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(Research Article)

A simple oxidative coupling-based spectrophotometric method for the determination of Mesalazine in pure form and pharmaceutical formulations

Noor Mansoor Ghaiballah *

Department of Chemistry, College of Science, University of Kirkuk, Kirkuk, Iraq.

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Abstract

A straightforward, rapid, precise, and sensitive spectrophotometric technique has been established for the quantification of mesalazine (MES) in both its pure form and within pharmaceutical tablet formulations. This method relies on the oxidative coupling reaction that occurs between mesalazine and the metol reagent, facilitated by potassium periodate (KIO_4) acting as the oxidizing agent and sodium hydroxide (NaOH) serving as the alkaline medium, leading to the formation of a stable purple-hued chromogen exhibiting maximum absorbance at 628 nm. The experimental conditions affecting color development were optimized, including reagent volume, oxidant volume, alkaline medium, order of addition, temperature, and reaction time. Beer's law was obeyed over the conc. range of 3.0-20.0 µg mL^{-1} with the regression equation A = 0.0386C + 0.1432 and a coefficient of determination (R^2) of 0.9991. The method showed good accuracy and precision, with recoveries of 98.87-101.20% for the pure drug and 98.13-101.00% for tablet analysis. The D.L was 0.1759 µg mL^{-1} and the limit of quantitation was 0.5863 µg mL^{-1}. The suggested approach was worked implemented to AwaSalazin® 500 mg tablets with no significant interference from common excipients, indicating its suitability for routine quality-control analysis.

Keywords: Mesalazine; Metol; Potassium Periodate; Oxidative Coupling Reaction; Spectrophotometry; Pharmaceutical Preparations

1. Introduction

Mesalazine (5-aminosalicylic acid, 5-ASA) is an aminosalicylate derivative widely used as a first-line agent for induction and maintenance of remission in ulcerative colitis and in selected clinical settings of Crohn's disease [1,2]. Chemically, mesalazine is 5-amino-2-hydroxybenzoic acid (Figure 1), with the Mw.F $C_7H_7NO_3$ and a Mwt of 153.14 g mol^{-1} [3,4]. It is characterized by ionizable amino, phenolic hydroxyl, and carboxyl groups, limited aqueous solubility, and amphoteric behavior, which influence its pharmaceutical formulation and analytical determination [3,4]. Its topical anti-inflammatory activity in the intestinal mucosa is associated with inhibition of inflammatory mediator pathways, free-radical scavenging, modulation of cytokine release, activation of peroxisome proliferator-activated receptor-gamma, and interference with nuclear transcription pathways involved in inflammation [5-7].

* Corresponding author: Noor Mansoor Ghaiballah

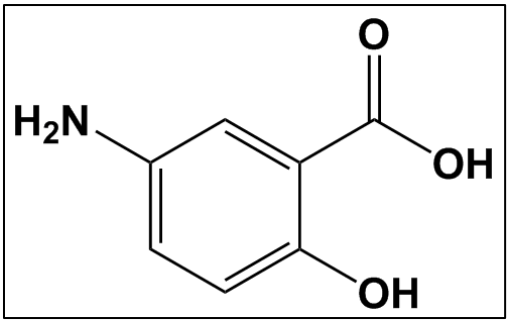

Figure 1 The structure of MES.

Several analytical methodologies have been reported for quantifying MES in medicinal dosage forms and biological specimens, including visible spectrophotometric assays based on oxidative coupling, diazotization, chelation, and related chromogenic reactions [8-12], spectrofluorimetric determination after solid-phase extraction [13], voltammetric procedures [14,15], and chromatographic, electrophoretic, LC-MS/MS, and HPLC methods for pharmaceutical or biological samples [16-18]. Although these methods are useful, many require expensive instrumentation, laborious sample preparation, or long analysis time.

Visible spectrophotometry remains attractive for routine quality-control laboratories because it uses relatively simple instrumentation, inexpensive reagents, and straightforward calibration and validation calculations compared with more sophisticated chromatographic and mass-spectrometric techniques [19-22]. Accordingly, the present method was designed as a simple oxidative-coupling spectrophotometric procedure using metol reagent in the presence of potassium periodate (KIO_4) and sodium hydroxide (NaOH), producing a colored product with abs. at 628 nm. The reaction variables were systematically optimized, and the technique was validated through linearity, accuracy, precision, detection limit, quantitation limit, and application to commercial tablets.

