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A COMPARATIVE STUDY OF HPLC AND UV SPECTROPHOTOMETRIC METHODS FOR METRONIDAZOLE AND MICONAZOLE NITRATE QUANTIFICATION IN PHARMACEUTICAL FORMULATIONS AND ECO-FRIENDLINESS

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ABSTRACT

Objective: The demand for environmentally friendly analytical methods in pharmaceutical analysis has led to the development of ecologically friendly chromatographic and spectrophotometric techniques. Using a sustained approach, this study investigates the simultaneous quantification of metronidazole and miconazole nitrate using high-performance liquid chromatography (HPLC) and UV spectrophotometry. **Methodology:** In vaginal gel and cream formulations, a reversed-phase high-performance liquid chromatography method has been developed that effectively detects metronidazole and miconazole nitrate. This technique uses a Zorbax SB-C8 column (150 × 4.6 mm, 3.5 µm) with isocratic elution and a mobile phase consisting of a solution of water and isopropyl alcohol. The simultaneous equation of the UV technique estimated drug quantity and the simultaneous equation for miconazole nitrate and metronidazole were calculated using wavelengths of 210 nm and 315 nm, respectively. **Result:** The developed techniques exhibited excellent linearity (r2 > 0.999) over the concentration range of UV spectrophotometry and HPLC methods of 18.75-112.5 µg/ml and 50-300 µg/ml for metronidazole and miconazole. The precision of the methods was characterized by ≤ 2 % and average recovery rates, which were 98.6–99.8 % for the spectrophotometric UV method and 99.6–100.6 % for the HPLC method. **Conclusion:** The developed methods were validated for accuracy, precision, linearity, and robustness, and their applicability in routine drug analysis was demonstrated. By minimizing the use of organic solvents and introducing environmentally friendly analytical protocols, this study highlights the benefits of green analytical chemistry (GAC) in pharmaceutical quality control.

KEYWORD:- Liquid chromatography, Metronidazole, Miconazole Nitrate, Method development, and Method validation.

1. INTRODUCTION

Antibiotics and antifungals are used to treat vaginal infections. An excess of bacteria and fungi can cause a vaginal infection, which is an infection of the vagina and vulva (The tissue at the vaginal opening). Inflammation, itching, thick discharge, color changes in discharge, burning, or pain during urination are symptoms of a vaginal infection. Metronidazole and miconazole are a combination of the antibiotic metronidazole and the antifungal miconazole. Metronidazole destroys bacteria by damaging their DNA. Miconazole stops the growth of fungi by preventing their protective covering from forming.[1]

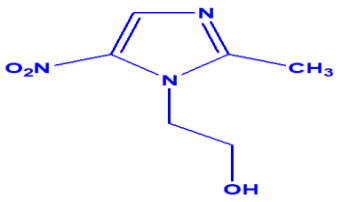
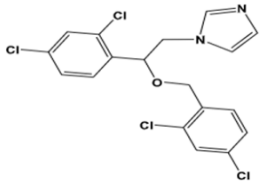
Metronidazole and miconazole nitrate are often used to treat bacterial and fungal infections. Traditional analytical methods often rely on organic solvents that contribute to environmental pollution.[2] Metronidazole is a nitroimidazole derivative with antibacterial and antiprotozoal activity. By entering microbial cells and being degraded by anaerobic organisms, they produce reactive intermediates that disrupt DNA synthesis, leading to cell death.[3] The United States Food and Drug Administration authorized it for the treatment of protozoal and anaerobic infections. Metronidazole exerts its antimicrobial action by generating free radicals that are toxic to microorganisms.[4]

Mechanism of action

Metronidazole is a prodrug that passively circulates in anaerobic bacteria and protozoa. Inside the cell, it was reduced by microbial enzymes (e.g., nitro reductases), forming reactive nitroso radicals.[5] These radicals cause DNA strand breakage, inhibition of replication, and cell

death. It selectively targets anaerobes because oxygen in aerobic cells prevents its activation. Used for anaerobic bacterial infections, protozoal infections (e.g., Giardia, Trichomonas), and H. pylori-associated conditions.[6,7]

