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New method for estimation of metronidazol by oxidative coupling reaction using phenothiazine reagent

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***Abstract*---**A Spectrophotometry method was developed to be faster and more sensitive to determine the purity of metronidazole compound. This method is relying on the reaction of oxidation and conjugation with phenothiazine in the acidic medium and with presence of N-bromo succinimide compound as oxidative factor to give a red product at the wavelength 514 nm. This is will be within the range of Beer's law (5-50 ppm), molar absorbance (2.0538 × 103 L.mol-1.cm-1), correlation coefficient (0.998), and Sandell's sensitivity (0.0833 µg/cm2). Limits of detection (LOD) (4.6 x 10-3 ppm). This method was successfully applied to pharmaceutical preparations.

***Keywords*---**estimation, metronidazole, oxidative phenothiazine.

Introduction

Metronidazole (MRZ) has a chemical formula $C_6H_9N_3O_3$, it is a chemically consists of 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol, it is soluble in the acetone and alcohols, but quite unsalable in water (Figure-1) [1][2][3][4].

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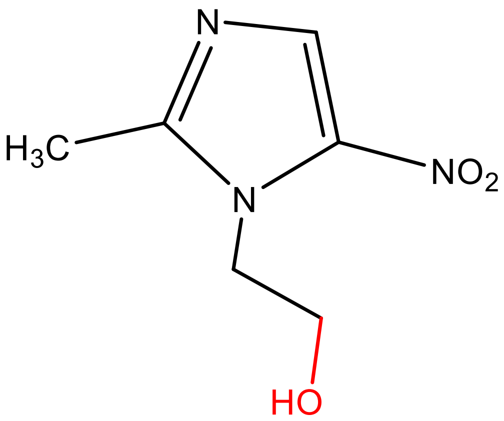


Figure 1. Structure of MRZ

Metronidazole used as an ant-parasitic and antibacterial agent [1]. Following the discovery of azomycin antibiotic the studies focused on the metronidazole as an anti-parasitic medicine. Previse studies showed that metronidazole has highly efficacy against the amoebae parasite in the host tissue as well as against the Entamoeba histolytic in vitro experiments [2]. In addition, metronidazole was used as bactericidal against anaerobic and facultative anaerobic like *peptostreptococcus*, *Trichomonas species*, *Fusobacterium* and *Clostridium species* [3]. It is also used as a drop to treat the eye infection that caused by *Bacterioides fragilis* [4]. Several techniques have been described for the determination of metronidazole in bulk and dosage forms or in biological fluids spectrophotometric method [5][6][7][8], HPLC methods. [9], and potentiometry [10][11].

Apparatus

The absorption and spectroscopic reading were achieved by using a Shimadzu UV/Vis 1800 spectrophotometer (Japan), and quartz cells with a light path of 1 cm. The weight of chemical compounds was measured using the sensitive scale (S 201 BL Sartorius). Heating process was carried out using the water bath (Karl Kolb-Germany).

Materials and chemical solutions

All the chemicals and analytical reagents were used in this study have the highest purity. As described below :

Standard Metronidazole solution, (100 μg/ml)

The amount of 0.01 g of pure metronidazole (General Company of Pharmaceuticals and Medical Appliances Samarra - Iraq) was dissolved within 5 ml of pure ethanol, then the mixture transferred to a volumetric bottle and volume was competed with ethanol up to 100 ml.

N-bromo succinamide solution (0.05 M)

This solution was prepared by dissolving 0.5 g of N-bromo succinamide in the distilled water with slight heating after that adding a distilled water up to 50 ml and kept in a dark bottle.

Phenothiazine reagent solution (5.018×10^{-4} M)

0.01 g of the Phenothiazine was dissolved in 5 ml of ethanol then the volume completed up to 100 ml.

Hydrochloric acid solution (M1)

8.5 ml of concentrated hydrochloric acid was diluted by adding a distilled water up to100 ml.

Solutions of surfactants (1.0%)

The solutions of surfactants (SDS, CPC, Triton 100-X) were prepared by dissolving 1.0 g of them in distilled water and the volume then completed up to a 100 ml.

