Flame Photometric and Molecular Absorption Determination of Metronidazole in pure form and Pharmaceutical preparations

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Abstract

Simple, rapid and sensitive flame atomic emission spectrophotometric method is described for the determination of trace amount of Metronidazole Benzoate in pure and its pharmaceutical preparations. The method was based on the oxidation of Metronidazole Benzoate by potassium permanganate in alkaline solution to form an intense yellow soluble product and the intensity emission measured of potassium at emission line 766 nm using flame emission spectrophotometer. Beer's law is obeyed over the concentration range of 5-45 µg/mL, and relative standard deviation RSD% were (1.645, 1.705), and detection limits were (1.844 , 1.635 µg/mL), for tablets and injections respectively. The proposed method has been successfully applied for the determination of Metronidazole Benzoate in bulk drug and pharmaceutical formulations. The common excipients and additives did not interfere in these method.

Keyword:- Metronidazole, Flame Photometric Emission.

تقدير الميترانيدازول بالمضوائية اللهبية و الامتصاصية الجزيئية بشكله النقي و مستحضراته الصيدلانية

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قسم الكيمياء -كلية العلوم - الجامعة المستنصرية

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الخلاصة

في هذا البحث وصفت طريقة انبعاث ذري لهبي طيفي،امتازت بالسهولة والسرعة والحساسية العالية لتقدير كميات ضئيلة من عقار بنزوات الميترونيدازول كمادة نقية وكذلك في مستحضراته الصيدلانية. تعتمد الطريقة على اكسدة بنزوات الميترونيدازول بوساطة برمنغنات البوتاسيوم في المحلول القاعدي كعامل مؤكسد ومن ثم قياس شدة انبعاث البوتاسيوم عند الطول الموجي 766 نانومتر باستخدام مطيافية الانبعاث اللهبي ووجد ان قانون بير ينطبق ضمن مدى التراكيز (5-45 مايكروغرام/لتر)، وكان الانحراف النسبي المئوي RSD% (1.645 و 1.705) وحدود الكشف (1.844 و 1.635 مايكروغرام/مل) للأقراص الدوائية ولمحاليل الشراب على التوالي. طبقت الطريقة بنجاح في تقدير بنزوات الميترونيدازول في حالته النقية وفي بعض مستحضراته الصيدلانية، كما وجد ان لا تأثير للمضافات الدوائية في الطريقتين المقترحة.

الكلمات المفتاحية: الميترونيدازرل ، التقدير بالمضواء اللهبي ،التقدير بالامتصاص الجزيئي

Introduction:

Metronidazole (MTZ) is chemically name 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (or 2-Methyl-5-nitroimidazole-1-ethanol) as shown in figure 1. Molecular formula: $C_6H_9N_3O_3$,Molecular weight: 171.16 , Percent Composition: C 42.11%, H 5.30%, N 24.55%, O 28.04%, properties: Cream-colored crystals, Melting Point: 158-160 ^oC , Sparingly soluble in DMF. Soluble in diluted acids, pH of standard Aqueous solution: 5.8, trade name Flagyl . It is a well-established antibacterial agent in the treatment of various bacterial infections and antiprotozoal agent. It is a therapeutic agent of choice for amoebiasis and is also used in combination with other antimicrobial drugs against yeast infections [1,2].

