International Journal of Applied
and Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 2, Issue 5, Nov 2013, 21-26


International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

# FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND NORFLOXACIN IN THEIR COMBINED DOSAGE FORM

**RAJYA LAKSHMI CH[1] & RAMBABU C[2]**

[1]Department of Chemistry, Vishnu Institute of Technology, Bhimavaram, Andhra Pradesh, India

[2]Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

## ABSTRACT

Derivative spectroscopy offers a useful approach for the analysis of drugs in mixtures. In this study First order derivative spectrophotometric method was developed for the simultaneous determination of Metronidazole (MET) and Norfloxacin (NOR) in bulk and combined tablet dosage form. The derivative spectra for both the methods were obtained in methanol and the linearity was obtained in the concentration range of 1.25 – 6.25 µg/ml for Metronidazole and 1.0 - 5.0µg/ml for Norfloxacin. The zero order spectra are obtained at wavelengths 311nm for Metronidazole and 283nm for Norfloxacin. First order derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP).

The determinations were made at 216 nm (ZCP of Norfloxacin) for Metronidazole and 209 nm (ZCP of Metronidazole) for Norfloxacin. The mean recovery was 99.8 and 100.7 for Metronidazole and Norfloxacin respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Metronidazole and Norfloxacin in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

**KEYWORDS:** Metronidazole, Norfloxacin, Zero Order Spectra, First Order Spectra, Zero Crossing Point Validation

## INTRODUCTION

Metronidazole (MET), chemically known as 2-(2-methyl-5-nitro-1*H*- imidazol-1-yl) ethanol (figure 1) is an antibiotic, antiprotozoal, amoebicidal, bactericidal and trichomonicidal. Metronidazole is used to treat certain infections of the urinary and genital systems caused by bacteria. Literature survey reveals that a few spectrophotometric[1], RP-HPLC[2, 3] methods are reported for the estimation of Metronidazole individually and in combination with other drugs. Norfloxacin (NOR) is a synthetic chemotherapeutic antibacterial agent used to treat urinary tract infections. Chemically it is 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-*1H*-quinoline-3-Carboxylic acid. Norfloxacin (figure-2) is a first generation synthetic fluoroquinolone.

The combination of Metronidazole and Norfloxacin used to treat diarrhea caused by various micro-organisms such as bacteria and protozoa. A survey of the analytical literature for Norfloxacin revealed that methods based on TLC-Densitometric [4], UV spectrophotometric methods [5-7] for its determination in pharmaceutical formulations individually and in combination with other drugs is reported. Few HPLC methods [8, 9] were reported for simultaneous estimation of above mentioned drugs. Literature survey does not reveal any simple first order derivative spectrophotometric method for simultaneous estimation of MET and NOR in combined dosage forms. The present communication describes a simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on derivative spectroscopy for simultaneous estimation of both drugs in bulk and combined dosage forms.

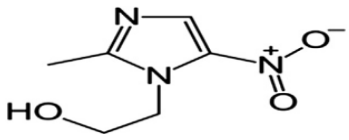

Figure 1

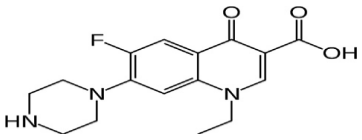

Figure 2

## MATERIALS AND METHODS

### Apparatus

A Lab India model 3000 double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Win system software. The zero order and first derivative absorption spectra were recorded over the wavelength range 200-400 nm against the solvent blank.

### Reagents and Materials

Metronidazole and Norfloxacin were obtained as gift samples from Dr. Reddy's Laboratories, Hyderabad. Sample tablet (Nor-metrogyl with Metronidazole 500 mg & Norfloxacin 400 mg) was purchased from local market. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

### Preparation of Standard Stock Solution

The standard stock solutions of both the drugs were prepared. 10mg of both the drugs were weighed separately and transferred into a 100 ml volumetric flask. The compounds are then dissolved separately in methanol and made up to the mark with methanol. Further, dilute 3.75 ml MET and 3 ml NOR into a 100 ml volumetric flask and make up to the mark with methanol

### Determination of Absorption Maxima

Standard stock solutions were scanned separately in the range of 200-400 nm to determine the maximum absorption for both the drugs. Metronidazole showed absorbance maxima at 311 nm and Norfloxacin at 283nm (Figure-3). The zero order spectra thus obtained was then processed to obtain first derivative spectra. The first order derivative spectra were overlain and it appeared that MET show zero crossing at 209 nm, while NOR showed zero crossing at 216 nm (figure-4). At the zero crossing point of MET, Norfloxacin showed a first derivative absorbance, whereas at the zero crossing point of NOR, Metronidazole showed a first derivative absorbance. Hence 216 and 209 nm was selected as analytical wavelengths for determination of MET and NOR, respectively. These two wavelengths can be employed for the determination of MET and NOR without any interference from the other drug in their combined dosage formulations.

