Spectrophotometric Determination Of Metronidazole And Secnidazole In Pharmaceutical Preparations With Alpha Naphtol As A Chromogenic Reagent

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ABSTRACT

A rapid and sensitive spectrophotometric method is proposed for determination of metronidazole and secnidazole. The method depends on the reduction of metronidazole and secnidazole molecule with zinc dust and hydrochloric acid flowed by diazotization and coupling with α-naphtol to give red colored chromogens easily measured spectrophotometrically which has $\lambda_{max} =$ 483 nm. The experimental conditions were optimized and Beer's law was obyed over the applicable concentration ranges.both techniques were applied successfully to a wide variety of pharmaceutical preparations. © 2007 Trade Science Inc. - INDIA

INTRODUCTION

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) and secnidazole (1-(2-methyl-5-nitroimidazole-1-yl)propan-2-ol) are used as antiprotozoal, antiamebic and antibacterial drugs[1]. Excellent reviews have been published on the activity and pharmacokinetics of these drugs.

Several methods have been reported for determination of metronidazole and secnidazole which includes potentiometric[2,3], polarographic[4,5], CPG[6], supercritical fluid chromatography[7], TLC[8], HPLC[9-12], voltammetric[13], derivative spectrophotometry[14-16], flow injection analysis[17] and spectrophotometry[18-29]. Most of the spectrophotometric methods reported suffer from the disadvantage, like narrow range of determination, requires heating or extraction, long time for the reaction to complete,

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use of non-aqueous systems, stability of the coloured product formed, etc.

This paper describes sensitive and simple spectrophotometric method for the determination of metronidazole and secnidazole in either pure form or in its pharmaceuticals formulations.The method is based on the reduction of metronidazole and secnidazole molecule with zinc dust and hydrochloric acid flowed by diazotization and coupling with α-naphtol.

The scientific novelty of the present work is that the reagents used in both the method are easily available and the chemistry of these reagents is already well established. The reactions involed with these reagents are simple, rapid and sensitve in their range of determination compared with other established methods. As Metronidazole and secnidazole are important class of imidazole compouds known for their antiamebic and antiprotozoal activity, their determination in pharmaceutical is of great importance.

EXPERIMENTAL

Instrumentation

A Perkin-Elmer 551 UV-Visible spectrophotometer with 1.0 cm matched cells was used.

Reagents

All chemicals used were of analytical-reagent grade. α-Naphtol was purchased from prolabo. Sodium nitrite was purchased from prolabo. Metronidazole and secnidazole were obtained as gifts from Aventis Pharma. All other reagents and solvents were of analytical-reagent grade.

Solutions

Accurately weighed (100 mg) metronidazole or secnidazole was transferred to a 100 ml beaker. Add 1g of zinc dust along with 20ml 1M hydrochloric acid. Stir well and wait for 1h at room temperature, filter and the filtrate was diluted with water to 100ml in a volumetric flask. The working standard solution of the reduced metronidazole and secnidazole containing $100 \mu g \ ml^{-1}$ was prepared by further dilution. A 1% α-naphtol solution in 1M NaOH and 10% solution of hydroxyde de sodium were kept in am-

ber-glass volumetric flasks.

A 1% sodium nitrite. solution and a 2% sulfamic acid solution were prepared separately in distilled water.

Procedure

Aliquots of the working standard solution of reduced metronidazole or reduced secnidazole were transfered into 10 ml calibrated flasks. 1ml of 1M HCl was added, cool in an ice bath and add 2ml of 1% $NaNO_2$, stir the solution for 2 min. Add 1ml of 2% sulfamic acid, stir the solution for 1 min and add 1 ml of 1% of α-naphtol. After 2min made up to the mark with 10% of NaOH solution.

Assay of pharmaceutical tablets

Twelve tablets were powdered and mixed thoroughly. An amout equivalent to 100 mg of the drug was reduced as mentioned in and the filtrate was made up to 100ml and an aliquot of this solution was treated as described above for pure sample in both the method.

