Spectrophotometric Determination of Mesalazine Via Arsenazo III – Cerium (III) Reaction

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الملخص

تم تطوير طريقة طيفية بسيطة وحساسة لتقدير الميزالازين (5-امينو حامض السالسيلك) بشكله النقي وفي مستحضره ألصيدلاني (الكبسول). اعتمدت الطريقة على تفاعل الأكسدة والاختزال بين الميزالازين وايون السيريوم الرباعي ثم يليه مفاعلة ناتج التفاعل ايون السيريوم الثلاثي مع الكاشف آرسين ازو III في وسط حامضي ليعطي معقدا اخضر مزرق اللون مستقر وذائب بالمحلول المائي ويعطي أعلى شدة امتصاص عند طول موجي 654 نانوميتر مقابل المحلول الصوري . كانت قيمة الامتصاص ية المولارية 2.103×10^5 لتر .مول $^{-1}$.سم $^{-1}$ واتبعت الطريقة قانون بير في مدى التركيز من 0.5 إلى 14 مايكروغرام من الميزالازين في حجم نهائي 25 مل. طبقت الطريقة بنجاح في تقدير الميزالازين في مستحضره الصيدلاني (الكبسول).

Abstract

A simple and sensitive spectrophotometric method has been developed for the determination of mesalazine (5-aminosalicylic acid) in pure form and in its pharmaceutical preparation(capsule). The method is based on the oxidation-reduction reaction between mesalazine and cerium (IV) ion, then reaction of cerium(III) with arsenazo III reagent in acidic medium to produce a green-blue complex, which is stable, water soluble and has a maximum absorption at 654 nm with a molar absorptivity of 2.103×10^5 l.mol^{-1}.cm $^{-1}$. Beerُ s law is obeyed in the concentration range of 0.5 to 14 µg of mesalazine in a final volume of 25 ml. The proposed method has been applied successfully for determination of mesalazine in pharmaceutical preparation (capsule).

Introduction

Mesalazine is 5-aminosalicylic acid (5-ASA). It is an anti-inflammatory drug structurally related to salicylates and active in inflammatory bowel disease[1], it is a first-line drug in pediatric inflammatory bowl disease (IBD), and is customarily used to induce and maintain remission in mild to moderate disease. In children pharmacokinetic data are scarce and dosage recommend actions are largely extrapolated from studies in adults [2].

Different methods have been reported for the determination of mesalazine. These methods include:
Amicellar electrokinetic chromatography with ion-pair reagent [3], high performance liquid chromatography (HPLC) methods[4-6], voltametry [7] and fluorometry [8]. Several spectrophotometric methods have been used for the determination of mesalazine, such as UV method[1], charge transfer complex [9], diazotization of mesalazine and coupling with N-(1-naphyl) ethylene diamine[10] or resorcinol [11].

Another spectrophotometric methods including oxidative coupling reaction with 2,6-xylinol in presence of sodium metaperiodate [12] or 4-amino antipyrine in presence of potassium hexacyanoferrateIII[13] have been reported.

Arsenazo III undergoes sensitive and selective reaction with several cations [14-15], such as reaction with cerium(III) ion [16-17]. This reaction can be used in the determination of some organic compounds which have the ability to undergo oxidation-reduction reaction with cerium(IV) ion [18-21].

The present work is described a spectrophotometric method for assay of mesalazine, based on the oxidation-reduction reaction of mesalazine with cerium (IV) ion, then a reaction of cerium(III) ion with arsenazo III reagent in acidic medium to give a coloured complex whose intensity is indirectly related to the concentration of mesalazine.

Experimental

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

Reagents

Standard solution of mesalazine,100µg.ml^{-1}.

This solution was prepared by dissolving 0.0100g of mesalazine in 10 ml absolute ethanol, and the volume was diluted to 100 ml with distilled water in a volumetric flask.

Working mesalazine solution, 10 µg.ml^{-1}.

