Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 August; 6 (9s): 1583-1589

Method Development And Validation Of Metronidazole In Bulk And Marketed Dosage Form

Supriya. S. Bhosale[1*], Smita A. Navale[2], Mahesh N. Pingale[3], Pooja M. Patil[4], Amruta R. Ghongade[5]

[1]*Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[2]Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[3]Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[4]Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[5]Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi

***Corresponding Author:** Supriya. S. Bhosale
E-mail: bhosalesupriya75@gmail.com

Abstract :

Development and Validation of a High-Performance Liquid Chromatographic Analytical Procedure for Determining of Metronidazole in a bulk and marketed dosage form is described in this paper. The Separation was made with a C_{18} Symmetry (4.6 X 150mm, 5µm) Column at ambient temperature, with isocratic mode and mobile phase Phosphate Buffer pH 2.8 : Acetonitrile 35:65 v/v. Eluent was monitored at 284 nm and the flow rate was 1.0 ml/min. Metronidazole was effectively separated with retention time (RT) of 5.1min. Within the selected chromatographic conditions the method was validated for Analytical Parameters:- Specificity, Linearity, Precision, Accuracy, and Limits of Detection and Quantitation. The Calibration curves were linear in the concentration range of 2-20 µg/ml for Metronidazole, and the Regression was found to be 0.997 respectively (NMT 0.999) for Metronidazole. The % recovery for 50%, 100% and 150% accuracy level of Metronidazole was found to be within the range of 99.13-100%. This Analytical Procedure is applicable for the Quality Control of drug formulations.

Keywords: Metronidazole, Stationary Phase, Mobile Phase, HPLC, Validation, Phosphate Buffer, Acetonitrile.

1. INTRODUCTION:

Metronidazole

Metronidazole has antibacterial and antiprotozoal effects and cures amebiasis, trichomoniasis, and giardiasis. Certain infections caused by anaerobic bacteria can be effectively treated with metronidazole. The majority of obligatory anaerobes have been demonstrated to be resistant to metronidazole's antibacterial effects, although investigations conducted in vitro have found that neither facultative anaerobes nor obligate aerobes are significantly affected. The antimicrobial cytotoxic actions of metronidazole, which harm microorganisms' DNA strands, are probably caused by anaerobic organisms reducing the nitro group of the antibiotic. An observation regarding carcinogenesis, neuropathy, and convulsions The potential side effects of metronidazole, particularly at higher doses, include peripheral neuropathy and seizures.

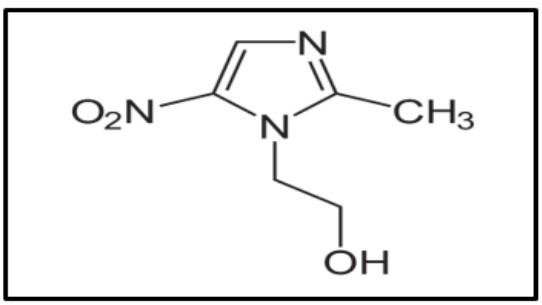

Fig. No. 01. Structure of Metronidazole

Metronidazole is an antibiotics, it works by preventing the bacterial cells from dividing and repairing, thereby killing the bacteria. Metronidazole kills parasites and anaerobic bacteria that cause infections by damaging their DNA. together, they treat harmful infections.

2. MATERIALS AND METHODS

Instrumentation

Agilent 1220 Infinity LC (G4288C) HPLC System used with a C_{18} Symmetry (4.6 x 150mm, 5µm, Make: Xterra) Column. Final chromatographic mobile phase for final optimization was Phosphate Buffer pH 2.8 : Acetonitrile 35:65 v/v. Detection carried out at 284nm.

Drug Sample

Metronidazole gift samples were offered by "Indoco Remedies Ltd. "A nearby drugstore provided the "MLevo M Suspension". Suspension contains (Metronidazole 500 mg).
Analytical grade chemicals and reagents were utilized.

Optimized Chromatographic conditions

The chromatographic conditions were optimized by using C_{18} column (150 x 4.6mm, particle size 5µ). Final chromatographic mobile phase for final optimization was Phosphate Buffer pH 2.8 : Acetonitrile 35:65 v/v. Detection was carried out at 284nm.

Preparation of standard stock solution

10 mg of Metronidazole working standards were accurately weighed and transferred into a 100 ml clean dry volumetric flask add about 70ml of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. (Stock solution) Further 3 ml of Metronidazole was pipetted from the above stock solution into a 10 ml volumetric flask and diluted upto the mark with diluent.