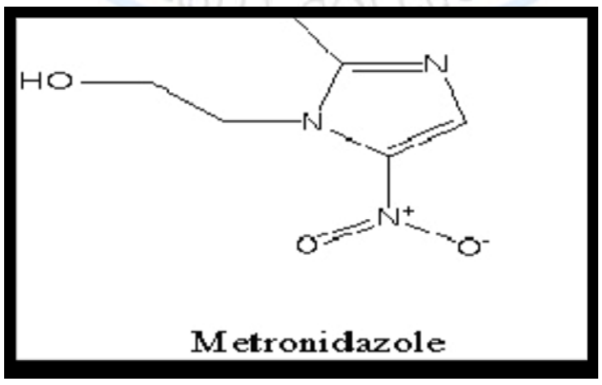


Figure 1. Chemical structure of Metronidazole

Inamdav , and Mody [3] , have studied the estimation of metronedazole based on precipitation of metronidazole–tungstiosilicic acid complex which extracted by $CHCl_3$-ethanol(5:1) medium . Liu [4] determined metronidazole in blood by UV spectrophotometry, the sample was treated with 3mL of H_3PO_4-KCl buffer solution(pH=9) and metronidazole was extracted into 10ml of $CHCl_3$ and the absorbance measured at 277nm. Sanyal et al [5] described a method for quantitative determination of metronidazole, raw materials, tablets, and injections, 0.35% sulphanilamide solution in 2.5M HCl was added followed by a 0.1% solution of N-(1-naphthyl) ethylene diamine dihydrochloride and the absorbance was measured at λ 583 nm after 10 min,. The recoveries of (0.005-0.025mg/mL) ranged from 98.8-100.5% **.** Assan et al [6] applied a difference in absorbance of nitro compound and its reduction products, sample solution was mixed with either aqueous 10% NH_4Cl, or H_2O and HCl followed by Zn powder. Nandipura et al [7] have determined metronidazole and tinidazole spectrophotometrically in pure as well as in dosage form by reduction of the nitro group of drugs using a reduction system comprising 10% Pd- C and formic acid and the resulting amine was then subjected to a condensation reaction with sodium 1,2-naphthaquinone-4-sulfonate to form red Schiff-base with an absorption maximum at 510 nm. Khalil [8] used kinetic method for the determination of metronidazole benzoate, based on the oxidation with $KMnO_4$ in alkaline medium in the presence of sodium dodecyl sulphate and the formed ion pooduct is spectrophotometrically measured at 610 nm. Zoest et al [9] applied HPLC analysis of metronidazole and nitromidazole separated on a column(10cm X 2mm) of hybersil ODS(5µm) at 28ºC , with a 9.5% acetonitrile containing 20mM Na acetate(pH=5)as mobile phase(0.5mL/min.)and detection at 320nm. Tong et al [10] determined metronidazole in human serum by HPLC in wich serum containing metronidazole was mixed with 1ml of ethanol, and analyzed on a column (25cm X 2.6mm) of YOG-$C_{18}H_{37}$(5µm) with 18% acetonitrile as a mobile phase (1mL/min.) and detection at 318nm. Avramova [11] had determined metronidazole in biological material and blood which was treated with acetonitrile and the mixture was centrifuged ,solution was evaporated under N_2 gas and the residue dissolved in the mobile phase of aqueous 58% acetonitrile ,and analyzed on a column(25cm X 4mm)of licosorb RP-18(5µm) at mobile phase flow rate 1mL/min and

detection at 318 nm . Zoest et al [12] applied central composite design to the optimization of HPLC analysis of metronidazole and nitromidazole on a column of Hamilton PRP-1 at 42^{o}C, the mobile phase was citrate-phosphate buffer containing 2-propanel and THF and detection was 272nm. Jaber et al [13] performed a reverse phase HPLC method to plasma levels of metronidazole , the separation and analysis on phenyl(300×4.6mm) column, with 5% acetonitrile in 0.1 M KH_2PO_4 buffer(pH = 4.5)at 324 nm. Dhanajay et al [14] ,used a high performance-thin layer chromatographic for the simultaneous quantification of metronidazole and miconazole nitrate in gel on silica gel 60 GF254 thin layer plates using mobile phase toluene: chloroform: methanol and the detection at 240 nm. Mahalingan and Rajarajan [15] ,used Reverse phase HPLC method for the estimation of metronidazole and norfloxacin in combined dosage forms, chromatography was carried out on zorbax C_8 column by gradients Lelution with mobile phase (Acetonitrile phosphate buffer) at flow rate of 1.5 ml/min, and UV response was monitored at 250 nm. Safwan and Nuha [16] determined metronidazole and miconazole nitrate simultaneously in tablets and vials by gas chromatography, using a capillary column SE-54 (15 m × 0.53 mm, i.d.) and nitrogen as a carrier gas at a flow rate of 9 mL min^{-1}, the oven temperature was programmed at 140°C to 250°C, the injector and detector port temperatures were maintained at 260°C, detection was carried out using flame ionization detector**.** Dalkara and et al [17] use differential pulse polarography for the determination benzoyl metronidazole in suspension, after reduction of nitro group at a static mercury drop electrode , the polarogram were recorded between 0.0-1.5V vs. silver-AgCl with scanning at 5mV S^{-1}, and the working pH was 6. Olajire and et al [18] determined metronidazole and tinidazole ,using coupling of diazotized nitroimidazoles with *p*-dimethylaminobenzaldehyde to form a greenish-yellow solution, coloured adducts of drugs showed shoulders at 406 nm and 404 nm. Thulasamma and Venkateswarlu [19] have used room temperature in methanol and the resulting amine was used to two based on oxidation coupling with 1,10-Phenanthrolin to form Orange red colored exhibiting absorption maxima spectrophotometric methods estimation of metronidazole in pure form and tablet dosage forms and based on the reduction of metronidazole with Zinc powder and 5N HCl at 510 nm and the second based on diazotization and coupling reaction with $NaNO_2$ and 4-Chloro-3-nitro Aniline to form Yellow

