World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2025
Volume: 4; Issue: 3
Page: 1567-1589

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RESMETIROM USING UV SPECTROSCOPY AND RP-HPLC

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Article Received: 22 May 2025 | | Article Revised: 12 June 2025 | | Article Accepted: 03 July 2025

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DOI: https://doi.org/10.5281/zenodo.15879822

How to cite this Article: *Shish A. Khan, Sabeeha A. Kamal, Amaan Khan, Megha Pandagale (2025) ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RESMETIROM USING UV SPECTROSCOPY AND RP-HPLC. World Journal of Pharmaceutical Science and Research, 4(3), 1567-1589. https://doi.org/10.5281/zenodo.15879822

ABSTRACT

The current research focuses on developing and validating precise, straightforward UV spectrophotometric and reverse-phase high-performance liquid chromatographic (RP-HPLC) methodologies for quantitative determination of Resmetirom in pure form. The UV spectrophotometric method was established using a phosphate buffer and acetonitrile mixture (50:50 v/v) as solvent. Maximum absorbance occurred at 225 nm. The linearity range was determined to be 16-24 microgram per millilitre, and the method was validated for parameters including linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) according to ICH guidelines. For the RP-HPLC procedure, separation was achieved using a Phenomenex Kinetex XB-C18 column (150 x 4.6 mm, 5 µm) with phosphate buffer and acetonitrile (60:40 v/v) as mobile phase, at a flow rate of 1.0 mL/min and detection at 225 nm. Resmetirom exhibited a retention time of 3.41 minutes. The method demonstrated excellent linearity (R^2 = 0.9998), precision (intra-day %RSD = 0.94, inter-day %RSD = 1.94) and accuracy (recovery values of 100.07%, 100.64% and 100.07%). LOD and LOQ were determined as 0.56 µg/mL and 1.69 µg/mL respectively. Both methodologies satisfied validation requirements, with the HPLC method showing superior accuracy, making it particularly suitable for routine quantitative analysis. These developed methods can be utilized to evaluate Resmetirom efficacy, as analytical data and validated results are currently scarce in the literature.

KEYWORDS: Resmetirom, UV Spectrophotometry; RP-HPLC; validation parameters.

INTRODUCTION

Resmetirom is a novel, selective thyroid hormone receptor-β (THR-β) agonist currently in investigation for the therapeutic effects on non-alcoholic steatohepatitis (NASH) and associated metabolic disorders. By selectively

targeting hepatic THR-β receptors, Resmetirom promotes lipid metabolism and reduces liver fat without eliciting the adverse cardiovascular effects associated with non-selective thyroid hormone activity. As research and clinical interest in Resmetirom continues to grow, the requirement of accurate, reliable, and validated analytical methods for its quantification has become increasingly important.[1]

Analytical techniques play a pivotal part in the development, quality control, and regulatory evaluation of pharmaceutical compounds. Among these, UV spectrophotometry is widely appreciated for its simplicity, cost-effectiveness, and rapid execution in the analysis of drug substances. However, UV methods are sometimes limited by their response in its sensitivity and its specificity, especially in complex integrates or at low concentrations.

The use of high-performance liquid chromatography (HPLC) especially in reverse-phase (RP-HPLC) format, has turned out to be the gold standard in pharmaceutical analysis because of its precision, can be replicated frequently, separates and quantifies effectively. even with contents of impurities or degradation products. The drug is very adaptable to many properties of drugs and is hence well adapted in routine and applications on research based thought.[2] There is not much information about Resmetirom even though it can become an important chemical. The well-proven methods of analysis of its quantitative analysis are in the public domain. In this study, there is an expectation to design and create solid UV spectrophotometric and RP-HPLC procedures were used in the estimation of Resmetirom drug in the pure form. The suggested techniques are considered with regard to International Council for Harmonisation (ICH) recommendations to keep them at par with its future viability of being applied in the aspects of pharmaceutical quality control research settings.[3]

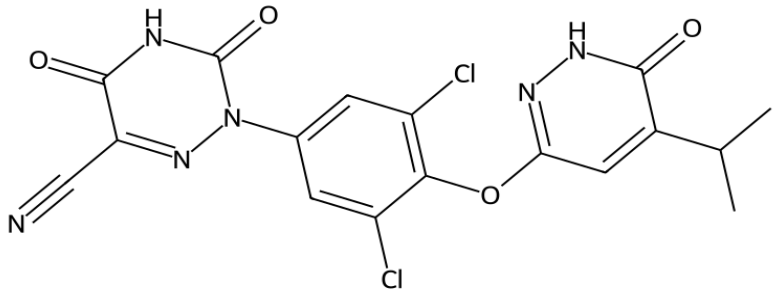

Fig. No. 1: Resmetirom Structure.

Drug Characterization

Resmetirom was initially characterized based on its organoleptic properties, including physical characteristics, colour, odour, and its taste. The drug is typically white to yellow powder and odourless, with a bitter taste. These physical attributes provide preliminary information about the drug's identity and purity. The melting point was also determined and found to be in close agreement with reported literature values, with an observed range of 320°C to 323°C compared to the reported 321°C to 324°C, confirming the sample's integrity and purity. Regarding solubility, Resmetirom is soluble in organic solvents which includes dimethyl sulfoxide (DMSO), ethanol, and dimethylformamide (DMF), but also exhibits poor solubility in water. These physicochemical characteristics are critical for developing suitable analytical methods and formulations.

MATERIALS AND METHODS

Instrumentation

The high-performance liquid chromatography (HPLC) analysis was performed using an Agilent Technologies 1260 Infinity II system with a 1260 Quaternary Pump VL (G7111A) that includes a degasser and mixer for solvent delivery. The sample injection was performed through a 1260 vial sampler with an autosampler unit (G7129A), ensuring consistent and accurate sample introduction. Detection was achieved using a 1260 Diode Array Detector (DAD WR, G7115A), which provided high sensitivity and precise wavelength monitoring.

Chromatographic isolation was accomplished using a Phenomenex Kinetex XB-C18 column (150 × 4.6 mm, 5 µm), supplied by Agilent Technologies. For sample and solvent filtration, a Qualisil Nylon membrane syringe filter (0.45 µm, 25 mm) was employed along with a Pall Nylon 6,6 membrane solvent filter (0.45 µm, 47 mm) for mobile phase preparation. Filtration was performed using a Borosil all-glass 47 mm filter assembly. Additional equipment used during sample preparation included a Labman Scientific ultrasonic bath for degassing and dissolving, and an Aczet CY224C electronic analytical balance for accurate weighing of materials.[4]

Chemicals and Reagents

The chemicals and reagents procured and used in the study were of analytical grade. Potassium dihydrogen phosphate and potassium hydroxide, both used for buffer preparation, were obtained from Thomas Baker (Chemicals) Pvt. Ltd. Acetonitrile, used as the organic phase in the mobile phase, was procured from Qualigen, Thermo Fisher Scientific India Pvt. Ltd. The reference standard of Resmetirom (pure drug) was supplied by BLD Pharmatech (India) Pvt. Ltd.

Preparation of Standard and Sample Solutions (UV Spectrophotometry)

A stock solution of Resmetirom was prepared by accurately weighing 10 mg of pure drug and transferring it to a 100 mL volumetric flask. The drug was initially dissolved in approximately 50 mL of a 1:1 proportion mixture of phosphate buffer and acetonitrile. After complete dissolution, the volume was adjusted to the calibration mark using the same solvent mixture to achieve a final concentration of 100 µg/mL. From this stock solution, a series of standard solutions was generated by pipetting appropriate volumes into separate 10 mL volumetric flasks and diluting to volume with the buffer-acetonitrile mixture to obtain concentrations ranging from 16-24 µg/mL. These solutions were utilized to construct a calibration curve and evaluate method linearity. For the sample solution preparation, a precisely weighed quantity of Resmetirom equivalent to 10 mg was transferred to a separate 100 mL volumetric flask and dissolved in the 1:1 phosphate buffer-acetonitrile mixture to a volume of 100 mL. The solution was subsequently filtered through a 0.45 µm nylon syringe filter and diluted appropriately to ensure the concentration fell within the established linearity range for UV-spectroscopy analysis. Absorbance measurements of both standard and sample solutions were determined at 225 nm using a UV-visible spectrophotometer against a blank prepared from the same solvent mixture.

Preparation of Standard and Sample Solutions for HPLC

Measurement of Standard Stock Solution (SSS-I)

Weigh correctly 2 mg of Resmetirom and add it to 10 mL volumetric flask. Add 5 mL of the diluent (a solution of phosphate buffer acetonitrile). and sonicate 5min in order to have a total dissolution. next, bring up the current volume to 10 mL using the same deuterium to acquire a goal stock concerning the stock solution. and at a concentration of 200 0g/mL. Using this stock solution (SSS-I), pipette 1.0 mL into 10 mL volumetric flask and add pipette 5 mL of diluent

and vortex the mixture followed by pipetting to the mark with the diluent to obtain a working standard solution of Resmetirom at 20 0g/mL concentration.

Mobile Phase Buffer preparation

Dissolve 6.8 g potassium dihydrogen phosphate in about 700 ml of water to prepare the buffer. 1 Litre of HPLC grade water stir vigorously and titrate the mixture to pH 5.7 with the aid of 5 M potassium hydroxide solution.

Preparation of Mobile Phase: Put 600 mL of the prepared phosphate buffer and 400 mL of HPLC-grade acetonitrile in a solvent bottle and then thoroughly mix. Sift the mixture on a 0.45 nu membrane filter to remove the particulates and sonicate at 15 min before use.[5]

Chromatographic Conditions

The chromatographic test was done utilizing a Phenomenex Kinetex XB-C18 the stationary phase as column (150 x 4.6m 5 mic. particle). The mobile phase was comprised of phosphate buffer and acetonitrile in 60:40 percent proportion. v/v ratio. It was detected at wavelength 225 nm with the help of diode array detector. The column was held at a flow rate of 1.0 mL/min. The oven temperature is being fixed at 30 °C. Each analysis was performed using a 10 0L injection volume. Sample and standard diluent was made up of a mixture of phosphate buffer and acetonitrile used in a proportion of 50:50 % v/v. Every run was done with cumulative 10-minute run time so as to provide a sufficient separation leaching of Resmetirom.

Optimization of Chromatographic Conditions

The chromatographic required specifications for Resmetirom was optimized by varying the mobile phase composition while keeping other parameters constant, such as column temperature (30°C), flow rate (1.0 mL/min), and detection wavelength (250 nm). Four different buffer-to-acetonitrile ratios were tested: 50:50, 55:45, 60:40, and 65:35 (% v/v). Resmetirom response on increasing the buffer proportion shows increment of the retention time (RT) of Resmetirom, allowing better separation. The retention time shifted from 1.90 minutes at 50:50 to 6.39 minutes at 65:35 buffer-to-acetonitrile ratio. Peak purity remained excellent (1.00) throughout all trials, indicating no interference or co-eluting peaks. Peak symmetry and tailing factors (TP and ASY) were acceptable in all trials, with slight improvements noticed at 60:40 buffer-to-acetonitrile ratio. However, at the highest buffer composition (65:35), peak broadness increased, leading to longer run times without significant gains in resolution. Therefore, the 60:40 mobile phase ratio was selected as the optimal condition, balancing efficient separation, peak shape, and reasonable analysis time.[6] Refer Table no. 1

Table No. 1: Optimization Trials of Mobile Phase Composition for Resmetirom by HPLC.

