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# A novel spectrophotometric method for the determination of mesalazine

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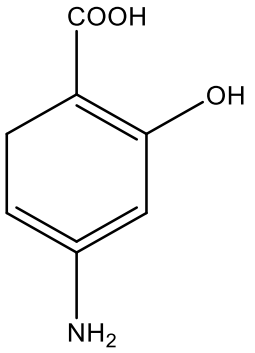
***Abstract*---**A sensitive, accurate and easy indirect spectrophotometric method was developed for the determination of mesalazine in its pure form and in pharmaceutical preparations (tablets, capsules). The method was based on the oxidation of the mixture of the drug compound with the hydralazine reagent by using the oxidizing agent potassium periodate in an acidic medium and in the presence of the surfactant triton (x-100), absorption was measured at 380 nm for the drug compound, and the molar absorbance of mesalazine was 1147.5 liters. Mol-1. cm-1. With a recovery rate of 99.38, the method was developed to be simple, inexpensive and fast because it does not include any solvent extraction. The method has been successfully applied to pharmaceutical preparations.

***Keywords*---**mesalazine, hydralazine, potassium periodate, determination.

## Introduction

Mezalazine or Mesalazine is known by its scientific name 5-Amino salicylic acid and its chemical name 5-Amino-2-hydroxy benzoic acid has a molecular dye $C_7H_7NO_3$ and a molar mass of 153.135 (g/mol) (1). It also has the synthetic dye shown in the figure below (2)




**The chemical structure of mesalazine**
**5-Amino-2-hdroxy benzoic acid**

Mesalazine is used in the treatment of inflammatory bowel diseases, especially in the treatment of gouty mucosal colitis. It is also used in the prevention of hemorrhagic eruptions that start from the colon to the anus (3) (4). Mesalazine is in the form of a white to slightly gray powder that melts at a temperature of 274 °C. Mesalazine is easily oxidized in the laboratory, and the temperature affects its stability in solution, the exposure to light and pH are factors that make the compound subject to oxidation. Mesalazine has some side effects such as gas, headache, stomach aches and diarrhea, and it has not been proven to have any symptoms for pregnant women, but its effect was found in the milk of lactating women, it is forbidden to give the drug to children under the age of two, and it is also forbidden to give mesalazine to kidney patients or people who are allergic to aspirin (6). Mesalazine is the active part of the drug Salazosulfapyridine (after fission occurs by colon bacteria) the pharmacological effect is due to mesalazine, as it was found that the side effects associated with Salazosulfapyridine diminish upon direct use of mesalazine (7). Several analytical methods were used for the determination of the drug mesalazine, including spectroscopic methods (9-12), electrochemical methods (13) in addition to chromatographic methods (14).

**Practical Part**
**The Devices Used**

Spectrophotometers were drawn and the absorbance measured in a dual-beam T92 + UV Spectrophotometer PGINSTRUMNTS equipped with a pair of 1 cm wide quartz cells. Samples were weighed with a BEL ENGINEERING scale and heated with a STAR WHITE heater.

**The Chemicals**

All chemicals and reagents were used with high purity

- **Mesalazine Standard Solution (100 µg/ml)**
  The mesalazine solution was prepared by weight (0.01 g) and dissolved in a small amount of ethanol and completing the volume with distilled water to the mark in a volumetric vial (100 ml).

- **Hydralazine reagent solution (100 µg/ml)**
  The reagent was prepared by dissolving (0.01 g) of Hydralazine in 2 mL of concentrated HCl and completing the volume with distilled water to the mark in a 100 mL volumetric vial.
- **Potassium periodate oxidizing agent solution (0.1 M)**
  The oxidizing agent was prepared with a weight (2.3 g) of KIO4 and dissolved in 2 ml of concentrated sulfuric acid and completing the volume to the mark in a 100 ml volumetric vial.
- **Solutions of surfactants**
  SDS and CTAB were prepared at a concentration of $5\times10^{-3}$ molarity and triton (x-100) at a concentration of 1%.
- **A solution of hydrochloric acid (1 M)**
  was prepared by diluting it with distilled water (8.33 ml) of concentrated hydrochloric acid with a percentage of 37% in a 100 ml volumetric vial.
- **A solution of sodium hydroxide (1 M)**
  was prepared by dissolving (4 g) in 100 ml of distilled water.

