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Spectrophotometric and HPLC determination of secnidazole in pharmaceutical tablets

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Abstract

Simple and accurate spectrophotometric and HPLC methods were developed for the determination of secnidazole in tablets dosage form. The first spectrophotometric method depends on the reduction of secnidazole molecule with zinc dust and hydrochloric acid followed by condensation with either *p*-dimethylaminobenzaldehyde or anisaldehyde to give colored chromogens having absorbance at 494 and 398 nm, respectively. The second method was based on the reaction of the drug with sodium nitroprusside in the presence or absence of hydroxylammonium hydrochloride. The formed colored chromogens were measured at 584 and 508 nm, respectively. The experimental conditions were optimized and Beer's law was obeyed over the applicable concentration ranges. The application of HPLC procedures depended on using either a conventional or microbore reverse-phase (C_{18}) column along with mobile phases consisting of water and methanol (30:70), at pH of 3.5. Both techniques were applied successfully for the analysis of secnidazole in tablets form. The results obtained from both procedures were statistically compared using the Student's-*t* and *F*-variance ratio tests. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Anisaldehyde; Conventional and microbore columns; *p*-Dimethylaminobenzaldehyde; Hydroxylammonium hydrochloride; High performance liquid chromatography; Secindazole; Tablets

1. Introduction

Secnidazole, 1-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol, is a relatively new antiprotozoal agent used in the treatment of amoebiasis and has also been tried in trichomoniasis [1]. The drug is not yet official in any pharmacopoeia. The literature revealed no visible spectrophotometric methods for the determination of secnidazole in pharmaceutical tablets. Two UV spectrophotometric procedures were reported, one was based on direct absorbance measurement [2] and the other on difference spectrophotometry [3]. Polarographic [4,5] and high performance liquid chromatography (HPLC) [6] methods have been also proposed. In blood, secnidazole was analyzed by gas–liquid chromatography (GLC) [7] and HPLC [8,9] methods.

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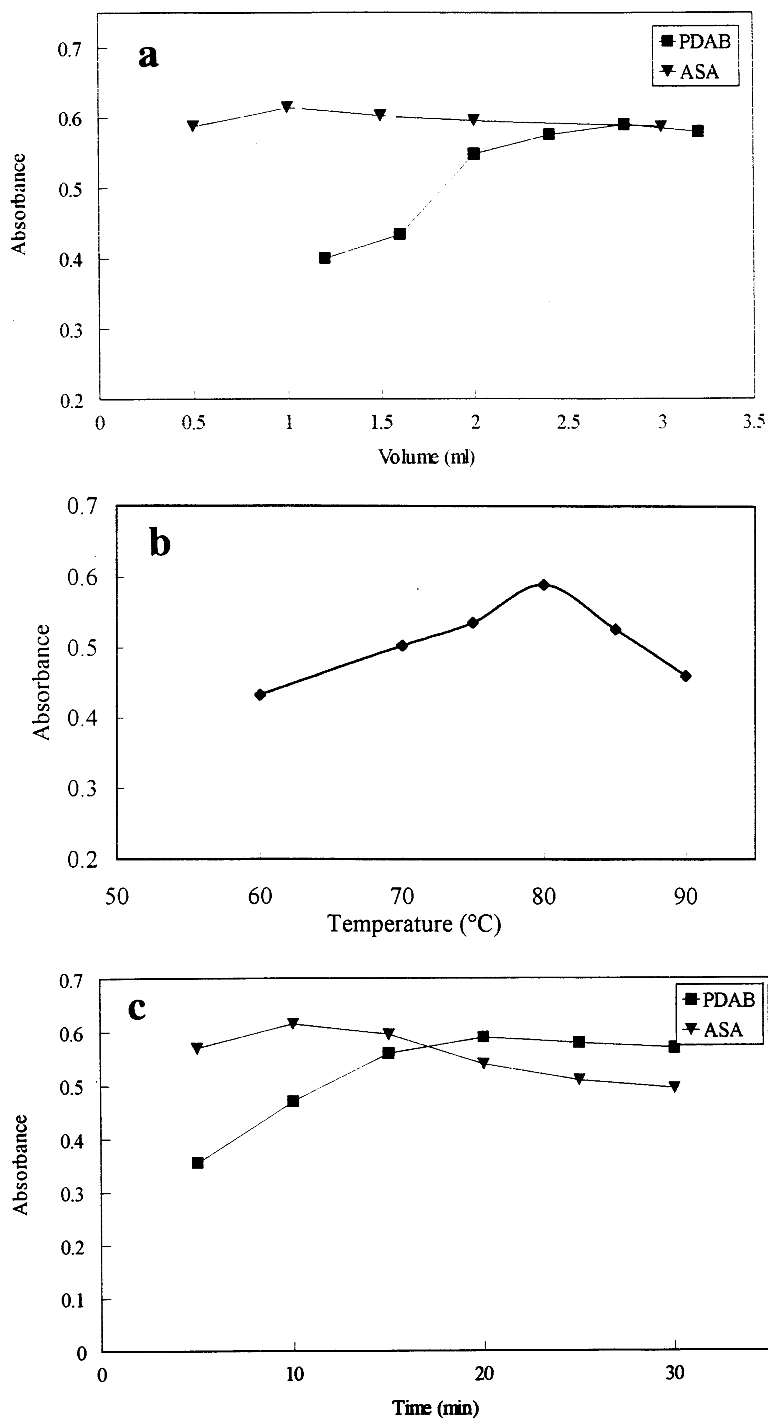


