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Research Article

Forced Degradation Studies to Assess the Stability of a Janus Kinase Inhibitor Using RPLC Method

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ABSTRACT

In optimization studies, it is important to study the retention behavior of the compounds containing the ionizable functional groups under the intended chromatographic conditions. In this study, the influence of pH and acetonitrile (ACN) composition in the mobile phase on chromatographic behavior of tofacitinib (TOF), a Janus kinase (JAK) inhibitor, was investigated in details. First, the chromatographic conditions were optimized using retention factors and pKa values. Then, the developed method was used for the stability studies under various stress conditions, and for the estimation of TOF concentration in tablets. Finally, the method was validated using the International Conference on Harmonization (Q2) guidelines and it was successfully used to separate the TOF degradation products. A linearity range, limit of detection and limit of quantification were determined as 2.0-12.0, 0.416, and 1.260 µg/mL, respectively. Between-day and within-day precisions were found to be as 0.290 and 0.462 for 4 µg/mL, respectively. The result indicates that the developed method is rather effective to separate the parent drug from the degradation products.

Keywords: Chromatography; Tofacitinib; RPLC; Method validation; Drug formulation; Forced degradation

INTRODUCTION

The Janus kinase (JAK) family consists of four nonreceptor protein tyrosine kinases: tyrosine kinase 2 (TYK2), JAK3, JAK2 and JAK1 [1]. JAK pathways are normally involved in development, survival, differentiation, and growth of a variety of cells, but are crucially important for hematopoietic and immune cells.

These pathways lead to cytokine production, which is responsible for the loop of inflammation. If proinflammatory cytokines produce redundantly, several autoimmune diseases arise.

Therefore, inhibitors of the JAK pathway are potential therapeutics as immunomodulator drugs including TOF (Figure. 1).

TOF is an oral JAK inhibitor for the therapy of ulcerative colitis [2], psoriasis [3], alopecia [4], and rheumatoid arthritis [5].

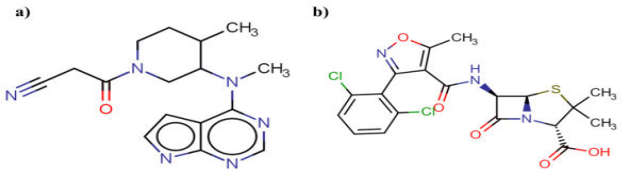

Figure 1: Chemical structure of a) TOF, b) Dicloxacillin.

There are a few approaches to predict the retention behavior of an ionizable drug molecule under chromatographic conditions. Today, reversed phase liquid chromatography (RPLC) is the most common way for drug analysis regardless of the ionic status of drug molecule . The RPLC based approach is preferred since it can provide structural information in a short time . During method optimization, the principal target is to ensure optimal separation conditions. For an ionizable analyte, column, mobile

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phase type, organic solvent composition, and pH are the most important factors targeted for optimization of chromatographic conditions [6].

The forced degradation study is a significant part of the drug development process as it provides knowledge about the degradation profile of drug molecules. It is also important for method validation studies . Since a Diode Array Detector (DAD) is very efficient in confirming peak purity, an HPLC instrument equipped with a DAD is routinely used to separate a drug from its degradation products under conditions including hydrolysis, oxidation, photolysis and thermal stress recommended by ICH [7].

For drugs with an ionizable functional group, a working range of pKa ± 1.5 for a chromatographic separation is recommended . The drug molecules at this pH range are ionized making the retention time much shorter at lower organic mobile compositions. There are several reports demonstrating the determination of TOF in biological samples and pharmaceutical formulations. The forced degradation studies of TOF were also reported using RPLC and LC/MS-MS methods. However, there is no procedure detailing the optimization using the chromatographic behavior of TOF in the literature. This study aims to fill this gap as an extension of my previous study with my colleague that we determined the pKa value of TOF in the water-ACN binary mixture . In this study, organic modifier content and pH of mobile phase on retention behavior of TOF and its degradation products was investigated with the aim of developing a more accurate, precise and reliable method. Then, the degradation products of TOF under forced conditions were investigated [8].

MATERIALS AND METHODS

Instrumentation and apparatus

The device used was a Shimadzu HPLC system (Japan), consisting of a pump (LC-20AD), a DAD (SPDM20A), degasser (DGU-20A3) and a column oven (CTO-10AS VP). Chromatographic determinations were realized on the Kinetex EVO C18 core-shell (150×4.6 mm, 5 µm, I.D.) column (Phenomenex, USA). HPLC grade water for preparation of solutions was obtained from Millipore (Direct Q3 UV, France). pH measurements were made by a glass electrode (In Lab 412) connected to a Mettler Toledo MA 235 pH meter (Switzerland) [9].

Chemicals and reagents

TOF and dicloxacillin (Figure 1) (internal standard, IS) were they purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (ACN), potassium hydrogen phthalate (KHP), hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), orthophosphoric acid, and uracil were analytical quality and supplied by Merck (Darmstadt, Germany). KHP is used as the standard buffer component for the calibration of the pH electrode in ACN-water mixtures [10].

Liquid chromatographic conditions

The effect of ACN in the binary mobile phase mixtures on the retention of TOF was carried out in three different percentages ranging from 30 to 40% (v/v). The pH of the mobile phase containing 25 mM orthophosphoric acid was adjusted to the desired value by the addition of 1.0 M NaOH. Considering of pKa value of the drug, the pH values of the mobile phase were adjusted to the values between 3.0 and 7.0. The chromatographic analysis was performed under the condition that the flow rate was 1.0 mLmin-1 and injection volume of 20 µL. The column temperature at which the peak symmetries were excellent was chosen as 30° C. The detection of wavelength was 287 nm.

