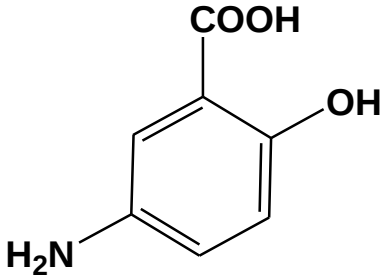
Spectrophotometric Determination of Mesalazine Via Oxidative Coupling Reaction

Intisar Adil Shihab
Chemistry Department, Education College for girls, Mosul University, Mosul , Iraq
(**Received:** 23 / 5 / 2010 ---- **Accepted:** 11 / 5 / 2011)

Abstract
A simple, rapid and sensitive new spectrophotometric method for the determination of mesalazine .The method is based on the oxidative coupling reaction of mesalazine in acidic medium with pyrocatechol in the presence of potassium chromate as oxidizing agent forming an intense stable purple–red water soluble dye, which exhibits maximum absorption at 530nm. The molar absorptivities ranged from 3685l.mol^{-1}.cm^{-1} for mesalazine. Beer's law was obeyed over the range of (0.4-10) ppm.The proposed method is applied for the determination of mesalazine in pharmaceutical preparations.

Introduction
Misalazine or 5- aminosalicylic acid(5-ASA) is used to treat nflammatory bowel diseases especially the acute ulcerative colitis diseases and to avoid developed hemorrhagic bleedings starting from colon until anus [1]. It is also considered as number one medication in treating inflammatory bowel diseases in children . it is used to meditate the severity of the disease in mind cases. The information of drug dynamics of this mediation in children are very few and the used doses were extracted from the studies that were made on adults [2]. Mizalazine represents the active part of the drug "salazosulfapyridine" after the fission done by colon bactria) to witch the drug effect is attributed. The side effects of " salazosulfapyridine " disappear or totally decrease when the misalazine is directly used [3]. masalazine has the following chemical structures.



Prolonged treatments as well as the need for clinical and pharmacological studies require fast and sensitive analytical techniques of the drug presence determination in several biological samples. Up to now, most common procedures for the determination of 5-ASA in pharmaceutical dosage forms [4and 5] and biological fluids [6, 7, 8, 9, 10, 11, 12 and 13] were based on chromatographic techniques. High-performance liquid chromatographic methods with UV [6, fluorescence [8,11and13] and electrochemical [10and12] detection were primarily used for the analysis of 5-ASA in biological samples. Spectrophotometry[14] and colorimetry[15] were also used for the compound quantitation. Electrochemical methods have been recently introduced in the analysis of this drug[16] Chromatographic methods need sophisticated equipment or require lengthy extraction and clean-up procedures. The purpose behind this chapter was to develop sensitive and accurate way to determine the quantity of misalazine inpharmaceuticals by using potassium chromete and coupling reagent with the pyrocatechol as an oxidative factor in the alkaline medium

Experimental
Apparatus
Shimadzu (UV-210) Double Beam Spectrophotometer with 1.0 cm silica cells was used to measure the absorbance and heating of solutions is carried out on a water bath of Frost Instruments LTD. The reading of pHs made on a PW 9420 pH meter supplied with an electrode type CE 10-12 pH. Weighing is carried out on a balance type of Mettler H 54 AR.

Reagents
All Chemicals used are of the highest purity available.

Pyrocatechol solution (**2.72×10^{-3}M**): This solution is prepared by dissolving 0.03g of Pyrocatechol in distilled water in 100ml volumetric flask.

Potassium chromate (4.6×10^{-3}M): 0.09g of pure potassium chromate was dissolved in 100ml distilled water.

Hydrochloric acid solution: A diluted (0.05M) was used.

Mesalazine (100 µgml^{-1}): 0.01g is dissolved in ethanol, solution is transferred into a 100 ml volumetric flask, and diluted to the mark with distilled water

Recommended procedure
٣ml of pyrocatechol (2.72×10^{-3}M) was added into a series of 25ml calibrated flask and 1ml of potassium chromate (4.6×10^{-3}M) followed by the addition of increasing volumes of (100 µgml^{-1}) mesalazine solution and followed by 2ml of (0.05M) hydrochloric acid. The solutions were diluted to the mark with distilled water and the reaction mixture was allowed to stand for ١٥minute. The absorbance of each solution was measured at 530nm versus blank prepared in the same manner but without mesalazine.

Results and Discussion
Study of the Optimum Reaction Conditions
The various parameters affecting and related to the above mentioned coloured product have been studied and optimum conditions were obtained.

