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Spectrophotometric determination of Mesalazine by a charge transfer complex

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***Abstract*---**A simple, developed, fast and accurate spectrophotometric method to determination Mesalazine (MES) in its pure form and pharmaceutical preparation (Pentasa). This method was based on the formation of a charge transfer complex between MES and the Iodine (IOD) reagent to give a purple color product that gives its highest absorption at the wavelength of 514 nm. The best conditions for complex formation were found (time, temperature, optimal reagent concentration and pH). The linearity of the method for the complex consisting ranged from 5-45 µg/ml, the Sandell's index was 0.03278 µg/cm2, the molar absorption coefficient was 4670.6175 L/mol.cm and the detection limit was 0.10245 µg /mL, the quantitative limit was 0.31045 µg/ml, the percent recovery range was Rec% between (103.145 - 97.311) % and the relative standard deviation range RSD% between (0.128 - 0.263) %. It was found that the method is accurate , precision and has been successfully applied to determinate MES in its pharmaceutical preparation, in direct methods and in multi standard additions.

***Keywords*---**charge transfer, Mesalazine, Iodine.

Introduction

Mesalazine is an anti-inflammatory drug that is used to treat inflammatory bowel disease, including inflammation of the colon or rectum, and protects against Crohn's disease by preventing the development of cancer in people with inflammatory bowel disease[1] Mesalazine is a white to pink crystalline powder, slightly soluble in cold water and alcohol, but highly soluble in hot water and hydrochloric acid [2] MEZ played an important role as an activating factor of the platelet. MEZ as an anti-inflammatory drug is used for treating the common disease of inflammatory bowel and Crohn's disease[3,4].Commonly reported 5-ASA

adverse effects to consist of headache, diarrhea, bloating, nausea, hypersensitivity with rare side effects include interstitial nephritis, pancreatitis, dermatitis, myocarditis and alopecia whereas nephrotoxi city is seen with any of 5-ASA compounds[5,6] . Figure (1) shown the chemical structure of mesalazine[7].

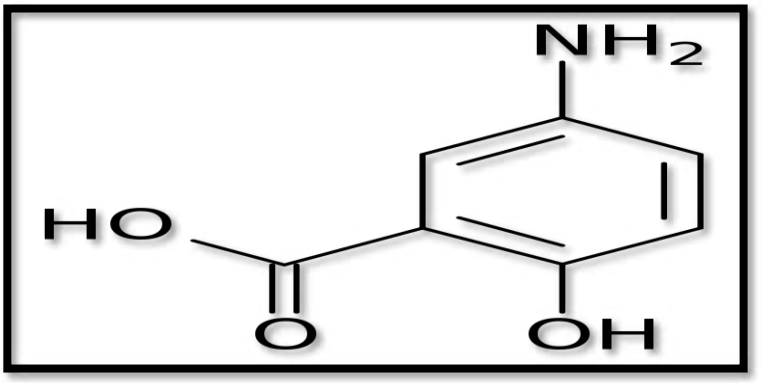

Figure 1. Mesalazine chemical structure [7]

MES is an antioxidant and derivate of salicylic acid that traps free radicals, which are potentially harmful species by-products of metabolism. It is a major metabolite present in blood with a half-life of up to 10 h [8] With its significance in the pharmaceuticals industry, quality control in pharmacopeia through various analytical methods has been used to analyze MSA in actual samples, such as liquid chromatography (LC) [9] With its significance in the pharmaceuticals industry, quality control in pharmacopeia through various analytical methods has been used to analyze MES in actual samples, such as chromatographic[10,11,12,13]. There are other methods which depend on the reaction of Schiff bases forming [14] and also reactions of forming charge transfer complexes [15] and chromatographic methods such as (UPLC)[17].Among these, electrochemical methods were found to be useful for rapid response, sensitive and selective determination of various pharmaceutical applications [16, 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 Single Beam, Spectrophotometer-200705044, China. pH-meter, Jenway-3310. Ultrasonic water bath, LabTech - Korea.

