ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Spectrophotometric Determination of Mesalazine Based on Schiff Base Formation with 2,4-dimethoxybenzaldehyde and 3,4-dihydroxybenzaldehyde

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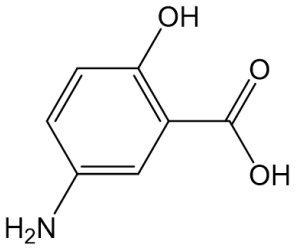
A B S T R A C T:
Two straightforward, fast, easy, accurate, and sensitive spectrophotometric methods were developed to measure mesalazine either in its pure form or in tablet dosage form The proposed procedures, specifically, rely on a condensation reaction between mesalazine and aromatic aldehydes, (method A) and (method B), to produce yellow color Schiff's bases. For methods A and B, the concentration ranges where Beer's relationship was observed were 0.4–10 µg mL^{-1} with a limit of detection of 0.1070 µg mL^{-1} and a limit of quantification of 0.1323 µg mL^{-1}, and 2–15 µg mL^{-1} with a limit of detection of 0.3244 µg mL^{-1} and 0.4010 µg mL^{-1}, respectively. The recovery percentage was 97.82% to 102.1% and 98.15% to 99.91% for the two proposed methods. The proposed methods have been used successfully to determine mesalazine in their tablet formulations.

KEY WORDS: 2,4-dimethoxybenzaldehyde; 3,4-dihydroxybenzaldehyde; spectrophotometric; mesalazine; Schiff's bases.
DOI: http://dx.doi.org/10.21271/ZJPAS.35.5.11
ZJPAS (2023) , 35(5);119-127 .

INTRODUCTION :

Mesalazine, also known as mesalamine, chemically is 5-amino-2-hydroxybenzoic acid. The chemical formula of mesalazine is $C_7H_7NO_3$ and its chemical structure is shown in Figure 1. In terms of solubility it is virtually soluble in alcohol but just slightly soluble in water. However, it is soluble in diluted solutions of hydrochloric acid and alkali hydroxide. Mesalazine is a light-darkening powder that ranges from creamy-white to off-white (Pavlović et al., 2010), (Remington, 2006) .



5-amino-2-hydroxybenzoic acid

Figure ١. Mesalazine strcucture

An anti-inflammatory drug called mesalazine is used to treat digestive tract inflammation, including ulcerative colitis and mild to moderate Crohn's disease. The therapeutic effects of mesalazine may be due to inhibition of lipoxygenase or inhibition of various inflammatory mediators. Reactions to the

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Article History:
Received: 09/01/2023
Accepted: 12/03/2023
Published: 25/10 /2023

stomach or hypersensitivity are mesalazine's most frequent adverse effects. (Beiranvand, 2021),(Bousquet et al., 2012). Mesalazine was formally determined by HPLC methods (Tang and Gillevet, 2003),(Okeke et al., 2000).There are many mesalazine assay methods which were published in the literature. Most of these methods use spectrophotometry, and related techniquies to spectrophotometry.(Acharjya et al., 2010). The other approaches use visible spectrophotometric techniques, like employing 7,6-dinitrobenzofuroxane as an analytical reagent to detect the presence of mesalazine in urine (Garmonov et al., 2012). There are two techniques to derivatize employing the charge transfer procedure and a reaction with 1,2-naphthoquinone in an alkaline medium. (Alasha Abdalla and Elbashir, 2014). Mesalazine and histidine undergo an oxidative coupling process in the presence of N-bromosuccinimide as an oxidizing agen(Al-Zakaria, 2019a), and also thymol reagent is used in a diazotization-coupling reaction for the determination of the drug.(Al-Enizzi et al., 2022). More techniques have been published such as the host-guest inclusion spectrofluorimetric complex of mesalazine and the supramolecular interaction of 18-crown-6-ether with mesalazin via cyclodextrin (Elbashir et al., 2015). Two dimensions ZnCr bilayer hydroxide/tungsten carbide composite was employed as an electrochemical electrode material for mesalazine detection (Kokulnathan et al., 2023).The mesalazine was determined with an electrode synthesized from polymerized congo red (Ganesh et al., 2022). Mesalazine in the plasma of the Beagle Dog was determined using the chromatographic technique LC-MS/MS (Qin et al., 2015). The liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method was developed to detect mesalamine in human plasma. (Pathan and Kshirsagar, 2021). An HPLC approach with fluorometric detection was used to find mesalazine in human plasma(Ceylan et al., 2022).Vanillin(Chandra et al., 2011), p-dimethylaminobenzaldehyde and p-anisaldehyde (Mohammed, 2010), and salicylaldehyde reagents have all been used to determine mesalazine through Schiff bases (Anumolu et al., 2019). The purpose of present study are developing two spectrophotometric techniques for the quantitative estimation of mesalamine based on the production of Schiff bases with 2,4-dimethoxybenzaldehyde (DMBA) and 3,4-dihydroxybenzaldehyde (DHBA).

