

EXTRACTIVE CLOUD POINT SPECTROPHOTOMETRIC DETERMINATION OF MESALAZINE USING BRILLIANT GREEN DYE

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(Received: August 23, 2022; Accepted for Publication: February 22, 2023)

ABSTRACT

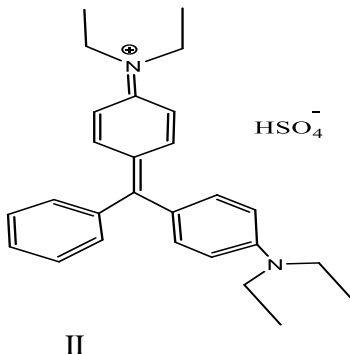
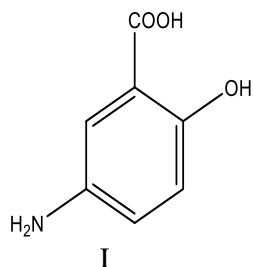
A simple and accurate cloud point-spectrophotometric method for determination of Mesalazine (MEZ) in pure form and pharmaceutical formulations is proposed. The procedure is based on the reaction of Mesalazine with brilliant green dye (BG) forming a pale-yellow ion-pair complex after extracting with Triton X-114 and measured at 361 nm. The experimental conditions for the phase separation were optimized. Beer's law was obeyed in the range 0.3-3.5 $\mu\text{g mL}^{-1}$ with molar absorptivity of $3.914 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$, average recovery % of 100.82 and average relative standard deviation (RSD) is 0.87. The method was applied successfully for determination of MEZ in its pharmaceutical formulations.

KEYWORDS: Ion-pair, Cloud point, Brilliant green, Mesalazine.

INTRODUCTION

Mesalazine (MEZ) is a commercial name for 5-amino salicylic acid (I), is used in inflammatory bowel diseases,

particularly in the treatment of ulcerative mucosal colitis, as well as for the prevention of advanced hemorrhagic eruptions from the colon to the anus (Desai & Kale, 2019), (Singh et al., 2010), (Wiersma et al., 2004).



Different kinds of analytical techniques have been described for the determination of Mesalazine in various formulations and some biological liquids. These involve HPLC (Gatkal et al., 2013), reversed-phase UPLC method (Kanubhai & R, 2011), Electrochemical (Teradale et al., 2017), (Desai & Kale, 2019) and spectrofluorometric methods (Elbashir et al., 2015). However, these techniques are expensive instruments, need experience and not available in all laboratories. The most widely used and applied methods are those based on spectrophotometry due to its sensitivity, specificity and simplicity. Spectrophotometric methods are based on different principles, are

described for estimation of Mesalazine, such as oxidative coupling reactions (Shehab & Muhammed, 2020), (Al-Zakaria, 2019), Schiff base formation reaction (Desai & Kale, 2019), charge transfer complex (Al-Obaidi & Al-Samarrai, 2022), (Abdalla & Elbashir, 2014) and diazotization and coupling principles (Madhavi et al., 2011), (Trung Dung et al., 2016). In addition, various reagents have been used for determination of Mesalazine spectrophotometrically, for example 1,2-naphthoquinone-4-sulphonate (NQS), p-dimethyl amino cinnamaldehyde (PDAC), (Gurupadayya, 2011), sodium nitroprusside with hydroxylamine hydrochloride (Al-Sabha &

Habeeb, 2015) and and 1,5-diphenyl carbzide (1,5-DPC)(Hamdoon, 2018). In the present study, a simple and sensitive method have been suggested for determination of Mesalazine in pure and pharmaceutical forms, the method based on the spectrophotometric determination of cloud point extraction (CPE) of ion-pair complex formation between Mesalazine and BG dye(II) in the presence of Triton X-114

Cloud Point Extraction, CPE

The Cloud Point Extraction (CPC) technique was described by Watanabe in 1978 (Watanabe & Tanaka, 1978). This technique relies on the preconcentration of metal ions in aqueous samples, and it is known that surfactant in the aforementioned technique have the ability to concentrate materials. The CPE technique receives great attention because of the simplicity and speed of the method, and the extraction process is useful by improving the experimental conditions by adjusting the pH, temperature, type of electrolyte, extraction time, and surfactant concentration (Zain et al., 2014). The surface tension factor leads to the separation of the phase called the surfactant-rich phase and the surfactant aqueous phase. Comparing the maximum temperature with the critical temperature is called the cloud point temperature, which will be then the substance to be analyzed is concentrated and the preconcentration factor is high (Liang & Yang, 2010).

