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Direct and Indirect Spectrophotometric Methods for Determination of Metronidazole in Pharmaceutical Formulations

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Abstract

Two easy and fast spectrophotometric methods were used to estimation of (Metronidazole) in pure form and its pharmaceutical preparations. The first method (Direct), depends diazotization coupling reaction, includes the reduction of Metronidazole with Iron metal and hydrochloric acid followed by reaction with Sodium nitrite and coupling reaction between the drug and 4-Aminoacetophenone, in a basic intermediate to form (pink-red) product, with highest absorption at the wavelength 510 nm. The second method (indirect), depended on the Oxidation of Metronidazole in presence of an acidic medium by a known excess of N-Bromosuccinimide and subsequent determination of unreacted oxidant by reacting it with (Phenosafranin dye) to form a (red) product, with the highest absorption at the wavelength 514 nm. Beer's law is obeyed at the concentrations range of (10-225) µg/ml and (5-200) µg/ml with a molar (absorptivity 5.86×10^4 L/mol.cm) and (7.27×10^4 L/mol.cm) for 4-Aminoacetophenone and Phenosafranin dye respectively. The limit of detections (LOD) was found to be 7.9×10^{-4} µg/ml and 5.9×10^{-4} µg/ml respectively. The suggested method was prosperity implement to the estimation of "This drug" in pure form and its pharmaceutical formulations.

Keywords: Spectrophotometry, Metronidazole, 4-Aminoacetophenone, Phenosafranin.

1. Introduction

Metronidazole (MZ), (Figure 1), is a commonly used antibiotic, belonging to the Nitroimidazole class of antibiotics. It is frequently used to treat gastrointestinal infections as well as trichomoniasis and giardiasis, and amebiasis which are parasitic infections. Metronidazole has been used as an antibiotic for several decades, with added antiparasitic properties that set it apart from many other antibacterial drugs, allowing it to treat a wide variety of infections. It is available in capsule form, tablet form, topical form, and suppository preparations for the treatment of various infections [1,2]. There are many methods for estimating of (MZ), including: HPLC[3], Flow-Injection[4], Quantitative NMR[5], UV. Spectroscopy[6]. Chemical Luminosity[7,8], IR [9], Chromatography methods[10,11], Electrochemical methods[12,13,14], and UV-Vis. spectrophotometry [15,16].

2. Aim of the study

The research aims to find simple, fast and economical spectrophotometric methods for the determination of Metronidazole, diazotized and coupling using 4-aminoacetophenone (4AP) in alkaline media, and phenosphrine (PS) dye through the use of an oxidizing agent (NBS).

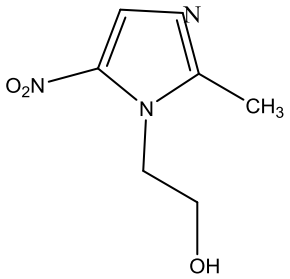

Fig. 1: Chemical structure of Metronidazole

The success of these two proposed methods for the determination of (MZ) drug in its pharmaceutical preparations in the form of tablets.

3. Apparatus

UV-VIS spectrophotometer "single beam from Genesys UV 10", Balance Kern 770 Gs/Gj from Sartorius BL 2105, Oven from memmert Schutzart DIN 40059-Ip20.

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3-1-1. Materials

Metronidazole 99% from (SDI Samarra. Iraq), 4-Aminoacetophenone 99%, Phenosafranin dye 99%, N-Bromosuccinimide 99%, Sodium Hydroxide 99% have purchased from Merck. Hydrochloric acid 36% from (Thomas baker), Iron 99% from (BDH), Ethanol 99.9% from (Scharlau).