2. Materials and Methods

2.1. Apparatus

All spectrophotometric analyses were executed using a T92 + double-beam UV-Visible spectrophotometer (China) with standardized quartz cells of 1.0 cm path length; the solution's pH was measured with a Jenway 3310 pH meter, and weighing was conducted using a Sartorius BL210S analytical balance (Germany).

2.2. Reagents and chemical materials

The mesalazine active pharmaceutical ingredient (certified purity 99.8%) was obtained from the State Company for Pharmaceutical and Medical Supplies (SDI), Samarra, Iraq. The commercially available tablet formulation, AwaSalazin® 500 mg, manufactured by Awamedica Ltd., Erbil, Iraq, was used for application studies. Metol (99.0% purity), potassium periodate, and sodium hydroxide were obtained from Fluka and BDH. All compouds were of analytical grade, and freshly DW was used throughout the study.

2.3. Solution Preparation

2.3.1. Mesalazine stock solution (1000 ppm)

It was prepared by defrost 0.1000 g of pure mesalazine in DW and diluting to 100 mL in a vial. A 25 mL aliquot of this solution was moved into a 100 mL volumetric flask and diluted to the mark with DW to obtain a working standard solution of 250 μg mL^{-1}.

Metol solution (1×10^{-3} M)

It was produced by defrosting 0.0344 g of metol in DW and diluting to 100 mL in a flask. The solution remained stable for at least five days when stored under appropriate conditions.

Potassium periodate solution (1×10^{-2} M)

It was produced by defrosting 0.230 g of KIO_4 in water and diluting to 100 mL in a vial. The solution was freshly prepared under suitable conditions away from direct light.

2.4. Sodium Hydroxide Solution

A 2×10^{-2} M sodium hydroxide solution was synthesized by melting 0.080 g of NaOH in DW and adjusting the volume to 100 mL in a flask.

Tablet sample solution (250 μg mL^{-1} as mesalazine)

It was prepared from AwaSalazin® 500 mg tablets. Ten tablets were accurately weighed and the total mass was 10.823 g, giving an average tablet mass of 1.0823 g. The tablets were finely powdered and mixed thoroughly. A portion of powder amouted to 25.0 mg of mesalazine was accurately weighed. Based on the declared content and average tablet mass, the required powder mass was 0.0541 g. This portion was transferred into a beaker, dissolved in distilled water with gentle stirring, filtered through a sintered glass funnel, and diluted to 100 mL with DW in a volumetric flask.

3. Results and Discussion

3.1. Analytical Principle of the Approach

The technique is depending on the effect of potassium periodate (KIO4) in an alkaline medium of sodium hydroxide (NaOH) on an oxidative coupling reaction between mesalazine and metol reagent. Metol is oxidized by potassium periodate to a reactive intermediate which then couples with mesalazine to form a stable purple-colored dye. The formed chromogen showed maximum absorption at 628 nm when measured against reagent blank. The color intensity is directly proportional to mesalazine concentration in the working range; therefore, the drug can be quantified in bulk and pharmaceuticals formulations.

3.2. Optimization of reaction conditions

3.2.1. Effect of metol reagent volume

The effect of metol reagent volume was investigated by varying the reagent volume from 0.5 to 2.0 mL while maintaining fixed volumes of mesalazine, potassium periodate, and sodium hydroxide. The highest absorbance was obtained with 1.0 mL of metol solution; therefore, this volume was selected for subsequent experiments.

Table 1 Effect of metol reagent volume.

Metol volume (mL)	Absorbance
0.5	0.492
0.8	0.531
1.0	0.563
1.5	0.520
1.8	0.489
2.0	0.462

3.2.2. Selection of oxidizing agent

Different oxidizing agents were examined under identical experimental conditions to select the most suitable oxidant for the oxidative coupling reaction. Potassium periodate (KIO_4) gave the highest and most reproducible absorbance and was therefore adopted as the oxidizing agent in the final procedure.

Table 2 Effect of oxidizing-agent type.

Oxidizing agent	Absorbance
NBS	0.422
KIO_3	0.505
KIO_4	0.562
$K_2S_2O_8$	0.531

3.3. Effect of potassium periodate volume

The effect of potassium periodate volume was examined by adding different aliquots of 1×10^{-2} M KIO_4 solution. Maximum absorbance was obtained with 1.0 mL, which was therefore selected as the optimum volume.

