

USING BENZIDINE REAGENT FOR SPECTROPHOTOMETRIC DETERMINATION OF MESALAZINE BY OXIDATIVE COUPLING REACTION

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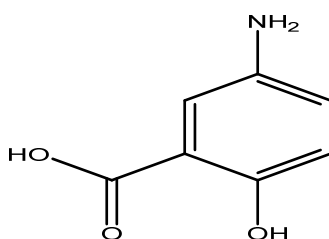
Abstract: A spectroscopic method has been developed to determine the mesalazine in both its pure form and pharmaceutical preparations. The procedure is characterized by its simplicity, rapidity, and high sensitivity. It relies on the oxidative coupling reaction between mesalazine and benzidine in the presence of ferric chloride as an oxidizing agent within a hydrochloric acid medium, leading to the formation of a brown-colored product exhibiting maximum absorbance at 480nm. The technique demonstrated adherence to Beer's law over a concentration range of (1–15) $\mu\text{g}.\text{mL}^{-1}$, with limits of detection (LOD) and quantification (LOQ) calculated at 0.0397 and 0.1323 $\mu\text{g}.\text{mL}^{-1}$, respectively. The molar absorptivity was found to be $7.2 \times 10^3 \text{ L}.\text{mol}^{-1}.\text{cm}^{-1}$, while the recovery rate reached up to 100.65%. The technique could be effectively applied to the analysis of pharmaceutical dosage forms such as tablets and capsules.

Keywords: Oxidative Coupling Reactions, Mesalazine, Benzidine, Spectroscopic determination.

Introduction

Mesalazine, systematically named 5-amino-2-hydroxybenzoic acid and also known as mesalamine or 5-amino-salicylic acid (5-ASA) [1], is marketed under several trade names, including Mesacol, Salofalk, and Pentasa [2]. It is an organic compound that appears as a white or grayish-white powder and may also crystallize. Mesalazine is partially soluble in alcohols, slightly soluble in water, and readily soluble in dilute solutions of alkaline hydroxides and hydrochloric acid [3, 4].

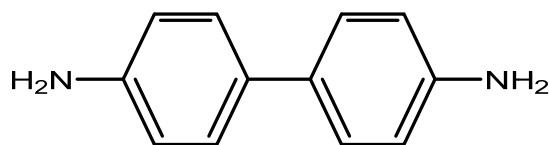
As an anti-inflammatory drug, mesalazine is widely used to treat inflammatory diseases of the gastrointestinal tract. It reduces colon inflammation by inhibiting the formation of arachidonic acid metabolites, which are elevated in patients with chronic intestinal inflammation [5]. Mesalazine is prescribed for Crohn's disease and for the treatment of acute ulcerative colitis, where it helps to reduce inflammation and bleeding in the distal gastrointestinal tract [6]. Its structural formula is shown in Scheme 1 [7].



Scheme 1. The structural formula of Mesalazine ($\text{C}_7\text{H}_7\text{NO}_3$). M.wt=153.135 g/mol

A variety of analytical techniques have been employed to determine mesalazine, including spectroscopic methods [8–18], chromatographic methods [19–25], and electrochemical techniques [26]. Benzidine has also been widely used as a reagent for the determination of numerous organic compounds [27] and pharmaceuticals [28]. This is attributed to its role as a chromogenic reagent in spectrophotometric analysis, owing to the presence of two amino groups that enable oxidative coupling reactions with pharmaceutical compounds containing amino or phenolic groups in the presence of an appropriate oxidizing agent. These reactions yield stable colored products that can be quantitatively measured by UV-Vis spectrophotometry. The method offers high sensitivity, rapid

response, and operational simplicity, making it particularly suitable for the accurate quantification of trace amounts of active pharmaceutical substances. However, due to the toxic and carcinogenic nature of benzidine, its use demands strict compliance with laboratory safety protocols. The structural formula of benzidine is shown in Scheme 2.



