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Synthesis of new Organic reagent by Vilsmeier – Haack reaction and estimation of pharmaceutical compounds (Mesalazine) containing aromatic amine groups

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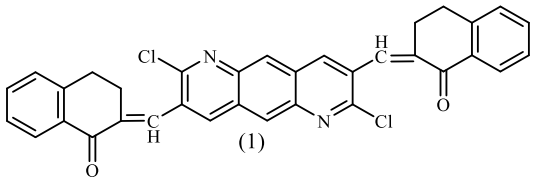
Abstract

In this paper Synthesis new heterocyclic compound from p-phenylenediamine by converting p-diacetanilide (1) and reacting with Vilsmeier-Haack reagent to give (2,7-dicloropyrido[2,3-g]quinoline-3,8-dicarbaldehyde) (2). The two carbaldehydes in compound (2) react with two moles 1-tetralone to give chalcone(3). This chalcone is used as the reagent to estimate the pharmaceutical compounds containing aromatic amino groups. Where a sensitive spectrophotometric method has been developed for the determination of mesalazine by diazotization and coupling reaction of mesalazine with chalcone reagent in basic medium to form a colored water-soluble and stable azo-dye. The product shows maximum absorption at 450 nm. Beer's law was obeyed over the concentration range 0.5-27.5 µg/ml with molar absorptivity of 9494.37 l.mol-1.cm-1. The limit of detection and quantitation is 0.161and 0.538µg.ml-1. The recovery was 101.48% with a relative standard deviation ≤ **3.265**%. Mesalazine and Chalcone reagent products were formed in the ratio of 2: 1 . Full-color development was described and the proposed method was successfully applied to determine mesalazine in the pharmaceuticals preparation. The proposed method was applied to pharmaceutical preparations containing mesalazine derivatives.

Keywords: Diazotization and Coupling, Spectrophotometry, Mesalazine, Chalcone reagent, Vilsmeier – Haack, quinoline

1. Introduction

The reaction of synthesis carbaldehyde substituted on the ring by Vilsmeier-Haack was very important [1,2] and has much application on antibacterial[3], antifungal[4], and the new theoretical studies have proven antivirals by using docking programs[5]. Chalcone compounds were used to estimate some drags containing (-NH2) groups[6]. The reaction involves adding an amine group to the carbon-carbon double bond in chalcone [7]. Chalcone compounds was -unsaturated ketone involving the reactive keto methylene group (CO-CH=CH-) which gave colored compounds due to the presence (CO-CH=CH) the chromophore group [8-12] Fig(1):



(1)

Fig.(1): Formula of Chalcone

The medicinal compound mesalazine

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Mesalazine [5-Amino salicylic acid; (5-ASA)]which is also known as mesalamine[13] is an anti-inflammatory drug used to treat inflammation of the digestive tract (Crohn's disease),[14].mesalazine is produced by bacterial action on sulfasalazine in the colon [15], and it is a first-line drug for the treatment of inflammatory bowel diseases[16]. It has a molecular formula (C7H7O3) and The chemical structure in the following figure-(2) [17].

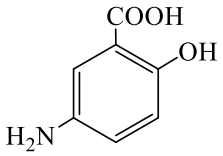


Fig.(2): Mesalazine Structure

Several methods have been used to determine Mesalazine of which are diazotization and coupling with various Reagents [18,19]. There are several Spectrophotometer methods that have been described using oxidative coupling reactions [20]. and also reactions of forming charge transfer complexes [21], other methods depend on the reaction of Schiff bases forming [22], or by oxidation-reduction reactions [23]. The chromatographic methods for the determination of mesalazine include high-performance liquid chromatography [24]. In this paper, a spectroscopic method was developed to determine mesalazine in its pure form and in its pharmaceutical preparations, based on coupling the isozymes drug mesalazine with the organic chalcone reagent in the base medium to give a stable iso-orange pigment with maximum absorption at 450 nm. In this paper, the quinoline compound was developed to act as a reagent for aromatic amines indirectly through diazonium salt reactions, and it was applied to the mesalazine compound as an application model.

2. Materials and Methods

2.1. EXPERIMENT

Apparatus:

All of the spectrophotometric measurements are carried out on "dual-beam photometric apparatus of the type Shimadzu UV-1800 duplex"Glass cells with a width of 1 cm were used, and the weight was carried out using a "Sensitive balance" type (ae ADAM) and the heating was carried out using the "water bath" type (Elektro. mag).

