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Spectrophotometric Determination of Mesalazine via Diazotization-Coupling Reaction by Using Thymol Reagent

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Abstract
A simple and sensitive spectrophotometric method has been developed to determine Mesalazine in an aqueous solution. The method depends on the reaction of Mesalazine with thymol reagent to afford a colored analogue that has an absorption band at 436 nm with molar absorptivity of $4.1x10^4$ $L.mol^{-1}.cm^{-1}$. The determination range was 2-70 µg/ml with a recovery of 102.4 and a relative standard of less than 1.5 %. The proposed method is applied for the determination of Mesalazine in pharmaceutical preparation.
Keywords: Spectrophotometric, Mesalazine, Diazotization- coupling reaction, thymol

1. Introduction

Mesalazine is 5-aminosalicylic acid (5-ASA) and is known as mesalamine, which t is a medication that used to treat inflammatory diseases in an unknown mechanism[1, 2].Generally , it is used in the treatment of mild to moderate severe diseases orally or rectally. However, the effective one is that taken by mouth (Figure 1).

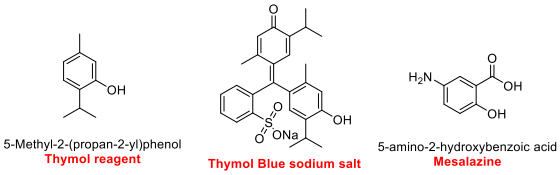

Figure 1: Thymol and Mesalazine

Primarily in preclinical development, drug determination measurements are used. Determination of the drugs in urine, saliva, blood so breathing samples can be done via various methods. Those of oral fluid are relatively little in comparison to urine.[3, 4]. Anyhow, various techniques used for drug determination are available.[5]

Thymol reagent, 2-isopropyl-5-methylphenol, IPMP (Figure 1), is a reagent that is found in oil of thyme with strong antiseptic properties. It is slightly soluble in water and strongly soluble in alcohol.[6, 7]

Also, Diazotization-Coupling Reaction occurred between the diazonium salt and phenolic compounds. It begins with the conversion of aromatic amines to diazonium salt which reacts with phenolic compounds to afford the Diazotization-Coupling analog.[8] Different analytical methods have been used to determine mesalazine which is represented by the protective diazotization.[9]

There are several methods for drugs determination; Sectrophotometric methods [10-13], Electrical, voltammetric and chromatographic methods that have been applied for the determination of the pharmaceutical formulation of a drug.[14-18]

According to the above survey, the aim of our work is to determine Mesalazine drug spectrophotometrically via using a diazotization-Coupling Reaction between the Thymol Reagent as a phenolic derivative and Mesalazine in an aqueous solution..

2. Experimental

2.1. Chemistry

2.1.1 Instruments

All chemicals used were of a high degree of purity.

Mesalazine solution (100ppm)

Dissolve 100mg mesalazine in 5 mL ethanol, then the volume was completed with ethanol until 100 ml volumetric flask.

Thymol solution (100 ppm)

Dissolve 100mg thymol in 5 mL ethanol, then the volume was completed with the distilled water until 100 ml volumetric flask.

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Sodium Hydroxide (1M)

Dissolving 4 g NaOH with distilled water, then the volume was supplemented with distilled water to the point of the mark in a 100 ml volumetric flask

Soduim Carbonate (1M)

Dissolving 10.600 g Na_2CO_3 in a volume of a distilled water, then the volume was supplemented with a distilled water to the point of the mark in a 100 ml volumetric flask.

Hydrochloric acid (1M)

Adding 8.3 ml of 11.8M acid to a volume of a distilled water, then the volume was completed to the extent of the mark with the distilled water.

Sodium nitrite (1%)

Dissolving 1.00 g of sodium nitrite in 100 ml of distilled water

Solutions of surfactants (0.5%)

Dissolving 0.5 g of surface active substances with a quantity of a distilled water, then the volume was supplemented with a distilled water to the point of the mark in a 100 ml volumetric flask.

Preparation of solution under investigation[19]

In a 10 ml flask, 1.0 ml of mesalazine was added then 1.0 ml of Hydrochloric acid (1M) and 1.0 ml of sodium nitrite (1%). This solution was left for 5 min at 0-5ºC. Then 1 ml of sodium carbonate (1M) was added with 1 ml of thymol reagent at a concentration of 100 µg / ml.