Scheme 2. The structural formula of benzidine ($C_{12}H_{12}N_2$). M.wt = 184.23 g/mol

In this study, mesalazine was estimated spectrophotometrically by oxidative coupling reactions using benzene reagent and in the presence of ferric chloride as an oxidizing agent in the acidic medium.

Experimental part

Used devices. A Shimadzu UV-1900i spectrophotometer was used to measure the solutions using cells made of glass with a width of 1 cm, and an electro-mag water bath was used to perform the heating process, using a sensitive balance type AE ADAM.

Chemicals Used. Mesalazine solution (100 $\mu\text{g/ml}$). The solution has been prepared by dissolving 0.0100 g of the pure substance in 10 ml of ethanol and transferring it to a volumetric flask of 100 ml, then the volume is completed to limit the sign with distilled water.

Benzidine solution (200 $\mu\text{g/ml}$). The solution was prepared by dissolving 0.0200 g of pure benzidine powder in absolute ethanol using a 100 ml volumetric flask.

Ferric chloride solution $FeCl_3$ (1×10^{-2} M). A ferric chloride solution was prepared by dissolving 0.1625 g of the substance in 100 ml of distilled water using a 100 ml volumetric flask.

Hydrochloric acid (1 M). The solution has been prepared by taking 8.3 ml from concentrated acid, which has a concentration of 12.06 M. Dilute it with distilled water to the mark in volumetric flask with a capacity 100 ml.

Optimal conditions for the reaction. To obtain the best conditions for the reaction of the mesalazine drug compound with benzidine reagent, the effect of different variables (reagent, oxidizing agent, medium) on the intensity of absorption of the products formed was studied. The study was conducted using 5 $\mu\text{g/ml}$ of mesalazine in a 10 ml volumetric flask.

Studying the effect of different concentrations of benzidine reagent. Concentrations of benzidine reagent were studied to find the best concentration that gives the highest absorption after 10 minutes of diluting the solution. Table 1 shows the best concentration is 200 $\mu\text{g/ml}$.

Table 1. Effect of different concentrations of reagent.

Concentration ($\mu\text{g/ml}$)	Absorbance
Absorbance of Benzidine(1ml)	
50	0.067
100	0.102
150	0.119
200	0.132
250	0.120

Studying the effect of different quantities of benzidine reagents. Different amounts of benzidine reagent (0.25-2 ml) were used with a concentration of 200 $\mu\text{g/ml}$. Fig. 1 shows that the best

quantity is 0.75 ml of the benzidine reagent used.

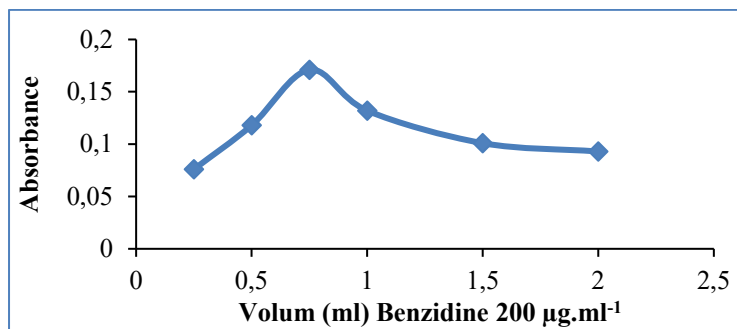


Fig. 1. Effect of different amounts of reagent on absorption

Study of different types of oxidizing agents (1×10^{-2} M). The effect of different types of oxidizing agents with a volume of 1 ml. Table 2 shows that ferric chloride, FeCl_3 , is the best oxidizing agent that gives the highest absorption at the wavelength of 480 nm.