This solution was prepared by appropriate dilution of standard solution.

Ammonium ceric sulphate solution ,1.45x10^{-4} M.

This solution was prepared by dissolving 0.0092g of ammonium ceric sulphate dihydrate in 100 ml of distilled water in a volumetric flask, this solution was freshly prepared daily.

Arsenazo III reagent solution ,5.44x10^{-4}M.

This solution was prepared by dissolving 0.0447 g of arsenazo III in 100 ml distilled water in volumetric flask.

Buffer solution, pH = 3.2

This solution was prepared by mixing 50 ml of 0.1M KHphathalate solution (1.02 g KHphathalate in 50 ml distilled water) with15.7 ml of 0.1N HCl, then the volume was completed to 100 ml of distilled water.

Mesacol capsules solution, 100 µg mesalazine/ 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 0.01 g mesalazine is dissolved in 10 ml of absolute ethanol and 30 ml of distilled water, after filtration of the solution, the volume was completed to 100 ml with distilled water in a volumetric flask**.**

Procedure and calibration graph

To a series of 25 ml calibrated flasks 0.05-1.2 ml of 10 µg.ml.$^{-1}$ mesalazine solution were transferred followed by addition of 4 ml of 1.45×10^{-4}M cerium(IV) ion solution and standing for 25 minutes, then 4 ml of buffer solution (pH 3.2) (KH phthalate- HCl) and 1.25 ml of 5.44×10^{-4}M arsenazo III reagent solution were added, then the volumes were completed to the mark with distilled water, the absorbance were measured at 654 nm against the reagent blank. Beer's law was obeyed over the range of concentration 0.5 to 14 µg mesalazine/25ml (Fig 1). The concentrations above 14 µg/25 ml gives a negative deviation from Beer's law .The molar absorptivity was 2.103×10^{5} l.mol^{-1} cm $^{-1}$.

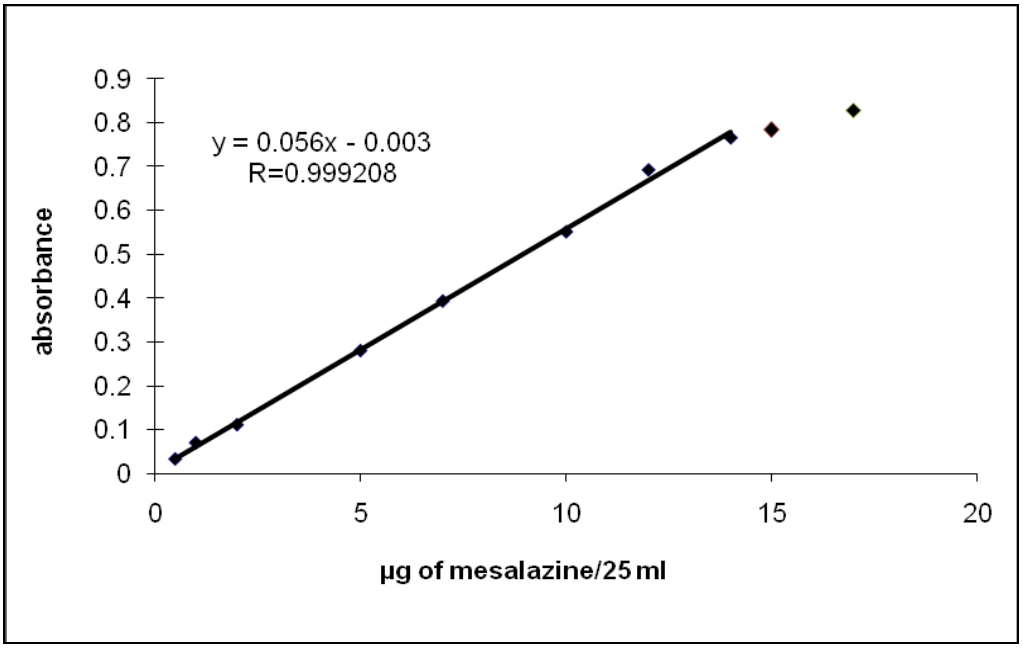

Fig. 1 : The calibration graph for mesalazine determination using the reaction between arsenazo III and cerium (III) .