**Working Method and Standard Curves**

(1 ml) of hydralazine reagent (100 µg) was added and increasing amounts of mesalazine (0.25-4.75 ml) were added to it at a concentration of 2.5 to 47.5 µg/ml, then (1 ml) of the oxidizing agent was added potassium periodate (0.1 M) and (1 ml) of hydrochloric acid (1 M) and (2 ml) of triton (x-100) and the solutions were left for 10 minutes, after which the volume was completed to the mark with distilled water in 10 ml volumetric vials and the absorbance of the solutions was measured against their mock solution at wavelength 380 Nanometer, the method followed Beer's law up to the mentioned extents, followed by a negative deviation from the law.

**Pharmaceutical solutions**
**The medicinal preparation tablets (pentasa - 500 mg/tablet)**

It was prepared by weighing 10 tablets, grinding them well and weighing equal to 0.01 g of mesalazine, dissolved in 5 ml ethanol and 50 ml distilled water. The solution was heated, then cooled, and filtered into a 100 ml volumetric vial, and the filter paper was washed 4-5 times.

**The medicinal preparation Capsule (Mesacol - 400 mg/capsule)**

It was prepared by weighing 10 capsules and weighing equal to 0.01 g of mesalazine and dissolved in 5 ml of ethanol and 50 ml of distilled water. The solution was heated, then cooled and filtered into a 100 ml volumetric vial. The filter paper was washed from 4-5 times.

**Results and Discussion**

The optimal conditions necessary for the formation of the mesalazine complex were studied by changing one of the conditions and stabilizing the other, and studying the effect of these conditions on the absorbance as shown in the following steps:

**Studying the effect of the amount of hydralazine reagent**

Different amounts of hydralazine reagent from (0.5-3.0) ml were studied. It was found that using (1) ml of the reagent with 1 ml of the oxidizing agent potassium periodate with 2 ml of the drug mesalazine gives the highest absorption as in the following table.

| Volume of ml Reagent 100 ppm | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 |
|---|---|---|---|---|---|---|
| Absorbance | 0.132 | 0.143 | 0.137 | 0.134 | 0.131 | 0.128 |

**Studying the effect of the type of oxidizing agent (potassium periodate)**

The effect of different types of oxidizing agents was studied, and it was found that the use of potassium periodate gave the highest absorption as shown in the table.

| oxidant 0.1 M | $KIO_4$ | $KIO_3$ | $K_2Cr_2O_7$ |
|---|---|---|---|
| Absorbance | 0.147 | 0.066 | 0.052 |

**Studying the effect of the best amount of oxidizing agent**

A study was conducted of different amounts of the oxidizing agent potassium periodate from (0.25-1.5 ml) and it was found that the volume of (1 ml) gave the highest absorption, so it was relied on in the subsequent measurements and the results showed as shown in the following table:

| Volume of $KIO_4$ 0.1M | 0.25 | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
|---|---|---|---|---|---|---|
| Absorbance | 0.120 | 0.139 | 0.152 | 0.155 | 0.151 | 0.140 |

**Study of the oxidation time**

The time required for oxidation of the reagent was studied using potassium periodate. The solution was left for 5 minutes, then 2 ml of mesalazine was added and the solution was left for different periods of time. It was found that after 10 minutes it gave the highest absorption as in the following table:

| Time/min | 1 | 5 | 10 | 15 | 20 | 25 | 30 | 35 |
|---|---|---|---|---|---|---|---|---|
| Absorbance | 0.085 | 0.159 | 0.170 | 0.170 | 0.169 | 0.168 | 0.166 | 0.165 |

