Research Article

Spectrophotometric Determination of Secnidazole Using Hydroxylamine and Sodium Carbonate

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Preparation of Standard Stock Solution

100 mg of secnidazole was accurately weighed and dissolved in 30ml of distilled water and treated with 10ml of 4N hydrochloric acid and 1.2gm of zinc dust was added in portions. After standing for 1hr at room temperature the solution was filtered through cotton wool, the residue was washed with 3×10ml portions of distilled water and the total volume of the filtrate was brought to 100ml with distilled water (solution I)

Absorption Spectra of Coloured Species

25ml of solution I was pipette into 100ml standard volumetric flask and was diluted to 100ml with distilled water. (solution II)

From the solution II, 2ml was pipettedout into a dry 25ml standard volumetric flask. 2.5ml of hydroxyl amine reagent, and 5ml of sodium carbonate solution were added and the volume was made upto 25ml with sodium hydroxide solution. The absorption spectrum was obtained by scanning between the wavelength 550-740 nm against the reagent blank.

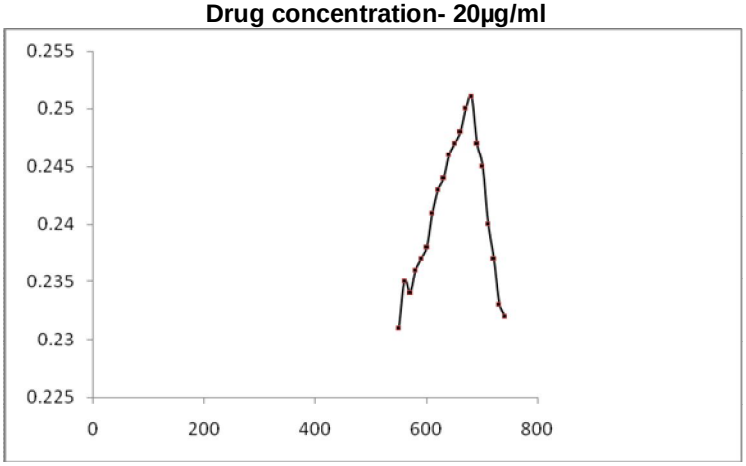
The readings are tabulated in table no 4.a and graphically shown in graph no 4.a.

Table 4a: Absorption Spectral Data of Coloured Species Using Hydroxylamine and Sodium Carbonate Reagents

Drug concentration 20µg/ml

Wavelength in nm	Absorbance
550	0.231
560	0.235
570	0.234
580	0.236
590	0.237
600	0.238
610	0.241
620	0.243
630	0.244
640	0.246
650	0.247
660	0.248
670	0.250
680	0.251
690	0.247
700	0.245
710	0.240
720	0.237
730	0.233
740	0.232

Graph 4a: Absorption Spectra For Drug With Hydroxylamine and Sodium Carbonate Drug concentration- 20µg/ml



e. Effect of Reagent Concentration

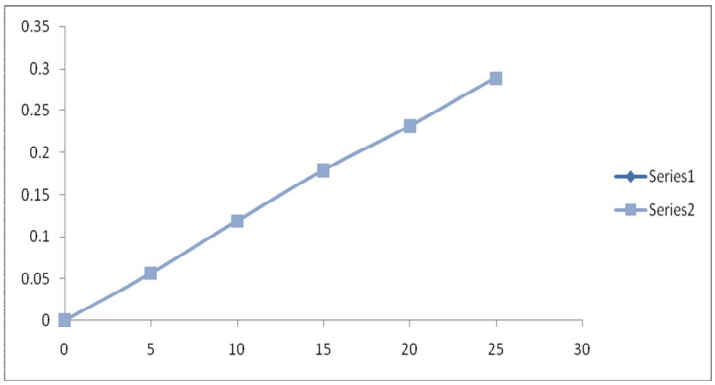
To find out the effect of different amounts of hydroxylamine reagent 1ml, 1.5ml, 2.0ml, 2.5ml of hydroxylamine reagent was added into 5, 10, 15, 20 & 25µg/ml concentration of drug. 5ml of sodium carbonate solution was also added. The absorbance was measured at 677nm against the reagent blank.

The readings are shown in table no 4.b and are graphically represented in graph no 4.b, 4.c, 4.c, 4.d, 4.e.

