

## Spectrophotometric Determination of Mesalazine in Pharmaceutical Preparations by Diazotization and Coupling with 2,6-Dihydroxytoluene as a New Coupling Agent

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### ABSTRACT

A simple and sensitive spectrophotometric method is proposed for the determination of mesalazine (MZ) in some of its pharmaceutical preparations. The method is based on the diazotization of mesalazine by reaction with sodium nitrite in an acidic medium and then coupling of diazonium salt corresponding to mesalazine with 2,6-dihydroxytoluene reagent in basic medium to produce an intense orange colored water-soluble and stable azo-dye for at least two hrs, and exhibits maximum absorption at 453 nm. Beer's law is obeyed in the concentration range of 2.5-100 µg of mesalazine in a final volume of 10 ml i.e., 0.25-10.0 ppm with a molar absorptivity of  $4.6 \times 10^4$  l. mol<sup>-1</sup>. cm<sup>-1</sup> and sandell sensitivity index of 0.00332 µg. cm<sup>-2</sup>. A relative error is -0.48 to -4.80 and relative standard deviation of ±0.01 to ±0.17 depending on the concentration level. The proposed method has been applied successfully to determine mesalazine in pharmaceutical preparations, tablets and suppositories.

**Keywords:** Mesalazine, diazotization and coupling, 2,6-dihydroxytoluene reagent spectrophotometry.

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التقدير الطيفي للميزالازين في المستحضرات الصيدلانية بالأزوتة والاقتران مع الكاشف 6،2-ثنائي

هيدروكسي تولوين كعامل اقتران جديد

### الملخص

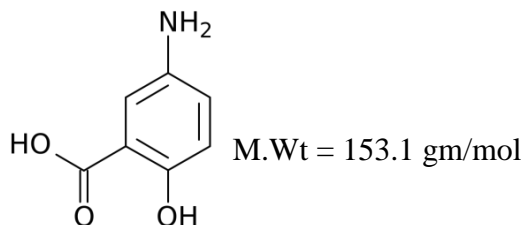
يتضمن البحث اقتراح طريقة طيفية بسيطة وحساسة لتقدير الميزالازين في بعض مستحضراته الصيدلانية. تعتمد الطريقة على أزوتة الميزالازين وذلك بتفاعله مع نترتيت الصوديوم في الوسط الحامضي يتبعه اقتران ملح الدايازونيوم المقابل للميزالازين مع الكاشف 6،2 - ثنائي هيدروكسي تولوين في الوسط القاعدي لتكوين صبغة ازوية برتقالية اللون ذائبة في الماء ومستقرة لمدة ساعتين على الأقل وتعطي اعلى امتصاص عند الطول الموجي 453 نانوميتر. انطبق قانون بير في مدى التراكيز 2.5 - 100 مايكروغرام من الميزالازين في حجم نهائي 10 مللتر (0.25 - 10) جزء لكل مليون، وكانت قيمة الامتصاصية المولارية للصبغة الناتجة  $4.6 \times 10^4$  لتر.مول<sup>-1</sup>. سم<sup>-1</sup>، وقيمة دلالة ساندل 0.00332 مايكروغرام.سم<sup>-2</sup>، والخطأ النسبي من -0.48 الى -4.80 والانحراف القياسي النسبي بين  $\pm 0.01$  و  $\pm 0.17$  اعتمادا على مستوى التركيز. تم تطبيق الطريقة المقترحة بنجاح لتقدير الميزالازين في الأقراص الدوائية والتحاميل.

**الكلمات الدالة:** الميزالازين، أزوتة و اقتران، كاشف 6،2-ثنائي هيدروكسي تولوين، طريقة طيفية.

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## 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 (Kanala *et al.*, 2013). Mesalazine is a first line drug for treatment of inflammatory bowel diseases. However, its mechanisms are not fully understood (Oh-oka *et al.*, 2017). Mesalazine is a polar compound and it exhibits amphoteric properties (Nobilis *et al.*, 2006), and chemically it is: 5-Amino-2-hydroxybenzoic acid and has the following chemical structure:



**Mesalazine (C<sub>7</sub>H<sub>7</sub>NO<sub>3</sub>)**

Mesalazine is a white or light grey or light pink powder or crystals, practically insoluble in alcohol, slightly soluble in water. It dissolves in dilute solutions of alkali hydroxides and in dilute hydrochloric acid (British pharmacopeia, 2013).

