Research Article

Identification of Impurities in the Drug Risankizumab Using RP-HPLC Method

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Abstract

Aim: To establish and validate a stability-indicating RP-HPLC method for the concurrent quantification of Risankizumab and its associated impurities in both bulk drug substances and pharmaceutical dosage forms.

Specific objectives:

- To develop a simple, rapid and specific RP-HPLC method for the estimation of Risankizumab and its impurities in bulk and combined pharmaceutical dosage forms.
- To validate the proposed methods in accordance with the analytical parameters mentioned in the ICH guidelines, such as system suitability, accuracy, precision, specificity, linearity, robustness, LOD and LOQ.
- To develop the method under the forced conditions such as acid, alkali, peroxide, reduction, thermal, hydrolysis and photo degradation.

A simple, rapid, precise, sensitive and reproducible Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the quantitative analysis of Risankizumab and its impurities in pharmaceutical dosage form. Chromatographic separation of Risankizumab and its impurities was achieved on waters alliance-e2695, by using Luna Phenyl Hexyl 150 mm × 4.6 mm, 5 μ column and the mobile phase containing 0.1% TEA pH-2.5/OPA and Acetonitrile in the ratio of 80:20% v/v. The flow rate was 1 ml/min; detection was carried out by absorption at 263 nm using a photodiode array detector at ambient temperature. The number of theoretical plates for Risankizumab and its impurities was not less than 2000 and the tailing factor did not exceed 2. The % Relative Standard Deviation (%RSD) of peak areas across all measurements remained below 2.0. The developed method was validated in accordance with ICH guidelines. It proved to be a simple, cost-effective, precise, accurate, robust and suitable approach for the quantitative determination of Risankizumab and its impurities, as well as for conducting stability studies.

Keywords: HPLC; Risankizumab drug; Impurity-1; Impurity-2

Introduction

A drug's Active Pharmaceutical Ingredient (API) is the component that, when combined with other ingredients, produces the drug's desired pharmacological effect [1]. Such substances are generally called drug substances and used to formulate the drug product which are consumed by the patients. These pharmaceuticals provide pharmacological activity or some other direct action to influence the structure and function of the body for the purpose of diagnosis, cure, mitigation, therapy or prevention of illness. Thus, pharmaceutical sector has two demarcated sections, one is manufacturing of API's and other is formulating them in the tablets, capsules, injectables, syrup, patches, etc. International Conference of Harmonization has adopted stringent rules to maintain quality of the API used in pharmaceutical product. Quality of the drug is nothing but compliance of the substance (API) with respect to set of quality parameters such as chemical purity, chemical assay, impurity profile, content of known and unknown and genotoxic impurities, inorganic contents, residual solvents, physical attributes such as polymorph, particle size, bulk density and etc. All these set of analytical examination is formatted together and formatted to release the material to end users is known as Certificate of Analysis (COA). Establishment of analytical chemist is multifold and critical during the period of product or process development. To assure the effectiveness, purity, stability and overall quality of API and developed therapeutic products, the pharmaceutical industry relies heavily on the work of analytical chemists [2]. Method validation of analytical methods is another milestone, which is critical to ensure that impurity levels are accurately and consistently reported throughout drug development.

Impurity profiling

Introduction to impurity profiling: Impurity profiling plays a crucial role in pharmaceutical analysis, focusing on the detection, characterization and measurement of impurities found in both active pharmaceutical ingredients and finished drug formulations. These impurities may arise during synthesis, formulation, storage or degradation.

Understanding and controlling impurities is essential to ensure the safety, efficacy and quality of pharmaceutical products. Regulatory authorities like the ICH (International Council for Harmonisation) provide guidelines for acceptable impurity levels, making impurity profiling an integral part of drug development and quality control processes [3-5].

