Research Article

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# STABILITY INDICATING UV VISIBLE SPECTROPHOTOMETRIC METHOD FOR TEZEPELUMAB DEVELOPMENT AND VALIDATION

**Ch.Ajay Kumar,M*, A.Naveena, D.Lalitha, K.Anand, Sk.Nagoor Basha.**

**Department of Pharmaceitical Analysis, A.M.Reddy Memorial College of Pharmacy, Narasaraopet, 522412, A.P, India**

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**Abstract**

Spectroscopy is the study of how electromagnetic radiation interacts with matter. Electromagnetic radiation is frequently released as matter relaxes back to its initial (ground) state after being energised (stimulated) by the application of thermal, electrical, nuclear, or radiant energy. The purpose of this research with the results of this work, we verify a stability-indicating UV approach for the measurement of tezepelumab in bulk and pharmaceutical formulations. The determination of the Tezepelumab dosage form was a good fit for the proposed UV-Spectrophotometric approaches. Every parameter of the created methods complied with the requirements of the ICH standards for method validation. It is claimed that the developed UV methods for the estimate of tezepelumab are quick, easy to use, precise, accurate, sensitive, affordable, and repeatable within the defined method parameter.

***Keywords:*** Spectroscopy, electromagnetic radiation, Tezepelumab**,** ICH.

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***Corresponding Author**

Ch.Ajay Kumar,M

Department of Pharmaceitical Analysis

A.M.Reddy Memorial College of Pharmacy

Narasaraopet, 522412, A.P, India

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## Introduction

The study of how electromagnetic radiation interacts with matter is known as spectroscopy. Electromagnetic radiation is frequently released when matter is energised (stimulated) by the introduction of thermal, electrical, nuclear, or radiant energy when the matter relaxes back to its initial (ground) state [1-4]. An emission spectrum is the range of radiation that an object emits after absorbing energy, and emission spectroscopy is the scientific discipline that studies emission spectra. Another common method for studying how electromagnetic radiation interacts with matter is to let a continuous stream of radiation (like white light) fall on a substance and then look at the frequencies that are absorbed by it. The material produces a spectrum that includes the initial range of radiation and dark regions that correspond to [5-10].

Table 1: Common solvents (UV) with their cut off wavelength [11-15]

| S.NO | Solvent | Cut off wave length (nm) |
|---|---|---|
| 1 | Acetonitrile | 190 |
| 2 | Water | 191 |
| 3 | Cyclohexane | 195 |
| 4 | Hexane | 201 |
| 5 | Methanol | 203 |
| 6 | 95%ethanol | 304 |
| 7 | 1,4-dioxane | 215 |
| 8 | Ether | 215 |
| 9 | Dichloromethane | 220 |
| 10 | Chloroform | 237 |
| 11 | Carbon tetrachloride | 257 |
| 12 | Benzene | 280 |

**Aim, Objectives**

**AIM:** To validate a stability indicating UV method for the estimation of Tezepelumab in bulk and pharmaceutical formulations.

**Specific objectives:**

- To develop a simple, rapid and specific UV method for the estimation of Tezepelumab in bulk and 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.

**Plan of Work**

To develop and validate an effective UV method for the determination of Tezepelumab in bulk and pharmaceutical dosage forms.

**The plan of work for the UV method is as follows:**

1. Gathering physical chemical properties of drug
2. Gathering physical chemical properties of drug
3. Selection of suitable solvent
4. From the Spectrophotometric analysis detection of λmax
5. Optimization of Chromatographic and Spectral conditions
6. Summarize methodology, finalize documentation.