Several methods have been reported for determination of mesalazine in pure form or in pharmaceutical preparations. A spectrophotometric methods based on the diazotization and coupling reactions (zakaria, 2009; Dung *et al.*, 2016), or based on the oxidative coupling reactions (Al-Fakhry, 2006; Salih and Al-Sharook, 2008; Shihab, 2011). Another spectrophotometric methods are based on formation of colored species (Al-Sabhaa and Habeeb, 2015) or by charge-transfer complex formation (Al-Enizzi *et al.*, 2012) or by oxidation-reduction reactions (Hamdoon, 2018). Also UV spectrophotometric methods have been reported (Rao *et al.*, 2015). The chromatographic methods for the determination of mesalazine include high performance liquid chromatography (Rao *et al.*, 2013), and electrochemical methods for the determination of mesalazine based on cyclic voltammetry at a glassy carbon electrode (Kumar *et al.*, 2017).

According to our knowledge and literature survey, the reagent 2,6-dihydroxytoluene has never been used in analytical chemistry. So, the aim of present work is the employing of this reagent for the determination of mesalazine in aqueous solution by diazotization and coupling reactions and application of proposed method to the pharmaceutical preparations.

## EXPERIMENT

### Instruments

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

All chemicals used are of analytical reagents grade.

### Standard solution of mesalazine (100 µg/ml)

This solution was prepared by dissolving 0.01g of pure mesalazine (Aldrich) in 5 ml ethanol and 40 ml distilled water with gentle heating, then made up to the mark with distilled water.

### Working mesalazine solution (25 µg/ml)

A 25 ml of (100 µg/ml) is diluted with distilled water to the mark in a 100 ml volumetric flask.

### Hydrochloric acid solution (1N)

This solution was prepared by diluting 8.6 ml of the concentrated acid (Thomas Baker) to the mark with distilled water in a 100 ml volumetric flask.

**Sodium nitrite solution (1%)**

This solution was prepared by dissolving 1.0g of sodium nitrite (BDH) in 100 ml distilled water in a volumetric flask.

**Sulphamic acid solution (1.5%)**

This solution was prepared by dissolving 1.5g of sulphamic acid (BDH) in 100 ml distilled water in a volumetric flask.

**2,6-Dihydroxytoluene solution (0.1%)**

This solution was prepared by dissolving 0.1g of 2,6-Dihydroxytoluene (Fluka) in 100 ml distilled water.

**Sodium hydroxide solution (1M)**

This solution was prepared 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 (25 µg/ml)**

The contents of 10 tablets (each tablet contains 500 or 400 mg mesalazine as Pentasa or Awasalazine formulations) were finely powdered, mixed thoroughly and weighed accurately to an amount equivalent to 0.01 g of mesalazine and was dissolved in 5 ml ethanol and 40 ml distilled water with gentle heating and after filtration of the solution, the volume was completed to 100 ml by distilled water in a volumetric flask. The working solution (25 µg/ml) was prepared by dilution.

**Suppositories (Asacol) solution (25 µg/ml)**

The content of three Asacol suppositories (each one contains 500 mg of mesalazine) are mixed well; an accurately weighed equivalent to 0.01 g of mesalazine was dissolved in 5 ml ethanol and 40 ml distilled water with gentle heating. After filtration of the solution, the volume was completed to 100 ml by distilled water in a volumetric flask. The working solution (25 µg/ml) was prepared by appropriate dilution.

**Procedure and calibration graph**

Accurately measured volumes containing 2.5-100 µg of MZ were transferred into a series of 10 ml calibrated flasks, followed by addition of 0.75 ml of 1N hydrochloric acid and 0.3 ml of 1% sodium nitrite solution with occasional shaking and standing for 3 min., a 0.5 ml of 1.5% sulphamic acid solution is added with occasional shaking and standing for 3 min. to remove the excess of sodium nitrite, 1ml of 0.1% 2,6-dihydroxytoluene reagent solution followed by addition of 2 ml of 1M NaOH and then the volumes are completed to the mark with distilled water; the absorbances are measured at 453 nm against the reagent blank solution. Beer's law is obeyed over the range of concentration 2.5 to 100µg of mesalazine/ 10 ml (0.25-100 µg/ml) and the concentration above 100µg/10 ml gives negative deviation Fig. (1). The apparent molar absorptivity referred to mesalazine has been found to be  $4.6 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ .

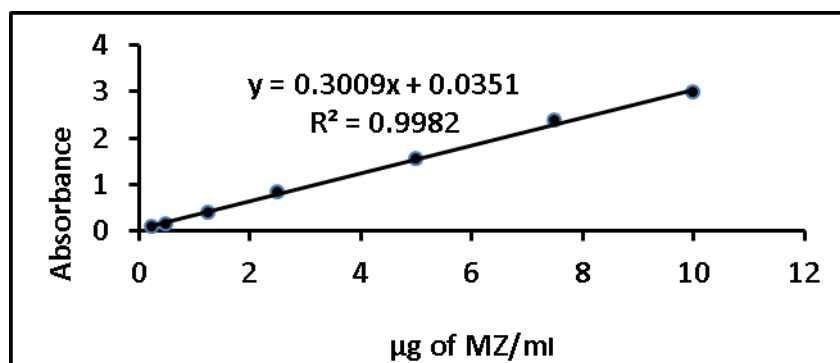


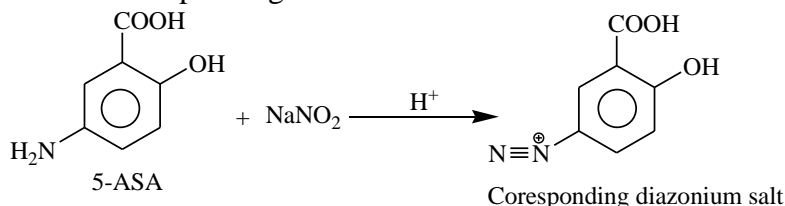
Fig. 1: Calibration graph for mesalazine determination using the proposed method

**RESULTS AND DISCUSSION**

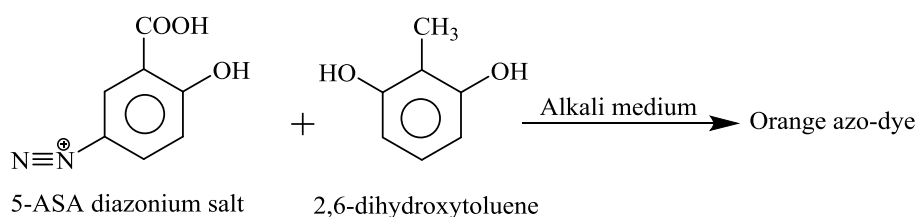
During the investigation, 25 µg of mesalazine was taken and the volumes are brought to 10 ml with distilled water.

### Principle of the method

The first step for the determination, based on the reaction of mesalazine with excess nitrite in acidic medium to form the corresponding diazonium salt:



Then the corresponding diazonium salt was coupled with 2,6-Dihydroxytoluene in alkaline medium to form an intensely orange colored azo-dye:



### Optimum reaction condition

The effect of various parameters on the intensity of the colored product has been studied and optimum conditions have been selected.

#### Effect of acid used for diazotization reaction

The effect of quality and quantity of acid on the absorbance of the colored azo-dye was examined. The results shown in (Table 1) indicate that 0.75 ml of 1 N HCl is considered as an optimum volume; therefore, it was recommended for subsequent experiments.

**Table 1: Effect of acid used for diazotization reaction**

Acid used soln. (1N)	Absorbance / ml of acid added				
	0.25	0.5	0.75	1.0	1.5
HCl	0.5098	0.5472	0.6168	0.6023	0.5601
HNO <sub>3</sub>	0.5683	0.5914	0.5730	0.5100	0.4577
H <sub>2</sub> SO <sub>4</sub>	0.5461	0.5656	0.5947	0.5419	0.5374
CH <sub>3</sub> COOH	0.3824	0.4115	0.4276	0.3966	0.3806

#### Effect of sodium nitrite amount and time

The diazotization process of mesalazine was investigated by addition of different amounts of 1% NaNO<sub>2</sub> solution for different times. The results in (Table 2) indicate that complete diazotization of MZ occurs after 3 min. when 0.3 ml of 1% NaNO<sub>2</sub> solution is added because it gives maximum absorbance; therefore, it has been selected for subsequent experiment.

