SPECTROPHOTOMETRIC DETERMINATION OF MESALAMINE USING SODIUM NITROPRUSSIDE AS CHROMOGENIC REAGENT

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A simple, sensitive and accurate spectrophotometric method has been developed for the determination of mesalamine in pure and commercial dosage forms. The method is based on the reaction of mesalamine with sodium nitroprusside in the presence of hydroxylamine hydrochloride in alkaline medium to give a highly green colored species which absorb maximally at 703 nm. Beer's law is obeyed in the concentration range of 0.0-30 µg mL^{-1} with molar absorptivity = 2.0367×10^4 L mol^{-1} cm^{-1}. The average recovery is 103.0 % and relative standard deviation is less than 1.5 % (n = 6). The proposed method has been applied successfully for determination of mesalamine in commercial pharmaceutical products such as tablet and suppositories. There is no interference from the excipients.

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Introduction

Mesalamine or 5-aminosalicylic acid, is an anti-inflammatory drug used to treat inflammation of the digestive tract (Crohn's disease) and mild to moderate ulcerative colitis. Mesalamine is a bowel-specific aminosalicylate drug that is metabolized in the gut and has its predominant actions there, thereby having fewer systemic side effects. Sulfasalazine is believed to be metabolized to mesalamine, which is considered as the active compound .[1]

Several techniques such as fluorimetry,[2] voltammetry,[3] coulometry,[4] chromatography[5-8] have been reported for the determination of mesalamine. These techniques require sophisticated instruments, expensive reagents, involve several manipulation steps and derivatization reactions. Literature survey revealed many spectrophotometric methods including different reactions have been reported for determination of mesalamine. These reactions are charge transfer complex formation,[9,10] diazotization and coupling,[11,12] oxidative coupling,[13,14] Schiff's base[15,16] and oxidation-reduction[17] reactions. Derivative formation[18] and UV spectrophotometric[19] methods have also been reported. Most of these methods are either insufficiently sensitive or tedious and required an extraction step or suffered from interferences.

The present work is a development of a simple, sensitive and selective spectrophotometric method for determination of mesalamine based on its reaction with sodium nitroprusside (SNP) in the presence of hydroxylamine hydrochloride (HAH) in alkaline medium.

Experimental

Apparatus

Shimadzu UV-1650 PC UV-Visible spectrophotometer equipped with a 1.0-cm path length silica cell and Philips PW (9421) pH-meter with a combined glass electrode were used. All calculations in the computing process were done in Microsoft Excel for Windows.

Reagents

Mesalamine was procured from State Company for Drug Industries and Medical Appliances, Sammara-Iraq, SDI. SNP solution of 0.2 % (w/v) strength was prepared by dissolving 0.2 g of SNP in 100 mL of distilled water. HAH solution (0.02 M) was prepared by dissolving 0.139 g of HAH in 100 mL of distilled water. Stock solution of mesalamine (100 µg mL^{-1}) was prepared by dissolving 0.01 g of pure mesalamine in a 5 mL of ethanol and diluted to the mark with distilled water in a 100 mL-volumetric flask. This solution was further diluted with water as per requirement. A 1.0 M solution of potassium hydroxide was prepared by dissolving 5.6098 g of the base in 100 mL in water and a 0.1 M solution was prepared by its dilution with distilled water.

Recommended procedure

Aliquots of mesalamine solution containing 0.2-25 µg mL^{-1} of the drug were pipetted into a series of 10 mL standard volumetric flasks. A 0.5 m1 of 0.02 M HAH followed by 1.0 mL of 1% SNP and 2.5 mL of 0.1 M KOH solutions were added to each flask and the contents were diluted to the mark with distilled water. The mixture was allowed to stand for 20 min and the absorbance of coloured product formed was measured at room temperature at 703 nm against a reagent blank prepared in a similar manner without mesalamine.

Determination form tablets of commercial preparations

The contents of 10 tablets (each tablet contains 400 or 500 mg mesalamine as Mesacol or Pentasa formulations) were powdered, mixed thoroughly and weighed accurately to an amount equivalent to 100 mg of mesalamine. The mixture was dissolved with 10 mL absolute ethanol and 40 mL distilled water, stirred well and filtered through a Whatmann No.42 filter paper. The residue was washed with distilled water for complete recovery of the drug. The filtrate and washings were diluted to 100 mL with water. It was further diluted according to the need and then analyzed by following the recommended procedure.