Preparation of sample solution

1 ml of Metronidazole suspension were weighed and transferred into volumetric flask. The 5 ml suspension equivalent to the amount of active ingredient present in 1 ml & Metronidazole 250 mg was transferred into a 100 ml clean dry volumetric flask, 70 ml of diluent was added to it and was shaken for 5 minutes. Then make up the final volume 100 ml with selected solvent (stock solution). 1 ml of stock solution was transferred to a 10 ml (250 µg/ml) volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system.

VALIDATION OF ANALYTICAL METHOD

Specificity

The chromatograms of standard and sample are identical with nearly same retention time. No interference due to placebo and sample at the retention time of analyte. There is no interference due to blank at the retention time of analyte. which shows that the method was specific. As shown in Fig. No. 02 & 03.

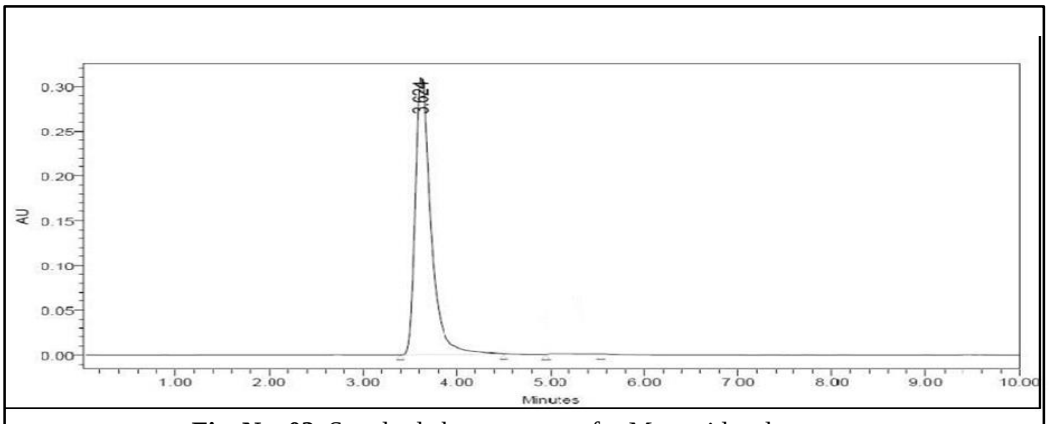

Fig. No. 02. Standard chromatogram for Metronidazole

Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 August; 6 (9s): 1583-1589

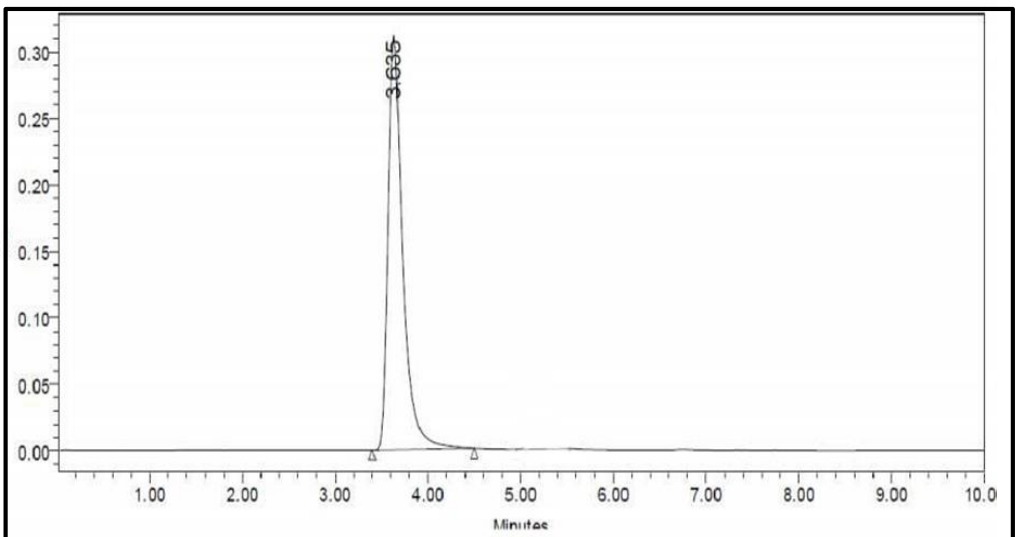

Fig. No. 03. Sample chromatogram for Metronidazole

There is no interference due to blank at the retention time of analyte, which shows that the method was specific.

