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Utilizing Trifluoperazine hydrochloride as an organic reagent in the spectrophotometric analysis of metronidazole

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Running Title: Utilization of Trifluoperazine hydrochloride as an organic reagent

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Abstract :

Background: A straightforward, precise, rapid, sensitive, and accurate spectrophotometric method has been developed for the estimation of metronidazole (MTZ) in pure, tablet, and super dosage forms. **Methods:** This method involves the reduction of metronidazole using zinc powder and 5N HCl at room temperature in methanol. **Results:** The resulting amine is utilized in the procedure. The method is based on the oxidation coupling with trifluoperazine hydrochloride, leading to the formation of an orange-red chromogen that exhibits an absorption maximum at 500 nm, with an apparent molar absorptivity of 2.6763 x 10^3 (L m^{-1} cm^{-1}), and adheres to Beer's law within a concentration range of 1-30 µg/ml. The assay results align well with the label claim. **Conclusions:** The proposed method is characterized by its simplicity, sensitivity, precision, accuracy, speed, and suitability for routine quality control.

Key words: Spectrophotometry, Metronidazole triflouperazine hydrochloride, Sodium persulphate, Zinc powder, pharmaceutical perpetrations.

INTRODUCTION

Metronidazole (MTZ) is chemically designated as 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol and possesses the molecular formula C6H9N3O3 (see Figure 1) [1]. This compound was initially synthesized in research laboratories in France in 1959 [2].

As a member of the 5-nitroimidazole family, MTZ is utilized in the treatment of bacterial infections [3]. The identification of the antitrichomonal effects of the antibiotic azomycin prompted the exploration of nitroimidazoles as potential antiparasitic agents [4]. MTZ functions as a nitroimidazole antiprotozoal and antibacterial medication, effective against anaerobic pathogens and infections caused by amoeba [5, 6]. The clinical investigations concerning MRZ have demonstrated its efficacy in treating amoebic liver abscess, amoebic invasive dysentery, as well as infections of the colon, small intestine, and vagina. Additionally, it has proven effective in the treatment of *Helicobacter pylori* associated with peptic ulcer diseases [7]. The officially endorsed methods for assessing MRZ encompass high-performance liquid chromatography (HPLC)[8-12], spectrophotometry[13-17], flow injection analysis[18,19], and polarographic analysis[20]. Most spectrophotometric techniques documented in the literature for quantifying metronidazole in the visible spectrum involve an initial reduction process using zinc powder and hydrochloric acid [16-23], followed by diazotization and coupling of the resultant amine. The reduction solutions of MTZ

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were subsequently transferred into 50 mL volumetric flasks and diluted to the designated mark with the same solvent. These solutions were then moved into 125 mL beakers, to which 20 mL of distilled water and 20 mL of hydrochloric acid were added for each MTZ reduction solution. More dilute solutions were prepared daily using suitable dilutions of distilled water.

This study outlines the creation of sensitive, straightforward, and precise spectroscopic techniques for oxidative coupling spectrophotometry aimed at quantifying metronidazole (MTZ), both in its pure state and within pharmaceutical formulations at a wavelength of 500 nm. The methodology involves the transformation of MTZ utilizing Trifluoperazine hydrochloride (TRF) as an organic reagent, resulting in the formation of a colored product complex in the presence of sodium persulfate, which acts as an oxidizing agent, along with dilute nitric acid. The objective of this research is to establish spectroscopic methods centered on metronidazole, whether as a standalone compound or incorporated in pharmaceutical products, and to apply these methods for quality control purposes.

MATERIALS AND METHODS:

The substances employed in this research are characterized by their affordability. The materials exhibited high purity and were utilized without the need for additional disinfection methods. A 1 M solution of nitric acid was prepared by dissolving 8.93 ml in 100 ml of deionized water. Additionally, 0.05 g of TRF was dissolved in 100 ml of deionized water, and 0.721 g of sodium persulfate was also dissolved in 100 ml of deionized water. The manufacturer supplied all volumetric vials containing the product in a consolidated manner, analytical grade BDH. The provided standard was a 500 ppm solution, which was subsequently used in the analysis. Following the dissolution of 0.639 g in 20

ml and 50 ml of bulk drug in 100 ml of water from the State Drug Industries and Medical Appliances Company (SDI), the process was applied to MTZ across four distinct pharmaceutical formulations in Iraq, as detailed in Table 1.