Table2. Effect type oxidizing agent

Type of oxidant(1×10^{-2} M) (1ml)	λ_{max} (nm)	Absorbance
Potassium permanganate (KMnO_4)	300	0.122
Sodium periodate (NaIO_4)	512	0.052
Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$)	534	0.023
Ferric chloride (FeCl_3)	480	0.176
Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	304	0.196
N-Chlorosuccinimide (NCS)	302	0.069
sodium nitroprusside $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$	304	0.136

Study the effect of different concentrations of the oxidizing agent FeCl_3 . The effect of different concentrations of the oxidizing agent FeCl_3 was studied. It was found that the best concentration of the oxidizing agent that gives the highest absorption is 1×10^{-2} M, as displayed in Table 3.

Table 3. Effects of different concentrations of the oxidizing agent

Concentration (M) of FeCl_3 (1ml)	Absorbance
1×10^{-3}	0.107
5×10^{-3}	0.121
1×10^{-2}	0.176
5×10^{-2}	0.096

Study of the effects different quantities of the oxidizing agent FeCl_3 1×10^{-2} M. Different volumes of the oxidizing agent were used, from 0 to 2 ml. It was found that 1.5 ml of the oxidizing agent was the best, as shown in Fig. 2.

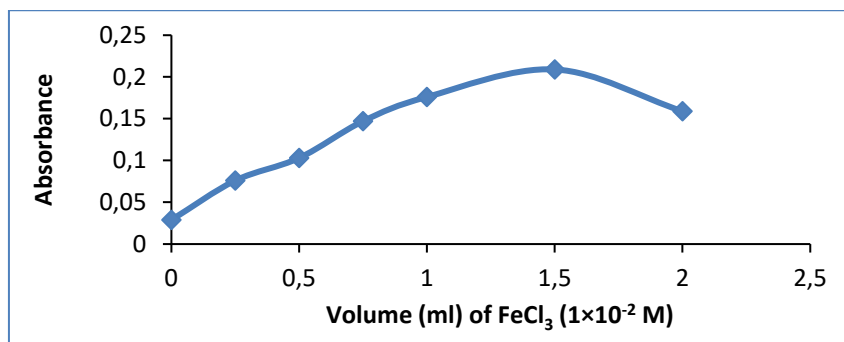


Fig. 2. Effects of different amounts of FeCl₃

Study the effects of different types of acids. The effect of different types of acids with a concentration of 1 M. Fig. 3 shows that the best acid is hydrochloric acid to give it the highest absorption.

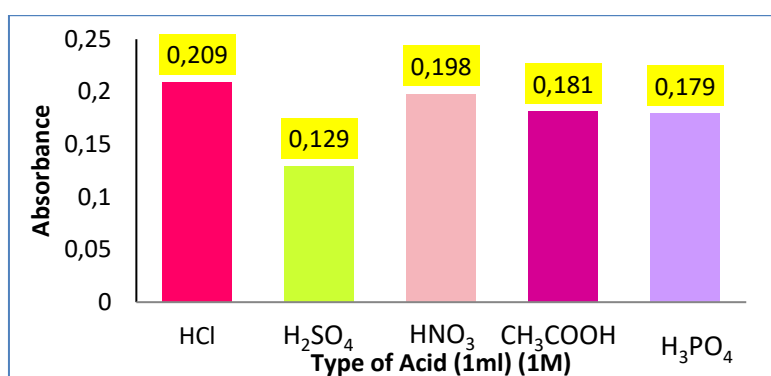


Fig. 3. Effects of different kinds of acids

Study effect of different amounts of acid HCl (1M). Different amounts (0-2 ml) of HCl were used and their effect on the absorption of the reaction product was observed. Fig. 4 shows that the best quantity is 1 ml, which gives the highest absorption.

