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Spectrophotometric Determination of Mesalazine in Pharmaceutical Preparations by Oxidative Coupling Reactions with *m*-Aminophenol and 2,6- Dihydroxybenzoic Acid

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Abstract

Tow simple, rapid and sensitive spectrophotometric methods for the determination of mesalazine in pharmaceutical preparations have been carried out. The proposed methods depend on oxidative coupling reaction of mesalazine with *m*-aminophenol in the existence of N-bromosuccinamide in alkaline medium (method A) and 2,6-dihydroxybenzoic acid in the existence of sodium metaperiodate in basic medium (method B) to produce colored products , show highest absorptions at 640 (nm) and 515 (nm), alternately. Beer's law was consistent in concentrations extent of 1.25-30 and 0.5-12.5 (µg.mL^{-1}) with molar absorptivity of 0.36×10^4 and 0.77×10^4 L.mol^{-1}.cm^{-1}, alternately. The limits of detection (LOD) were 0.0144 and 0.0829 µg.mL^{-1}, while limits of quantitation (LOQ) were 0.0483 and 0.2766 (µg.mL^{-1}), alternately. The suggested methods have been applied successfully to the determination of mesalazine in its pharmaceutical preparations as tablets and suppositories.

Key words: Mesalazine, Oxidative coupling, *m*-aminophenol, 2,6-dihydroxybenzoic acid, Spectrophotometry.

Introduction:

Mesalazine (MZ), also known as mesalamine or 5-aminosalicylic acid (5-ASA), is an anti inflammatory drug used to treat inflammation of the digestive tract (Crohn's disease) and mild to moderate ulcerative colitis (1). The mechanism of action of MZ is unknown, but appears to be topical rather than systemic. Mesalazine acts as a scavenger of oxygen-derived free radicals, which are produced in greater numbers in patients with inflammatory bowel disease (2). Mesalazine is administered orally or rectally in the treatment of ulcerative colitis and Crohn's disease (3). The chemical structure of mesalazine as it is shown in Fig. 1 :

M.Wt = 153.1 g/mol
($C_7H_7NO_3$)

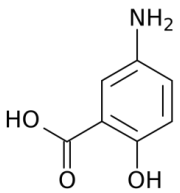

Figure 1. Chemical structure of mesalazine

Mesalazine consist of white to pinkish crystals, decomposed at about 280 °C, slightly soluble in cold water, alcohol more soluble in hot water and in hydrochloric acid (4) .

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A number of analytical methods have been reported for the assay of mesalazine in pharmaceutical dosage forms. These methods include different techniques such as: spectrophotometry (5-10), spectrofluorometry (11), high-performance liquid chromatography (12-14) and voltammetry (15,16). However these methods generally require expensive equipment, provision for use and personal skills , so it still seems to be that the spectrophotometric methods are more convenient than other techniques due to their inherent simplicity , high sensitivity , low cost and wide availability in most laboratories .To the best of our knowledge, the reagents *m*-aminophenol and 2,6-dihydroxybenzoic acid were not previously used in oxidative coupling reactions, so the aim of this project is to employ these reagents for the assay of mesalazine in its pharmaceutical preparations .

Materials and Methods:

Apparatus

All spectrophotometric measurements were carried out on Jasco V-630 UV-Visible spectrophotometer with 1.0 cm matched glass cells, pH measurements were performed by pH meter type TRANS BP 3001.

Reagents and chemicals

All reagents and chemicals used were analytical grade .

Mesalazine solution, (100 µg/mL)

The solution was attended by dissolving 0.01 g of pure mesalazine (Aldrich) in 5 mL ethanol and 40 mL distilled water with gentle heating, then the volume was completed to the mark with distilled water .

N-bromosucinamide solution, (0.1%)

0.1 g of pure substances (Reidel-Haen) was dissolved in enough amount of distilled water, then the volume was completed to 100 mL by distilled water in avolumetric flask .

***m*-Aminophenol reagent solution, (0.1%)**

The solution was attended by dissolving 0.01g of *m*-aminophenol (Fluka) in enough amount of distilled water with gentle heating and the volume was completed to (100) mL by distilled water in a volumetric flask .