Absorption spectrum

Absorption spectrum of the coloured complex formed from the reaction between cerium(III) ion with arsenazoIII in acidic medium against its corresponding reagent blank shows maximum absorption at 654 nm contrast to the arsenazo III reagent blank(Fig. 2).

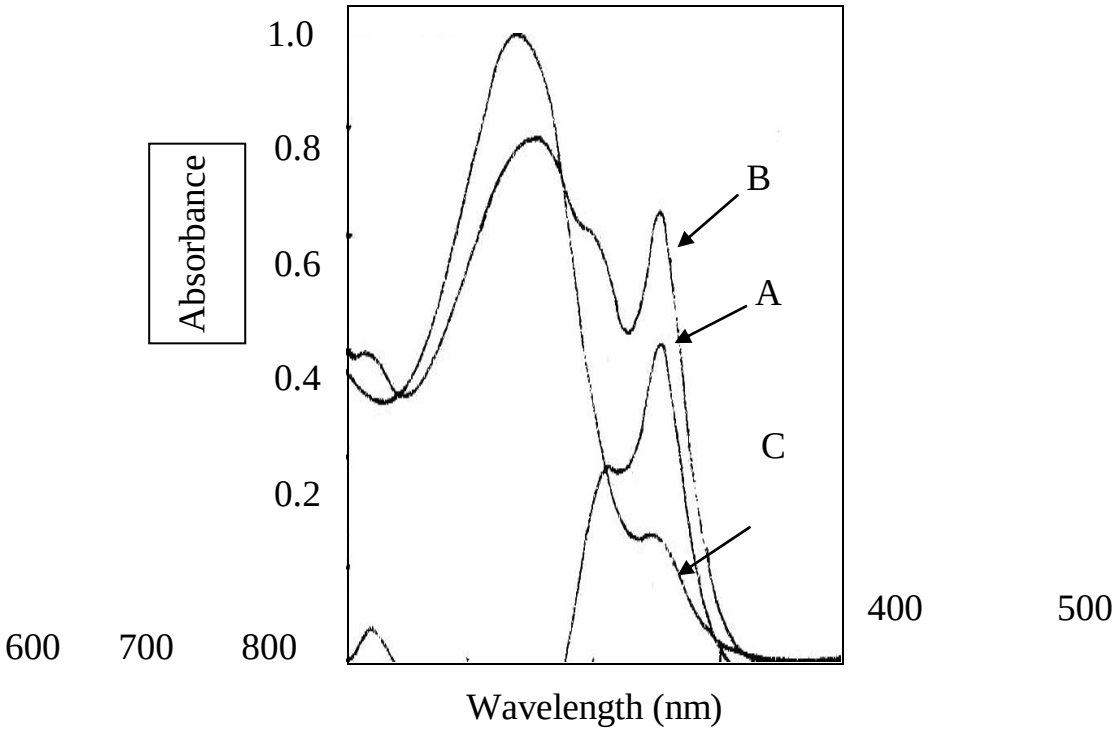

Fig.2 Absorption spectra of 10µg mesalazine/25ml treated according to the recommended procedure and measured against(A) reagent blank,(B) distilled water and(C) blank measured against distilled water

Optimization of variables

The effect of various parameters on the absorption intensity of the coloured complex was studied and the reaction conditions have been optimized.

Effect of pH

The effect of pH on the intensity of the coloured complex was examined. Different volumes (0.01- 4 ml) of 0.05M hydrochloric acid solution were added to an aliquot of solution reaction containing 10 µg of mesalazin. The results are shown in Table 1.

