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Analytical Method Development and Validation for Assay of Mesalmine Delayed Release Capsules by Using HPLC

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ABSTRACT:

A simple, specific, accurate, cost effective & time efficient RP-HPLC method was developed for the determination of Mesalamine, using Syncronis C18 (250x4.6)mm,5µm column. A mobile phase consisting of Buffer (Dilute 0.5ml of orthophosphoric acid in to 1000ml with millq water) and Methanol in the ratio of 90:10 (V/V) was employed in this study. The flow rate was kept at 0.8 mL/min and the injection volume was 5 µL. Column temperature was maintained at 40^{o}c. Eluents were monitored by PDA detector wavelength set at 230 nm. Total run time of run is 8mins.The developed method was statistically validated for the linearity range from 12.5 to 180% (5-72 µg/ml) and observed the correlation coefficient 0.999 with regression coefficient slope=11722 and intercept 88800, the results of precision for 100% was %RSD= 0.6, accuracy, robustness, specificity, solution stability are well within the limits. % Recovery was performed in three different range 20%,100% and 150% observation found to be 99.1%,98.6% and 99.8% respectively. The specificity of the method was ascertained by force degradation studies by acid hydrolysis, alkali hydrolysis and degradation by oxidation. The degraded products were well resolved from the analyte peak with significant difference in their RT values.

Key words: Mesalamine, Method development and validation, Forced degradation.

Introduction:

Mesalamine is used to treat inflammatory bowel disease and some forms of arthritis. It acts by inhibiting the production of cyclo-oxygenase and prostaglandin, thromboxane synthetase, platelet activating factor synthetase, and interleukin-1 by macrophages. so reduces the acute inflammatory response in inflammatory bowel disease. Production of immunoglobulin by plasma cells is also decreased. It also used to treat ulcerative colitis, proctitis and crohn's disease. Mesalamine is chemically known as 5-amino-2-hydroxy benzoic acid. Molecular formula of the drug is $C_7H_7NO_3$ and molecular weight is 153.14. It is white powder slightly soluble in water, soluble in dilute HCL and in dilute alkali hydroxides. Practically insoluble in chloroform, ether and butyl alcohol. It should be preserved in tight, light resistant container. Absorption of mesalamine is similar in fasted and fed subjects. The absorbed mesalamine is rapidly acetylated in the gut mucosal wall and by the liver. It is excreted mainly by the kidney as N-acetyl-5-aminosalicylic acid. Mesalamine is available in tablet dosage forms (400 mg) and is an official drug of USP.

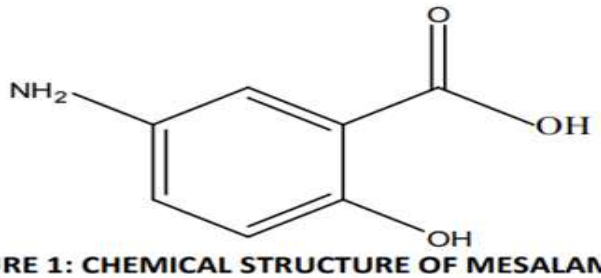


FIGURE 1: CHEMICAL STRUCTURE OF MESALAMINE

Comprehensive literatures for measure the quality of mesalamine delayed-release product by HPLC have long run time, limited solution stability, less efficiency (N), higher limit for unknown single maximum impurity, lack of related retention time for all impurities and less resolution, as such there is lack of a suitable procedure for the quantification and estimation for them. Therefore, the aim of the present work was to develop and validate a simple, precise, accurate, short runtime and specific method for the quantification and separation of 5-ASA and its impurities/degradation product by reversed-phase UPLC method, in mesalamine delayed-release formulation.

Materials:

Standard: Mesalamine from Divis Laboratories Limited and mesalamine product in-house material. And other references material are purchased from sigmaArchid

Chemicals : Methanol(HPLC grade), Acetonitrile(HPLC grade), potassium dihydrogen phosphate(HPLC grade),1-Octane sulphuric acid sodium salt(HPLC grade), hydrochloric acid(AR grade) and orthophosphoric acid (AR grade) purchased from rankem and sodium hydroxide (Emparta),Hydrogen peroxide(Emparta), Perchloric acid (Emparta) are purchased from Merck.

