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Spectrophotometric determination of Mesalazine by formation of copling with reagent

Mustafa Hamed Mahmood
Department of chemistry, college of education, university of Sammrra, Sammrra, Iraq
Corresponding author email: mustafa.hamad.iq@gmail.com

Eman Thiab Al-Sammrrai
Department of chemistry, college of education, university of Sammrra, Sammrra, Iraq

***Abstract*---**A simple, developed, fast and accurate spectrophotometric method for determination of Mesalazine (MSL) in its pure form and pharmaceutical preparation (Pentasa). This method was based on the formation of complex between MSL and the reagent 2,6-Dichloro indophenol sodium (DIS) to give a red color product that gives ats highest absorption at the wavelength of 520 nm. The best conditions for complex formation were found (time, temperature, optimal reagent concentration, pH). The linearity of the method for the complex consisting ranged from 5-30 µg/ml, the Sandell's index was 0.0354µg/cm^2, the molar absorption coefficient was 4318.40L/mol.cm and the detection limit was 0.252µg /ml , the quantitative limit was 0.766 µg/ml , the percent recovery range was Rec% between (98.900-101.715)% and the relative standard deviation rate RSD% between (0.00306- 0.00696)%. It was found that the method was accurate and precision and has been successfully applied to estimate the MSL in its pharmaceutical preparation, in direct methods and in multi standard additions.

***Keywords*---**mesalazine, reagant, 2,6-dichloro indophenol sodium.

Introduction

According to the British nomenclature is known as Mesalazine , is known as Mesalazine according to the American nomenclature and 5-amino-2-hydroxybenzoic acid according to the regular label [(1).] Mesalazine is an anti-inflammatory drug. It is used in the treatment of inflammatory bowel disease, such as ulcers of the colon, anus or rectum, and protects against Crohn's disease

through the development of cancer in people who suffer from inflammatory bowel disease[2] . Figure (1) shown the chemical structure of Mesalazine[3].

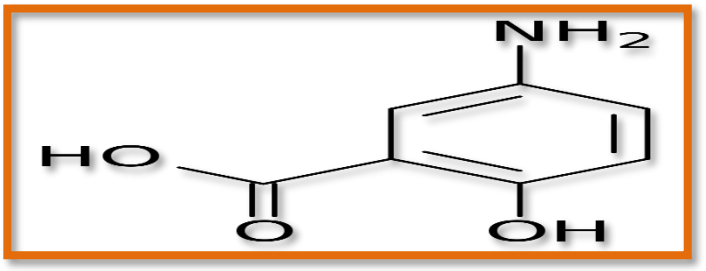

Figure (1) Mesalazine chemical structure[3]

MSA can reduce the production of pro-inflammatory prostaglandins and leukotrienes [4] Whereas ulcerative colitis is a condition that causes long-lasting inflammation along with sores (ulcers) in the large intestine (colon) and rectum [5] Commonly effects results with colon cancer, skin, eye, and joint inflammation, this usually occurs due to IBD flare-ups [6,7] . MSL also exhibits various significance in controlling mucosa by inhibiting bacterial peptides and cell injury through trapping most reactive oxygen species resulting in reducing its toxicity thereby blocking the production of prostaglandins [8,9] . With its significance in the pharmaceuticals industry, quality control in pharmacopeia through various analytical methods has been used to analyze MSL in actual samples, such as chromatographic [10,11.12,15], fluorescence spectroscopyic [13], electrochemical [14,16] and spectrophotometric [15] methods. Among these, electrochemical methods were found to be useful for rapid response, sensitive and selective determination of various pharmaceutical applications[17].

Practical Part
Apparatuses Used

Many devices were used in this method: Sensitive balance (with four digits) Sartorius- Germany. Uv-Vis Spectrophotometer Double Beam, Shimadzu -1650-Japan. Uv-Vis Spectrophotometer Double Beam, Spectrophotometer-200705044, China. pH-meter, Jenway-3310. Ultrasonic water bath, LabTech - Korea.

