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Quantitative determination of a Mesalazine using reversed phase high- performance liquid chromatography (RP-HPLC)

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*---**Mesalazine was quantified in a fast, accurate and sensitive way using reversed-phase high-performance liquid chromatography (RP-HPLC) method. In this method, a separation column of porous silica particles L10 (300 × 4.6mm) was used, the flow rate is 0.8 ml/min, the column temperature is 25 °C, the reagent wavelength is 230 nm, and the injection volume is 20 µl. As for the mobile phase, it consisted of acetonitrile 10% 0.1 and phisphoric acid 0.1% and 90% water and the pH was 4. The method was validated by calculating the recovery that ranged between (95.0457-102.280%), and the correlation coefficient (0.9991) and linearity (20-160 µg/ml).The method was successfully applied for the quantitative determination of the mentioned drug.

***Keywords*---**mesalazine, RP-HPLC, quantitative determination.

Introduction

Mesalazine (MSL), also known as mesalamine or 5-aminosalicylic acid (5-ASA), is an anti inflammatory drug used to treat inflammation of the digestive tract (Crohn's disease) and mild to moderate ulcerative colitis [(1)] . Mesalazine acts as a scavenger of oxygen-derived free radicals, which are produced in greater numbers in patients with inflammatory bowel disease. Figure (1) Shown the chemical structure of Mesalazine[(3)].

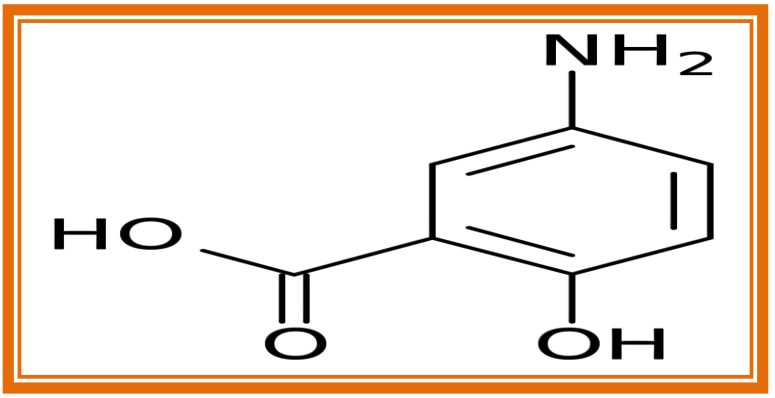

Figure (1) Mesalazine chemical structure[3]

Mesalazine is administered orally or rectally in the treatment of ulcerative colitis and Crohn's disease [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 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 [14,16,17] and spectrophotometric [15] methods.

Materials and reagents

MSL used as a standard solution is of Germany origin, acetonitrile is supplied by Supelco (USA), and distilled water is used by the SDI Company, and the same substances mentioned throughout the experiment was used after they were purchased from the local Iraqi market. As for the pharmaceutical preparations, they contained Mesalazine 500 mg Pentasa; High-purity materials were used: Mesalazine (Sigma-Aldrich).

Chromatographic Conditions

A HPLC device was used, type Shimadzu, Japan, Kyoto, which consists of a pump type LC-20AD and a reagent SPD-20A, and used a separation column type Porous silica particles (230mm), and the working conditions were: flow rate 0.8ml/min; column temperature 25°C; The wavelength of the reagent is 230 nm; The injection volume was 20µl, the mobile phase was made of acetonitrile 10% 0.1 and phisphoric acid 0.1% and 90%, and the pH was adjusted at 4. Then also used Whatman filter paper No. 0.41 to remove impurities so that the prepared and used solutions were clear and measurable.

Standard Solutions of mobile phase

The standard solution was prepared by dissolving the drug in the mobile phase solution, and then it was diluted to the required concentration. The mobile phase was prepared from acetonitrile10**%** Phsphoric acid 0.1% Water90% .

Preparation of Standard Solutions

Mesalazine standard solution (1000 µg/ml)was Prepared by dissolving 0.1 g of the drug in an appropriate volume of solvent, then fill the volume up to 100 ml of the same solvent in a suitable volumetric flask, transfer 10 ml standard stock solutions with a concentration of 1000 µg/ml of the drug into 100 volumetric flask. The volume was supplemented with the mobile phase to obtain a concentration of 100 µg/ml of the drug.

