Research Article

Development and Validation of A UV Method for Quantitative Estimation of Satralizumab

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Received: 08-10-2025; **Revised:** 28-12-2025; **Accepted:** 06-01-2026; **Published online:** 20-01-2026.

ABSTRACT

We introduce an innovative and affordable UV spectrophotometric method for accurately measuring Satralizumab in pharmaceutical products. Through thorough investigation, we identified Satralizumab's absorption peak at 275.1 nm. The technique demonstrates remarkable linearity across 7.5-45 µg/mL, boasting a correlation coefficient of 0.999. Precision was confirmed by both intra- and inter-day studies, with %RSD values consistently under 2%, reflecting exceptional reliability. Following ICH Q2 (R1) guidelines, the method achieved 98.8% recovery and 99.5% assay accuracy. This approach not only fulfills regulatory requirements but also ensures rapid, precise, and reproducible quality assessment of Satralizumab in pharmaceutical formulations.

Keywords: Satralizumab, Validation, Ultraviolet spectroscopy, Method development.

INTRODUCTION

Satralizumab[1-9], a humanized IgG2 monoclonal antibody, is used to manage Neuromyelitis Optica Spectrum Disorder (NMOSD)[1-8], a rare autoimmune condition. It works by acting as an interleukin-6 (IL-6) receptor antagonist[8-9]. It binds to both membrane-bound and soluble forms of IL-6R, thereby preventing interleukin-6[8-9] from engaging its receptor and activating downstream intracellular signaling pathways such as JAK/STAT, MAPK, and PI3K–Akt. Inhibition of IL-6 signaling[8-9] reduces B-cell differentiation into antibody-producing plasma cells, thereby decreasing the production of pathogenic aquaporin-4 immunoglobulin G (AQP4-IgG) autoantibodies. It reduces astrocyte injury, complement-mediated inflammation, and relapse frequency in NMOSD[1-8].

The UV spectrophotometric technique employed in this study offers a cost-effective and straightforward approach**.** A meticulous examination of the existing literature reveals a notable absence of UV methods for quantifying Satralizumab. Motivated by this gap, we have chosen to develop a novel, uncomplicated, and expeditious UV spectrophotometric analytical method for the accurate measurement of Satralizumab in both bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrument

A double-beam LAB INDIA UV-Visible spectrophotometer UV 3200, equipped with two matched quartz cells with a 1 cm light path, was used to measure the absorbance of Satralizumab (0.1 mg sensitivity). An electronic balance IN-600 was used for weighing. The ultrasonic bath Sonicator, Model No. 1.5L 50H, PCI Ltd., Mumbai, was used in the present study.

Chemicals and reagents:

Satralizumab was procured from Hetero Drugs Ltd., Hyderabad, India. ACN and CH_3OH were obtained from E. Merck Specialties Pvt. Ltd., Mumbai, India.

Selection of solvent:

Plentiful trials were executed to find a suitable solvent system for dissolving Satralizumab. Solvents such as acetonitrile, methanol, and distilled water were tested based on the drug's solubility. Satralizumab is soluble in the solvent acetonitrile. Thus, acetonitrile was selected as the solvent.

Method Development

Determination of λ max:

To determine the optimal λmax for Satralizumab, a 30 µg/mL solution was prepared in acetonitrile and scanned over the ultraviolet spectrum from 200 to 400 nm. The drug exhibited its maximum absorbance at 275.1 nm, which was selected as the detection wavelength for analysis. The UV spectrum of Satralizumab is shown in Fig. 1.

