**A REVIEW ON RP - HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TOFACITINIB**

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**ABSTRACT**

This study presents a review on the development and validation of a robust analytical method for the estimation of tofacitinib, a JAK inhibitor used in the treatment of autoimmune diseases. The method employs high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection, optimizing key parameters such as mobile phase composition, flow rate, and column temperature to achieve the desired resolution and sensitivity. The validation process adheres to ICH guidelines, evaluating specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Results indicate a linear response over a concentration range of X to Y µg/mL, with acceptable LOD and LOQ values. The method demonstrates high accuracy and precision, making it suitable for routine quality control and pharmacokinetic studies of tofacitinib in pharmaceutical formulations and biological samples. This method provides a reliable tool for clinicians and researchers to ensure therapeutic efficacy and safety in patients receiving tofacitinib therapy.

**Keywords:** RP-HPLC, Tofacitinib, Mobile phase, ICH guidelines, Validation.

**INTRODUCTION**

Tofacitinib is an oral Janus kinase (JAK) inhibitor primarily used to treat autoimmune conditions like rheumatoid arthritis, ulcerative colitis, and psoriatic arthritis. By targeting specific enzymes involved in the inflammatory process, tofacitinib helps to reduce inflammation and improve symptoms in patients.

Approved by the FDA in 2012, it offers a different mechanism of action compared to traditional biologic therapies, allowing for greater flexibility in treatment. Common side effects include infections, elevated liver enzymes, and gastrointestinal issues, necessitating monitoring during treatment.

Overall, Tofacitinib represents an important option in the management of chronic inflammatory diseases, particularly for patients who may not respond to conventional therapies.

**Structure of TOFACITINIB**

**Chemical Name :** 3-(2-(4-(methylthio)phenylamino)-6-methylpyrimidin-4-yl) phenol

**Chemical Formula:** C16H20N6O

**Molecular Weight:** 312.37 g/mol**.**

**Category:** Several Inflammatory conditions like Rheumatoid Arthritis ,Psoriatic Arthritis, Ulcerative Colitis.

**Mechanism of action:**

Tofacitinib works by inhibiting Janus kinase (JAK) enzymes, specifically JAK1 and JAK3. Here’s how it functions:

**Targeting JAK Enzymes**: JAKs are crucial for the signaling pathways of various cytokines and growth factors that are involved in immune responses and inflammation.

**Inhibition of Cytokine Signaling**: By blocking JAK1 and JAK3, Tofacitinib prevents the phosphorylation and activation of signal transducer and activator of transcription (STAT) proteins. This disrupts the signaling pathways that lead to inflammation and immune responses.

**Reduction of Inflammatory Mediators**: With cytokine signaling inhibited, there is a decrease in the production of pro-inflammatory mediators, leading to reduced inflammation and relief of symptoms in conditions like rheumatoid arthritis and ulcerative colitis.

**Oral Administration**: Tofacitinib is taken orally, providing a convenient option for patients compared to some injectable biologics.

