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Research Article

WHITE ANALYTICAL CHEMISTRY DRIVEN GREEN AND SENSITIVE UV SPECTROSCOPIC METHOD FOR ESTIMATION OF RISANKIZUMAB

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ABSTRACT

Risankizumab is a humanized monoclonal antibody used for the treatment of autoimmune diseases such as plaque psoriasis. A simple, sensitive and precise UV spectroscopic method was developed and validated for the quantification of Risankizumab in pharmaceutical formulations. The maximum absorbance, was obtained at 289 nm. The validation parameters, including linearity, accuracy, precision, and robustness, were evaluated according to the International Conference on Harmonisation (ICH) guidelines. The method showed excellent linearity ($R^2 > 0.999$) over the concentration range of 20-100 µg/mL. The accuracy of the method was demonstrated by performing recovery studies from 98-102% of the nominal concentration, with a relative standard deviation (RSD) of <2%. The precision studies were assessed for system, method, intra and inter day, ruggedness was assessed yielding acceptable relative standard deviations (% RSD). Additionally, the robustness of the method was evaluated by altering parameters, confirming its reliability under different conditions. The development and validation of an analytical method was successfully applied to the analysis of Risankizumab in pharmaceutical formulations, demonstrating its suitability for identification and routine quality control analysis. To develop an eco-friendly UV spectroscopic method for estimating risankizumab, guided by white analytical chemistry principles. The method involves selecting an optimal wavelength for risankizumab detection, choosing a suitable solvent to ensure its stability with minimal interference, and validating the method for accuracy, precision, linearity, and robustness. ComplexGAPI uses a hexagonal pictogram to assess analytical methods. Analytical GREEness is based on the 12 principles of Green Analytical Chemistry (GAC), converting them into a 0–1 scale using user-friendly freeware software was developed to generate these pictograms.

Keywords: Risankizumab, UV spectroscopic method development, White analytical chemistry, Complex GAPI, AGREE.

INTRODUCTION

The development of sustainable, efficient, and reliable analytical methods is crucial in pharmaceutical analysis. White Analytical Chemistry (WAC), which integrates the principles of Green Analytical Chemistry with analytical performance and cost-effectiveness, offers a comprehensive framework for such methods (Armenta *et al*., 2008; Mohamed, 2015; Tobiszewski *et al*., 2009). In this context, a WAC-driven UV-Visible spectroscopic method presents a promising approach for the estimation of Risankizumab. Risankizumab is a human monoclonal antibody that targets the p19 subunit of IL-23, a cytokine involved in inflammation and immune responses (Haugh *et al*., 2018; Pang *et al*., 2024). IL-23 plays a critical role in the pathogenesis of psoriasis by promoting the differentiation of TH-17 cells, which produce IL-17 leading to inflammation, keratinocyte activation, and plaque formation. Blocking IL-23 disrupts this cascade, making Risankizumab an effective treatment for psoriasis (Ohtsuki *et al*., 2019; Slueiman *et al*., 2018).

UV-Visible Spectroscopy works on the principle that molecules absorb light at specific wavelengths, leading to electronic transitions between energy levels. This interaction helps identify chemical structures and properties of organic/inorganic compounds in various states (Patil *et al*., 2018; Raja Reddy *et al*., 2011; Roopa Sirisha *et al*.,

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2019). White Analytical Chemistry (WAC) is an emerging approach that integrates ecological (green), analytical (red), and practical (blue) aspects of analysis to improve the detection of hazardous compounds like pesticides, heavy metals, and pharmaceuticals in food, biological, and environmental samples (Armenta *et al*., 2008; Sajid & Plotka-Wasylka, 2022). Traditional analytical methods face challenges related to selectivity, efficiency, cost, and sustainability.

WAC extends Green Analytical Chemistry (GAC) by including broader sustainability (social, financial, environmental) and performance criteria (Pena-Pereira *et al*., 2020; Tobiszewski *et al*., 2020). It combines the green principles which minimize toxic chemicals, solvents, waste, and energy use. Red principles ensure analytical accuracy, precision, and detection capability. Blue principles assess cost and time efficiency, and simplify procedures (Dogan & Tobiszewski, 2020; Kokilambigai & Lakshmi, 2022). This holistic model helps develop sustainable, high-performing, and cost-effective analytical methods promising better safety, reliability, and eco-friendliness in various real-world applications (Chemat *et al*., 2013; Farré *et al*., 2010; Melchert *et al*., 2012). The RGB model uses red (analytical performance), green (eco-friendliness), and blue (practical efficiency) to evaluate methods. The final "color" reflects the balance of these aspects, and a score called “method brilliance” quantifies overall quality.