**Studying the effect of the types of acids**

The effect of different types of acids was studied using 1 ml of each acid, and it was found that hydrochloric acid gave the highest absorption as shown in the following table.

| Acid 1M | $CH_3COOH$ | $H_2SO_4$ | HCl | $HNO_3$ |
|---|---|---|---|---|
| Absorbance | 0.168 | 0.165 | 0.171 | 0.142 |

**Studying the effect of surfactants**

A study was conducted of different types of surfactants (positive, negative and neutral) to find out their effect on the stability of the formed product. It was found that using (2 ml) of triton (x-100) gave the highest absorption as shown in the table below.

| 2 ml of surfactant | λmax | Absorbance |
|---|---|---|
| without | 380 | 0.170 |
| triton(x-100) | 380 | 0.177 |
| SDS | 380 | 0.160 |
| CTAB | 380 | 0.155 |

**Study the effect of temperature**

The effect of time and three different temperatures on the absorbance and stability of the formed product was studied, and it was found that the best temperature is the laboratory temperature, which is equal to (21°C), which gave the highest and stable absorption for more than a quarter of an hour, as shown in the table below.

| time/min | RT | 35 $c^o$ | 50 $c^o$ |
|---|---|---|---|
| 5 | 0.164 | 0.160 | 0.132 |
| 10 | 0.177 | 0.152 | 0.126 |
| 15 | 0.176 | 0.148 | 0.114 |
| 20 | 0.176 | 0.140 | 0.110 |
| 25 | 0.175 | 0.132 | 0.108 |
| 30 | 0.161 | 0.125 | 0.097 |
| 35 | 0.159 | 0.120 | 0.082 |

**Studying the effect of the addition sequence**

The effect of the best addition sequence on the absorption of the formed product was studied. It was found from Table (8) that the order (III) gave the highest absorption as shown in the table.

| order number | order of addition | Absorbance |
|---|---|---|
| I | D+O+R+A+T | 0.143 |
| II | R+O+D+A+T | 0.153 |
| III | R+D+O+A+T | 0.176 |

D = mesalazine R = hydralazine O=$KIO_4$
A= HCL , T= Triton x-100

**Final Absorption Spectrum**

The absorption spectrum of the reaction, after fixing the optimal conditions for the reaction of the hydrolyzine reagent with the oxidizing agent potassium periodate, by adding 2 ml of mesalazine at a concentration of 100 µg/ml, showed

that the highest absorption of the formed product was at a wavelength of 380 nm, while the absorption of the mock solution was weak as shown in the figure ( ).

