

Spectrophotometric Assay of Mesalazine in Pharmaceutical Preparations Via Oxidative coupling reaction with o-cresol and sodium metaperiodate

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Abstract:

In this search, two methods were used to include the watermark in the video. The first method was based on DCT (Discrete Cosine Transform), the second method was based on an algorithm SVD (Singular Value Decomposition) for the purpose of converting video to frequency domain. The process of embedding the watermark in both methods was done after the original video was divided into a set of frames, and one frame was divided into a block of 8 x 8 and the DCT on each block when using the first method and the SVD algorithm when using the second method. And then include the Bit Binary for the watermark inside the center of the cluster. Random selection of video frames and rows of watermark images has been adopted in both ways. The performance of the two methods was assessed using the experimental tests PSNR, MSE and NC. The experimental results show that both methods have achieved a good understanding and high resistance against various attacks, adopted Matlab 2013a language.

Keyword: Digital Video, watermark.

التقدير الطيفي للميزالازين في المستحضرات الصيدلانية بواسطة تفاعل الاقتران التاكسدي مع اوثو-كريسول وميتابيريوات الصوديوم

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الملخص

تم وصف طريقة طيفية بسيطة وسريعة ودقيقة وحساسة لتقدير الميزالازين بهيئته النقية وفي المستحضرات الصيدلانية. اعتمدت الطريقة على أكسدة المركب الدوائي بواسطة ميتابيريونات الصوديوم يتبعها تفاعل الاقتران التأكسدي مع اورثو-كريسول في وسط قاعدي لتكوين صبغة الاندوفينول الزرقاء يقاس أقصى امتصاصها عند 645 نانوميتر. كانت حدود تطبيق قانون بير ضمن مدى التراكيز 0.4 – 12 مايكروغرام / ملتر وبدقة وتوافق مرضيين، إذ بلغ معدل نسبة الاسترجاع 101.38% والانحراف القياسي النسبي اقل من 3.5%. بلغت الامتصاصية المولارية 22435 لتر. مول⁻¹. سم⁻¹ بحد كشف 0.101 مايكروغرام / ملتر و0.338 مايكروغرام / ملتر كحد كمي. طبقت الطريقة بنجاح في تقدير الميزالازين في مستحضراته الصيدلانية بشكل أقراص مغلفة معويًا وكبسولات مديدة التأثير، إذ وجد أن نتائج الطريقة المطورة متوافقة احصائياً مع الطريقة القياسية المعتمدة في دستور الادوية البريطاني.

الكلمات المفتاحية : التقدير الطيفي، ميزالازين، اورثو-كريسول، تفاعل الاقتران التأكسدي، الطيف الفوتومتري.

Introduction

Mesalazine known as mesalamine as well. It is known chemically as 5-aminosalicylic acid (Figure 1. It is used for the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease [1].

Mesalazine has been revealed to be a potent scavenger of reactive oxygen species that play a important role in the pathogenesis of inflammatory bowel disease, inhibit of natural killer cell activity, inhibition of antibody synthesis, inhibition of cyclo-oxygenase and lipoxygenase pathways and impairment of neutrophil function [2][3]. It is considerably soluble in water and basically insoluble in alcohol. It can be dissolve as well in dilute solution of alkali hydroxide and acidic solution [4].