Bacterial isolates are gathered and identified.

Multidrug-resistant pathogenic bacterial isolates (MDRs) encompass a variety of organisms, particularly gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These bacteria have been isolated from diverse specimens, including burns, fecal samples, synovial fluids, wounds, blood, and urine. Additionally, *Staphylococcus aureus* and other gram-positive bacteria have been detected in similar samples. This research was conducted in the laboratories of the Department of Biology at Kufa University College of Science. These isolates underwent sub-culturing Brain Heart Infusion Agar (BHIA) and incubated for 24 hours at 37°C before their application.

The chemical samples enumerated below were prepared for use:

In Tube 1, a mixture was prepared by adding 1 ml of MTZ, 1 ml of TRF, 1 ml of Na2S2O8, and 1.3 ml of HNO3, along with deionized distilled water, to reach a total volume of 25 ml. In Tube two, the composition included 1 ml of TRF, 1 ml of Na2S2O8, 1.3 ml of HNO3, and deionized distilled water, totaling 25 ml. Tube three contained 1 ml of MTZ and deionized distilled water, adjusted to a final volume of 25 ml.

Antibacterial activity was tested:

The procedure for producing bacterial suspensions is outlined [24-28]as follows: The effectiveness of the antimicrobial test tubes against the isolated bacterial strains is evaluated through the agar diffusion technique. To evaluate the biological activity of the sample tubes, Mueller-Hinton agar (MHA) is

employed and compared with isolated bacteria [26-28].

The execution of the agar diffusion test wells:

A suspension of bacteria was prepared following the 0.5 McFarland standard, utilizing 100 µl of Brain Heart Infusion Broth (BHIB). This bacterial suspension was uniformly spread across the surface of the Müller-Hinton agar (MHA) plates, employing a sterile cork borer to create wells in all experimental plates. In the central well of each plate, 100 µl of Gentamicin was introduced as a positive control, while another well received 100 µl of Dimethyl sulfoxide (DMSO) as a negative control. The remaining wells were filled with 100 µl of test samples. Subsequently, the plates were incubated for 24 hours at 37 degrees Celsius. The diameter of the inhibition zones surrounding the wells was measured in millimeters. Each experiment was conducted in triplicate [25-30].

Apparatus:

The primary equipment utilized in this study comprises the following: (i) T80 UV-Visible Spectrophotometer from PG Instruments Ltd. (double beam), (ii) 303 PD UV-Visible Spectrophotometer from Apel, Japan (single beam), (iii) UV-1650PC UV-Visible Spectrophotometer by SHIMADZU (double beam), (iv) Electric Balance from Matter Toledo, Switzerland, and (v) a shaking water bath, model VS-1205 WL, from Scientific CO, LTD. Additionally, a pH meter from thephaw, Spinbot, was employed.

Method for calibrating curves

Various volumes of (MTZ) were introduced into 25 mL volumetric flasks, with amounts varying from 0.05 mL to 2.2 mL. Following this, 1 mL of TRF, 1 mL of Sodium persulfate, and 1.3 mL of Nitric Acid were added. Deionized distilled water was subsequently included to bring the total volume to 25 mL. After a 15-minute incubation at 20 degrees Celsius, the absorption of each solution was measured at 500 nm, using a blank solution from each volumetric flask as a reference.

