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Spectrophotometric Dertermination of Mesalazine by 8-Hydroxyquinoline and N-(1-naphthyl)ethylenediamine dihydrochloride Reagents in Bulk and Capsule Dosage Forms

Rawaa A. Zakaria
Department of Chemistry
College of Education
University of Mosul

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ABSTRACT

Two simple and sensitive visible spectophotometric methods have been developed for the quantitative determination of mesalazine (MEZ) in bulk drug and pharmaceutical preparation (capsules). The proposed methods are based on oxidative coupling reaction of mesalazine with 8-hydroxyquinoline (method A) and N-(1-naphthyl)ethylendiamine (N-NED) (method B) in the presence of sodium periodate as oxidizing agent in alkaline medium to form coloured products, exhibiting maximum absorptions at 644 nm and 543 nm, respectively. Beer's law was obeyed in the concentrations range of 10-300 and 10-180 µg /25ml with molar absorptivity of 1.7223×10^4 and 0.6545×10^4 $l.mol^{-1}cm^{-1}$, respectively. The relative error ranged between -0.4% to +0.16% and - 0.9% to - 0.31% with relative standard deviation of ±1.31% to ±0.39% and ±0.88% to ±0.32% for methods A and B, respectively. The optimum conditions for full colour development are described and the proposed methods were applied successfully to the assay of MEZ in pharmaceutical preparation (capsules).

Keywords: spectrophotometry, oxidative coupling, mesalazine, 8-hydroxyquinoline, N-NED

التقدير الطيفي للميزالازين باستخدام 8-هيدروكسي كوينولين و ن-(1-نفثيل) اثيلين ثنائي الأمين بشكله الحر وفي الكبسول

الملخص

تم تطوير طريقتين طيفيتين اتصفتا بالسهولة والحساسية للتقدير الكمي للميزالازين بشكله الحـر وفـي مستحضره الدوائي (الكبسول) اعتمدت كلتا الطريقتين على تفاعل الاقتـران التأكـسدي للميـزالازين مـع 8-هيدروكسي كوينولين في الطريقة A و ن-(1- نفثيل) اثلين ثنائي الأمين (N-NED) فـي الطريقـة B بوجود العامل المؤكسد بيريودات الصوديوم في الوسط القاعدي لتكوين نواتج ملونة تعطي أعلى امتـصاص عند الطول الموجي 644 نانوميتر و543 نانوميتر على التوالي. لقد أمكن تطبيق قانون بيـر ضـمن مـدى

التراكيز10-300 مايكروغرام/ 25 مـل و10-180 مـايكروغرام/ 25 مـل وبامتـصاصيات مولاريـة 1.7223×10^{4} و0.6545×10^{4} لتر.مول$^{-1}$.سم$^{-1}$ وتراوح الخطأ النسبي مـن 0.40 - % إلـى 0.16+% و0.90-% إلى 0.31-% وقيمة الانحراف القياسي النسبي من1.31 ±% إلى 0.39 ± % و0.88 ± % إلـى 0.32 ± % في الطريقتين A وB على التوالي. تمت دراسة الظروف المثلى للتفاعـل وطبقـت الطريقتـان بنجاح في تقدير الميزالازين في مستحضره الدوائي (الكبسول).

INTRODUCTION

Mesalazine (MEZ) is chemically known as 5-amino-2-hydroxybenzoic acid, it is a white to pinkish, crystalline powder, slightly soluble in cold water and alcohol, more soluble in hot water, soluble in hydrochloric acid. It is an anti- inflammatory drug used to treat and also maintain the remission of mild to moderate ulcerative colitis or Crohn's (Madhavi *et al*., 2011 ; Singh *et al*., 2010).

Various methods have been reported for the determinations of MEZ as pure and in pharmaceutical preparations, these include:

High performance liquid chromatography (HPLC) (Darak *et al*., 2012) and (Nobilis *et al*., 2006), reversed-phase-HPLC (RP-HPLC) (Majji *et al*., 2008) have been also used in the determination of MEZ in pharmaceutical preparations and blood plasma .

A Square wave voltammetric method (SWV) was applied to the determination of MEZ in pharmaceuticals by using pencil graphite electrodes (Uliana *et al*., 2010).

A Fluorimetric method has been employed for the determination of MEZ in blood serum, the fluorescence intensities were measured at 480 nm with excitation at 340 nm (Zadeh and Kolansal, 2012).

UV- spectophotometric methods were developed for the determination of MEZ in pure form and pharmaceuticals based on the measurement of absorbance at 210 nm in methanol and 303 nm in 0.5N HCl, respectively (Singh *et al*., 2010 ; Moharana *et al*., 2011).

