Research on the development and validation of UV-spectrophotometric and HPLC method determination of metronidazole in bulk and formulation.

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ABSTRACT: The objective of this work was to create an HPLC analytical technique that is quick, easy to use, and sensitive for quantifying metronidazole in pharmaceutical formulations. Chromatographic separation has been carried out using a C18 column (4.6 X 250) as the stationary phase and 0.1 ml OPA (pH 3) and methnol,water (62+ 38 percent v/v) as the mobile phase at a flow rate of 0.7 ml/ UV detection was carried out at a wavelength of 318 nm. According to the ICH guidelines, linearity, accuracy, range, and robustness were all within acceptable limits. This approach provides a high level of resolution (swartz, 2007)
Keywords: HPLC, UV Spectroscopy, Metronidazol ,validation method

I. INTRODUCTION :

Metronidazole has antibacterial and antiprotozoal effects and cures amebiasis, trichomoniasis, and giardiasis. anaerobic infectious diseases respond well to metronidazole therapy.[1] 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.[2]

Metronidazole is a commonly utilised antibiotic to treat infections caused by protozoa, microaerophilic bacteria, and anaerobic bacteria. It is cytotoxic to facultative anaerobic microorganisms.[3]

Antibiotic and antiprotozoal medication metronidazole. It is 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanol chemically. [4] formula C6H9N3O3, the bioavailability of the medication is 80% oral, 60% rectal, and 25% vaginal. and drug excretion was 77% in the urine and 14% in the faeces. used in medicine to treat amoebiasis, pelvic inflammatory illness, intra abdominal infections, and bacterial vaginosis. [5]

The goal of the current work is to provide a precise and trustworthy HPLC technique for simultaneously estimating metronidazole in solid dose form.[6]

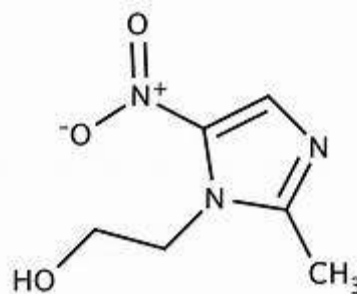Chemically, metronidazole is 2-methyl-5-nitroimidazole1-ethanol and has the structural formula as shown on Fig1

Figure no.1 of Structure of Metronidazole

II. MATERIALS AND METHODS

Chemicals and reagents:HPLC grade water, acetonitrile, methanol, and OPA from Merck.ltd were used in this study. An analytically pure metronidazole working standard was obtained from Swapnarorp Drug & Pharmaceutical.

A local store provided metronidazole 200mg tablets

Instrumentation: The Agilent Tech. Gradient System with Auto injector, equipped with a Quaternary Gradient (G130A) S.NO. DE9180834 pump, a 20-l injection loop, and a UV (DAD) G13148 S.NO. DE71365875 Absorbance detector,

and running CHEMSTATION 10.1 software, was used to analyse the drug.

Selection of wavelength: In a 10 ml volumetric flask, weigh and transfer 10 mg of the metronidazole working standard before adding diluted methanol to the desired amount. After 15 minutes of Sonication to dissolve the 1000g/ml standard (stock solution), 0.2 ml was transferred to a 10 ml volumetric flask and the volume was brought up to the mark using methanol. **Figure 2** shows that when the solution was scanned in the 200–400 nm range, the absorbance of metronidazole was discovered at 318 nm.

Preparation of standard drug solution : With regard to diluents, precisely weigh and transfer 10 mg of the metronidazole working standard into a 10 ml volumetric flask. Completely dissolve the

1000 g/ml standard in methanol and make the volume up to the mark with the same solvent. After 15 minutes of Sonication to dissolve the standard, the resulting stock solution (0.1 ml) was transferred to a 10 ml volumetric flask and the volume was made up to the mark with mobile phase. Methanol: 4 (0.1 percent OPA)ml of water were used as the solvent in 6 ml of MEOH and (0.1 percent OPA)water.