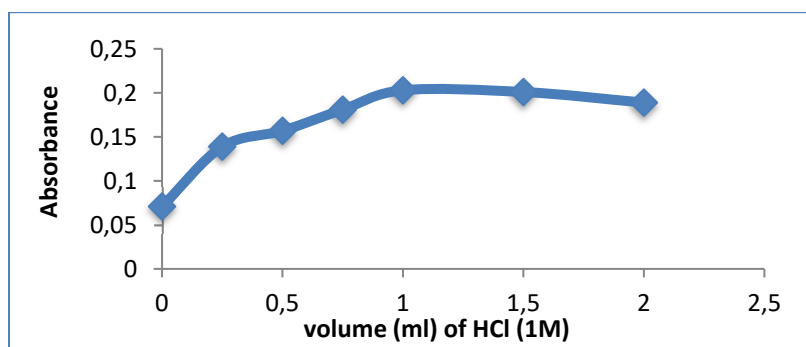


Fig. 4. Effects of different amounts of acid

Study the effect of oxidation time. Different time periods (1-10 minutes) required for the oxidation of the mesalazine drug compound by the oxidizing agent FeCl₃ were studied. Table 4 shows that 1 minute is the best time for oxidation.

Table 4. Effect of oxidation time

Time of Oxidation (min)	Absorbance
1	0.233
3	0.203
5	0.203

7	0.183
10	0.161

Study the effect exerted by temperature and settling time in the reaction product. The effect of exerted temperatures (R.T. (20) – 50°C) on the strength of absorption of the resulting product was studied. The outcomes shown in Fig. 5 display that the best absorption is at a laboratory temperature of 20°C, with a formation time of 15 minutes and a settling time of more than 100 minutes.

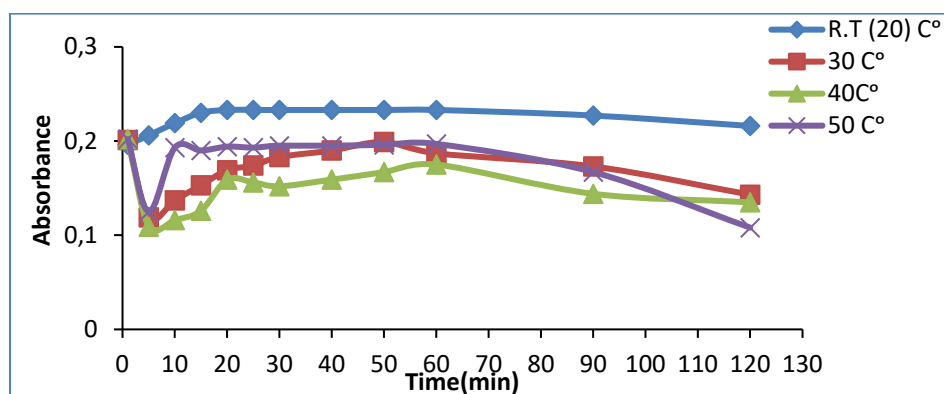


Fig. 5. Effect of temperature

The results obtained to establish the optimal conditions were listed in Table 5.

Table 5. Summary of optimal conditions

Experimental Conditions	Results
$\lambda_{\max}$ (nm)	480
Benzidine 200 $\mu\text{g}.\text{ml}^{-1}$ (ml)	0.75
FeCl_3 1×10^{-2} (ml)	1.5
HCl (1M) (1ml)	1
Temperature(°C)	R.T (20)
Development Time (min)	15
Stability Period (min)	100

Study of the final absorption spectrum. The final absorption spectrum of mesalazine was plotted after setting the optimal conditions for it, at the specified wavelength of 480 nm, against its mock solution as displayed in Fig. 6.