RESULTS AND DISCUSSION

The spectrophotometric method for the determination of metronidazole and secnidazole is based on the reduction of the nitro to an amino group with zinc dust and hydrochloric acid flowed by diazotation and coupling with a-naphtol to give red colored product.

Spectral characteristics and reaction mechanism

The absorption spectra of the red coloured product with $\lambda_{max} = 483$ nm are shown in. The reagent blank has pratically negligible absorption at this wavelength. The stochiometric equation derived was shown in SCHEME 1.

Optimization of reactions conditions

The factors affecting color development, reproducibility, sensitivity, and conformity with Beer's law were investigated.

For the maximum formation of azodye, a volume of 1% $NaNO_2$ solution was optimized. The results are shown in TABLE1. As can be seen, 2ml of nitrite solution was found to be the optimum vol-

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SCHEME 1: Reaction sequence for the formation of red azo colored product
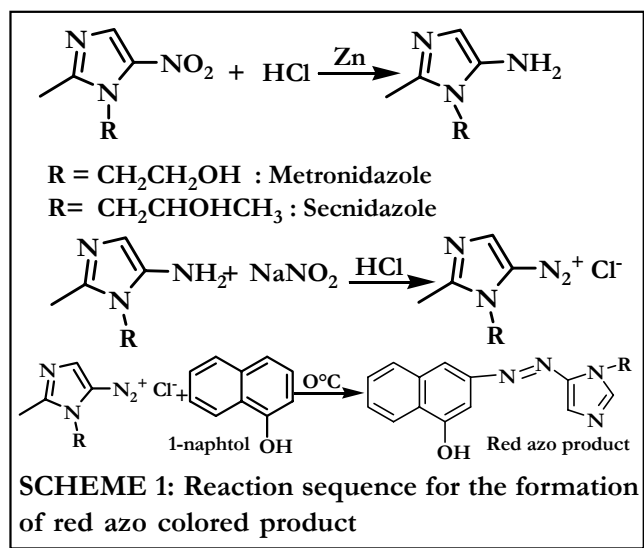
TABLE 1: Optimisation of the volume of nitrite solution for the formation of azodye

Serial no.	Vol. of nitrite solution (ml)	Absorbance
1	0.5	0.476
2	1	0.517
3	2	0.520
4	3	0.518
5	4	0.509
6	5	0.488
Conditions		
λ_{max}	483 nm	-
Metronidazole solution	10 ppm	-
α-naphtol (1%)	1 ml	-
HCl (1N)	1 ml	-
Sulfamic acid (2%)	1 ml	-

ume for maximum azodye formation.

The volume of 1M hydrochloric acid was also optimized. The results are shown in TABLE 2. As can be seen, 1ml of HCl was found to be the optimum volume.

In the next step, the amount of the coupling reagent, α-naphtol, was optimized (see TABLE 3). As seen in TABLE 3, 3ml of 1% α-naphtol solution was found to be the optimum volume.

The excess of nitrite sodium could be removed by the addition of sulfamic acid. Therefore, the volume of 2% sulfamic acid solution was optimized (the results are given in TABLE 4). As can be seen, 1ml of sulfamic acid was found to be the optimum volume.

TABLE 2: Optimisation of the volume of HCl solution for the formation of azodye

Serial no.	Vol. of HCL solution (ml)	Absorbance
1	0.5	0.513
2	1	0.518
3	2	0.516
4	3	0.501
5	4	0.491
Conditions		
λ_{max}	483 nm	-
Metronidazole solution	10 ppm	-
α-naphtol (1%)	1 ml	-
NaNO$_2$ (1%)	2 ml	-
Sulfamic acid (2%)	1 ml	-

TABLE 3: Optimisation of the volume of 1-naphtol solution for the formation of azodye

Serial no.	Vol. of α-naphtol (ml)	Absorbance
1	0.5	0.430
2	1	0.515
3	2	0.517
4	3	0.519
5	4	0.505
6	5	0.466
Conditions		
λ_{max}	483 nm	-
Metronidazole solution	10 ppm	-
NaNO$_2$ (1%)	2 ml	-
HCl (1N)	1 ml	-
Sulfamic acid (2%)	1 ml	-

Quantification

Beer's law is obeyed over the metronidazole concentration range of 1-15 µg/ml. Similarly, for secnidazole, Beer's law is obeyed over the concentration range of 1-15 µg/ml The proposed procedure is validated by determining various optical parametrs, which are listed in TABLE 5.