Shireen.othman@uod.ac.Hamdoon, 2018
B.G dye in the presence of Triton X-114.**Experimental part Instruments**

Absorbance measurements were carried out by JENWAY double beam UV-visible equipped (model 6800) with a 1.0-cm quartz cell. KERN & Sohn GmbH Electronic balance was used for weighing the samples. A thermostatic water bath from memmert. A centrifuge model 80-2 was used for separation surfactant-rich phase and aqueous phase. A JENWAY digital pH meter model 3505 with combined glass electrode was used for measuring the pH. Statistically, Excel 2010 software has been used.

Reagents

The chemical materials used were from Fluka and BDH companies. MEZ was provided by SDI company-Iraq, and $100 \mu\text{g mL}^{-1}$ was prepared by dissolving 0.01 g in 100 ml distilled water in volumetric flask. BG concentration of 0.1% was prepared by diluting 0.1 g in 100 ml distilled

water in volumetric flask. Triton X-114 (4%) was prepared by mixing 4 ml of the surfactant with distilled water and diluted to 100 ml in a volumetric flask.

General cloud point extraction (CPE) procedure for Mesalazine

An aliquot containing $0.3\text{-}3.5 \mu\text{g mL}^{-1}$ of MEZ were added into 10 mL volumetric flasks followed by addition of 0.4 mL of 0.1M NaOH, 1 mL of 0.1% BG and 0.8 mL of 4 % v/v Triton X-114. The mixture was mixed and diluted to the mark with distilled water. Then, placed in a thermostatic bath at 50°C for 20 minutes. The contents of a turbid solution formed were transferred to a 10 mL centrifuge tube. The two phases were separated by centrifugation (3500 rpm for 15 minutes) and then cooled in an ice bath for 10 minutes to increase the viscosity of the surfactant-rich phase and to stabilize the micelle layer at the bottom of the tube. Then by inverting the tube, the aqueous phase was easily poured. Finally, 1 mL of ethanol was added for dissolve the micelle layer, and the absorbance of the complex was measured at 361 nm against a reagent blank prepared under similar conditions.

Analysis of pharmaceutical formulation

Six capsules or tablets were properly grounded and a quantity equal to one capsule or tablet was carefully weighed separately and dissolved in deionized water. The solution was filtered through a Whatman no. 42 filter paper and the transparent solution was transferred to a 1000 ml volumetric flask and diluted to the mark with deionized water. Working solutions were prepared by suitable dilution and aliquots containing amounts within the calibration graph were treated according to the general procedure.

RESULTS AND DISCUSSION

In the preliminary investigation, it was found that MEZ reacts with BG dye in basic medium forming a pale-yellow ion-pair complex, which extractable in Triton X-114, having λ_{max} at 361 nm (Fig.1). A result of the reaction, whereas the blank have low absorbance at this wavelength. To achieve the sensitivity and stability of the colored complex of cloud point extraction for drug assay, a number of experiments have been carried out in order to investigate the impact of various important factors.

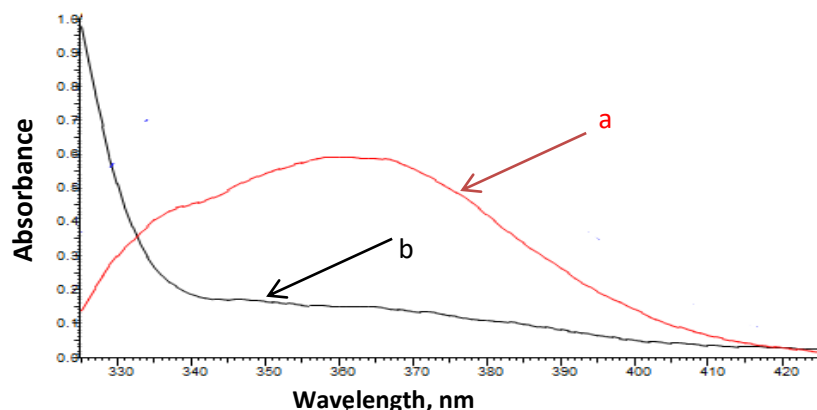


Fig.(1):Absorption spectra of $2 \mu\text{g mL}^{-1}$ of MEZ in the presence of 1mL of 0.1% BG and 0.4 mL of 0.1 M NaOH

(a) against reagent blank (b) under the optimum conditions.