Effect of oxidant amount to the reagent (pyrocatechol)

The reaction of oxcidant amount to pyrocatechol reagent was studied. The absorbent was measured at different periods of time and at 530 nm. versus blank

Table (1): Effect of oxidant amount to the pyrocatechol

ml of K_2CrO_4 (4.6×10^{-3} M)	Absorbance at time				
	0min	5min	10min	20min	Blank
0.5	0.190	0.192	0.191	0.191	0.078
1	0.460	0.459	0.457	0.452	0.099
2	0.323	0.320	0.322	0.321	0.110
3	0.297	0.298	0.297	0.298	0.149
4	0.310	0.311	0.314	0.309	0.167
5	0.300	0.305	0.303	0.301	0.188

The result shows that the dye formation reached the maximum with 1ml of potassium chromate**.**

Effect of different acids on absorbance

In order to select the most suitable acid, the oxidative coupling reaction was carried out using various acids (hydrochloric, sulphuric, acetic, formic and phosphoric acids). The absorbance was measured at ٥٣٠nm versus reagent blank. Table (2) shows that hydrochloric acid was the most suitable acid for the reaction.

Table (2): Effect of different acids on absorbance

ml of acid	**Absorbance**				
	HCl (0.05M)	H_2SO_4 (0.05M)	CH_3COOH (0.05M)	HCOOH (0.05M)	H_3PO_4 (0.05M)
0.5	0.090	0.047	0.054	0.089	0.054
1.0	0.194	0.049	0.177	0.079	0.090
1.5	0.200	0.098	0.195	0.123	0.123
2.0	0.460	0.120	0.182	0.134	0.135
3.0	0.220	0.160	0.124	0.178	0.198
4.0	0.199	0.173	0.234	0.230	0.236
5.0	0.188	0.190	0.245	0.245	0.236

Effect of reagent concentration

This effect was studied by placing different volume of pyrocatechol (2.72×10^{-3}M) into a series of 25ml calibrated flask. The absorbances were measured at 530 nm versus blank. The results obtained in Table (3) indicate that the use of 3ml of (2.72×10^{-3}M) pyrocatechol reagent gave the maximum colour intensity.

Table (3): Effect of the concentration of reagent on absorbance.

Reagent conc.(ml)	**Absorbance**	
	(sample)	**(blank)**
0.5	0.119	0.090
1.0	0.156	0.089
2.0	0.206	0.078
2.5	0.306	0.060
3.0	0.460	0.058
4.0	0.278	0.120
5.0	0.157	0.198

Effect of temperature

The effect of temperature on the absorbance of the coloured product was studied. This was implemented by placing into three 25ml calibrated flasks, 3ml of (2.72×10^{-3}M) pyrocatechol, 1ml of (4.6×10^{-3}M) potassium chromate, followed by 2ml of (100 μgml^{-1}) mesalazine, solution and 2ml of (0.05M) hydrochloric acid solution. The solution was diluted to the mark with distilled water and the first flask was allowed to stand for increasing time at room temperature, the second was at 0°C and the third in water bath at 45°C. The absorbance was measured at 530nm at

different periods versus blank prepared in the same way but containing no mesalazine. The results obtained in Table (4) indicated that the absorbance of the coloured product was decreased when the reaction was carried out at 0°C or 45C therefore; it is recommended that the reaction mixture should be carried out at room temperature (28 **ºC**)

Table (4): Effect of temperature on absorbance of coloured product.

Temp. ºC	Absorbance/minutes							
	0	5	10	15	20	25	30	40
0.0	0.082	0.091	0.095	0.099	0.105	0.119	0.122	0.123
R.T.	0.450	0.457	0.476	0.486	0.489	0.490	0.498	0.489
45	0.050	0.058	0.053	0.053	0.051	0.050	0.049	0.060

Stability of the product.

This was studied by placing 3ml of (2.72×10^{-3}M) pyrocatechol, into a series of 25ml calibrated flasks, followed by ١ml of (4.6×10^{-3}M) potassium chromate and 2ml of (100 µgml⁻¹) mezalazine and 2ml of (0.05M) hydrochloric acid. The solution was diluted to the mark with distilled water and the absorbance was measured at 530nm at different periods versus reagent blank. The results obtained in Table (5) show that the product needs 15minutes to attain maximum absorbance and it remains stable for about 30minutes.

Table (5): Rate of reaction and stability of product.

Time (min)	0	5	15	20	25	30	35	40	45	50	55	65
Absorbance	0.429	0.467	0.499	0.498	0.497	0.498	0.499	0.499	0.497	0.499	0.498	0.498

Order of addition of reagents.

The reagent 3 ml of (2.72×10^{-3}M) pyrocatechol (**R**), the oxidant ١ml of (4.6×10^{-3}M) (**ox**) and the sample 2ml of (100 µgml⁻¹) mezalazine solution(D), followed by 2ml hydrochloric acid (0.05M)(**A**) were mixed in various orders as is shown in Table (6). Here this table shows that (III) is the best so it has been depended with the coming measures.