Visible spectophotometric methods were used for MEZ determination using different reagents including: 1,2-naphthoquinone-4-sulphonate in alkaline medium, p-dimethylaminobenzaldehyde, 2,2-bipyridyl or potassium ferric cyanide in the presence of ferric chloride (Narala and Sarawathi, 2010), 3-methyl-2-benzothiazolinone hydrochloride in the presence of Fe(III) in an acidic medium (Narala and Saraswathi, 2011). Also, MEZ has been determined in pharmaceuticals using the mixture of potassium iodate and potassium iodide (Sloka *et al*., 2010), 7-chloro-4, 6-dinitrobenzofuroxane (Garmonov *et al*., 2011), Bratton-Marshall and Gibb's reagent (Patel *et al*., 2010).

The present work describes two simple and accurate spectrophotometric methods for the determination of MEZ in pure and pharmaceutical preparation (capsules).

EXPERIMENTAL

Instruments

Spectrophotometric measurements were carried out using Shimadzu UV-160 and UV-Visible spectrophotometer CECIL-CE 1021 digital single beam using 1-cm silica cells. pH meter type Philips PW 9420 was used for pH reading.

Reagents

All chemicals used in this investigation were analytical grade reagents.

Working MEZ solution, 100 µg/ml. A 0.01g of MEZ (Fluka) was dissolved in 10 ml of absolute ethanol to increase solubility and diluted to 100 ml with distilled water in a volumetric flask.

N-(1-naphthyl)ethylenediaminedihydrochloride (N-NED) solution, 0.005M. This solution was prepared by dissolving 0.1295g of N-NED (Fluka) in 100 ml distilled water.

8-hydroxyquinoline solution, 0.01M. This solution was prepared by dissolving 0.145g of 8- hydroxyquinoline (Fluka) in absolute ethanol in a 100 ml volumetric flask.

Sodium periodate solution, 0.015M. This solution was prepared by dissolving 0.321 g of sodium periodate in distilled water and made up to 100 ml in a volumetric flask with the same solvent.

Sodium hydroxide solution, 1N. This solution was prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water to 1000 ml in a volumetric flask and then transferred to a plastic bottle.

Mesacol (capsules) solution, 100 µg /ml MEZ solution from Mesacol Capsules. This solution was prepared by weighing and mixing the contents of ten capsules (each one contains 400 mg MEZ), an accurately weighed amount of powder equivalent to 0.01g MEZ was dissolved in 10 ml absolute ethanol and 30 ml distilled water, after filtration of the solution, the volume of filtrate was completed to 100 ml with distilled water in a volumetric flask.

General procedure and calibration graph

Method- A

To a series of 25-ml volumetric flasks, 0.1- 4 ml of MEZ solution (100 µg /ml) were transferred, followed by the addition of 2 ml of 8-hydroxyquinoline (0.01 M), 0.5 ml of sodium periodate (0.015M) and 2 ml sodium hydroxide(1N). The solutions were left to stand for 10 minutes before the volumes were completed to the mark with distilled water. After 15 minutes, the absorbances were measured at 644 nm against the reagent blank. The calibration graph was linear over the concentration range of 10-300 µg MEZ /25 ml (0.4- 12 ppm), a concentration above 300 µg /25 ml gave negative deviation from Beer's law (Fig.1). The molar absorptivity is 1.7223×10^{4} $l.mol^{-1}cm^{-1}$.

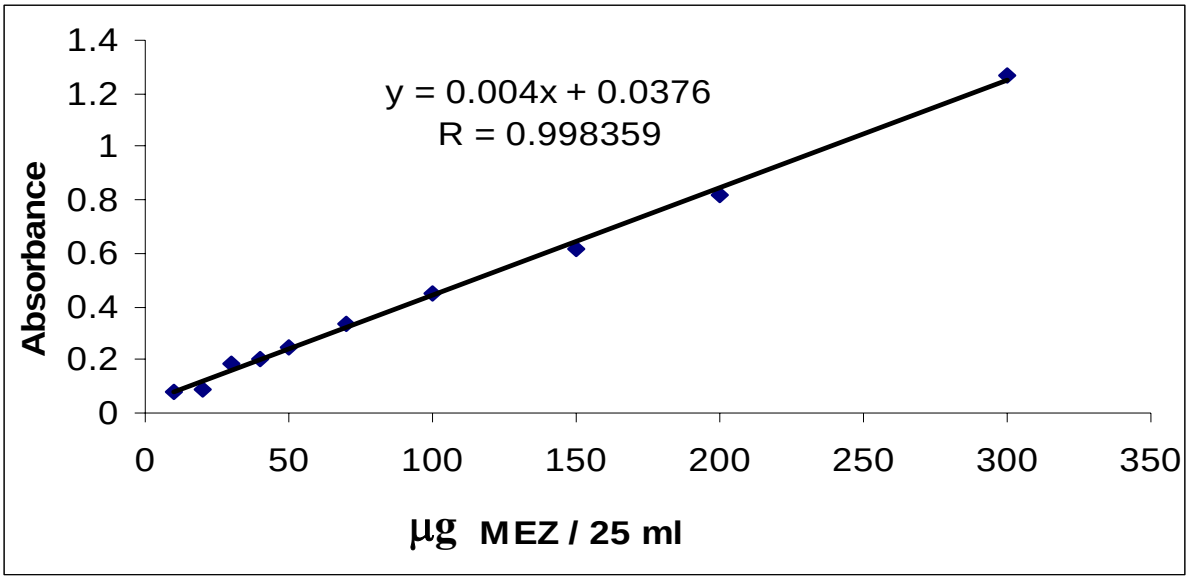


Fig. 1: Calibration graph for MEZ based on reaction with 8-hydroxyquinoline reagent

