International Journal of Scientific Engineering and Research (IJSER)
www.ijser.in
ISSN (Online): 2347-3878
Volume 2 Issue 6, June 2014

Development and Validation of HPLC Assay Method for Determination of Mesalamine in Bulk Drug and Tablet Formulation

K. Sivarami Reddy, B. Ramachandra, N. V. S. Naidu*

Department of Chemistry, S. V. University, Tirupati-517502, A.P., India

Abstract: ***The objective of the current study was to develop simple, precise and accurate RP-HPLC assay method was developed and validated for rapid assay of Mesalamine in bulk and tablet dosage form. Isocratic elution at a flow rate of1.0 mL/min was employed on Waters (alliance) HPLC C18, 100X4.6, 5µ.column using mobile phase of mixed buffer and Acetonitrile (65: 35 v/v) at UV detector. The UV detection wavelength was 258 nm and 20 µL of sample was injected, retention time for Mesalamine was 3.214 min. The method was linear in the drug concentration range of 10-60 µg/ml with a correlation coefficient 0.998. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability and also found to be robust as indicated by the % RSD values which are less than 2%. The method was validated as per the ICH guidelines, successfully applied for routine analysis of Mesalamine in bulk samples and its formulations.***

Keywords: Mesalamine, assay validation, RP-HPLC Method.

1. Introduction

5-Aminosalicylic acid (Mesalamine, 5-ASA) is a drug widely used in the treatment of inflammatory bowel diseases (IBDs) such as ulcerative colitis and Crohn's diseases [1–3]. 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; Mesalamine is available in tablet dosage forms and is an official drug of USP. Mesalamine is also thought to be an antioxidant that traps free radicals, which are potentially damaging byproducts of metabolism [4]. Mesalamine is considered the active moiety of Sulfasalazine, which is metabolized to Sulfapyridine and Mesalamine [5]. Its mechanism of action is not yet fully understood: it seems to act locally, at the colonic mucosa level, since systemic concentrations following oral dosing is very low [6]. 5-ASA inhibits local prostaglandin and leukotriene synthesis in the gastrointestinal mucosa [7]. Other studies have proved that 5-ASA is a potent-free radical scavenger, thus suppressing toxicity of reactive oxygen species [8–10]. All these properties seem to play an important role in reducing the acute inflammatory response. When orally administered, 5-ASA is rapidly absorbed, although with low efficiency, from the upper gastrointestinal tract [11]. The knowledge of pharmacokinetic and metabolism of 5-ASA from Mesalamine-containing drugs is mandatory when new drug formulations are developed for the treatment of IBDs, and therefore validated analytical methods are needed for bioequivalence studies. Due to the presence of the primary aromatic amino group (–NH3+ p*K*a = 6), carboxylic group (–COOH p*K*a = 3) and phenolic group (–OH p*K*a = 13.9) in the molecule [15], 5-ASA exhibits amphotericproperties, which, together with its high polarity, complicate its extraction, separation and detection [14]. A number of analytical methods have been developed for the analysis of 5-ASA and its metabolite in many biological matrices, e.g. plasma [14, 16–21], serum [22], feces [22], urine [16, 17, 20–22], stones [23] and rectal biopsies [21, 22]. These methods include HPLC combined with UV [14, 23–25], fluorescence (FL) [12–14, 16, 18, 26, 27], and electrochemical (EC) [19] detections. The preanalytical step for human plasma analysis includes protein precipitation with acetonitrile [13], methanol [19, 24, 26 & 28] or perchloric acid [12, 14& 27]. Most of the previously developed HPLC methods required a preanalytical derivatization step [14, 16, 26 &27], consisting in acylation of the primary amino group followed by liquid–liquid or solid phase extraction [14], resulting in time-consuming procedures potentially affected by variability of reaction yield. The direct analysis were carried out using ion pair methods [19& 25] and, although affected by poor retention of 5-ASA, reversed-phase chromatography [18 & 23].HPLC combined with mass spectrometry (MS) using electro-spray interface is now recognized as a powerful tool for both confirmatory and quantitative analyses, owing to its high sensitivity and selectivity, and therefore highly recommended for pharmacokinetic studies in complex matrices [29]. Multiple reaction monitoring (MRM) modes with triple quadruple tandem mass spectrometry (MS/MS) enable selective and accurate analyses over a wide linear range. In recent years, GC coupled with electron impact ionization-ion trap MS [23] and LC with electro spray (ESI) source-ion trap MS [14].