Instruments: HPLC(Waters Alliance Separation Module 2695 Detector PDA 2996/UV 2487),pH meter (LabIndia-PICO+), Orbital Shaker(Neolab), Milli-Q-Water system(Miilipore Elix- Gradient), Semi Micro Balance(Radwad-XA 82/220/2X), Pan Balance(Radwag-PS600 R2), Sonicator(PCI analytics), Centrifuge(Eltek), Water bath(VLS), Photo stability chamber(Tempo/ Ti-9ii), Hot air oven(VTS), Vaccum pump(Rocker.610).

Methodology:

Preparation of Reagents:

1) Preparation of 1N sodium hydroxide solution: Weigh and transfer 4.0 g of sodium hydroxide 100mL ultrapure water and mix well.

2) Preparation of Sodium dihydrogen phosphate buffer: Weigh and transfer about 13 g of Sodium dihydrogen phosphate in 1900 mL of ultrapure water and mix well. Adjust pH to 6.1 ±0.05 with 1N sodium hydroxide solution and make upto 2000 mL volume with ultrapure water. Filtered through 0.2µm membrane filter.

3) Preparation of mobile phase-A : Mix pH 6.1Sodium dihydrogen phosphate buffer and methanol in ratio of 60:40 (v/v) respectively and sonicate for 5 minutes.

4) Preparation of mobile phase-B: Mix ultrapure water and Acetonitrile in ratio of 300:700 (v/v) respectively and sonicate for 5 minutes.

5) Preparation of diluent (0.01N HCI): Transfer 1.72 mL of concentrated HCI into 2000 mL ultrapure water and mix well.

6) Preparation of 1N HCI: Transfer 43 mL of Concentrated HCI into 500 mL ultrapure water and mix well.

7) Preparation of 0.25N HCI: Transfer 25 mL of 1N HCI into 100 mL ultrapure water, make up with water and mix well.

8) Preparation of standard stock solution (About 500 ppm): Weigh and transfer about 50 mg of Mesalamine API into 100 mL volumetric flask. Add 5 mL of 0.25 N HCI and sonicate to dissolve for 10 minutes. Make upto volume with ultrapure water and mix well.

9) Preparation of standard stock solution (About 100 ppm): Weigh and transfer about 10 mg of Mesalamine API into 100 mL volumetric flask. Add 5 mL of 0.25 N HCI and sonicate to dissolve for 10 minutes. Make upto volume with ultrapure water and mix well.

10) Preparation of standard solution (About 40 ppm): Pipetted out 4 mL of standard stock solution into 50 mL volumetric flask, make upto volume with ultrapure water and mix well.

11) Preparation of sample: Weigh and transfer 5 capsules into 500 mL volumetric flask, add 10 mL of 1N hydrochloric acid and sonicate for 5 minutes. Add 50 mL of Methanol, sonicate for 5 minutes with intermittent shaking. Then add 40 mL of 1N hydrochloric acid and sonicate for 20 minutes. Add 200 mL of ultrapure water, sonicate for 10 minutes. Make upto volume with ultrapure water and mix well. Centrifuge a portion of above solution at 5000 rpm for 5 minutes. Pipette out 2 mL of above supernatant solution into 200 mL volumetric flask and make upto volume with diluent solution and mix well. Filter through a 0.45 µm nylon syringe filter by discarding initial 3 mL of filtrate and remaining filtrate was collect for analysis.

Optimized Chromatographic conditions: After several trails Methanol with dilute orthophosphate. The separation of the standard peak from baseline achieved by using Syncronis C18 (250x4.6)mm,5µm column with mobile phase Methanol with dilute orthophosphate 90:10 ratio v/v%(pH adjusted by dilute orthophosphate), using a flow rate 0.8 mL/min and column temperature was maintain at 40^{o}C.Detection was achieved at 200 to 400 nm (PDA).

Method Validation:

Validation of analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application. As per ICH guidelinesQ2(R1), system suitability, specificity, linearity, precision, accuracy, lod, loq, Robustness and roggudness this method was validated.

1. System Suitability: Any chromatographic system must consider system performance, which assesses several factors like tailing factor, intensity factor, reference resolution, and number of theoretical plates. This value is contrasted with the typical system specifications. The column was equilibrated with mobile phase for 30 minutes at a flow rate of 0.8 milliliters per minute prior to analysis. In addition, the first injection of blank solution was followed by six injections of 40 ppm mesalamine standard into the HPLC apparatus.

