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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF MESALAMINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Mesalamine (USAN) or 5-aminosalicylic acid (5-ASA), is an amino salicylate anti-inflammatory drug used to treat inflammatory bowel disease, including ulcerative colitis, or inflamed anus or rectum, and to maintain remission in Crohn's disease. A simple, selective, precise, accurate and cost effective reverse phase HPLC method has been developed and validated for estimation of Mesalamine in extended release tablet dosage form. In the chromatographic conditions, Zorbax Rx-C8 (150 X 4.6 mm, 5µ) stationary phase with mobile phase consisting of potassium phosphate monobasic with 0.1% of Sodium 1- octane sulfonate buffer (pH 2.2 ± 0.05): methanol (70: 30 v/v) was used at a flow rate of 1.5 mL/min. and column temperature was maintained at 30°C. Mesalamine was detected at 220 nm. The chromatographic procedure separated Mesalamine and potential interfering peaks in an analysis time of 15 min. with Mesalamine eluting at about 8 min. The assay method was found linear in the concentration range of 50 to 400 µg/mL with a correlation coefficient of 0.9999. The percentage recovery of assay was found between 100.1 and 100.9. The developed method was validated with respect to specificity, linearity, accuracy, precision, sensitivity, robustness and solution stability as per ICH guidelines. The proposed method can be used for routine analysis of Mesalamine formulations in quality control laboratories.

KEY WORDS

Mesalamine, HPLC, Validation, Dissolution, Extended Release

INTRODUCTION:

Mesalamine delayed-release tablet for oral administration contains 800 mg of mesalamine, an aminosalicylate. *Mesalamine* delayed-release tablets contain an outer protective coat consisting of a combination of acrylic based resins, Eudragit S (methacrylic acid copolymer B, NF) and Eudragit L (methacrylic acid copolymer A, NF)[1]. The inner coat consists of an acrylic based resin, Eudragit S, which dissolves at pH 7 or greater, releasing mesalamine in the terminal ileum and beyond for topical anti-inflammatory action in the colon[2].

Reverse Phase HPLC:

In this chromatographic technique, the stationary phase is non-polar, and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.[3]

Steps involved in development of RP-HPLC method:

Selection of chromatographic method:

The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drug selected is polar and ionic hence reverse phase

chromatography was used because of its simplicity and suitability.[4]

Selection of stationary phase:

Matching the polarity of sample and stationary phase and using a mobile phase of different polarity will achieve a successful separation.[5]

Selection of mobile phase:

Reverse phase bonded packing, when used in conjunction with highly polar solvents; approach is ideal and is a universal system for liquid chromatography. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.[6]

Selection of suitable detector:

Detector is the eye of HPLC system that measures the compounds after their separation on the column. There are basically two types of detectors- the bulk property detectors and solute property detectors. Detectors, in order of their popularity are UV, fluorescent, conductivity, polarimeter and refractive index detectors. UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest versions of equipment's are available with photo diode- array detectors (PAD or DAD).

Method optimization:

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and shape, plate count asymmetry, capacity, elution time, detection limits, limit of quantization, and overall ability to quantify the specific analyte of interest.

Literature search reveals, that that very few methods were developed for the estimation of mesalamine 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 Mesalamine 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 quantization of Mesalamine in coated tablets[10]. In the current work we have made an attempt to develop simple, robust, cost effective and high throughput analytical method for the determination of Mesalamine in tablet dosage form. The method uses UV detection with a run time of 15 min. The method has several advantages like simple mobile phase, low injection volume, less run time over the reported methods. The developed method was successfully validated as per ICH guidelines[11-16] and can be used in routine quality control analysis.

MATERIALS AND METHODS

Drug profile of *Mesalamine*

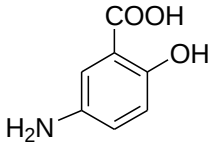

Fig.1: Structure of *Mesalamine*

IUPAC Name:5-amino-2-hydroxybenzoic acid

Chemical formula: $C_{20}H_{27}N_5O_2$

Molecular weight: 153.137

Description: White to pinkish crystals or purplish-tan powder.

