Development and Optimization of RP-UHPLC Method for Mesalamine Through QbD Approach

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ABSTRACT: The current study aimed at developing and optimizing a prompt, simple and efficient RP-UHPLC method based on Quality by Design (QbD) for analyzing mesalamine. Experimental design for developing the method was performed capitalizing a 3^2 full factorial design in Design Expert® software (Version 12, Stat-Ease Inc., USA) where the percentage of methanol in the mobile phase and flow rate of the mobile phase were considered as independent factors and studied at three levels. Retention time, tailing factor and theoretical plate count were recorded as responses to the experiment. Mesalamine was analyzed using a reversed-phase C_{18} column (5µm, 150 ×4.6 mm) supported by a photodiode array plus (PDA+) detector with detection at 214 nm. The optimized method involved the use of a mobile phase of *p*H=7.4 phosphate buffer: methanol (63.5: 36.5, v/v) and a flow rate of 1.1 ml/min. Responses recorded during experimentation exhibited an error of -0.24%, 0.376% and -0.659% from predicted values of retention time, tailing factor and theoretical plate count, respectively. Experimental models adopted for the development of the method were found statistically significant (*p*-value <0.05). According to ICH Q2 (R1) guidelines, the method was also found to be robust, highly sensitive, specific, accurate, precise and linear.

Keywords: Mesalamine, QbD, flow rate, retention time, RP-UHPLC

INTRODUCTION

Ulcerative Colitis (UC), a type of inflammatory bowel disease (IBD), can cause swelling, ulcerating and loss of function of the large intestine leading to colon cancer.[1-4] First-line treatment option for mild to moderate UC involves the use of mesalamine, also known as 5-aminosalicylic acid (5-ASA) or mesalazine (Figure 1).[1]

The International Conference on Harmonisation (ICH) has implicated the term QbD in 2009 for human use and since then it has gained much popularity in pharmaceutical development.[5,6] Continuous improvement is the basis of the QbD approach.[7,8] To obtain robust processes, QbD helps to establish a design space.[7] The aims of using the QbD approach are to give analytical scientists a clear concept of the process parameters and their working principles.[8]

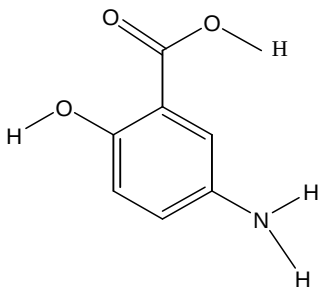

Figure 1. Structure of mesalamine (5-aminosalicylic acid or mesalazine).

The chromatographic analytical method recommended for mesalamine in USP could be criticized due to higher cost, time-consuming and involvement of complex procedures.[9] Apart from the USP method, several other methods have been also developed for the analysis of mesalamine.[11,12] Earlier, the retention time (RT) of mesalamine has been reported as 5.92 min by S. Gatkal *et al.*[13] Limit of detection (LOD) and limit of quantification (LOQ)

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were reported as 0.807 µg/ml and 2.44 µg/ml, respectively, by M. Banarjee *et al.* in their method.[11] The current study focuses on developing an optimized, easy, rapid and more sensitive analytical method through QbD approach for the analysis of mesalamine. The optimized method was validated according to ICH Q2 (R1) guidelines.[14]

MATERIALS AND METHODS

Chemicals and reagents. Mesalamine powder (assay by HPLC 100.5%; BEC Chemicals, India) was gifted generously by the UniMed UniHealth Pharmaceuticals Ltd. (Dhaka, Bangladesh). Methanol used in the experiment for preparation of the mobile phase was of HPLC grade and purchased from Scharlau, Spain. Monobasic and dibasic potassium phosphate, employed for the preparation of phosphate buffer, were also procured from Scharlau, Spain. Water used in the process was purified using the LaboStar® RO DI system (Evoqua, Germany).