Chemical Materials Used

High-purity materials were used: Mesalazine (Sigma-Aldrich), Iodine(Sigma-Aldrich) , 2-Bromobenzaldehyde C_7H_5BrO (Sigma-Aldrich) , Toluene C_6H_5-CH_3(GCC-England), Ethanol (GCC-England), Hydrochloric acid (BDH-U.K) and Sodium hydroxide (Fluka-Switzerland).

Solutions Perpetration
Preparation of Schiff Bases

Schiff's base was prepared from condensing aldehyde 2-Bromobenzaldehyde with primary amines 5-amino-2-hydroxybenzoic acid using a microwave device then blunt the reaction by adding (2-3) a drop of glacial acetic acid and placing the solution in a microwave device for 10 minutes, then placing the substance in a beaker for 24 hours to evaporate the largest amount of solvent, then washing with water to re-crystallization and filtering by Whatman No.42 filter paper . Then the precipitate was taken, dried and stored.

Standard Mesalazine Solution (1000 µg/ml)

Standard solution 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 becomes 1000 µg/ml as a working solution.

Iodine (IOD) reagent solution (1000 µg/ml)

It was prepared by dissolving 0.1g of the reagent in a specific volume of toluene in a 100 mL volumetric flask, then complete the volume to the limit of the mark with the ethanol, to being the concentration to 1000 µg/ml as a working solution.

Hydrochloric Acid Solution (0.05M) with an Approximate concentration

The solution was prepared by diluting 0.42 ml of concentrated acid (11.86M) in volumetric flask with a capacity of 100 ml, then complete the volume up to the mark with distilled water.

Sodium Hydroxide Solution with an Approximate Concentration (0.05M)

Solution was prepared by dissolving 0.2 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 (Pentasa) (1000 µg/ml)

10 tablets of Mesalazine produced by FERRING International Center SA, Chemin de la Vergognausaz 50,1162 St-Prex, Germany were crushed using a ceramic mortar, and the average weight of one tablet was (0.750) g, which contains 500 mg of the active substance mesalazine. Dissolve the powder in a certain volume of ethanol with stirring, then add (0.4) ml of aldehyde (2-bromobenzaldehyde) diluted with a certain amount of ethanol to the preparation with continuous stirring where the color change to yellow, then the reaction by adding (2-3) drops of glacial acetic acid and the solution was placed in a microwave device for 10 minutes, then placed in a baker for 24 hours to evaporate the largest amount of solvent, The precipitate was washed with water and filtered by a filter paper Whatman No.42. and dried, The powder was placed in a volumetric flask of 500

ml and dissolved in a certain amount of ethanol completed the volume with water to the mark, then the solution was filtered by a Whatman No.42 filter paper. The filtrate containing 1000 µg/ml of mesalazine .

Preparation of the Charge Transfer Complex

Prepare the charge transfer complex for Mesalazine by mixing 1 ml of Mesalazine Schiff base solution (1000 µg/ml) with 1 ml of IOD reagent (1000 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 scan, and the resulting complex gave a new peak at 514 nm, which was adopted in subsequent experiments.

Study of Experimental Conditions
Optimum Concentration of Reagent

In order to choose the best reagent concentration with the resulting complex gives the highest absorption, increased concentration (0.5-3 µg/ml) of standard IOD reagent solution (1000 µg/ml) were added in 10 ml volumetric flask containing a fixed volume of 1 ml of standard Mesalazine Schiff base solution (1000µg/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 concentration for the Charge transfer complex of MES

Conc.of IOD µg/ml	Absorbance
0.5	0.211
1	0.241
1.5	0.231
2	0.218
2.5	0.205
3	0.186

The table(1) shows that(10 µg/ml) was the optimum reagent concentration through which the resulting complex gives the highest absorption, so it was chosen as the best reagent concentration.

Effect of pH

A study was conducted to choose the optimum pH at which the complex formed gives the highest absorption. This study was conducted at different pH values ranging between (5.9-9.8) and the absorption values for the complex formed at each of these values were recorded and shown in Table (2).

