USP Emerging Standards: Methods for the Analysis of Tofacitinib Oral Solution

Introduction:

To jump-start the standard development process and have earlier stakeholder engagement, USP is piloting a new approach for developing and sharing information with our stakeholders. Through a collaboration between USP's Small Molecules Department and the Global Analytical Development Laboratory, methods will be developed and validated for drug substances and drug products for which no monographs are currently available. The Emerging standards are intended to improve USP's official standards elaboration process by increasing transparency and allowing for broader stakeholder participation by publishing on the USP website prior to formal notice and comment through publication in the Pharmacopeial Forum.

Tofacitinib Oral Solution has been evaluated and shown to be a suitable candidate for development under this new program. The methods, in this document, are being published to help analyze Tofacitinib Oral Solution. Additional method development and validation information is provided to justify the use of method parameters.

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Available Resources:

Pharmaceutical Analytical Impurities (PAIs):

- Tofacitinib Amide (25 mg)

Background:

Tofacitinib is in a class of medications called janus kinase (JAK) inhibitors. Tofacitinib, often in the salt form Tofacitinib Citrate, is the active ingredient in Tofacitinib Oral Solution. It is used to treat rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis by inhibiting janus kinase enzymes that play a role in joint inflammation[1].

The *USP-NF* does not contain a drug substance or drug product monograph for Tofacitinib Citrate. As part of the emerging standards initiative, it was decided to develop methods for Tofacitinib Oral Solution starting with the conditions from high-performance liquid chromatography (HPLC) with ultraviolet (UV) methods from a submission for drug substance.

This document describes methods and includes supporting chromatographic system data for peak retention time match and photodiode array (PDA) spectral match, which may be suitable for identifying tofacitinib in the presence of various impurities and excipients. HPLC method and supporting validation data, which may be suitable for determining strength and purity, are also described.

Tofacitinib citrate and related impurities are shown in **Figures 1** and **2**.

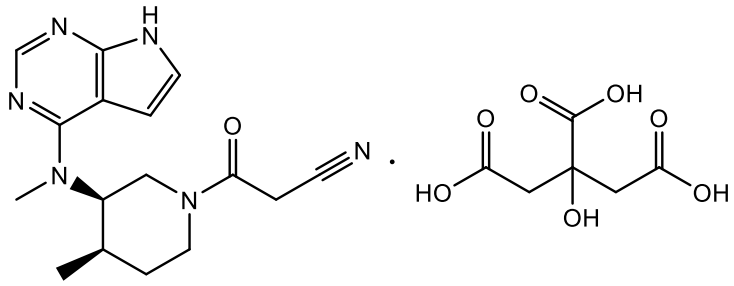

Figure 1. Tofacitinib Citrate

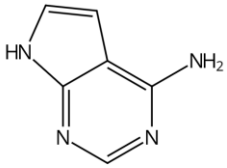

Figure 2a. 7-Deazaadenine

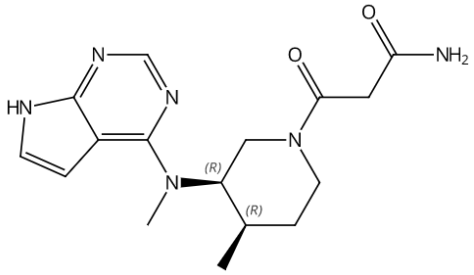

Figure 2b. Amide Tofactinib (Amide-TOFT)

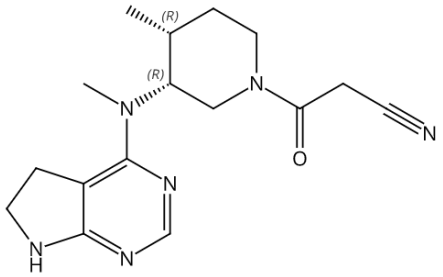

Figure 2c. Dihydrotofacitinib (Dihydro-TOFT)

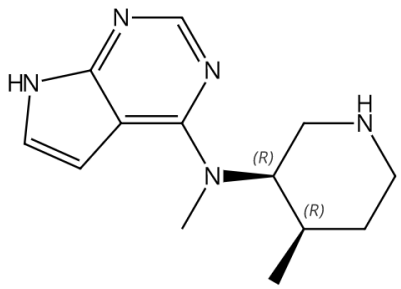

Figure 2d. Descyanoacetyl Tofacitinib (Descyanoacetyl-TOFT)

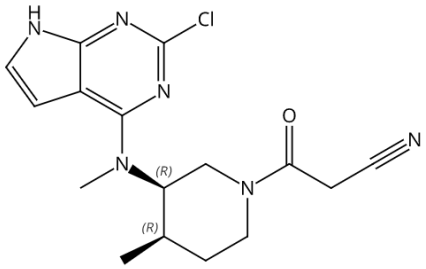

Figure 2e. Chlorotofacitinib (Chloro-TOFT)

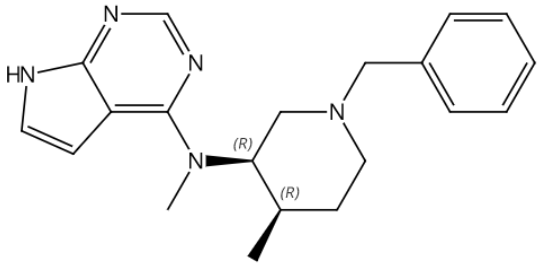

Figure 2f. Benzyl Tofacitinib (Benzyl-TOFT)

Figure 2. Tofacitinib citrate related impurities

EXPERIMENTAL

Materials:

Tofacitinib citrate and the related impurities 7-Deazaadenine, Amide Tofacitinib (Amide-TOFT), Dihydrotofacitinib (Dihydro-TOFT), Descyanoacetyl Tofacitinib (Descyanoacetyl-TOFT), Chlorotofacitinib (Chloro-TOFT), and Benzyl Tofacitinib (Benzyl-TOFT) were procured from commercial sources.

Tofacitinib Oral Solution with 1 mg tofacitinib/mL label claims was procured from commercial sources and was used to evaluate identification by ultraviolet (UV) spectral match and retention time match,

assay, and organic impurities using methods described in this document. As a USP reference standard (RS) for tofacitinib citrate is not currently available, the Tofacitinib Citrate active pharmaceutical ingredient (API) was procured and qualified as a standard.

