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Spectrophotometric Determination of Metronidazole via Diazotization Reaction with p-Hydroxy Benzaldehyde as a Coupling Reagent

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Abstract: A new, simple and sensitive spectrophotometric method for the determination of Metronidazole (MN) has been developed. The method is based on reduction of nitro group of MN 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 (p-HB) to yield, insoluble water yellow dye indicated at 410 nm. The linearity of the method was between 5.8×10^{-5}-52.2×10^{-5} M, the molar absorptivity was 7131.121 L/mol/cm and Sandell index was 0.0240 μg cm^{-2}. The method has been successfully applied to the assay of MN in pure and pharmaceutical forms.

Key words: Spectrophotometric, determination, diazotization reaction, p-hydroxy benzaldehyde, metronidazol, Iraq

INTRODUCTION

Metronidazole [1-(2-hydroxyethyl) 2-methyl-5-nitroimidazole] has been used $C_6H_9N_3O_3$ (M_r = 171.12) as a therapeutic drug for at least 30 years. It has strong antiprotozoal and bactericidal action (El-Hawary *et al*., 1985). Several methods were used to the determination of MN, including Electrochemical Methods (La-Scalea *et al*., 1999; Edwards, 1993); Chromatographic Methods (Akay *et al*., 2003; Rizk *et al*., 2002), Spectrophotometric Techniques (Parimoo *et al*., 1996; Chen *et al*., 2006; Deepika *et al*., 2008; Siddappa *et al*., 2008) and HPTLC Method.

MATERIALS AND METHODS

Experimental instrumentals: Spectrophotometer, 721-2000, Jenway 3310 pH meter, Precisa XB 220 A sartorius balance and Jenway Hot plate magnetic stirrer.

Chemicals: All the chemical substances were of analytical grade. Metronidazole (MN) was kindly supplied by state company for Drug Industries and Medical appliance (SDI) Samarra, Iraq. Metronidazole tablets were purchased from a local market, para Hydroxy Benzaldehyde (p-HB), Sodium Nitrite (SN), Sulfamic Acid (SA), Sulfuric acid and Zn dust were supplied by Fluka company. All water used was Double Distilled (DDW).

Stock solutions: Pure Metronidazole (MN) solution (5.844×10^{-3} M) was prepared by dissolving 100 mg in 100 mL ethanol (1000 ppm). Sulfuric acid 2% was prepared by diluting 2 mL of concentrated sulfuric acid to 100 mL with DDW. Sodium Nitrite (SN) solution 1% was prepared by dissolving 1 g of pure substance in DDW and completed the volume to the mark with 100 mL volumetric flask. Sulfamic Acid (SA) solution 2% was prepared by dissolving 2 g of pure Sulfamic acid in DDW and completed the volume to the mark in 100 mL volumetric flask. para-Hydroxy Benzaldehyde (p-HB) solution (5.8×10^{-3} M) was prepared by dissolving 0.0071 g from pure substance in ethanol and completed the volume to the mark in a 10 mL volumetric flask.

Reducing of MN: Reduced Metronidazole (RMN) was prepared by adding 0.1 g of Zinc dust and 3 mL of concentrated hydrochloric acid to 10 mL of stock solution of MN, diluted to 70 mL and refluxed up to 75°C for 15 min with stirring after cooling, the solution was filtrated and completed to the volume in a 100 mL volumetric flask with DDW to obtain the standard solution with concentration of 100 ppm.

Developing procedure: About 1 mL of 2% Sulfuric acid was added to 5 mL of (100 ppm) RMN in 25 mL volumetric flasks then 1.5 mL of 1% SN was added to the solution at <5°C in ice bath, the solution was stirred for 5 min then 2.5 mL of 2% SA was added and stand for 5 min in order to distributes the residual of SN. Then 1 mL of p-HB (5.8×10^{-3} M) was added as a coupling reagent, the solution was made up to the mark with DDW, mixed thoroughly, stand up for 18 min and the pH was adjusted to 1.83. The absorbance of formed yellow dye was measured at 410 nm at 25°C against blank.