AGREE (Analytical GREEnness) and ComplexGAPI are recent tools developed to evaluate the sustainability of analytical methods (Pena-Pereira *et al*., 2020; Plotka-Wasylka & Wojnowski, 2021). Since there were no analytical methods in the literature reported for Risankizumab by UV-Visible Spectroscopy to date, we aimed to develop and validate a stability-indicating, ecofriendly UV-Visible Spectroscopy technique as per International Conference on Harmonisation (ICH) guidelines.

MATERIALS AND METHODS

Materials

Pharmaceutical grade of Risankizumab was gifted by Glenmark Pharmaceuticals, Hyderabad, India. Ethanol used was of analytical grade and double distilled water was prepared in the laboratory.

Instruments

A Shimadzu Weighing balance (ATX124) was used for weighing all materials. UV-Vis Double beam Spectrophotometer (LABINDIA – UV3200) with UV analyst software was used with 1cm matched quartz cuvettes.

Preparation of stock solution

Weigh accurately about 10mg of Risankizumab and transfer to 10mL volumetric flask. Then dissolve the drug by adding solvent / diluent (5mL each of ethanol and distilled water) with vigorous shaking for 3 to 5 min. Then the final volume was made up to the mark to obtain concentration of 1mg/mL or 1000µg/mL.

Preparation of working standard solution

From stock solution, pipette out 1mL and transfer to a 10mL volumetric flask and make up to the mark with diluent to obtain concentration of 100µg/mL.

Determination of λ max

The above standard solution (100µg/mL) was scanned in UV-VIS Spectrophotometer in the range of 200-400nm using mixture of ethanol and distilled water as blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 289 nm shown in figure 1.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its pre-determined specifications and quality characteristics. The method was validated for various parameters like linearity, accuracy, precision, ruggedness and robustness.

Linearity

Linearity of Risankizumab was determined at 5 levels with concentration of 20, 30, 50, 80, 100 µg/mL which is prepared from standard solution. Then the samples were scanned in UV-VIS Spectrophotometer using distilled water as blank. The absorbance of solution was measured at 289 nm. The calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. Linearity was obtained from correlation coefficient. The results obtained were shown in table 1 and figure 2. Limit of detection (LOD) and Limit of quantification (LOQ) values were calculated from the calibration curve and shown in table 2.

Accuracy

Accuracy of proposed method was assessed by recovery studies which were carried out at three different levels i.e. 20%, 30%, 40% in which standard drug solution was added to pre-analysed sample solution. The solutions were prepared in triplicates and absorbance was recorded. The accuracy was indicated by % drug content. The % recovery was calculated and shown in the table 3.

Precision

Generally, Precision is of two different kinds which includes, System Precision and Method Precision.

System precision

Standard solution of concentration 100 µg/mL was prepared from stock solution in a 10mL volumetric flask.

The absorbance was measured six times against blank. The % RSD was calculated and shown in table 4.

Method precision

Precision of method was demonstrated by performing intra-day and inter-day variation studies The results were indicated by % RSD and values are shown in table 5.

Inter-day precision

Standard solutions of concentration 100, 200 and 300 µg/mL were prepared from stock solution in 10mL volumetric flasks and were measured thrice in three consecutive days on same time. Now the absorbance of these solutions was noted. Then % RSD was calculated and values are shown in table 6.

Ruggedness

Standard solution of concentration 100 µg/mL was prepared from stock solution in a 10mL volumetric flask. The study was performed by taking the absorbance values of the standard preparation in two different UV -Vis spectrophotometers instruments and by change in analyst. The results were indicated by % RSD and values are shown in table 7 and 8.

Robustness

Robustness was obtained by performing the analysis at two different wavelengths with a deviation ± i.e. 288 and 290 nm from set wavelength of 289 nm for standard solution of concentration 100 µg/mL. The respective absorbances were noted. The result was indicated by % RSD and values are shown in table 9 (Raja Reddy *et al*., 2011; Suma *et al*., 2022).