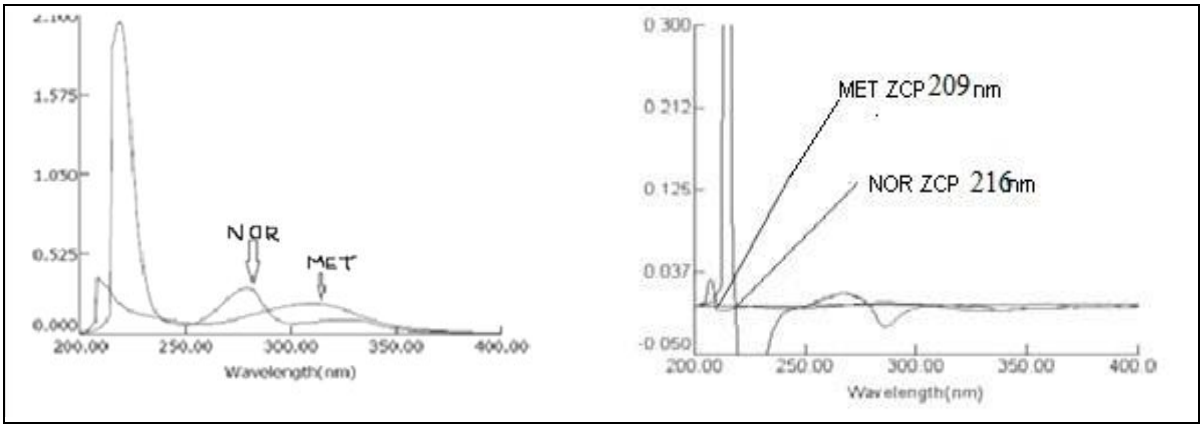


Figure 3: Zero Order Spectra




Figure 4: Overlain First Order Spectra

**Validation of the Proposed Method**

The proposed methods were validated according to the International Conference on Harmonization ICH guidelines

**Linearity**

The calibration curves were plotted over a concentration range of 1.25 – 6.25 µg/ml for Metronidazole and 1-5 µg/ml for Norfloxacin. Accurately measured standard solutions of MET (1.25, 2.5, 3.75, 5.0 and 6.25ml) and NOR (1.0, 2.0, 3.0, 4.0 and 5.0ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. First order derivative absorbance was measured at 216 and 209 nm for MET and NOR respectively. The calibration curves were constructed by plotting absorbance versus concentrations and the regression equations were calculated (figure- 5, 6).