2,6-Dihydroxybenzoic acid solution, (0.1 %)

This solution was attended by dissolving 0.1 g of pure reagent (Fluka) in enough amount of distilled water with gentle heating and the volume was completed to (100) ml by distilled water in avolumetric flask .

Sodium metaperiodate solution, (0.1%)

The solution was attended by dissolving 0.1g of $NaIO_4$ in (100) mL distilled water .

Sodium hydroxide solution, (1M)

This solution was attended by the appropriate dilution of the concentrated volumetric (BDH) solution with distilled water and then transferred to a plastic bottle.

Tablets (Pentasa and Awasalazine) solution, (100 µg.mL^{-1})

The contents of ten tablets (each tablet contains 500 or 400 (mg) mesalazine as Pentasa or Awasalazine preperations) were finely grinded, mixed well and weighed accurately to a quantity equal to (0.01 g) of mesalazine was dissolved in (5 mL) ethanol and (40 ml) distilled water with gentle heating and after filtration of the solutions, the volumes were made up to (100 ml) by distilled water in a volumetric flask .

Suppositories (Asacol) solution, (100 µg.mL^{-1})

The content of three Asacol suppositories(each one contains 500 mg of mesalazine) are mixed well, an accurately weighed equivalent to (0.01 g) was dissolved in (5 ml) ethanol and (40 ml) distilled water with gentle heating, then the solution filtrate using a filter paper and the volume was made up to (100 ml) by distilled water in a volumetric flask .

Recommended procedures

(Method A)

Accurately measured volumes containing (25-600) µg of mesalaszine were transferred into a series of 20 ml calibrated flasks, followed by the addition of 1.0 ml of 0.1% N-bromosuccinamide and 0.5 ml of 0.1% *m*-aminophenol ,then adding 1.0 mL of 1.0 M sodium hydroxide and the volumes completed to the mark with distilled water and then measure the absorbance at 640 nm versus the reagent blank solution .

(Method B)

Accurately measured volumes containing 10-250 (µg) of mesalaszine were transferred into a series of 20 ml calibrated flasks, followed by the addition of 1.5 ml of 0.1% 2,6-dihydroxybenzoic acid, 1.5 ml of 0.1% sodium periodate and 1.5 mL of (1.0 M) sodium hydroxide and the volumes were completed to the mark with distilled water and then the absorbance was measured at 515 (nm) versus the reagent blank solution .

Results and Discussion:

The effect of different factors on the color evolution of (100 µg) of mesalazine was investigated in 20 ml total volume and the absorbance measurements were carried out at 640 and 515 nm for the methods A and B , alternately .

Effect of *m*-aminophenol and 2,6-dihydroxybenzoic acid reagents amounts

The effect of m-aminophenol and 2,6-dihydroxybenzoic acid reagents amounts on the absorbance of the colored product in method A and B , alternately was studied . The results in Table 1 indicate that 0.5 mL of 0.1% m-aminophenol and 1.5 mL of 0.1% 2,6-dihydroxybenzoic acid give the highest absorbance which were chosen for the next experiments.

Table 1.The effect of reagents quantities

Reagent added (mL)	Absorbance	
	m-Aminophenol (0.1%)	2,6-DHBA(0.15%)
0.25	0.233	--------
0.50	0.297	0.155
1.00	0.285	0.206
1.50	0.283	0.208
2.00	---------	0.198

Effect of oxidizing agent amount

The effect of oxidizing agent amount on the absorbance of the formed dyes for both methods hase been investigated. The results noticed from Table 2 that 1.0 mL of 0.1% N-bromosuccinamide and 1.5 mL of 0.1% sodium metaperiodate were the

ideal volumes, so they were select for the next experiments.

Table 2.The effect of oxidizing agents amounts

Oxidizing agent (mL)	Absorbance	
	NBS (0.1%)	$NaIO_4$ (0.1%)
0.5	0.223	0.184
1.0	0.287	0.247
1.5	0.271	0.268
2.0	0.274	0.208

Effect of bases type and its quantities

The initial tests revealed that mesalazine produced colored dyes for two methods (A and B) just in alkaline medium, so the effect of different bases on the absorbance was investigated and the obtained results are illustrated in Table 3. They indicate that sodium hydroxide forms the intense dye for both methods compared with the other bases, so different amounts of sodium hydroxide were added and the obtained results reveal that 1.0 mL and 1.5 mL of 1M of NaOH are the optimum volumes for method A and B, respectively .

