ISSN: 0975-5772

Lalit Jejurkar et al / Journal of Pharmaceutical Science and Technology Vol. 4 (1), 2012, 792-797

HPLC METHOD OF DETECTION FOR 5ASA IN PURE AND IN TABLETS

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ABSTRACT:

A simple, Rapid and Reproducible HPLC method has been developed for the estimation of 5ASA in bulk drug and its Pharmaceutical dosage forms using RP C_{18} column. The mobile phase consists of Phosphate Buffer pH 6.8 and was pumped at a flow rate of 1ml/min at 26±1°c. The detection was carried out at 331nm and the calibration curve was linear in the range of 5-100µg/ml, Retention time was found to be 2.89 min for run time of 5min. The method was statistically validated for its linearity, Precision and accuracy. Intra and Inter-day variation study was carried out and found to be less than 3% showing reasonable precision of the assay method. Parameters of validation obtained prove the accuracy of the method and its applicability for the determination of 5- ASA in tablet dosage formulations.

INTRODUCTION

5- ASA (5-aminosalicylic acid, Fig 1) is used for its local effects in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease [1-2]. Despite the fact that it has been used for over 50 years, the mechanism of action of this drug remains uncertain. 5-ASA has been shown to be a potent scavenger of reactive oxygen species that play a significant role in the pathogenesis of inflammatory bowel disease, inhibition of natural killer cell activity, inhibition of antibody synthesis, inhibition of cyclo-oxygenase and lipoxygenase pathways and impairment of neutrophil function [3-4]. Literature reveals that that very few methods were developed for the estimation of 5- ASA in pure and pharmaceutical dosage form. A HPLC method adopted by the British Pharmacopoeia (BP) is based on the mobile phase containing glacial acetic acid, methanol and methyl isobutyl ketone (10: 40: 50 v/v)[5]. A HPLC method available in United States Pharmacopoeia (USP) is based on the mobile phase containing tetrabutylammonium hydrogen sulphate as an ion-pairing agent, which shortens column life. Moreover, mobile phase preparation requires tedious procedures [6]. The spectrophotometric method was developed for the determination of MES in pure and its pharmaceutical formulations [7-8]. Very few HPLC methods were developed for simultaneous determination of 5-aminosalicylic acid and its metabolite in human plasma [9] and nitrosation method for the quantitization of MES in coated tablets [10]. In this present study, we developed simple and sensitive spectrophotometric method for 5- ASA.

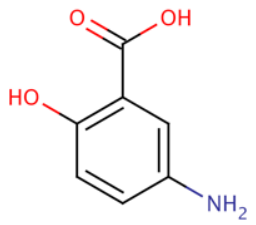

Figure 1: 5- ASA

EXPERIMENTAL

Instrumentation

A Shimadzu HPLC instrument, equipped with a Luna C_{18} reverse phase column (250mm x 4.6mm.,5µ), an LC-20AT pump and variable wavelength programmable UV/visible detector SPD-20A, was employed in this study. A 20 µl Hamilton injection syringe was employed for sample injection. Degassing of mobile phase was done by using a ultrasonic bath sonicator, electronic balance was used for weighing the materials.

Chemicals:

Disodium hydrogen phosphate, Potassium dihydrogen phosphate and Water were of HPLC grade and purchased from S.D fine Chemicals, Mumbai. 5- ASA was obtained as gift sample from Wallace Pharmaceuticals, Goa. Mesacol; marketed preparation of 5- ASA was obtained from local market.

Chromatographic Condition:

The mobile phase used was Phosphate buffer pH 6.8. The mobile phase was filtered before use through 0.45µ membrane filter. The flow rate of the mobile phase was maintained at 1 ml/min. The column temperature was maintained at 26±1^{o}c and detection was carried out at 331 nm by UV detector. The run time was set at 5 min and the volume of the injection loop was 50 µl. Prior to injection of the drug solution the column was equilibriated for at least 30 min with mobile phase flowing through the system.

Procedure:

About 25 mg of 5- ASA was accurately weighed and dissolved in HPLC grade water in a 25 ml volumetric flask so as to give 1mg/ml. subsequent dilutions of solution was made with mobile phase to get concentration of 5-100µg/ml of 5- ASA. The standard solutions prepared above are injected 5 times in to the column at a flow rate of 1ml/min. The peak areas of drug concentration were calculated. The regression equation was used to estimate the amount of 5- ASA in pharmaceutical dosage forms (Tablets). 5- ASA solution containing 10, 20 and 30µg/ml were subjected to the proposed HPLC analysis for finding out intra and inter-day variations. The recovery studies were carried out by adding known amount of 5- ASA to the pre analyzed samples and subjecting them to the proposed HPLC method.