Reagent preparation methods :

2.2. Synthesis of acetanilide (1) :

In around beaker, put 10 grams of (p-Phenylenediamine) and add 10 ml of acetic acid anhydride to it, then 10 ml of glacial acetic acid. The mixture was refluxed by low heating for 20 minutes. The yield cools before the condenser is lifted. The cold reaction mixture was added to 10% of the dilute sodium hydroxide with stirring (10 g of NaOH - 100 ml of distilled water). The product was filtered, dried, and recrystallized with ethanol (Scheme-1-). The Physical Constant and chemical and spectra data ofthecompound are given in The IR spectroscopy for compound (1) shows the band 3296-3169 cm^{-1} for NH amides, 3050 cm^{-1} for C-H Aromatic, 1710 cm^{-1} for C=O amide.

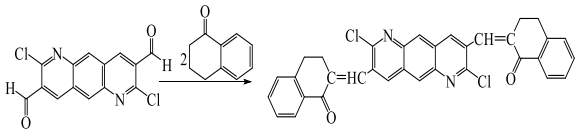
2.3. Synthesis of (2,7-dicloropyrido[2,3-g]quinoline-3,8-dicarbaldehyde)(2):

To solution of (0.01mole) of N,N-diacetyl,1,4,phenylenediamine in (0.3 mole) dry DMF withstirring $POCl_3$ (0.12mole) at (0-5ºC) was added dropwise. The reaction mixture was heated at80ºC for about 16 hrs with stirring. The reaction mixture was poured into crushed ice and the precipitated solid was filtered and washed with an excess of cold water and dried and recrystallized from ethyl alcohol(Scheme-1-).The Physical Constant and chemical and spectra data

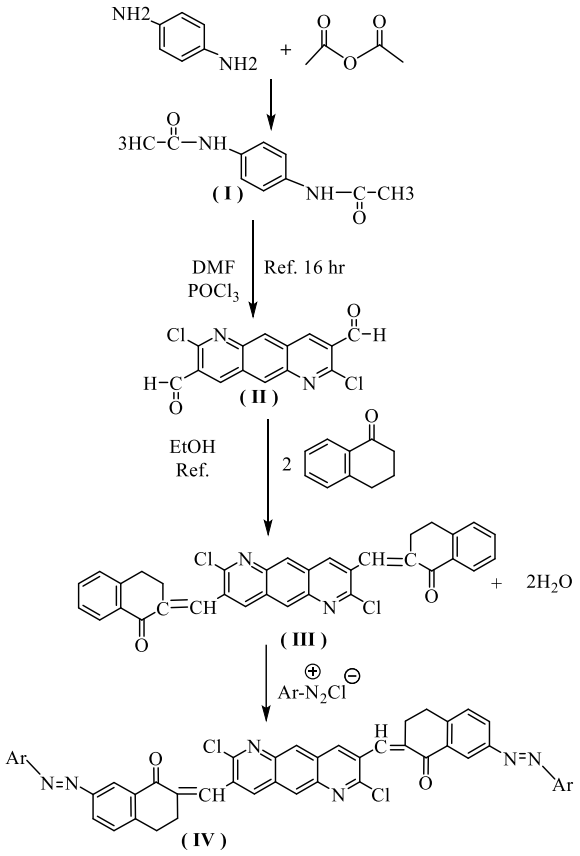
ofthecompound are given in The IR spectroscopy for compound (2) shows the band 3079 cm^{-1} for C-H Aromatic, 2780 cm^{-1} for CH aldehydes, 1658 cm^{-1} for the C=O group. The H^1NMR spectra show bands at 7.69δ (d , C5 & C10, 2H), 8.71δ (d , C4 & C9 , 2H), 10.38δ (s , CHO , 2H).

2.4. Synthesis of chalcone(3):

A compound (2) (0.1 mmol) was dissolved with (0.2 mol) of alpha tetralone in a small amount of ethanol and added (6 milliliters) of sodium hydroxide 10% (the preparation is made by dissolving 1 gram of NaOH in 10 milliliters of distilled water and stirring the mixture. For a period of two hours at laboratory temperature, then the mixture is added to 20 ml of ice water and kept in the refrigerator for 24 hours, after which the sediment is filtered and washed with distilled water, then the product is dried and recrystallized using ethanol (Scheme-1-). The Physical Constant and chemical and spectra data ofthecompound are given:



The IR spectroscopy for compound (3) shows the band 3055 cm^{-1} for C-H Aromatic, 2991 cm^{-1} for C-H Aliphatic, 1635 cm^{-1} for C=O group, 1670 cm^{-1} for C=C. The H^1NMR spectra show bands at 7.94δ (d, C5 & C10, 2H) quinolone fused ring, 8.06δ (d, C4 & C9, 2H) quinolone fused ring, 7.70δ (s, C3 benzene ring, 2H), 7.65δ (s, C4 benzene ring, 2H), 7.62δ (s, C5 benzene ring, 2H), 7.8δ (s, C6 benzene ring, 2H), 3.30δ (s, C=CH, 2H), 2.68δ (d, CH_2, 4H) tow methylene group substituted α Carbone in Chalgone, 2.55δ (d, CH_2, 4H) tow methylene group substituted after α Carbone in Chalgone.



Scheme – 1 –

2.5. Preparation of chemical solutions:

All chemicals used are of the highest purity available

1. The standard mesalazine solution 100µg/ml :

This solution is prepared by dissolving 0.0100 g of pure mesalazine is dissolved in 5 mL of ethanol with heating and added in a 100 mL volumetric flask and diluted to the mark with distilled Ethanol.

2. The approximate Hydrochloric acid solution 1M:

This solution is prepared by diluting 8.3 ml of concentrated acid to 100 ml with distilled water.

3. Sodium nitrite solution 1%:

This solution is prepared by dissolving 1.00g of sodium nitrite in 100 ml of distilled water in a volumetric flask.

4. chalcone Reagent Solution (0.5%):

This solution is prepared by dissolving 0.500g of chalcone in 100ml distilled water in a volumetric flask.

5. The approximate Sodium hydroxide solution 1M : This solution was prepared by dissolving 4.00g of sodium hydroxide in 100ml distilled water in a volumetric flask.

3. Results and discussion

Quinoline compounds are widely spread and have many applications, one of the most important methods of preparing them using the Valismeier-Haack reagent, which consists of the reaction of dimethylformamide with phosphorous oxychloride as in the equation:

Which reacts with acetanilide to give the quinoline compound, which substitutes aldehyde and chlorine at positions 2 and 3.

When identifying compound (2), we notice in the infrared spectrum the appearance of the aldehyde C-H stretch band, as well as the disappearance of any band higher than 3100 (the amide group bands) present in compound (1).

While when identifying the compound (3), we notice the appearance of several distinct bands in the infrared spectrum, the most important of which is the decrease in the stretching of carbonyl groups due to the resonance, the disappearance of the stretching band of C-H aldehyde.

The nuclear magnetic resonance spectrum also shows the aromatic protons, which are characterized by different displacements according to the neighboring groups and are identical to the measurements in the references with which they were compared.

Spectroscopic measurements were also used to determine the identity of the product of the reaction of the diazonium salt with chalcone, and through nuclear magnetic resonance spectrometry, the binding site of the diazonium salt with chalcone was determined at position 3 for the benzene ring fused with cyclohexanone.

We note the IR and H^1NMR spectrum values of the resulting compound: The IR spectroscopy for compound (4) show the band 3157 cm-1 for O-H phenol, 3054 cm-1 for C-H Aromatic, 2988 cm-1 for C-H Aliphatic, 2863 cm-1 for O-H acid, 1700 cm-1 for C=O acid,1678 cm-1 for C=O carbonyl group, 1622 cm-1 for C=C chalcone, 1283 cm-1 for C-O acid, 1223 cm-1 for C-O phenol. The H1NMR spectra show bands at 7.34δ (d, C5 & C10, 2H) quinolone fused ring, 8.23δ (d, C4 & C9, 2H) quinolone fused ring, 7.53-7.56δ (m, C3,5 benzene fused ring & C2,3,6 azobenzene ring, 10H), 7.87δ (s, C6 benzene ring, 2H), 3.35δ (s, C=CH, 2H), 2.93δ (d, CH2, 4H) tow methylene group substituted α Carbone in Chalgone, 2.59δ (d, CH2, 4H) tow methylene group substituted after α Carbone in Chalgone.

