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A NEW BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RESLIZUMAB IN BULK AND FORMULATION BY LC-MS

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ABSTRACT

An easy, quick, precise, active and reproducible LC-MS/MS bio analytical Method was developed in rat plasma for the estimation of Reslizumab using Avelumab as internal standard.

This article summarizes the recent progress on bioanalytical LC-MS/MS method developed by using Inertsil ODS column (250x4.6 mm, 5µ) with organic mobile phase of 5mM Ammonium Formate: Methanol (60:40) of pH-5.5 with flow rate of 1ml/min and the run time was 7min with detected wavelength of 225nm by using avelumab as an internal standard. The Retention time of Reslizumab and internal standard of Avelumab was found to be 2.281min and 4.436min. The analyte was analyzed by mass spectrometry in the multiple reaction monitoring mode. A Turbo-Ion spray source was interfaced between the HPLC and triple quadrupole mass spectrometer (MDS Sciex API 4000). Where the acquired The MRM transitions were m/z 476.2 → 134.1masses for Reslizumab and Avelumab was m/z 313.3 → 245.1 (ISTD) were used for quantification of an analyte and its IS. The calibration curve was linear in the range of 5-100 ng/ml for Reslizumab. The method was validated in terms of accuracy, precision, selectivity, recovery, freeze-thaw stability, bench-top stability, stock solution stability and re-injection reproducibility. The mean recovery

for drug was obtained 99.8%, where as the mean recovery of IS was 99.4%. The %RSD value at higher concentration and lower concentration in all stability experiments was within 15%. This method is free from ion suppression, ion enhancement and any type of abnormal ionization. The method denotes all the parameters of system suitability, specificity, linearity and accuracy are in good agreement with USFDA guidelines and applied effectively for the investigation of pharmacokinetic studies in rat plasma for the determination of reslizumab in bulk and formulation using Avelumab as internal standard.

Keywords: Reslizumab, Avelumab (IS), LC-MS/MS, Validation, Rat plasma

INTRODUCTION

Interleukin-5 **[1]** monoclonal antibody **[2, 3]** reslizumab is humanised (IL-5) **[4]**. Eosinophil **[5, 6]** development, maturation, recruitment, and activation are all dependent on IL-5, which Reslizumab particularly binds to. By interacting with human IL-5, it inhibits its biological function, which results in decreased eosinophil survival and activity. Using reslizumab, individuals with severe eosinophilic asthma **[7, 8]** (blood eosinophil count 400 cells/L) and at least one prior asthma exacerbation in the preceding year may benefit from reduced exacerbation rates and improved lung **[9]** function and asthma-related quality of life. Anaphylactic **[10, 11]** responses, myalgia, and elevated blood creatine phosphokinase **[12]** are the most prevalent adverse effects. Patients with a history of severe asthma episodes (exacerbations) despite using their existing asthma medications may be eligible for Cinqair treatment. Reslizumab is available for intravenous infusion as a chilled, sterile, single-use solution devoid of preservatives. Less common adverse effects include musculoskeletal pain **[13, 14]**, neck pain **[15, 16]**, muscle spasms, extremity pain, muscle fatigue **[17, 18]**, anaphylaxis, malignancy **[19]**.

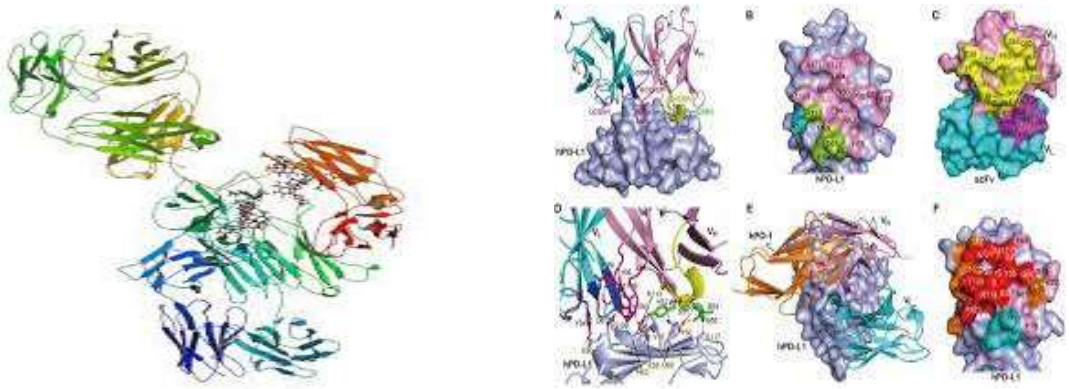

Figure 1.01: Structure of Reslizumab and structure of Avelumab (Internal standard)

The Literature survey revealed there is only one RP- HPLC method reported for the quantification of Reslizumab in bulk and pharmaceutical formulation. The present work aims to develop a new bioanalytical method for the estimation of Reslizumab and its application to pharmacokinetics studies by LCMS.

MATERIALS AND METHODS

Chemicals and reagents

The pure sample of Reslizumab and Avelumab were obtained from Glenmark pharmaceuticals pvt ltd, Andheri, Mumbai, India. HPLC grade methanol, ammonium format, ortho phosphoric acid, water and all other chemicals were obtained from Merck chemical division, Mumbai. The rat plasma was obtained from Vivo Bio Tech Ltd., Hyderabad stored at 2-8°C until use.

Blank plasma

Specimens were calibrated and controlled using buffered blank plasma obtained from a rat. Vivo Bio Tech Ltd., Hyderabad, provided plasma samples for the specificity check experiment in method development, validation and also used for study analysis for the preparation of calibration standards and quality control samples. The plasma was collected from healthy rat for research purpose, through an informed consent.

Equipment

Details of instruments used in the current study are reported below. Data acquisition and handling on LC-MS/MS system was performed using analyst software version 1.4.2. As a part of this experiment, we employed an HPLC system of the Waters alliance type E2695 coupled to the QTRAP 5500 triple-quadrupole instrument (sciex). The procedure was performed using Empower 2.0. After optimization, the following are the mass spectrometry working parameters: ion spray voltage is 5500 volts, the temperature source is 550 degrees Celsius, the drying gas temperature is 120 to 250 degrees Celsius, the collision gas is nitrogen, the pressure is 55 psi, and the drying gas flow rate is 5 millilitres per minute.

Pharmacokinetic Study

Selection of animals

In vivo pharmacokinetic studies, 6 healthy rats (250-300 g) were obtained from Biological E Limited, Hyderabad, India. The protocol of animal study was approved by institute of animal ethics committee (Reg.No:1074/PO/Re/S/05/CPCSEA).

Chromatographic conditions

Chromatographic separation, using inertsil ODS (250 x 4.6 mm, 5 micron) columns, was administered in isocratic mode at room temperature. As a mobile phase, a mix

of 5mM Ammonium format of pH-5.5 with OPA and Methanol at 60:40 v/v with a flow of 1.0 ml/min was used. 10µl was the injection rate and the run time was 7 minutes.

Preparation of standard and internal control samples

Standard stock solution preparation

Dissolve 5 mg Reslizumab standard in a 10ml volumetric flask and add 7ml of diluent and sonicate for 10 minutes and the volume was made upto the mark with diluent. Further dilute 0.1 ml into 10 ml volumetric flask and further transfer 0.4 ml of the above solution into a 10ml volumetric flask and the volume make upto the mark with diluent.

Internal Standard preparation

Dissolve 5mg of Avelumab internal standard into 10ml of volumetric flask and sonicate the solution for ten minutes. Transfer 0.1 ml of the above stock solution into 10 ml volumetric flask volume made upto the mark with diluent. Transfer 0.4 ml of the above solution is transferred into 10ml volumetric flask and diluent is added to make up the volume upto the mark.

Sample stock solution preparation

5 mg of Reslizumab sample is transferred into a 10ml volumetric flask and add 7ml of diluent and sonicate for 10 minutes for dissolve and the volumetric flask is make upto the mark with diluent. Further dilute 0.1 ml of above solution into 10 ml volumetric flask. Transfer 0.4 ml of the above solution is put into a 10ml volumetric flask and diluent is added to make upto the volume.

Standard solution preparation

In a centrifuge tube, 200 µl of plasma and 300 µl of methanol were combined, and then 500 µl of stock solution, 500 µl of IS, and 500 µl of diluting solutions were added and vortexed for 10 minutes. This was followed by a ten-minute centrifugation of the centrifuge tube. These samples were then spun at 2000 rpm for 30 minutes in a centrifuge. After being filtered via a 0.45 nylon syringe filter, the clear solution was placed in a vial and introduced into the system.