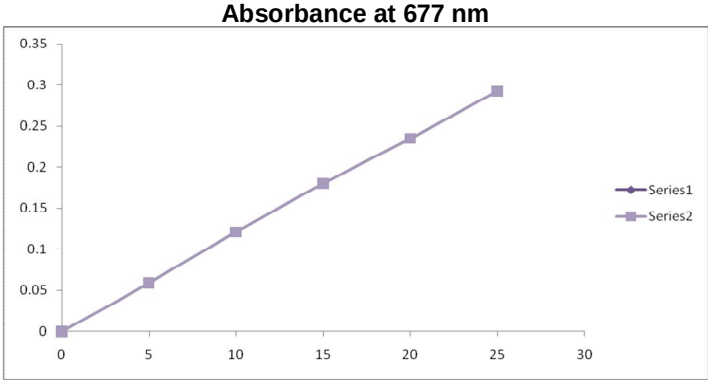
Table 4b: Data for the Calibration Curve with Different Volumes of Hydroxylamine Reagent

S.NO	Drug concentration in µg/ml	Volume of sodiumcarbonate reagent added	Volume of hydroxylamine reagent added & Absorbance			
			1ml	1.5ml	2ml	2.5ml
1	5	5ml	0.057	0.059	0.061	0.062
2	10	5ml	0.119	0.121	0.122	0.124
3	15	5ml	0.179	0.180	0.181	0.185
4	20	5ml	0.231	0.235	0.241	0.244
5	25	5ml	0.289	0.292	0.298	0.302

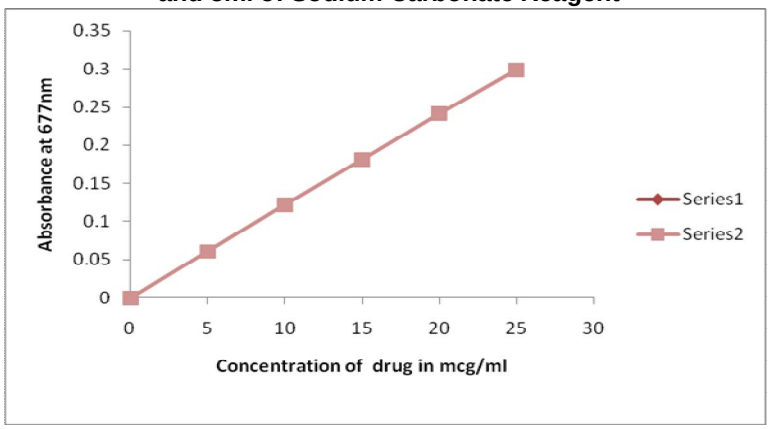
Graph 4b: Calibration Curve for Various Concentration of Drug With 1ml of Hydroxylamine Reagent & 5ml of Sodium Carbonate Reagent Absorbance at 677 nm



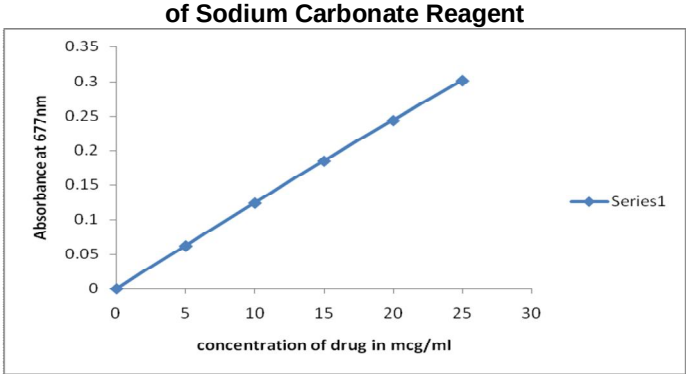
Graph 4c: Calibration Curve for Various Concentration of Drug With 1.5ml of Hydroxylamine Reagent & 5ml of Sodium Carbonate Reagent Absorbance at 677 nm



Graph 4d: Calibration Curve for Various Concentration of Drug With 2ml of Hydroxylamine Reagent and 5ml of Sodium Carbonate Reagent



Graph 4E: Calibration Curve for Various Concentration of Drug With 2.5ml of Hydroxylamine Reagent & 5ml of Sodium Carbonate Reagent



f. Fixation of Various Parameters λ_{max} (maximum wavelength)

The spectral data shows that the maximum absorbance was observed at 677nm and that was selected as working maximum.

2. Stability of colour

Stability of the colour of this system was tested by using 5, 10, 15, 20 & 25 mcg/ml concentration of drug with 2.5ml of hydroxylamine reagent and 5ml of sodium carbonate solution. Absorbance was measured at 677nm against the reagent blank. The readings were taken at 15min time interval upto 1hr. The results are shown in table 4.d.