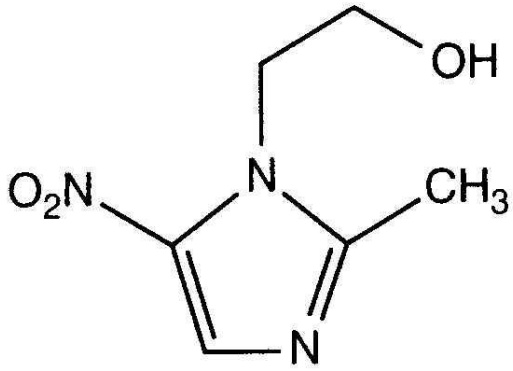

Figure 1. Chemical structure of metronidazole

Table 1. The examination of pharmaceutical formulations was conducted.

Samples of drug formulations	Composition defined	Corporation
Midagyl	Per tablet 500 mg Metronidazole	Pioneer Co. Pharmaceuticals and Chemical Industry Inc. – Iraq
Midagyl Oral Suspension	Per 5 ml contains 200 mg Metronidazole	Pioneer Co. Pharmaceuticals and Chemical Industry Inc. – Iraq
Flazi MDI Oral Suspension	Per 5 ml contains 200 mg Metronidazole	Moderen C0., Pharmaceutical Industries, Baghdad - Iraq.
Metronidazole Injection (BP)	Per 100 ml contains 500 mg Metronidazole	Shandong Qidu Pharmaceutical Co., Ltd. No.17, Hongda Road, Linzi District, Zibo City, Shandong Province, China.

RESULTS AND DISCUSSION

Absorption Spectra The colorless liquid of the MTZ drug (C) and blank solution (B) consisted of $Na_2S_2O_8$, TRF, HNO_3, and deionized distilled water for dilution. The analysis of the dilution reactions involving MTZ, TRF, $Na_2S_2O_8$, HNO_3, and deionized water was conducted using UV-Vis spectroscopy. During this process, samples of the orange product (A) and its reactants were examined. The spectra of the pure water phase MTZ solution, the blank sample, and the colored product obtained through the gradual addition of MTZ, TRF, Na2S2O8, HNO3, and deionized water are presented in Figures 2 A, B, and C. The orange product exhibits an absorption peak at 500 nm, distinctly different from the reactants. This observable color change can serve as a method for determining the presence of MTZ.

The reaction conditions have been enhanced, and different volumes of Na2S2O8 influence the outcome.

The study investigates the effects of varying amounts of sodium persulfate needed to achieve maximum absorption. The procedure used volumes ranging from 0.1 ml to 2.2 ml of $Na_2S_2O_8$ (0.004 M), as illustrated in Figure 2. Maximum absorption was observed with the application of 1 ml; subsequently, 1.0 ml of Sodium persulfate (0.01 M) was employed for this method.

The effect of different quantities of Trifluoperazine hydrochloride (TRF).

The effects of varying volumes of TRF on achieving maximum absorption were comprehensively investigated. The procedure was conducted within a range of 0.1 mL to 2.2 mL of TRF (0.015 M). Maximum absorption was attained with the use of 1 mL; consequently, as illustrated in Figure 3, 1.0 mL of TRF (0.015 M) was selected for the technique, as this volume was determined to be optimal. Additionally, the effects of different TRF volumes on the formation of (MTZ) were examined, which included volumes of 0.1, 0.4, 0.7, 1, and up to 2.2 mL.

(0.015 M) within a volume range of 0.1 ml to 2.2 ml. Maximum absorption was observed at a volume of 1 ml; subsequently, 1.0 ml of Trifluoperazine hydrochloride (0.015 M) was employed for this method, identifying 1 ml as the ideal quantity of the base form. The impact of varying TRF volumes on the formation of (MTZ) was analyzed, specifically at volumes of 0.1, 0.4, 0.7, 1, and 2.2 ml. As illustrated in Figure 4, the peak absorption was recorded at 0.1 ml. In this procedure, 2.2 ml of Trifluoperazine hydrochloride was utilized as a stabilizing agent to mitigate the aggregation of oxidative coupling. The reaction between Sodium persulfate and the analytes generates H+ ions, and a reduction in H+ concentration can facilitate the formation of Na-TR. This process establishes Sodium ions by forming Na (TRF)+ complexes in the TRF solution while concurrently removing the H+ ions generated during the oxidation phase that are produced through the formation of H(TRF) [31].