OPTIMIZATION OF CPE CONDITIONS

Effect of base, pH and buffer solution

In this study, 0.5ml of (1M) from different bases (KOH, NaOH, Na_2CO_3 and NH_4OH) were examined. It was found that NaOH has the highest absorption intensity (Fig.2). As a result, sodium hydroxide was chosen as a convenient, and the effect of its concentration was investigated in the range (0.06-1 M). As shown in (Fig.3), a base concentration of 0.1M was the best and chosen for subsequent experiments. The

effect of different volumes of 0.1 M NaOH (0.2-1mL) was also examined. The maximum intensity was obtained when the volume of NaOH was 0.4mL at pH 11.22, (Fig. 4). However, different buffers including phosphate, carbonate, and borate adjusted at pH 11.22 were examined. As seen in (Fig.5), a decrease in the absorbance of the complex was observed. Therefore NaOH has been recommended in this method.

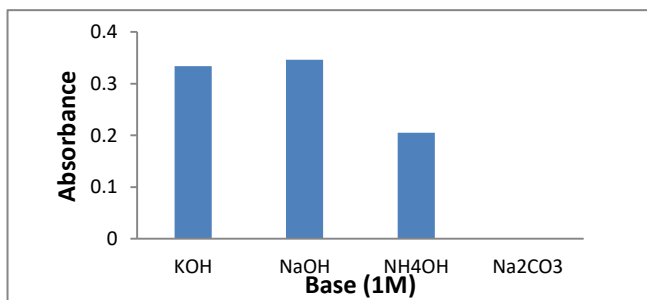


Fig.(2): Effect of different bases on the absorbance of $2 \mu\text{g mL}^{-1}$ MEZ

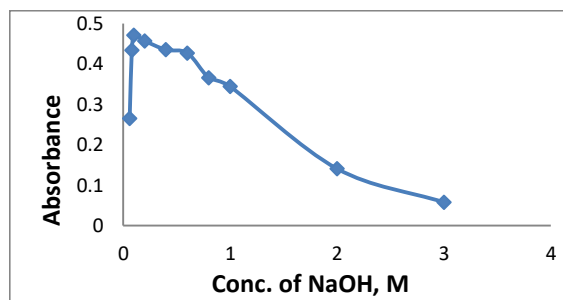


Fig.(3): Effect of different conc. of NaOH on the absorbance of $2 \mu\text{g mL}^{-1}$ MEZ

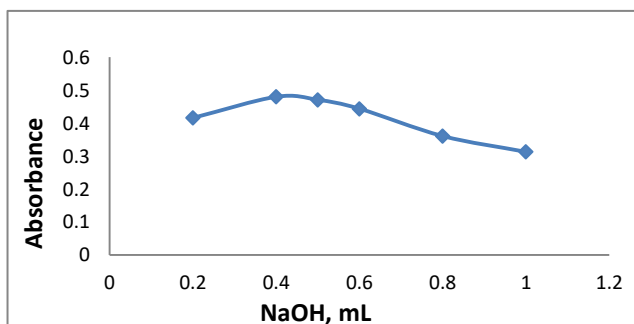


Fig. (4): Effect of different volumes of 0.1M NaOH

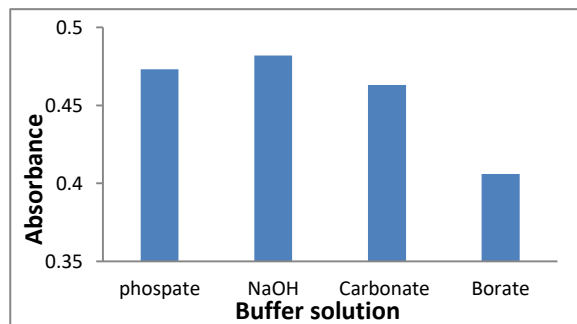


Fig. (5): Effect of buffers

Effect of brilliant green dye (B.G) concentration and volume

The effect of B.G concentration on the formation of colored complex in Triton X-114 medium was investigated using different concentrations of BG ranging from 0.06 % to 0.5 %; the results are shown in (Fig.6). It was pointed out that the absorbance reached a maximum at 0.1 %, then decreased at high