Preparation of sample solution: To quantify the amount of metronidazole in commercially available tablets, the average weight of 20 tablets was computed. Tablets were triturated, and 10 mg of metronidazole was measured in the powder. Using 10 mL of methanol, the medication was extracted from the tablet powder. It was sonicated for 15 minutes in order to achieve thorough extraction. After that, mobile phase was used to dilute 0.3 mL of supernatant by up to 10 mL.

Table no 1: Different Trials of Chromatographic Condition

Fig. No.	Column used	Mobile phase, Flow Rate and Wavelength	Inj. Vol.	Observation	Conclusion
1	Agilent C18 (250 ×4.6mm, 5µ)	0.1% (OPA) Water+ Methanol (20+80 % v/v) PH-3. Flow Rate 0.7.318nm	20µl	Sharp Peaks were not obtained	Hence rejected
2	Agilent C18 (250 ×4.6mm, 5µ)	0.1% (OPA) Water+ Methanol (25+75 % v/v) PH-3. Flow Rate 0.7.318nm	20 µl	Sharp Peaks were not obtained	Hence rejected
3.	Agilent C18 (250 ×4.6mm, 5µ)	Methanol+0.1 %(OPA) water, (62+38% v/v) PH-3 Flow Rate 0.7 ml. 318nm.	20 µl	Sharp Peaks were obtained	Hence selected

Thus, from the above, it has been observed that, using mobile phase of Methanol+0.1% (OPA)water,(62:38 % v/v),PH3.,318nm, Flow rate 0.7ml gave adequate retention at 4.569 min with good peak shape (Theoretical plates Metronidazole 3933).

Validation Method: According to ICH method validation criteria, analytical method validation

was completed Q2 (R1). in relation to a number of factors, including linearity, accuracy, precision, and resilience.

Linearity: Different working standard solutions (10-50 g/ml for HPLC and (5 g/ml -25 g/ml for UV) were made from metronidazole standard stock solution in the mobile phase. Twenty litres of sample solution were then injected into the

chromatographic system using a mixed volume loop injector. There were chromatograms made. The area for each concentration was measured, and the calibration curve is depicted in **Table no. 2 and fig. 2** accordingly.

Accuracy : Recovery experiments were carried out to verify the suggested method's accuracy. A specific concentration of the standard medication (80 percent, 100 percent, and 120 percent) was added to the previously examined Tablet solution, and the recovery was then examined. Statistical verification of the recovery trials depicted in **Table No. 3**

Precision: The procedure was developed by the examination of several replicated standards for metronidazole. In order to track any intra-day and inter-day variations in the final outcome, every solution was examined three times. According to **Table No. 4**, the result achieved for intraday is displayed.

Repeatability: The sample was used to evaluate the system's precision. Two replicates of a sample solution containing 40 mg/ml Metronidazole were injection, and peak areas and percent RSD were computed.shown in respectively **Table no5.**

Robustness: A method's robustness is its capacity to withstand minor, intentional changes in the parameters. Small but intentional changes were made to the optimal method parameters to test the suggested technique's resilience. Changes in the mobile phase's composition and flow rate, as well as the impact of wavelength on retention time and the drug peak's tailing factor, were investigated.shown in respectively **Table no 6.**

Limit of detection: The lowest amount of analyte in a sample that can be detected but not always precisely measured is known as a person's performance procedure's detection limit.
The detection limit (DL) was determined using the S.D. of the response and the slope of the calibration curve.

$$DL = \frac{3.3\sigma}{S}$$

Limit of quantification: The quantitation limit of a single analytical technique is the least number of analytical in a sample that can be quantitatively determined with adequate precision and accuracy.The quantitation limit (QL) was computed using the response S.D. and the slopes of the calibration curve.