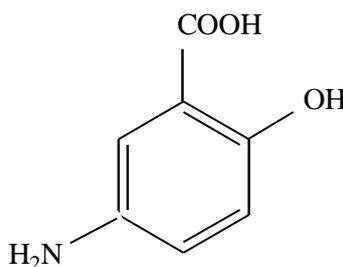


Figure 1. Chemical Structure of mesalazine

Several approaches have been stated for the determination of mesalazine, including UV-spectrophotometry [5],[6], fluorimetry [7], chemiluminescence [8], galvanostatic coulometry [9], square wave voltammetry at pencil graphite electrodes [10], RP-HPLC [11-14] and micelle- electrokinetic capillary chromatography with ion-pair reagent [15]. A simple colorimetric method was developed for the determination of mesalazine in pure and its pharmaceutical preparations using various reagents such as sodium Nitroprusside [16], thymol [17], 1,2-naphthoquinone-4-sulphonate, p-dimethylaminocinnamaldehyde [18], p-hydroxybenzaldehyde, Folin-Ciocalten [19], phloroglucinol [20], the ferric-ferricyanide reagent in aqueous solution, methylene blue and Rhodamine- B dyes in the presence of chloramine-T [21], 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tetracyanoethylene [22], and o-chloranil [23]. The drug and its formulations are officially listed in British Pharmacopeia which suggests potentiometric [4] and HPLC [24] methods for its assay.

The current work aims to create and validate a new and simple spectrophotometric method for the detection of mesalazine in bulk and pharmaceutical dosages. The proposed method is based on the oxidative coupling reaction between mesalazine, o-cresol, and sodium metaperiodate in an alkaline medium to form a stable dye with blue color.

Experimental

Apparatus

A double beam spectrophotometer Shimadzu model UV-160A with 1.0^{cm} matched quartz cells was used to carry out all spectral and absorbance measurements.

Reagent

All chemicals used were of analytical grade and used without further purification. Mesalazine was provided from SDI-Iraq, o-cresol, sodium metaperiodate and sodium hydroxide were obtained from Fluka company.

Standard solution of Drug (mesalazine 100 µg/ml):

was prepared by dissolving 0.0100 g of pure drug in a minimum amount of ethanol and the mixture diluted to the mark with distilled water in a 100 ml volumetric Flask.

o-Cresol solution (0.01 M)

The reagent was prepared by dissolving 0.1081 g of o-cresol with the smallest volume of ethanol and diluted up to the mark (100 ml) with distilled water. In addition, Sodium metaperiodate (0.015M) was prepared by dissolving 0.8021g in 250 ml with DW.

Sodium hydroxide solution (0.1 M)

Alkaline er solution was prepared by dissolving 0.400 g in 100 ml of distilled water.

Recommended procedure:

To a series of 25 ml volumetric flasks, 2.5 ml of 0.01M of the o-cresol solution was added followed by the addition of 1 ml of 0.015 M sodium metaperiodate solution and 5 ml of 0.1 M sodium hydroxide solution. Aliquots of standard solution (10-300 µg) were transferred to the flasks respectively. The contents were diluted up to the mark with DW and left for 10 min. After that, the absorbance was measured at 645 nm contrary to blank. A calibration curve was drawn and the regression equation was calculated (Figure 2).

