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Spectrophotometric Determination of Metronidazole by Prior Reduction and Subsequent Diazotisation and Coupling with N-(1-naphthyl)ethylenediamine–Application to Pharmaceutical Preparations

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method for the determination of microgram amounts of metronidazole (MZ) has been proposed. The method is based on the reduction of metronidazole with iron metal and hydrochloric acid followed by diazotisation and coupling with N-(1-naphthyl)ethylenediamine in acidic medium to give a pinkish-red colored azo dye which has maximum absorption at 503 nm. Beer's law is obeyed in the concentration range of 20-500 µg of metronidazole in a final volume of 25 ml, i.e., 0.8-20 ppm, with a molar absorptivity of 4.673×10^3 $l.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.0366 $\mu g.cm^{-2}$, a relative error of – 1.73 to +1.58% and relative standard deviation of ±0.47to ±1.32 %, depending on the concentration level. The method has been applied successfully to the determination of metronidazole in pharmaceutical preparations.

Keywords: Metronidazole, reduction, diazotization-coupling, N-(1-naphthyl) ethylenediamine reagent, spectrophotometry, assay, pharmaceutical preparations.

التقدير الطيفي للميترونيدازول بالاختزال المسبق متبوعاً بالازوتة والاقتران مع كاشف N- (1- نفثيل) اثيلين ثنائي امين والتطبيق على المستحضرات الصيدلانية

الملخص

تم اقتراح طريقة بسيطة و سريعة وحساسة لتقدير كميات مايكروغرامية من دواء الميترونيدازول. وتعتمد الطريقة على اختزال المركب الدوائي بفلز الحديد وحامض الهيدروكلوريك، متبوعة بعملية ازوتة واقتران مع كاشف N- (1- نفثيل) اثيلين ثنائي امين في محيط حامضي لتكوين صبغة وردية محمرة لها اعلى امتصاص عند الطول الموجي 503 نانوميتر. وكان قانون بير خاضعا في مدى التركيز (20 - 500)

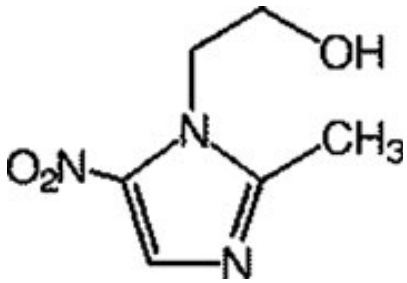
مايكروغرام من الميترونيدازول في حجم نهائي 25 مل، وهذا يعني 0.8-20 جزء/مليون بمعامل امتصاص مولاري مقداره 4.673×10^{3} لتر. مول$^{-1}$.سم$^{-1}$ ودلالة ساندل تساوي 0.0366 مايكروغرام.سم$^{-2}$، الخطأ النسبي من 1.73 – إلى +1.58% والانحراف القياسي النسبي من ±0.47 إلى±1.32 اعتماداً على مستوى التركيز. تم تطبيق الطريقة بنجاح في تقدير الميترونيدازول في المستحضرات الصيدلانية.

الكلمات الدالـة : الميترونيدازول، اختزال، ازوتـة – اقتـران، كاشـف N- (1- نفثيـل) اثيلـين ثنـائي الامين، طريقة طيفية، تطبيقات على مستحضرات صيدلاني

INTRODUCTION

Metronidazole (MZ) has a bacterial action against anaerobic bacteria, its spectrum of activity includes the anaerobic gram negative bacilli which include most of Bacteroides such as *Fusobacterium* and *Veillonella*, anaerobic gram positive cocci such as *Peptococcus* and *Peptidostreptococcus* species and anaerobic gram positive bacilli which include *Clostridium* and *Eubacterium* species. (Block *et al*., 2004).

Chemically MZ is 1 -(β- hydroxyethyl) -2-methyl-5- nitroimidazole (Sweetman, 2009)



$C_6 H_9 N_3 O_3$, M.wt = 171.16 g.mol^{-1}

Different methods have been reported for the determination of metronidazole and most of which have been applied to assay in dosage forms. These including: voltammetry (Manohara *et al*., 2010), spectrophotometry (Adegoke and Bourne, 2009), chromatography (Nora, 2007), polarography (Bartlett, 2005), gas chromatography (Safwan, 2010), and flow injection analysis.(Simone, 2006). The non aqueous titration method using perchloric acid as titrant and determining the end –point potentiometrically for the assay of metronidazole has also been described (British pharmacopoia, 2000).