Chemical Materials Used

High-purity materials were used: Mesalazine (Sigma-Aldrich), Reagant, 2,6-Dichloro indophenol sodium, Ethanol (GCC-England), Hydrochloric acid (BDH-U.K) and Sodium hydroxide (Fluka-Switzerland).

Solutions Perpetration
Standard Mesalazine Solution (1000 µg/ml)

It was prepared by dissolving 0.1 g of Mesalazine in a specific volume of hot distilled water in a 100 ml volumetric flask, then complete the volume to the limit of the mark with the same solvent so that the concentration of MSL was 1000 µg/ml as a stock solution. Then 10 ml of the solution was withdrawn and

transferred to a 100 ml volumetric flask, and the solution was diluted with distilled water to the limit of the mark, so that the concentration was 100 µg/ml as a working solution.

Reagent Solution (100 µg/ml)

It was prepared by dissolving 0.1g of the reaggent in a specific volume of ethanol in a 100 mL volumetric flask, then complete the volume to the limit of the mark with the same solvent, to being the concentration to 1000 µg/ml as a stock solution. Then 30 ml of the solution was transfer and transferred to a 100 ml volumetric flask, and the solution was diluted with ethanol to the limit of the mark, so that the concentration was 200 µg/ml as a working solution**.**

Hydrochloric Acid Solution with an Approximate concerts(0.01M)

This solution was prepared by diluting 0.08 ml of concentrated acid (11.86M) in volumetric flask, then complete the volume up to the mark with distilled water.

Sodium Hydroxide Solution with an Approximate Concentration (0.01M)

Solution was prepared by dissolving 0.04g of solid sodium hydroxide in a specific volume of distilled water in a 100ml volumetric flask, then complete the volume up to the mark of the same solvent.

Pharmaceutical Solution = (1000 µg/ml)

10 Tablets of Pentasa, and the average weight of one tablet containing 500mg of Mesalazine was taken, the powder was placed in a volumetric flask of 500 ml and added methanol and the volumetric flask was shaken and put it in an ultrasound water bath for 10 min. Complete the volume with the same solvent and filter it with Whatman No.42 filter paper. From the filtrated solution (1000 µg/ml Mesalazine) transferred 10ml to a 100 ml volumetric flask and diluted with methanol up to the mark so that the concentration was 100 µg/ml.

Preparation of the Complex

Prepare the complex for Mesalazine by mixing 1 ml of Mesalazine (100 µg/ml) with 1 ml of DIS reagent(300 µg/ml) in a 10 ml volumetric flask, then complete the volume to the mark with Ethanol. A range of wavelengths ranged from 190 to 800 nm were surveyed, and the resulting complex gave a new peak at 520 nm, which was adopted in subsequent experiments.

Study of Experimental Conditions
Optimum Concentration of Dye

In order to choose the best reagent volume with the resulting complex gives the highest absorption, increased volues DIS (0.2-2.4ml) of standard DIS reagent solution (300 µg/ml) were added in 10 ml volumetric flask containing a fixed volume of 1 ml of standard Mesalazine solution (100µg/ml), complete the volume

with ethanol up to the mark level. The absorption values for the complex formed versus the blank solution were recorded, as shown in Table (1).

Table (1) the optimum reagent volume for complex of MSL

Concentration of DIS (µg/ml)	Absorbance
2	0.055
4	0.068
6	0.167
8	0.269
10	0.276
12	0.292
14	0.403
16	0.412
18	0.422
2	0.464
22	0.382
24	0.362

The above table shows that 2 ml(300 µg/ml) was the optimum reagent volume through which the resulting complex gives the highest absorption, so it was chosen as the best reagent concentration.

Effect of pH

The optimum pH at which the complex formed was studied that gives the highest absorption. A different pH values ranging between (6.7-9.2) and the absorption values for the complex formed at each of these values were recorded and shown in Table (4).

Table (4) the effect of Ph on the complex of MSL

Addition	Volume(ml)	Absorbance	pH
HCl (0.05)M	0.05	-0.002	4.71
	0.1	-0.005	4.48
	0.2	-0.009	4.22
Without add.		0.462	7.7
NaOH (0.05)M	0.05	0.00974	9.75
	0.1	0.0043	10.32
	0.2	0.0033	10.55

It was found from the results above that adding the acid and base led to a decrease in the absorption so we are avoided them , and the natural pH of the complex was adopted (7.7) without any addition.

