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Spectrophotometric Determination of Mesalazine by Diazotisation-Coupling Method with Resorcinol

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ABSTRACT

A simple spectrophotoemtric method for the determination of mesalazine [5-aminosalicylic acid; (5-ASA)] in aqueous solution is achieved. The method is based on the reaction of mesalazine, 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 is coupled with resorcinol reagent in basic medium to produce, an intense orange coloured water-soluble and stable azo-dye which exhibits maximum absorption at 471nm. Beer's law is obeyed in the concentration range of 10-300µg of mesalazine in a final volume of 25 ml i.e., 0.4-12 ppm with a molar absorptivity of 2.9480×10^4 $l.mol^{-1}.cm^{-1}$ and Sandell sensitivity index of 0.0051µg.cm^{-2}, a relative error of -0.96 to -0.23% and relative standard deviation of ±1.05 to ±0.37% depending on the concentration level. The proposed method has been applied successfully to determine mesalazine in pharmaceutical preparation (capsules).

التقدير الطيفي للميزالازين باستخدام طريقة الأزوتة والاقتران مع الريسورسينول

الملخص

يتضمن البحث طريقة طيفية بسيطة لتقدير الميزالازين [5-أمينو حامض السالسيلك، (5-ASA)] في الوسط المائي بطريقة الأزوتة والاقتران. تعتمد الطريقة على مفاعلة الميزالازين مع زيادة من النتريت في وسط حامضي لتكوين ملح الدايازونيوم المقابل وإزالة النتريت الفائض باستخدام حامض السلفاميك، ثم يتم اقتران الميزالازين المؤزوت مع الكاشف ريسورسينول في وسط قاعدي ليعطي صـبغة آزويـة ذات لون برتقالي مستقرة وذائبة في الماء وتعطي اعلى شدة امتصاص عند الطول المـوجي 471 نـانوميتر. كانت حدود قانون بير في مدى التركيز من 10الى 300 مايكروغرام ميزالازين في حجم نهائي 25 مللتر أي 0.4-12 جزء/مليون وكانت الامتصاصية المولارية للصبغة الناتجة 2.9480×10^4 لتر.مول$^{-1}$.سم$^{-1}$ ودلالة ساندل للحساسية 0.0051 مايكروغرام.سم$^{-2}$. الخطأ النسبي من -0.96 الى -0.23% والانحراف

القياسي النسبي من ±1.05 الى ±0.37% اعتماداً على مستوى التركيز وتم تطبيق الطريقة بنجـاح فـي تقدير الميزالازين في مستحضر دوائي (الكبسول).

INTRODUCTION

Mesalazine [5-Amino salicylic acid; (5-ASA)] is an agent widely used in the treatment of inflammatory bowel disease (IBDs), is metabolized in organism to the principal biotransformation product, N-acetyl-5-ASA, is a polar compound and besides it exhibits amphoteric properties (Liu et al., 1995 and Nobilis *et al*., 2006).

Different methods have been reported for the determination mesalazine including: liquid chromatographic technique which was used to determine 5-ASA and its metabolite N-acetyl-5-aminosalicylic acid in plasma and urine by using a spectrofluoimetric detector, excitation at 311nm. The lower limit of detection was 20 ng/ml and a relative standard deviation was below 6.7% (Bystrowska *et al*., 2000).

High performance liquid chromatography (HPLC) method was employed to measure 5-ASA and its metabolities in blood plasma. Chromatographic analysis were performed on a 250-4mm column with UV photodiode-array and fluorescence detectors (Nobilis *et al*., 2006).

A micellar electrokinetic chromatographic (MEKC) has been used for the estimation of mesalazine and its major impurities, the method used capillary chromatography column select fused-silica with a buffer solution (pH 10.20), methanol, sodium dodecyl sulfate (SDS), tetrabutylammonium bromide (TBAB) as a mobile phase. (Gotti *et al*., 2001).

