Spectrophotometric determination of Metronidazole via diazotization reaction with p-Hydroxy Benzaldehyde as a coupling reagent

Khalaf Faris Alsamarrai
department of chemistry, College of Education Samarra, Tikrit University ,Samarra, Iraq. *correspondence E.mail of author: Khalaf_58@yahoo.com*
(K . J . Pharm . Sci)

(Received April 2011 , Accepted Nov. 2011)

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 410nm. The linearity of the method was between 5.8×10^{-5}M-52.2×10^{-5}M, the molar absorptivity was 7131.121 $L.mol^{-1}.cm^{-1}$ and Sandell index was 0.0240µg.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, Metronidazole.

التقدير الطيفي للميترونيدازول بوساطة تفاعلات الازوتة والاقتران في المستحضرات الصيدلانية باستخدام بارا هيدروكسي بنزالديهايد كعامل اقتران

خلف فارس السامرائي

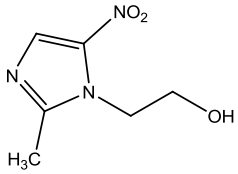
قسم الكيمياء، كلية التربية سامراء، جامعة تكريت، سامراء، العراق
العنوان الالكتروني المعتمد للمراسلة : E-Mail; *Khalaf_1985@yahoo.com*

الخلاصة

تم تطوير طريقة طيفية جديدة وسريعة وحساسة لتقدير الميترونيدازول. تستند الطريقة على اختزال مجموعة نيترو NO_2– الميترونيدازول الى مجموعة امين باستعمال مسحوق الزنك وحامض الهيدروكلوريك المركز في الايثانول الساخن والمحرك, ثم حضر ايون الدايزونيوم من العقار المختزل وتم اقترانه مع بارا هيدروكسي بنزالديهايد (P–HB) لتكوين صبغة صفراء اعطت اعلى امتصاصية عند 410 نانومتر وكانت خطية الطريقة مابين 5.8×10^{-5} مولاري ـ 52.2×10^{-5} مولاري والامتصاصية المولاري 7131,121 لتر.مول$^{-1}$. سم$^{-1}$ وحساسية ساندل 0.0240 مايكروغرام.سم^{2} طبقت الطريقة بنجاح في تقدير الميترونيدازول في المستحضرات النقية والصيدلانية.

Introduction

Metronidazole[1-(2-hydroxyethyl) 2-methyl-5-nitroimidazole] has been used as a therapeutic drug for



$C_6H_9N_3O_3$ (M_r=171.12)

at least 30 years, It has strong antiprotozoal and bactericidal action[1] .Several methods were used to the determination of MN, including electrochemical methods[2-6],chromatographic methods[7-9], spectrophotometric techniques[10-15] and HPTLC method[16].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

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 100mg in 100ml ethanol (1000ppm).

-Sulfuric acid 2% was prepared by diluting 2ml of concentrated Sulfuric acid to 100ml with DDW .

-Sodium nitrite (SN) solution 1% was prepared by dissolving 1gm of pure substance in several ml of DDW and completed the volume to the mark in 100ml volumetric flask.

-Sulfamic acid (SA) solution (2)% was prepared by dissolving 2gm 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 0.0071gm from pure substance in ethanol and completed the volume to the mark in a 10ml volumetric flask.

Reducing of MN:

Reduced Metronidazole (RMN) was prepared by adding 0.1gm of Zinc dust and 3ml of concentrated hydrochloric acid to 10ml of stock solution of MN, diluted to 70ml and refluxed up to 75^{o}C for 15 minutes 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 100ppm.

Developing procedure:

One ml of 2% Sulfuric acid was added to 5 ml of (100ppm) RMN in 25 ml volumetric flasks, then 1.5 ml of 1% SN was added to the solution at less than 5^{o}C in ice bath, the solution was stirred for 5 minutes, then 2.5 ml of 2% SA was added and stand for 5 minutes in order to distributes the residual of SN. Then 1ml 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 minutes and the pH was adjusted to 1.83. The absorbance of formed yellow dye was measured at 410 nm at 25^{o}C against blank.