Trial No.	Mobile Phase (Buffer: ACN, % v/v)	Diluent (Buffer: ACN, % v/v)	Column Temp (°C)	Wave length (nm)	Flow Rate (mL/ min)	RT (min)	Tailing Factor (TP)	Asymmetry (ASY)	Peak Purity	Observation
1	50:50	50:50	30	250	1.0	1.90	4820	0.97	1.00	Peak eluted early before 2 minutes
2	55:45	50:50	30	250	1.0	2.35	5125	1.02	1.00	RT, TP and ASY increased with 5% more uffer
3	60:40	50:50	30	250	1.0	3.41	5282	1.05	1.00	RT increased by ~1 min; TP and ASY table
4	65:35	50:50	30	250	1.0	6.39	5238	1.04	1.00	RT increased ~3 min; peak broadness increased

RESULTS AND DISCUSSION

A. UV spectrometry

1. Determination of λmax

The λmax (Wavelength) of pure drug Resmetirom was found to be at 225 nm in the Diluent.

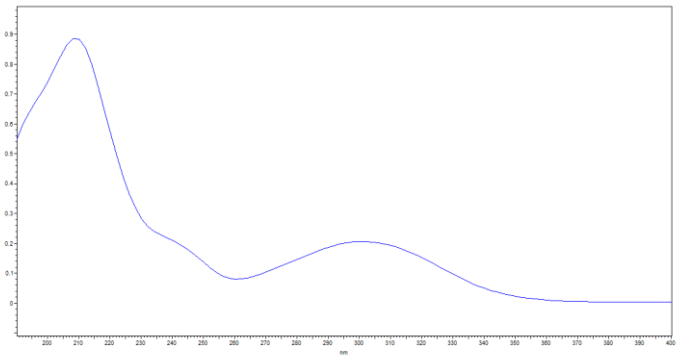

Fig. No. 2: Spectrum of pure Resmetirom Drug.

2. Linearity and Range

From the standard stock solution of Resmetirom, various working standard of solutions (16 – 24 µg/ml) were prepared using ethanol as solvent and the absorbance at 225 nm was taken, a linear table is shown. presented in table no. 2. The calibration curves are visualized fig no.3.

Table no. 2: Linearity Study.

% Level	Conc (ug/ml)	Absorbance
80	16	0.262
90	18	0.341
100	20	0.419
110	22	0.508
120	24	0.596

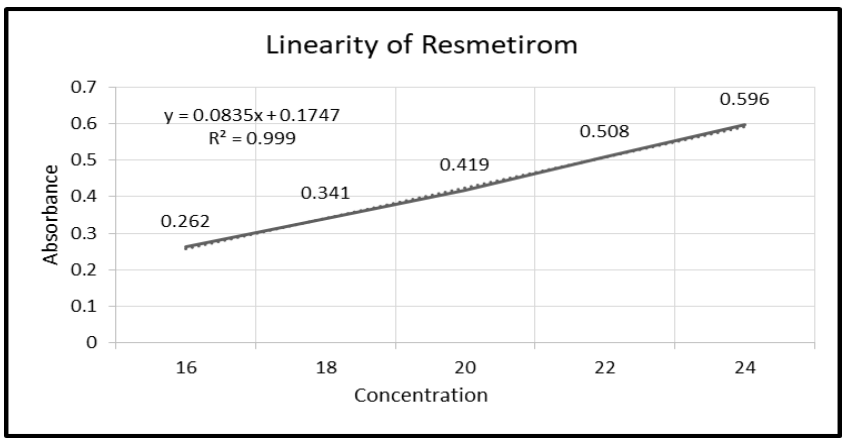

Fig. no. 3: Linearity of Standard Resmetirom.

3. Accuracy

Recovery investigations were conducted to confirm the accuracy of the established methodology. Initial sample analysis was performed, followed by the introduction of specified standard drug concentrations (80%, 100%, and 120%), after which recovery assessment was undertaken. (Table no.3)

Table no. 3: Recovery studies of Resmetirom.

Sample ID	Reps	Spiked Conc. (ng/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	15.84	0.262	12.58	79.39	79.60	0.349909	0.44
	Rep 2	15.84	0.262	12.58	79.39			
	Rep 3	15.84	0.264	12.67	80.00			
100%	Rep 1	19.80	0.412	19.78	99.88	99.88	0.242424	0.24
	Rep 2	19.80	0.413	19.82	100.12			
	Rep 3	19.80	0.411	19.73	99.64			
120%	Rep 1	23.76	0.596	28.61	120.40	120.27	0.233273	0.19
	Rep 2	23.76	0.596	28.61	120.40			
	Rep 3	23.76	0.594	28.51	120.00			

4. Precision

The analytical method was developed through examination of various Resmetirom standard replicates. Each solution underwent triplicate analysis to document any intraday and interday result variations. The results obtained intraday and interday are presented respectively in Table No.13.

Table no. 4: Intra-day & Inter-day precision of Resmetirom

Condition	Sample ID	Absorbance
Morning	WS	0.415
Evening	WS	0.411
AVG		0.413
STDEV		0.0028
% RSD		0.68
Day 2	WS	0.402
AVG		0.407
STDEV		0.0067
% RSD		1.63

5. Limit of Detection & Limit of Quantification

Regression Statistics	
Multiple R	0.999510561
R Square	0.999021361
Adjusted R Square	0.998695148
Standard Error	0.004771443
Observations	5

ANOVA

Table no. 5: Result of LOD & LOQ.

	df	SS	MS	F	Significance F
Regression	1	0.0697225	0.0697225	3062.481698	1.29972E-05
Residual	3	6.83E-05	2.27667E-05		
Total	4	0.0697908			

	Coefficients	Standard Error	t Stat	P-value
Intercept	-0.4098	0.015238766	-26.89194063	0.000112836
X Variable 1	0.04175	0.000754431	55.3396937	1.29972E-05

LOD & LOQ of Resmetirom	**LOD**	1.20	ug/ml
	LOQ	3.65	ug/ml

6. Robustness

The robustness studies development was established through analysis conducted at various λ max values, with results presented as percentage relative standard deviation (%RSD). The findings from this robustness investigation are documented in the referenced table(6).

Table no. 6: Robustness study.

Variation in wavelength		
Condition	**Sample ID**	**Absorbance**
223 nm	WS	0.415
225 nm	WS	0.417
227 nm	WS	0.415
AVG		0.416
STDEV		0.0012
% RSD		0.28

7. Repeatability

Table no. 7: Repeatability study.

Sample ID	Absorbance
100% Rep 1	0.412
100% Rep 2	0.413
100% Rep 3	0.411
100% Rep 4	0.414
100% Rep 5	0.413
100% Rep 6	0.412
AVG	0.413
STDEV	0.001048809
% RSD	0.25

B. HPLC

1. Chromatographic Optimization of Mobile Phase Composition for Resmetirom Using HPLC

The exploration of the complete medium consisting of mobile phases having different ratios of adjusting the buffer to water was done to get the best chromatographic separation of Resmetirom acetonitrile. The changes in the retention time (RT), tailing factor (TP), asymmetry (ASY), and peak purity were observed diligently to determine the changes. statements which give the optimum trade-off between resolution and analysis time. These trial results are tabulated in Table1 and plotted in Figure 4. accompanying graphs.

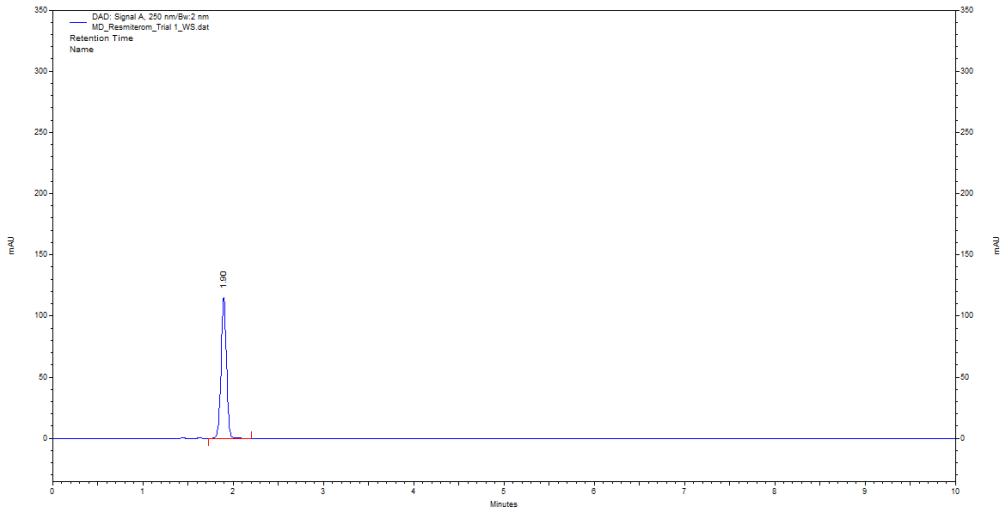

Fig. no. 4: Trial 1.

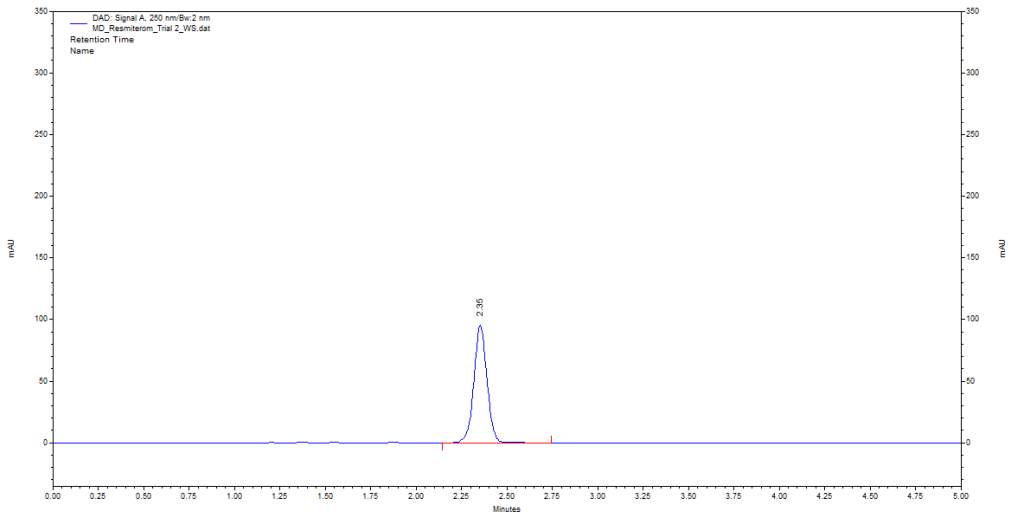

Fig. no. 5: Trial 2.