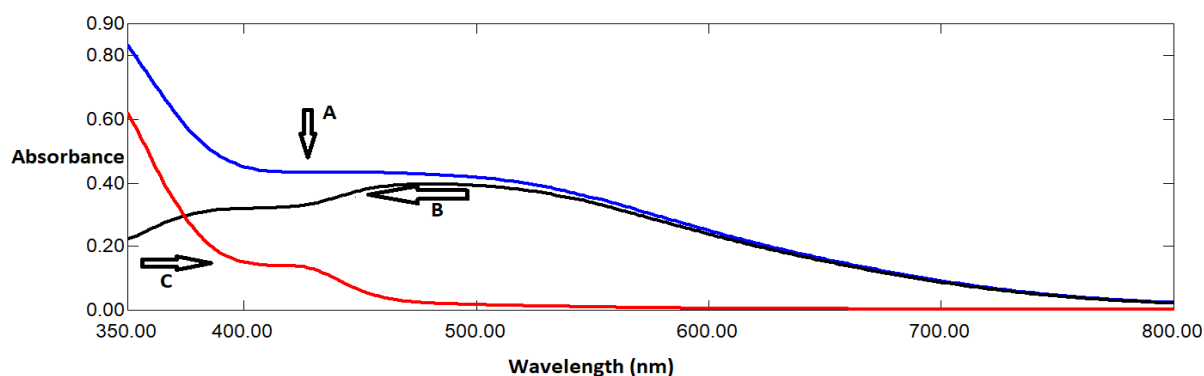


Fig. 6. Spectrum of the reaction product of mesalazine (8 $\mu\text{g}/\text{ml}$) and benzidine. **A:** Mesalazine-benzidine reaction product vs. distilled water. **B:** The product of the mesalazine-benzidine reaction vs. the blank solution. **C:** blank solution vs. distilled water

Study of the standard curve. The standard curve for mesalazine was obtained following Beer's law at concentrations (1-15 $\mu\text{g/ml}$) as in Fig. 7, where the molar absorptivity reached $7.24 \times 10^3 \text{ L.mol}^{-1}.\text{cm}^{-1}$. LOD is 0.0397 $\mu\text{g/ml}$ and LOQ is 0.1323 $\mu\text{g/ml}$.

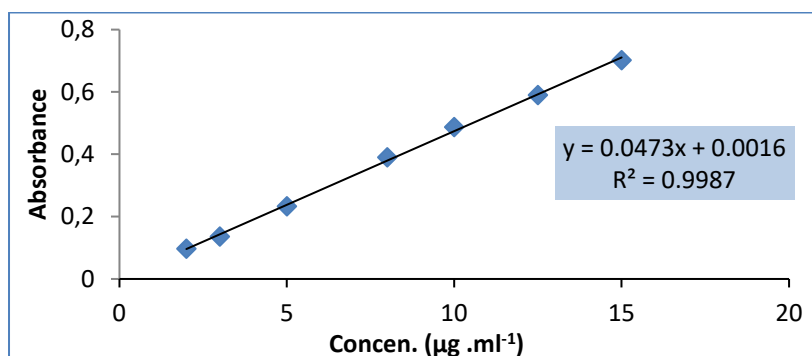


Fig. 7. Standard curve for estimation of the mesalazine drug compound

Study of accuracy and compatibility. Table 6 shows that the method has good accuracy and compatibility.

Table 6. Accuracy and compatibility

Compound	Amount added ($\mu\text{g.ml}^{-1}$)		Recovery* (%)	Average Recovery (%)	RSD* (%)
	Taken	Found			
Mesalazin	3	2.98	99.33	100.65	0.697
	8	8.23	102.87		0.051
	12.5	12.47	99.76		0.040

*Average of Five determinations

Study the nature of the products formed. To determine the reaction rate in estimating the studied drug compound, the molar ratio method and the slope method were applied to the complex formed between mesalazine and the benzidine reagent in the presence of the oxidizing agent ferric chloride in a medium of hydrochloric acid.

Molar ratio method. The molar ratio method is one of the most common methods for determining the nature of the resulting product [29]. When the benzidine reagent interacts with the mesalazine drug compound using a fixed concentration for both the reagent and the drug at a concentration of $6.5 \times 10^{-4} \text{ M}$. The results obtained show that the ratio is 1:1 (drug: benzidine reagent) as shown in Fig. 8.