Table 3.Choice of the base type

1 mL of (1M) Based used	Method- A		Method- B	
	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)
$NaHCO_3$	0.089	490	0.010	473
Na_2CO_3	0.120	640	0.026	449
NaOH	0.318	640	0.268	515
KOH	0.285	638	0.170	495

The effect of sequence of addition

The effect of sequence of addition on the absorbance of colored products was investigated. The results listed in Table 4 indicate that order (I) for method A and order (II) for method B have been chosen for the next experiments because they gave high sensitivity .

Table 4. The effect of sequence of addition

Addition serial	Order of addition	Absorbance	
		Method A	Method B
I	MZ+Oxidant+Reagent+Base	0.315	0.230
II	MZ+Reagent+Oxidant+Base	0.072	0.270
III	MZ+Reagent+Base+Oxidant	0.061	0.255
IV	MZ+Base+Reagent+Oxidant	0.057	0.264
V	MZ+Base+Oxidant+Reagent	0.020	0.223

Relationship between temperature and absorbance

The role of temperature on the absorbance of the formed dyes was studied by applying the procedure for methods A and B under optimum conditions at three different temperatures .The obtained results listed in Table 5 reveal that room temperature (20±2 °C) gave high value of absorbance, while conducting the reaction at (0 °C) or in (40 °C) reduced the sensitivity of the methods, therefore it is better to do the reaction in room temperature due to its better sensitivity and simplicity.

Table 5. Effect of temperature on absorbance of colored product

Temperature (℃)	Absorbance	
	Method A	Method B
0 (ice bath)	0.267	0.243
R.T	0.276	0.312
40 (water bath)	0.266	0.230

Effect of surfactants

The results obtained from the investigation of three types of surfactants (CTAB, SDS and Triton X-100) on the sensitivity of proposed method A and B revealed that there is no improvement in the intensity of the formed dyes, so it is unfavorable to use it .

Effect of time on color development

The effect of time development and stability period of the colored product was investigated under the optimum conditions. From Table 6, it is noticed that the formed dyes in method(A and B) reached maximum absorbance immediately after the addition of the base and stayed stable about 2 hrs. in which many measurements can be done .

Table 6. Effect of time on color development

Time (min.)	Absorbance / 100 µg of MZ	
	Method A	Method B
After addition	0.309	0.279
5	0.309	0.279
10	0.308	0.277
15	0.308	0.276
20	0.306	0.275
25	0.306	0.275
30	0.305	0.275
35	0.305	0.274
40	0.305	0.274
45	0.304	0.273
50	0.304	0.273
55	0.303	0.273
60 (1 hr.)	0.302	0.272
120 (2 hrs.)	0.300	0.271

calibration graphs for proposed methods

According to the obtained optimum conditions the calibration curves for method (A) and (B) Fig.2, were linear over the concentrations extent (1.25-30 $\mu g.mL^{-1}$), and (0.5-12.5 $\mu g.mL^{-1}$), respectively .The apparent molar absorption referred to mesalazine has been found to be 0.36×10^4 and 0.77×10^4 $L.mol^{-1}.cm^{-1}$.

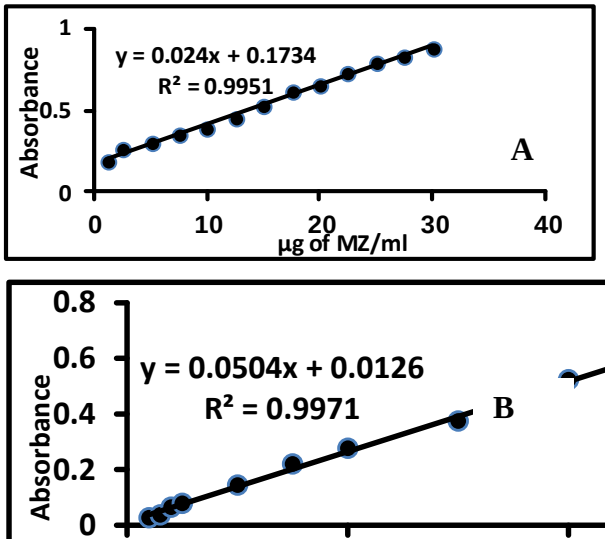


Figure 2.Calibration graphs for MZ determination (method A & B)

