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Indirect Spectrophotometric Determination of Mesalazine via Chromate-1,5-Diphenyl carbazide Complex

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ABSTRACT

A simple and rapid indirect spectrophotometric method for the determination of mesalazine is developed. The method is based on the rapid reduction of known large amount of chromate CrO_4^{-2} in the presence of mesalazine in acidic medium of 2N H_2SO_4. The excess amount of chromate is measured after it's reaction with 1,5-diphenylcarbazide which finally gives a pink-violet, water soluble and stable complex, which exhibit a maximum absorption at 546 nm. Beer's law is obeyed in the concentration range from (5-900) µg of mesalazine in a final volume of 25 ml (0.2-36 ppm) with a molar absorbtivity of 1×10^5 $l.mol^{-1}.cm^{-1}$, Sandell's sensitivity index of 0.0015 µg $.cm^{-2}$ and relative standard deviation ± 0.0848 to ± 0.3155 depending on the concentration level. The proposed method is applied successfully to the assay of mesalazine in its pharmaceutical prepearation (capsules).

Keywords: Mesalazine, Spectrophotometic Method, Chromate-DPC, 1,5-Diphenylcarbazide.

التقدير الطيفي غير المباشر للميزالازين باستخدام معقد الكروم–5,1–ثنائي فنيل كاربازيد

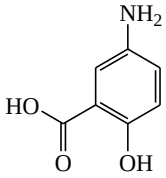
الملخص

يتضمن البحث تطوير طريقة طيفية غير مباشرة بسيطة وسريعة لتقدير الميزالازين. تعتمد الطريقة على اختزال الكرومات CrO_4^{-2} بوساطة الميزالازين في الوسط الحامضي باستخدام حامض الكبريتيك (2 عياري) ومن ثم تقدير الكمية غير المختزلة من الكرومات بقياس طيف امتصاص المعقد الملون المتكون بين الكرومات غير المختزلة وبين الكاشف العضوي 5,1– ثنائي فنيل كاربازيد عند الطول الموجي 546 نانوميتر. كانت حدود قانون بير في مدى التركيز (5-900) مايكروغرام من الميزالازين في حجم نهائي 25 مللتر، (0.2-36 ppm). كانت الامتصاصية المولارية 1×10^5 لتر.مول$^{-1}$.سم$^{-1}$، دلالة ساندل للحساسية 0.0015 مايكروغرام. سم$^{-2}$ والانحراف القياسي النسبي في مدى ±0.0848 إلى ±0.3155 اعتمادا على مستوى التركيز. تم تطبيق الطريقة المقترحة بنجاح في تقدير الميزالازين في مستحضره الدوائي (الكبسول).

الكلمات الدالة: الميزالازين، التقدير الطيفي، الكرومات DPC، 5,1– ثنائي فنيل كاربازيد.

INTRODUCTION

Mesalazine, also known as mesalamine or 5-aminosalicylic acid (5-ASA), is an anti-inflammatory drug used to treat inflammatory bowel disease, such as ulcerative colitis and mild-to-moderate Crohn's disease. Mesalazine is a bowel-specific aminosalicylate drug that acts locally in the gut and has its predominant actions there, thereby having few systemic side effects. As a derivative of salicylic acid, mesalazine is also thought to be an antioxidant that traps free radicals, which are potentially damaged by products of metabolism. Mesalazine is the active moiety of sulphasalazine, which is metabolized to sulphapyridine and mesalazine. Mesalazine is formed from the prodrug balsalazide along with the inert carrier molecule 4-aminobenzoyl-beta-alanine. (Liu *et al*., 1995; Sandborn *et al*., 2007 and Kruis *et al*., 2001)



