Spectrophotometric Determination of Mesalazine with Phloroglycinol in Pharmaceutical Preparation

Enam A. Hamdon , Safaa A. Zakaria , Nassim M.AL-Hamdany
Department of chemistry, College of Science , University of Mousl , Mousl , Iraq
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Abstract
A simple sperctrophotometric method for the determination of mesalazine in aqueous solution is achieved. The method is based on the reaction of mesalizine, with excess nitrite, in an acidic medium, to produce the corresponding diazonium salt. After the removal of residual nitrite with sulphamic acid, the diazonium salt was coupled with phlorogycinol reagent in basic medium to produce, an intense yellow-orange coloured water-soluble and stable azo dye which exhibits maximum absorption at 430 nm. Beer's law is obeyed in the concentration range of 5-300 µg of mesalazine in a fina0.34l volume of 25 ml i.e., 0.2-12 ppm with a molar absorptivity of 3.02×10^{4}l.mol^{-1}.cm^{-1},a relative error of –o.56 to 0.19% and a relative standard deviation of± 0.34 to ±0.91 % depending on the concentration level. The proposed method has been applied successfully to determine mesalazine in pharmaceutical preparation as capsules.
Keyword: Mesalazine, Phloroglycinol, Pharmaceutical Preparation.

Introduction
Mesalazine is chemically, 5-aminosalicylic acid (5-ASA), which is used as a gastrointestinal anti-inflammatory drug for the treatment of inflammatory bowel diseases[1] and active ulcerative proctitis[2,3] , is metabolized in organism to the principle biotransformation product, N-acetyl-5-ASA, is a polar compound and besides it exhibits amphoteric properties [4]**.** Different methods have been reported for the determination of 5-ASA including: mass spectroscopy [5], high performance liquid chromatography [6], micellar electrokinetic chromatography [7] , reverse phase-high performance liquid chromatography [8], differential pulse voltammetry [9].Various spectrophotometric methods are used for the determination of 5-ASA these include oxidative coupling with 2,6-xylenol in the presence of sodium metaperiodate [10].Another method include reaction of 5-ASA with 1,2-naphthoquinone-4-sulphonate (NQS) in the presence of sodium hydroxide [11], a mixture of potassium iodate and potassium iodide were used for the determination of 5-ASA in bulk and pharmaceutical formations [12] , also, 5-ASA was determined by formation of three coloured chromogens, which were measured at 552 nm, 440 nm, and 494 nm respectively [13].A colouremetric method has been developed for the determination of 5-ASA in urine and feces using Bratton-Marshall reaction [14], also, 5-ASA has been determined in pharmaceutical preparations by galvanostatic coulometric method using reactions of electrogenerated bromide and chlorine with 5-ASA [15], the objective of the investigation reported in this paper is to develop of spectrophotometric method for the determination of 5-ASA based on the diazotization of 5-ASA and coupling with phloroglycinol reagent and applying the method to the determination of 5-ASA in pharmaceutical preparation as capsules.

Experimental Apparatus
All spectrophotometric measurements are performed on shimadzu UV-Visible recordingspectrophotometer UV-160 using 1-cm silica cells. pH meter types Philips PW 9420 is used for pH reading.

Reagent
All chemicals used are of the highest purity available.
working 5-ASA solution,50 µg.mL^{-1}. This solution was prepared by dissolving 0.01g of 5-ASA supplied by (Fluka) in 10 ml distilled water, and the volume was completed to 200 ml in a volumetric flask.
Hydrochloric acid solution, 1 N. This solution was prepared by diluting 8.5 ml of the concentrated acid to 100 ml with distilled water.
Sodium nitrite solution, 1%. This solution was prepared by dissoslving 1 g of sodium nitrite in 100 ml distilled water in a volumetric flask.
Sulphamic acid solutions, 3%. This solution was prepared by dissolving of 3 g of sulphamic acid in 100 ml distilled water.
Phloroglycinol solution, 0.1%. This solution was prepared by dissolving 0.1 g of phloroglycinol in distilled water in a 100 ml volumetric flask.
Sodium hydroxide solution, 1N. This solution was prepared by appropriate dilution of the concentrated (Fluka) solution with distilled water and then transferred to a plastic bottle.
Mesacol capsules solution 50 µg.ml^{-1} . Weight and mix the contents of ten capsules (each one containe 400 mg 5-ASA), an accurately weighed amount of powder (0.0111 g) equivalent to 0.01 g 5-ASA was dissolved in 10 ml of absolute ethanol and 30 ml distilled water, after filtration of the solution, the volume was completed to 200 ml of distilled water in a volumetric flask to prepare a solution of 50 ppm 5-ASA.