$$QL = \frac{10\sigma}{S}$$

Assay :
Calculate the average weight of 20 Metronidazole tablets, weigh them precisely, and then transfer the sample—equivalent to 15.75 mg of Metronidazole—into a 10 ml volumetric flask. About 10 ml of diluents should be added. Sonicate the mixture to thoroughly dissolve it before adding diluent to the appropriate proportion. Mix well and run through 0.45 m filter. Pipette 0.3 ml of the aforementioned stock solution into a volumetric flask with a 10 ml capacity, then diluent it to the appropriate level. (30 µg/ml). The straightforward metronidazole test chromatogram By extrapolating the area from the calibration curve, the quantities of metronidazole per tablet shown were estimated. Five times the analysis process was carried out using the tablet formulation. **Table No. 7** shows the Tablet Assay for Percent Lable Claim for Percent RSD Calculated.

III. RESULT :
Research in the pharmaceutical industry is faced with the requirement to validate an analytical method on an almost daily basis, because sufficiently validated methods are required for approved regulatory submission.
The unique and simple have been created for the determination of metronidazole in satisfactory to provide a well retained, sharp and symmetric peak at 4.569min, with an average number of therotical plates of 3933, indicating the column's efficient performance. The linear detector response was found to be linear over the concentration range of 10-50g/ml, with a correlation coefficient of 0.9994 and a regression equation of y=103.24x69.449. The method's accuracy was found to be 98-102 percent, indicating that there was no interference from excipients. The intermediated precision study was determined using intra-day and inter-day data received from the proposed method of evaluating metronidazole
The approach was adequately resistant for generally expected variations in chromatographic conditions, such as wavelength, solvents, and flow rate, as defined by the ICH guideline. For metronidazole, the developed HPLC method is simple, specific, accurate, and exact.

IV. CONCLUSION:
The results of all validation parameters are well within the acceptance criteria defined by the ICH guideline for the validation of analytical techniques, leading to the conclusion that the

suggested HPLC,UV analytical method for the quantitative determination of metronidazole is simple, rapid, sensitive, accurate, precise, and robust.
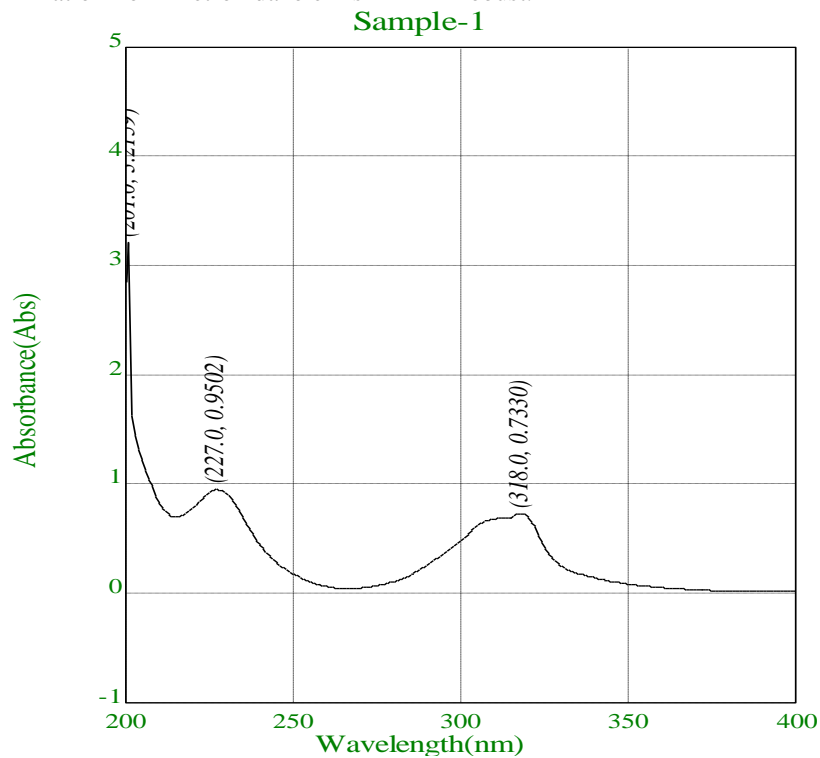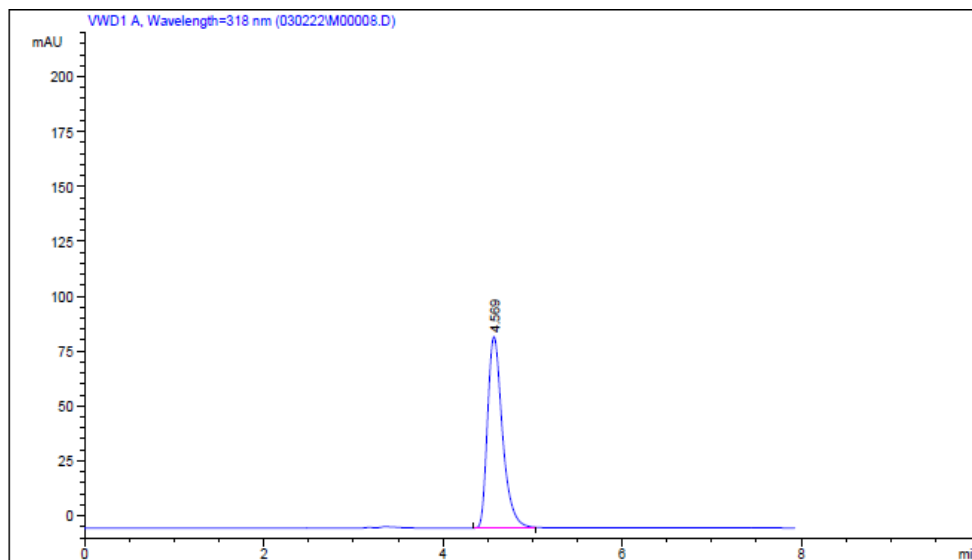