Drug profile of Risankizumab

Drug profile: Risankizumab

Generic name: Risankizumab-rzaa

Brand name: Skyrizi

Drug class: Humanized monoclonal antibody-Interleukin-23 (IL-23) inhibitor

ATC code: L04AC19

Molecular formula: $C_{17}H_{17}N_3O_6S$

Molecular weight: 391.0838 g/mol

Regulatory status

FDA approved: Yes (April 2019)

EMA approved: Yes

Prescription status: Rx-only

Mechanism of action: Risankizumab selectively binds to the p19 subunit of Interleukin-23 (IL-23), a cytokine involved in inflammatory and immune responses. By inhibiting IL-23, it downregulates the inflammatory pathway and reduces the release of pro-inflammatory cytokines and chemokines, which are implicated in
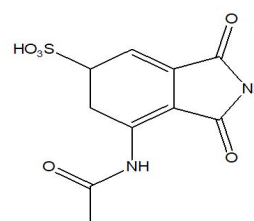
conditions like psoriasis and inflammatory bowel disease (Figures 1-3 and Table 1).
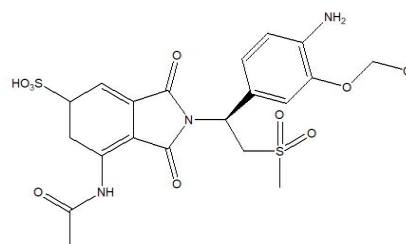
Figure 1: Molecular structure of Risankizumab
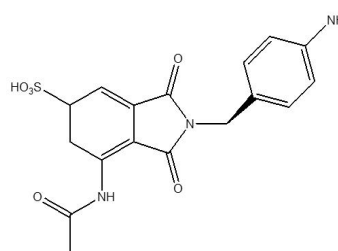
Figure 2: Molecular structure of impurity-1. Note: Drug profile of impurity-1 (Risankizumab impurity-1)

Figure 3: Molecular structure of Impurity-2. Note: Drug profile of impurity-2 (Risankizumab impurity-2)

Table 1: Drug profile of Risankizumab

IUPAC name	7-acetamido-2-((S)-1-(4-amino-3-(chloromethoxy)phenyl)-2-(methylsulfonyl)ethyl)-2,3,5,6-tetrahydro-1,3-dioxo-1H-isoindole-5-sulfonic acid
Molecular formula	$C_{6476}H_{9992}N_{1720}O_{2016}S_{44}$
Molecular weight	145611.84 g·mol^{-1}
Description	Patients with moderate-to-severe plaque psoriasis who may also benefit from phototherapy (ultraviolet light treatment) are given an injection of Risankizumab-rzaa. Psoriasis plaques are persistent red spots covered with silvery white scales.
Therapeutic category	The monoclonal antibody Risankizumab-rzaa is a kind of drug.

Uses of Risankizumab

In people whose plaque psoriasis is too severe to be treated with topical medicines alone, an injection of Risankizumab-rzaa is used to alleviate the symptoms of the illness [6].

Interleukin-23 (IL-23) is a pro-inflammatory cytokine that plays a key role in the development of various chronic inflammatory disorders. Its interaction with the IL-23 receptor activates the IL-23/Th17 pathway, which regulates T-cell driven immune responses and inflammation. This activation leads to the release of pro-inflammatory cytokines and chemokines, such as IL-17 and supports the differentiation of Th17 and Th22 cells. Although the IL-23/Th17 pathway is essential for host defence against

certain pathogens, it is also associated with the progression of chronic autoimmune inflammatory conditions. IL-23 consists of a unique p19 subunit and a p40 subunit, the latter of which it shares with IL-12. Risankizumab selectively targets and binds with high affinity to the p19 subunit of IL-23, effectively neutralizing its activity. By doing so, it prevents IL-23 from binding to its receptor and blocks the subsequent intracellular signalling events [7-10].

Materials and Methods

The basic parts of a HPLC instrumentation

The pump, injector, column, detector and data system make up the rest of the HPLC apparatus. A schematic diagram of a typical HPLC instrumentation is shown in Figure 4.
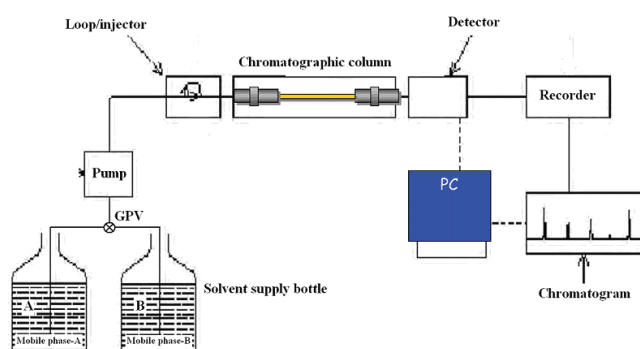
Figure 4: Basic HPLC instrumentation

Analytical methods/techniques used for impurity profiling study: The identification, characterization and quantification of impurities (including degradation products) in APIs and pharmaceutical formulations is a vital part of modern pharmaceutical analysis. Impurities in medications may pose serious health hazards, hence the employment of selective methods for identifying and determining impurities has recently gained popularity.

