ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG544

Metronidazole Analysis: Method Development and Validation by using UV Spectroscopy

Tadikonda Rama Rao*[1]; Jadhav Bharath[2]
Department of Pharmaceutical Analysis,
CMR College of Pharmacy, Hyderabad, Telangana, India

*Address of Corresponding Author: Tadikonda Rama Rao
Professor & Principal, CMR College of Pharmacy,
Kandlakoya Village, Medchal Road, Hyderabad – 501401, Telangana, India

Orcid Number: 0000-0003-1746-2167

Abstract:- The current study examines the creation of UV Spectrophotometric technique for metronidazole. Metronidazole in tablet dosage form can now be estimated using an easy to use, precise and economical spectrophotometric approach. The ideal circumstances were determined for medication analysis. It was discovered that 275 nm was the maximum wavelength (λ_{max}). Metronidazole percentage recovery fell between 98-102%. In the 5-40 µg/ml concentration range, beers law followed. A true correlation between optical density and intensity can be seen in calibration curve. Linearity, accuracy, precision, LOD, and LOQ were all validated in accordance with ICH requirements.

Keywords:- *UV Determination, Method Development, Validation, Metronidazole.*

I. INTRODUCTION

Medications belonging to the class of 5-nitroimidazole end product, such as metronidazole (MND), ornidazole, tinidazole, etc., are heavily represented in the global pharmaceutical market[1–4]. The most often used medication in this class is metronidazole. Many adverse effects, including an unpleasant (metallic) taste in the mouth, vomiting, diarrhoea, stomach discomfort, headaches, depression, violent reactions, and itching, are seen when metronidazole is administered[5-12]. Due to metronidazole's inhibition of the enzyme's acetaldehyde dehydrogenase and alcohol dehydrogenase, when combined with alcohol, a strong intoxication syndrome is observed. This includes severe vomiting, persistent nausea, a sharp headache, and other symptoms; additionally, a "disulfiram-like response" occurs, which is characterized by a sudden rush of blood to the head and upper body, breathing difficulties, tinnitus, and a sharp decrease in blood pressure, tachycardia, and death anxiety[13-18].

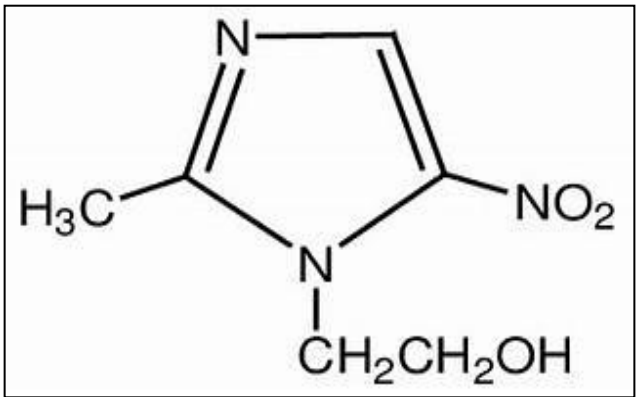

Fig 1: Structure of Metronidazole

Several spectrophotometric[19-21], [RP]-HPLC [22- 24] are documented and designed for the quantification of metronidazole alone and in grouping with other medications, according to a study of the literature survey. This work presents new visual spectrophotometric technique for the measurement of metronidazole and in drugs that are highly sensitive, straightforward, accurate, precise, fast, repeatable, and affordable.

➢ *Objective*

Finding a straightforward, accurate, and targeted spectrophotometric approach to identify MND in pharmaceutical tablet formulation is the goal of the current effort.

➢ *Instrument and Materials:*

The UV/Visible Spectrophotometer and the PG UV 1600 analytical balance were the instruments utilized. The pure medication metronidazole was utilized without additional purification after being given as a gift sample by RPG Life sciences Ltd., Mumbai. It had a 99.99% w/w assay value. Analytical grade materials and reagents were utilized in every instance.

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II. STANDARD SOLUTION OF METRONIDAZOLE

Weigh out exactly 100 mg of metronidazole (API), then pour it into a 100 ml volumetric container and dilute it with 0.1N HCl to the appropriate level. Pipette 5 ml of the above standard solution, then place it into a 100 ml volumetric flask and top it off with the same solvent. The wavelength of greatest concentration (λ_{max}) of a medicine in a diluent solution at a concentration of 10 µg/ml existed measured among the 200 to 400 nm range using a UV-Visible spectrophotometer. We observed the λ_{max} at 275 nm.