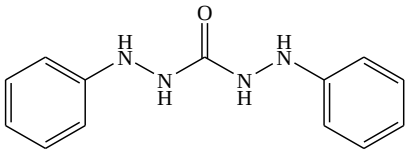
5-Amino-2-hydroxybenzoic acid

$C_7H_7NO_3$

M.wt.= 153.135 g/mol

Different spectrophotometric methods have been reported for the determination mesalazine in bulk and tablet dosage forms based on diazodtization and coupling reaction with resorcinol (Madhavi *et al*., 2011), other depend on three ways: first based on the reduction of tungstate and/or molybdate in Folin ciocalteu's reagent, second describes the coupling between the diazotized drug and α-naphthol, and the third one based on the reaction of the drug with vanillin in acidic medium (Chandra *et al*., 2011), two simple spectrophotometric methods for the assay of mesalamine in bulk and pharmaceutical formulation have been carried out, first method based on the reaction of mesalamine with 1,2-naphthoquinone-4-sulphonate in the presence of sodium hydroxide, second method based on a condensation reaction between mesalamine and acidic solution of p-dimethyl amino cinnamadehyde (Narala1 and Saraswathi, 2010). Other spectrophotometric methods were applied for the determination of mesalazine based on oxidative coupling with: 2,6-xylenol (Al-Fakhry, 2006), 4-aminoantipyrine (Lupett *et al*., 2004). UV spectrophotometric methods are used for the determination of mesalamine in bulk and tablet dosage form at 330 nm (Gatkal *et al*., 2013), while the drug reacts with 0.5N HCl shows absorption maximum at 303 nm (Moharana *et al*., 2011), Finally, determine the pharmacokinetic parameters of mesalamine 400 mg tablets (Kanala, *et al*., 2013).

1,5-Diphenylcarbazide (DPC) is an organic compound usually used in analytical chemistry for colorimetric measurements. It exhibits many useful properties, it is used as an artificial donor during charge separation in photochemical reactions and also photosynethesis electron transport (Sandell,1950).



1,5-Diphenyl carbazide
$C_{13}H_{14}N_4O$
M.wt. = 242.28

It is well known that chromate-1,5-diphenylcarbazide chelate shows an intense pink-violet colour at pH 0.2 (Sandell, 1950; Kostakis *et al*., 2013), on the other hand mesalazine reduced chromate to chromium (III) then the excess of chromate reacted with 1,5-diphenylcarbazide.

The purpose of this work is to make use of these fact to develop a simple, sensitive and rapid spectrophotometric method for the determination of mesalazine, without requiring an expensive instrumentation, without extraction of product or temperature control, and the possibility of application of the proposed method to determination of mesalazine in its pharmaceutical preparation (capsules).

EXPERAMENTAL

Apparatus

Spectral and absorbance measurements are carried out using double-beam Spectrophotometer (CECIL 7200). In all measurements 1-cm matched quartz cells are used. The pH measurements are carried out using HANA pH meter.

Chemicals

All chemicals used are of analytical reagent grade.

Working mesalazine (5-ASA), (100 µg/ml):

A 0.0100 g of 5-ASA is dissolved in 10 ml distilled water, and the volume is completed to 100 ml in a volumetric flask with same solvent.

Potassium chromate solution (1.72×10^{-3} M):

This solution is prepared by dissolving 0.1672 g of potassium chromate (Fluka) in 100 ml distilled water in a volumetric flask. The solution is transferred to a dark bottle and it is stable for at least one month.

1,5-diphenylcarbazide (DPC) solution (1×10^{-3} M):

This solution is prepared by dissolving 0.0606 gm of 1,5-diphenylcarbazide (BDH) in enough amount of pure acetone, then the volume is completed to the mark with 250 ml distilled water in a volumetric flask.

Sulphuric acid (2N):

This solution is prepared by adding 28.1 ml of concentration sulphuric acid solution to the distilled water then complete the volume to the mark with 250 ml distilled water in a volumetric flask.

Mesacol capsules solution (100 µg/ml.):

Weight and mix the contents of ten capsules (each one contains 400 mg mesalazine), an accurately weighed amount of powder (0.0111 g) equivalent to 0.0100 g mesalazine is dissolved in 10 ml of absolute ethanol and 30 ml distilled water, after filtration of the solution, the volume is completed to 100 ml with distilled water in a volumetric flask to prepare a solution of 100 ppm.