Table of1. System Suitability Data of Mesalamine

Name	Retention time(t_r)	Area
Standard-1	4.040	4006801
Standard-2	4.036	4007767
Standard-3	4.033	4011299
Standard-4	4.034	4016914
Standard-5	4.034	4020496
Standard-6	4.034	4005981
Mean		4011523
Standard Deviation		5945.5
%RSD		0.1

Observation:

Name of Component	Evaluation parameters				
	Avg.Area	%RSD	Retention time (t_r)	Tailing factor (Tf)	Plate count (N)
Mesalamine	4011543	0.1	4.040	1.4	7563

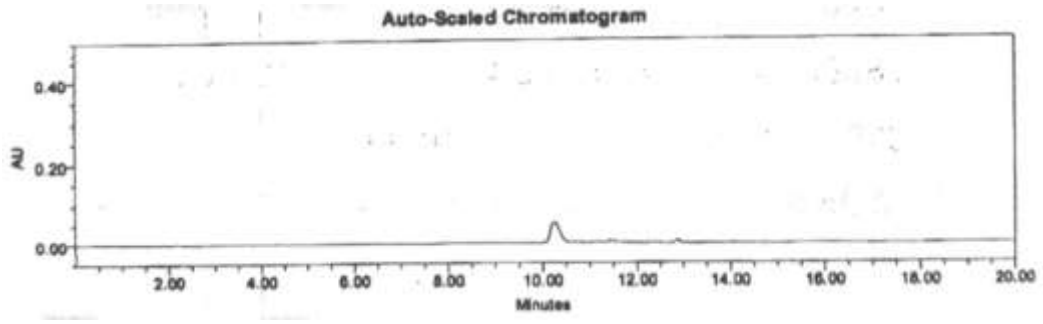


Figure.2. Chromatogram of Blank

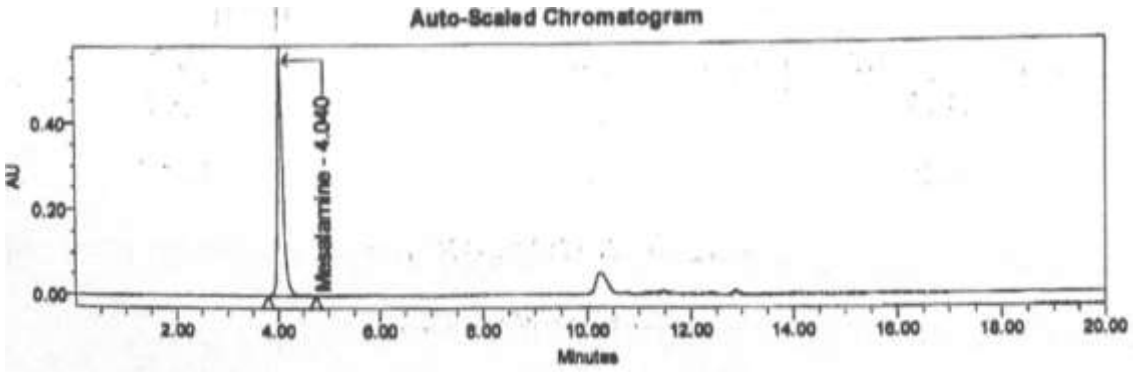


Figure.3. Chromatogram of Standard

Acceptance Criteria: The USP tail coefficient for the maximum level of mesalamine should not exceed 2.0. Mesalamine column efficiency(plate count) is more than 2000. After six injections of the standard solution, the mesalamine peak area %RSD should be less than 0.85.

Conclusion: System suitability parameters were acceptable and met the criteria.

2. Specificity: Specificity refers to the capacity to performance conclusively evaluate an analyte despite without being influenced by external factors. Analytical approaches like specificity can be employed to ascertain the impact of excipients and other additives in a formulation. To serve this objective, both placebo and blank test solutions were integrated into the HPLC system by utilizing a standard solution containing a concentration of 40 ppm.

