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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF MESALAMINE IN BULK AND TABLET FORMULATION

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ABSTRACT

A simple and cost effective spectrophotometric method is described for the determination of mesalamine in pure form and in pharmaceutical formulations. When the drug reacts with 0.5N HCl shows absorption maximum at 303 nm and obeys beer's law in the concentration range 10-50µg mL-1. The absorbance was found to increase linearly with increasing concentration of MSZ, which is corroborated by the calculated correlation coefficient value of 0.999 (n=6). The apparent molar absorptivity and sandell sensitivity were 1.63 X $10^{10}$and 0.0402 µg cm^{-2} respectively. The slope and intercept of the equation of the regression line are 0.025 and 0.002 respectively. The limit of detection and limit of quantification was found to be 0.04914 µg mL^{-1} & 0.14893 µg mL^{-1}. The validity of the described procedure was assessed. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of MSZ in pharmaceutical formulations without any interference from common excipients. The relative standard deviations were ≤0.6536%, with recoveries of 98.65% - 101.05%.

Keywords: Mesalamine (MSZ), UV spectrophotometry, Validation, Pharmaceuticals.

INTRODUCTION

Mesalamine (5-aminosalicylic acid) is an anti-inflammatory agent, structurally related to the salicylates, which is active in inflammatory bowel disease and active ulcerative proctitis. It is a tan to pink crystalline powder, relatively insoluble in chloroform, ether, n-hexane and ethyl acetate and freely soluble in dil.HCl and alkali hydroxides[1, 2]. Mesalamine is available in tablet dosage forms (400 mg) and is an official drug of USP. The chemical structure of Mesalamine is shown in Figure 1.

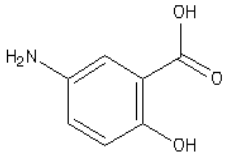

Fig 1: Chemical Structure of Mesalamine

Literature survey reveals that, some study about HPLC determination of Mesalamine and its degradation metabolite in plasma[3,4,5,67], determination of 5-aminosalicylic acid in pharmaceutical formulation by differential pulse voltammetry[8]. To the best of our knowledge, there is no UV method for the analysis of MSZ in pharmaceutical formulations has been reported in literature. The aim of this study is to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of MSZ in bulk drug and commercial pharmaceutical formulations as tablet and its validation.

EXPERIMENTAL

Instruments

A Jasco UV-Visible Spectrophotometer (Jasco V-630) with a matched pair of 10 mm quartz cells were used for experimental purpose.

Materials

Mesalamine was procured as gift sample from Wallace pharma, Goa. Freshly prepared 0.5N HCl and all other chemicals and reagents were of analytical grade. The commercially available two marketed tablet brands containing Mesalamine, 400 mg in each tablet have been used for estimation.

Preparation of standard stock solution

The standard stock solution was prepared by dissolving Mesalamine in 0.5N HCl to make final concentration of 100 µg/ml. Different aliquots were taken from stock solution and diluted with 0.5N HCl separately to prepare series of concentrations from 10-50 µg/ml. The λ_{max} was found by UV spectrum of Mesalamine in 0.5NHCl, in the range of 200-400 nm and it was found to be 303 nm. Absorbance was measured at 303 nm against 0.5N HCl as blank. The calibration curve was prepared by plotting absorbance versus concentration of Mesalamine.

Application of the proposed procedure for the determination in tablets

The proposed procedure was adopted for determination of Mesalamine in tablets in following manner. The marketed tablet formulations of Mesalamine were used for the purpose of analysis. Twenty tablets were weighed and average weight was calculated, crushed to fine powder. The powder equivalent to 5 mg of Mesalamine was transferred in 50 ml volumetric flask and dissolved in 0.5N HCl by sonicating for 10 minutes. The volume was made up to mark to get final concentration of 100µg/ml. The solution was then filtered through Whatmann filter paper (no.41). This solution was used as stock solution.

The working solution of drug (40µg/ml) was prepared from standard stock solution in 0.5N HCl. The absorbance of working solution was measured and amount of Mesalamine was calculated from the calibration curve. The readings were taken in six times and same procedure was repeated with another marketed tablet formulation.

All the marketed tablet formulations contain excipients which are added along with active pharmaceutical ingredient. These substances may cause some interference during estimation of active pharmaceutical ingredient. Recovery study was carried out on marketed tablet formulations and the results obtained showed that, there was no interference from excipients. From the results of recovery study it can be claimed that, the method can be used for estimation of Mesalamine in tablet dosage forms. The results obtained are shown in Table 2.