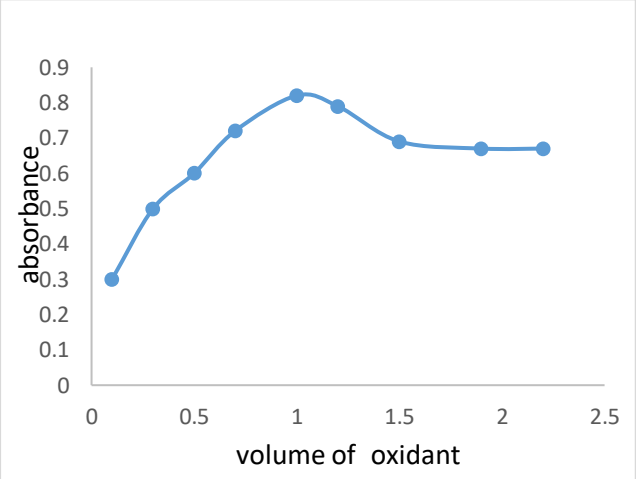

Figure 2. Action of $Na_2S_2O_8$

Effect of various volumes of Nitric Acid

The effects of various acids, specifically HNO_3, H_2SO_4, HCl, and CH_3COOH, on the generation of colored products were examined as illustrated in Figure 4. To evaluate the impact of the selected acids on the formation of (MTZ), a volume of 1 mL (1 M) was utilized. Among the acids tested, HNO_3 demonstrated the most favorable results, as indicated by the highest absorbance of the colored solution. The influence of different volumes of HNO_3 on (MTZ) formation was analyzed, with volumes of 0.1, 0.3, 0.7, 1, and up to 2.2 mL being assessed. The maximum absorption was achieved with the application of 1.3 mL of Nitric Acid (1 M); therefore, 1.3 mL of Nitric Acid (1 M) was employed in this process.

Series of addition

The intensity of color in the resulting compounds is significantly influenced by the varying concentrations of solutions employed during the synthesis of the oxidative coupling under investigation. Consequently, a series of experiments were conducted with different additive combinations, leading to the selection of the optimal sequence for all interactions examined. This selection resulted in the maximum uptake of the ultimate components, as presented in Table 2.

Table 2 outlines the sequence of reagent addition as follows: MTZ (A), TRF (B), Sodium persulphate (C), HNO_3 (D), followed by Dilution using purified water (E) to the designated mark. The combination of (A + B + C + D + E) yields the maximum absorption, indicating that this order is most effective for product formation.

The ideal enhancement

The table displayed the order of all oxidative coupling reactions and was employed in subsequent research.

The impact of temperature on the coloration of fruits and vegetables

The velocity of oxidative coupling was examined concerning temperature, as illustrated in Figure 5, covering the temperature spectrum of 0° to 60° Celsius (11). An increase in temperature to 20° Celsius resulted in stable absorption, potentially indicating stabilization at the optimal temperature. A temperature of 50° Celsius was selected for the synthesis of oxidative coupling, these specific temperatures were determined to facilitate the interactions of oxidative coupling in subsequent experiments.

The effect of time

The effect of time on the synthesis of colored compounds has been examined, specifically focusing on the production of oxidative coupling. Measurements were taken every five minutes under optimal conditions established in prior experiments, with durations varying from 5 to 120 minutes. The oxidative coupling produced exhibited significant stability, lasting over one hour, which facilitates further investigation of these interactions. As illustrated in Figure 6, a time interval of 15 minutes has been identified as the optimal duration within the overall procedure.