Chromatographic conditions. The RP-UHPLC system consisted of Perkin Elmer Flexar series (UK) integrated with auto-sampler, FX-15 binary pump, vacuum degasser, column oven and PDA plus detector was used to analyze mesalamine. The data were analyzed using Chromera Manager Software (Version 4.0). Chromatographic separation was achieved with the isocratic elution technique on the Brownlee Analytical C_{18} column with a 5 µm particle size and 150 mm length × 4.6 mm internal diameter. The injection volume was 12 µL and the column temperature was fixed at 30˚C. The detection wavelength was at 214 nm. The mobile phase used for elution is composed of a combination of *p*H=7.4 phosphate buffer and methanol at the ratio of 63.5: 36.5, v/v and a flow rate of 1.1 ml/min, with a total run time of 15 minutes.

Standard stock solution preparation. Mesalamine stock solution was prepared at a concentration of 500 µg/ml. Accurately weighed 25 mg of mesalamine was taken in a 50 ml volumetric flask, and about 30 ml of solution vehicle containing phosphate buffer and methanol (60:40, v/v) was added. The drug was dissolved by sonicating for 5 minutes, and the volume was adjusted to the mark with the same vehicle. The stock solution thus prepared was further diluted with the same solvent system to get the desired concentrations.

Preparation of mobile phase. Accurately weighed 3.636 gm of monobasic potassium phosphate and 1.242 gm of dibasic potassium phosphate were transferred to a 1000 ml beaker, and the volume was adjusted to the mark with distilled water. Filtration of the solution was done using 0.45 µm filter paper followed by degassing through sonication. The HPLC grade methanol was also filtered and degassed in the same way.

Design of experiment. Design Expert® software (Version 12, Stat-Ease Inc., USA) was used for the experimental design and statistical analysis of data. The independent variables were the methanol concentration in the mobile phase (A, % v/v) and flow rate (B, ml/min). And the dependent variables were retention time (RT) (R1, min), tailing factor (TF) (R2) and theoretical plate count (TP) (R3, N). Utilizing 3^2 full factorial design, nine experimental runs were constructed considering the independent factors at three levels (i.e. low, medium and high). During optimization of the method, RT was kept at minimum constraints level to develop a rapid analytical method for mesalamine. The tailing factor, a measure of peak tailing, was also kept at minimum constraint levels to obtain sharp, symmetric and sensitive peaks. Theoretical plate count, an index of column efficiency, was kept at maximum constraint levels during optimization, as its larger values indicate higher column efficiency. All the levels and constraints were determined from sufficient preliminary trials. Optimization of the developed method and the effect of variables on responses were determined with the aid of Design Expert® software.

Method validation. Validation of the developed method was performed as per ICH Q2 (R1) guidelines. The system suitability, specificity, linearity, accuracy, precision, sensitivity, ruggedness and robustness were reported.

RESULTS AND DISCUSSION

Analysis of responses. For response 1 (RT), after analyzing the experimental values a linear mathematical model was recommended by Design Expert® software. The model F-value of 414.36 implies the significance of the model and there is a minimal (0.01%) possibility of this large F-value occurring due to noise. *P*-values confirm that factor B plays a significant role in the retention time of mesalamine (Table 1). The projected R^2 of 0.982 is in reasonable agreement with the adjusted R^2 of 0.990, as the difference is less than 0.2. The signal-to-noise ratio was determined by adequate precision. A ratio of 42.487 indicates an adequate signal, as a ratio greater than 4 is highly desirable. The effects of variables on response 1 are presented in Figures 2(a) and 2(b).

Table 1. ANOVA and regression equation of responses.