METHOD DEVELOPMENT

Multivariate method development software was used to model and optimize the resolution between tofacitinib and related compounds, following Quality by Design (QbD) principles, using an HPLC-UV method provided to USP by a donor as the starting point. Chromatographic method conditions, including column type, organic mobile phase composition, column oven temperature, gradient time, initial isocratic hold time, and flow rate, were evaluated using a mixture solution containing tofacitinib and six related impurities at 5 µg/mL in water and methanol (50:50). Column and oven temperature, organic mobile phase concentration, and gradient time significantly affected peak retention and resolution. The modeling evaluation of trend responses that met defined criteria (total number of peaks, peaks with a USP resolution value of $\geq$ 1.5 and 2.0, and USP tailing) was applied to determine the final proposed method conditions. The proposed chromatographic conditions were utilized on an Agilent 1260 instrument with a PDA detector. The Waters Xbridge BEH Shield RP18, 4.6 mm x 150 mm, 2.5 µm column was used for analysis, and the results were processed using Empower (Waters software). The separation was achieved by a gradient program as listed in **Table 1**. Mobile phase A contained 2.72 g/L potassium phosphate monobasic and 1 g/L sodium 1-octanesulfonate monohydrate, adjusted to pH 5.5 with 1% potassium hydroxide solution. Mobile phase B contained 90% methanol and 10% acetonitrile by volume. The flow rate was 0.8 mL/min. The column temperature was maintained at 45°C and the autosampler temperature at 4°C. The PDA detector was set at a wavelength range of 200–400 nm, with detection at 280 nm. The injection volume was 10 µL. An example chromatogram of the mixture solution analyzed with the proposed chromatographic conditions is shown in **Figure 3**.

Table 1. Gradient Program

Time (min)	Solution A (%)	Solution B (%)
0	77	23
3	77	23
28	45	55
31	45	55
32	38	62
37	38	62
38	77	23
42	77	23

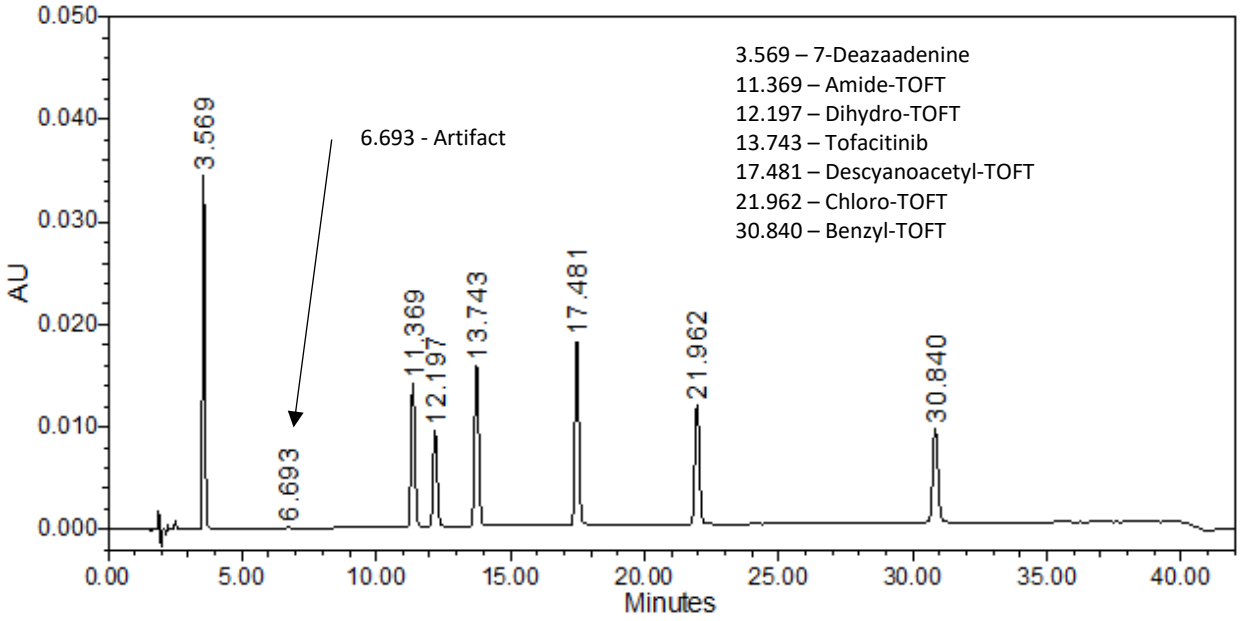

Figure 3. *Mixture solution* containing tofacitinib and related impurities at 5 µg/mL separated under the proposed chromatographic conditions.

Forced Degradation Study

A forced degradation study was performed by exposing Tofacitinib Citrate standard from a commercial source to acid, base, oxidation [hydrogen peroxide(H_2O_2) and 2, 2'-Azobis(2-methylpropionitrile)(AIBN)], heat, heat/humidity, and UV/visible light. The analysis results (average of n=2 injections) carried out under the proposed HPLC conditions are included in Table 2.

Table 2. Forced Degradation Study Results

Sample	Condition	Tofacitinib % w/w	Tofacitinib %TDA	Descyanoacetyl-TOFT (RRT 1.3) %TDA	Unknown %TDA (RRT)
Control	Freshly prepared (unstressed)				
Aged Control	Aged for 3 days	100.3	99.8	ND	
Acid Stress	0.1 N HCl for 3 days	102.7	98.9	0.8	
Base Stress	0.05 N NaOH for 10 minutes	75.3	76.7	3.7	17.4 (1.2)

H_2O_2 Oxidative Stress	3% H_2O_2 for 3 days	112.0	99.5	ND	
AIBN Oxidative Stress	0.5 mg/mL AIBN for 3 days at 40°	100.9	99.0	ND	
Heat Stress	105° for 3 days	100.1	99.8	ND	
Heat/ Humidity Stress	80° and 80% RH for 3 days	100.1	99.8	ND	
Photolytic Stress	600-watt hours/m² of UV light and 1.2 million lux hours of Visible light	100.0	99.9	ND	

Tofacitinib %w/w determined against average peak response of five replicate injections of freshly prepare control sample.
ND – Not Detected

No major degradation was detected under oxidative, heat, heat/humidity, and photolytic stress conditions. The base stress was first performed for 3 days with 0.1 N NaOH and almost 100% degradation was observed. The base stress was repeated for 10 minutes using 0.05 N NaOH and about 25% degradation was observed, resulting in two major degradants (descyanoacetyl-TOFT and an unknown peak), with resolution between peaks ≥1.5. Both degradant peaks were well resolved from the API and other specified impurities. Minor amounts (< 1.0%) of descyanoacetyl-TOFT were observed in the acid stressed sample. The PDA of the API data from 200–400 nm showed homogeneity of the UV spectrum for the tofacitinib peak, indicating the lack of coelution.

