

Assay of Metronidazole from Different Manufacturing Sources in Iraqi Markets

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الخلاصة

دواء الميترونيدازول هو من مجموعة المضادات البكتيرية المستخدمة في علاج الالتهابات البكتيرية اللاهوائية والذي يمكن ان يتحلل بواسطة (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لمعرفة وزن المادة الفعالة للدواء في الصيغة الدوائية المعينة.

في هذه الدراسة تم استخدام نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لتحديد الكمية الدقيقة من الميترونيدازول في العينات، وهذه العينات عبارة عن دواء الميترونيدازول 200 غرام على الشكل الصيدلاني (الأقراص) من الشركات الدوائية المختلفة والمتوفر في السوق الصيدلاني العراقي ومقارنتها مع النسبة المئوية المقررة حسب دستور الأدوية الادوية البريطاني.

الدراسة جرت بتشكيل منحنى التحديد الناتج من استخدام محاليل قياسية بتركيز مختلفة من المعيار الخارجي الميترونيدازول وتحقق هذه المحاليل في نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لتعطي قراءات مختلفة لمساحة ما تحت القمة ومن معرفة التراكيز ومساحة كل واحد منها يمكننا رسم المنحنى الذي سيتبع معادلة الخط المستقيم.

الميترونيدازول استخلص من الأقراص وبعد ذلك حقن في نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لمعرفة مساحة ما تحت القمة ومن المساحة الناتجة يمكن معرفة التركيز وبعد ذلك وزن المادة الفعالة في القرص الواحد بواسطة معادلة الخط المستقيم الناتجة.

أن النتائج التي تم الحصول عليها تبين أن جميع العينات الخمسة هي ضمن النسبة المئوية المقبولة حسب دستور الأدوية البريطاني (95%-105%).

Abstract

Metronidazole is an antibacterial agent that is effective in treatment of anaerobic infection. It can be assayed using HPLC to estimate the weight of the active constituent of the drug in the specific dosage form.

In this work we used the HPLC techniques to estimate the exact amount of Metronidazole in samples, which are Metronidazole tablet 200 mg from different pharmaceutical companies in Iraq pharmaceutical market to evaluate the content of drug in dosage form in these samples.

The experimental work includes making calibration curve using standard solutions of different concentrations of the external standard metronidazole USP 2002. Different readings of the area under the peak were obtained following the straight line equation.

The result show that all of five samples are within maximum percentage of the differences allowed (95-105%) according to the B.P 2003.

Introduction

Metronidazole [2-methyl-5-nitroimidazole-1-ethanol] is white or creamy-white crystalline powder with a slight odor and a bitter slightly saline taste and it darkens on exposure to light.

It is practically soluble as 1 g in 100 ml of water; 1 g in 200 ml of alcohol; 1 g in 250 ml of chloroform and slightly soluble in ether [1].

Metronidazole is one of theazole group antimicrobial agents as shown in figure (1), which is effective against anaerobic (but not aerobic) microorganism [2]. The infections are often polymicrobial that is the anaerobic bacteria are found in mixed infection with other anaerobes [3]. The anaerobic bacteria include gram-positive, gram-negative, cocci and bacilli.

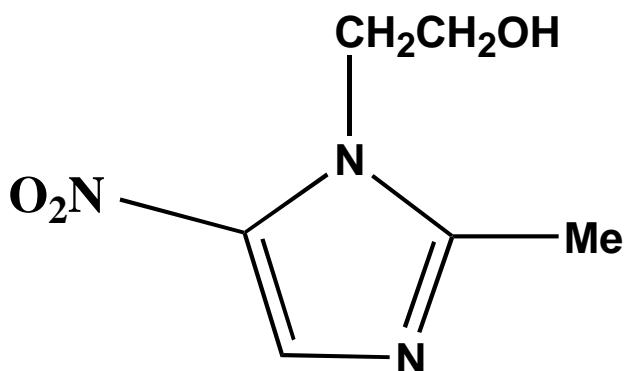


Figure 1: Chemical structure of Metronidazole

Pharmacokinetics:

Metronidazole is formulated as a tablet, suppositories and infusion [3], it is readily absorbed from Gastro-intestinal tract and widely distributed in body tissues.

Maximum concentration occurs in serum after 1 to 2 hours and traces detected after 24 hours.