Assay of 5- ASA in Tablets:

Five tablets each containing 400mg were taken weighed and powdered. A tablet powdered equivalent to 100 mg was accurately weighed and transferred to 100ml volumetric flask containing 50ml HPLC grade water. The contain of flask was sonicated for 20min to dissolve 5-ASA and the volume was made up to the 100ml with water and resulting solution was filtered through 0.45µ membrane filter. 4ml of this solution was taken diluted to 100ml with mobile phase. This (40µl) was injected 5 times into the column. The mean peak areas of 5 such determinations were calculated and the drug content in the tablets was quantified using regression equation.

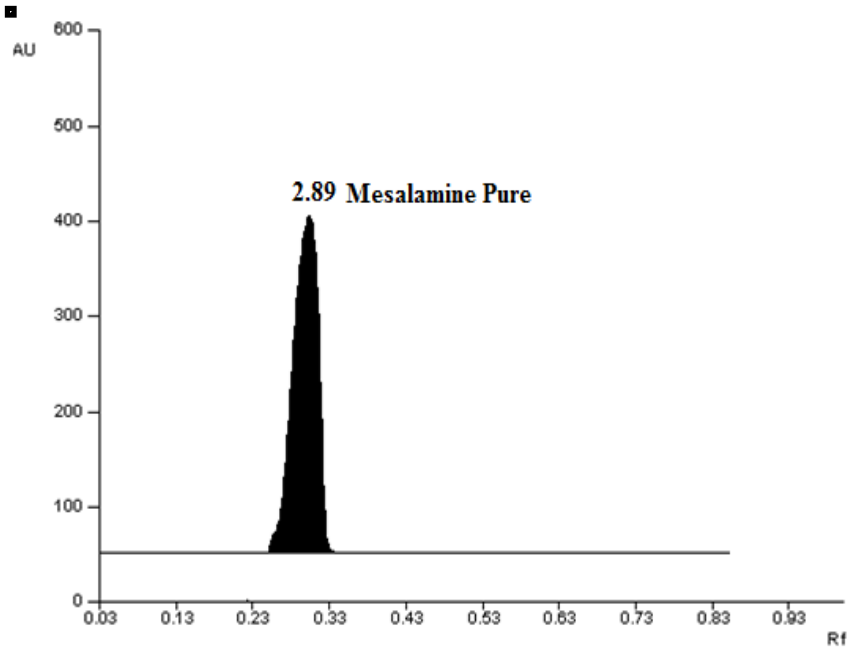

Figure 2: The Typical Chromatogram of 5- ASA Standard 40 mcg/ml.

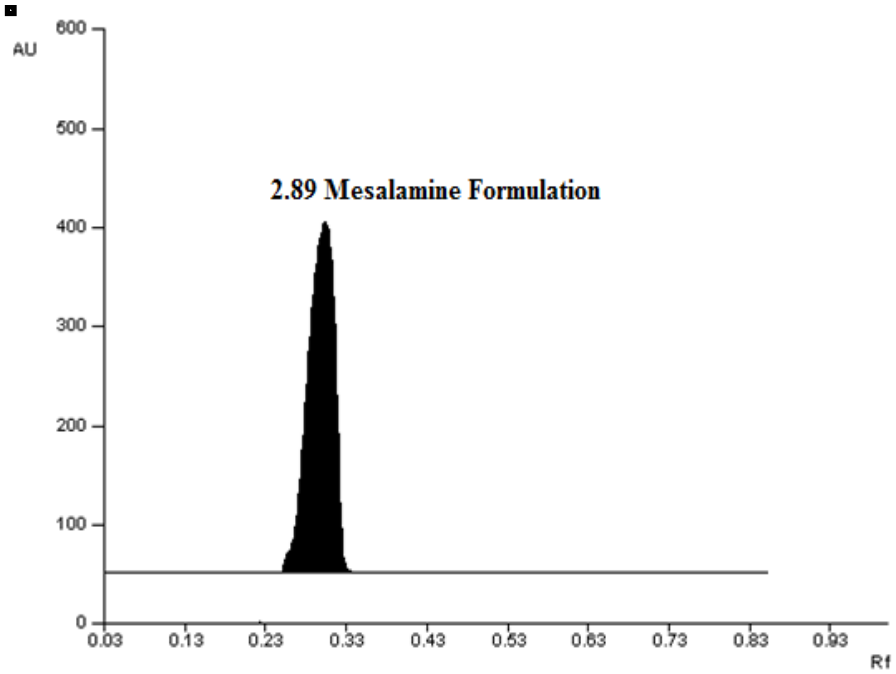

Figure 3: The Chromatogram of 5- ASA Formulation 40 mcg/ml.