**UG PROFILE OF TEZEPELUMAB**

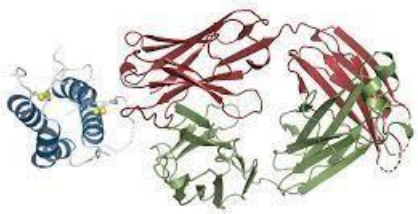

**Fig No. 01: Molecular structure of Tezepelumab**

**Table 3**: **Drug profile of Tezepelumab**

| | |
|---|---|
| Molecular Formula: | $C_{6400}H_{9844}N_{1732}O_{1992}S_{52}$ |
| Molecular Weight: | 144590.40 g·mol$^{-1}$ |
| Description: | Tezepelumab is a human monoclonal IgG2λ thymic stromallymphopoietin (TSLP)-blocking antibody for add-on maintenance therapy in severe asthma. |
| Category: | Tezepelumab is a first-in-class human monoclonalantibody that binds to TSLP, thus inhibiting its interaction with TSLP receptor complex. |
| Half life: | Tezepelumab has an elimination half-life of ~26 days. |

**Uses**

Tezepelumab, is a human monoclonal antibody used for the treatmentof asthma. Tezepelumab blocks thymic stromal lymphopoietin (TSLP), an epithelial cytokine that has been suggested to be critical in the initiation and persistence of airway inflammation.

**Mechanism of action**

Asthma is a heterogeneous chronic obstructive respiratory disease characterized by reduced airflow, chronic inflammation, and airway remodelling. Generally, asthma can be divided into "type 2" (T2, including allergic and eosinophilic presentations) and T2-low (including neutrophilic and paucigranulocytic presentations) endotypes, each driven by distinct underlying pathways. Thymic stromal lymphopoietin (TSLP) is an innate pleiotropic IL-2- family cytokine distantly related to IL-7; two forms of TSLP exist, with a short isoform (sfTSLP, 60 amino acids long) and a long isoform (lfTSLP, 159 amino acids long). The short isoform appears to be constitutively expressed, especially by lung and gut epithelial cells, while lfTSLP is upregulated in response to proinflammatory stimuli. While the role of sfTSLP is still unclear, lfTSLP has emerged as an upstream alarmin central to the pathophysiology of inflammatory disorders including asthma, atopic rhinitis, chronic obstructive pulmonary disease, eosinophilic esophagitis, and atopic dermatitis.

Under normal conditions, lfTSLP interacts with its cognate receptor TSLPR, and IL-7Rα in a ternary complex with three contact sites labelled site I (TSLP:TSLPR), site II (TSLP:IL- 7Rα), and site III (TSLPR:IL-7Rα). The assembly of the ternary complex is stepwise, as TSLP does not interact appreciably with IL-7Rα until after it has bound TSLPR. Complementary electrostatic surfaces on TSLP and TSLPR mediate initial high affinity formation of a TSLP:TSLPR complex ($K_D$ of 32 nM and $k_a$ of 1.7 x $10^5$ $M^{-1}s^{-1}$). This initial binding induces a restructuring of the π-helical turn in the TSLP αA helix and structuring of the AB loop to facilitate binding of TSLP to a hydrophobic patch on IL-7Rα to form the ternary complex ($K_D$ of 29 nM and $k_a$ of 1.23 x $10^5$ $M^{-1}s^{-1}$). The complete ternary complex is stabilized by additional interactions between TSLPR and IL-7Rα at site III near the transmembrane domain of each receptor.

Formation of the ternary complex activates JAK1/2, which, through downstream pathways involving STAT3/5, NF-κB, PI3K, and MAPK, induces the expression of Th2 cytokines including IL-4, IL-5, IL-9, and IL-13. TSLP can induce Th2 cytokine production by stimulating dendritic cells and ILC2 cells (primarily in T2 asthma). Furthermore, TSLP has been implicated in steroid resistance of ILC2 cells. In neutrophilic asthma, TSLP induces dendritic cells to drive the development of Th17 cells, which secrete IL-17A to recruit neutrophils and drive inflammation. In paucigranulocytic asthma, TSLP mediates cross-talk between mast cells, smooth muscle cells, and fibroblasts. Hence, despite different underlying pathways, TSLP appears to function as a critical upstream driver across asthma endotypes.