EXPERIMENTAL

1-Apparatus

Spectra and absorbance measurements were taken using a Shimadzu UV-1900 spectrophotometer with a 1 cm matched quartz cell.

2-Material

Mesalazine was graciously donated by the Awa Medical pharmaceutical company in Hawler, Iraq. All of the reagents used were of analytical grade.

3-Reagents

3.1- Standard mesalazine solution (100 g/mL)

0.01 g of pure form of mesalazine was dissolved in 100 mL of 100% ethanol and transffeed quantitatively into volumetric flask, a standard stock solution of mesalazine (100 g/mL) was prepared.

3.2-DMBA and DHBA preparation

DMBA (0.1%) and DHBA (0.2%) solutions were prepared by dissolving 0.1 and 0.2 g each in a sufficient amount of ethanol, the volume was then completed with ethanol in a volumetric flask to final volume of 100 mL.

3.3 Acid solution

The concentrated acid solutions of sulfuric acid (36M), hydrochloric acid (11.8M), glacial acetic acid (17.4M), and nitric acid (15.54M) were employed in accordance with conventional methods.

3.4-Drug preparation

Twenty tablets of mesalazine were powdered and weighed precisely an amount of powder equal to 100 mg of mesalazine was dissolved in 10 mL ethanol, filtered, and transferred into a 100 ml volumetric flask.

3.5-Preliminary Tests

A suitable amount of 100 µg/mL drug reference solution was quantitatively transferred into a 5 mL volumetric flask. For method A, 0.4 mL of 0.1% DMBA and 0.1 mL of sulfuric acid solution was added and thoroughly combined. For method B, 0.4 mL of 0.2% DHBA and 0.2 mL of sulfuric acid solution were added and mixed thoroughly. After five minutes of heating in a water bath at 40 °C, the solution was cooled to room temperature for both operations. After cooling, the volume was

modified with glacial acetic acid, and each species' absorbance was measured after one minute. The same procedure was used to prepare blank reagents without the medication.

RESULTS AND DISCUSION

1- Absorption spectra

The absorbance/wavelength spectra of the colored Schiff base solutions were scanned between the ranges of 350-550 nm relative to the blank solution under the preliminary test settings of the suggested procedures. Maximum absorbance was seen in Schiff's base colored solutions at 388 nm for Method A and 400 nm for Method B. The reagent blank showed no absorbance at 388 nm and 400 nm as shown in Figure 2.