Fig. 2.Uv spectrum of Metronidazole

Fig No 3: Result of Chromatogram Trial

Table no2 Result of linearity studies

Regression Equation Data Y=mx+c	
Slope(m)	103.2
Intercept(c)	69.44
Correlation Coefficient	0.999
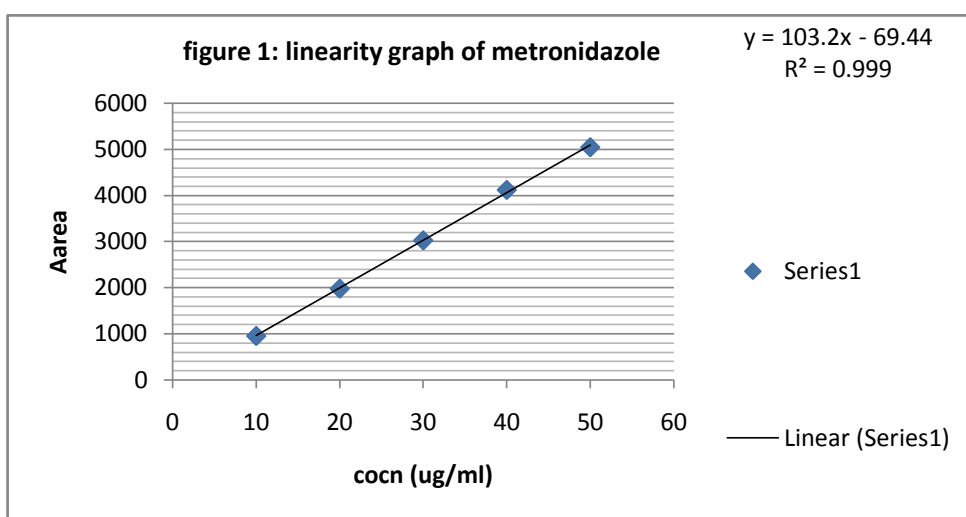
Figure 4:linearity graph of metronidazole