Tezepelumab is a human monoclonal IgG2λ antibody that binds to TSLP with a dissociation constant of 15.8 pM. Specifically, the variable heavy chain domain ($V_H$)

complementarity determining regions (CDRs) of tezepelumab bind TSLP at the AB-loop region and C-terminal region of the αD helix, obstructing the TSLPR binding region while leaving the IL-7Rα binding region unobstructed. As TSLP is incapable of binding IL-7Rα prior to its inclusion in the TSLP:TSLPR dimer, tezepelumab effectively blocks the assembly of the ternary complex and resulting downstream signalling. Furthermore, unlike existing therapies that act on specific downstream effector molecules, targeting TSLP ensures effective upstream blockade and is expected to be efficacious against multiple asthma endotypes.

**Absorption**

When administered subcutaneously, tezepelumab reaches $C_{max}$ in approximately 3-10 days with an estimated absolute bioavailability of 77%, regardless of injection site choice.

Tezepelumab displays dose-proportional pharmacokinetics over a range of 2.1-420 mg (0.01- 2 times the recommended dose) following a single subcutaneous dose. With a 4-week dosing schedule, tezepelumab achieves steady-state kinetics after 12 weeks with a 1.86-fold $C_{trough}$ accumulation ratio.

There are no clinically meaningful changes expected for tezepelumab pharmacokinetics in patients across patient populations, including those with renal or hepatic impairment.

**Indications**

Tezepelumab is indicated as an add-on maintenance treatment for patients aged 12 years and older with severe asthma. In Europe, it is reserved for patients who are inadequately controlled despite maintenance treatment with high-dose inhaled corticosteroids plus another drug.

**Experimental Work**

**UV Experimental Work**

**Solubility test and selection of solvent:** Solubility of the drugs was checked in differentsolvents and the drug was found to be soluble in Acetonitrile. From the solubility analysis, Acetonitrile was selected as solubilising agent for methoddevelopment.

**Method Development**

**(a) Determination of λ max**

**Method A**: A solution of 25µg/mL of Tezepelumab was scanned against acetonitrile blank inthe range of 200-400 nm. The $\lambda_{max}$ was found to be 275nm for Tezepelumab**.**

**(b) Preparation of standard solution**

The pure drug of 25mg of Tezepelumab was weighed and transferred in to a 100mL volumetric flask. The drug was dissolved completely in Acetonitrile and made up to the final volume with the same solvent to get a stock solution of concentration 250µg/mL. Aliquots of standard stock solution were pipette out 5 ml to 50ml and diluted suitably with acetonitrile to get the final concentration of standard solutions. (25 µg/mL)

**Selection of analytical concentration range**

Appropriate aliquots were pipette out from the standard stock solution in to a series of 100mL volumetric flasks. The volume was made up to the mark with water to obtain a series of dilutions of concentration range, ranging from 6.25-37.50 µg/mL of Tezepelumab. Absorbance of the above solutions were measured at 275nm and 275nm and converted to zero order spectra calibration curve of absorbance against concentration were plotted. The regression equation and correlation coefficient was determined. Beer Lambert's law was obeyed in the concentration range of 6.25-37.50µg/ml for Tezepelumab.

**(c) Analysis of tablet formulation**

Weigh 0.23ml of Tezepelumab sample and taken in a 100mL volumetric flask and it was dissolved in acetonitrile and made up to the mark with same solvent. Then the solution was filtered using Whitman filter paper No.40. From this filtrate, dilute 5ml to 50ml volumetric flask was made with water to obtain the desired concentration (25µg/ml of Tezepelumab). These solutions were analyzed in UV and the result was indicated by % assay.

**Method Validation**

The methods were validated as per ICH guidelines for different parameters like Linearity, Accuracy, Precision, Robustness and Ruggedness.

**Linearity**

Fresh aliquots were prepared from standard stock solution ranging from 6.25-37.50 µg/mL and the absorbance values of Tezepelumab concentration was recorded at 275nm for zero order using acetonitrile as blank. The drugs show linearity between 6.25-37.50µg/mL for Tezepelumab. The correlation co efficient was found to be 0.999 for method.

**Preparation of stock solution**

Accurately weigh and transfer 25mg of Tezepelumab working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume upto the mark with the same solvent. (Stock solution)

**Preparation of Level – I (6.25ppm of Tezepelumab)**

1.25 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up tothe mark with diluent.