Pentasa suppository

The content of Pentasa suppository (containing 1 g of mesalamine) was transfer to a 1 L volumetric flask containing 500 mL of absolute ethanol and 250 mL of distilled water, sonicated for 5 min and diluted to the mark with distilled water. The resulting solution was filtered through a Whatmann No.42 filter paper. From this solution suitable dilutions were made to obtain the working solutions and then analyzed by following the recommended procedure.

Results and discussion

The method is based on the reaction of mesalamine with SNP in the presence of HAH and potassium hydroxide to form a faint brown colour which after 20 min became a deep green coloured complex, having maximum absorption at 703 nm where as blank reagent show no absorbance at this wavelength (Figure 1).

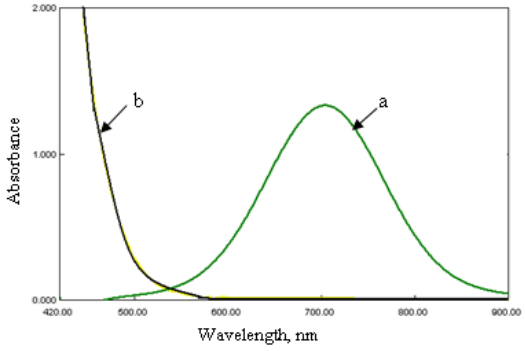


Figure 1. Absorption spectra of (a) (12 μg mL^{-1}) mesalamine with SNP and HAH against reagent blank (b) reagent blank against water (Conditions as mentioned in the recommended procedure).

The effect of various parameters on the absorption intensity of the complex formed was studied and the reaction conditions are optimized.

Effect of pH and Buffer Solutions

The reaction takes place in alkaline solution, therefore, to test the efficacy of different bases, 1.0 mL of 0.1 M solution of different bases were used in the recommended procedure. The results (Figure 2) indicated that KOH gave the maximum absorbance. The effect of pH was examined by addition of different volumes of 0. 1.0 M solution of KOH in the range of 0.0-3.0 mL. It was found that 2.5 mL KOH gave maximum absorbance and the optimum pH was found to be 11.93 (Figure 3). The effect of different buffer solutions, such as carbonate and phosphate of pH 11.93, were also studied and found to have no effect. Therefore 2.5 mL of 0.1 M KOH was used in other experiments.

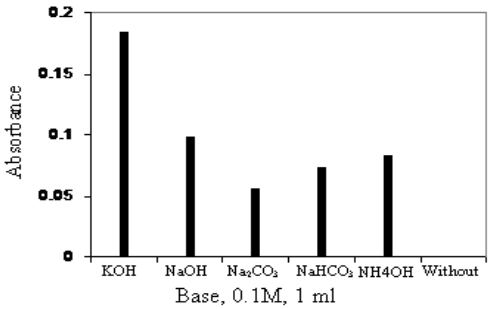


Figure 2. Effect of bases on the absorption of 2 μg ml^{-1} mesalazine with SNP.

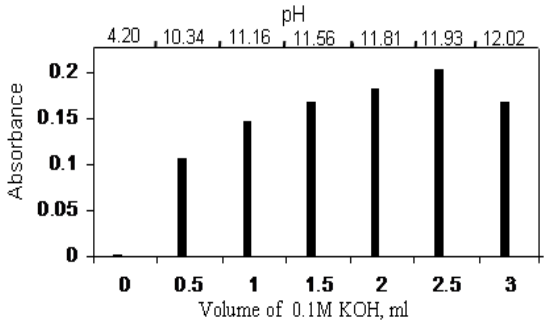


Figure 3. Effect of KOH and pH on the absorbance of 2 μg mL^{-1} mesalamine with SNP.

Variation of SNP concentration

The effect of changing the SNP concentration on the absorbance of solution containing a fixed amount of the drug, KOH and HAH was studied, It was found, that absorbance increases with increasing SNP concentration and reached the maximum value on using 1.0-2.0 mL of 1 % solution and further increase causes a gradual decrease in absorbance. Therefore 1.0 mL of 1 % solution was used in subsequent experiments.

Variation of HAH concentration

The effect of various volumes of a 0.02 M solution of HAH in the range of 0.0 to 3.0 mL on the intensity of absorption was investigated. It was observed that a volume of 0.5 mL of 0.02 M solution of HAH gave the highest absorbance and this concentration was chosen for further work.