About one-half of Metronidazole does excreted in the urine as Metronidazole and its metabolite, including an acid oxidation product and a glucuronide [4].

Precaution:

Metronidazole should not be used in patients with blood dyscrasias or with active diseases with central nervous system, during pregnancy. Metronidazole may provoke reaction in some individuals when given in conjunction with alcohol [4].

Toxic effect:

Side effect of metronidazole include gastrointestinal discomfort, anorexia, nausea, coated tongue, dryness of mouth and unpleasant taste, headaches, skin rashes, vertigo, depression, insomnia and darkening of urine.

Occasionally there may be a temporary decrease in the total white-cell count[5].

Uses:

Metronidazole has antiprotozoal action and it is effective against *Trichomonas vaginalis* and other protozoa. It is used in the treatment of trichomoniasis of the genitor-urinary tract in male and female.

In amoebiasis, it is effective at all sites of infections.

Metronidazole also used in the treatment of giardiaasis and of Vincent's infection.

In trichomoniasis, the usual dose for adult and children over 10 years is 200 to 250 mg three times daily after food for 7 to 10 days.

In elderly women the Metronidazole used in combination with hormonal therapy to clear vaginitis, in such case Metronidazole is given as vaginal pessaries in a dosage of 500 mg daily for 10 to 20 days.

In treatment of acute and chronic hepatic and intestinal amoebiasis, a single daily dose of 2 to 2.4 mg of Metronidazole is given for 2 to 3 consecutive days.

In addition, Metronidazole is used for eradication of cysts in symptom less carries, treatment with 400 to 800 mg of Metronidazole three times daily for 5 to 10 days [6].

In our study, Metronidazole was extracted from the tablets and injected into HPLC system to measure the area under the peak. From which we calculate the concentration, then we evaluate the weight of the active constituent of the drug.

Aim of the study:

The aim of this study was to investigate Metronidazole from different pharmaceutical companies in Iraqi pharmaceutical market to:

1. To prove that the weight of each tablet is within the range of maximum difference allowed.
2. Assay the active constituent of different samples using HPLC-UV method and comparing the results to obtained the most potent one form the tested samples

Materials and Methods

Material:

Methanol of HPLC grade (lot 643458 HN) is obtained from Panreac Quimica S.A., European Union. Metronidazole B.P/USP as a standard powder is manufactured by Aerti Drug LTD (India). All the other chemical were used of pharmacopoeial grade.

Apparatus:

The HPLC with liquid delivery system, WATERS 2795, Separation model (alliance HT) equipped with autosampler system. UV-visible spectrophotometric detector, WATERS.

PH meter microprocessor HANA type 211 and Ultrasonic mixer FRITSCH Laboratte type 17. 202.

Study design:

Samples:

The raw material that used for preparation of stock solution obtained from SAMARWA DRUG INDUSTRY (SDI) and tested in Iraqi National Center for quality control by reference standard and the result of quality was (98%). But the samples (Metronidazole tablet) were taken from the Iraqi pharmaceutical market and table (1) explain the data obtained concerning the proprietary name , source , M.D, E.D, number and average tablet weight of each sample.