The aim of the present work is to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of Mesalamine bulk and in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the determination of Mesalamine in bulk and tablet dosage forms the chemical structure as shown in figure-1.

2. Materials and Methods

Mesalamine sample was obtained from. Rantus Pharma Pvt. Ltd Hyderabad. Mesalamine tablet was purchased from local market. The solvent used Potassium dihydrogen orthophosphate and dipotasssium hydrogen phosphate (HPLC grade), Acetonitrile (AR grade), these chemicals were purchased from Merck Chemicals (Tirupati, (AP) India).

Selection of Mobile Phase

Chromatographic separation studies were carried out Waters HPLC 2 2695 series consisting 4 pump, C-18, column on the working standard solution of Mesalamine (10µg/ml). Initially, trials were carried out using Mixed Phosphate Buffer and Acetonitrile in various proportions along with varying pH, to obtain the desired system suitability parameters. After several trials, Mixed Phosphate Buffer: Acetonitrile (pH adjusted to 6.5 with Potassium dihydrogen orthophosphate and dipotasssium hydrogen phosphate) (65: 35 v/v), was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Chromatographic Conditions

Column	: C18, 100 X 4.6, 5µ.
Flow Rate	: 1.0 ml/min
Wave length	: 258 nm
Column temperature	: 30°C
Injection volume	: 20 µL
Diluent	: Mobile Phase
Elution type	: Isocratic
Needle wash solution	: Water: Acetonitrile (90:10)

Preparation of Standard Stock Solution

40mg of Mesalamine reference standard was weighed accurately and transferred in 100ml volumetric flask. Drug was dissolve in Mixed Phosphate Buffer and Acetonitrile (65: 35 v/v) and volume was made up to 100ml with same solvent. So as to get the concentration 100µg/ml. 1ml standard stock solution of Mesalamine was then diluted in 10ml Mixed Phosphate Buffer and Acetonitrile (65: 35 v/v) to get working standard solution 10µg/ml.

Preparation of Mobile Phase

Mobile phase was prepared by Mixed Phosphate Buffer and Acetonitrile (pH adjusted to 6.5 with Potassium dihydrogen orthophosphate and dipotasssium hydrogen phosphate) (65: 35v/v), filtered through 0.45µ membrane filter paper and then sonicated on ultra sonic water bath for 30min.

Selection of Detection Wavelength

From the standard stock solution further dilutions were done using Mixed Phosphate Buffer and Acetonitrile (65: 35 v/v) and scanned over the range of 200 - 400nm and the spectra was obtained. It was observed that Mesalamine showed considerable absorbance at 258 nm.

Chromatogram of Mesalamine

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate).Standard solution of Mesalamine was injected to get the chromatogram. The retention time for Mesalamine was found to be 3.214 min. Chromatogram of Mesalamine is shown in (Figure- 3)

Validation of Analytical methods

The validation for HPLC method development was performed using parameters like Linearity, Precision, Accuracy, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness.

Linearity

The standard stock solution containing 100µg/ml of Mesalamine to prepare range of standard solutions containing six different concentrations of analyte. The linearity of the relationship between peak area and concentration was determined by analyzing six standard solutions over the concentration range 10-60µg/ml. The results obtained are shown in (table 1). The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure -2).

Precision

The precision of the method was demonstrated by intraday and inter-day variation studies. In the inter day studies, 3 different concentrations 30, 40 and 50µg/ml were injected in stabilized chromatographic conditions and were analyzed in triplicate. The percentage RSD was calculated. The result obtained for intraday variations are shown in (table- 3 & 4). In the inter day variation studies, 30, 40 and 50µg/ml were injected in stabilized chromatographic conditions and were analyzed. This procedure was repeated once a day for three consecutive days. The percentage RSD was calculated. The result obtained for inter-day variations are shown in (table-5& 6).

Accuracy

To check accuracy of the method, recovery studies were carried out by mixing standard drug solution to pre analyzed sample solution at three different levels 50%, 100% and 150%. Basic concentration of sample chosen was 20µg/ml of Mesalamine bulk drug solution to which 40and 60 µg/ml of Mesalamine tablet solution was added. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms.The drug concentrations of Mesalamine were calculated by using linearity equation. The results obtained are shown in (table -4).