Table no 3: Result of Accuracy

HPLC	80%	101.83	0.50	0.49
	100%	102.34	0.42	0.41
	120%	100.94	0.14	0.13

Table no 4:Result of Precision

Conc^n (µg/ml) HPLC	Intraday Precision			Interday Precision		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	983.27±3.78	102.00	0.38	977.84±0.53	101.40	0.05
20	2019.7±9.49	101.22	0.38	2008.21±4.40	100.66	0.22
30	3046.9±12.49	100.66	0.41	3042.81±3.48	100.53	0.11

Table no 5: Result of Repetability

Sr.No.	Concentration of Metronidazole(mg/ml)	Peak area	Amount found (mg)	% Amount found
1	40	4069.3820	40.10	100.26
2	40	4069.1000	40.09	100.24
		Mean	40.10	100.25
		SD	0.04	0.04
		%RSD	0.001	0.001

Table no 6: Result of Robustness :

Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Mob-phase composition(61ml+39ml)Methanol + 0.01% (OPA)water	40	4140.8±0.35	0.01
Mob-phase composition(63ml+37ml) Methanol + 0.01% (OPA)water	40	1419.72±0.77	0.05
Wavelength change317nm	40	4296.6±4.23	0.10
Wavelength Change 319nm	40	3927.91±0.89	0.02
Flow rate change(0.6ml)	40	4582.11±2.51	0.05
Flow rate change(0.7ml)	40	3422.14±4.25	0.12

Table no 7:Result of Tablet Dosage form

Conc	Area		
	I	Amt Found	% Label Claim
30.00	3064.768	30.37	101.23
30.00	3045.089	30.17	100.60
Mean	3054.93	30.27	100.91
SD	13.91	0.01	0.01
%RSD	0.46	0.01	0.01

REFERENCE :
[1]. Bandawane, A., & Saudagar, R. (2019). Analytical Method Development: A Review. Journal of Drug Delivery and Therapeutics, 9(3), 522-524.

[2]. Patil, K., Patil, P., Patil, J., & Pawar, S. (2012). A basic approach on sustained release drug delivery system. Am J PharmTech Res, 2, 213-231.

[3]. Pharmaceutical process validation, Nacre and Watcher AH, CBS publishers and Distributors, Newdelhi

[4]. Modern Pharmaceutical analysis , Volume1-4, SatishAhuja, CBS publishers and Distributors, Newdelhi

[5]. Crowther JB. Validation of pharmaceutical test methods. In: Handbook of modern pharmaceutical analysis, Academic press, New York, 2001, pp. 415–443.

[6]. Soman, A., Qiu, Y., & Chan Li, Q. (2008). HPLC-UV method development and validation for the determination of low level formaldehyde in a drug substance. Journal of Chromatographic science, 46(6), 461-465.

[7]. Sarkar, M., Khandavilli, S., & Panchagnula, R. (2006). Development and validation of RP- HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms. Journal of Chromatography B, 830(2), 349-354.

[8]. Sadasivudu, P., Shastri, N., & Sadanandam, M. (2009). Development and validation of RP-HPLC and UV methods of analysis for fluconazole in pharmaceutical solid dosage forms. International Journal of ChemTech Research, 1(4), 1131-1136.

[9]. Das, J., & Dhua, M. (2014). UV-spectrophotometric assay method development and validation of metronidazole in bulk and tablet formulation. Journal of pharmasciTech, 3(2), 106-109.

[10]. Mishra, A. K., Yadava, R., Mishra, A., Verma, A., & Chattopadhyay, P. (2010). Development and validation of UV spectrophotometric method for the determination of metronidazole in tablet formulation. International journal of Pharmaceutical research and development, 2(6), 001-004.

[11]. Klimenko, L. Y., Shkarlat, G. L., Shovkova, Z. V., Yaremenko, V. D., & Shpychak, O. S. (2018). Development and validation of HPLC/UV-spectrophotometric procedures for metronidazole quantitative determination.