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DEVELOPMENT AND VALIDATION OF COLORIMETRIC METHOD FOR THE ESTIMATION OF MESALAMINE

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ABSTRACT

The objective of the study is to develop simple, precise, authentic and cost-effective analytical method for the estimation of anti-inflammatory drug mesalamine according to ICH guidelines. Accurate and precise analytical method was developed for the analysis of mesalamine. The drug mesalamine is active against inflammatory bowel disease. The colorimetric method was developed by using 5-25µg/ml of mesalamine. The absorption maxima λmax was found to be 576nm. In this method mesalamine shows correlation coefficient of 0.9997. Developed method is valid as per ICH guidelines.

KEYWORDS: Mesalamine, UV -Visible spectrophotometry.

INTRODUCTION

Mesalamine (5-aminosalicylic acid, 5-ASA) is an anti-inflammatory drug primarily used to treat inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease. It works by inhibiting pro-inflammatory mediators, such as cytokines and leukotrienes, thereby reducing inflammation in the gastrointestinal (GI) tract.[1]

The drug exerts a local effect on the colonic mucosa, with minimal systemic absorption, making it a preferred treatment for mild-to-moderate cases of ulcerative colitis. Various formulations, including oral, rectal suppositories, enemas, and controlled-release tablets, have been developed to improve drug delivery and adherence.[2]

Analytical method development is the process of creating and optimizing a procedure to accurately and reliably measure the presence, concentration, or characteristics of a substance in a given sample. It involves selecting

appropriate techniques, reagents, and instruments to ensure precision, accuracy, specificity, sensitivity, and reproducibility of the analysis. This process is crucial in pharmaceuticals, chemistry, and quality control to ensure regulatory compliance and reliable results.

Ultraviolet spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200-400 nm. Colorimetry/Visible spectroscopy is concerned with the study of absorption of visible radiation whose wavelength ranges from 400-800nm. When a radiation is passed through a substance, it absorbs energy moving from ground state to an excited state. Absorption only occurs if the photon's energy matches the energy difference between these states, and this energy absorption is quantized. This is done using instruments called UV-visible spectrophotometer, which measures the ratio or a function of the ratio of the intensities of two light beams in the UV-visible spectrum.[3]

The main principle behind UV-Visible spectrophotometry is Beer-Lambert's law. The quantitative analysis by UV-Visible spectrophotometry is governed by the Beer-Lambert's law, it states that, "When a beam of monochromatic light is passed through a transparent cell containing a solution of an absorbing substance, reduction of intensity of the light may occurs; the rate of reduction in intensity with the thickness of the medium is proportional to the intensity of the light and the concentration of the absorbing substance.

$A = \log I_o / I_t = abc$

Transmittance (T): It is the ratio of intensity of transmitted light to that of incident light.

$T = I_t / I_o$

Absorbance(A): It is the negative algorithm of transmittance to the base 10.

$A = - \log_{10} T = \log_{10} I_o / I_t$

$A = abc$

Absorptivity (a) is given by,

$a = A/bc$

where A = absorbance, b = pathlength, c = concentration in gm/100ml

Molar absorptivity: When concentration "c" in equation A =abc is expressed in mol/lit and cell length in cm, the absorptivity is called as molar absorptivity.

$\varepsilon = A/bc$

EXPERIMENTAL APPARATUS

1 Electronic Balance Samson

2 UV Visible Spectrophotometer Systronics

REAGENTS AND MATERIALS

1. MESALAMINE Yarrow chem products, Mumbai.
2. HYDROCHLORIC ACID Research lab fine chem. industries, Mumbai.
3. BASIC FUCHSIN Medlise chemicals.

EXPERIMENTAL PROCEDURE

Selection of wavelength range for estimation

Mesalamine were dissolved in 0.5N HCL, And appropriate dilutions were prepared by taking aliquots from the stock solution. The drug solutions were scanned from 400-800 nm and from that wavelength ranges are selected for estimation of drugs.

MATERIAL AND METHODS

Preparation of standard stock solution (1000µg/ml)

An accurately weighed quantity of MSL (0.1g) were transferred to a 100ml of Volumetric flask. 0.5 N HCL is used to dissolve the drug, and the volume was made up to the mark with HCL to get the solution having a concentration of 1000µg/ml. The solution is used as the **Stock A**, from that further dilution carried out.