Preparation of pharmaceutical Solution

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 dissolved in the mobile phase (acetonitrile10% phsphoric acid 0.1% water90%) was shaken and put it in an ultrasound water bath for 5 min. Complete the volume with the same solvent and filter it with Whatman No.42 filter paper dissolve then completed the volume in the mobile phase to the mark to obtain 1000 µg/ml of MSL transferred 10ml to a 100 ml volumetric flask and diluted with same mobile phase up to the mark so that the concentration was 100 µg/ml.

Results and Discussion

The Optimal Conditions for the Experiment

The optimum chromatographic conditions were improved by changing the wavelength, as it was found that the best wavelength gives the high peak and retention time it was 230 nm, Figure 3, Table 1.

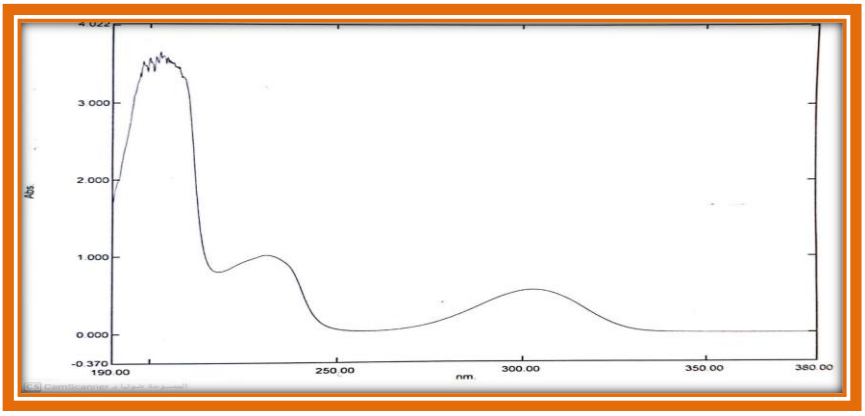

Figure: The spectrum of MSL

Table (1): The wavelengths effect

Wavelength(nm)	AUP mV	Rt min.
230	5628903	1.941
240	2853159	1.958
300	896947	1.464

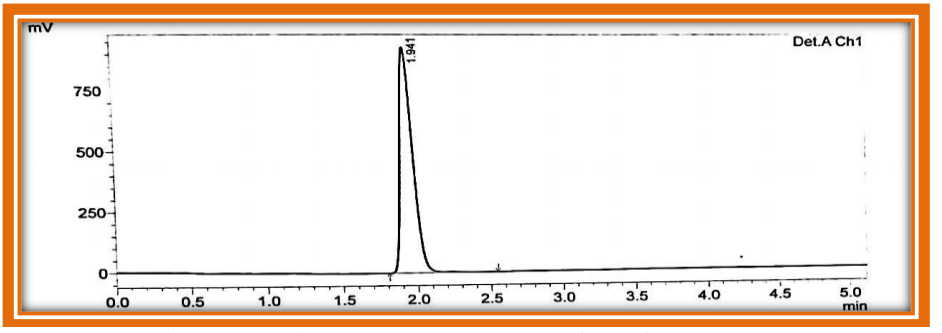

Figure 3: Chromatogram at wavelength 230 nm.

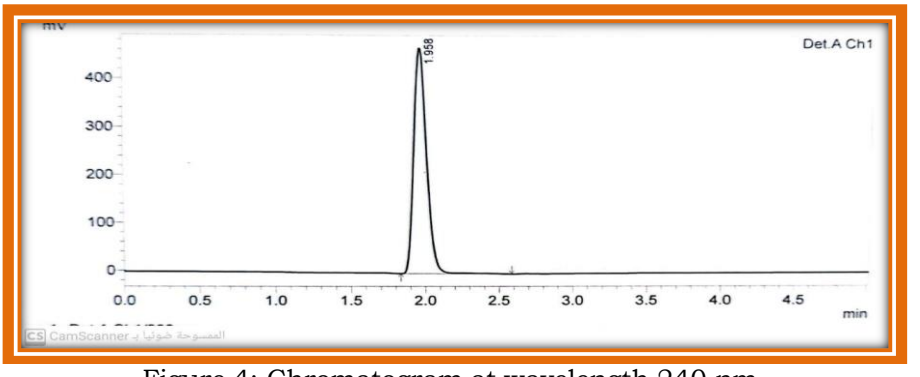

Figure 4: Chromatogram at wavelength 240 nm.