Table 3 Effect of potassium periodate volume

KIO_4 volume (mL)	Absorbance
0.5	0.490
0.8	0.532
1.0	0.564
1.5	0.519

3.4. Effect of alkaline medium

The oxidative coupling reaction requires an alkaline medium for maximum color development. Sodium hydroxide provided the best response with stable color intensity and was selected as the optimum alkaline medium.

Table 4 Effect of alkaline-medium type.

Alkaline medium	Absorbance at 628 nm
NH_4OH	0.468
Na_2CO_3	0.340
NaOH	0.564

3.5. Effect of sodium hydroxide volume

Different volumes of sodium hydroxide solution (2×10^{-2} M) were tested under constant experimental conditions. The highest absorbance was observed at 1.5 mL, corresponding to a pH of 11.55. Therefore, 1.5 mL was selected as the optimum volume for further experiments.

Table 5 Effect of sodium hydroxide volume.

NaOH volume (mL)	pH	Absorbance
0.5	10.94	0.476
1.0	11.10	0.498
1.3	11.26	0.531
1.5	11.55	0.563
1.8	11.81	0.522
2.0	12.20	0.489

The influence of reagent-addition sequence on the absorbance of the colored product was investigated. The sequence oxidant (O), mesalazine (M), base (B), and reagent (R) produced the highest absorbance and was adopted as the optimum addition order.

Table 6 Effect of order of addition.

Order No.	Order of addition*	Absorbance
1	O1 + R1 + M1 + B1	0.544
2	O1 + M1 + B1 + R1	0.563
3	M1 + R1 + O1 + B1	0.512
4	M1 + O1 + R1 + B1	0.527
5	R1 + B1 + M1 + O1	0.532
6	O1 + B1 + M1 + R1	0.540
7	O1 + R1 + B1 + M1	0.466

*M1 = mesalazine; R1 = metol reagent; O1 = potassium periodate (KIO_4); B1 = sodium hydroxide (NaOH).

The stability of the colored chromogen was evaluated by measuring the absorbance of three mesalazine concentrations at different time intervals under the optimized conditions. The formed dye was stable for up to 50 minutes, after which a slight decrease in absorbance was observed.

Table 7 Stability of the colored product over time**.**

Time (min)	Absorbance at 5 µg mL^{-1}	Absorbance at 10 µg mL^{-1}	Absorbance at 15 µg mL^{-1}
0	0.260	0.53	0.84
10	0.260	0.55	0.85
20	0.260	0.54	0.85
30	0.260	0.54	0.85
40	0.260	0.54	0.85
50	0.260	0.54	0.85
60	0.240	0.52	0.82

3.5.1. Effect of temperature

Table 8 Effect of temperature on absorbance intensity.

Temperature (°C)	Absorbance at 628 nm
5	0.470
10	0.490
15	0.520
20	0.560
25	0.540
30	0.530
35	0.510
40	0.490
45	0.470
50	0.460

The effect of temperature on absorbance was studied in the range of 5-50 °C. Maximum color intensity was obtained at 20 °C, while lower and higher temperatures decreased the absorbance. Consequently, room temperature (20 °C) was selected for subsequent measurements.

3.5.2. Absorption spectrum

The following procedure was conducted under the optimum conditions to obtain the absorption spectrum of the colored product: 1.0 mL of MES solution, 1.0 mL of metol reagent, 1.0 mL of potassium periodate solution, and 1.5 mL of NaOH solution. The mixture was left at 20 °C for 5 min and then diluted to 25 mL with water dist. The colored derivative of the product was found to have maximum abs. at 628 nm, using the reagent blank. However, the blank was found to be negligible at the analytical wavelength.