colored exhibiting absorbance maximum at 480 nm. Khalaf [20] determined Metronidazole spectrophotometricaly by reduction of nitro group to amine group by Zn dust and concentrated hydrochloric acid in hot ethanol under stirring, then the diazonium ion was prepared and coupled with para hydroxyl benzaldehyde to yield Insoluble water yellow dye detected at 410nm. Saffaj and et al [21] proposed spectrophotometric method for **determination** of Metronidazole which depended on the reduction of Metronidazole molecule with zinc dust and hydrochloric acid flowed by diazotation and coupling with 8-quinolinol to give colored chromogens easily measured at λmax= 437 nm. Adegoke and et al [22] determined metronidazole and tinidazole spectrophotometricaly which based on the charge-transfer complexation reaction of reduced forms of metronidazole and tinidazole with chloranilic acid to form a purple-colored complex with a new absorption band at 520 nm. Hesham and his groups [23] used chromatographic methods for determination of metronidazole and diiodohydroxyquin in pharmaceutical preparation, a mixture of chloroform, toluene, ethanol and acetic acid was used as the developing solvent for TLC-densitometry, a mixture of methanol and acetonitrile was used as a mobile phase for HPLC and UV detection at 254nm. The linearity 0f 0.5-10 μg $spot^{-1}$ for DIQ and 1-20 μg $spot^{-1}$ for MET in TLC-densitometry and 0.005-0.5 mg mL^{-1} for DIQ and 0.01-0.5 mg mL^{-1} for MET in HPLC. Kadam, and et al [24] have been developed quantitative estimation of poorly water-soluble drugs involves use of organic solvents, using three procedures for simultaneous method, absorbance ratio method and dual wavelength method which showed a maximum absorbance at a wavelength of about 325&285 nm respectively and isobestic point is observed at 296 nm. Siddappa and et al [25] determined metronidazole in pure form or in their tablets by two spectrophotometric methods. The methods were based on the reduction of the nitro group to amino group of the drug, the reduction of metronidazole was carried out with zinc powder and 5 N hydrochloric acid at room temperature in methanol, the resulting amine was then subjected to a condensation reaction with aromatic aldehyde namely, vanillin and p-dimethyl amino benzaldehyde PDAB to yield yellow colored Schiff's bases, the formed Schiff's bases are quantified spectrophotometrically at their absorption maxima at 422 nm for vanillin and 494 nm for PDAB. The aim of this work determination of metronidazole

benzoate in pure form and its pharmaceutical preparations for controls quality of drugs in many companies and countries.

Expermental parts

1. **Instruments and Equipment:-**
   - **1.1.** Flame emission spectrophotometer: (CORNIN G400) was used for emission measurements.
   - **1.2.** Double-beam UV-Visible spectrophotometer: Varian Gary 100 UV-Vis spectrophotometer.
   - **1.3.** Analytical balance: DENVER Instrument Max 220 gm, d=.0001g.
2. **Chemicals**

Metronidazole Benzoate standard material, Metronidazole tablets (500mg) and Metronidazole Syrups (200mg/5mL) were a supplied from the State Company for Drug Industries and Medical Appliances (Samara-IRAQ-SDI).

3. **Preparation of Metronidazole Solutions:-**
   - **3.1.** Stock solution of Metronidazole 1000 µg/mL was prepared by dissolving 0.1 g of Metronidazole Benzoate standard material by dissolving in 10 ml 0.5 M HCl and transferred to 100 mL volumetric flasks, diluting to mark with distilled water DW. Other standard solutions were prepared by subsequent dilution of stock solution.
   - **3.2.** Metronidazole solution 100 µg/mL was prepared by diluting 10 mL of Stock solution to 100 mL DW in volumetric flask, this solution was used for recorded UV-Vis spectrum.
   - **3.3.** Standard solutions for calibration curve were prepared by diluting Metronidazole solution 100 µg/ml to (5-50 µg/mL)**.**
   - **3.4.** Potassium permanganate solution 0.01 mole L^{-1} prepared by dissolving 0.158 g of $KMnO_4$ analytical reagent in 100 mL volumetric flasks.
   - **3.5.** Sodium hydroxide solution 2.5 mole L^{-1} prepared by dissolving 10 g of NaOH analytical reagent in 100 mL volumetric flasks.