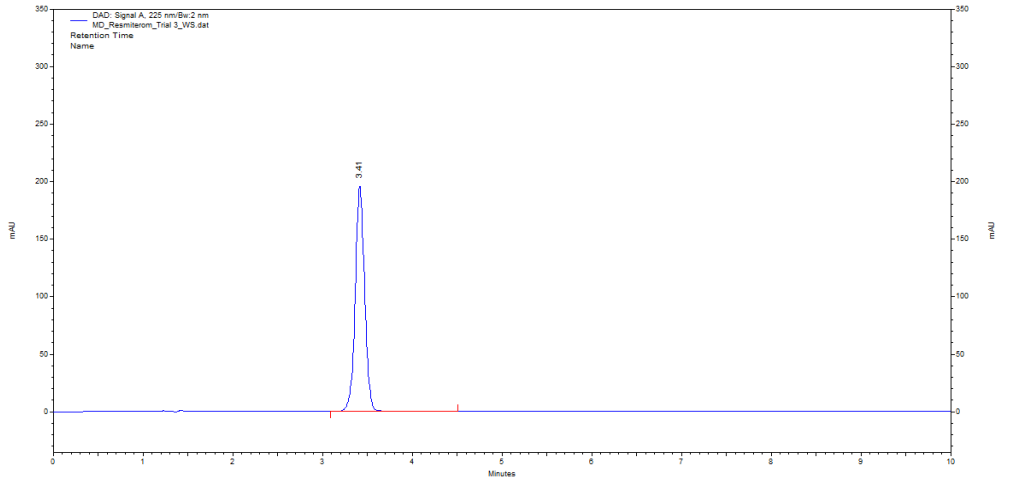

Fig. no. 6: Trial 3.

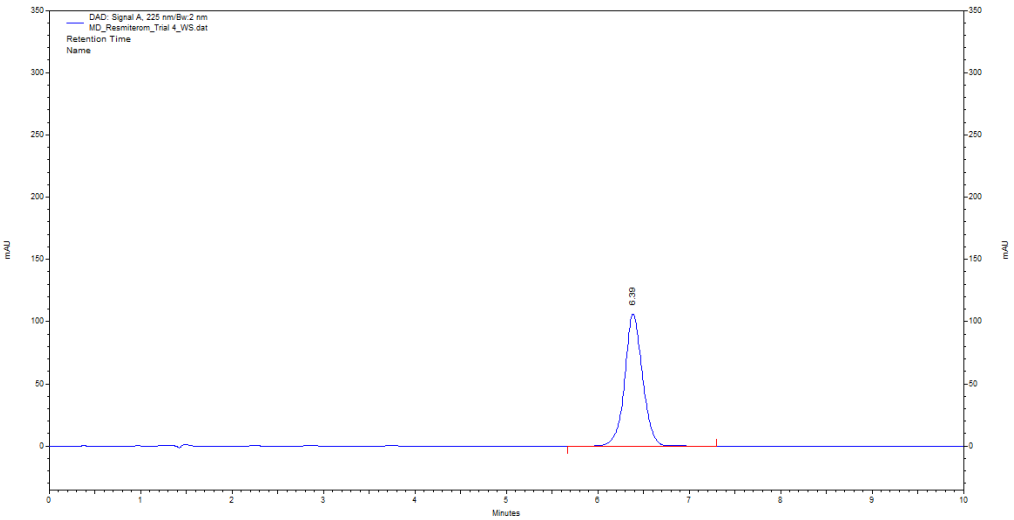

Fig. no. 7: Trial 4.

2. System suitability

Table no. 8: System suitability parameters for Resmetirom.

Sample	Resmetirom				
	RT	Area	TP	Asymmetry	Peak Purity
Blank	-	-	-	-	-
Working Standard	3.38	2990068	5355	1.02	1.00

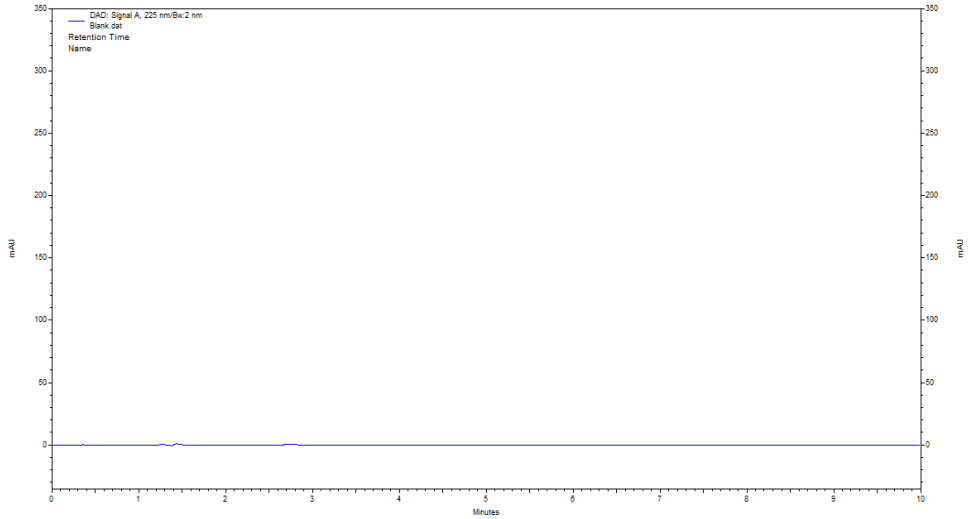

Fig. no. 8: Blank.

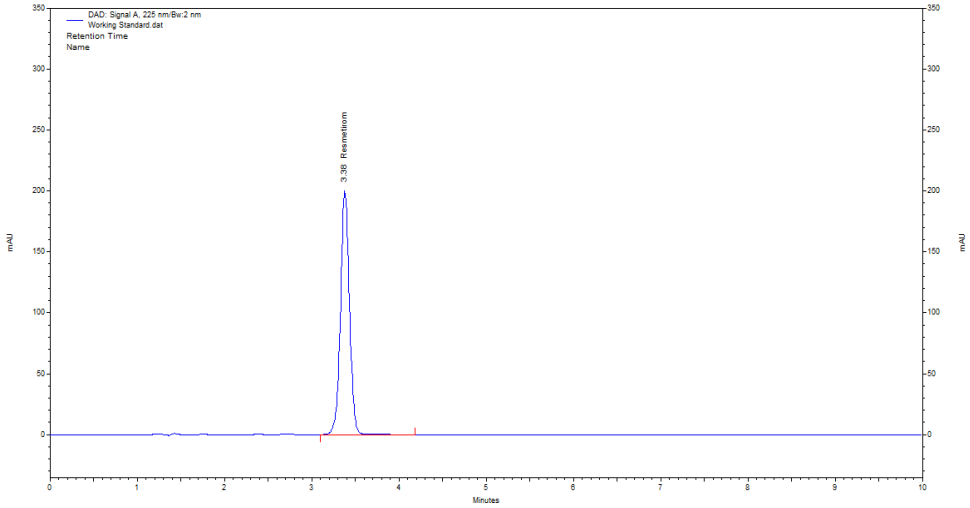

Fig. no. 9: Working Standard.

3. Linearity

The results obtained are shown in Table (9) for Resmetirom

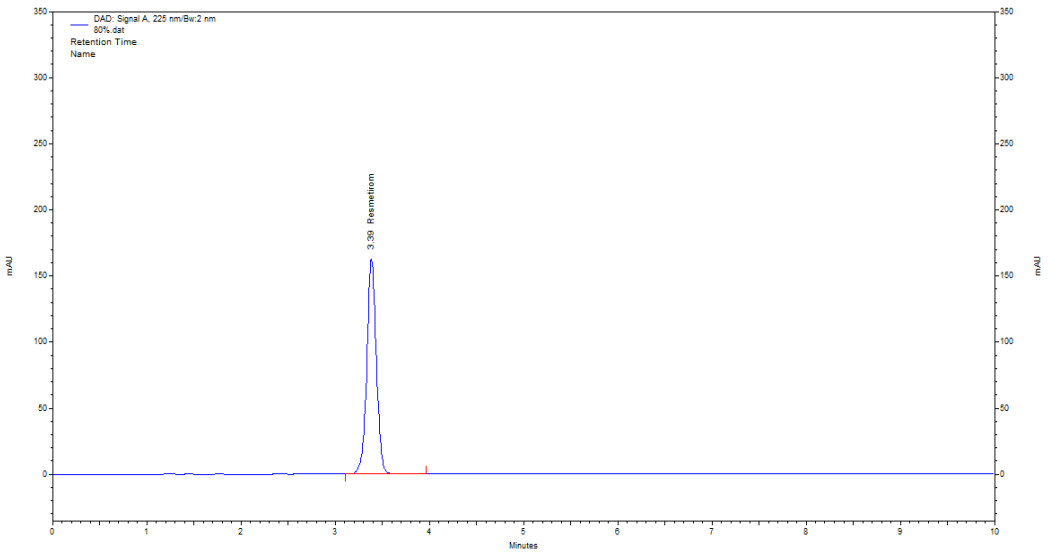

Fig. no. 10: Linearity (80%).

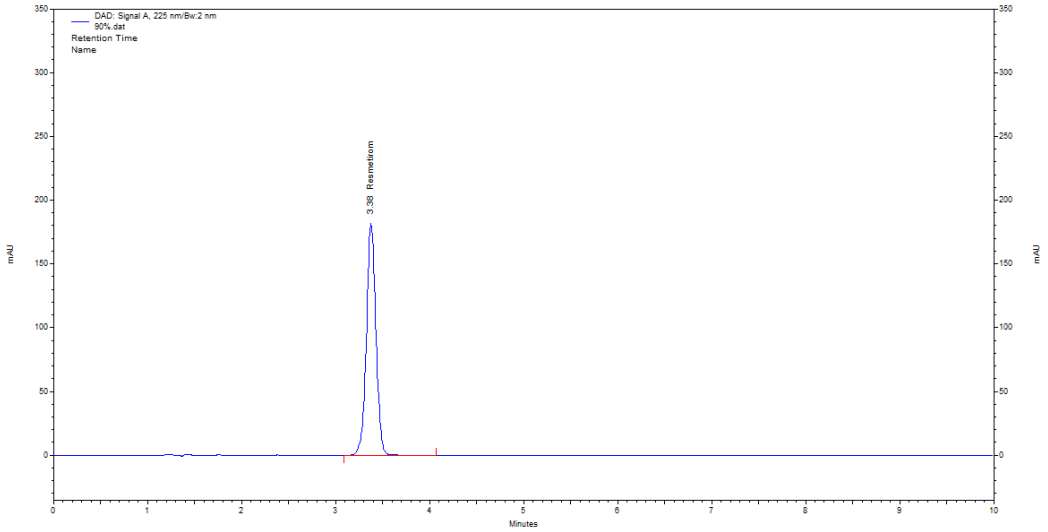

Fig. no. 11: linearity (90%).