Resolute & Dissociation

Effect amount of acid

Hydrochloric acid (1M) as standard acid was used in various volumes to obtain the optimal volume. As depicted in table 1, the results revealed that the best volume is 1.0mL HCl in the range of 0.25-25mL (Table 1). This optimal volume was adopted in the subsequent experiments. Table (1)

Table 1: effect of HCl volume

X mL HCl	0.25	0.5	0.75	1.0	1.5	2.0	2.5
Absorbance	0.099	0.111	0.215	0.236	0.116	0.069	0.045

Effect of sodium nitrite

At a 10-ml volumetric flask, the amount of sodium nitrate (ranging from 0.25-2.5mL) is increased gradually with a recording of absorbance (Table 2) to get the optimal volume of sodium nitrite that was adopted in the subsequent studies**.**

Table 2: Effect of the Volume of sodium nitrite

X mL $NaNO_3$	0.25	0.5	0.75	1.0	1.5	2.0	2.5
Absorbance	0.23	0.25	0.24	0.23	0.19	0.18	1.17
nce	0	7	8	6	6	0	2

Effect of acid

Adding 1 ml of acetic, nitric, hydrochloric and sulfuric acids with (1M). The absorption was measured at λ = 436 nm. The results revealed that the hydrochloric acid has its highest absorption band in comparison with the acids, so it was relied upon by the subsequent experiments (Figure 2).

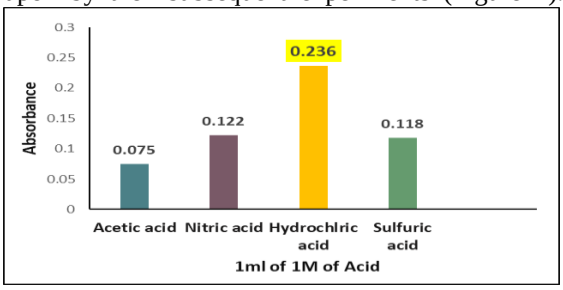


Figure 2: Effect of acid type

Effect of the type of base

This study was conducted to find out the type of the suitable base. Several different bases with a concentration of 1M were studied. The Equal volumes of 1.0 ml were added to a series of 10 ml volumetric flask, and figure (3) shows that the absorption resulting from Na_2CO_3 It was the best absorption so it was approved in subsequent trials

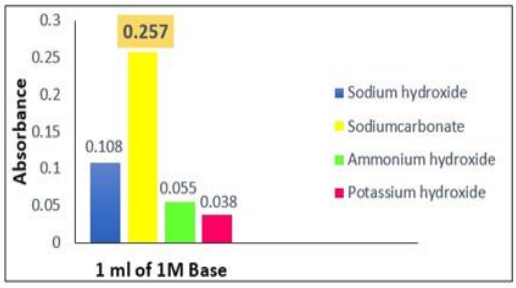


Figure 3: Effect of the base typeEffect of the base size

This study was conducted to find out the best volume of sodium carbonate solution (Na_2CO_3) at a concentration of 1M. Table (3) shows the best volume being adopted in the subsequent experiments.

Table 3: Effect of the base size

X mL Na_2CO_3	0.25	0.5	0.75	1.0	1.5	2.0	2.5
Absorbance	0.080	0.118	0.244	0.257	0.260	0.223	0.160

Effect the volume of the reagent

The effect of the amount of the thymol reagent on the product composition of the mesalazine compound was studied by adding increasing volumes of its solution ranging from 0 to 2.5 ml

with a concentration of 100 µg / ml. Table (4) shows the best volume of the reagent[20].

Table 4: Effect the volume of the reagent

X mL Thymol	without	0.25	0.5	0.75	1.0	1.5	2.0	2.5
Absorbance	0.124	0.268	0.285	0.274	0.260	0.237	0.231	0.224

Effect the temperature and time

The effect of the temperature and the product stability time at (0-55 ° C) was studied in the presence of a constant amount of mesalazine (10 µg / ml) and the optimum quantities of hydrochloric acid, sodium nitrite, base and reagent, then the volume completed up to the mark with a distilled water to measure the absorption at 436 nm. Figure (4) indicates that the complex has the highest sensitivity and the color contrast after 10 min of the additions at laboratory temperature (9 ºC) and the complex remains stable for more than 90 min. It is commonly known that the diazotization reaction is done at low temperatures, i.e. between 0 and 10 degrees Celsius. Low temperatures should be available to maintain diazonium salts in aqueous solution. However, if the temperatures are too low, crystallization can occur[21].