The separation, identification and determination of impurities to lowest possible level in drug substance are done by various techniques. The separation techniques include various chromatographic techniques. These methods rely on differential migration to separate many species present in a single sample. Different spectroscopic (mass spectrometry and nuclear magnetic resonance spectroscopy) and chromatographic (TLC, HPLC, GC, LC-MS and GC-MS) methods are used nowadays for impurity profiling investigations in medicinal compounds. The types of contaminants and Active Pharmaceutical Ingredients (APIs) to be removed determine which methods may be used. The pharmaceutical business requires a method of quantifying contaminants in medicinal ingredients [11-13].

Results and Discussion

A Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed for the simultaneous estimation of Risankizumab and its impurities, utilizing a Luna Phenyl Hexyl column (250 × 4.6 mm, 5 µm). The mobile phase consisted of 0.1% Triethylamine (TEA) adjusted to pH 2.5 with Orthophosphoric Acid (OPA) and mixed with acetonitrile in a ratio of 80:20 (v/v). The flow rate was maintained at 1.0 mL/min, with the column operated at ambient temperature. Detection was carried out at a wavelength of 263 nm.

Drugs samples

Glenmark was the source for both Risankizumab and its contaminants. Samples of Risankizumab from a commercial pharmaceutical formulation claiming to contain 15 mg were analyzed [14,15].

Method development

Selection of the wavelength for simultaneous estimation: Scanned absorption spectra of Risankizumab and its impurities informed the selection of the detection wavelength used in the creation of the test procedure. An accurately weighed 15 mg quantity of Risankizumab was transferred into a 10 mL volumetric flask. After dissolving the substance in a suitable buffer, the volume was brought up to the mark using the same solvent. This resulted in a stock solution with a concentration of 1500 µg/mL. From this stock, 1 mL was further diluted with solvent in another 10 mL volumetric flask to achieve the desired concentration. Put 5 milligrammes of impurity-1 and 5 milligrammes of impurity-2 into a 10 millilitre volumetric flask. Dissolve the chemical in 7 ml of diluent (mobile phase) and add more diluent until the desired volume is reached. Take 1 millilitre of the aforesaid solution and transfer it to a clean 10 millilitre volumetric flask, which should then be filled to the mark with diluent. Then, transfer 1 millilitre of the aforementioned solution to a 10 millilitre volumetric flask and dilute to the desired concentration. After setting up the auto sampler, we automatically injected the solution into the HPLC with PDA detector. Here is the obtained spectrum (Figure 5).
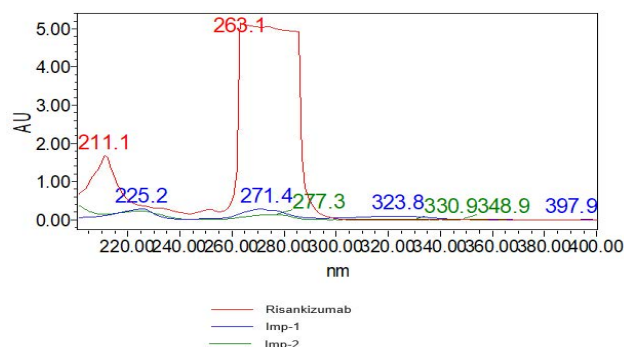

Figure 5: PDA spectrum

From the spectra shown above, the wavelength at which both medications exhibited highest absorbance was chosen. We settled on 263 nm as the optimal wavelength.

Selection of chromatographic method

The sample's composition, molecular weight and solubility all play a role in determining which chromatographic technique to use. Because medicines are so polar, the reverse phase chromatographic method was used for this investigation [16].
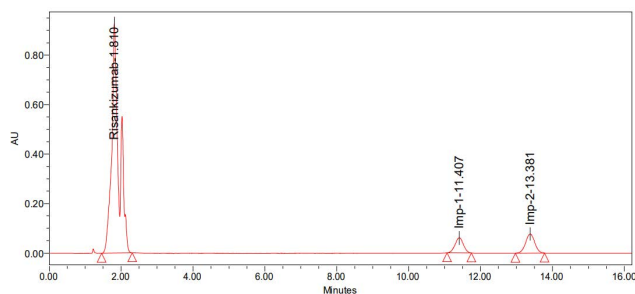
Trail-1