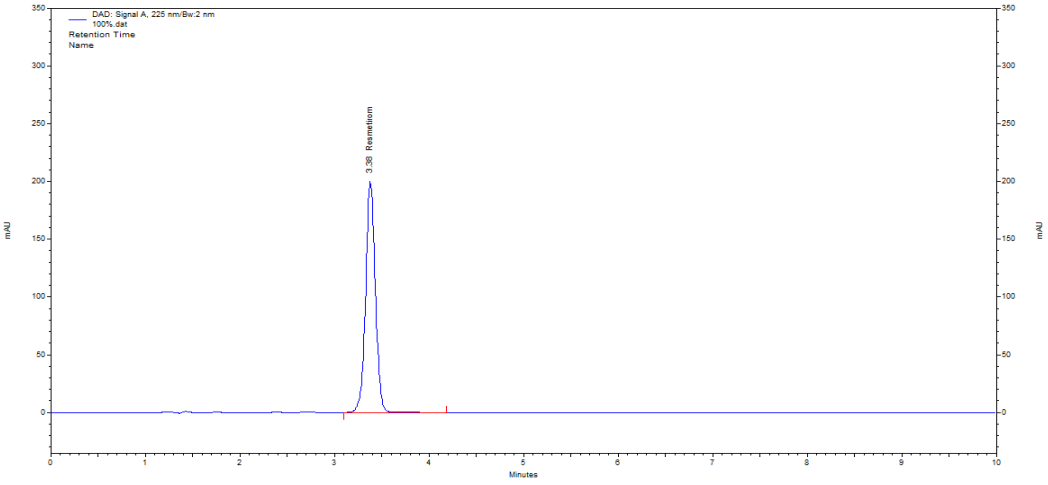

Fig no. 12: Linearity (100%).

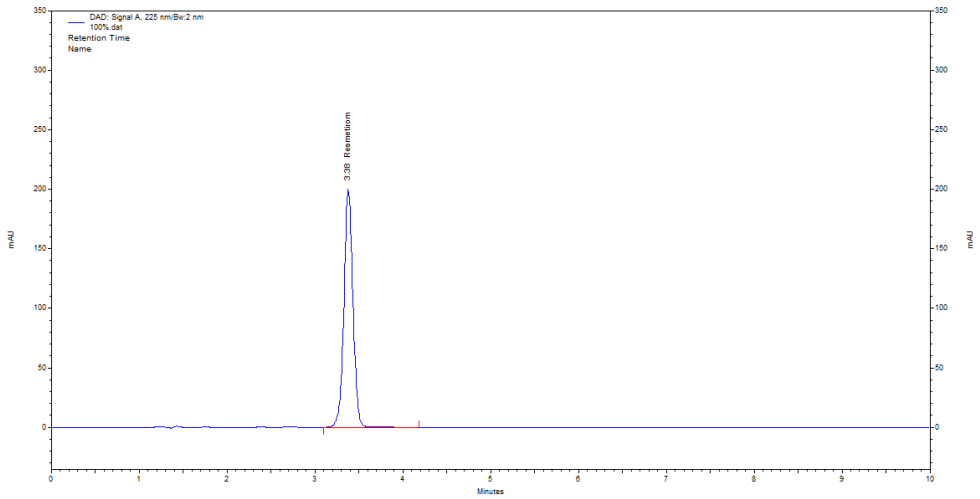

Fig. no. 13: Linearity (110%).

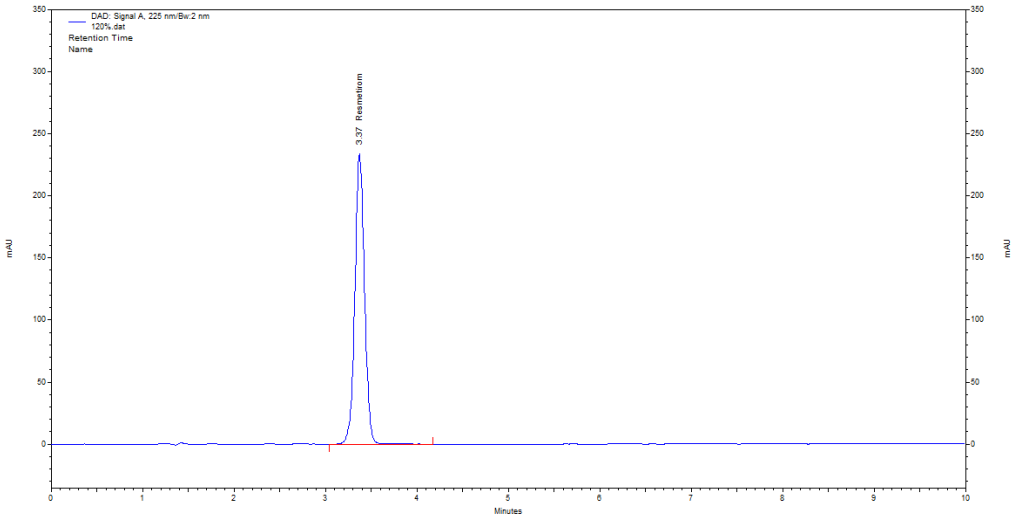

Fig. no. 14: Linearity (120%).

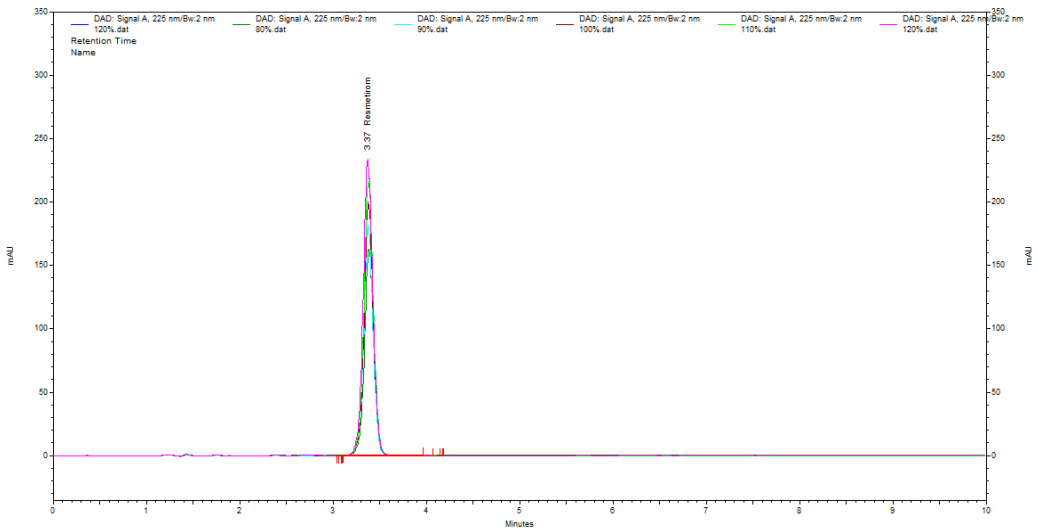

Fig. no. 15: Linearity Overlay.

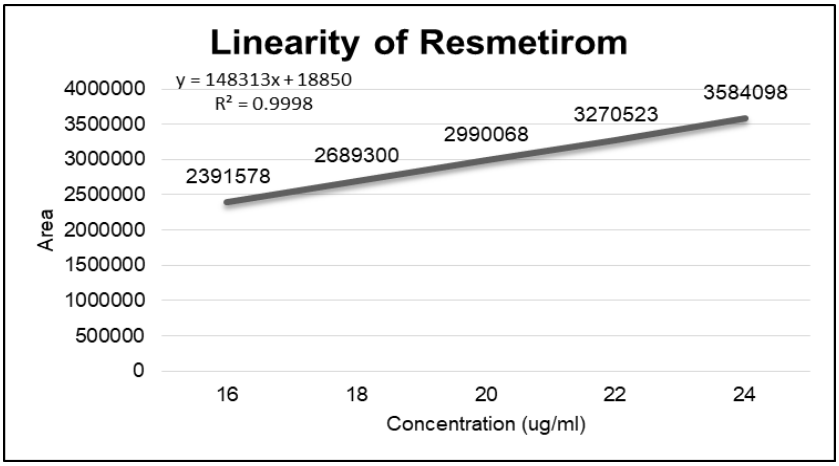

Fig. no. 16: Overlay graph of Linearity for Resmetirom.

Table no. 9: HPLC: Linearity study of Resmetirom.

% Level	Conc (ug/ml)	Area
80	16	2391578
90	18	2689300
100	20	2990068
110	22	3270523
120	24	3584098

4. Range

The analytical method's range represents the interval between the highest and lowest analyte concentrations that have been demonstrated to possess adequate precision, accuracy, and linearity.

Resmetirom = 16-24 µg/ml

5. Precision

The data obtained on intraday and interday change are very accurate, highly repeatable, and %RSD values are within acceptable limits. Table no. 10 represent the results for intraday and interday study for Resmetirom.

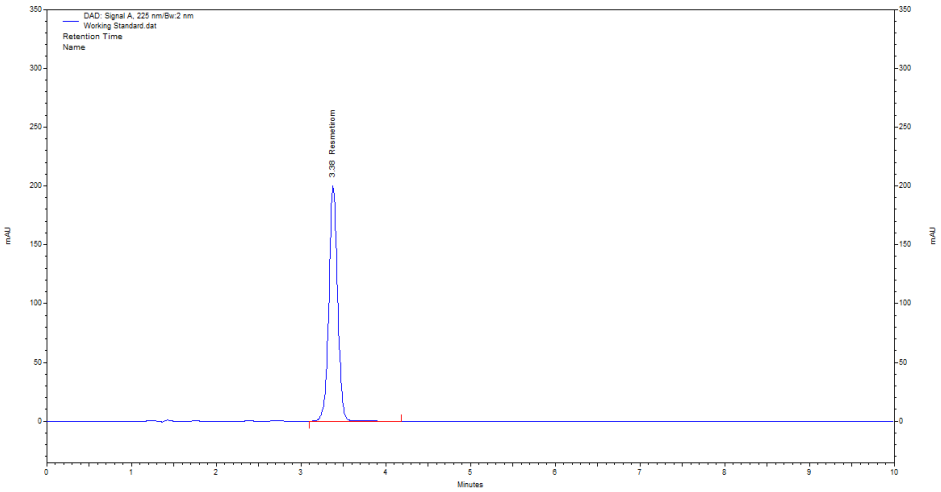

Fig no. 17: Morning Day 1.