Interference Table

Injection ID	Name of Impurities	Retention time(t_r)	Resolution	Purity angle	Purity Threshold	Interference (Yes/No)
Blank	Mesalamine	Not detected	NA	NA	NA	No
Placebo	Mesalamine	Not detected	NA	NA	NA	No
STD	Mesalamine	3.870	NA	0.153	0.440	No

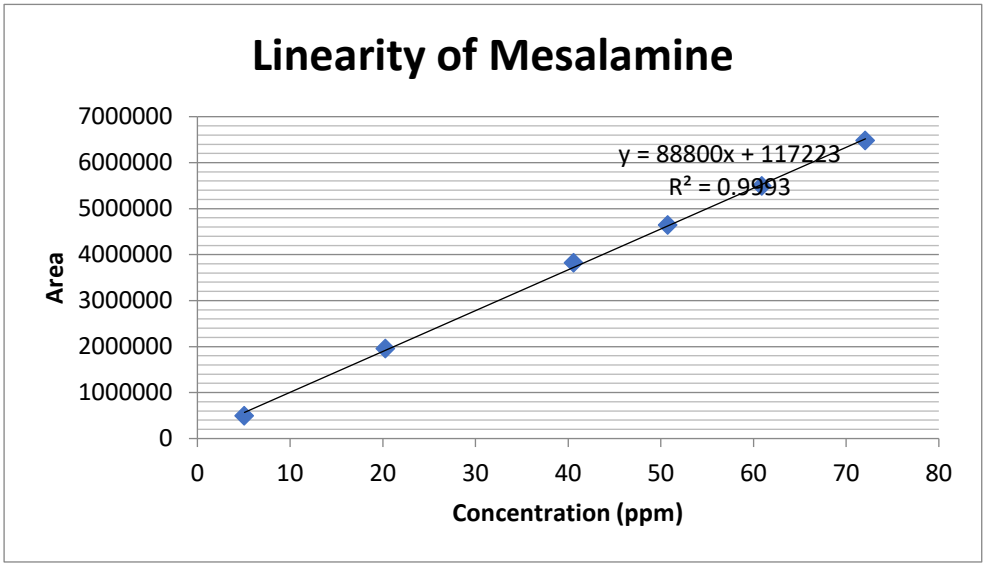
Control sample	Mesalamine	3.866	NA	0.137	0.390	No
Spiked sample with impurity G	Mesalamine	3.865	NA	0.164	0.391	No
	Impurity -G	5.132	NA	0.706	1.244	No
Individual Impurities	Impurity-K	10.658	NA	0.485	1.091	No
	Impurity-L	6.294	NA	0.575	1.738	No
	Impurity-M*	10.174	NA	17.575	0.456	Yes
	Impurity-N*	10.198	NA	6.979	0.394	No
	Impurity-O	2.923	NA	0.392	0.436	No
	Impurity-P	10.198	NA	6.979	0.385	No
	Impurity-J	2.923	NA	2.573	3.410	No
	Impurity-R*	10.199	NA	7.521	0.421	No
	Impurity-G	5.150	NA	0.361	0.592	No
	Impurity-H*	10.207	NA	6.813	0.397	Yes
	Impurity-D	3.595	NA	0.343	0.362	No
	Impurity-E	3.535	NA	0.694	1.397	No
	Impurity-F	4.504	NA	0.210	0.413	No
	Impurity-A	4.731	NA	1.092	1.336	No
	Impurity-B	8.060	NA	0.335	0.528	No
	Impurity-C*	10.192	NA	7.130	0.428	Yes
Note: From the above table few process impurities M,N,R,H and C shows interference among one another and eluted at the similar RT about 10.2 minutes.						

Acceptance Criteria: Each peak needs to be properly resolved. Do not interfere with the Mesalamine peak of other peaks. NLT 0.99 is the recommended maximum purity for Mesalamine.

Conclusion: No interference peak was observed in the RT of standard peak.

3. Linearity: The achievement of linearity in an analytical method is determined to ensure a balanced relationship between the concentration of an analyte and the peak area of that analyte. Assessment of linearity was performed by carefully examining the signal and concentration plot of the sample analytes. Six solutions were analyzed over a concentration range of 5–72 µg/ml to confirm linearity.

S. No	**%Level**	**Concentration (ppm)**	**Peak Area**
01	12.5	5.076	496147
02	50	20.304	1956466
03	100	40.608	3819070
04	125	50.76	4639243
05	150	60.912	5491153
06	180	72.079	6478043
Correlation coefficient(r)			1.000
Regression coefficient (R^2)			0.999
Slope			88800
Y-intercept			117227
Residual sum squares			18686337681
Bias at 100%			3.07



Acceptance Criteria: Correlation coefficient should not be less than 0.999.

