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RESEARCH ARTICLE

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Development of a green high-performance liquid chromatography method for tofacitinib quantification in pharmaceutical formulations and degradation studies

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Abstract

A new high-performance liquid chromatography (HPLC) method was applied for the quantification of the active substance of tofacitinib. Analysis was performed on a Chromasil 100 C18 (100.0 × 4.0 mm, 3.5 µm) stationary phase. The mobile phase consisted of acetonitrile:0.2% phosphoric acid in water (12:88, v/v). The prepared sample (20.0 µL) was injected into the system. A detection wavelength of 285.0 nm was chosen for the compound, and the flow rate was 0.8 mL/min. The experiment was completed in 5.0 min. The analysis temperature was set to 40.0°C. The method was evaluated using green chemistry. The method was validated according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines. For linearity studies calibration curves were constructed in the range of 10.0–200.0 µg/mL. The recovery values were calculated at 97.66% and 105.68%. The method developed for the analysis of the active substance had a short analysis time and was cost-effective. It is an environmentally friendly method due to the mobile phase content used. The technique can be used in laboratory analysis and bioequivalence experiments.

KEYWORDS
determination, greenness, HPLC, tofacitinib, validation

1 | INTRODUCTION

Rheumatoid arthritis is an inflammatory autoimmune disease that causes the immune system to attack the joints. This is a disabling and painful inflammatory form that causes significant loss of movement due to pain and joint wear. The main goal in the remedy of rheumatoid arthritis is to control the inflammation in the joint. Tofacitinib (TFC), 3-(3*R*,4*R*)-4-methyl-3-[methyl(1*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)amino]-1-piperidinyl-3-oxo propane nitrile (Figure 1), is an effective inhibitor of the Janus kinase family, and the drug is used to treat rheumatoid arthritis (Howland et al., 2006; Strand & Khanna, 2010).

TFC was determined using various methods in the literature: liquid chromatography (Abdelhameed et al., 2017; Dixit et al., 2019; Gorantla et al., 2021a; Kadi et al., 2016; Kim et al., 2020; Wang et al., 2022) and spectrophotometry (Gorantla et al., 2021b). It was quantified both alone and in the presence of different active ingredients.

In the proposed method, the high-performance liquid chromatography (HPLC) technique was developed, and the optimum experimental parameters were provided. Analysis could be applied for the assay of TFC in pharmaceutical preparation for every laboratory drug analysis. The aim of this study was to determine the active substance quickly without the need for pre-separation. Because the method has

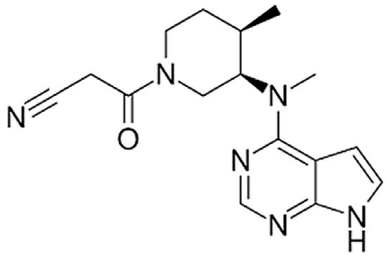


FIGURE 1 Chemical formula of tofacitinib.

a short analysis time, the amount of waste is low, and it is sensitive to the environment. Another advantage of this method is that devices that consume large amounts of energy are not used. Thus, analyses were carried out by reducing energy consumption. Because the method consumes less energy and the analysis is completed in a short time, it was evaluated for various parameters, including assessment of green profile (AGP), analytical eco-scale, analytical greenness calculator (AGREE), and green assessment of procedure index (GAPI), using green chemistry.

2 | METHODS AND MATERIALS

2.1 | Instrument

HPLC determination was performed using an Agilent 1100 series (Agilent Technologies, CA, USA) liquid chromatographic system. TFC was separated using a Kromasil 100 C18 (100.0 × 4.0 mm, 3.5 µm) column (Nouryon, Bohus, Sweden). The mobile phase system consisted of acetonitrile:0.2% phosphoric acid in water (12:88, v/v). A detection wavelength of 285.0 nm was preferred for the compound.

2.2 | Used materials

All the materials used were of HPLC quality. TFC-active substance was purchased from Sigma-Aldrich (Darmstadt, Germany, CAS-No:540737-29-9). LC quality phosphoric acid and acetonitrile were purchased from Merck (Darmstadt, Germany). For HPLC, the used solvent system was filtered using a 0.45-µm membrane filter.