Recommended procedure for the determination of 5-ASA:

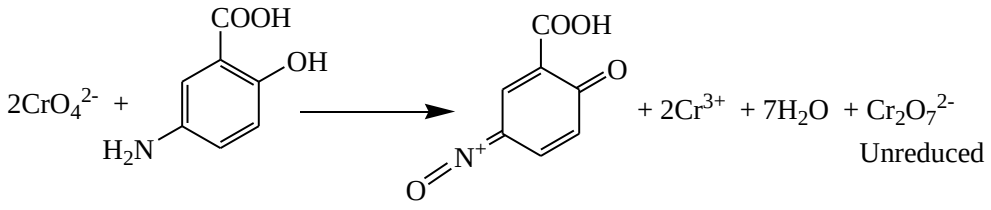
An aliquots of standard 5-ASA solution covering the range 5-900 µg is transferred in to a series of 25 ml volumetric flask. To each flask, 2.0 ml of sulphuric acid (2N), 1 ml of chromate solution 1.72×10^{-3} M, and 2 ml of DPC 1×10^{-3} M, were added. The solution is diluted to the mark with distilled water and mixed well. After 2 minutes, the absorbance is measured against a similarly prepared reagent blank at 546 nm. The colour is stable for at least 3.0 hours.

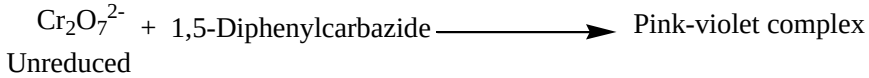
RESULTS AND DISCUSSION

For subsequent experiments, 100 µg/25 ml of 5-ASA was taken.

Colour reaction

In aqueous solution chromate reduced in acid medium by 5-ASA, then the unreduced chromate is reacted with DPC to give an intense pink-violet complex. The reaction sequence is postulated as follows:





Absorption spectra

When 5-ASA is treated according to the recommended procedure, the absorption spectrum shows a maximum absorption at 546 nm, characteristic of the chromate-DPC colour.

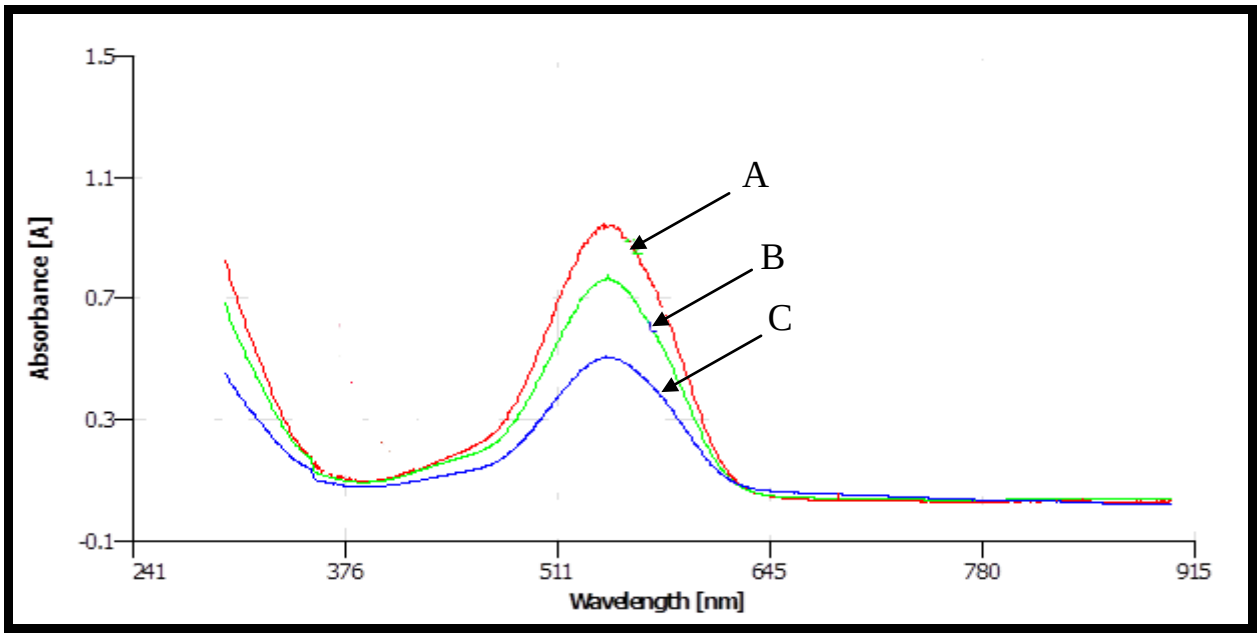


Fig. 1: Absorption spectra of (A) chromate against reagent blank, (B) 100 µg of 5-ASA /25 ml measured against reagent blank (C) 400 µg of 5-ASA /25 ml measured against reagent blank

Study of optimum conditions

The effect of various parameters on the intensity of the coloured complex have been studied and optimum conditions have been selected.