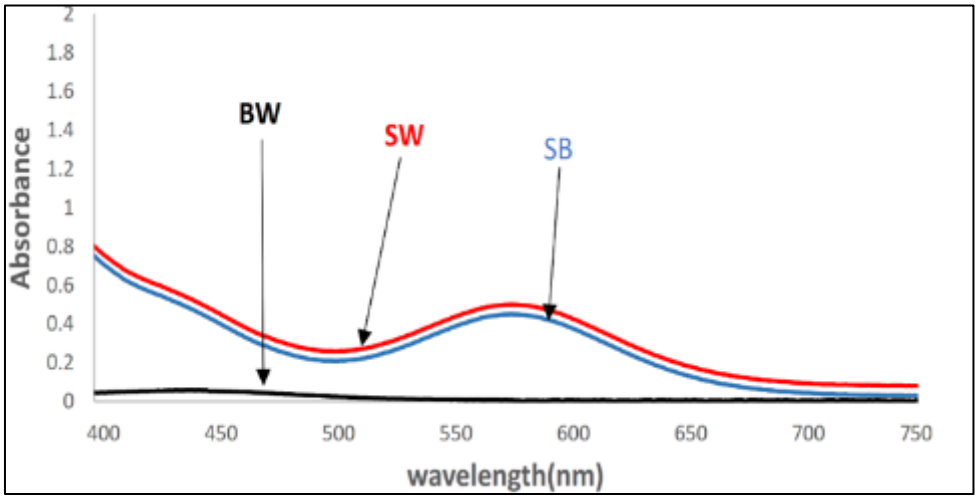

Figure 2 Absorption spectrum for MES Vs water (SW), MES Vs blank solution (SB), and blank solution Vs water (BW).

Table 9 Summary of optimum experimental conditions**.**

Parameter	**Optimum condition**
λmax	628 nm
Mesalazine solution	1.0 mL
Potassium periodate (1×10^{-2} M)	1.0 mL
Sodium hydroxide (2×10^{-2} M)	1.5 mL
Temperature	20 °C
Development time	5 min
Dilution solvent	Distilled water
Final volume	25 mL

3.6. Calibration graph and analytical sensitivity

Under optimal conditions, mesalazine working solution volumes (0.3-2.0 mL) were pipetted into a sequential of 25 mL volumetric flasks to get final concentrations of 3.0-20.0 µg mL^{-1}. Potassium periodate , sodium hydroxide and metol reagent were successively added and the absorbance was read at 628 nm versus the reagent blank. Beer's law was obeyed in the concentration range investigated with the regression equation. The molar absorptivity, derived from the calibration slope using 1.0 cm cell and molecular weight 153.14 g mol^{-1}, was 5.91 × 103 L mol^{-1} cm^{-1}. Sandell's index = 0.0259 µg cm^{-2}. These analytical sensitivity parameters were estimated by using standard spectrophotometric relations [20,21].

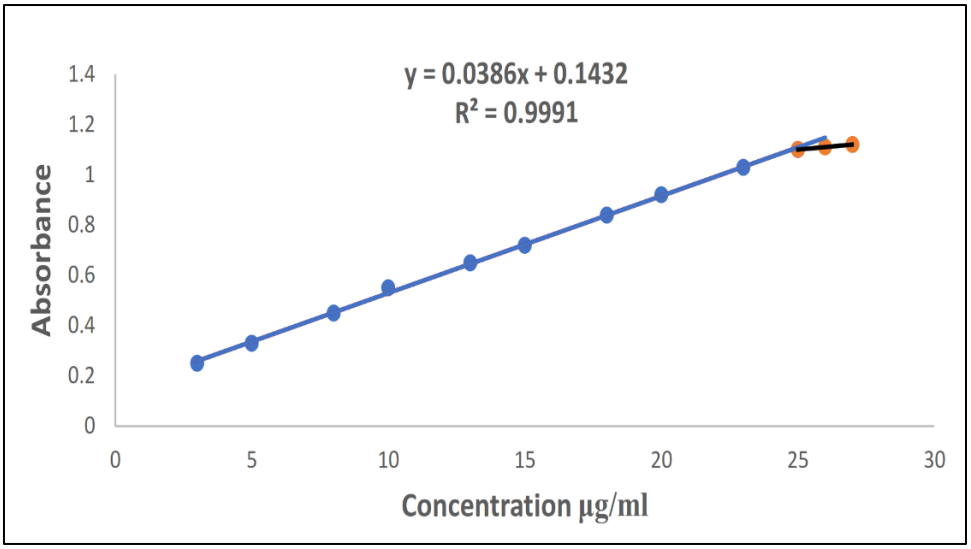

Figure 3 Calibration graph for the determination of mesalazine using the proposed method.

3.7. Accuracy, precision, and detection limits

Method reliability was checked using three selected concentrations: 5, 10, and 15 µg mL^{-1}. Each concentration was measured six times, and the recovery percentage, (RE%), and RSD% were calculated. The results demonstrate acceptable accuracy and precision for quantitative analysis.