The final of absorption spectra

Using the recommended procedures in both methods (A and B), the formed dyes show a final absorption spectra with maximum absorbance at 640 nm (method A) and at 515 nm (method B), against the blank solutions as shown in Fig. 3 and 4.

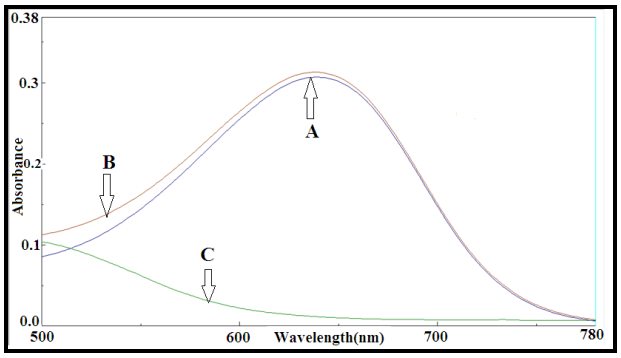


Figure 3. Absorption spectra of MZ with m-aminophenol (Method A) formed dye versus blank, (B) formed dye versus the distilled water (c) blank versus distilled water

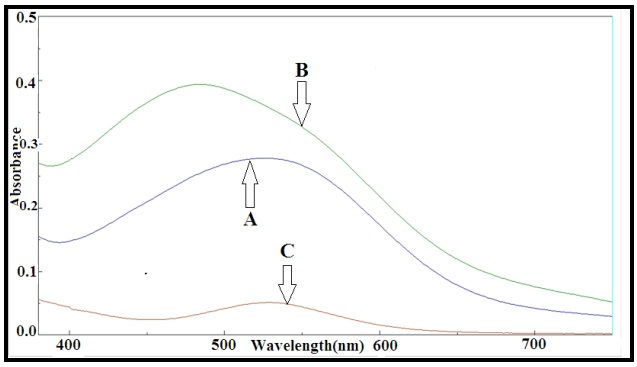


Figure 4. Absorption spectra of MZ with 2,6-DHBA (Method B) (A) formed dye versus blank , (B) formed dye versus the distilled water (c) blank versus distilled water

Nature of the formed dyes

To estimate the reaction ratio of MZ to m-aminophenol and 2,6-dihydroxybenzoic acid reagents, Job's method was used, which indicates that the formed dyes in both methods have a composition ratio 1:1 as shown in Fig. 5 and 6.

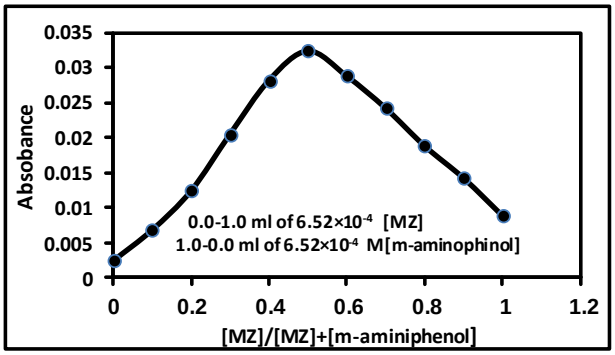


Figure 5. Job's plot for MZ -m-Aminophenol

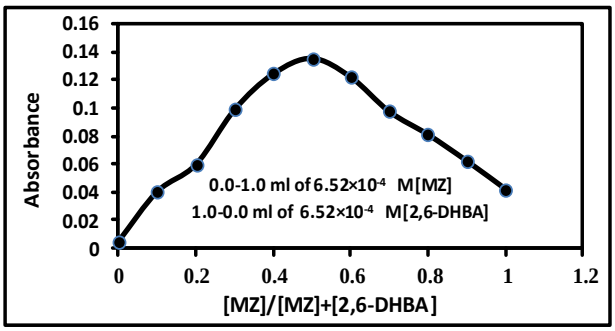
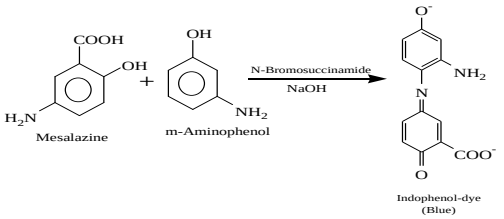