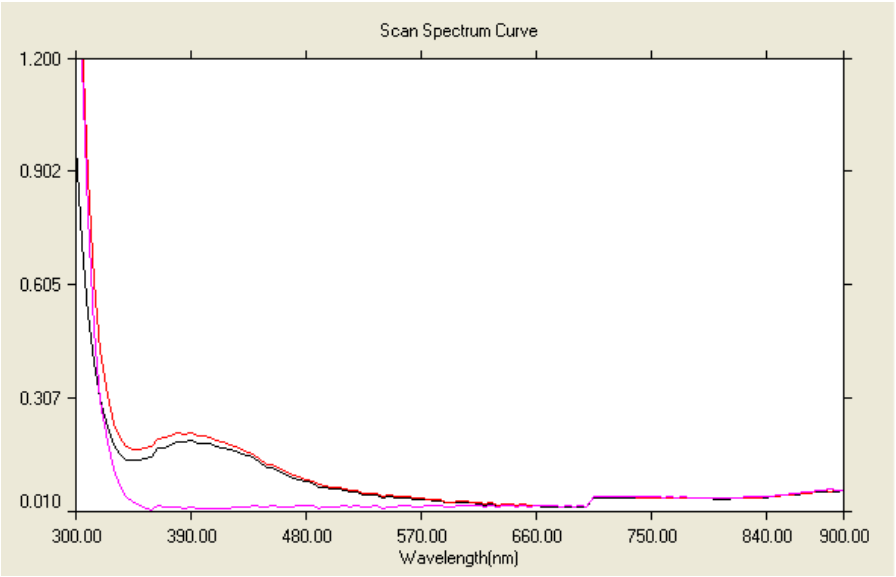
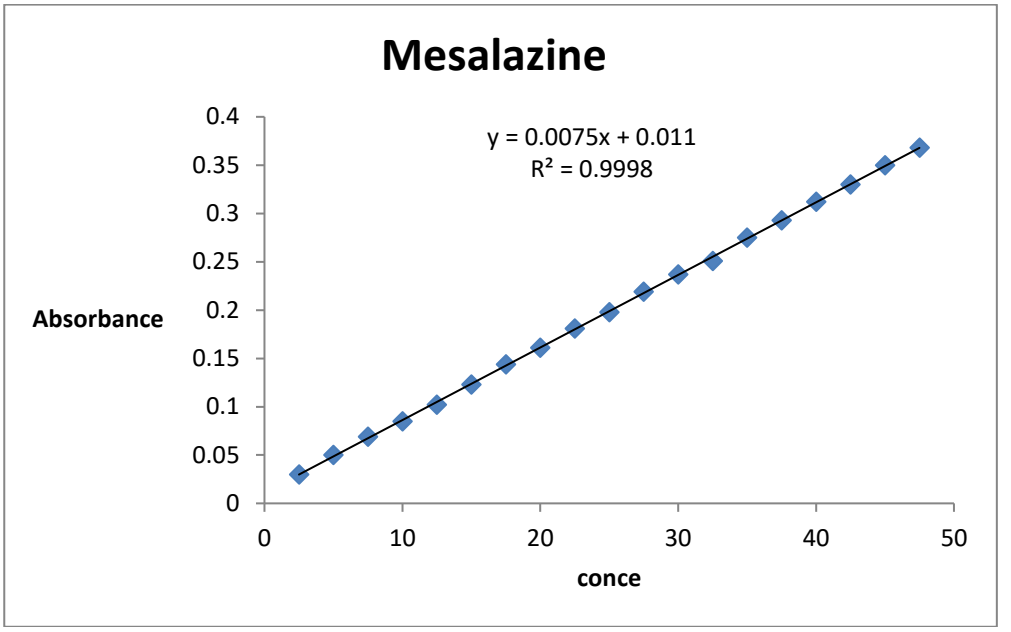



Figure ( ) shows the standard curves for the drug compound, which indicates the possibility of estimating (2.5-47.5) µg/ml of mesalazine and that there is a negative deviation after the upper estimated limits if the value of the estimation factor for the standard curves reaches (0.9991) for the drug, which indicates that it has excellent linear specifications While the molar absorbance reached (1147.5) liters.mol$^{-1}$.cm$^{-1}$ for the drug compound, which indicates the high sensitivity of the method. The diagram ( ) shows the linear specifications, estimated range, molar absorbance, limits of detection and quantitative estimation in the estimation of mesalazine.




(Standard curve for mesalazine estimation)

The limits of detection and quantitative estimation were calculated by taking replicates of Planck and measuring their absorption against distilled water and by applying equation (LOQ = $\frac{10\,\sigma B}{S}$) and equation (LOD = $\frac{3\,\sigma B}{S}$) as **σ** represents the standard deviation of the absorption of the mock solution and **S** represents the slope of the standard curve.

**Method accuracy and compatibility**

The accuracy and compatibility of the method was calculated by calculating the relative standard deviation and the recovery rate for five replicates at three different concentrations, as shown in the table ( ), where it showed that the method has good accuracy and compatibility, as the average recovery rate was 99.544, while the relative standard deviation rate RSD was 1.334%.