3-1-2. Solutions

- Metronidazole solution (1000 µg/ml): Prepared by weight (0.1000 gm) of (MZ) "standard" dissolved in 100 ml Ethanol. Dilute solutions were prepared for experiments.
- Metronidazole reducing solution (1000 µg/ml): Exactly (0.1000 gm) of "standard" (MZ) was dissolved in (20 ml) ethanol with (10 ml) of Hydrochloric acid 0.5 gm of Iron was added, then the solution was shaken and left for a 1.0 hour filtered washed completed to 100 ml with Ethanol. From it, dilute solutions were prepared for experiments.
- 4-Aminoacetophenone (1×10^{-2} M): 0.1352 gm in 100 ml of Ethanol.
- Phenosafranin dye (1×10^{-4} M): 0.0032gm in 100ml distilled water.
- $NaNO_2$ (1×10^{-2} M): Prepared by dissolving 0.0689 gm in 100 ml distilled water.
- N-Bromosuccinimide (1×10^{-2} M):It was prepared by dissolving 0.1779 gm in 100 ml Ethanol.
-NaOH (1 M): Was prepared by dissolving 4 gm in 100 ml distilled water.
-HCl (1 M): Prepared at an approximate concentration of (1.0 molar) by diluting 8.6 ml of concentrated acid (11.64 M) to (100 ml) distilled water.
- Excipients solutions (1000 µg/ml): Prepared by dissolve (0.1000) gm for each substance in 100 ml of Ethanol[17].

3-2. Procedures

3-2-1. (I) Determination of (MZ) by 4-Aminoacetophenone:

After conducting preliminary tests and experiments, optimal conditions were reached by adding (1 ml) of (MZ reducing solution) (500 µg/ml) to a volumetric flask (10 ml), followed by adding (1 ml) of $NaNO_2$ at a concentration (1×10^{-2} M), Then (1 ml) of (1 M) HCl, and waiting for 10 minutes, followed adding (3 ml) of (4AP) at a concentration of (1×10^{-2} M) and (0.5 ml) of (1 M) NaOH, then the volume was completed to (10 ml) from absolute Ethanol, whereby the maximum absorption of the resulting product (pink-red) was at λ_{max}. 510 nm at room temperature**.**

3-2-2. Application of proposed methods

"Ten tablets" were taken from the pharmaceutical preparations and weighed, then the weights were calculated, then these tablets were ground into a fine powder.

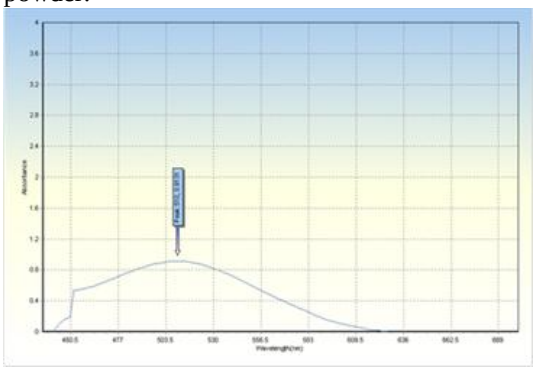

Fig. 2: Absorption spectrum of (MZ-4AP) product against blank

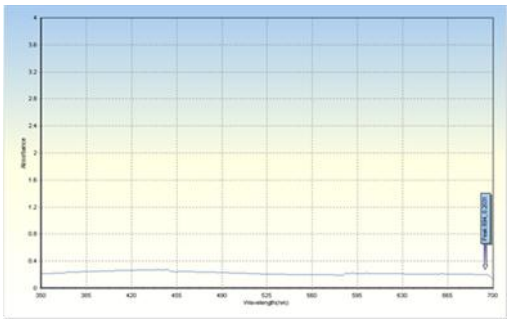

Fig. 3: Absorption spectrum of blank against ethanol

A finely weighted amount of powder was transferred to a beaker, shaken with 50 ml of solvent (Ethanol), filtered, then washed in a 100 ml "volumetric flask". This filtered and wash to the mark with solvent to obtain the final concentration of 500 µg/ml, the proposed method for the determination of (MZ) in pharmaceutical preparations have been successfully applied.

4. Results and Discussion

4-1. "Optimal Conditions"

4-1-1. Effect of reagent volumes

When studying the effect of adding increasing volumes of the reagent (4AP) on the absorption values of the product, it was found that the volume (3ml) was the best, as shown in figure 4.