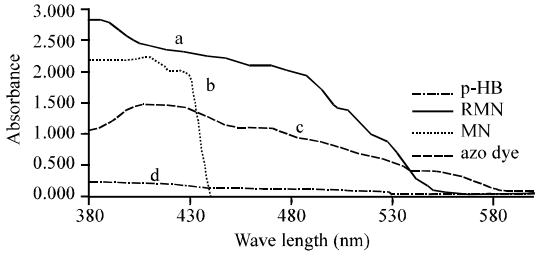

Fig. 1: Absorbance spectra of: a) RMN; b) MN; c) azo dye and d) p-HB against blank solutions and according to the concentrations stock solutions preparation

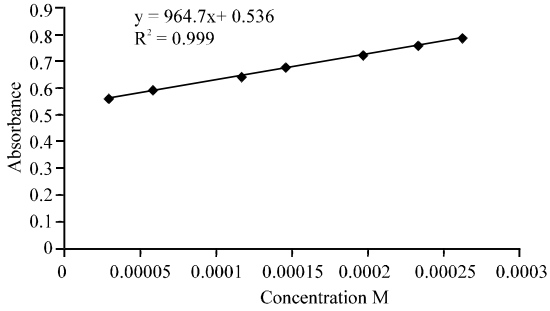

Fig. 2: The calibration curve of MN

Assay of MN tablets: The solution of MN tablets either of (SDI) (Medazol 500 mg) form (1) or of state company for Drug Industries and Medical appliance Nenawa-Iraq (NDI) (Medazol 200 mg) form (2) was prepared separately by ground up ten tablets of each pharmaceutical preparation. An accurate quantity of powder (0.01305 g) of form (1) and (0.0204 g) of form (2) were weighed. The reduction of MN was carried out as mentioned in developing procedure. The resulting filtrate solutions transferred to a 10 mL volumetric flasks and made up to the mark with DDW then 7.6 mL of solution (1) and 0.58 mL of solution (2) were pulled and diluted with DDW to the mark in 10 mL volumetric flask. These solutions equivalent to 0.00762 M and 0.01 M of RMN according to forms (1) and (2), respectively.

Absorbance spectra: The wavelength of the yellow azo dye, p-HB, MN and RMN were investigated against suitable blank solutions. The results in Fig. 1 showed the maximum absorbance of azo dye was at 410 nm while MN, RMN and p-HB were <400 nm.

Calibration graph: The calibration graph was prepared by added 1 mL of 5.8×10^{-3} M of P-HB to a diazonium ions which prepared by aliquot volumes (0.1-0.9 mL) of 5.8×10^{-3} M of RMN and brought up the volumes to 10 mL with DDW in volumetric flasks. The absorbance was measured at 410 nm against a blank and plotted the absorbance against the concentration as in Fig. 2. Beer's law is obeyed over the concentration range (5.8×10^{-5}-52.2×10^{-5}).

The molar absorptivity was 7131.121 L/mol/cm and Sandell index was 0.0240 μg cm^{-2} referred to good sensitivity of the developed method.

RESULTS AND DISCUSSION

Optimization of variable conditions: In order to fixing the optimum conditions inquiry to the determination of MN some experiments were achieved as following.

Effect of reduction acid volume and concentration: Different volumes of hydrochloric acid were used to detected the suitable volume which required to reduce the nitro group of MN to amino group, the study showed that 3 mL of concentrated hydrochloric acid gave maximum absorbance as in Table 1. So, it is used for this purpose.

Zn dust amount effect: The effect of Zn dust mount as a reduction reagent on the dye color was studied. The results in Table 2 expressed 0.1 g of Zn dust gave clear solution when used another weights, the solutions did not be clear, although it were gave absorbance. So, 0.1 g dependent as one of optimal conditions.

Heating and time effect of reduction of MN: To investigate the temperature and time of MN reduction, some experiments were done by using different temperatures over 70-90°C and time over 10-30 min as in Table 3. The results showed 75°C at 15 min were the suitable temperature and time.