Table
Method accuracy and compatibility

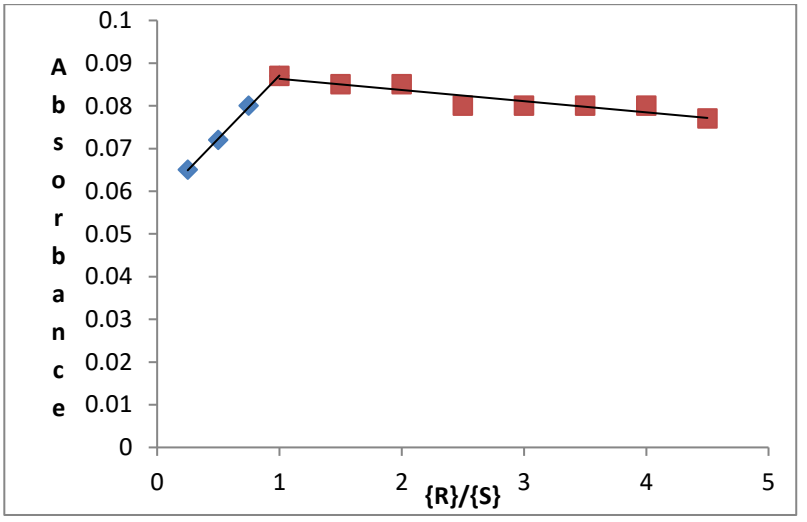
| Compound | Amount added ($Mg.ml^{-1}$) | | Recovery % | Average Recovery % | RE % | RSD % |
|---|---|---|---|---|---|---|
| | Taken | Found | | | | |
| Mesalazine | 20 | 20.01 | 99.38 | 99.544 | 0.62 | 2.138 |
| | 30 | 29.61 | 99.575 | | -0.425 | 0.799 |
| | 40 | 39.88 | 99.679 | | -0.321 | 1.066 |

Table ( ) Linear specifications, range, slope, molar absorbance, limits of detection and quantification in the estimation of mesalazine

| The measured values of the proposed method | |
|---|---|
| Beer's law limits (Mg.ml$^{-1}$) | 2.5 – 47.5 |
| Molar absorptivity (1.mol$^{-1}$.cm$^{-1}$) | 114.5 |
| LOD (Mg.ml$^{-1}$) | 0.3346 |
| LOQ (Mg.ml$^{-1}$) | 1.1146 |
| Correlation coefficient ($R^2$) | 0.9995 |
| Intercept | 0.0119 |
| Slop | 0.0075 |
| Sandells sensitivity (Mg.cm$^{-2}$) | 0.1333 |