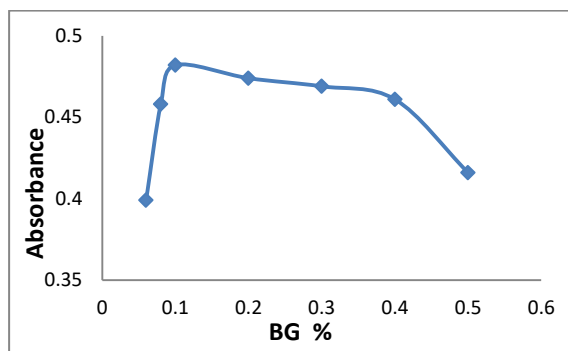


Fig. (6): Effect of BG concentration

concentrations. During the CPE procedure, the variation of the absorption signal as a function of BG amount was investigated in the range of (0.2-2.2 mL). The results shown in (Fig.7) show that the analytical response increased linearly with increasing BG dye volume, reaching a maximum at 1mL, indicating sufficient for ion-pair complex formation and selected in this investigation.

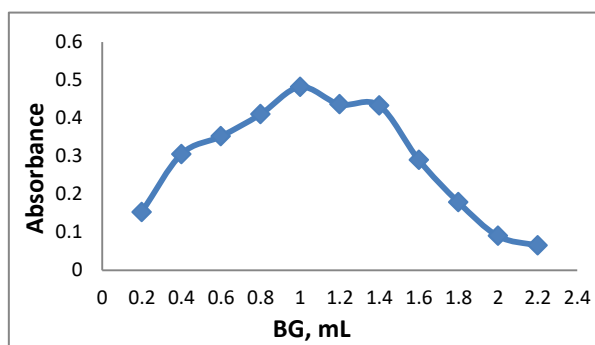


Fig. (7): Effect of volume of 0.1% BG

Effect of Triton X-114 concentration and volume

To obtain the optimal concentration of Triton X-114, the effect of its concentration on the absorbance of the extracted phase was investigated. The results shown in (Fig.8), indicated that 4% (v/v) more extraction occurred. This was chosen as the optimal concentration value. (Fig.8), depicts the effect of Triton X-114 amount on the preconcentration ion-pair complex. The absorbance signal increases dramatically and reaches a maximum at 0.8 mL of 4% Triton X-114 before abruptly

decreasing. The absorbance for ion-pair complex is low at low surfactant concentrations, perhaps at low surfactant amount, the absorbance for ion-pair complex is low, possibly due to a lack of assembly formation entrapping the complex quantitatively. While using a higher amount of surfactant, the extraction efficiency is low, most likely due to the increase in surfactant-rich phase volume, which causes the analyte to become more diluted, resulting in poor sensitivity and thus valueless extraction efficiency. However, 0.8 mL of 4% (v/v) Triton X-114 was used in the next experiments.

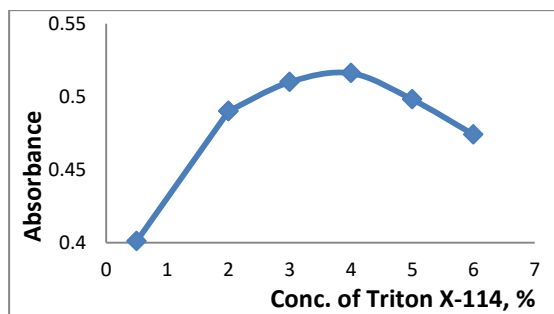


Fig. (8): Effect of Triton X-114 concentration

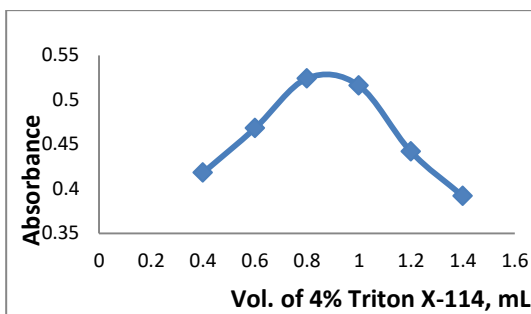


Fig. (9): Effect of different volumes of 4% Triton X-114

Effect of temperature and time of extraction

It is critical to optimize the extraction temperature and incubation time in order to achieve efficient phase separation and easy preconcentration. To investigate the effect of temperature on the extraction of colored complex, the incubation temperature ranging

from 15 to 70°C for 15 minutes has been studied. The maximum absorbance for complex was at 50°C and chosen (Fig.10). The CPE method typically requires a sufficient period of time to achieve equilibrium between the surfactant-rich and aqueous phases. The effect of incubation time on extraction efficiency was investigated at

50°C for 5-90 minutes. The separation process required only 20 minutes of incubation time (Fig. 11). A 15-minute centrifuge time period

was chosen as optimal because complete separation occurred within this time period Table 1.

