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RP-HPLC Method Development and Validation of for Resmetirom in Bulk & Formulation

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Abstract

Analytical monitoring of the pharmaceutical products, or of specific ingredients within the product, its necessary to insure it's safely and efficacy throughout all phases of its shelf-life, including storage, distribution and use. Essentially, the techniques of chromatography is based on the differences in the rate at which the components of the mixture move through a porous medium (called stationary phase) under the influence of same solvent or gas (called mobile phase). Methanol: water (0.1%Acetic Acid), (68:32) v/v, pH 3.was used as the mobile phase for the method. The detection wavelength was 220 nm and flow rate was 0.9 ml/min. In the developed method, the retention time of Resmetirom was found to be being 3.1 min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. The method has been found to be better than previously reported method, because of its less retention time, isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks

Keywords: RP-HPLC; Analytical Chemistry; Linearity; Precision; ICH Guidelines; Resmetirom

1. Introduction

Analytical chemistry is mainly concerned with determining the qualitative and quantitative composition of material under study[2]. Analytical monitoring of the pharmaceutical products, or of specific ingredients within the product, its necessary to insure it's safely and efficacy throughout all phases of its shelf-life, including storage, distribution and use. Analysis is important in every product, but it is especially vital in medicine as it deals with life. In the manufacture of drug, it is necessary to follow certain recommended practices for overall control to ensure that consumer receives drug of high quality.

Qualitative analysis: it deals with the identification of substances. It is concerned with what elements or compounds are present in the sample.

Quantitative analysis: it provides numerical information concerning the quantity of the same species (the analyte) in a measured amount of matter (the sample). Various techniques employed for analysis and method development are as follows:-

Spectrophotometric method : - UV

Chromatographic method: - HPLC, HPTLC C]

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Other methods : - LC-MS, GC-MS

Essentially, the techniques of chromatography is based on the differences in the rate at which the components of the mixture move through a porous medium (called stationary phase) under the influence of same solvent or gas (called mobile phase).

The chromatographic method of separation, in general, involves the following steps

- Retention of a substance or substances on the stationary phase
- Separation of the adsorbed substances by the mobile phase
- Recovery of the separated substances by a continuous flow of the mobile phase
- Qualitative and quantitative analysis of the eluted substance

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirement for the intended analytical applications.

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. It, is mainly an investigation of the intrinsic stability of the molecule providing the foundation for developing and validating the analytical methods and for developing the stable formulations. Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug product.

1.1. Drug profile

Resmetirom is a thyroid hormone receptor-beta (THR-beta) agonist. On March 14, 2024, it was approved by the FDA as the first treatment of liver fibrosis due to no cirrhotic non-alcoholic steatohepatitis (NASH), which is a form of non-alcoholic fatty liver disease (NAFLD).

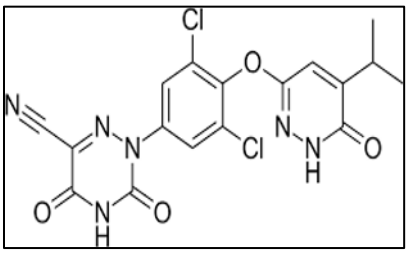

Figure 1 Structure of Resmetirom

Molecular formula: $C_{17}H_{12}Cl_2N_6O_4$

Molecular weight: 435.2 g/mol

Description: White crystalline powder

Category: Resmetirom is a thyroid hormone receptor-beta (THR-beta) agonist.