Method- B

To a series of 25 ml volumetric flasks, 0.1- 2 ml of MEZ solution (100 µg /ml) were transferred, followed by the addition of 0.5 ml of sodium periodate (0.015M), 1ml of N-NED (0.005 M) and 1ml of sodium hydroxide (1N). The solutions were left to stand for 15 minutes before the volumes were completed to the mark with distilled water. After 15 minutes the absorbances were measured at 543 nm against the reagent blank .The calibration graph was linear over the concentration range of 10-180 µg MEZ /25 ml (0.4-7.2 ppm), a concentration above 180 µg /25 ml gave negative deviation from Beer's law Fig. (2). The molar absorptivity was $0.6545 \times 10^4 l.mol^{-1}cm^{-1}$.

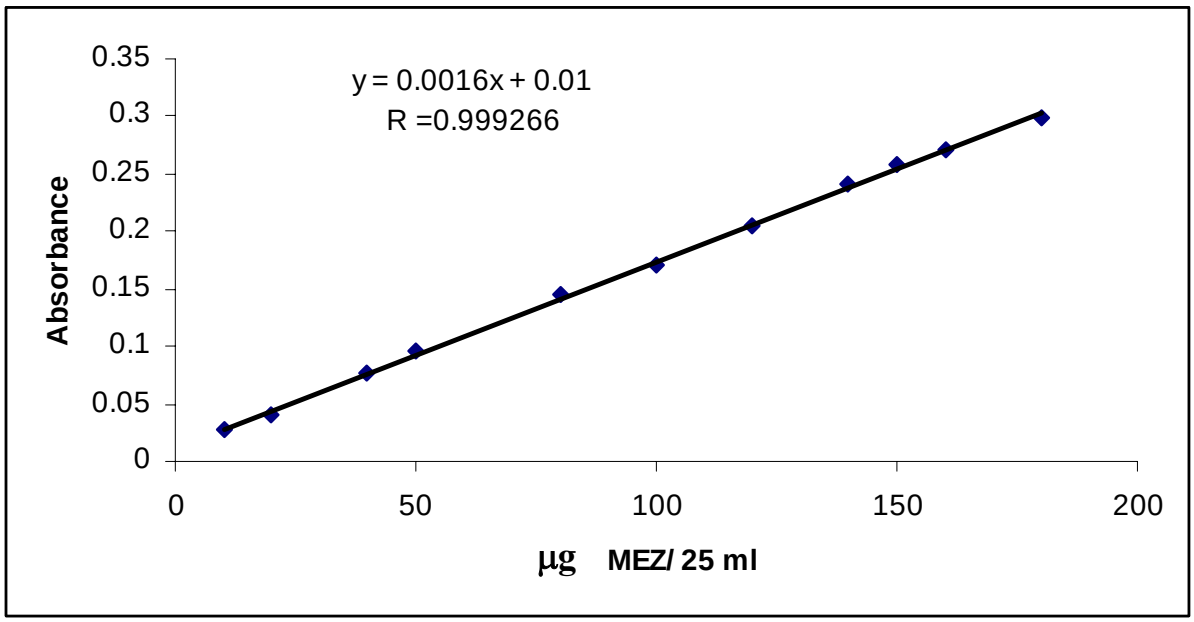


Fig. 2: Calibration graph for MEZ based on reaction with N-NED reagent

RESULTS AND DISCUSSION

The effect of various parameters on the colour development of 100 µg of MEZ were considered in 25 ml final volume and the absorbance measurements were performed at 644 and 543 nm for the methods A and B, respectively.

Choice of oxidizing agent and its concentration

Different types of oxidizing agents were used for the purpose of producing intense coloured product and strong colour contrast [Table (1)].

Table 1: Selection of oxidizing agent

1ml Oxidizing agent (0.015M) soln.	Method A		Method B	
	Absorbance	Δλ*	Absorbance	Δλ *
$NaIO_4$	0.298	301.5	0.146	221
KIO_3	0.120	300.5	No colour contrast	
K_2CrO_4	0.445	18	No colour contrast	
$K_2Cr_2O_7$	No colour contrast		No colour contrast	
N-Chlorosuccinimide	0.210	264	Turbid	…….
N-Bromosuccinimide	0.139	282.5	Turbid	…….
Ammonium ceric sulphate	Turbid	…….	Turbid	…….