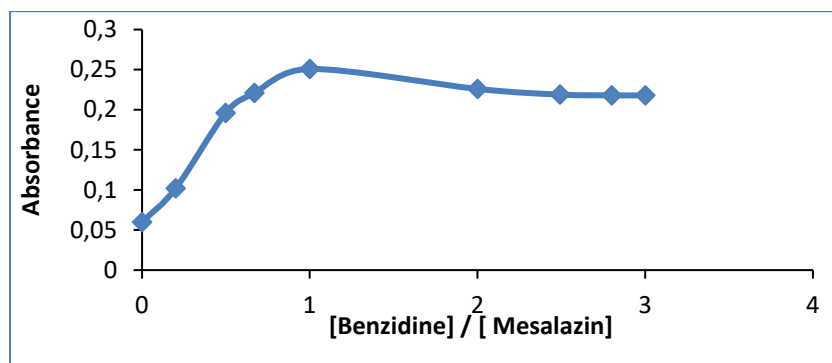


Fig. 8. Molar ratio of mesalazine with benzidine reagent

Slope method. To ensure the ratio is correct. In the molar ratio method, the slope ratio method was applied to the drug mesalazine with the benzidine reagent. Fig. 9 shows that the slope value in the two forms of the straight-line equation is that the ratio is 1:1 (drug: benzidine reagent), which

confirms what was achieved in the molar ratio method.

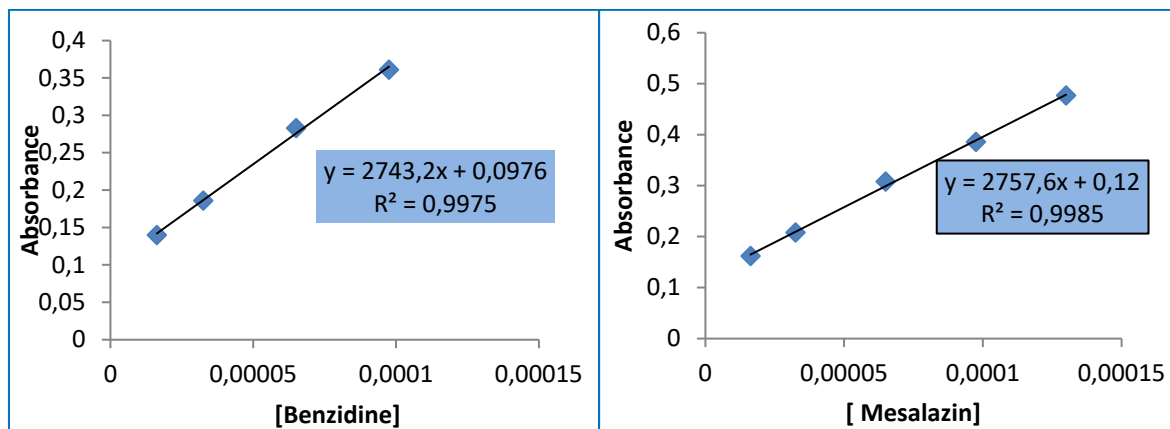


Fig. 9. The slope ratio of the mesalazine-benzidine product

Constant stability of the resulting colored materials. The stability constant was calculated through the following equation for the 1:1 ratio:

$$K_{st} = \frac{1 - \alpha}{\alpha^2 C}$$

Table 7. Stability constant for the resulting compound

Compound	Conc. (mol.l ⁻¹)	Absorbance		α	K_{st} (l.mol ⁻¹)	Average Kst (l.mol ⁻¹)
		As	Am			
Mesalazin	3.25×10^{-5}	0.157	0.231	0.320	2.04×10^5	3.04×10^5
	6.5×10^{-5}	0.420	0.541	0.224	2.37×10^5	
	9.75×10^{-5}	0.575	0.666	0.137	4.71×10^5	