Forced degradation studies

Forced degradation studies are done as per the ICH guidelines such as: ICH Q1A: Stability Testing of New Drug Substances and Products. ICH Q1B: Photo Stability Testing of New Drug Substances and Products. Degradation studies are performed under acidic, basic hydrolysis, oxidation, thermal and photolytic conditions (Ferraz *et al*., 2014; Raja Reddy *et al*., 2011).

Acid degradation

10 mg of Risankizumab was weighed and transferred into a 10 mL volumetric flask. A diluent [ethanol:water (1:1)] was added, and the solution was diluted. The volume was then made up to 10 mL with 0.1N HCl, and the solution was allowed to stand for 24 hours. Afterward, 1 mL of the prepared solution was pipetted into another 10 mL volumetric flask. 5 mL of the diluent was added, and the solution was neutralized with 0.1N NaOH up to the mark. The absorbance of the solution was measured at 289 nm, and the amount of drug degraded was calculated.

Alkali degradation

10 mg of Risankizumab was weighed and transferred into a 10 mL volumetric flask. A diluent [ethanol:water (1:1)] was added, and the solution was diluted. The volume was then made up to 10 mL with 0.1N NaOH, and the solution was allowed to stand for 24 hours. Afterward, 1 mL of the prepared solution was pipetted into another 10 mL volumetric flask. 5 mL of the diluent was added, and the solution was neutralized with 0.1N HCl up to the mark. The absorbance of the solution was measured at 289 nm, and the amount of drug degraded was calculated.

Oxidative degradation

10 mg of Risankizumab was weighed and transferred into a 10 mL volumetric flask. A diluent [ethanol:water (1:1)] was added, and the solution was diluted. The volume was then made up to 10 mL with H_2O_2 (Hydrogen peroxide), and the solution was allowed to stand for 24 hours. Afterward, 1 mL of the prepared solution was pipetted into another 10 mL volumetric flask. 5 mL of the diluent was added, and the volume was made up to 10 mL with H_2O_2. The absorbance of the solution was measured at 289 nm, and the amount of drug degraded was calculated.

Thermal Degradation

10 mg of Risankizumab was weighed and transferred into a petri dish, then exposed to a temperature of 60°C in a hot air oven. After 24 hours, the required amount of drug was taken, and a 100 µg/mL solution was prepared using the diluent. The absorbance of the solution was measured at 289 nm, and the amount of drug degraded was calculated.

Photolytic Degradation

10 mg of Risankizumab was weighed and transferred into a petri dish, then exposed to UV light for 24 hours. Afterward, the required amount of drug was taken, and a 100 µg/mL solution was prepared using the diluent. The absorbance of the solution was measured at 289 nm, and the amount of drug degraded was calculated**.** The % degradation obtained was less than 10% showing the preparations were stable for 24 hours under different stress conditions. The results obtained are shown in table 10.

Whiteness Proile Assessment

The method development parameters and conditions were applied in the AGREE and ComplexGAPI software apps to prove the method developed for estimation of Risankizumab API by UV-Vis spectroscopic technique is an environment friendly and sustainable white analytical method. The results obtained are shown in table 11 and figures 3 and 4. AGREE: Green Analytical Chemistry promotes direct analysis, minimal sample use, in situ measurements, and integrated, automated, and miniaturized processes to save resources and energy. It avoids derivatization, emphasizes multianalyte methods, waste reduction, and the use of degradable, non-toxic reagents. Overall, it ensures sustainability and operator safety

without compromising analytical accuracy (Pena-Pereira *et al*., 2020; Sajid & Plotka-Wasylka, 2022).

Complementary Green Analytical Procedure Index

The complex green analytical procedure index (ComplexGAPI) is a tool that covers all aspects of an analytical procedure, from sample collection, its transport, preservation, and storage to sample preparation and final analysis, but also these aspects and processes which are performed prior to the general analytical methodology (Plotka-Wasylka & Wojnowski, 2021; Armenta *et al*., 2019; Farré *et al*., 2010).

