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U.V SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF MESALAZINE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS

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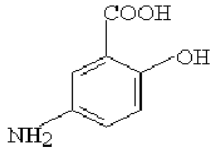
ABSTRACT

Mesalazine is used, either orally or rectally, as an anti-inflammatory agent in treating ulcerative colitis and, to a lesser extent, Crohn's disease. Mesalazine is a white to pinkish crystalline powder. It is slightly soluble in cold water and alcohol; more soluble in hot water, soluble in hydrochloric acid. A simple UV spectrophotometric method was developed for the determination of Mesalazine (MEZ) in pure and its pharmaceutical formulations. Mesalazine exhibiting max absorbance at 210 nm in methanol and obeyed linearity in the concentration range of 0.2-50 μg/ml. The proposed method was statistically validated.

INTRODUCTION: The scope of developing and validating analytical methods is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. Chemically Mesalazine (MEZ) is 5-amino salicylic acid. It is an anti-inflammatory drug structurally related to salicylates and active in inflammatory bowel disease. Tablet formulations containing 250, 400 and 500 mg MEZ are available in the market. Literature survey revealed that Mesalazine is estimated by HPLC and micellar electrokinetic chromatography. No UV spectrophotometric methods have been reported for estimation of MEZ in single component formulation. Hence, an attempt has been made to develop new UV methods for its estimation in pharmaceutical formulations with good accuracy, simplicity, precision and economy.



5-Amino-2-hydroxybenzene-1 carboxylic acid [Mesalazine or 5-Amino salicylic acid (ASA)]

EXPERIMENTAL:

Instrumentation: Spectral and absorbance measurements were made on an ELICO SL-159 UV-Vis spectrophotometer by using 1 cm quartz cells. Dhona balance was used for weighing the samples. Commercially available tablets of Mesalazine were procured from the local market and estimated.

Reagent used:

1. Disodium hydrogen phosphate anhydrous (Merck)
2. Citric acid
3. Double distilled water
4. Mesalazine, Sun pharma ltd., (India)

Optimization: Scanning and determination of maximum wavelength (λ_{max}):

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, different solutions of the drugs (10 µg/ml and 20 µg/ml) in methanol were scanned using spectrophotometer within the wavelength region of 200 – 380 nm against Phosphate buffer (pH- 3.6) as blank. The resulting spectra were shown in fig 1 and the absorption curve showed characteristic absorption maxima at 298 nm for Mesalazine.

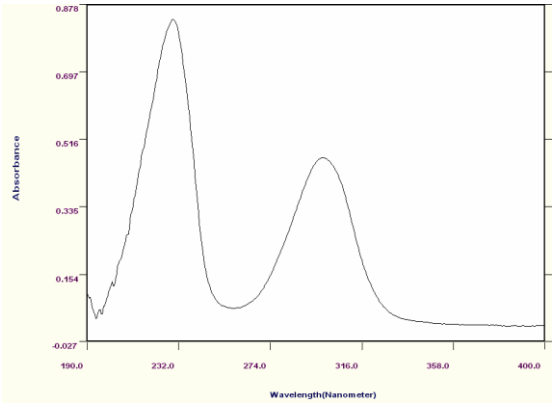


Fig 1: UV Spectrum of Mesalazine in Phosphate buffer (pH- 3.6)

Sample: Mesalazine (10 µg/ml)

Reference: Phosphate Buffer (pH-3.6)

Instrument: Elico SL 159 UV –Visible Spectrophotometer.

METHOD

Preparation of Phosphate buffer solution (pH-3.6): 0.9g of anhydrous disodium hydrogen phosphate and 1.298g of citric acid monohydrate were weighed accurately, mixed and volume made with double distilled water (1000ml).

Preparation of Stock Solutions: Standard stock solution was prepared by dissolving 25 mg of each drug in 25 ml of Phosphate Buffer (pH-3.6) to get concentration of 1mg/ml (1000 µg/ml) solutions.