RESULTS AND DISCUSSION:

The present study was carried out to develop simple sensitive, precise and accurate reverse phase HPLC method for the analysis of 5- ASA in pharmaceutical dosage forms. The column pressure varied from 60 Atm. The retention time for 5- ASA was 2,89 min for a runtime of 5min. Each sample was injected 5 times in to the column and same retention time was obtained in all cases. The peak areas of different concentration set up as above were calculated and presented in Table 1. A good linear relationship (r=0.997) was obtained between concentration of 5- ASA and the respective peak areas. Calibration curve was found to be in the concentration range of 5-100µg/ml (Table-2). when solution containing 10, 20 and 30µg/ml were analyzed by the proposed HPLC method for finding out intra and inter-day variations. A low % RSD variation was observed. (Table-3). This shows the present HPLC method was highly precise. The amount of 5- ASA from preanalysed sample containes known amount of the drug are shown in Table 4 about 99.93% 5- ASA could be recovered from the pre analyzed sample indicating high accuracy of the proposed method. The absence of additional peak indicates no interference of the excipients used in the tablet formulations. The tablets were found to contain 99.72% (Table-5). The low %RSD indicates reproducibility of the assay in the tablet dosage forms. The proposed method is highly accurate, precise and simple.

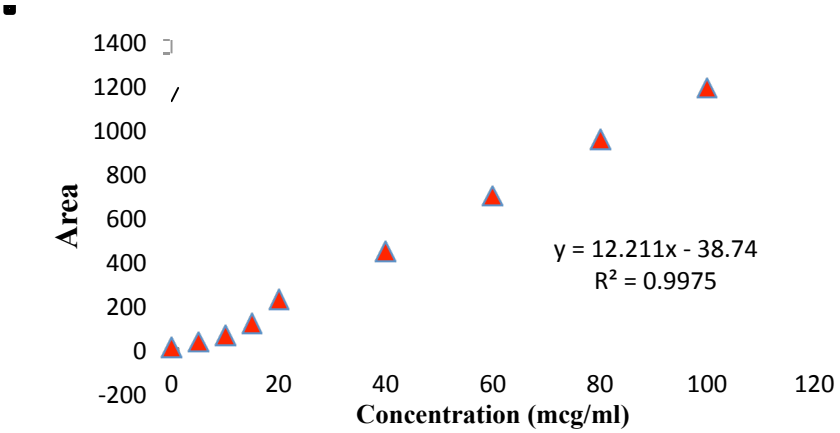

Figure 4: Linearity Plot

Sr. No	Concentrations (mcg/ml)	Mean Peak Area (n=3)
1	0	0
2	5	27.42
3	10	56.14
4	15	111.24
5	20	220.83
6	40	441.81
7	60	691.85
8	80	650.41
9	100	1181.42

Table 1: Linearity Table

Linearity Range	20-100 mcg/ml
Regression equation (Y*)	
Slope (a)	12.21
Intercept (b)	-38.74
Correlation Coefficient (r)	0.997
Standard Deviation	0.007
% RSD	0.00025
Confidence Limits	
95% Confidence Limit	0.0043
99% Confidence Limit	0.0057

Table 2: Method Characteristics

*** Y= a + bC where C is the concentration of 5- ASA and Y is the peak area**

Concentration (mcg/ml)	Intra-day Precision			Inter-day Precision		
	Mean amount found(n=3)	Percent amount found	Percent RSD	Mean amount found(n=3)	Percent amount found	Percent RSD
10	9.12	91.2	0.19	9.10	91.00	0.21
20	19.24	96.2	0.42	19.20	96.00	0.17
30	29.15	97.14	0.23	29.11	97.03	0.26

Table 3: Intra- Inter Day Precision Study.

Amount Taken (mcg/ml)	Amount Found (mcg/ml)	Percent Recovery	Mean % Recovery	%RSD
10+10=20	19.94	99.7	99.7	0.0015
10+10=20	19.91	99.55		
10+10=20	19.97	99.85		
10+20=30	29.95	99.83	99.85	0.00068
10+20=30	29.94	99.80		
10+20=30	29.98	99.93		
10+30=40	39.93	99.82	99.88	0.0005
10+30=40	39.96	99.91		
10+30=40	39.97	99.92		

Table 4: Accuracy Study.

Formulation	Labeled amount (mg)	UV Method		
Mesacol (Tablets)	400 mg	Mean ± s.d (amount mg recovered by Proposed Method)	%Drug recovered	% RSD
		398.88±1.134	99.72±0.274	0.274

Table 5: Recovery Study

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 5- ASA in pure samples and pharmaceutical formulations.

ACKNOWLEDGEMENTS

The authors thank Wallace Pharmaceuticals, Goa for providing the gift sample of 5- ASA

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