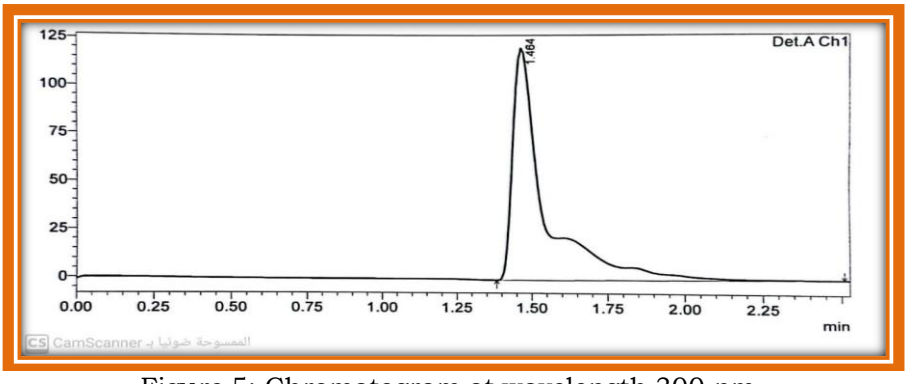

Figure 5: Chromatogram at wavelength 300 nm.

Column selction

four colums were used column for the separation (L1,L3,L7,L10) the best column for the separation was studied and it was of the type L10, which gave the best results, Figure 3 and Table 2.

Table (2): Platelet count values for each column

Column	AUP mV	Rt min.
L1	5628903	1.941
L3	5494402	3.119
L7	5554882	2.124
L10	**5594964**	**3.428**

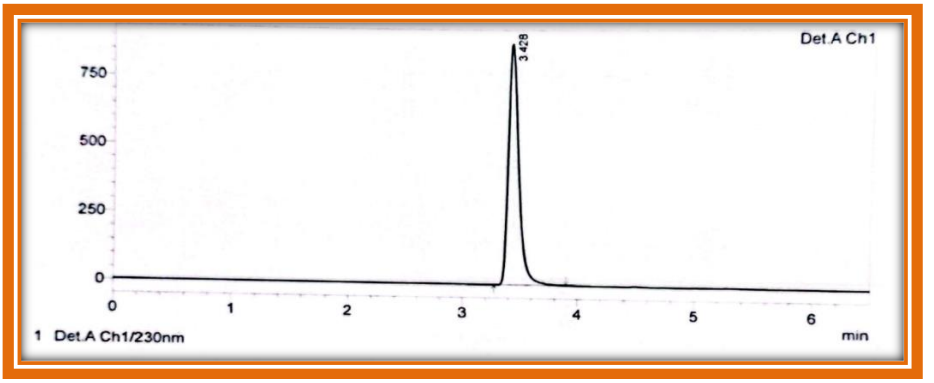

Figure 6: Chromatogram of the best column

Selection the mobile phase solution

It was also found that the best mixture ratio for the mobile phase was (10:90) because it was give best peak aera and retention time.

Table (3): Platelet count values for each mix ratio.

Ratio of Act:0.1%GAA	AUP mV	Rt min.
10 : 90	**5858366**	**3.350**
30 : 70	5602937	2.247
40:60	5734185	2.064
70 : 30	5837393	1.837
20 : 80	5696841	2.539

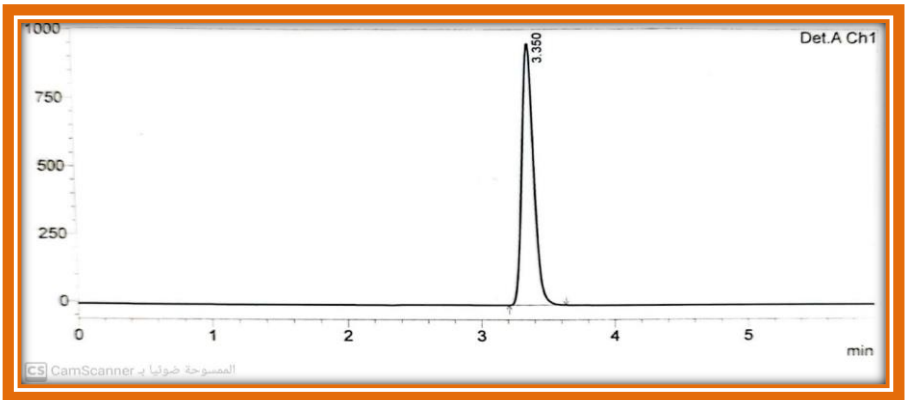

Figure 7: Chromatogram of the best mixture ratio

Effect of pH

The pH had a significant effect on the shape of the chromatogram as well as it was found that the pH was 4 gives the best results as in Figure 5 and Table 4.