Assay:

Standard Préparation:

Transfer 10 ml of standard stock solution in to 100 mL volumetric flask and make up to volume with diluent.

Sample Preparation:

Transfer sample quantitatively equivalent to 40 mg of Mesalamine in to 100 mL volumetric flask add 100 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure:

Inject 20 µL of blank solution, standard solution, and sample solution record the chromatogram. And calculate percentage of assay the results are shown in table-9.

Assays result
Mesalamine = 100.07 %

Limit of Detection (LOD)

LOD is calculated from the formula:

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = standard deviation of response for the lowest conc. In the range
S = slope of the calibration curve.

LOD =Mesalamine: **0.7999 µg/ml**

Limit of Quantification (LOQ)

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\sigma}{S}$$

LOQ = Mesalamine: **2.4241µg /ml.**

Ruggedness:

The ruggedness of test method is demonstrated by carrying out precision studies with different analysts and on different days. % of RSD on Day-1& Day-2.The % of RSD of areas from six injections should not be more than 2.0%.The results shown in table -8.

Robustness

Robustness was performed by injecting the Mesalamine standard solution in to the HPLC by altering the flow rate and column oven temperature from the normal chromatographic conditions. The results are tabulated in (table-7).Summary of validation parameters or Mesalazine

Validation Parameter Mesalamine

Linearity Equation	Y=40955x + 30988
(r2)	0.998
Range	10 – 60µg/ml
Precision (% RSD)	
Intraday	0.35%
Inter day	0.238%
Accuracy (% recovery)	98.98%, 99.15%, 98.74%
LOD	0.0302µg/ml
LOQ	0.0916µg/ml

3. Results and Discussion

The developed method was found to be precise as the %RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (98% to 102%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the %recoveries ranging from 98% to 102%. The LOD and LOQ were found to be 0.7999µg/ml and 2.4241µg/ml indicating the sensitivity of the method. The method was also found to be robust as indicated by the % RSD values which are less than 2%.

4. Conclusion

All the above factors lead to the conclusion that the proposed method as accurate, precise, simple, sensitive, robustsness and cost effective and can be applied successfully for the estimation of Mesalamine bulk and pharmaceutical formulation.

Acknowledgements

Thanks to Department of Chemistry, S.V University for providing laboratory facilities.

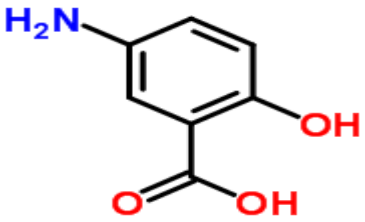


Figure 1: Chemical Structure of Mesalamine

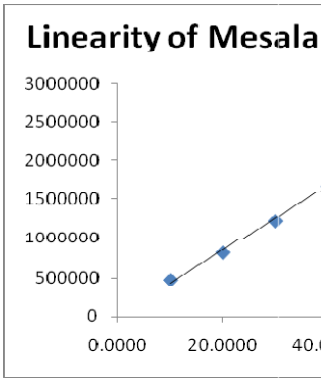
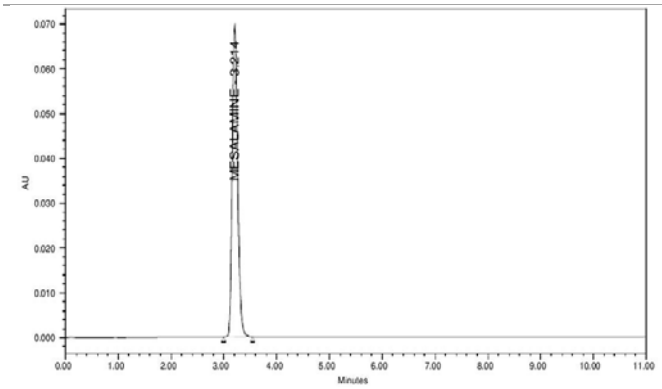


Figure 2: Linearity of Mesalamine



	Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count
1	MESALAMINE	3.214	480938	69626	100.000	1.17	5048

Figure 3: Chromatogram of Mesalamine

Table 1: Linearity of Mesalamine

%	*Conc(mcg)*	*Area*
25	10.0000	480938
50	20.0000	815491
75	30.0000	1228631
100	40.0000	1679153
125	50.0000	2088232
150	60.0000	2494042

