Development and validation of RP-UHPLC procedure for estimation of 5-amino salicyclic acid in 5-amino salicyclic acid rectal suppositories

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Development and validation of RP-UHPLC procedure for estimation of 5-amino salicyclic acid in 5-amino salicyclic acid rectal suppositories

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Abstract. The present study describes a simple and robust reverse phase ultra performance liquid chromatography (RP-UPLC) method for the quantification of 5-amino salicyclic acid in 5-amino salicyclic acid rectal capsules. Successful separation of Mesalamine peak from excipient peaks and diluent were achieved on a Acquity C8 (50 × 2.1 mm, 1.7 µm) and UV detector at 254 nm, 0.3 mL/min as a flow rate, and 3 µL as an injection volume. For the RP-UPLC method, phosphate buffer and methanol was used as mobile phases at ratio of 83:17 and the column temperature was 25 °C. Percentage recovery obtained in the range of 98.7 – 99.7 % and the method is linear for Mesalamine for specified concentration range with coefficient of variation (r) not less than 0.99. The proposed RP- UPLC method was found to be specific, linear, precise, accurate and robust.

1. INTRODUCTION

5-Amino salicylic acid is otherwise named as Mesalamine or Mesalazine or USAN or 5-ASA. Sulfasalazine is a fine renowed drug for colitis (ulcerative) and ileo-colitis. 5-Amino salicylic acid and sulfapyridine contributes the Sulfasalasine structure. The sulfapyridine part of the drug is responsible for majority of side effects [1]. Novel drugs which are derivatives of salicylic acid and its salts were developed for the treatment of colitis with minimum side effects. These are 5-ASA derivatives that don't comprise of side effect causing sulfapridine moiety. Mesalazine is also one such 5-ASA based drug being prescribed for treatment of UC in recent times. Chronic inflammation of large bowel and most usually rectum, extending in a unceasing manner are indications of UC . The etiology of these indications are still unclear. The indication such as chronic inflammation of small bowel (swelling and soreness of inner lining of terminal ileum) and colon [2].

The US FDA on August 06, 2006 approved Canasa rectal suppositories for Forest laboratories to treat Ulcerative colitis [3].

The solvents required for High Performance Liquid Chromatography are expensive, a high pure solvents are preferred to avoid interference, hence HPLC techniques are considered, particularly when procedures involving longer run times. A technique where low solvents consumed are always preferred, UHPLC is ultra performance liquid chromatography where sample can be analysed in seconds to few minutes

with reduced flow rate and solvent consumption. Number of users and support for UHPLC methods were increased in pharmaceutical industries and other food industries manifold. [4].

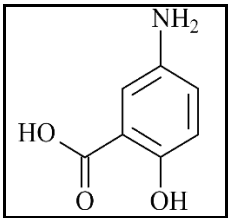

Figure 1. Structure of 5-ASA

5-Aminosalicylic acid being a small molecule with amine at position 5, hydroxy group at position 2 and an acid group (Fig.1)

Table 1. Solubility of 5-amino salicylic acid[5]

S.No	Solvent	Solubility	S.No	Solvent	Solubility
1.	Dilute acid	Soluble	2.	n-butyl alcohol	Practically insoluble
3.	Dilute alkali	Soluble	4.	Chloroform	Practically insoluble
5.	Water	Slightly soluble	6.	Ether	Practically insoluble
7.	Methanol	Very slightly soluble	8.	Ethylacetate	Practically insoluble
9.	Ethanol	Very slightly soluble	10.	n-hexane	Practically insoluble
11.	Acetone	Very slightly soluble	12.	Dichloromethane	Practically insoluble

Considering the strength of dosage form (1000 mg per suppository) and the limited solubility in aqueous and organic solvents the extraction of drug was found to be challenging for estimation of drug substance in any dosage form and particularly in a wax, hard fat based formulation like suppositories. 0.8 mg is soluble per mL in aqueous solutions at 25°C and 1.41 mg/mL at 37°C. 5-amino salicylic acid shows 2 pKa , pKa1 at 2.30 and pKa2 at 5.69, solubility of the drug is increased when the pH is less than 2.0 and more than 5.5., minimum solubility observed between pH 2.0 and 5.5. Increase in temperature will support the solubility but chances of degradation of 5-ASA to 4 amino phenol and 2-amino phenol are reported [6].