Table (4): Platelet count values for each pH

pH	AUM mV	Rt min.
3	4987366	3.413
3.5	4998946	3.287
3.8	5035643	3.313
4	**6674969**	**2.798**
5	6356963	2.738
6	6031042	2.806
6.5	5573452	3.044

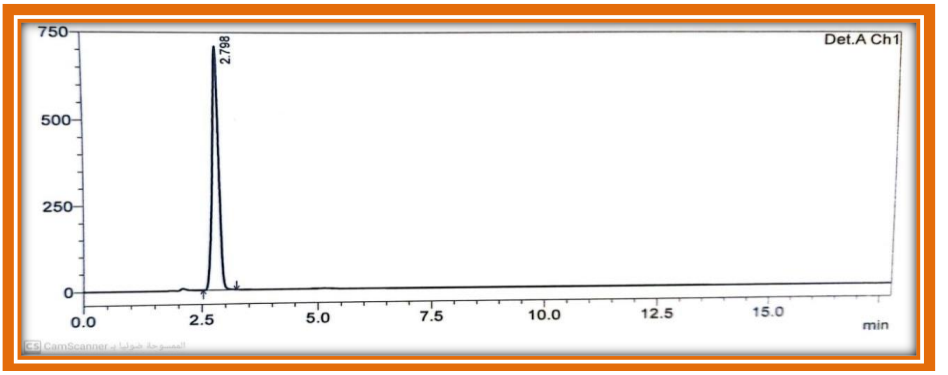

Figure (8): chromatogram of the best pH

The flow rate

The flow rate average was studied, and depending on the optimal conditions above In Figure 4, and Table 3 it was found that the best flow rate is 0.8 ml/min.

Table (5): Platelet count values for each flow rate

Flow Rate ml/min.	AUP mV	Rt min.
0.8	**7542576**	**3.763**
1.0	5858366	3.350
1.2	39922713	2.857

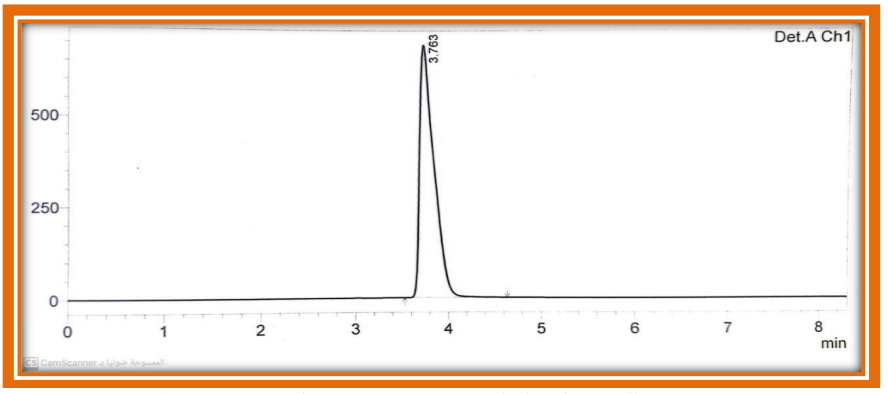

Figure (9): chromatogram of the best flow rate

Temperature

The table(6) show that effect of temperature to the area and retention time. It was also found that the best temperature it was 25 ^{O}C because it was give best peak aera and retention time.