Table 2
The effect of PH the charge transfer complex of MES

Addition	Volume(ml)	Absorbance	pH
HCl (0.01)M	0.1	0.221	7.6
	0.2	0.206	7.2
	0.3	0.178	5.9
with out addition	-----	0.240	8.5
NaOH (0.01)M	0.1	0.297	8.8
	0.2	0.314	9.0
	0.3	0.306	9.5
	0.4	0.282	9.8

The results of this study showed that the addition of the acid led to a decrease in the absorption of the colored product, so it was avoided. As for the role of the base, it led to an increase in the absorption of the complex formed well, so it was adopted in subsequent experiments. and the PH was (9.0).

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 ^{o}C which was shown in Table (3).

Table 3
Effect of temperature on the charge transfer complex of MES

Temperature	Absorbance
5	0.287
10	0.298
15	0.313
20	0.312
25	0.315
30	0.316
35	0.311
40	0.302
45	0.289
50	0.271
55	0.245
60	0.216

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 absorption of the colored product formed was decrease when the temperature is increased is observed. Therefore, the laboratory temperature was adopted in subsequent experiments.

Effect of Time

A study was conducted to find the constancy and stability of the complex formed between MES and the IOD reagent by choosing the optimal time at which the complex formed gives the highest absorption, and Table (4) shows the values of complex absorption at different times, ranging from the beginning of forming the complex to 60 min.

Table 4
Effect of time on the charge transfer complex of MES

Time(min)	Absorbance
0	0.314
5	0.315
10	0.315
15	0.314
20	0.313
25	0.312
30	0.286
35	0.273
40	0.262
45	0.256
50	0.247
55	0.241
60	0.233

It was found from the table (4) The best absorption is given by the complex from the moment of interaction until 25 minutes, after which the absorption gradually decreases up to 60 minutes .

Stoichiometric Ratio of Complex

A study was conducted to determine the ratio of the drug to the ratio of the (IOD) reagent in the complex, 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).

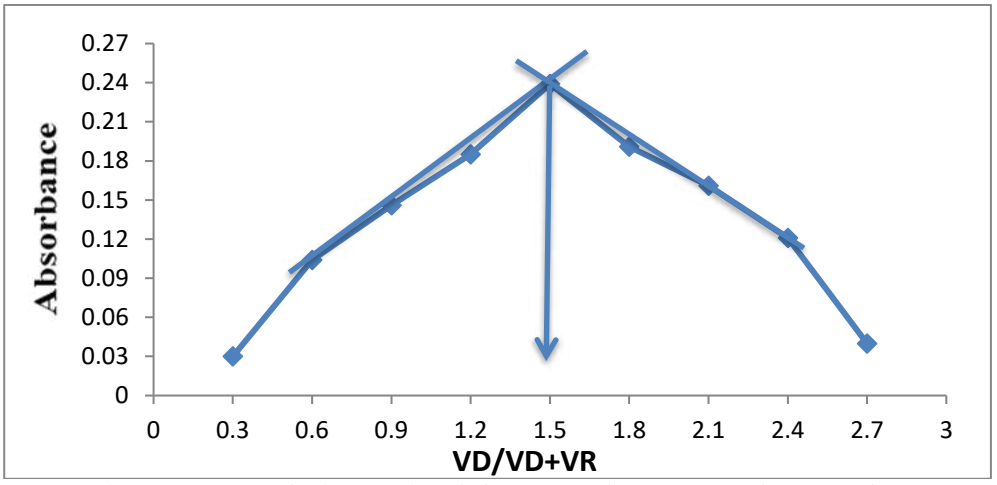

Figure 2. Correlation ratio of the MES charge transfer complex

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.

Results and Discussion

Calibration Curves of Complexes

The calibration curve for the MES charge transfer complex with IOD was constructed under the best conditions. The linearity of the method was between 5-45 µg/ml, the Sandell's index was 0.03278 µg/cm^2 and molar absorption coefficient was 4670.6175 L/mol.cm. The detection limit was 0.10245µg/ml and the quantitative limit was 0.31045 µg/ml. Figure (3) shows the calibration curve for the MES charge transfer complex.

