AL-Qadisiya Journal for science Vol. 15 No. 2 year 2010

Spectrophotometric methods for the determination of mesalazine using *p*-dimethylaminobenzaldehyde and p-anisaldehyde.

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Abstract:-

Two simple, sensitive and accurate spectrophotometric methods have been developed for the determination of mesalazine in pure form and commercial dosage form. These methods are based on the reaction of mesalazine with *p*-dimethylaminobenzaldehyde in method A and *p*-anisaldehyde in method B to form an Schiff base which is a yellow compound coloured complex species in glacial acetic acid which are absorb maximally at 442 and 370 nm respectively. Beer's law is obeyed in the concentration ranges 0.1-6.25 and 0.5-12.5µgml^{-1} with high apparent molar absorptivities of 4.6248×10^4 and 1.6539×10^4 for methods A and B respectively. The proposed methods are applied successfully for determination of mesalazine in commercial pharmaceutical formulation. There is no interference from the excipients.

Introduction:-

5-Aminosalicylic acid (5-ASA, mesalazine) is an anti-inflammatory drug widely used in the therapy of bowel diseases, such as Crohn's disease and ulcerative colitis [13]. It has been shown to be a potential scavenger of oxygen free radicals that play a significant role in the pathogenesis of inflammatory bowel disease [4]. Orally administered 5-ASA is rapidly and completely absorbed from the upper gastrointestinal tract and therefore an enteric-coated formulations have been designed for drug release in the terminal ileum and colon [1]. After absorption, 5-ASA is metabolized mainly to its N-acetyl derivative.Prolonged treatments as well as the need for clinical and pharmacological studies require fast and sensitive analytical techniques of the drug presence determination in several biological samples. Up to now, most common procedures for the determination of 5-ASA in pharmaceutical dosage forms [6 and14] and biological fluids [2,5,7,8,9,12,15,16, 17] were based on chromatographic techniques.

Keywords: *p*-dimethylaminobenzaldehyde; P-anisaldehyde; Schiff base; mesalazine

High-performance liquid chromatographic methods with UV [5], fluorescence [2,7 and15] and electrochemical [8 and 12] detection were primarily used for the analysis of 5-ASA in biological samples. Spectrophotometry [11] and colorimetry [18] were also used for the compound quantitation. Electrochemical methods have been recently introduced in the analysis of this drug [3,10] Chromatographic methods need sophisticated equipment or require lengthy extraction and clean-up procedures. The aim of present work to produce reliable methods which can be used for the spectrophotometric determination of mesalazine in commercial pharmaceutical formulation.

Experimental

Apparatus

All spectral measurements were performed on Shimadzu U.V-visible recording spectrophotometer (U.V-160) with 1 cm quartz cells.

Reagents

All reagents used were of analytical grade and obtained from Fluka and BDH companies. Mesalazine was provided from State Company for Drug Industries and Medical Appliances, Sammara-Iraq, SDI.

Stock standard solution of mesalazine (1000 $\mu g\ ml^{-1}$)

An accurately weighed 0.1000 g standard sample of mesalazine was dissolved in glacial acetic acid, transferred into 100 ml standard flask and diluted to the mark with glacial acetic acid and mixed well. The solution was stable for at least 1 month at 5°C. The stock standard solution was diluted to 100 mg ml^{-1} before being used. This solution was used in experiment as a standard solution, it was stable for at least 1 week at 5°C.

***p*-Dimethylaminobenzaldehyde solution, 1%**

An accurately weighed 0.5 g of *p*-dimethylaminobenzalhyde was dissolved in 25 ml glacial acetic acid and diluted to the mark in a 50 ml standard flask with glacial acetic acid.

p-Anisaldehyde,1%

An accurately weighed 0.5 g of p-Anisaldehyde was dissolved in 25 ml glacial acetic acid and diluted to the mark in a 50 ml standard flask with glacial acetic acid.

surfactants,0.1%

An accurately weighed 0.025 g of surfactants was dissolved in glacial acetic acid and diluted to the mark in a 25 ml standard flask with glacial acetic acid.