Table (6): Effect of order of addition on the absorbance of the coloured product.

Reaction components	Order number	Absorbance at 530nm
D+A+O+R	I	0.390
D+O+A+R	II	0.336
D+R+O+A	III	0.487
D+A+R+O	IV	0.343

D : Drugs , A; Acid , O :Oxidant , R : Reagent

Final absorption spectra.

Using the optimum conditions described above, the mezalazine -pyrocatechol complex formed has an absorption spectrum ranging between 400 and 600nm with a maximum absorption at 530nm in contrast to the reagent blank which shows small absorption at λmax. Therefore, the 530nm wavelength of maximum absorption has been selected for subsequent work.

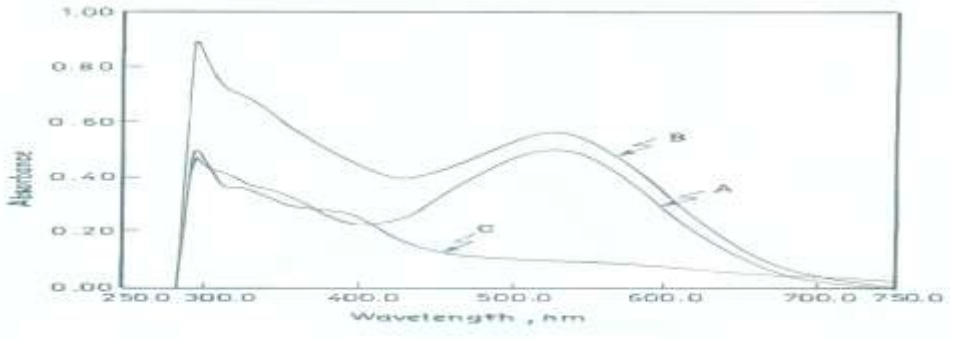


Fig. (1): Absorption spectra of 8 µgml^{-1} mesalazine measured, (A) Against blank, (C) blank against distilled water.(B)mesalazin Against water.

Quantification

Having thus establishing optimum reaction conditions, a calibration graph is constructed by plotting absorbance versus concentration. Beer's law is obeyed over the range (0.4-10)µg /ml of the solution Fig (2). Negative deviation from Beer's law occurred beyond the upper determination limits.

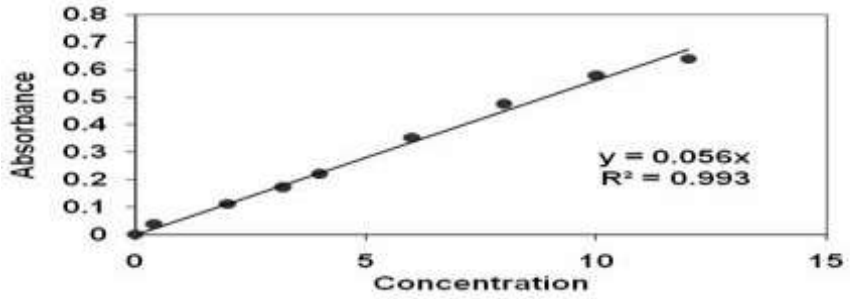


Fig. (2) : Calibration graph for the determination of mesalazine.

Accuracy and precision of the method.

To check the accuracy and precision of the method, mesalazine has been determined at three concentrations. The results are shown in Table (7) indicated that the method is performing well.

Table (7): Accuracy and precision of the method

Amount of mesalazine taken, µg	Relative error, % *	Relative standard deviation, % *
100	+1.24	±1.55
200	+0.95	±1.31
300	-0.84	±0.49

Nature of the product

The stoichiometry of the reaction between mesalazine and pyrocatechol in the presence of potassium chromate was investigated by the mole–ratio method. In this experiment 3ml of pyrocatechol (2.72×10^{-4}M) were added into a series of 25ml calibrated flask followed by the addition of increasing volumes of (2.72×10^{-4}M) pyrochatecol and 1ml of potassium chromate (4.6×10^{-4}M) followed by 2ml of (0.05M) hydrochloric acid. The solutions were diluted to the mark with distilled water and the reaction mixture was allowed to stand for 15 minutes. The absorbance of each solution was measured at 530 nm versus blank. The results obtained in fig (3) showed the existence of a 1:1.