Detection

Detection was done by turbo ion spray (API) positive mode with unit resolution. Quantification was by MRM, the MRM transitions were m/z 476.2 → 134.1masses for Reslizumab and Avelumab was m/z 313.3 → 245.1 (ISTD) were used for quantification of an analyte and its IS. where the acquired masses for Chromatographic conditions Chromatographic separation was achieved with Methanol:20mM Ammonium formate (40:60%v/v), gave the best peak shape and low baseline noise was observed using the Inertsil ODS column (250x4.6 mm, 5µ) with detection wavelength of 225nm.The

total analysis run time was 7min and flow rate was set to 1.0 mL/min. The temperature was set to 40°C for the column oven. The sample volume for the injection into mass spectrometry was adjusted to 10 µL for better ionization and chromatography.

Table 1.01: Assay results of Reslizumab

S. No.	Formulation (Capsule)	Labelled amount (mg/Tab)	Amount Found	%Assay
1	Reslizumab	10mg/ml	9.85mg	98.5%

Bio analytical Method validation

The validation was performed as per FDA guidelines to evaluate the method in terms of linearity response, sensitivity, selectivity, precision and accuracy (within-batch and between-batch/inter-day), stabilities (freeze-thaw, bench top, short- term and long-term stock solutions, working solutions and long term stability in matrix), carryover effects, recovery, dilution integrity, matrix effect, matrix factor, autosampler re-injection reproducibility and ruggedness experiment.

System suitability

System suitability was carried out to check the system performance. In This validation parameter system suitability was carried out by two injections of low standard and six injections of high standard analyte. Reslizumab is injected to ensure system conditions. The low standard concentration was to check the peak shape. The % CV of area ratios (analyte/ISTD for the analyte) high standard should be less than 4. The found percentage CV values are in the range of 0.83-0.84 for entire validation. injecting six replicates of the standard solutions of the MQC-1 to MQC-6. The system suitability results and the chromatograms are shown in the **Table 1.02** and **Figure 1.02-1.07**.

Benchmark for approval: In the proposed approach the %CV of the RT should be less than 2.00 percent. The percentage CV of the response ratio should be ≤ 5.00 percent.

Table 1.02: System suitability Results of Reslizumab

Samlple Name MQC (50ng/ml)	Analyte Response (cps)	Analyte RT (min)	ISTD Response (50ng/ml)	ISTD RT (min)	Response Ratio
MQC-1	$3.521x10^5$	2.881	$3.334x10^5$	4.436	0.9469
MQC-2	$3.584x10^5$	2.886	$3.364x10^5$	4.439	0.9375
MQC-3	$3.562x10^5$	2.889	$3.35x10^5$	4.442	0.9405
MQC-4	$3.581x10^5$	2.892	$3.350x10^5$	4.446	0.9355
MQC-5	$3.59x10^5$	2.896	$3.320x10^5$	4.446	0.9248
MQC-6	$3.53x10^5$	2.898	$3.334x10^5$	4.451	0.9445
Mean	$3.561x10^5$	2.89	$3.342x10^5$	4.443	0.9383
SD	0.02943	0.00635	0.01565	0.00543	0.00785
%CV	0.83	0.22	0.47	0.12	0.84

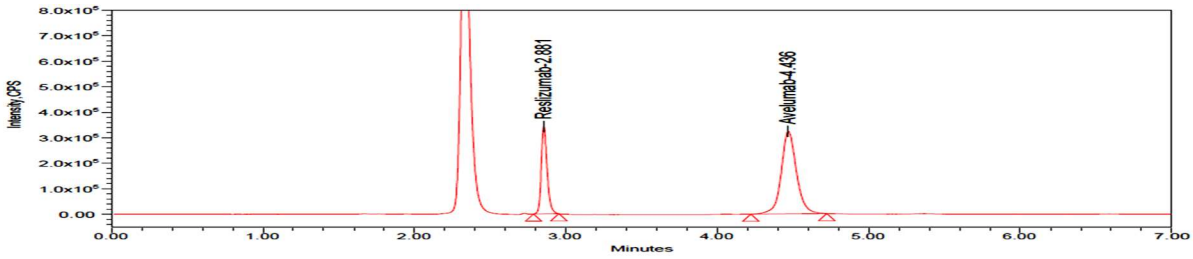

Figure 1.02: Standard-1 Chromatogram

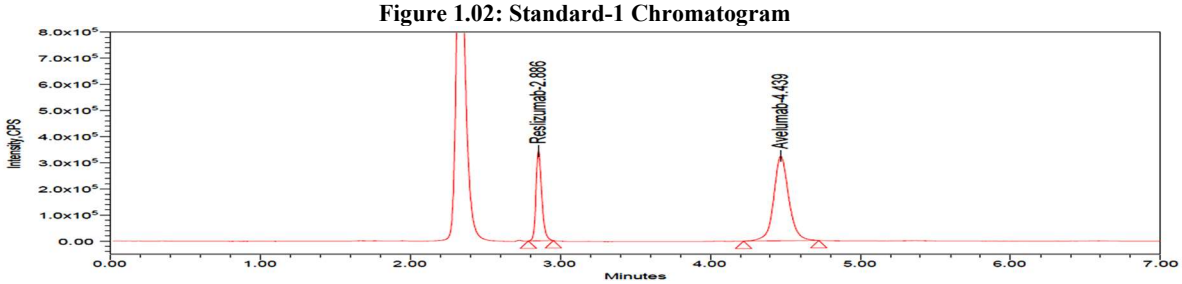

Figure 1.03: Standard-2 Chromatogram

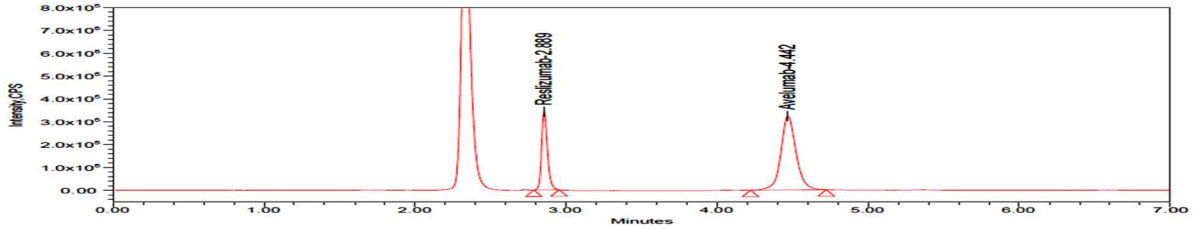

Figure 1.04: Standard-3 Chromatogram

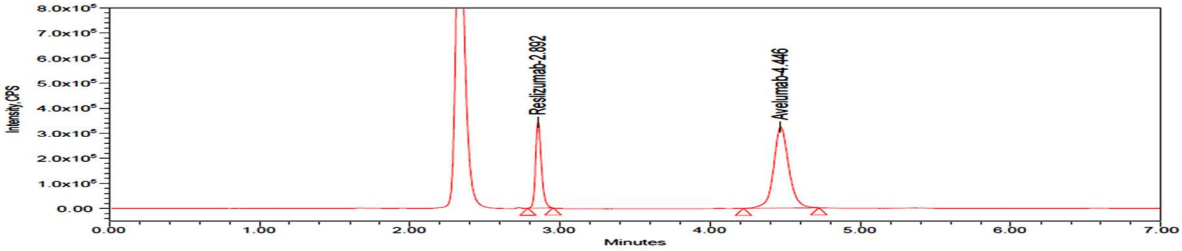

Figure 1.05: Standard-4 Chromatogram

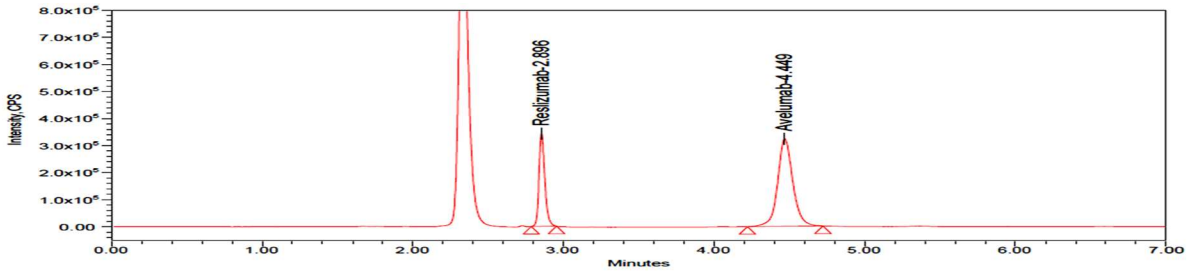

Figure 1.06: Standard-5 Chromatogram

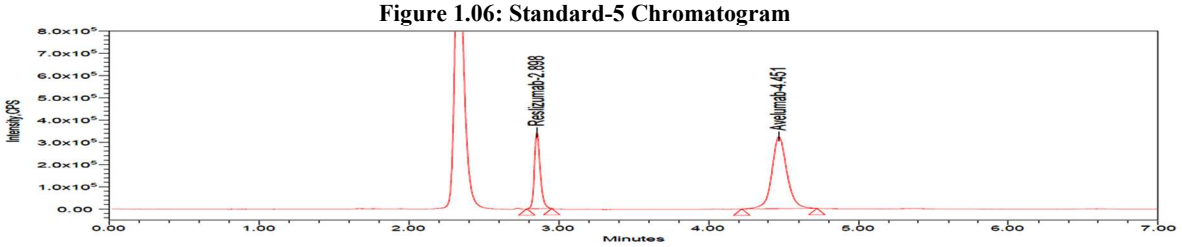

Figure 1.07: Standard-6 Chromatogram

Specificity and sensitivity

Specificity is the capability of the developed method to differentiate the analyte with the other endogenous components in the sample. Potential interfering substances in a biological matrix include endogenous matrix components, metabolites and decomposition products.