Robustness Study

The robustness study was conducted with a Robustness solution (0.2 mg/mL Sample solution of Tofacitinib Oral Solution spiked with 0.5 µg/mL of each specified impurity). The *Robustness solution* was analyzed under proposed condition and deliberately changed conditions including flow rate ±10% (0.8 ± 0.08 mL/min), column temperature ±3° (45° ± 3°C), isocratic hold time ±0.5 min (3.0 min ± 0.5 min), post gradient ±2% absolute of *Solution B* (55% ± 2% at 28 min), pH of *Solution A* (5.5 ± 0.2), and concentration of ion-pairing reagent in *Solution A* (1.0 g/L ± 0.1 g/L sodium 1-octanesulfunoate monohydrate). The system suitability target criteria of USP resolution of NLT 2.0 from tofacitinib peak and NLT 1.5 between impurities were achieved across all evaluated robustness conditions. An example chromatogram of the *Robustness solution* analyzed under the proposed conditions is shown in **Figure 4**.

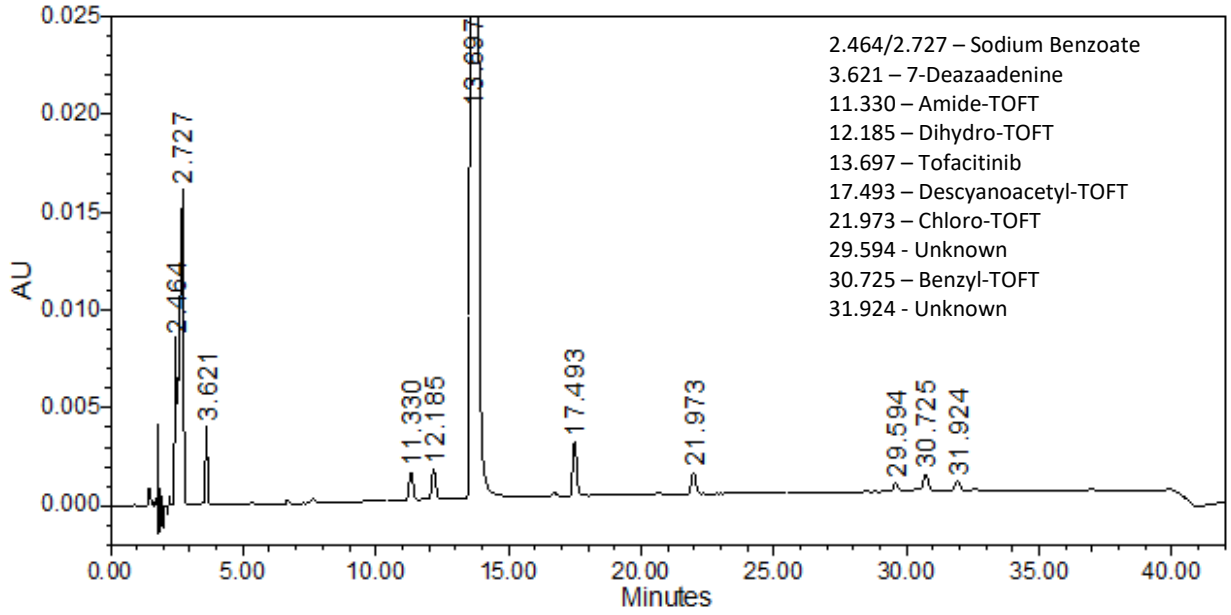

Figure 4. Example chromatogram of Robustness solution

IDENTIFICATION

Identification of tofacitinib in Tofacitinib Oral Solution was evaluated using HPLC-UV with PDA detector and chromatographic HPLC retention time match.

A. HPLC-UV with PDA Detector:

The HPLC assay procedure with PDA detector was used as the chromatographic identification procedure. See the *Assay* section for the method conditions and solution preparations. The validation parameter and results are summarized in **Table 3,** and representative UV spectra of the tofacitinib citrate standard and sample are shown in **Figures 5** and **6**.

Table 3. Summary of Validation Experiment, Samples, and Results for HPLC-UV with PDA Detector

Parameter	Samples and Procedure	Results
Spectral Agreement	Collect PDA data from 200–400nm for the *Standard solution* and *Sample solution*	The UV spectrum of the tofacitinib peak from the *Sample solution* matched the spectrum of tofacitinib in the *Standard solution* and exhibited maxima and minima only at the same wavelengths as the *Standard solution.*