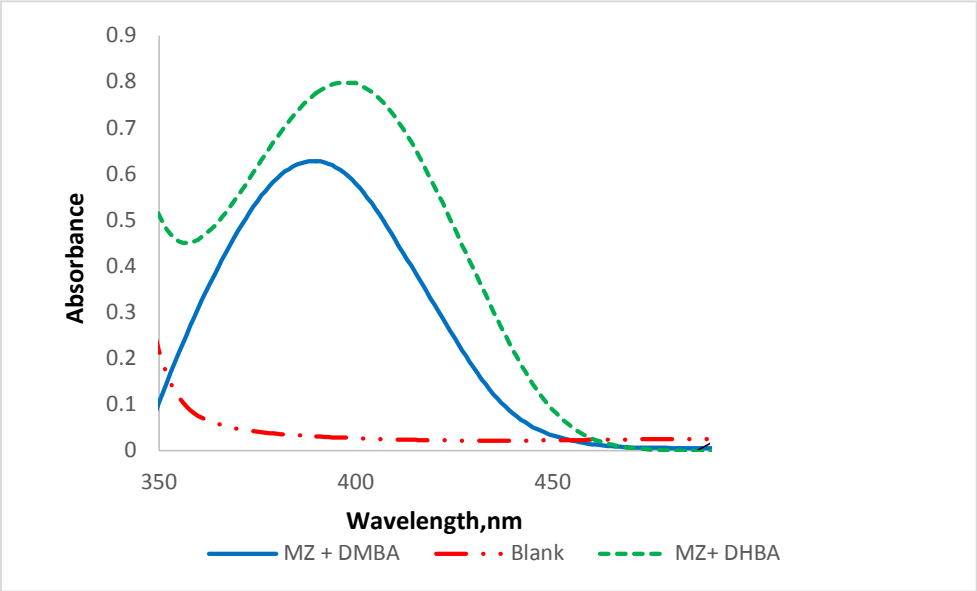
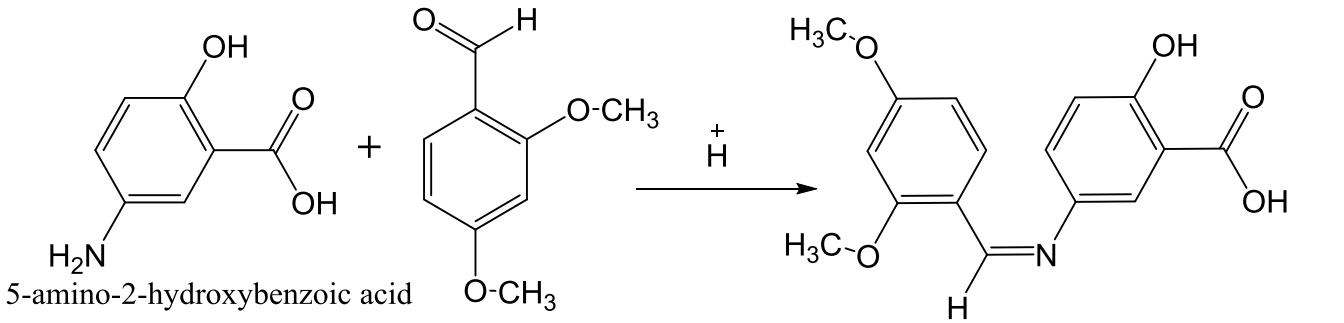


Figure 2. Absorption spectra of mesalazine (5 µg mL^{-1}) with DMBA and DHBA Schiff base against reagent blank.

2. Reaction Mechanism

Schiff base is synthesized by the condensation of carbonyl groups with primary amines. The principal aromatic amine in the mesalazine molecule (colorless solution), which is a nucleophile, combines with the aldehyde to create the unstable addition product known as a carbinolamine. Alcohol carbinolamine is dehydrated by an acid catalyst to form a colorful Schiff base species. The Schiff base formation mechanism is shown in Scheme 1.

Method A:

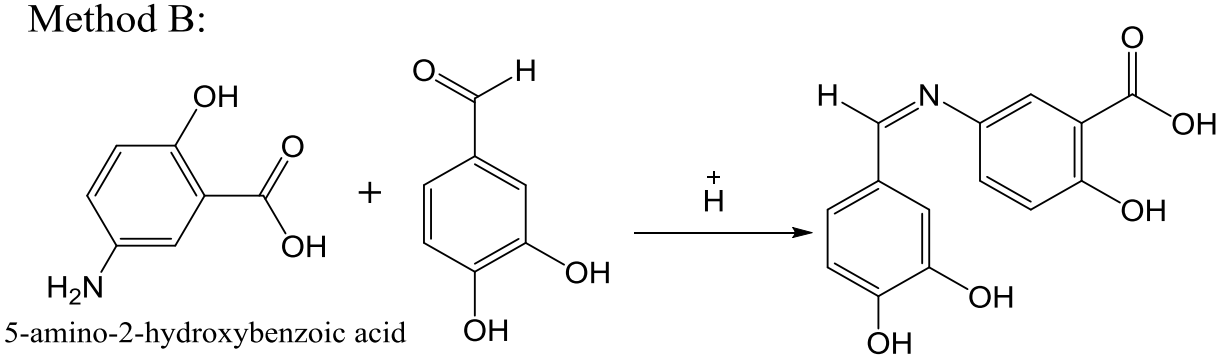


5-amino-2-hydroxybenzoic acid

2,4-dimethoxybenzaldehyde

5-((2,4-dimethoxybenzylidene)amino)-2-hydroxybenzoic acid

Method B:



5-amino-2-hydroxybenzoic acid

3,4-dihydroxybenzaldehyde

5-((3,4-dihydroxybenzylidene)amino)-2-hydroxybenzoic acid

Scheme 1: Reactions that may well produce colorful Schiff bases in an acidic media.

3. Optimal Situation

The following variables were examined in order to create the ideal circumstances for the creation of Schiff bases.

3.1- Effects of acid type

Hydrochloric acid, sulfuric acid, glacial acetic acid, and nitric acid have been used to study the impact of acid type on the production of colored Schiff bases. It was discovered that sulfuric acid recorded the highest color intensity and absorption. The results are shown in Table 1.

Table 1. Effect of the type of acid.

Type of acid	method A absorbance	method B absorbance
HCl	0.189	0.053
H_2SO_4	**0.582**	**0.651**
CH_3COOH	0.421	0.589
HNO_3	0.321	0.091

3.2-Effect of volume of acid

The impact of various volume of sulfuric acid was investigated. The amount of sulfuric acid solution needed for methods A and B to achieve maximum absorption was 0.1 mL and 0.2 mL, respectively, as shown in Table 2.

3.3-Effect of 0.1 %DMBA, and 0.2 %DHBA volume

In the volume range of 0.0 mL to 1.6 mL, the effects of aldehyde volume on the color intensity of Schiff bases was examined. As outlined in Table 2, The optimum volume for the formation of the colored Schiff base was determined to be 0.2 mL of 0.1% DMBA (method A) and 0.8 mL of 0.2%DHBA (method B) since the absorbance of Schiff bases was directly proportional to the volume aldehyde.

Table 2. Effects of the volume of acid and amount of reagent, on the formation of Schiff bases.

		Method A absorbance		Method B absorbance	
		Acid	0.1% DMBA	Acid	0.2% DHBA
Volume (mL)	0.0	0.131		0.252	
	0.05	0.525		0.432	
	0.1	0.567	0.158	0.623	
	0.2	0.443	0.624	0.654	
	0.3	0.494	0.611	0.651	
	0.4	0.355	0.588	0.617	0.301
	0.5	0.222	0.459	0.612	0.332

	0.6	0.222	0.447	0.187	0.415
	0.8				0.568
	1.0				0.482
	1.2				0.445
	1.4				0.436
	1.6				0.197

3.4- Effect of the Order of Addition

Various series of experiments have been carried out to determine the most suitable additional order of the reacting substances as show in Table 3.