ANOVA for the responses									
	R1			R2			R3		
Source	SS*	F	P	SS	F	P	SS	F	P
Model	0.210	414.3	$< 0.1^4$	0.096	38.76	0.006	60931.3	53.69	0.0039
A	0.0004	1.65	0.2465	0.032	64.57	0.004	22940.1	101.08	0.002
B	0.209	827.1	$< 0.1^4$	0.0007	1.50	0.307	400.17	1.76	0.276
AB				0.003	6.65	0.082	2652.2	11.69	0.042
A^2				0.060	121.02	0.002	34936.1	153.93	0.001
B^2				0.000	0.049	0.838	2.72	0.012	0.920
Residual	0.0015			0.002			680.86		
Cor Total	0.211			0.098			61612.2		
Fit statistics				Regression equation					
Source	R1	R2	R3	R1 =	+3.782-0.002A-1.867B				
Std. Dev.	0.016	0.022	15.06	R2 =	$+8.816-0.443A+1.201B-0.058AB+0.007A^2+0.35B^2$				
Mean	1.86	1.47	2378.5						
C.V. %	0.856	1.52	0.633	R3 =	$-2837.3889+330.933A-1487.5B+51.5AB-5.287A^2-116.667B^2$				
AP**	42.48	15.12	17.865						

*SS-Sum of squares; **AP-Adequate precision.

For response 2 (TF), a quadratic mathematical model was suggested based on maximum adjusted R^2 and predicted R^2 values. The significance of the model is confirmed by the model F-value of 38.76. There is only a 0.63% possibility of occurring this large F-value from noise. From *p*-values, it was found that both models A and A^2 are significant terms (Table 1). The difference between the predicted R^2 (0.820) and adjusted R^2 (0.959) is less than 0.2, indicating a reasonable agreement between them. The value of adequate precision (15.123) reflects an optimal signal intensity. The effects of variables on response 2 are presented in Figures 2(c) and 2(d).

For response 3 (TP), a quadratic model was also suggested. Here, the F-value of the model (53.69) entails that the model is significant. The possibility of occurring of this large F-value from noise is only 0.39%. From *p*-values, it was found that in this case models A, AB, and A^2 are significant (Table 1). Likewise in responses 1 and 2, the predicted R^2 value (0.869) found here is in rational agreement with the adjusted R^2 value (0.971). An adequate precision value (17.865) shows a sufficient signal intensity. The effects of variables on response 3 are highlighted in Figures 2(e) and 2(f).

Optimization of the method. Out of 12 solutions provided by Design Expert® software, method involving the use of variables A as 36.56% and B as 1.1 ml/min with the desirability of 0.991, which was the highest among others, was taken as the

optimized method. Experimental values of A and B were set to 36.5% and 1.1 ml/min, respectively. Experimental values of the responses observed during the study and percent error from predicted values are mentioned in Table 2.