Fig. 1. Effect of (a) aldehyde volume; (b) heating temperature and (c) heating time on Schiff's bases formation using 10 and 25 μg ml^{-1} of secnidazole with PDAB and ASA, respectively.

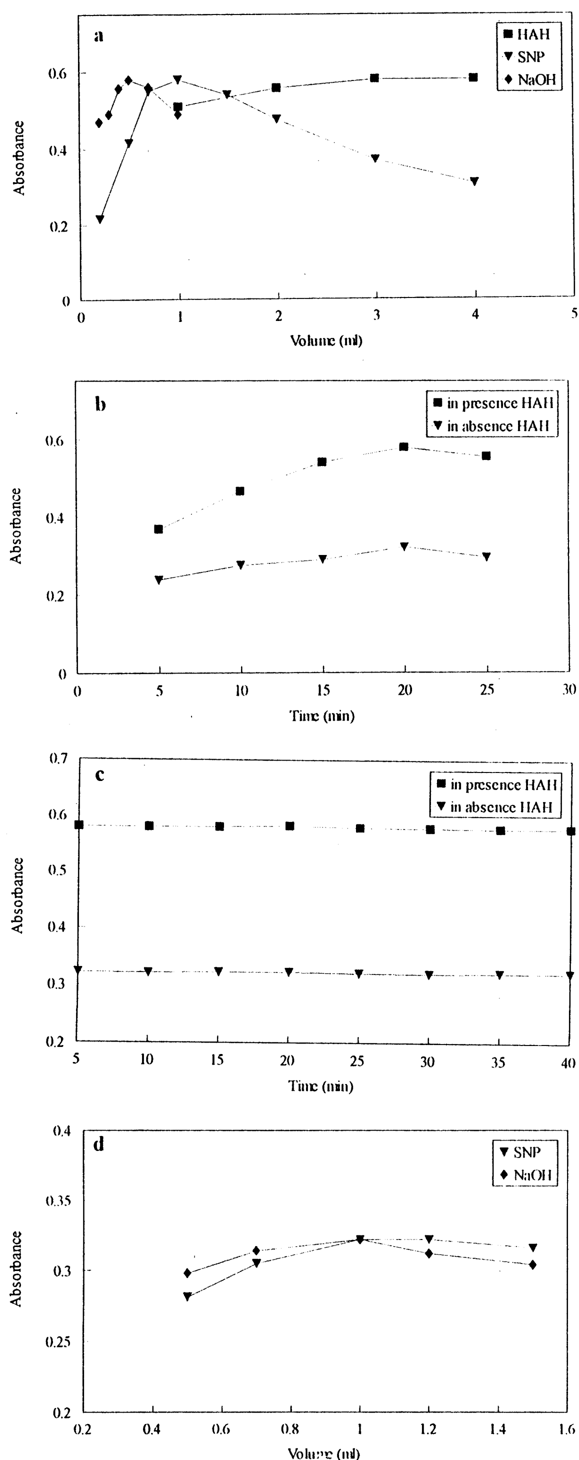


Fig. 2.

This paper describes simple colorimetric and HPLC procedures for the determination of secnidazole in tablet dosage form. The first spectrophotometric procedure depends on the reduction of the nitro to an amino group which then reacted with either *p*-dimethylaminobenzaldehyde (PDAB) or anisaldehyde (ASA) to form colored Schiff's base. The second procedure utilized sodium nitroprusside in the presence or absence of hydroxyl ammonium hydrochloride to form colored chromogens easily measured spectrophotometrically. In both procedures, the effect of the reagents concentration, heating temperature and the time on the nature of the formed chromogens were investigated.

In view of the fact that there is no spectrophotometric method (VIS region) for secnidazole, sensitive and precise method would greatly aid in the determination of secnidazole in bulk samples or pharmaceutical tablets form. Although the reported UV spectrophotometric procedures [2,3] are simpler but the proposed colorimetric methods are free from interference of tablet excipients as absorbance measurements are performed at longer wavelength. In order to assure a rapid, reproducible and above all selective analytical procedure for determination of secnidazole, two HPLC procedures have been developed for the drug determination and to act as reference methods for the spectrophotometric procedures. The microbore HPLC system offers a good advantage with respect to the used volume of mobile phase. The results obtained from both techniques are compared statistically using Student's-*t* and *F*-variance ratio tests.