The specificity of the method was tested by analyzing blank samples of the appropriate biological matrix from at least six sources. Each blank should be tested for the interference of other substances and selectivity should be ensured at LLOQ. Specificity is ensured at LLOQ level. Aqueous samples of LLOQ were injected six times. Spiked samples of LLOQ were injected six times along with one injection of blank plasma sample.

Retention times of Reslizumab and its ISTD may not interfere with the blank rat plasma specimens. The results of specificity and their chromatograms of lower limit of quantification should be shown in the **Table 1.03** and the calibration curve and Linearity chromatograms are shown in the **Figure 1.08-1.10**.

Table 1.03: Results of Specificity study

Name of the solution	Retention time
Blank rat plasma	No peak
Placebo	No peak
Reslizumab	2.867 min
ISTD	4.451 min

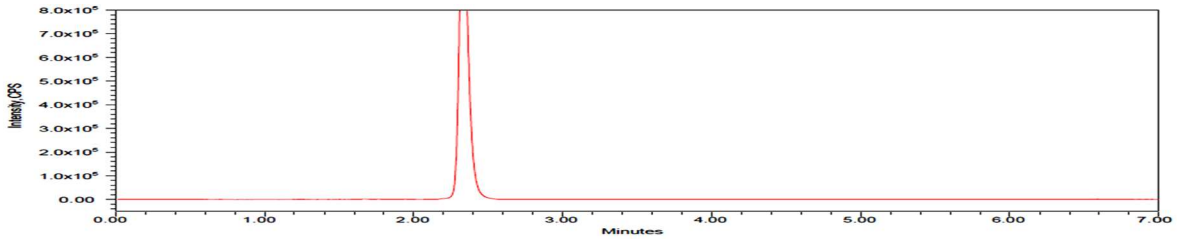

Figure 1.08: Blank rat plasma Chromatogram

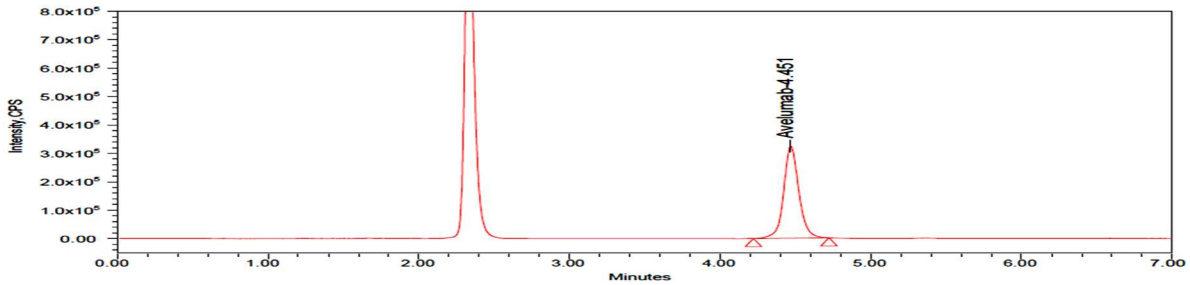

Figure 1.09: Blank and ISTD Chromatogram

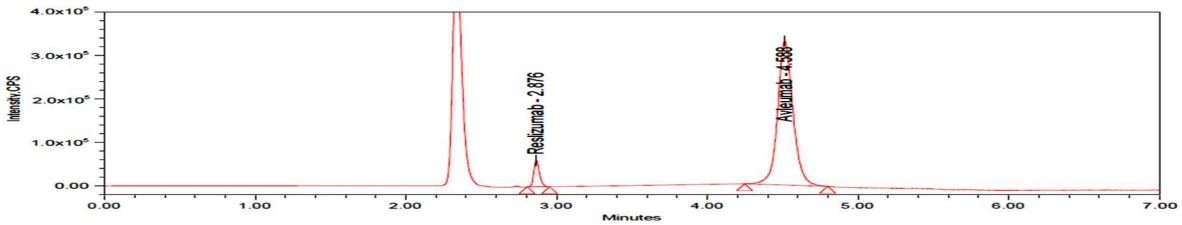

Figure 1.10: LLOQ Chromatogram

Matrix effect

The signal suppression or enhancement via ionization should be studied in mass spectrometric detection methods. To prove method is free from matrix effects and method diversity, post extraction responses from 10 different lots (including each two lots of hemolytic and lipemic plasma) was compared with aqueous samples. The matrix effect was evaluated at LQC, HQC levels by calculating matrix factor of analyte and ISTD. Later ISTD normalized matrix factor was calculated by using matrix factor of analyte and ISTD. Matrix factor value 1 indicates there is no suppression or enhancement. The acceptable limits for IS normalized matrix factor 0.94-1.03. Matrix effect was also evaluated by direct quantification method. Each three replicates of LQC and HQC from six plasma lots along with each two hemolytic and lipemic lots were prepared. More than 2/3 rd. or 67 % of processed QC's were accepted from all lots which indicates that there was no significant effect due to plasma constituents (matrix) on quantification method.

Sample response after plasma extraction and analyte compatibility at identical intensities are examined in this research. A chromatographic technique is used to detect the plasma and ionised ions in the extracted plasma. The matrix effect results are shown in the **Table 1.04 and Figure 1.11-1.12** gives matrix effect chromatograms.

The benchmark for approval: Two out of three samples had a percent average exactness of 85-115. 80% of the matrices must fulfil the standards for approval is 98.46% and HQC is 98.55% percent for numerous biologic-matrix back-measured concentration accuracy percentages.

Linearity

The eight-point calibration curve was constructed by plotting the peak area ratio on Y-axis and the nominal concentration on X-axis. Three calibration curves were processed and injected. From the back calculated concentrations of each curve, regression equation calculations for weighing factors 1/X, 1/X2 were performed to determine the best linear fit. Finally, the weighing factor selected was 1/X2. The obtained linear

regression equation for Reslizumab is y = 0.029820x + 0.00351. The correlation coefficient for all calibration curves generated during the validation was 0.999. The mean back calculated concentration results of calibration standards for Reslizumab were shown in the **Table 1.05** and the linearity graph and chromatograms are shown in the **Figure 1.13-1.21**. The linear relationship for reslizumab is in between the concentration of 5-100 ng/ml. It had an average coefficient of 0.999. The analyte peak to IS peak ratio was used to determine the samples' concentrations.

Table 1.04: Matrix effect data Results of Reslizumab (HQC-75ng/ml, LQC-25ng/ml)

Blank plasma lots	Internal standard normalized matrix factor	
	LQC	HQC
LOT-1	0.99	0.98
LOT-2	0.98	0.97
LOT-3	0.98	0.94
LOT-4	0.97	0.94
LOT-5	1.02	0.95
LOT-6	0.99	0.94
LOT-7 hemolytic	1.03	0.95
LOT-8 hemolytic	0.98	0.98
LOT-9 Lipemic	0.99	0.99
LOT-10 Lipemic	0.95	0.94
Mean	0.994	0.985
SD	0.196	0.0395
%CV	2.0	4.0
%Mean accuracy	98.46%	98.55%