**Table 2: Effect of sodium nitrite amount and time**

ml of NaNO <sub>2</sub> soln. (1%)	Absorbance / standing time (min.)			
	1	3	5	7
0.1	0.3866	0.5129	0.4922	0.4441
0.2	0.5033	0.5615	0.4990	0.4750
0.3	0.5404	0.6210	0.6196	0.5428
0.5	0.5271	0.5380	0.4750	0.4340
0.7	0.4752	0.4857	0.4721	0.4301

### Effect of sulphamic acid with the time

The excess sodium nitrite must be removed by sulphamic acid because of its undesirable reactions (Clayden *et al.*, 2001). The results in (Table 3) indicate that complete destruction of nitrite occurs after 3 min. by addition of 0.5 ml of 1.5% sulphamic acid solution.

**Table 3: Effect of sulphamic acid with the time**

ml of sulphamic acid soln.(1.5%)	Variable	Absorbance / Standing time (min.)				
		1	2	3	4	5
0.1	S	0.0922	0.0931	0.3751	0.4580	0.3598
	B	1.0928	1.8016	0.0251	0.0325	0.0352
0.2	S	0.2545	0.2999	0.4670	0.5772	0.3137
	B	0.0439	0.0511	0.0305	0.0217	0.0388
0.3	S	0.4004	0.4579	0.5947	0.5872	0.4667
	B	0.0362	0.0325	0.0268	0.0276	0.0304
0.4	S	0.3900	0.5325	0.5789	0.5640	0.4718
	B	0.0301	0.0333	0.0266	0.0227	0.0300
0.5	S	0.4011	0.5956	0.6255	0.5813	0.4660
	B	0.0366	0.0209	0.0200	0.0266	0.0335
0.6	S	0.5434	0.5058	0.5309	0.5547	0.4350
	B	0.0267	0.0205	0.0287	0.0282	0.0355

S = Sample ; B = Blank

### Effect of reagent amount

The coupling reaction between diazotized mesalazine and 2,6-dihydroxytoluene (DHT) reagent was investigated by adding different amount of reagent. From (Table 4), it can be observed that 1ml of 0.1% (2,6-DHT) is the more suitable amount which gives highest value of determination coefficient.

**Table 4: Effect of reagent amount**

ml of 0.1% 2,6-DHT soln.	Absorbance / $\mu\text{g}$ of mesalazine					$R^2$
	12.5	25	50	75	100	
0.25	0.2510	0.5322	1.2205	1.8942	2.4578	0.9987
0.5	0.2496	0.6285	1.1935	1.9638	2.4579	0.9956
1.0	0.2433	0.6363	1.2657	1.8787	2.5412	0.9991
1.5	0.2145	0.6356	1.2379	1.8700	2.4477	0.9977
2.0	0.2402	0.5854	1.1894	1.9744	2.4688	0.9958

### Effect of quality and quantity of base

The preliminary experiment had shown that azo-dye formation occurs just in alkaline medium; so, the coupling reaction has been carried out with strong and weak bases. The results in (Table 5) show that sodium carbonate gave better sensitivity than sodium hydroxide and the formed azo-dye has a good stability; therefore, 2.5 ml of 1M  $\text{Na}_2\text{CO}_3$  was selected for next experiments.

**Table 5: Effect of quality and quantity of base**

ml of base used (1M)	Absorbance / ml of base added					
	0.5	1.0	1.5	2.0	2.5	3.0
<b>NaOH</b>						
$\lambda_{max}$	470	487	485	487	490	491
pH	4.90	12.14	12.56	12.70	12.84	13.17
A	0.5662	0.6352	0.6843	0.6997	0.7034	0.6713
<b>KOH</b>						
$\lambda_{max}$	407	454	472	482	487	490
pH	4.64	9.49	12.39	12.83	13.05	13.16
A	0.5111	0.6901	0.6812	0.6662	0.6852	0.6539
<b>Na<sub>2</sub>CO<sub>3</sub></b>						
$\lambda_{max}$	452	452	453	452	453	453
pH	5.85	8.95	9.64	10.61	10.16	10.20
A	0.7176	0.7774	0.7782	0.7888	0.8256	0.8250
<b>NaHCO<sub>3</sub></b>						
$\lambda_{max}$	440	442	448	453	454	454
pH	1.59	5.98	6.51	6.89	6.92	7.05
A	0.2116	0.3457	0.3428	0.3537	0.4919	0.5178

**Effect of surfactants**

The obtained results from the investigation of three types of surfactants (CTAB, SDS and Triton X-100) on the sensitivity of proposed method revealed that there is no improvement in the intensity of the colored azo-dye. Therefore, it has been recommended to eliminate their use in the subsequent experiments.