Abbreviations- PDA: photodiode array; UV: ultraviolet

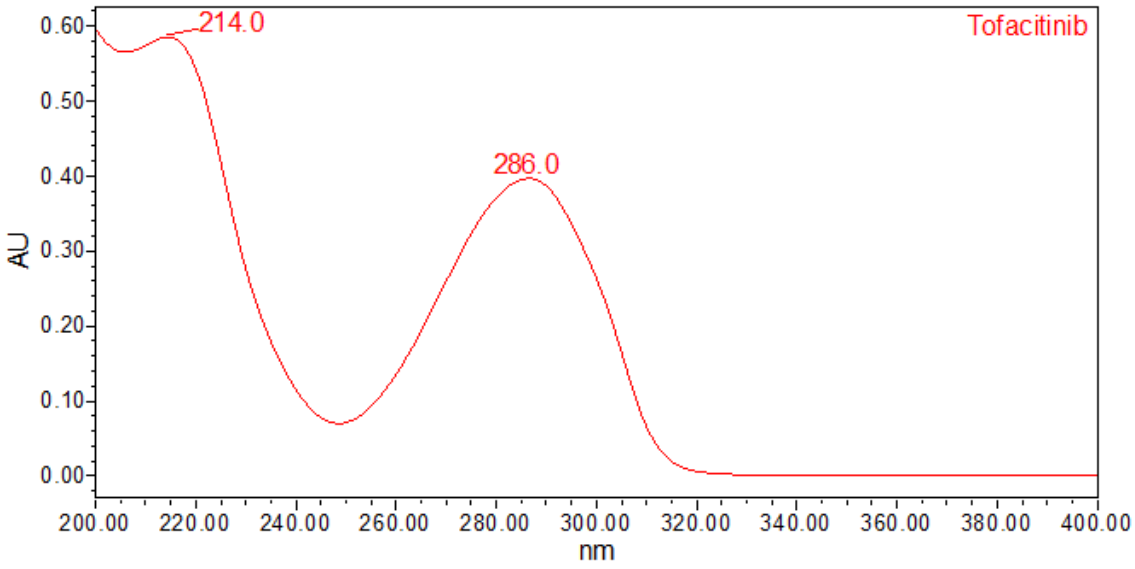

Figure 5. UV spectrum of tofacitinib from the *Standard solution*.

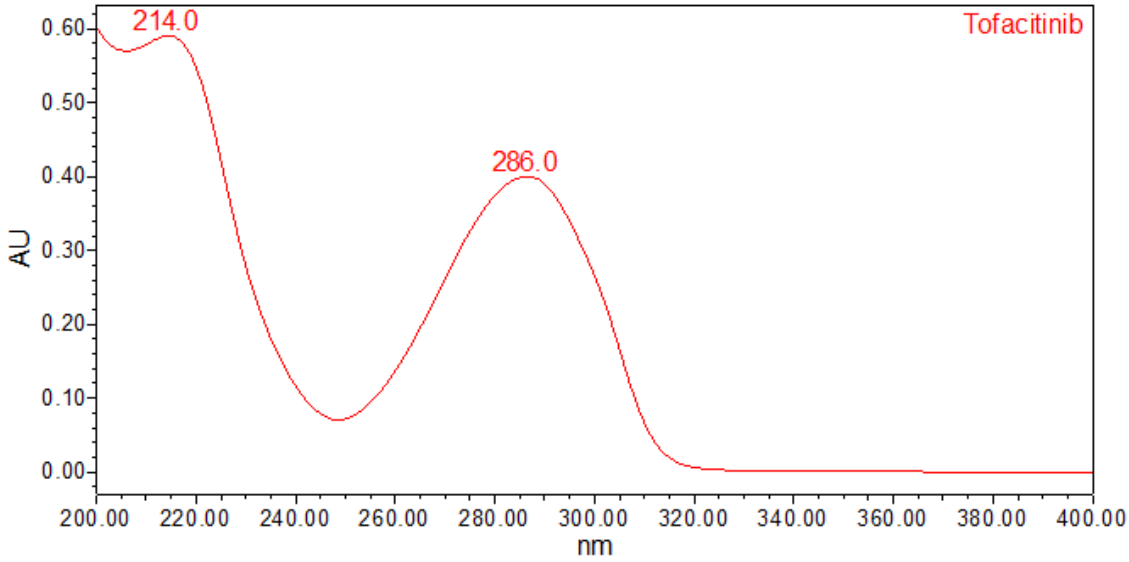

Figure 6. UV spectrum of tofacitinib from the *Sample solution*.

B. <u>Retention Time Match:</u>

The chromatographic retention time is used as an identification method. The HPLC assay procedure was utilized for this identification test. Refer to the *Assay* section for the method conditions and solution preparations.

The validation parameter and results are summarized in **Table 4**, and the example chromatograms for the *Standard solution* and *Sample solution* are shown in **Figures 7** and **8**, respectively.

Table 4. Summary of Validation Experiment, Samples, and the Results for Retention Time Match

Parameter	Samples	Results
Retention Time Match	*Standard solution* and *Sample solution*	The relative standard deviation (%RSD) of the tofacitinib peak retention time for all injections of the *Standard solution* and *Sample solution* was <1.0.