Fig. 2. (a) Effect of volume of sodium nitroprusside (SNP), hydroxyl ammonium hydrochloride (HAH) and sodium hydroxide (1 M) on the chromogen formation with 40 μg ml^{-1} secnidazole; (b) effect of the time on the reaction between sodium nitroprusside and secnidazole (40 and 100 μg ml^{-1}) in the presence and absence of hydroxyl ammonium hydrochloride (HAH), respectively; (c) absorbance stability at room temperature of 40 and 100 μg ml^{-1} secnidazole chromogens with sodium nitroprusside in the presence and absence of hydroxyl ammonium hydrochloride (HAH), respectively; (d) effect of sodium nitroprusside (SNP) and sodium hydroxide (1 M) on the chromogen formation with 100 μg ml^{-1} secnidazole.

2. Experimental

2.1. Instrumentation

A Perkin-Elmer Model 550S double-beam spectrophotometer (Norwalk, CT, USA) with matched 10 mm quartz cells and attached to a Hitachi Model 561 recorder with scan speed of 60 nm min^{-1} was employed for all absorbance measurements. For HPLC procedures, a Hewlett-Packard (Avondale, PA, USA) chromatograph Model 1090 equipped with UV-diode array detector, binary DR 5 solvent delivery system and interfaced with HP-85B personal computer was used. The peak area integration was performed using a HP-3392A integrator. The samples were injected automatically using an auto-injection system. The second HPLC procedure was carried out using a chromatograph consisting of Isco syringe pump Model 100 DM, with Isco absorbance detector Model S500. The chromatograph was controlled and the peak areas were quantitated through Axxiom Chromatography data system Model 727. A thermostated water-bath accurate to 0.5°C was utilized throughout the work.

2.2. Materials

Pharmaceutical grade secnidazole (Rhone-Poulenc Rorer, France) was kindly supplied by Alexandria Pharmaceutical Co. (Alexandria, Egypt) and was certified to contain 99.50%. The drug used without further purification. *p*-Dimethylaminobenzaldehyde and anisaldehyde (Aldrich Chemical Company, Milwaukee,WI, USA) were used as 0.2 and 10% solutions in 2% methanolic trichloroacetic acid medium. Sodium nitroprusside and hydroxyl ammonium hydrochloride (Sigma Chemical Company, Milwaukee,WI, USA) were prepared as 2 and 5% solutions in water. Naproxin and benzyl metronidzole were used as internal standards for the HPLC procedures when using the conventional and microbore columns, respectively. They were an in-house standard and their purity were certified to be 99.20 and 99.50%, respectively. Methanol (Romil Chem. Limited, UK) was of HPLC grade; water was doubly distilled from all glass apparatus. All other chemicals were analytical reagent grade.

2.3. Chromatographic conditions

2.3.1. Conventional HPLC conditions

Chromatographic separation was carried out at ambient temperature on a 10 μm Hypersil C-18 column (250 × 4.6 mm i.d.) (Shandon Scientific Ltd., Cheshire, UK). The compounds were sepa-

Table 1
Spectrophotometric characteristics and statistical data of the regression equations

Parameter[a]			
Schiff's base procedure		Sodium nitroprusside procedure	
PDAB	ASA	With HAH	Without HAH
Concentration range (μg ml^{-1})			
2.5–15	5–25	15–40	60–100
Apparent molar absorptivity (1 mol^{-1} cm^{-1})			
10 907	4553	2685	601
Sandell's sensitivity (μg cm^{-2} per 0.001 A)			
1.70E-2	4.07E-2	6.83E-2	0.311
Regression equation			
Intercept (a)			
−1.00E-3	−3.30E-4	3.18E-3	−2.81E-3
tS_a[b]			
8.63E-3	3.10E-3	5.94E-3	6.61E-3
Slope (b)			
5.89E-2	2.46E-2	1.45E-2	3.25E-3
tS_b[c]			
8.86E-4	2.01E-4	2.06E4	8.15E-4
Correlation coefficient (r)			
0.9999	0.9999	0.9999	0.9998
Variance (S_0^2)			
1.11E-5	1.55E-6	2.42E-6	9.05E-7
Linearity ($S_{b\ rel}$ %)[d]			
0.54	0.29	0.51	0.90
Detection limit (μg ml^{-1})			
0.14	0.13	0.27	0.73
Relative sensitivity[e]			
1.08	1.00	2.08	5.62

[a] PDAB, *p*-dimethyl aminobenzalde; ASA, anisaldehyde; HAH, hydroxyl ammonium hydrochloride.
[b] Confidence intervals of the slopes ($P = 0.05$).
[c] Confidence intervals of the intercepts ($P = 0.05$).
[d] Linearity is the RSD of the slopes.
[e] Calculated relative to Shiff's base formation using ASA.