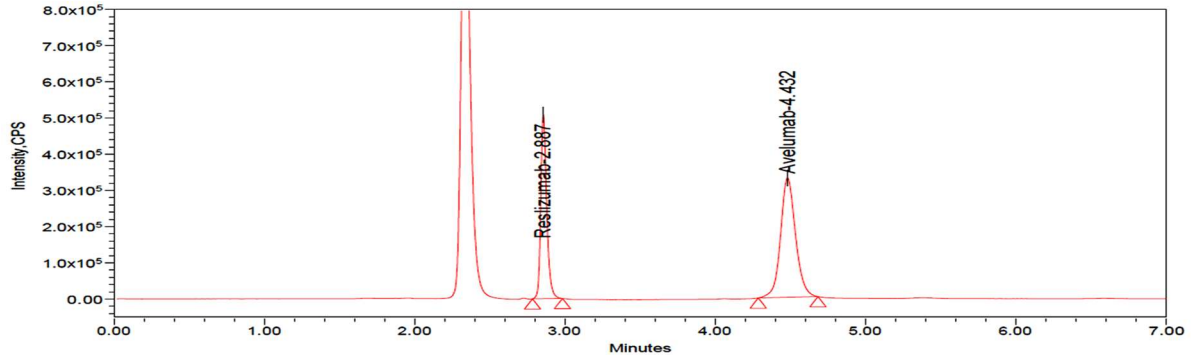

Figure 1.11: Matrix effect chromatogram of HQC

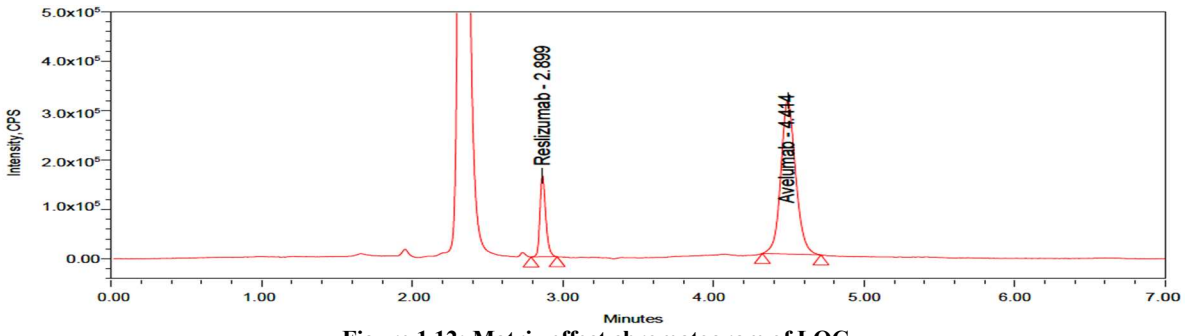

Figure 1.12: Matrix effect chromatogram of LQC

Table 1.05: Summary of Reslizumab Calibration standards

Nominal concentration(ng/ml)	Mean concentration found(ng/ml)	%Accuracy	%Relative error
5	**4.98**	**99.7**	**0.3**
12.50	**12.4**	**99.2**	**0.8**
25.0	**24.82**	**98.3**	**1.7**
37.50	**36.07**	**97.5**	**2.5**
50.00	**48.25**	**96.5**	**3.5**
62.50	**59.39**	**95.8**	**4.2**
75.00	**71.1**	**94.8**	**5.2**
100.00	**92.9**	**92.9**	**7.1**

Acceptance criteria: The linearity regression coefficient should be 0.999. It was accepted