Pharmaceutical solution (100 µg/ml)

The pharmaceutical compound which containing metronidazole is available as a tablets form (General Company for Pharmaceuticals and Medical Appliances Samarra - Iraq (SDI)). 5 tablets were weighted about (3.1943 g), each tablet contains (500 mg) of metronidazole. To prepare a solution with a concentration of 100 µg /ml, the tablets were crushed and mixed well and then approximately (0.0127 g) of the crushed powder was dissolved in 5 ml of ethanol. After that the solution was filtered by using filter paper and the pellet was washed with ethanol, then the volume completed with ethanol up to 100 ml.

Preliminary study

0.5 ml of (1 M) Hydrochloric acid was added to 1 ml of metronidazole solution (100 µg / ml), then 0.5 ml of the oxidizing agent N-bromo succinimide (0.05 M) was added in a volumetric bottle with a capacity of 5 ml and waiting for a while to complete the oxidation process, then 0.5 ml of phenothiazine reagent with a concentration of 5.018×10^{-4} molar was added. This process resulted in the following figure.

Add order

The effect of multiple addition orders on the absorption of the colored product was investigated to get the best results, and these results showed when adding order that provides the maximum absorption value (sample solution, Hydrochloric acid solution, N-bromo succinimide, Phenothiazine reagent solution).

Effect of time on product stability

This study focused on the stability of the product that formed over time. After using the optimal conditions that were previously studied, the samples were measured at different time periods at the wavelength (514 nm). It was found that the stability of the formed product is approximately 60 minutes. This time is considered sufficient to make the necessary measurements.

final absorption spectrum

Based on the previous experiments, the optimum conditions of studying the absorption spectrum of Metronidazole oxidation by N-bromo succinamide in acidic medium were taken in the presence of the phenothiazine coupling reagent in Figure 2.

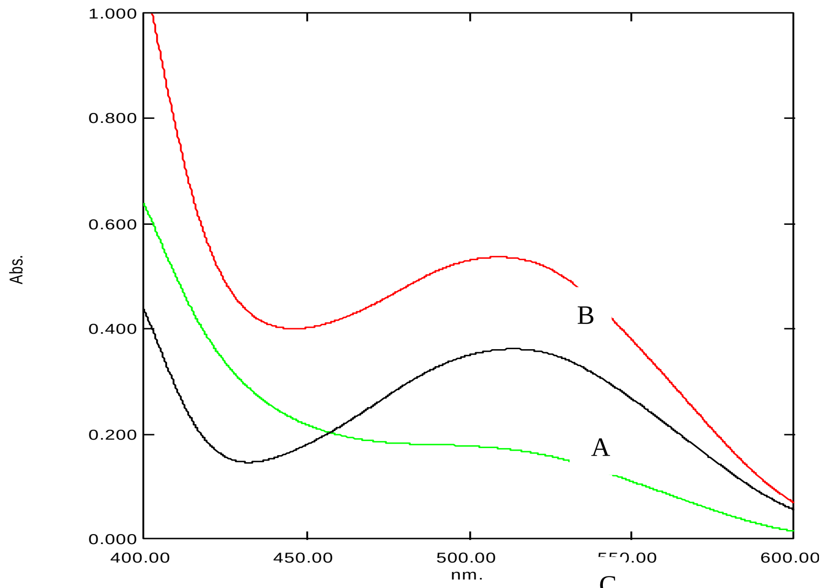


Figure 2. Shows the absorption spectrum in its final [illegible]n of (20 µg /ml) solution of Metronidazole

A-versus blank, B- versus distilled water, C-blank versus distilled water
Calibration curve creation procedure

According to the optimum conditions in the previous experiments, the standard curve was prepared by taking a series of different volumes (0.25 - 2.5 ml) in a volumetric vial 5 ml of metronidazole solution. Then 0.5 ml of concentrated Hydrochloric acid (1 M) was added, then 0.5 ml of the oxidizing agent N-bromo succinamide (0.05M) was added, then the solution was left for (5 minutes). After that 0.4 ml of phenothiazine reagent solution was added and the volume supplemented to the mark with ethanol solvent. The absorbance was measured at a wavelength of 514 nm. Figure 3 shows the standard curve follows Beer's law within the concentrations (5-50 µg /ml) and there was a negative deviation in the concentration that higher than 50 µg/ml. It was found through the value of the correlation coefficient that the linear features of the curve coefficient are excellent.