. $\Delta\lambda^* = \lambda_{max}\,S - \lambda_{max}\,B$ S = the dye B=Blank

The results in Table 1 indicated that $NaIO_4$ gave the highest intensity with a good colour contrast for coloured product in both methods A and B.

The effect of different volumes (0.3-2 ml) of $NaIO_4$ solution (0.015M) on the colour intensity has been studied, it was observed that 0.5 ml of $NaIO_4$ for both methods is the most suitable volume therefore it was chosen for the subsequent experiments.

Effect of 8-hydroxyquinoline and N-NED reagents concentration

The effect of 8-hydroxyquinoline and N-NED concentrations on the absorbance of the complex in method A and B, respectively were investigated. It was found that 2 ml of 8-hydroxyquinoline (0.01M) and 1ml of N-NED (0.005M) gave maximum absorbance which were then used in subsequent experiments Table (2).

Table 2: Effect of reagent amount

ML of Reagent	Absorbance	
	8-hydroxyquinoline(0.01M)	N-NED(0.005M)
0.5	0.324	0.129
1	0.341	0.157
2	0.380	0.146
3	0.351	0.140

Choice of the base and its amount

The preliminary experiments have shown that MEZ gave an intense coloured dye with 8-hydroxyquinoline (method A) and N-NED (method B) in the presence of sodium periodate in alkaline medium, so that different bases are examined Table (3).

Table 3: Selection the of base

1ml of 1N Base	Method A		Method B	
	Absorbance	Δλ *	Absorbance	Δλ *
NaOH	0.268	306	0.156	219
KOH	0.266	308	0.143	219
Na_2CO_3	0.166	160	0.137	219
$NaHCO_3$	0.171	184	0.126	214

$\Delta\lambda^* = \lambda_{max}\tilde{S} - \lambda_{max}B$

The results shown in Table 3 indicated that NaOH gave the highest colour intensity of the product and a good colour contrast for both methods .The effect of different volumes (0.5-4 ml) of 1N NaOH solution on the colour intensity was studied then, a 2 ml and 1 ml of 1N NaOH with a final solution pH of 11.8 and 12.48 gave the highest intensity of the formed products for methods A and B, respectively.

Effect of time on oxidative coupling reaction

The time required for complete oxidation was tested in both methods. The results indicated that 10 minutes (method A) and 15 minutes (method B) were needed to give complete oxidation process before dilution with distilled water in both methods.

Effect of surfactant

The effect of different surfactants on the colour intensity were studied by using 1 ml of various types of surfactants. The results reveal that non of the surfactants give useful results from the analytical point of view. Therefore, it has been recommended to eliminate their use in the subsequent experiments in both methods [Table (4)].

Table 4: Effect of surfactant

1 ml Surfactant solution	Method A		Method B	
	Absorbance	Δλ *	Absorbance	Δλ *
CTAB 1×10^{-3}	0.438	31	0.174	149.5
SDS 1×10^{-3}	0.394	44	0.176	135
Triton x-100 1%	0.387	34.5	0.175	140
With out	0.450		0.178	

$\Delta\lambda^* = \lambda_{max}\tilde{S} - \lambda_{max}B$

Effect of the order of addition

The effect of the order of addition on the absorbance of the product was studied under the optimum experimental conditions [Table (5)].

Table 5: Effect of the order of addition

Order number	Order of addition	Method A	Method B
		Absorbance	
I	S+O+R+B	0.378	0.177
II	S+R+O+B	0.455	0.175
III	S+O+B+R	0.450	0.072
IV	B+O+R+S	0.451	0.121
V	B+O+S+R	0.453	0.155

Assuming that: S = sample, R = reagent, B = base, O = oxidizing agent

From the results above, order II for method A and order I for method B have been used for subsequent experiments due to the highest sensitivity.

Effect of temperature on the absorbance

The effect of temperature on the colour intensity of the resulting product was investigated. In practice, the high value of absorbance was obtained when the colour was developed at room temperature (25 C° ±1), but when the volumetric flasks were placed in an ice-bath at (0 C°) or in a water-bath at (50 C°) a loss in colour intensity and stability were observed. It is therefore recommended that the colour reaction should be carried out at room temperature for both methods.

Development time and stability period

The effect of time development and stability period of the coloured complex was investigated under the optimum conditions of the reaction. The stability of the colour intensity was reached after about 15- 20 minutes and the absorbance of the colour complex remained constant for at least one hour for both methods. [Table (6)].

Table 6: Effect of colour stability time

µg of MEZ	Method	Absorbance/ min. standing time							
		5	10	15	20	30	40	50	60
50	A	0.241	0.245	0.245	0.246	0.247	0.246	0.246	0.247
	B	0.095	0.098	0.103	0.103	0.102	0.102	0.101	0.101
100	A	0.442	0.447	0.449	0.450	0.451	0.450	0.449	0.449
	B	0.163	0.169	0.172	0.173	0.173	0.173	0.172	0.171

Final absorption spectrum

When MEZ was treated according to the recommended procedure, the absorption spectrum showed a maximum absorption at 644 nm (method A) and 543 nm (method B) versus the blank (Fig. 3 and 4).