Effect of sulphuric acid volume

In order to choose the optimum amount of sulphuric acid on the reaction of 5-ASA with chromate, and the formation of a stable coloured complex between chromate and DPC, different amounts (0.5-4.0) ml of sulphuric acid (2N) were tested. The results are shown in Table 1 indicate that 2.0 ml of 2N H_2SO_4 is considered optimum, as it gives the more stable coloured complex (Sandell,1950), therefore it is recommended for subsequent experiments.

Table 1: Effect of sulphuric acid

Time, Minutes	**Absorbance/ ml of sulphuric acid 2N**							
	0.5	1.0	1.5	**2.0**	2.5	3.0	3.5	4.0
5	0.831	0.747	0.735	**0.717**	0.731	0.726	0.729	0.719
10	0.843	0.751	0.744	**0.718**	0.739	0.736	0.730	0.724
15	0.859	0.755	0.753	**0.717**	0.744	0.751	0.744	0.753
20	0.867	0.762	0.760	**0.718**	0.757	0.758	0.751	0.755
25	0.870	0.769	0.773	**0.717**	0.772	0.764	0.766	0.763
30	0.882	0.771	0.784	**0.717**	0.781	0.769	0.771	0.767
Final pH	2.25	2.14	1.90	**1.88**	1.74	1.66	1.60	1.53

Effect of time

The effect of different time on the reducing chromate CrO_4^{-2} to Cr(III) by 5-ASA on the absorbance of the resulting coloured complex has been investigated. Also, the development and stability of coloured complex for 100 µg of 5-ASA is investigated. The results showed that 2 minutes reaction time is optimum and recommended for the subsequent experiment and the reaction is stable at least 60 minutes.

Table 2: Effect of time

µg of 5-ASA	Absorbance / minute									
	1	2	3	5	10	20	30	40	50	60
100	0.729	0.717	0.718	0.719	0.718	0.718	0.719	0.718	0.719	0.719

Effect of 1,5-diphenylcarbazide amount

It is found from the experimental results (Table 3) that 2 ml of DPC 1×10^{-3}M is optimum (correlation coefficient = 0.99953), and recommended for the subsequent experiments.

Table 3: Effect of sulphuric acid

mlof DPC (1×10^{-3}M)	Absorbance/ µg of 5-ASA								R^2
	25	50	100	200	300	400	500	600	
0.5	0.545	0.499	0.407	0.388	0.361	0.319	0.268	0.184	0.96728
1.0	0.655	0.593	0.503	0.466	0.423	0.376	0.299	0.201	0.97844
1.5	0.722	0.691	0.601	0.577	0.491	0.433	0.323	0.247	0.99232
2	0.771	0.755	0.719	0.619	0.547	0.458	0.364	0.288	0.99953
2.5	0.753	0.711	0.688	0.612	0.572	0.458	0.364	0.288	0.99475
3.0	0.801	0.786	0.701	0.674	0.589	0.449	0.368	0.285	0.99338

Effect of surfactants

The presence of surfactants in a coloured mixture solution frequently does not lead to an increase in the absorbance or a shift in the wavelength to higher values. Then a test of the effect of surfactant addition to the reaction mixture. In this respect, sodium dodecyl sulfate (SDS) (anionic surfactant), cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CPC) (cationic surfactants) and Triton X-100 (non-ionic surfactant) have been introduced. The results indicated that addition of surfactants gave no useful effect. Therefore, omitted in this study.

Effect of order of addition:

The different orders of addition were studied. The results shown in table 4 indicate that the first order is optimum because it gives lowest absorbance value, therefore recommended for the subsequent experiment.

Table 4: Effect of order of addition

Reaction component	Order number	Absorbance
5-ASA+H+C+R	I	0.718
5-ASA+C+R+H	II	0.788
5-ASA+H+R+C	III	0.763
5-ASA+R+C+H	IV	0.761
5-ASA+R+H+C	V	0.731

5-ASA= Mesalazine, C=Chromate, H= Sulphuric acid, R= DPC.