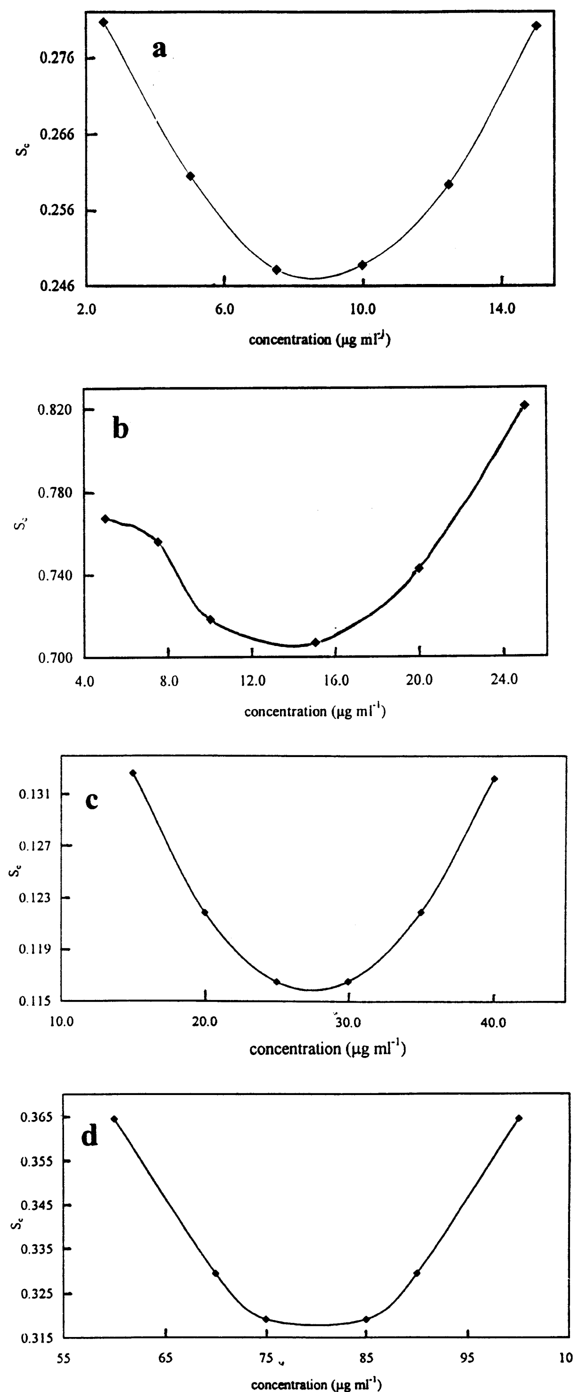


Fig. 3. Errors (S_c) in the determination of the concentration of secnidazole using (a) PDAB; (b) ASA; (c) sodium nitroprusside with hydroxyl ammonium hydrochloride and (d) sodium nitroprusside.

rated isocratically with a mobile phase consisting of a mixture of methanol–water (30:70,v/v) with the pH of the water adjusted to 3.5 with acetic acid. The flow rate was 1 ml min^{-1}. The mobile phase was degassed for 15 min by passing helium gas. The analysis usually started after the passage of 20–30 ml of mobile phase to reach equilibrium. The injection volume was 5 μl. The eluted analytes were detected at 310 nm with 10 nm band width. The automatic integrator conditions were set as follows. Attenuation, 3; threshold, 3; peak width, 0.04 and chart speed, 0.5 cm min^{-1}.

2.3.2. Microbore HPLC conditions

The separation was carried out at ambient temperature on a 5 μm Hypersil C-18 column (250 × 1.0 mm i.d.) (Shandon Scientific Ltd., Cheshire, UK). The mobile phase was methanol–water, and the pH was adjusted to 3.5 with acetic acid, in a ratio of 70:30 (v/v) at a flow rate of 75 μl min^{-1}. The injection volume was 0.1 μl and the column effluent was monitored at 310 nm with sensitivity of 0.05 AUFS. Benzyl metronidazole was used as internal standard. The chem. station conditions were set as follows. Threshold, 1000; peak width, 0.2; area rejection, 2000; slope, 1 and drift, 1.

In both HPLC procedures, the calibration graph was obtained by plotting peak area ratios of secnidazole to the internal standard (IS) versus the concentrations.

2.4. Standard solution

A solution of 0.5 mg ml^{-1} was prepared by dissolving secnidazole in distilled water. The solution was stable for at least 3 days if it had been stored in a cool (< 20°C) and dark place.

2.5. General procedures

2.5.1. Schiff's base formation

Transfer 25 ml from the standard secnidazole solution to a 100 ml Erlenmyer flask, add 5 ml 1 N hydrochloric acid and 2 g of zinc dust. With occasional shaking, allow the flask to stand for 1 h at room temperature. Through a wetted filter paper (Whatman No. 41) and to a 50 ml volumetric flask, filter the reaction mixture, wash the filter