Table 10 Accuracy and precision for mesalazine determination in pure form.

Taken (µg mL^{-1})	Measured(µg mL^{-1})	Recovery (%)	RE (%)	RSD (%)
5.00	5.05	101.00	+1.00	1.42
10.00	10.12	101.20	+1.20	1.55
15.00	14.83	98.87	-1.13	0.96

Limit of detection (LOD) was calculated based on the blank response at the optimized conditions was 0.1759 µg mL^{-1}. The related LOQ, 10σ/S, was 0.5863 µg mL^{-1}, where σ is the standard deviation of the blank and S is the slope of the calibration plot. These calculations are in line with accepted validation of analytical and statistical procedures [19,22].

Table 11 Analytical figures of merit of the proposed method.

Parameter	Value
Linearity range	3.0-20.0 µg mL^{-1}
Regression equation	A = 0.0386C + 0.1432
Coefficient of determination (R^2)	0.9991
Molar absorptivity	5.91×10^3 L mol^{-1} cm^{-1}
Sandell sensitivity	0.0259 µg cm^{-2}
LOD	0.1759 µg mL^{-1}
LOQ	0.5863 µg mL^{-1}

3.8. Stoichiometric ratio of mesalazine and metol

The stoichiometric composition of the colored product formed between mesalazine and metol reagent was studied using Job's method of continuous variation. The maximum absorbance occurred at a mole fraction of 0.5, indicating a 1:1 stoichiometric ratio between mesalazine and the reagent [20].

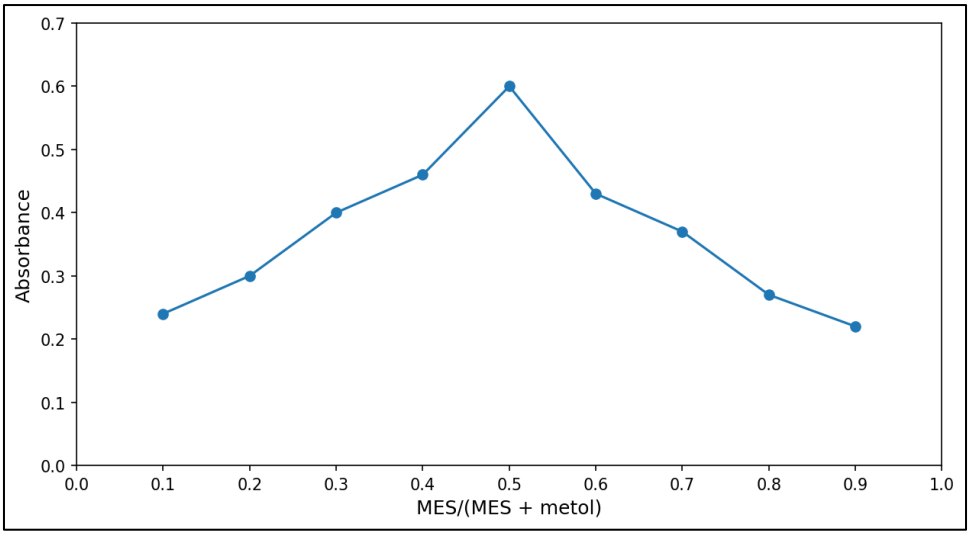

Figure 4 Continuous variation plot for the purple-colored product.

The suggested reaction mechanism involves oxidative activation of metol by potassium periodate followed by coupling with mesalazine in alkaline medium to produce the purple-colored dye shown in Figure 5

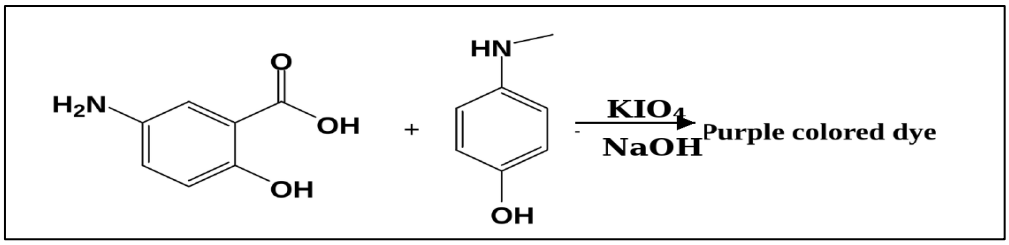

Figure 5 Suggested mechanism for the oxidative coupling reaction.