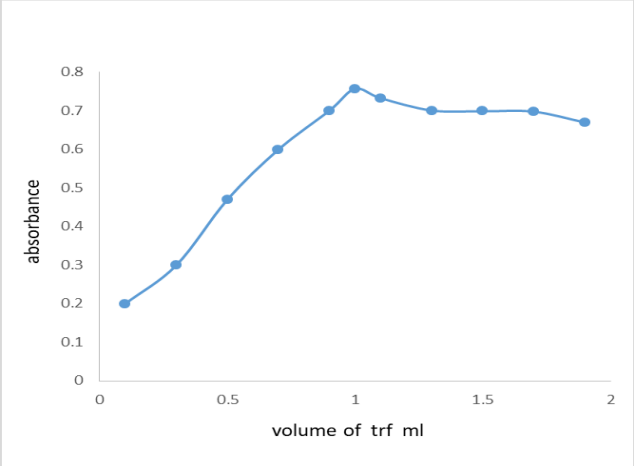

Figure 3. Impacts of Trifluoperazine hydrochloride (TRF) in various volumes

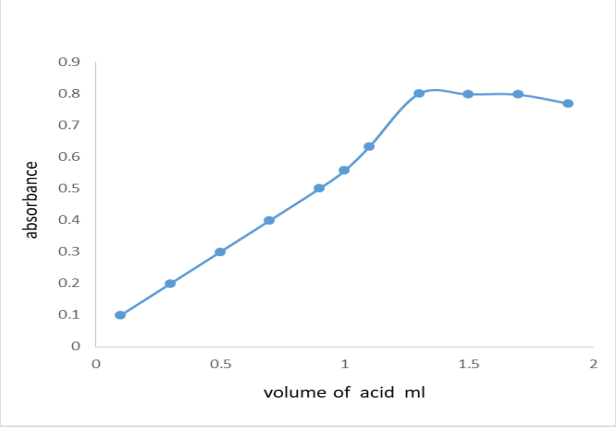

Figure 4. The influence of different volumes of HNO3

Table 2. The impact of incorporating variations in gradation.

Sequence	B+A+D+C+E	A+B+C+D+E	B+C+A+D+E	A+C+B+E+D	C+A+B+D+E	C+B+A+E+D	D+B+A+C+E
Absorbance	0.655	0.767	0.646	0.561	0.609	0.595	0.612

Standardization curve

Under the optimal conditions previously outlined, a standard curve was established for the colored solution. Additionally, various analytical attributes, including the calculations illustrated in Figure 7, along with the data presented in Table 3, improve the effectiveness of this analytical technique for measuring metronidazole at minimal concentrations.

Precision and accuracy

To evaluate the suggested to ensure the precision and accuracy of the method, nine replicates were performed, conducted at concentrations of 2, 14, and 24 $\mu g.ml^{-1}$ of Metronidazole. The accuracy for the three different concentrations of Metronidazole was the calculations were performed, and the findings are displayed in Table 4, which demonstrates the effectiveness of the method for determining Metronidazole is both precise and acceptable [32].

Interferences effect

The examination of the impact of excipients (interferents) is essential to ensure the reliability of the method for routine analyses of specific samples, particularly in the context of pharmaceutical formulations containing metronidazole. These excipients are present in concentrations that are The concentration was ten times higher than that of the drug compound under investigation. This assessment was carried out using spectral analysis of the expected pharmaceutical compounds, in addition to the independent incorporation of these substances into the solutions being examined, following the same approach used for the calibration curve.. The procedure involved the addition of 1 ml of the medication (500 ppm), followed by TRF, $Na_2S_2O_8$, and HNO_3, and subsequently 1 ml of each ingredient's concentration (500ppm), completed with distilled water. Optimal conditions were then established, and the absorbance of the resulting product was measured, allowing for the calculation of recovery and error ratios. Interfering effects are deemed acceptable if the error rate remains below 2%, particularly when Taking into account the dilution of the resulting 25 mL A solution utilizing filtered water (in which each value reflects the average of three measurements), it can be concluded that the inclusion of these additives does not negatively impact the estimation methods for metronidazole, as evidenced by the recorded Values about the percentage of errors and the percentage of recovery. The influence of these modifications on the absorption of the colored component is illustrated in Table 7. The impact of excipients on the metronidazole Assessment method can be assessed derived from the data on percentage error and percentage recovery.

