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Research

Analytical Method Development And Validation Of Metronidazole In Bulk And Tablet Formulation By Uv-Spectrophotometer

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Abstract

Metronidazole, a nitroimidazole, remains a front-line choice for treatment of infections related to inflammatory disorders of the gastrointestinal tract including colitis linked to Clostridium difficile. Metronidazole was first described in 1959 as a therapeutic agent to treat Trichomonas vaginalis infection; over time, metronidazole has been used against Giardia lamblia, Entamoeba histolytica, Clostridium difficile, Helicobacter pylori and ana- aerobic bacteria. Brand names: Acea, Anabact, Flagyl, Metrogel, Metrosa, Rosiced, Rozex, Vaginyl, Zidoval, Zyomet. Metronidazole is classified as a synthetic antibacterial and antiprotozoal agent and is administered by the intravenous route. Analysis of the UV spectra of Metronidazole enables to determine a maximum of the analytical quantification of the medicine.

Keywords: Metronidazole, ultraviolet spectroscopy, validation, Linearity.

INTRODUCTION

Metronidazole is one of the mainstay drugs for the treatment of anaerobic infections. It is approved by the US Food and Drug Administration for the treatment of anaerobic and protozoal infections. Metronidazole exerts its antimicrobial effects through the production of free radicals that are toxic to the microbe. Metronidazole is an antibiotic that is used to treat a wide variety of infections. [1] It works by stopping the growth of certain bacteria and parasites. This antibiotic treats only certain bacterial and parasitic infections. It will not work for viral infections (such as common cold, flu). Metronidazole was first described in 1959 as a therapeutic agent to treat Trichomonas vaginalis infection; over time, metronidazole has been used against Giardia lamblia, Entamoeba histolytica, Clostridium difficile, Helicobacter pylori and ana- aerobic bacteria. Brand names: Acea, Anabact, Flagyl, Metrogel, Metrosa, Rosiced, Rozex, Vaginyl, Zidoval, Zyomet.[2,3] Find out how metronidazole treats infections and how to take it. Metronidazole is soluble in organic solvents such as ethanol, DMSO, and dimethyl

formamide (DMF). The solubility of metronidazole in ethanol is approximately 5 mg/ml and approximately 15 mg/ml in DMSO and DMF. The drug metronidazole was initially developed by Rhône-Poulenc in the 1950s and licensed to G.D. Searle. Searle was acquired by Pfizer in 2003.[4,5] Metronidazole is available under the following different brand names: Flagyl, Flagyl ER, and Flagyl IV RTU. Metronidazole is a 5-nitroimidazole derivative. White to pale yellow crystalline powder. Melting point 158–160° C. Each 100 mL contains 14 mEq sodium, pH 5.8 (4.5 — 7.0). Metronidazole is classified as a synthetic antibacterial and antiprotozoal agent and is administered by the intravenous route. Analysis of the UV spectra of Metronidazole enables to determine a maximum of the analytical quantification of the medicine. The maximum absorbance wavelength of metronidazole is between 310-330 nm, similar to a previous report. The UV procedure proved to be superior with respect to sensitivity (detection limits: 0-1 microgram/ml serum for both compounds), speed and precision. It discriminates between metronidazole and its two major active metabolites and quantitates the total amounts present. Metronidazole Soluble in water (10 mg/ml at 20° C), ethanol (5 mg/ml), methanol, chloroform.[6,7]

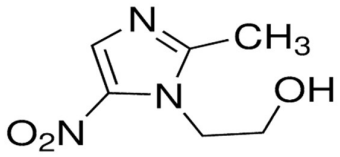

Fig1: Chemical Structure of Metronidazole

MATERIALS AND METHODS

Materials

Metronidazole 400mg tablets were procured from market. Solvents like methanol were purchased from Merck solvents.

Instruments Used

UV-Visible spectrophotometer with UV Win software and make was PG-Instruments. Weighing balances and matching quartz cells with a 1 cm cell path length were utilized along with the mentioned equipment, which had automatic wavelength accuracy of 0.1 nm.

METHOD DEVELOPMENT

STANDARD STOCK SOLUTION PREPARATION

Standard stock solution was prepared by dissolving 100mg of metronidazole API into 100ml volumetric flask to get 1mg/ml concentrations. Pipette out 10ml from the above solutions was further diluted to 100ml. Transfer 1ml of above solution into a 10ml volumetric flask. 10µg/ml of the solution was scanned 200nm-400nm and absorption was found to be 235nm.

PREPARATION OF CALIBRATION CURVE

The contents of the blank solution were filtered before use through 0.45µm filter paper, and prepare a series of solutions for standard drug (Metronidazole) ranging from 1 µg/ml to 7 µg/ml. Then the prepared series of dilutions were scanned for its maximum absorbance and the maximum absorbance of Metronidazole both for sample and the standard was found to be 235 nm. The calibration curve of Metronidazole is plotted by taking concentration (µg/ml) on X-axis and absorbance on Y-axis. From the calibration curve correlation coefficient value for Metronidazole was found to be 0.999.