3.9. Application to pharmaceutical tablets

3.9.1. Direct method

The above method was executed for AwaSalazin® 500 mg tablets at 3 levels of concentration. The results demonstrated that the method can be used for the routine determination of commercial tablets of mesalazine without any unwanted interference from the tablet excipients.

Table 12 Direct determination of mesalazine in tablet formulation.

Taken (µg mL^{-1})	Measured (µg mL^{-1})	Recovery (%)	RE (%)	RSD (%)
3.00	2.99	99.67	-0.33	1.41
8.00	7.85	98.13	-1.88	1.63
16.00	15.70	98.13	-1.88	0.84

3.10. Standard addition method

To verify that there was no interference from excipients, the tablet formulation was subjected to the standard addition method. A fixed volume of the sample solution was spiked with a known quantity of standard MES, and the absorbance after standard additions was determined under the optimized conditions. The recoveries obtained were near 100%, which indicates the accuracy and selectivity of the suggested method.

Table 13 Standard addition results for mesalazine in tablets.

Taken (µg mL^{-1})	Measured (µg mL^{-1})	Recovery (%)	RE (%)	RSD (%)
6.00	6.06	101.00	+1.00	0.23
15.00	15.08	100.53	+0.53	0.09

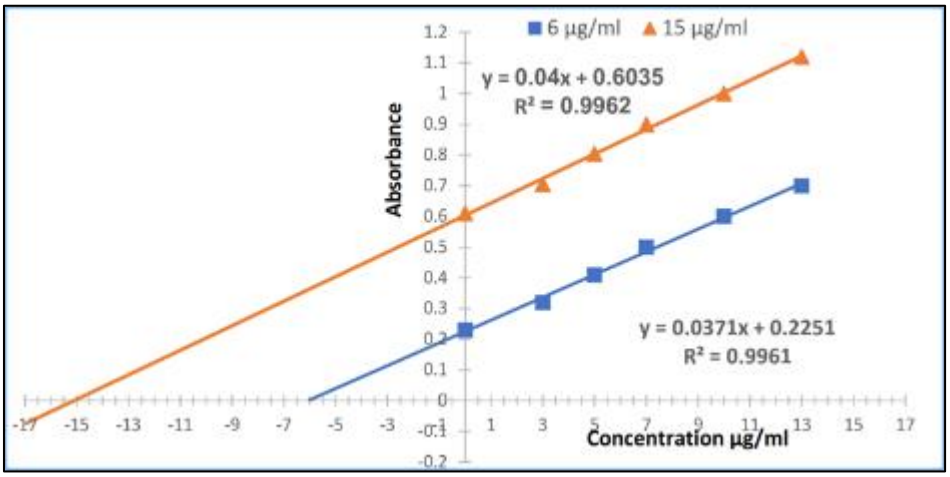

Figure 5 Standard addition curve for mesalazine determination in tablets.

3.11. Comparison with reported methods

Table 14 Comparison between the proposed method and selected reported methods.

Approach	Linearity (µg mL^{-1})	LOD (µg mL^{-1})	RSD (%)	Reference
Spectrophotometry	0.4-12	0.101	1.54	[8]
Differential pulse voltammetry	0.3-5.31	0.124	1.35	[14]
Spectrofluorimetry	2.07-94.2	0.160	2.66	[13]
HPLC	0.25-1.5	0.075	<5.92	[18]
Proposed method	3.0-20.0	0.1759	0.84-1.63	This work

The suggested method was evaluated with some of the previously mentioned methods for the determination of mesalazine. The developed method provides a simple and sensitive visible spectrophotometric method with good recovery and low RSD values, which can be routinely used for pharmaceutical quality control.

4. Conclusion

A simple, selective and fast spectrophotometric method was developed for the estimation of mesalazine in bulk and in medicinal tablets. An oxidative coupling reaction among mesalazine and metol in the exitance of potassium periodate (KIO_4) and sodium hydroxide (NaOH) results in the generation of a stable purple-colored chromogen with maximum absorbance at 628 nm. The method exhibited good linearity (3.0-20.0 µg mL^{-1}), accuracy, precision and detection limit. It was also successfully applied to a commercial preparation, i.e., AwaSalazin® tablets, to demonstrate its potential application in routine quality control of mesalazine in pharmaceutical formulations.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no competing interests.

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