Most of the spectrophotometric methods found in the literature for the determination of metronidazole are in the visible region and involve initial reduction by treatment with Zn powder and HCl (Manohara *et al*., 2010), followed by the diazotisation and coupling of the resulting amine with different coupling agents such as: 4-chloro-3-nitroaniline (Thulasamma and Venkateswarlu, 2009), 3-methylbenzothiazolin-2-one hydrazone (Nagaraja *et al*., 2002), p-benzoquinone (Atta *et al*., 2005), 8-quinolinol (Saffaj *et al*.,

2004), and β-naphthol (Charrouf *et al*., 2004). Various chromatographic methods have been reported for the analysis of metronidazole in pharmaceutical dosage form and biological fluids (Mustapha *et al*., 2006). High performance liquid chromatography (HPLC) method was employed to measure metronidazole in human plasma and application to single dose pharmacokinetic and bioequivalence studies (Jaber *et al*., 2006). The objective of the investigation reported in this paper was to evaluate a simple spectrophotometric method for the determination of metronidazole; the method involves the diazotisation of reduced metronidazole followed by subsequent coupling with N-(1-naphthyl)ethylenediamine dihydrochloride (N-NED) to form a highly coloured dye.

EXPERIMENTAL

Apparatus

CECIL CE7200 UV-Visible Recording Spectrophotometer UV-160 with 1.0 cm matched quartz cells were used for all absorption measurements.

Reagent

All chemicals used were of analytical-reagent grade.

Reduced metronidazole solution (500 µg /ml).

A 0.05g of metronidazole (NDI,Iraq) is dissolved in about 10 ml distilled water followed by addition of 0.4 g of powdered iron and 5 ml of concentrated HCl then the solution is cooled, and filtered, the clear mixture is then transferred to a 100 - ml volumetric flask and is completed to the mark with distilled water . The solution is kept in a brown bottle, where it is stable for 2 days.

Reduced working metronidazole solution (200 µg /ml). A 40 ml of (500 ug/ml) is diluted with distilled water to the mark in a 100- ml volumetric flask.

Hydrochloric acid solution(1M). This solution is prepared by diluting 8.47 ml of the concentrated acid (Fluka) to the mark with distilled water in a 100- ml volumetric flask.

Sodium nitrite solution(1%). This solution is prepared by dissolving 1g of sodium nitrite (BDH) in distilled water and the volume is completed to the mark in a 100-ml volumetric flask.

Sulphamic acid solution(3%). This solution is prepared by dissolving 3 g of sulphamic acid (Fluka) in distilled water and the volume is completed to the mark in a 100-ml volumetric flask.

N-(1-naphthyl)ethylenediamine dihydrochloride (N-NED) solution(0.1%). This solution is prepared by dissolving 0.1 g of the compound (Molekula, UK) in distilled water and the volume is completed to the mark in a 100-ml volumetric flask.

Reduced metronidazole tablets solution (500 µg /ml). Weigh and mix the contents of ten tablets(each one contains 500 mg MZ), then an accurately weighed amount of powder equivalent to 0.05 g MZ was dissolved in 10 ml of distilled water, then the procedure of

reduction is followed as above. After filtration of the solution, a suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug.

Reduced metronidazole suspension solution(500 µg /ml). The suspension solution is shaken very well and 1.25 ml was taken (equivalent to 200 mg MZ) with 10 ml distilled water and the procedure for reduction was followed as above; a suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug.

Reduced metronidazole injection solution (500 µg /ml). 10 ml of the solution was taken (equivalent to 500 mg MZ) with 10 ml distilled water and the procedure for reduction was followed as above. a suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug.

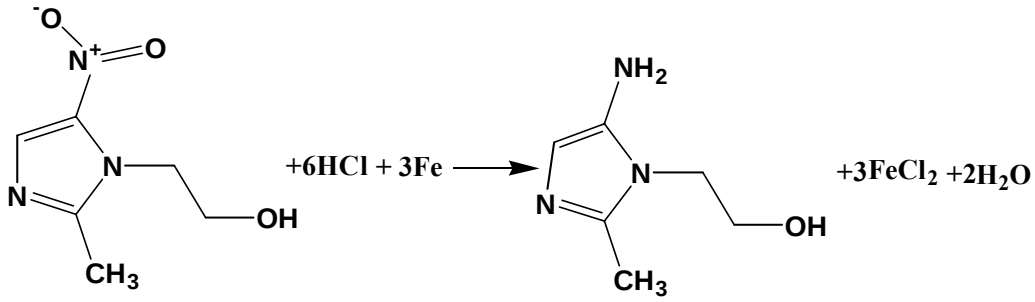
RESULTS AND DISCUSSION

For the subsequent experiments, 200 µg of metronidazole is taken in 25 ml final volumes and absorbance measurements are performed at 503 nm.

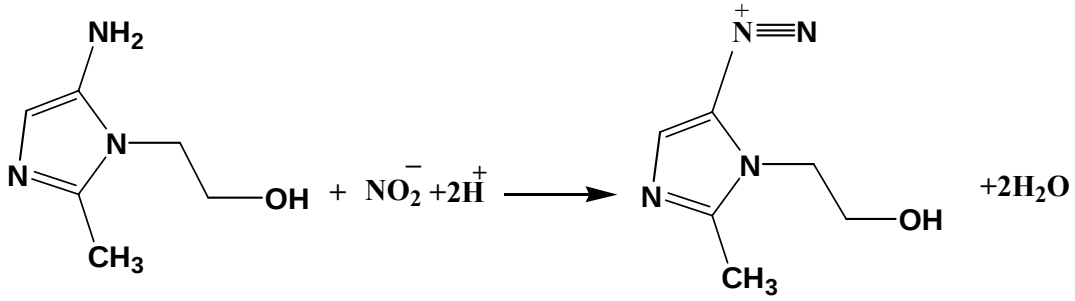
Principle of the method

The method included the following steps:

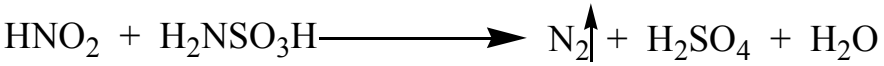
Reduction of metronidazole (Denniston *et al*., 2004):



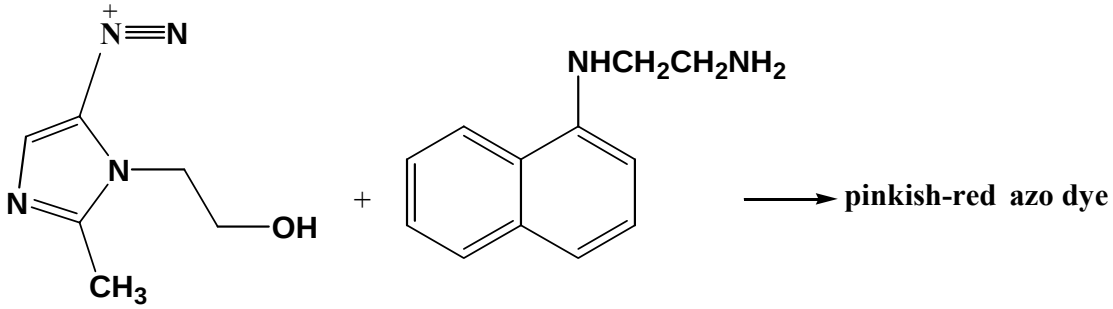
Reduced metronidazole is reacted with excess nitrite in acidic medium to form the diazonium ion :



The residual nitrite (as nitrous acid) which was undesirable due to its side reaction, such as, nitrosation of coupling agent (Bladyga *et al*., 1999), was be removed by sulphamic acid:



The colored solution is formed by coupling diazotized MZ with N-NED in acidic medium.



Study of the optimum reaction conditions

The various parameters affecting the colour intensity of the dye have been studied and optimum conditions are selected.

Choice of coupling agent

Different coupling agents are used for the reaction with diazotized reduced metronidazole in acidic medium. The results in Table 1 show that N-NED gives the more sensitive reaction ($\varepsilon = 4.986 \times 10^3$ l.mol^{-1} .cm^{-1}) in acidic medium.

Table 1: Selection of coupling agent.

Reagents 1%	Absorbance	λmax(nm)	* Δλnm	ε (l.mol^{-1}.cm^{-1})
4-aminobenzophenone	0.06	435	45	1.284×10^3
4-aminoantipyrine	0.102	335	25	2.183×10^3
3,5-diaminobenzoic acid	**	**	**	**
1,5-naphthalenediamine	**	**	**	**
N-NED	0.233	503	99	4.986×10^3
promethazine	**	**	**	**

*Δλ= colour contrast = λmax_S - λmax_B , Where S= The dye , B=Blank

**: no colour contrast

Effect of N-NED amount

The effect of different N-NED amount on the colour intensity of the dye has been studied (Table 2).

Table 2: Effect of N-NED amount.

ml of N-NED (0.1%)soln.	Absorbance/ µg of reduced metronidazole					R^2
	50	100	200	300	500	
1.0	0.038	0.076	0.138	0.197	0.310	0.9982
3.0	0.025	0077	0.154	0.201	0.360	0.9933
5.0	0.062	0.119	0.229	0.331	0.558	0.9997
7.0	0.051	0.109	0.219	0.288	0.491	0.9962
10.0	0.053	0.11	0.21	0.281	0.448	0.9956

From the results, it can be observed that 5 ml of 0.1% N-NED solution is the more suitable amount which gives the highest value of formed azo dye absorbance and also it gives highest determination coefficient (R^2) values.

Effect of acids on the diazotization

The effect of the amount of different acids (weak and strong) used for the diazotisation of reduced MZ has been investigated. The results indicated that 2 ml of 1M HCl gives the highest colour intensity therefore, it has been selected in subsequent experiments (Table 3).

Table 3: Effect of acid type and its amount on absorbance of dye.

Acid used (1M)	Absorbance / ml of acid used						
	0	**0.5**	**1**	**2**	**3**	**4**	**5**
HCl	0.044	0.161	0.178	0.226	0.189	0.181	0.179
H_2SO_4	0.046	0.133	0.143	0.140	0.139	0.134	0.130
HNO_3	0.044	0.123	0.135	0.131	0.121	0.129	0.122
CH_3COOH	0.043	0.138	0.148	0.141	0.138	0.137	0.134
H_3PO_4	0.045	0.144	0.156	0.145	0.137	0.135	0.133

Effect of nitrite amount and time

The colored dye reached its maximum intensity when using 1 ml of 1% sodium nitrite solution after 5 minutes as a reaction standing time (Table 4).