2. Material and methods

Table 1 Drug and Drug Supplier

Name of Drug	Drug Supplier
Resmetirom	Swapnroop drug and pharmaceutical

Table 2 List of Reagents and Chemicals used

Sr. No.	Name of chemicals	Manufacturer.
1.	Acetonitrile (HPLC grade)	Merck Ltd., India
2.	Methanol (HPLC)	Merck Ltd., India
3.	water (HPLC grade)	Merck Ltd., India
4.	0.1% OPA (HPLC grade)	Merck Ltd., India
5	0.1% Acetic Acid (HPLC grade)	Merck Ltd., India

2.1. Selection of formulation:

Table 3 List of brand names of combined formulations of Resmetirom

Sr.No	Brand name	Formulation	Available strength	Address of manufacturer
1.	ReMASH 80	Tablet	Resmetirom-80 mg	Azista

Table 4 Instrument (HPLC) Details used during Method Development

	Name of Instrument	Company Name
1	HPLC Instrument	Agilent with auto sampler(DAD Detector) (chem-station)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C_8)	AgilentC_{18} (150mmX 4.6mm,5µm) :
4	pH meter	VSI pH meter(VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance.
6	Sonicator	Ultrasonic electronic instrument

2.2. Experimental work.

2.2.1. HPLC

Selection of Analytical Technique.

HPLC was selected as analytical technique for estimation of Resmetirom.

2.2.2. Instruments

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector, UV (DAD) & Gradient Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 150mm; 5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software.

2.2.3. Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table 5 Chromatographic conditions (HPLC) details used during method

Sr	Instrument	Details
1.	HPLC	Agilent Tech. Gradient System With Auto injector, UV Detector
2.	Software	chemstation 10.1
3.	Column	(Agilent) C18 column (4.6mm x 150mm
4.	Particle size packing	5 m
5.	Stationary phase	C-18 (Agilent)
6.	Mobile Phase	Methanol : 0.1 % Acetic acid (68 : 32)
7.	Detection Wavelength	220 nm
8.	Flow rate	0.9 ml/min
9.	Temperature	Ambient
10.	Sample size	20 l
11.	Ph	3.2
12.	Run Time	15 min
13.	Filter paper	0.45 m

UV-VIS Spectrophotometer:

UV-VIS Spectrophotometer was selected as analytical technique for estimation of Resmetirom .UV absorbance range of 200-400nm.

- Analytical column : Agilent C18 Column (150mm x 4.6mm), 5µm particle size.
- Injection volume : 20µl
- Flow rate : 0.9 ml/min
- Detection : 220 nm
- Run Time : 15 min

Following Mobile phase were tried:

Validation of method for analysis of Resmetirom:

The developed method was validated as per ICH guidelines.

2.3. Analytical Method validation

Analytical method validation was carried out as per ICH method validation guidelines Q2 (R1).

2.3.1. Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyst in samples within a given range, The Result are studied

2.3.2. Accuracy (recovery)

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyst have been added. The accuracy is calculated from the test results as the percentage of analyst recovered by the assay, The Result are studied.

2.3.3. Accuracy

The accuracy was determined by Resmetirom (equivalent to 5 mg of Resmetirom) (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed Tablet.

2.3.4. Repeatability

Precision of the system was determined with the sample. Two replicates of sample solution containing 5 µg/ml of Resmetirom were injected and peak areas were measured and %RSD was calculated it was repeated for two times result are studied

2.3.5. Precision

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test.

2.3.6. Intra-day precision

Sample solutions containing 5 mg of Resmetirom three different concentration (10µg/ml, 15µg/ml, 20µg/ml) Resmetirom were analyzed three times on the same day and %R.S.D was calculated. The Result is shown studied.

2.3.7. Inter-day precision

Sample solutions containing 5 mg of Resmetirom were analyzed three times on different concentration (10µg/ml, 15µg/ml, 20µg/ml) Resmetirom different days and % R.S.D was calculated. It is usually expressed as standard deviation or relative standard deviation. The Result is studied.

2.3.8. Robustness

The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion of Methanol in the mobile phase composition (±1 ml/ min^{-1}**)** and the change in detection wavelength (±1 ml/ min^{-1}**)** and the effect of the results were examined

2.3.9. Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

$$DL=3.3\ \Sigma s$$

Where,

σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

The result is shown in **section 8.**

2.3.10. Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

$$QL=10\ \sigma S$$

Where,

σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

2.4. Analysis of marketed formulation

To determine the content of Resmetirom in marketed Tablet Take 6.26 mg of Tablet dissolve in 10 ml methanol, to ensure complete extraction it was sonicated for 15 min. 0.5 mL of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted**.**

Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of Resmetirom in the sample was calculated. The amount of Resmetirom was obtained from the regression equation of the calibation curve as described in analysis of formulation are studied.