**Preparation of Level – II (12.50ppm of Tezepelumab)**

2.5 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up tothe mark with diluent.

**Preparation of Level – III (18.75ppm of Tezepelumab)**

3.75 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up tothe mark with diluent.

**Preparation of Level –IV (25ppm of Tezepelumab)**

5 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up to the mark with diluent

**Preparation of Level – V (31.25ppm of Tezepelumab)**

6.25 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up tothe mark with diluent**.**

**Preparation of Level – VI (37.50ppm of Tezepelumab)**

7.5 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up tothe mark with diluent.

**Accuracy**

Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges 50%, 100%, 150% each one in triplicate and the accuracy was indicated by % recovery. The %RSD for accuracy of Tezepelumab in this method was found to be less than 2. The % recovery was in the range of 100.0 for Tezepelumab. According to ICH guidelines the statistical results were within the acceptance range

**Precision**

Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, six solutions of 25µg/mL of Tezepelumab were prepared and analyzed three times in a day and the respective absorbances were noted. The results were indicated by % RSD. In the inter-day variation study, six solutions of 25µg/mL of Tezepelumab were prepared and analyzed three times for three consecutive days and the respective absorbances were noted. The results were indicated by % RSD.

The %RSD for intraday and inter day precision of Tezepelumab in this method was found to be less than 2. According to ICH guidelines, the %RSD should less than 2 (within the acceptance criteria).

**Robustness**

Robustness of the method was determined by carrying out the analysis at two different wavelengths (±5nm). The respective absorbances were noted and the results were indicated by % RSD. The % RSD values were found to be within the acceptance criteria.

**Degradation Studies**

**Preparation of stock**

Accurately weigh and transfer 25mg of Tezepelumab working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

**Acid degradation**

Pipette 5 ml of above solution into a 50ml volumetric flask and 1 ml of 1N HCl was added. Then, the volumetric flask was kept at 60ºC for 1 hour and then neutralized with 1 N NaOH and make up to 50ml with diluent.

**Alkali degradation**

Pipette 5 ml of above solution into a 50ml volumetric flask and add 1ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 1 hour and then neutralized with 1N HCl and make up to 50ml with diluent.

**Thermal degradation**

Tezepelumab sample was taken in petridish and kept in Hot air oven at 105$^0$ C for 24 hours. Then the sample was taken and diluted with diluents.

**Peroxide degradation**

Pipette 5 ml above stock solution was added to a 50 ml vacuum flask, 1 ml of 3 percent w/v hydrogen peroxide was added to the flask and the volume was built up to the mark using diluent. The vacuum flask was then maintained at 60$^o$C for 1hour.After that, the vacuum flask was left at room temperature for 15 minutes.

**Reduction degradation**

Pipette 5ml of above-stock solution was added to a 50ml vacuum flask, 1ml of 10% Sodium bisulphate was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60$^o$C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes.

**Photolytic degradation**

Tezepelumab sample was placed in sun light for 24 hours. Then the sample was takenand diluted with diluents.

**Hydrolysis degradation**

Pipette 5ml of above-stock solution was added to a 50ml vacuum flask, 1ml of HPLC grade water was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60˚C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes.

Tezepelumab is not indicated for the relief of acute bronchospasm or status asthmaticus.

**Results and Discussion**

**UV Spectrophotometric Method of Tezepelumab:**

**Selection of analytical wavelength**

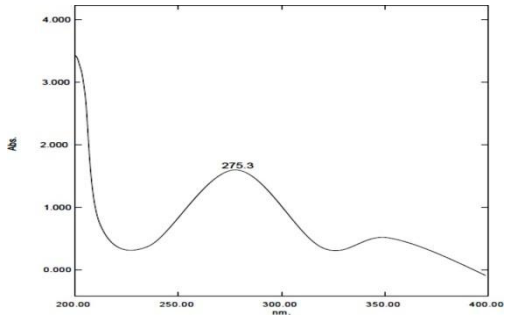


Figure 6: UV spectrum of Tezepelumab (275 nm)