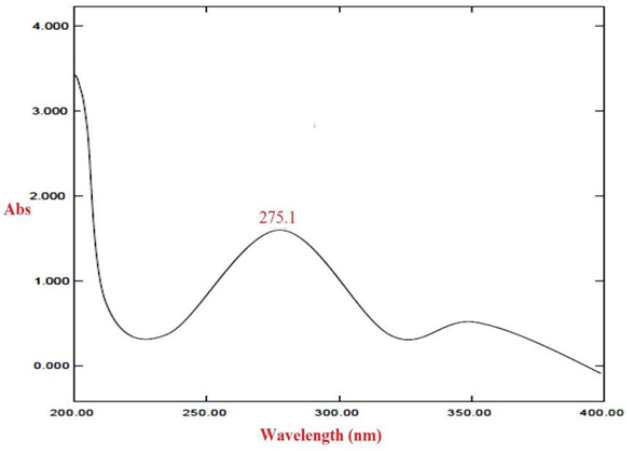

Figure 1: UV spectrum of Satralizumab

Preparation of standard solution:

The pure drug, 30 mg of Satralizumab, was weighed and transferred into a 100 mL volumetric flask. The drug was completely dissolved in Acetonitrile and diluted to the final volume with the same solvent to obtain a stock solution at 300 µg/mL. Aliquots of the standard stock solution were pipetted out from 5 mL to 50 mL and diluted suitably with acetonitrile to obtain a final concentration of 30 µg/mL.

Selection of analytical concentration range:

Appropriate aliquots were pipetted out from the standard stock solution into a series of 100 mL volumetric flasks. The volume was made up to the mark with solvent to obtain a series of dilutions of concentration range, ranging from 7.5-45 µg/mL of Satralizumab. The absorbance of the above solutions was measured at 275.1nm, and a zero-order calibration curve of absorbance versus concentration was plotted.

The regression equation and correlation coefficient were determined. Beer-Lambert's law was obeyed in the concentration range of 7.5-45 µg/mL for Satralizumab.

Preparation of Calibration curve:

Dilutions from the Satralizumab stock solution were made to final concentrations of 7.5, 15.00, 22.50, 30.00, 37.50, and 45.00 µg/mL. Absorbance was measured at λmax, 275.1 nm. The average of five measurements was used for the calibration plot. The calibration curve used Satralizumab concentration (x-axis) versus absorbance (y-axis) between 7.50 and 45.00 µg/mL. The calibration curve of Satralizumab is displayed in Fig. 2. The calibration data of Satralizumab is presented in Table 1 and the linear regression data is presented in Table 2. The Summary Output Regression ANOVA data for Satralizumab is presented in Table 3.

Table 1: Calibration data of Satralizumab

S. No	Concentration (µg/mL)	Absorbance
1	7.50	0.339
2	15.00	0.695
3	22.50	1.034
4	30.00	1.382
5	37.50	1.736
6	45.00	2.059

Table 2: Linear regression data

Parameter	Results
Detection wavelength (λ_{max})	275.1 nm
Beer's law limits (µg/mL)	7.5 - 45 µg/mL
Molar absorptivity (L. mole^{-1}cm^{-1})	459.555555555
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.0217601547388
Regression equation (y= mx+ c)	0.046x+0.0002
Slope (m) & Intercept (c)	0.046 & 0.0002
Standard error of slope (S_m)	0.000198
Standard error of intercept (S_c)	0.005346656
Standard error of estimate (S_e)	0.007847
Correlation coefficient (r^2)	0.9999

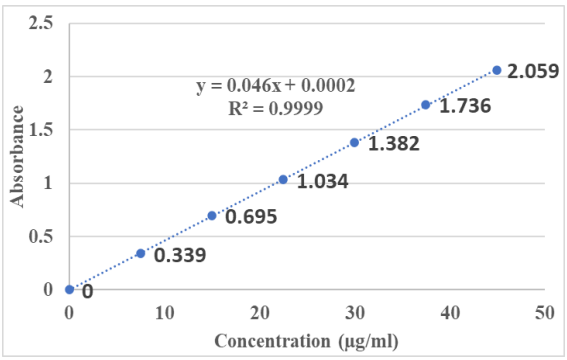

Figure 2: Calibration curve of Satralizumab

Table 3: Summary output regression ANOVA data of Satralizumab

	A	B	C	D	E	F	G	H	I
1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.999954							
5	R Square	0.999908							
6	Adjusted R	0.999889							
7	Standard E	0.007847							
8	Observatio	7							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*ignificance F*			
12	Regression	1	3.33132	3.33132	54104.97	2.79E-11			
13	Residual	5	0.000308	6.16E-05					
14	Total	6	3.331628						
15									
16		*Coefficients*	*tandard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	0.000214	0.005347	0.040078	0.969582	-0.01353	0.013958	-0.01352973	0.013958304
18	X Variable	0.04599	0.000198	232.6047	2.79E-11	0.045482	0.046499	0.04548222	0.04649873