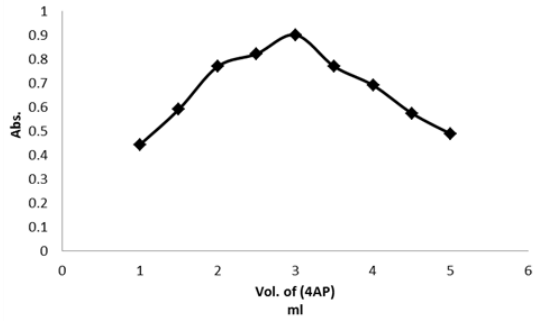

Fig. 4: Effect of (4AP) volumes on product

4-1-2. Effect of the $NaNO_2$ volumes

As shown in Figure (5), when using different volumes of $NaNO_2$, it was found that the best added volume was (1.0), ml which was used in subsequent experiments.

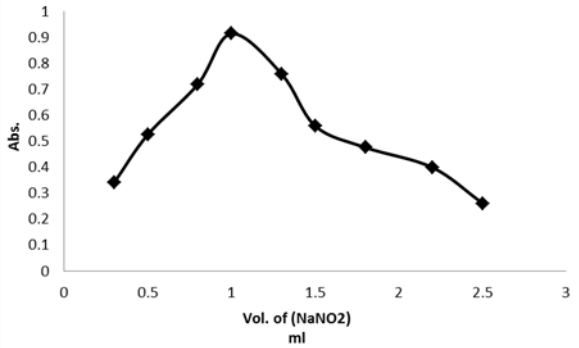

Fig. 5: Effect of $NaNO_2$ volumes on product

4-1-3. Effect of using different acids

Different acids were used such as (HCl, H_2SO_4, HNO_3, CH_3COOH), with a concentration of 1.0 M for each, and the volume was added to 1.0 ml, to find out which acid gave the highest absorption value when forming the product, and it turned out that hydrochloric acid was the best, and as shown in the table (1).

Table (1): Effect of different acids on the product

Acid	Absorbance
HCl	0.9129
H_2SO_4	0.3428
HNO_3	0.2792
CH_3COOH	0.1306

4-1-4. Effect of acid volumes

Different and increasing volumes of hydrochloric acid with a concentration of 1.0 M were used, to find out which volume gives the highest absorption value of the formed product, and the optimum volume is 1.0 ml, and when an increase in volumes is added, the color of the product is gradually disappearing, and this is clear in Figure (6).

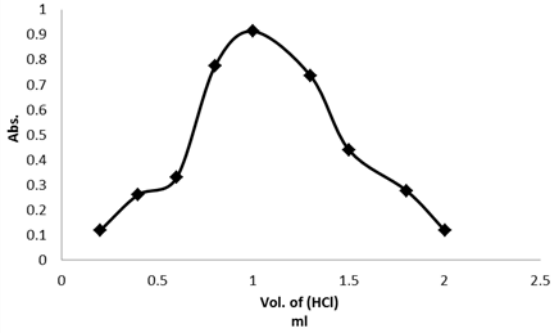

Fig. 6: Effect of HCl volumes on product

4-1-5. Effect of using different bases

Different bases (NaOH, KOH, NH_4OH) were used with a concentration of (1.0 M) for each, and by adding volume (0.5 ml) to find out which base gives the best absorption when forming the product, and table (2) shows that the best base used to form the product is sodium hydroxide.

Table (2): Effect of different types of bases

The base used	Absorbance
NaOH	0.9142
KOH	0.483
NH_4OH	0.186

4-1-6. Effect of base volumes

Different and increasing volumes of NaOH at a concentration of (1 M) were used, to find out which volume gave the best absorption, and as shown in Figure (7), the optimum volume added from the base is 0.5 ml.

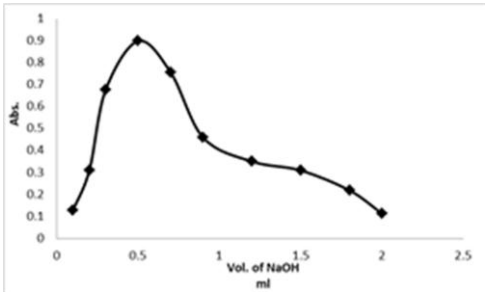

Fig. 7: Effect of NaOH volumes on product

4-1-7. The stability time

Table (3) shows the stability of the absorbance values for the product formed at the $\lambda_{max.}$ 510 nm with time, where the stability time was about 60 minutes, (1.0 hour).