Table 1. Effect of pH on absorbance

Ml of 0.05 M HCl	Absorbance	Final pH
0.01	0.211	3.974
0.1	0.248	3.647
0.3	0.226	3.423
0.5	0.238	3.284
0.7	0.270	3.222
1	0.215	3.120
2	0.200	2.876
4	0.159	2.543

The results in

Table 1 indicate that the pH of 3.22 is considered optimum. A pH 3.2 is selected for subsequent experiments because of good sensitivity. Four buffer solutions of pH 3.2 with different composition have been tested, glycine-HCl (B_1) KH phathalate-HCl (B_2), acetic acid-KOH (B_3) and citric acid-Na_2HPO_4 (B_4). The results are shown in Table 2.

Table 2. Effect of buffer solutions on absorbance

Ml of buffer solution	Absorbance/ ml of buffer added			
	B_1	B_2	B_3	B_4
1	0.250	0.239	0.237	0.215
2	0.255	0.248	0.230	0.201
3	0.245	0.257	0.251	0.174
4	0.259	0.261	0.235	0.186
5	0.260	0.261	0.241	0.140

The results in Table 2 indicate that the buffer solution B_2 give the highest intensity for the absorbance of coloured complex, and 4 ml of B_2 has been recommended for subsequent experiments.

Effect of oxidizing agent [cerium (IV) ion] amount

Different amounts of cerium(IV) ion solution were added and the optimum amount which give high colour intensity of complex and higher value of correlation coefficient, therefore 4 ml of 1.45×10^{-4} M cerium(IV) ion solution recommended for subsequent experiments(Table 3).

Table 3. The effect of cerium(IV) ion amount on absorbance

Ml of cerium(IV) ion solution (1.45×10^{-4} M)	Absorbance/µg mesalazine in 25 ml						Correlation coefficient
	1	3	5	7	10	13	
1	0.034	0.082	0.146	0.205	0.251	0.254	0.9568
2	0.033	0.081	0.142	0.221	0.300	0.391	0.9984
3	0.045	0.103	0.170	0.239	0.348	0.425	0.9987
4	0.057	0.113	0.189	0.252	0.353	0.449	0.9991
5	0.041	0.094	0.161	0.229	0.275	0.358	0.9940

Effect of time on reduction of cerium(IV) ion

The effect of time needed to complete the reduction of cerium(IV) ion to cerium(III) ion was studied by standing of the solutions after adding cerium(IV) ion solution for different times, then the other reagent were added and the absorbance measured against the reagent blank (Table 4).

Table 4. Effect of time on reduction process

Time (minute)	0	5	10	15	20	25	30	35
Absorbance	0.164	0.371	0.474	0.482	0.492	0.571	0.534	0.482

The results indicate that complete reduction of cerium(IV) ion occurred after 25 minutes and the intensity decreased above 25 minutes, therefore the standing time 25 minutes was recommended for the subsequent experiments.

Effect of arsenazo III reagent amount

The effect of the amount of arsenazo III reagent on maximum formation of the coloured complex is investigated. The results shown in Table 5.

Table 5. Effect of arsenazo III reagent amount on absorbance

Ml of Arsenazo III reagent ($5.44x10^{-4}$ M)	Absorbance / µg mesalazine in 25 ml						Correlation coefficient
	1	3	5	7	10	13	
0.5	0.090	0.195	0.280	0.328	0.338	0.342	0.8323
1.0	0.080	0.175	0.286	0.401	0.545	0.677	0.9884
1.25	0.092	0.191	0.290	0.394	0.558	0.781	0.9994
1.5	0.068	0.176	0.275	0.386	0.522	0.735	0.9985
2.0	0.058	0.143	0.257	0.349	0.479	0.721	0.9989

The results shown in Table 5 indicate that 1.25 ml of arsenazo III reagent solution give the higher absorbance and the higher value of correlation coefficient, therefore it has been selected for the subsequent experiments.

Effect of time

The effect of time on the development and stability period of the coloured complex for different amounts of mesalazine was investigated under the optimum experimental conditions established. The coloured formation occurs immediately after arsenazoIII added for the reaction mixture and the absorbance remained constant at least for one hour.