Table 3. Effect of addition order

Order number	Method A		Method B	
	Addition	Absorbance	Addition	Absorbance
I	$H^{*}+R^{*}+S$	0.466	H+R+S	0.455
II	R+H+S	0.452	**R+S+H**	**0.644**
III	**R+S+H**	**0.571**	S+H+R	0.492

*H= acid, *R= reagent, *S=sample

3.5-Solvent selection

Influence of solvents (ethanol, methanol, glacial acetic acid, chloroform, n-butanol and water) on the formation of colored Schiff bases was studied. The highest absorbance of colored Schiff bases was obtained in glacial acetic acid. Table 4 lists the absorbance values for each solvent. Glacial acetic acid was chosen as the most suitable solvent for the formation of Schiff bases**.**

Table 4. Effect of different solvents

Solvents	Method A absorbance	Method B absorbance
Ethanol	0.484	0.563
Methanol	0.362	0.354
Acetic acid	**0.607**	**0.798**
Chloroform	0.125	0.781
n-butanol	0.491	0.727
Water	0.223	0.051

3.6- Effect of temperature

The effect of a temperature of 25-60°C on the absorption of the colored Schiff base product formed was studied Table 5. The optimal temperature selected was 35 °C for method A and 40 °C method B, and were therefore used in subsequent experiments.

Table 5. Effect of temperature on the absorption of the colored Schiff base product

Temperature	Method A absorbance	Method B absorbance
25	0.416	0.411
30	0.504	0.502
35	**0.704**	0.596
40	0.678	**0.71**
45	0.568	0.363
50	0.573	0.321
55	0.561	0.356
60	0.552	0.152

3.7- Effect of reaction time

The effect of the reaction time on the development of Schiff bases and its stability was examined. The absorbance of Schiff base of method A was directly measured after mixing (1 min), and of method B was measured after 4 minutes. Table 6 exhibits the Schiff base's absorption over various time periods.

Table 6. Stability of Schiff bases.

Time (min)	Method A absorbance	Method B absorbance	Time (min)	Method A absorbance	Method B absorbance
0	0.711	0.617	25	0.232	0.573
1	**0.769**	0.641	30	0.222	0.551
2	0.725	0.653	35	0.219	0.531
3	0.681	0.66	40	0.217	0.512
4	0.615	**0.665**	45	0.216	0.494
5	0.579	0.664	50	0.217	0.477
10	0.362	0.647	55	0.217	0.464
15	0.291	0.618	60	0.218	0.456
20	0.252	0.597	65	0.219	0.454

4-Validation of proposed method

4.1-Linearity

Under the optimum experimental conditions table 7. The absorbance vs. concentration plot was found to be linear as illustrated in Figure 3.

Table 7. The optimum experimental conditions

	Acid type	Volume(mL) acid	Volume (mL) 0.1% DMBA	Volume(mL) 0.2% DHBA	Solvent	Temperature	Time (min)
Method A	H_2SO_4	0.1	0.2		Glacial acetic acid	35	1
Method B	H_2SO_4	0.2		0.8	Glacial acetic acid	40	4