Interference

The extent of interference by common ions were determined by mesuring the absorbance of a solution containing 20 µg ml^{-1} of metronidazole or secnidazole

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TABLE 4: Optimisation of volume of sulfamic acid for the formation of azodye

Serial no.	Vol. of Sulfamic acid (ml)	Absorbance
1	0	0.418
2	0.5	0.499
3	1	0.518
4	2	0.515
5	3	0.524
Conditions		
λ_{max}	483 nm	-
Metronidazole solution	10 ppm	-
$NaNO_2$ (1%)	2 ml	-
HCl (1N)	1 ml	-
α-naphtol (1%)	1 ml	-

TABLE 5: Parameters for the spectrophotometric determination of metronidazole and secnidazole

Parameters\Characteristics	Secnidazole	Metronidazole
Color	Red	Red
λ_{max} (nm)	483	483
stability (in days)	3	3
Beer's law range (μg ml^{-1})	1-15	1-15
Molar absorptivity (l mol^{-1}cm^{-1})	1.27 10^4	1.27 10^4
Regression equation[a]		
Slop(a)	0.0519	0.0545
Intercept (b)	-0.001	-0.023
Correlation coefficient	0.9997	0.9996
R.S.D.(%)[b]	0.50	0.55

a. y= ax + b where x is the concentration of Metronidazole or Secnidazole in μg ml^{-1} ; b. five replicates

and various amouts of diverse species. Majority of the common ions do not interfere. An error of 2% in the absorbnce readings was considered tolerable. Some of the common excipients, which often accompany the pharmaceutical preparations, do not interfere in the present method.the results are given in TABLE 6.

Analysis of pharmaceutical preparation

Application of the proposed method to the determination of metronidazole and secnidazole drugs in its dosage forms was successfully made; the results are presented in TABLE 7. The excellent

TABLE 6: Determination of metronidazole and secnidazole in presence of excipients

Excipients	Amount (mg)	Recovery of MNZ[a], %(± RSD[b])	Recovery of SCN[a], %(± RSD[b])
Magnesium stearate	40	100,4± 0,60	100,3± 0,50
Carboxy methylcellulose	50	101,5± 0,54	101.1± 0,75
Lactose	30	99.5± 0,78	99.9± 0,75
Glucose	30	100.4± 0,93	98.3± 0,45
Sorbitol	50	99.9± 0,65	101.1± 0,40
Hypromellose	50	100.9± 0,90	100.7± 0,56
Povidone	50	101.3± 0,70	101.1± 0,89
Talc	50	99.7± 0,45	98.7± 0,90
Cellulose	30	99.8± 0,60	98.1± 0,71

a. 10 μg ml^{-1} of metronidazole and secnidazole taken; b. average of five determination

TABLE 7: Analysis of Metronidazole and secnidazole in pharmaceutical preparation

Commercial formulations analyzed	Content	Label claim in mg	Recovery[a], %(± RSD[b])
Flagyl® 250	Metronidazole	250/tablet	100.8(±1.33)
Flagyl® 500	Metronidazole	500/tablet	99.2(± 1.40)
Nidazol® 500	Metronidazole	500/tablet	100.9(±1.10)
Synthetic mixture	Metronidazole	500	98.8(±1.20)
Flagentyl®500	Secnidazole	500/tablet	99.7(±1.25)
Synthetic mixture	Secnidazole	500	100.1(±0.80)

recoveries obtained indicated the absence of any interference from the excipients.

CONCLUSION

The method is found to be simple, economical, selective and more sensitive than most of the spectrophotometric methods reported. The statistical parametrs and recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of the authentic samples containing metronidazole and secnidazole showed no interference from the common excipients. Hence, this approach could be considered for the determination of metronidazole and secnidazole in the quality control laboratories.

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