**Assay**:

Table 4: Results of analysis of Formulation by UV-Spectrophotometry

| Name | Wave Lengthnm | Label claim (mg/tab) | Standard absorbance | Test absorbance | Amount found (mg/mL) | % recovery |
|---|---|---|---|---|---|---|
| Tezepelumab | 275 | 110 | 1.628 | 1.621 | 24.9 | 99.6 |

**Method validation of Tezepelumab by UV spectrophotometryLinearity & Range:**

Table 5: Linearity of Tezepelumab

| S. No | Tezepelumab | |
|---|---|---|
| | Conc.(µg/mL) | Absorbance |
| 1 | 6.25 | 0.464 |
| 2 | 12.50 | 0.845 |
| 3 | 18.75 | 1.236 |
| 4 | 25.00 | 1.631 |
| 5 | 31.25 | 2.019 |
| 6 | 37.50 | 2.458 |
| Regression equation | y = 0.06x+0.03 | |
| Slope | 0.06 | |
| Intercept | 0.03 | |
| $R^2$ | 0.99969 | |

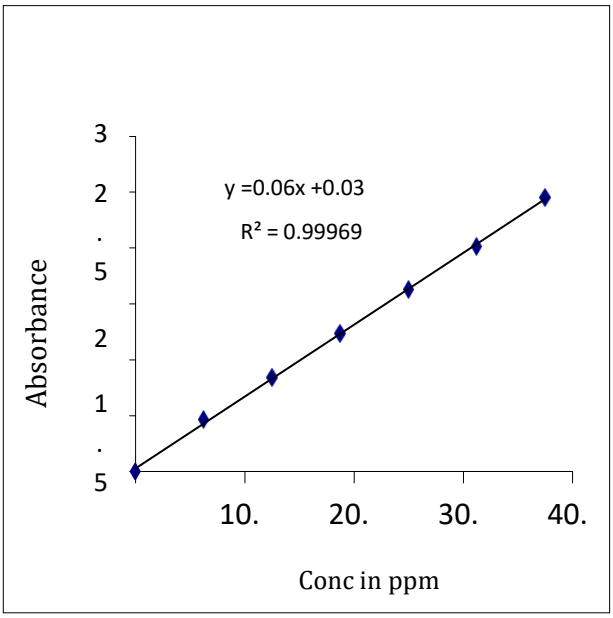


**Figure 7: Calibration curve for first order**

**Accuracy**

**Table 6: Accuracy data of UV Method**

| Method | Amount of µg/mL | | % of drug added | % Mean recovered | % RSD |
|---|---|---|---|---|---|
| | L C | Pure drug | | | |
| Method-A | 110 | 12.5 | 50 | 100.9 | 0.85 |
| | | 25.0 | 100 | 100.0 | |
| | | 37.5 | 150 | 99.2 | |

**Precision**

**Table 7: Intraday precision of Tezepelumab**

| Analytical method | Method Precision | Absorbance | % Assay |
|---|---|---|---|
| METHOD A | 1 | 1.627 | 99.3 |
| | 2 | 1.634 | |
| | 3 | 1.621 | |
| | 4 | 1.637 | |
| | 5 | 1.641 | |
| | 6 | 1.652 | |

**Table 8: Inter day precision of Tezepelumab**

| Analytical method | Intermediate Precision | Absorbance | % Assay |
|---|---|---|---|
| METHOD A | 1 | 1.628 | 99.2 |
| | 2 | 1.623 | |
| | 3 | 1.618 | |
| | 4 | 1.633 | |
| | 5 | 1.661 | |
| | 6 | 1.642 | |

**Robustness**

Table 9: Robustness results of Tezepelumab

| Parameter | Concentration 15 (µg/mL) | % Assay Method-A |
|---|---|---|
| Robustness Change in λmax (± 5nm) | λ+ : 280 nm | 99.1 |
| | λ - : 270 nm | 98.7 |