RESULTS AND DISCUSSION

Determination of λmax of Risankizumab:

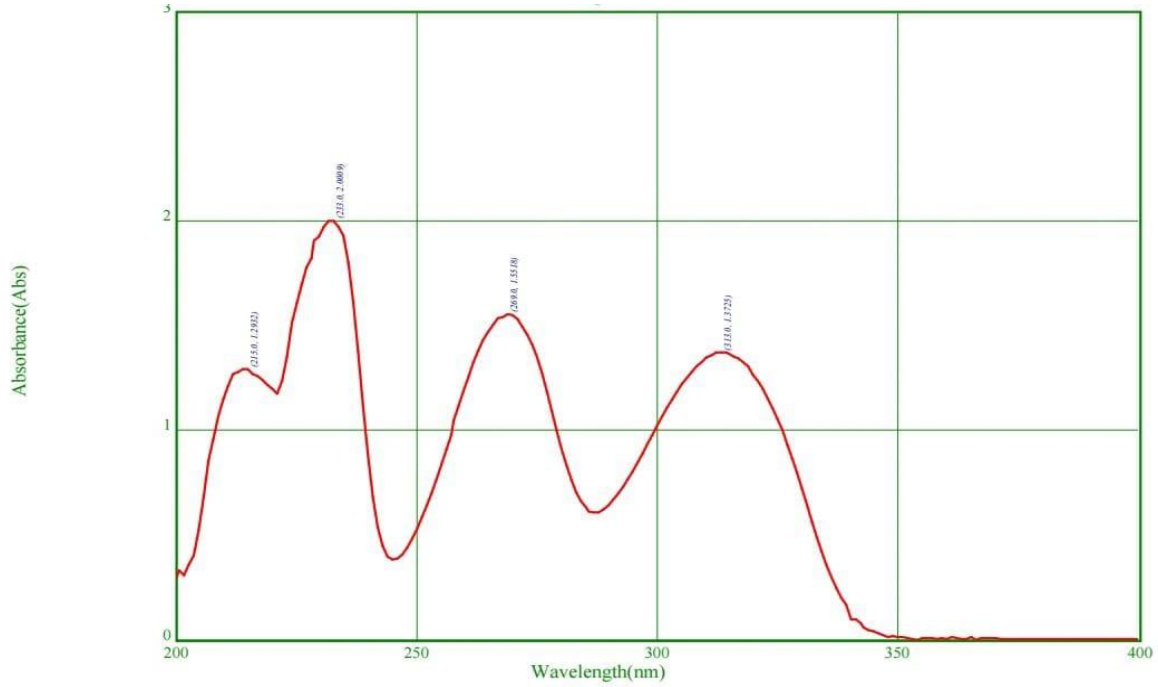

Figure 1. UV Spectra of Risankizumab**.**

Linearity:

Table 1. Linearity by method of calibration curve.

S. No	Concentration	Absorbance
1	10	0.2391
2	20	0.5274
3	30	0.8189
4	40	1.0899
5	50	1.3799

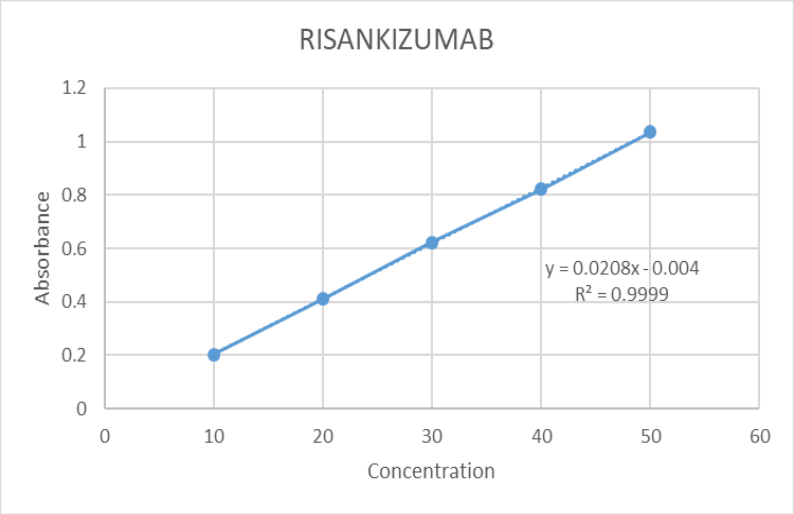

Figure 2. Linearity by method of calibration curve.

Table 2. Limit of Detection and Quantification.

S. No	LOD	LOQ
1.	2.998µg/ml	8.6538µg/ml

Accuracy:

Table 3. Results for accuracy.

Concentration	**Amount spiked**	**Observed values**	**Theoretical values**	**Mean absorbanceof observed values (n=3)**	**% Recovery**	**%RSD**
10	10	0.2034	0.204	0.2057	99.7%	1.2
20	10	0.4111	0.412	0.4120	99.7%	1.3
30	10	0.6231	0.62	0.6369	100.5%	1.1

Precision:

Table 4. Results for system precision.