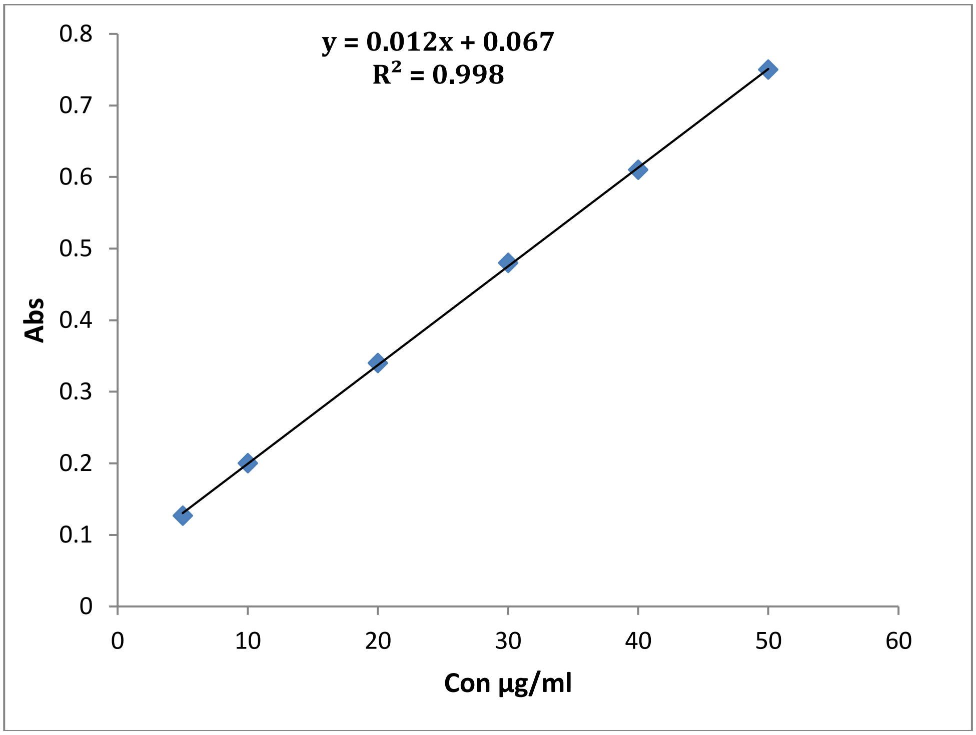


Figure 3. Shows the standard curve for determination of Metronidazole

Accuracy and compatibility

Accuracy and compatibility were studied by taking five replicates of three different concentrations (5, 10, 20) µg/ml of metronidazole solution according to Beer's law about the calibration curve. As shown in Table: 1 the recovery rate was about 99.79 %, and the relative standard deviation rate did not exceed 0.254 %, which indicates that the method has good accuracy and good concordance.

Table 1
Accuracy and precision

Con. MTZ µg/ml	Recovery %*	Relative Error %*	RSD %*
5	100.78	-0.78	0.225
10	98.76	1.24	0.345
20	99.85	0.15	0.192
	Ave of Rec% 99.79		Ave RSD %* 0.254

Table 2
Summary of optimal conditions

Material solution	Optimum amount
Drug 20 ppm	1 ml
HCl 1 M	0.5 ml
N–Bromo succinimid 0.05 M	0.5 ml
Phenothiazine M5.018×10^{-4}	0.4 ml
λ max (nm)	514 nm
Temperature C°	R. T

Table: 3 shows the values of λ_{max}, LOD, LOQ, Sandal sensitivity, and correlation coefficient. It is clear from the max values that the technique is good, and the correlation coefficient value reveals the high linear specifications of the caliber.

Table 3
Analytical parameters of the proposed method

Linearity range(µg\ml)	5-50
LOD (µg\ml)	0.408
LOQ (µg\ml)	1.36
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	2.0538×10^3
Sandell's sensivity ($µg\backslash cm^2$)	0.0833
Slope	0.012
Intercept	0.067
Correlation coefficient	0.998

Study the nature of the product formed
Continuous changes method (Job`s method)

This study focused on the nature of the formed product by applying the Job method [12] Same concentrations of metronidazole solution and phenothiazine reagent solution (5.8×10^{-4} M) were prepared. Different volumes of metronidazole solution and reagent were taken, with keeping the final for both of them as 3 ml. By applying the same optimal conditions which shown in Figure. 3 a volumetric vial 5 ml was used after the dilution up to the mark, then the reading was done at a wavelength 514 nm. Figure 3 shows that the conjugation ratio between metronidazole the phenothiazine reagent is 1:1.