Table 4: The effect of sodium nitrite amount and time on dye absorbance.

ml of(1%)$NaNO_2$ solution	Absorbance / minute standing time						
	0	**1**	**2**	**3**	**5**	**7**	**10**
0	0.004	0.006	0.005	0.004	0.007	0.006	0.006
0.1	0.125	0.155	0.161	0.176	0.171	0.163	0.159
0.3	0.145	0.166	0.172	0.175	0.168	0.163	0.158
0.5	0.147	0.155	0.161	0.166	0.169	0.162	0.159
0.7	0.153	0.163	0.169	0.174	0.173	0.167	0.160
1.0	0.169	0.187	0.198	0.210	0.228	0.192	0.188
1.5	0.151	0.161	0.164	0.166	0.168	0.162	0.155

Effect of sulphamic acid amount and time

The presence of unreacted nitrite is undesirable in diazotisation reaction. Therefore, it should be removed by sulphamic acid which rapidly reacts with nitrite. The results indicated that 1 ml of 3% sulphamic acid solution with 3 minutes standing time are considered to be the most suitable (Table 5), and therefore are selected subsequently.

Table 5: The effect of sulphamic acid amount and time on the dye absorbance.

ml of sulphamic acid solution(3%)	Absorbance / minute standing time						
		0	**1**	**2**	**3**	**5**	**10**
0.0	**Sample**	*	*	*	*	*	*
	Blank=B	1.848	1.475	1.328	1.322	1.292	1.21
0.1	**S**	*	*	*	*	*	*
	B	1.77	1.35	1.26	1.24	1.21	1.19
0.3	**S**	*	*	*	*	*	*
	B	1.54	1.51	1.44	1.36	1.32	1.30
0.5	**S**	0.078	0.112	0.120	0.129	0.115	0.109
	B	0.089	0.067	0.061	0.062	0.065	0.068
0.7	**S**	0.123	0.133	0.138	0.141	0.136	0.134
	B	0.033	0.035	0.038	0.030	0.041	0.043
1.0	**S**	0.135	0.167	0.189	0.226	0.201	0.186
	B	0.020	0.019	0.018	0.019	0.017	0.018
1.5	**S**	0.122	0.128	0.132	0.131	0.128	0.119
	B	0.016	0.017	0.017	0.016	0.018	0.019
2.0	**S**	0.123	0.129	0.120	0.113	0.103	0.110
	B	0.017	0.016	0.018	0.019	0.017	0.015

* negative absorbance values.

Effect of time and amount of MZ on absorbance

The effect of time on the development and stability period of the formed colored dye was investigated under optimum experimental conditions described before. The formation of colored dye being complete after mixing the components of the reaction and the absorbance of the colored species remained constant for at least 75 minutes (Table 6).

Table 6: The effect of time on absorbance.

Time, minutes	Absorbance/µg of MZ/ 25 ml		
	100	**200**	**500**
5	0.126	0.240	0.563
10	0.122	0.232	0.558
15	0.121	0.231	0.559
20	0.121	0.232	0.559
25	0.120	0.231	0.558
30	0.121	0.233	0.559
35	0.122	0.232	0.560
40	0.121	0.230	0.558
45	0.120	0.231	0.560
50	0.121	0.231	0.561
55	0.120	0.232	0.559
60	0.121	0.230	0.560
90	0.121	0.231	- - - -

Effect of order of addition

The results indicated that the effect of order of addition (Table 7) of reagents is important:

Table 7: Effect of order of addition of reagents.

order	absorbance
MZ + HCl + $NaNO_2$ + sulphamic acid + N-NED	0.229
MZ + $NaNO_2$ + HCl + sulphamic acid + N-NED	0.163
MZ + HCl + sulphamic acid + $NaNO_2$ + N-NED	0.044
MZ + sulphamic acid + HCl + $NaNO_2$ + N-NED	0.023
MZ + $NaNO_2$ + sulphamic acid + HCl + N-NED	0.145
MZ + $NaNO_2$ + sulphamic acid + N-NED + HCl	0.213(λ_{max} = 460)

From the orders cited above, we can conclude that the reaction of nitrite with sulphamic acid is faster than the reaction of nitrite with reduced metronidazole and hence an absorbance decrease is observed. The last order reveals that nitrite will nitrosate N-NED to form the nitroso derivative which might be yellow to orange in colour, depending on the nature of the amine.

Final absorption spectra

Absorption spectra of the colored dye formed by coupling of diazotized reduced MZ with N-NED reagent in acidic medium was recorded against its corresponding reagent blank and show a maximum absorption at 503 nm in contrast to the N-NED reagent blank which shows no absorption in the visible region (Fig. 1).

