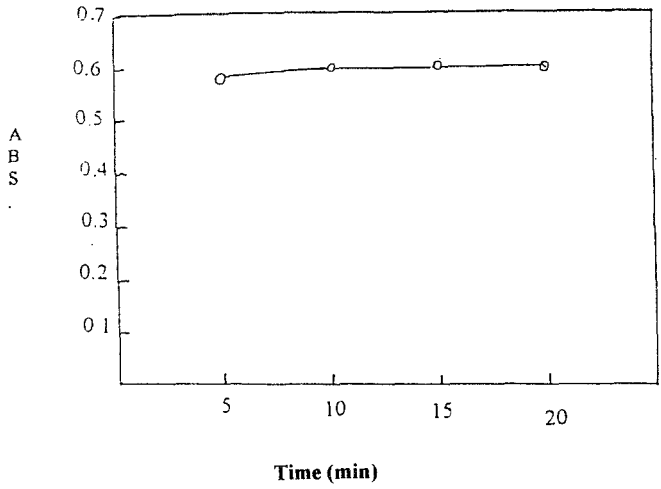


Figure 4 : Kinetics of hydrolysis of 3.8 μg m^{-1} secnidazole in 2M NaoH , and color formation due to related nitrate ions .

To assess the stability- indicating efficiency of the proposed methods, the degradation products of secnidazole were mixed with its intact sample in different ratios and analyzed by the proposed methods . The results obtained are shown in Table 1 . It is clear that the accuracy of the proposed methods are not affected by 70 % in the derivative spectrophotometeric method and up to 90 % in HPLC, densitometric and colorimetric methods . The proposed methods were successfully applied for the analysis of secnidazole in its dosage forms, and its validity was further assessed by applying the standard addition technique. Results obtained are presented in Table 2.

Table 2 :Comparison between the proposed methods and reported one for determination of secnidazole in pure form and in its pharmaceutical formulations.

Preparation	First derivative	Standard addition	RP-HPLC	Standard addition	Densitometric method	Standard addition	Colorimetric method	Standard addition	Reported method[3]
	Found % ± C.V.	Recovery* % ± C.V.	Found % ± C.V.	Recovery* % ± C.V.	Found % ± C.V.	Recovery* % ± C.V.	Found % ± C.V.	Recovery* % ± C.V.	Found % ± C.V.
Pure samples	99.87±0.56		100.12±0.80		99.33±0.50		99.68±0.39		99.36±0.32
Flagentyl tablets B.N.6900008	98.57±0.94 F=1.17(6.4) t=1.49(2.306) n=5	100.42±0.35	100.11±0.60 F=1.73(6.4) t=2.06(2.306) n=5	100.52±0.66	99.70±0.52 F=1.69(6.4) t=1.4(2.306) n=5	100.21±0.67	99.71±0.72 F=0..30(6.4) t=1.25(2.306) n=5	1oo.12±0.63	99.52±0.87
Flagentyl granules for oral suspension B.N.8314000	99.52±0.31 F=2.30(6.4) t=1.75(2.306) n=5	99.94±0.51	99.81±0.69 F=2.82(6.4) t=0.93(2.306) n=5	99.90±0.79	99.40±0.63 F=1.1(6.4) t=1.72(2.306) n=5	99.51±0.45	100.12±0.62 F=1.18(6.4) t=0.03(2.306) n=5	100.00±0.51	100.01±0.47

***The average of five determinations .**
Ref [3] : UV Spectrophotometry in methanol at 311 nm.

Table3 : Statistical comparison between the determination of secnidazole by the proposed methods and the reported one .

	First derivative method	RP-HPLC method	Densitometric method	Colorimetric method	Reported method
Range of concentration	4 - 30 $\mu g.ml^{-1}$	2 - 20 μ g.ml^{-1}	4 - 18 μg	0.8 - 6.0 μ g.ml^{-1}	5 - 25 μ g.ml^{-1}
C.V.	0.56	0.80	0.5	0.39	0.32
Correlation coefficient	0.9998	0.9999	0.9999	0.9969	
Variance	0.31	0.64	0.25	0.15	0.1
n	5	5	5	5	5
F	3.04 (6.4)*	6.27(6.4)*	2.45(6.4)*	1.49(6.4)*	
t	1.59 (2.306)**	2.00(2.306)**	0.12(2.306)**	1.45(2.306)**	

* Theoretical value F = 6.4 at 95% confidence level.