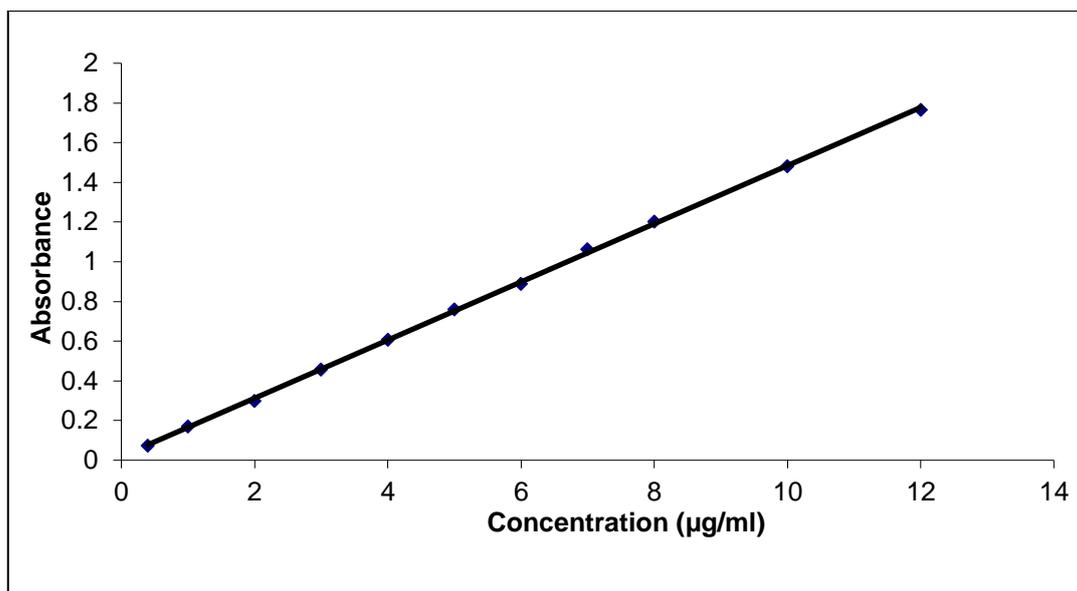


Figure 2: Calibration graph of mesalazine.

Procedure for pharmaceutical preparations:

Tablets:

Ten tablets of mesacol (enteric-coated tablets) were weighted and crushed into a powder. An accurately weighed quantity equivalent to 400 mg of the pure drug (mesalazine) was dissolved in 10 ml of ethanol and completed to 100 ml with DW. The resulting solution was shaken well and filtered. A solution of 100 µg/ml of the drug was prepared by appropriate dilution and the measurement was carried out as described earlier under the recommended procedure.

Capsules:

The contents of ten mesacol capsules (extended-release capsules) were weighted, powdered, and mixed well. A weighted quantity equivalent to 400mg of pure mesalazine was dissolved in 10 ml ethanol and 0.2 M sodium hydroxide (5:5) to increase the solubility. After that, transferred into 100 ml and completed up to the mark with DW. The final solution was filtered and treated as mentioned previously.

Results and discussion:

Characteristic of Absorption Spectral

A blue indophenol dye has been formed when o-cresol react with mesalazine in an alkaline medium in the presence of sodium metaperiodate. The resulting product has maximum absorption at 645 nm as shown in Figure 3. However, the reagent blank has no absorbance at the same wavelength.

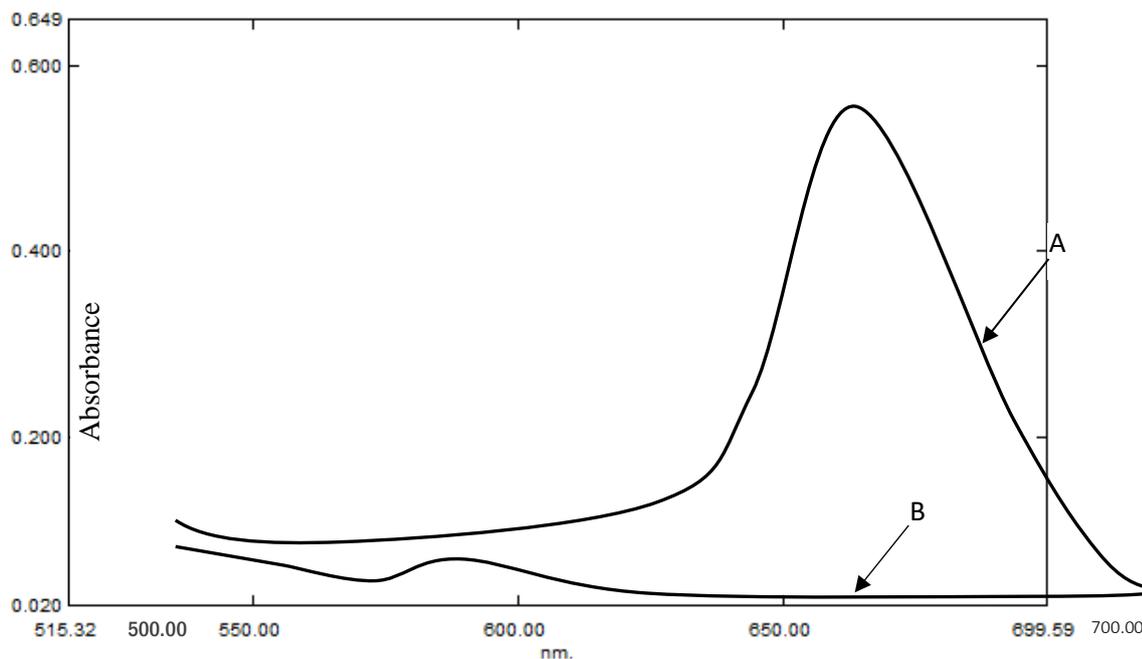


Figure 3: Absorption spectra (A) of 4 µg/ml of mesalazine with 0-cresol in alkaline medium versus blank (B) Reagent blank versus distilled water.