**Effect of time on the color development**

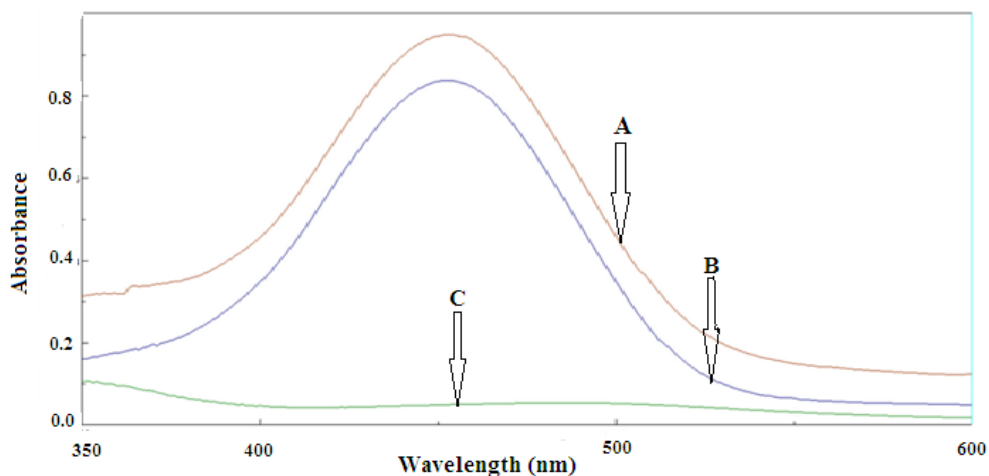
The effect of time on color development of the formed azo-dye for two concentrations of MZ was investigated under the optimum experimental conditions. It has been noticed in (Table 6) that the azo-dye reached maximum absorbance immediately after the addition of the base, and stayed stable at least for 2 hrs. in which many measurements can be done.

**Table 6: Effect of time on color development**

Time, minutes	Absorbance / $\mu\text{g}$ of MZ per 10 ml	
	25	75
<b>After dilution</b>	0.8294	2.3474
<b>5</b>	0.8288	2.3470
<b>10</b>	0.8279	2.3467
<b>15</b>	0.8269	2.3458
<b>20</b>	0.8264	2.3453
<b>25</b>	0.8236	2.3450
<b>30</b>	0.8220	2.3449
<b>35</b>	0.8207	2.3448
<b>40</b>	0.8196	2.3446
<b>45</b>	0.8194	2.3440
<b>50</b>	0.8190	2.3435
<b>55</b>	0.8188	2.3421
<b>60 (1hr.)</b>	0.8189	2.3418
<b>120 (2hrs.)</b>	0.8145	2.3411

### Final absorption spectra

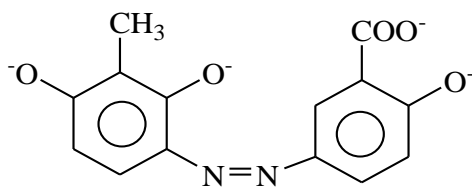
The orange azo-dye formed between diazotized MZ and DHT in presence of sodium carbonate shows a maximum absorption at 453 nm, while the reagent blank has a slight absorption at this wavelength Fig. (2).



**Fig. 2 : Absorption spectra of 25 µg of MZ / 10 ml treated according to the optimum conditions and measured against (A) distilled water (B) blank (C) blank measured against distilled water**

### Nature of the azo-dye

To determine the reaction ratio of MZ to DHT reagent, Job's and mole-ratio methods have been applied. The results obtained indicate that the azo-dye has a composition ratio 1:1, hence it may have the following structure:



Orange azo dye

### Interference of additives species

The effect of some added compounds (excipients) which are often found in pharmaceutical preparations were studied by adding different amounts of this additives to 25 µg of mesalazine using the recommended procedure and the results are given in (Table 7) indicating that none of added compounds can introduce significant interference.