Solubility: Slightly soluble in water, alcohol; more soluble in hot water; soluble in hydrochloric acid

Category: Anti-inflammatory

λ_{max}: 220 nm

Drugs used:

Table: 1. List of Drugs used

S. No.	Drugs	Manufacturer
1.	*Mesalamine*	Hetero Drugs Ltd
2.	Mesacol DR 800 mg Commercial Tablets	Sun Pharma Ltd

Reagents Used:

Table: 2. List of Reagents used

S. No.	Chemicals	Manufacturer name	Grade
1	Water	Merck	HPLC
2	Methanol	Merck	HPLC
3	Acetonitrile	Merck	HPLC
4	Potassium phosphate, (Monobasic)	Merck	G. R
5	Sodium 1- octane sulfonate	Merck	HPLC

Equipment and Apparatus Used:

Table 3: Equipment and Apparatus Used

S. No.	Instrument Name	Model Number	Software	Manufactures Name
1	HPLC	Alliance UV-Visible detector-2487	Empower	Waters
2	U.V Double beam spectrophotometer	SL 210	-	ELICO
3	Digital weighing balance (Sensitivity 5 mg)	BL-200H	-	SHIMADZU
4	PH-meter	LI-120	-	ELICO
5	Sonicator	3305013	-	SISCO

Preparation of mobile phase:

A combination of mobile phase containing potassium phosphate monobasic with 0.1% of Sodium 1- octane sulfonate buffer (pH 2.2 ± 0.05): Methanol (70: 30 v/v) was mixed and degassed in ultrasonic water bath for 5 minutes finally filtered through 0.45µ membrane filter. This prepared solution was used as mobile phase.

Diluent:

0.1 N HCl was used as diluent.

Preparation of standard solution: (0.2mg/ml)

Accurately weighed 25 mg of *Mesalamine* working standard into a 25 mL volumetric flask, added 20 mL of diluent, mixed sonicate for 10 minutes to dissolve and made up the volume with diluent. Further dilution was made by diluting 10.0 mL of the stock solution to 50 mL.

Preparation of sample solution: (0.2mg/ml)

20 tablets were crushed to powder, weighed and transferred the tablet powder equivalent to 400 mg of *Mesalamine* into 500 mL volumetric flask added 300 mL of diluent, sonicated for 15 minutes and stir for 15 minutes and diluted to volume with diluent. Filtered the solution through 0.45 µ nylon filter. Diluted 12.5 mL of the sample solution to 50 mL with diluent, prior to injection on chromatographic system.

Wavelength selection:

About 0.25 mg/mL of *Mesalamine* solution was accurately prepared by dissolving the standard in water. The Mesalamine solution was scanned in the 200-400 nm UV region. The wavelength maximum (λ_{max}) was observed at 250 nm and this wavelength was adopted for absorbance measurement.

Optimized chromatographic conditions:

Column: Zorbax Rx-C8 (150 X 4.6 mm, 5µ)

Column temperature: 30°C.

Wave length: 220nm

Mobile phase ratio: Potassium phosphate monobasic with 0.1% of Sodium 1- octane sulfonate buffer (pH 2.2 ± 0.05): methanol (70: 30 v/v)

Flow rate: 1.5 min/ml

Injection volume: 20µl

Run time: 15minutes

Validation of developed RP-HPLC method:

As per the International conference on harmonization (ICH) guidelines the method validation parameters such as linearity, precision, accuracy, system suitability, limit of detection and limit of quantitation were optimized.

Assay

Sample and standard solutions were injected into the chromatographic system and measured the area for Mesalamine and calculated the % assay by using the below formula.

Calculation:

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where:

Avg.wt = average weight of tablets

P = percentage purity of working standard

LC = label claim of *Mesalamine* mg/ml

RESULTS AND DISCUSSION:

Optimized method:

It was performed on Zorbax Rx-C8 (150 X 4.6 mm, 5µ) with a mobile phase composition of potassium phosphate monobasic with 0.1% of sodium 1- octane sulfonate buffer (pH 2.2 ± 0.05): Methanol (70: 30 v/v) at a flow rate of 1.5 min/ml. 20 µl of sample was injected and the run time was 15 min.

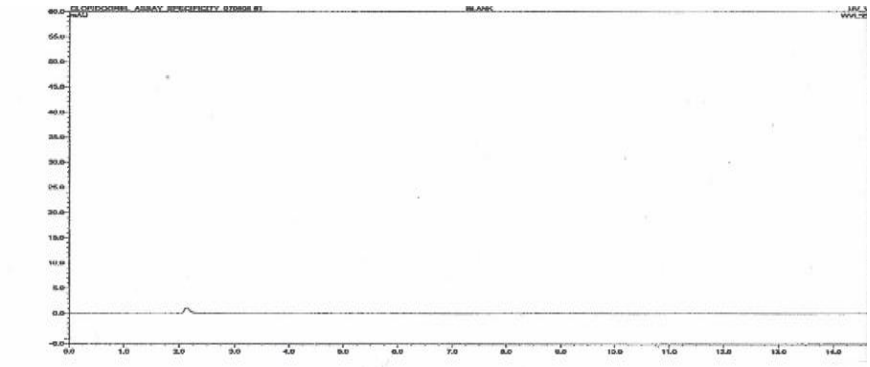

Fig. 2: Chromatogram showing blank preparation (mobile phase)