Method of molar proportions

In this method, the volume of metronidazole solution (5.8×10^{-4} M) is fixed [12]. 0.5 ml of metronidazole solution was taken, while taking variable volumes of the reagent solution from (0.25 - 3 ml) and following the optimal conditions that previously described in the Figure 4. Then it was diluted with ethanol to the desired level in a 5 ml volumetric vial, then the absorption was measured at 514 nm. It was found that the products were formed in a ratio of (1:1).

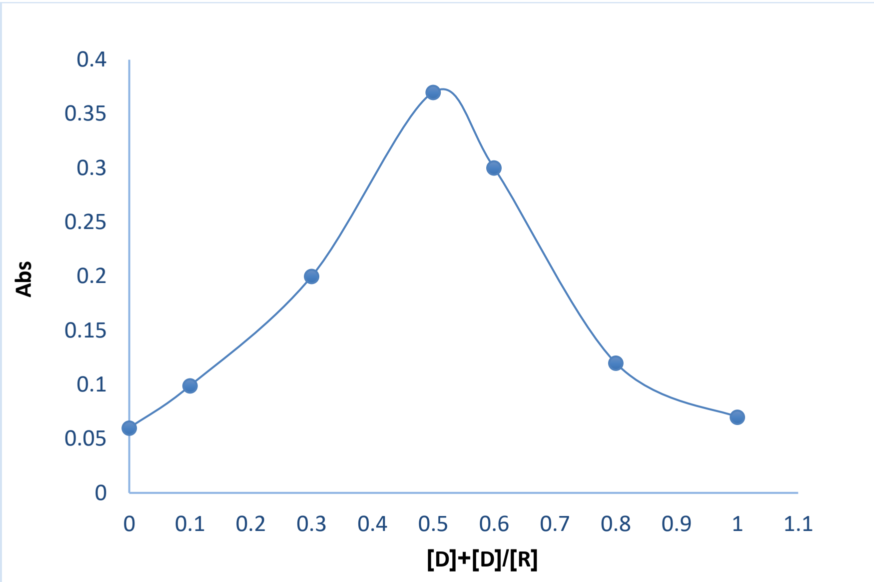


Fig. 4. Job's method for the oxidative coupling reaction of metronidazole with phenothiazine

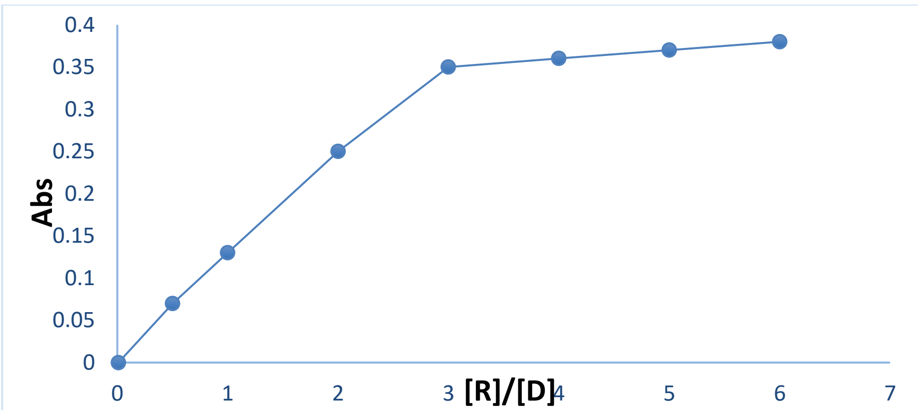


Figure. 5. Molar ratios method for the oxidation coupling reaction of metronidazole

Application in pharmaceutical preparations
Direct method

This method was achieved by taking three different concentrations of the metronidazole tablets. The procedure was applied depending on the optimal conditions that described previously in table 2. The average of five readings was calculated for each concentration against the versus blank solution at 514 nm.