**Table 1: Chromatographic** **methods reported in the literature for the determination of Tofacitinib.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Author Name/ Journal Name** | **Title Of The Journal** | **Chromatographic Conditions** | **Results** |  |
| BadithalaSivasaikiran Sundaranjan Raja(2018)  Research Journal of life sciences, Bioinformatics,Pharmaceutical of Tofacitinib | RP-HPLC Method Development and Validation for the Quantification of Tofacitinib | Column: Phenomnex Lunac C18 column ODS (250 \*4.6mm,5μm) mobile phase :methnol and water 45:55(V/V), Flow rate 1.0 ml/min UV detector at 254 nm injection volume 20μL run time 10 min | L O D :2.22μg/mL  L O Q: 6.73μg/mL  Linearity:15-90μg/mL  Retention time:4.35min |  |
| Sampath Kumar Reddy Govind, Nagaraju Ch.V.S.a, Rajan S.T.a, Eshwaraiah S.a Kishore M.a and Chakravarthy I.E.b  Scholars Research Library, Der Pharma Chemica,(2014) | Stability indicating HPLC method for the quantifcation of Tofacitinib citrate and its related substances\* | Acetonitrile 90:10v/v asmobilephase-A, and Acetonitrile:Buffer in the ratio of 70:30v/v as mobile phase-B.Coluuent ,mn temperature 25 C. flow rate:1.0mLmin-1 10μL injection volume water and acetonitrile 8:2v/v was used as diluent,kromasil c-18 column with the dimension of 2590mm x 4.6 mm and 5μm | The stability data confirmed that sample soutions were stable up to 48hrs.  Theoretical plates should not be less han 3000. |  |
| Hitaishi panchal1, Digesh patel2, Foram patel3, Hetal Patel4,Chirag Patel5  IJSART-Volume 6 Issue 2-FEBUARY(2020 | Development And Validation of Stability Indicating Assay Method For Estimation of Tofacitinib Citrate In Its Extended Release Tablets | Diluent:Water and acetonitrile  (50:50) %v/v.buffer solution:acetonitrile 65:35)%v/v.Column:Inertsil O D S-3V (150mm x 4.6 mm.5μm) detector : 287nm Flow rate : 1.0ml/min Injection volume :5μL Coiumn oven temperature : 400C Run Time :7mins Wash vial -purified water :ACN (50:50)%v/v sampler temperature :2 50 C | Recovery at 50% level: % RSD 0.6  Recovery at 100% level: % RSD 1.8  Recovery at 150% level: % RSD 0.6  Linearity 20 -160 correlation coefficient (r2) 0.999 | 7 |
| Krishna prasad Narapereddy 1,Devi srawanthi Alladi2  Journal of medicinal and chemical sciences (2023) | Tofacitinib pharmaceutical solid dosage form dissolution study :Development and Validation of RP-HPLC method | Column :waters X -bridge shiedld RP-18 (150 x406 mm 5mm) injection volume 20 μL flow rate 1.0 mL/min , mobile phase : 85:15 (%v/v ) triethylamine & acetonitrile | Retention time :7.21,  Tailing factor : 1.10  Theoretical plates 7610,  correlation coefficient (r2) 0.999 ,system precision %RSD 0.07 | 8 |
| A. S. K. Sankar, B. Datchayani, N. Balakumaran, Mohammed Rilwan, R. Subaranjani  Research Journal of pharmacy and technology | Developement of validated Reverse Phase liquid Chromatrographic Assay Method for Determination of Tofacitinib in pure form and in Physical Admixtures | Mobile phase Methanol: water (50:50)  Column C18 Diluents Methanol Column temperature 30 C Wavelength 285 nm  Injection volume 20 L  Flow rate 1.0 ml / min  Run time 10 min  Retention time 6.62  Theoretical plates 7072.58 Asymmetry 1.22  Capacity factor 2.79 | correlation coefficient (r2) 0.998  Recovery 20% 1.717  Recovery 40% 1.689  Recovery 60% 1.645  The limit of detection (LOD)  and limit of quantification (LOQ) for Tofacitinib were  found to be 0.053 μg/ml and 0. 163 μg/ml respectively. | 9 |

**CONCLUSION**

The development and validation of analytical methods for the estimation of tofacitinib are critical to ensure accurate and reliable measurement in pharmaceutical formulations and biological matrices. Through systematic method development, including optimization of parameters like mobile phase composition, pH, and column selection, a robust method can be established.

Methods such as High-Performance Liquid Chromatography (HPLC), UPLC, or LC-MS/MS have proven effective for quantifying tofacitinib. Selection should be based on the required sensitivity and specificity for the intended application.A comprehensive validation process is essential, following guidelines such as ICH Q2(R1). Parameters including specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness must be thoroughly evaluated.Validation results should confirm that the method is reliable for routine analysis, providing consistent results across different conditions and sample types.Validated methods facilitate pharmacokinetic studies, ensuring proper dosing regimens and monitoring of therapeutic levels in patients.Continued advancements in analytical technology may offer improved sensitivity and faster analysis times, further enhancing the monitoring of Tofacitinib in clinical settings. In conclusion, a well-developed and validated analytical method for Tofacitinib is crucial for ensuring drug safety and efficacy, contributing to better patient outcomes in therapeutic use. Regular updates and adherence to regulatory standards will be vital as new methodologies emerge.

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