No. of readings	**Absorbance**
1	0.7089
2	0.7015
3	0.7021
4	0.7262
5	0.7272
6	0.7226
Average	0.71475
Standard deviation	0.010936
% Relative Standard Deviation (% RSD)	1.53

Table 5. Results for method precision.

Concentration	Absorbance	Mean absorbance +/- SD	% RSD
20	0.3457 0.3389 0.3521	0.34556 +/- 0.00539	1.5
30	0.5844 0.5749 0.5853	0.5815 +/- 0.0047	0.8
40	0.7356 0.7222 0.7167	0.7248 +/- 0.0079	1.09

Table 6. Results for inter-day precision.

Inter-day	Sample absorbance	Mean absorbance +/- SD	% RSD
Day 1	0.3842 0.3748 0.3853	0.3814 +/- 0.0047	1.23
Day 2	0.7455 0.7223 0.7342	0.734 +/- 0.0094	1.2

Table 7. Results of ruggedness for variation in analyst.

Analyst	Sample absorbance	Mean absorbance +/- SD	%RSD
Analyst 1	0.4156 0.4149 0.4168	0.4157 +/- 0.0007	0.18
Analyst 2	0.4285 0.4356 0.4191	0.4277 +/- 0.0067	1.5

Table 8. Results of ruggedness for variation in instrument.

Instrument	Sample absorbance	Mean absorbance +/- SD	%RSD
Instrument 1 (Lab India)	0.4394 0.4249 0.4196	0.4279 +/- 0.0083	1.95
Instrument 2 (Analytical UV)	0.4229 0.4169 0.4277	0.4225 +/- 0.0044	1.0

Robustness:

Table 9. Results for robustness.

Wavelength (in nm)	Absorbance
288	0.4173 0.4268 0.4275
289	0.4183 0.4276 0.4184
290	0.4058 0.4279 0.4289
Mean absorbance +/- SD	0.4220 +/- 0.0072
% RSD	1.7

Table 10. Results of forced degradation studies.

S. No	Degradation parameters	Absorbance	% degradation
1	Control	0.0324	____
2	Acid	0.1543	13.31
3	Alkali	0.1561	10.54
4	Peroxide	0.154	5.63
5	Thermal	0.1405	8.88
6	Photo light (UV light)	0.1559	4.589

Table 11. Applying White analytical chemistry - using AGREE software.

S. No	Testing Principle	Input parameters	AGREE Score
1	Sampling procedure	At line analysis	0.6
2	Amount of sample in mL	2	0.55
3	Positioning of Analytical device	At-line	0.33
4	Major distinct steps in the sample preparation procedure	3 or fewer	1.0
5.1	Degree of automation	automatic	0.5
5.2	Sample preparation	Not miniaturised	0.5
6	CAS lookup	nil	1.0
7	Amount of waste in mL or G	0	1.0
8.1	Number of analytes determined in a single run	1	0.94
8.2	Sample throughput (sample analysed per hour)	60	0.94
9	Total power consumption of a single analysis in KWh	0.0167	1.0
10	Type of reagents	Bio-based	1.0
11	Does this method involve the use of toxic reagents or solvents	No	1.0
12	Threats which are not avoided	nil	1.0

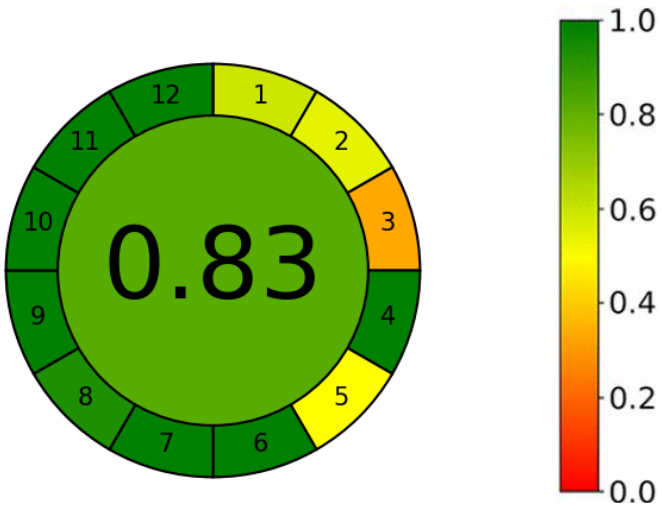

Figure 3. Generic result of assessment obtained (left) and the corresponding color scale for reference (right).