Miconazole nitrate is an imidazole antifungal that inhibits ergosterol synthesis, a major component of fungal cell membranes, leading to increased permeability and cell death. It is used in dermatophytosis, candidiasis, and systemic fungal infections.[8] It has been used to treat fungal infections of the mucous membrane, including oral and vaginal infections. Although miconazole was no longer available for intravenous administration, a wide variety of suppository, cream, gel, and tablet products were available. Miconazole was thought to primarily inhibit fungal CYP450-14α-lanosterol demethylase activity[9] and is commonly used to treat fungal mucosal infections, including oral and vaginal infections. Although intravenous miconazole was no longer available, suppositories, creams, gels, and tablet-based products were widely available. Metronidazole has a molecular formula of $C_6H_9N_3O_3$ and a molecular weight of 171–154 mg. and Miconazole has a molecular formula $C_{18}H_{14}Cl_4N_2O$ and a molecular weight of 416.129 mg. The structure of metronidazole and miconazole is shown in Figure 1.

Structure of Metronidazole	Structure of Miconazole
	

Mechanism of action

Miconazole contains an imidazole antifungal that destroys fungal cells by disrupting the integrity of fungal cell membranes.[10] Miconazole inhibits lanosterol 14α-demethylase, a cytochrome P450-dependent enzyme. The conversion of lanosterol to ergosterol, an important component of fungal cell membranes, requires this enzyme.[11] Inhibition of ergosterol synthesis leads to accumulation of methylated sterols, which disrupt membrane structure and function. This increases membrane permeability, causing leakage of essential intracellular components. Miconazole also affects other cytochrome P450-dependent enzymes, further disrupting fungal metabolism and growth.[12]

The literature was reviewed to investigate existing techniques for the analysis of pharmaceutical formulations or other drug combinations in biological fluids, including UV detection, HPTLC, and HPLC.[13-21] gas chromatography[22] spectrophotometry[23-25] supercritical fluid chromatography[26,27] Many of the methods in this area still have problems related to more toxic organic solvents, buffers, long running time and the challenge that these methods are lengthy procedures and very time-consuming.

The older approaches are progressively showing their limitations and consequently, there is an urgent need for an economical and reliable technology. The aim of this article is to describe a straightforward, precise, and accurate reversed-phase high-performance liquid chromatography (RP-HPLC) and UV spectroscopic method for the simultaneous quantification of metronidazole and miconazole combined dosage gel forms. Various parameters of the developed method will be validated as per ICH guidelines.

2. MATERIALS AND METHODS

Reagents and Chemicals

Metronidazole and miconazole gel formulation dosage forms were purchased from Encube Ethical Pvt Ltd. The active ingredients metronidazole and miconazole were purchased from commercial sources. Isopropyl alcohol (HPLC grade) was purchased from Merck (India) Ltd.

Instrumentation and Chromatographic conditions

A Thermo Fisher Scientific double-beam UV spectrophotometer with a wavelength of ± 0.5 nm and a spectral bandwidth of one nm was used to perform spectrophotometric measurements. It was placed in a 1 cm quartz cell to measure absorbance and analyzed using Thermo Insight software. High-performance liquid chromatography (HPLC) analysis was performed using Empower 3 software version on a Waters Alliance e2695 HPLC system equipped with a 2998 PDA detector to obtain chromatographic information. The column adopted is Zorbax SB C8 (150 x 4.6 mm x 3.5 µm) and detection was performed at 210 nm. The injection volume was 10 µl ml and the column temperature was kept at 30 °C. In addition, it lasted six minutes. Isopropyl alcohol and water (70:30 v/v) form an isocratic mobile phase. The mobile phase was degassed and passed through a 0.45 µm membrane filter before use.

Preparation of the standard solution

Accurately weighed and transferred 15 mg metronidazole and 40 mg miconazole as working standards into clean and dried 20 ml measuring flasks. A small portion of the mobile phase was added and treated by ultrasound to completely dissolve all the components and labeled with the mobile phase. Further, 5 ml of the stock solution prepared above was transferred to a precisely cleaned and dried 50 ml volumetric flask

containing the mobile phase. (75 µg/ml metronidazole or 200 µg/ml miconazole)

Preparation of sample solution

Weighed and transferred a 1000 mg gel sample that contained (0.75 % metronidazole and 2% Miconazole) into a dry and clean 50 mL volumetric flask. Add approximately 30 mL of mobile phase, sonicate for up to 30 minutes to completely dissolve all components, and fill the volume to the mark with diluent and mix well. Allow to cool at room temperature for a few minutes and filter through a 0.45-µ PTFE injection filter. (75 µg/ml metronidazole or 200 µg/ml miconazole).