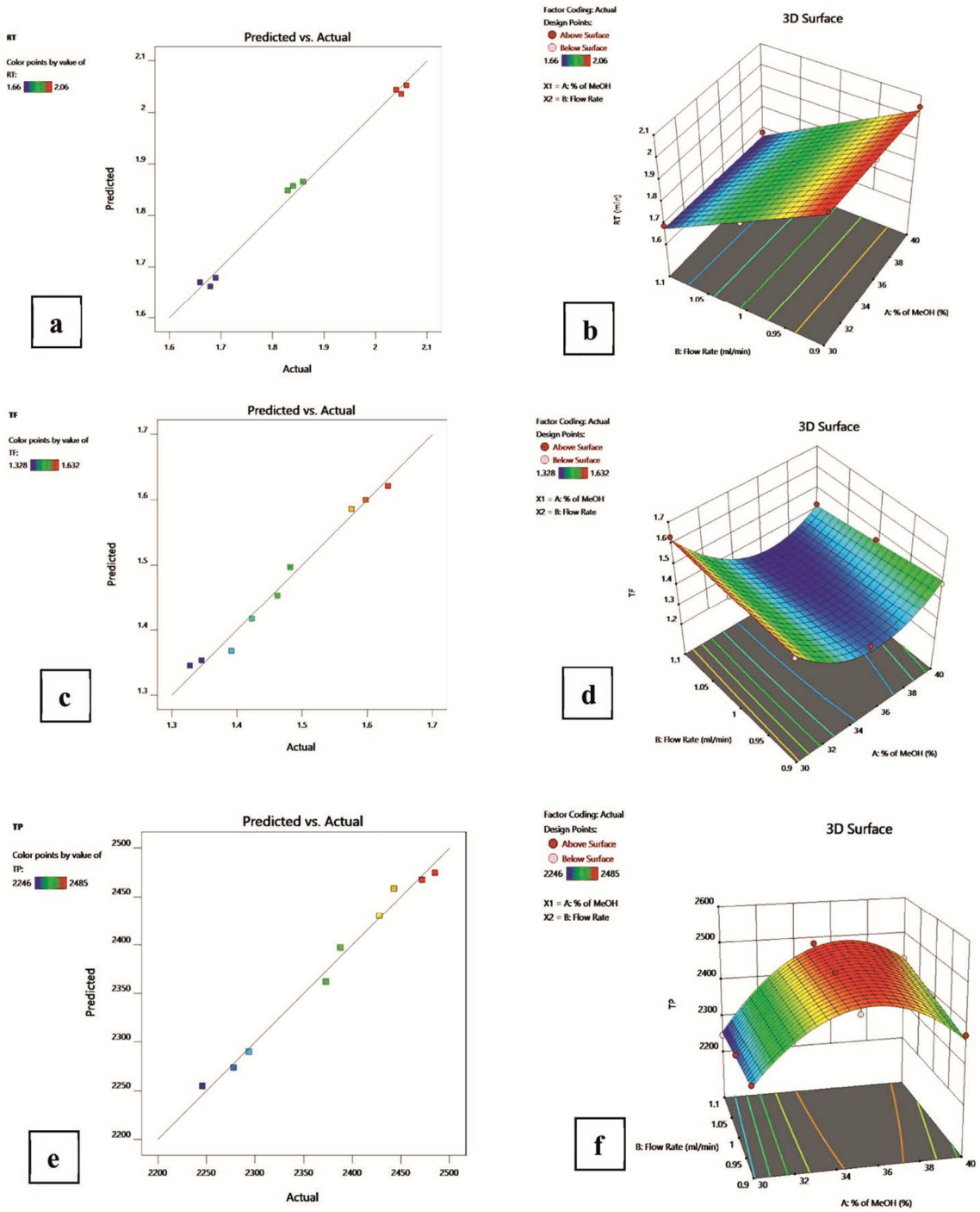

Figure 2. (a) Predicted vs actual plot for response 1 (RT). (b) 3D surface plot depicting the effect of independent variables on response 1 (RT). (c) Predicted vs actual plot for response 2 (TF). (d) 3D surface plot for the effect of independent variables on response 2 (TF). (e) Predicted vs actual plot for response 3 (TP). (f) 3D surface plot showing the effect of independent variables on response 3 (TP).

Table 2. Optimized method and predicted error of responses.

Method	A (%)	B (ml/min)	R1 (RT, min)	R2 (TF)	R3 (TP)
Predicted values	36.562	1.1	1.667	1.330	2488.91
Experimental values (Mean±%RSD)	36.5	1.1	1.663 ± 0.21	1.335 ± 0.367	2472.5 ± 0.425
*Predicted error (%)			-0.240	0.376	-0.659

*Predicted error = (Experimental values – Predicted values)×100 / Predicted values.

Validation study. Results obtained from the validation study are discussed below.

System suitability. The system suitability of the method was measured using values of six replicates of working standard solution of mesalamine at a concentration of 100 µg/ml. The results were presented as Mean±% RSD. The chromatographic parameters were 8390132±0.098, 1.335±0.367, 2472.5±0.425 and 1.663±0.21 for peak area, tailing factor, theoretical plate count and retention time, respectively. The % RSD values for all the parameters were within the acceptable limits (<2%).

Specificity. A good resolution was obtained for mesalamine in working standard and sample solution under different conditions, and the chromatogram of the blank ensured no interference within the sample retention time (Figures 3a and 3b).