Mesalazine was determined by three different methods: the first method (HPLC) was carried out with a C_{18} column and a mobile phase was constituted of 30 mmol/l monobasic phosphate buffer (pH 7.0) and methanol (70:30; v/v), with 25% tetrabutylammonium hydrogen sulphate used ultraviolet detection at 254 nm, the second method used 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) at 517nm and using 100 mmol/l acetate buffer, pH 5.5, ethanol and 250 µmol/l ethanolic solution of DPPH and the third method nitrosation was accomplished using a platinum electrode and standard 0.1 mol/l sodium nitrite as titrant solution, the experimental recoveries were between 72.5 and 99.9%. All proposed methods can be used for the reliable quantitation of 5-ASA in pharmaceutical dosage forms (Rafael *et al*., 2007).

A differential pulse voltametry method was employed to determine 5-ASA in tablet using a glassy carbon electrode in Britton-Robinson buffer (pH 1.81), the peak current gave a linear relationship in the concentration range 1×10^{-4} and 2×10^{-6} molarity, the recovery was 101.23% with a relative standard deviation of 1.35% (Nigovic and Imunic, 2003).

A spectrophotometric method for the determination of microgram amounts (0.16-8µg/ml) of mesalazine based on the oxidative coupling with 2,6-xylenol in the presence of sodium metaperiodate in alkaline medium to form a blue indophenol dye which has maximum absorption at 610 nm with a molar absorptivity is 13316$l.mol^{-1}.cm^{-1}$ (Al-Fakhry, 2006).

Another spectrophotometric method was applied for the determination of phenols based on a multicommuted flow system. It was based on oxidative coupling of phenolic

compounds with 4-amino-antipyrine in alkaline medium containing potassium hexacyanan ferrate (III). The detection limit was 1µg/l phenol. (Lupetti *et al*., 2004).

A colouremetric method has been developed for the determination of 5-ASA in urine and feces using Bratton-Marshall reaction to form a violet product with absorption at 560 nm. Beer's law was obeyed in the concentration range 0-70µg/ml (Pieniaszek and Bates, 1975).

Also, mesalazine has been determined by galvanostatic coulometric method in pharmaceutical preparations using reactions of electrogenerated bromide and chlorine with mesalazine. The end-point of coulometric titration was determined aperometrically with two polarized platinum electrodes. Procedures for the galvanostatic coulometric determination of 2.4 to 19.2 µg/ml with relative standard deviation varied from 1 to 5% (Abdullin *et al*., 2002).

The objective of the investigation reported in this paper is to introduce spectrophotometric method for the determination of 5-ASA. Based on the diazotization of 5-ASA and coupling with resorcinol reagent and applying the method to the determination of 5-ASA in pharmaceutical preparation (capsules).

EXPERIMENTAL

Spectral absorbance measurements are carried out on double beam spectrophotometric Shimadzu (UV-160A) and UV-visible spectrophoto-meter CECIL-CE 1021 digital single beam using 1 cm silica cells.

Reagents

All chemicals used are of the highest purity available.

Working mesalazine (5-ASA) solution, 50µg/ml. A 0.01g of mesalazine supplied by (Fluka) is dissolved in 10 ml distilled water, and the volume is completed to 200 ml in a volumetric flask.

Hydrochloric acid solution, 1N. This solution is prepared by diluting 8.5 ml of the concentrated acid to 100 ml with distilled water.

Sodium nitrite solution, 1%. This solution is prepared by dissolving 1g of sodium nitrite in 100 ml distilled water in a volumetric flask.

Sulphamic acid solution, 3%. A 3g of sulphamic acid is dissolved in 100ml distilled water.

Resorcinol solution, 0.1%. This solution is prepared by dissolving 0.1g of resorcinol in distilled water in a 100 ml volumetric flask.

Sodium hydroxide solution, 1N. This solution is prepared by appropriate dilution of the concentrated (Fluka) solution with distilled water and then transferred to a plastic bottle.

Mesacol capsules solution 50µg/ml. Weight and mix the contents of ten capsules (each one contains 400 mg mesalazine), an accurately weighed amount of powder (0.0111g)

equivalent to 0.01g mesalazine is dissolved in 10 ml of absolute ethanol and 30 ml distilled water, after filtration of the solution, the volume is completed to 200 ml of distilled water in a volumetric flask to prepare a solution of 50 ppm mesalazine.