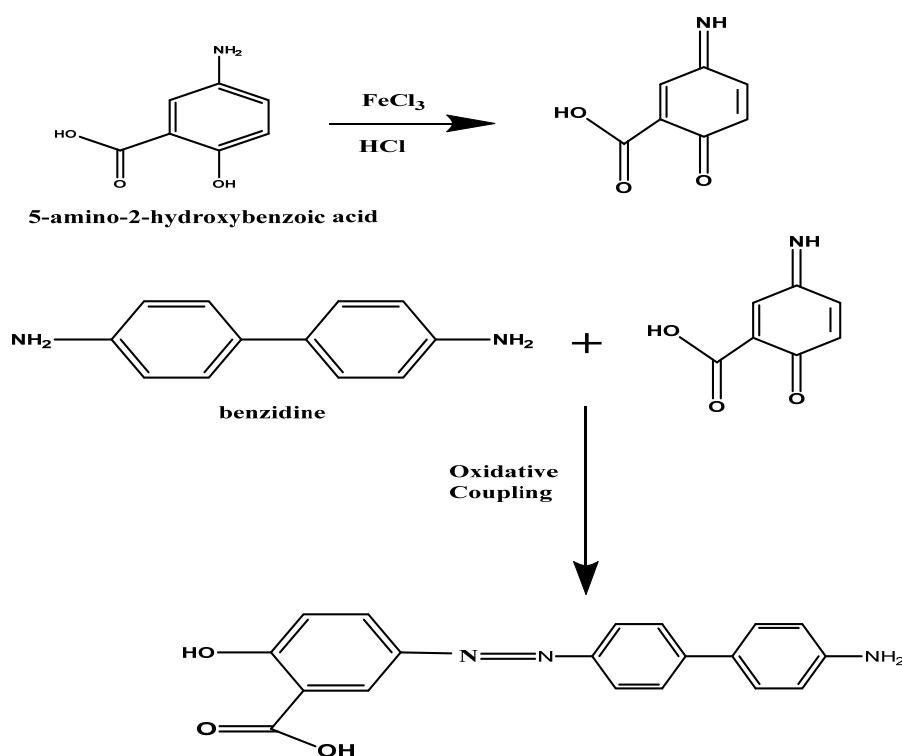


Fig. 10. Mechanism of the proposed chemical reaction

Proposed chemical reaction. The compound mesalazine reacts with the benzidine reagent in a ratio of 1:1, leading to the formation of a brown product in the presence of ferric chloride as an oxidizing agent in the acidic medium. Fig. 10 shows the proposed mechanism.

Applying the proposed method to a pharmaceutical preparation. The developed method was applied to estimate the pharmaceutical compound (mesalazine) on the pharmaceutical preparation in the form of compressed tablets and capsules, as shown in Table 8.

Analysis of pharmaceutical preparation (Pentasa and Misacol). Five tablets of the two drug models were weighed accurately, then ground and mixed well. The equivalent of one tablet of powder was weighed and dissolved with a small amount of ethanol and supplemented with distilled water. After that, the solution was filtered, and the volume was increased to 100 ml with distilled water, then a solution with 100 µg/ml concentration was prepared from it. As the standard curve of the drug in its pure form was used to find the concentrations of the pharmaceutical preparation, the developed method was applied for estimating the mesalazine pharmaceutical compound in its pharmaceutical preparation, as shown in Table 8.

Table 8. Estimation of the medicinal compound in the pharmaceutical preparation

Pharmaceutical Preparation	Certified Value (mg)	Amount Present (µg/ml)		Drug content found(mg)	Recovery *%	Average Recovery %
		Taken	Found			
Pentasa tablets	500	3	2.93	488.30	97.66	97.93
		8	7.89	493.10	98.62	
		12.5	12.19	487.60	97.52	
Mesacol capsule	400	3	2.89	385.32	96.33	96.65
		8	7.83	391.48	97.87	
		12.5	11.97	383.04	95.76	

*Average of Five Determination

Evaluation of the Proposed Method Using the Standard Addition Technique. To verify the efficiency and reliability of the proposed spectrophotometric method for estimating mesalazine, the standard addition technique was applied. As shown in Fig. 11 and Table 9, the results obtained using the standard addition method are in good agreement with those of the proposed procedure. This consistency confirms the method's accuracy and demonstrates its good selectivity for mesalazine determination.