Table (6): Platelet count values for each temperature

Temperature C°	AUP mV	Rt min.
Lab.temp	**7454912**	**3.763**
30°	4769945	3.368
40°	4743907	3.245
50°	4706354	3.140

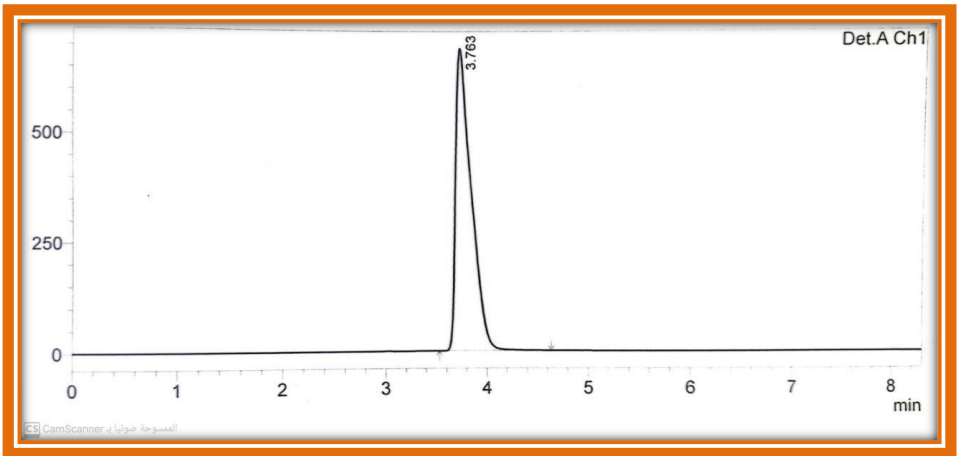

Figure (10): chromatogram of the best Figure temperature

Calibration Curve

The calibration curve of Mesalasine was built according to the optimal conditions above and it was found that the range of concentrations was (20-160 µg/ml). Fgure 6 shows the linearity and correlation coefficient of the drug, and also shows that the method has good agreement through the RSD was btween(0.00237-0.0509 %) study.

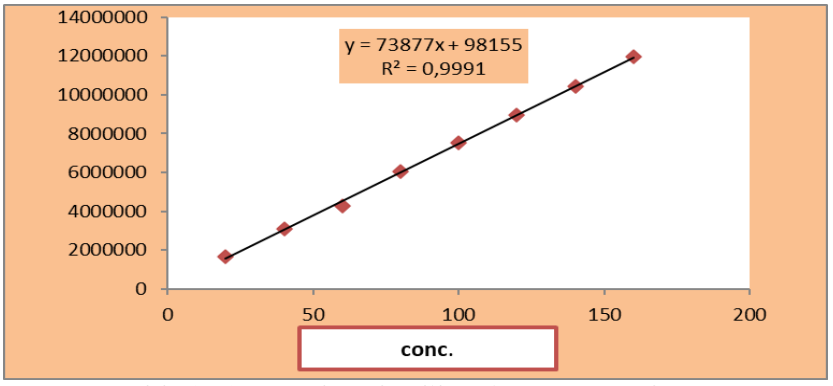

Table (11): Results of calibration curves of MSL

Method Accuracy

The recoverability was calculated to show the accuracy of the drug estimation method. Table 7 shows that the proposed method for instantaneous drug estimation has high accuracy, as the recovery of mesalazine was btween (95.045-102.280), and therefore the proposed method can be adopted for quantitative determination.

Table 7. Results of the Recovery Tests for the Drugs*

Conc. µg/ml	Average AUP mV	Founded mg/ml	Rec%
20	1684993.2	21.479	102.280
40	3076018	40.308	100.770
60	4311171	56.769	95.0457
80	6019553	80.152	100.190
100	7454912	99.581	99.581
120	8782349	117.549	97.957
140	10342404	138.666	99.0473
160	11600085	155.690	97.3064
Slope	73877	Intercept	98155
LOD	0.157 mg/ml	LOQ mg/ml	0.476
R^2	0.9991	RSD%	(0.00237- 0.0509)

Method Application

The proposed method was applied to some pharmaceutical forms available in the local market, where it was found that the method is effective, as the Recovery percentage (95.242-98.766 %), Table 8 show that s Application of the method to some pharmaceutical forms containing MSL.

Table (8): Application of the method to some pharmaceutical forms containing MSL

Sample Drug	Conc. µg/ml	AUP mV	Found µg/ml	Rec%
Mesalasine	60	4476175	59.260	98.766
	100	7123507	95.423	95.423
	140	9948976	133.340	95.242

Conclusion

This method can be used for the quantitative determination of MSL in pharmaceutical forms. The method was validated and found to have high accuracy and precision. This method can also be used in the quality control department to test the pharmaceutical forms that Mesalasine contain.

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