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Short communication

# Spectrophotometric determination of Metronidazole and Secnidazole in pharmaceutical preparations based on the formation of dyes

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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 β-naphtol to give red colored chromogens easily measured spectrophotometrically which has $\lambda_{max} = 510$ nm. The experimental conditions were optimized and Berr's law was obeyed over the applicable concentration ranges. Both techniques were applied successfully to a wide variety of pharmaceutical preparations.


*Keywords:* Metronidazole; Secnidazole; Diazotization; β-Naphtol; Spectrophotometry

## 1. 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 [18–26]. 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, use of non-aqueous systems, stability of the colored 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 β-naphtol.

The scientific novelty of the present work is that the reagents used in both the method is easily available and the chemistry of these reagents is already well established. The reactions involved with these reagents are simple, rapid and sensitive in their range of determination compared with other established methods. As Metronidazole and Secnidazole are important class of

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imidazole compounds known for their antiamebic and antiprotozoal activity, their determination in pharmaceutical is of great importance.

## 2. Material and methods

### 2.1. Instrumentation

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

### 2.2. Reagents

All chemicals used were of analytical-reagent grade. β-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.

### 2.3. Solutions

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

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

### 2.4. Procedure

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

### 2.5. Assay of pharmaceutical tablets

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

## 3. 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 diazotization and coupling with β-naphtol to give red colored product.

### 3.1. Spectral characteristics and reaction mechanism

The absorption spectra of the red colored product with $\lambda_{max} = 510$ nm are shown in. The reagent blank has practically negligible absorption at this wavelength. The stochiometric equation derived was shown in Scheme 1.

### 3.2. Optimization of reaction conditions

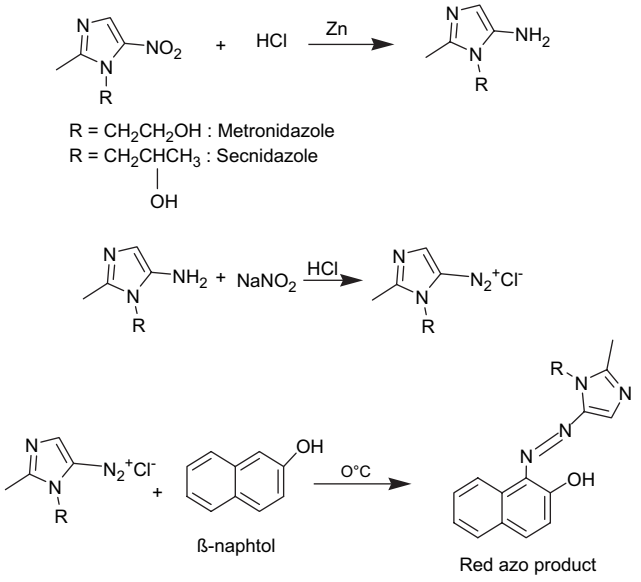
The factors affecting color development, reproducibility, sensitivity, and conformity with Beer's law were investigated.

It was found that, 0.5–3 ml of 1 M HCl, 1–3 ml of 1% $NaNO_2$ solution and 1–2 ml of 1% β-naphtol solution were necessary to achieve maximum color intensity.

The excess of sodium nitrite could be removed by the addition of 1 ml of 2% sulfamic acid solution. An excess of sulfamic acid has no effect on the color intensity of the product formed.

### 3.3. Quantification

Beer's law is obeyed over the Metronidazole concentration range of 5–50 μg $ml^{-1}$. Similarly, for Secnidazole,




Scheme 1. Reaction sequence for the formation of red azo colored product.

Table 1
Parameters for the spectrophotometric determination of Metronidazole and Secnidazole

| Parameters\characteristics | Secnidazole | Metronidazole |
|---|---|---|
| Color | Red | Red |
| $\lambda_{max}$ (nm) | 510 | 510 |
| Stability (in days) | 2 | 2 |
| Beer's law range ($\mu g\,ml^{-1}$) | 2–30 | 5–50 |
| Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$) | $6.60 \times 10^3$ | $2.68 \times 10^3$ |
| Regression equation[a] | | |
| Slope ($a$) | 0.0370 | 0.0162 |
| Intercept ($b$) | −0.019 | −0.018 |
| Correlation coefficient | 0.9992 | 0.9994 |
| R.S.D. (%)[b] | 0.65 | 0.49 |

[a] $y = ax + b$ where $x$ is the concentration of Metronidazole or Secnidazole in $\mu g\,ml^{-1}$.
[b] Five replicates.

Beer's law is obeyed over the concentration range of 2–30 $\mu g\,ml^{-1}$. The proposed procedure is validated by determining various optical parameters, which are listed in Table 1.