PREPARATION OF SAMPLE SOLUTION OF METRONIDAZOLE

Accurately weigh and transfer 100mg of metronidazole in volumetric flask and make up to 100ml with methanol solution. From the above solution transfer 10ml and make up to 100ml with methanol solution. Pipette out 1ml and make up to 10ml with methanol solution, mix well and measure the absorbance at 235nm.

Method validation

According to ICH Q2 (R1) and USP criteria, the suggested technique was validated for a number of parameters, including linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness[8,9].

Linearity

Absorbance was plotted on the y-axis, and concentration was plotted on the x-axis, to create the calibration curve.

Precision

By taking six replicate measurements from the homogeneous solution, repeatability (intraday precision) and intermediate precision (interday precision) for the reference solution (6 µg/mL) were used to determine the method's precision. Three replicates were introduced into the system on the same day to increase the method's accuracy, and after calculating the RSD percentage, the results were expressed as a percentage of the measurement.

LOD and LOQ

The smallest amount of analyte in a sample that can be detected but not necessarily measured as an exact value is the detection limit of an individual's analytical method. The lowest amount of analyte in a sample that can be quantitatively measured with sufficient accuracy and precision is the quantification limit of a specific analytical process.

Robustness

By assessing the 6µg/mL metronidazole standard solution at various maximums (i.e. ±1nm) of the actual maximum, the robustness of this procedure was ascertained and absorbance was measured.

Stability

Stability of metronidazole sample solution was determined initially and 24 hours later, the % assay was compared.

Accuracy

The standard addition method was used to assess accuracy. A known quantity of standard solution was added to the sample solution in three different amounts—50%, 100%, and 150% of triplicate—before the solution was tested and the percentage recovery was computed.

RESULTS AND DISCUSSION

LINEARITY

From the graph it was found that metronidazole obeys Beer's law and the linearity concentrations lies between 2-18µg/ml. The linearity data calibration curve was shown in figure. The correlation coefficient, intercept and slope were calculated for metronidazole.

Table 1: Linearity data of metronidazole

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE
1	1	0.0385
2	2	0.067
3	3	0.095
4	4	0.125
5	5	0.153
6	6	0.1825

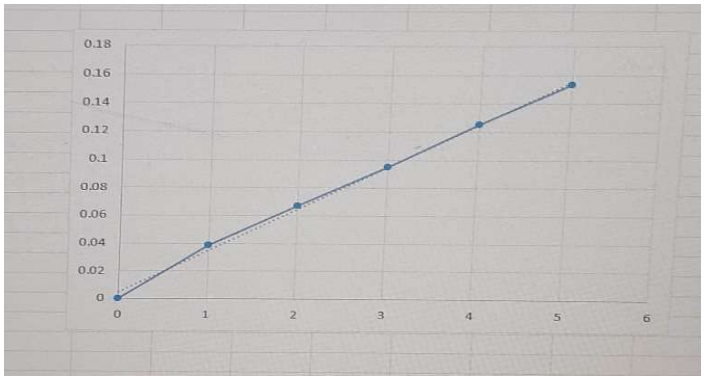

Fig 2: Calibration curve of metronidazole

OPTICAL CHARACTERISTICS OF METRONIDAZOLE

Table 2: Optical characteristics of metronidazole

Parameters	Metronidazole
Max	310
slope	0.030071
Linearity	1 to 6 **µg/ml**
Correlation coefficient	0.9985
Intercept	0.004571

Calibration curve was plotted and correlation coefficient was found to be 0. 998.so, there was a good relation between absorbance and concentration

PRECISION

Intraday and Interday precision was shown in table 3 and 4 respectively.

Table 3: Intraday precision data of metronidazole

CONCENTRATION(µg/ml)	ABSORBANCE
4	0.123
4	0.122
4	0.125
4	0.120
4	0.128
4	0.121
MEAN	0.123167
STD.DEVIATION	0.0068
%RSD	1.38

Table 4: Interday precision data of metronidazole

S.NO	CONCENTRATION(µg/ml)	INTRADAY ABSORBANCE DAY-1	INTERDAY ABSORBANCE DAY-2
1	4	0.123	0.125
2	4	0.122	0.120
3	4	0.125	0.129
4	4	0.120	0.123
5	4	0.128	0.120
6	4	0.121	0.120
	MEAN	0.123167	0.123117
	STD.DEVIATION	0.0068	0.0045
	%RSD	1.38	0.93

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

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

LOD and LOQ was calculated and shown in table 5.

Table 5: LOD and LOQ data of metronidazole

PARAMETERS	METRONIDAZOLE(µg/ml)
LOD	0.19
LOQ	0.58

LOD and LOQ values of metronidazole was found to be 0.19 µg/ml and 0.58 µg/ml. It indicates that the method was sensitive.