Preparation of working standard solution (50µg/ml)

From the above prepared stock solution of MSL (A), 5ml were transferred to 100ml of volumetric flask to obtain working standard solution having a concentration of 50µg/ml. The solution is used as the **Stock B.**

From the above working standard solution of Mesalamine (1,2,3,4,5 ml) aliquots were transferred in a series of 10 ml volumetric flask, to get a concentration range of 5-25 µg/ml of Mesalamine. To this 2 ml of colouring reagent (Basic Fuchsin) was added and the volume was adjusted to the mark with HCL. The absorbance of the solution was measured as function of wavelength from 400-800 nm against blank prepared in same manner.

METHODOLOGY

The working standard solutions of Mesalamine were scanned in uv from the range of 400-800 nm and it shows 576nm as the wavelength having maximum absorbance and these wavelengths are selected for the quantitative estimation of Mesalamine.

Linearity and Range: Different dilutions of concentration 5,10,15,20,25 µg/ml of Mesalamine were prepared.
The calibration curve was plotted and interpreted in terms of correlation coefficient and equation of line.

Method precision (Repeatability): The precision of the instrument was checked by repeated scanning and absorbance of solution of (n = 6) MSL (50µg/ml) without changing the parameters of the developed methods.

Reproducibility: The intraday and interday precision was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solution of Mesalamine (10,15,20 µg/ml). Relative standard deviation (% RSD) was used to report the results.

Accuracy (% Recovery): Accuracy can be reported in terms of % recovery. The percentage accuracy levels are 80,100 and 120%, About 50µg/ml of mesalamine were used for the study.

Limit of detection and Limit of quantification (LOD & LOQ): The LOD and LOQ were calculated by the equation method.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$ Where, σ = the standard deviation of the response

S = slope of the calibration curve.

RESULTS AND DISCUSSION

Colorimetric method for mesalamine was developed by dissolving 0.1g of mesalamine in 100ml of 0.5 N HCL. Pipette out 5 ml from above solution and then make up to 100ml by using 0.5 N HCL. Pipetted out (1,2,3,4,5) ml of above solution and an unknown concentration into the series of 10 ml volumetric flask. To this 2 ml of colorimetric reagent was added and made up to the mark by HCL. The absorbance was measured at 576 nm using reagent blank and graph was plotted between absorbance obtained and the concentrations of the solutions.

Beer-Lambert's law was obeyed with the concentration range 5-25µg/ml at 576nm.

Linearity: Different dilutions of concentration 5,10,15,20,25 µg/ml of mesalamine were used to record the absorbance of each solution at its respective wavelength (576 nm) and the calibration curve was recorded.

LOD and LOQ: According to ICH guideline there are several methods for the determination of LOD and LOQ in the present study the LOD and LOQ were calculated by equation.

LOD and LOQ of Mesalamine was found to be 0.476& 1.443 respectively.
Precision (Repeatability): Here the % RSD is less than 2 indicates the method is repeatable.
Reproducibility (Intermediate Precision): Here the % RSD was found to be below 2% indicates the reproducibility of the developed analytical method.

Accuracy: Here the recovery results indicates the accuracy of the proposed method. The accuracy was calculated by recovery studies in various levels.

Table 1: Regression analysis data and summary of validation parameters from the calibration plot.

Parameter	Mesalamine
Absorption maximum	576 nm
Linearity range(µg/ml)	5-25
Correlation coefficient	0.9997
Regression equation	y= 0.00988x + 0.5206
slope	0.00988
Y intercept	0.5206

Precision Analysis

Table 2: Result of Precision study by developed method.

Concentration MSL (10); n=6	Absorbance
1	0.624
2	0.626
3	0.624
4	0.623
5	0.623
6	0.625
MEAN	0.624
SD	0.001169
RSD%	0.1872

Reproducibility analysis

Table 3: Result of Reproducibility study by developed method.

Drug n=3	Conc (µg/ml)	INTRADAY Absorbance Found		INTERDAY Absorbance Found	
		MEAN±SD	% RSD	MEAN±SD	% RSD
MSL	10	0.624±0.001	0.224	0.625±0.001	0.16
	15	0.686±0.0015	0.222	0.685±0.0015	0.222
	20	0.742±0.0015	0.205	0.741±0.0015	0.205