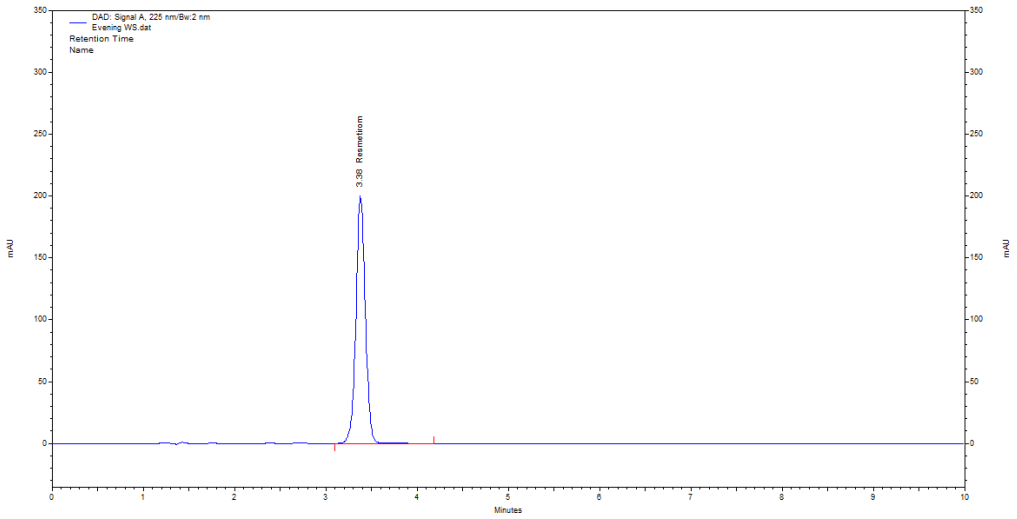

Fig no. 18: Evening Day 1.

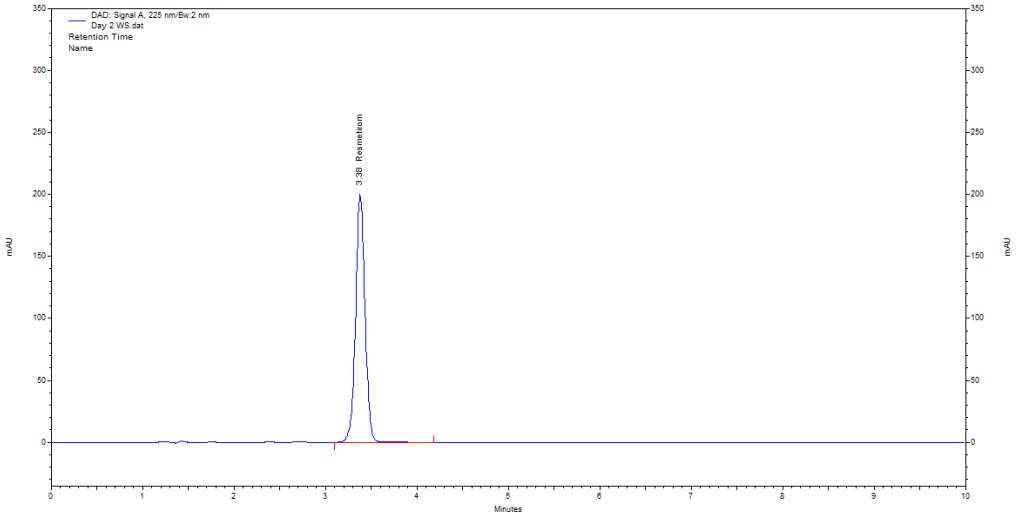

Fig no. 19: Day 2.

Table no. 10: Intra-day & Inter-day precision of Resmetirom.

Condition	Sample ID	RT	Area	TP	Asymmetry	Peak Purity
Morning	WS	3.38	2990068	5355	1.02	1.00
Evening	WS	3.38	2950574	5225	1.03	1.00
AVG		3.38	2970321			
STDEV		0.0000	27926.4752			
% RSD		0.00	0.94			
Day 2	WS	3.38	2877552	5271	0.99	1.00
AVG		3.38	2939398			
STDEV		0.0000	57084.4970			
% RSD		0.00	1.94			

6. Accuracy

Accuracy of the method were carried out for drug (Resmetirom) was determined at 80%, 100%, 120% respectively. Results obtained were in terms of % Recovery, results obtained are mentioned in Table No. 1.1

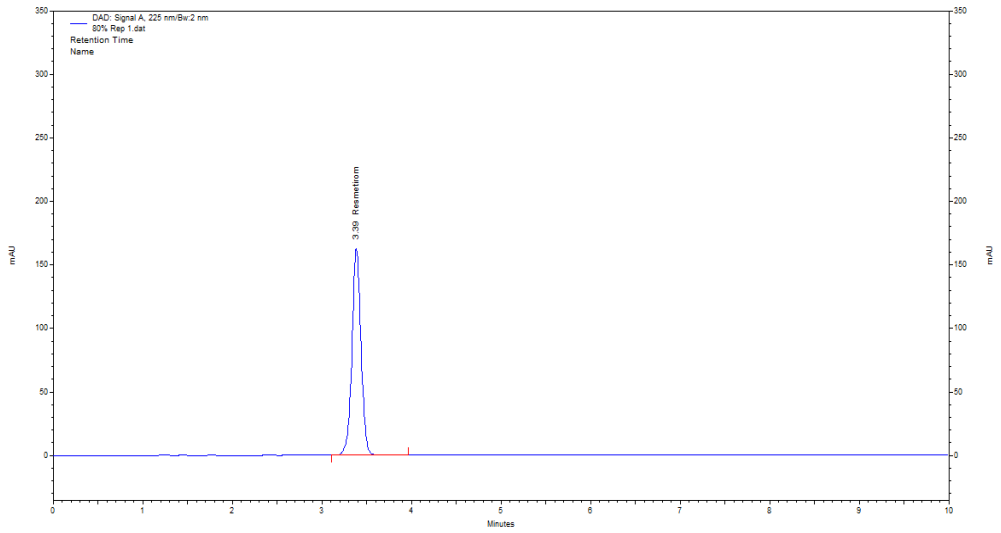

Fig. no. 20: 80% REP.

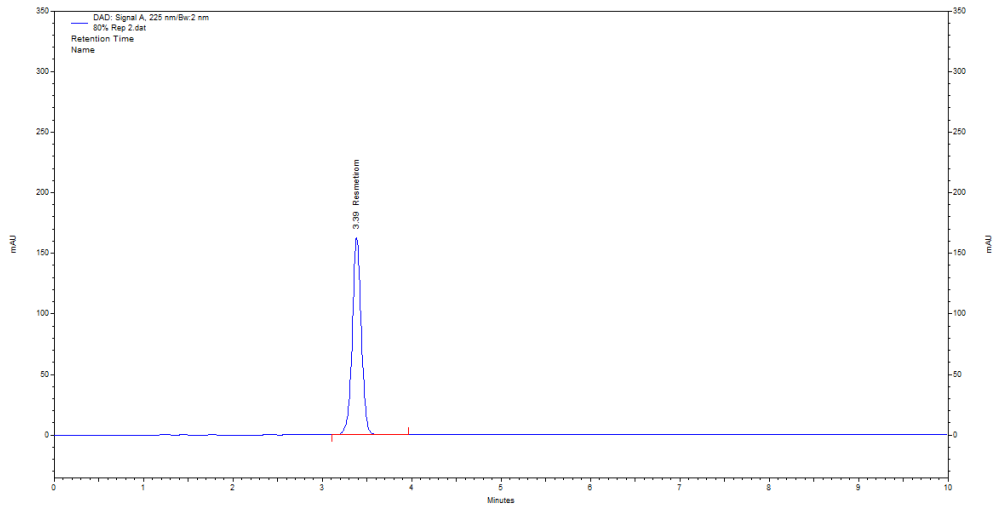

Fig. no. 21: 80 % REP 2.

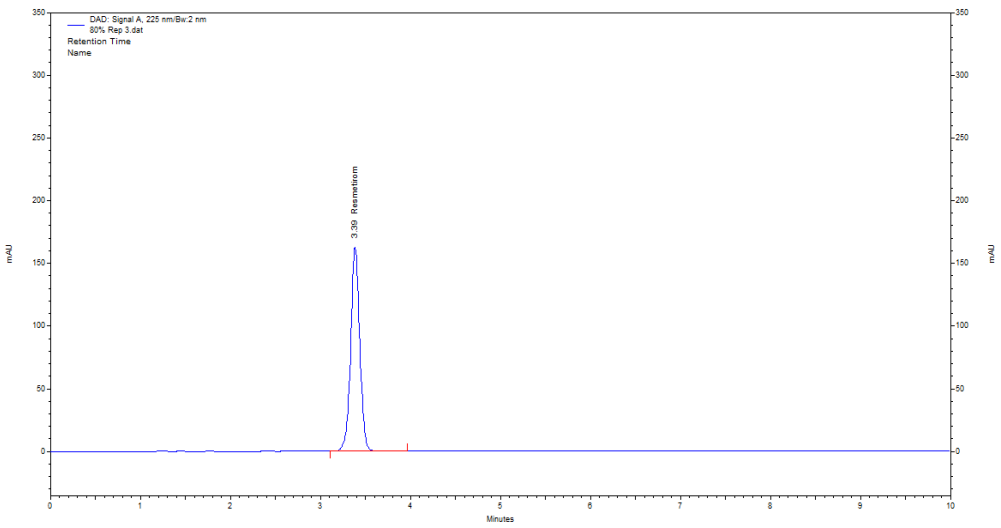

Fig. no. 22: 80% REP 3.

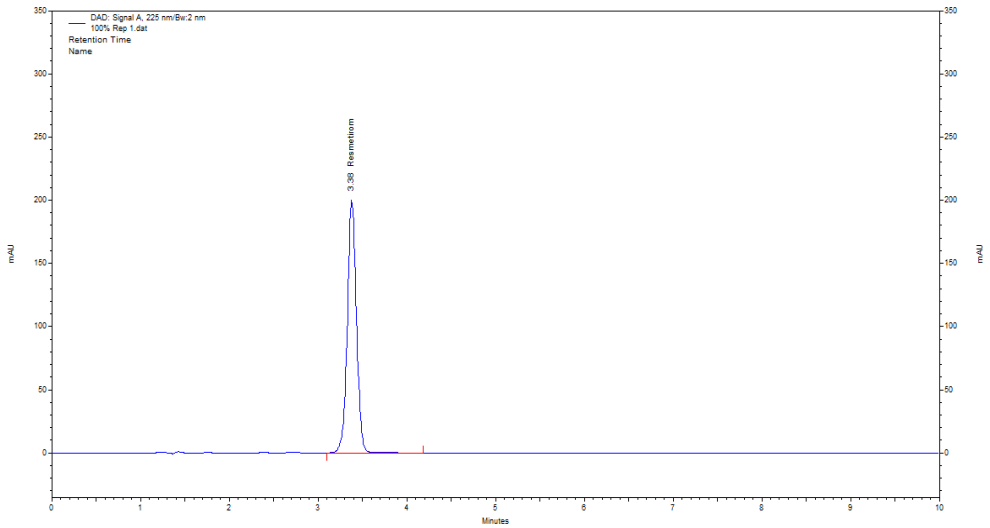

Fig no. 23: 100% REP 1.