Development time and stability period

To test the effect of time on the absorbance of the coloured complex at the wavelength of maximum absorption at 546 nm, under the optimum experimental conditions, the absorbance is

measured at different intervals of time. The experimental results showed that the coloured complex develops immediately and the absorbance remains maximum and constant for at least 3 hr.

Beer's law, molar absorptivity and sensitivity

The linearity of the change in absorbance with variation of the amount of 5-ASA present is tested by reacting aliquots of the standard solution containing 5.0-900 µg of 5-ASA followed by 2.0 ml of sulphuric acid (2N), 1 ml of chromate solution (1.72×10^{-3}M), and 2 ml of DPC (1×10^{-3} M) in a final volume of 25 ml and measuring the absorbance at 546 nm. Beer's law is obeyed over the range of 0.2-36 ppm of 5-ASA as illustrated in Fig. 2. The molar absorptivity being 1×10^{5} l.mol^{-1}.cm^{-1}, and the Sandell's sensitivity is 0.0015 µg .cm^{-2}.

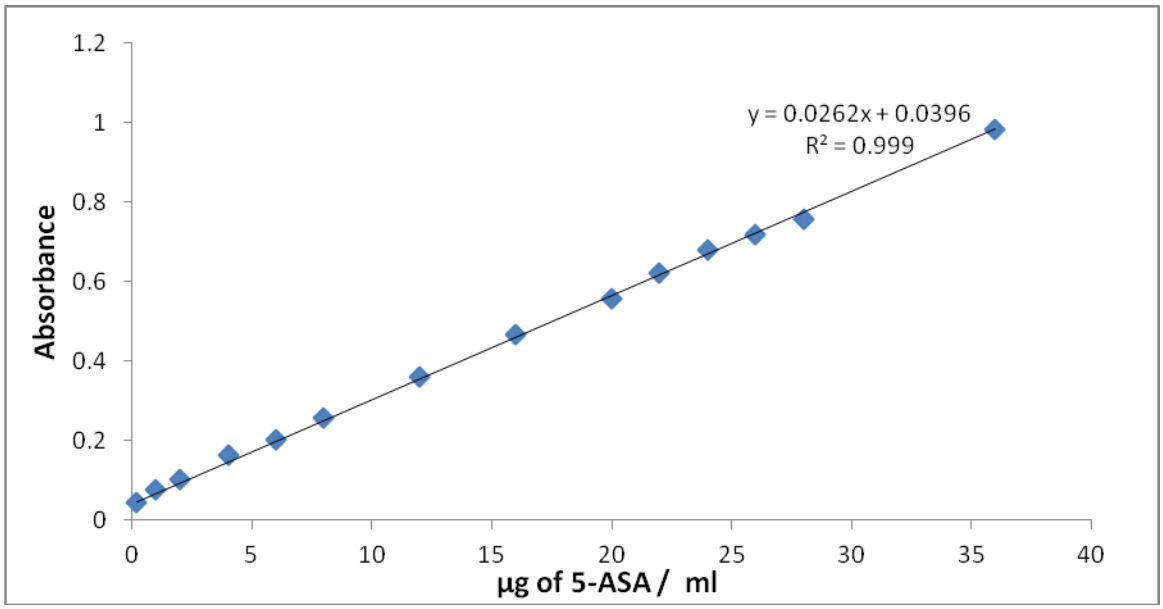


Fig. 2 : Calibration graph for 5-ASA determination

Accuracy and precision

To check the accuracy and precision of the method, 5-ASA is determined at four concentrations. The results shown in table 5 indicated that the proposed method is reliable.

Table 5 : Accuracy and precision

Amount of 5-ASA taken, µg/25ml	Recovery*, %	Relative standard deviation*, %
50	99.97	± 0.0848
100	99.89	± 0.1588
200	100.26	± 0.1835
500	100.33	± 0.3155

* Average of five determinations.

Nature of the reaction between chromate and (DPC) reagent

Job's method (Delvie, 1997) has been used in the determination of the reaction ratio of 5-ASA with chromate. The obtained results showed that 1:2 5-ASA to chromate ratio is obtained. Also Job's method has been used in the determination of the reaction ratio of chromate with DPC reagent, the obtained results in Fig. 3 showed that the ratio is 1:2 chromate to DPC reagent.