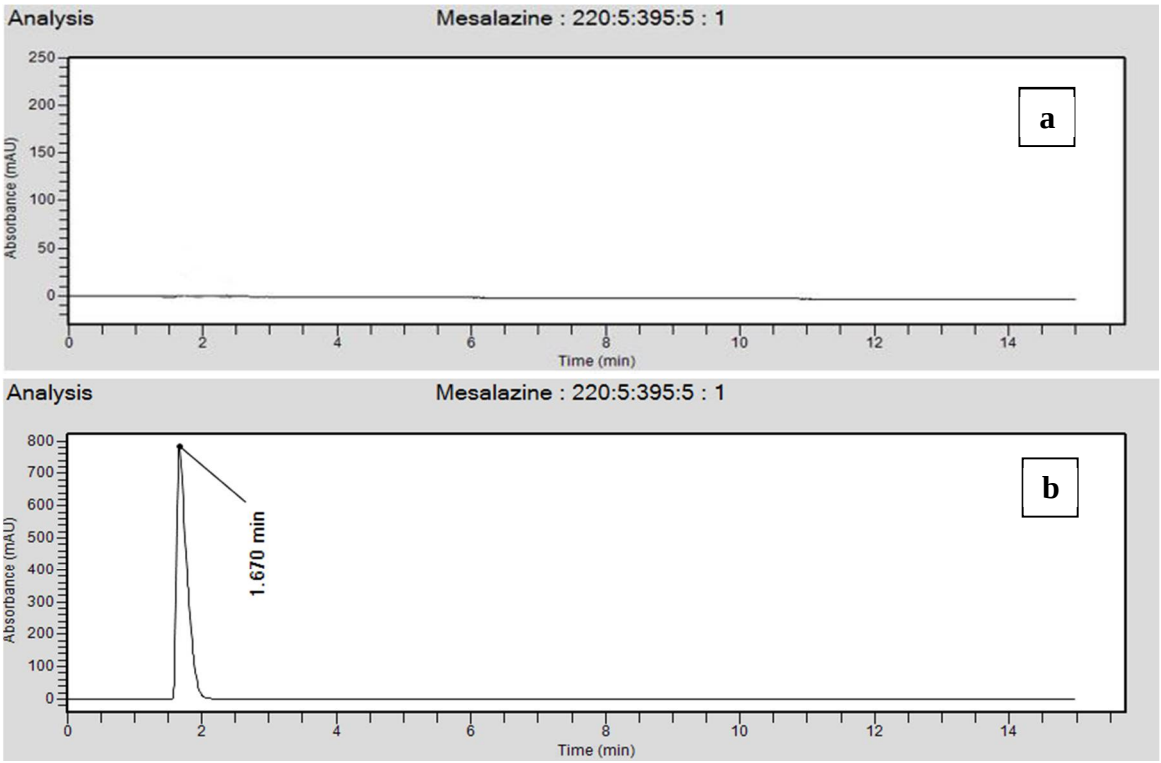

Figure 3. (a) Chromatogram of a blank sample. (b) Chromatogram of the standard solution of mesalamine (100 µg/ml).

Linearity. The linearity study was performed for mesalamine within the concentration range of 80-120 µg/ml. The correlation coefficient (R^2) value was 0.9987, which confirms the linearity of the method (Figure 4).

Accuracy. The accuracy of the method was investigated by % recovery experiments. The calculated recovery values of mesalamine (99.89 ±0.34% to 100.365±0.14%) were within the acceptable limits (Table 3).

Precision. The intra-day and inter-day precision analyses are listed in Table 3. The results confirm no significant differences between assay results within a day or between days.