Method Validation:

The methods were validated in accordance with ICH Q2 (R1) guidelines[10-15], for various parameters, including linearity, accuracy, precision, robustness, ruggedness, and forced degradation.

Linearity:

Fresh aliquots of the standard stock solution were prepared with concentrations ranging from 7.5 to 45 µg/mL. The absorbance of Satralizumab was measured at 275.1 nm using acetonitrile as the blank. The drug exhibited linearity across the concentration range of 7.5 to 45 µg/mL. The method's correlation coefficient was 0.999. The method was validated for parameters including specificity, linearity, range, accuracy, precision, LOD, LOQ, robustness, and system suitability. The linearity of Satralizumab is presented in Table 4.

Preparation of Stock Solution:

Weigh 30 mg of Satralizumab standard and put it in a clean, dry 100 mL volumetric flask. Add diluent, sonicate until dissolved, and make up to volume with the same solvent.

Preparation of Satralizumab Calibration Levels:

Level I (7.5 ppm): Accurately transfer 1.25 mL of the stock solution into a 50 mL volumetric flask. Dilute the solution to the mark with the diluent and mix thoroughly.

Level II (15.0 ppm): Accurately transfer 2.5 mL of the stock solution into a 50 mL volumetric flask. Dilute the solution to the mark with the diluent and mix thoroughly.

Level III (22.5 ppm): Accurately transfer 3.75 mL of the stock solution into a 50 mL volumetric flask. Dilute the solution to the mark with the diluent and mix thoroughly.

Level IV (30.0 ppm): Accurately transfer 5 mL of the stock solution into a 50 mL volumetric flask. Dilute the solution to the mark with the diluent and mix thoroughly.

Level V (37.5 ppm): Accurately transfer 6.25 mL of the stock solution into a 50 mL volumetric flask. Dilute the solution to the mark with the diluent and mix thoroughly.

Level VI (45.0 ppm): Accurately transfer 7.5 mL of the stock solution into a 50 mL volumetric flask. Dilute the solution to the mark with the diluent and mix thoroughly.

Table 4: Linearity of Satralizumab

	Conc.(µg/mL)	Absorbance
1	7.50	0.338
2	15.00	0.693
3	22.50	1.091
4	30.00	1.384
5	37.50	1.737
6	45.00	2.057
Regression equation	y = 0.046x + 0.0002	
Slope	0.046	
Intercept	0.0002	
R^2	0.9999	

Accuracy:

The accuracy of the developed method was validated through recovery studies conducted at three different concentration levels, 50 %, 100 %, and 150 % with each level analyzed in triplicate. Accuracy was assessed based on % recovery, and the % RSD for Satralizumab was <2 %. The % recovery for Satralizumab ranged from 99.9 %. These statistical results met the acceptance criteria as per ICH guidelines. The accuracy data for the UV method is tabulated in Table 5.

Table 5: Accuracy data of UV Method

Amount of µg/mL		% of drug added	% recovered	% Mean recovered	% RSD
LC	Pure drug				
120	0.125	50	99.8	99.86	0.50
	0.250	100	100.4		
	0.375	150	99.4		

Precision:

System precision was evaluated by taking six replicate absorbance measurements at 22.50 µg/mL and 275.1 nm on the same day. The reproducibility of the measurements was ensured, and the resulting data were used to calculate the mean, standard deviation (SD), and % RSD.

Method precision:

The precision of the method was evaluated through intra-day and inter-day variation studies. For the intra-day study, six solutions of Satralizumab at 30 µg/mL were prepared and analyzed three times on a single day, with absorbance recorded at each analysis. Precision was quantified using % RSD.