3. Result

3.1. Preliminary studies on Resmetirom.

Melting point: The procured reference standard of Resmetirom was found to melt in the range of 163-165^{0}C respectively.

Solubility: The drug was found to be soluble in Methanol, DMSO, DMF,Insoluble in water.

UV Spectroscopy: 220 nmλmax

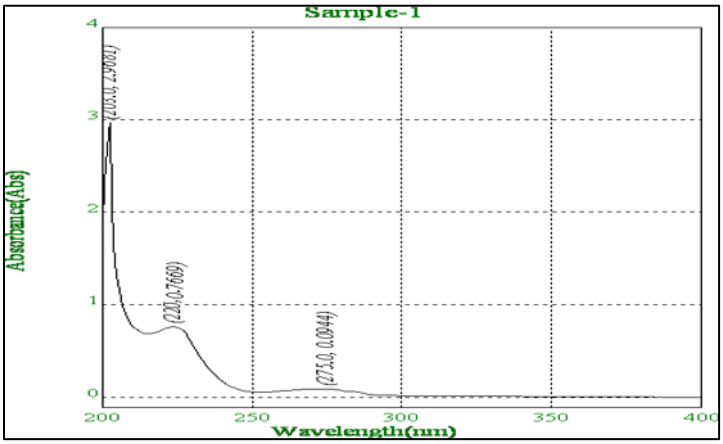

Figure 2 UV spectrum of Resmetirom

3.2. Studies on the chromatographic behavior of Resmetirom.

The final chromatographic conditions selected were as follow:

- Analytical column : Agilent C_{18} Column (150mm x 4.6 mm, 5µm particle size).
- Injection volume : 20µl
- Flow rate : 0.9 ml/min
- Mobile phase : Methanol : 0.1% (Acetic acid) water (68: 32 % V/V)
- Detection : 220 nm
- Run Time : 15 min
- Observation: Sharp peak were obtained, so method was selected.

Chromatogram of Final selected

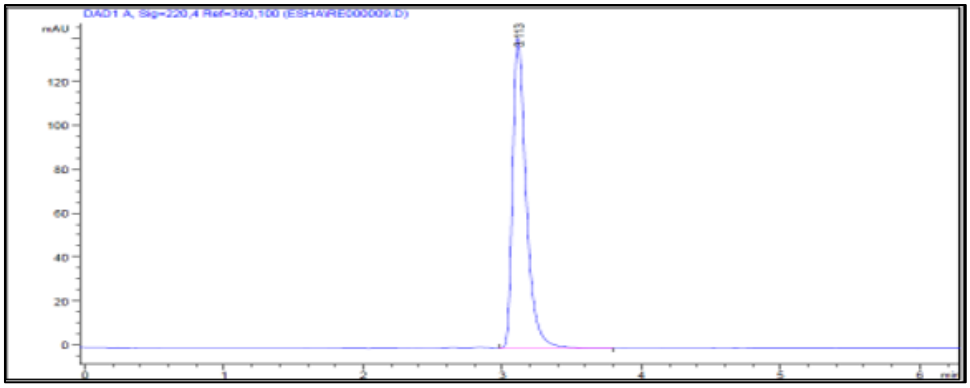

Figure 3 Chromatogram of final selected chromatographic conditions

3.3. Analytical of Method Validation: Linearity:

From Resmetirom standard stock solution, different working standard solution (5-25 µg/ml) for HPLC were prepared in mobile phase 20µl of sample solution was injected into the chromatographic system using mixed volume loop injector. Chromatograms were recorded. The Calibration curves are shown in