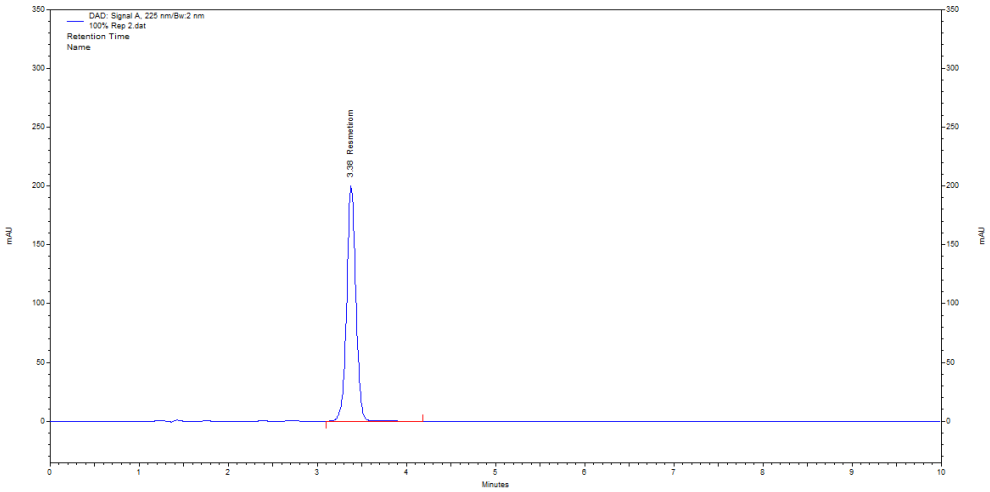

Fig no. 24: 100%REP 2.

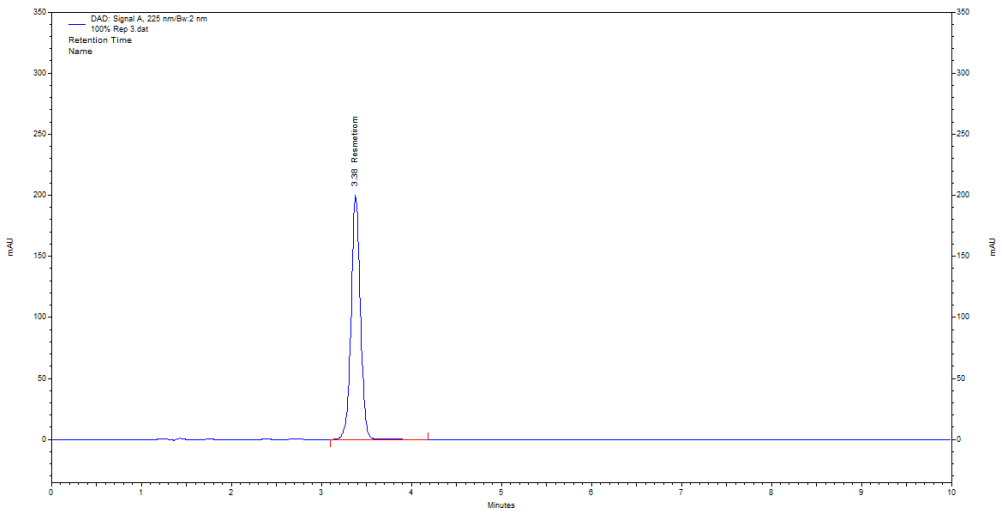

Fig no. 25: 100% REP 3.

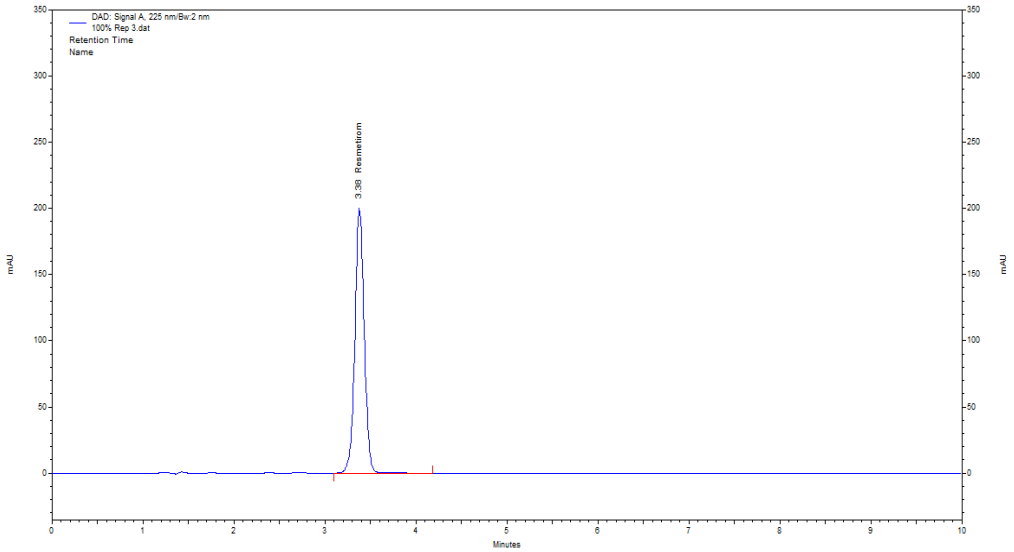

Fig no. 26: 120% REP 1.

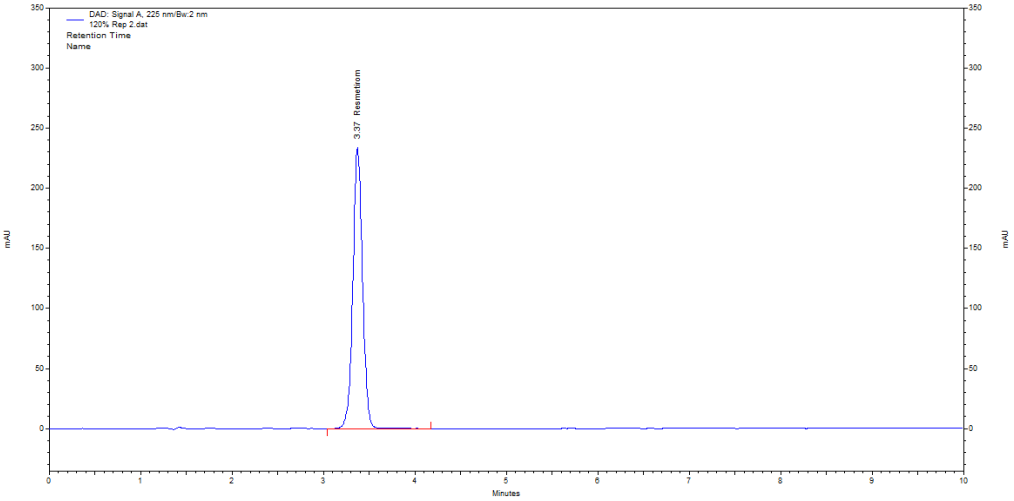

Fig. no. 27: 120% REP 2.

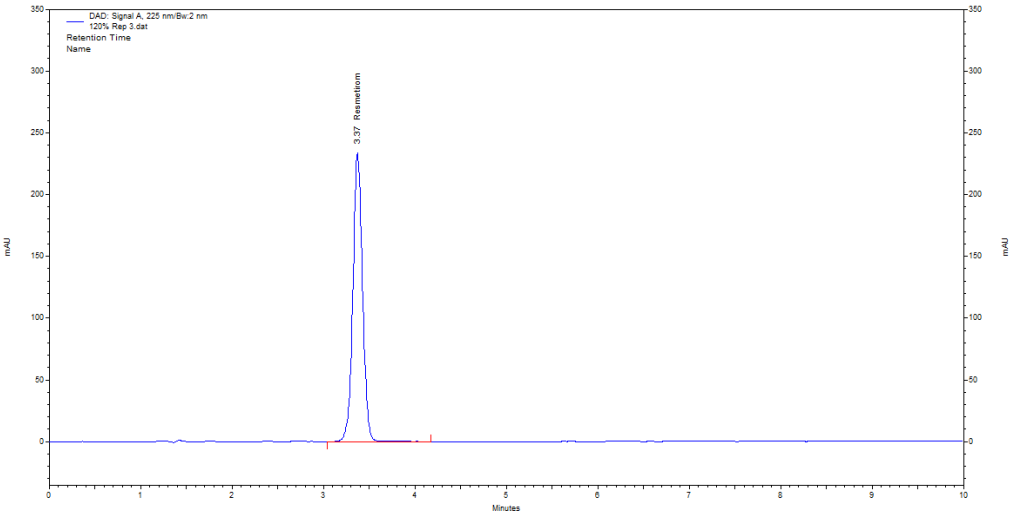

Fig no. 28: 120% REP 3.

Table no. 11: Recovery studies of Resmetirom.

Sample ID	Reps	Spiked Conc. (ng/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	15.84	2391578	15.97	100.82	100.74	0.072172	0.07
	Rep 2	15.84	2388254	15.95	100.68			
	Rep 3	15.84	2389205	15.95	100.72			
100%	Rep 1	19.80	2990068	19.97	100.84	100.19	0.638642	0.64
	Rep 2	19.80	2952254	19.71	99.57			
	Rep 3	19.80	2969347	19.83	100.15			
120%	Rep 1	23.76	3584098	23.93	100.73	100.66	0.070517	0.07
	Rep 2	23.76	3581220	23.91	100.65			
	Rep 3	23.76	3579099	23.90	100.59			

7. Limit of Detection (LOD) & Limit of Quantification (LOQ)

Regression Statistics	
Multiple R	0.999895479
R Square	0.999790969
Adjusted R Square	0.999721292
Standard Error	7830.688056
Observations	5

ANOVA

Table no. 12: Result of LOD and LOQ.

	df	***SS***	***MS***	***F***	***Significance F***
Regression	1	8.79872E+11	8.79872E+11	14348.92818	1.28272E-06
Residual	3	183959026.3	61319675.43	-	-
Total	4	8.80056E+11		-	-

	Coefficients	***Standard Error***	***t Stat***	***P-value***
Intercept	18850.4	25009.21209	0.75373826	0.505768917
X Variable 1	148313.15	1238.140495	119.7870117	1.28272E-06

LOD & LOQ of Resmiterom	LOD	0.56	ug/ml
	LOQ	1.69	ug/ml

8. Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength and column temeperature were altered and the effects on the area were noted. The results obtained are shown in Table 13.

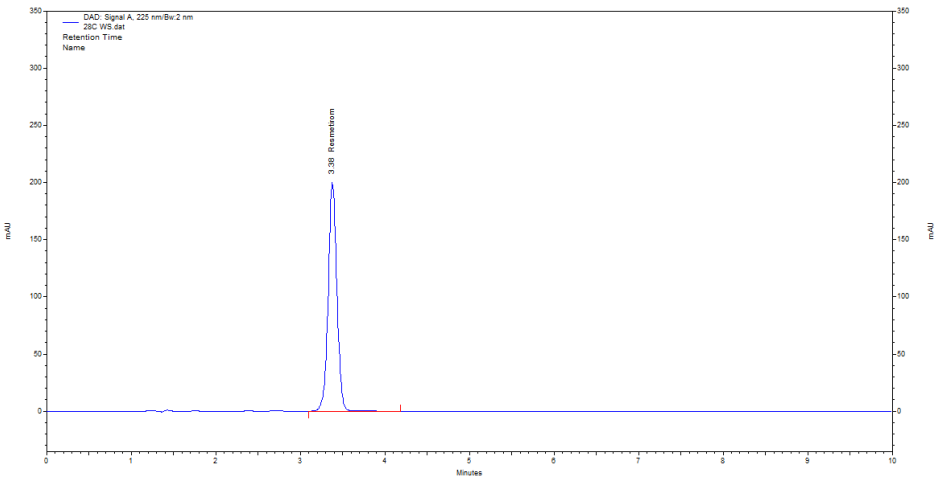

Fig no. 29: Robustness 28°C.