Chromatographic conditions:

- **Column:** Agilent Eclipse XDB (250 mm × 4.6 mm 5 µ)

- **Mobile phase:** Acetonitrile and 0.1% formic acid (50:70)

- **Injection volume:** 10 µl

- **Flow:** 1 ml/min

- **Detection:** PDA 200-400 nm (Figure 6 and Table 2)
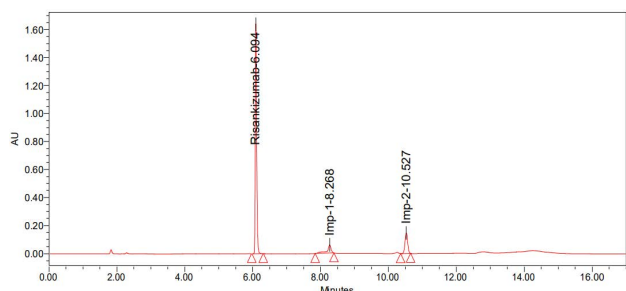
Table 2: Results obtained from trail 1

S. no	Name	RT	Area	USP resolution	USP tailing	USP plate count
1	Risankizumab	1.81	12856587	-	1.29	833
2	Imp-1	11.407	972273	25.98	1	10893
3	Imp-2	13.381	1358308	4.3	1.06	12887
Note: The observed conditions for system appropriateness are beyond the allowed range						

Figure 6: Graph obtained in trail 1

Trail-2

Chromatographic conditions:

- **Column:** Agilent eclipse XDB (250 mm × 4.6 mm 5 μ)
- **Mobile phase:** Acetonitrile and 0.1% formic acid (40:60)
- **Injection volume:** 10 μl
- **Flow:** 1 ml/min
- **Detection:** 263 nm (Figure 7 and Table 3)

Table 3: Results obtained from trail 2

S. no	Name	Retention time	Area	USP resolution	USP tailing	USP plate count
1	Risankizumab	6.094	4723743	-	1.29	106049
2	Imp-1	8.268	470478	15.03	2.16	52289
3	Imp-2	10.527	790108	19.12	1.01	83023
Note: Observation: Peak shape is not good						

Figure 7: Graph obtained in trail 2

Trail-3

Chromatographic conditions:

- **Column:** Luna phenyl hexyl (250 mm × 4.6 mm, 5 μ)
- **Mobile phase:** Acetonitrile and 0.1% formic acid (30:70)
- **Injection volume:** 10 μl
- **Flow:** 1 ml/min
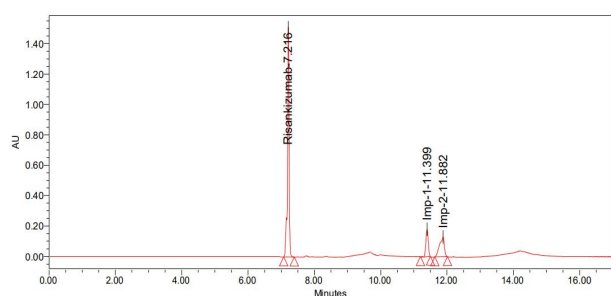- **Detection:** 263 nm (Figure 8 and Table 4)

Table 4: Results obtained from trail 3

S. no	Name	Retention time	Area	USP resolution	USP tailing	USP plate count
1	Risankizumab	7.216	4971381	-	0.79	15159
2	Imp-1	11.399	885211	16.08	0.99	11877
3	Imp-2	11.882	1260859	2.28	0.73	25954
Note: Observation: Unknown peaks are observed						

Figure 8: Graph obtained in trail 3

Table 5: Results obtained from trail 4

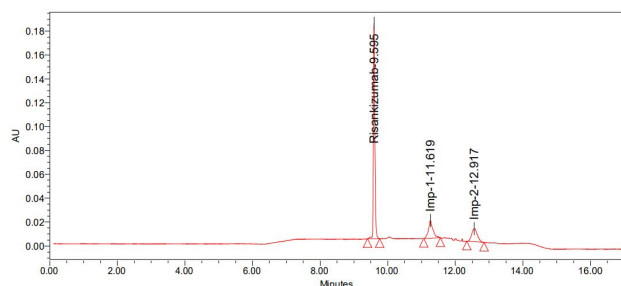| S. no	Name	Retention time	Area	USP resolution	USP tailing	USP plate count
1	Risankizumab	9.595	542429	-	1.33	24571
2	Imp-1	11.619	136207	4.22	1.21	17994
3	Imp-2	12.917	122233	2.89	1.27	11870
Note: Observation: Base line is not sufficient						

