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METHOD DEVELOPMENT AND VALIDATION OF MESALAMINE BY COLORIMETRIC METHOD

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ABSTRACT

A novel, simple, and precise colorimetric method was developed and validated for the quantitative estimation of mesalamine. The method relies on the reaction of mesalamine with an alkaline potassium hydroxide (KOH) solution leading to the formation of a green-colored chromophore. The developed method demonstrated high accuracy, with an average recovery of 99.16% and a relative standard deviation (RSD) within acceptable limits. Sensitivity analysis determined the limit of detection (LOD) and limit of quantification (LOQ) to be 0.07619 and 0.23089, respectively. The proposed method offers a reliable, cost-effective, and efficient approach for the routine analysis of mesalamine in pharmaceutical formulations.

KEYWORDS: Mesalamine, Sodium nitroprusside, Potasium hydroxide.

INTRODUCTION

Analytical chemistry is a field within chemistry that focuses on the separation, identification, and quantification of components in a material sample. This discipline primarily involves two types of analysis: qualitative, which detects the presence of compounds, and quantitative, which determines the amounts of these compounds. Qualitative methods provide information about the identity of atomic or molecular species or functional groups within a sample. In contrast, quantitative methods offer numerical data regarding the relative amounts of one or more components. The techniques used in analytical chemistry are divided into two main categories: classical methods and instrumental methods. Modern analytical chemistry integrates both classical and instrumental methods, combining precision and advanced

technologies to address complex analytical challenges in various scientific and industrial fields.

Spectroscopy

Spectroscopy is a scientific discipline that examines how electromagnetic radiation interacts with matter. A key principle of this interaction is that matter absorbs or emits energy in discrete quantities known as quanta. As one of the most effective methods for investigating atomic and molecular structures, spectroscopy is employed to analyze a diverse array of samples. The field of optical spectroscopy encompasses the portion of the electromagnetic spectrum ranging from 100 Å to 400 nm.

UV-Visible spectrophotometry is a specific type of absorption spectroscopy that focuses on the ultraviolet region of light (200-400 nm). In this technique, molecules absorb ultraviolet radiation, causing electrons to transition from their ground state to higher energy levels. The energy of the absorbed ultraviolet radiation corresponds exactly to the energy difference between these two states.

Colorimetry

Colorimetry is a technique that accurately measures a substance's concentration in a solution by assessing its color intensity and light absorption between 400-800nm. This technique is based on Beer-Lambert's law, which posits that a solution's light absorption is directly related to the substance's concentration.

Beer's law

Intensity of beam of monochromatic radiation decrease exponentially with increase in the concentration of absorbing species.

$$\mathbf{It = I0\ e^{-KC}} \quad (1)$$

Lambert's law

Rate of decrease of intensity of monochromatic light with thickness of medium is directly proportional to intensity of incident light.

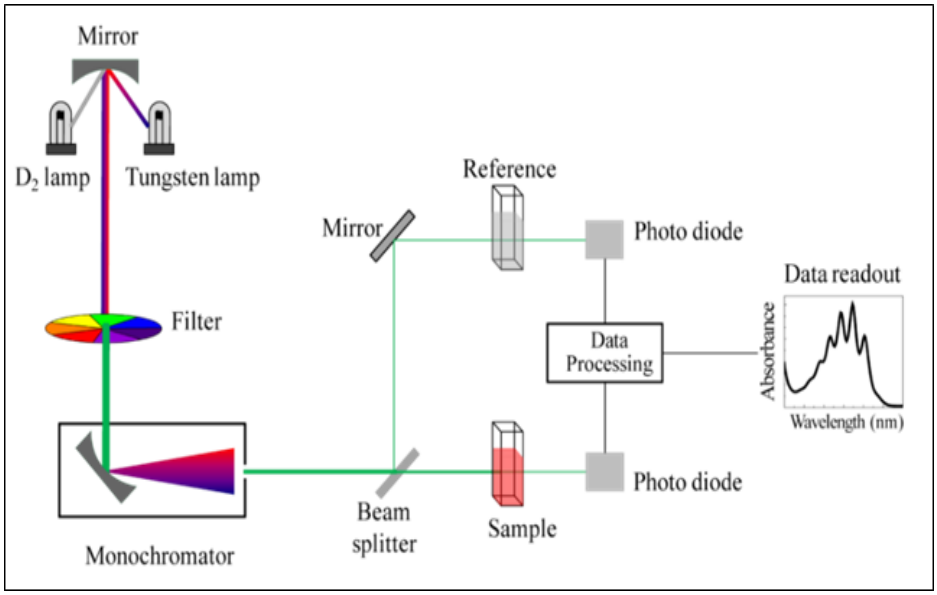
$$\mathbf{It = I0\ e^{-Kb}} \longrightarrow (2)$$

Beer's- Lambert's Law

Absorption of solution is directly proportional to thickness/path length as well as concentration of the solution. Combine eq:(1)&(2)

A= log (I0/It) = ε C l

Instrumentation



Method validation

As per ICH Guideline "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desirable result or product meeting its predetermined specification and quality characteristics".