4. Recommended procedure:-

Metronidazole standard solution covering the working concentration range from 5 to 50 µg mL^{-1} were transferred into 20 mL volumetric flasks and 6.0 mL of 0.01 mole L^{-1} potassium permanganate followed by 5.0 mL of 2.5 mole L^{-1} NaOH were added and shacked well, then make up to the mark with water. The reaction mixture allowed to stand for 10 min. Atomic emission intensity for potassium was measured directly at 766 nm against appropriate blank sample, and the values of the emission intensity against the final concentration in µg mL^{-1} to get the calibration curve were plotted.

5. Determination of Metronidazole in pharmaceutical preparations:-

5.1. Tablets:-Twenty Metronidazole tablet 500 mg were mixed, grinded and weighted, 0.1 g of this powder was weighted and dissolved in 100mL of DW in volumetric flask. 10ml was transferred to 100mLvolumetric flask and diluted to the mark with water. 2.0, 4.0 and 6.0 mL of these solutions was transferred to 20ml volumetric flasks. Then followed as described under the above recommended procedure.

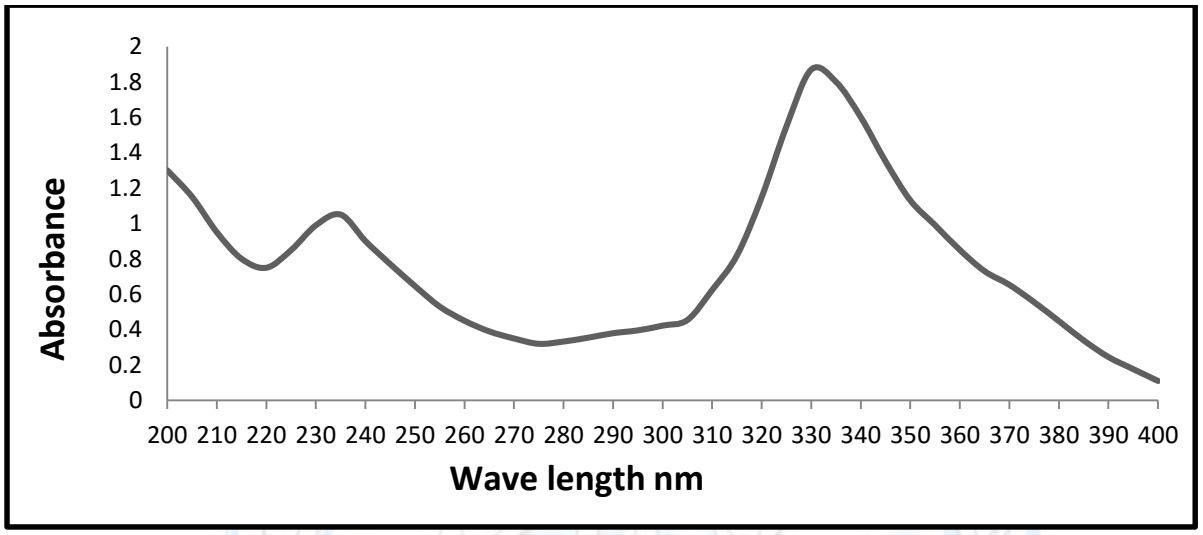
5.2. Syrups:- An accurately measured 2.0 mL aliquot of the mixed contents of three syrups was transferred into a 5 mL test tube containing 1 mL 0.5 M of NaOH and then centrifuged at a rate of 5000 rpm for 5 min. The residue was washed at least three or more times with mild alkaline solution, then was quantitatively transferred into a 100 mL calibrated flask, and after the complete dissolution in 0.6 M HCl, diluted to the mark. 2.0, 4.0 and 6.0 mL of these solutions was transferred to 20mL volumetric flasks.Then followed as described under the above recommended procedure.