Table 2: System Precision of Mesalamine

S No	Name	RT	Area
1	Injection-1	3.237	1763478
2	Injection-2	3.238	1786713
3	Injection-3	3.238	1765368
4	Injection-4	3.239	1769023
5	Injection-5	3.241	1775435
6	Injection-6	3.243	1759085
Avg		3.239	1769850
Std Dev		0.002	9927.9
% RSD		0.069	0.56

Table 3: Method Precision of Mesalamine

S No	Name	RT	Area
1	Solution-1	3.235	1685389
2	Solution-2	3.237	1690156
3	Solution-3	3.239	1693424
4	Solution-4	3.24	1701543
5	Solution-5	3.242	1695635
6	Solution-6	3.241	1698905
Avg		3.239	1694175
Std Dev		0.003	5877.1
% RSD		0.081	0.35

Table 4: Accuracy of Mesalamine

Accuracy-50%		Accuracy-100%		Accuracy-150%	
S No	Area	S No	Area	S No	Area
Injection -1	835385	Injection -1	1672098	Injection -1	2499132
Injection -2	835690	Injection -2	1673319	Injection -2	2501354
Injection -3	835902	Injection -3	1674178	Injection -3	2502210
Avg	835659	Avg	1673198	Avg	2500898.667
amt Recovered	49.49	amt Recovered	99.15	amt Recovered	148.11
%Recovery	98.98	%Recovery	99.15	%Recovery	98.74

Table 5: Ruggedness of Mesalamine day1

S No	Name	RT	Area
1	Injection-1	3.235	1685389
2	Injection-2	3.237	1690156
3	Injection-3	3.239	1693424
4	Injection-4	3.24	1701543
5	Injection-5	3.242	1695635
6	Injection-6	3.241	1698905
Avg		3.239	1694175
Std Dev		0.003	5877.1
% RSD		0.081	0.35

Table 6: Ruggedness of Mesalamine day2

S No	Name	RT	Area
1	Injection-1	3.231	1658905
2	Injection-2	3.233	1659683
3	Injection-3	3.235	1663065
4	Injection-4	3.237	1664324
5	Injection-5	3.23	1660964
6	Injection-6	3.239	1669689
Avg		3.234	1662772
Std Dev		0.003	3952.3
% RSD		0.108	0.238

Table 7: Robustness of Mesalamine

S. No	Peak Name	RT	Area	Height	%Area	USP Tailing	USP Plate Count
1	Mesalamine	3.871	1996189	229383	100.000	1.30	4652
2		3.214	1427701	202976	100.000	1.20	3605
3	Mesalamine	3.242	1668274	217834	100.000	1.24	4213
4		3.241	1681028	220686	100.000	1.23	4239

Table 8: Ruggedness of Mesalamine D1&D2

S No	Name	RT	Area
1	Injection-1	3.235	1685389
2	Injection-2	3.237	1690156
3	Injection-3	3.239	1693424
4	Injection-4	3.24	1701543
5	Injection-5	3.242	1695635
6	Injection-6	3.241	1698905
7	Injection-7	3.231	1658905
8	Injection-8	3.233	1659683
9	Injectoion-9	3.235	1663065
10	Injection-10	3.237	1664324
11	Injection-11	3.23	1660964
12	Injection-12	3.239	1669689
	AVG	3.237	1678473.50
	STDEV	0.00387	17081.028
	%RSD	0.12	1.02

Table 9: Assay of Mesalamine

1686733	40	10	100	100	99.5	682	100.000	Result
1690478	100	100	68	10	100	400		100.07 %