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 (0.01305gm) of form (1) and (0.0204gm) of form (2) were weighed. The reduction of MN was carried out as mentioned in developing procedure. The resulting filtrate solutions transferred to a 10ml volumetric flasks and made up to the mark with DDW, then 7.6ml of solution (1) and 0.58ml of solution (2) were pulled and diluted with DDW to the mark in 10ml volumetric flask. These solutions equivalent to 0.00762M and 0.01M 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 Figure (1) showed the maximum absorbance of azo dye was at 410 nm while MN,RMN and p-HB were less than 400nm .

Calibration graph:

The calibration graph was prepared by added 1ml of 5.8×10^{-3} M of P-HBA to a diazonium ions which prepared by aliquot volumes (0.1ml-0.9ml) 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 figure (2). Beer's law is obeyed over the concentration range (5.8×10^{-5}M - 52.2×10^{-5}M). The molar absorptivity was 7131.121L.mol^{-1}. cm^{-1} and Sandell index was 0.0240µg.cm^{-2} referred to good sensitivity of the developed method.

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 3ml of concentrated hydrochloric acid gave maximum absorbance as in table (1), So it 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 gm of Zn dust gave clear solution, when used another weights, the solutions didn't be clear although it were gave absorbance, So 0.1gm 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^{o}C and time over 10-30 minutes as in

table (3). The results showed 75^{o}C at 15 minutes were the suitable temperature and time.

Reducing MN volume effect:

The effect of RMN volumes on the reaction which increase or decrease the color intensity was studded, So different volumes (1ml-10ml) of 100 ppm of MN were used in preparing of azo dye as in table (4). The results showed 5ml 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 (0.5ml-3ml) of 1% SN to form diazonium salt at less than5^{o}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 ml - 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 minutes by 2.5 ml of SA.

Coupling reagent's time and volume effect:

Different volumes of 5.8×10^{-5}M P-HB (0.75 ml - 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 minutes by using 1 ml of P-HBA, 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^{o}C. The dye attained maximum color intensity between 20-25^{o}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.

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}M, 11.6×10^{-5}M

and 23.2×10^{-5}M) according to the calibration curve.

The results showed good precision through low values of RSD percentage (low than 1%) and good accuracy through error percentage(-2.3% – +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}M, 15.2×10^{-5}M and 22.8×10^{-5}M) and (10×10^{-5}M , 20×10^{-5}M and 30×10^{-5}M) belong to forms 1 and 2 respectively.

The results in table (12) shows low RSD percentage (less than 1%) which referred to good precision, Err percentage (-3.25% - +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 (scheme 1).

Conclusion

The developed method is simple, reliable, economical, have a good sensitive (ε =7131.121 $L.mol^{-1}.cm^{-1}$, Sandell index = 0.0240$\mu g.cm^{-2}$) and practically free from interferences for the determination of MN in pharmaceutical forms .

References

1.El-Hawary, M.B.; Khayyall, M.T.; Isaak, Z. Hand Book of Pharmacology. The Scientific Book Center, S.O.P. Press, Cairo, **1985.**

2.Mauro, A. La-Scalea.; Silvia, H.P. Serrano.; Ivano, G. R. Gutz. *J. Braz . Chem. Soc.* **1999**, *10*, 127.

3. Edwards, D. I. *J.Antimicrob . Chemothr.***1993**, *31*, 9.

4. Edwards, D. I. In Comprehensive medicinal Chemistry, Hansch, C., Ed.; Pergamon Press, New York, vol. 2, 5th ed.,**1990**, p.725.

5.Knox,R.I.;Knight, R.C.;Edwards,D.I. *Biochem. Pharmac.* **1983**, *32*, 2149.

6.Mohamed, R.G.; Parviz, N.; Farnoush, F.; Hooshag,P.*J. Chin. Chem. Soc*, **2007,***54*,55-61.