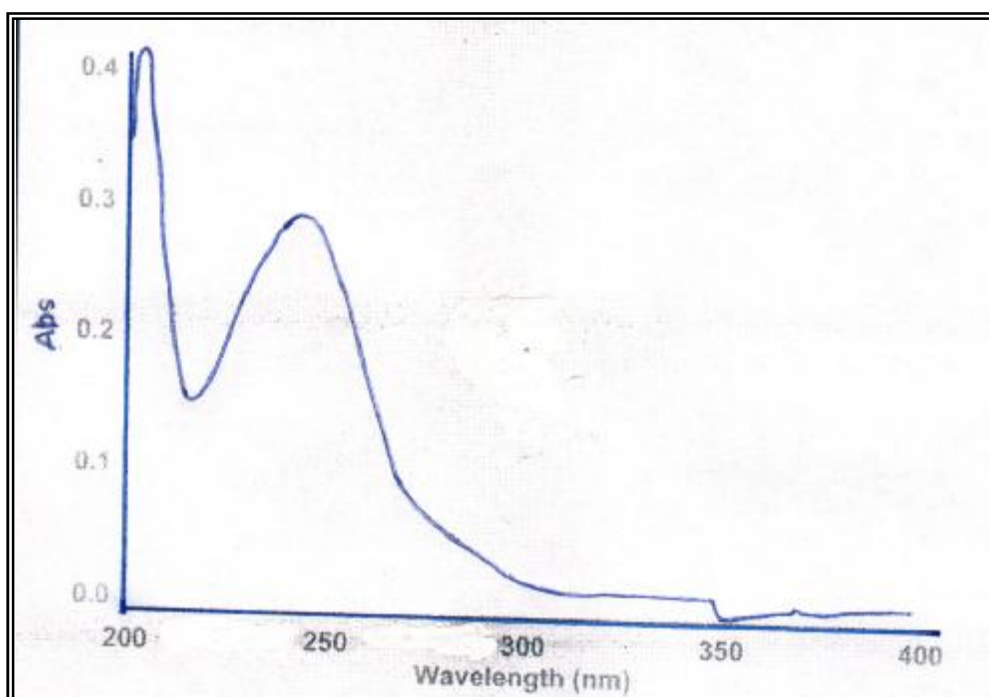
Type	Company	M.D	E.D	Number	Average tablet wt g.
A MEDAZOLE	SDI	09/2004	09/2007	B-12	392.5
B METROSULE	Ajanta Pharma	08/2005	07/2008	PAO21 5H	367.2
C METRONIDAZOLE	IPI	07/2005	07/2008	B-3-7	343.9
D SAFAGYL	SAFA	03/2005	03/2009	B-3	340.5
E METROZOLE-200	MACLEODS	10/2005	09/2009	MT20-505	385.7

Table 1: Metronidazole 200 mg tablets in the Iraqi market.

UV Spectrophotometer for Qualitative Analysis:

Measured The Ultraviolet absorption spectrum of a 1 in 50,000 solution of it in a 1v in 350 ml solution of sulfuric acid in methanol exhibit a maxima and minima at the same wavelength as that of the similar solution of the USP Metronidazole RS, concomitantly measured [7].

The following data obtained shown in figure (2).



Method:

First, we prepare the calibration curve or standard curve, after that we can test the metronidazole 200 mg tablet of many samples from different pharmaceutical companies.

For the preparation of the calibration curve, we use external standard Metronidazole USP 2002 for the preparation of stock solution and from this stock solution, we make different dilution, which are then injected into the HPLC to obtain the (AUP) of that concentration [8].

The chromatographic procedure is carried out by using:

- 1- Stainless steel column (15 cm, 4.6 mm) packed with (LI 7).
- 2- Mobile phase, which is composed of (80:20) water to methanol respectively.
- 3- UV detector with 254 nm wavelength.

Preparation of Standard Solution & Calibration Curve:

Dissolve an accurately weighed quantity (50mg) of USP Metronidazole standard powder in (100 ml) of the mobile phase to obtain a solution having a known concentration of about 0.5mg /ml [8].

After that, we transfer different volume from this stock solution into 10 ml volumetric flask to prepare solutions of different concentration by complete the volume to 10 ml. with the mobile phase, then each one of these dilutions was injected into HPLC and then take the result by the area under the curve (AUP) method. As shown in tablet (2).

The volume taken from the stock solution	The concentration (x-axis)	The AUP mm a
0 mls	0 mg/ml (0 mmole/l)	0.00
4 mls	0.2 mg/ml (1.1684 mmole/l)	8135.00
6 mls	0.3 mg/ml (1.752 mmole/l)	11974.00
8 mls	0.4 mg/ml (2.336 mmole/l)	15000.00
10 mls	0.5 mg/ml (2.921 mmole/l)	19050.00
12 mls	0.6 mg/ml (3.503 mmole/l)	23000.00

Table 2: Dilutions of Metronidazole and their peak area.

The calibration curve is obtained by plotting the concentration of Metronidazole versus the peak area as in figure (3).