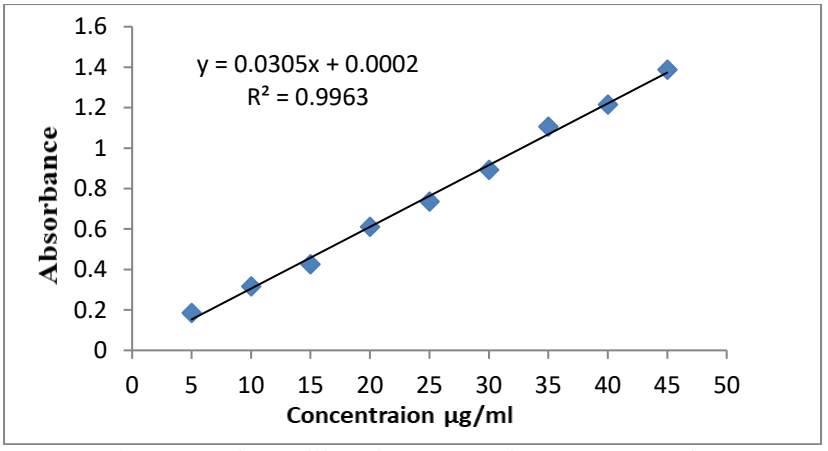

Figure 3. the calibration curve for MES complex

Accuracy and Precision

A study was conducted to calculate the accuracy and precision of the proposed method, by calculating the Rec% value to express the accuracy of the results, and the RSD% for expressing the precision of the results and for three concentrations (10,20,30µg/ml) of the calibration curve, and by performing six readings for each measurement process conducted. Where the values of Rec% ranged between (97.310 -103.140) % and the values of RSD% between (0.128 - 0.263) %. Table (5) shows that.

Table 5
Accuracy and precision of MES charge transfer complex

Conc.of MES taken µg/ml	Conc.of MES found µg/ml	Rec %	RSD %
10	10.314	103.140	0.263
20	19.986	99.930	0.178
30	29.193	97.310	0.128

Applications
Method

The proposed method was applied at pharmaceutical preparation (Pentasa), with different concentrations (20,30,40) 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 (99.431-102.360)%, and to express the precision of the results, used RSD% and it was between (0.182-0.262)%, which is shown in Table (6).

Table 6
Application of the direct method for complex charge transfer of the drug

Conc.of MES taken µg/ml	Absorbance	Conc.of MES found µg/ml	Rec %	RSD %
20	0.624	20.472	102.360	0.182
30	0.910	29.829	99.431	0.232
40	1.235	40.485	101.213	0.262

Standard Additions Method

Mesalazine was determinate in the Pentasa pharmaceutical preparation using the multiple standard additions method. (0.5) ml of the prepared solution of the pharmaceutical preparation (1000 µg/ml) (described in paragraph 3-6) was added to a series of volumetric flasks (seven flasks) of 10 ml capacity. Increasing volumes of MES standard solution (1000 µg/ml) ranged from (0.5-3) ml were added, and the seventh volumetric flask was left without addition. Then add a fixed volume of 1 ml of the standard solution of the reagent, add 0.2 ml of 0.05 M NaOH solution and fill the volume with ethanol to the mark. Plot the concentration of the added solution against absorption at 514 nm wavelength.

The results were showed in Figure (4). The Rec% and RSD% were (103.684) % and (3) % respectively, which indicates that the method is accurate, precision.