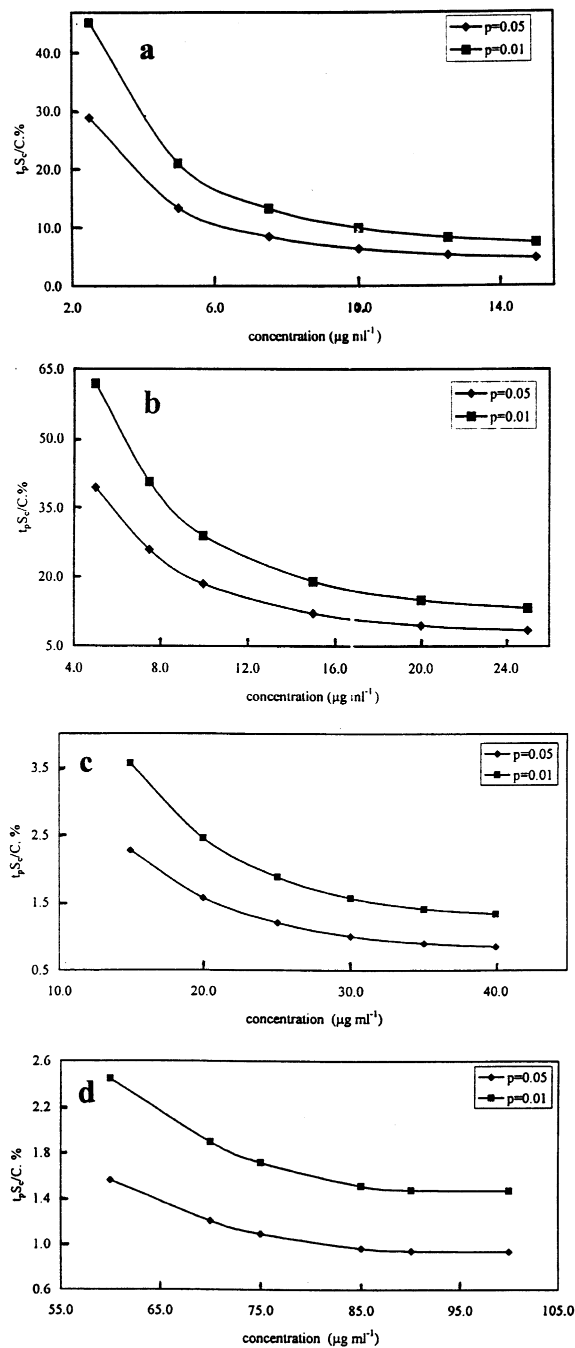


Fig. 4. Variation of confidence limits at $P = 0.05$ and $P = 0.01$ of significance, presented as percentage of uncertainty on the secnidazole concentration using (a) PDAB; (b) ASA; (c) sodium nitroprusside with hydroxyl ammonium hydrochloride and (d) sodium nitroprusside.

paper twice with water and then complete the flask to volume with water.

An x ml volume of the reduced secnidazole standard solution ($x = 0.10–0.60$ or $0.20–1.00$ ml for PDAB and ASA, respectively) was pipetted into two sets of 10 ml volumetric flasks. Complete the volume to 1.00 ml with 0.1 N hydrochloric acid. To each flask, add 2.8 or 1.0 ml of PADBA or ASA, respectively. For PADBA procedure, heat the flasks for 20 min at 80°C, while, for ASA procedure heat in boiling water-bath for 10 min. Cool the flasks and complete to volume with methanol. Measure the absorbance of the flasks at 494 or 398 nm for PABDA or ASA, respectively, against blank prepared simultaneously and substituting the drug with 0.1 N hydrochloric acid.

2.5.2. Sodium nitroprusside procedure (with or without hydroxyl ammonium hydrochloride)

An x ml volume of the working solution of secnidazole ($x = 0.30–0.80$ or $1.20–2.00$ ml in case of with or without using hydroxylammonium hydrochloride) was pipetted into two sets of 10 ml volumetric flasks. Adjust the volume in the flasks to 2 ml using water. A 3 ml volume of hydroxyl ammonium hydrochloride solution was added, only, to the first set of the flasks. To both sets, add 1 ml of sodium nitroprusside solution, shake for 2 min and then add 0.5 or 1.0 ml of sodium hydroxide to the first and second flask sets, respectively. Leave the flasks to stand for 20 min and complete to volume with water. The absorbances were measured at 584 and 508 nm with and without hydroxyl ammonium flasks, respectively, against blank prepared simultaneously with water replacing secnidazole solution.

2.6. Assay of pharmaceutical tablets

Ten tablets were powdered and a quantity of the powder equivalent to 100 mg of secnidazole was extracted by shaking with 50 ml water, followed by another two extractions each with 20 ml of water. The extracts were filtered through a Whatman No. 41 filter paper into a 100 ml volumetric flask and then diluted to volume with water. The assay for secnidazole content was completed as described in Section 2.5.