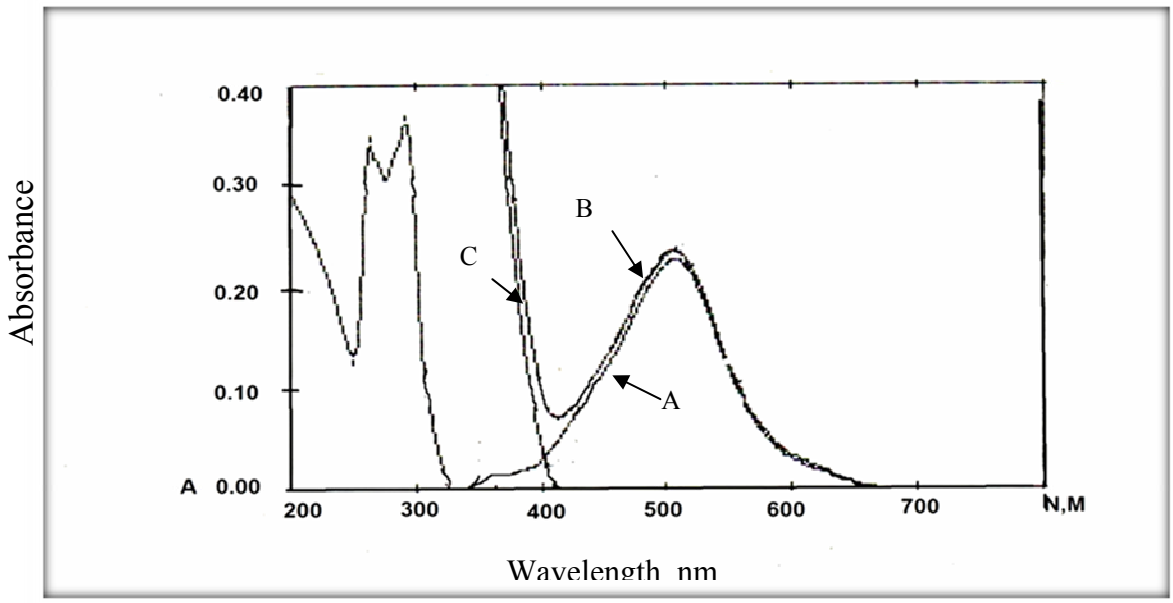


Fig. 1: Absorption spectra of 200 µg MZ treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water

Procedure and calibration graph :

To a series of 25-ml calibrated flasks, an aliquot of aqueous solution containing 20 – 500 µg 0f reduced MZ are transferred, 1 ml of 1 M hydrochloric acid is added and the mixture is shaken, then 1 ml of 1% sodium nitrite solution is added and the mixture is allowed to stand for 5 minutes then 1 ml of 3% sulphamic acid solution is added with occasional shaking for 3 minutes. A 5 ml of 0.1% N-NED solution is added and the volumes are completed to the mark with distilled water, the absorbances are read at 503 nm against blank. The colour was stable for at least 1 hour. The calibration graph is linear over the range 0.8-20 ppm (Fig.2). The apparent molar absorptivity, referred to MZ, has been found to be 4.673×10^3 l.mole^{-1}.cm^{-1}.

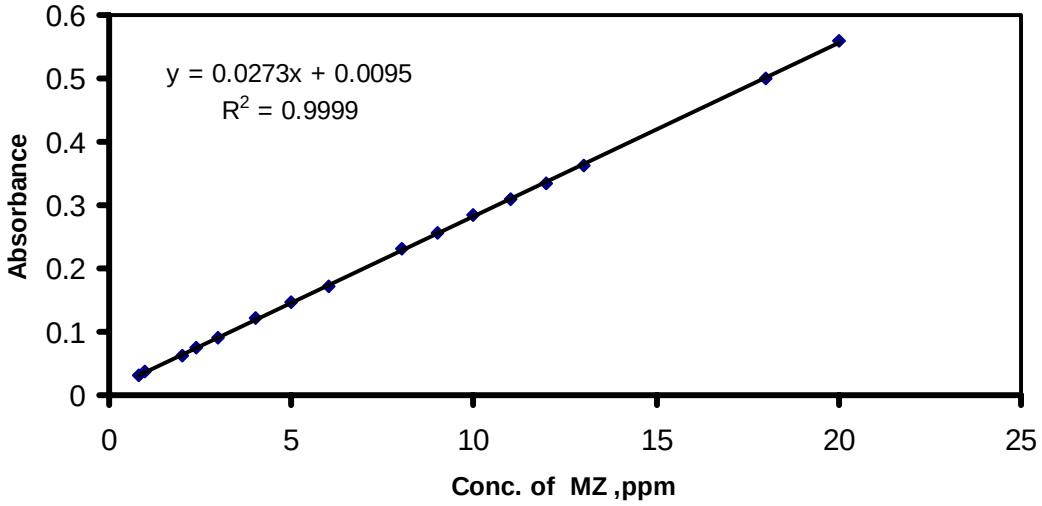


Fig. 2: Calibration graph for MZ determination using N-NED as coupling reagent.

Accuracy and precision

To check the accuracy and precision of the calibration curve, MZ was determined at three different concentrations. The results illustrated in Table 8 indicate that the method is satisfactory.