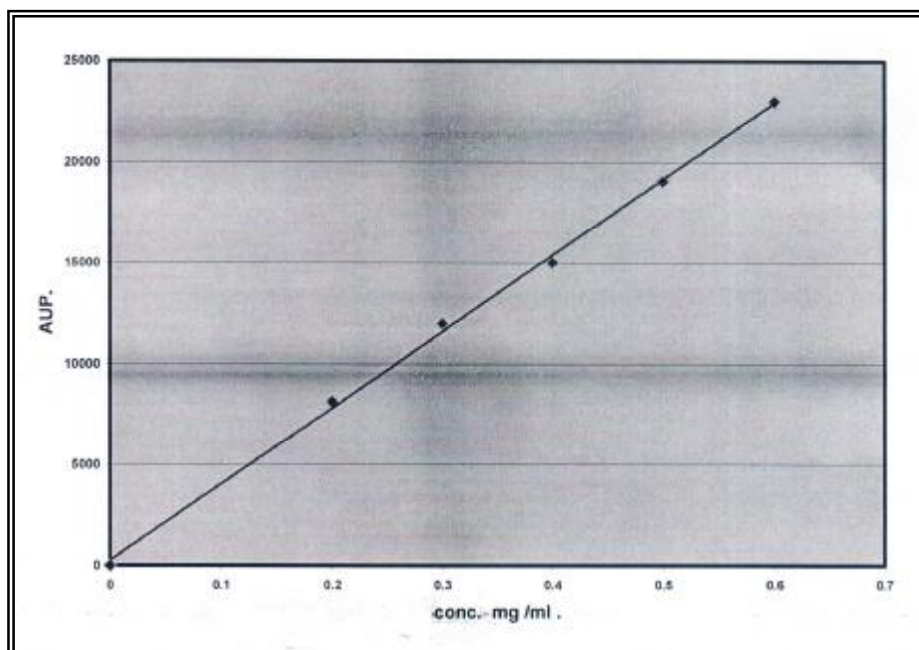


Figure 3: Calibration Curve for Metronidazole.

We find that the calibration curve will follow the straight-line equation ($Y = a + bX$). and by substitution the statistical application we get the following data:

$a = 253.36$ the intercept obtained to be applied in the equation.

$b = 37819$ the slope or regression coefficient.

$r^2 = 0.9986$ the coefficient of determination.

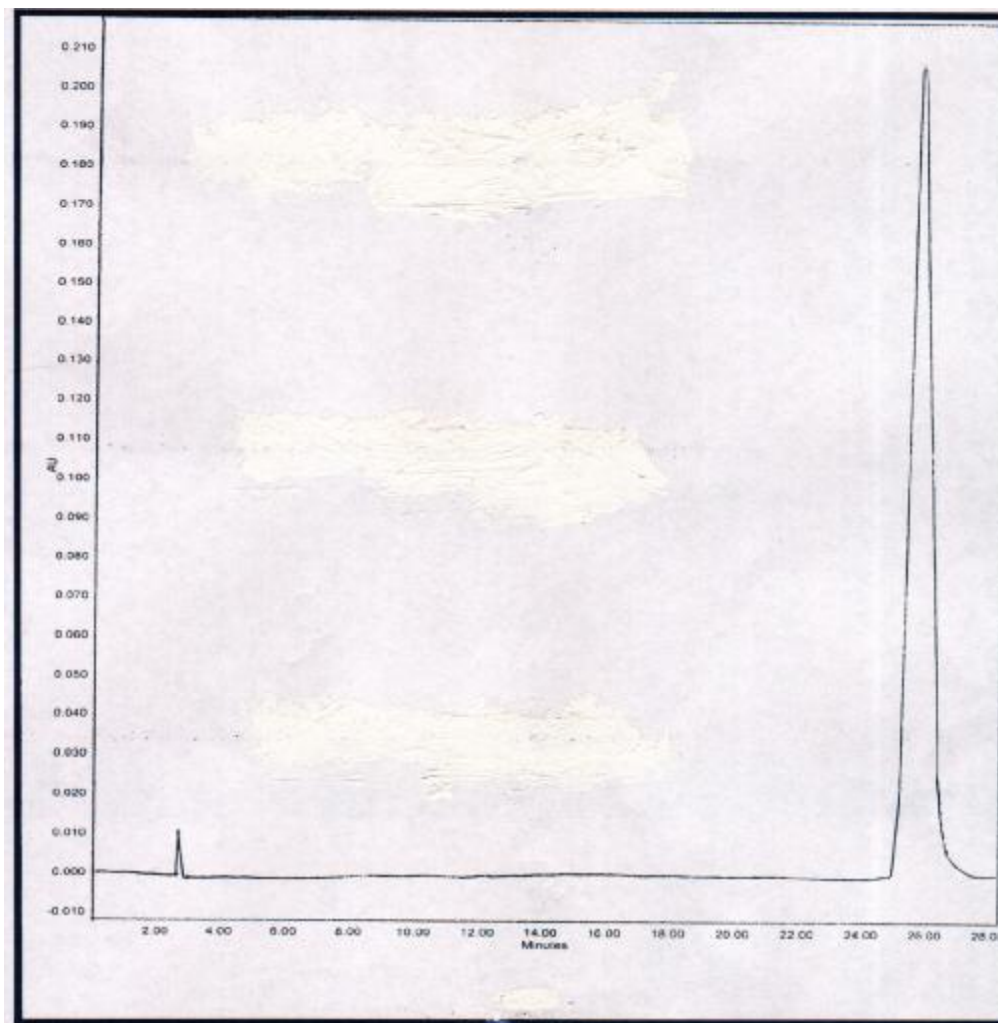
$r = 0.9992$ the correlation coefficient.