Formation and stability of product II

According to the procedure, the absorbance of mixture solution of 5 mg ml^{-1} of mesalazine was measured after standing for different times. The results are shown in Table 1. The tests showed that mesalazine reacts immediately with *p*-dimethylaminobenzaldehyde at room temperature. However, absorbance decreased gradually. The absorbance was no longer changed after standing for 17 min.

Recommended procedures

***Method A*.**

Increasing volumes of the 50μg/ml mesalazine solution were transferred into a series of 10 ml an standard volumetric flasks to cover the range 0.1-6.25μg ml^{-1}, 2 m1 of *p*-dimethylaminobenzaldehyde solution were added to each flask and the contents were diluted to the mark with glacial acetic acid. The solutions were left for 15 min the absorbance of colored product formed was measured at 442 nm against the reagent blank prepared with same reagent concentration but no mesalazine.

***Method B*.**

Aliquots of 50μg/ml mesalazine solution were pipetted into a series of 10 ml standard volumetric flasks. to cover the range 0.5-12.5μg ml^{-1} .A 2.5 m1 of p-Anisaldehyde solution followed by 1.5 ml of 0.1% SDS were added to each flask and the contents were diluted to the mark with glacial acetic acid. The absorbance of colored product formed was measured at room temperature after 5 min standing time at 370 nm against the reagent blank prepared simultaneously.

Results and Discussion:-

Absorption spectra

Mesalazine reacted with *p*-dimethylaminobenzaldehyde and p-Anisaldehyde reagents in glacial acetic acid and produced a yellow color with *p*-dimethylaminobenzaldehyde having maximum absorption at 442 nm and yellow color with p-Anisaldehyde having maximum absorption at 370 nm against their respective reagent blank (Fig. 1 and 2).

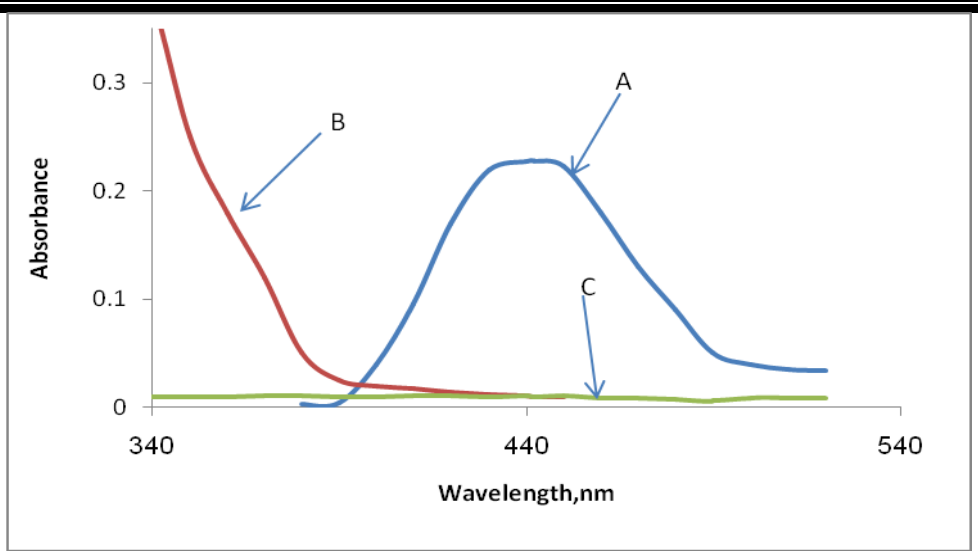


Fig.1 . Absorption spectra of (a) Absorption spectra of mesalazine compound (0.5µg/ml) with *p*-dimethylaminobenzaldehyde against reagent blank. (b)Absorption of *p*-dimethylaminobenzaldehyde against glacial acetic acid. (c). Absorption spectra of mesalazine compound against glacial acetic acid