Recommended Procedure and Calibration Graph

To a series of 25ml volumetric flasks aliquots covering the range of 10-400µg (0.4-16 ppm) of mesalazine are transferred, 0.5ml of 1N HCl is then added and the mixtures are shaken. Then 0.5ml of 1% sodium nitrite solution is added and the mixtures are allowed to stand for 3 minutes.

Then 0.3 ml of 3% sulphamic acid solution is added and the mixtures are after that 1.5ml of sodium hydroxide solution (1N) is added, then the volumes are completed to the mark with distilled water. After 10 minutes the absorbance are read at 471nm against blank solution, using 1cm matched cells. (Fig. 1) shows the calibration curve which indicates that Beer's law is obeyed over the concentration range 10-300 µg/25ml final volume, i.e., 0.4-12 ppm and concentration above 300µg/25ml gives negative deviation. The molar absorptivity is 2.9480×10^4 $l.mol^{-1}.cm^{-1}$.

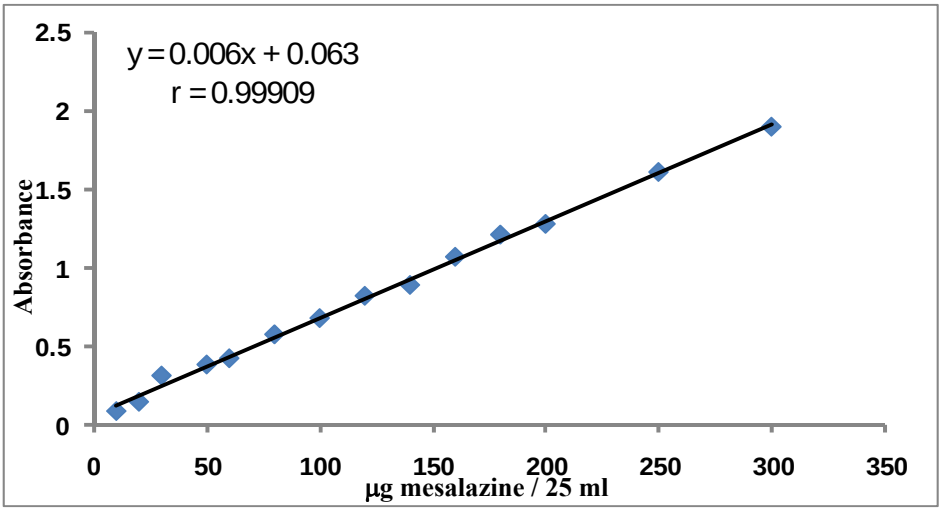


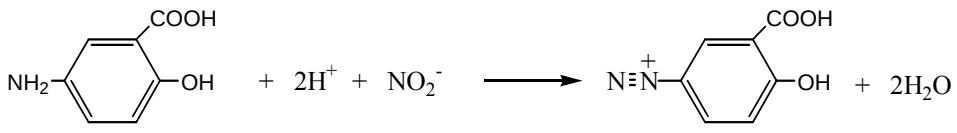
Fig.1: Calibration graph for mesalazine determination using resorcinol as coupling reagent

RESULTS AND DISCUSSION

For the subsequent experiments, 50µg of mesalazine is taken in 25 ml final volumes and absorbance measurements are performed at 471 nm.

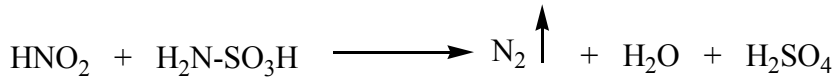
Principle of the method

Mesalazine is reacted with excess nitrite in acidic medium to form the corresponding diazonium salt:

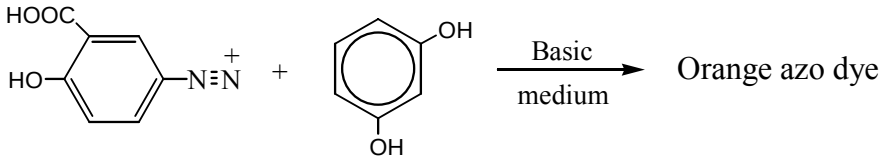


5-ASA Diazotised 5-ASA

The residual nitrite (as nitrite acid) which was undesirable due to its side reaction, such as, nitrosation of coupling agent (Bladyga *et al*., 1999). Therefore, it should be removed by sulphamic acid which reacts more fast than urea:



The colored solution formed by coupling diazotized 5-ASA with resorcinol in alkaline medium.