Table (3): The stability time for the product

Time (min.)	Absorbance
5	0.9129
10	0.9130
15	0.9131
20	0.9131
25	0.9132
30	0.9132
35	0.9131
40	0.9130
45	0.9131
50	0.9130
55	0.9129
60	0.9128
65	0.9123
70	0.9120

4-1-8. Effect of the sequence additions

Several tests were conducted to find out the effect of changing the sequence of adding reactants on the absorption values of the formed product, and it was found that the addition

sequence No. (1) has the highest absorption value, as in table (4).

Table (4) Effect of the sequence additions

Order number	Order of addition	Absorbance
1	D + N + A + R + B	0.9128
2	D + N + B + A + R	0.4112
3	D + A + N + R + B	0.2864
4	R + A + N + B + D	0.1987
5	R + B + N + D + A	0.3498
6	R + D + A + N + B	0.1623
7	R + N + A + D + B	0.3448

(MZ)=D, (4AP) = R, $NaNO_2$ = N, NaOH = B, HCl =A

4-1-9. Effect of excipients

A study was conducted to verify the effect of the additives on the formed product by adding two concentrations for each substance, and it was confirmed that there was no significant effect on the absorption values, as shown in table (5).

Table (5): Effect of excipients

Excipients	Added con. µg/ml	%RE	Added con. µg/ml	%RE
Cellulose	100	0.32	200	-2.23
Magnesium stearate	100	0.63	200	3.48
Lactose	100	1.69	200	2.96
Starch	100	-2.54	200	-1.55
Povidone	100	-1.72	200	0.73

4-1-10. Calibration curve

The calibration curve of (MZ) was studied by adding increasing volumes to form the product using the reagent (4AP) to find out the concentrations obeying Beer's law, where the linearity of the formed product appeared in the range of concentrations (10-225) µg/ml, as shown in Figure (8).

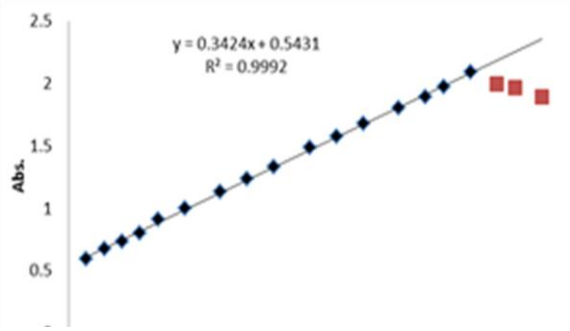

Fig. 8: Calibration curve of (MZ) product

4-1-11. Study the application of the proposed method

Table (6), shows the results obtained from the estimation of (MZ) in its pharmaceutical preparations (in the form of tablets).

Table (6): Determination of (MZ) (as tablet)

Pharmaceutical preparation	Content (µg/ml) declared	Found (µg/ml) by proposed method	%Recovery
Flagyl (250) mg	40	40.04	100.10
	60	59.24	98.73
	80	79.78	99.73
Flagyl (500) mg	40	40.18	100.45
	60	60.85	100.42
	80	79.39	99.24

4-1-12. Equivalence study

The equivalence ratio between the drug (MZ) and the reagent (4AP) was studied by applying the optimal proven conditions, using the molar ratio method and continuous changes (Job method), using the same initial concentration of the drug and the reagent (5×10^{-3}) M for both methods, and figures (9) and (10) show that the equivalence ratio is 1:1.