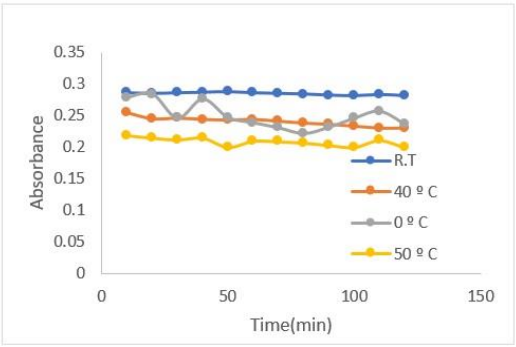


Figure 4: Effect the temperature and time

Effect of surface tension materials

Typical amounts of the acid, sodium nitrite, base and reagent were added to 1.0 of drug., then 1 ml of negative, positive and neutral surfactants were added to the prepared solution in a 10 ml volumetric flask. It is found that their effect was negative on the absorbents, so they were excluded. Table (5).

Table 5: Effect of surface tension materials

1.0mL of 5% surfactant	**absorbance**
Without surfactant	0.285
Cetyl trimethylammonium bromide (CTAB)	0.204
Sodium dodecyl sulfate (SDS)	0.245
Cetavlone	0.260

Absorption spectra

The absorption spectrum was plotted for the mesalazine complex with the thymol at the wavelengths ranging from 380 to 600 nm.

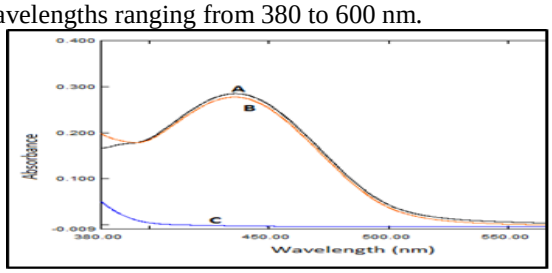


Figure 5: Absorption spectra of (10 µg / ml)
A Mesalazine versus the blank. B: Mesalazine versus distilled water. C: The blank solution versus distilled water

Study the standard curve

The standard curve was prepared by adding the incremental volumes (0.1-3.5) ml to a series of 10 mL volumetric bottles of a solution with a concentration of 200 µg / mL of mesalazine, i.e. a final concentration between 2 and 70 µg / ml and the volume was completed to the mark with a distilled water and the samples were left for 10 minutes at the laboratory temperature and measure the absorbance against the form solution. The results were included in Figure (6) and Table (6).

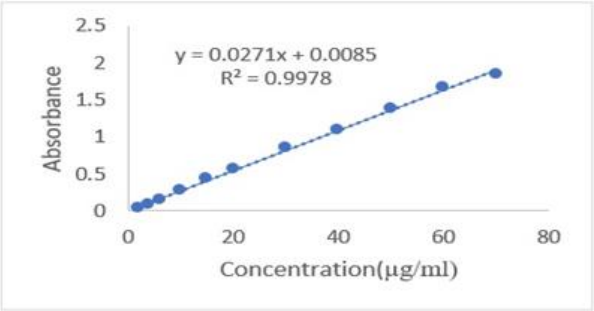


Figure 6: Calibration graph for the determination of Mesalazine.

Table 6: Summary of optical characteristics and statistical data for the proposed method

Linearity range (µg.mL^{-1})	Molar absorptivity (L.mol^{-1}.cm^{-1})	LOD* (µg.mL^{-1})	LOQ* (µg.mL^{-1})	Slope	Intercept	Correlation coefficient
2-70	4149.95	0.0972	0.3243	0.0271	0.0085	0.9978

*average of ten determination

Accuracy and precision

The accuracy and the compatibility of the method was then checked by calculating the recovery rate and the standard relative deviation (RSD) by using five readings of three different concentrations of mesalazine compound. The results table (6) below indicates that the method has a good accuracy and a good compatibility as the recovery rate is reached[22].

Table 6: Accuracy and precision of the method

compound	Added amount ($\mu g.mL^{-1}$)	Recovery* (%)	Average recovery (%)	RSD* (%)
Mesalazine	10	101.2	102.4	0.55
	20	103.5		0.37
	40	102.5		1.3

*average of five determination

The stoichiometry of the product

The mole Ratio method was followed to find out the rate of interaction of mesalazine with the thymol reagent using the dilute solutions of the medicinal compound and the reagent by following the method shown below. The Dilute solutions of mesalazine and the thymol were prepared by adding volumes of the thymol reagent (0-3) ml to a fixed volume of mesalazine 1 ml, and the volume was then completed up to the mark of 10 ml with a distilled water.