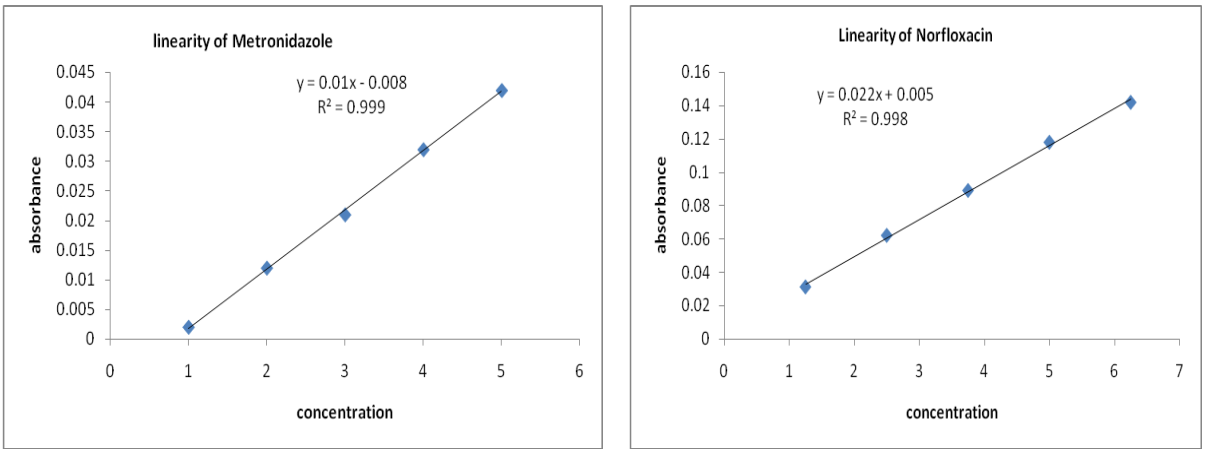


**Figure 5, 6: Calibration Curves of MET and NOR**

**Accuracy (Recovery Studies)**

The accuracy of the method was determined by calculating recoveries of MET and NOR by the standard addition method. Known amounts of standard solutions of MET and NOR were at added at 50, 100 and 150% level to pre quantified sample solutions. The amounts of MET and NOR were estimated by applying obtained values to the respective regression line equations (Table-1)

**Table 1: Recovery Data of MET and NOR by Proposed Method**

| % of Concentration (at Specification Level) | Amount Added (mg) | | Amount Found (mg) | | % of Recovery | | Mean Recovery | |
|---|---|---|---|---|---|---|---|---|
| | MET | NOR | MET | NOR | MET | NOR | MET | NOR |
| 50 | 5.0 | 5.0 | 4.95 | 5.06 | 99.03 | 101.3 | 99.8% | 100.76% |
| 100 | 10.0 | 10.0 | 9.90 | 10.11 | 99.04 | 101.1 | | |
| 150 | 15.0 | 15.0 | 15.23 | 14.97 | 101.5 | 99.8 | | |

**Precision and Intermediate Precision**

The Intraday (repeatability) and Inter day precisions (reproducibility) of the proposed UV spectrophotometric method were determined by estimating the corresponding response three times on the same day and on different day for concentration of Metronidazole (3.75µg/ml) and Norfloxacin (3.0µg/ml) and the results are reported in terms relative standard deviation. The results of precision studies are reported in Table -3 and values of standard deviation less than 2% indicates high degree of precision.

**Limit of Detection and Limit of Quantification**

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the five replicate determinations, standard deviation (SD) of the responses was calculated. From these values, the parameters Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined on the basis of standard deviation and slope of the regression equation.$LOD = 3.3 \times \sigma/S$; $LOQ = 10 \times \sigma/S$

Where, $\sigma$ = the standard deviation of the response and S = slope of the calibration curve

**Analysis of MET and NOR in Combined Tablet Dosage form (Assay)**

The average weight of tablets is determined with the help of weight of 20 tablets. A portion of powder equivalent to 10 mg was accurately weighed and was transferred into a dry 100 ml volumetric flask diluted up to mark with methanol. The solution was filtered and sonicated for 5 min. Then 3.75 ml of sample stock solution is taken in a 100 ml volumetric flask and diluted with methanol up to the mark. Similar method as above was applied to the determination of MET and NOR in a commercial tablet formulation. The results are shown in table-2. The results obtained were in satisfactory agreement with the label claims, within the acceptable limits.

**Table 2: Assay of MET and NOR in Tablet Formulations**

| Drug | Label Claim (mg) | Amount Found (mg) | % of Recovery |
|---|---|---|---|
| Metronidazole | 500 | 504.5 | 100.9 |
| Norfloxacin | 400 | 395.56 | 98.89 |

## RESULTS AND DISCUSSIONS

The standard solutions of MET and NOR were scanned separately in the UV range 200-400 nm. Zero order spectra (figure - 3) thus obtained was then processed to obtain first derivative spectra. Sampling wavelengths selected for quantification were 216 nm (ZCP of Norfloxacin) for Metronidazole and 209 nm (ZCP of Metronidazole) for Norfloxacin. First derivative spectra give good quantitative determination of both the drugs at their respective without any interference from the other drug in their combined dosage formulation. The proposed method is validated as per ICH guidelines. Both the drugs obey the Beer's law with the concentration ranges of 1.25 – 6.25 µg/ml for Metronidazole and 1.0-5.0 µg/ml for Norfloxacin. For this method regression equations generated were Y= 0.01x + -0.008 ($R^2$=0.999) and Y= 0.022x + 0.005 ($R^2$=0.998) for MET and NOR respectively. The low percentage of RSD values of inter day (0.8 and 0.2) and intraday (0.4 and 1.7) for MET and NOR respectively, reveals that the proposed method is precise. LOD and LOQ values were found to be 0.14 and 0.45 µg/ml for MET and 0.05 and 0.15µg/ml for NOR, respectively. These shows that proposed method is sensitive. The % mean recovery was found to be 99.8% and 100.76% for MET and NOR respectively, shows that the method is accurate. The % assay was found to be 100.9% and 98.89% for MET and NOR, respectively. No interference of the excipients with the absorbance appeared. Hence the method is applicable for the routine simultaneous estimation of MET and NOR in pharmaceutical dosage form. Summary of validation parameters for MET and NOR by first order derivative spectrophotometric method was shown in Table – 3