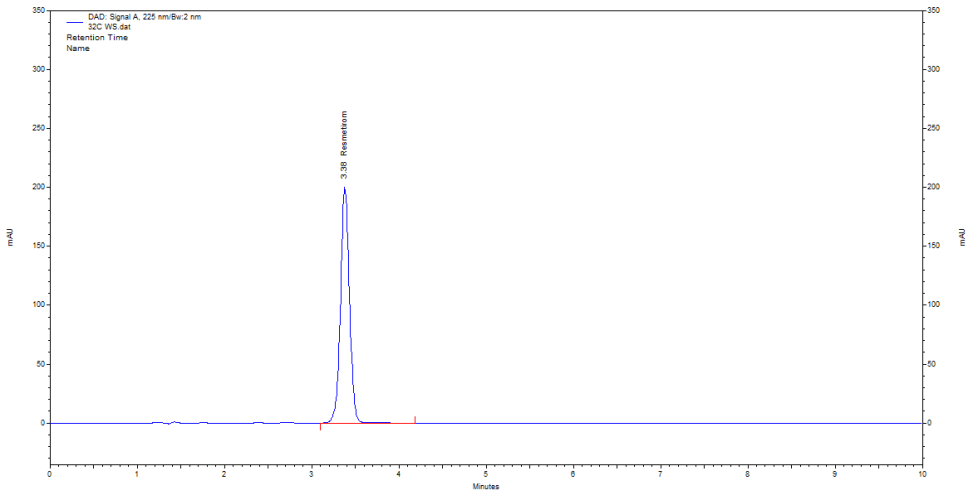

Fig no. 30: Robustness 32°C.

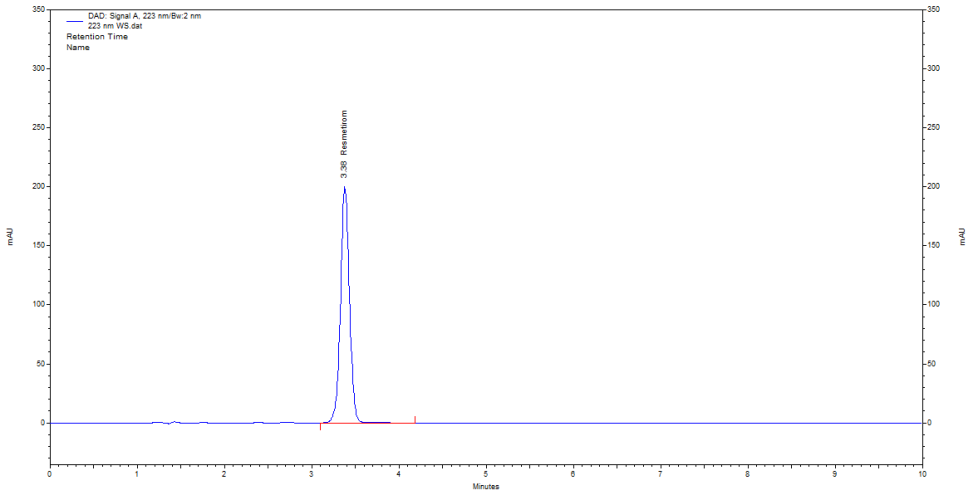

Fig. no. 31: Robustness 223 nm.

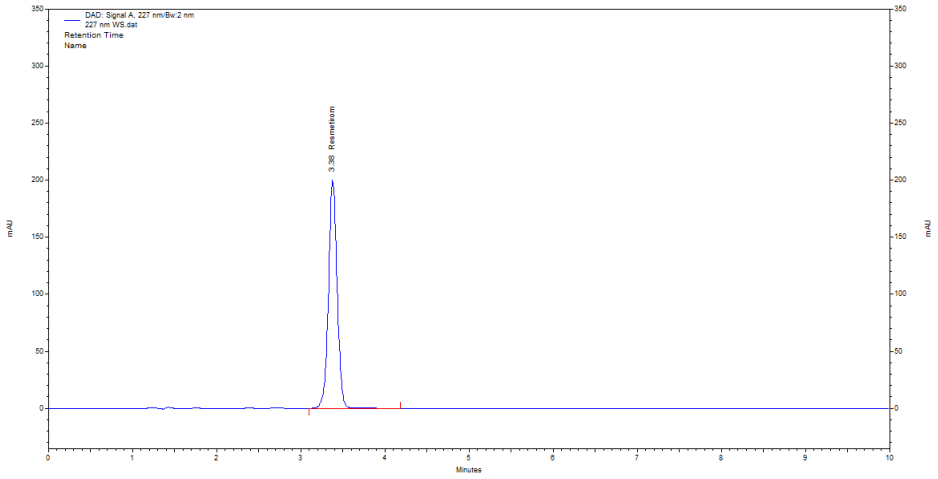

Fig. no. 32: Robustness 227 nm.

Table no. 13(a): Robustness study.

Variation in Column temperature						
Condition	**Sample ID**	**RT**	**Area**	**TP**	**Asymmetry**	**Peak Purity**
28C	WS	3.38	2982241	5288	1.01	1.00
30C	WS	3.38	2990068	5355	1.02	1.00
32C	WS	3.38	2980074	5371	1.02	1.00
AVG		3.38	2984128			
STDEV		0.0000	5257.3418			
% RSD		0.00	0.18			

Table no. 13(b): Robustness study.

Variation in wavelength						
Condition	**Sample ID**	**RT**	**Area**	**TP**	**Asymmetry**	**Peak Purity**
223 nm	WS	3.38	2986057	5367	1.03	1.00
225 nm	WS	3.38	2990068	5355	1.02	1.00
227 nm	WS	3.38	2979977	5219	1.04	1.00
AVG		3.38	2985367			
STDEV		0.0000	5080.7283			
% RSD		0.00	0.17			

9. Repeatability

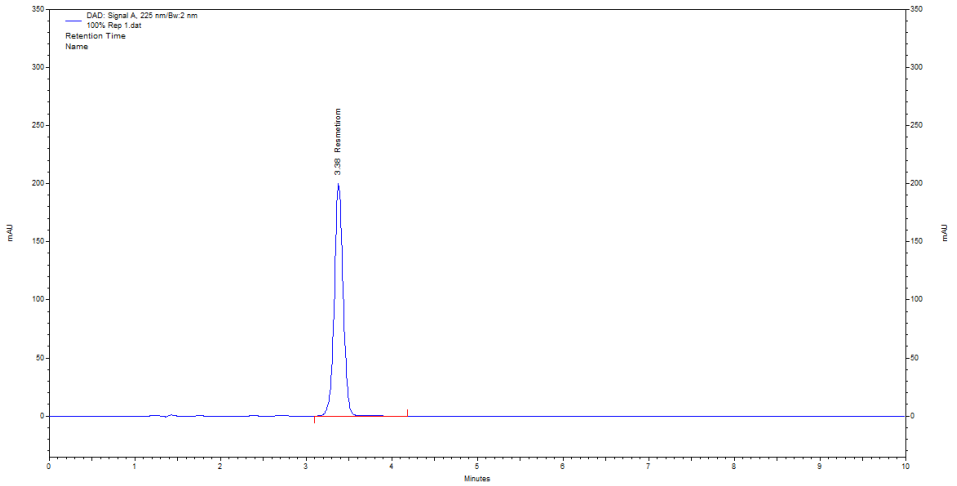

Fig. no. 33: Repeatability 100% Rep 1.

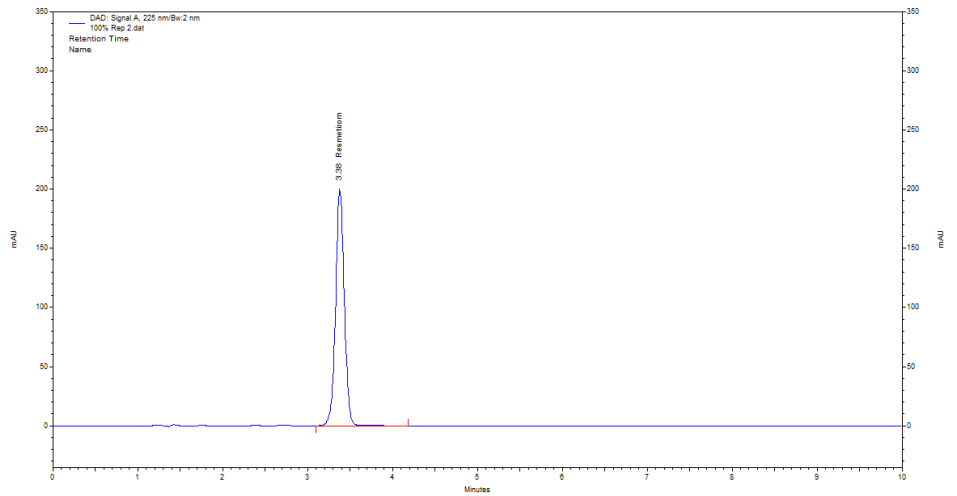

Fig. no. 34: Repeatability 100% Rep 2.

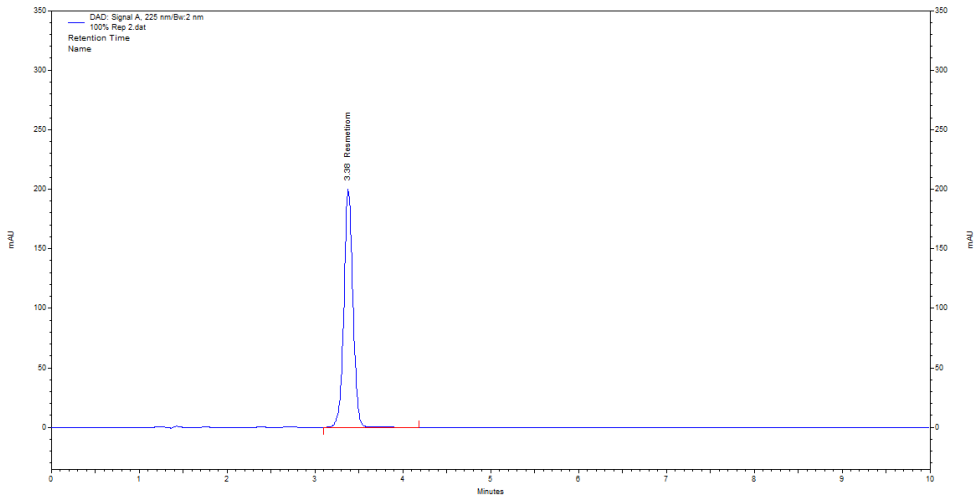

Fig no. 35: Repeatability 100% Rep 3.