Table 2
Evaluation of the accuracy and precision of the two proposed procedures

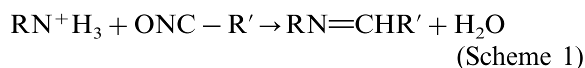
Proposed method	Added[d]	Found S.D.[a]	RSD (%)	SAE[b]	Confidence limit[c]
Shiff's base formation					
PDAB	3.00	3.00 ± 0.040	1.35	0.018	0.050
	5.00	5.00 ± 0.059	1.18	0.026	0.073
	7.00	7.00 ± 0.083	1.19	0.037	0.104
ASA	10.00	10.05 ± 0.210	2.09	0.094	0.262
	15.00	15.08 ± 0.314	2.08	0.141	0.391
	20.00	20.04 ± 0.463	2.31	0.207	0.575
Sodium nitroprusside					
With HAH	15.00	15.02 ± 0.218	1.45	0.098	0.271
	20.00	20.03 ± 0.288	1.44	0.129	0.358
	25.00	25.03 ± 0.363	1.45	0.162	0.451
Without HAH	60.00	59.99 ± 0.283	0.47	0.127	0.352
	80.00	79.97 ± 0.504	0.63	0.225	0.626
	100.00	100.09 ± 0.560	0.56	0.251	0.697

[a] Mean ± S.D. for five determinations.
[b] SAE, standard analytical error.
[c] Confidence limits at $P = 0.05$ and four degrees of freedom.
[d] Concentration in μg ml^{-1} (final concentration).

3. Results and discussion

3.1. Spectrophotometric method

The utilization of a variety of chromogenic reagents after the reduction of the aryl nitro group was the basis for the sensitive spectrophotometric determination of many nitro groups containing pharmaceutical compounds [10]. The developed method depends on reducing the nitro to amino group, reacting with either *p*-dimethylaminobenzaldehyde (PDAB) or anisaldehyde (ASA) and measuring the yellow colored Schiff's base produced at 494 and 398 nm, respectively. The reaction of the reduced secnidazole with the two aldehydes to form the colored Schiff's base was carried out in the presence of trichloroacetic acid in methanol (Scheme 1).



$$RN^{+}H_3 + ONC - R' \rightarrow RN{=}CHR' + H_2O$$
(Scheme 1)

The reaction of the primary aromatic amine and PDAB and ASA was assumed to take place through the condensation of the protonated primary amino group with the carbonyl group of the reagent to produce the imminium salt (Schiff's base) [11].

Investigations were carried out to establish the most favorable conditions for the reaction and to achieve maximum color development in the quantitative determination of secnidazole. For the reduction procedure, the use of 5 ml of 1 M hydrochloric acid with 2 g zinc dust, at room temperature (22°C ± 2°C), was found to be optimal. The reduction time was established by increasing it in intervals of 5 min, and it was found that one hour is sufficient to yield maximum absorbance. The optimum volume of the reagent (aldehyde) was found to be 2.8 and 1.0 ml of 0.2 and 10% solutions of PDBA and ASA, respectively, as shown in Fig. 1a and heating for 20 and 10 min at 80°C and boiling water bath (Fig. 1b and c) for PDBA and ASA, respectively. The yellow color produced was proved to be stable for about 40 and 30 min for PDBA and ASA, respectively.

Sodium nitroprusside is a valuable reagent for the qualitative and quantitative determination of a wide variety of nucleophilic compounds [12]. The reactivity of the nitroprusside is based on the positive nitrosyl group, reacting with the nucleophilic species in alkaline medium. Generally, most of the formed addition compounds are

highly colored and stable to be used as a basis for the spectrophotometric analysis. On the above basis, secnidazole was found to form water-soluble colored complex with sodium nitroprusside in the presence of hydroxyl ammonium hydrochloride under alkaline conditions. The complex exhibited wavelength of maximum absorbance at 584 nm. Unfortunately, the reaction mechanism is not that simple, but it may be suggested that the nitroprusside $\{[Fe(CN)_5NO]^{2-}\}$ undergoes decomposition and reduction of the iron(III) to iron(II) with alkaline hydroxyl ammonium hydrochloride, leading to the formation of $\{[Fe(CN)_5H_2O]^{3-}\}$. So, since secnidazole (SEC) develops color under the above mentioned conditions, the color may be due to the formation of $\{[Fe(CN)_5(SEC)]^{3-}\}$ [13].

Table 3
Determination of secnidazole in commercial tablets using the proposed procedures compared statistically with the conventional HPLC procedure results

Recovery (CV %)[a]			
Proposed spectrophotometric procedures		HPLC methods	
Shiff's base formation		Microbore	Conventional[b]
PDAB	ASA		
Flagentyl tablets[f] (BN. 8507002)			
99.76 (0.98)	99.51 (0.90)	99.27 (0.56)	9.41 (0.64)
$t=0.66$	$t=0.20$	$t=0.37$[c]	
$F=2.42$	$F=2.01$	$F=1.27$[d]	
Recovery[e]			
100.35 (0.76)	99.65 (0.98)	100.51 (0.72)	100.22 (0.39)
Sodium nitroprusside procedure			
With HAH	Without HAH		
98.98 (0.69)	99.07 (0.63)		
$t=1.04$	$t=0.87$		
$F=1.15$	$F=1.01$		
Recovery			
99.55 (1.45)	100.30 (0.67)		

[a] Mean ± coefficient of variation (CV%) of five determinations.
[b] Used for statistical comparison.
[c] Tabulated t-value for $P=0.05$ and eight degrees of freedom is 2.31.
[d] Tabulated F-value for $P=0.05$ and $f1=f2=4$ is 6.39.
[e] Standard addition of 100% of the nominal content.
[f] Each tablet contains 500 mg of secnidazole and manufactured by Alexandria Pharm. Co., Alexandria, Egypt, under licence of Rhone Poulenc Rorer, Paris, France.