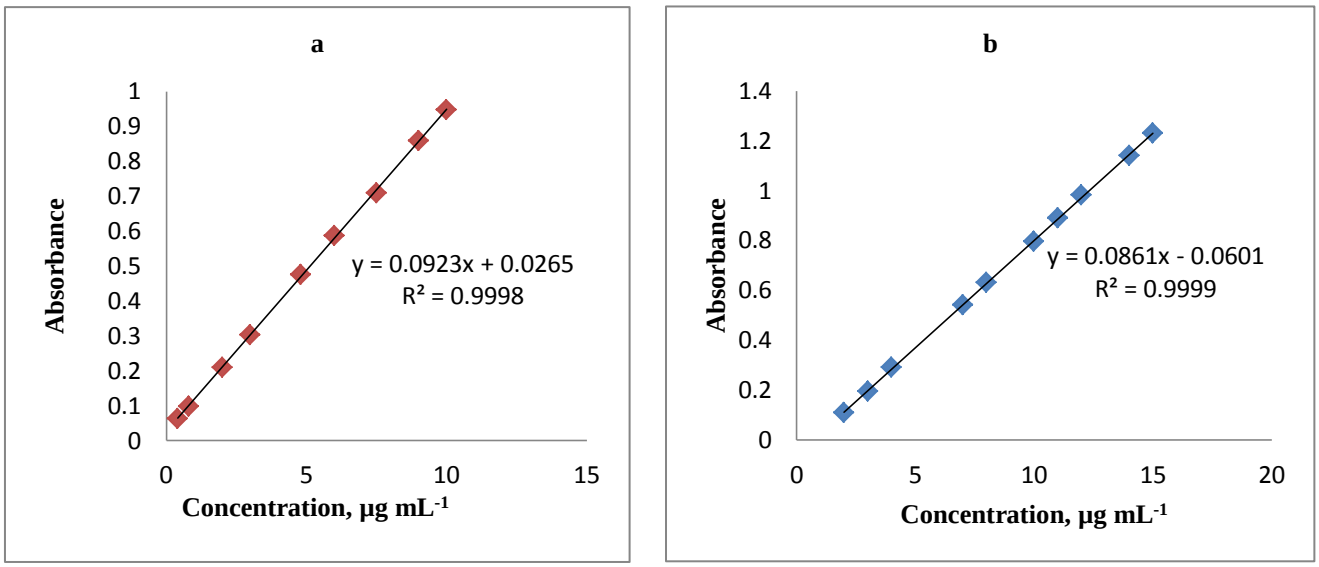


Figure 3. Calibration curve of a - mesalazine-DMBA Schiff base b- mesalazine-DHBA Schiff base

According to the calibration curve, the extracted color system corresponds with Beer's Law for concentrations between 0.4 and 10 g mL^{-1} for technique A and 2 to 15 g mL^{-1} for method B. The significant correlation coefficient value (r^2=0.9998, 0.9999) served as evidence for the calibration curves' linearity. Because the calibration curve's interception was so low, the determined and expected concentrations within the examined range using the suggested approach did not differ systematically. Sandell's sensitivity and apparent molar absorptivity (ε) were calculated and found to be 7.077×10^{-5} µg/cm^2,14.129×10^3 L/mol.cm (method A), and 7.599×10^{-5}µg/cm^2,13.158×10^3 L/mol.cm (method B) respectively. The detection and quantification limits for methods A and B, respectively, were found to be 0.1070 µg mL^{-1} and 0.3244 µg mL^{-1}, 0.1323 µg mL^{-1}, and 0.4010 µg mL^{-1}. The proposed methods compared with other spectrophotometric methods, are shown in Table 8. Method (A) and method (B) have an acceptable range of determination and sensitivity compared with the most up to date spectrophotometric method (Al-Obaidi and Al-Samarrai)[1],(Salih and Science, 2020)[2]. The results of this study indicates the high sensitivity of the suggested approach. The statistical data of the calibration curves for the spectrophotometric measurement of mesalazine is shown in Table 8.

Table 8. Statistical data of the calibration curve for spectrophotometric determination of mesalazine

Statistical data	Method A	Method B	Other Method [1]	Other Method [2]
λ nm	388	400	556	645
Linearity (µg mL^{-1})	0.4-10	2-15	5-45	0.4 – 12
Detection Limit (µg/mL),n=6	0.1070	0.1323	0.0377	0.101
Quantitation limit (µg/mL)	0.3244	0.4010	0.1143	0.338
Correlation coefficient,r^2	0.9998	0.9999	0.9981	0.9990
Sandell's sensitivity, µg/cm^2	7.077×10^{-5}	7.599×10^{-5}	1.934×10^{-2}	6.8×10^{-3}
Molar absorptivity, L/mol.cm	14.129×10^{3}	13.158×10^{3}	7.9173×10^{3}	2.2435×10^{4}

4.2-Composition of the Schiff base

The composition of Schiff's base was investigated by continuous variations of Job's method using varying concentrations of MA and aldehyde. The result in Figure 4 shows a 1:1 ratio for mesalazine-DMBA and mesalazine -DHBA Schiff base.