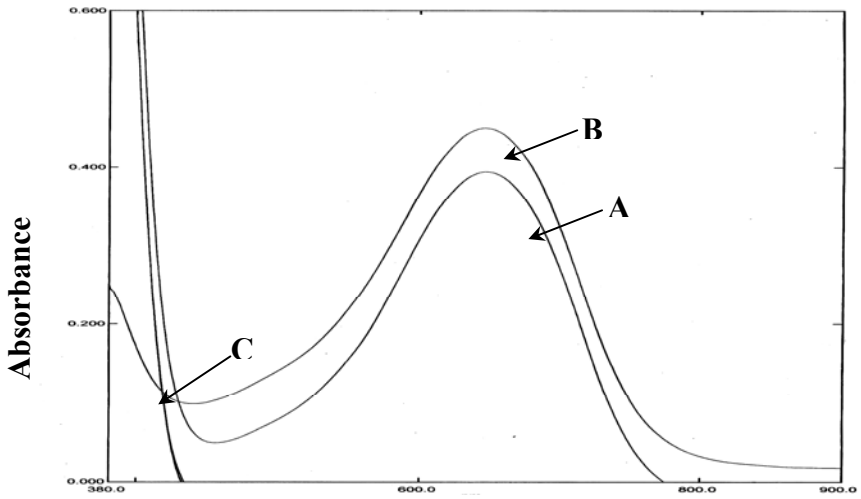


Fig. 3 : Absorption spectrum of MEZ with 8-hydroxyquinoline at 644nm

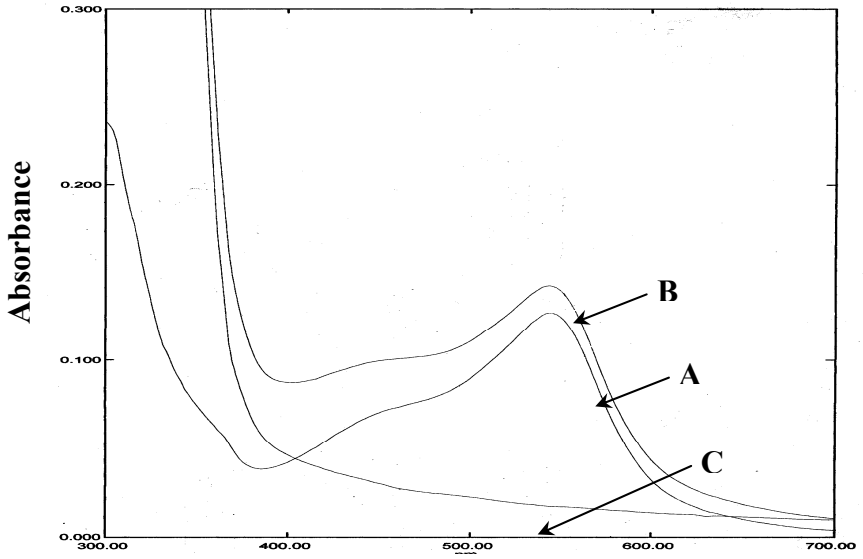


Fig. 4 : Absorption spectrum of MEZ with N-NED at 543nm

(A) the complex against blank, (B) the complex against distilled water, (C) the blank against distilled water.

Accuracy and precision

To check the accuracy and precision of the calibration graph, MEZ was determined at three different concentrations. The results shown in Table (7), indicate that a satisfactory accuracy and precision could be obtained with the proposed methods.

Table 7: Accuracy and precision of the proposed methods

MEZ (µg /25ml)	Relative error, %		Relative standard deviation, %*	
	Method A	Method B	Method A	Method B
50	-0.40	-0.90	±1.31	±0.88
100	0.35	-0.11	±0.25	±0.76
150	0.16	-0.31	±0.39	±0.32

*Average of five determinations

The nature of the reaction product

Mole ratio method indicates that the coloured product has a composition of 1:1 MEZ to 8-hydroxyquinoline reagent at 644 nm (Fig. 5) and 2:1 MEZ to N-NED reagent at 543 nm (Fig. 6).