Study of the Optimum Reaction Conditions

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

Effect of acids on the diazotization

The effect of the amount of different acid (weak and strong) for the diazotization of 5-ASA, have been investigated. The results are indicated that 0.5ml 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 acid on absorbance

solution **1N acid used**	**Absorbance(A)/ml of acid used**					
	0	**0.2**	**0.5**	**0.7**	**1**	**1.5**
	A	**A**	**A**	**A**	**A**	**A**
HCl	0.305	0.324	0.350	0.344	0.300	0.309
HNO_3	0.309	0.317	0.343	0.337	0.297	0.243
H_2SO_4	0.319	0.315	0.322	0.319	0.262	0.219
CH_3COOH	0.314	0.298	0.317	0.295	0.224	0.132

Effect of nitrite amount and time

The color is reached maximum intensity when using 0.5ml of 1% (w/v) sodium nitrite solution with 3 minutes reaction time, it seems that diazotization of 5-ASA is fast

Table 2: The effect of sodium nitrite amount and time on absorbance

ml of 1% (w/v) $NaNO_2$ solution	Absorbance/minute standing time					
	0	1	2	3	4	5
0.1	0.303	0.357	0.372	0.380	0.374	0370
0.2	0.300	0.335	0.340	0.372	0.367	0.362
0.3	0.290	0.345	0.372	0.376	0.370	0.368
0.5	0.331	0.360	0.376	0.386	0.382	0.360
0.7	0.211	0.287	0.243	0.238	0.233	0.221

Effect of sulphamic acid amount and time

The presence of unreacted nitrite is undesirable in diazotization reaction. Therefore, it should be removed by sulphamic acid which fastly reacts with nitrite. The results indicated that 0.3 ml of 3% sulphamic acid solution with 3 minutes standing time are considered to be the most suitable(Table 3), and therefore are selected subsequently.

Table 3: The effect of sulphamic acid amount and time on absorbance

ml of sulphamic		Absorbance/minute standing time					
		0	1	2	3	4	5
0	S	0.030	0.036	0.133	0.162	0.183	0.212
	B	0.114	0.152	0.157	0.158	0.154	0.142
0.1	S	0.072	0.090	0.341	0.335	0.301	0.343
	B	0.116	0.180	0.163	0.183	0.169	0.155
0.2	S	0.120	0.293	0.346	0.367	0.382	0.361
	B	0.165	0.149	0.158	0.165	0.160	0.143
0.3	S	0.336	0.325	0.358	0.387	0.370	0.360
	B	0.076	0.067	0.063	0.060	0.057	0.045
0.5	S	0.389	0.346	0.337	0.376	0.375	0.320
	B	0.077	0.075	0.021	0.030	0.018	0.083
0.7	S	0.360	0.297	0.319	0.343	0.368	0.318
	B	0.084	0.026	0.017	0.044	0.018	0.016

Effect of resorcinol amount

The effect of resorcinol amount on the color intensity of the dye has been studied. From the results, it can be observed that 4 ml of 0.1% resorcinol is the more suitable amount which gives the highest value of absorbance for the azo-dye formed and the highest value of correlation coefficient (Table 4).

Table 4: The effect of resorcinol amount

ml of resorcinol (0.1%)	Absorbance / µg of 5-ASA						
	20	**50**	**70**	**100**	**150**	**200**	**r**
1	0.142	0.382	0.397	0.528	0.804	1.098	0.993326
2	0.153	0.375	0.395	0.577	0.869	0.166	0.996388
3	0.165	0.380	0.409	0.604	0.884	0.1204	0.99692
4	0.179	0.385	0.436	0.674	0.914	0.1216	0.998218
5	0.122	0.308	0.413	0.545	0.900	0.104	0.997061

Effect of time on color development

The effect of time on the development and stability period of the coloured dye is investigated under the optimum conditions described above for 5-ASA. From the experimental data, it has been noticed that the azo-dye reached maximum absorbance after 10 minutes and remains stable at least for another 50 minutes when the concentrations of 5-ASA was ≤ 50 µg/25ml. But it was stable for only 30 minutes when the concentration of 5-ASA was ≥100 µg/25ml. However, several measurements can be performed in both cases (Table 5).