Reaction conditions:

In order to improve the suggested method, the effect of some experimental variables was considered. The optimization was done using a sample solution of mesalazine (4µg/ml) at 645 nm.

Effect of pH:

It was observed that the blue dye product formed only in an alkaline medium, therefore, the effect of different alkaline solutions (pH 9-12) and sodium hydroxide (pH > 12) were studied. It was confirmed that sodium hydroxide gave the best and highest color intensity of the dye product for mesalazine (Table 1) and the optimum amount of this base was found to be 5-7ml and 5ml which was used in the subsequent experiments (Figure 4).

Table 1: Effect of pH on the sensitivity of the product.

Type of buffer solution *	pH	$\lambda_{max}(nm)$	Absorbance
$C_2H_5NO_2 + NaOH$	9	630	0.288
$Na_2[B_4O_5(OH)_4] \cdot 8H_2O + NaOH$	10	640	0.311
$NaHCO_3 + NaOH$	11	640	0.339
$NaH_2PO_4 + NaOH$	12	645	0.350
$NaOH (0.1M)$	>12	645	0.378

* 2 ml added.

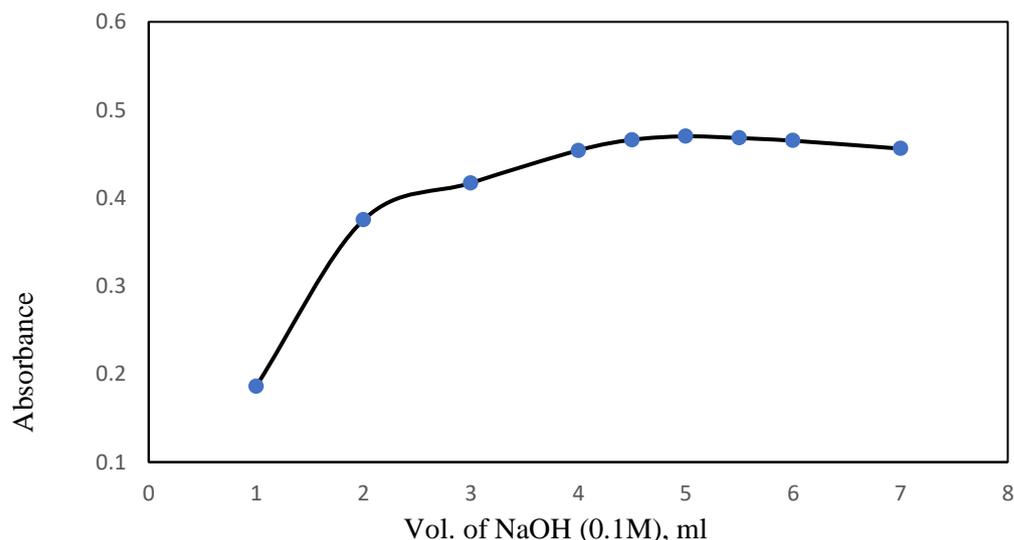


Figure 4: Effect of the amount of sodium hydroxide.

Effect of o-cresol concentration

The influence of the volume (1-5 ml) of 0.01M of the o-cresol solution was examined. The obtained results showed that 2.5ml of o-cresol gave a higher color intensity of the dye product and was recommended for all measurements (Figure 5).