** Theoretical value t = 2.306 at 95% confidence level.

Table 3 shows statistical comparison of the results obtained by the proposed methods and the reported one. The data permits one to conclude, with 95% confidence, that there is no significant difference between the proposed methods and the reported one .

The degradation products of secnidazole were prepared in the laboratory by photodegradation in methanol and separated by TLC using ethyl acetate as a developing system . Three spots appeared, one for secnidazole at Rf = 0.23, and two others for its degradation products at Rf= 0.42, zero for 2-methyl-5-nitroimidazole and hydroxy propanol ,

respectively Using HPLC method, two distinct peaks were obtained other than the intact drug, Figure 3. The suggested degradation product, 2-methyl-5-nitroimidazole was confirmed by NMR spectrum which showed the appearance of singlet signal at δ 7.95 ppm. due to imidazole C4-H in addition to the signal due to CH_3 protons at δ 3.25 ppm.The other degradation product which was suggested to be hydroxypropanol showed NMR spectrum with signals at the region δ 0.9-3.22 ppm. Due to aliphatic and hydroxy protons as shown in Figures 5 -7 .

CONCLUSION

The suggested methods are simple, accurate, rapid, selective and equally sensitive with no significant difference of the precision. Application of the proposed methods to the analysis of secnidazole in its pharmaceutical formulations shows that neither the excipients usually formulated in these market preparations nor the degradation products interfere with the determination. This is indicated by the percentage recovery of added standard secnidazole to the pharmaceutical formulations. The advantage of the proposed RP-HPLC, densitometric and colorimetric methods over the first derivative method is that they can determine the intact drug in the presence of up to 90% of its degradation