A. *Planning of Calibration Curve:*

Calibration curve was made in 0.1N HCl next to λ_{max} 275 nm using UV/Visible Spectrophotometer starting this stock solution of 50 µg/ml. Suitable dilutions were made using this stock to get the solutions in the choice of 5 µg/ml to 40 µg/ml. The calibration curve occurred.

B. *Technique for Assay of Medication Formulation:*

Metronidazole marketed preparations containing ten tablets existed evaluated and ground into powder. A 100 ml volumetric container was filled with 0.1N HCl after a amount of tablet grind equal to 40 mg of Metronidazole was added. Next, a Whatman filter paper grade 1 was used to filter the mixture. More suitable dilution of the filtrate was applied. The quantit of MND was calculated from the calibration plot of the resultant solution's absorbance, which was measured at 275 nm.

$$\%\ \text{Assay} = \frac{\textbf{Sample absorbance}}{\textbf{Standard absorbance}} \times \frac{\textbf{Wt. of Std}}{\textbf{Dilution of std}} \times \frac{\textbf{Wt. of Sample}}{\textbf{Dilution of Sample}} \times \frac{\textbf{Purity}}{\textbf{100}} \times \frac{\textbf{Wt. of Tablet}}{\textbf{Lable claim}} \times 100$$

% Purity = 98.40

Table 1: Evaluate of Metronidazole Formulation

Metronidazole	Quantity of Tablet (mg)		% Label accusation	Mean	Standard Deviation	% RSD
	Labeled	Found				
	40	39.34	98.40	0.287	0.005657	1.971029

III. VALIDATION OF METHOD PARAMETERS[25-27]

➢ *Linearity*

Linearity was located with in the range of 1–40 µg/ml. The method of least square regression was used to calculate the linearity.

➢ *Precision*

The measure of arrangement involving several quantities assembled after repetitive samples of the identical consistent material in required circumstances is said as the precision of an systematic order. Precision followed decided by intra-day and inter-day study. It remained evaluated by Executing assay 3 occasions on the equivalent day and intermediate precision was assessed by holding out the assay on 3 days for the experiment resolution. The percentage relative standard deviation (% RSD) was calculated.

➢ *Accuracy*

Recovery findings at three stages, involving the addition of a given quantity of analyte and three duplicates of the recovery process, will be used to assess accuracy. The percentage recovery and each concentration level were computed (80%, 100% and 120%) by normal accumulation technique and the tests were examined in triplicate by the anticipated method.

➢ *Ruggedness and Robustness*

Different laboratory conditions and analysts were used to analyze the solutions under various analytical conditions.

- LOD and LOQ: The lowest concentration of analyte in a test that can be detected except may not always be completely determined is known as the detection limit of a particular analytical method. The smallest intensity of analyte in an experiment that can be quantitatively detected with assume precision and accuracy is told as the quantitation limit of a specific analytic method.

$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$

Where,
σ = Standard deviation of the response, and
S = Slope of the calibration curve.

IV. RESULTS AND DISCUSSION

A. *Linearity*

The linearity of the method was tested with in the limit of 5–40 µg/ml of the target concentration. A 100-milliliter volumetric flask was filled with a precisely weighed 100 milligram pure medication. Pipette out 5 ml of that and use 0.1N HCl solution to make up to 50 ml. From the previously produced material, dilutions 5, 10, 15, 20, 30 and 40 µg/ml were created. The calibration curve for metronidazole determines the correlation coefficient. The calibration graph is made by plotting the obtained absorbance readings against the concentration of metronidazole. The linearity of the approach shown in Table 2 was determined, and the correlation coefficient is less than 2.

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Table 2: Linearity of Metronidazole

Concentration (µg/ml)	Absorbance
5	0.139
10	0.291
15	0.413
20	0.556
30	0.795
40	1.073