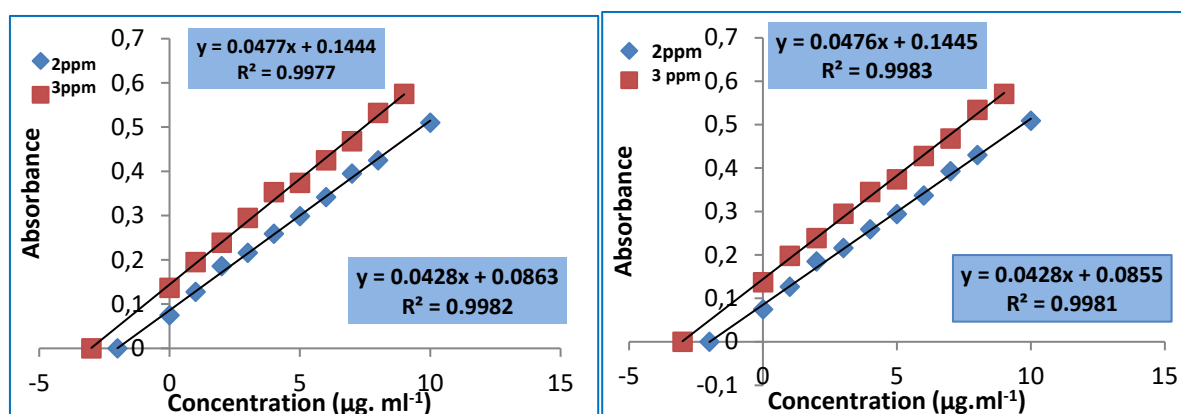


Fig. 11. Application of the standard addition method to the pharmaceutical preparation. A: Pentasa tablets; B: Mesacol cap

Table 9. Determination of mesalazine by the standard addition method

Compound	Pharmaceutical preparation	Certified Value (mg)	Amount Present ($\mu\text{g/ml}$)	Recovery (%)	Drug content found (mg)
Mesalazin	Tablets	500	2	100.81	504.05
			3	100.90	504.50
	Capsule	400	2	99.88	399.52
			3	101.19	404.76

Comparison of Methods. The proposed oxidative coupling method for mesalazine determination using benzidine as a reagent was compared with an existing spectrophotometric method. The comparative results are summarized in Table 10. The proposed method demonstrated superior analytical performance, characterized by a higher detection wavelength and improved sensitivity.

Table 10. Comparison of the proposed method with other spectroscopic methods

Analytical parameters	Present method	Literature method [30]	Literature method [17]	Literature method [14]
Type of method	Oxidative coupling reaction	Oxidative coupling reaction	Charge-transfer complex	Oxidative coupling reaction
Reagent	Benzidine	Hisitidine	p-chloranil	PXN
λ max (nm)	480	459	346	549
Oxidant Agent	FeCl_3	NBS	-----	Potassium iodate
Beer's law range($\mu\text{g.ml}^{-1}$)	1-15	2.5-37.5	1-30	0.2-30
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	7.24×10^3	3.368×10^3	4.60×10^3	4.8×10^3
Temperature ($^\circ\text{C}$)	R.T (20)	R.T (20)	40	R.T (20)
Develupment Time(min)	15	-----	20	5
Stability Period (min)	100	240	50	120
Recovery %	100.65	-----	98.80	100.9
RSD %	<0.7	<1.25	<2.07	<3.1

Conclusion

A sensitive and accurate spectrophotometric method was developed for the determination of mesalazine in an acidic medium using benzidine in the presence of ferric chloride as an oxidizing agent, based on an oxidative coupling reaction. The method enables the quantification of mesalazine in microgram concentrations (1–15 $\mu\text{g/mL}$), with a molar absorptivity of $7.24 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 480 nm and a recovery rate of 100.65%. The standard deviation was less than 0.7%, confirming the precision of the procedure. The method was successfully applied to the determination of mesalazine in pharmaceutical preparations, including compressed tablets and capsules.

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