7.Frasey, A.M.; Anne, B.G;.; Pouget, M.P.; Vennat, B.L.; Galmier, M.J. *Micro.Acta*.**2004**,*144*,171-176.

8.Akay, C.; Ozkan, S.A.; Senturk, Z.; Cevheroglu, S. *Farmac,* **2003**, *57*, 953-957.

9.Rizk,M.; Belal, F.; Ibrahim, F.; Ahmed,S.;Sheribah,Z.A.*J.pharmaceut. Biomed. Anal*, **2002**, *27*, 813-820.

10.Nagaraja, P.; Sunitha, K.R.; Vasantha, R.A.; Yathirajan, H.S.*J. Pharm. Biomed. Anal*, **2002**, *28*,527-535.

11.Parimoo, P.; Prasad, C.V.N.; Gautam, A. *Imter. J. Pharm*, **1996**, *28*, 527-535.

12. Parimoo, P.; Prasad, C.V.N.; Vineeth, R. *J. Pharm. Biomed. Anal*, **1996,** *14*, 389-393.
13.Chen, H.; Gong, Z.; Zhang, Z, *J. Pharm. Biomed. Anal*.**2006**, 41, 1412-1417.
14.Deepika, M.; Anurekha, J.; Maheshwari, RK.; Vidyasagar, P. *Research J. Pharm. and Teach.* **2008,** *1*, 357-361.
15.Siddappa, K.; Mallikarjun, M.; Reddy, P.T.; Tambe, M.*Ecl. Quim.Sao Paulo,***2008**, *33*, 41-46.
16.Dhananjay, B.M.; Shashikant, B.B.; Madhukar, R.T. *Pak. J. Pharm. Sci*, **2009**, 22, 323-

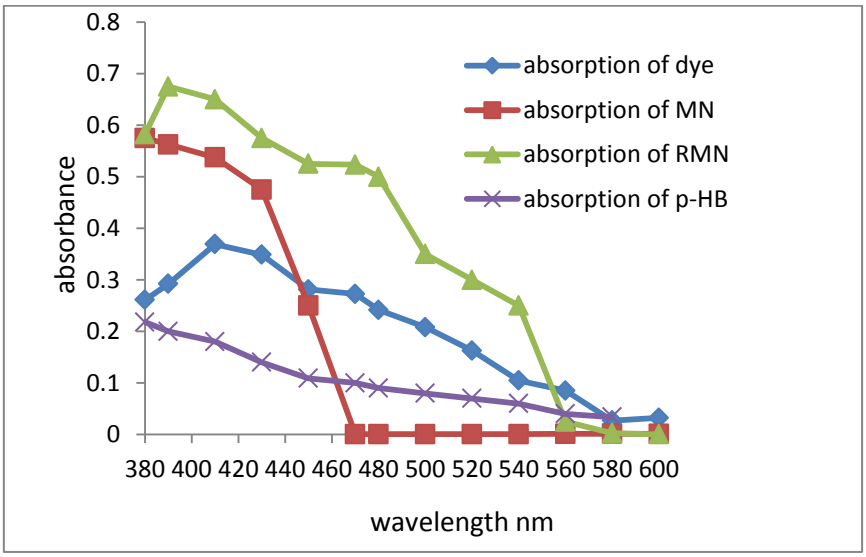


Figure (1) absorbance spectra of RMN (**a**), MN (**b**), azo dye (**c**) and p-HB **(d)** against blank solutions and according to the concentrations stock solutions concentrations for **a, b** and **d** and 10ppm for **c**