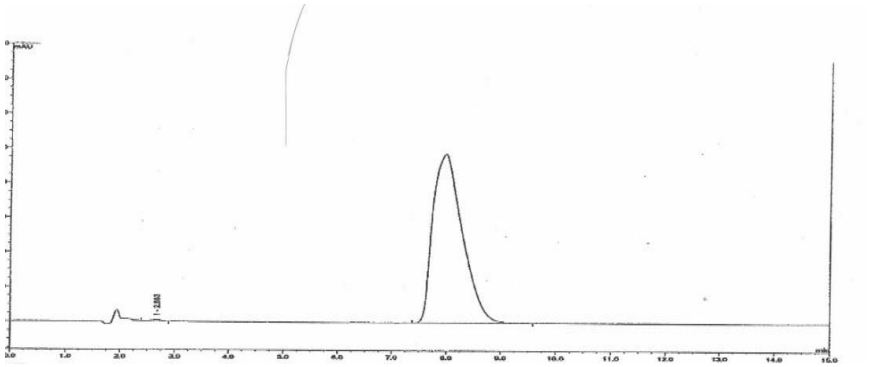

Fig. 3: Chromatogram of *Mesalamine* standard peak

Linearity:

50, 100, 200, 300, 400 µg/ml was injected into the chromatographic system and peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and Y-axis peak area) and the correlation coefficient was calculated.

Acceptance criteria:

Correlation coefficient should be not less than 0.999.

Table 4: Showing the results for the Linearity

Conc.(µg/ml)	RT	Area
50	8.026	16808
100	8.028	33609
200	8.022	67206
300	8.021	84103
400	8.024	135016
Co efficient of correlation(R^2)		0.999

Precision:

The standard solution (0.1 mg/ml) was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria:

The %RSD for the area of five standard injections results should not be more than 2.

Table 5: Showing the results for Precision

S. No	Conc.((mg/ml)	RT	Area
1	0.2	8.034	68006
2	0.2	8.066	68906
3	0.2	8.043	68356
4	0.2	8.086	68243
5	0.2	8.028	68186
Mean			68339
SD			341.06
% RSD			0.0

Accuracy:

The standard solution of concentration 100, 200 and 300 µg/ml were injected into chromatographic system.

CALCULATED % RECOVERY AND MEAN %RECOVERY OF MESALAMINE

Acceptance criteria:

The % recovery for each level should be between 98.0 to 102.0%.

Table 6: Showing Accuracy results for *Mesalamine*

S. No	Conc(µg/ml)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean% recovery
1	100	33821	10	10.09	100.9%	
2	200	68001	20	20.02	100.1%	100.1%
3	300	134919	30	30.03	100.1%	

System suitability:

The standard I solution was injected one time and standard II solution was injected 5 times.

Table 7: Showing system suitability results for *Mesalamine*

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	1.0	9675	1.0
2	1.2	9866	1.0
3	1.4	9952	1.1

Limit of detection (LOD)

From the above preparation 1ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluent.

Table 8: Showing results for Limit of Detection

Drug name	y-Intercept	Slope(s)	LOD(µg/ml)
Mesalamine	1256	3134065	5.54

Limit of quantitation (LOQ)

From the above preparation 0.5ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluent.

Table 9: Showing results for Limit of Quantitation

Drug name	y-Intercept	Slope (s)	LOQ (µg/ml)
Mesalamine	1256	3134065	16.66

Assay:

The developed and validated method was applied to the determination of Mesalamine in marketed tablets containing 800 mg of drug per tablet. Three injections of sample were injected into chromatographic system. Assay % was calculated by using the formula mentioned above and it was found to be 99.8%.

Table 10: Showing the results of assay

S. No	Name	RT	Area
1	Mesalamine	8.043	68243
2	Mesalamine	8.086	68243
3	Mesalamine	8.028	68243

CONCLUSION:

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of *Mesalamine* in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for *Mesalamine* within a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy. Thus, the method can be proposed for routine analysis laboratories and for quality control.

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