Preparation of Working Standard Solutions and construction of standard graph: The prepared stock solution was further diluted with Phosphate Buffer (pH-3.6) to get working standard solutions of 10 µg/ml and 100 µg/ml of mesalamine to construct Beer's law plot for pure drug, different aliquots of Mesalazine were taken and diluted to 10 ml with Phosphate Buffer (pH-3.6). The absorbance was measured maximum at 245 nm, against Phosphate Buffer (pH-3.6) as blank. The results were shown in table (1). The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Fig. (b) The drug has obeyed Beer's law in the concentration range of 0.2-50µg/ml.

Table 1: Linearity table of Mesalazine in Working Standard

Concentration (µg /ml)	Absorbance
0.2	0.009
0.5	0.017
1	0.032
2	0.06
5	0.128
10	0.25
20	0.486
30	0.737
40	0.982
50	1.205

Table 2: Optical characteristics

Beer's Law limit (µg/mL)	0.2-50
Sandell's sensitivity (µg/cm2/0.001absorbance unit)	0.0390
Molar extinction coefficient (1 mole-1 c.m-1)	4.90×10^3
% Relative standard deviation	1.1083
Confidence limits	
95% Confidence limits	0.189209
99% Confidence limits	0.249058
Correlation coefficient	0.999
Regression equation (Y*)	
Slope (a)	0.024
Intercept (b)	0.0077

* Y= a + bC where C is the concentration of mesalamine and Y is the peak area

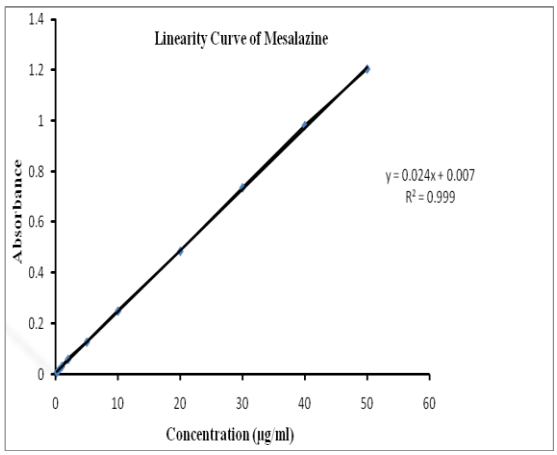


Fig 2: Standard graph of Mesalazine

Table 3: Recovery from the formulation:

Formulation	Labeled amount (mg)	UV- method*		
		Mean ± s. d (amount mg recovered)	%Drug recovered	% RSD
Mesacol (tablets)	400	395.58±1.144	98.895±0.286	0.289

* Each value is average of three determinations ± standard deviation.

Validation:

Precision: The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From this absorbance, Mean, Standard deviation, % RSD was calculated. The readings were shown in Table 4.

Table 4: Precision readings:

Concentrations (µg/ml)	Absorbance	Statistical analysis
10	0.246	Mean = 0.2456
10	0.251	SD = 0.002722
10	0.247	%RSD = 1.1083
10	0.245	
10	0.242	
10	0.246	
10	0.245	
10	0.243	

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of mesalamine within the linearity range were taken and added to the pre-analyzed formulation of concentration 20µg/ml. From that percentage recovery values were calculated. The results were shown in Table 5.

RESULTS AND DISCUSSION: From the optical characteristics of the proposed method, it was found that mesalazine obeys linearity within the concentration range of 0.2-50 µg/ml. From the results shown in Table (4) it was found that the % RSD is less than 2, which indicates that the method has good reproducibility. From the results shown in accuracy Table 5, it was found that

the percentage recovery values of pure drug from the preanalyzed solution of formulation were in between 98.676 – 101.397, which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

Table 5: Accuracy table

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis	
	Pure drug	Formulation			
S_1 : 80 %	8	10	101.29	Mean	101.397
S_2 : 80 %	8	10	101.52	SD	0.1159
S_3 : 80 %	8	10	101.38	% RSD	0.1143
S_4 : 100 %	10	10	99.046	Mean	99.3253
S_5 : 100 %	10	10	99.57	SD	0.2637
S_6 : 100 %	10	10	99.36	% RSD	0.2655
S_7 : 120 %	12	10	98.819	Mean	98.6757
S_8 : 120 %	12	10	98.328	SD	0.3026
S_9 : 120 %	12	10	98.880	% RSD	0.3067

CONCLUSION: The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Mesalazine in pure samples and pharmaceutical formulations.

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