Table 1: Volume and concentration acid effect

	HCl conc.				
V of HCl (mL)	2 M	4 M	6 M	8 M	C*
Absorbance					
1	0.08	0.087	0.090	1.000	1.059
2	0.11	0.128	0.133	0.141	0.150
3	0.13	0.133	0.127	0.143	0.156
4	0.14	0.131	0.140	0.145	0.155
5	0.13	0.110	0.130	0.138	0.140

C* = Concentrated acid, Conc. = Concentration, V = Volume

Table 2: Effect of Zn dust

Zn (g)	Absorbance
0.05	0.100
0.10	0.145
0.25	0.137
0.50	0.105
0.75	0.102
1.00	0.103

Table 3: Heating and time effect of reduction of MN

	Temperature (°C)				
Time (min)	70	75	80	85	90
Absorbance					
10	0.34	0.44	0.36	0.39	0.48
15	0.62	076	0.59	0.48	0.49
20	0.41	0.30	0.14	0.26	0.28
25	0.30	0.18	0.01	0.18	0.24
30	0.20	0.14	0.01	0.15	0.17

Table 4: The effect of volume RMN effect

RMN volume (mL)	Absorbance	RMN volume mL	Absorbance
1	0.099	6	0.67
2	0.210	7	0.45
3	0.170	8	0.53
4	0.320	9	0.45
5	0.780	10	0.52

Table 5: Effect of H_2SO_4

Vml H_2SO_4	Absorbance
0.25	0.560
0.5	0.581
0.75	0.640
1	0.890
1.25	0.550
1.5	0.210

Table 6: SN volume effect

Absorbance	SN volume (mL)
0.83	0.5
0.89	1.0
0.91	1.5
0.62	2.0
0.74	2.5
0.74	3.0

Reducing MN volume effect: The effect of RMN volumes on the reaction which increase or decrease the color intensity was studded. So, different volumes (1-10 mL) of 100 ppm of MN were used in preparing of azo dye as in Table 4. The results showed 5 mL gave a deeper intensity color.

Sulfuric acid effect: The preparation of diazonium ion occurred in acidic medium, so variant volumes of 2% sulfuric acid were used to improved the favorite volume which achieved this aim. The results showed 1 mL gave maximum absorbance as in Table 5, so it depended in optimal conditions.

Sodium nitrite amount effect: In order to fixed the amount of SN requires to diazotized of RMN throw achieve maximum absorbance, several experiments were done by using different volumes (0.5-3 mL) of 1% SN to form diazonium salt at <5°C, Table 6 showed 1.5 mL of SN was employed for diazotization of RMN.

Sulfamic acid volume and distributing time effect: In order to indicated the volume of SA requiring to distributed the unreacted amount of SN, different volumes (0.75-3 mL) of (2%) SA were added, otherwise the time of distributing of SN was investigated too. The result in Table 7 showed the residual amount of SN was distributed completely after 2 min by 2.5 mL of SA.

Table 7: SA volume and distributing time effect

	SA (mL)					
Time (min)	0.75	1	1.5	2	2.5	3
Absorbance						
1	0.60	0.52	0.55	0.53	0.59	0.41
2	0.56	0.58	0.77	0.61	0.94	0.43
3	0.52	0.39	0.25	0.37	0.71	0.38
4	0.40	0.35	0.11	0.29	0.69	0.33
5	0.30	0.28	0.01	0.16	0.26	0.20

Table 8: Coupling reagent's time and volume effect

	0.75	1	1.5	2	2.5	3
Time (min)	----------(mL)----------					
Absorbance of 5.8×10^{-5} p-HB						
1	0.68	0.68	0.59	0.50	0.68	0.46
5	0.66	0.60	0.47	0.67	0.57	0.45
10	0.52	0.60	0.30	0.54	0.41	0.32
15	0.44	0.78	0.32	0.46	0.39	0.35
18	0.32	0.87	0.22	0.25	0.33	0.33
20	0.07	0.60	0.19	0.21	0.35	0.23

Table 9: Effect of temperature

Absorbance	Temperature
0.45	20
0.79	25
0.42	30
0.36	35
0.28	40
0.25	45
0.25	50

Coupling reagent's time and volume effect: Different volumes of 5.8×10^{-5} M p-HB (0.75- 3 mL) were used to react with diazonium ion to give azo dye, otherwise the necessary time to complete this reaction was studied by measured maximum absorbance as indicator at variant time. The study showed the reaction was completed after 18 min by using 1 mL of p-HB then these two variants were included with optimal conditions. Table 8 explains the results of the study.