Table 6: Results of system precision

S. No	Absorbance
1	0.695
2	0.697
3	0.691
4	0.699
5	0.693
6	0.696
Mean	0.695166667
Standard deviation	0.002857738
% Relative Standard deviation	0.411086747

In the inter-day study, the same concentration was prepared and analyzed three times per day over three

consecutive days, and the absorbance readings were recorded. The % RSD for both intra-day and inter-day precision was below 2 %, in compliance with ICH guidelines, which require a % RSD of less than 2 % to be considered within acceptable limits. The results of system precision are depicted in Table 6 and the results of method precision for inter-day and intra-day are shown in Table 7 and 8 respectively.

Table 7: Results of Method precision (Inter-day precision)

Intermediate Precision	Absorbance	% Assay
1	1.388	100.2
2	1.393	
3	1.381	
4	1.386	
5	1.376	
6	1.395	

Table 8: Results of method precision (Intra-day precision)

Method Precision	Absorbance	% Assay
1	1.362	99.7
2	1.378	
3	1.372	
4	1.387	
5	1.394	
6	1.383	

Robustness:

The robustness of the method was evaluated by performing the analysis at two different wavelengths (± 5 nm). The absorbance values were recorded, and the results were expressed as % RSD. The % RSD values were within the acceptable range. The robustness results are provided in Table 9.

Table 9: Robustness results of Satralizumab

Parameter	Concentration 15 (µg/mL)	% Assay
Robustness Change in λmax (± 5nm)	λ+ : 280 nm	100.4
	λ - : 270 nm	99.9

Solution Stability:

The stability of Satralizumab solution at 45.00 µg/mL was evaluated at ambient temperature. Absorbance measurements were taken at 8, 16, 24, 32, and 48 hours. The absorbance values remained consistent, with variations of less than 2 % throughout the testing period. The solution stability studies are shown in Table 10**.**

Table 10: Solution Stability Studies of Satralizumab

Time (hrs)	Absorbance 45.00 µg/mL Standard in Ambient Conditions
0	2.059
8	2.058
16	2.059
24	2.060
32	2.059
48	2.058

Analysis of Marketed Formulation:

Take 0.25 mL of the Satralizumab sample and transfer it into a 100 mL volumetric flask. Dissolve it in acetonitrile, then bring the volume to the mark with the same solvent. Filter the solution using Whatman filter paper No. 40. From the filtrate, dilute 5 mL to a final volume of 50 mL with same solvent to achieve the desired concentration of 30 µg/mL Satralizumab. Analyze these solutions using UV spectroscopy and determine the % assay. The sample's percentage recovery was 98.8%, indicating good agreement with the formulation's label claim. The data are shown in Table 11.

Table 11: Analysis of Marketed Formulation

Name	Wavelength (nm)	Label claim (mg/tab)	Standard absorbance	Test absorbance	% recovery
Satralizumab	275.1	120	1.383	1.366	98.8

Degradation studies:

Preparation of Stock Solution:

Take 0.25 mL of the Satralizumab sample and transfer it to a clean, dry 100 mL volumetric flask. Add a diluent and sonicate until the sample is fully dissolved. Adjust the volume to the mark with the same solvent to prepare the stock solution.

Acid degradation:

Pipette 5 mL of the prepared stock solution into a 50 mL volumetric flask and add 1 mL of 1N hydrochloric acid (HCl). Heat the flask at 60 °C for 1 hour, then neutralize with 1N sodium hydroxide (NaOH). Dilute the solution to 50 mL with the diluent.

Alkali degradation:

Pipette 5 mL of the stock solution into a 50 mL volumetric flask and add 1 mL of 1N sodium hydroxide (NaOH). Heat the flask at 60°C for 1 hour, then neutralize with 1N hydrochloric acid (HCl). Dilute the solution to 50 mL with the diluent.

Thermal degradation:

Place the Satralizumab sample in a petridish and incubate in a hot air oven at 105°C for 24 hours. Afterward, cool the sample and dilute it with the diluent.