Linearity

Linearity study was performed in the concentration range of 1-50 µg/ml. The Calibration curve for the linearity are shown in Fig. No. 04 for Metronidazole.

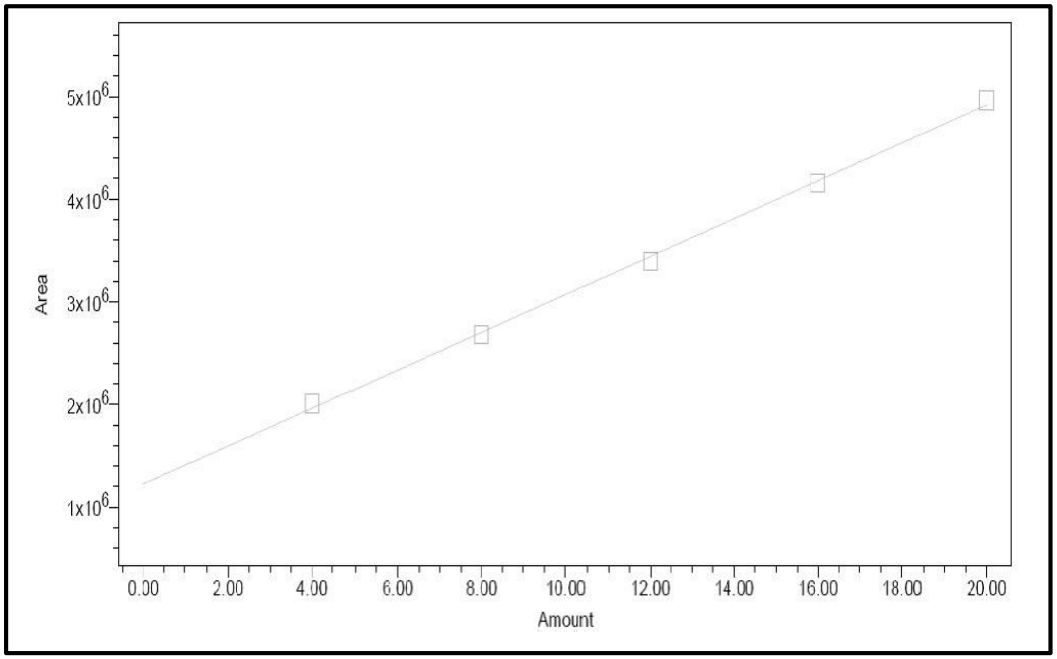

Fig. No. 04. Calibration curve of Metronidazole

Correlation co-efficient of Metronidazole was found to be 0.997 respectively (NMT 0.999).

Accuracy

The percentage recoveries of pure drug from the analyzed solution of formulation are calculated in the recovery range from 50% to 150%. The summary of accuracy results are tabulated in Table No.01.

Table No. 01. % Recovery results for Metronidazole

Sample No.	Spike Level	Amount (µg/ml)added	Amount (µg/ml)found	% Recovery	Mean %Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	14.8	14.72	99.4%	99.69%
		14.8	14.79	99.9%	
		14.8	14.77	99.79%	

The % recovery for 50%, 100% and 150% accuracy level of Metronidazole was found to be within the range of 99.13-100 % respectively (98.0 to 102.0%).

Precision

The RSD of % Recovery for Metronidazole chromatogram of repeatability precision and intermediate precision is calculated.

1. Repeatability

Table No. 02 Sample values for repeatability of Metronidazole

Metronidazole		
Injection No.	**Peak Area**	**% Recovery**
1	323863	99.2%
2	325248	99.8%
3	322052	99.2%
4	328133	99.4%
5	328655	100%
Mean	325590	99.52
SD	2802.3	0.33
% RSD	**0.86**	**0.36**

The % RSD for area of five standard injections of repeatability of Metronidazole was found to be 0.36.

2. Intermediate precision (analyst to analyst variability)

Comparison of both the results obtained for two different analysts shows that the assay method was rugged for analyst-analyst variability. The results of intermediate precision (Ruggedness) were found to be within the limits and are tabulated in Table given below.

Table No. 03 Intermediate precision results for Metronidazole

Parameter	**Peak Area**	**% Assay**
Avg*	3281662	99.98%
% RSD*	0.98	0.49

The % RSD for the area of five standard injections for intermediate precision of and Metronidazole was found to be 0.36 for day-1, analyst-1 and 0.26 for day-2, analyst-2 respectively.