Accuracy analysis

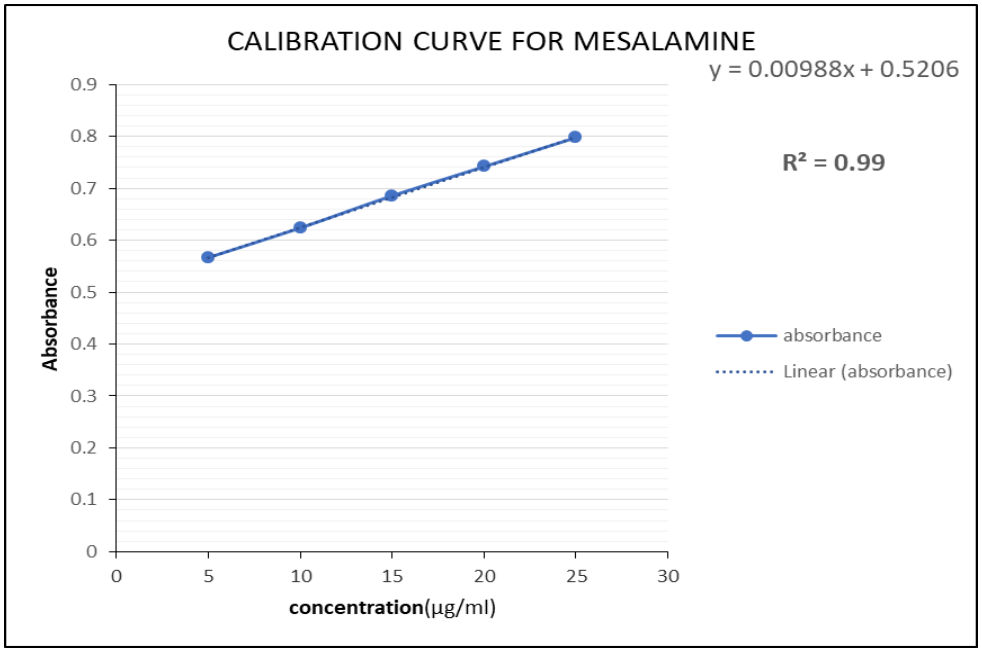
Table 4: Result of Recovery study by developed method.

Drug	Accuracy level %	Amount			% Recovery	MEAN ± SD	% RSD
		Actual (µg/ml)	Added (µg/ml)	Found (µg/ml)			
MSL	80%	20	16	35.80	99.4%	99.5 ± 0.2081	0.20907
	100%	20	20	39.82	99.5%		
	120%	20	24	43.92	99.8%		

Assay of sample

Table 5: Assay of sample at 576 nm.

SL. No	Drug	Sample solution Concentration µg/ml	Amount found	Drug content (%) ± SD
1	MSL	12	11.9	99.1±0.091



y= 0.00988x + 0.5206

R^2= 0.9997

R L.NO	TYPE	CONCENTRATION	ABSORBANCE
1	STANDARD	5	0.567
2	S ANDARD	10	0.624
3	STANDARD	15	0.686
4	STANDARD	20	0.742
5	STANDARD	25	0.798
6	SAMPLE		

Figure 1: Calibration curve of Mesalamine.

MESALAMINE (λmax =576 nm)

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Date: 15.11.2024 **Spectrum scan** **Time: 02:48:43**

Organisation Name : KCP PALAKKAD

Chemist Name : SOSAMMA CICY EAPEN

Instrument ID : 2202TS

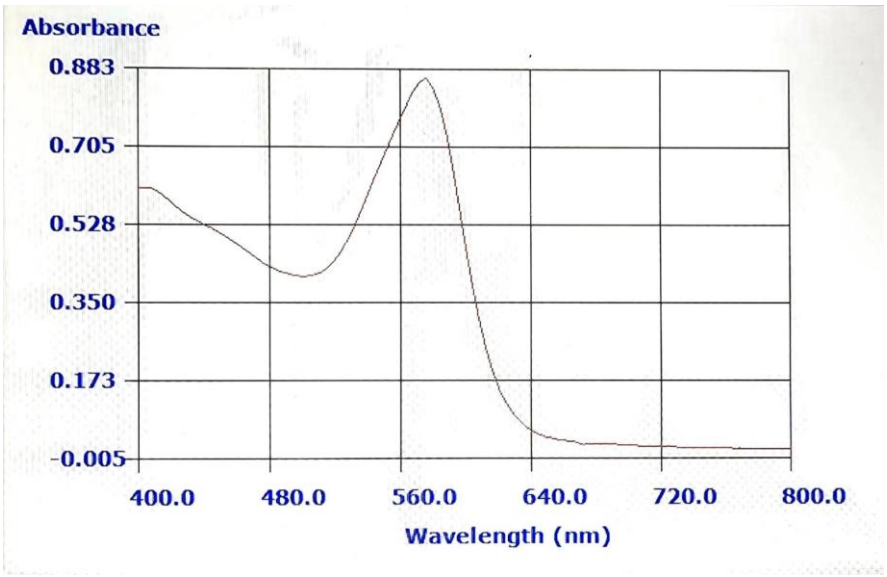

Figure 2: Absorption spectra of pure drug mesalamine at 576 nm.

