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# METHOD DEVELOPMENT AND VALIDATION OF METRONIDAZOLE BY RP-HPLC METHOD

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**ABSTRACT**

A simple, precise, accurate and reproducible RP-HPLC method for simultaneous estimation of Metronidazole tablet. Isocratic elution at a flow rate of 1.0 mL/min was carried on YMC Pack Pro C18 column (250 mm x 4.6mm x 5µ) on using mobile phase of sodium dihydrogen phosphate buffer and methanol (65:35 v/v).The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 50-150 mg/mL,the correlation coefficient was found to be 0.999, the limit of detection (LOD) was 0.743 the limit of quantification (LOQ) was 2.475 & the relative standard deviation (RSD) of six replicates is less than 2%. This HPLC method is applied successfully to the simultaneous quantitative analysis of Metronidazole in commercial tablets.

## INTRODUCTION

Metronidazole chemically is 2-(2-methyl-5-nitro-1Himidazol-1-yl) ethanol. Metronidazole is a nitro imidazole antibiotic drug used against anaerobic organisms, amoebozoa infections and antiprotozoal. It is frequently used for mild-to moderate Clostridium difficile infection. Metronidazole is also used to treat bacterial vaginosis, pelvic inflammatory disease, pseudo membranous colitis, aspiration pneumonia, rosacea, fungating wounds, intra-abdominal infections, lung abscess, gingivitis, amoebiasis, giardiasis, trichomoniasis, and infections caused by susceptible anaerobic organisms such as Bacteroides fragilis spp, Fusobacterium spp, Clostridium spp, Peptostreptococcus spp and Prevotellaspp. It has a molecular formula of $C_6H_9N_3O_3$and a molecular weight of 171.15 g/mol.

## MATERIALS AND METHOD:

### Chemicals and Reagents

Metronidazole was purchased from S D Fine Chemicals Ltd., Mumbai. Sodium dihydrogen phosphate and methanol of HPLC grade was purchased from Merck (India) Ltd., Mumbai. Ortho phosphoric acid of analytical reagent grade was obtained from S D Fine Chemicals Ltd., Mumbai. Distilled water was used throughout the process.

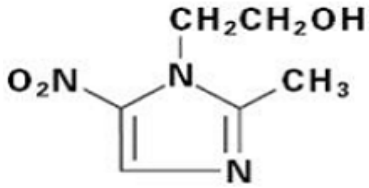


**Fig No.1 Structure of Metronidazole**

### Instrument and Analytical Conditions:

The HPLC analysis was carried on Shimadzu Lc-2010 CHT system with UV-Visible detector with Auto sampler. The analytical column used to achieve chromatographic separation was

YMC Pack Pro C18, (250 mm × 4.6; 5µm) column. The mobile phase consisting of sodium dihydrogen phosphate buffer (pH 5.8) and methanol was degassed and pumped from the solvent reservoir in the ratio of 65:35 v/v. The flow rate was 1.0mL/min. The column temperature was maintained at 30°C. The detection was performed at 270 nm and the run time was 12 min. Injection was carried out using a 10 µL loop.

**Standard Solution:**

400mg of Metronidazole was accurately weighed, dissolved in mobile phase and diluted to volume in a 100 mL volumetric flask. Pipette out 2.5 mL of the above standard stock standard stock into 100 mL volumetric flask and dilute to volume with mobile phase.

**Sample Solution:**

Accurately weigh 915.50 mg of sample. Transfer the sample powder into 100 mL volumetric flask. Add 10 mL mobile phase and sonicate for 20 minutes. The resulting solution was made up to the volume with mobile phase. Filter through the 0.45 µm Whatman filter paper. Transfer 2.5 mL of the above solution into a 100 mL volumetric flask and made up to the volume with mobile phase.

**METHOD VALIDATION [4-10]**

**System Suitability**

System suitability tests are an integral part of liquid chromatographic method. System suitability was checked on each day of validation to evaluate the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established are number of theoretical plates, resolution and tailing factor.

**Linearity**

The linearity of the proposed method was constructed for Metronidazole standard solutions by plotting the concentrations of the compound versus peak area response. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

**Accuracy and Precision**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried on the selected drugs at three different concentration levels (50%, 100% and 150%). The percentage recovery and standard deviation of the percentage recovery were calculated. The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage relative standard deviation were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage relative standard deviation were calculated.

**Robustness**

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as composition of mobile phase ratio and temperature of the column, and studying its effects on the performance of the method.

**LOD and LOQ**

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Metronidazole. The LOQ and LOD values were calculated by using the following formula

1. $LOQ = 10\ \sigma / S$
2. $LOD = 3.3\ \sigma / S$

Where $\sigma$ = residual standard deviation of response; S = slope of the calibration curve.

**Results:**

**System Suitability Studies**

The column efficiency, resolution and tailing factor were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System suitability parameters may fallwithin ±2 % Relative standard deviation range during routine performance of the method

**Linearity** : The linearity of the method was determined at five concentration levels. The calibration curve was plotted by plotting response factor against concentration of drugs 50-150 µg/mL .The regression equations was

$y = 64152x - 35747 (R2 = 0.9999)$

Where y = peak area and x = concentration of the drug in µg/mL. The results show an excellent correlation exists between areas and concentration of drugs. The results for calibration data are shown in Table no.2 Accuracy and Precision

The results of accuracy of proposed methods at three different concentration levels are summarized in Table. The chromatograms at three different levels are shown in Figure. From the results obtained, added recoveries of standard drugs were found to be accurate.