Figure 6. Job's plot for MZ -2,6-DHBA

Hence, the formation of colored dyes according to berthelot or indophenol reaction (17) may probably take place as shown in Fig. 7:

Method-A:



Method-B:

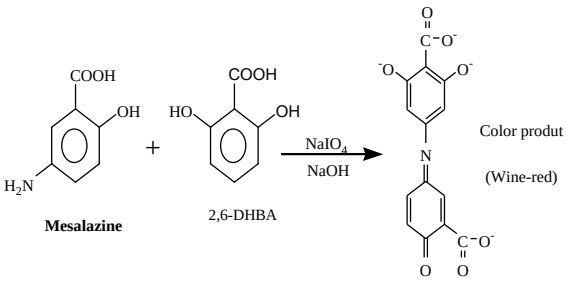


Figure 7. The suggested reactions for color product formation

Effect of additives species

The effect of some added compounds which are often found in the pharmaceutical formulations was examined by adding various quantity of this additives to (100 µg) of mesalazine using the recommended procedures. The results in Table 7 indicate that none of added compounds can introduce significant interference.

Table 7. Effect of additives species on mesalazine recovery

Added compound	Recovery % of 100 µg mesalazine/ µg of added compound					
	Method A			Method B		
	250	500	1000	250	500	1000
Starch	98.0	98.6	99.1	97.5	95.4	95.7
Glucose	95.8	97.7	98.3	97.4	95.3	97.6
Lactose	97.7	96.7	98.2	96.7	95.9	95.9
Arabic gum	98.6	99.0	99.0	96.1	97.7	96.3

Application of the proposed methods

To test the applicability of the proposed methods, they the determination of mesalazine in its pharmaceutical preparations (tablets and suppositories) has been applied. The results shown in Table 8 indicate that the proposed methods have good accuracy, precision and recovery.

Table 8. Application of the proposed methods

Pharmaceutical preparation	Method	Amount taken, µg	Amount measured, µg	Recovery* (%)	Relative error*, %	Relative standard deviation*,%
Pentasa, 500mg/tablet (Ferring,Germany)	**A**	50	48.95	97.90	-2.10	±0.22
		100	98.99	98.99	-1.01	±0.13
		200	197.05	98.50	-1.50	±0.11
	B	50	47.85	95.70	-4.30	±0.33
		100	96.60	96.60	-3.40	±0.14
		150	142.72	95.14	-4.86	±0.05
AwaSalazine, 400 mg/tablet (Awamedica,Iraq)	**A**	50	48.60	97.20	-2.80	±0.19
		100	99.30	99.30	-0.70	±0.12
		200	195.10	97.55	-2.45	±0.28
	B	50	47.97	95.94	-4.06	±0.29
		100	96.84	96.84	-3.16	±0.14
		150	142.79	95.19	-4.81	±0.11
Asacol, 500mg/suppository (Tillotts Pharma AG, Switzerland)	**A**	50	47.81	95.62	-4.38	±0.38
		100	98.00	98.00	-2.00	±0.57
		200	193.20	96.60	-3.40	±0.10
	B	50	47.99	95.98	-4.02	±0.24
		100	96.89	96.89	-3.11	±0.10
		150	142.78	95.18	-4.82	±0.11

***Average of five estimations**

Evaluation of the proposed methods

The efficiency of the proposed methods was checked by calculating the t-test for the present methods compared with the standard method of British pharmacopeia (18) for 95% confidence level with eight degree of freedom. The results in Table 9 indicate that the t-values were less than the critical value (2.306), which mean there are no real differences between the present methods and standard method for mesalazine determination.