Effect of surfactants

Addition of various surfactants viz., tween-80, triton x-100, cetyltrimethylammonium bromide and sodium dodecyl sulphate decreases the absorbance.

Effect of temperature and developing time

The reaction time was determined by following the color development at room temperature and in thermostatically controlled water-bath at 40 °C. The absorbance was measured against reagent blank treated similarly. It was observed that the sensitivity reached maximum after 20 min at room temperature (25 °C) and was stable for more than 100 min. Room temperature was therefore, chosen for the subsequent experiments.

Effect of order of addition

It was observed that the best results are obtained when the reagents are mixed in the order described in the recommended procedure. Changes in the order result in loss of color intensity.

Analytical parameters

Under the optimum experimental conditions described in the recommended procedure, a calibration graph of the coloured product was constructed by plotting absorbance versus concentration. The correlation is good (r = 0.9918). Beer's law is obeyed in the range of 0.05-30 µg mL^{-1}, and the molar absorptivity (ε) value indicated a high sensitivity of the method. The optical characteristics and statistics for the proposed method are summarized in Table 1.

Table 1. Summary of optical characteristics and statistics for the proposed method.

Parameter	Value
λ_{max}	703 nm
Linear range (µg mL^{-1})	0-30
Limit of detection (µg mL^{-1})	0.0248
Limit of quantification (µg mL^{-1})	0.0826
Slope	0.113
Intercept	0.053
Correlation coefficient (r)	0.9918
Sandell's sensitivity (µg cm^{-2})	0.0084
ε (L mol^{-1} cm^{-1})	2.0367×10^4

Precision and accuracy

The accuracy and precision of the proposed method were estimated by measuring the content of mesalamine in pure form at three different concentration levels within the Beer's law limit in six replicates. The relative standard deviation (representing precision) in the range of 0.23 to 1.4 and mean percent recovery (representing accuracy) of 98.74 obtained by the proposed method can be considered to be satisfactory.

Interference

The extent of interference by some excipients, viz., sucrose, glucose, fructose, sodium chloride and starch, which are often found in pharmaceutical preparations, was studied by measuring the absorbance of solutions containing 2 µg mL^{-1} of mesalamine and 100, 500 and 1000 µg mL^{-1} of excipients in the final volume of 10 mL. It was found that these excipients do not interfere in the present method. The range of recovery is between 96.2 and 102.1 %. We consider that this variation is acceptable.

Applications

Application of the proposed method to the assay of pharmaceutical sample of mesalamine as tablet and pentasa suppository gave reproducible and accurate results as shown in Table 2. The obtained results were compared statistically by a Student's *t*-test for accuracy and a variance ratio *F*-test for precision by the standard method[20] at the 95% confidence level with six degrees of freedom, as mentioned in Table 2. The results showed that the experimental *t*-test and *F*-test were less than the theoretical value (t= 2.57, F= 4.284, n=6), indicating that there was no significant difference between the proposed method and official method.

Stoichiometry, stability constant and reaction mechanism

The mole ratio of the reaction product formed between the mesalamine and SNP was investigated by applying the Job's method of continuous variation[21] using equimolar solutions (5×10^{-3} M) of the drug and SNP. The results shown in Figure 4 indicated that the product was formed in the ratio of 1:2 for mesalamine : SNP. The stability constant (K_c) of the product was determined by applying Eqn. (1).

$$K_c = 1 - \alpha / 4\alpha^3 C^2 \qquad (1)$$

where

K_c is the stability constant,

α is the degree of dissociation and

C is the concentration of the product which is equal to the concentration of drug.

The value of K_c (average of measurements at three concentrations) was found to be 2.79×10^5 L^2 mol^{-2}. This indicated that the product is highly stable.