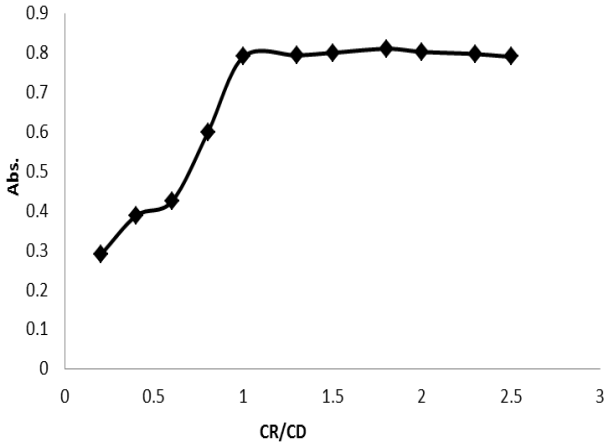

Fig. 9: Mole-ratio method of (MZ-4AP)

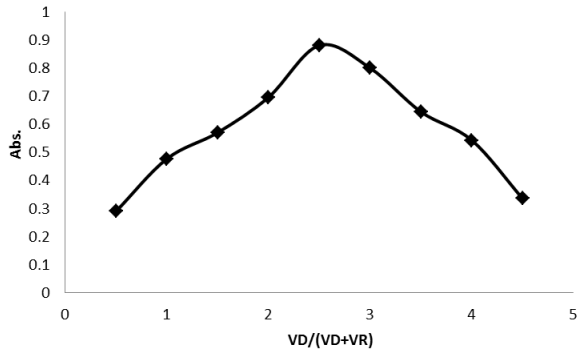

Fig. 10: Continuous variations method of (MZ-4AP)

5. (II) Determination of (MZ) by Phenosafranin dye:

To reach the optimal conditions, several experiments and preliminary tests were conducted, where (1.5 ml) of 500 µg/ml (MZ) was transferred to a 10 ml volumetric flask, followed by the addition of 1.5 ml of (NBS). After waiting for (5) minutes, (0.5 ml) of 1.0 M HCl acid was added. After 10 minutes, with continuous shaking, (4.0 ml) of (1×10^{-4} M) of (PS) was added, and after 15 minutes, the volume was supplemented with Ethanol to 10 ml, which gave the highest absorption value of the (red) product formed and at room temperature is at $\lambda_{max.}$ 514 nm.

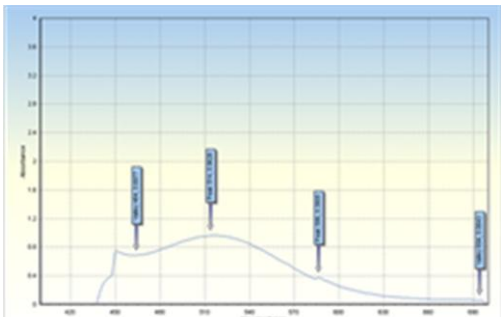

Fig. 11: Absorption spectrum of (MZ-PS) product against blank

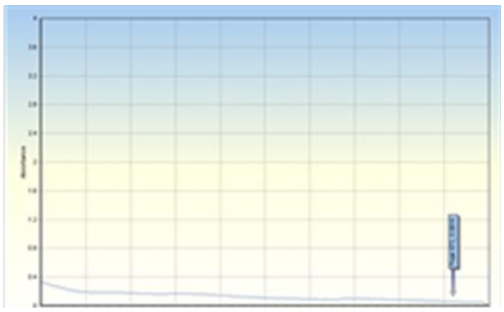

Fig. 12: Absorption spectrum of blank against ethanol

5-1. "Optimum Conditions"

5-1-1. Effect of reagent volumes (Dye)

The effect of increasing volumes of reagent (PS) on the reaction of formation of the formed product was studied, as it was observed that the highest absorption value was at 4.0 ml volume, as shown in Figure (13).