2.3 | Preparation of active substance solution

The analyte was dissolved in a methanol solution. The prepared analyte solutions were diluted using the solvent system as calibration for HPLC trials using TFC concentrations of 10.0–200.0 µg/mL. Phosphoric acid was prepared using ultrapure water with a concentration of 0.2%.

2.4 | Pharmaceutical preparation

XELJANZ (5.0-mg TFC) is a film-coated tablet used to treat rheumatoid arthritis. This tablet was manufactured by Pfizer Industries Limited. The tablets were bought from local pharmacies in Turkey.

2.5 | Preparation of pharmaceutical solution

Ten tablets were weighed on a precision balance and powdered in a mortar. The amount equal to the weight of one tablet was weighed and was taken 50.0-mL volumetric flask containing methanol. The mixture was kept in an ultrasonic bath for ∼20.0 min. Then the mixture was diluted to a concentration of 50 µg/mL using the mobile phase. A syringe filter was used to filter the prepared tablet solutions.

3 | VALIDATION PARAMETERS OF THE TECHNIQUE

The presented technique was validated according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (Q2B) guidelines for accuracy, precision, specificity, and linearity (Committee ICH, 1996).

3.1 | Accuracy

Synthetic laboratory mixtures and pharmaceutical preparation were used to quantify the accuracy of the method. The recovery of active ingredients was calculated.

3.2 | Specificity

Specificity is the determination of the intended components in the analytical technique in the presence of any interference.

3.3 | Precision

To determine the precision of the trials, intra- and inter-day analyses were performed at different times and on different days.

3.4 | Linearity

For HPLC determination, a calibration graph with concentration versus area was constructed.

3.5 | Robustness

Robustness experiments were performed to observe the effect of small changes using the applied method.

4 | DEGRADATION EXPERIMENTS

Degradation experiments were performed to determine the stability of the active substance under acidic, alkaline, oxidative, and photolysis conditions. A stock active substance solution was prepared by dissolving 10.0 mg of active ingredient in 50.0 mL of methanol; 1.0 mL of the stock solution was transferred, and 0.1 M acid or base was added in equal milliliters to make up to 10.0 mL. The solution was stirred. After degradation, the solutions were neutralized and injected into the system. For oxidation experiments, 10.0% hydrogen peroxide solution was mixed with the active substance solution, and the chromatogram was obtained. For photodegradation studies, the solution of the active substance was kept in direct sunlight for 48 h.

5 | RESULTS AND DISCUSSION

5.1 | Chromatographic procedure

Different mobile phases, column types, and column temperature conditions were optimized to provide the best separation. The system's suitable parameters are presented in Table 1. The analyte was separated using an Xbridge C18 HPLC column (3.0 × 50.0 mm, 2.5 µm). The column was first conditioned for ~20.0 min using the solvent system. The flow rate was 0.8 mL/min. The mobile phase consisted of acetonitrile:0.2% phosphoric acid in water (12:88, v/v). The specimens were injected into the system at a volume of 20.0 µL. The experiment was performed at different wavelengths 230.0, 240.0, 260.0, and 280.0 nm; 285.0 nm was chosen for the active ingredient because TFC exhibits its maximum absorbance at this wavelength. The chromatogram of the active ingredient (50.0 µg/mL) and tablet (50.0 µg/mL) is shown in Figures 2 and 3.

5.2 | Calibration graph and limits of quantification and detection

Calibration curves were constructed in the 10.0–200.0 µg/mL concentration ranges of TFC. The limit of detection is the minimum concentration at which the determined substance is detected, however not inside the quantitative limits. The lowest linearity concentration at which the quantity of the tested material can be accurately and consistently measured is known as the limit of quantification (LOQ). The calculated parameters are presented in Table 2.

5.3 | Recovery results of the HPLC method

The HPLC method was used for calculating the recovery results for the pharmaceutical dosage form and standard addition. The calculated values are presented in Tables 3 and 4.