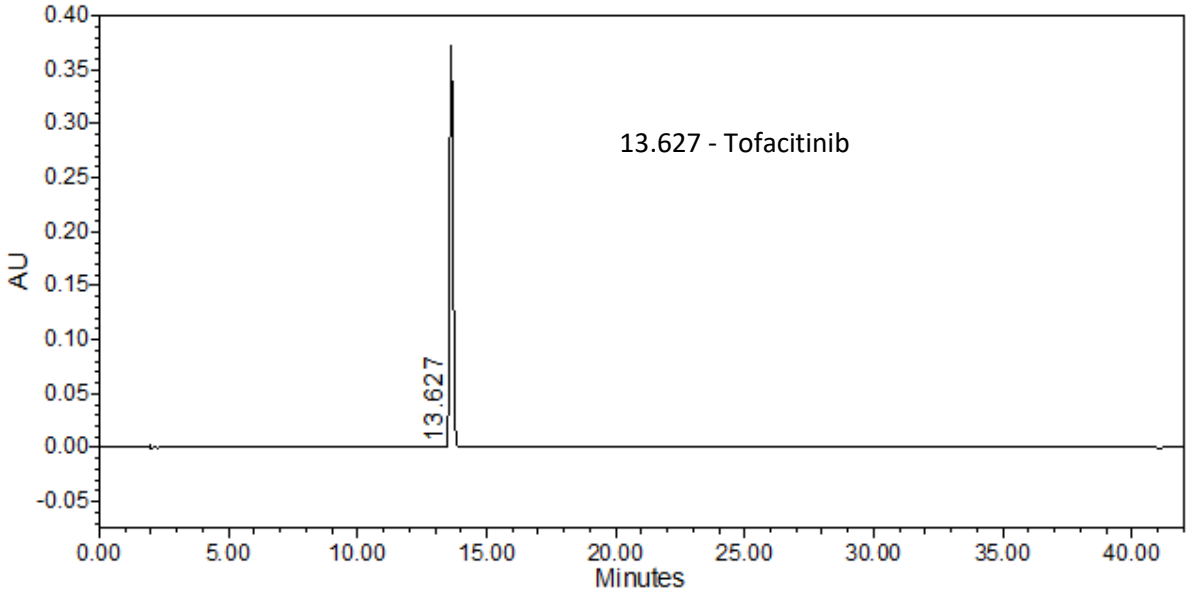

Figure 7. Chromatogram of *Standard solution* using HPLC assay procedure

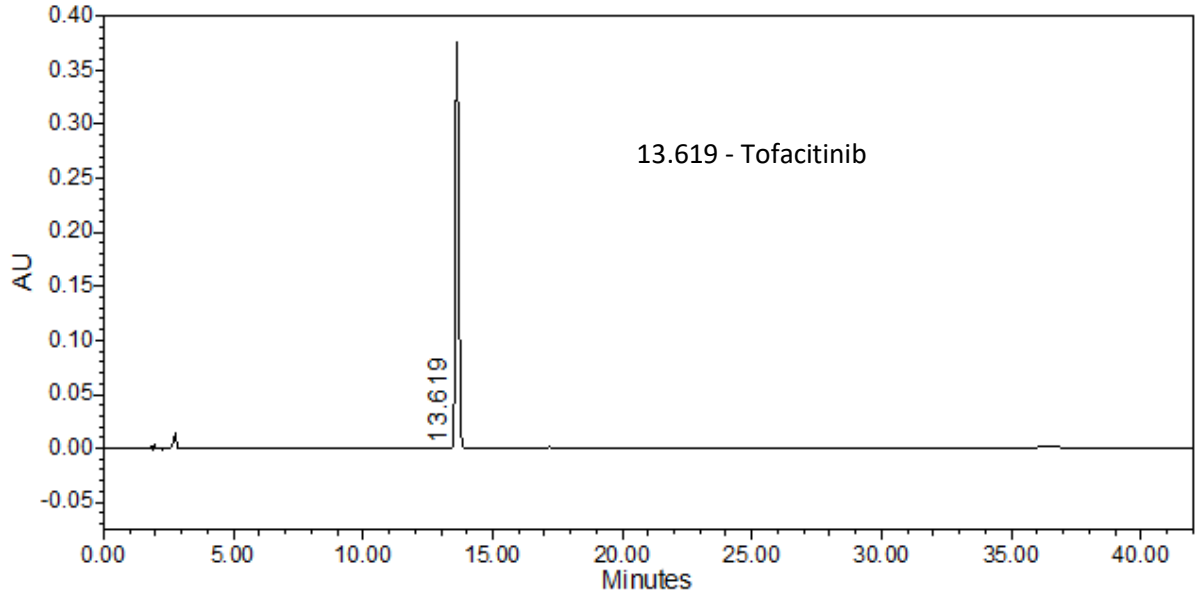

Figure 8. Chromatogram of *Sample solution* using HPLC assay procedure

ASSAY

A gradient reversed-phase HPLC procedure was developed for the assay of Tofacitinib Oral Solution. The procedure was validated using the criteria described in USP General Chapter <1225> *Validation of Compendial Procedures*[2] and found to be specific, linear, accurate, precise, robust, and free from interference for the sample evaluated.

Chemicals:

Potassium phosphate monobasic (ACS grade), potassium hydroxide (ACS grade), acetonitrile (Optima LC/MS grade), methanol (Optima LC/MS grade), and sodium 1-octanesulfunoate were obtained from Fisher Chemicals. Ultrapure water was obtained from a Milli-Q water purification system.

Instruments and method:

The analysis of Tofacitinib Oral Solution was performed using Agilent 1260 and Waters Alliance 2695 instruments with PDA detector, and the results were processed using Empower (Waters software). The Waters XBridge BEH Shield RP18, 4.6-mm x 150 mm, 2.5 µm column was used for analysis. The analysis was performed at 45°C, with a flow rate of 0.8 mL/min and 10 µL as the injection volume. Autosampler temperature was kept at 4°C. The PDA detector was set at 200–400 nm wavelength, and the detection of chromatogram was at 280 nm. The separation was achieved by a gradient program as listed in **Table 1**.

Solutions:

1% Potassium hydroxide solution: Dissolve 1.0 g of potassium hydroxide to 100 mL with water.

Solution A: Accurately weigh 5.44 g of potassium phosphate monobasic and 2 g of sodium 1-octanesulfonate monohydrate and transfer to a glass bottle. Add 2000 mL of water and mix well to dissolve solids. Adjust pH to 5.5 with 1% Potassium hydroxide solution.

Solution B: Mix 1800 mL of methanol and 200 mL of acetonitrile.

Diluent: Mix 500 mL of water with 500 mL of methanol.

Impurity stock solution: Accurately weigh and transfer 2 mg each of amide-TOFT and dihydro-TOFT to a 10-mL volumetric flask. Add about 8 mL of *Diluent* and mix well then sonicate for 5 minutes to dissolve solids. Dilute to volume with additional *Diluent* and mix well.

System suitability solution: Accurately weigh 16 mg of tofacitinib citrate standard (equivalent to 10 mg of tofacitinib) and transfer to a 100-mL volumetric flask. Pipette 1000 µL of *Impurity stock solution* into the flask and add about 80 mL of *Diluent*. Mix well then sonicate solution for 5 minutes to dissolve solids. Dilute to volume with *Diluent* and mix well.

Standard solution: Accurately weigh 16 mg of tofacitinib citrate standard (equivalent to 10 mg of tofacitinib) and transfer to a 100-mL volumetric flask. Add about 80 mL of *Diluent* and mix well then sonicate solution for 5 minutes to dissolve solids. Dilute to volume with *Diluent* and mix well.

Sample solution: Nominally 0.1 mg/mL of tofacitinib from Tofacitinib Oral Solution was prepared by pipetting 5 mL of Tofacitinib Oral Solution, equivalent to 5 mg of tofacitinib, to a 50-mL volumetric flask, diluting to volume with *Diluent*, and mixing well.

Analytical parameters and validation:

The system suitability requirements are summarized in **Table 5**. The validation parameters, solutions, and results for Tofacitinib Oral Solution are summarized in **Table 6**. The example chromatograms for the *Assay Standard solution* and *Sample solution* are shown in **Figures 7** and **8**, respectively.

