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Spectrophotometric Determination of masalazine Via Oxidative Coupling Reaction

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ABSTRACT— The current study looks into a new spectrophotometric approach for determining masalazine in depth. The method is based on an oxidative coupling reaction of the chemical with trihydroxy benzoic acid in an acidic medium in the presence of potassium chromate as an oxidant to produce a stable – Brown coloured product with maximum absorbance at 390 nm. 2863.62 l.mol-1.cm-1 molar absorptivity. In the range of (0.4 -24) ppm, Beer's law was followed.

KEYWORDS: Spectrophotometry, masalazine, Oxidative coupling, trihydroxy benzoic acid

1. INTRODUCTION

Mesalazine is chemically, 5-aminosalicylic acid (5 ASA), which is used as a gastrointestinal antiinflammatory drug for the treatment of inflammatory bowel diseases [1] and active ulcerative proctitis [2], [3] Because mesalazine is swiftly and completely absorbed from the upper gastrointestinal tract, enteric-coated formulations for drug release in the terminal ileum and colon have been developed [4]. A variety of analytical methods for determining MSZ in medicinal dose have been published [5], [6] spectrophotometric [7- 15], HPLC [16- 22] Voltmeter voltage difference [23] chromatography [24] Phosphorus spectroscopy [25] Fig (1) 5-Amino-2-hydroxybenzene-1 carboxylic acid [Mesalazine]

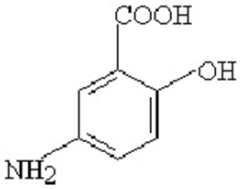

Fig (1) Mesalazine

2. Experimental

2.1 Apparatus

All spectrum and absorbance measurements were performed with matched 1-cm quartz cells using a Shimadzu UV-VIS 1800 digital double-beam recording spectrophotometer (Kyoto, Japan).

2.2 Reagents

All Chemicals used are of the highest purity available.
Mesalazine (100 $\mu g ml^{-1}$): 0.01g is dissolved in ethanol, solution is transferred into a 100 ml volumetric flask, and diluted to the mark with distilled water
Hydrochloric acid solution: A diluted (0.05M) was used
Trihydroxy benzoic acid solution (2.72×10^{-3}M): This solution is prepared by dissolving 0.03g of

Trihydroxy benzoic acid in distilled water in 100ml volumetric flask.
Potassium chromate (4.6×10^{-3}M): 0.09g of pure potassium chromate was dissolved in 100ml distilled water.

2.3 Pharmaceutical solution

The contents of ten tablets of the medicinal preparation Pentasa Mesalazine were weighed, crushed and mixed well, then weighed the equivalent of one tablet (500 mg of mesalazine) and dissolved in a small amount of ethanol and water and then treated in the same manner described in the tablets analysis.

3. Results and discussion

2 ml of trihydroxy benzoic acid (2.72×10^{-3}M) were added into a series of 25ml calibrated flask and 2 ml of potassium chromate (4.6×10^{-3}M) followed by the addition of increasing volumes of ($100\mu gml^{-1}$) masalazine solution and followed by 1 ml of (0.05M) hydrochloric acid. The solutions were diluted to the desired concentration with distilled water, and the reaction mixture was set aside for 5 minutes. Each solution's absorbance was measured at 390nm in comparison to a blank prepared in the same method but without masalazine.

3.1 The Investigation of Optimal Reaction Conditions

The different parameters that affect and are related to the aforementioned colored product have been investigated, and the best circumstances have been chosen.

3.2 Effect of the potassium chromate

This effect was studied The absorbance was measured at 390nm versus blank

Table (1): Effect of the potassium chromate

ml of K_2CrO_4 (4.6×10^{-3} M)	Absorbance/min.						Blank
	0	5	10	15	20	30	
1	0.064	0.065	0.065	0.065	0.065	0.065	0.018
1.5	0.099	0.099	0.098	0.099	0.098	0.099	0.024
2	0.121	0.121	0.120	0.121	0.121	0.121	0.042
2.5	0.076	0.076	0.077	0.077	0.077	0.077	0.038
3	0.025	0.025	0.025	0.026	0.027	0.027	0.007

The result shows that the dye formation reached the maximum with 2ml of potassium chromate.