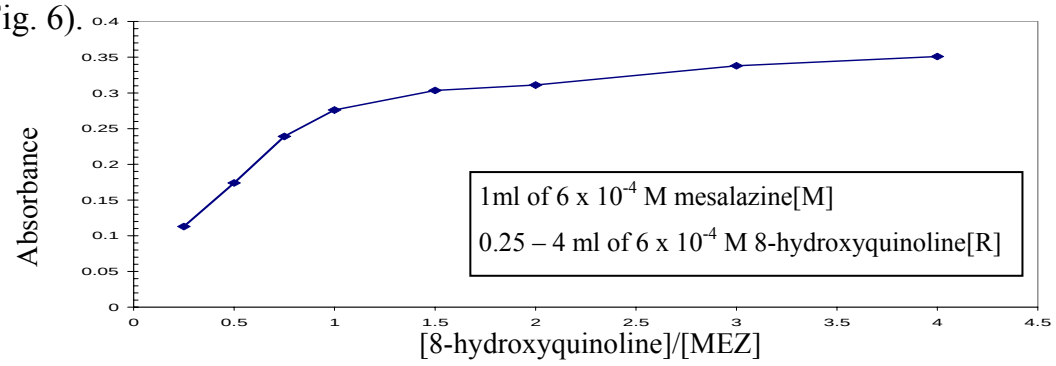


Fig. 5: Mole ratio's plot of MEZ- 8-hydroxyquinoline coloured product