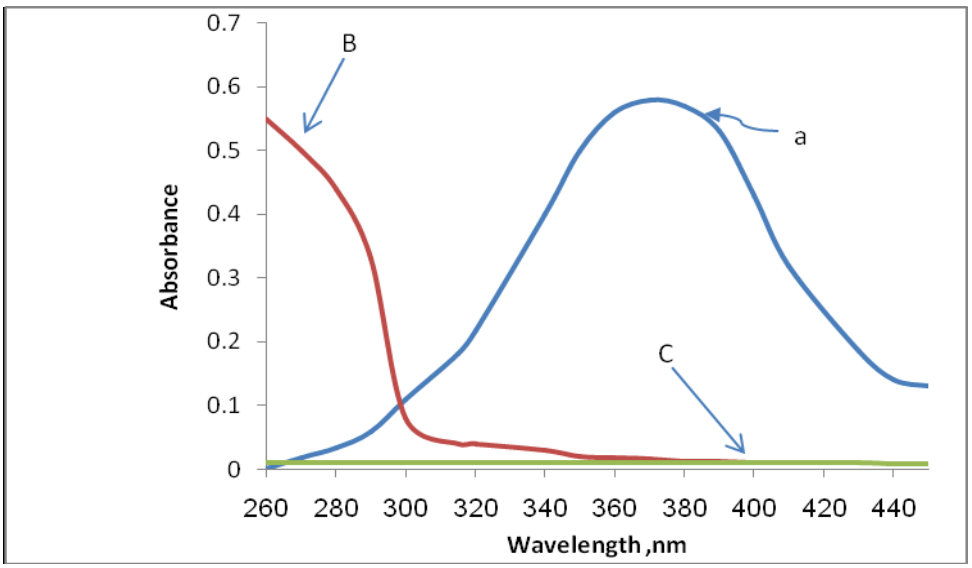
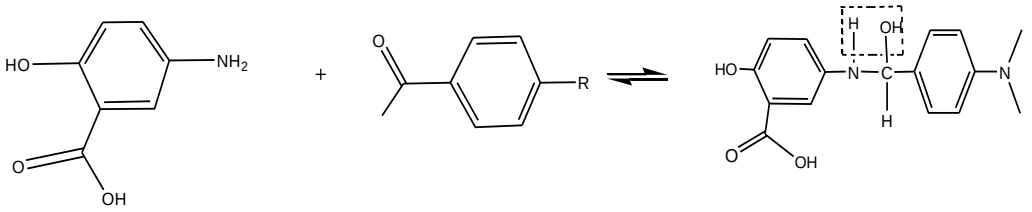


Fig.2 . Absorption spectra of (a) Absorption spectra of mesalazine compound (0.5µg/ml) with *p*-anisaldehyde against reagent blank. (b)Absorption of *p*-anisaldehyde against glacial acetic acid. (c). Absorption spectra of mesalazine compound against glacial acetic acid

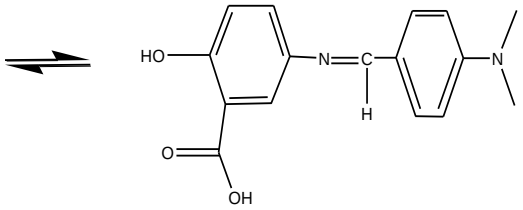
Mechanism of the reaction and effect of reaction medium

The amino group of mesalazine reacted with the aldehyde group of *p*-dimethylaminobenzalhyde and p-anisaldehyde in method A and B respectively, The chemical reactions of mesalazine with *p*-dimethylaminobenzalhyde and p-

anisaldehyde were influenced by reaction medium. Tests found that mesalazine does not form a colored compounds with *p*-dimethylaminobenzaldehyde or p-anisaldehyde in water, but the color formation of the mesalazine compound is fast in glacial acetic acid. In addition, Hence, we proposed reactions mechanism of mesalazine with *p*-dimethylaminobenzalhyde and p-anisaldehyde based on a Schiff reaction. The reactions equation reads as follows:



5-amino salicylic acid



R = $N(CH_3)_2$ *Method A*
R = OCH_3 *Method* B

Study of the optimum reaction conditions

1. *Effect of temperature*

The reaction time was determined by following the colour development at room temperature and in thermostatically controlled water-bath at different temperatures. The absorbance was measured against reagent blank treated similarly. It was observed that the absorbance reached maximum after 15 min at room temperature (28°C) after addition of the reagent solution in method A and remain constant for more than 60 minutes and fading was observed thereafter. In method B, the maximum colour intensity of the reaction mixture was attained after 5 minutes at 28°C and remain constant for 20 min at this temperature and fading was observed thereafter which may be attributed to the destruction of the complex, (Figure 3). These temperatures and reaction time were chosen for colour development in both methods.