Conclusion: The Correlation coefficient is 0.999 with slope 88800 and Y-intercept 11722. Therefore, the HPLC method for Mesalamine is linear.

Accuracy: The accuracy of an analytical method refers to its ability to ensure the precise values of analytical results. To measure the accuracy of the analytical method, a solution must be applied to a sample that is completely free of the compound and analyzed according to the specified method. The accuracy of the analytical method was evaluated by mixing 20% to 150% of different levels of standard substances and checking the recovery rate of mesalamine.

S. No	Recovery Level	Sample	mg/mL added	mg/mL found	% Recovery	Average % recovery	%RSD
1	20%	Sample-1	8.005	7.979	99.7	99.1	0.6
		Sample-2	8.006	7.942	99.2		
		Sample-3	8.003	7.886	98.5		
2	100%	Sample-1	40.011	39.500	98.7	98.6	0.1
		Sample-2	40.007	39.431	98.6		
		Sample-3	40.009	39.450	98.6		
3	150%	Sample-1	60.010	59.761	99.6	99.8	0.2
		Sample-2	60.012	59.936	99.9		
		Sample-3	60.006	59.992	100.0		

Acceptance criteria: It should be between 98% and 102% of the recovery level.

Conclusion: According to the results of this evaluation, the accuracy of this method is 20 to 150% level

Precision: Precision is determined by the degree of agreement in a series of measurements obtained by repeatedly sampling the same sample under specified conditions. To evaluate precision parameter, one blank injection and follower by six standard drug injections were performed.

Table of Precision Data of Mesalamine

S. No	Sample Name		%Assay
01	Sample-1		100.4
02	Sample-2		101.9
03	Sample-3		101.8
04	Sample-4		101.9
05	Sample-5		100.7
06	Sample-6		101.3
Average		:	101.3
STDEV		:	065
%RSD		:	0.6

Acceptance Criteria: The % RSD should not be more than 2%

Conclusion: % RSD of precision was found to be 0.2

Stability solution: To obtain accurate and reliable results, it is important to properly store the test solutions, indicators, and reagents used in HPLC methods for a certain period of time. A day, a week or a month depending on special conditions. These procedures are designed to maintain operational integrity. First, standard and sample solutions are introduced into the HPLC system to evaluate the stability of the solutions, and new standards are determined after 24, 48, and 72 hours. A pattern similar to the first pattern was found in the surface response of the mesalamine peak during the standard preparation after 24 ,48 and 72 hrs.

Table of Standard solution stability Data of Mesalamine

Condition	Weight (mg)	Avg. Area	Similarity Factor
Fresh	50.22	3986345	---
Room temperature (After 24hrs)(25±3ºC)	50.23	4023339	1.01
Refrigerator (After 24hrs)(25±3ºC)	50.23	3984742	1.00
Fresh	50.34	3917251	0.98
Room temperature (After 48hrs)(25±3ºC)	50.23	3918545	0.98
Refrigerator (After 48hrs)(25±3ºC)	50.23	3929906	1.00
Fresh	50.38	3960320	1.01
Room temperature (After 72hrs)(25±3ºC)	50.23	4027536	1.02
Refrigerator (After 72hrs)(25±3ºC)	50.23	4052194	1.03

Condition	%Assay	Difference
Initial	100.4	--
Room temperature (After 24hrs)(25±3ºC)	100.9	0.5
Refrigerator (After 24hrs)(25±3ºC)	100.7	0.3
Room temperature (After 48hrs)(25±3ºC)	98.6	1.8
Refrigerator (After 48hrs)(25±3ºC)	99.6	0.8
Room temperature (After 72hrs)(25±3ºC)	99.9	0.5
Refrigerator (After 72hrs)(25±3ºC)	99.8	0.6

Acceptance Criteria: The % standard deviation from the peak area is limited to a maximum of 2.0.

Conclusion: From the observations, it was concluded that this pattern is stable for 48 hours at room temperature (25 ± 3ºC) and refrigerated (2ºC to 8ºC).