The key components in this process include

1. Linearity
2. Accuracy
3. Precision
4. Limit of Detection (LOD)
5. Limit of Quantitation(LOQ)

Experimental apparatus

1. Electronic Balance: Samson
2. UV Spectrophotometer: 2202TS SYSTRONICS
3. Heating mandle: Rotek
4. Sonicator:

Reagents and Materials

1. MESALAMINE YARROW CHEM®, Mumbai.

2. POTTASIUM HYDROXIDE NICE CHEMICALS, Manimala.
3. SODIUM NITROPRUSSIDE FORTUNE Chemicals Melmuri.
4. Distilled water

Experimental procedure

Spectral Characterization of MZL

Determination of wave length maxima of MZL solution for set up the UV-Visible Spectrophotometer, turn on the spectrometer and allow it to warm up (typically 15-30 minutes). Set the baseline by using pure solvent in reference cuvette. For scan the wavelength range place the various concentration of MZL in sample cuvette. Perform a wavelength scan across the relevant range, usually 400-800nm. Observe the spectrum obtained, the peak with highest absorbance corresponds to λ max of MZL.

Preparation of 1%Sodium nitroprusside as Chromogenic agent

Weight out 1g of Sodium nitroprusside using analytical balance, transferred to 100mL volumetric flask and add distilled water to make the total volume up to 100mL. Mix thoroughly and the solution is filtered by Whatsmann filter No.41. Stored in air tight container protecting from light.

Preparation of 0.1N KOH as Alkaline Medium: Accurately weigh 1.4g of KOH pellets, transferred to 100mL volumetric flask and made up the final volume to 100mL by using distilled water then the solution is filtered by Whatman filter No.41.

Preparation of Reference standard of MZL: Accurately weigh 0.1 g (100 mg) of MZL and dissolve it in a small volume of hot distilled water. Transfer the solution to a 100 mL volumetric flask and dilute to the mark with hot distilled water, placed on sonicator for 10 minutes ensuring complete dissolution. This forms a 1000µg/Ml (1mg/mL) stock solution. And then the solution is filtered by filter No.41.

Preparation of working standard

Using a pipette, transfer 1 mL of the stock solution (1000µg /mL) into a 100 mL volumetric flask. Dilute to the mark with distilled water to obtain a 100µg /mL (0.1mg/mL) working standard solution.

Preparation of calibration standards

Pipette 0.25, 0.5, 0.75, 1.0, and1.25 ml aliquots from the 100µg/mL working standard

solution into separate 10 mL volumetric flasks. These volumes correspond to 0.25, 0.5, 0.75, 1, and 1.25µg of MZL, respectively. To each flask, add 1 mL of SNP solution as a chromogenic agent. Dilute each solution with KOH. Also prepare an blank using chromogenic agent and KOH. The KOH provides an alkaline medium necessary for the colorimetric reaction between MZL and SNP.

METHODOLOGY

The calibration standard solutions of MZL were scanned in UV-Visible spectrophotometer from the range of 400- 800nm.Where MZL shows 704nm as the wavelength having maximum absorbance. And this wavelengths are selected for the quantitative estimation of MZL. From the absorbance value the calibration curve were plotted and concentration of MZL were be determined.

Method validation

Linearity

Linearity between response of analytical instruments (techniques) and concentrations of drug substances or content. Various dilutions of calibration standard 0.25, 0.5, 0.75, 1, and 1.25µg/mL of MZL were scanned in UV-Visible Spectrophotometer in wavelength ranges from 704 nm and plot an graph by absorbance and concentration. Linearity can be tested by verifying additivity homogeneity, graphing for a straight line or using linear regression for a high R^2.

Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentration levels relative to the target concentration of 1 µg/mL, in accordance with ICH guideline, by replicate analysis(n=3).Low level (80%),Medium level (100%), High level (120%).

Precision

Degree of closness of data values to each other for number of measurements under the same analytical conditions, results are presented as %RSD. The study of precision encompassed both inter-day and intra-day analyses.