Application of techniques.

The approaches employed to guarantee the effectiveness of the suggested methods necessitated the incorporation of a diverse range of pharmaceutical compositions (MTZ) in the context of the pharmacy solutions involved. A diluted preparation was prepared at a concentration of 500 ppm. The precision of all analyzed reactions corresponds with the findings derived from multiple pharmaceutical formulations, as illustrated in Table 5. This was achieved by employing three different volumes of each prepared sample while maintaining consistent operational procedures during the standard calibration preparation. Furthermore, all analyzed reactions correspond to the outcomes recorded for several therapeutic preparations, as presented in Table 6. The effectiveness and efficiency of the suggested approach for administering pharmaceutical formulations are illustrated, with each entry in the

table reflecting three measurements taken on a medium. To facilitate a comparison of results, Analytical methods were established based on the outcomes of a reliable and well-established Procedure for Isolated Compounds, as recognized in the pharmacopoeias of the UK and the USA. It is noteworthy that numerous pharmaceutical formulations are available in the market, with the theoretical values for F and T being 0.302 and 0.714, respectively [33-36].

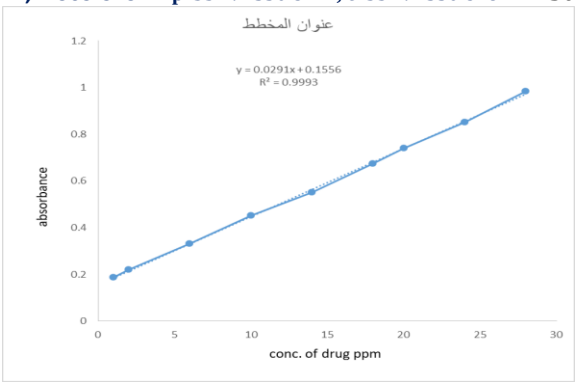

Figure 7. Standard calibration curve for Metronidazole.

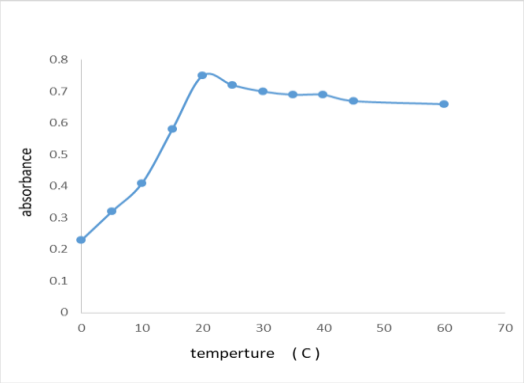

Figure 5. Impact of temperature on pigmented products.

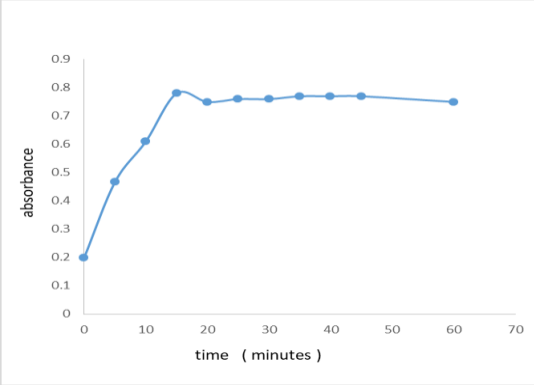

Figure 6. Time impact on colored product created

Table 3. Parameter for analytical determination metronidazole

Variable	Result
The Law of Beer Limits at (ppm)	1-30
absorptivity constant (L/mol.cm)	2.0043 x 10^4
Liner equation	Y=0.0291 X + 0.1556
Sandell's Sensitivity (µg /cm^2)	0.00014
Detection Limits (LOD) ppm	0.234
Quantitative Limits (LOQ) ppm	0.671
Coefficient of Correlation	0.9993
Slope(b)	0.0291
Intercept(a)	0.1556

Table 4. The incorporation of additives at a concentration of 25 ppm affects Metronidazole absorption