Robustness: The robustness of an analytical methodology denotes its capacity to maintain stability despite any proposed modifications to its parameters, even those that are minor, and to exhibit consistency throughout its practical application.

Parameteres	Changes	Avg.Area	RT	Tailing Factor	USP Plate	%RSD	%Assay
Flow rate	1.0mL/min	3904023	4.005	1.3	7429	0.3	100.4
	0.8mL/min	5009003	5.019	1.3	8770	0.1	99.3
	1.2mL/min	3299274	3.405	1.3	7343	0.3	99.4
Mobile phase Composition	MP-A (490:10) M-B(700:300)	3904023	4.005	1.3	7429	0.3	100.4
	MP-A (495:5) MP-(670:370)	3964363	3.910	1.2	6124	0.4	99.3
	MP-A (485:15) MP-(730:270)	3920744	4.033	1.2	6917	0.3	101.4
Mobile phase pH	pH.4.4	3904023	4.005	1.3	7429	0.3	100.4
	pH.4.6	369502	3.866	1.3	8845	0.0	101.5
	pH.4.8	4107254	3.971	1.3	7254	1.1	99.3
Acceptance criteria		NA	NA	NMT 2.0	NLT 2000	NMT 0.85	

Acceptance Criteria: The % amount found should be between 98% to 102%. % relative standard deviation should not be more than 2.0%

Conclusion: The test method was found to be robust against changes in flow rate, buffer pH, column temperature, and organic ratio.

Ruggedness : Two analyst conducted individual preparations and evaluations of 40 µg/mL solutions of mesalamine to validate the consistency of environmental and operational factors on different days. The peak areas of solutions with identical concentrations were measured on six separate occasions.

Acceptance Criteria: % amount found should be between 98% to 102%. % relative standard deviation should not be more than 2.0%

Ruggedness Conclusion:

Based on the observations made, it can be concluded that the developed high performance liquid chromatography HPLC technique is found to be effecti ve in quantifying mesalamine concentrations**.**

Forced Degradation Studies:

Additional evaluation of method specificity for Assay parameter was performed by peak purity testing of Drug product and Drug substance. Samples were treated under relevant stress conditions; this study allows verifying the effect of accelerated testing on the purity of the active ingredient, Mesalamine excluding the presence of extraneous co-eluting peaks.

The test is based on the evaluation of two key parameters of chromatographic software:

Purity Angle: A measure of spectral homogeneity calculated by comparing the spectra from each data point in an integrated .Peak against the peak apex spectrum. Purity Angle can range from 0 to 90°; small values indicate homogeneity

Purity Threshold: The Threshold values indicate the non-ideal contributions that affect Peak Purity testing, not due to an actual component absorbance difference in spectral shape but caused by noise and/or solvent. Purity Threshold can range from 0 to 90°; the larger the value, the lower is the sensitivity of the measurement.

If the value of Purity Angle is less than the Purity Threshold, the spectra are homogeneous within the noise of the measurements; therefore the peak is spectrally pure.

The active ingredient was well resolved (Resolution > 1.5) from any other interference and its chromatographic peak was found spectroscopically pure (Purity Angle < Purity Threshold).

Table of Degradation Study Results Summary

S. No	Stress	Condition	%Assay	%Degradation	Purity Angle	Purity Threshold
01	Control	Unstressed	100.2	0	0.292	0.756
02	Acid	0.1N,80oC,2hr	98.5	1.5	0.421	0.443
03	Base	0.1N,80oC,2hr	89.9	10.1	0.276	0.323
04	Peroxide	6%,80ºc,2hr	81	19	0.282	0.416
05	Water	80ºC,2hr	99.4	0.6	0.309	0.411
06	Thermal	80ºC,2hr	99.5	0.5	0.301	0.407

Observation and Conclusion:

The proposed method gave good resolution of Mesalamine and its degradants. The method is confirmed to be stable indicating capability of distinguishing the active pharmaceutical ingredient (API) from any degradation (decomposition products) formed during the defined storage conditions during the stability testing period.