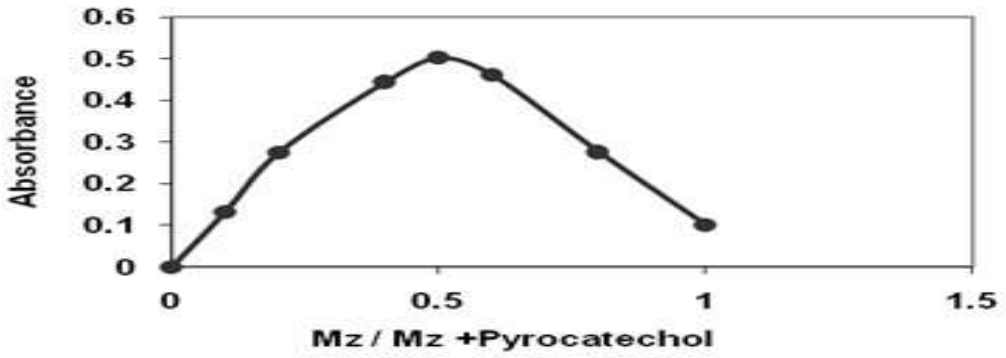


Fig. (3):Job´s method plot for mesalazine to the pyrocatechol reagent in the presence of potassium chromate.

Application of the method: Analyzing Mesacol Tablets

Ten tablets of drug sample (**mesacol**) were weighed accurately .After they have been ground and mixed well ,the equivalent of one enteric coated tablet(400mg of mesalazine) of the powder was weighed and dissolved in a certain quantity of ethanol and the amount was completed to100ml by distilled water. Then, the solutionwas filtered and used to prepare a solution with a concentration of100 µg /ml .Different amounts were taken from the last solution to get the concentration of 2, 6,10 $\mu g ml^{-1}$)and they were treated according to the work method described in the intem mesalazine concentration was found in the tablet by using the standard curve of the drug compound in its pure form and the results obtained was listed in the table (8).

Table (8): Assay of mesalazine drug in commercial pharmaceutical formulation by the proposed method

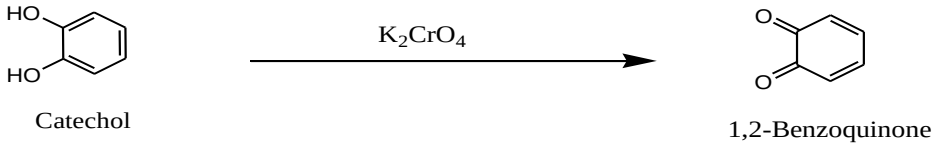
Pharmaceutical perparation	Amount added (µg/ml)	Recovery*(%)	Average recovery(%)
Tablet	2	98.10	98.93
	6	98.20	
	10	100.50	
capsules	2	100.90	99.71
	6	97.14	
	10	101.71	

* Every reading is an average of three determination

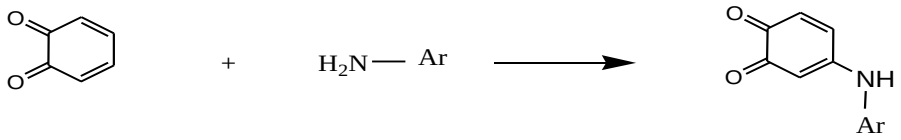
Analyzing Mesacol Capsules:

The content of ten capsules of drug sample was weighed ,crushed, ground and mixed. Then ,the equivalent of one mesalazine extended release capsule was weighed(400mg of mesalazine)and treated by the same method described in analyzing mesacol tablets. The table(8)includes the results we obtained.

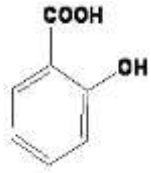
A suggested chemical reaction [17]



Catechol 1,2-Benzoquinone



Ar is the following structure :



Conclusion

A new spectrophotometric method has been proposed for the determination of mesalazine in aqueous solution. The method is based on coupling of mesalazine with pyrocatechol reagent in the presence of potassium chromate to form a coloured dye which exhibits maximum absorption at 530 nm. The molar absorptivity is 3685 $l.mol^{-1}cm^{-1}$. The proposed method is applied for the determination of mesalazine in two pharmaceutical preparations .

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التقدير الطيفي للميزالازين بوساطة الاقتران ألتأكسدي

انتصار عادل شهاب

قسم الكيمياء ، كلية التربية للبنات ، جامعة الموصل ، الموصل ، العراق

(تاريخ الاستلام: ٢٣ / ٥ / ٢٠١٠ ---- **تاريخ القبول:** ١١ / ٥ / ٢٠١١ **)**

الملخص

يتضمن البحث طريقة طيفية جديدة وبسيطة وسريعة وحساسة لتقدير الميزالازين . تعتمد الطريقة على تفاعل الأقتران التأكسدي للميزالازين مع البايروكاتيكول بوجود كرومات البوتاسيوم كعامل مؤكسد في الوسط الحامضي ليكون ناتج مستقر ذو لون احمر وردي ، له أعلى امتصاص عند 530نانوميتر. معامل الامتصاصية 3685 لتر.مول$^{-1}$.سم$^{-1}$. وكانت حدود قانون بير (٠,٤-١٠) جزء بالمليون.تم تطبيق الطريقة بنجاح لتقدير الميزلازين في مستحضراته الصيدلانية.