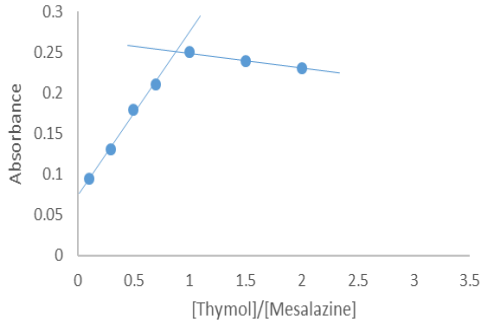
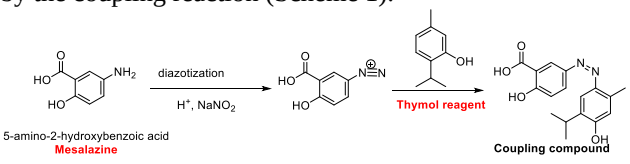


Figure 7: Mole ratio for the product of Mesalazine with thymol reagent

Diazotization and Coupling reaction of Mesalazine with thymol (17)

Mesalazine could be reality Diazotized in an acidic medium and the resultant diazotization would then react with the coupling reagent, thymol. The proposed sequence of the diazotization followed by the coupling reaction (Scheme 1).



Scheme (1): synthesis of coupling compound between Mesalazine and thymol reagent.

The stability constant of the complex formed

The component output stability constant 1: 1 (reagent: drug) was calculated separately using the following formula:

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

Where C is the concentration of the complex and its unit (mol / liter), and α is the degree of dissociation, and kst is the stability constant of the formed complex, as the results in Table (7) indicate the high stability of the formed product[23].

Table 7: The stability constant of the complex formed

compound	Conc. ($mol.L^{-1}$)	Absorbance		α	Average K_{st} ($L.mol^{-1}.cm$)
		As	Am		
Mesalazine	$12*10^{-6}$	0.032	0.044	0.272	$4.13*10^{5}$
	$24*10^{-6}$	0.074	0.108	0.314	
	$36*10^{-6}$	0.092	0.144	0.361	

Study the effect of cross linkers

The effect of interferers was studied by using several interferers in the absorption (10µg/ mL) of mesalazine. The different volumes of interferers were added to the mesalazine solution where the absorption was measured using the optimal conditions for the mesalazine complex at the wavelength of 436 nm. Table (8) shows that the results indicate the selectivity of the method and the absence of the interference by the additives.

Table 8: Effect of surfactants

Foreign compounds	**Recovery % of 10µg.mol^{-1} of mesalazine per µg/ml foreign added**			
	100	500	750	1000
Glucose	100.2	99.5	101.2	97
Starch	101	102.1	103.8	99
Talc	98.8	99.5	102.3	96.3
Mg-stearate	100.5	96.9	99.1	97.8

Analysis of pharmaceutical preparation tablets

10 tablets of the medicinal form (Mesacol) were weighed, crushed and then mixed well. The equivalent of one tablet was weighed and dissolved in an amount of ethanol 25 ml and the volume was completed to 500 ml. Out of 800 µg / ml filtered solution 200 µg / ml was prepared. Different volumes were taken from this solution to obtain the concentrations of (10, 20, 40 and 60) µg / ml. It is found the these concentrations of mesalazine in comparison with the standard curve in its pure form , included in the table below, is of high accuracy and it is consistent with the original content of mesalazine in the pharmaceutical preparation.

Table 9: Assay of Mesalazine in pharmaceutical preparation.

Pharmaceutical preparation	Certified value	Amount (ppm)	Drug content (mg)	Recovery %	Average recovery %
Mesacol tables Syria	400mg	10	396.84	99.21	99.85
		20	400.4	100.1	
		40	399.6	99.9	
		60	400.8	100.2	

Evaluate the results of the proposed method

It could be concluded that the experimental F values are less than their tabular value of 9.28 at a 95% confidence level and for three degrees of freedom, and this indicates that there is no clear difference between the accuracy of the two methods. The deviation is due to a random error. Also, it was found that the experimental t values are less than the tabular t value of 3.182 at a 95% confidence level and for three degrees of freedom, indicating that there is no clear difference between the accuracy of the two methods and the deviation resulting from a random error.

Conclusion

A sensitive, rapid, selective and simple spectrophotometric method has been developed for the determination of mesalazine with thymol by diazotization-coupling reaction. The proposed method does not involve a solvent extraction step.

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The efficiency and the success of the proposed method in estimating the medicinal compound mesalazine in pharmaceutical preparations goes hand in hand with the standard method approved in the British Pharmacopeia as shown in table 10.

Table 10: Efficiency and success of the proposed method

Pharmaceutical preparation	Recovery %		F-test	T-test
	Present method	Standard method		
Mesacol tables Syria	**99.85**	**99.61**	**1.05**	**0.89**

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