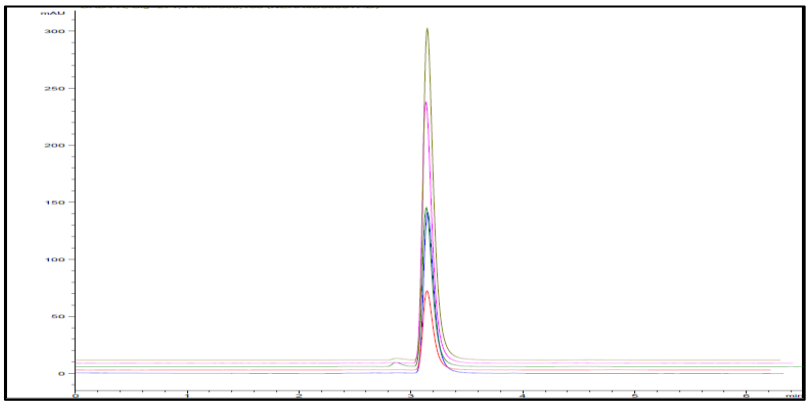

Figure 4 Chromatogram of Overlay

Table 6 Linearity of Resmetirom (HPLC)

Sr.No.	Concentration µg/ml	Area Resmetirom
1	5	466.534
2	10	932.358
3	15	1415.934
4	20	1836.235
5	25	2321.008

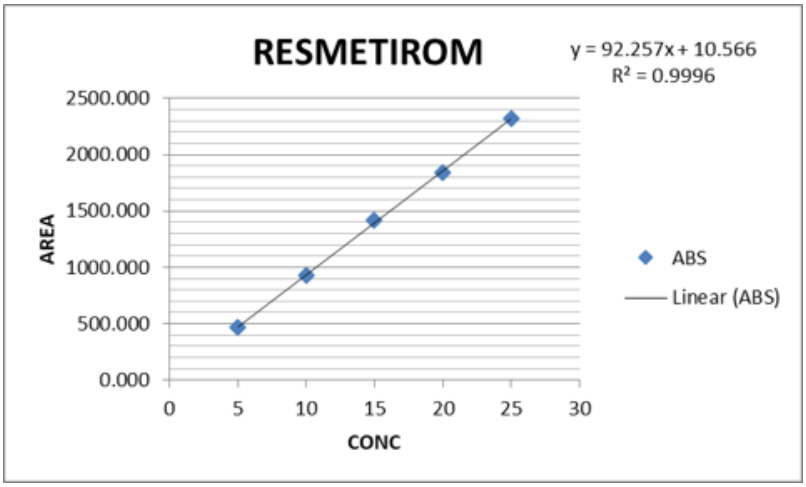

Figure 5 Calibration curve of Resmetirom (HPLC)

Table 7 Regression equation data for Resmetirom

Regression Equation Data Y=mx+c	
Slope(m)	92.25
Intercept(c)	10.56
Correlation Coefficient	0.999

Linearity of Resmetirom was observed in the range of 5-25 µg/ml. Detection wavelength used was 220 nm. ; The calibration curve yielded correlation coefficient (r^2) 0.999 & 0.999 for Resmetirom respectively.

3.4. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed Tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed .Statistical validation of recovery studies shown in **Table**