Table 5: The effect of time on absorbance

µg of 5-ASA/25 ml	Absorbance / minute standing time							
	0	**5**	**10**	**20**	**30**	**40**	**50**	**60**
20	0.136	0.140	0.144	0.145	0.145	0.146	0.146	0.145
50	0.379	0.382	0.385	0.385	0.384	0.383	0.383	0.380
100	0.665	0.673	0.677	0.676	0.645	0.636	0.625	0.618

Effect of surfactant

The results indicated that addition of different types with different amounts of surfactants give no useful effect. Therefore, it has been recommended to eliminate their use in the subsequent experiments (Table 6).

Table 6: Effect of surfactant.

Surfactant solution	Absorbance*/order**of addtion											
	I		II		III		IV		V		VI	
	A	Δλ, nm	A	Δλ, nm	A	Δλ, nm	A	Δλ, nm	A	Δλ, nm	A	Δλ, nm
CTAB 1×10^{-3}M	0.381	100	0.323	155	0.372	110	0.339	118	0.384	151	0.380	153
SDS 1×10^{-3}M	0.343	146	0.375	160	0.351	157	0.363	109	0.307	109	0.352	149
Tritonx-100	0.312	160	0.373	168	0.361	168	0.346	146	0.325	147	0.305	143

* A=0.385 without surfactant and Δ =153nm

** I. Mesalazine (M) +Surfactant(S) +HCL(H)+$NaNO_2$(N) +Sulphamic acid(F)

+Resorcinol(R) +NaOH(B)
II. M+H+S+N+F+R+B
III.M+H+N+S+F+R+B
IV.M+H+N+F+S+R+B
V.M+H+N+F+R+S+B

Effect of base

The preliminary experiments have shown that diazotized 5-ASA gave colored dye of highest intensity with resorcinol in alkaline medium, therefore the coupling reaction has been carried out with different (strong and weak) bases and the results show that sodium carbonate and sodium bicarbonate gave better sensitivity than sodium ydroxide and potassium hydroxide. But the later bases gave better color contrast (Δλ), and the azo-dye formed has good stability compared with weak bases, so that 1.5ml of 1N sodium hydroxide solution has been recommended for the subsequent experiments (Table7).

Table 7: The effect of base on the absorbance and colour contrast

Solution 1N base used	Variable	Absorbance / ml of base use						
		0.5	**1**	**1.5**	**2**	**2.5**	**3**	**4**
NaOH	A	0.330	0.329	0.387	0.331	0.251	0.233	0.194
	Δλ *, nm	128	153	154	163	168	149	143
KOH	A	0.304	0.298	0.309	0.313	0.294	0.267	0.221
	Δλ	136	152	152	169	165	147	146
Na_2CO_3**	A	0.649	0.640	0.726	0.732	1.042	0.811	0.739
	Δλ	8	18	27	26	27	11	36
$NaHCO_3$**	A	0.831	0.503	0.629	0.618	0.601	0.495	0.482
	Δλ	17	25	23	23	29	30	34

Δλ *= $\Delta\lambda_{maxS}$- $\Delta\lambda_{maxB}$S = The dye B=Blank
**Gives unstable azo-dye

Final absorption spectra

When mesalazine is treated according to the recommended procedure, the absorption spectrum shows a maximum absorption at 471 nm, characteristic of the orange dye .The reagent blank shows nill absorption at the wavelength of maximum absorption (Fig. 2).