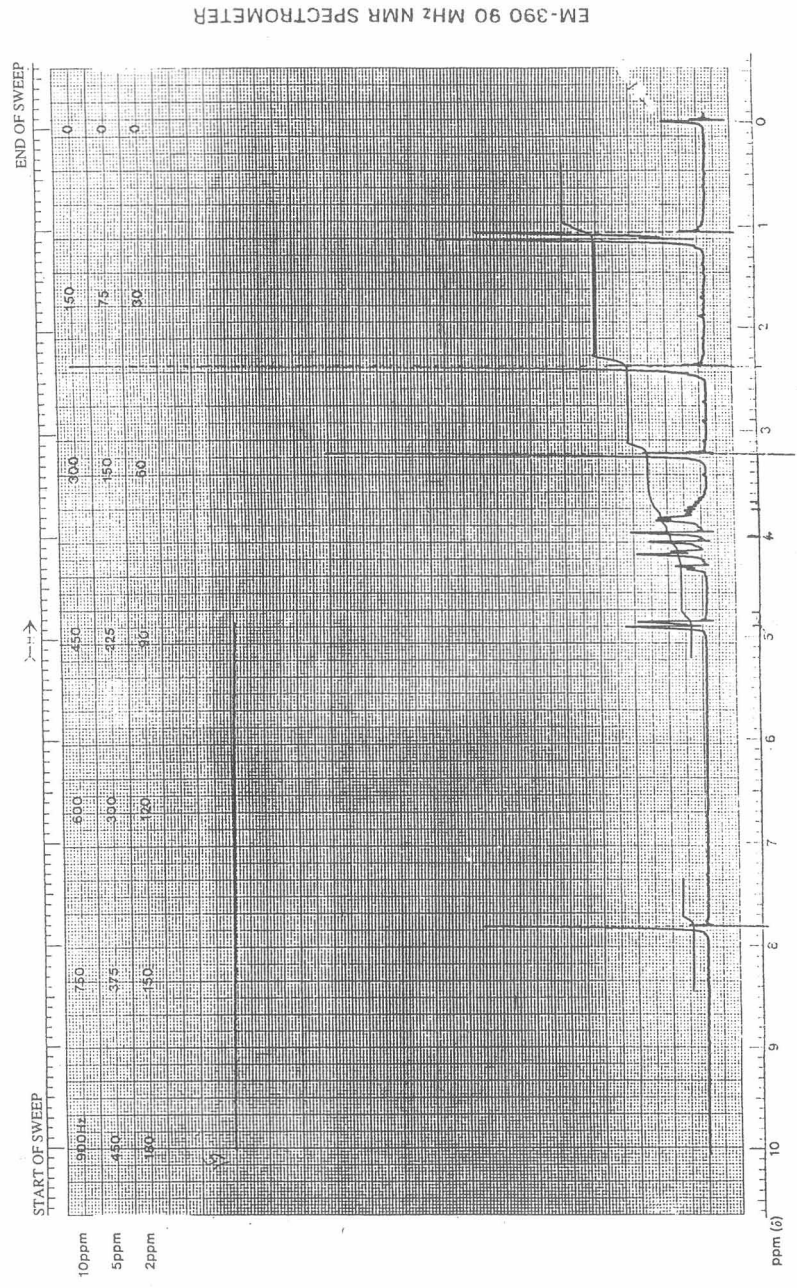


Figure 5 : NMR spectrum of secnidazole .

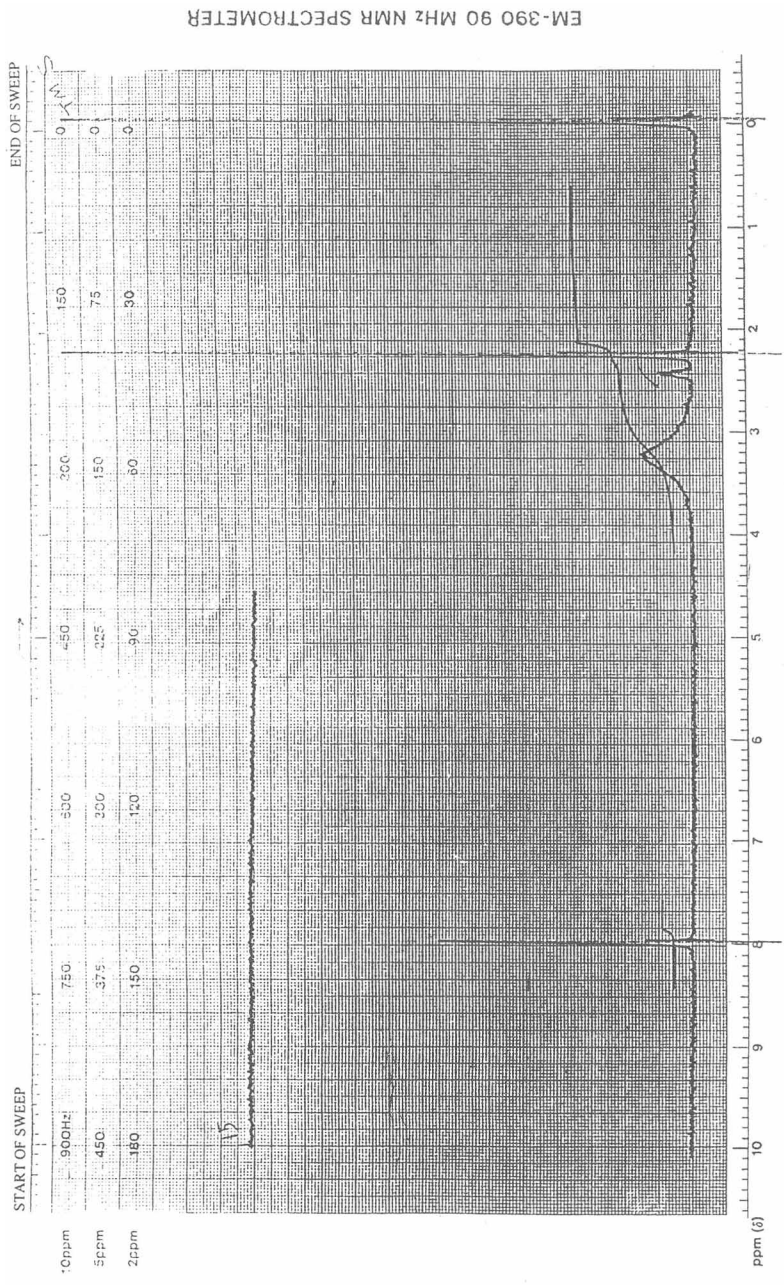


Figure 6 : NMR spectrum of 2-methyl-5-nitroimidazole .