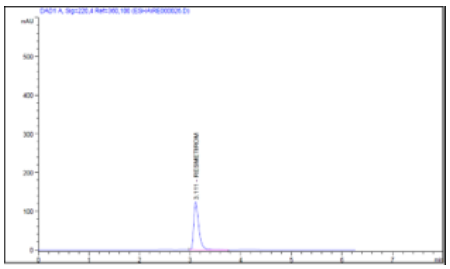

Figure 6 Chromatogram of Accuracy 80%

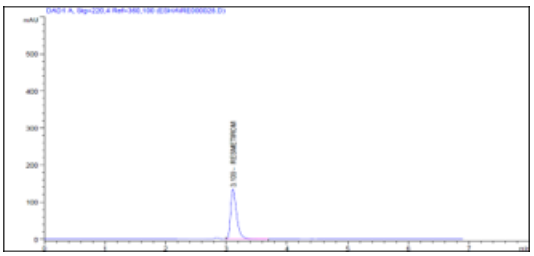

Figure 7 Chromatogram of Accuracy 100%

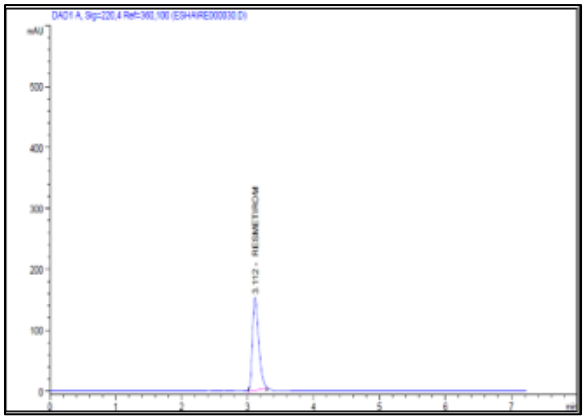

Figure 8 Chromatogram of Accuracy 120%

Table 8 Result of Recovery data for Resmetirom

Drug	Sr No.	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml)	Amt found* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean*± S.D.
HPLC method	1	80%	5	4	8.96±0.01	3.96± 0.01	99.10±0.03
	2	100%	5	5	10.00±0.01	5.0±0.01	100.05± 0.20
	3	120 %	5	6	10.98±0.01	5.98±0.018	99.69± 0.30

*mean of each 2 reading.

Table 9 Statistical Validation of Recovery Studies Resmetirom

	Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
HPLC	80%	99.10	0.03	0.03
	100%	100.05	0.20	0.20
	120%	99.69	0.30	0.30

*Denotes average of three determinations.

Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-102%

3.5. System suitability parameters :(Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Resmetirom system suitability parameters were studied. The result shown in below

3.5.1. Result for Repeatability (SST):

Table 10 Repeatability studies on Resmetirom (HPLC)

Sr.No.	Concentration of Resmetirom (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	5	467.44	4.95	99.03
2	5	467.24	4.92	99.05
		Mean	4.94	99.04

		SD	0.14	0.14
		%RSD	0.03	0.03

Repeatability studies Resmetirom was found to be 101.04%, the %RSD was less than 2, which shows high percentage amount found in between 100% indicates the analytical method that concluded.

3.5.2. Precision

The method was established by analyzing various replicates standards of Resmetirom. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in respectively

Table 11 Result of Intraday and Inter day Precision for Resmetirom HPLC

Concⁿ (µg/ml) HPLC	**Intraday Precision**			**Interday Precision**		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	933.05±0.02	100.00	0.00	932.93±8.02	99.99	0.86
15	1418.87±0.52	101.77	0.04	1387.20±13.24	99.49	0.95
20	1841.23±0.78	99.22	0.04	1846.26±16.63	99.50	0.90

*Mean of each 3 reading

Intraday and Inter day Precision for Resmetirom which shows the high precision % amount in between 98% to 102% indicates to analytical method that concluded.

3.6. Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied

Table 12 Result of Robustness Study of Resmetirom

Parameters	**Conc.(µg/ml)**	**Amount of detected(mean±SD)**	**%RS D**
Mob-phase composition(67ml+33ml)Methanol + 0.1% (Acetic Acid)water	5	468.53±0.01	0.001
Mob-phase composition(69 ml+31 ml) Methanol + 0.1% (Acetic Acid)water	5	468.57±0.16	0.03
Wavelength change 219 nm	5	460.9±0.81	0.18
Wavelength Change 221 nm	5	474.70±0.01	0.00

3.6.1. Robustness Study of Resmetirom :

The changes were did mobile phase composition (±1 ml/ min^{-1}), and Wavelength.

%RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded.

3.6.2. Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

= 3.3 X 7.82/ 92.25

= 0.27974

Where, SD = Standard deviation of Y intercept S = Slope

The LOD of Resmetirom was found to be **0.27974** (µg/mL) analytical methods that concluded.