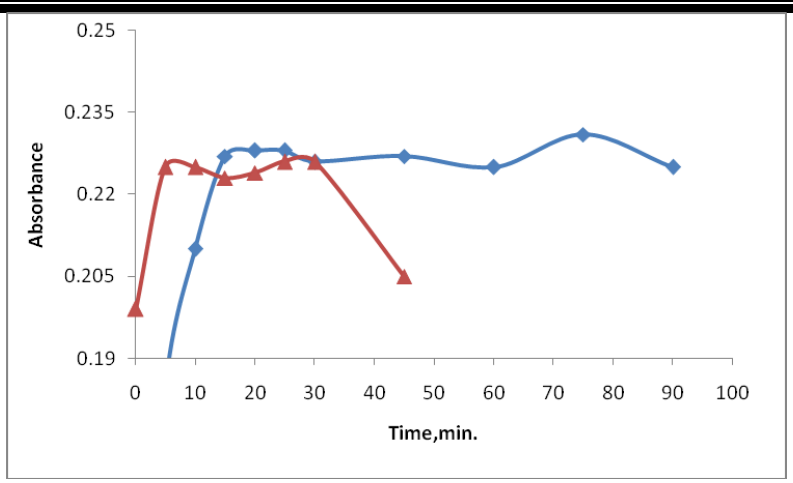


Fig.3 . Effect of the time and temperature on the absorbance of 0.5 µg/ml and 5µg/ml mesalazine in method A (■) and B (▲) respectively

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2. *Effect of reagent concentration*

The effect of changing the reagent concentration on the absorbance of solution containing a fixed amount of the drug was studied, It was found, as shown in Figure 4, that absorbance increases with increasing p-dimethylaminobenzaldehyde concentration in method A and p-anisaldehyde concentration in method B and reached their maximum value on using 2ml of 1% and 2.5ml of 1% of p-dimethylaminobenzalhyde and p-anisaldehyde respectively which were used in subsequent experiments.

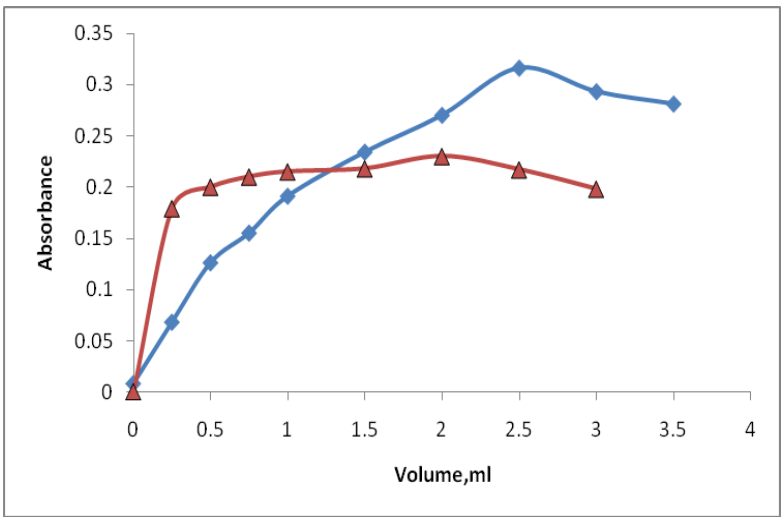

Fig.4 . Effect of 1% p-dimethylaminobenzalhyde (▲) amount on the absorption intensity of the reaction product of 0.5µg/ml mesalazine and Effect of p-Anisaldehyde (■)

amount on the absorption intensity of the reaction product of 5µg/ml mesalazine.

3. ***Effect of surfactants***

A. p-Dimethylaminobenzalhyde : The effect of surfactant on the colour intensity has been examined. The results reveal that the presence of the surfactants has no remarkable effect on the intensity of the colour. Therefore ,the proposed method has been carried out without using surfactants.