Table 8: Accuracy and precision of the calibration curve.

ugMZ Present/ 25 ml	µg MZ measured/25ml	Recovery,%*	Relative error, %*	Relative standard deviation, %*
50	50.1	100.2	+1.58	±1.32
100	99.8	99.8	-0.83	±1.08
200	199.3	99.6	-1.73	±0.47

*Average of five determinations

Nature of the Dye

Job's method (Hargis, 1988) indicates that the azo dye has a composition of 1:1 [MZ] to N-NED reagent (Fig.3).

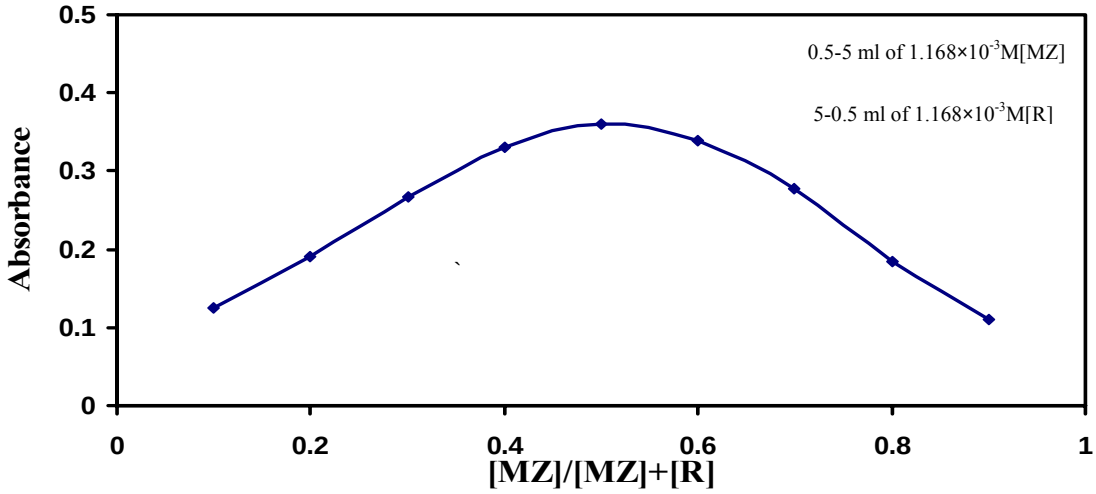
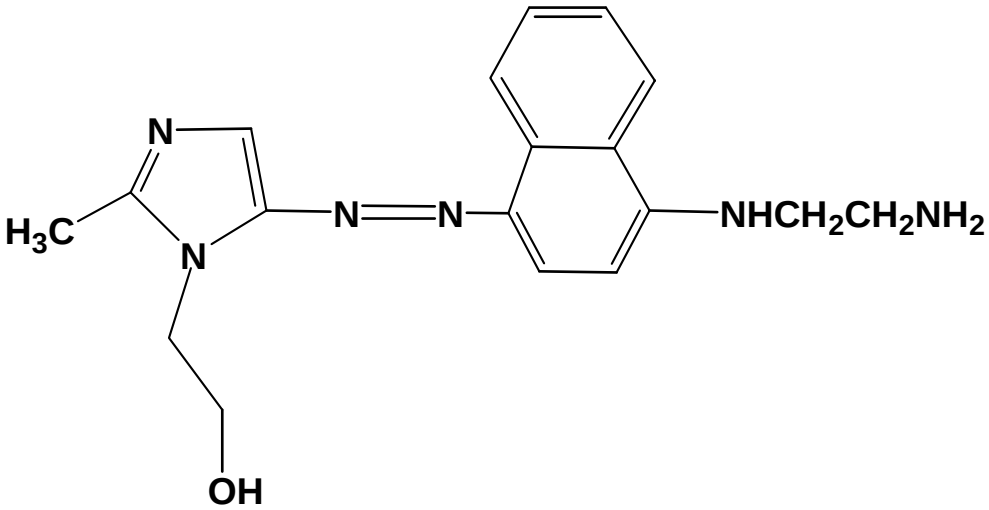


Fig.3: Job's plot for MZ –N-NED.

Which may have the following structure



Interference

The effect of some foreign compounds, which often accompanied pharmaceutical preparations, was studied by adding different amounts to 200 µg metronidazole in a final volume 25 ml (Table 9).

Table 9: Effect of excipients on assay of metronidazole

Interferences	Recovery / µg of Interferences			
	100	200	500	1000
Starch	100.2	99.5	99.6	98.5
Glucose	100.1	100.2	99.5	98.7
Arabic Gum	99.8	98.3	97.6	96.8
Lactose	100.1	100.3	98.9	98.1

The results in Table 9 indicated that the studied foreign compound do not interfere in the determination of metronidazole using the proposed method.

Effect of organic solvents

Different organic solvents have been examined to evaluate their effects on the spectrum of the resulting azo dye, the results are given in Table 10 and Fig. 5.

Table 10: Effect of organic solvents on optical properties of azo dye.