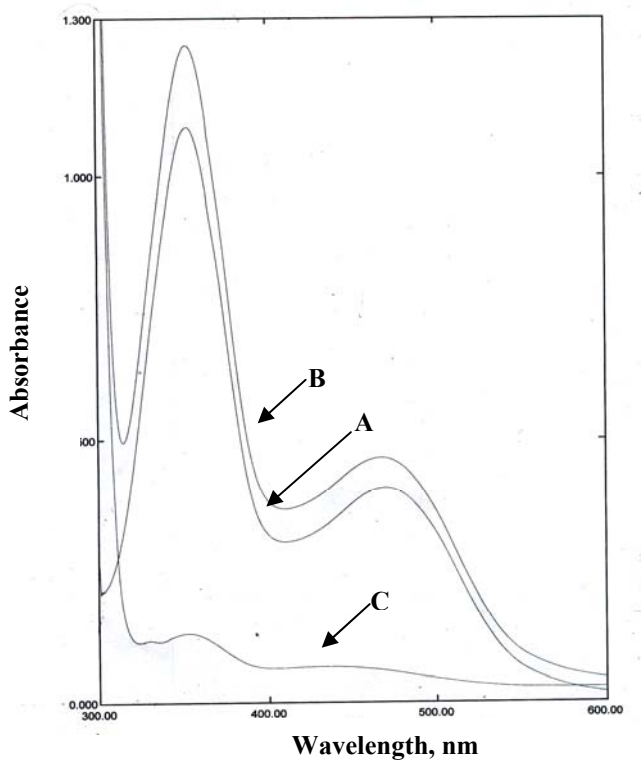


Fig. 2: Absorption spectra of 50 µg mesalazine / 25 ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water

Accuracy and Precision

Three different concentrations of 5-ASA are used in the determination of the accuracy and precision of the method, the results shown in Table 8 indicate that the method has good accuracy and precision.

Table 8: Accuracy and precision of the method

Amount of mesalazine taken, µg	Relative error, %*	Relative standard deviation, %*
20	-0.96	±1.05
50	-0.83	±0.49
100	-0.23	±0.37

* Average of five determinations

Nature of the Dye

The composition of the intense orange dye that results from the reaction ofdiazotized 5-ASA with resorcinol has been established using the continuous variations and the mole-ratio methods, the results indicate that the dye has a combination 1:1 ratio of diazotised 5-ASA to recorcinol (Fig. 3 and 4).