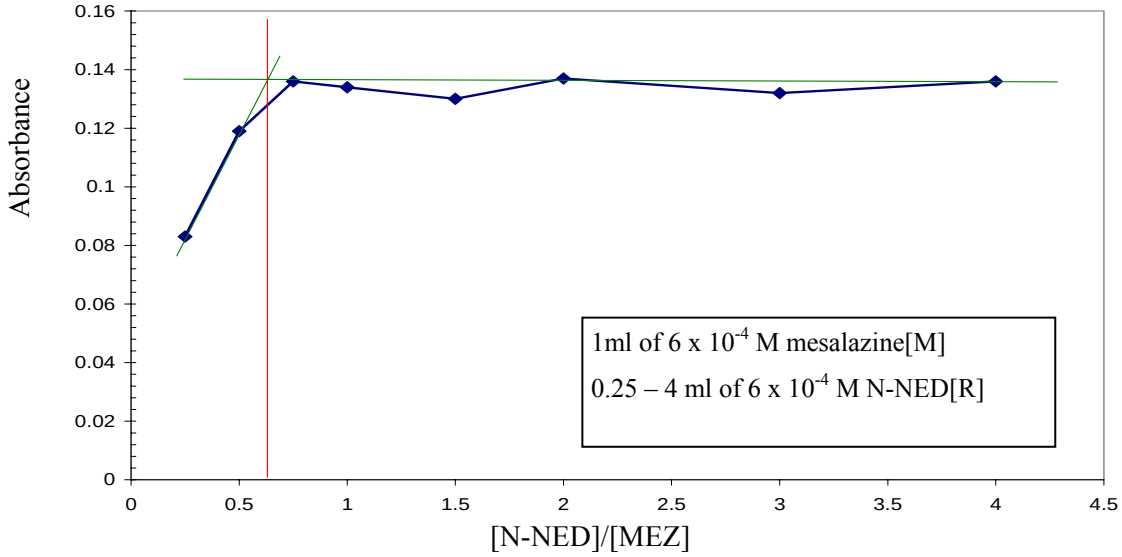
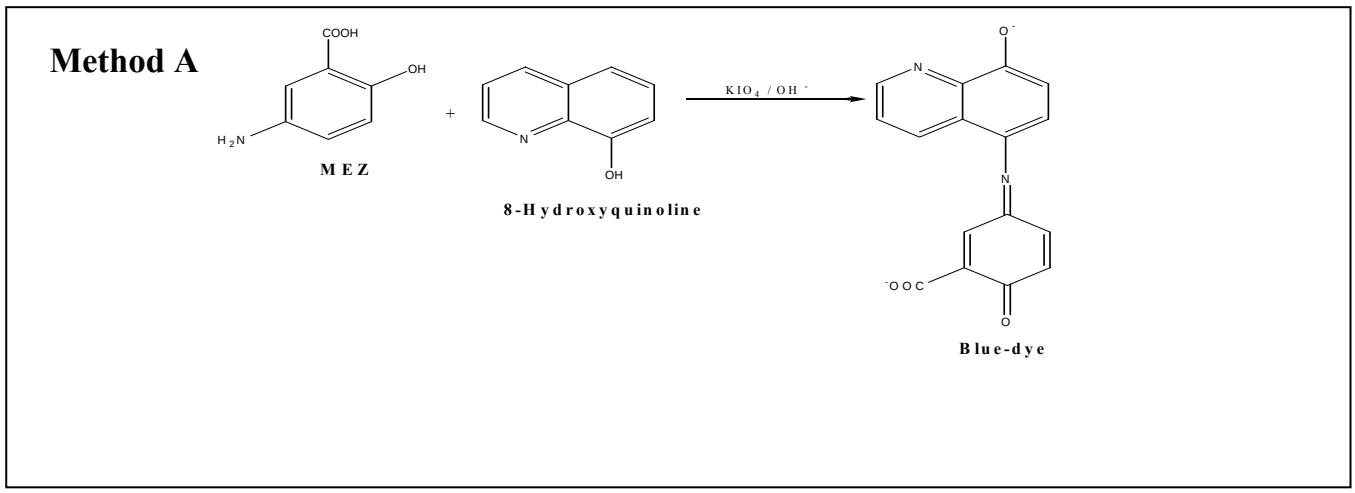
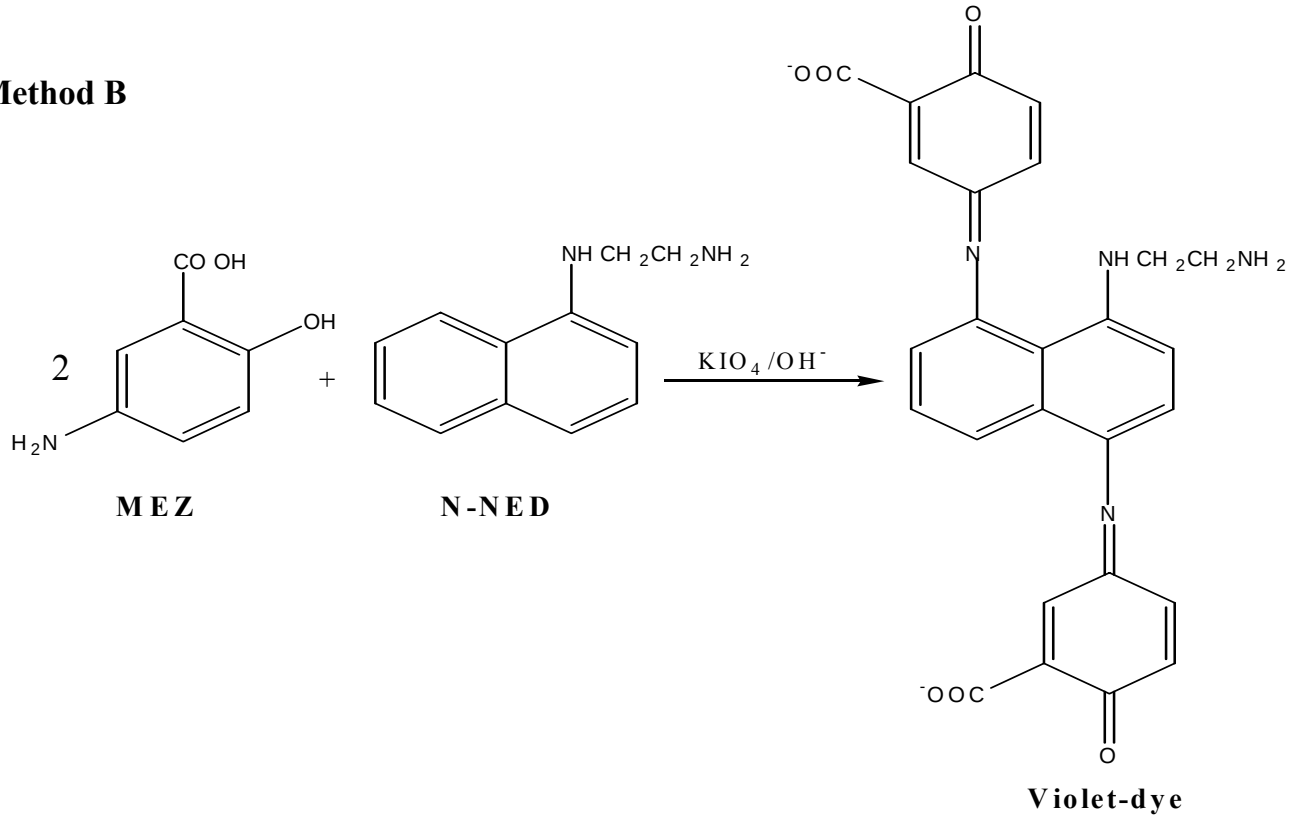