Repeatability

Repeatability is the ability to achieve consistent results under the same conditions.

Reproducibility (Intraday precision)

Three duplicate sample solutions were derived from the stock solution. To evaluate intra-day precision, the drug's absorbance was measured three times within a single day, with 2-hour intervals between measurements.

Reproducibility (Inter-day Precision)

For the inter-day precision assessment, the drug's absorbance was measured across three different days.

Robustness

The method's reliability was evaluated by conducting analyses under varying temperature conditions. The absorbance at 0.75mg/ml was recorded, and the outcome was expressed as %RSD.

Ruggedness

The method of determining ruggedness involved conducting the analysis with different analysts. The respective absorbance of 0.75 µg/ml.

Limit of detection and Limit of quantification (LOD & LOQ): The LOD and LOQ were calculated by the equation method. LOD = 3.3 × σ/S LOQ = 10 × σ/S Where, σ = the standard deviation of the response S = slope of the calibration curve.

RESULTS AND DISCUSSION

In this method different dilute solutions of MZL were scanned from 400-800nm.The wavelengths 704 nm is the absorbance maxima of MZL.

Method validation

Linearity: As per ICH Guidelines for Validation Characteristic Assessment Criteria, linearity should be $r^2 \geq 0.999$, similar response ratio. The correlation coefficient is 0.9992 with slope 0.0099 and Y-intercept 0.0102. Therefore the colorimetric method for MZL is linear. (Fig1).

Accuracy

As per ICH Guidelines, it should be between 98% and 102% of the recovery level.

Precision

Degree of closeness of data values to each other for number of measurements under the

same analytical conditions, results are presented as %RSD.

Repeatability

As per ICH Guidelines % RSD should not be more than 2%.

Reproducibility (Intra Precision)

As per ICH Guidelines % RSD should not be more than 2%.

Reproducibility (Inter-day Precision)

As per ICH Guidelines % RSD should not be more than 2%.

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 .The LOD and LOQ of MZL was found to be0.07619 &0.23089 respectively.

Fig. 1: Color variation of MZL at different concentration.

Table 1: Calibration Data of MZL at 704 nm.

Sr. No	Concentration (µg/ml)	Absorbance
1	0.25	0.253
2	0.5	0.499
3	0.75	0.763
4	1	1.011
5	1.25	1.229

Table 2: Result of accuracy.

Drug	Accuracy % level		Amount		% Recovery	MEAN SD	%RSD
		Actual	Added	Found			
	80%	100	80	179.3	99.611		
MZL						99.761 ±.1695	0.169982
	100%	100	100	199.89	99.945		
	120%	100	120	219.4	99.727		

Precision

Table 3: Result of repeatability.

Concentration MZL (0.75 µg/ml): n=6	Absorbance
1	0.763
2	0.761
3	0.762
4	0.761
5	0.763
6	0.764
MEAN	0.762333
SD	0.001211
RSD	0.158862

Table 4: Result of Reproducibility (Intra Precision).

Concentration (µg/ml)	Absorbance1	Absorbance2	Absorbance3	Average %RSD
0.75	0.761	0.763	0.763	
0.75	0.763	0.761	0.761	
0.75	0.765	0.765	0.762	
0.75	0.766	0.765	0.766	0.25491533
0.75	0.762	0.766	0.763	
0.75 Mean	0.767 0.764	0.763 0.76367	0.764 0.763167	
SD	0.002366	0.001751	0.001722	
RSD%	0.309742	0.229313	0.225691	

Table 5: Result of Reproducibility (Inter-day Precision).

Concentration(µg/ml)		%RSD		MEAN	STD	%RSD
	Day 1	Day 2	Day 3			
0.75	0.763	0.764	0.762	0.763	0.001	0.131062

Table 6: Result of robustness.

Sr. No	Concentration(µg/ml)	Absorbance	
		Room temperature	18°C
1	0.75	0.764	0.762
2	0.75	0.763	0.761
3	0.75	0.765	0.764
4	0.75	0.767	0.766
5	0.75	0.762	0.763
6	0.75	0.761	0.765
7	MEAN	0.7634	0.7635
8	SD	0.002074	0.001871
9	%RSD	0.271633	0.245033

Table 6: Result of ruggedness.

Sr. No	Concentration(µg/ml)		Absorbance	
		Analyst1	Analyst2	Analyst3
1	0.75	0.763	0.763	0.763
2	0.75	0.765	0.761	0.761
3	0.75	0.766	0.764	0.765
4	0.75	0.762	0.766	0.766
5	0.75	0.762	0.763	0.763
6	0.75	0.767	0.765	0.765
7	Mean	0.7646	0.763667	0.763833
8	STD	0.002074	0.001751	0.001835
9	RSD	0.271206	0.229313	0.240216

Table 7: Result of LOD and LOQ.