MESALAMINE (Unknown concentration spectrum at λmax= 576 nm

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UV-VIS DOUBLE BEAM SPECTROPHOTOMETER- 2202 TS

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Date: 04.01.2025 Spectrum scan Time: 12:11:22

Organisation Name : KCP PALAKKAD

Chemist Name : SOSAMMA CICY EAPEN

Instrument ID : 2202TS

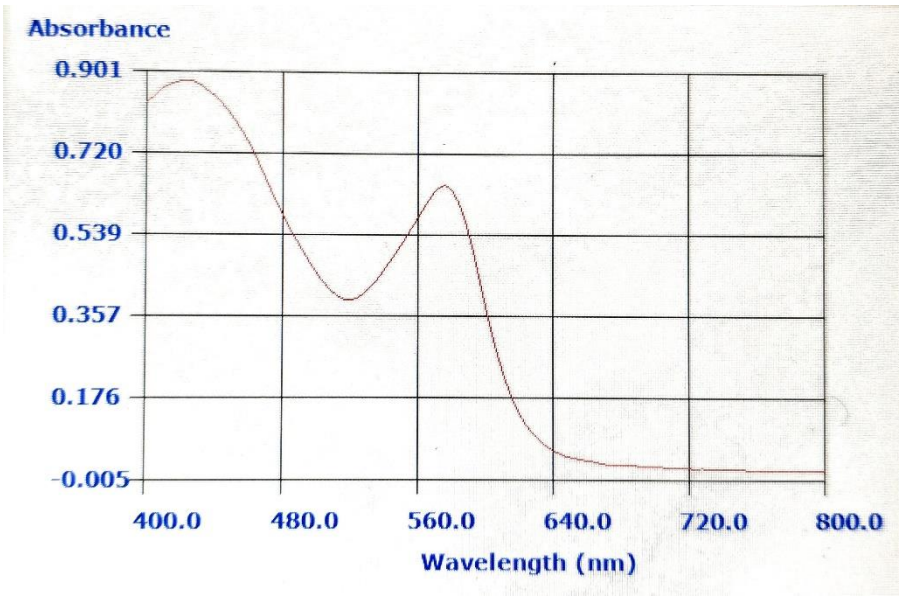

Figure 3: Absorption spectrum of sample.

CONCLUSIONS

The work aimed to develop and validate a reliable analytical method for mesalamine quantification.

A Colorimetric method for mesalamine quantification was successfully developed.

The solvent and reagent used in colorimetric method for estimation of mesalamine was 0.5 N HCL and Basic Fuchsin.

The absorption maxima of mesalamine was found to be 576 nm.

Mesalamine shows linearity from 5-25 µg/ml

REFERENCES

1. Boyle M. et al., Adherence to Rectal Mesalamine in Patients with Ulcerative Colitis. Inflammatory Bowel Diseases, 2015; 21(12): 2873-2878. DOI: 10.1097/MIB.0000000000000551
2. Mahantesh Kunchanur et al., Design, Characterization, and Stability Studies of Mesalamine-Loaded Solid Lipid Nanoparticles. Research Journal of Pharmacy and Technology, 2023; 16(10): 4767-4773. DOI: 1052711/0974-360X.2023.00773.

3. Shah RS, Shah SA, Devani MB, Soni KP. Spectrophotometric determination of mesalamine and its dosage forms. Indian Drugs, 1994; 31: 34. 258.
4. Al-Sabha TN, Habeeb NN. Spectrophotometric determination of mesalamine using sodium nitroprusside as chromogenic reagent. J Pharm Res., 2015; 14(1): 15.
5. Mahmoud NT, Shaban A. Validation of mesalamine by spectrophotometric method in pharmaceutical formulations. Int J Pharm Pharm Sci., 2018; 10(2): 254-9.
6. VR Darak, AB Karadi, MD Arshad, Colourimetric estimation of mesalamine in bulk and pharmaceutical dosage form. Journal of current pharma-2011- academia.edu.