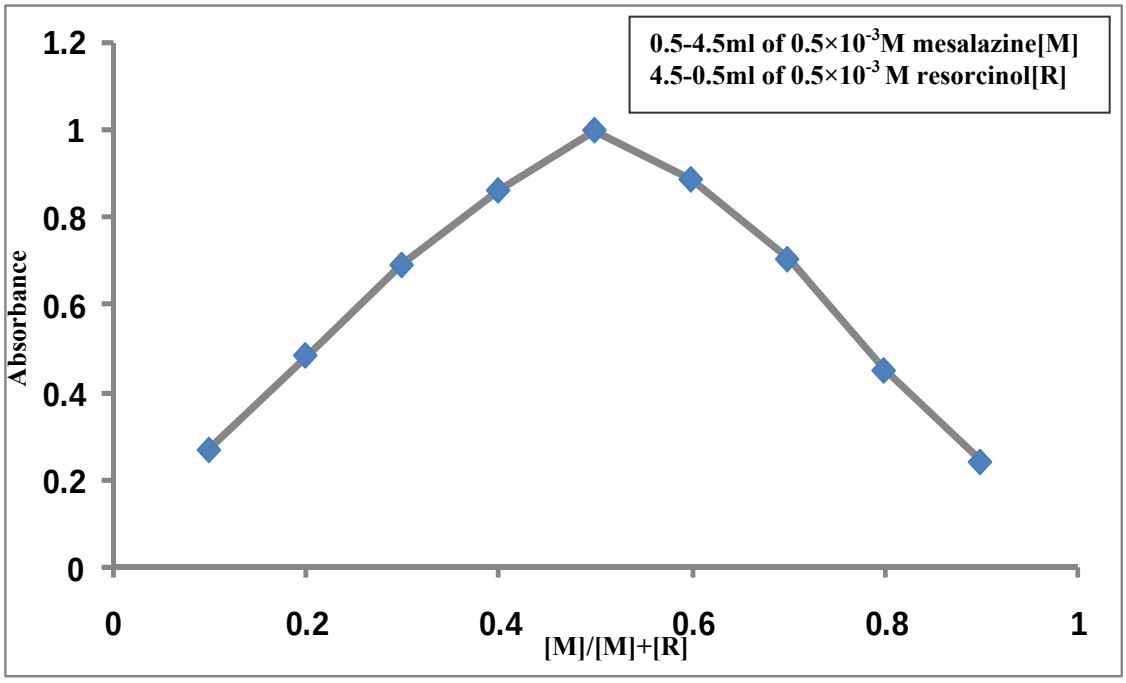


Fig. 3: The continuous variations plot for diazotized mesalazine to resorcinol

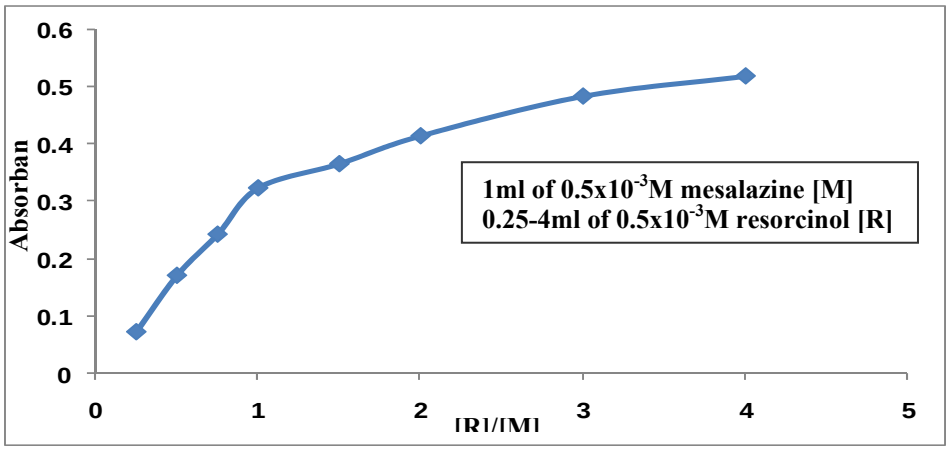
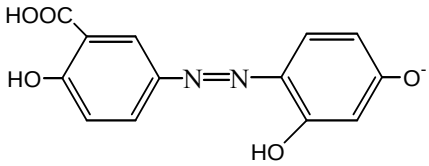


Fig. 4: The mole-ratio plot for diazotized mesalazine to resorcinol

Hence the dye may have the following suggested structure:



Orange azo-dye

Interference

The effect of some foreign compounds which often accompanied pharmaceutical preparations were studied by adding four different amounts (50, 200, 500 and 1000µg) to 50µg mesalazine in a final volume 25ml (Table 9).

Table 9: Effect of foreign compounds for assay of mesalazine

Foreign Compound	**Recovery(%) of 50µg mesalazine per µg Foreign compound added**			
	50	**200**	**500**	**1000**
Glucose	95.5	95.2	95.4	96.1
Lactose	98.3	97.2	95.4	100.7
Starch	94.7	97.2	100.3	95.5
GumArabic (Acacia)	99.4	95.5	105.1	96.1

The results in table indicated that the studied foreign compounds do not interfere in the determination of mesalazine using the proposed method. An error not more than of 5.1% in the absorbance readings is considered tolerable from that of the mesalazine alone.

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 10.

Table 10: Application of the method

Drug	µg mesalazine present/25ml	µg mesalazine measured/25ml	Recovery*, %
Mesacol Extended release capsules 400 mg Universal pharmaceutical Industries-unipharma-Damascus-Syria	20	21.2	106.12
	50	50.26	100.52
	100	100.73	100.73

* Average of five determinations

Evaluation of the proposed method

Because there is no standard method in the literature for determination mesalasine, so that this standard addition method applied in order to prove that the proposed method can be used in the determination of mesalazine without interferences. (Table 11 and Fig. 5).