Procedure and calibration graph
To a series of 25 ml volumetric flasks aliquots covering the range of 5-400 µg (0.2-16 ppm) of 5-ASA were transferred followed by addition of 0.7 ml of 1N HCl then the mixtures were shaken. Then 0.3 ml of 1% sodium nitrite solution was added and the mixtures were allowed to stand for 3 minutes. Then 0.1 ml of 3% sulphamic acid solution was added then left for 4 minutes , 2 ml of sodium hydroxide solution

(1N) was added, The volumes were completed to the mark with distilled water and the absorbance were read after 5 minutes at 430 nm against blank solution. (Fig.1) shows the calibration curve which indicates that Beer's law was obeyed over the concentration range 5-300 µg / 25 ml final volume, i.e., 0.2-12 ppm and concentration above 300 µg /25 ml gives negative deviation. The molar absorptivity was 3.02×10^4 l.mol^{-1}.cm^{-1}.

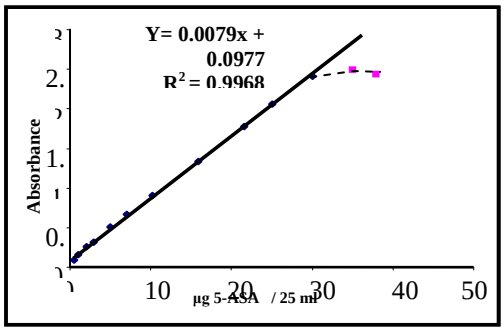
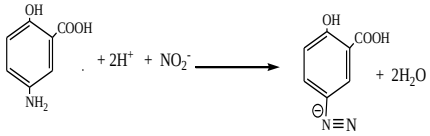


Fig.1: Calibration graph for 5-ASA determination using phloroglucinol as coupling reagent.

Results and disscusion

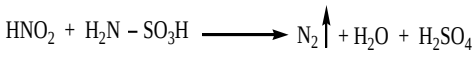
Principles of the method

5-ASA was reacted with excess nitrite in acidic medium to form the corresponding diazonium salt:

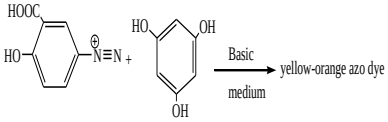


Diazotised 5-ASA

The residual nitrite (as nitrite acid) which was undesirable due to its side reaction, such as, nitrosation of coupling agent. Therefore, it should be removed by sulphamic acid which reacts more fast than urea[16]:



The coloured solution formed by coupling diazotized 5-ASA with phloroglycinol in alkaline medium:



Study of the optimum reactions conditions

The various parameters effecting and related the colour intensity of the dye have been studied and optimum conditions were selected.

Effect of acids on the diazotization

The effect of the amount of different acid (week and strong) for the diazotization of 5-ASA, have been investigated. The results were indicated that 0.7 ml of 1N HCl produces the highest intensity for the dye, so it has been selected in the subsequent experiments (Table 1).