Results and Discussion

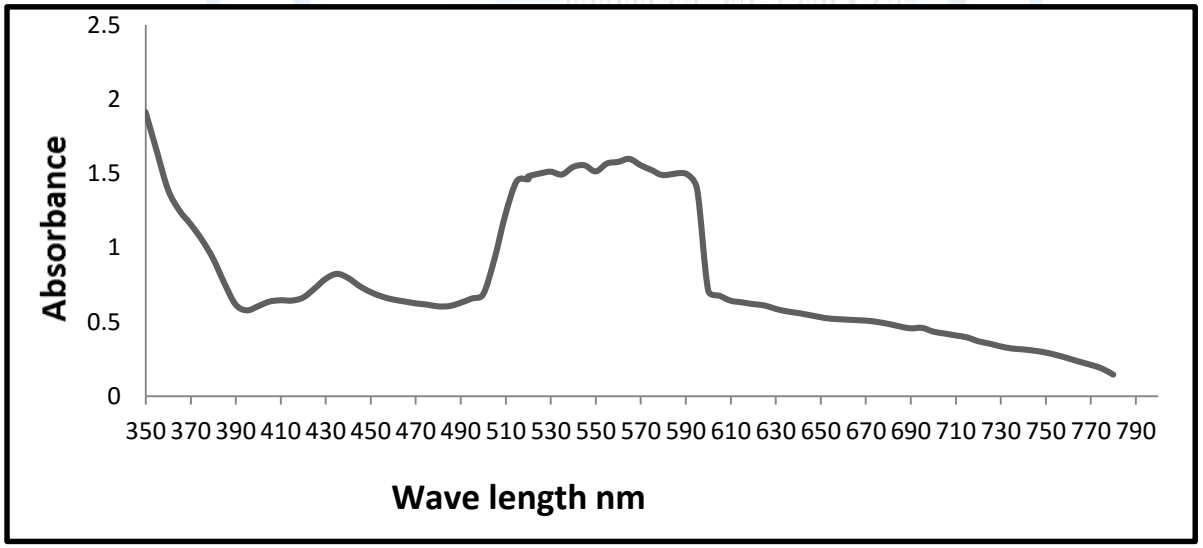
Molecular (UV-Vis) spectra

Two peaks at 235 and 330 nm for 100 µg/mL Metronidazole solution shows in normal UV-Vis spectrum in (Figure 2). $KMnO_4$ in basic medium shows an absorption bands at 510, 530 and 550 nm (Figure 3). The addition of aqueous solution of Metronidazole to $KMnO_4$ solution

in basic medium causes a change in the absorption spectrum of $KMnO_4$ (producing of green color) with a new characteristic bands at 610 nm for manganite ion (Figure 4).



Figure(2): Molecular spectrum of 100 µg/mL Metronidazole solution.



Figure(3): Molecular spectrum of $KMnO_4$ in alkaline medium.