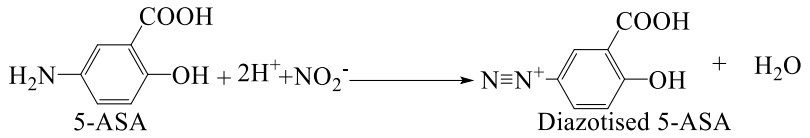
3.1. Reaction Stapes :

1. Initial tests:

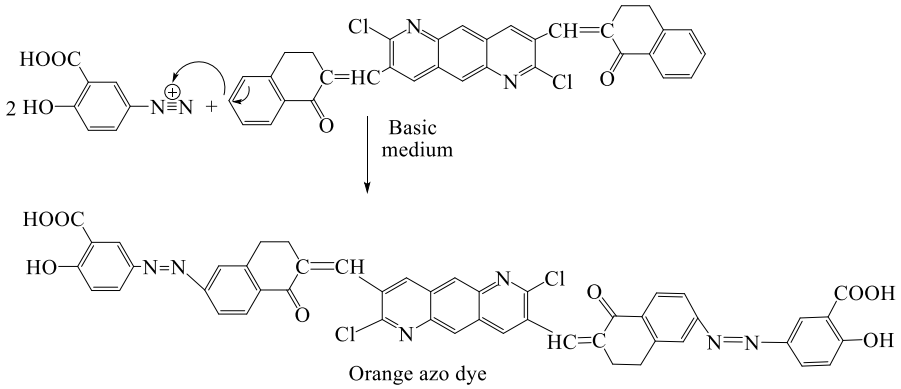
For the subsequent experiments, 5µg/ml of mesalazine is taken in 10 ml final volumes and absorbance measurements are performed at 450 nm.

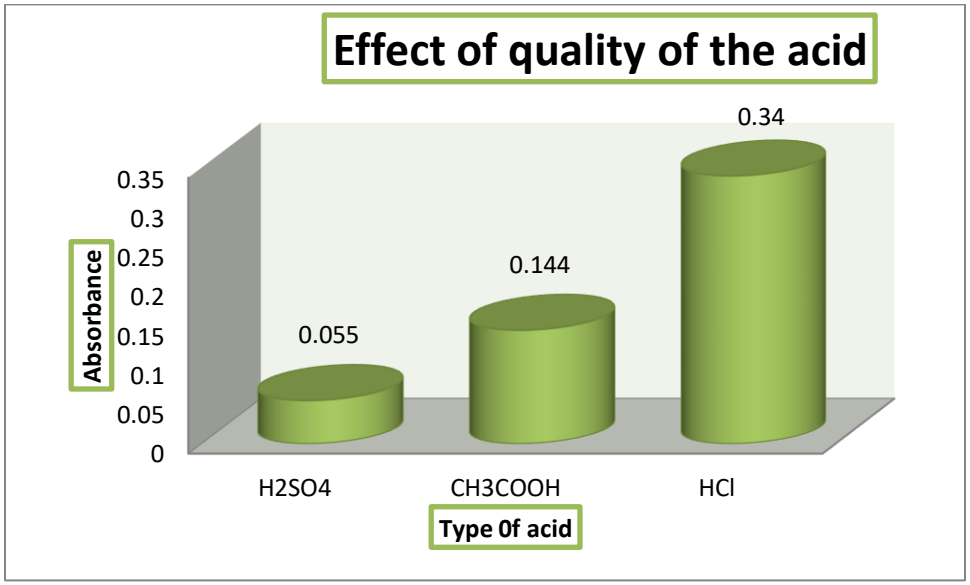
2. Principle of the method

Mesalazine is reacted with excess nitrite in an acidic medium to form the corresponding diazonium salt:



The colored solution formed by coupling diazotized 5-ASA with chalcone in an alkaline medium.





Fig(3): Effect of type acid used for diazotization reaction

3.2. Effect of the hydrochloric acid amount 1 M:

This effect was studied using increasing volumes (0.25 - 2.0 ml) of hydrochloric acid at a concentration of 1 molar to obtain the diazonium salt, and it was noted from the results in Table (1) that 0.5 ml of hydrochloric acid is the amount to obtain the highest of the colored product and this volume was used in subsequent experiments.

Table 1: Effect of hydrochloric acid amount

X ml of	0.25	*0.5*	0.75	1.0	1.5	2.0
HCl (1M)						
Absorbance	0.287	*0.340*	0.215	0.196	0.099	0.058

3.3. Effect of sodium nitrite amount 1%:

The diazotization process of mesalazine was investigated by the addition of different amounts of 1% NaNO2 solution The results in Table (2) indicate that adding 0.5 ml of a 1% sodium nitrite solution

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gives the maximum absorption limit, which was chosen in the subsequent experiments.

Table 2: Effect of sodium nitrite amount

X ml of $NaNO_2$(1 %)	0.25	*0.5*	0.75	1.0	1.5	2.0
Absorbance	0.307	*0.340*	0.305	0.320	0.225	0.163

3.4. Effect of reaction time required to complete The diazotization process:

The diazotization process of mesalazine was investigated for different times The results in (Table 3) indicate that complete diazotization of mesalazine occurs after 3 min and gives maximum absorbance; therefore, it has been selected for a subsequent experiment.