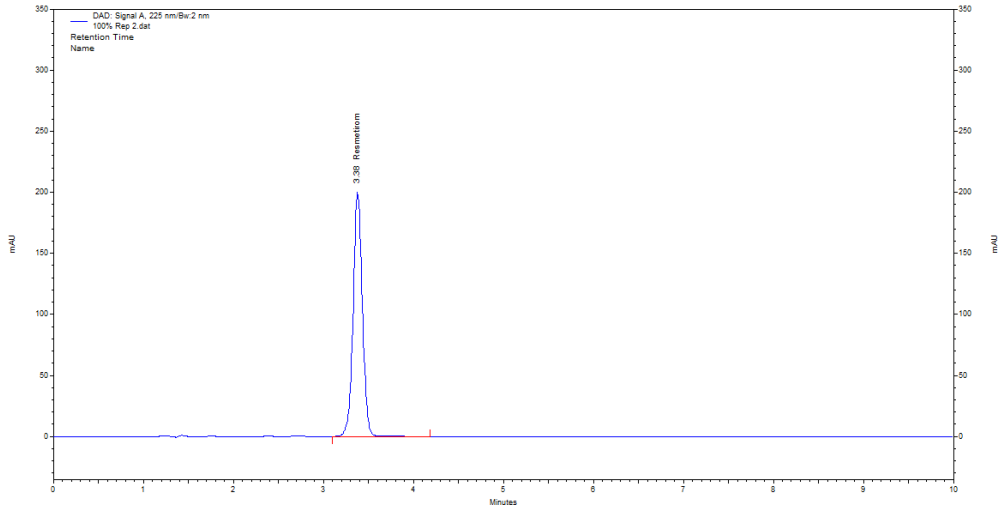

Fig. no. 36: Repeatability 100% Rep 4.

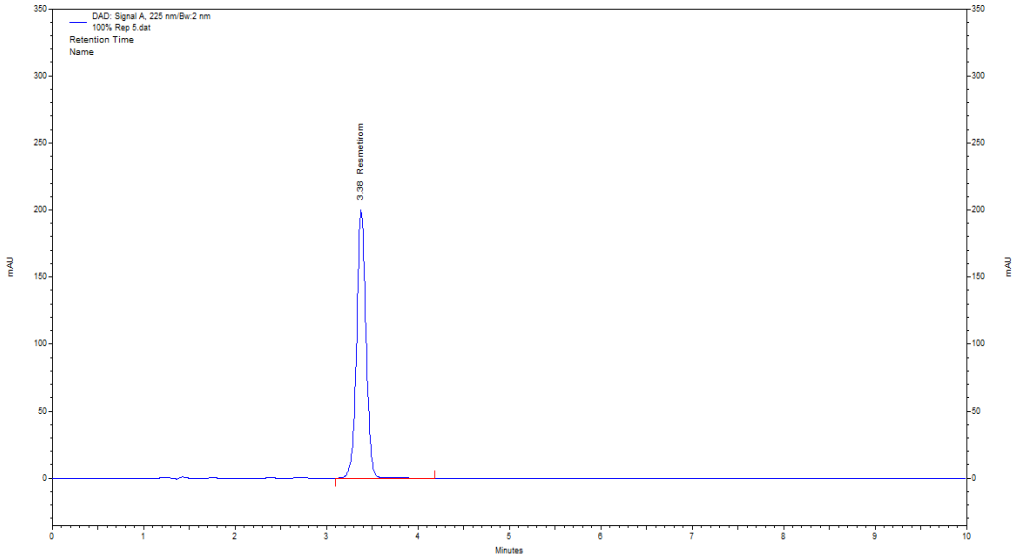

Fig. no. 37: Repeatability 100 % Rep 5.

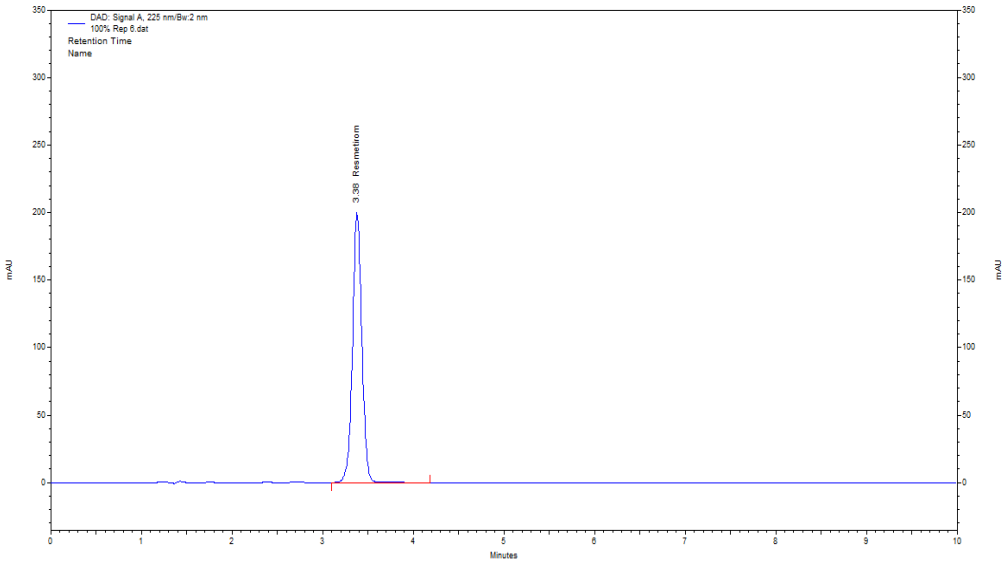

Fig. no. 38: **Repeatability 100% Rep 6.**

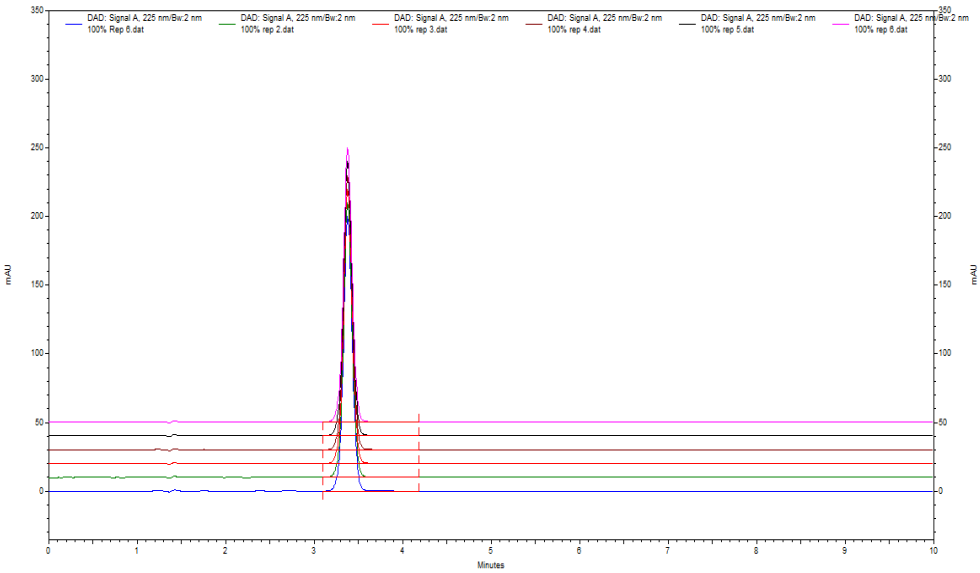

Fig no. 39: **HPLC Chromatogram Repeatability Overlay.**

Table no. 14: Repeatability study.

Sample ID	Area	RT	TP	Asymmetry	Peak Purity
100% Rep 1	2990068	3.38	5355	1.02	1.00
100% Rep 2	2952254	3.38	5168	1.05	1.00
100% Rep 3	2969347	3.38	5722	1.03	1.00
100% Rep 4	2944701	3.38	5529	0.99	1.00
100% Rep 5	2978971	3.38	5375	1.01	1.00
100% Rep 6	2954899	3.38	5319	1.02	1.00
AVG	2965040	3.38			
STDEV	17429.98	4.86E-16			
% RSD	0.59	0.00			

Summary

A. UV: Summery of validation study.

Table no. 15: Summary of Resmetirom by UV spectroscopy method.

Sr. No	Validation Parameter	Results	Pass / Fail
1	Linearity equation	Y=0.0835x + 0.1747	Pass
	R 2	0.999	
	Range	16 - 24 µg/ml	
2	Accuracy	Mean ±%RSD	Pass
	80	79.60±0.44	
	100	99.88±0.24	
	120	120.27±0.19	
3	Precision	%RSD	Pass
	Intraday Precision	0.68	
	Interday Precision	1.63	
4	LOD	1.20 µg/ml	Pass
5	LOQ	3.65 µg/ml	Pass
6	Robustness	%RSD	Pass
		0.28	
7	Repeatability	%RSD	Pass
		0.25	

B. HPLC: Summary of validation study

Table No. 16: Summary of Resmetirom by HPLC Method.

Sr. No.	**Validation Parameter**	**Results**	Pass / Fail
1.	Linearity	y = 148313x – 18850 R^2 = 0.998	Pass
	Range	16 - 24 µg/ml	Pass
2.	Precision	%RSD	Pass
	A) Intraday precision	00.94	
	B) Interday precision	1.94	
3.	Accuracy	% Recovery ± % RSD	Pass
	80%	100.74 ± 0.07	
	100%	100.19 ± 0.64	
	120%	100.66 ± 0.07	
4.	LOD	0.56 µg/ml	Pass
5.	LOQ	1.69 µg/ml	Pass
6.	Specificity	Specific	Pass
	Robustness	% RSD	Pass
7.		0.18 0.17	
8.	Repeatability	%RSD	Pass
		0.59	

CONCLUSION

In this investigation, sturdy UV spectrophotometric and RP-HPLC procedures were adequately achieved. established and produced the quantitative estimation of Resmetirom in its pure drug-form. The two techniques showed good linearity and precision. accuracy, sensitivity and specificity as per the ICH Q2(R1) guidelines. UV method was linear over the range of 16-24 µg/mL with the LOD 1.20 µg/mL. single and less expensive method of routine analysis. The RP-HPLC approach, using a column Phenomenex Kinetex XB-C18 and a 60:40 phosphate buffer : acetonitrile as mobile phase, registered a retention time of 3.41 minutes and showed high level of accuracy (recoveries between 98 % and 102 %) and precision. Specificity in the presence of possible excipients or impurities (2%) and no more than 2 percent RSD. All validation parameters, such as LOD (0.56 µg/mL), LOQ (1.69 The determination of µg/mL, robustness, and repeatability; all met with the acceptance rates; this indicates the reliability of the HPLC assay. Given

clinical interest that is growing in Resmetirom, and minimal, published analytical data, these methods are validated and fill a significant gap by offering reliable guidelines of quality control and additional investigations. The UV method can be adopted so that it can take just a quick screening whereas RP-HPLC method can take place. in a routine and regulatory testing because it is more sensitive and accurate. All in all, the procedures that were verified make sure that Resmetirom can be preventatively assessed in a study and drug-based environment, helping in future formulation and therapeutic monitoring.

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