**Table 7: Interference of additives species**

Excipients	Recovery (%)			
	100 µg	250 µg	500 µg	1000 µg
starch	97.6	99.1	99.3	98.3
Glucose	97.7	98.3	98.7	97.7
Lactose	97.3	97.6	98.2	97.3
Arabic gum	97.6	97.7	98.6	97.4

### Application of the Method

To test the applicability of the proposed method, it has been applied to the determination of mesalazine in some of its pharmaceutical preparations (tablets and suppositories). The results which are shown in (Table 8) indicate that the proposed method has good accuracy, precision and recovery.

**Table 8: Application of the method**

Pharmaceutical preparation	Amount taken, $\mu\text{g}$	Amount measured, $\mu\text{g}$	Recovery* (%)	Relative error*, %	Relative standard deviation*, %
Pentasa, 500mg/tablet (Ferring, Germany)	12.5	12.02	96.16	-3.84	$\pm 0.17$
	25	24.80	99.20	-0.80	$\pm 0.13$
	75	72.79	97.05	-2.94	$\pm 0.03$
AwaSalazine, 400 mg/tablet (Awamedica, Iraq)	12.5	11.90	95.20	-4.80	$\pm 0.02$
	25	24.82	99.28	-0.72	$\pm 0.03$
	75	72.68	96.90	-3.09	$\pm 0.06$
Asacol, 500mg/suppository (Tillotts Pharma AG, Switzerland)	12.5	11.91	95.28	-4.72	$\pm 0.13$
	25	24.88	99.52	-0.48	$\pm 0.05$
	75	72.65	96.86	-3.13	$\pm 0.01$

\*Average of five determinations

**Evaluation of proposed method**

The performance of the proposed method was checked by estimation of t-test compared with the standard method (Potentiometric titrations, British Pharmacopeia, 2013) for 95% confidence level with eight degrees of freedom. The results in (Table 9) showed that the t-value was less than the critical value, which means there is no significant difference between the present method and standard method for determination of mesalazine.

**Table 9: Evaluation of proposed method by t-test analysis**

Pharmaceutical preparation	Recovery* (%)		t-test (experimental)
	Present method	Standard method**	
Pentasa, 500 mg/tablet (Ferring, Germany)	97.06	98.29	$\pm 0.206$
AwaSalazine, 400 mg/tablet (Awamedica, Iraq)	99.27	102.57	$\pm 0.704$
Asacol, 500mg/suppository (Tillotts Pharma AG, Switzerland)	96.87	99.51	$\pm 0.436$

\*Average of five determinations

\*\*British pharmacopeia method (2013)

**Comparison with other spectrophotometric methods**

The proposed method has been compared favorably with other reported spectrophotometric methods. As shown in (Table 10), the proposed method is more sensitive with acceptable width-scale compared with other mentioned methods.

**Table 10: Comparison with other spectrophotometric methods**

Analytical parameters	Present method	Literature method*	Literature method**
Reagent	2,6-DHT	Resorcinol	Pyrocatechol
Medium of reaction	Aqueous	Aqueous	Aqueous
pH	10.16	12.38	----
Temperature	R.T	R.T	R.T
Development time, (min.)	After dilution	10	15
$\lambda_{\text{max}}$ (nm)	453	471	530
Beer's law range ( $\mu\text{g} \cdot \text{ml}^{-1}$ )	0.25-10	0.4-12	0.4-10
Molar absorptivity ( $\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ )	$4.6 \times 10^4$	$2.9 \times 10^4$	$0.36 \times 10^4$
Stability of the dye (hr.)	2 (at least)	1	$\frac{1}{2}$
Colour of the dye	Orange	Orange	Purple-red
Application of the method	Tablets and suppositories	Capsules	Tablets and capsules

\*(Zakarria, 2009) ; \*\*(Shihab, 2011)



## CONCLUSION

A simple, rapid and sensitive spectrophotometric method was described for the determination of mesalazine in aqueous solution. The method was based on diazotization-coupling reactions to produce orange azo-dye which is water-soluble and stable for at least 2 hrs. The proposed method doesn't need temperature control or extraction process, and was applied successfully for the assay of mesalazine in its pharmaceutical preparations.

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