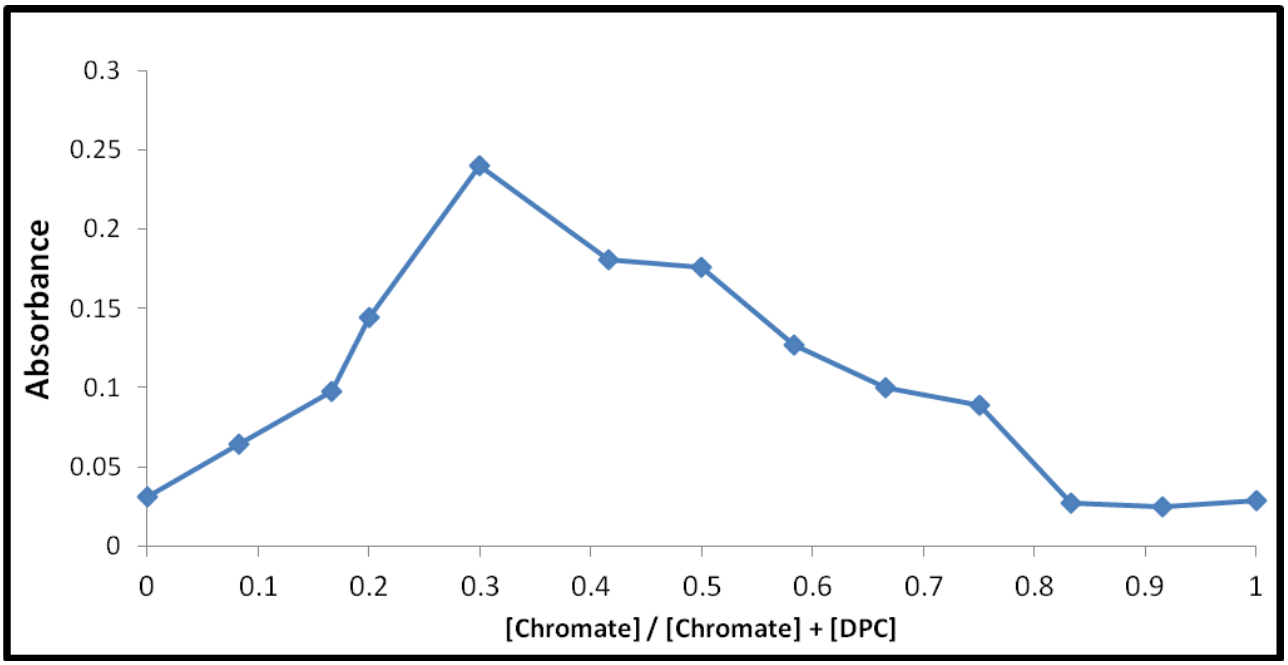


Fig. 3: Job's plot for chromate – DPC

Also the stoichiometry of the reaction is investigated using the mole ratio method under the optimized conditions. The obtained results in Fig.4 showed that a 1:2 chromate to DPC reagent is obtained.