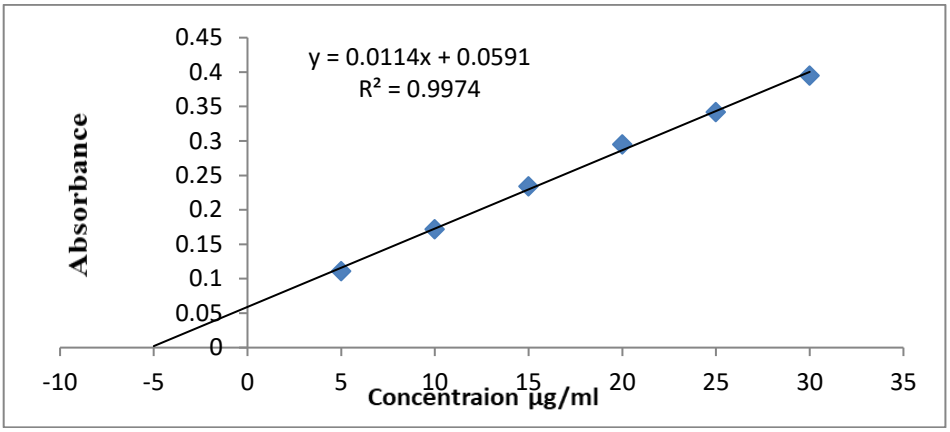

Figure 4. Standard additions curve

Final Absorption Spectrum

According to the optimum conditions obtained, the final absorption spectrum of the MES complex versus the blank solution was recorded to confirm the result, as a new peak of the complex appeared at the wavelength 514 nm while the value of (λ_{max}) of the pigment IOD was 360 nm and MES 298 nm. It is shown in Figure (5).

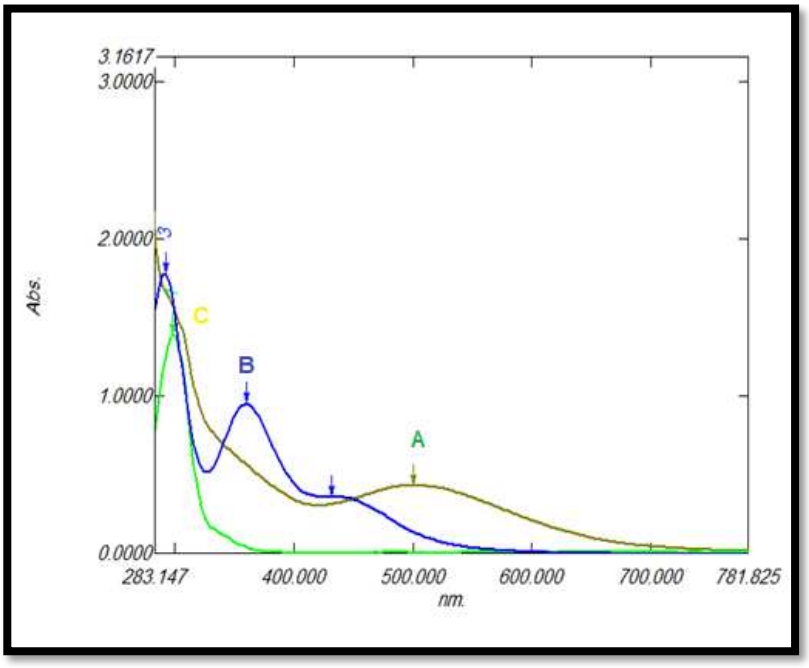

Figure 5. Absorption spectrum of charge transfer complex (A), IOD spectrum (B) and MES spectrum (C) versus blank

Method Comparison

The proposed method was compared with another spectral 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)	514	346
Beer's law range (µg/ml)	5-45	0.48-12
T (ºC)	15-30	40
L.O.D (µg/ml)	0. 10245	0.053
L.O.Q (µg/ml)	0. 31045	0.176
Correlation coefficient (R^2)	0.9963	0.9987
Sandell's index (µg/cm^2)	0. 03278	0.02356
ε (L/mol.cm)	4670.6175	6500
Rec% Average	100.1306	98.04
RSD%	0.1280- 0.2636	1.70

Conclusions

The charge transfer method was used to determine MES in its pure drug and Pentasa pharmaceutical form. This method based on the reaction of MES with IOD reagent to formation of a purple complex. The highest absorption was given at 514nm 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 MES.

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