Figure 9: Graph obtained in trail 4

Table 6: Results obtained from trail 5

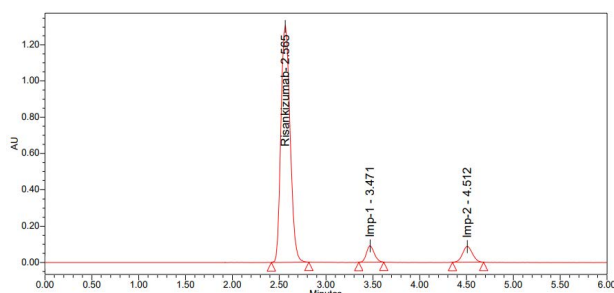| S. no	Name	Retention time	Area	USP resolution	USP tailing	USP plate count
1	Risankizumab	4.917	36987592	-	1.03	9123
2	Imp-1	9.74	1362972	25.63	0.94	41704
3	Imp-2	10.379	3582134	3.52	0.98	36916
Note: Observation: Response of the peaks are very high						

Figure 10: Graph obtained in trail 5

Table 7: Results obtained from trail 6

S. no	Name	RT	Area	USP resolution	USP tailing	USP plate count
1	Risankizumab	2.565	8845928	-	1.17	15214
2	Imp-1	3.471	531542	4.71	1.05	8602
3	Imp-2	4.512	641033	6.23	1.20	13679
Note: Observation: This technique may be used for validating hypotheses						

Trail 4

Chromatographic conditions

- **Column:** Luna phenyl hexyl (250 mm × 4.6 mm, 5 µ)
- **Mobile phase:** Acetonitrile and 0.1% TEA pH-2.5/ OPA (10:90)
- **Injection volume:** 10 µl
- **Flow:** 1 ml/min
- **Detection:** 263 nm (Figure 9 and Table 5)

Trail-5

Chromatographic conditions

- **Column:** Luna phenyl hexyl (250 mm × 4.6 mm, 5 µ)
- **Mobile Phase:** Acetonitrile and 0.1% TEA pH-2.5/ OPA (15:85)
- **Injection Volume:** 10 µl
- **Flow:** 1ml/min
- **Detection:** 263 nm (Figure 10 and Table 6)

Trail 6 (Optimized chromatogram)

Chromatographic conditions:

- **Column:** Luna phenyl hexyl (250 mm × 4.6 mm, 5 µ)
- **Mobile phase:** Acetonitrile and 0.1% TEA pH-2.5/ OPA (20:80)
- **Injection Volume:** 10 µl
- **Flow:** 1 ml/min
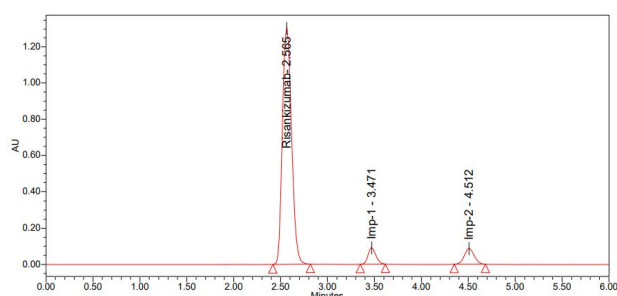- **Detection:** 263 nm (Figure 11 and Table 7)

Figure 11: Graph obtained in trail 6

Inference: This experiment was optimised due to the positive outcomes and shorter retention period.

Optimized method

Preparation of mobile phase: The buffer and acetonitrile mixture was filtered and degassed and the final concentration was 80:20.