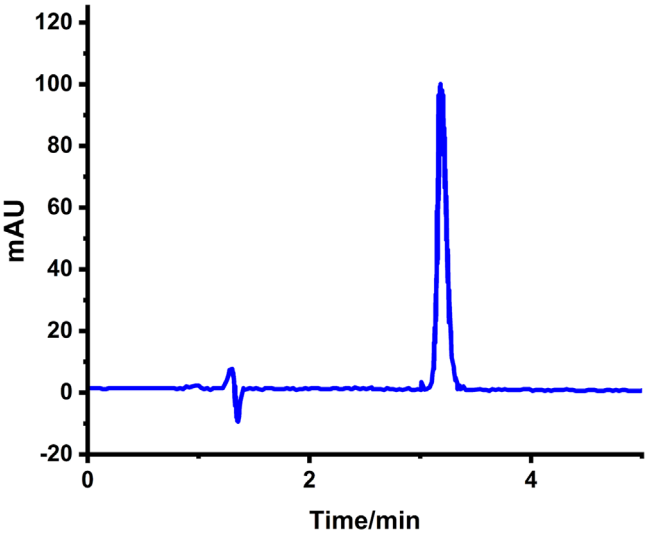


FIGURE 2 Chromatogram of the 50-µg/mL TFC (tofacitinib).

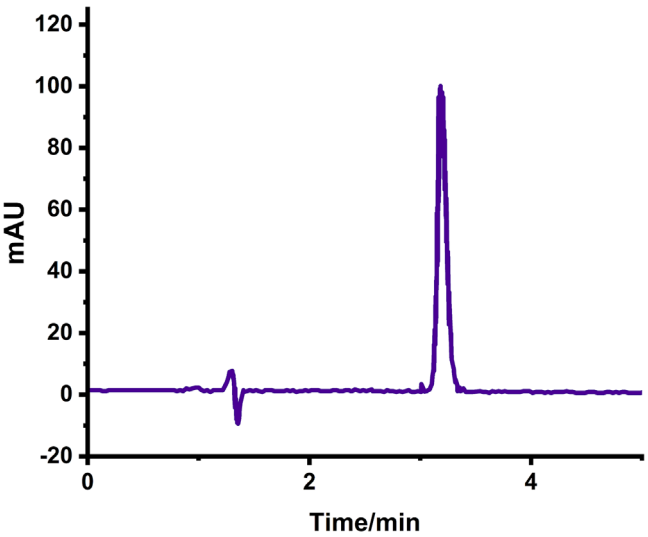


FIGURE 3 Chromatogram of the tablet.

TABLE 1 System-suitable parameters of the HPLC method.

Parameters	t_R (retention time)	*N* (column efficiency)	*T* (tailing factor)
TFC	2.286	3694	1.6

Abbreviation: TFC, tofacitinib.

6 | PRECISION RESULTS OF THE HPLC METHOD

Precision experiments were carried out using HPLC techniques. The calculated values are presented in Tables 5 and 6.

6.1 | Robustness results of the HPLC method

Experiments were carried out using different mobile phase ratios, flow rates, and column temperatures. There was no change in the method from the changes made. The findings of the robustness trials are presented in Table 7.

TABLE 2 The parameters of the HPLC method.

Parameters	TFC
Concentration range (μg/mL)	10.0–100.0
Wavelength (nm)	285.0
Intercept value	−0.6123
Slope value	62.9973
Correlation coefficient, R^2	0.9998
LOD (μg/mL)	1.33
LOQ (μg/mL)	4.40

Abbreviations: HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; TFC, tofacitinib.

TABLE 3 The results of recovery for pharmaceutical preparation.

Sample number	Tofacitinib XELJANZ
Sample 1	102.7
Sample 2	102.6
Sample 3	102.7
Sample 4	102.7
Sample 5	102.7
Mean %	102.7
SD	0.1
RSD %	0.1

Abbreviations: RSD, relative standard deviation; SD, standard deviation.

TABLE 4 Standard addition method.

Sample number	Added concentration (μg/mL) Tofacitinib	Found concentration (μg/mL) Tofacitinib	Recovery (%) Tofacitinib
Sample 1	5.00 + 20.00	24.69	98.8
Sample 2	5.00 + 44.00	49.63	101.3
Sample 3	5.00 + 69.00	74.17	100.2
Mean %			100.1
RSD %			1.25

Abbreviation: RSD, relative standard deviation.

6.2 | Results of degradation studies

Decomposition experiments were carried out under different conditions. As a result of the degradation experiments, the active substance remained stable in photodegradation and acid conditions and degraded in oxidation and alkaline conditions (Figure 4).