B. p-Anisaldehyde: The effect of surfactant on the colour intensity has been examined. The results given in Figure 5 reveal that the presence of the Sodium dodecyl sulphate (SDS) increase the intensity of the colour product.

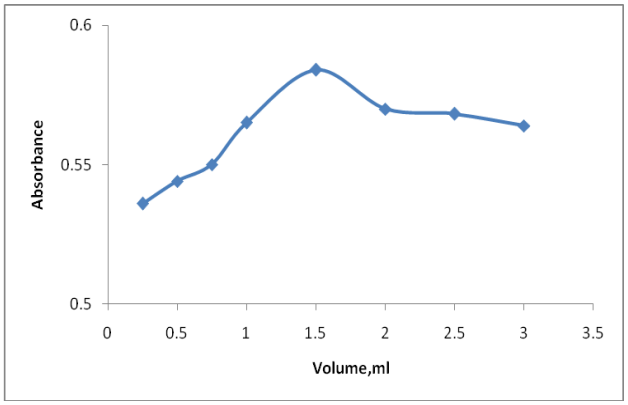

Fig. 5. Effect of SDS amount on the intensity of mesalazine – p-anisaldehyde complex

Analytical parameters

Under the optimum experimental conditions described in recommended procedures, standard calibration curves of shiff- base complexes for mesalazine with p-dimethylaminobenzalhyde and p-Anisaldehyde regents were constructed by plotting absorbance versus concentration (Figure6). The correlation coefficients are 0.998 and 0.999 for method A and B respectively, indicating good linearity. Beer's law is obeyed in the ranges as cited in Table 1, and the molar absorptivity values indicating the high sensitivity of both methods.

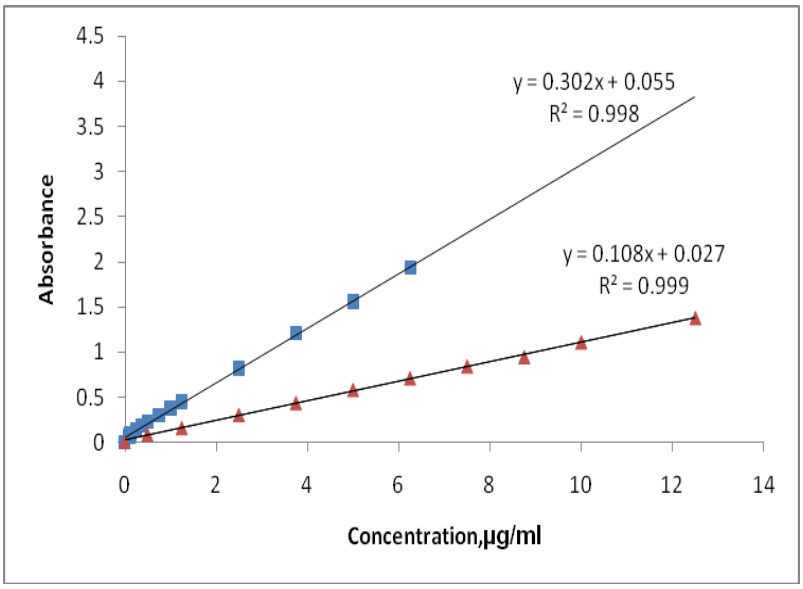

Fig.6 .Calibration graphs for mesalazine
(■) method A
(▲) method B

Table 1; Summary of optical characteristics and statistics for the proposed methods.

Parameters	Method A	Method B
λ_{max}	442	370
Linear range(µg/ml)	0.1-6.25	0.5-12.5
Slope	0.302	0.108
Intercept	0.055	0.027
Correlation coefficients	0.998	0.999
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	4.6248×10^4	1.6539×10^4

Precision and accuracy

The accuracy and precision of the proposed methods were estimated by measuring the content of mesalazine in pure form at three different concentration levels (low, medium and high) within the Beer's law limit in five replicates , (Table2). The relative standard deviation (representing precision) and mean percent recovery (representing accuracy) obtained by the proposed methods can be considered to be satisfactory.