Method validation

Following the procedure described in ICH Guidelines Q2 (R1) for Validation of Analytical Methods, the optimized spectrophotometric and chromatographic techniques were fully validated.[28]

Specificity

Chromatography was loaded with standard and sample gel solutions that were prepared over the test concentration range to screen for any potential interference peaks. The UV spectrum of this solution was recorded between 200 and 400 nm for spectrophotometric analysis and between 210 nm for miconazole and 315 nm for metronidazole to assess the presence of any possible interference bands.

Linearity and Range

An analytical method is said to be linear if it can produce test results that are exactly proportionate to the analyte concentration in the sample, within a given range. Using serial dilutions of a working standard stock solution, the linearity of the detector response for metronidazole and miconazole was ascertained. Five concentrations such as 18.75 µg/ml (25%), 37.5 µg/ml (50%), 60 µg/ml (80%), 75 µg/ml (100%), 90 µg/ml (120%), 112.5 µg/ml (150%) for metronidazole and 50 µg/ml (25%), 100 µg/ml (50%), 160 µg/ml (80%), 200 µg/ml (100%), 240 µg/ml (120%), 300 µg/ml (150%) miconazole prepared for UV spectrophotometric and HPLC, respectively. The percent Y-intercept and correlation coefficient must be in range. The area of the residual sum of squares at each level, the slope of the regression line, the correlation coefficient, and the percent Y-interference were calculated.

Accuracy

By mixing a known quantity of working standard with a placebo that matched the accuracy level, accuracy was determined. Metronidazole was prepared in triplicate in three different solutions at 50%, 100%, and 150 % of the predetermined concentration (37.5, 75, 112.5 µg/mL). Miconazole was made in triplicate at 50%, 100%, and 150 % of the specified concentration (100, 200, and 300 µg/mL). The mean percentage and recovery for each method were calculated.

Method precision (Repeatability)

Repeatability was achieved by employing both techniques to analyze the sample solution six times in a single day at 100% test concentration. Similarly, gel samples were analyzed on the same day and on different days at different times to assess intra-day and inter-day precision. Metronidazole and miconazole concentrations, along with the relative standard deviation (R.S.D.) value were calculated.

Robustness

The robustness of an analytical method is a measure of its ability to remain unchanged by small but deliberate fluctuations in the parameters of the method and also indicates its reliability in general use. It was observed that column oven temperature, flow rate mobile phase composition, etc.

3. RESULTS AND DISCUSSION

Method Development and Optimization

Metronidazole and miconazole nitrate were completely soluble in the water and Isopropyl alcohol mixture in the ratio of (70:30 v/v) and Hence water and isopropyl alcohol mixture was chosen as solvent for metronidazole and miconazole to obtain UV spectrum in the range of 200–400 nm. (Figure 2). After spectrum evaluation, wavelengths of 210 nm for miconazole and 315 nm for metronidazole were selected for measurement due to adequate molar absorption of metronidazole and miconazole in this region.

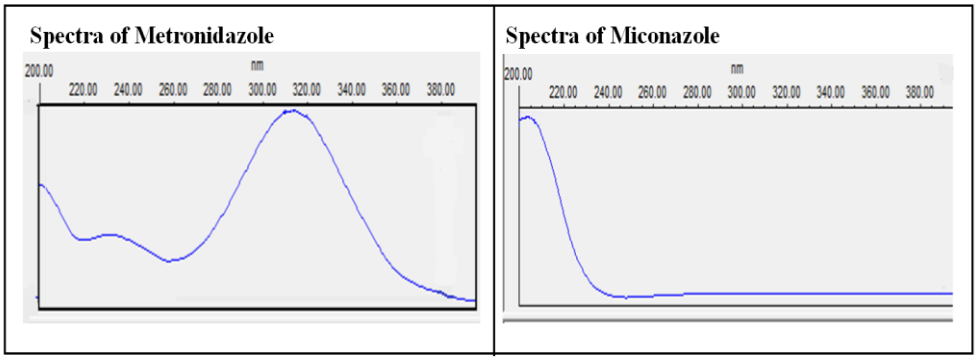


Figure 2: Spectra of metronidazole and miconazole.

The chromatographic method was optimized by modifying the mobile phase, flow rate and column design. Finally, development was carried out using a C8 column and a mobile phase of water and isopropyl alcohol (70:30 v/v) at a flow rate of 1.0 ml/min. The eluent was monitored at 210 nm and short run times were achieved as demonstrated in the chromatogram shown in Figures 3, 4, 5, and 6 of the blank, Standard, metronidazole Peak ID and miconazole Peak ID the peak identification of each analyte. In Table 1, the system suitability parameters are displayed.