Table 5. Summary of System Suitability Parameters and Results for the Assay

Parameter	Solutions	Results
Retention time of tofacitinib	*Standard solution*	13.6 min
Tailing factor	*Standard solution*	1.2
Resolution between: Amide-TOFT and Dihydro-TOFT Dihydro-TOFT and Tofacitinib	*System suitability solution*	 3.3 6.3
System Precision (for 5 replicate injections)	*Standard solution*	0.1%

Abbreviation: RSD, relative standard deviation; NMT, not more than; NLT, not less than

Table 6. Summary of Validation Parameters and Results for the Assay

Parameter	Solutions	Results
Specificity (Chromatographic Separation) **Peak Purity Analysis**	*Diluent*, *Standard solution*, and *Sample solution*	Any peak from the *Standard solution* and *Sample solution* was separated from the tofacitinib peak by a resolution ≥ 2.0.

(Spectral Homogeneity)		The PDA data from 200–400 nm showed homogeneity of UV spectrum for the tofacitinib peak, indicating the lack of coelution.
Linearity	Linearity solutions from 50% to 150% of the nominal concentration (0.05, 0.075, 0.1, 0.125, and 0.15 mg/mL of tofacitinib)	The correlation coefficient (r) was not less than 0.999. The bias of the linearity curve due to the intercept not being zero was within ±2.0%.
Accuracy	Accuracy solutions from 110–130% of the nominal concentration prepared in triplicate: 110% (0.11 mg/mL), n=3 120% (0.12 mg/mL), n=3 130% (0.13 mg/mL), n=3	The average recovery at each level was within 100 ± 3.0%.
Repeatability	Repeatability solutions*:* 6 *Sample solutions*	The %RSD of assay results was NMT 2.0 (n=6).
Intermediate Precision	Intermediate precision was done by a different analyst, on a different day by using a different instrument and different column lot number.	The %RSD of assay results was NMT 2.0 (n=6). The %RSD of assay results was NMT 3.0 for the combined data of the first and second analysts (n=12).
Solution Stability	*Standard solution* and *Sample solution*	*Standard solution* and *Sample solution* were stable for 24 hours at a sample cooler temperature of 4°C.
Sample Assay Test	*Sample solution*	98.0% (for 1 mg/mL of tofacitinib in Tofacitinib Oral Solution) for the one source of the drug product tested.

ORGANIC IMPURITIES

The HPLC method used for the analysis of the organic impurities is the same procedure described in the *Assay* section. The method can be used to quantitate degradants in Tofacitinib Oral Solution as well as potential process impurities. The procedure was validated using the criteria described in USP General

Chapter <1225>[2] and found to be specific, linear, accurate, precise, robust and free from interference for the samples evaluated. The validation study showed that the method was suitable for the evaluation of the organic impurities in Tofacitinib drug product.

Solutions:

1% Potassium hydroxide solution, Solution A, *Solution B*, *Diluent*, and chromatographic conditions were prepared and followed as per the *Assay* procedure.

Impurity stock solutions: Accurately weigh and transfer 5 mg each of each impurity material (7-deazaadenine, amide-TOFT, dihydro-TOFT, descyanoacetyl-TOFT, and benzyl-TOFT) except for chloro-TOFT to individual 25-mL volumetric flasks. Add about 20 mL of *Diluent* and mix well then sonicate for 5 minutes to dissolve solids. Dilute to volume with additional *Diluent* and mix well.

Chloro-TOFT stock solution 1: Accurately weigh 5 mg of chloro-TOFT and transfer to a 25-mL volumetric flask. Add about 20 mL of methanol and mix well then sonicated for 5 minutes to dissolve solids. Dilute to volume with additional methanol and mix well.

Chloro-TOFT stock solution 2: Pipette 5 mL of *Chloro-TOFT stock solution 1* and 5 mL of water into a flask. Mix well.

Tofacitinib stock solution 1: Accurately weigh 6.5 mg of tofacitinib citrate standard (equivalent to 4 mg of tofacitinib) and transfer to a 20-mL volumetric flask. Add about 16 mL of *Diluent* and mix well then sonicate solution for 5 minutes to dissolve solids. Dilute to volume with *Diluent* and mix well.

Tofacitinib stock solution 2: Pipette 0.5 mL of *Tofacitinib stock solution 1* into a 20-mL volumetric flask. Dilute to volume with *Diluent* and mix well.

2.5% Impurity solution: Pipette 2500 µL of *Chloro-TOFT stock solution 2* and 1250 µL of each other *Impurity stock solution* into a 50-mL volumetric flask and dilute to volume with *Diluent*. Mix well.

Preparation of System suitability solution: Accurately weigh 6.5 mg of tofacitinib citrate standard (equivalent to 4 mg of tofacitinib) and transfer to a 20-mL volumetric flask. Pipette 8 mL of *2.5% Impurity solution* into the flask and add about 8 mL of *Diluent*. Mix well then sonicate solution for 5 minutes to dissolve solids. Dilute to volume with *Diluent* and mix well.

Preparation of Sensitivity solution (prepared as a Standard solution at 0.1% nominal sample concentration): A solution containing 0.2 µg/mL of tofacitinib, and each impurity material was prepared by pipetting 60 µL each of *Tofacitinib stock solution 2* and *2.5% Impurity solution* and 1380 µL of *Diluent* to an HPLC vial and mixing well.

Preparation of Sample solution: Nominally 0.2 mg/mL of tofacitinib from Tofacitinib Oral Solution was prepared as by pipetting 2 mL of Tofacitinib Oral Solution, equivalent to 2 mg of tofacitinib, into a 10-mL volumetric flask, diluting to volume with *Diluent*, and mixing well.

Analytical parameters and validation:

The method chromatographic conditions were validated by evaluating specificity, linearity, accuracy, repeatability, intermediate precision, and sample analysis as described in USP General Chapter <1225>[2]. Linearity was established for tofacitinib and related impurities, whereas accuracy and repeatability were established for tofacitinib-related impurities.

The limit of quantitation (LOQ) was established at 0.10% of sample concentration. The system suitability requirements are summarized in **Table 7**. The validation parameters and testing results are summarized in **Table 8**. The relative response factors calculated from the linearity study are presented in **Table 9**. The example chromatograms of *Diluent*, *System suitability solution*, *Sensitivity solution*, *Sample solution*, and *Sample solution* with spiked impurities (LOQ level) are presented in **Figures 9–13**, respectively.