Then the ratio of recall, relative error and relative standard deviation were calculated.

Table 4
Estimation of Metronidazole in pharmaceutical preparations

Pharmaceutical preparation	Amo. taken µg	Amo. measured µg	Rec (%)*	RE (%)*	RSD (%)*
Metronidazole 500mg\Tablets S.D.I. IRAQ	5	4.79	95.9	4.01	0.851
	10	9.86	98.6	1.4	0.811
	20	19.36	96.8	3.2	0.239

*Average of five determinations.

Standard addition method

This study applied the standard addition method to the metronidazole. That fixed volumes (0.25 and 0.5 ml) of metronidazole solution (100 µ / ml) were added to 5 ml bottles, and then increasing volumes of Standard metronidazole solution (0.25-2.5 ml) at a concentration (100 µg/ml) were taken. The solutions which mentioned above were treated as a same step to the standard curve method. The absorbance was measured at 514 nm against the mock solution. The results are shown in Figure 6.

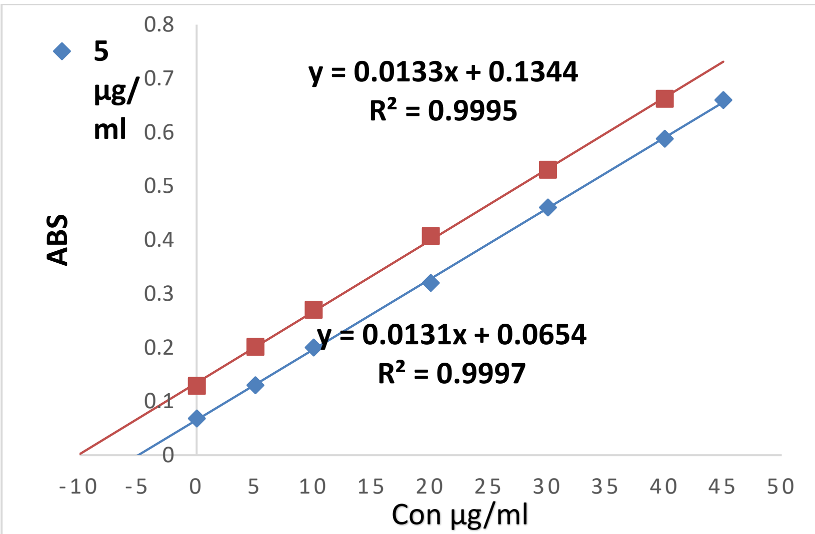
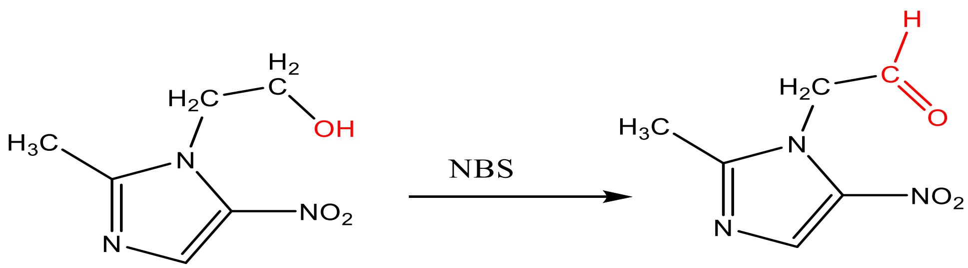


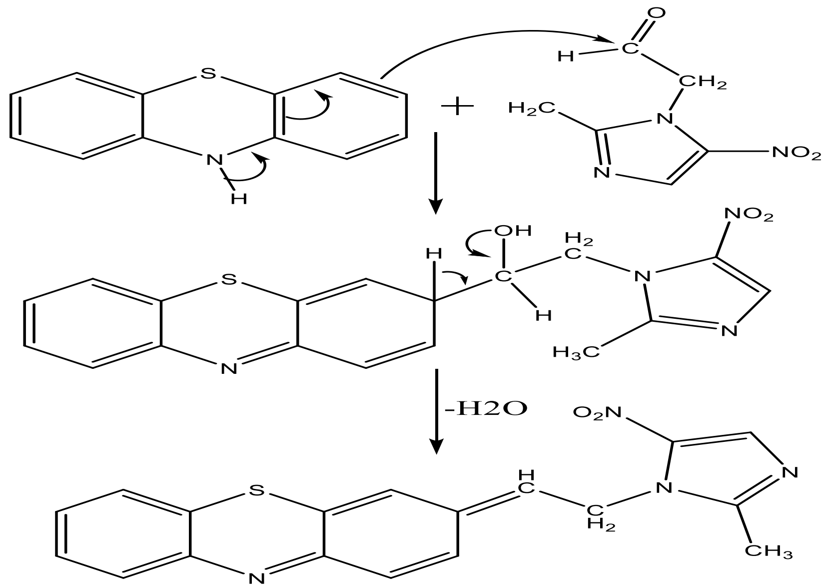
Fig. 6. Standard addition curve for the determination of Metronidazole in the pharmaceutical preparation at concentration (5, 10 µg/ml).