3.6.3. Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the

S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

=10 X 7.82/ 92.25

= 0.8476

Where, SD = Standard deviation Y intercept

S = Slope

The LOQ of Resmetirom was found to be **0.8476** (µg/mL) analytical methods that concluded.

Analysis of formulation:-

Brand Name : ReMASH (80MG)

Total weight of 20 tab Powder wt. = 2.512gms

Average Powder Weight = 125.6 mg /Tab Eq.Wt for 5 mg = 5 x 125.6 /100

= 6.28 mg

Take 6.28 mgs in 10 ml Methanol i.e= 500µgm/ml RESMETIROM -TAB STOCK II

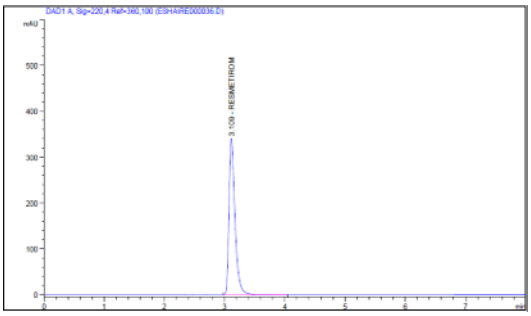

Figure 9 Chromatogram for Formulation 01

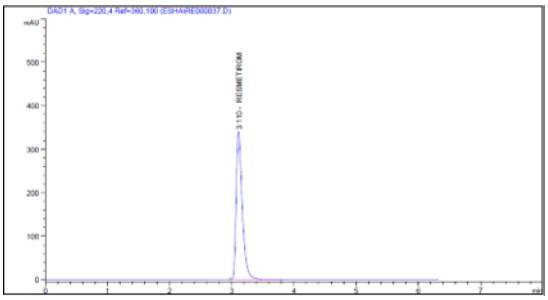

Figure 10 Chromatogram for Marketed Formulation Marketed 02

Table 13 Analysis of marketed formulation. (HPLC)

Sr.no	Amount present in mg	Area(I)	Amount found in mg	% Label claim
	RMT	RMT	RMT	RMT
1	25.00	2314.2	24.97171	99.89
2	25.00	2313.56	24.96477	99.86
Mean		2313.88	24.97	99.87
SD		0.453	0.005	0.020
%RsD		0.020	0.020	0.020

Analysis of marketed formulation were also %Lable Claim was found to be 98-101% Satisfactory are concluded**.**

Table 14 Tablet for %Lable claim

Sample	Label claimed	%Label claimed± SD	%RSD
ReMASH	Resmetirom =80mg	99.87± 0.020	0.020

Tablet Assay for %Lable claim for were also was found to be 98% and 102% RSD are less than 2 satisfactory result that concluded.

3.6.4. Ruggedness

The degree of reproducibility of test result obtains by the analysis of same sample under variety of Condition. Such as different analyst, laboratory Different instrument

Table 15 Analysis of Ruggedness.

Assay	Drug	Conc	%Amt Found	SD	%RSD
Resmetirom	Analyst1	10.00	99.77	0.019	0.19
	Analyst2	10.00	100.04	0.19	0.19

4. Discussion

Attempts were made to develop RP-HPLC method for simultaneous estimation of Resmetirom from Tablet.

Methanol: water (0.1%Acetic Acid), (68:32) v/v, pH 3.was used as the mobile phase for the method. The detection wavelength was 220 nm and flow rate was 0.9 ml/min. In the developed method, the retention time of Resmetirom was found to be being 3.1 min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible.

So the proposed methods can be used for the routine quality control analysis Resmetirom in bulk drug as well as in formulations.

5. Conclusions for HPLC method

This developed RP-HPLC method for estimation of Resmetirom is accurate, precise, robust and specific.

The method has been found to be better than previously reported method, because of its less retention time, isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

Availability of data and materials

Supplementary material that is available on the publisher's website along with the published article contains spectroscopic data of all compounds.

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