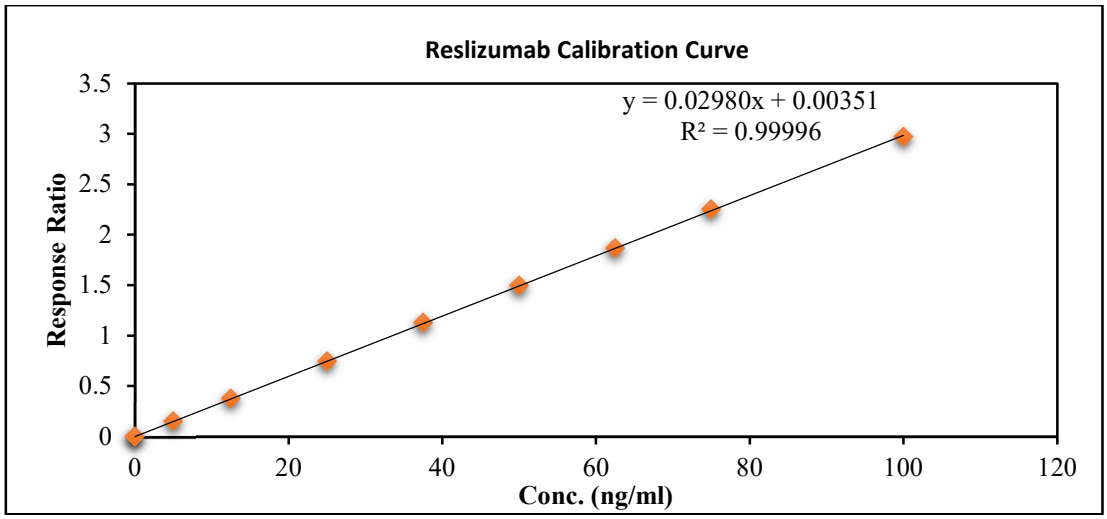

Figure 1.13: Calibration plot for concentration Vs area ratio of Reslizumab

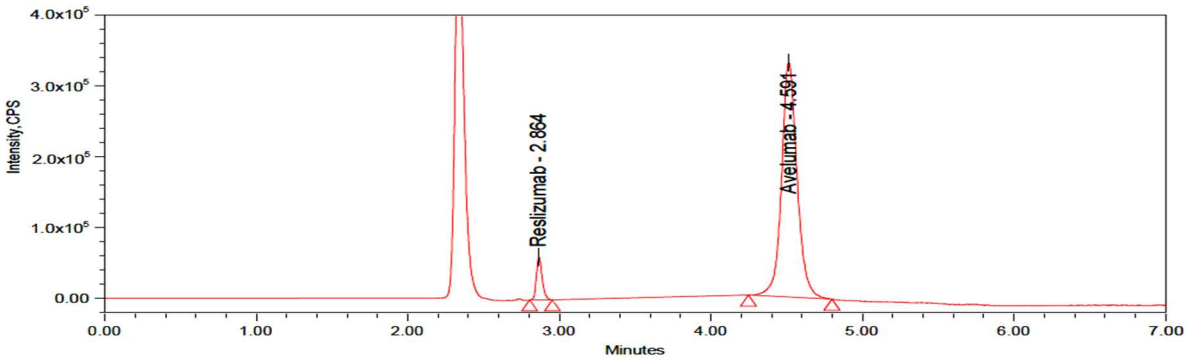

Figure 1.14: Chromatogram of linearity-1

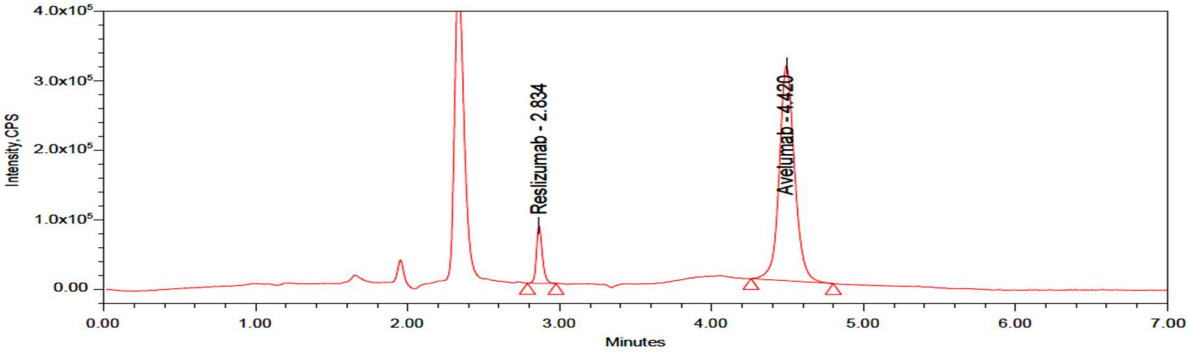

Figure 1.15: Chromatogram of linearity-2

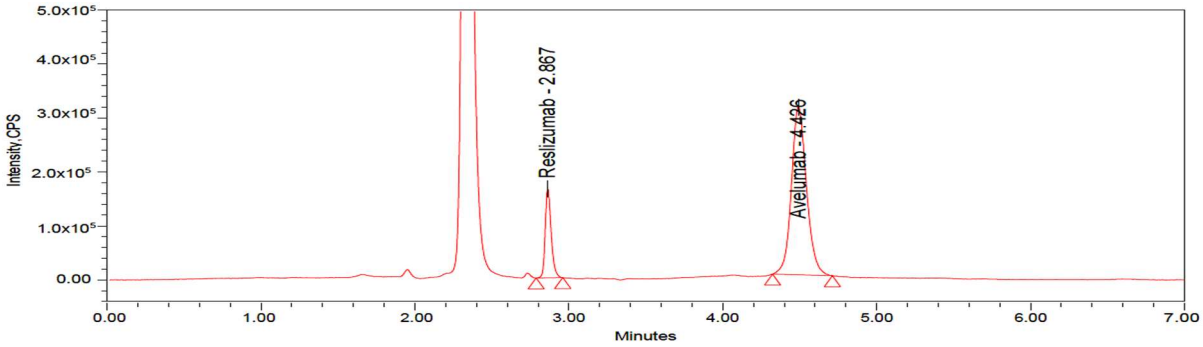

Figure 1.16: Chromatogram of linearity-3

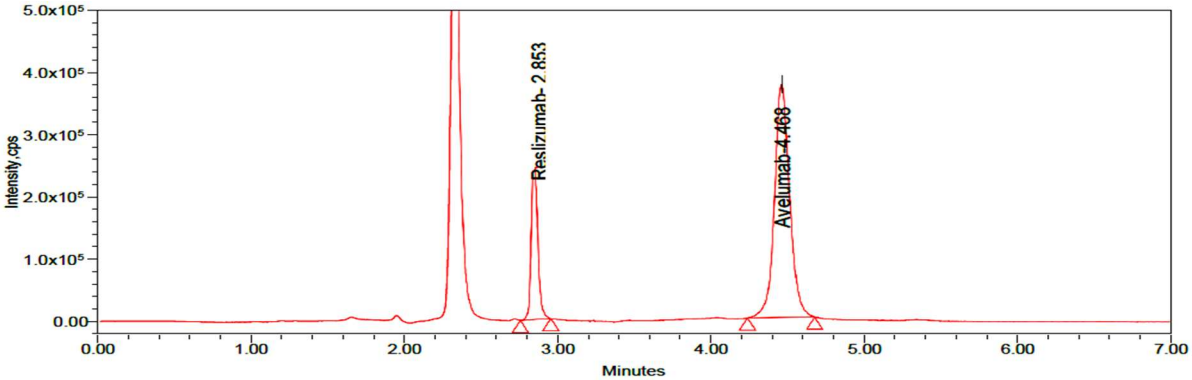

Figure 1.17: Chromatogram of linearity-4

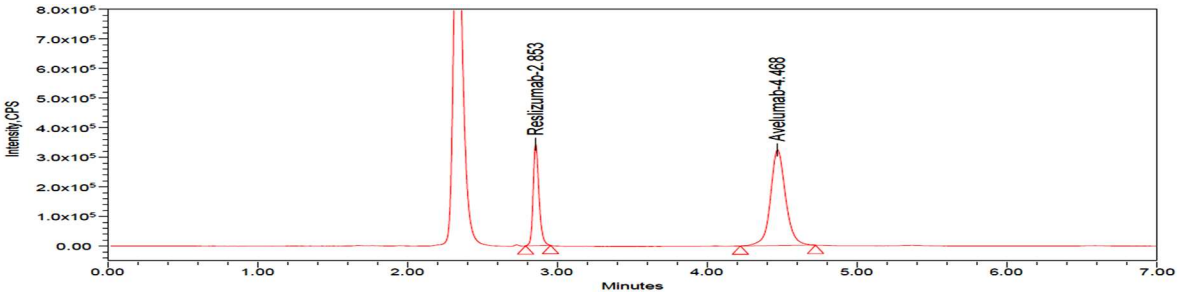

Figure 1.18: Chromatogram of linearity-5

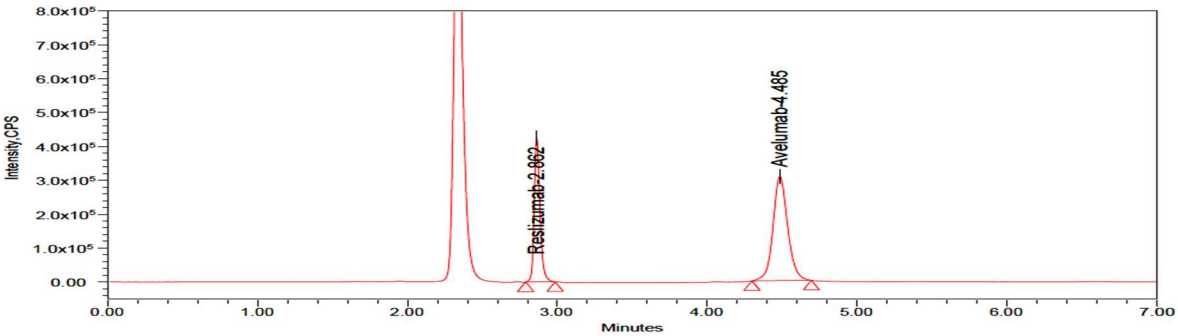

Figure 1.19: Chromatogram of linearity-6

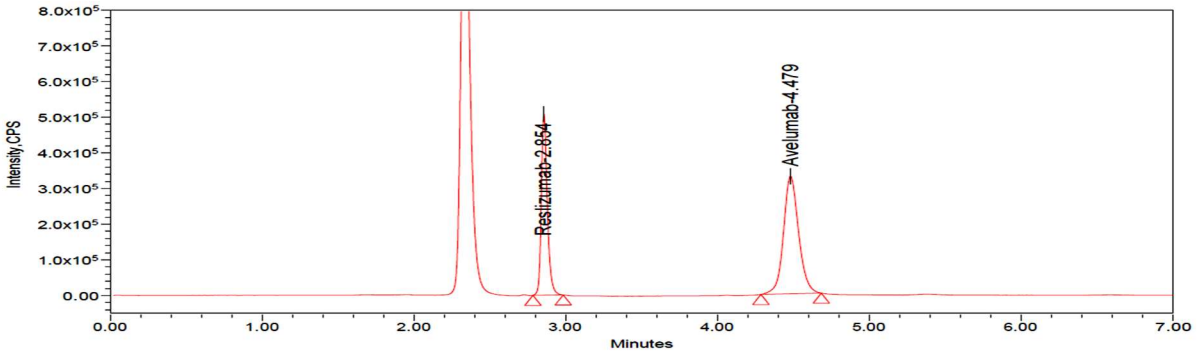

Figure 1.20: Chromatogram of linearity-7

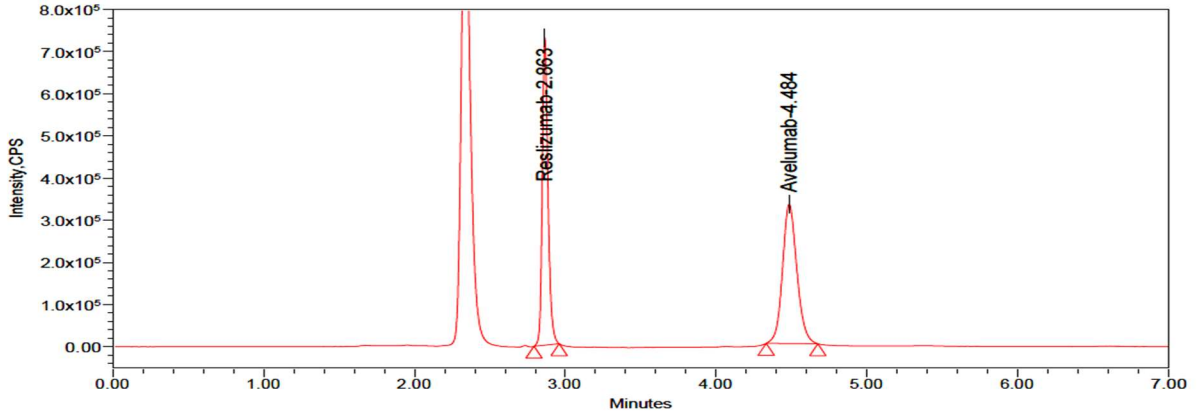

Figure 1.21: Chromatogram of linearity-8

6.5.2.5. Recovery of analyte

Qualitative and quantitative assessments of medication and IS recovery were performed at three distinct concentrations (low, medium and high). By comparing the sample's reaction to a normal solution, recovery is determined. The amount of analyte utilised affects the extraction's effectiveness, and the findings are equivalent to those obtained by analytical methods. Following are the extracted and un-extracted chromatograms for the chromatograms of LQC, MQC, and HQC.The Recovery results are shown in the **Table-1.06** and the recovery chromatograms are shown in the **Figure 1.22-1.33**.

The benchmark for approval: Specific stages such as QC and ISTD must have a recovered percentage (percent RSD) of less than or equal to 15%. Replicas of QC should provide net average recovery findings of less than or equal to 20%.

Table 1.06: Accuracy Results of Reslizumab

Sample name	%Mean recovery Abosolute	%Mean recovery Relative
LQC	98.16%	105.14
MQC	99.94%	102.5
HQC	98.47%	100.57
ISTD calculated at MQC level		99.92