Suggested Reaction Mechanism

Alcohol is converted by N-Bromo succinamide (NBS) to Aldehydes and Ketones as shown in the following reaction [13]:

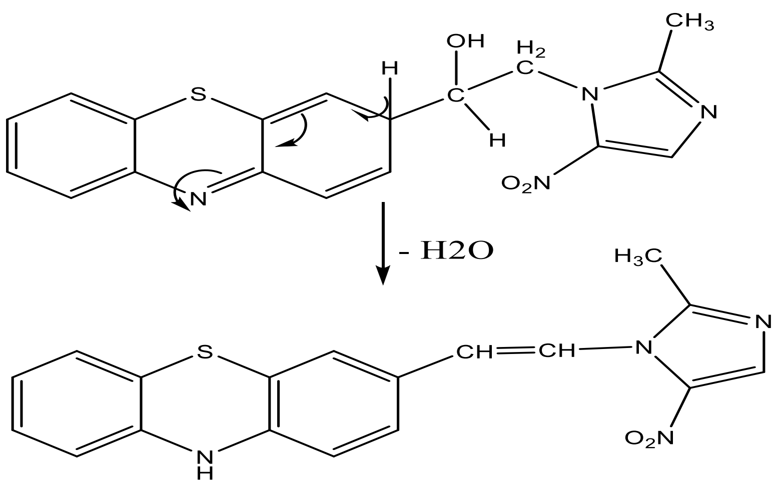


The reaction of this Aldehyde with phenothiazine is as follows:



A colored solution exchange system

Or the proton is removed from occurs outside the ring



This line eliminates a water molecule from a proton outside the ring is the best, as the ring restores the aromatic character and also a long reciprocal system that gives the color.

The Proposed Method Comparison

Some spectral changes for the suggested method to determine the metronidazole were compared with the same spectral variables to another spectroscopic method [7]. The results are recorded in the following table.

Table 4
Comparing the proposed method for the determination of metronidazole with another method

Parameter	Present method	Literature method*
Reagent	Phenothiazine	p-benzoquinone
Color of the product	Red	Purple
y max	514	526
Medium of reaction	Acidic	Acidic
Beer’s law range (ppm)	5-50	15-190
Molar absorptivity (L/mol.cm)	2.0538×10^{3}	1.09×10^{3}
Sandell's sensitivity (µg ⁄Cm^2)	0.0833	0.125
Slope	0.012	0.0061
Intercept	0.067	0.0016
Correlation coefficient(R)	0.9989	0.9998
LOD (µg/ml)	4.6×10^{-3}	___________
LOQ (µg/ml)	0.01518	___________
RSD %	0.333	0.625
Recovery %	99.79	__________
Stability of Colour (min)	60	20

Conclusion

In this study a simple spectrophotometric method was developed to determine the metronidazole which rely on the oxidative conjugation reaction of metronidazole with the phenothiazine in the presence of the detector N-bromo succinamide and within acidic medium which resulted in red complex at 514 nm. This method recognized as a simple, cheaper, sensitive and fast method. Concentrations between 5-50 µg/ml were determined by this method and reverse average was about 99.79 %, and the relative standard deviation coefficient about 0.254 %, and the Sandell sensitivity about 0.0833 µg/cm^2, the detection level is $4.6\text{x}10^{-3}$ µg/ml. This method was successfully applied to determine of metronidazole in pharmaceutical compounds.

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