Increase in peak tailing was observed commonly in salicylic acid derivatives and tailing of more than 1.5 was reported in estimation methods by HPLC [7]. Continuous analysis of samples with peak tailing of more than 1.5 will result in reduced column life and usage increasing the quality control expenditure for procuring new columns. Further longer run time HPCL methods, usage of organic solvents in mobile phase are factors which increases cost of running quality control department.

The objective of this development is to advance a method which facilitates the usage of chromatographic column for more number of samples by decreasing the tailing factor, injection volume, run time, organic usage by decreasing usage of organic solvent and reduced flow in the method. Optimization of sample preparation procedure by overcoming the solubility limitation without compromising in using the representative number of sample units for assay.

A new fast LC-UPLC method was developed using Acquity C8 50mm length, 2.1mm ID, 1.7µm particle size, mobile phase flow of 0.3 mL and 4.0 minutes run time. Method was validated as per ICH Q2 guidelines for analytical method validation [8].

2. Reagent and preparations

2.1 Materials and Instruments

Methanol, Sodium dihydrogen phosphate, disodium hydrogen phosphate, Tetrabutyl ammonium hydroxide solution 0.1M, Hydrochloric acid was obtained from Merck. The instrument used was Shimadzu Nexera 1 series UHPLC system consisting of a pump, a 2010 LC prominence Diode array detector and empower3 data analysis software. Waters Acquity C8 50mm length, 2.1mm ID, 1.7µm particle size were employed.

2.2 Standard solution

About 25 mg of Mesalamine was weighed in 50 mL volumetric flask, dissolve in 10 mL of 1N HCl and dilute to volume with same. Further dilute with mobile phase to get a concentration of 50 ppm.

2.3 Test solution

About 5 suppositories were transferred in to 1000 mL flask, added 20 mL of tetrahydrofuran, sonicated for 15 mins or until the fat content gets dissolved, added 700 mL of 1N HCl stirred using magnetic stirrer for 15 minutes, sonicated for 15 minutes, made up to volume with 1N HCl, diluted 2 mL of this solution to 200 mL with mobile phase, mixed and filtered through 0.45µ nylon membrane filter.

3. Discussion with results

3.1 chromatographic conditions Optimization

Few HPLC columns such as octadecyldimethyl-silane (C18) and octyldimethylsilane (C8), polar group embedded C18 stationary phases with changing buffer pH, organic modifiers were used, Mesalamine peak shape and interference from blank, placebo were monitored in all trials, the results were tabulated in table.2.

Waters Acquity C8 50mm length, 2.1mm ID, 1.7µm was found to be showing less tailing of about 1.2 (Figure 2). The trial chromatograms using different columns are given in Figure 1.

For the RP-UPLC, mobile phase prepared by dissolving 18 g of mono basic sodium hydrogen phosphate, 21 g of dibasic sodium hydrogen phosphate and 22.5 mL of tertrabutyl ammonium hydroxide solution (0.1M solution) was added t0 3000 mL of water. A buffer, methanol ratio of 83:17 was used in isocratic pump as mobile phase. Column oven temperature was maintained at 25℃. The wavelength was set at 254nm. A nominal flow of was 0.7 mL/ minute was maintained and 3 µL of the standard and sample solutions were injected.

3.2 Validation of procedure

3.2.1 Specificity

Blank as well as placebo solutions were injected to evaluate specificity. No interference due to blank and placebo were observed at retention time of 5-Amino Salicylic acid. Peak purity evaluated using Diode array detector with empower 3 chromatographic data management system shows a purity of above 99 %, found to be specific. This shows the LC method is capable of separating all other placebo matrix of the formulation from the active component.

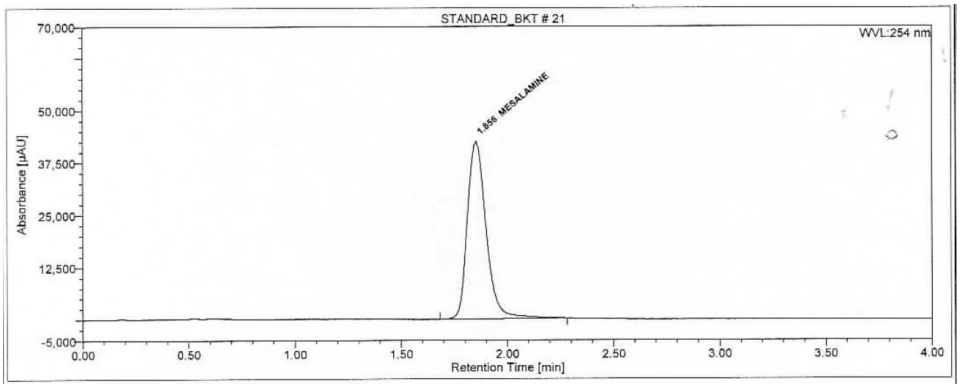

Figure 2. Assay chromatogram.