Then the straight-line equation that used in the calculation is rearranged to:

$$Y = 253.36 + 37819 X$$

The highly significant linear correlation of the area on the concentration is indicated by the high value of r & r^2 , which close to the highest value of perfect correlation; this will ensure that accuracy of the work and qualification of the HPLC device.

The chromatogram of 0.4 mg/ml conc. Of standard solution of Metronidazole is shown in figure (4).



Procedure for Sample Handling:

Transfer to 200 ml volumetric flask grounded 10 tablets, then add methanol mix with aid of ultrasound for 30 min then dilute with methanol to volume and allow the solution to stand until the insoluble material has settled [9].

Pipette 5.0 ml of clear supernatant liquid into a 100 ml volumetric flask, dilute with mobile phase to volume and mix. Filter the solution, the filtrate is ready to be injected into HPLC system.

Each one of the five samples tested using the same condition that used in external standard in HPLC system to get AUP, the equation of straight line is applied to calculate Metronidazole concentration and its weight.

The result AUP and the concentration the samples are shown in table (3).

Let	Type of Metronidazole	Max% difference allowed	AUP mm2 (y) axis	Cone. mg/ml ((x) axis
A	MEDAZOLE	412.125-372.875	19265.4	0.508 (2.967
B	METROSULE	385.560-348.850	19938.1	0.5205 (3.041 mmole/1)
C	METRONIDAZOLE	361.095-326.705	17952.6	0.468 (2.734 mmole/1)
D	SAFAGYL	357.525-323.475	18822.5	0.491 (2.868 mmole/1)
E	METROZOLE-200	404.985 - 366.415	18482.1	0.482 (2.816 mmole/1)

Table 3: The AUP and concentration of the five samples.

Results

From the data, obtain in table (5) in which the concentration of each AUP was determined, we can calculate the weight and recovery percent of each sample compared to the standard weight, which is 200 mg as shown in table (4).

No.	Let	Drug Source	Weight of Metro. Of each	Error %	Recovery
1	B	Metrosule	208.2 mg	4.1	104.1 %
2	A	Medazole	203.5 mg	1.75	101.75%
3	D	Safagyl	196.4mg	-1.8	98.2 %
4	E	Metrozoie-200	193.0mg	-3.5	96.5 %
5	C	Metronidazole	187.2mg	-6.4	93.6 %

Table 4: Data represent the weight of Metronidazole & recovery %.

Discussion and Conclusion

- 1- All of the tested tablets were within the range of the "Maximum % difference allowed".
- 2- The quantitative analysis was performed using HPLC with external standard method. The recoveries were closed to 100 % with acceptable accuracy and precision.
- 3- The results indicate that Metronidazole is accepted within the normal percentage (95% -105-%) according to USP 2002.
- 4- The HPLC quantitative analysis procedure is fast and accurate for Metronidazole analysis and can be used for routine work.
- 5- The HPLC method is multiple method which performed for the identification, separation and analysis of drug.

- 6- For compares of the result obtained from the tested five samples was found that Medazole (SDI) is the most potent one and closed to 100 % recovery as shown in table (4).

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