Effect of Temperature

In order to choose the optimum temperature at which the resulting complex gives the highest absorption, the measurement process was performed for the complex with a temperature range of 5-60 °C which was shown in Table (3).

Table (3) effect of temperature on the complex of MSL

Temperature	Absorbance
0	0.322
5	0.345
10	0.356
15	0.464
20	0.465
25	0.468
30	0.461
35	0.462
40	0.461
45	0.459
50	0.453
55	0.454
60	0.459

It is clear from the results of this study and shown in Table (3) that the maximum absorption was at the laboratory temperature while the decrease in the absorption of the product formed when the temperature is increased is observed. Therefore, the laboratory temperature was adopted in subsequent experiments.

Effect of Time

The stability and constracy of the complex formwere studied by choosing the optimal time at which the complex formed gives the highest absorption, and Table (2) shows the values of complex absorption at different MSL, ranging from the beginning of preparing the complex to a limit of 60 min.

Table (2) effect of time complex of MSL

Time(min)	Absorbance
0	0.467
5	0.466
10	0.456
15	0.463
20	0.459
25	0.445
30	0.465
35	0.461
40	0.463

45	0.458
50	0.465
55	0.459
60	0.454

It was found from the above table that there is no significant effect of the time factor on the formation process of the complex, that is, the compound was almost stable from the moment of the reaction until 60 min, and the time of the reaction moment was adopted in subsequent experiments.

Stoichiometric Ratio of Complex

The ratio of the composition the drug to the reagent was studied according to the Job method for continuous changes. The absorption values for the complex formed were measured against the blank's solution as shown in Figure (2).

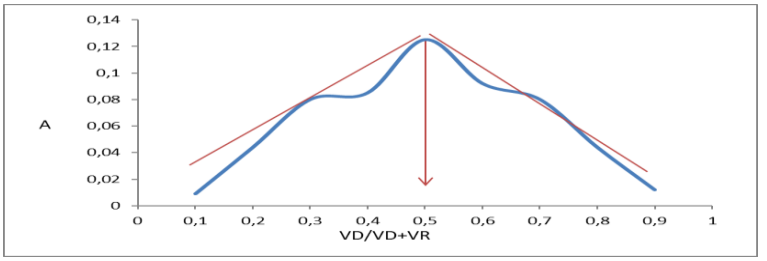

Figure (2) correlation ratio of the MSL complex

Through the results obtained from the Job method, it was found that the complex formed under the best conditions is composed of equal molar ratios of the drug and the reagent at a ratio of (1:1), respectively.

Calibration Curves of Complex

The calibration curve for the MSL complex with DIS was constructed under the best conditions. The linearity of Figure (3) the Calibration curve for MSL complex

Accuracy and Precision

A study was conducted to calculate the accuracy and precision of the proposed method were calculate the Rec% value to express the accuracy of the results, and the RSD% for expressing the precision of the results and for three concentrations (15,25,45µg/ml) of the calibration curve, and by performing six readings for each measurement. Where the values of Rec% ranged between (98.900-101.715)% and the values of RSD% between (0.00306- 0.00696)% . Table (5) shows that.

Table (5) accuracy and precision of MSL complex

Conc.of MSL taken µg/ml	A	Conc.of MSL found µg/ml	Rec %	RSD %
10	0.468	10.095	100.950	0.00696

20	0.757	20.343	101.715	0.00479
30	1.020	29.670	98.900	0.00306

Applications

Direct Method

The proposed method was applied at pharmaceutical preparation (Pentasa), with different concentrations (15,25,30) µg/ml of the drug, and by performing six readings for each measurement. To express the accuracy of the results, used Rec%, where it was between (98.900-102.733)%, and to express the precision of the results, used RSD% and it was between (0.00255- 0.00503)%, which is shown in Table (6).