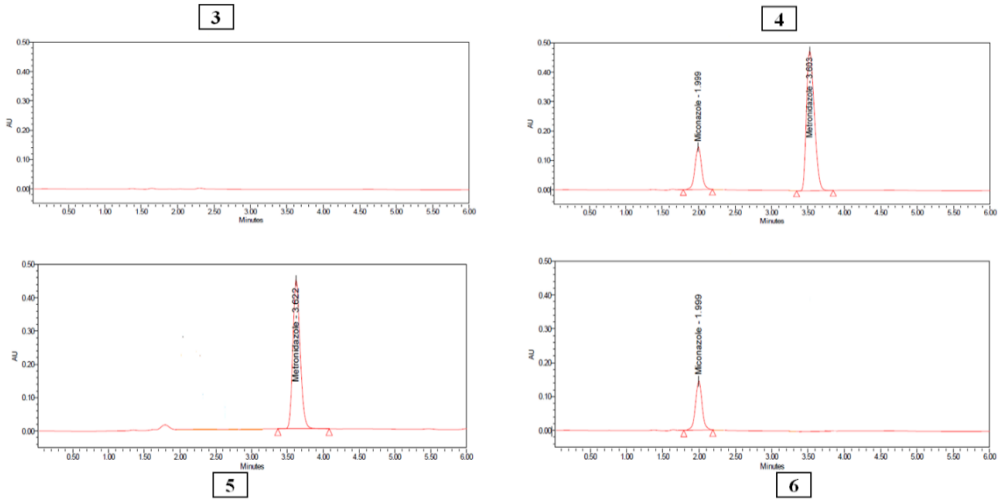


Table 1: System suitability parameters.

Parameter	Metronidazole	Miconazole	Acceptance criteria
% RSD (Peak area)	0.09	0.12	NMT 2.0%
Tailing Factor	1.3	1.1	NMT 2.0
Theoretical plate	2685	3429	NLT 2000

Linearity & Range

The concentration and response of UV and HPLC methods were linearly related. The linearity study was carried out using five concentrations such as 18.75 µg/ml - 112.5 µg/ml for metronidazole and 50 µg/ml - 300 µg/ml for miconazole. Plots of the method's concentration in µg/mL (x-value) against absorbance (y-axis) or response (area observed) are displayed in Figure 7. Table 2 shows the slope of the regression line, the correlation coefficient, the percentage Y-intercept, and the residual sum of squares results.

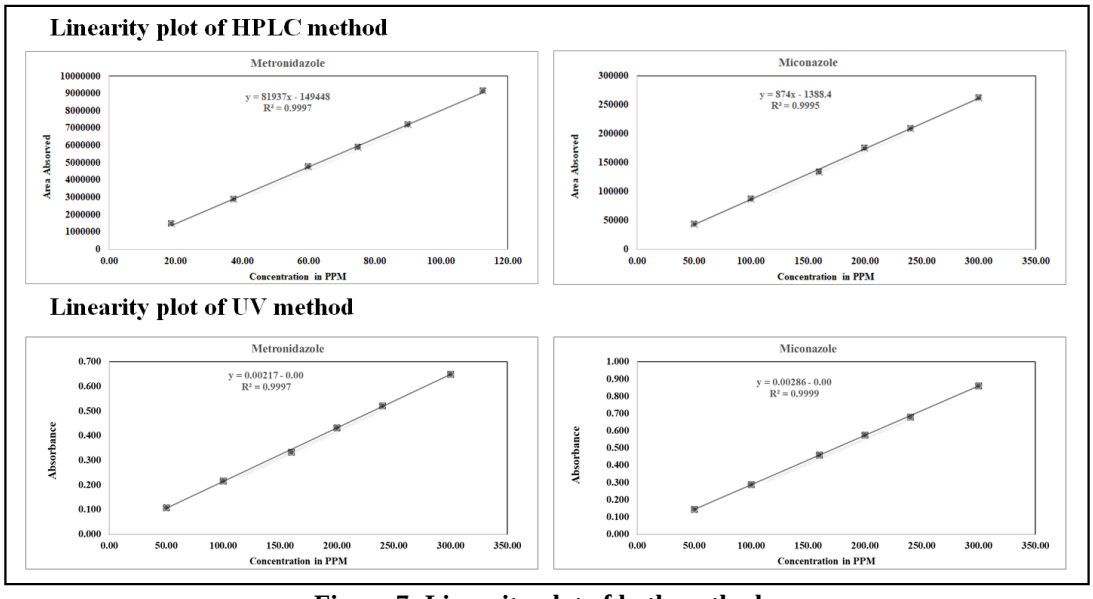


Figure 7: Linearity plot of both methods.