Table 1: Effect of diazotization acid on absorbance

Acid used (1N)	Absorbance (A)/ ml of acid used					
	0	0.2	0.5	0.7	1.0	1.5
HCl	0.342	0.430	0.439	0.447	0.157	0.103
H_2SO_4	0.328	0.419	0.432	0.441	0.203	0.162
HNO_3	0.355	0.420	0.424	0.436	0.444	0.107
H_3PO_4	0.258	0.389	0.390	0.406	0.248	0.195
CH_3COOH	0.394	0.377	0.361	0.362	0.140	0.087

Effect of nitrite amount and time

The colour was reached maximum intensity when using 0.3 ml of 1% (w/v) sodium nitrite solution within 3 minutes reaction time(Table 2), it seems that diazotization of 5-ASA was fast

Table 2: Effect of nitrite amount and time on absorbance .

Amount of 1% $NaNO_2$ solution	Absorbance / minute of standing time					
	0	1	2	3	4	5
0.1	0.419	0.424	0.428	0.431	0.418	0.413
0.2	0.315	0.436	0.424	0.435	0.436	0.437
0.3	0.431	0.434	0.432	0.449	0.431	0.436
0.5	0.433	0.435	0.434	0.435	0.438	0.436
0.7	0.310	0.430	0.429	0.433	0.432	0.400

Effect of sulphamiac acid amount and time

The presence of unreacted nitrite was undesirable in diazotization reaction. Therefore, it should be removed by sulphamic acid which fastly reacts with nitrite. The results indicated that 0.1 ml of 3% sulphamic acid solution with 4 minutes standing time were considered to be the most suitable (Table 3), and therefore were selected subsequently.

Table 3: Effect of sulphamic acid and time on absorbance

Amount of 3% sulphamic acid (ml)		**Absorbance / minute standing time**					
		0	**1**	**2**	**3**	**4**	**5**
0	**S**	0.026	0.033	0.018	0.108	0.225	0.152
	B	0.135	0.150	0.147	0.147	0.149	0.154
0.1	**S**	0.428	0.377	0.459	0.465	0.481	0.455
	B	0.057	0.096	0.028	0.041	0.013	0.008
0.2	**S**	0.415	0.459	0.441	0.453	0.443	0.450
	B	0.021	0.005	0.006	0.005	0.004	0.005
0.3	**S**	0.441	0.445	0.436	0.446	0.437	0.446
	B	0.012	0.008	0.012	0.012	0.013	0.012
0.5	**S**	0.428	0.437	0.437	0.442	0.437	0.442
	B	0.012	0.012	0.012	0.012	0.002	0.002

Effect of phloroglycinol amount

The effect of phloroglycinol amount on the colour intensity of the dye has been studied. From the result, it can be observed that 4 ml of 0.1% phloroglycinol was the more suitable amount which gives the highest value of correlation coefficient (Table 4).

Table 4: The effect of phloroglycinol amount

Amount of 0.1% phloroglycinol (ml)	**Absorbance / µg of 5-ASA**						
	20	**50**	**70**	**100**	**150**	**200**	**r**
1	0.226	0.446	0.617	0.858	1.255	1.646	0.9999
2	0.216	0.430	0.605	0.844	1.208	1.621	0.9995
3	0.276	0.493	0.647	0.892	1.252	1.620	0.9997
4	0.270	0.486	0.648	0.887	1.288	1.687	0.9998
5	0.206	0.489	0.650	0.869	1.279	1.761	0.9982

Effect of base

The effect of different type of base solution (which is necessary for developing the chromophore) on the

colour intensity and the colour contrast was investigated. The colour of azo dye became most intense and stable with high colour contrast when 2 ml of 1N sodium hydroxide solution (pH = 12.08) was used (Table5).

Table 5: The effect of base on the absorbance and colour contrast

Base used (1N)	variables	Absorbance / ml of base used						
		0.5	1	1.5	2	2.5	3	4
NaOH	A	0.489	0.488	0.495	0.513	0.508	0.495	0.487
	Δλ*, nm	40	45	52	57	50	49	45
KOH	A	0.455	0.446	0.452	0.487	0.472	0.453	0.418
	Δλ, nm	48	44	50	62	47	47	48
Na_2CO_3**	A	0.549	0.540	0.626	0.632	0.942	0.711	0.639
	Δλ, nm	18	10	30	24	27	11	36
$NaHCO_3$**	A	0.721	0.421	0.525	0.518	0.599	0.482	0.475
	Δλ, nm	17	25	25	25	30	29	32

$\Delta\lambda^* = \lambda max_S - \lambda max_B$, S= the dye; B= blank. ** Gives unstable azo-dye.