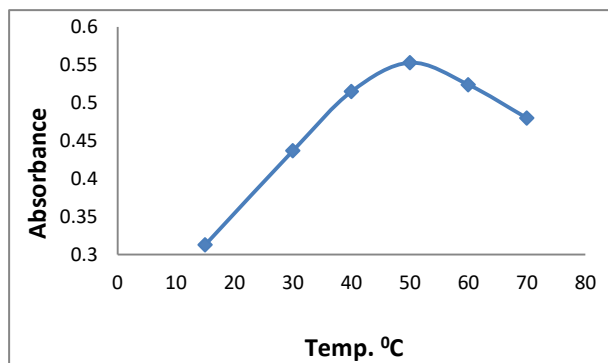


Fig. (10): Effect of equilibration temperature on the absorption of 2 µgmL⁻¹ for MEZ- BG complex

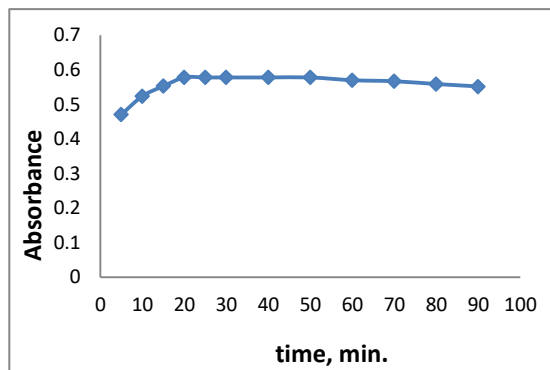


Fig. (11): Effect of incubation time

Table (1): Effect of centrifuge time

Time/min	1	3	5	7	10	15	20	25	30
Abs.	0.353	0.352	0.372	0.435	0.578	0.588	0.546	0.461	0.372

Effect of sequence additions.

The effect of sequence additions has been studied depending on the same procedure applied in CPE under optimal conditions but in a different order. The results show that the order

of (drug + base + reagent + surfactant) is optimal because it produces the highest absorbance signal, and this order is fixed in subsequent experiments, as shown in Table 2.

Table (2): Effect of Sequence of additions

Sequence of Additions	Abs at λ _{max} 361 nm
D+B+R+S	0.588
D+R+B+S	0.545
D+ B + S+R	0.315
D+ S+B+R	0.425
R+B+D+S	0.023
D+R+S+B	0.500

D: Drug (Mesalazine), **B:** Base (NaOH), **R:** reagent (BG), **S:** surfactant (Triton X-114)

Quantification

The calibration graph was created under ideal experimental conditions. In this method, optical properties such as Beer's law limits, molar absorptivity, Sandell's sensitivity, limit of detection (LOD), and limit of quantitation

(LOQ) were calculated. Furthermore, the regression characteristics slope (b), intercept (a), and correlation coefficient (r) were calculated and are presented in Table 3, and Figure 12 depicts the calibration curves. Specifically, excellent linearity within the concentration

range used. The limit of detection (LOD) was calculated using $LOD = 3 (S_B / b)$ and the limit of quantification (LOQ) using $LOQ = 10 (S_B /$

$b)$, where S_B is standard deviation of blank solution ($N=5$) and b is the slope of the calibration graph.

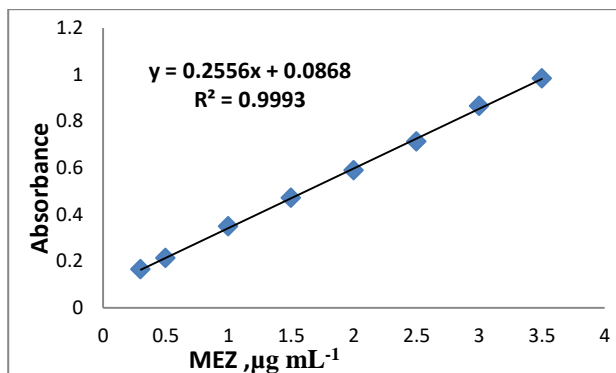


Fig. (12): Calibration graph of Mesalazine

Table (3): Characteristic parameters of the proposed method

Parameter	Value
λ max (nm)	361
Linearity range ($\mu\text{g mL}^{-1}$)	0.3-3.5
Correlation coefficient(R)	0.9993
Molar absorptivity, ϵ ($\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)	3.914×10^4
Regression equation	$Y=0.2556 X +0.0868$
Intercept (a)	0.0868
Slope (b)	0.2556
*LOD ($\mu\text{g mL}^{-1}$)	0.0532
*LOQ ($\mu\text{g mL}^{-1}$)	0.1772

* Average of four determinations

Accuracy and Precision

To check the accuracy and precision of the method, the recovery % and the relative standard deviation (RSD) were calculated for four replicates and for three different concentrations

of MEZ. The results shown in Table (4) indicate that the method has good accuracy, as the average recovery% is 100.82 and good precision as the average RSD is 0.87.