Solvent	Absorbance	λ_{max}, nm	ε, l.mol^{-1}.cm^{-1}
Water	0.228	503	4.88×10^{3}
Acetone	Turbid	--------	--------
Acetic acid	0.001	517.5	0.21×10^{2}
Ethanol	0.18	510	3.852×10^{3}
Formic acid	0.10	511	1.926×10^{3}
Methanol	Turbid	--------	--------
N,N-Dimethylformamide	Turbid	--------	--------
Tetrahydrofuran	0.008	511.5	1.71×10^{2}

It can be seen from the above table that water is the best solvent from both economic as well as analytical points of view. Therefore, water is still chosen in the subseqent experiments.

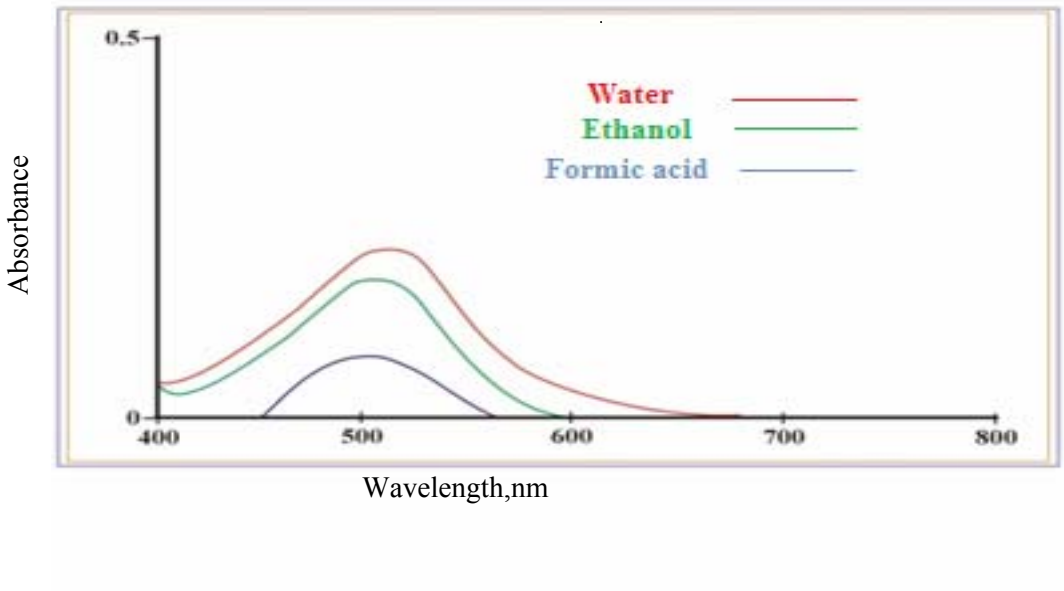


Fig. 5: Effect of organic solvents on optical properties of azo dye

Application of the Method

The proposed method was successfully applied to determine metronidazole in its pharmaceutical preparations (Table 11). The performance of the proposed method was assessed by calculation of the t-test compared with the standard method (British Pharmacopeia, 2000) (non aqueous titration with perchloric acid) for 95% confidence level with four degrees of freedom. The results showed that the t-value was less than the critical value=2.306, indicated that there was no significant difference between the proposed and standard method for metronidazole (Table 12).

Table 11. Analytical applications of the proposed method.

Pharmaceutical preparation	gMZµ Present/ 25 ml	gMZµ measured/ 25 ml	Recovery%*
Metron 500 mg Injection Alkem (INDIA)	50	50.16	100.3
	100	99.5	99.5
	200	197	98.5
Flazol fort 200 mg(suspenstion) ASIA (SYRIA)	50	49	98
	100	98.3	98.3
	200	200.2	100.1
Negazole 500 mg Tablet Julphar (UAE)	50	48.8	97.6
	100	99.8	99.8
	200	197	98.5
MEDAZOLE 500mg Tablet S.D.I IRAQ	50	49.7	99.4
	100	100.2	100.2
	200	195.6	97.8

*Average of five determinations.

Table 12: The results of t-test analysis.

Pharmaceutical preparation	Recovery%		t. exp
	Present method	British Pharmacopeia method	
Metron 500 mg Injection Alkem (INDIA)	98.5	99.4	1.16
Negazole 500 mg Tablet Julphar (UAE)	98.5	99.2	1.84
MEDAZOLE 500mg Tablet S.D.I IRAQ	97.8	98.9	1.6

Comparison of Method

Table 13 shows the comparison between the analytical variable obtained from the present method with those of recent spectrophotometric method.

Table 13: Comparison of the methods.