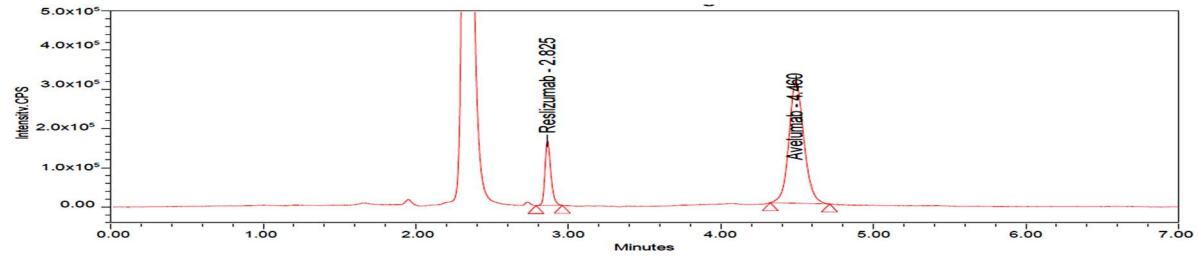

Figure 1.22: Chromatogram of recovery extracted LQC

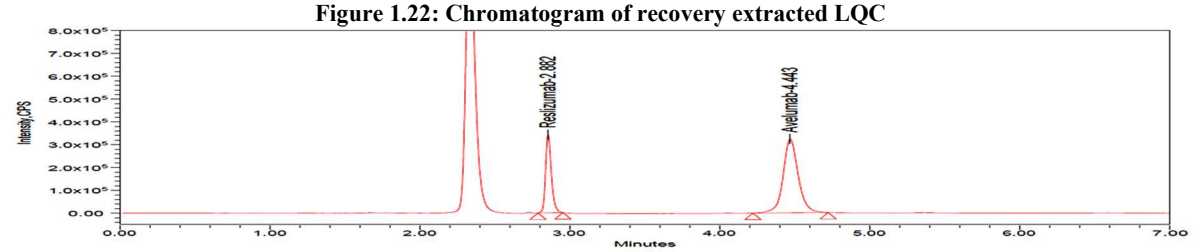

Figure 1.23: Chromatogram of recovery extracted MQC

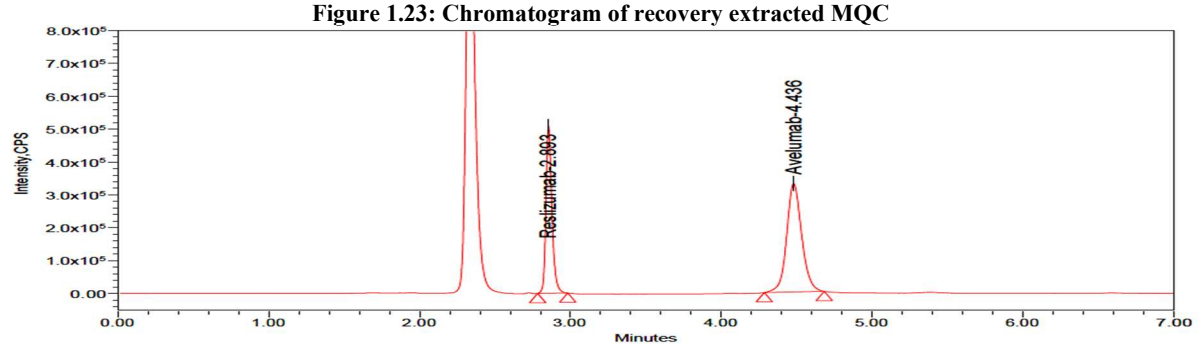

Figure 1.24: Chromatogram of recovery extracted HQC

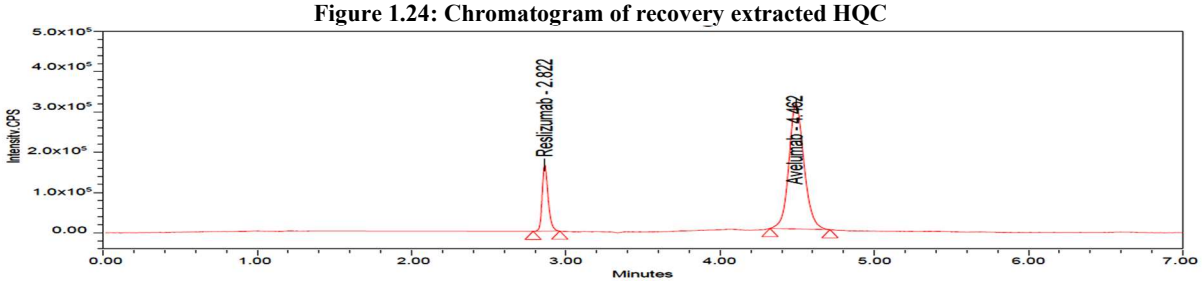

Figure 1.25: Chromatogram of recovery Un-extracted LQC

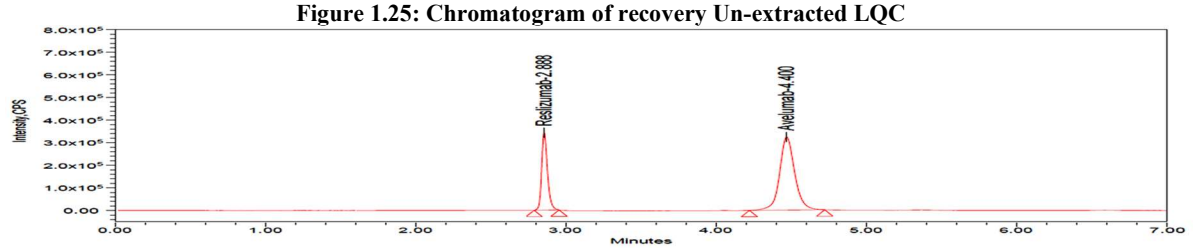

Figure 1.26: Chromatogram of recovery Un-extracted MQC

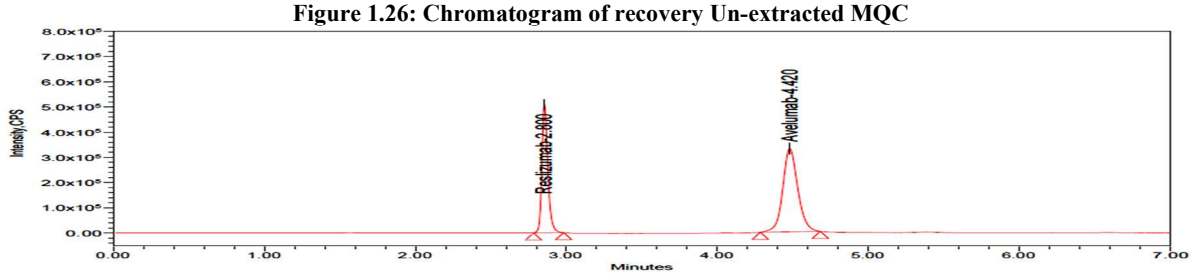

Figure 1.27: Chromatogram of recovery Un-extracted HQC

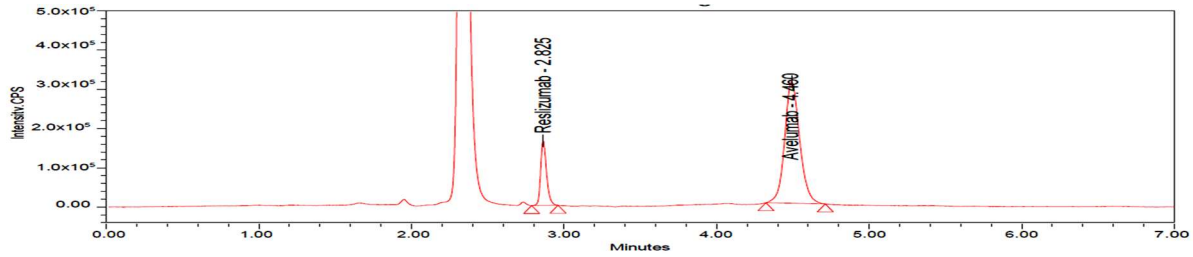

Figure 1.28: Chromatogram of recovery extracted LQC

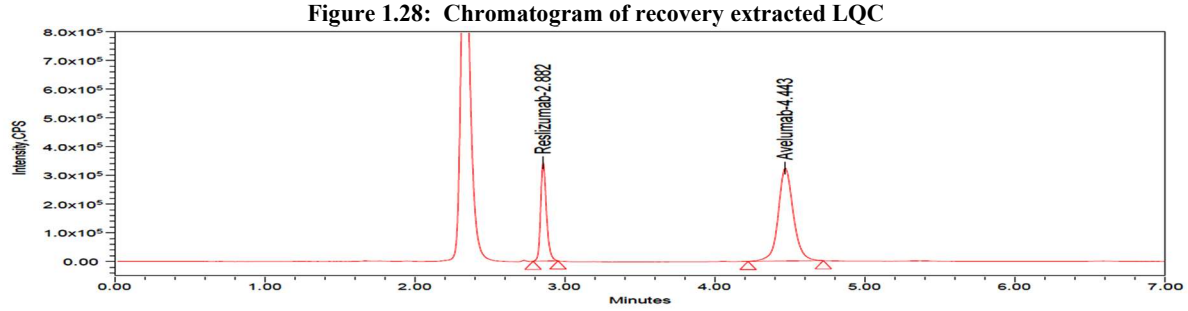

Figure 1.29: Chromatogram of recovery extracted MQC

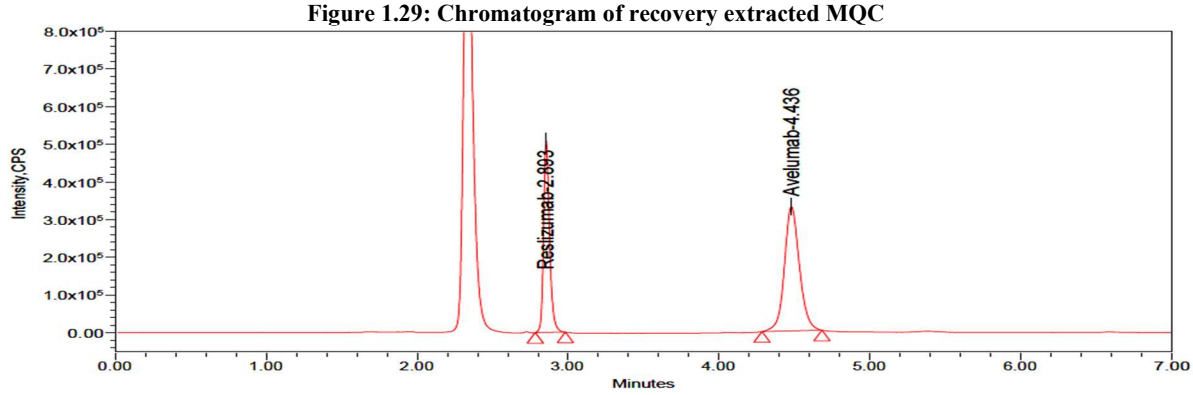

Figure 1.30: Chromatogram of recovery extracted HQC

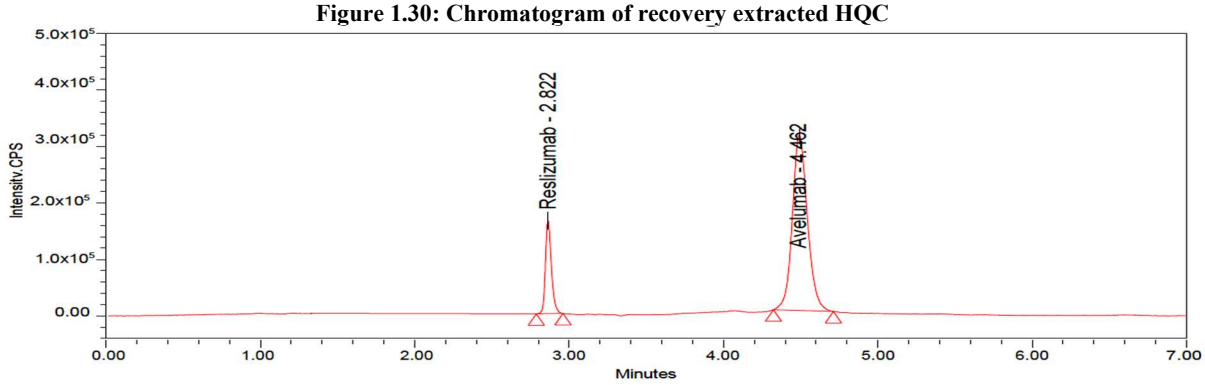

Figure 1.31: Chromatogram of recovery Un-extracted LQC

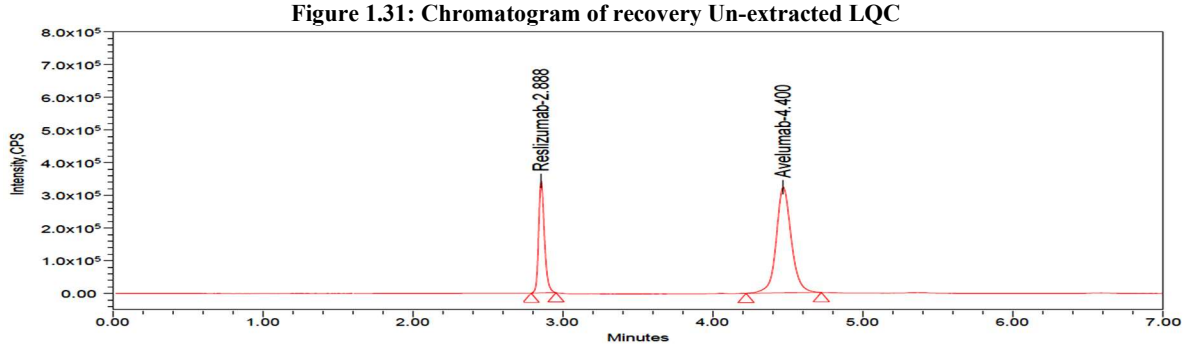

Figure 1.32: Chromatogram of recovery Un-extracted MQC

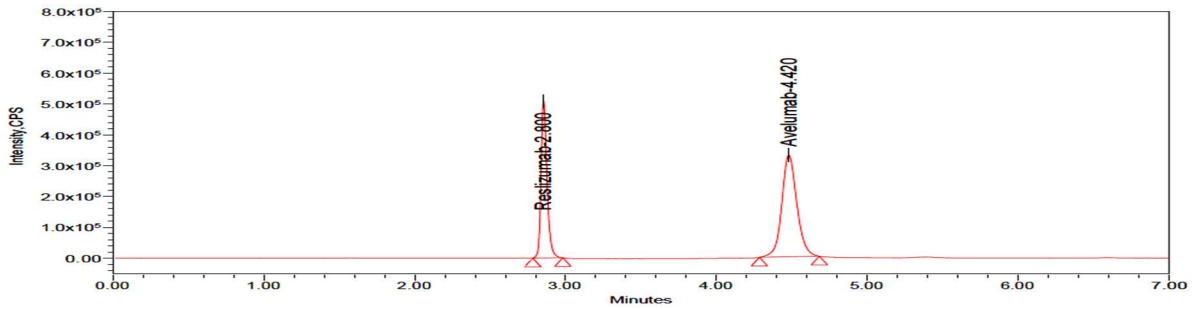

Figure 1.33: Chromatogram of recovery Un-extracted HQC

6.5.2.6. Precision and Accuracy results of Reslizumab (Inter and Intra-day)

Accuracy and precision were determined by performing different analytical runs for six continuous days. Each analytical run consists of calibration curve along with six replicates of QCs at five concentration levels. For Reslizumab the precision was found to be in between 0.4-1.3% for intra-day, whereas for inter day the precision was in between 0.2%-0.84% respectively. The accuracy for intra-day and inter-day was in the range of 98.3%-99.4% for Reslizumab. The results illustrate that the method was accurate, robust and judged to be reproducible over the calibration range for both analytes. The accuracy and precision results were shown in **Table 1.07** and the chromatograms are shown in the **Figure 1.34-1.37**.

The benchmark for approval: A accuracy of 15% for LQC, MQC, and HQC samples is acceptable, whereas LLQC samples should have a precision of 20% is acceptable under the present technique.

Table 1.07: Accuracy and Precision Results of Reslizumab

Sample name	INTRA-DAY(n=6)		INTER-DAY(n=12)	
	Reslizumab			
	% Accuracy	%CV	% Accuracy	%CV
LLOQQC	99.4	0.9	98.5	0.83
LQC	98.46	1.03	99.2	0.22
MQC	98.3	0.74	99.47	0.47
HQC	98.5	0.4	99.41	0.84