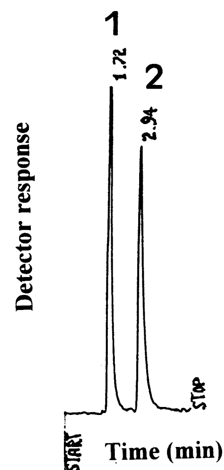


Fig. 5. Sample chromatogram using the conventional system, peaks1 and 2 are secnidazole and naproxin, respectively.

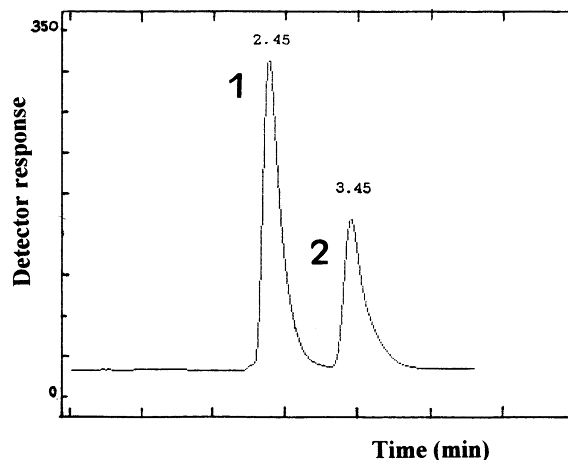
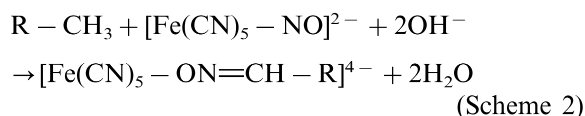


Fig. 6. Sample chromatogram using the microbore system, peaks 1 and 2 are secnidazole and benzyl metronidazole, respectively.

A study of the effect of the reagents concentration, shaking time, the degree of alkalinity and the role of the hydroxyl ammonium chloride with respect to the maximum sensitivity and obedience to Beer's law was carried out. A volume of 1.0 ml of sodium nitroprusside of 2% solution in the presence of 3 ml hydroxyl ammonium chloride 5% solution (Fig. 2a) was found to be optimum volumes for the color development. The time re-

quired for complete complex formation, at room temperature (22 ± 2°C), was found to be 20 min (Fig. 2b) and with alkalinity developed by the addition of 0.5 ml of 1 M sodium hydroxide (Fig. 2a). The color developed under the above conditions was found to be stable for 40 min (Fig. 2c). The stability was evaluated by recording the VIS spectrum (λ_{max} at 584 nm) every 5 min, in which, the molar absorptivity remained unchanged for at least 20 min. Omitting the hydroxyl ammonium chloride from the reaction mixture caused a hypsochromic shift (maximum at 508 nm) and hypochromic effect. At the same time, it was found that the optimum volume needed from 1 M sodium hydroxide was also changed to be 1.0 ml (Fig. 2d). Literally, it was reported that compounds which contain an active methylene group, or in which an active methylene group can arise through the shifting of hydrogen atom, yield colors with alkaline nitroprusside [14]. Secnidazole molecule belongs to the imidazole family with additional methyl and nitro groups substituted in the 2 and 5 positions of the imidazole ring. Therefore, the methyl group can be considered as a good source for the formation of active methylene, under alkaline condition; this was attributed to the electron deficiency around it, which is intensified by the presence of the nitro group at position 5 [15]. So, on the basis of the literature background and what is mentioned above, a proposed scheme for the reaction of secnidazole with nitroprusside in alkaline medium could be outlined as in Scheme 2.



$$R-CH_3 + [Fe(CN)_5-NO]^{2-} + 2OH^- \rightarrow [Fe(CN)_5-ON{=}CH-R]^{4-} + 2H_2O$$

(Scheme 2)

In the above experimental conditions the calibration graphs obtained, from both methods, by plotting absorbance at the specified wavelengths against concentrations were found to be linear over the Beer's law ranges given in Table 1. The molar absorptivities, limits of detection, variances, slopes, intercepts and correlation coefficients obtained by linear least squares treatment of the results were also given in Table 1. The excellent linearity of the calibration graphs is clear from the correlation coefficients and at the same time, the intercepts are close to zero. The values of the correlation coefficients were not sufficient to evaluate the linearity of the calibration graphs. The linearity was evaluated by the relative standard deviation (RSD %) of the slope ($S_{b\ rel}$ %) [16] (Table 1). The values of the detection limits and variances are evidence of the sensitivity of both methods and the negligible scatter of the points with respect to the line of regression.