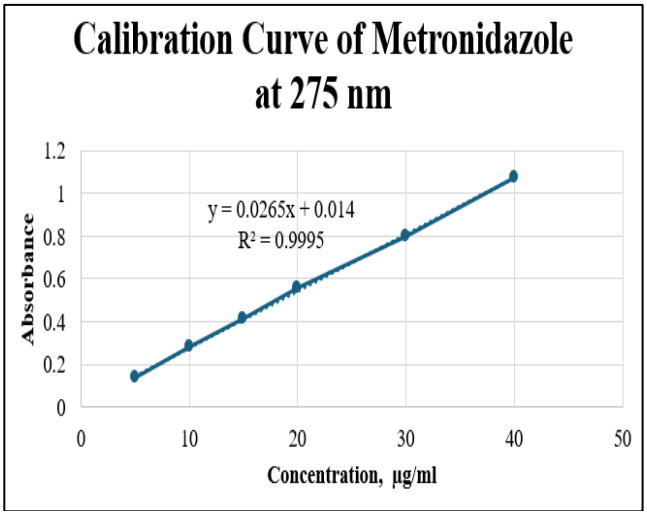

Fig 2: Calibration Curve of Metronidazole

B. Limit of Detection:

LOD for Metronidazole is given on the reaction and slope of the regression coefficient.

LOD = 3.3 × σ/S

Where,
σ = Standard deviation,
S = Linearity curve slope.

C. Limit of Quantization

Limit of quantization for Metronidazole is given on the reaction and slope of the regression coefficient.

LOQ =10 × σ/S

Table 3: LOD & LOQ Values of Metronidazole

Parameters	Metronidazole (µg/mL)
LOD	0.19
LOQ	0.58

- **Discussion:** LOD and LOQ values for Metronidazole were found to be 0.19 µg/mL and 0.58 µg/ml. It shows that the process was sensitive.

D. Precision

When an analytical approach is used constantly to several samplings of uniform samples. The intensity of agreement between the results of each individual test is used to determine precision. It was stated as a coefficient of variation and gives an indicator of the random errors.

E. Intra and Inter-Day Precision

An analysis was conducted on variations in outcomes and variations between days (interday). Metronidazole was analysed three times at 275 nm to assess the intraday precision. The medication was analysed once a day for three days at a wavelength of 275 nm to assess the interday precision.

Table 4: Intraday Precision of Metronidazole

Concentration (µg/mL)	Absorbance
10.0	0.265
10.0	0.270
10.0	0.267
10.0	0.271
10.0	0.272
10.0	0.273
mean	0.2696
Std. deviation	0.003077
%RSD	1.140962

Table 5: Interday Precision data of Metronidazole

S. No.	Concentration (µg/mL)	Interday Absorbance Day-1	Interday Absorbance Day-2
1	10.0	0.275	0.273
2	10.0	0.277	0.279
3	10.0	0.278	0.275
4	10.0	0.273	0.277
5	10.0	0.271	0.273
6	10.0	0.271	0.278
MEAN		0.274	0.2758
STD.DEV		0.002994	0.002563
%RSD		1.092861	0.929134

- **Discussion:** The % RSD for Intraday and Interday precision was found to be < 2%. It indicates that the method was precise.

F. Accuracy

Recovery studies: Recovery studies were carried out by run through the samples solution with regular solution at 80%, 100%, and 120% at 3 replicates and data was shown in Table 6.

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Table 6: Accuracy Data of Metronidazole

Sample (%level)	Amount taken (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% recovery	Average
80	10	8	7.94	99.25	99.25
80	10	8	7.98	99.75	
80	10	8	7.90	98.75	
100	10	10	10.05	100.5	100.3
100	10	10	9.98	99.8	
100	10	10	10.06	100.6	
120	10	12	12.01	100.1	100.5
120	10	12	12.10	100.8	
120	10	12	12.08	100.6	

- **Discussion:** The % recovery of Metronidazole was discovered to remain in between 98-102%.

G. Robustness

Table 7: Robustness of Metronidazole

S. NO.	Wavelength (λmax)	Absorbance
1	273	0.286
2	275	0.290
3	277	0.288

- **Discussion:** Here remained nope greatly change in the absorbance including modification in wavelength.

Table 8: Results of Metronidazole Ruggedness

S. No.	ANALYST	Absorbance	Mean	Standard Deviation	%RSD
1	ANALYST 1	0.270			
2	ANALYST 2	0.265	0.2675	0.003536	1.321695

V. CONCLUSION

The proposed UV - Spectrophotometric method is exceptionally direct, correct and reproducible for the estimation of Metronidazole. The made spectrophotometric methodology was endorsed for estimation of Metronidazole utilizing exactness, precision, linearity, expand and quality, % RSD for all parameters were found to be less than 2 which illustrates the authenticity of procedure. Test comes approximately showed up the accuracy of proposed procedure for estimation of Metronidazole. This procedure can be accommodatingly associated for the plan examination and the quality control of Metronidazole in bulk and formulation.

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ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG544

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