3.3 Effect of different acids on absorbance

In order to select the most suitable acid, the oxidative coupling reaction was carried out using various acids (hydrochloric, sulphuric, acetic acids). This has been done by placing into a series of 25 ml calibrated flasks, 2ml of trihydroxy benzoic acid solution (2.72×10^{-3}M) 2ml of (4.6×10^{-3}M) of potassium chromate, followed by 2ml of ($100\mu g\ ml^{-1}$) of mesalazine and different volume of (0.05M) of acid (hydrochloric, sulphuric, acetic acid). After the solutions were diluted to the marks using distilled water, the reaction mixture was allowed to settle for 5 minutes. The absorbance at 390 nm was measured in contrast to a regent blank. The most suitable acid for the reaction was hydrochloric acid, as shown in table (2).

Table (2): Effect of different acids on absorbance

ISSN: 1343-4292
Volume 140, Issue 02, May, 2022

Ml of acid	HCl (0.05M)	H_2SO_4 (0.05M)	CH_3COOH (0.05M)
0.5	0.052	0.034	0.009
1	0.085	0.067	0.026
1.5	0.103	0.092	0.063
2	0.123	0.081	0.094
2.5	0.095	0.046	0.072

The result shows that the dye formation reached the maximum with 2ml of hydrochloric acid

3.4 Effect of reagent concentration

This effect was studied by placing different volume of trihydroxy benzoic acid (2.72×10^{-3}M) into a series of 25ml calibrated flask. The absorbances were measured at390 nm versus blank. The results obtained in Table (3) indicate that the use of 0.5 ml of (2.72×10^{-3}M) trihydroxy benzoic acid reagent gave the maximum colour intensity.

Table (3): Effect of the concentration of reagent on absorbance.

Reagent conc.(ml)	Absorbance
0.2	0.083
0.5	0.123
1	0.091
1.5	0.072
2	0.057
2.5	0.030

The result shows that the dye formation reached the maximum with 0.5ml of trihydroxy benzoic acid

3.5 Effect of amount of hydrochloric acid (0.05M).

The effect of the amount of hydrochloric acid was studied by placing into a series of 25-ml calibrated flasks, 0.5ml of (2.72×10^{-3}M) trihydroxy benzoic acid, 2ml of (4.6×10^{-3}M) potassium chromate, followed by 2ml of (100µgml^{-1}) and different volume of (0.05M) hydrochloric acid. The solutions were diluted to the mark with distilled water. The absorbances were measured at390nm versus blank.

Table (4): Effect of hydrochloric acid amount on absorbance of the coloured product.

Hydrochloric acid solution(0.5N)	Absorbance
0.5	0.052
1	0.085
1.5	0.103

2	0.123
2.5	0.095

Because the results in Table (4) showed that using 2ml resulted in the most intense color, 2ml was chosen in all following studies.

3.6 Effect of temperature

The impact of temperature on the coloured product's absorbance was investigated. This was implemented by placing into three 25ml calibrated flasks,0.5ml of (2.72×10^{-3}M) trihydroxy benzoic acid, 2 ml of (4.6×10^{-3}M) potassium chromate, followed by 2ml of ($100\mu gml^{-1}$) Mesalazine solution, and 2ml of (0.05M) hydrochloric acid solution. The solution was diluted to the desired concentration with distilled water, and the first flask was left to stand at room temperature for an increasing period of time, the second at 5°C, and the third in a water bath at 50°C. At 390nm, the absorbance was measured over time versus a blank prepared in the same method but without Mesalazine. The results obtained in Table (5) indicated that the absorbance of the coloured product was decreased when the reaction was carried out at 0°C or 50°C As a result, it is advised that the reaction mixture be carried out at room temperature.

Table (5): Effect of temperature on absorbance of coloured product.