Table 3: Effect of reaction time on the absorption spectrum to form the resulting azo dye

Time(min)	1	2	*3*	5
Absorbance	0.312	0.351	*0.368*	0.340

3.5. Effect of reagent amount

The coupling reaction between diazotized mesalazine and chalcone reagent was investigated by adding different amounts of reagent. it was found from the results in Table (4) that 0.5ml of 0.5% of chalcone reagent is the quantity sufficient to complete the reaction and give high absorption values.

Table 4: Effect of reagent amount

Xml of Reagent(0.5%)	0.25	0.5	0.75	1.0	1.5	2.0
Absorbance	0.317	0.368	0.309	0.270	0.261	0.225

3.6. Effect of quality of the base

The preliminary experiment had shown that azo-dye formation occurs just in an alkaline medium; so, the coupling reaction has been carried out with three types of bases. The results in (fig. 4) show that sodium hydroxide1M gave the highest absorption and for this, it was adopted in subsequent experiments.

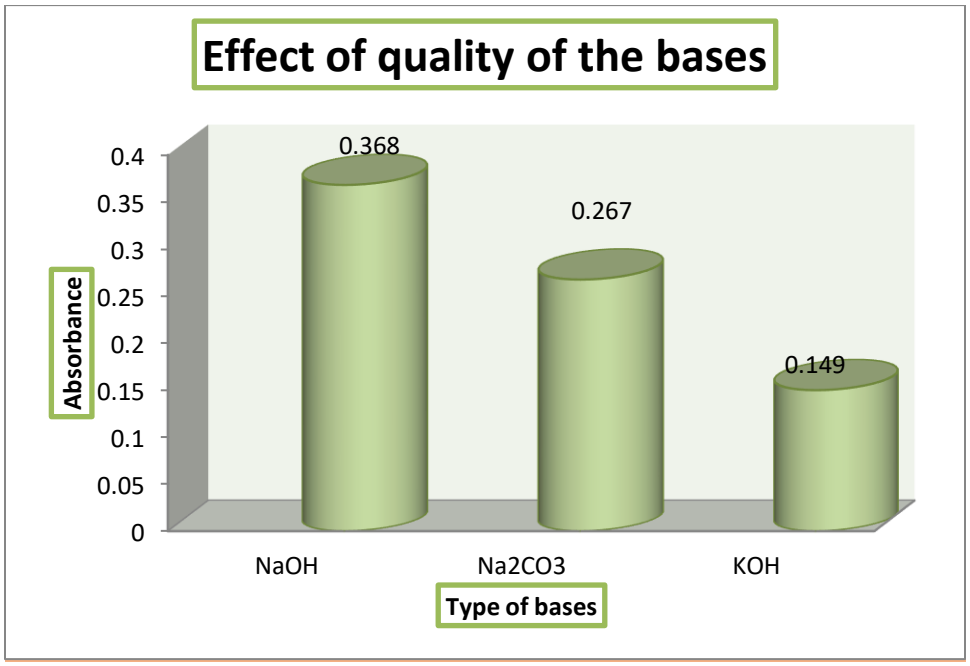

Fig.(4): Effect of quality of the base

3.7. Effect of quantity base:

The effect of different types of base solution (which is necessary for developing the chromophore) on the color intensity was investigated of sodium hydroxide solution, which was chosen as the best base, was used. Therefore, the effect of the quantity of the base used on the absorption of the colored product was studied. The results in Table (5) show that 0.5 ml of the base is a sufficient amount to obtain the highest absorption of the colored product.

Table 5: Effect of quantity base

X ml of NaOH (1M)	0.25	*0.5*	0.75	1.0	1.5	2.0
Absorbance	0.062	0.368	0.296	0.255	0.248	0.230

3.8. Effect of time on color development:

The color of the formed azo dye of mesalazine was investigated under the optimum conditions described for mesalazine. it was observed in Fig. (5) that the azo dye reached its maximum absorption after 15 minutes in a water bath at 25 ° C after dilution, and it was stable for more than 1 hrs.

The effect of time (formation and stability) and temperature on the color formation of azo dye formed under optimal conditions was studied. By taking different temperatures. Figure (5) shows that the temperature of 25 ° C is the best among the other degrees, as the dye was formed after 15 minutes and this dye remains stable for more than an hour.