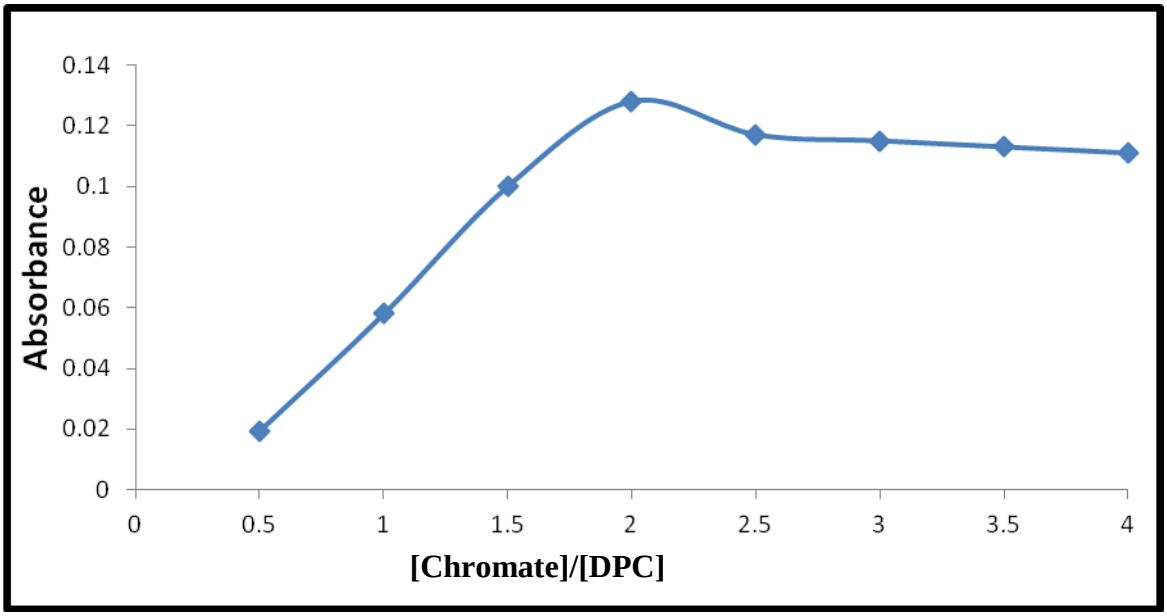


Fig. 4: Mole ratio plot for chromate-(DPC) reagent complex.

Effect of interference

The effect of some foreign compounds which often accompanied pharmaceutical preparation were studied by adding four amounts (50, 200, 500 and 1000 µg) to 100 µg mesalazine in a final volume 25 ml (Table 6).

Table 6: Effect of interference

Foreign compound	Recovery (%) of 100 µg mesalazine per µg foreign compound added			
	50	200	500	1000
Glucose	100.13	100.27	100.41	100.69
Lactose	99.31	99.03	99.86	99.72
Starch	99.58	99.44	99.72	99.86
Gum Arabic (Acacia)	99.86	99.72	99.45	99.59

The results in Table 5 indicated that the studied foreign compounds do not interfere in the determination of mesalazine by using the proposed method.

Application of the method

To test the applicability of the present method, it has been applied to the determination of 5-ASA in pharmaceutical preparation (capsules). On applying proposed procedure, good recovery is obtained as shown in Table 7

Table 7: Determination of mesalazine in capsule

Mesalazine, µg	Recovery*, % capsule 400 mg/capsule Unipharma-Syria
50	100.37
100	100.27
200	100.70

Comparison of the method

Table 8 shows the comparison of spectrophotometric methods for 5-ASA determination.

Table 8: Comparison of the method

Analytical parameters	Present method	Literature method (Rawa, 2009)
Reaction	Indirect oxidation reaction	Diazotization
pH	1.98	12.38
λ_{max} (nm)	546	471
Reaction time (min)	2	10
Beer's law range (ppm)	0.2-36	0.4-12
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1×10^5	2.9479×10^4
R.S.D. (%)	±0.0848 - ±0.3155	±1.05 to ±0.37
Colour of the product	Pink-violet	Orange
Application of the method	Pharmaceutical preparation (capsules)	Pharmaceutical preparation (capsules)

The present method seems to be rapid, sensitive, fair and can be applied to the determination of 5-ASA in pharmaceutical preparation.

The performance of the proposed method is assessed by calculating the student t-test (Christian, 2004) with the literature method (Rawa, 2009). At the 95% confidence limit for five degree of freedom, the calculated t-value don't exceed the theoretical value. The value of experimental t-test (0.02529) compared to the theoretical (t-test) (2.571) indicating that there is no significant difference between the proposed method and the literature method (Rawa, 2009).

CONCLUSION

A simple, sensitive, selective and inexpensive spectrophotometric method for the determination of 5-ASA in pharmaceutical preparation has been carried out by the rapid reduction of known amount of chromate CrO_4^{-2} in the presence of 5-ASA in acidic medium of sulphuric acid. Then the excess of chromate is measured when it reacts with 1,5-diphenylcarbazide as a reagent which finally gives a pink-violet, water soluble and stable complex, which exhibit maximum absorption at 546 nm. The present method has been applied for determination of 5-ASA in pharmaceutical preparation (capsules).

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