**Table 3: Regression Analysis Data and Summary of Validation Parameters for MET and NOR by First Order Spectrophotometric Method**

| Parameters | MET | NOR |
|---|---|---|
| Wave length (nm) | 209 | 216 |
| Beer's law limit (µg/ml) | 1.25 - 6.25 | 1.0 – 5.0 |
| Slope | 0.01 | 0.022 |

**Table 3: Contd.,**

| | | |
|---|---|---|
| intercept | -0.008 | 0.005 |
| Regression equation | Y= 0.01x + -0.008 | Y= 0.022x + 0.005 |
| Correlation coefficient | $R^2$=0.999 | $R^2$=0.998 |
| LOD (µg/ml) | 0.14 | 0.05 |
| LOQ (µg/ml) | 0.45 | 0.15 |
| Accuracy (% Recovery) | 99.8% | 100.7% |
| Precision (%RSD) | | |
| Inter day | 0.84 | 0.24 |
| Intra day | 0.49 | 1.71 |

## CONCLUSIONS

The proposed first order derivative spectrophotometric method for simultaneous estimation of Metronidazole and Norfloxacin in their combined dosage form is novel, accurate, precise and reproducible. Moreover the method is simple, rapid, and economic and does not involve the use of complex instrument such as HPLC, which is expensive in both the hardware and chromatographic reagents hence can be employed for routine analysis in quality control laboratories.

## REFERENCES

1. Nagaraja P, Sunitha K R, Vasantha RA, Yathirajan HS. Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations. J Pharm Biomed Anal. 2002; 28(3-4): 527-535.
2. Tashtoush Bassam M, Jacobson Elaine L, Jacobson Myron K. Validation of a simple and rapid HPLC method for determination of metronidazole in dermatological formulations. Drug Dev Ind Pharm. 2008; 34(8):840-844.
3. Sebai Mahmoud M, El-Shanawany Abdullah A, El-Adl Sobhy M, Abdel-Aziz Lobna M, Hashem Hisham A. Rapid RP-HPLC method for simultaneous estimation of sparfloxacin, gatifloxacin, metronidazole and tinidazole. Asian J Pharm Res. 2011; 1(4):119-125.
4. Saleh Gamal A, Askal Hassan F, Refaat Ibrahim H, Abdelaal Fatma AM. Stability indicating assay and kinetic study for norfloxacin using TLC-densitometric method. J Liq Chromatogr Relat Technol. 2013; 36(4):454-469.
5. Maheshwari R K. Application of hydrotropic solubilization phenomenon in spectrophotometric estimation of Norfloxacin in tablets. Indian J Pharm Educ Res. 2006; 40(4): 237-240.
6. Wankhede SB, Prakash A, Chitlange SS. Simultaneous spectrophotometric estimation of norfloxacin and ornidazole in tablet dosage form. Int J Chem Tech Res. 2009; 1(4):937-940.
7. Maliwal Deepika, Jain Anurekha, Maheshwari R K, Patidar Vidyasagar. Simultaneous spectrophotometric estimation of metronidazole and norfloxacin in combined tablet formulations using hydrotropy. Res J Pharm Technol. 2008; 1(4): 357-361.
8. Ghante Minal R, Pannu Harpreet K, Loni Amruta, Shivsharan Tejashree. Development and validation of A RPHPLC method for simultaneous estimation of Metronidazole and norfloxacin in bulk and tablet dosage form. Int J Pharm Pharm Sci. 2012; 4(4):241-245.
9. Mahalingam K, Rajarajan S. Estimation of metronidazole and norfloxacin in formulations by reverse phase HPLC method. Arch Pharm Sci Res. 2009; 1(2):162-165.