Table 4d: Data For Stability of Colour

Concentration of drug in mcg/ml	Time interval & Absorbance at 677nm				
	0 mins	15 mins	30 mins	45 mins	60 mins
5	0.062	0.062	0.062	0.062	0.059
10	0.122	0.122	0.122	0.122	0.124
15	0.181	0.181	0.181	0.181	0.178
20	0.242	0.242	0.242	0.242	0.244
25	0.303	0.303	0.303	0.303	0.298

3. Specific Extinction co-efficient

This was found by

= A/Lc = 125.5

4. Beer's law plot

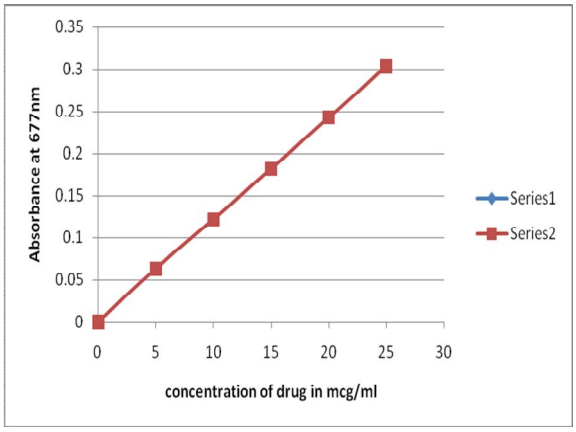
Beer's law plot was constructed by measuring the absorbance of various concentration of drug solution against the reagent blank.

From solution II, 1ml, 1.5ml, 2ml, 2.5ml & 3ml were taken into five different dry 25ml standard volumetric flasks. To that 2.5ml of hydroxylamine reagent and 5ml of sodium carbonate were added respectively. The colour was allowed to develop, the volume was made up to the mark by slowly adding sodium hydroxide solution. The absorbance was measured at 677nm against the reagent blank. The readings are shown in table no 4.d graphically plotted in graph no 4.f.

Table 4d: Data for Beer's Law Plot

Concentration of drug in mcg/ml	Absorbance at 677 nm
5	0.063
10	0.122
15	0.182
20	0.243
25	0.304

Graph 4f: Beer's Law Plot for Secnidazole with Hydroxylamine and Sodium Carbonate at 677nm



g. Assay of Tablet

Three different brands of secnidazole (Ambiform, Seczol DS, Seclong DS) were taken for analysis.

Total weight of each brand of tablet was determined. Powdered tablet equivalent to 100mg was accurately weighed and dissolved in 30ml of distilled water and treated with 10ml of 4N hydrochloric acid and 1.2 gm of zinc dust was added in portions. After standing for 1hr at room temperature the solution was filtered through cotton wool. The residue was washed with 3×10 ml portions of distilled water and the total volume of the filtrate was brought to 100ml with distilled water (solution I)

From solution I, 25ml was pipetted out into a dry 100ml standard volumetric flask and the volume was made upto mark with distilled water (Solution II)

From solution II, 1.5ml was pipetted out into a dry 25ml standard volumetric flask. To that 2.5ml of hydroxylamine reagent and 5ml of sodium carbonate were added and the volume made upto mark with sodium hydroxide solution. The absorbance was measured at 677nm against the reagent blank.

The results are shown in table no: 4.e

The same procedure was repeated five times for each brand of tablet powder.

Standard Dilution

100mg of pure Secnidazole → (Zinc/Hydrochloric acid) → 100ml(1mg/ml)

→ With distilled water

25ml → 100ml(250mcg/ml)

1.5ml → 25ml(15mcg/ml)

Test Dilution

Quantity of powder equivalent to 100mg of drug → 100ml(1mg/ml)

→ With distilled water

25ml → 100ml(250mcg/ml)

1.5ml → 25ml(15mcg/ml)

*2.5ml Hydroxylamine reagent & 5ml Sodium Carbonate reagent

The contents of drug was determined by using the formula

$$= \frac{\text{Test absorbance}}{\text{std. absorbance}} \times \frac{\text{std.wt}}{100} \times \frac{25}{100} \times \frac{1.5}{25} \times \frac{100}{\text{test wt}} \times \frac{100}{25} \times \frac{25}{1.5} \times \text{Total wt. of tablet}$$

h. Recovery Studies

The recovery experiments were performed for three different brands of secnidazole tablets. (Ambiform,Seczol DS,Seclong DS)

Powdered tablet equivalent to 100 mg was weighed accurately and transferred into a 250ml conical flask. A known quantity of pure drug was added to the flask and dissolved in 30 ml of distilled water and then treated with 10ml of 4N hydrochloric

acid and 1.2gm of zinc dust was added in portions. After standing for 1 hour at room temperature, the solution was filtered through cotton wool, the residue was washed with 3×10 ml portions of distilled water and the total volume of the filtrate was brought to 100 ml with distilled water.
The procedure for the assay of the tablet was followed. The absorbance was measured at 677nm against reagent blank.
The experiment was repeated five times for each brand of tablet. The results shown in the table no 4.f
The percentage of recovery was calculated by using the formula.

$$\text{percentage of recovery} = \frac{\text{Average content from recovery studies} - \text{Average content from assay}}{\text{Amount of std. solution added}} \text{X100}$$