Preparation of standard solutions

The solubility of TOF is first studied in a water-ACN binary mixture since its solubility is critical for qualitative and quantitative analysis. The main stock solutions of IS and TOF prepared at 50 µgmL−1 is protected from light and stored 4°C. For the calibration curve, the TOF solutions in that of range of 2.0 to 12.0 µgmL−1 are prepared. The concentration of IS is kept constant at 1.0 µgmL−1 throughout the study.

Sample solution

Ten TOF (Xeljanz™ 5 mg) tablets are thoroughly crushed in a porcelain mortar and an amount equivalent to 100.0 mg of drug was transferred to 100 mL volumetric flask containing 50 mL acetonitrile-water binary mixture and sonicated for 30 min. Then, the volume was brought to 100 mL with the same binary mixture and filtered to prepare the stock solution. Then, IS was added to this solution and the new concentration was calculated.

Forced degradation conditions

Hydrolytic degradation of TOF was separately carried out under acidic and basic conditions by mixing 2 mL of TOF stock solution with 1 mL HCl (0.1 M) and NaOH (0.1 M), respectively. These experiments were repeated at room temperature for 2, 6, 12, 24, 48 hours. For oxidative stress study, 2 mL of TOF stock solution was treated with 1 mL of 3% (v/v) H_2O_2 at room temperature for 2, 6, 12, 24, 48 hours. For that thermal and photolytic degradation studies, the drug substance in solid form was used. For the thermal stress experiments, accurately weighed 0.0001 g of TOF was taken in a glass dish and kept in hot air oven for both 2 and 4 hours at 30, 40, 50°C. After the heating, a certain amount was transferred into that of 25 ml flask containing acetonitrile-water binary mixture to make the concentration at 25 µgmL−1. For the photolytic degradation, TOF was exposed to 254 nm UV light in a UV cabinet for 2, 4, 6 hours at room temperature. All the stressed samples were diluted with the acetonitrile-water binary mixture to get a final concentration of TOF at 10 µgmL−1 and injected in the RPLC system. Acid and base degradation samples were neutralized with an equal molar concentration of base and acid prior to RPLC analysis.

RESULTS

Qualitative determination of degradation products

The specificity of the developed method was determined by forced degradation experiments. The degradation behavior of TOF under various stress conditions (mild) was investigated using ICH Q1A (R2) guidelines .

Hydrolytic degradation

Under the hydrolytic conditions (0.1 M HCl or 0.1 M NaOH), the aqueous solution of TOF at constant temperature and at a certain time interval was subjected to degradation. Since temperature and pH have an important effect on degradation, the degradation studies were carried out at room temperature and neutral pH. The decomposition data of TOF in solution exposed to 0.1 M HCl and 0.1 M NaOH is given in and the chromatograms are provided in . When the chromatograms were examined, only one peak was observed in the degradation in the acidic hydrolysis while more than one peak appeared in the case of alkaline hydrolysis. After 48 hours, the TOF showed more degradation in the basic medium. The observed difference can be explained with different hydrolysis mechanisms under acidic and basic conditions. It is possible that hydrolysis takes place at one site under the acidic conditions while it takes place at multiple sites under the basic conditions .

Oxidative degradation

When the TOF solution was exposed to 3% (v/v) H_2O_2 at room temperature, the degradation was quite fast as consistent with the report by Wu et al . the chromatogram obtained from the TOF solution exposed to H_2O_2. As seen, there are six degradation peaks in addition to the TOF peak.

Photolytic and thermal degradation

In the stress studies, the solid form of TOF was exposed to UV light and heat under various conditions. When TOF was exposed 254 nm light for 2, 4 and 6 hours, only one degradation product was observed, and the % degradation increased over time When TOF was exposed to increasing temperatures (30, 40, 50°C) for the 2 and 6 hours, it showed increased degradation behavior at higher temperatures, especially at 50 °C In this study, the peak impurity of TOF was determined using a DAD detector and found to be higher than 99.9% under all conditions.

In this study, the degradation of products was determined qualitative. In the study conducted by Wu et al. , degradation products of TOF were defined in the degradation conditions studied. It was found that TOF was prone to hydrolytic under acidic and basic conditions at the amide and cyano position of the 3-oxopropanenitrile moiety. TOF was especially sensitive to the oxidation degradation at the pyrrole ring double bond with the major degradation products .

DISCUSSION AND CONCLUSION

There is no procedure detailing the optimization using the chromatographic behavior of TOF in the literature. This is the first study on the chromatographic optimization of TOF. A suitable analytical procedure was developed and validated for the quantification of TOF in pharmaceutical dosage with an HPLC instrument equipped with a DAD detector. The method validation produced perfect results in terms of linearity, specificity, precision, accuracy, LOD, and LOQ. The developed method showed no interference with the formulation excipients. The behavior of TOF under the stress conditions in acidic, alkaline, thermal, photolytic and oxidative media was studied and the peaks originating from degradation products were well resolved from TOF peak. The experimental model which describe the capacity factors in different pH and ACN concentration conditions was successfully applied to degradation studies of TOF under different conditions. The highest degradation of TOF was realized in the oxidative condition and the least degradation was in the photolytic condition.

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