Table 2 : Test of precision and accuracy of the proposed methods

Proposed method	Amount added (µg/ml)	Recovery*(%)	Average recovery (%)	RSD*
A	0.25	99.8		0.80
	0.75	100.5		0.48
	2.5	101.6	100.40	0.17
B	1.25	98.0		0.18
	6.25	100.17		1.42
	10	101.40	99.85	1.12

*** Average for five determinations**

Interference

The potential interference by the excipients in the dosage form was also studied. Samples were prepared by mixing known amount (5µg in method A and 50 µg in method B) of mesalazine with various amounts of the common excipients in final volume of 10 ml. The results cited in Table 5 revealed that no interference was observed from any of these excipients with the proposed methods.

Table 3: Analysis of mesalazine in the presence common excipients by the proposed methods.

Ingredient	Amount added (µg)	Recovery[a] (%)	
		Method A	Method B
Paracetamol	25	101.98	100.23
	50	102.06	101.00
	100	104.00	102.01
Benzamide	25	99.14	101.37
	50	100.01	99.96
	100	101.13	100.62
Starch	25	99.45	101.21
	50	98.03	100.04
	100	96.00	101.00
Urea	25	99.97	100.01
	50	101.05	99.97
	100	102.00	101.00
Talc	25	100.06	99.05
	50	101.51	100.65
	100	102.03	102.15
Mg - stearate	25	100.00	99.00
	50	99.28	98.46
	100	98.54	96.00

[a] Average for three determinations

Applications

Application of the proposed methods to the assay of pharmaceutical sample of mesalazine gave reproducible and accurate results as shown in Table 3. The results obtained showed that the mean recoveries for method A and B respectively, which can be considered to be very satisfactory, Table 4.

Table 4: Assay of mesalazine drug in commercial pharmaceutical formulation by the proposed methods

Method	Pharmaceutical preparation	Certified value(mg)	Amount present µg/ml	Recovery*	Average recovery	Drug content found(mg)
A	Mesacal Enteric Coated tablets	400	0.5 1.0 2.5	105.00 95.50 96.00	98.83	399.4
	Mesacol Extended release Capsules	400	0.5 1.0 2.5	97.00 102.50 94.00	97.83	397.2
B	Mesacal Enteric Coated tablets	400	2.5 5.0 7.5	105.00 103.00 99.33	102.44	398.5
	Mesacol Extended release Capsules	400	2.5 5.0 7.5	94.00 99.00 95.33	96.11	396.7

Conclusion

The proposed spectrophotometric methods are simple, accurate and more sensitive. In addition, they can be applied to the quality control analysis of the mesalazine in its pharmaceutical formulation. Method A (used *p*-dimethylaminobenzaldehyde) was found to be more sensitive compared to method B (used *p*-anisaldehyde) for the assay of mesalazine. The methods are successfully applied for the determination of pure mesalazine and in pharmaceutical formulation. The advantage of the proposed methods is that no prior separation procedure is required.

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طرق طيفية لتقدير الميزالازين باستخدام بارا امينوا بنزالديهايد وبارا أنيسلديهايد

الخلاصة :-

تم تطوير طريقتان طيفيتان تميزتا بالبساطة والحساسية والدقة في تقدير الميزالازين في هيئته النقية وفي مستحضراته الصيدلانية ، اعتمدت الطريقتان على تفاعل الميزالازين في هيئته النقية ومستحضراته الصيدلانية مع بارا – ثنائي اثيل امينوا بنزالديهايد في الطريقة(أ) وبارا –انيسلديهايد في الطريقة(ب) ليكون معقدات شف صفراء اللون في حامض ألخليك الثلجي تمتلك أقصى امتصاص عند الأطوال الموجية 442 و 370 نانوميتر على التوالي. أمكن تطبيق قانون بير للتراكيز 0,1- 6,25 و0,4- 12,4 مايكروغرام/مللتر وبامتصاصيات مولارية 4,6248 ×410 و 1,6539 ×410 لتر.مول-1سم-1 للطريقتين (أ و ب) على التوالي. تم تطبيق الطريقتين بنجاح في تقدير الميزالازين في مستحضراته الصيدلانية.