Fig. 6: Mole ratio's plot of MEZ- N-NED coloured product

Therefore, the probable reaction path might be written as follows :



Method B



Interferences

In order to asses the possible analytical application of the proposed methods, the effect of some foreign substances which often accompany the pharmaceutical preparations were studied by adding different amounts of these foreign substances to 100 µg MEZ /25 ml. It was found that the studied foreign species did not interfere in the present methods Table (8).

Table 8: Effect of interferences

Foreign compound	Recovery % of 100 µg MEZ /25 ml per µg of foreign compound added					
	Method A			Method B		
	100	500	1000	100	500	1000
Glucose	101.7	103.7	99.7	102.2	101.1	104.3
Lactose	104.1	104.6	104.1	101.5	97.6	103.5
Glycine	103.2	104.8	103.0	102.3	102.0	101.9
Gum Arabic	103.2	100.6	96.7	95.0	100.1	100.4
Starch	104.8	103.2	103.2	104..7	103.5	103.2

Analytical applications

The proposed methods were successfully applied to the determination of MEZ in its pharmaceutical preparation (capsules) as good recoveries were obtained Table (9).

Table 9: Application of methods

Drug	Method	µg MEZ present /25ml	µg MEZ measured /25ml	Recovery*, %
Mesacol Extended release capsules 400mg Universal pharmaceutical Industries-Unipharma-Damascus-Syria	**A**	40	39.3	98.2
		100	99.4	99.4
		120	121.9	106.6
	B	50	47.7	95.4
		100	100..6	100.6
		150	150.9	100.6

*Average of five determinations

Evaluation of the proposed methods

Because the standard method for the determination of MEZ involves potentiometric titration and according to difficulties of availability of using it, standard addition method was used in order to prove that the proposed methods can be used in the determination of MEZ in pharmaceutical preparation (capsules) Table (10) and Fig.(7)

Table 10:The results of standard addition method

Drug	Method	µg MEZ present /25ml	µg MEZ measured /25ml	Recovery %*
Mesacol Extended release capsules 400mg Universal pharmaceutical Industries-Unipharma-Damascus-Syria	A	20	19.08	95.4
		40	39.42	98.5
	B	20	19.44	97.2
		40	40.52	101.3

- Average of three determinations

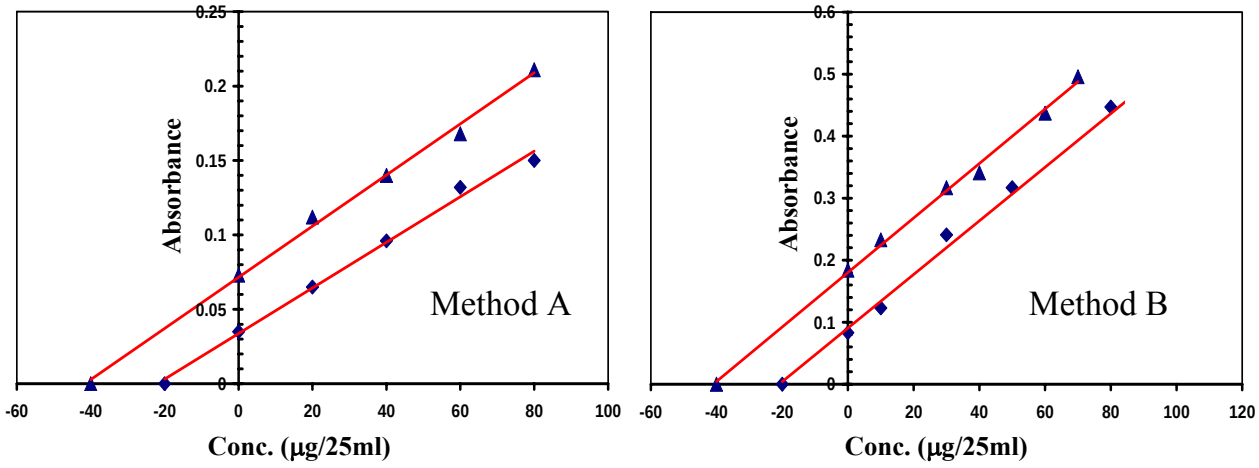


Fig.7: Calibration standard addition graphs for the determination of MEZ in capsules for both methods

The results in Table (10) and Fig. (7) indicated that the proposed methods are reliable and can be used to determine MEZ in Pharmaceutical preparation (capsules) with satisfactory results.

Comparison of Methods

Table (11) shows the comparison between the analytical variables from the present methods with that of a recent spectrophotometric method.

Table 11: Comparison of the methods

Analytical parameters	**Present methods**		**Literature method***
	A	**B**	
Temperature(C°)	At room temperature	At room temperature	At room temperature
Development time (minutes)	15	15	5
λmax (nm)	644	543	610
Medium of method	Aqueous	Aqueous	Aqueous
Type of reaction	Oxidative coupling	Oxidative coupling	Oxidative coupling
Reagent	8-hydroxyquinoline	N-NED	2,6-Xylenol
Beer's law range (ppm)	0.4-12	0.4-7.2	0.16-8
Molar absorptivity l.mol^{-1}cm^{-1}	1.7223×10^4	0.6545×10^4	1.3116×10^4
RSD(%)	±1.31 to ±0.39	±0.88 to ±0.32	±0.1.23 to ±1.01
Nature of the dye	1:1	2:1	1:1
Applications	Determination of MEZ in capsules	Determination of MEZ in capsules	Determination of MEZ in two drug forms (capsules and tablets)

*Al-Fakhry, M.H.(2006), M.Sc. Thesis, Mosul University, pp. 64-80.

CONCLUSION

The proposed visible spectrophotometric methods for the estimation of MEZ are simple, sensitive, accurate and precise. Method A (used 8-hydroxyguinoline) was found to be more sensitive compared to method B (used N-NED) for the assay of MEZ and can be used for the routine quality control of the drug in bulk as well as in a pharmaceutical preparation (capsules).

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