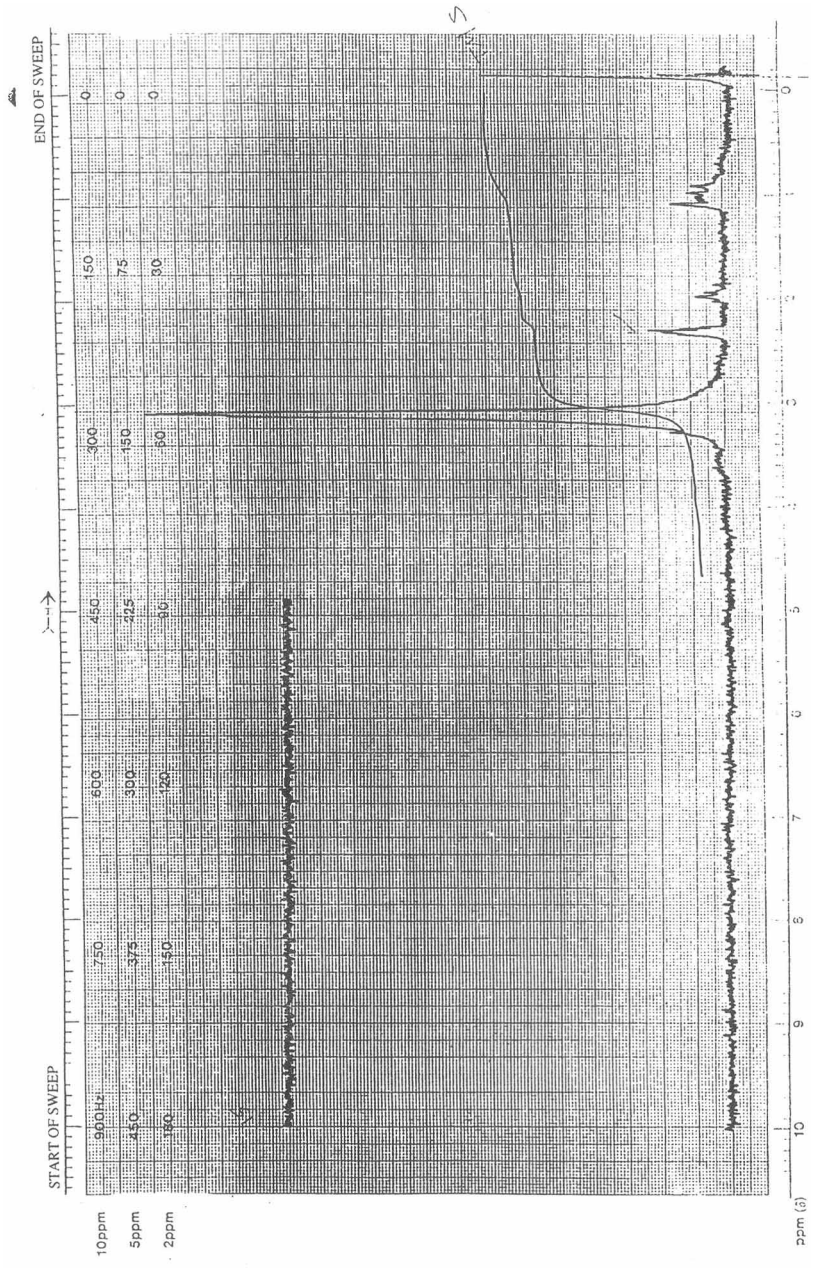


Figure 7 : NMR spectrum of hydroxy propanol .

products . The reported methods can not be used as a stability indicating method.

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Date Received: December 23, 1998
Date Accepted: June 15, 1999