6.3 | Evaluation of the developed techniques in terms of greenness

Experimental conditions are optimized by choosing environmentally safe solvents and chemicals. Minimizing the chemicals used is

TABLE 5 Results of intra-day values.

Added concentrations (μg/mL)	Time	Found concentrations (%)
Method	HPLC	
50.0 μg/mL TFC	0. Minute	99.4
	1. Hour	99.5
	2. Hour	99.2
	3. Hour	99.3
	4. Hour	99.3
	5. Hour	99.2
RSD %		0.12

Abbreviations: HPLC, high-performance liquid chromatography; RSD, relative standard deviation.

TABLE 6 Results of inter-day values.

Added concentrations (μg/mL)	Time	Found concentrations (%)
Method	HPLC	
	1. Day	102.7
50.0 μg/mL TNF	2. Day	102.2
	3. Day	101.3
	4. Day	101.1
RSD %	5. Day	100.8
		0.78

Abbreviations: HPLC, high-performance liquid chromatography; RSD, relative standard deviation.

TABLE 7 Results of robustness experiments.

Condition	Value	Retention time	Tailing factor	Column efficiency
Flow rate (mL/min)	0.7	2.9	1.6	3817
	0.9	2.7	1.6	3530
	0.8	2.5	1.6	3694
Mobile phase ratio	93:7	8.4	2.1	3883
	83:17	1.7	1.4	3680
	88:12	2.5	1.6	3694
Temperature of column oven	37	2.9	1.7	3789
	43	2.8	1.7	3816
	40	2.5	1.6	3694

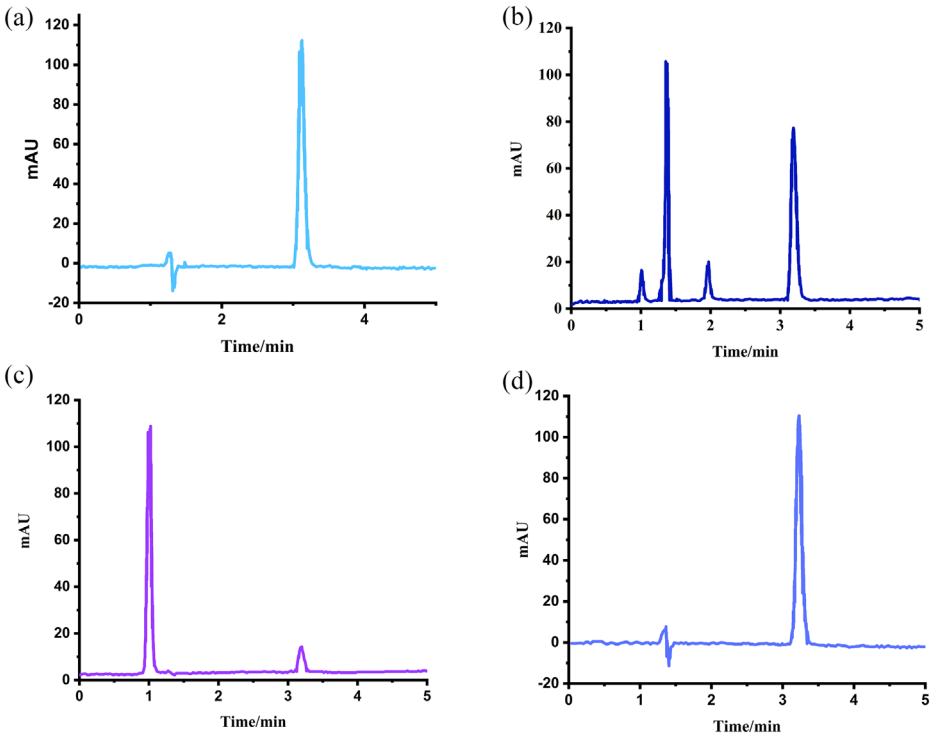


FIGURE 4 Chromatograms of the TFC: (a) Acid, (b) Alkaline, (c) Oxidation, and (d) Photolytic conditions.

important for the environment. Additionally, environmentally friendly methods can be developed using nanomaterials (Ali et al., 2023; Boher et al., 2023; Lanjwani et al., 2024). Chemicals that are less harmful to humans and the environment are those that are less toxic to organisms when they are accumulated in the body as well as in the environment.