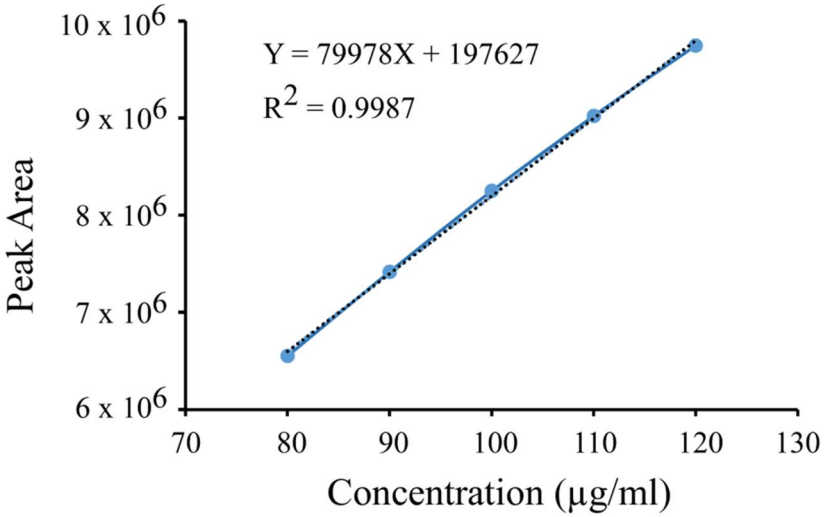

Figure 4. Linearity of the quantitation of mesalamine from standard solutions (range: 80-120 µg/ml).

Table 3. Validation study of the developed method for analysis of mesalamine.

Type of study	Amount added (µg/mL)			Mean % recovery	% RSD
Accuracy	80			100.36	0.14
	90			100.013	0.17
	100			99.991	0.04
	110			99.991	0.08
	120			99.893	0.34
Type of study	Spike level (%)	Type of precision		Mean % recovery	% RSD
Precision	100	Intra-day		99.96	0.04
		Inter-day	Day 1	99.98	0.05
			Day 2	100	0.056
			Day 3	99.96	0.03
Type of study	Type of ruggedness	Amount added (µg/ml)		Mean % recovery	% RSD
Ruggedness	Analyst 1	100		99.916	0.147
	Analyst 2	100		99.675	0.464
Type of study	Parameters	Variations	Amount added (µg/ml)	Mean % recovery	% RSD
Robustness	Mobile phase flow rate (ml/min)	0.9	100	99.83	0.26
		1	100	99.61	0.56
		1.1	100	99.88	0.19
	Mobile phase (Buffer : Methanol)	60:40	100	99.673	0.32
		65:35	100	99.945	0.098
		70:30	100	99.733	0.351

Sensitivity. The limit of detection (LOD) and limit of quantification (LOQ) of mesalamine by the proposed method were found to be 0.04 µg/ml and 0.13 µg/ml, respectively, through several trials with a diluted working standard solution of mesalamine USP (Figures 5a and 5b).

Ruggedness. The ruggedness of the mesalamine quantitation method was conducted by performing the analysis with different analysts. The % RSD of the ruggedness study varied from 0.147% to 0.464%, indicating that the current method was rugged (Table 3).

Robustness. The robustness of the proposed method was assessed by changing the mobile phase composition and flow rate parameter. The percentage of RSD values were within the acceptable ranges (0.098% to 0.56%), showing that the current method was robust (Table 3).