Analytical parameters	Present method	Literature method*
Temperature (˚C)	At room temperature	At room temperature
λ_{max}(nm)	503	480
Medium of method	Aqueous	Aqueous
Reducing agent	Iron metal	Zinc metal
Coupling Reagent	N-NED	4-chloro3-nitro Aniline
Beer's law range(ppm)	0.8-20	5-60
Molar absorptivity (l.mol-1.cm^{-1})	4.673×10^3	2.71×10^3
RSD(%)	±0.47to ±1.08	2.5
Sandell's sensitivity(µg/cm^2)	0.0366	0.0632
Nature of the dye	1:1	1:1
Color of the dye	Pinkish-red	Yellow
Application of the method	Has been applied to the assay of metronidazole in pharmaceutical preparation (tablets, injection and suspenstion)	Has been applied to the assay of metronidazole in pharmaceutical preparation example: Metrozyl

* Thulasamma and Venkateswarlu (2009)

The application of the present method is of wider scope.

REFERENCES

Adegoke, O. A.; Umoh O. E. (2009). A new approach to the spectrophotometric determination of metronidazole and tinidazole using p-dimethylaminobenzaldhehyde. *Acta Pharm*., **59**(4), 407-419.

Atta - Ur- Rehnan, A. S. Ijaz ; Raza. (2005). Spectrophotometric determination of metronidazole and secnidazole in pharmaceutical preparation. *J. Iranian Chem. Soc.*, **2**(3), 197-202.

Bartlett, P. N.; Choneim, E.; El-hefnawy, G.; Hallag, I. EL. (2005). Voltammetry and determination of metronidazole at a carbon fiber microdisk electrode. *Talanta* , 66 (4), 869-874.

Bladyga, J.; Bourne, J. R. (1999). "Turbulent Mixing and Chemical Reactions." John Wiley and Sons, Inc., New York. 644p.

Block, H. J.; Beale, M. J.; Wilson and Gisvold, S. (2004). "Text-book of Organic Medicinal and Pharmaceutical Chemistry". 11th edn., 260p.

"British pharmacopoia on CD-ROM", (2009). 5th edn., System Simulation Ltd, the Stationary Office, London, pp. 3952-3955.

"British pharmacopoia on CD-ROM". (2000). 3rd edn., System Simulation Ltd, the Stationary Office , London, pp. 917-918.

Charrouf, M.; Abourriche, A.; Aboud, Y.; Bennamara, A.; Saffj, T. (2004). Spectrophotometric determination of metronidazole and secnidazole in pharmaceutical preparations. *Laboratoire Dechimie Orgaique Biomoleculare*, **2**(1), 164-168.

Denniston, K. J.; Topping, J. J.; Caret, R. L. (2004). "General, Organic, and Biochemistry", 4th edn., The Mc Graw-Hill, New York, 466p.

Hargis, L.G. (1988). "Analytical Chemistry: Principles and Techniques". Prentice- Hill, Inc., New Jersey, pp. 424-427.

Jaber, E.; Neda, G.; Hamed, H. (2006). A rapid and sensitive HPLC method for the metronidazole in human plasma: application to single dose pharmacokinetic and bioequivalence studies. *Daru*., **14** (1), 15-21.

Manohara, Y. N.; Venkatesha, R.; Revathi, R.; Bahlul, Z. (2010). Novel and rapid estimation of metronidazol in tablets. *Der Pharma Chemical*, **2**(3), 148-151 .

Mustapha, K.; Odunola, M.; Garba, M.; Obodozie, O. (2006). Rapid, cost-effective liquid chromatograghic method for the determination of metronidazole in biological fluids. *African J. Biotechnol* . **5** (13), 1188-1191.

Nagaraja, P.; Sunitha, K. R.; Vasantha, R. A.; Yathirajan, H. S. (2002). Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations. *J. Pharm. Biomed. Anal.*. **28**, 527-535

Nora, H. Al-shaalan, (2007). Determination of diloxanide furoate and metronidazole in binary mixture using first derivative of the ratio- spectra and high-performance liquid chromatography-UV methods. *Amer. J. Appl. Sci.* , **4**(2), 66-72 .

Saffaj, T.; Charrouf, M.; Abourriche, A.; Aboud, Y.; Bennamara, A.; Maoufoud, S.; Berrada, M. (2004). Spectrophotometric determination of metronidazole in pharmaceutical preparations. *IL Farmaco***. 59** (10) 843-846.

Safwan, A.; Nuha, K. (2010). Simultaneous determination of miconazole nitrate and metronidazole in different pharmaceutical dosage forms by gas chromatography and flame ionization detector (GC-FID). *Intern. J. Biomed. Sci*. **6**(1), 13-18 .

Simone, S. S.; Everaldo, P. M.; Edvaldo, N. G.; Wellington, S. L.; Pablo, N. T.; Mario, C. U.; Edvan, C. S.; Valbere, N. (2006). Flow injection determination of metronidazole through spectrophotometric measurement of the nitrite ion produced upon alkaline hydrolysis. *J. Brazilian Chem. Soc*. **17**(3).

Sweetman, S. C. (2009). "Martindale, The Complete Drug Reference",. 36th edn. London. pp. 837-841.

Thulasamma, P.; Venkateswarlu, P. (2009). Spectrophotometric method for the determination of metronidazole in pharmaceutical pure and dosage forms. *Rasayan J. Chem*. **2**(4), 865-868.