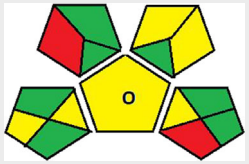
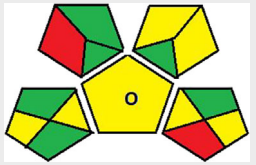
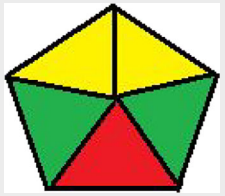
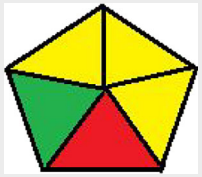
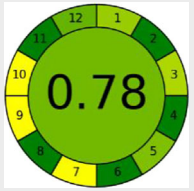
If the method used in the analysis consumes less energy, uses fewer chemicals, and produces less chemical waste, it is among the preferred methods in terms of green chemistry (https://www.epa.gov/greenchemistry/basics-green-chemistry#definition).

When chemical analysis methods are used, solvents, reagents, and energy are needed, and waste is produced. Regarding analytical chemistry, to preserve the environment, waste must be prevented, safer solvents and chemicals must be used, and the analysis time must be reduced for energy efficiency. By using instrumental methods in analysis, samples can be prepared at the microscale level and waste generation can be reduced (Koel & Kaljurand, 2006).

The analysis time of the developed HPLC method was 5.0 min. A short analysis time ensures that the amount of solvents and chemicals used is reduced. A comparison of the suggested technique and the analysis in the literature (Kim et al., 2020) is presented in Table 8. The eco-scale value of the developed analysis was found to be higher. The method is more environmentally friendly. Additionally, another study in the literature reveals that the analysis time is 8 min (Gorantla et al., 2021a).

According to the GAPI, the methods were evaluated based on criteria such as sample preparation, agents and chemicals used, and instruments used, and their harm to the environment was examined. Fifteen different parameters were evaluated while constructing the graph. The AGREE metric was used for calculating the 12 parameters of green chemistry. The calculation is presented in Table 8 Also, according to the AGP, the damage caused by the methods to the environment was examined by evaluating five different parameters, which are presented in Table 8 (Kannaiah et al., 2021; Polatka-Wasylka, 2018).

TABLE 8 Penalty points, GAPI, and AGP of methods.

Analytical eco-scale	Penalty points		GAPI AGP	Penalty points		GAPI AGP
	Proposed HPLC method			Reference Kim et al. (2020)		
Reagent	Acetonitrile	4		Acetonitrile	4	
	Phosphoric acid 85%	2		Ammonium acetate	4	
Instrument	Liquid chromatography	1		Liquid chromatography	1	
	Sonicator	1		Sonicator	1	
	Occupational hazards	0		Occupational hazards	0	
	Waste	3		Waste	5	
Overall penalty points		11			15	
Eco-scale		89			85	

Abbreviations: AGP, assessment of green profile; GAPI, green assessment of procedure index; HPLC, high-performance liquid chromatography.

7 | CONCLUSION

The suggested HPLC technique was applied for the quantification of TFC in their tablet form and laboratory-prepared solutions. The proposed technique was found to be simple, rapid, green, and precise without the need for any pre-preparation or derivatization. This method does not need any sophisticated equipment; simple software programs are used. Various analyses were performed on different samples in previous studies. These methods, such as LC–MS/MS, were performed using expensive and energy-consuming devices (Abdelhameed et al., 2017; Dixit et al., 2019). It was shown that the proposed technique performed better for both effectiveness and greenness using penalty points of the method. A novel HPLC method, which does not require pretreatment, has been improved for the quantification of active substances. The developed HPLC method has a short analysis time (5.0 min), and the flow rate was only 0.8 mL/min. The short analysis time of the method is superior to other studies in terms of green chemistry. Degradation studies were carried out for the active substance under different conditions. The active ingredient remained stable in acid and photolytic decomposition. It was observed that the active substance did not remain stable when treated with hydrogen peroxide in oxidation and alkaline conditions. Thus, under which conditions the substance decomposes and it remains stable were determined. In addition, the short analysis time results in less solvent and less energy consumption, thus reducing the amount of waste. The developed method can be applied quickly and practically in routine analysis and has also paved the way for the analysis of the active substance in biological fluids such as plasma.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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