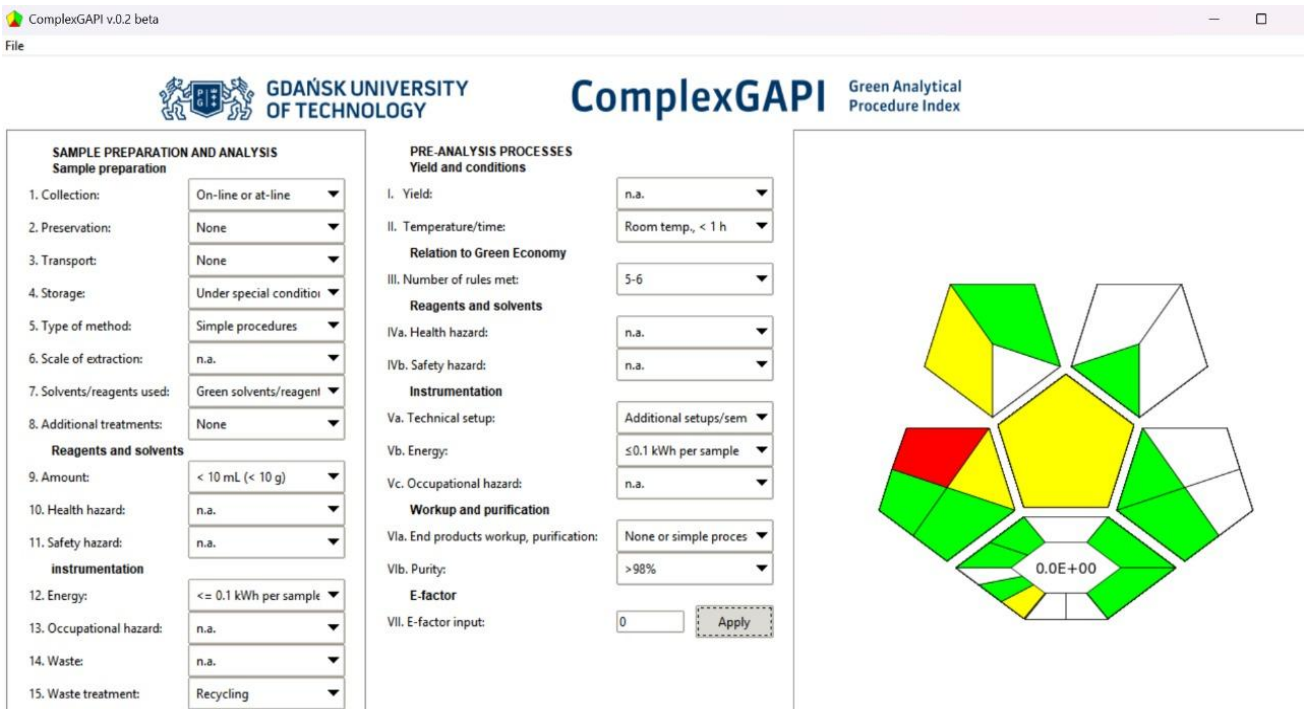

Figure 4. Results obtained by using ComplexGAPI Software.

AGREE is based on the 12 principles of Green Analytical Chemistry (GAC), converting them into a 0–1 scale using user-friendly software. The value obtained 0.83 is closer to 1, proving the method is a green method. It offers a simple, visual way to assess method greenness. ComplexGAPI uses a hexagonal pictogram showing more green hexagons a few yellow hexagon, a single red hexagon and a e factor value of "0" proving the method is a green method. These results obtained by these models led to the concept the developed method was a White Analytical Chemistry (WAC) driven UV Spectroscopic method.

CONCLUSION

A green and sensitive UV spectroscopic method was successfully developed and validated for the estimation of Risankizumab, in accordance with White Analytical Chemistry principles. The method exhibited excellent linearity, accuracy, precision, and robustness as per ICH guidelines, with λ_max at 289 nm. The method's performance was further confirmed through stress stability studies, showing less than 20% degradation and ensuring analytical reliability. Greenness and whiteness evaluations using AGREE and ComplexGAPI confirmed the method's eco-friendly and sustainable nature. This approach offers a reliable, cost-effective, and environmentally responsible alternative for routine quality control of Risankizumab in bulk and formulations.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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