Table (6) applying the direct method of the ionic complex of the drug

Conc.of MSL taken µg/ml	A	Conc.of MSL found µg/ml	Rec %	RSD %
15	0.618	15.41	102.765	0.00503
25	0.888	24.989	99.95	0.00462
30	1.020	29.670	98.900	0.00255

Multi Standard Additions Method

Mesalazine was determinate in the Pentasa pharmaceutical preparation using the multiple standard additions method. The results shown in Figure (4) showed the accuracy and precision of the proposed method, Rec% (98.000)% and RSD% (0.00836)% were obtained, and this indicates that the method is accurate, precision, and free of interferences.

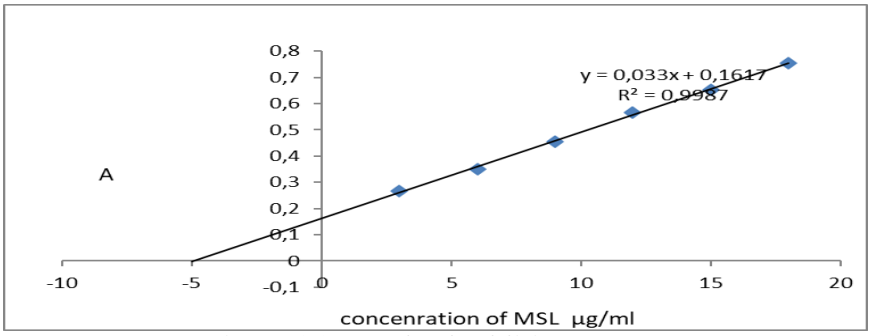

Figure (4) standard additions curve

Final Absorption Spectrum

According to the optimum conditions obtained, the final absorption spectrum of the MSL complex versus the blank solution was recorded to confirm the result, as a new peak of the complex appeared at the wavelength 520 nm while the value of (λ_{max}) of the DIS was 620 nm and MSL 298 nm. It is shown in Figure (5).

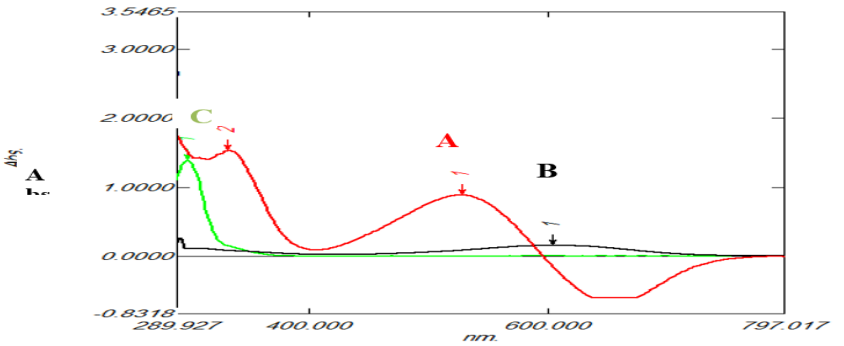

Figure (5) absorption spectrum complex (A), DIS spectrum (B) and MSL spectrum (C)

Method Comparison

The proposed method was compared with another spectroscopic method is shown in Table (7).

Table (7) comparing the proposed method with another spectral method

Parameters	Present Method	Other Method[18]
λ_{max} (nm)	520	346
Beer's law range (µg/ml)	5-30	0.48-12
T (ºC)	25	40
L.O.D (µg/ml)	0.252	0.053
L.O.Q (µg/ml)	0.766	0.176
Correlation coefficient (R^2)	0.9885	0.9987
Sandell's index (µg/cm^2)	0.0354	0.02356
ε (L/mol.cm)	4318.407	6500
Rec% Average	98.900-101.715	98.04
RSD%	0.00306- 0.00696	1.70

Conclusions

The method was used to determine MSL in its pure drug and Pentasa pharmaceutical . This method based on the reaction of MSL with DISreagent to formation of a purple complex. The highest absorption was given at 520 nm wavelength, and the results obtained showed the percentile recoveries values, the relative standard deviation, the detection limit, and the quantitative limit that the method is accurate and precision, which indicates the success of the proposed method for determination of MSL.

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