Table 6. Effect of time on the absorbance of complex

µg of 5-ASA in 25 ml	Absorbance*/ min. standing time							
	0	5	10	20	30	40	50	60
2	0.108	0.105	0.106	0.104	0.106	0.107	0.109	0.109
5	0.284	0.285	0.283	0.284	0.283	0.283	0.286	0.286
10	0.545	0.547	0.545	0.547	0.550	0.553	0.553	0.553

*After 25 minutes time to the reaction of mesalazine with Ce(IV)

Accuracy and precision

Three different concentrations of mesalazine were used in the determination of the accuracy and precision of the method, the results shown in Table 7 indicate that the method has good accuracy and precision.

Table 7. The accuracy and precision

Amount of 5-ASA taken (µg /25ml)	Relative error,%*	Relative standard deviation,%*
2	1.48	±1.65
5	2.71	±1.38
10	2.83	±2.25

* Average of five determinations

Interferences

In order to assess the possible analytical application of the proposed method, the effect of some foreign substances which often accompanied with pharmaceutical preparations were studied by adding different amounts of foreign substances to 10 µg mesalazine/25 ml. It is found that the studied foreign species do not interfere in the present method (Table 8).

Table 8. Effect of interferences

Foreign compound	Recovery % of 10µg 5-ASA /25 ml per µg of foreign compound added		
	200	500	1000
Glucose	104.2	105.7	106.9
lactose	106.0	105.5	106.8
Acacia	100.0	105.0	105.8
Starch	99.0	104.2	102.3
Sodium chloride	97.7	99.0	100.0

Applications

The proposed method is applied to determine mesalazine in pharmaceutical preparation (capsules), the results in Table 9 indicated that good recoveries.

Table 9. Analytical applications

Drug	µg mesalazine present/25 ml	µg mesalazine measured/25 ml	Recovery*, %
Mesacol Extended release capsules 400 mg**	2	1.96	98.00
	5	4.89	97.80
	10	9.92	99.20

*Average of five determinations

**Universal Pharmaceutical Industries-Unipharma- Damascus-Syria

Evaluation of the proposed method

There is no standard method in the literature for determination of mesalazine, so the standard addition procedure applied in order to proposed method used in the determination of mesalazine without interferences, Table 10 and Fig. 3

Table 10. The results of standard addition method

Drug	µg mesalazine present/25 ml	µg mesalazine measured/25 ml	Recovery*,%
Mesacol Extended release capsules 400mg	2	1.92	96.0
	4	3.85	96.2