Table 11: The results of standard addition method

Drug	µg mesalazine present/25ml	µg mesalazine measured/25ml	Recovery*, %
Mesacol Extended release capsules 400 mg Universal pharmaceutical Industries-unipharma-Damascus-Syria	20	19.7	98.5
	40	40.1	100.25

* Average of three determinations

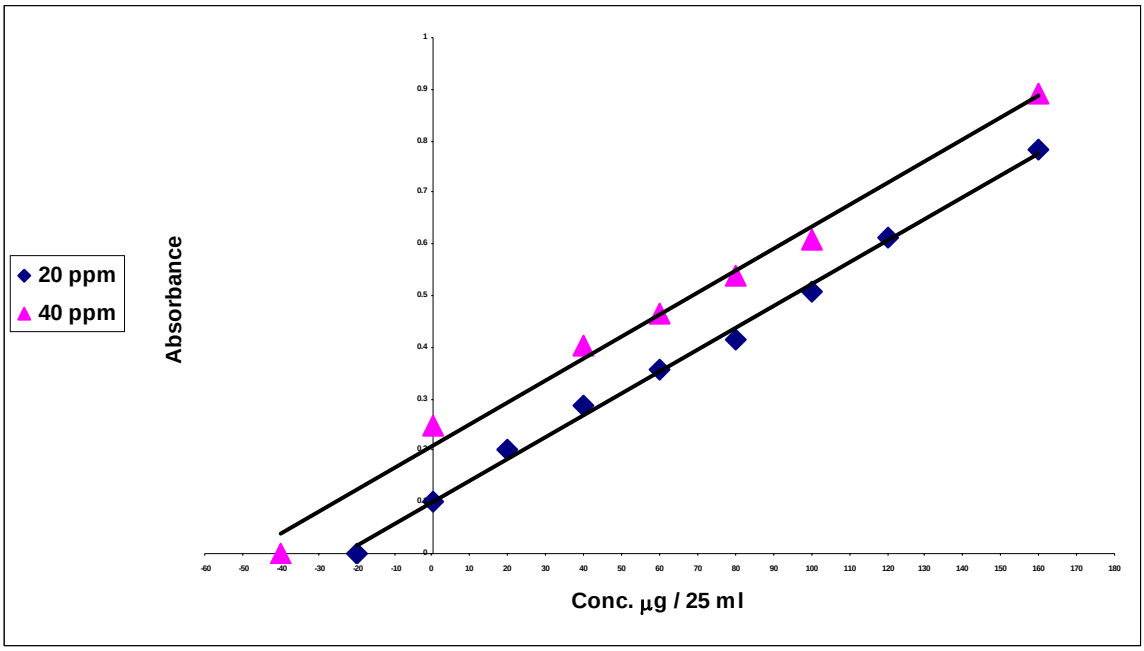


Fig.5: Graphs of standard addition method for the determination of mesalazine in pharmaceutical preparations (capsules).

The results in Table 11 and Fig. 5 indicated that the proposed method can be used to determine mesalazine in pharmaceutical preparation (capsules) with satisfactory results.

Comparison of Methods

Table 12 shows the comparison between the analytical variables obtained from the present method with those of recent spectrophotometric method.

Table 12: Comparison of the methods

Analytical parameters	**Present method**	**Literature method***
pH	12.38	$\geq$ 12
Temperature (^oC)	At room temperature	At room temperature
Development time (minutes)	10	5
λ_{max} (nm)	471	610
Medium of method	Aqueous	Aqueous
Type of reaction	Diazotisation	Oxidative coupling
Reagent	Resorcinol	2,6-xylenol
Beer's law range (ppm)	0.4-12	0.16-18
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	2.9479×10^4	1.3116×10^4
RSD (%)	±1.05 to ±0.37%	±1.23o ±1.01%
Color of the dye	Orange	Blue
Nature of the dye	1:1	1:1
Application of the method	Determination of mesalazine in capsules	Determination of mesalazine in two drugs (capsules and tablets)

Al-Fakhry M.H. 2006. M.Sc., Thesis, Mosul University, 64-80.

The proposed method is simple, rapid, sensitive and do not need any pretreatment of mesalazine or extraction of the dye formed and has good precision.

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