Peroxide degradation:

Pipette 5 mL of the stock solution into a 50 mL flask, add 1 mL of 3 % hydrogen peroxide, and dilute to the mark with the diluent. Heat the flask at 60°C for 1 hour, then allow it to return to room temperature for 15 minutes.

Reduction degradation:

Pipette 5 mL of the stock solution into a 50 mL flask, add 1 mL of 10 % sodium bisulphate, and adjust the volume to 50 mL with the diluent. Heat the flask at 60°C for 1 hour, then allow it to cool to room temperature for 15 minutes.

Photolytic degradation:

Expose the Satralizumab sample to sunlight for 24 hours. After exposure, dilute the sample with the diluent.

Hydrolysis degradation:

Pipette 5 mL of the stock solution into a 50 mL flask, add 1 mL of HPLC-grade water, and dilute to 50 mL with the diluent. Heat the flask at 60 °C for 1 hour, then let it cool to room temperature for 15 minutes.

Forced degradation:

The data regarding the forced degradation results of Satralizumab are tabulated in Table 12.

Table 12: Forced degradation results of Satralizumab

Results: % Degradation results	Satralizumab	
	Absorbance	% Degradation
Control	1.383	0
Acid	1.214	12.2
Alkali	1.229	11.1
Peroxide	1.192	13.8
Reduction	1.207	12.7
Thermal	1.238	10.5
Photolytic	1.356	2.0
Hydrolysis	1.361	1.6

The overall summary of the validation and optical characteristics of Satralizumab is depicted in Table 13.

Table 13: Summary of Validation & Optical Characteristics

Parameter	Results of Satralizumab
Beer's law limit (µg/mL)	7.5 - 45
Linear regression equation	y = 0.046x+0.0002
Linearity indicated by correlation coefficient	0.9999
Precision indicated by % RSD	
Intraday precision	0.22
Inter day precision	0.82
Accuracy indicated by % recovery	0.53
Robustness indicated by % recovery	99.4-100.4 %
Wave Minus	99.9 %
Wave Plus	100.4 %

RESULTS AND DISCUSSION

The UV spectrophotometric estimation was done using the Lab India 3200 UV-Visible Spectrophotometer. The estimation of Satralizumab was performed using Acetonitrile as the solubilizing agent. The ultraviolet spectrum of Satralizumab was scanned between 200 and 400 nm, and the λmax was determined to be 275.1 nm using the calibration curve method. The response to Satralizumab was found to be linear over the concentration range of 7.50 - 45.00 µg/mL, with a good correlation coefficient r^2 = 0.999. The system precision and intermediate precision results, i.e., intra-day and inter-day precision are tabulated in Tables 5 and 6 respectively. The % RSD is less than 2 in all precision results, which indicates that the method was precise. Recovery and study accuracy were determined using a standard addition method at three concentration levels (50 %, 100 %, and 150 %). The mean percentage was 99.86%, which is well within the acceptance criteria, indicating that the method is accurate. Ruggedness was assessed to evaluate reproducibility; the % RSD was less than 2, indicating the method was rugged. The mean assay was 98.8%. The UV spectrophotometric estimation uses Acetonitrile as a solubilizing agent and is validated for linearity in accordance with ICH guidelines. The results were found well within the limits, indicating that the developed method was simple, rapid, accurate, precise, robust, and economical.

CONCLUSION

The proposed UV-spectrophotometric methods were suitable for the determination of Satralizumab dosage form. All the parameters of the developed methods met the ICH Q2 (R1) guidelines for method validation. The developed UV methods for the estimation of Satralizumab are said to be rapid, simple, precise, accurate, sensitive, effective, and reproducible within the specified method parameters and can be effectively applied for the routine analysis of Satralizumab in bulk and formulations.

Acknowledgment

The authors would like to thank Hetero Labs for providing the Satralizumab samples. We are highly grateful to Dr. L. Rathaiah, Honourable Chairman, Vignan Group of Institutions, Vadlamudi, Guntur, for providing the necessary facilities to carry out this research work.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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