Table 9. Evaluation of proposed method by t-test analysis

Pharmaceutical preparation	Recovery* (%)			t-test (experimental)	
	Present method		Standard method		
	Method A	Method B		Method A	Method B
Pentasa, 500 mg/tablet (Ferring, Germany)	98.99	96.60	98.29	±0.11	±0.28
AwaSalazine, 400 mg/tablet (Awamedica, Iraq)	99.30	96.84	102.57	±0.69	±1.22
Asacol, 500mg/suppository (Tillotts Pharma AG, Switzerland)	98.00	96.89	99.51	±0.34	±0.43

***Average of five determinations.**

Present methods compare

The proposed methods compared with other spectrophotometric methods, are illustrated in Table 10. Method (A) has a wide scale while method (B) has an acceptable range of determination and sensitivity compared with a recent spectrophotometric method.

Table 10. Comparison with other spectrophotometric method

Analytical parameters	Method A	Method B	Literature method(19)
Reagent	m-Aminophenol	2,6-DHBA	1-Naphthol
Medium of reaction	Aqueous	Aqueous	Aqueous
Temperature	R.T	R.T	R.T
Development time, (min.)	After dilution	After dilution	15
λ_{max} (nm)	640	515	616
Beer's law range ($\mu g.ml^{-1}$)	1.25-30	0.5-12.5	0.2-20
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	0.36×10^4	0.77×10^4	1.20×10^4
Stability of the dye (hr.)	2 (at least)	2 (at least)	About 2
Colour of the dye	Blue	Wine-red	Blue
Application of the method	Tablets and suppositories	Tablets and suppositories	Tablets and capsules

Conclusion:

A simple, rapid and sensitive spectrophotometric methods are described for the assay of mesalazine in its pharmaceutical formulations as tablets and suppositories .The suggested methods depend on oxidative coupling reactions with *m*-aminophenol in the presence of N-bromosuccniamide as an oxidant agent in basic medium (method A) and with 2,6-dihydroxybezoic acid in the presence of sodium metaperiodate as oxidant agent in basic medium (method B) to form colored dyes were stable for at least 2 hrs at room temperature. The present methods do not need to control the temperature or extraction process.

Conflicts of Interest: None.

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التقدير الطيفي للميزالازين في المستحضرات الصيدلانية بوساطة تفاعلات الاقتران التأكسدي مع ميتا- امينوفينول و6،2- ثنائي هيدروكسي حامض البنزويك

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الخلاصة :

يتضمن البحث اقتراح طريقتين طيفيتين اتصفتا بالسهولة والسرعة والحساسية لتقدير الميزالازين في مستحضراته الصيدلانية. اعتمدت الطريقتين المقترحتين على تفاعلات الاقتران التأكسدي للميزالازين مع الكاشف ميتا-امينو فينول بوجود العامل المؤكسد N- بروموسكسيناميد في الوسط القاعدي (الطريقة A) والكاشف 6،2- ثنائي هيدروكسي حامض البنزويك بوجود العامل المؤكسد بيريودات الصوديوم في الوسط القاعدي (الطريقة B) ، لتكوين نواتج ملونة تعطي أعلى امتصاص عند الطول الموجي 640 نانوميتر و515 نانوميتر على التوالي . انطبق قانون بير ضمن مدى التراكيز 1.25- 30 مايكروغرام.مللتر$^{-1}$ و0.5- 12.5 مايكروغرام.مللتر$^{-1}$ وبامتصاصية مولارية 0.36 ×10^{4} و 0.77×10^{4} لتر.مول$^{-1}$.سم$^{-1}$ على التوالي. وكانت حدود الكشف تساوي 0.0144 و0.0829 مايكروغرام.مللتر$^{-1}$ على التوالي . تم تطبيق الطريقتين المقترحتين بنجاح لتقدير الميزالازين في مستحضراته الصيدلانية بشكل اقراص وتحاميل .

الكلمات المفتاحية: الميزالازين، الأقتران التأكسدي، ميتا- امينوفينول، 6،2- ثنائي هيدروكسي حامض البنزويك، طرائق طيفية .