Limit of detection

The limit of detection was calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve.

Calculation of S/N ratio -

a. Average baseline noise obtained from blank - 52 µV
b. Signal obtained from LOD solution (0.25% of target assay concentration) - 154 µVS/N = 154/52 = 2.96

Calculation of S/N ratio

a) Average baseline noise obtained from blank - 52 µV
b) Signal obtained from LOD solution (0.3% of target assay concentration) 155 µVS/N = 155/52 = 2.98.
Limit of detection was found to be 2.98 for Metronidazole.

Limit of Quantification (LOQ)

The limit of quantification was calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve.

Metronidazole

Calculation of S/N Ratio -
Average Baseline Noise obtained from blank : 52 µV
Signal Obtained from LOQ solution (1.0% of target assay concentration: 519µVS/N = 519/52 = 9.98
Limit of detection was found to be 2.98 for Metronidazole.

Robustness

01. Effect of variation in flow rate
As the % RSD of retention time and asymmetry were within limits for variation in flow rate (± 0.1 ml). Hence the allowable flow rate should be within 0.4 ml to 0.6 ml. The results of robustness for effect of variation in flow rate are tabulated in Table given below.

Table No.05. Robustness results for Metronidazole

Drug Sample	Sr. No	Flow rate (ml/min)	System suitability results	
			USP Plate count	USP Tailing
MTZ	1	0.5	3330.4	1.52
	2	0.7	3437.6	1.47
	3	0.9	3228.7	1.47

The % RSD of retention time and asymmetry were within limits for variation in flowrate (± 0.1 ml).

2. Effect of variation in mobile phase composition

The results of robustness for effect of variation in mobile phase composition are tabulated in Table given below.

Table No. 05. Results for variation in mobile phase composition

Drug Sample	Sr. No	Change in organic composition in the mobile phase	System suitability results	
			USP Plate count	USP Tailing
MTZ	1	10% less	3887	1.42
	2	*Actual	3437	1.42
	3	10% more	3985	1.51

The % RSD of retention time and asymmetry were within limits for variation in composition of mobile phase. Hence the method was found to be robust.

System Suitability

% RSD of retention time was found to be 0.2, % RSD of peak area was found to be 0.2. Theoretical plates were found to be more than 3500. USP tailing factor was found to be 1.52 for Metronidazole. All the parameters were within the limit.

Table No.06. Chromatogram values for system suitability of Metronidazole

Injection	Retention time	Peak Area	USP Plate count	USP Tailing
1	5.197	475301	4330.4	1.52
2	5.181	479658	4337.6	1.47
3	5.188	476736	4228.7	1.47

Mean	5.188	477231.7	4298.9	1.486
SD	0.008	2220.38	60.90148	0.028
% RSD	0.154	0.4652	1.416	1.941

% RSD of retention time was found to be 0.2, % RSD of peak area was found to be 0.2. Theoretical plates were found to be more than 3500. USP tailing factor was found to be 1.52 for Metronidazole. All the parameters were within the limit.

SUMMARY OF RESULTS:

Sr. No	Parameter	Requirement	Results	Acceptance Criteria
			MET	
1.	Specificity	No interference	Pass	No interference
2.	Linearity	Correlationcoefficient	0.9997	NLT 0.999
3.	Accuracy	50% recovery	100%	100 ± 2.0%
		100% recovery	99.13%	
		150% recovery	99.69%	
4.	Precision (repeatability)	%RSD	0.36	NMT 2%
5.	Intermediate precision	%RSD	0.89	NMT 1%
6.	Robustness	%RSD	0.36	NMT 1%
7.	System suitability	RT	5.181	-
	a.	Tailing factor	1.4	NMT 2
	b.	Plate count	3330	NLT 3000
	c.	Assay value	98.8%	100 ± 2.0%

Table No.09. Summary of Results.

Conclusion:

- The current analytical method has been verified using the ICH standards and complies with the necessary standards for acceptance.
- For determining Metronidazole the method is specific, linear, rapid, accurate, and economical.
- A more recent technique has been developed for HPLC.
- It is economical method because the amount and expenditure of the solvent utilized are less than what is currently reported in other publications.
- Without the presence of excipients, Metronidazole in their dose form can be routinely analyzed using HPLC techniques.
- It was determined that the procedure offered sufficient evidence for the drug's label claim.