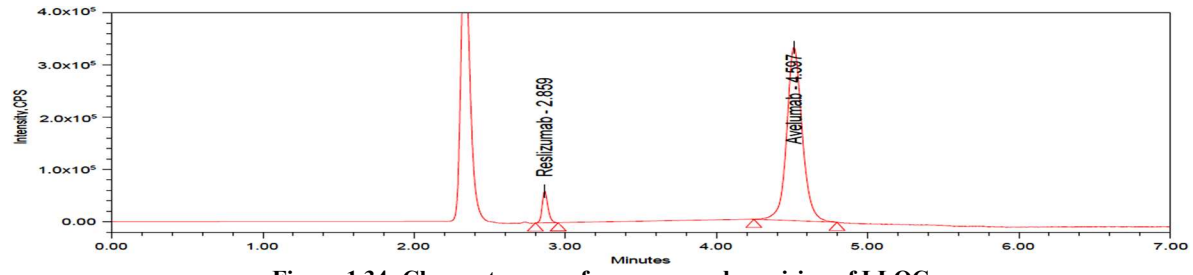

Figure 1.34: Chromatogram of accuracy and precision of LLQC

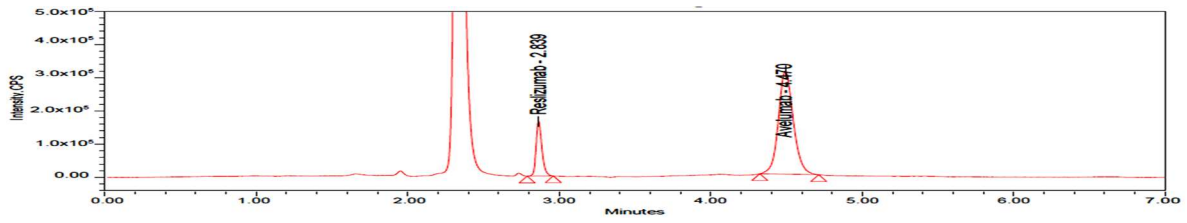

Figure 1.35: Chromatogram of accuracy and precision of LQC

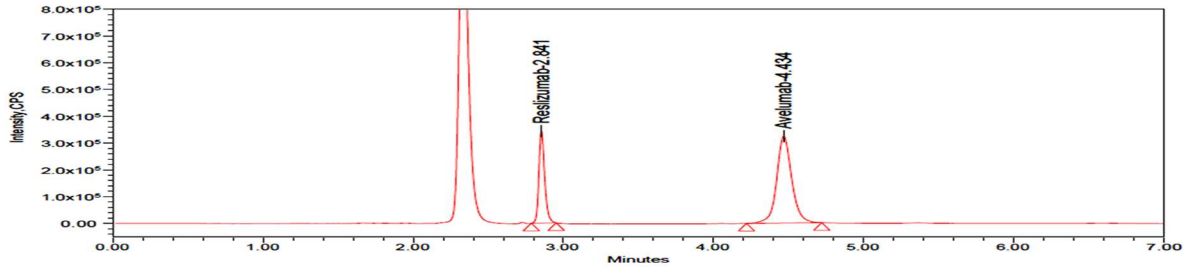

Figure 1.36: Chromatogram of accuracy and precision of MQC

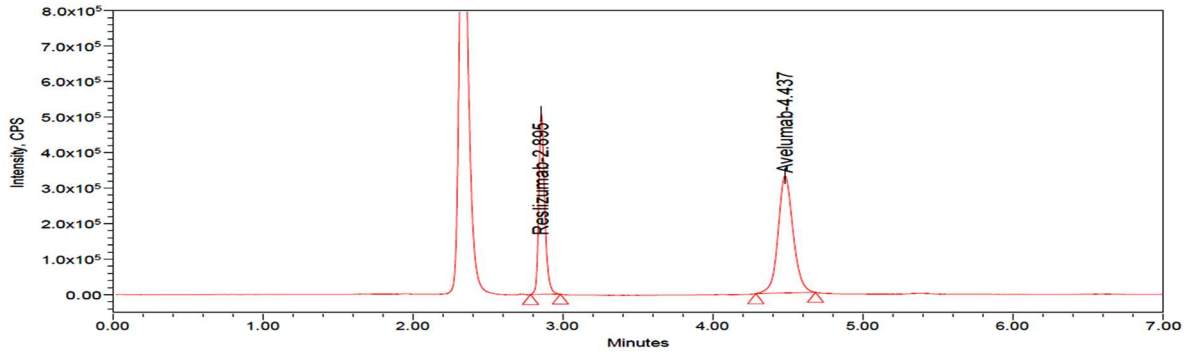

Figure 1.37: Chromatogram of accuracy and precision of HQC

The benchmark for approval: Specific stages such as QC and ISTD must have a recovered percentage (percent RSD) of less than or equal to 15%. Replicas of QC should provide net average recovery findings of less than or equal to 20%.

6.5.2.7. Stability

Based on method extraction conditions and regulatory requirements different stability experiments were conducted like bench-top stability (17 hrs) auto sampler stability (41hrs), repeated freeze–thaw cycles (five cycles) refrigerated stability (39 hrs) and long-term stability at -70°C for 32 days and whole blood stability (2.55 hrs) to evaluate analyte stability in plasma at proposed conditions. The mean percentage stability for Reslizumab was found to be within ±15% at LQC and HQC levels. Short term room temperature stability for stock solution and working dilutions were evaluated and the mean percentage stability results were found to be within ±10% for 23 hours. The results and stability conditions were represented in **Table 1.08**.

Table 1.08: Stability conditions and Results of Reslizumab

Stability experiment	Stability condition	%Mean stability of Reslizumab	
		LQC	HQC
Auto Sampler stability	41hrs at 5°C	98.46%	98.5%
Free and thaw stability	5cycles at -70°C±15°C	99.2%	99.4%
Dry extract Stability	52hrs at 2-8°C	98.3%	98.6%
Room Temperature Stability	16hrs at room temperature at 25°C±5°C	98.1%	"{
Long term stability	32 days at -70°C±15°C	99.92%	102.5%
Stability in blood	2.5h room temperature at 25°C±5°C	99.94%	100.5%

6.5.2.7 Pharmacokinetic Studies:

C_{max}, T_{max}, $T_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, were computed and the data is displayed. Reslizumab sample was introduced into six different rats and collected samples at various time intervals like 0.5, 1, 4, 8, 12, 16, 20, 24 and 28 Days. Afterwards, the samples are prepared according to the test technique, and the values are recorded in the chromatographic system.

Reslizumab pharmacokinetics were studied in healthy South Indian male participants (n = 6). The Independent Ethics Committee in the area accepted the study's protocol, which included written informed permission from all of the participants. All participants received a single dosage of Reslizumab pill (10mg/vial) and blood samples were obtained at 0.5, 1, 4, 8, 12, 16, 20, 24 and 28 Days after the medication. Each time point, a 5 ml sample of blood was taken in K2 EDTA vacutainer tubes. A predose sample was also taken to rule out any plasma-induced interferences. The IS was added to plasma samples and then processed with QC samples at four different concentrations. WinNonlin (Version 5.2) software was used to determine Reslizumab's pharmacokinetic parameters. **Table 1.09** gives pharmacokinetic parameter results and **Figure 1.38** gives recovery plots.

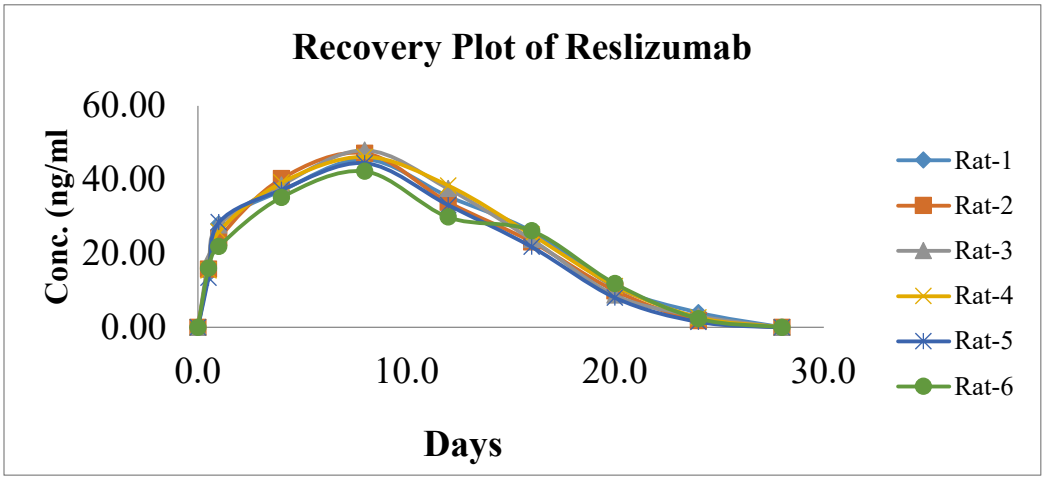

Figure 1.38: Recovery plot of Reslizumab

Table 1.09: Pharmacokinetic Results of Reslizumab

Pharmacokinetic parameters	Mean value of Reslizumab
t_{max}	0.83
C_{max}(ng/ml)	45.6 ng/ml
AUC_{0-t} (ng/ml)	1459 ng-hr/ml
$t_{1/2}$(h)	4.94
$AUC_{0-\infty}$	1459 ng-hr/ml
MRT	6.34

6.6. CONCLUSION

A novel method was developed for estimation of Reslizumab by using positive electrospray ionization technique, which is relatively more selective over the negative electrospray ionization. So far, no report was available for the estimation of Reslizumab in rat plasma. The method was validated as per current USFDA, ANVISA and EMA guidelines. The Q1 mass spectra for Reslizumab and ISTD were obtained individually and the most abundant product ions were selected for MRM analysis for high signal intensity. The MRM transitions were m/z 476.2 → 134.1 (Reslizumab), m/z 313.3 → 245.1 (ISTD) with a dwell time of 200 ms per transition. The MS/MS conditions for each transition were optimized to achieve the maximum signal transduction with low back ground noise. Based on the physicochemical properties and LC-MS/MS compatibility, Avelumab was selected as ISTD and its selection will not compromise the accuracy of analytical results during application of the method for clinical study. The analytes were extracted using LLE and chromatographed on Inertsil ODS (250 x 4.6 mm, 5 micron) analytical column. Reslizumab has a retention time of 2.881 minutes and Avelumab has a retention time of 4.436 minutes, for a total chromatographic runtime of 7.0 minutes No significant interferences were observed at retention times of Reslizumab and ISTD in the blank plasma samples including hemolytic and lipemic samples.

It is essential to have simple, rapid and rugged LC-MS/MS method was for the determination of Reslizumab concentration in clinical samples. The method applicable for calculating pharmacokinetics of bioequivalence/bioavailability and therapeutic drug monitoring studies in rat plasma.

Reslizumab has a dynamic linear range of 5-100 ng/mL, with a correlation value of r2-0.999. The method was successfully applied to a bioavailability study to evaluate the pharmacokinetics of Reslizumab injection (10 mg/ml). The reproducibility of the method was demonstrated with accepted results of incurred samples reanalysis.

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