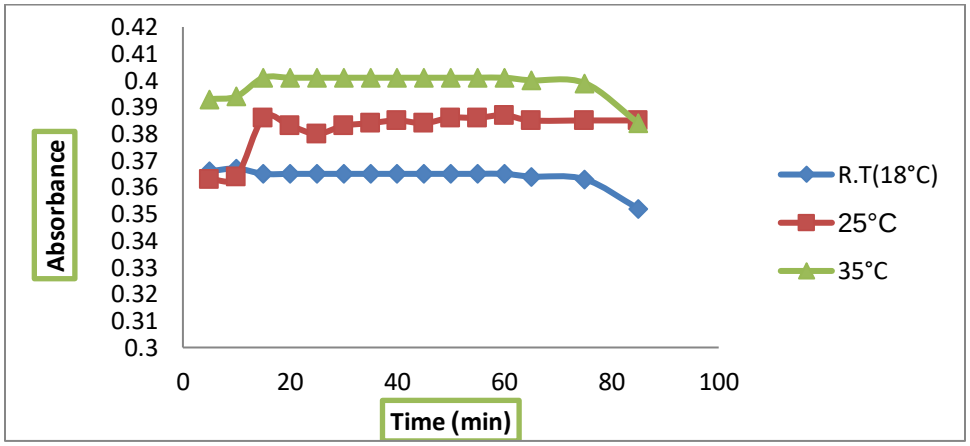

Fig.(5): Effect of time on color development

3.9. Final absorption spectra:

The orange azo-dye formed between diazotized mesalazine and chalcone reagent in presence of sodium hydroxide shows maximum absorption at 450 nm, while the reagent blank has a slight absorption at this wavelength Fig. (6)

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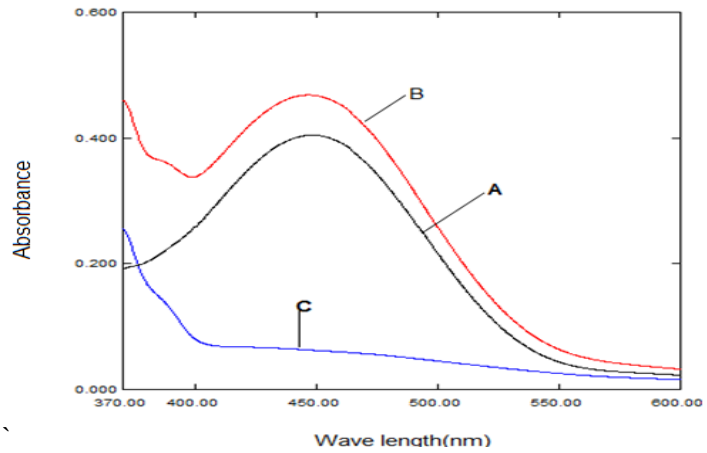

Fig.(6): Absorption spectra of 5 µg of 5-ASA /ml treated according to the optimum conditions and measured against ((A)Sample vs Blank), ((B) sample vs distilled water), ((C) blank measured against distilled water).

3.10. Procedure and calibration graph:

By using the optimal reaction conditions To a series of 10ml volumetric flasks covering the range of 0.5- 27.5 µg.ml-1 of Mesalazine are transferred, followed by the addition of 0.5 ml of 1M hydrochloric acid than 0.5 ml of 1% sodium nitrite solution with leave the solutions for 3 min in the ice bath. and addition 0.5ml of 0.5% chalcone reagent solution followed by the addition of 0.5 ml of 1M NaOH and then the volumes are completed to the mark with distilled water, and the solutions were left for 15 min in a water bath at a temperature of 25 °C the absorbance are measured at 450 nm against the blank solution. Beer's law is obeyed over the range of concentration 0.5 to 27.5 µg of mesalazine/ml, as shown in figure (7), the molarity absorption value 9494.37 l. mol -1. Cm-1, and the limit of detection LOD (0.161) µg. ml-1 and the limit of quantity LOQ (0.538) µg .ml-1.