Temperature effect: The effect of temperature on the colored dye developed was examined by measured the absorbance against temperature over 20-50°C. The dye attained maximum color intensity between 20-25°C as in Table 9, after that the compound of azo dye began degradation.

pH effect: The effect of pH on the azo dye formation was studied by adjustment the medium by sulfuric acid, the results in Table 10 showed the acidic medium of the solution at pH = 1.88 was the suitable medium to complete the reaction, otherwise under basic mediums the dye was precipitated.

Table 10: pH effect

Absorbance	pH	Absorbance	pH
0.060	1.81	0.033	1.95
0.029	1.78	0.025	1.91
0.019	1.76	0.368	1.88
0.010	1.60	0.331	1.85

Table 11: The accuracy and precision of the method

Present conc×10^{-5}	Found conc×10^{-5}	RSD %	Err %	Rec %
5.80	5.700	0.88	-1.720	98.280
11.6	12.06	0.61	+3.97	103.97
23.2	22.70	0.53	-2.300	98.700

Table 12: Determination of MN in pharmaceutical forms

Pharmaceutical forms	Present conc ×10^{-5} M	Found conc ×10^{-5} M	RSD %	Err %	Rec %
Medazol produced by SDI	7.60	7.800	0.75	+2.63	102.63
	15.2	15.25	0.51	+0.33	100.33
	22.8	22.06	0.14	-3.250	96.750
Medazol produced by NDI	10.0	9.940	0.63	-0.600	99.400
	20.0	19.50	0.84	-2.500	97.500
	30.0	30.15	0.44	+0.50	100.50

Accuracy and precision of method: After the conditions of the proposed method had been optimized, the accuracy and precision of the method were ascertained by performing five replicate analysis of MN in pure forms at three concentration (5.8×10^{-5}, 11.6×10^{-5} and 23.2×10^{-5} M) according to the calibration curve.

The results showed good precision through low values of RSD percentage (<1%) and good accuracy through error percentage (-2.3 to +3.97%) and recovery percentage (97.28-103.97%), Table 11 shows these results which indicated the capability of the application of the proposed method successfully.

Application of method: The proposed method was applied to the determination of MN in two pharmaceutical forms purchased from local market by using the procedure described in the experimental section. Three concentrations of each form were used (7.6×10^{-5}, 15.2×10^{-5} and 22.8×10^{-5} M) and (10×10^{-5}, 20×10^{-5} and 30×10^{-5} M) belong to forms 1 and 2, respectively. The results in Table 12 shows low RSD percentage (<1%) which referred to good precision, Err percentage (-3.25 to +2.63%) and Rec percentage (96.95-102.63%) which referred to good accuracy showed there are no interaction of the excipients and the good sensitivity of the method signed to capable applied the developing method successfully to determination of MN in pharmaceutical preparations.

Pathway of the reaction: The proposed pathway of the reaction of the developed method may be occurred as a follow reaction equation (Fig. 3).

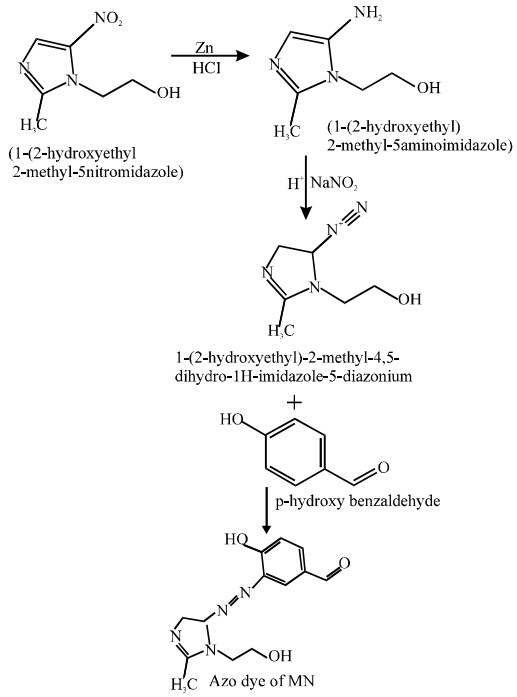

Fig. 3: The proposed mechanism of the reaction

CONCLUSION

The purpose of the present study was investigate the utility of diazotization of reduced MN and coupled it with p-Hydroxy Benzaldehyde, The method was applied to assay of MN in pure and pharmaceutical formulations.

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