Assay of Secnidazole in Tablet Formulations by Fluorometry and Spectrophotometry

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Two accurate and sensitive methods are described for the determination of secnidazole in bulk drug and tablet formulations. The methods are based on the reduction of secnidazole with zinc dust and sodium hydroxide. In method I, fluorescence of the reduced product was measured. The excitation and emission wavelengths were observed at 360 nm and 425 nm respectively. A linear relationship between relative fluorescence intensity and concentration was obtained for 1 to 10 mcg of secnidazole per ml. The reduced product was diazotized and coupled with Bratton and Marshal's reagent in method II to get a chromogen showing maximum absorbance at 502 nm.

Secnidazole, 1-(2-hydroxyl-1-propyl)-2-methyl-5-nitro imidazole is the first nitro-imidazole derivative to prove effective in single dose on protozoans and is well tolerated by patients. Secnidazole is not official in any profile or pharmacoepia but the literature cites a differential pulse polarography[1], reversed phase HPLC[2] and UV spectrophotometric[3] methods for the analysis of secnidazole, but no fluorimetric or colorimetric method is reported.

Secnidazole was obtained as a gift sample from Rhone-Poulenc India Ltd., Mumbai and was used as such. Zinc dust used for the reduction of secnidazole was AR grade (Glaxo Ltd.) and it was also used as such. The reduction of secnidazole was carried out by taking a weighed amount of secnidazole (25 mg) in a test tube and heating it with 1-2 g of zinc dust and 10 ml of 10% sodium hydroxide solution. After boiling for five minutes the solution was cooled to room temperature, filtered, washed and diluted either with distilled water to get a stock solution of 25 mcg/ml (method I) or with 10% sodium hydroxide to get a 250 mcg/ml solution (method II). All other solutions required for method II were prepared in the usual way.

A Shimadzu U-540 spectrofluorophotometer with a recorder was used for all the fluorescence measurements whereas, Hitachi U-2000 UV-visible spectrophotometer was used for spectral measurements in visible range.

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Different aliquots of the secnidazole solution (25 mcg/ml) were further diluted with distilled water to get working standards in the range of 1 to 10 mcg of secnidazole per ml.

The fluorescence data revealed the excitation wave length at 360 nm whereas the emission peak was recorded at 415 nm. The calibration curve was obtained by measuring relative fluorescence intensity of all the standard solutions at the emission wavelength.

Different aliquots of the reduced stock solution 250 mcg/ml were taken in different 25 ml volumetric flasks. These solutions were acidified with 5 ml of 6 N HCL. The flasks were cooled in ice-bath and then 3 ml of 0.5% w/v sodium nitrite was added to the flask. After 5 minutes, 5 ml 10% w/v ammonium sulphamate followed by 1 ml of 0.5% w/v (1-Napthyl) ethylenediamine dihydrochloride (NED) solution were also added. The solutions were diluted with distilled water upto the mark after through shaking. A reagent blank was also prepared simultaneously in the same manner. The absorbance of all the standard solutions were measured against reagent blank at 502 nm. The calibration curve was prepared by plotting the absorbance against concentration of secnidazole.

Twenty tablets of six different brands each were powdered and powder equivalent to 25 mg of secnidazole was heated with 1-2 g of zinc dust and 10 ml of 10% sodium hydroxide solution for two minutes. The solution

Table 1 - Recovery data for Method I and II

Formulations	Amount Analyzed (mg)	Amount found*		% Recovery**	
		Method I (mg)	Method II (mg)	Method I	Method II
Tablet 1	25	24.79	25.05	103.83	102.2
Tablet 2	25	24.38	24.65	104.33	98.6
Tablet 3	25	25.38	24.79	102.50	99.2
Tablet 4	25	24.63	24.31	100.50	97.2
Tablet 5	25	24.75	24.09	100.67	96.1
Tablet 6	25	24.75	22.90	103.17	91.6

* Average of three determinations ** After spiking the previously analyzed sample

Table - 2 Optical Characteristics and Other Parameter for Method I

Parameter	Result
Excitation wavelength	360 nm
Emission Wavelength	415 nm
Regression equation Slope Intercept	Y-9.119 X+0.495 9.119 0.495
Correlation coefficient	0.9987
Precision (% RSD)	1.330

Table 3 - Optical characteristics and other parameter for Method II

Parameter	Result
λmax	502 nm
Beer's law range	1 to 20 mcg/ml
Molar excitation coefficient	3.195×10^3 L/mole/cm
Sandell's sensitivity	0.0579 mcg/cm²/001 absorbance unit
Regression equation Slope Intercept	Y=0.0174 x +(-0.001) 0.0174 -0.001
Correlation coefficient	0.9994
Precision (% RSD)	1.326

was cooled to room temp and filtered in a 25 ml volumetric flask. The filter paper was washed and the volume was made upto the mark with distilled water (method I) or 10% sodium hydroxide solution (method II). From this an aliquot equivalent to 15 mcg/ml was used. The solution was further diluted with distilled water to measure the fluorescence intensity at 415 nm (method I). An aliquot of the sample was acidified, diazotized and coupled in the same manner as standards. The amount of secnidazole was computed from the calibration curve.

The proposed methods were found to be accurate, rapid, and simple for routine analysis of the drug. The nitro group present in secnidazole is reduced to nitroso, hydroxylamine and finally to amine group. The amino product was a fluorescent compound. Method I is a simple method and can be employed for the routine analysis of secnidazole in tablet formulations. As the emission peak of the fluorescence spectrum lies in the visible region, a simple fluorimeter with proper filter can be used for analysis. The optical characteristics and other parameters are given in Table-2.

Table 4 - Comparision of the proposed methods with the reported methods

Method	Linearity range (µg/ml)	% Recovery	RSD
DPP	1.04-8.32	-	-
HPLC (RP)	20-100	100	0.1-1.16
UV	5-20	-	-
Method I	> 0.1	102.5	1.330
Method II	1-20	97.5	1.326

The second method is the typical colorimetric method where diazotization and coupling is carried out after reducing the nitro group to amino group. Then the optimum pH required for diazotization, effect of sodium nitrite, ammonium sulphamate and NED reagent concentration were studied with respect to maximum sensitivity, adherence to Beer's law and colour stability, these conditions incorporated in the procedure. The optical characteristics and other constants are given in Table-3.

The results of analysis using these methods are given in Table-1. The recovery studies were also carried out by spiking the previously analyzed sample thrice with 1 mcg of pure drug. These results are stated in Table-1. The proposed methods are also compared with reported methods in Table-4 indicating the sensitivity of these methods. The value of standard deviation were satisfactorily low and recovery was close to 100% indicating the reproducibility and accuracy of both the methods.

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