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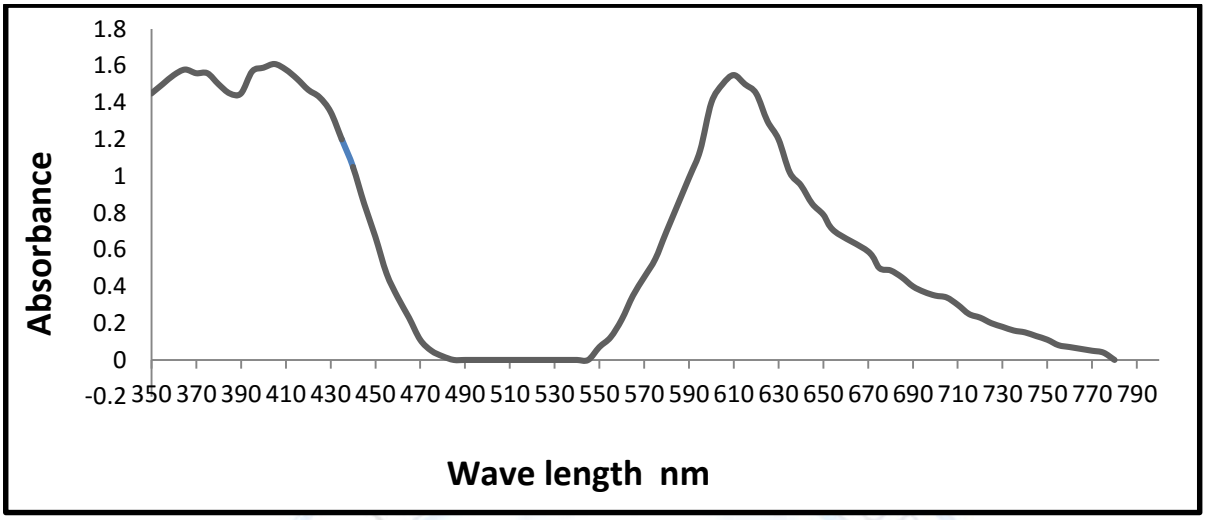
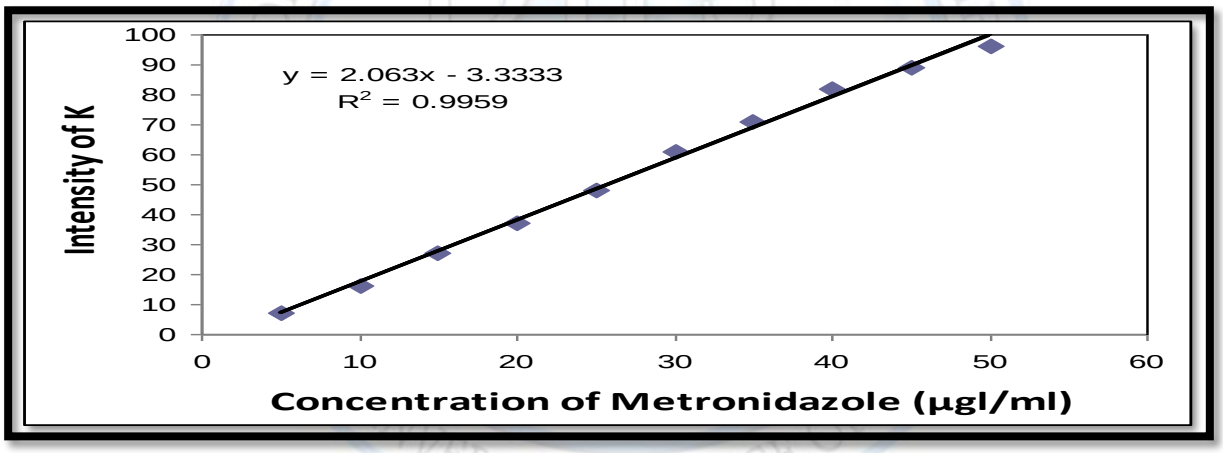


Figure(4): Molecular spectrum of $KMnO_4$ with Metronidazole in alkaline medium.



Figure(5): Calibration curve for Metronidazole with $KMnO_4$ in alkaline medium by flame atomic emission.

Table (1): The analytical data for Determination of Metronidazole.

Formula-tion type	linearity (µg/mL)	Regression equation	correlation coefficient r^2	Recovery%(Rec.%)	Detection Limit (DL) (µg/mL)	RSD%	Relative Error (RE%)
Tablets (500mg)	5-45	Y=2.063 X+3.333	0.995	95.761%	1.844	1.645	-4. 239
Syrups (200mg/5mL)	5-45	Y=2.063 X+3.333	0.995	95.873%	1.635	1.705	-4.127

Discussion

When Metronidazole reacting with $KMnO_4$ in basic medium the three bands (510, 530, 550 nm) were disappeared with appearance of one peak at 610 nm with changing purple color of permanganates to blue for manganite ion [26].

$$\mathbf{MnO_4^{-1} + OH^- + e \Longleftrightarrow MnO_4^{-2} \quad E=+0.564\ V}$$

Potassium permanganate considered strong oxidant reagent in reaction of Metronidazole, it oxidized either nitrogen atom in imidazole ring to nitro group [27], or hydroxyl group to form a carbonyl group [28] ,or methyl group to carboxyl group [29]. We applied a new flame photometric method for determination of Metronidazole in bulk and its pharmaceutical preparations, we obtained the concentration of Metronidazole in dosage form was closely for the value recorded (labeled) on the Metronidazole tablets and suspension (478.805, 191.746 µg/mL) for tablet and syrup respectively. In this research we applied other method for determination of Metronidazole in its dosage form in addition to normal spectrophotometric method and we obtained the concentration of Metronidazole in dosage form was closely for the value recorded (labeled) on the Metronidazole tablets and syrups.

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