Conclusion:

For routine analysis, it is useful to establish methods that allow rapid testing of large numbers of samples without the need for initial isolation. Lots of high-level data and very powerful and comprehensive analysis tools at your fingertips. The aim of this study was to develop a simple, precise, rapid and accurate HPLC method for the quantitative evaluation of mesalamine in mesalamine hydrochloride injections. The protocol involved the use of a 5 µm (250 × 4.6) mm Syncronis C18 column to equilibrate the stationary phase and the mobile phase in methanol with a buffer containing 0.5 mL of orthophosphoric acid diluted in 1000 mL of water in a ratio of 90:10 (V / V). The experimental procedure required a default gradient mode with a flow rate of 0.8 mL/min, a column temperature of 40 °C, and an injection volume of 5 µL. To identify mesalamine, photoelectric array detection (PDA) was performed in the 200–400 nm wavelength spectrum. The analysis takes only 8 minutes to complete. Additional assessments are conducted to assess the integration, visibility, accuracy, precision, accuracy, independence and reliability of the management system. This approach resulted in a linear relationship with a correlation coefficient (R2) of 0.999, a rise of 88,800, an intercept of 11,722, and a concentration range of 5 g/ml to 72 g/ml. The recovery of mesalamine ranged from 98.6 to 99.8. The precision of this method is estimated to be 0.65 standard deviation and 0.6 relative standard

deviation (RSD). One of the most popular features of this technology is its compatibility with mobile devices and stationary devices. The variability of variables, wavelengths, and differences in test results between different observers show the robustness and reliability of the approach. Coupling studies involving acidic, alkaline hydrogen, and oxidizing agents confirmed the specificity of the pathway. The measurements are different and different from the filter peaks, which show a significant difference in the retention time (RT) values. As a result, the proposed technology ensures full accuracy, precision, selectivity and availability of mesalamine determination at quality control (QC) level.

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References:

1. P.D. Sethi, Quantitative Analysis of drugs in Pharmaceutical Formulations,3rdedition,CBS publishers and distributors, New Delhi, 1997, 17-19.
2. K. A. Connors, A Text book of Pharmaceutical Analysis, 3rd edition, Interscience Publication, New York, 1999, 611.
3. Nuzhath Fathima*, Brahmaiah Marineni1 , Dr. G. Abdul Huq2 and Prof. P. Ravindra Reddy.Development and Validation of RP-HPLC Method for Determination of Related Substances of Medetomidine in Bulk Drug. ijprpjuly 2016 Vol.:6, Issue:4.
4. Trivedi rakshit kanubhai*, patel mukesh c and kharkar amit r.,Determination of Mesalamine Related Impurities from Drug Product by Reversed Phase Validated UPLC Method. E-Journal of Chemistry http://www.e-journals.net 2011, 8(1), 131-148
5. Darak, Venugopal; Karadi, Arvind B.; Appal raju, S.; Arshad, M. D.; Ganure, Ashok L.,
6. Development and validation of hplc method for determination of mesalamine in tablet dosage forms. : Pharma Science Monitor . Jan2012, Vol. 3 Issue 1, p74-81. 8p,
7. S. H. Gatkal*, P. R. Mhatre, V. V. Chopade and P. D. Chaudhari.,Development and validation of a stability indicating assay method of mesalamine by using different stress degradation conditions, IJPSR, 2013; Vol. 4(1): 401-406, 5. P Ravi; Padmavathi, Y; Niveditha, P; N. Raghavendra Babu,
8. Development and Validation of Difference Spectrophotometric Method for the Quantitative Estimation of Mesalamine in Bulk Drug and Dosage formskumar.,Asian Journal of Pharmaceutical Analysis; Raipur Vol. 7, Iss. 4, (Dec 2017),
9. Fatima Altayib Alasha Abdalla and Abdalla Ahmed Elbashir,Development and Validation of Spectrophotometric Methods for the Determination of Mesalazine in Pharmaceutical Formulation, Med chem 2014, 4:3,
10. . a.k. moharana*, m. banerjee, s. panda, j.n. muduli.,Development and validation of uv spectrophotometric method for the determination of mesalamine in bulk and tablet formulation,IJPPS, Vol 3, Issue 2, 2011,
11. alok kumar moharana*1, mrityunjay banerjee2, chinmaya keshari sahoo3 and nalini kanta sahoo .,development and validation of rp-hplc method for mesalamine,ajpcrvol 4, suppl 2, 2011,
12. Nalini Kanta Sahoo a, Madhusmita Sahu a , Podilapu Srinivasa Rao a , Goutam Ghosh., Validation of stability indicating RP-HPLC method for the estimation of mesalamine in