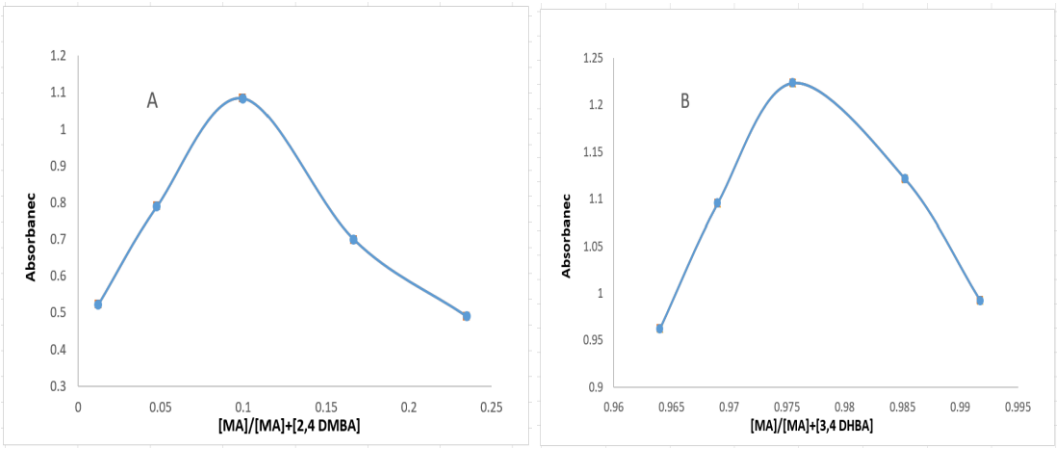


Figure 4. Job's plot for A) MA -2,4-DMBA, B) MA -3,4-DHBA the composition of Schiff's bases

4.3-Accuracy and precision

Using the suggested method, the analysis was repeated at three different mesalazine concentrations of 0.4, 4.8, and 10 g mL^{-1} (method A) 2, 8, and 15 g mL^{-1} (method B). The percentage recovery obtained was between 97.82% to 102.1% and 98.15% to 99.91 for methods A and B respectively. Over a brief period of time, the precision was assessed under the same operational circumstances. RSD% was discovered to range from 0.1018 to 1.281% and 1.144% to 4.29%. The value is under 2%, showing that the process might be repeated. Table 9 displays the outcomes of the accuracy and precision data.

Table 9. Accuracy and precision data for mesalazine obtained using the proposed methods.

Concentration *taken*, µg mL^{-1} Method A	Concentration Found, µg mL^{-1} Method A	Accuracy		Precision *RSD %*
		%Recovery	%Relative Error	
0.4	0.39	97.82	-2.17	4.29
4.8	4.86	101.25	1.25	1.14
10	10.21	102.10	2.10	2.02
Concentration *taken*, µg mL^{-1} Method B	Concentration Found, µg mL^{-1} Method B	Accuracy		Precision *RSD %*
		%Recovery	%Relative Error	
2	1.96	98.15	-1.85	1.28
8	8.02	99.71	0.28	0.81
15	14.98	99.91	-0.09	0.101

5- Application

The optimized methods were applied to measure Mesalazine in three separate commercial samples and compared with the literature (Aziz and Sultan, 2019) [(3)],(Al-Ramadhani et al., 2019) [(3)], (Al-Zakaria, 2019b) [(3)]. The results are presented in Table 10.

Table 10. Determination of mesalazine concentration in pharmaceutical samples.

No	Commercial name	Chemical name	Name of company	Labl claim mg	Method A		Method B		Other Methods [(3)]	
					Found mg	Recovery%	Found mg	Recovery %	Found mg	Recovery%
1	PENTASA	Mesalazine	Germany	500	511.98	102.39	507.12	100.42	494.95	98.99
2	PENTASA	Mesalazine	Sariyer, Istanbul , Turkey	500	493.96	98.79	518.63	103.72	494	98.8
3	METAZA	Mesalazine	Awamedica, Erbil, Iraq	500	503.98	100.79	506.13	101.22	503.0	100.6

CONCLUSION

Two new spectrophotometric methods for the measurement of mesalazine were developed in the current investigation. A yellow dye product was obtained from the Schiff base formation of mesalazine with DMBA and DHBA. The suggested techniques can be used to determine pure and pharmaceutical samples without any interference and are accurate, precise, sensitive, easy to use, and economical.

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