Table (4): Accuracy and precision of the CPE for determination of MEZ

Amount taken ($\mu\text{g mL}^{-1}$)	Amount Found ($\mu\text{g mL}^{-1}$)	Recovery* (%)	Average Recovery (%)	RSD %	Average RSD %
1	1.0207	102.07	100.82	1.26	0.87
2	1.9644	98.22		0.66	
3	3.0653	102.18		0.69	

*Average of four determinations

Selectivity

The selectivity of the developed CPE ion-association method has been examined by application of the standard addition procedure for Mesalazine in commercial formulation as tablet and capsule. The results showed in

Fig.13(a & b) indicate the method is free from the effect of additive interactions in its pharmaceutical preparations. Where it was found that the recovery% values are 98.8% and 98.07% for tablet and capsule respectively.

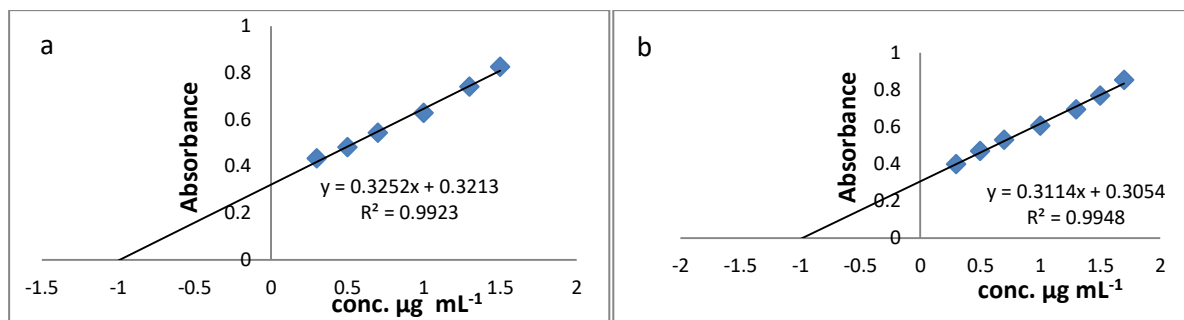


Fig. (13): Standard addition plots for determination of 1 µg mL⁻¹ Mesalazine in tablet (a) and capsule (b)

Validation of the method

The developed method was successfully applied for determination of MEZ, as an active ingredient, only in two commercial

pharmaceuticals available (capsule and tablet). The results, cited in Table 5, indicated good accuracy and precision and showed no interferences with the excipients.

Table (5): Determination of MEZ in the pharmaceutical formulations

Pharmaceutical formulation	Amount Taken (µg.mL ⁻¹)	Recovery ^a %	Average Recovery%	RSD%	Average RSD %	Certified value (mg)	Recovery value (mg)
Metaza ^b tablet	1	103.53	100.72	1.24	1.6	500	503.6
	2	97.36		1.36			
	3	101.28		2.2			
Mesacol ^c capsule	1	103.17	101.61	2.53	2.30	400	406.44
	2	97.57		2.08			
	3	104.10		2.30			

^a Average of four determinations

^b Manufactured by Awamedica company

^c Manufactured by Unipharma company

Stoichiometric, stability constant and suggested mechanism of CPE complex

The continuous variation (Job's method) and mole ratio procedures were applied to assess the stoichiometry of MEZ: BG ratio. The two procedures are depended on using same concentration (1×10^{-3} M) for both drug and reagent in the presence of NaOH and Triton X-114, and following the general procedure. The results, as show in Fig.14 (a&b), indicated that the ratio is 1:2 MEZ : BG in the complex. However, the conditional stability constant of

the complex was calculated depending 1:2 ratio using the following equation:

$$K_{st} = \frac{1 - \alpha}{4\alpha^3 C^2} \quad \text{and} \quad \alpha = \frac{Am - As}{Am}$$

Where K_{st} is the stability constant (L.mol^{-1}), α is the dissociation degree, C is the molar concentration of the complex, Am is the excessive amount of BG and As is the stoichiometric amount of BG. The result is $3.4 \times 10^9 \text{ L.mol}^{-1}$ indicated the complex have good stability.