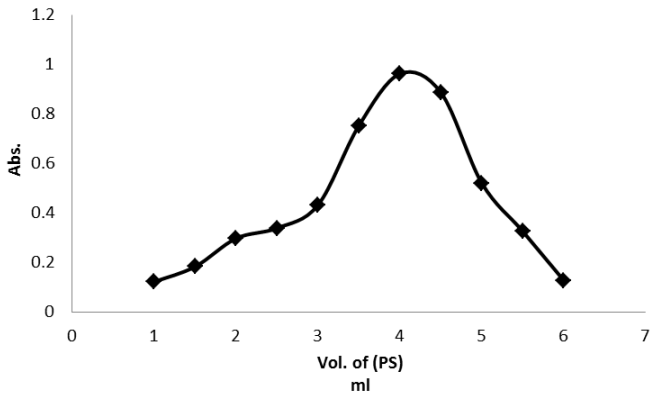

Fig. 13: Effect of (PS) dye volumes on product

5-1-2. Effect of using different acids

Various acids were used such as (HCl, H_2SO_4, HNO_3, CH_3COOH), with a concentration of 1.0 M for each, and the volume was added to 0.5 ml, to find out which acid gave the highest absorption value when forming the product, and it turned out that hydrochloric acid was the best, and as shown in the table (7).

Table (7): Effect of different acids on the product

Acid	Absorbance
HCl	0.9624
H_2SO_4	0.2719
HNO_3	0.1998
CH_3COOH	0.1025

5-1-3. Effect of acid volumes

Different and increasing volumes of hydrochloric acid with a concentration of 1.0 M were used, to find out which volume gives the highest absorption value of the formed product, and the optimum volume is 0.5 ml, and when an increase in volumes is added, the color of the product is gradually disappearing, and this is clear in Figure (14).

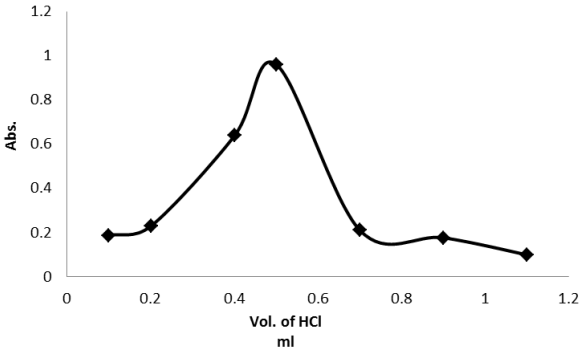

Fig. 14: Effect of HCl volumes on product

5-1-4. Effect of oxidizing agent volumes

Increased volumes of the oxidizing agent (NBS) were added to see its effect on the absorption value of the colored product, as it was found that the best added volume was 1.5 ml, as shown in Figure (15).

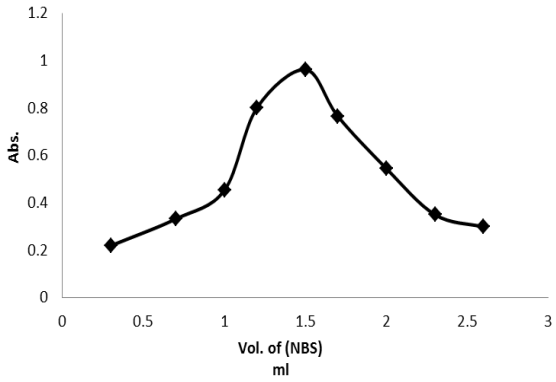

Fig. 15: Effect of (NBS) volumes on product

5-1-5. Stability study of the formed product

The stability of the formed product has been studied for the importance of knowing the time in which it remains constant, by applying the optimal conditions, as it was found that the product remains stable for 24 hours.

5-1-6. Calibration curve[18]

The calibration curve of (MZ) was studied by adding increasing volumes to form the product using the reagent (PS) dye to find out the concentrations obeying Beer's law, where the linearity of the formed product appeared in the range of concentrations (5-200) µg/ml, as shown in Figure (16).

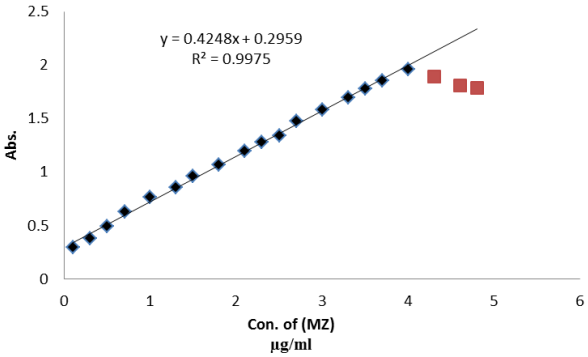

Fig. 16: Calibration curve of (MZ-PS) product

5-1-7. Study the application of the proposed method

Table (8), shows the results obtained from the estimation of (MZ) in its pharmaceutical preparations (in the form of tablets).