**Forced Degradation:**

Table 10: Forced degradation results of Tezepelumab

| Results: % Degradation results | Tezepelumab | |
|---|---|---|
| | Absorbance | % Degradation |
| **Control** | 1.647 | 0 |
| **Acid** | 1.444 | 12.4 |
| **Alkali** | 1.429 | 13.3 |
| **Peroxide** | 1.392 | 15.5 |
| **Reduction** | 1.477 | 10.4 |
| **Thermal** | 1.468 | 10.9 |
| **Photolytic** | 1.626 | 1.3 |
| **Hydrolysis** | 1.641 | 0.4 |

**Table 11: Summary of Validation & Optical characteristics**

| Parameter | Results of Tezepelumab |
|---|---|
| Beer's law limit (µg/mL) Linear regression equation<br>Linearity indicated by correlation coefficientPrecision indicated by %RSD<br>Intraday precision Inter day precision<br>Accuracy indicated by % recovery<br>Robustness indicated by % recovery<br>Wave Minus<br>Wave Plus | 6.25-37.5<br>y=0.06x+0.03<br>0.999<br>0.32<br>0.66<br>0.95<br>99.2-100.9%<br><br><br>98.7%<br>99.1% |

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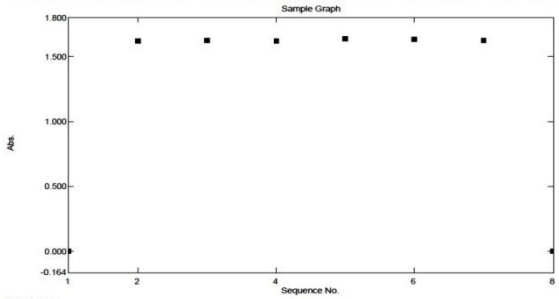


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| 4 | Standard 3 | 1.623 |
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| 6 | Standard 5 | 1.631 |
| 7 | Standard 6 | 1.627 |
| 8 | Blank 1 | 0.000 |
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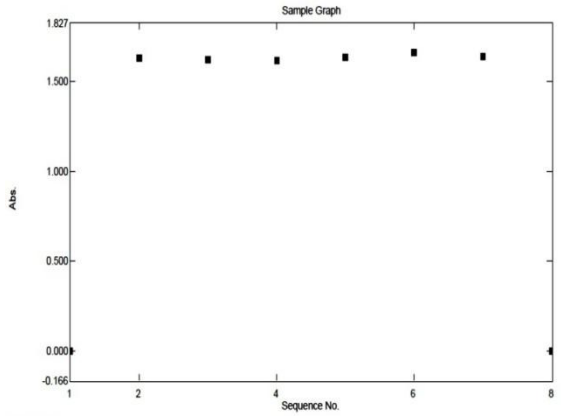


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| 4 | Intermediate Precision 3 | 1.618 |
| 5 | Intermediate Precision 4 | 1.633 |
| 6 | Intermediate Precision 5 | 1.661 |
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| 9 | | |

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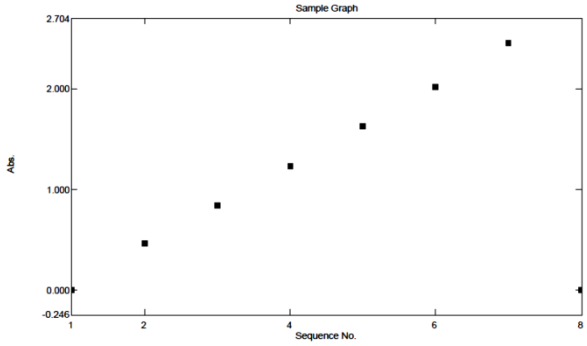


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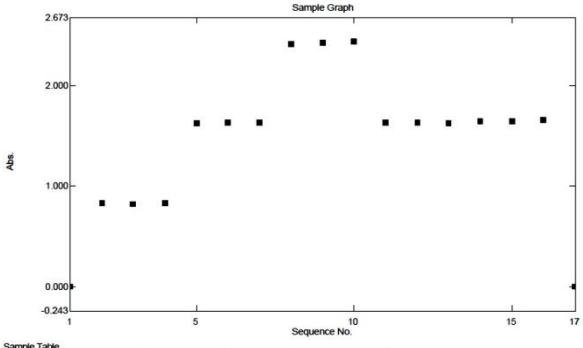