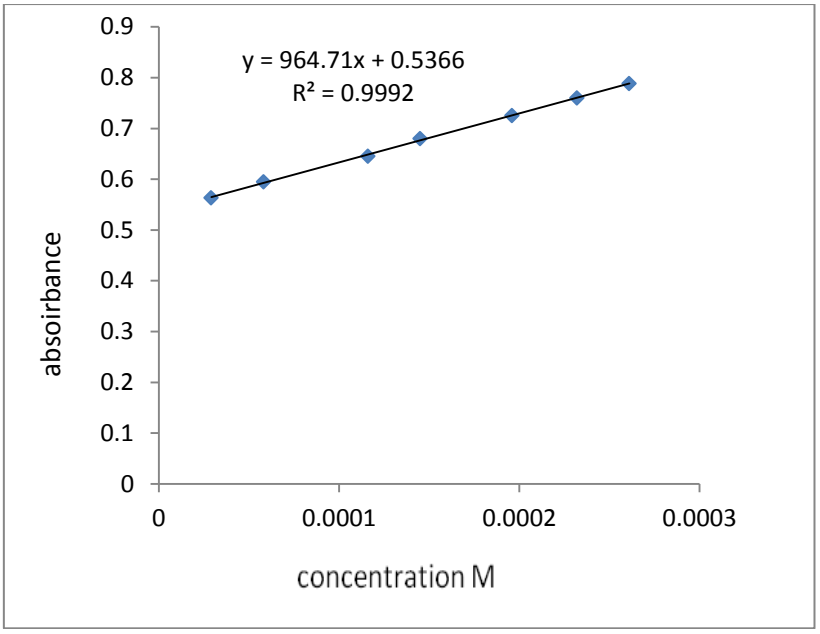


Figure (2) the calibration curve of MN

Table(1) Volume and concentration acid effect

V of HCl ml \ HCl conc		2M	4M	6M	8M	C*
1	Absorbance	0.08	0.087	0.09	1	1.059
2		0.11	0.128	0.133	0.141	0.15
3		0.13	0.133	0.127	0.143	**0.156**
4		0.14	0.131	0.14	0.145	0.155
5		0.13	0.11	0.13	0.138	0.14

C* = concentrated acid, conc = concentration, V = volume

Table(2) effect of Zn dust

Zn gm	Absorbance
0.05	0.1
0.1	**0.145**
0.25	0.137
0.5	0.105
0.75	0.102
1	0.103

Table(3) Heating and time effect of reduction of MN:

Temperature°C / time minute		70	75	80	85	90
10	Absorbance	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	0.21	7	0.45
3	0.17	8	0.53
4	0.32	9	0.45
5	**0.78**	10	0.52

Table (5) Effect of H_2SO_4

Vml H_2SO_4	Absorbance
0.25	0.56
0.5	0.581
0.75	0.64
1	**0.89**
1.25	0.55
1.5	0.21

Table (6) SN volume effect

SN volume ml	Absorbance
0.5	0.83
1	0.89
1.5	**0.91**
2	0.62
2.5	0.74
3	0.74

Table (7) SA volume and distributing time effect

Time min \ Vml of SA		0.75	1	1.5	2	2.5	3
1	Absorbance	0.6	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		0.4	0.35	0.11	0.29	0.69	0.33
5		0.3	0.28	0.01	0.16	0.26	0.2

Table (8) Coupling reagent's time and volume effect:

Time min \ volume ml		0.75	1	1.5	2	2.5	3
1	absorbance of 5.8×10^{-5} P-HBA	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