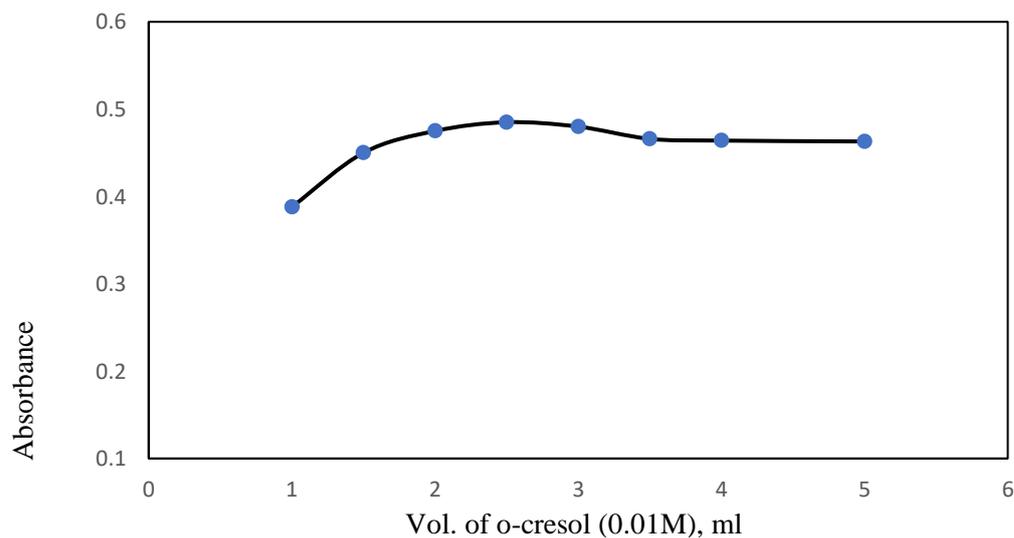


Figure 5: Effect of o-cresol concentration

Effect of oxidizing agent:

Different oxidizing agents were tested such as N-bromosuccinimide, ferric chloride, potassium hexacyanoferrate(III), sodium metaperiodate, and sodium nitroprusside. The obtained results show in Figure6 that sodium metaperiodate gave a stable blue color dye product with maximum intensity compared to other oxidizing agents. It was found that 1.0ml of 0.015M sodium metaperiodate was sufficient to get the highest color intensity (Figure 7).