Table 2. The system suitability results for different mobile phase and column scouting.

Mobile phase	Ratio	Column	Mesalamine		Diluent and placebo interference
			Rt	Tailing factor	
Sodium phosphate buffer+TBAH with methanol	85:15	Waters Acquity C8 50mm length, 2.1mm ID, 1.7µm	2.0 min	1.3	Interference found nil
Sodium phosphate buffer+TBAH with methanol	85:15	Waters Acquity C18 50mm length, 2.1mm ID, 1.7µm	0.8 min	1.5	Interference observed
Sodium phosphate buffer+TBAH with methanol	85:15	Waters Acquity Shield RP18 50mm length, 2.1mm ID, 1.7µm	1.3 min	1.6	Interference found nil
TEA (0.1%) and methanol	75:25	Waters Acquity C8 50mm length, 2.1mm ID, 1.7µm	3.8 min	1.8	Interference found nil
0.1%OPA and methanol	75:25	Waters Acquity C8 50mm length, 2.1mm ID, 1.7µm	5.6 min	1.0	Interference observed
Sodium phosphate buffer+TBAH with methanol	83:17	Waters Acquity C8 50mm length, 2.1mm ID, 1.7µm	1.8 min	1.2	Interference found nil

3.2.2 Precision

Consistency in repeated response by the chromatographic system and sample preparation procedure was evaluated by injecting six replicate preparations of sample solution and six replicate injections of standard solution. The % RSD for Mesalamine peak area was found to be 0.5% for six sample preparation and 0.5% for six standard replicate injections. The precision result shows the reliability and repeatability of the method in the ideal laboratory conditions.

3.2.3 Linearity

To evaluate the linearity in detector response the components were injected from 5 µg/ mL to 75 µg/ mL concentration and the correlation coefficient was found to be not more than 0.99. The % Y intercept difference value was found to be 0.2% from the area obtained for 100% sample concentration. The detector response is found to be linear for a concentration range of 10% to 150% of the working concentration, this provides flexibility to analyse different dosage strengths and dilutions.

3.2.4 Accuracy

Accuracy was demonstrated by spiking Mesalamine drug substance in placebo at 50 %, 100 % and 150 % levels. The % recovery was calculated from the amount added and amount found. The results are presented in Table 3.

Table.3. Recovery results

S.No	Level	"mg" added	"mg" found	% recovery
1	50%	2502.1	2490.1	99.5
2	100%	5003.1	4987.1	99.7
3	150%	7508.3	7412.1	98.7

The recovery result shows that the method is capable of giving accurate results from concentration range of 50% to 150% of working concentration.

3.2.5 Robustness

Robustness of the method was evaluated by injecting standard. Theoretical plate count, tailing factor of 5-amino salicylic acid and %RSD for replicate injections of standard solution were monitored. The method was found to be robust for the below mentioned conditions.

I. ± 0.05 mL flow rate
II. ± 5 °C column temperature
III. ± 10 % difference in organic ratio

Theoretical plate count was found to be more than 4000, tailing factor was found to be less than 1.2 and the % RSD of replicate injections of standard solution was found to be less than 2.0% in all the above stated robustness conditions.

4. Conclusions

A fast liquid chromatographic method was developed for estimation of 5-amino salicylic acid in suppositories formulation, time required for estimation is not more than 4.0 minutes, using very minimum organic solvents. In contrast with the other LC methods this method shall be employed for analysing large number of sample counts by spending very less time and resources. The peak shape, repeatability, column life for multiple analysis were found to be rugged. The method is considered validated, satisfies the needs of a ideal chromatography method such as specificity, accuracy, robustness, and linearity. This RP-UHPLC method shall be employed for quality monitoring of rectal suppositories at quality control units of pharmaceutical industries.

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