**Precision:**

The precision of the method was demonstrated by inter-day and intra-day variation studies. The results of the precision studies are tabulated in the Table . From the results obtained, the developed method was found to be precise.

**Robustness**

Robustness of the method was determined by making slight changes in the chromatographic conditions such as column temperature and mobile phase flow rate. It was observed that there were no marked changes in the analytical performance of the method. The results are shown in Table 7. The results demonstrated that the proposed method is robust.

**Limit of quantification and limit of detection**

Limit of quantification (LOQ) and limit of detection (LOD) gives information about the sensitivity of the method. The LOD and LOQ values are presented in Table . The results indicated that the proposed method possess sufficient sensitivity.

**Table No.1 System Suitability**

| Parameters | Metronidazole Sample |
|---|---|
| Retention time | 7.915 |
| Theoretical Plate | 4762 |
| Tailing Factor | 1.30 |
| % RSD | 0.9 |

**Table 2: Linearity data**

| Sr No. | Conc.of drug µg/mL | Peak Area |
|---|---|---|
| 1 | 50 | 3191470 |
| 2 | 75 | 4746135 |
| 3 | 100 | 6381099 |
| 4 | 125 | 7987977 |
| 5 | 150 | 9589540 |

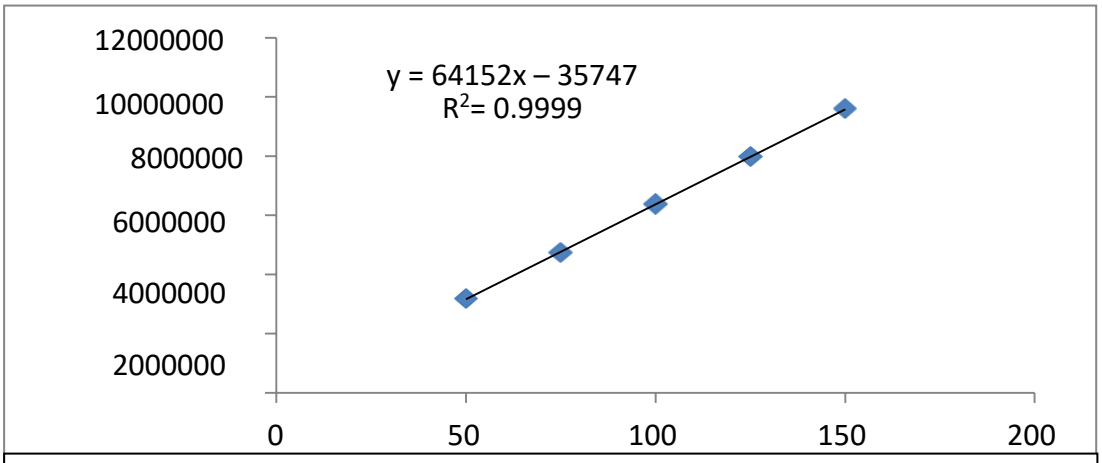


**Figure 2: Linearity curve for Metronidazole**

**Table 3: Accuracy for Metronidazole**

| Accuracy level | Sample weight | µg/mL added | µg/mL found | % Recovery | % Mean |
|---|---|---|---|---|---|
| 50% | 457.75 | 49.500 | 49.74 | 100 | 100 |
| | 457.75 | 49.500 | 49.75 | 101 | |
| | 457.75 | 49.500 | 49.65 | 100 | |
| 100% | 915.50 | 99.000 | 99.40 | 100 | 100 |
| | 915.50 | 99.000 | 99.35 | 100 | |
| | 915.50 | 99.000 | 99.43 | 100 | |
| 150% | 1373.25 | 148.500 | 149.23 | 100 | 100 |
| | 1373.25 | 148.500 | 149.24 | 100 | |
| | 1373.25 | 148.500 | 149.16 | 100 | |

**Table 4: Precision of the method**

| Sample No. | Peak Area | % Assay |
|---|---|---|
| 1 | 6388399 | 99 |
| 2 | 6385175 | 99 |
| 3 | 6383473 | 99 |
| 4 | 6385571 | 99 |
| 5 | 6386220 | 99 |
| 6 | 6388474 | 99 |

**Table 5: Robustness of the method**

| Sample No. | Sample Name | Retention Time | Peak Area | Theoretical Plates | USP Tailing |
|---|---|---|---|---|---|
| 1 | Temp-1 | 9.670 | 6269893 | 3209 | 1.11 |
| 2 | Temp-2 | 8.776 | 6348317 | 3725 | 1.15 |
| 3 | Flow-1 | 12.009 | 7866323 | 4458 | 1.09 |
| 4 | Flow-2 | 7.957 | 5206920 | 3517 | 1.21 |

**Table 6: LOD and LOQ**

| Sample Type | Sample Name | Retention Time | Peak Area | Value |
|---|---|---|---|---|
| LOD | METZ 1 | 9.600 | 2404260 | 0.745 |
| LOQ | METZ 2 | 9.612 | 3410520 | 2.455 |

## CONCLUSION

The proposed HPLC method developed and validated was found to be simple, precise, accurate and sensitive. Hence, this method can easily and conveniently adopt for routine quality analysis of Metronidazole in pure and in its tablet form.

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