References:

1. Veeshma, S. Priyanka, K. Praveen Kumar and K. Sirisha Simultaneous Estimation of Ciprofloxacin and Metronidazole in Bulk and Tablet Formulation by UV Spectrophotometry, International Journal of Pharmaceutical Sciences and Research 2021, 12, 2247-2256.
2. Mahrouse A. Development and validation of a UV spectrophotometric method for the simultaneous determination of Ciprofloxacin hydrochloride and Metronidazole in binary mixture. Journal of Chemical and Pharmaceutical Research, 2012, 4(11), 4710-4715.
3. Maslarskaa V, Tsvetkovab B, Lily Peikovab, Bistra Kostovac, Dimitar Rachevc, Stanislav Bozhanova, Nelina Zareva. HPLC Assay of Model Tablet Formulations Containing Metronidazole, and Ciprofloxacin International Journal of Pharmacy and Pharmaceutical Sciences, 2016 (8) 1052-1060.
4. S.S. Khadabadi and Meenakshi G. Devkara. Validated RP-HPLC Method for Simultaneous Estimation of Metronidazole and Ciprofloxacin Hydrochloride in Pharmaceutical Dosage form. International Journal of Pharmaceutical Sciences and Research. 2013, 4736-4740.
5. Ramzia El-Bagarya, Asmaa Ahmed El-Zaherb, Ehab Elkadyb, Asmaa Abdelkerim Mandoura. Simultaneous Determination of Ciprofloxacin Hydrochloride and Metronidazole in Spiked Human Plasma by Mass Spectroscopy, Journal of Applied Pharmaceutical Science, 2016, 6, 041-047.
6. Kamboj PC. Pharmaceutical analysis. 2nd Edition. Vallabh Publications; 2007.
7. Erwing GW. Instrumental Methods of Chemical Analysis, Mc Graw Hill Boo Company, Inc; 1960.
8. Lavanya G, Sunil M, Eswarudu M M, Eswaraiah M C, Harisudha K, Spandana B N. Analytical Method Validation: An updated Review. International J Pharm Sci Res 2013; 4(4):1280.

9. Connors KA: A text book of pharmaceutical analysis. 3rd edition, John Wiley and Sons; 1999.
10. Francis Rouessac, Annick Rouessac, Chemical Analysis Modern Instrumentation Methods and Technique Chemical Analysis Modern Instrumentation Methods and Technique, John Wiley & Sons Inc. Second Edition 63-91.
11. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare. The Indian Pharmacopoeia Commission, Ghaziabad; 2010, 3. 1815-1827.
12. United State Pharmacopoeia. 32nd edition. United States Pharmacopieal Convention. Inc. Rockville ; 2009 ; 3200-3139.
13. British Pharmacopoeia. Published by The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA); 2011(2): 1643, 1598.
14. Tripathi KD. Essential of Medical Pharmacology. 6th edition. New Delhi: J P Brothers Medical Publishers Pvt Ltd; 2010.
15. Patel, S. Development and validation of HPLC Method for simultaneous estimation of Ofloxacin and Metronidazole from pharmaceutical formulation, Int J Pharm Front Res, 2011, 1, 2, 68-74.
16. Bind B, Lokhande R, Munigela N, Kolhal S, & Gupta, A. RP-HPLC Method for the Simultaneous Determination of Metronidazole, Tinidazole, Ornidazole, Secnidazole and Ofloxacin in Bulk and Pharmaceutical Dosage Form, Int. J. Pharm. Sci. Rev. Res, 2015, 34, 61-67.
17. Hesham, S. Spectrofluorimetric, Atomic Absorption Spectrometric and Spectrophotometric Determination of Some Fluoroquinolones. American Journal of Applied science, 2005, 2, 719-729.
18. Natesh G. A. New analytical method development and validation for estimation of ciprofloxacin and metronidazole by ISO absorption method by using UV spectrophotometer. J Chem Biol Phys Sci. 2013, Vol-3, 1663-1670.
19. Sawal V S, Dr D. Umamaheshwari, A Review on Novel Analytical Techniques used in Method Development and Validation of Pharmaceuticals, J. Pharm. Sci. & Res. 2020, 12, 321-328.
20. Miniyar, P. B., Milani, A I, Dhange, A A, Gawande, V. T, & Kashid, A. M. Development and Validation of RPHPLC Method for Simultaneous Determination of Ofloxacin and Metronidazole in Infusion. American J. of Pharm Tech Research, 2012, 2, 382-390.