Table 2: Linearity plot of Metronidazole and Miconazole.

Parameter for Linearity	HPLC method		UV Method	
	Metronidazole	Miconazole	Metronidazole	Miconazole
Correlation coefficient R	0.9997	0.9995	0.9997	0.9999
Slope	81936.68	873.62	0.00217	0.00286
Y Intercept	149447.55	1388.44	0.00	0.00
%Y – axis intercept	2.50	0.80	0.72	0.38

Accuracy

Recovery studies were conducted using the developed methods to examine the accuracy. To determine the analyte using spectrophotometric and chromatographic methods, varying concentrations of standard analyte (50, 100, and 150 %) were added to the sample solution and analyzed in the same manner. the percent, which is the mean percentage recovery rate. The results of mean percentage recovery rate, %RSD and standard error are shown in Table 3.

Table 3: The percentage recovery of Metronidazole (MTZ) and Miconazole (MIC).

Method	Spiked Level (%)	Mean Recovery (%)		% Overall Recovery		% RSD	
		MTZ	MIC	MTZ	MIC	MTZ	MIC
HPLC Method	50	99.9	99.6	100.3	99.8	0.56	0.28
	100	100.5	100.1				
	150	100.6	99.7				
UV Method	50	98.9	98.6	99.3	98.7	0.37	0.72
	100	99.8	99.5				
	150	99.2	98.8				

Method Precision (Repeatability)

The precision of these methods was determined by repeatability (intraday) and intermediate precision (interday). Both methods were reported as %RSD of a measurement sequence. Detailed study data are provided in Table 4. The obtained results show good intraday accuracy.

Table 4: Method Precision and Intermediate precision data.

Precision	HPLC Method		UV Method	
	Metronidazole	Miconazole	Metronidazole	Miconazole
Area of Standard	3100051	194643	0.433	0.576
% Assay	100.1	99.3	99.4	99.1
% RSD	0.31	0.65	0.64	0.83
Intermediate Precision				
Area of Standard	3156046	195952	0.484	0.536
% Assay	100.3	99.8	98.9	99.5
% RSD	0.77	0.51	0.48	0.39

Robustness

As all the tested robustness parameters satisfied the system suitability criteria, the method was found to be robust. Deliberately changing the parameters cannot affect the performance of the method, which shows the robustness of the developed RP-HPLC method. The results are shown in Table 5.

Table 5: Robustness data of Metronidazole (MTZ) and Miconazole (MIC).

Parameter		%RSD (Area Response)		% Assay	
As per method		MTZ	MIC	MTZ	MIC
		0.09	0.12	100.1	99.3
Change in column temperature	25 °C.	0.20	0.18	100.5	100.2
	35 °C.	0.42	0.36	99.7	99.7
Change in flow rate	0.9 mL/min	0.38	0.31	99.9	99.9
	1.1 mL/min	0.67	0.59	100.2	99.6

CONCLUSION

In the study, HPLC and UV spectrophotometric methods for the simultaneous quantification of metronidazole and miconazole nitrate were successfully developed and validated. These environmentally friendly techniques are in line with sustainable pharmaceutical analysis and offer a reliable alternative to traditional methods while reducing environmental impact. The use of environmentally friendly solvents such as isopropyl alcohol and water mixtures significantly reduces the

hazardous footprint of analytical processes. In general, UV spectrophotometric methods do not require complicated operations and procedures. It is cost-effective and takes less time. Compared to the LC method, the UV method has advantages in these situations. In terms of statistics, the LC method surpasses the UV method in terms of method precision and recovery. In addition, the developed methods offer high accuracy, precision, and robustness, making them suitable for pharmaceutical quality control applications. Consequently, routine analysis of metronidazole and miconazole in pharmaceutical gel form may benefit from this technique.

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Ethical approval

The work described has not been previously published and it is not under consideration for publication elsewhere. This publication is approved by all authors and the responsible authorities where the work has been carried out.

Conflict of interest

The authors declare that there is no conflict of interest.

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