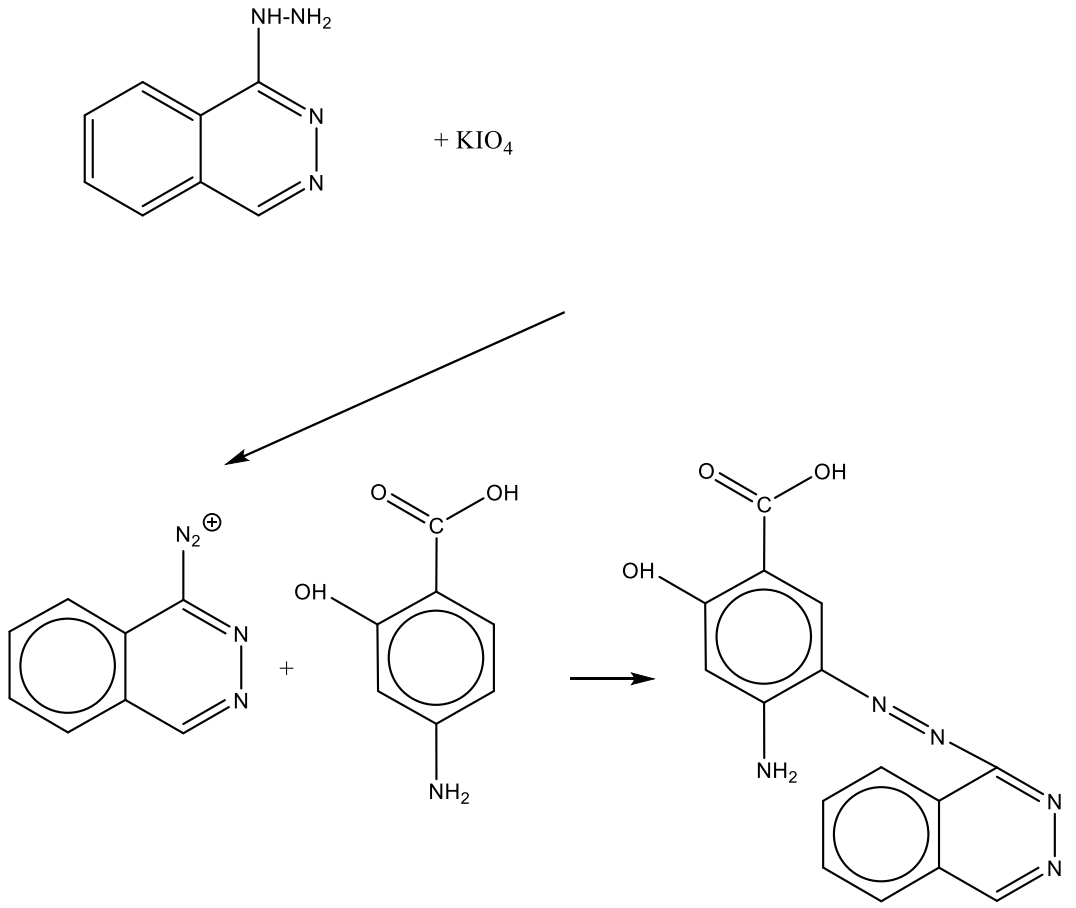
**Ratio of complex composition and stability constant**

The ratio of the composition of the formed complex between the drug compound mesalazine and the reagent hydralazine, was studied by applying the molar ratio method, using solutions of equal concentration ($6.5\times10^{-4}$ M), the obtained result indicates that the complex is formed in a 1:1 ratio of mesalazine to the reagent hydrolazine as shown in the following figure.




**Suggested Interaction**

The reagent hydralazine reacts with the medicinal compound mesalazine to form a compound and this complex is oxidized using potassium periodate as an oxidizing agent in the aqueous solution in a similar way to its oxidation with other oxidizing agents to form a brown-coloured complex, its bond ratio was 1:1 by the molar ratio method, and it has the highest absorption at 380 nm. Through previous studies, the proposed chemical equation is similar to the following formulas:




**Applying of the developed method to pharmaceutical preparations for the drug compound**

Table
Determination of drug compound on pharmaceutical preparations

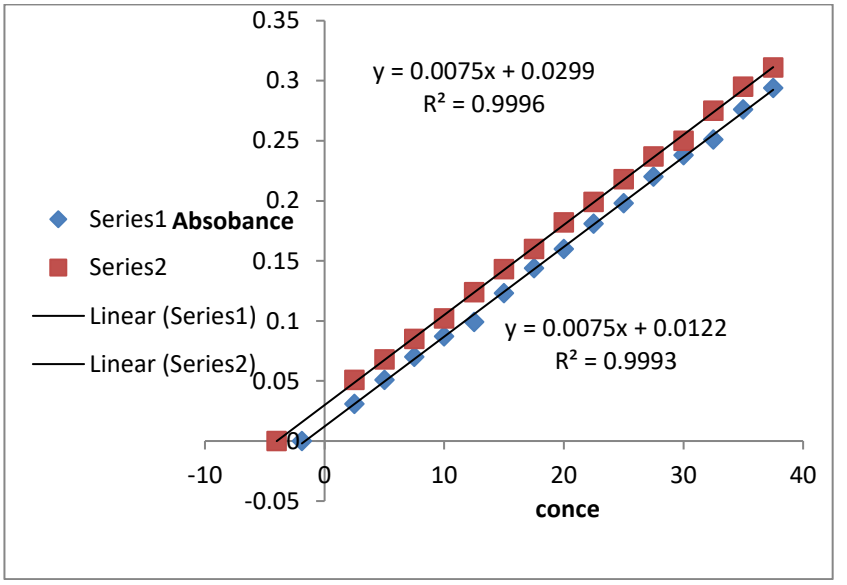
| Pharmaceutical preparation | certified value (mg) | Amount present (Mg/ml) | | Drug content found* | Recovery% | Average recovery % |
|---|---|---|---|---|---|---|
| | | Taken | Found | | | |
| Tablets | 500 | 10 | 10.13 | 494.12 | 98.824 | 99.25 |
| | | 20 | 19.86 | 496.89 | 99.379 | |
| | | 30 | 30 | 497.85 | 99.57 | |
| capsules | 400 | 10 | 10.9 | 395.28 | 98.82 | 99.23 |
| | | 20 | 19.76 | 397.24 | 99.31 | |
| | | 30 | 30.97 | 398 | 99.5 | |

**Evaluate the results of the proposed method**

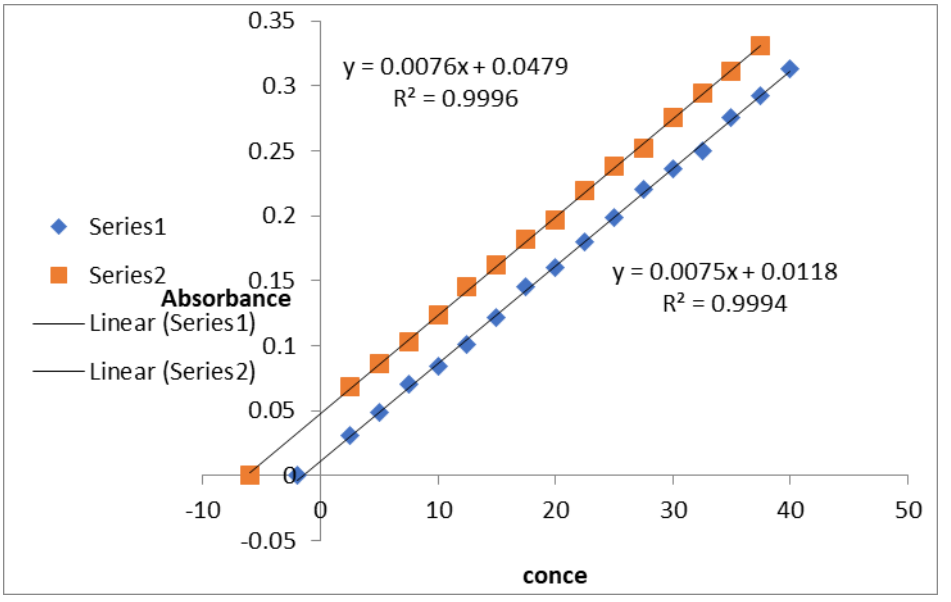
In order to prove the results of the spectroscopic method and its success in estimating the studied drug compound and that it is free from the interference of

additives by manufacturers in pharmaceutical preparations and because of the lack of requirements and devices of the standard method of the British Constitution (8) the standard addition method was used in the proposed method on pharmaceutical preparations as in the figure ( ) which includes a preparation Tablets and figure ( ), which includes the capsule preparation.