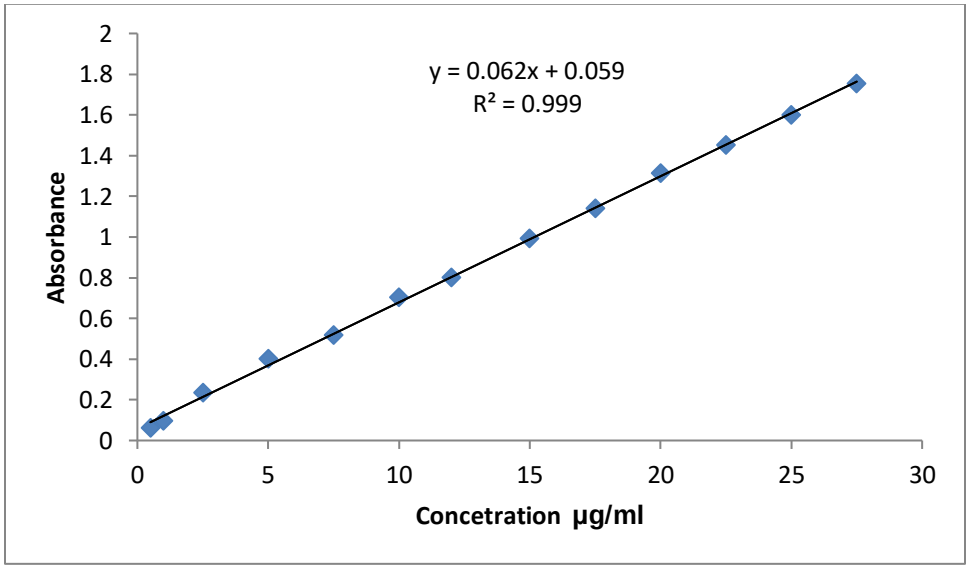

Fig. (7): Calibration graph for mesalazine determination using the proposed method

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3.11. Accuracy and Precision

Three different concentrations of 5-ASA are used in the determination of the accuracy and precision of the method, the results are shown in Table (8) indicate that the method has good accuracy and precision.

Table (8) Accuracy and compatibility of the method

Compound	Amount added ($\mu g.ml^{-1}$)	Recovery * (%)	Average recovery (%)	RSD* (%)
mesalazine	5	103.16	101.48	3.265
	10	101.48		1.285
	15	99.80		0.619

Average of Five Determinations.*

3.12. Nature of the Dye:

The composition of the intense orange dye that results from the reaction of diazotized mesalazine with chalcone reagent has been established using the continuous variations and the mole-ratio methods, the results indicate that the dye has a combination 2:1 ratio of diazotized mesalazine to chalcone reagent (Fig 8 and 9).

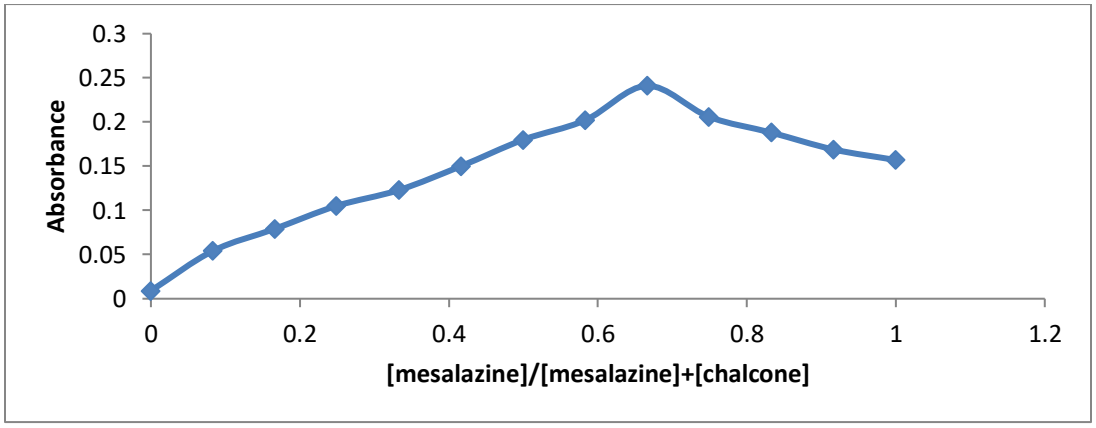

Fig. (8): The continuous variations plot for diazotized mesalazine to chalcone reagent