Effect of time on colour development

The colour of the formed azo dye reached the full intensity within not more than 5 minutes and it was stable for at least 1 hour (Table 6).

Table 6: Effect of time on the intensity of azo-dye.

µg of 5-ASA / 25 ml	Absorbance / minutes standing time							
	0	5	10	20	30	40	50	60
20	0.254	0.252	0.252	0.252	0.252	0.252	0.252	0.256
50	0.511	0.513	0.513	0.513	0.513	0.513	0.513	0.510
100	0.900	0.895	0.894	0.894	0.894	0.894	0.894	0.893
150	1.348	1.351	1.334	1.334	1.334	1.332	1.332	1.328

Final absorption spectra

When 5-ASA was treated according to the recommended procedure, the absorption spectrum shows a maximum absorption at 430 nm, characteristic of the yellow-orange dye. The reagent blank shows no absorption at this wavelength (Fig 2).

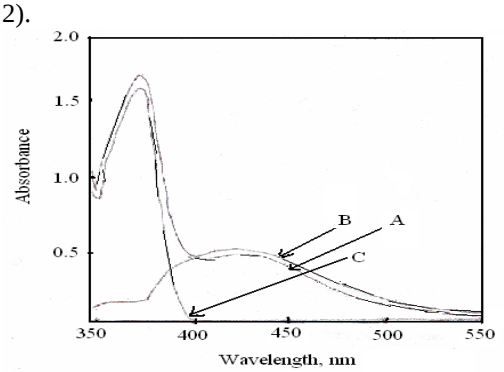


Fig.2: Absorption spectra of 50 µg 5- ASA / 25 ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.

Accuracy and precision

Three different concentrations of 5- ASA are used in the determination of the accuracy and precision of the method, the result, shown inTable 7 indicate that the method has good accuracy and precision.

Table 7: Accuracy and precision of the method.

Amount of 5- ASA taken, µg	Relative error, %*	Relative standard deviation, %*
20	- 0.50	± 0.91
50	-0.19	± 0.77
100	-0.56	± 0.34

* Average of five determinations

Nature of the dye

The composition of the intense yellow-orange dye that results from the reaction of diazotized 5-ASA with phloroglycinol has been established using the continuous variations method, the results indicate that the dye has a combination 1:1 ratio of diazotized 5-ASA to phloroglycinol (Fig.3).

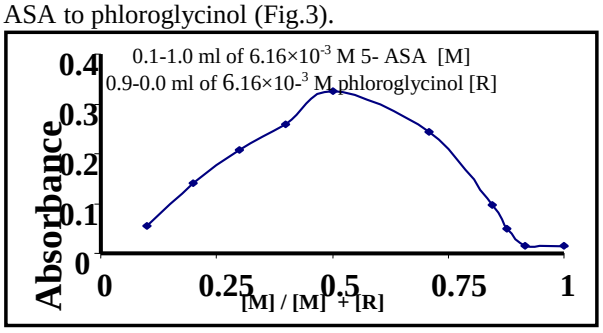
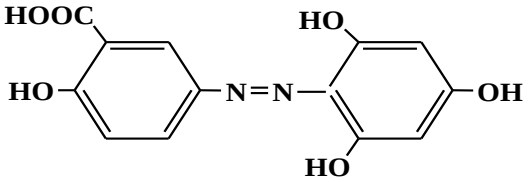


Fig.3: The continuous variation plot for diazotized 5-ASA to phloroglycinol

Hence the dye may have the following suggested structure:



Yellow-orange azo-dye

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 was obtained as shown in Table 8.