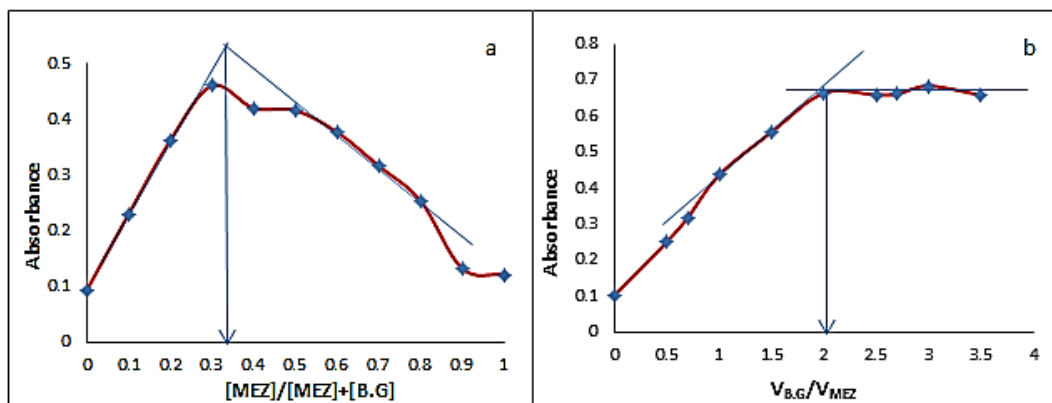
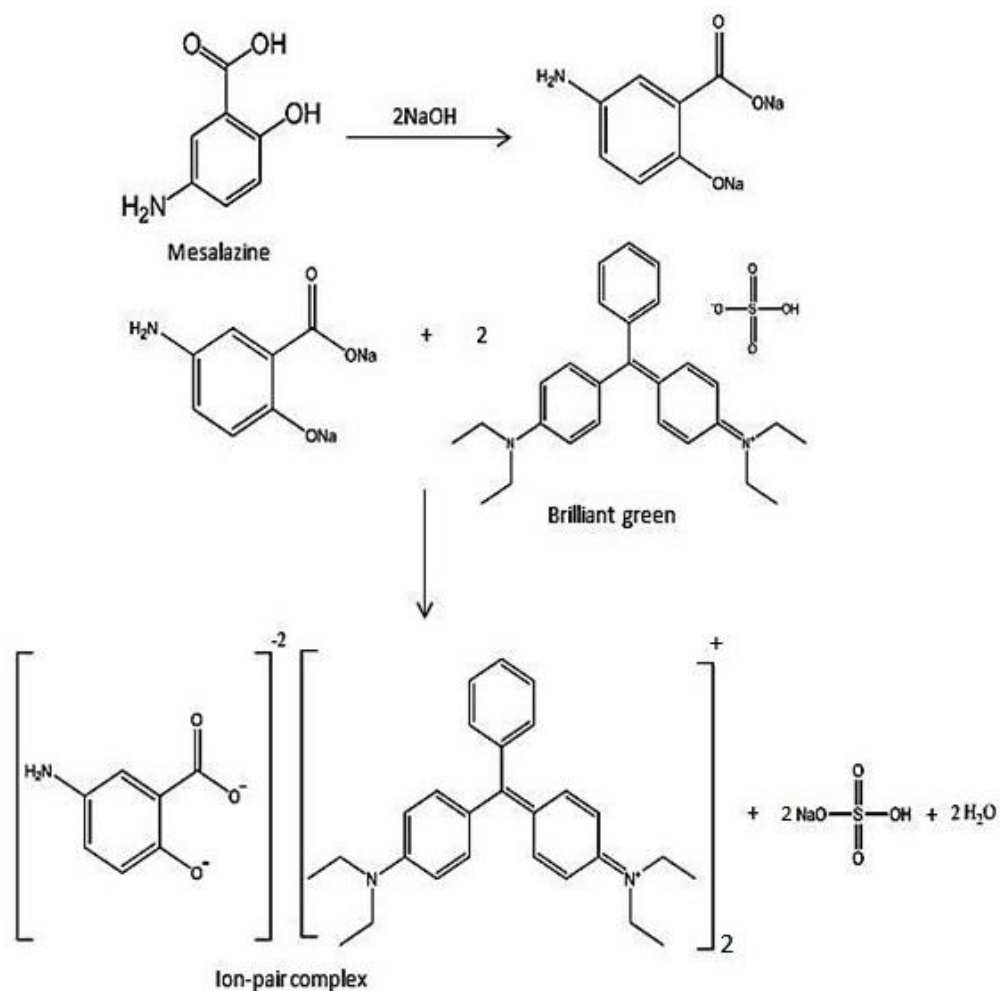


Fig. (14): Plots of continuous variation (a) and mole ratio (b) for MEZ-BG complex

According to the of Job's and mole ratio results obtained above, the mechanism of the MEZ-BG ion- pair complex formation reaction have been suggested as shown in scheme 1 (Ahmida et al., 2009).