* Average of five determinations

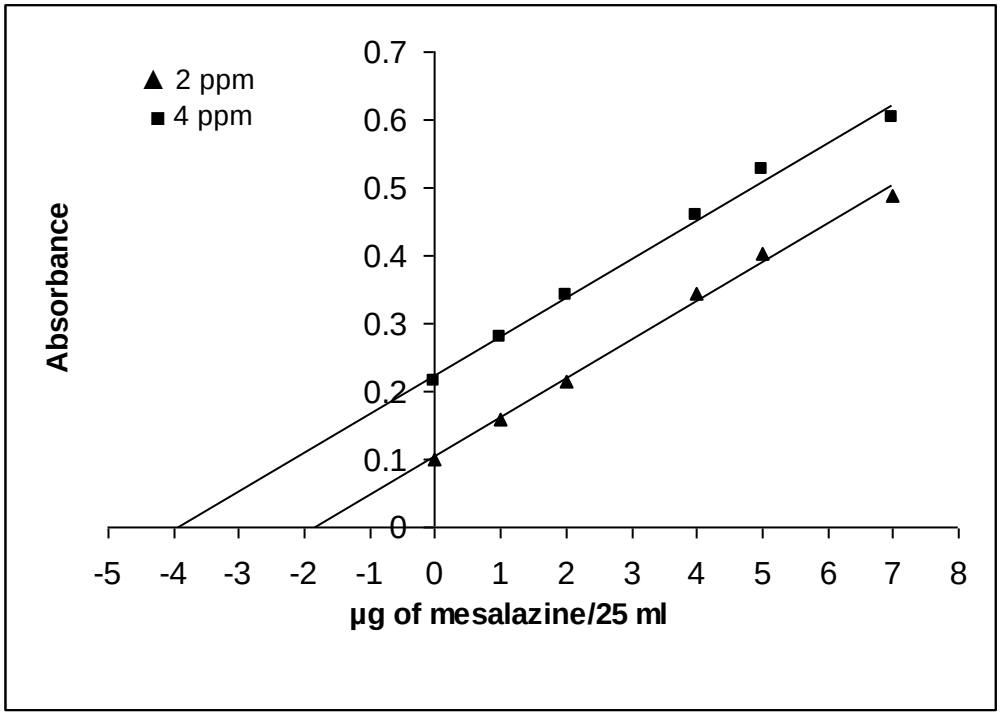

Fig.3 Standard addition method for the determination of mesalazine in pharmaceutical preparation(capsules)

The results in Table 10 and Fig. 3 indicated that the proposed method can be used to determine mesalazine in pharmaceutical preparation (capsules) with satisfactory results.

References

1) Praksh A., Lone KD., Shikla A., Mandloi R. and Ghosh V., Asian J. Research Chem., 1, 80-82, (2008).

2) Wiersma H. Escher J.C. and Dilger K., Inflammatory Bowel Disease, 10,626-631, (2004).

3) Gotti R., Pomponio R.,Bertucci C. and Cavrini V., J.Chromatogr. A,4,175-183, (2001).

4) Nobilis M., Vybiralova Z., Sladkova K., Lisa M., Holcapek M. and Kvetina J., J. Chromatogr. A,15, 299-308,(2006).

5) Palumbo G., Carlucci G., Mazzeo P., Pimpom T. and Fanini D., J. Pharm. Biomed. Anal.,14,175-180, (1995).

6) Rafael J.A., Jabor., Casagrande R., Georgetti S.R., Borin M.F. and Fonseca M.V., J Pharm. Sci.,43,97-103, (2007).

7) Nigovic B. and Imunic B., J.Pharm. Biomed. Anal., 31,169-174, (2003).

8) Feng-ling C., Li-Xia Q., Fang L. and Hong-Xia L., J. Molecular Modeling, 14,1111-1117, (2008).

9) Al-Sabha T.N. and Altaee O.A, J. Edu. & Sci., 21,49-62, (2008).

10) Norwitz G. and Keliher P., Talanta, 33,311-4, (1986).

11) Zakaria R. A., Raf. J. Sci., 20,90-104, (2009).

12) Al-Fakhry M. H., M.Sc., Thesis. College of Edu. University of Mosul (2006).

13) Lupetti K.O., Rocha F.R. and Filho O.F., Talanta, 62,463-467, (2004).

14) Ivanov VM. and Ermakova NV., J. Anal. Chem.,56,519-523,(2001).

15) Muhammad H.K, Peter W. and Nich E., Chemosphere, 63,1165-1169, (2006).

16) Shamsipur M., Yousefi M., Ghasemi Z., Hagiagha-Babaei L. and Ganjali MR., Sep. Sci. Technol., 37,3525-3534 (2002).

17) Nandkumar V.D., and Shripad M.K., Analyst., 114,105-107(1989).

18) Bubl F. and Sroka B.S., Chem. Anal., 46,69, (2001).

19) Al-Abdaly Z.Z., M.Sc. Thesis. College of Sci. University of Mosul (2005).

20) Othman N.S. and Abdul Aleem S., Tikrit J. of Pure Sci., 13,214-218, (2008).

21) Othman N.S. and Awadis L.S., Raf. J. Sci., 20,8-20, (2009).