- **Column:** Luna phenyl hexyl (250 × 4.6 mm, 5 μ)

- **Injection volume:** 10 μl

- **Wavelength:** 263 nm

- **Flow rate:** 1.0 ml/ min

- **Temperature:** Ambient

- **Diluent:** Same as mobile phase

About 2.565, 3.471 and 4.512 minutes passed between doses of Risankizumab, Risankizumab impurity-1 and Risankizumab impurity-2, respectively (Figures 12-14).
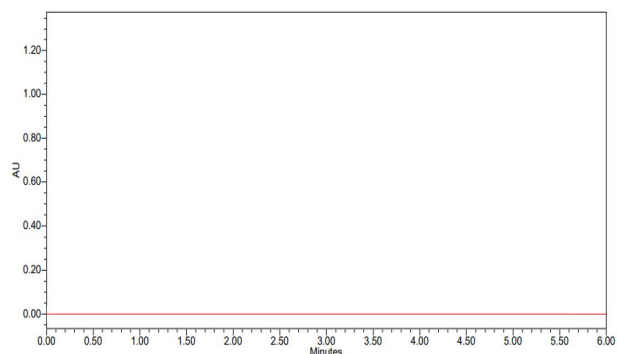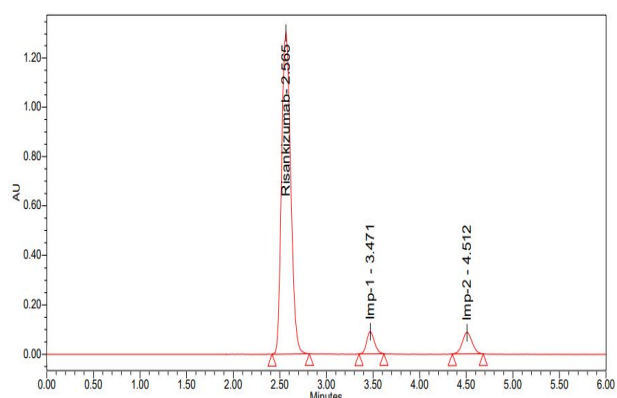

Figure 12: Chromatogram of blank

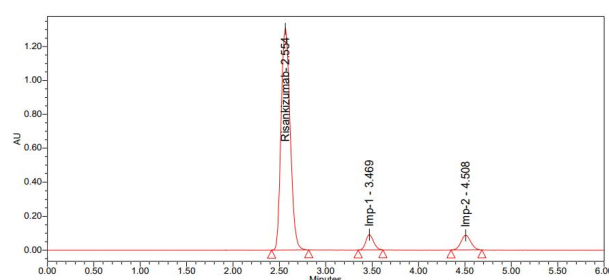**Figure 13:** Chromatogram of standard

Figure 14: Chromatogram of sample

Method validation and its parameters

To confirm the suitability of the HPLC method for the quantification of Risankizumab, impurity-1 and impurity-2, various parameters were evaluated as per the established protocol. All validation studies were performed in compliance with ICH guidelines to ensure the method meets its intended analytical purpose [17-20].

Accuracy: The closeness of agreement between the value found and the true value or accepted reference value.

Precision: The closeness of agreement among a series of measurements obtained from multiple samplings of the same homogeneous sample.

It includes:

- **Repeatability:** Same analyst, same equipment, short time interval.

- **Intermediate precision:** Different days, analysts or equipment within the same lab.

- **Reproducibility:** Different labs.

Ruggedness: The degree of reproducibility of test results under normal, but variable, conditions such as different laboratories, analysts, instruments and environmental conditions.

Robustness: A measure of the method's capacity to remain unaffected by small but deliberate variations in method parameters.

Linearity: The ability of the method to produce results that are directly proportional to the concentration of analyte in the sample across a given range.

Limit of Detection (LOD): The lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under stated experimental conditions [21-23].

Limit of Quantification (LOQ): The lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

Specificity: The ability to assess the analyte unequivocally in the presence of components that may be expected to be present, such as impurities, degradants or matrix components.

Conclusion

A stability-indicating RP-HPLC method was successfully developed and optimized for the simultaneous estimation

of Risankizumab and its impurities. Through systematic trials (Trail 1 to Trail 6), various chromatographic parameters were evaluated. Among them, Trail 6 provided optimal resolution, peak symmetry and retention times, demonstrating high precision and reproducibility. The method was validated following ICH guidelines by assessing key parameters such as accuracy, precision, specificity, linearity, robustness, as well as the Limits of Detection (LOD) and Limit of Quantification (LOQ). The use of Luna Phenyl Hexyl column and mobile phase containing 0.1% TEA pH 2.5/OPA and acetonitrile (80:20) resulted in clear separation with retention times of 2.565 min (Risankizumab), 3.471 min (impurity-1) and 4.512 min (impurity-2). This optimized method is simple, rapid and reliable, making it suitable for routine quality control and impurity profiling in pharmaceutical formulations.

Acknowledgements

Conflicts of Interest

The authors declare no conflict of interest.

Funding Clearance

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