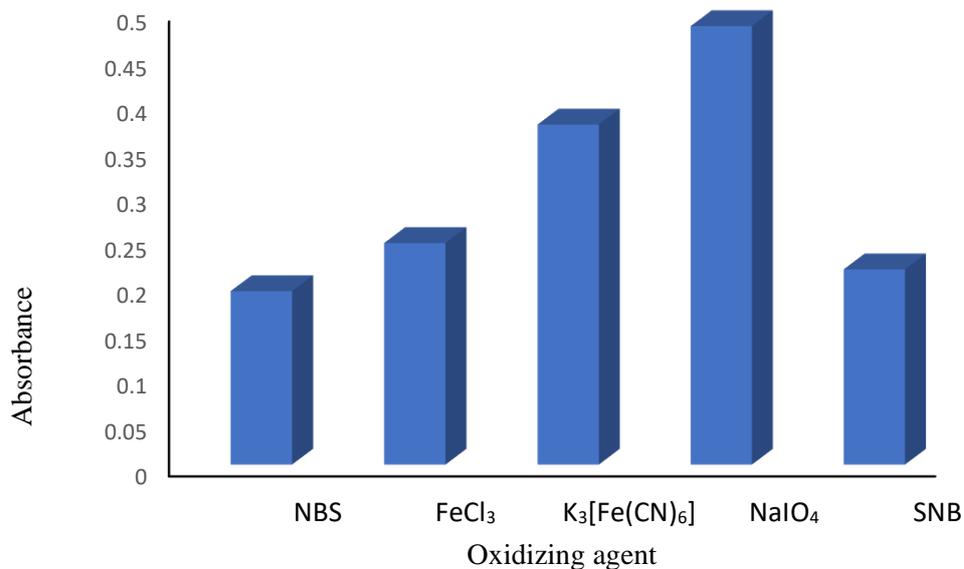


Figure 6: Effect of oxidizing agents

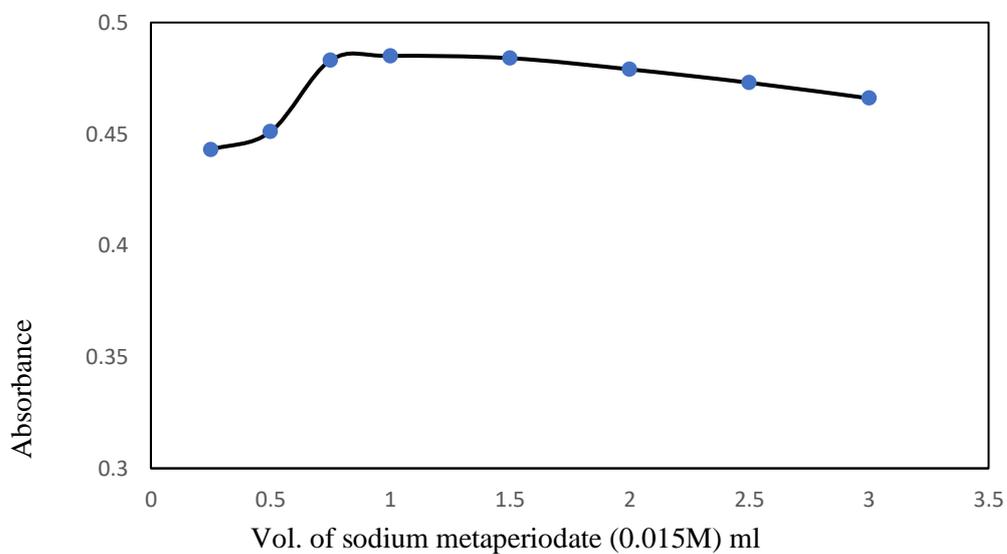


Figure 7: Effect of the amount of oxidizing agent.

Effect of temperature

After optimization of chemical variables, the effect of temperature on the reaction of mesalazine and o-cresol in the presence of sodium metaperiodate in the range of 0-50°C was studied. Practically, high absorbance was achieved at room temperature (23±2°C) as shown in Table 2.

Table 2: Effect of temperature on the color intensity and product absorbance.

Temp. (°C)	Absorbance/ min standing time													
	0	5	10	15	20	25	30	35	40	50	60	90	120	Over night
0	0.390	0.430	0.441	0.445	0.450	0.451	0.449	0.449	0.450	0.452	0.448			
R.T	0.440	0.460	0.489	0.488	0.488	0.487	0.489	0.489	0.487	0.487	0.488	0.485	0.483	0.479
40	0.420	0.439	0.445	0.447	0.449	0.451	0.455	0.457	0.457	0.455	0.456			
50	0.411	0.422	0.422	0.425	0.426	0.427	0.423	0.422	0.420	0.419	0.415			

Development time and stability period.

The color intensity of the dye formed reached a maximum after 10 min and was stable for at least 4hr at room temperature.

Order of addition of the reactants:

To achieve high color intensity, the order of addition of reactant should be followed serial No.III of as shown in Table 3, otherwise a loss in product color intensity was experimentally detected.

Table 3: Effect of the order of reactants addition.

Order number	Reaction component	absorbance
I	S+R+O+B	0.488
II	S+O+R+B	0.369
III	R+O+B+S	0.587
IV	R+O+S+B	0.433
V	R+B+S+O	0.442
VI	O+B+S+R	0.448

S=Sample, R=o-cresol, O=NaIO₄, B=NaOH

Method validation

Linearity of the method and sensitivity:

Under the optimum conditions, a linear relationship was reached between color intensity and concentration of mesalazine in the range 0.4-12µg/ml. The correlation coefficient, intercept, and slope for the calibration data was calculated in Table 4.

In addition, sensitivity parameters such as Sandell index and molar absorptivity, Limit of detection (LOD), and Limit of quantitation (LOQ) are calculated as presented in Table 4 [26].