Temp. °C	**Absorbance/minutes**							
	0	**5**	**10**	**15**	**20**	**25**	**30**	**40**
5	0.021	0.027	0.032	0.034	0.038	0.041	0.048	0.059
R.T.	0.123	0.123	0.123	0.123	0.123	0.123	0.124	0.124
50	0.402	0.472	0.451	0.443	0.438	0.415	0.401	0.388

*R.T.=Room temperature=25 C

3.7 Stability of the product.

This was studied by placing0.5ml of (2.72×10^{-3}M) trihydroxy benzoic acid, into a series of 25ml calibrated flasks, followed by 2. ml of (4.6×10^{-3}M) potassium chromate and 2ml of($100\mu gml^{-1}$) mesalazine and 2ml of (0.05M) hydrochloric acid. The solution was diluted to the mark with distilled water and the absorbance was measured at 390nm at different periods versus reagent blank. The results obtained in Table(6). show that the product needs 5 minutes to attain maximum absorbance and it remains stable for about 45 minutes.

Table (6): Rate of reaction and stability of product.

Time (min)	0	5	15	20	25	30	35	40	45	50	55	65
Absorbance	0.120	0.123	0.123	0.123	0.123	0.124	0.124	0.124	0.125	0.123	0.122	0.120

3.8 Order of addition of reagents.

The reagent 0.5ml of (2.72×10^{-3}M) trihydroxy benzoic acid (**R**), the oxidant 2ml of (4.6×10^{-3}M) (**ox**) and the sample 2ml of ($100\mu gml^{-1}$) solution masalazine, followed by 2ml hydrochloric acid (0.05M) were mixed in various orders as is shown in Table (7).

Table (7): Effect of order of addition on the absorbance of the coloured product.

ISSN: 1343-4292
Volume 140, Issue 02, May, 2022

Reaction components	Order number	Absorbance at 390nm
R+OX+S+HCL	I	0.123
R+S+OX+HCL	П	0. 076
S+R+HCL+OX	III	0.121
OX+S+R+HCL	IV	0. 092

The results indicate that order (I) gives higher absorbance of the product and therefore it was selected in all subsequent experiments.

3.9 Final absorption spectra.

The mesalazine-trihydroxy benzoic acid complex generated under the ideal conditions exhibits an absorption spectrum extending between 350 and 450nm, with a maximum absorption at 390nm, in contrast to the reagent blank, which has a tiny absorption at max. As a result, the maximum absorption wavelength of 390nm has been chosen for future research.

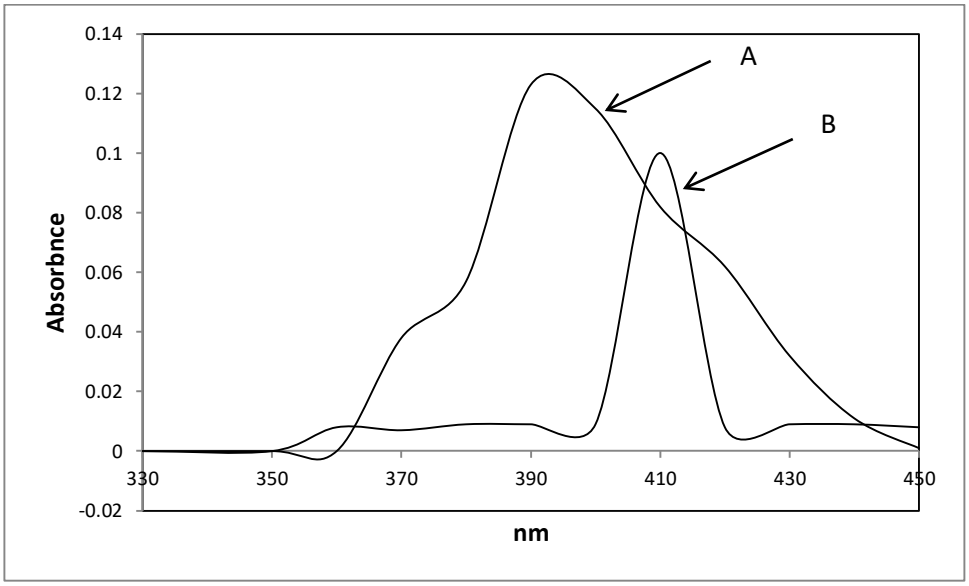

Fig. (2): Absorption spectra of 8µgml-1 mesalazine measured, (A)Against blank, (B) blank against distilled water.