DRUG	LOD (µg/ml)	LOQ (µg/ml)
MZL	0.07619	0.23089

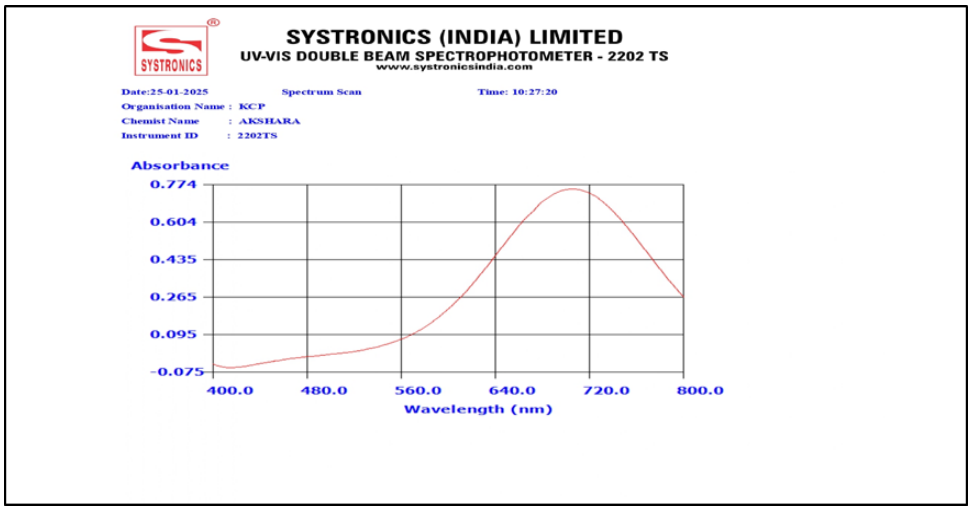


Fig. 2: Absorption spectra of MZL at 704nm.

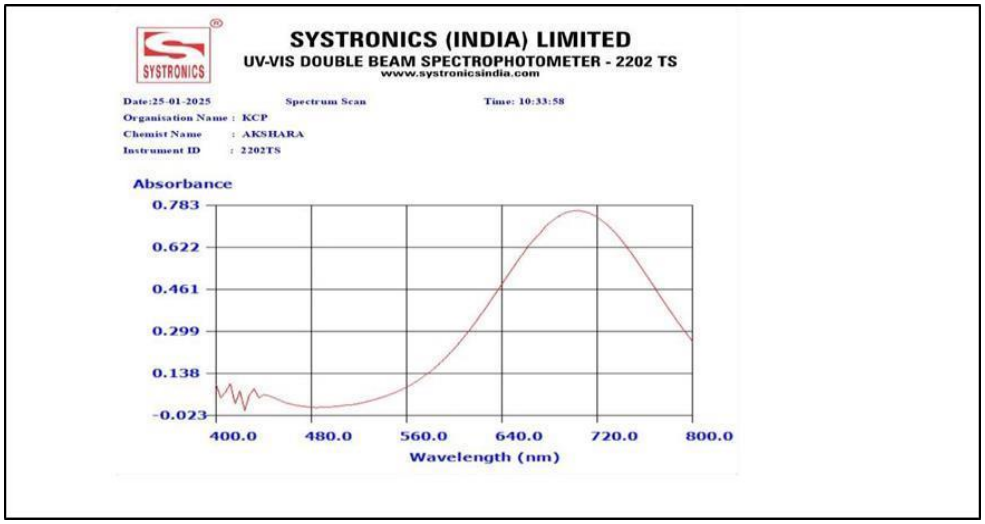


Fig. 3: Absorption spectra of MZL in Unknown concentration at 704nm.

CONCLUSIONS

- The study successfully established a validated, accurate, and precise colorimetric method for the determination of mesalamine. The method's adherence to ICH guidelines underscores its reliability for pharmaceutical analysis.
- The maximum absorbance of mesalamine by colorimetry was found to be 704nm.
- The given method will shows the linearity from 0.25-1.25 µg/ml.
- Linearity indicated by correlation coefficient was found to be 0.9992.
- Precision indicated by %RSD found to be <1,Accuracy indicated by % RSD found to be 0.169982,Limit of Detection 0.07619,Limit of Quantification 0.23089

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