Scheme (1): Suggested mechanism for MEZ-BG ion-pair complex

Comparison of the CPE method with literature methods

The developing method has been compared with other literature methods, for the determination of Mesalazine, but all of these methods cited in Table 6 suffer from limitations

involving for instance, heating, hydrolysis, low sensitivity or tedious.

Table

Analytical parameters	Present method		Literature method	
	BG	o-Chloranil (Mohammed S. Al-Enizzi, Theia'a N. Al-Sabha*, 2012)	Fe-bipyridyl (Desai & Kale, 2019)	Vanillin (Chandra et al., 2011)
$\lambda_{\max}(\text{nm})$	361	571.5	520	320
pH	11.22	9.8	-	Acidic
Temp.(°C)	50	25	100	R.T
Development time (min)	20	5	15	-
Stability period (min)	90	45	-	120
Beer's law range ($\mu\text{g mL}^{-1}$)	0.3-3.5	1.25-30	4.0-24	2.0-30
Molar absorptivity ($\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)	3.914×10^4	3.40×10^3	0.38×10^3	1.283×10^4
Recovery(%)	100.82	100.44	99.93	100.34
RSD(%)	0.87	1.67	0.684	0.409
Application	Tablet, Capsule	Tablet, Capsule	Tablet	Tablet

(6):.

Comparison of the suggested method with the literature methods

CONCLUSION

A new spectrophotometric method, depending on CPE of MEZ-BG ion pair complex in the presence of NaOH and Triton X-114, has been developed for the determination of Mesalazine in pure and pharmaceutical forms. The proposed method was sensitive and reliable with good precision and accuracy. The method was applied successfully for determination Mesalazine in its commercial forms as tablet and capsule. The suggested method is superior to the previously reported spectrophotometric methods in terms of sensitivity and stability.

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پوخته

ئەف توپزینە وەپە باسی لە ریکا شەبەنگی نوێ وئاسان وھویربەین بو دیار کرنا میسالا زین (Mesalazine) دناف دەرمانا . ئەف ریکە لاسەر بنە مایئ پوختهکردنی خالی عەوری جووتە ئایونی وپیکھاتنا ئاویتەبی ئالوزی لئاڤهرا کارلیکی میسالازین دگەل بویاغی بریلینت سەوز , (Brilliant green dye) وپوخته کرن ژبەر رووکرژی ترایتون (Triton X - 114) وپیقانا جووتە ئایونی زەری قە بی لە 361 نانومیتەرکە بلند ترین ئاستی مژیناوی وپاسایئ بیرھاتە بجھئینان لە مەودای (5.3- 30) میکروگرام مل-1 وەھاوکوگەیی مژینا مولاری 104×3.914 لترمول 1-سم-1 دگەل لادانا ستاندەر (RSD=0.87) وھویربەینی بریکا تیکرایی قەگەریانا 100.82%.

الخلاصة

تم وصف طريقة طيفية بسيطة ودقيقة لتقدير الميزالازين في شكله النقي وفي مستحضره الصيدلاني. تعتمد الطريقة على استخلاص النقطة السحابية للمزدوج الأيوني، الناتج من تفاعل الميزالازين مع صبغة البريلينت الخضراء (brilliant green dye)، بواسطة عامل الشد السطحي ترايتون (Triton X-114). حيث تم قياس المزدوج الأيوني الأصفر الفاتح عند 361 نانومتر. تم دراسة الظروف المثلى لفصل الطور. اتبعت الطريقة قانون بير ضمن مدى التراكيز 0.3-3.5 مايكروغرام مل⁻¹ بامتصاصية مولارية 3.914×10^4 لترمول⁻¹ سم⁻¹، وبمعدل نسبة استرجاع 100.82% وتوافقية (انحراف قياسي نسبي 0.87، تم تطبيق الطريقة بنجاح في تقدير الميزالازين في مستحضره الصيدلانية.