References

[1] D.K. Podolsky, N. Engl. J. Med. 347 (2002) 417
[2] R. Bergman, M. Parkes, Aliment. Pharmacol. Ther. 23 (2006) 841
[3] W.J. Sandborn, S.B. Hanauer, Aliment. Pharmacol. Ther. 17 (2003) 29
[4] Tripathi KD. Drugs for constipation and diarrhoea. Essentials of medical Pharmacology. 5th Ed., New Delhi:Jaypee Brothers Medical Publishers(P) Ltd 2003; 621.
[5] Stretch GL, Campbell BJ, Dwarakanath AD, Yaqoob M, Stevenson A, Morris AI and RhodesJM. 5-amino salicylic acid absorption and Metabolism in ulcerative Colitis patients receiving Maintenance Sulphasalazine, Osalazine orMesalazine. Aliment Pharmacol Ther.1996; 941-947
[6] L. Stærk Laursen, M. Stokholm, K. Bukhave, J. Rask-Madsen, K. Lauritsen, Gut 31(1990) 1271
[7] S.M.Greenfield, N.A. Punchard, J.P. Teare, R.P.H. Thompson, Aliment. Pharmacol.Ther. 7 (1993) 369
[8] W.H. Betts, M.W. Whitehouse, L.G. Cleland, B. Vernon-Roberts, J. Free Radic.Biol. Med. 1 (1985) 273
[9] I. Ahnfelt-Ronne, O.H. Nielsen, A. Christensen, E. Langholz, V. Binder, P. Riis, Gastroenterology 98 (1990) 1162.
[10] P. Gionchetti, C. Guarneri, M. Campieri, A. Belluzzi, C. Brignola, P. Iannone, M.Miglioli, L. Barbara, Digest. Dis. Sci. 36 (1991) 174
[11] B. Myers, D.N.W. Evans, J. Rodhes, B.K. Evans, B.R. Hughes, M.G.Lee, A. Richens,D. Richards, Gut 28 (1987) 196.
[12] I.R. Wilding, C. Behrens, S.J. Tardif, H.Wray, P. Bias,W. Albrecht, Aliment. Pharmacol.Ther. 17 (2003) 1153
[13] J. Tjørnelund, S.H. Hansen, J. Chromatogr. Biomed. Appl. 570 (1991) 109
[14] M. Nobilis, Z. Vybíralová, K. Sládková, M. Lísa, M. Holcapek, J. Kvetina, J. Chromatogr.A 1119 (2006) 299.
[15] H. Allgayer, J. Sonnenbichler, W. Kruis, G. Paumgartner, Arzneim. Forsch. 35(1985) 1457
[16] M. Brunner, E. Lackner, P.S. Exler, H.C. de Fluiter, K. Kletter, M. Tschurlovits, R.Dudczak, H.G. Eichler, M. Müller, Aliment. Pharmacol. Ther. 23 (2006) 137.
[17] K. Dilger, D. Trenk, M. Rössle, M. Cap, A. Zähringer, V. Wacheck, C. Remmler, I.Cascorbi,W. Kreisel, G. Novacek, Eur. J. Clin. Invest. 37 (2007) 558
[18] P. Gandia, I. Idier, G. Houin, J. Clin. Pharmacol. 47 (2007) 334
[19] G. Palumbo, S. Bacchi, L. Primavera, P. Palumbo, G. Carlucci, Biomed. Chromatogr.19 (2005) 350
[20] W.J. Sandborn, S.B. Hanauer, A. Buch, Aliment. Pharmacol. Ther. 19 (2004) 1089
[21] G. Aumais, M. Lefebvre, C. Tremblay, A. Bitton, F. Martin, A. Giard, M. Madi, J.Spénard, Aliment. Pharmacol. Ther. 17 (2003) 93
[22] F.N. Hussain, R.A. Ajjan, K. Kapur, M. Moustafa, S.A. Riley, Aliment. Pharmacol.Ther. 15 (2001) 53
[23] M. Orioli, C. Marinello, R. Cozzi, L.P. Piodi, M. Carini, J. Pharm. Biomed. Anal. 35(2004) 1263
[24] S.H. Hansen, J. Chromatogr. 226 (1981) 504
[25] V.S. Chungi, G.S. Rekhi, L. Shargel, J. Pharm. Sci. 78 (1989) 235
[26] F.N. Hussain, R.A. Ajjan, M. Moustafa, J.C. Anderson, S.A. Riley, J. Chromatogr. B:Biomed. Sci. Appl. 716 (1998) 257
[27] B. Bystrowska, J. Nowak, J. Brandys, J. Pharm. Biomed. Anal. 22 (2000)341
[28] J. Tjørnelund, S.H. Hansen, J. Chromatogr. Biomed Appl. 570 (1991) 224
[29] A. Roda, L. Sabatini, A. Barbieri, M. Guardagli, M. Locatelli, F.S. Violante, L.C.Rovati, S. Persiani, J. Chromatogr. B 844 (2006) 119