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| 3 | Accuracy 50% 2 | 0.814 |
| 4 | Accuracy 50% 3 | 0.826 |
| 5 | Accuracy 100% 1 | 1.623 |
| 6 | Accuracy 100% 2 | 1.627 |
| 7 | Accuracy 100% 3 | 1.634 |
| 8 | Accuracy 150% 1 | 2.407 |
| 9 | Accuracy 150% 2 | 2.426 |
| 10 | Accuracy 150% 3 | 2.430 |
| 11 | Method Precision 1 | 1.627 |
| 12 | Method Precision 2 | 1.634 |
| 13 | Method Precision 3 | 1.621 |
| 14 | Method Precision 4 | 1.637 |
| 15 | Method Precision 5 | 1.641 |
| 16 | Method Precision 6 | 1.652 |
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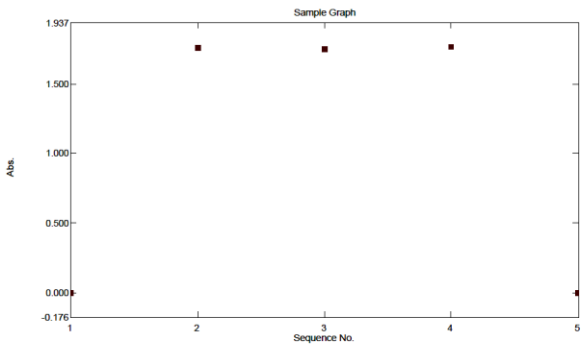


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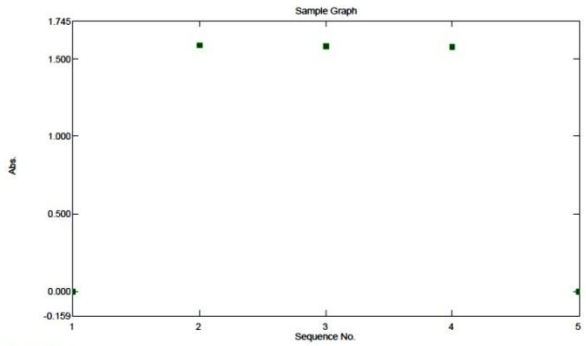


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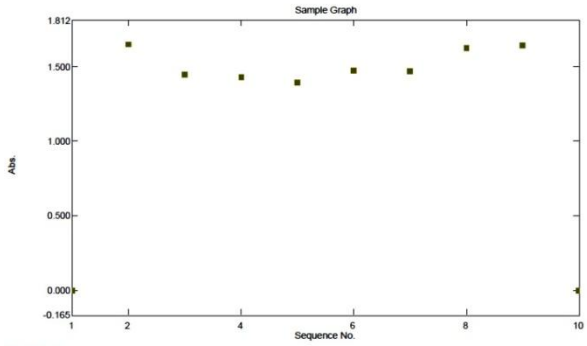


Sample Table

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| 3 | Acid deg | 1.444 |
| 4 | Alkali deg | 1.429 |
| 5 | Peroxide deg | 1.392 |
| 6 | Reduction deg | 1.477 |
| 7 | Thermal deg | 1.468 |
| 8 | Photolytic deg | 1.626 |
| 9 | Hydrolysis deg | 1.641 |
| 10 | Blank 1 | 0.000 |
| 11 | | |

## Summary and Conclusion

### Summary

The UV Spectrophotometric estimation was done by using Shimadzu 1700 UV Visible spectrophotometer. The estimation of Tezepelumab is done by using Acetonitrile as a solubilising agent and the $\lambda_{max}$ was found to be 275nm for calibration curve method and first order derivative. The UV Spectrophotometric estimation uses Acetonitrile as solubilising agent, and validated according to ICH guidelines for linearity, results were found well within the limits, indicating that the developed method was simple, rapid, accurate, precise, robust and economical.

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## Funding

Nil

## Conflict of Interest

Yes

## Informed Consent

Yes

### Ethical Statement

Not Applicable

## Author Contribution

All authors are contributed equally.

## Conclusion

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

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