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

Table (10) pH effect

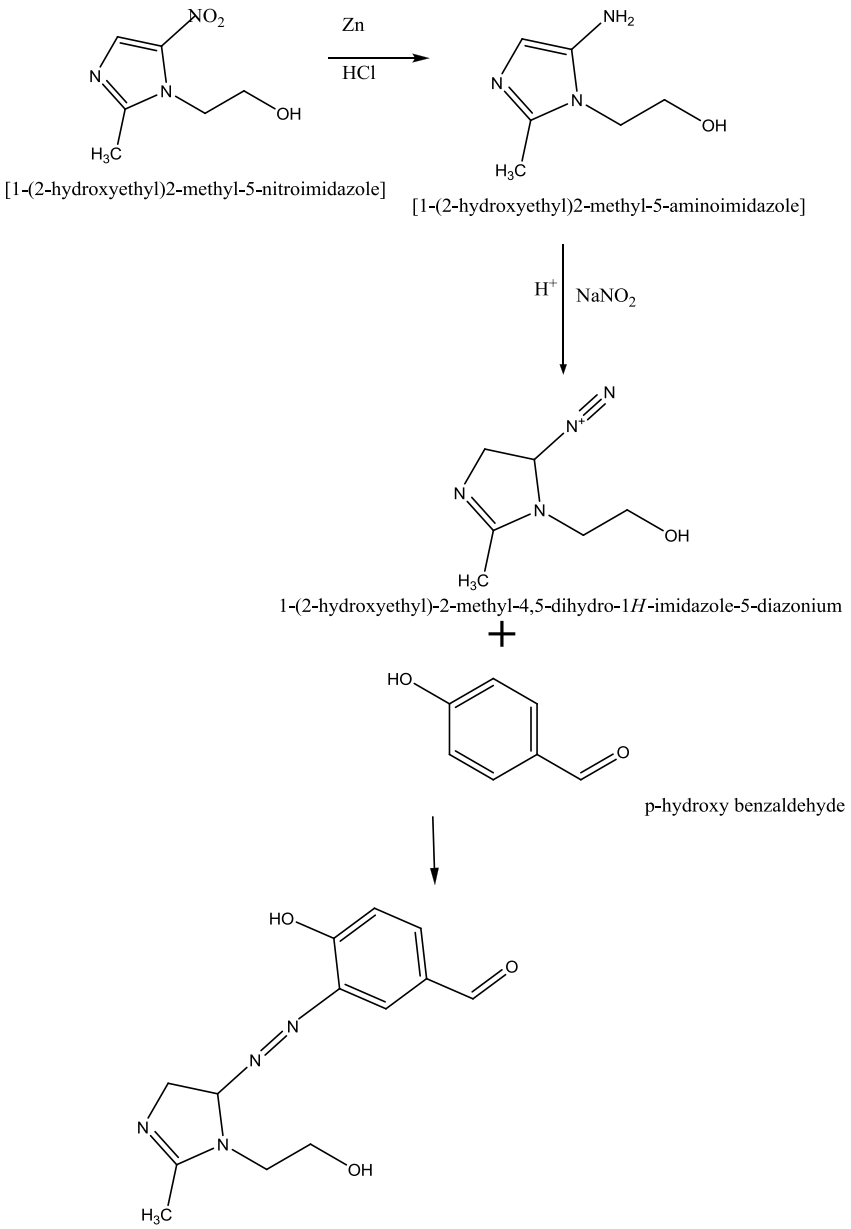
PH	absorbance	PH	Absorbance
1.95	0.033	1.81	0.06
1.91	0.025	1.78	0.029
1.88	**0.368**	1.76	0.019
1.85	0.331	1.6	0.01

Table (11) the accuracy and precision of the method

Present conc$\times 10^{-5}$	Found conc $\times 10^{-5}$	RSD%	Err%	Rec%
5.8	5.7	0.88	-1.72	98.28
11.6	12.06	0.61	+3.97	103.97
23.2	22.7	0.53	-2.3	98.7

Table (12) determination of MN in pharmaceutical forms

Pharmaceutical forms	Present conc $\times 10^{-5}$M	Found conc $\times 10^{-5}$M	RSD%	Err%	Rec%
Medazol produced by SDI	7.6	7.8	0.75	+2.63	102.63
	15.2	15.25	0.51	+0.33	100.33
	22.8	22.06	0.14	-3.25	96.75
Medazol produced by NDI	10	9.94	0.63	-0.60	99.40
	20	19.5	0.84	-2.50	97.50
	30	30.15	0.44	+0.50	100.50



Azo dye of MN

Schem1. The proposed mechanism of the reaction