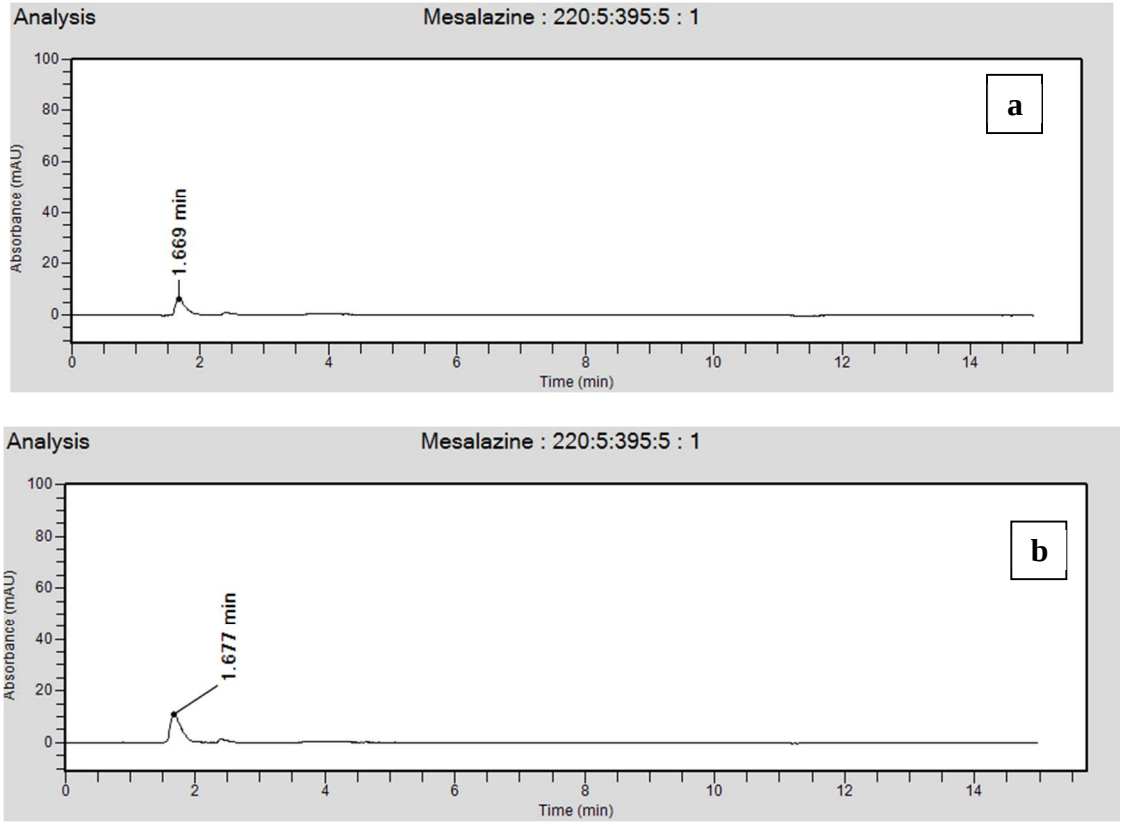

Figure 5. (a) Chromatogram for LOD (0.04 µg/ml). (b) Chromatogram for LOQ (0.13 µg/ml).

In this study, an RP-UHPLC analytical method is developed for mesalamine through the QbD approach which has not been reported previously so far by others. Retention time obtained from the developed method was 1.663±0.21 min, with an estimated error of -0.24% from the predicted value. The errors observed from the predicted values for all other responses were also within the acceptable limit (<2%). Moreover, the method was found to be highly sensitive with LOD and LOQ values of 0.04 µg/ml and 0.13 µg/ml, respectively. So, this could be claimed that the developed method provides a faster, more sensitive and structured way for the analysis of mesalamine by the RP-UHPLC system.

CONCLUSION

A rapid, selective and sensitive RP-UHPLC analytical method is developed through the QbD approach for the analysis of mesalamine. The

optimized method requires the use of 36.5% methanol in the mobile phase and a flow rate of 1.1 ml/min. The optimized method was found to be specific, robust, linear and precise with low detection and quantitation limits. This suggests that the developed method could be suitable for the analysis of mesalamine in bulk drug, and pharmaceutical formulations and could also be used for *in vivo* quantitation.

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AUTHORS CONTRIBUTIONS

The conceptualization, supervision, manuscript editing and fund acquisition were done by ASSR. The investigation, methodology, data curation, original manuscript writing and fund acquisition were performed by DKS. The statistical analysis, data curation and manuscript preparation (review and editing) were performed by UK, MAH, and RR.

CONFLICT OF INTEREST

The authors declare 'no conflict of interest'.

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