Table (8): Determination of (MZ) (as tablet)

Pharmaceutical preparation	Content (µg/ml) declared	Found (µg/ml) by proposed method	%Recovery
Flagyl (250) mg	40	39.55	98.88
	60	60.38	100.63
	80	79.99	99.99
Flagyl (500) mg	40	40.28	100.70
	60	59.63	99.38
	80	80.05	100.06

5-1-8. Effect of excipients

A study was conducted to verify the effect of the additives on the formed product by adding two concentrations for each substance, and it was confirmed that there was no significant effect on the absorption values[19-20], as shown in table (9)

Table (9): Effect of excipients

Excipients	Added con. µg/ml	%RE	Added con. µg/ml	%RE
Cellulose	100	-1.34	200	1.93
Magnesium stearate	100	2.98	200	2.74
Lactose	100	0.27	200	1.21
Starch	100	-1.83	200	-2.29
Povidone	100	1.54	200	-1.72

5-1-9. Equivalence study

The equivalence ratio between the drug (MZ) and the reagent (PS) dye was studied by applying the optimal proven conditions, using the molar ratio method and continuous changes (Job method), using the same initial concentration of the drug and the reagent (5×10^{-4}) M for both methods, and figures (17) and (18) show that the equivalence ratio is 1:1.

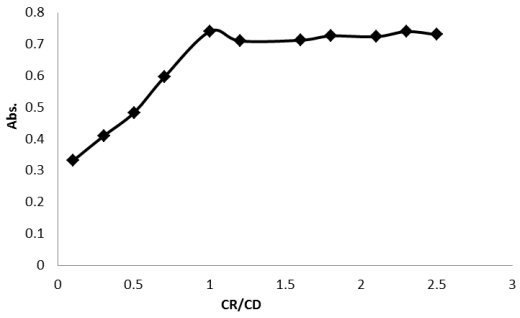

Fig. 17: Mole-ratio method of (MZ-PS)

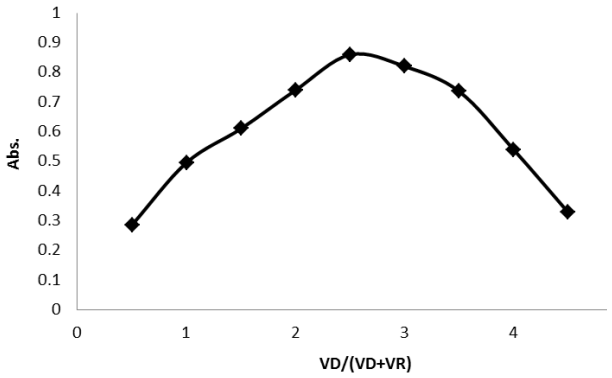

Fig. 18: Continuous variations method of (MZ-PS)

6. Establish optimal conditions

The optimum conditions that were installed for both of the proposed methods were clarified and as shown in table (10).

Table (10): Proven Optimal Conditions Spectral product identification (MZ)

Parameters	Value (4AP)	Value (Ps dye)
$\lambda_{max.}$ (nm)	510	514
Beer's law (µg/ml)	(10-225)	(5-200)
Molar absorptivity (L/mol.cm)	5.86×10^{4}	7.27×10^{4}
Correlation coefficient (r)	0.9996	0.9987
Limit of Detection (µg/ml)	7.9×10^{-4}	5.9×10^{-4}
Slope	0.3424	0.4248
Intercept	0.5431	0.2959
RSD%	0.014	0.026

7. Conclusions

Two simple, fast and economical methods were used, that do not require the use of special working conditions, as in other spectroscopic methods, where colored and stable results were formed with available and inexpensive reagents. The estimation can also be carried out at room temperature and does not require heating or studying the effect of temperature. The proposed methods for the determination of MZ can be applied pharmaceutical preparations (as tablets).

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