### 3.4. Interference

The extent of interference by common ions were determined by measuring the absorbance of a solution containing 20 $\mu g\,ml^{-1}$ of Metronidazole or Secnidazole and various amounts of diverse species. Majority of the common ions do not interfere. An error of 2% in the absorbance 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 2.

### 3.5. 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 3. The excellent recoveries obtained indicated the absence of any interference from the excipients.

Table 2
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 | 101.4 ± 0.50 | 99.3 ± 0.62 |
| Carboxy methylcellulose | 50 | 99.5 ± 0.46 | 101.5 ± 0.70 |
| Lactose | 30 | 100.5 ± 0.66 | 99.6 ± 0.75 |
| Glucose | 30 | 99.4 ± 0.85 | 99.3 ± 0.38 |
| Sorbitol | 50 | 100.9 ± 0.65 | 100.1 ± 0.40 |
| Hypromellose | 50 | 99.9 ± 0.50 | 100.7 ± 0.45 |
| Povidone | 50 | 100.3 ± 0.40 | 101.1 ± 0.30 |
| Talc | 50 | 99.7 ± 0.66 | 99.7 ± 0.90 |
| Cellulose | 30 | 100.8 ± 0.80 | 99.1 ± 0.75 |

[a] 20 $\mu g\,ml^{-1}$ of Metronidazole and Secnidazole taken.
[b] Average of five determination.

Table 3
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 | 99.8(±1.1) |
| Flagyl® 500 | Metronidazole | 500/tablet | 99.2(±1.5) |
| Nidazol® 500 | Metronidazole | 500/tablet | 100.3(±0.8) |
| Synthetic mixture | Metronidazole | 500 | 99.1(±1.7) |
| Flagentyl® 500 | Secnidazole | 500/tablet | 99.7(±0.7) |
| Synthetic mixture | Secnidazole | 500 | 100.5(±1.3) |

[a] Average of five determination.
[b] Relative standard deviation.

## 4. Conclusion

The method is found to be simple, economical, selective and more sensitive than most of the spectrophotometric methods reported. The statistical parameters 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.

## References

[1] Martindale the extra pharmacopoeia. 27th ed. London: The Pharmaceutical Press; 1977. p. 1570.
[2] The United States pharmacopoeia. 24th ed. Rockville, MD 20852: United States Pharmacopeial Convention, Inc.; 1999. p. 1104.
[3] The United States pharmacopoeia. 20th ed. Rockville, MD 20852: United States Pharmacopeial Convention, Inc.; 1980. p. 530.
[4] Gratteri P, Cruciani G. Analyst 1999;124:1683.
[5] El-Sayed GO. Microchem J 1997;55:110.
[6] Wang JH. J Chromatogr A 2001;918:435.
[7] Bari Viddesh R, Dhorda UJ, Sundaresan M. Anal Chim Acta 1998;376:221.
[8] Zarapkar SS, Kanyawar NS. Ind Drugs 1999;36:293.
[9] Nevin E, Levent M, Altun M. J Pharm Biomed Anal 2001;25:115.
[10] Daeseleire Els, De Ruyck H, Van Renterghem R. Analyst 2000;125:1533.
[11] Ravisankar S, Vasudevan M, Nanjan MJ, Gandhimathi M, Suresh B. Ind Drugs 1998;35:359.
[12] Raj SV, Kapadia SU, Argekar AP. Ind Drugs 1997;34:585.
[13] Warowna M, Fijalek Z, Dziekanska A, Korzeniewska A. Acta Pol Pharm 1991;48:17.
[14] Vega E, Sola N. J Pharm Biomed Anal 2001;25:523.
[15] Erk N. STP Pharma Sci 1996;6:312.
[16] Galal SM, Bedair MM, El-Sayed MA. J Pharm Belg 1991;45:315.

[17] Palomeque M, Garcia Bautista JA, Garcia Mateo JV, Martinez Calatayud J. Anal Chim Acta 1999;401:229.
[18] Nagaraja P, Sunitha KR, Vasantha RA, Yathirajan HS. J Pharm Biomed Anal 2002;28:527.
[19] El Walily A, Abdine HH, Abdel Razak O, Zamel S. J Pharm Biomed Anal 2000;22:887.
[20] Jadhav GP, More HN, Mahadik KR. Ind Drugs 1998;35:475.
[21] Das TK, Halder D. Ind Drugs 1992;29:165.
[22] Chatterjee PK, Jain CL, Sethi PD. Ind Drugs 1987;24:264.
[23] Srinath V, Bagavant G. Ind Drugs 1986;24:173.
[24] Sastry CSP, Aruna M, Rao ARM. Talanta 1988;35:23.
[25] Chatterjee PK, Jain CL, Sethi PD. Indian J Pharm Sci 1986;48:195.
[26] Talwar SK, Sharma SC, Das S. J Pharm Biomed Anal 1986;4:511.