Table 7. Summary of System Suitability Parameters and Results for Organic Impurities Test of Tofacitinib Oral Solution

Parameter	Solution	Results
Resolution Resolution between: Amide-TOFT and Dihydro-TOFT Dihydro-TOFT and Tofacitinib	*System suitability solution*	 3.3 5.8 (see **Figure 10**)
Retention Time Tofacitinib	*Sensitivity solution*	14.0 min
Relative Retention Time 7-Deazaadenine Amide-TOFT Dihydro-TOFT Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	*Sensitivity solution* and *System suitability solution*	 0.3 0.8 0.9 1.3 1.6 2.2 (See **Figure 10** and **11**)
System Precision **(%RSD of 6 replicate injections)** 7-Deazaadenine Amide-TOFT Dihydro-TOFT Tofacitinib Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	*Sensitivity solution*	 0.5% 1.0% 0.9% 0.6% 0.7% 1.4% 2.2%

USP Signal-to-Noise Ratio 7-Deazaadenine Amide-TOFT Dihydro-TOFT Tofacitinib Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	*Sensitivity solution*	 > 433 > 110 > 64 > 33 > 110 > 65 > 75

Table 8. Summary of Validation Parameters and Results for Organic Impurities Test of Tofacitinib Oral Solution

Parameter	Solutions	Results
Specificity	Blank (*Diluent*), *System suitability solution*, *Sensitivity solution*, *Sample solution*, and spiked *Sample solution*	No interference with peaks of interest. Any peak ≥0.1% total area was separated from the tofacitinib peak by a resolution of ≥2.0, and from adjacent specified impurity peaks by a resolution of ≥1.5.
LOQ (0.10%) 7-Deazaadenine Amide-TOFT Dihydro-TOFT Tofacitinib Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	*Sensitivity solution* and accuracy solution spiked with impurities at the LOQ level. Experimentally determined using signal-to-noise values, and meeting accuracy, and repeatability criteria at that concentration.	All concentration values met a signal-to-noise criterion of ≥ 10 (refer to **Table 7**). See below for accuracy and repeatability.
Linearity 7-Deazaadenine Amide-TOFT Dihydro-TOFT Tofacitinib Descyanoacetyl-TOFT Chloro-TOFT	Linearity solutions From LOQ (0.10%) to 1.0% of the nominal concentration of tofacitinib	The correlation coefficients of the linear curves for tofacitinib and the impurities were not less than 0.99.

Benzyl-TOFT		
Relative Response Factor (RRF) Values	Linearity solutions From LOQ (0.10%) to 1.0% of the nominal concentration of tofacitinib	For results, refer to **Table 9**.
Accuracy 7-Deazaadenine Amide-TOFT Dihydro-TOFT Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	Accuracy solutions: impurities spiked in *Sample solution* at 3 levels: LOQ (0.10%): n=6 0.50%: n=3 1.0%: n=3	The average recovery for each specified impurity at each level were observed to be within: 0.10%: 100 ± 20.0% 0.50%: 100 ±10.0% 1.0%: 100 ± 5.0%
Repeatability 7-Deazaadenine Amide-TOFT Dihydro-TOFT Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	Repeatability solutions: 6 spiked *Sample solutions* at the LOQ level	The %RSD of the recovery was ≤ 10.0 (n=6). See **Figure 12** for example chromatogram.
Intermediate Precision 7-Deazaadenine Amide-TOFT Dihydro-TOFT Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	Repeatability solutions: 6 spiked *Sample solutions* at the LOQ level, prepared and evaluated by a different analyst on a different day, using a different instrument and different column serial number	The average recovery at LOQ was within 100 ± 20.0% (for second analyst) %RSD of the 6 results at LOQ was ≤ 10.0 (for second analyst). %RSD of the 12 results at LOQ from both analysts was ≤ 15.0.
Sample Analysis	*Sample solution*	See **Figure 13.**
Solution Stability 7-Deazaadenine Amide-TOFT Dihydro-TOFT Tofacitinib Descyanoacetyl-TOFT Chloro-TOFT Benzyl-TOFT	*Sensitivity solution* and spiked *Sample solution* at LOQ level freshly prepared and analyzed periodically for over 24 hours while stored at 4°C in the autosampler.	Observed changes in the peak area for each impurity and tofacitinib from both solutions were within ± 10% of the initial values.

Table 9. Relative Response Factor of Tofacitinib and Related Impurities

Impurity Name	RRF
7-Deazaadenine	1.36
Amide-TOFT	0.88
Dihydro-TOFT	0.60
Descyanoacetyl-TOFT	1.15
Chloro-TOFT	0.85
Benzyl-TOFT	0.81
Tofacitinib	1.00

RRF values of the impurities were calculated by dividing the slope of the linearity curve for each impurity by the slope of the linearity curve for tofacitinib.