Table 4: Sensitivity and regression parameters.

Parameter	Value
λ max, nm	645
Beer's low limit, $\mu\text{g.ml}^{-1}$	0.4 – 12
Molar absorptivity, $\text{L.mol}^{-1}.\text{cm}^{-1}$	22435
Sandell sensitivity, $\mu\text{g.cm}^{-2}$	0.0068
LOD, $\mu\text{g ml}^{-1}$	0.101
LOQ, $\mu\text{g ml}^{-1}$	0.338
Intercept	0.019
Slope	0.146
Correlation coefficient (R^2)	0.9990

Precision and accuracy:

The intraday precision and accuracy of the proposed method were examined by carrying out six replicates determination at a fixed concentration of mesalazine (within Beer's law range). The RSD (less than 3.5%) and R^2 (101.38%) were found to be in the acceptable range (Table 5)

Table 5: Precision and accuracy of the proposed method.

The concentration of mesalazine ($\mu\text{g/ml}$)	Recovery* (%)	RSD* (%)
1.0	103.42	3.05
4.0	100.75	1.54
8.0	101.25	0.37
10	100.10	0.53

* Average of six determinations.

Nature and stability constant of the dye product

Job's method [27] under optimized conditions was applied to calculate reaction stoichiometry. The obtained results showed a 1:1 mesalazine to o-cresol at 645 nm (Figure 8).

The average stability constant [30] of the formed dye was $1.37 \times 10^5 \text{ l.mol}^{-1}$ which indicates the high stability of the product.

Interference

To evaluate the selectivity of the suggested method, the effect of common excipients for instance glucose, lactose, sucrose, starch, talc, acacia, magnesium stearate, and sodium chloride was tested by analyzing synthetic sample solutions containing $4 \mu\text{g/ml}$ of mesalazine and excess amount (10-20-fold excess) of each excipient. The results are listed in Table 6 and show no interference caused by the presence of these excipients.

Table 6: Effect of interference

Foreign compound	Fold excess	Recovery (%)
Glucose	20	97.27
Lactose	20	99.67
Sucrose	20	98.18
Starch	20	100.65
Talc	10	99.20
Acacia	10	97.27
Magnesium stearate	10	101.37
Sodium chloride	10	96.92

Applications:

The recommended method was effectively applied for the determination of mesalazine in its dosage forms. The obtained results were compared with certified values (Table 7) and statistically to those obtained by the official method [4] utilizing t- and F- tests at 95% confidence level (Table 8). The results indicated that there was no significant difference between the suggested and official methods in terms of accuracy and precision (Table 8).

Table 7: Determination of mesalazine in pharmaceutical preparation using the proposed method :

Pharmaceutical preparation	Certified value (µg)	Amount present (µg/ml)	Recovery * (%)	Drug content found (µg)
Mesacol **		1.0	96.06	384.24
Enteric-coated tablets	400	4	100.57	402.28
		6	97.86	391.44
		8	101.46	405.84
		10	99.23	396.92
Mesacol **		1.0	95.98	383.92
Extended release capsules	400	4.0	102.02	408.08
		6	100.39	401.56
		8	102.35	409.40
		10	100.80	403.20

* Average of three determinations.

** Marked by Universal Pharmaceutical Industries- Unipharma- Damascus- Syria.

Table 8: Determination of mesalazine in pharmaceutical preparations using the proposed and official methods:

Pharmaceutical preparation	Certified value (µg)	Recovery * (%)		t-test	F-test
		Proposal method	Official method		
Mesacol enteric coated tablets	400	99.04	98.84	0.186	3.948
Mesacol extended release capsules	400	100.31	100.05	0.203	3.958

* Mean value of five determinations

Tabulated t- and F- testes at 95% confidence level are 2.31 and 6.39 respectively.

Conclusion:

The proposed method is economic, accurate, simple, and reproducible and can be used as routine analysis of mesalazine in bulk form and to analyze the pharmaceutical preparations (tablets and capsules).

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