Interference	% Error	% Recovery
Lactose	1.2	101.2
Talc	1.3	103
Starch	-1.22	99.78
Cacl2	0/56	100.56
Sucrose	1.52	101.52
Glucose	-0.28	99.72
sodium citrate	0.65	100.65
Benzoic acid	-0.45	99.55
Paracetamol	-0.90	99.1
Caffeine	0.89	100.89
Sodium di sufate	1.7	101.7
Cholesterol	0.76	100.76
ascorbic acid	1.34	101.34

Table 5. The accuracy and precision of the chemical compound Metronidazole.

Concentration of metronidazole (ppm)		Relative error percentage	% Recoverability	% R.S.D
Present	Found			
2	1.97	-1.5	98.5	0.912
14	14.17	1.2	101.2	0.821
24	24.23	0.95	100.95	0.542

Table 6. The reactions of applications (van) involving various drug substances and F, t are compared to a conventional method for synthesizing nanoparticles. This analysis focuses on the interaction between sodium ion nanoparticles (MTZ).

Including (MTZ) preparation	Deliberated processes				The official procedure			
	Conc. of (MTZ) (ppm)		Rec %	R.S.D%	Conc. of (MTZ) (ppm)		Re%	R.S.D%
	Present	Found			Present	Found		
Midagyl tablet	2	1.94	97	0.967	2	1.93	96.50	0.543
	14	14.5	103.57	0.801	14	14.25	101.78	0.706
	24	24.55	102.29	0.517	24	24.3	101.25	0.347
Midagyl Oral	2	1.95	97.5	1.067	2	1.92	96	0.963
Suspension	14	14.6	104.2	0.828	14	13.9	99.30	0.501
	24	24.35	101.45	0.651	24	24.33	101.30	0.879
Flazi MDI Oral	2	1.96	98	0.825	2	1.93	96.50	0.331
Suspension	14	14.8	104.7	0.254	14	13.87	99.08	0.208
	24	24.45	102.57	0.788	24	24.35	101.40	0.725
Metronidazole	2	1.99	99.5	0.926	2	1.96	98	1.006
Injection (BP)	14	14.8	104.70	0.441	14	13.78	98.50	0.412
	24	24.25	101.041	0.178	24	24.45		0.804
							101.80	
Pure (MTZ)	2	1.97	98.5	0.912	2	1.94	97	0.912
	14	14.17	101.2	0.821	14	14.2	101.42	0.821
	24	24.23	100.95	0.542	24	24.8	103.30	0.542
F- value	0.302							
t-value								

Table 7. Demonstrates the antibacterial properties of silver nanoparticles against both Gram-negative and Gram-positive pathogenic microorganisms.

Types of Bacterial	Pure Antibiotic	Blank only	Color product modified antibiotic
Klebsiella pneumoniae	28	12	35
Staphyllococcus aureus	14	5	20
Pseudomomnas auroginosa	22	10	26

Conclusion

In summary, the interaction between Metronidazole and Sodium persulphate in reduction and oxidation processes has led to the development of a straightforward and rapid spectroscopic method for the quantification of Metronidazole. This method is relevant to both its natural state and its pharmaceutical formulations, employing altered nanoparticles as colorimetric sensors. The suggestion put forward the spectroscopic technique demonstrated commendable sensitivity, low detection limits, and a broad operational range. The colorimetric results exhibited remarkable stability in aqueous solutions, underscoring the method's high accuracy and precision. Notably, this approach does not require model primary treatments or solvent extraction.

The method proved effective in evaluating Metronidazole within pharmaceutical formulations, with Outcomes aligning closely Statistical evaluations with the anticipated content, including t and f tests, indicated Not substantial discrepancies in the dependability and precision of this approach in relation to conventional methods, thereby affirming the validity of its analytical application. Furthermore, the synthesized material was tested against various bacteria, demonstrating its effectiveness in reducing bacterial cell wall resistance.

Conflict of interest: NIL

Funding: NIL

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