Statistical analysis of the calibration graphs allowed the calculation of the error, S_c, in the determination of given concentration [17]. The graphs of S_c against concentrations are shown in Fig. 3a–d. The error reached minimum when the actual absorbance was equal to the average absorbance in the calibration graph. The quantity of S_c also allows the determination of confidence limits. These results are shown graphically in Fig. 4a–d in the form of percentage uncertainty on the concentration [18] (at levels of significance $P =$ 0.01 and 0.05). This is a useful way of representing the confidence limits because it allows a direct calculation of the relative uncertainty on concentration over the full range of the concentration tested, and hence it is a guide to the level of precision that may be expected from the application of both analytical procedures.

In order to determine the accuracy and precision of both procedures, solutions containing three different concentrations of secnidazole were prepared and analyzed in five replicates. The analytical results obtained from the investigation are summarized in Table 2. The standard deviations (S.D.), the relative standard deviations (RSD %) and the standard errors (SAE) can be considered to be very satisfactory.

Table 3 shows the results obtained for the determination of secnidazole in pharmaceutical tablets by means of both proposed methods and the reference method (conventional HPLC procedure).

There were no significant differences between the two sets of results. Tablet excipients (wheat statch, explotab, calcium phosphate dibasic, gelatine, magnesium stearate, avicel) did not interfere.

3.2. HPLC methods

The described reverse-phase HPLC methods were developed to provide specific procedures suitable for rapid quality control determination of secnidazole and as reference methods for the developed spectrophotometric methods. A satisfactory separation, from the used internal standards, with reasonable retention times were obtained with mobile phase consisting of water–methanol (30:70) for both HPLC systems (conventional and microbore). Practically and from Figs. 5 and 6, it was found that at acidic pH (3.5), good separation from the internal standard, with sharp peaks were obtained. Naproxin and benzyl metronidazole were chosen as internal standards for the conventional and microbore systems, respectively. Under the specified conditions (Section 2.3.), the average retention times $\pm$ S.D. for secnidazole, naproxin for conventional and secnidazole and benzyl metronidazole for microbore systems were 1.72 ± 0.08; 2.92 ± 0.012 and 2.45 ± 0.06; 3.45 ± 0.02 min, for 10 replicates, respectively. Quantitation was achieved with UV detection at 310 nm, which represents a wavelength with reasonable and nil absorbance for secnidazole and tablet excipients, respectively. Linearity of the detector responses were determined by preparing the calibration graphs. The plot of peak area ratios (PAR) versus concentrations were linear over the range 10–50 μg ml^{-1} for both systems. The least square regression equations were

$$\text{PAR (conventional)} = 6.80 \times 10^{-3} + 2.48 \times 10^{-2}\ C$$

$$\text{PAR (microbore)} = 3.33 \times 10^{-3} + 3.14 \times 10^{-2}\ C$$

With correlation coefficient, r, of 0.9999 and 0.9991 for the conventional and microbore systems, respectively. To check the linearity range of the calibration graphs, linearity plots were constructed by plotting sensitivity against the corresponding concentration [18]. Two parallel control lines representing the acceptable upper and lower limits (5%) for the analysis were placed on the plot to determine the acceptable working range. It was found that all the data points fall within the control lines indicating good linearity. Individual examination of the tablet excipients, under the described chromatographic conditions, indicated no interfering responses. The accuracy of both procedures was evaluated by the fortification of the samples with secnidazole at the 100% level and assayed by the proposed procedures. The recovery values ranged from 97.50 to 100.05% and from 97.25 to 100.45% with an overall average of 99.40 and 99.32% for the conventional and microbore systems, respectively (Table 3).

The methods precision was evaluated by repeated assays of the commercial formulation over separate periods of 1 day and 1 week. The within-day precision was determined by performing seven consecutive assays within a period of eight hours. The day-to-day repeatability of the method was determined by analyzing the same sample (single operator) on seven consecutive days. These measurements of precision provided coefficient of variation (CV %) values of 0.334 and 1.059% for the conventional system and 0.540 and 1.125% for the microbore system, respectively. A comparison of the assay values presented in Table 3 indicates that both HPLC methods provide similar results. The microbore HPLC system offers a good advantage with respect to the used volume of the mobile phase.

4. Conclusion

Under the experimental conditions described, the linearity and the sensitivity were the best with the Shiff's base formation procedure (no significant difference in using PDAB and ASA). At the same time, the reaction with sodium nitroprusside in the presence of hydroxyl ammonium hydrochloride showed almost double sensitivity. The sodium nitroprusside methods are simpler, do not need preliminary reduction and are performed at room temperature. The data in Tables 1–3 indicate rectilinearity, precision and reproducibility of the proposed procedures. Both HPLC procedures offer several advantages with respect to stability studies and overall versatility. But, because of the low cost and ease of carrying out the spectrophotometric methods, the proposed procedures are

likely to be very suitable for the analysis of secnidazole in tablets dosage form.

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