RESULTS AND DISCUSSION

Statistical evaluation of analysis and recovery study was carried out. The data obtained from the proposed method showed accuracy of method. The values of standard deviation and coefficient of variation were satisfactorily low. The percentage recovery of 99% to 101% was indicative of accuracy of method.

Validation of method

The method was validated with respect to linearity and range, accuracy and precision, limit of detection (LOD), limit of quantitation (LOQ), selectivity and robustness.

Linearity and range

The prepared aliquots (10-50 µg/ml) were scanned for absorbance at λ_{max} value 303 nm. The absorbance range was found to be 0.2470-1.2506. These solutions obeyed Beer-Lambert's law in above concentration range with regression of 0.999.

Accuracy and precision

Accuracy and precision were investigated by analyzing three concentrations of Mesalamine (i.e. 80, 100 and 120% of 400 mg Mesalamine tablet) in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision). The data evaluated was summarized in Table 4.

Intra-day and Inter-day relative standard deviation (RSD) values and also the low RSD values obtained from the analysis of the pharmaceutical formulations (Table 4) indicated good intermediate precision of method.

To validate prediction ability of suggested method, different concentrations of Mesalamine samples were prepared and analyzed. The results were satisfactory. Using standard addition technique, the method was further validated. The standard addition technique was carried out by adding excipients (lactose, starch, magnesium stearate, talc etc.), with the addition of Mesalamine at 80% (20 µg/ml), 120% (25 µg/ml) and 180% (30 µg/ml), respectively in sample solution. The percent recoveries of the three concentrations were found to be close to 100%, indicative of high accuracy. The high percent recoveries indicate no interference from ingredients and excipients that might be present in different formulations. The data evaluated was summarized in Table 2.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD (k=3.3) and LOQ (k=10) of the method was established according to ICH definitions (*$C1=kS_0/S$, where C1 is LOD or LOQ, S_0 is the mean standard deviation of blank determination, S is the slope of standard curve and k is the constant related to confidence interval*). LOD and LOQ of method reported in Table 1.

Robustness

Repeatability is based on the results of the method operating over short time interval under same conditions. The low RSD values of intra-day precision (Table 4), recovery (Table 2), and pharmaceutical preparations (Table 3) showed high repeatability. Making deliberate small changes in concentration of solvents used tested the robustness of method (Table 1).

The proposed UV method is simple, accurate, precise, specific and highly sensitive; developed and validated for the determination of Mesalamine in bulk and tablet dosage form. The method is economical rapid and do not require any sophisticated apparatus in contrast to chromatographic methods. Hence, the proposed method can be successfully used for routine quality control analysis of drug in marketed preparations.

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Table 1: Data for calibration curve of Mesalamine

Sr. No.	Parameters	In 0.5NHCl
1.	Absorbance maximum (λ_{max}) in nm	303
2.	Beer's law limit (µg/ml)	10-50
3.	Molar Absorptivity ($L/mol^{-1}/cm^{-1}$)	1.63×10^{10}
4.	Sandell's sensitivity, $\mu g/cm^2/0.001A.U$	0.0402
5.	Slope	0.025
6.	Intercept	0.002
7.	Correlation coefficient	0.999
8.	LOD (µg/ml)	0.04914
9.	LOQ (µg/ml)	0.14893

LOD: Limit of Detection; LOQ: Limit of Quantitation

Table 2: Results of recovery and precision

Formulation	Amount taken	Amount added		Recovery* (%±S.D)	RSD* (%)
		%	µg/ml		
Tablet-1	25	80	20	98.94±0.2858	0.2888
		100	25	99.81±0.6524	0.6536
		120	30	99.80±0.2217	0.2221
Tablet-2	25	80	20	99.79±0.59778	0.59903
		100	25	99.53±0.49038	0.49269
		120	30	100.69±0.36465	0.3621

* Percentage RSD of six samples

Table 3: Results of the marketed Mesalamine

Formulation	Label claim (mg)	Amount of Drug Found In Tablet (mg)	Drug content (%±S.D)
Tablet 1	400	397.86	99.46±0.81608
Tablet 2	400	397.77	99.44±0.8106

** Average of six determinations, S.D.; Standard Deviation*

Table 4: Results for determination of intraday & inter day precision

Formulation	Intraday precession* (RSD %)	Interday precession* (RSD %)		
		Day 1	Day 2	Day 3
Tablet 1	0.25777	0.47697	0.31408	0.32766
Tablet 2	0.48187	0.3363	0.282	0.3023

* Percentage RSD of six samples

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