Table 8: Application of the method.

Drug	µg 5-ASA present / 25 ml	µg 5-ASA measured / 25 ml	Recovery*, %
Mesacol extended release capsules 400 mg universal pharmaceutical industries-unipharma-Damscus-Syria	20	20.50	102.50
	50	50.21	100.42
	100	100.43	100.43

* Average of five determinations.

Evaluation of the proposed method

Because there is no standard method in the literature for determination 5-ASA, so that this standard addition method applied in order to prove that the proposed method can be used in the determination of 5-ASA without interferences. (Table 9 and Fig.4).

Table 9: The result of standard addition method

Drug	µg 5-ASA present / 25 ml	µg 5-ASA measured / 25 ml	Recovery*, %
Mesacol extended release capsules 400 mg universal pharmaceutical industries-unipharma-Damscus-Syria	10	9.96	99.6
	20	20.10	100.5

* Average of five determinations.

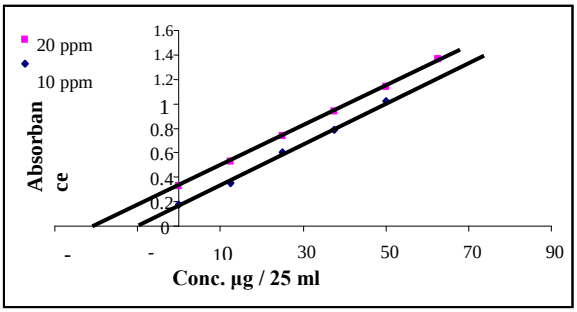


Fig. 4: Standard addition graphs for the determination of 5-ASA in pharmaceutical preparation (capsules).

The results in Table 10 and Fig.4 indicated that the proposed method can be used to determine 5-ASA in pharmaceutical preparation (capsules) with satisfactory results.

Conclusion

A simple and sensitive spectrophotometric method for the determination of micro amounts of 5-ASA in aqueous solution, based on the coupling of 5-ASA diazotized with phloroglycinol in basic medium, has been developed. The proposed method has been applied to the determination of 5-ASA in pharmaceutical preparation (capsules) with satisfactory results.

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التقدير الطيفي للميزالازين مع الفلوروكليسينول في مستحضر صيدلاني

انعام احمد حمدون ، صفاء عبد العليم ، نسيم ميسر

قسم الكيمياء ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق

(تاريخ الاستلام: ١٥ / ٥ / ٢٠١١ ---- **تاريخ القبول:** ٢٦ / ١٠ / ٢٠١١)

الملخص

يتضمن البحث طريقة طيفية بسيطة لتقدير الميزالازين [5-امينو حامض الساليسلك ، (5-ASA)] في الوسط المائي بطريقة الازوته والاقتران. تعتمد الطريقة على مفاعلة الميزالازين مع زيادة من النتريت في وسط حامضي لتكوين ملح الدايازونيوم المقابل وازالة النتريت الفائض باستخدام حامض السلفاميك، ثم يتم اقتران الميزالازين المؤزوت مع الكاشف فلوروكليسينول في وسط قاعدي ليعطي صبغة آزوية صفراء-برتقالية اللون مستقرة وذائبة في الماء وتعطي اعلى شدة امتصاص عند الطول الموجي 430 نانوميتر. كانت حدود قانون بير في مدى التركيز من 5-300 مايكروغرام ميزالازين في حجم نهائي 25 مللتر أي 0.2-12 جزء/ مليون وكانت الامتصاصية المولارية للصبغة الناتجة 3.02×10^4 لتر.مول$^{-1}$.سم$^{-1}$. تراوح الخطا النسبي من -0.56 الى – 0.19% والانحراف القياسي النسبي من الى ±0.34 ± 0.91% اعتمادا على مستوى التركيز وتم تطبيق الطريقة بنجاح في تقدير الميزالازين في مستحضر دوائي (الكبسول).