ACCURACY

Recovery studies were conducted by spiking the sample solution with standard solution at concentrations of 80%, 100%, and 120% for three repetitions. The results are presented in Table 6.

Table 6: Accuracy data of metronidazole

SAMPLE (%level)	AMOUNT TAKEN (µg/ml)	AMOUNT ADDED (µg/ml)	AMOUNT RECOVERED (µg/ml)	% RECOVERY	AVERAGE
80	4	2	8.94	98.3	
80	4	2	8.92	98.8	99.7
80	4	2	8.90	98.1	
100	4	4	12.18	99.5	
100	4	4	12.20	99.6	101.3
100	4	4	12.26	99.1	
120	4	6	15.12	100.4	
120	4	6	15.10	100.1	100.2
120	4	6	15.18	100.3	

The average% recovery of metronidazole was found to be in between 98-103%.

ROBUSTNESS

Robustness data was shown in table 7.

Table 7: Robustness data of metronidazole

S.NO	WAVELENGTH	ABSORBANCE
1	309	0.340
2	310	0.342
3	311	0.341

There was no much variation in the absorbance with change in wavelength.

STABILITY

Sample solution of metronidazole containing 4 µg/ml was taken in the solution stability. Stability data was shown in table 8.

Table 8: Stability data of metronidazole

TIME	%ASSAY
INITIAL	99.5%
24 HOURS	99.2%

It was observed that the difference in the results was not more than 2%

ASSAY

Table 9: Assay of metronidazole

LABLE CLAIM	AMOUNT FOUND	ASSAY%=SD
4oomg	99.5%	99.5%0±.04

Metronidazole was 99.5 % compared with label claim amount. It shows that UV-Visible method developed was successful in determining metronidazole from the tablet dosage form.

CONCLUSION

A simple spectroscopic method has been developed, validated and degradation studies of metronidazole in bulk and its dosage forms, the method was found to be specific along with qualitative and quantitative determination of metronidazole in pharmaceutical preparations. The proposed method was found to be accurate, economical so can be used for routine analysis of metronidazole in bulk and its dosage form.

REFERENCES

1. Shailesh W, Tukaram K, Shivraj S, Sima L, Supriya K. Development and Validation of Stability Indicating UV Spectrophotometric Method for Simultaneous Estimation of Amoxicillin Trihydrate and Metronidazole In Bulk And In-House Tablet. World Journal of Pharmaceutical and Medical Research. 2017;3(8):312-8.
2. Mishra AK, Kumar A, Mishra A, Mishra HV. Development of ultraviolet spectroscopic method for the estimation of metronidazole benzoate from pharmaceutical formulation. Journal of Natural Science, Biology, and Medicine. 2014 Jul;5(2):261. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4121894/
3. Jain R, Jain N, Jain DK, Patel VK, Rajak H, Jain SK. Novel UV spectrophotometer methods for quantitative estimation of metronidazole and furazolidone using mixed hydrotropy solubilization. Arabian Journal of Chemistry. 2017 Feb 1;10(2):151-6. https://www.sciencedirect.com/science/article/pii/S1878535213002992
4. Thulasamma P, Venkateswarlu P. Spectrophotometric method for the determination of metronidazole in pharmaceutical pure and dosage forms. Rasayan J. Chem. 2009;2(4):865-8. https://rasayanjournal.co.in/vol-2/issue-4/15.pdf
5. Miljkovic VM, Kostic DA, Bojanic ZZ, Kalicanin BM, Kocic GM. Development and application of kinetic spectrophotometric method for the determination of metronidazole. Tropical Journal of Pharmaceutical Research. 2014 May 27;13(3):417-22. https://www.ajol.info/index.php/tjpr/article/view/103744
6. Patel KN, Patel JK, Patel MP, Rajput GC. A validated method for development of atovaquone as API and tablet dosage forms by UV spectroscopy. Pharmaceutical methods. 2010 Oct 1;1(1):61-4. https://www.sciencedirect.com/science/article/pii/S2229470810110103
7. Chakraborty S, Sharmin S, Rony SR, Ahma SA, Sohrab H. Stability-indicating UV/Vis Spectrophotometric Method for Diazepam, Development and Validation. Indian Journal of Pharmaceutical Sciences. 2018 Mar 1;80(2). Doi: doi.org/10.4172/pharmaceutical-sciences.1000366
8. Omprakash GB, Madhuri VP, Sachin BG. Development, Validation and Stability Study of UV Spectrophotometric Method for Determination of Nabumetone in Bulk and Pharmaceutical Dosage Forms. Journal of Pharmaceutical Research. 2017 Dec 1:291-6. https://ischolar.sscldl.in/index.php/JPRKCP/article/view/171672
9. Kansagra P, Sanghvi G, Purohit P, Vachani A, Sheth N, Vaishnav D. Development and validation of stability indicating UV spectrophotometric method for the determination of Ketoconazole both in bulk and marketed dosage formulations. Pharm Analysis & Quality Assurance. 2013;1:1-5.