3.10 Quantification

Having thus establishing optimum reaction conditions, a calibration graph is constructed by plotting absorbance versus concentration. Beer's law is obeyed over the range (0.4-24)µg /ml of the solution(Fig3).

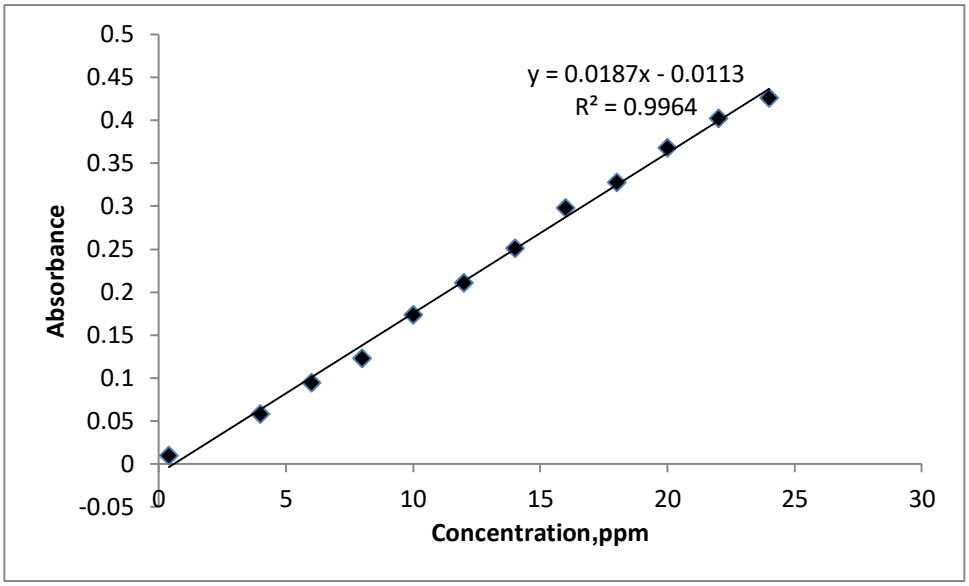

Fig. (3): Calibration graph for the determination of mesalazine

3.11 Accuracy and precision of the method.

To check the accuracy and precision of the method, mesalazine has been determined at three concentrations. The results are shown in Table (8) indicated that the method is performing well.

Table (8): Accuracy and precision of the method

Amount of mesalazine taken, µg/ml	Amount of mesalazine faund, µg/ml	Relative error, % *	Relative standard deviation, % *
10	9.9	+1.0	±1.41
16	16.13	-0.81	±0.607
22	21.9	+0.45	±0.575

* Rate 6 replicates

3.12 Product Characteristics

The mole–ratio approach was used to evaluate the stoichiometry of the reaction between mesalazine and trihydroxy benzoic acid in the presence of potassium chromate. In this experiment, 0.5 ml trihydroxy benzoic acid (2.7210-4M) was poured to a succession of 25ml calibrated flasks, followed by increasing quantities of (2.7210-4M) mesalazine and 2 ml potassium chromate (4.610-4M), and finally 2 ml (0.05M) hydrochloric acid. The reaction mixture was allowed to stand for 5 minutes after the solutions were diluted to the desired concentration with distilled water. At 390nm, the absorbance of each solution was compared to a blank. The results in fig. (4) revealed the presence of a 1:1 ratio of mesalazine to trihydroxy benzoic acid.

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Volume 140, Issue 02, May, 2022