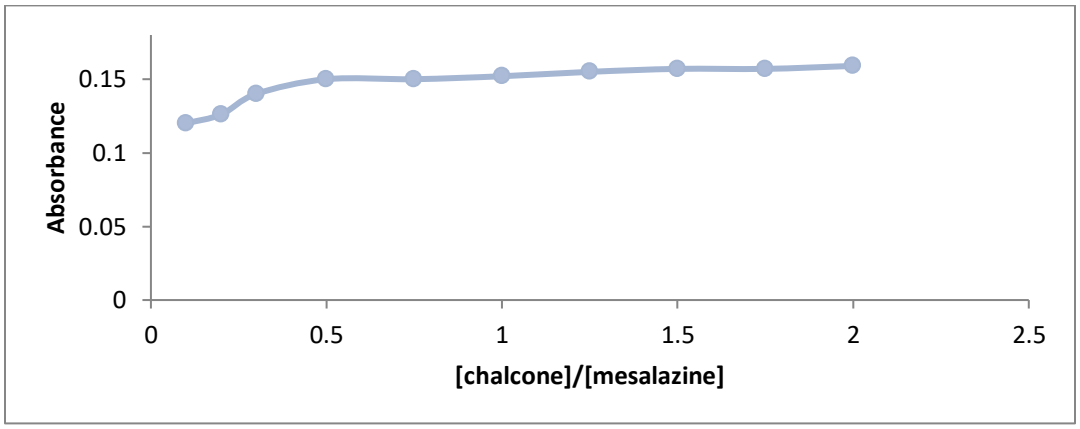

Fig.(9): The mole-ratio plot for diazotized mesalazine to chalcone reagent

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Table (9) Complex stability constant with chalcone reagent:

Compound	Conc. ($mol.l^{-1}$)	Absorbance As	Absorbance Am	∝	Average K_{st} ($l^2.mol^{-2}$)
Mesalazine	3.2×10^{-5}	0.089	0.103	0.1359	3.2×10^{10}
	6.5×10^{-5}	0.138	0.178	0.2247	
	9.7×10^{-5}	0.225	0.259	0.1312	

3.13. Interference of additives species:

The effect of some added compounds(excipients) which are often found in pharmaceutical preparations was studied by adding different amounts of these additives to 5µg/ml mesalazine using the recommended procedure. The results are given in (Table 10)below indicate the Selectivity of the method, the absence of interference additives

Table (10): Interference of additives species

Foreign compound				Recovery(%) of 5μg/ml of Mesalazine perμg/ml Foreign added
	100	500	750	1000
Glucose	103.5	101.9	101.6	98.1
Lactose	102.5	102.9	101.3	100.3
Sucrose	101.9	102.2	104.1	99.7
Talc	97.7	98.7	101.9	103.2

3.14. Application of the Method:

To test the applicability of the proposed method, it has been applied to the determination of mesalazine in pharmaceutical preparations (tablets). The results which are shown in (Table 11) indicate that the proposed method has good accuracy, precision, and recovery.

Table (11): Application of the method

Drug	Pharmaceutical Preparation	Certified Value	Amount present (ppm)	Drug content Found *	Recovery* (%)	Average recover (%)
Mesalazine	Asacol tablets Syria	400 mg	5	399.9	99.98	99.85
			10	403.72	100.93	
			15	394.2	98.65	
	Pentasa tablets Turkey	500 mg	5	505.8	101.16	99.93
			10	494.3	98.86	
			15	498.9	99.78	

Average of five determinations*

3.15. Evaluation of the proposed method

The performance of the proposed method was checked by estimation of t-test compared with the standard method (British Pharmacopeia),. The results in (Table 12) showed that the t-value was less than the critical value, which means there is no significant difference between the present method and the standard method for the determination of mesalazine.

Table (12): Evaluation of proposed method by t-test analysis

	Pharmaceutical Preparation	Recovery (%)		t_{ext}	F_{test}
		Present Method	Standard Method		
Drug	Pentasa tablets Turkey	101.16	99.52	0.189	2.776
		98.86	100.43		
		99.78	99.21		
		99.58	99.21		

4. Biological Importance of Chalcones:

Reports and research on Chalcones confirm that it has broad biological efficacy against fungi, against infections, against cell division (antimitotic), antioxidants, has an inhibitory effect in leukemia patients, against high blood sugar, and as a pain reliever, and some of its derivatives inhibit the spread of breast cancer, And also against antitrichomonal bacteria[25-27].

5. Conclusions

Synthesis of new quinoline derivatives containing chalcone, which have many medical applications, including anti-bacterial and also used as a reagent for aromatic amines indirectly. the sensitive spectroscopic method was developed for and applied to pharmaceutical preparations. for the determination of micro amounts of mesalazine in aqueous solution, based on the coupling of mesalazine diazotized with chalcone reagent in basic medium, The proposed method has been applied to the determination of mesalazine in pharmaceutical preparations With sensitivity, ease, and no need for prior extraction

6. **Acknowledgments:**

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