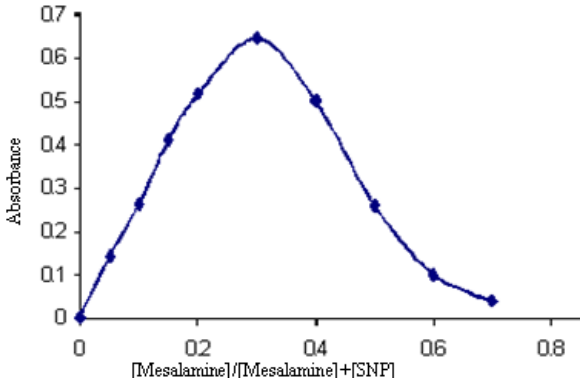


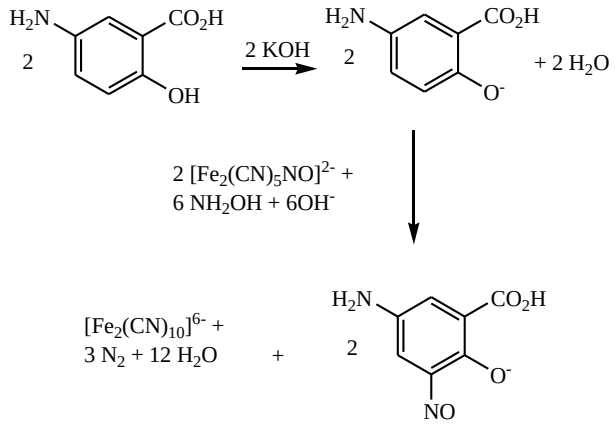
Figure 4. Continuous variation plot of mesalamine-SNP complexation.

Table 2: Assay of mesalamine in pharmaceutical preparations using the proposed method and its comparison with the official method.

Procedure applied	Pharmaceutical preparation	Drug amount present (μg mL^{-1})	Recovery[a] (%)	Drug content found (mg)	Average recovery	Certified value
Proposed method	Mesacol tablet [b]	2 5 10 15	99.9 101.2 98.9 101.5	399.6 404.8 395.6 406.0	401.5 mg (2.04, 2.15)[e]	400 mg
	Pentesa tablet [c]	2 5 10 15	99.5 97.8 103.3 97.7	397.5 489.0 516.5 488.5	497.9	500 mg
	Pentesa suppository[d]	2 5 10 15	99.5 96.2 102.3 99.8	0.995 0.962 1.023 0.992	0.993 g (2.32, 1.96)	1.0 g
British Pharmacopoeia	Pure mesalamine	50	101.06	50.532	-	50 mg

[a]Average of six determinations. [b]Manufactured by Universal Pharmaceutical Industries unipharama–Damascus – Syria; [c]Manufactured by Ferring AS, INHOUSE PHARMACY.BIZ., [d]Manufactured by Ferring Leciva, Czech Republic. [e]Figures in parenthesis are the calculated values for *t*, and *F* respectively.

The formation of the complex between SNP and mesalamine is an aromatic electrophilic reaction, which is represented by Scheme 1. In a strongly alkaline medium, phenol is converted to phenolate ion. Hence the electrophilic reaction can take place more easily because the benzene ring is activated due to the presence of anionic oxygen. It is suggested, the nitroso group of the SNP attacks the phenolate moiety in mesalamine at ortho-position. The electronic properties of the substituents in the benzene ring affect the extent of reaction, and consequently the sensitivity of the method.[22]



Scheme 1. The mechanism of reaction between mesalamine, sodium nitroprusside and hydroxylamine hydrochloride in a basic medium.

Comparison with other spectrophotometric methods

The proposed method compares favorably with other reported methods. As shown in Table 3, the proposed method is more sensitive than other methods, needs no heating and the product is stable for a longer time.

Table 3. Comparison of the proposed method with other spectrophotometric methods

Analytical parameter	SNP	Ref. 9	Ref. 16	Ref. 17
λ_{max} (nm)	703	571.6	320	520
pH	11.9	9.8	Acidic	-
Temp. (°C)	R.T.	25	R.T.	100
Development Time (min)	20	5	-	15
Stability Period (min)	>100	45	120	-
Beer's law (μg mL^{-1})	0.05 – 30	1.25-30	2.0-30	4.0-24
ε (L mol^{-1} cm^{-1})	2.03 x 10^4	3.4 x 10^3	1.283 x 10^4	0.38 x 10^3
Recovery (%)	98.74	100.44	100.34	99.93
RSD (%)	≤1.44	1.67	0.409	0.684
Application	Tablet, Enema	Tablet, Capsule	Tablet	Tablet

Conclusion

The proposed spectrophotometric method is simple, accurate and more sensitive than the other methods. No significant difference in the recovery between the proposed and official method was obtained. The method does not require any pre-treatment or extraction steps and was applied successfully for the assay of the pharmaceutical preparations for tablets and pentasa suppositories of mesalamine.

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