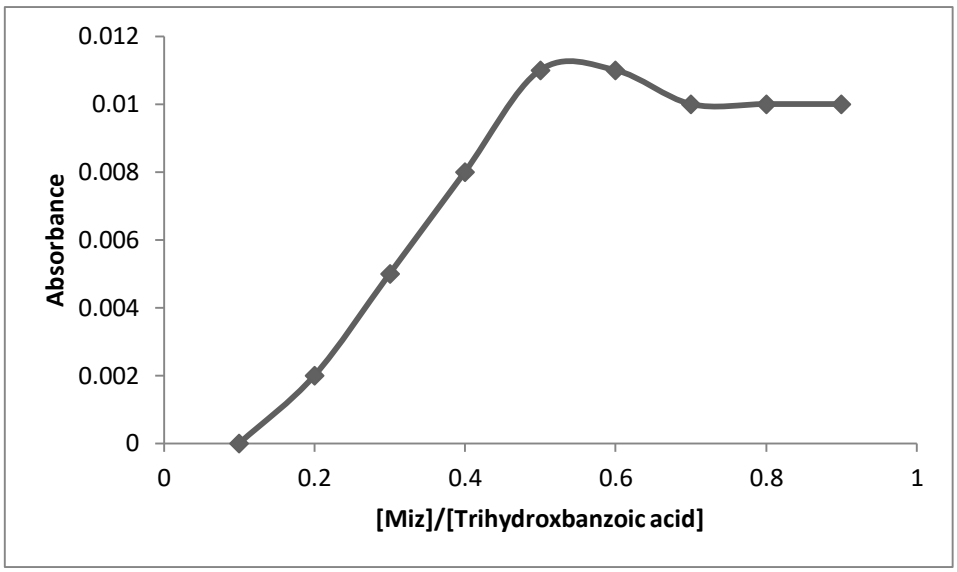

Fig. (4): Mole–ratio plot for mesalazine to the trihydroxy benzoic acid reagent in the presence of potassium chromate

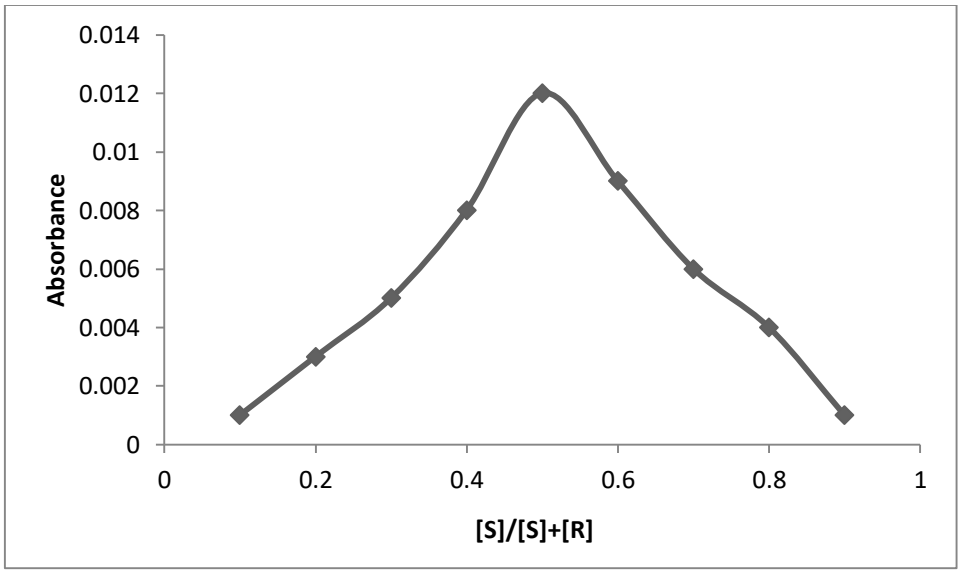

Fig (5) Jops method for measalazine to the trihdroxy benzoic acid reagent in the presence of potassium chromate

Application of the method:

Table (9): Accuracy and precision of Application of the method

Pharmaceutical formulation	Amount of mesalazine taken, µg	Relative error, % *	Relative standard deviation, % *
Pentasa tablets Messalazine	10	1.3	±1.02
	16	- 0.06	±0.299
	22	0.9	±0.147

* Rate 6 replicates

4. Conclusion

For the determination of mesalazine in aqueous solution, a new spectrophotometric approach has been presented. The procedure involves combining mesalazine with the trihdroxy benzoic acid reagent in the presence of potassium chromate to produce a colored dye with a maximum absorption wavelength of 390 nm. The molar absorption coefficient is 2863.62 $l.mol^{-1}cm^{-1}$ The proposed approach is used to determine mesalazine concentrations in two synthesized pharmaceuticals.

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