**The standard method for Pentasa**




**The standard method for Mesacol**



## Conclusions

An accurate, sensitive and easy indirect spectrophotometric method has been proposed for the determination of mesalazine, which is based on the reaction of the reagent hydralazine with mesalazine with the oxidizing agent potassium periodate in the acidic medium, the method has high accuracy (recovery rate (99.54%)) and good compatibility, RSD≥1.33. Also, the proposed method was successfully applied for the determination of mesalazine in its pharmaceutical preparations in the form of tablets and capsules.

## References


1. Mesalazine drug info (Data base available on internet): Drug bank available from Pubchem ncbi-nlm.nih.gov/compound/5-amino salicylic 20% acid.
2. British Pharmacopoeia (2013). on CD rom, system simulation 1td., the stationary office, London.
3. M-Brunner, S. Ziegler, A.F.D. Di Stefano and Dehyhanyar, (2006), “Gastrointer stinal transit, release and Plasma Pharma Co Kinetic of a new oral budesonide formulation”, British J. Clin, Pharm, 61,31-38.
4. H. Wiersma, J.C. Escher and K. Dilger, (2004), “Pharma Co Kinetics of mesalazine Pellets in children with in flammatroy bowel disease” Inflammatory Bowel Disease, 10, 626-631.
5. Safa Tahmasebi, Mohamed A El-Esawi, Zaid Hameed Mahmoud, Anton Timoshin, Hamed Valizadeh, Leila Roshangar, Mojtaba Varshoch, Aydin Vaez, Saeed Aslani, Jamshid G Navashenaq, Leili Aghebati-Maleki, Majid Ahmadi., ‘Immunomodulatory effects of Nanocurcumin on Th17 cell responses in mild and severe COVID-19 patients’., Journal of cellular physiology, 236(7):2325-2338, 2021.
6. US National Library of Medican 8600 Reckvill Pike, Bethesda, MD 20894 US Department of Health and Human service National Institutes of Healt Page last updated May 2018.
7. A.B. Hawthorne (2009), "A Review of multimatrix system (MMX) mesalazine in the management of ulcerative colitis” Clin. Med. Insights: Thera Pentics, 1, 1423-1436.
8. D Kaduim, Z Mahmoud, F Mousa, “Green Biosynthesis of Iron Oxide Nanoparticles and Testing Their Inhibitory Efficacy Against Some Pathogens”., Asian Journal of Water, Environment and Pollution, 18(4):119-123, 2021. doi: 10.3233/AJW210051
9. Z. A. M. Al-Shrefy (2012) “ Development of spectrophotometric and fluorophotometric methods for determination of phenolicandnitrogenic drug compounds”, M.Sc. Thesis Mosul University, 47-66.
10. H. H. Abdu-Allah ; A. N. A. El-Shorbagi ; S. G. Abdel-Moty. R. El-Awady and A. A. M. Abdel-Alim (2016) “5-Aminosalyicacid (5-ASA) A unique anti-inflammatory salicylate”, Med. Chen, Chen, 6(5), 306-315.
11. Sama, N. S. ; Gurupadayya, B. M. and Kumar, C. A. (2011) “Spectrophotometric determination of mesalamine by PDAC and NQS reagents in bulk and tablet dasage form Journal of Pharmacy Research, 4(1), PP. 39-41.

12. Al-Nuaimi, K. (2017) Development of spectrophotometric and flourophotometric methods for determination of some drug compounds M.Sc. Thesis, University of Mosul, Iraq. (in Arabic).
13. Kumar, A. A. ; Rani, T. S. ; Ganesh, P. S. and Swang B. E. K. (2017) Electrochemical oxidation of mesalazine drug at poly (glutamic acid) modified glassy carbon electrode. Anal. Bioanal. Electrochem, 9(3), PP.328-339.
14. NANDPATI, S. ; REDDY, D. V. K. and UBA, S. (2013) Avalidated ultra performance liquid chromatography method for assay determination of masalmine. In conference on Harmonization (ICH) guidelines (Vol.14, P.15).
15. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
16. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202