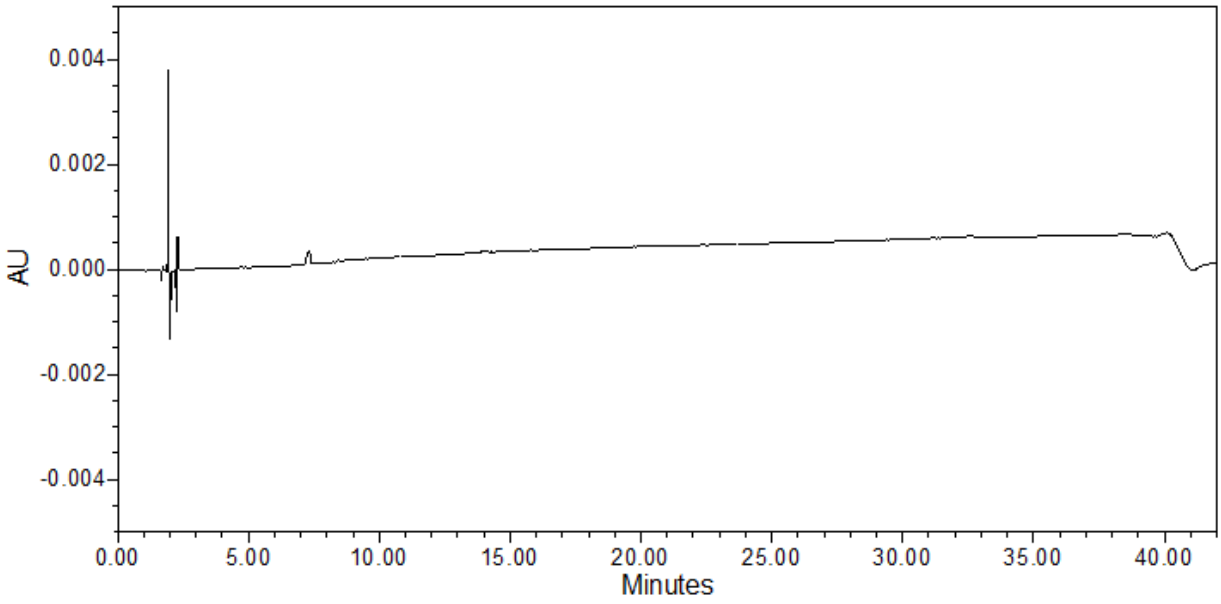

Figure 9. Chromatogram of *Diluent* (blank).

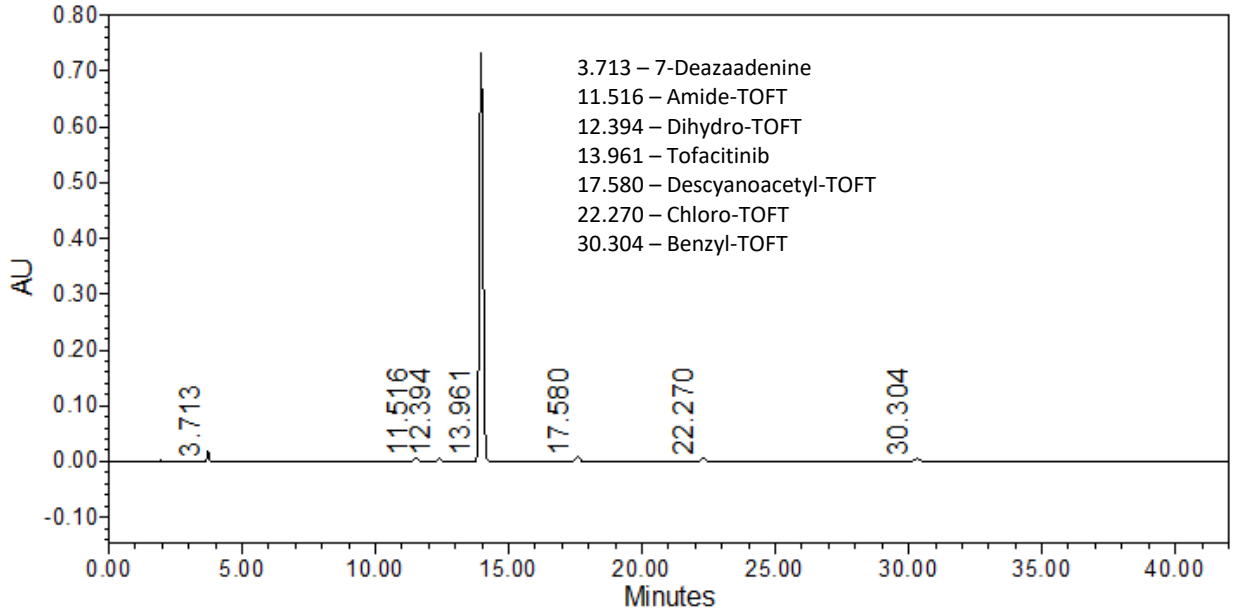

Figure 10. Chromatogram of *System suitability solution*.

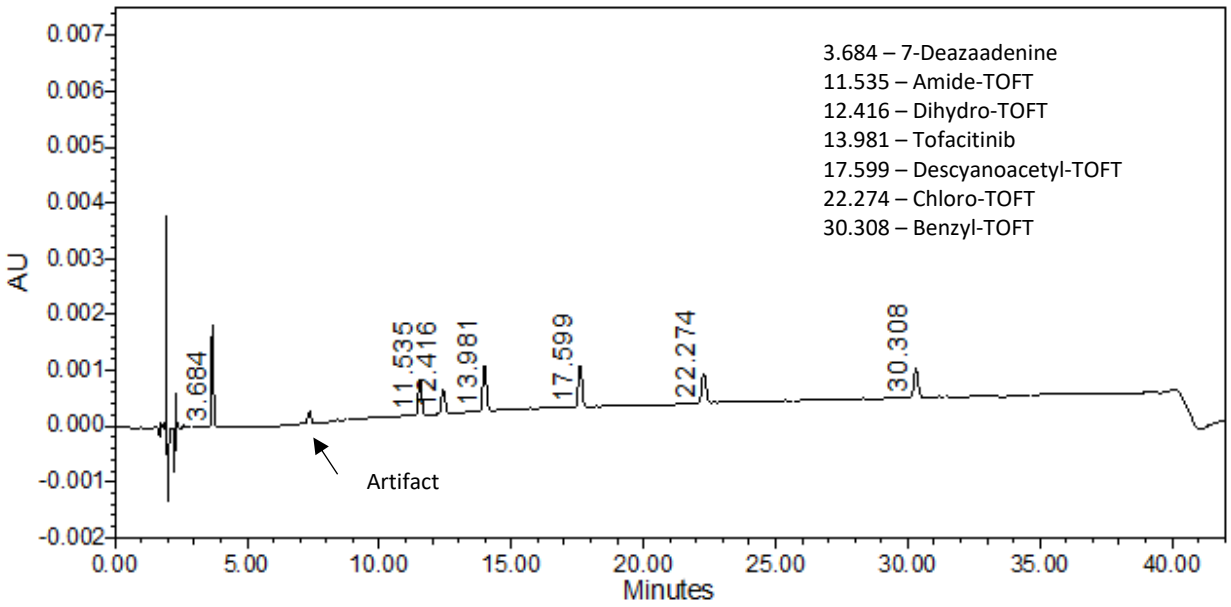

Figure 11. *Sensitivity solution* at LOQ level (0.10%).

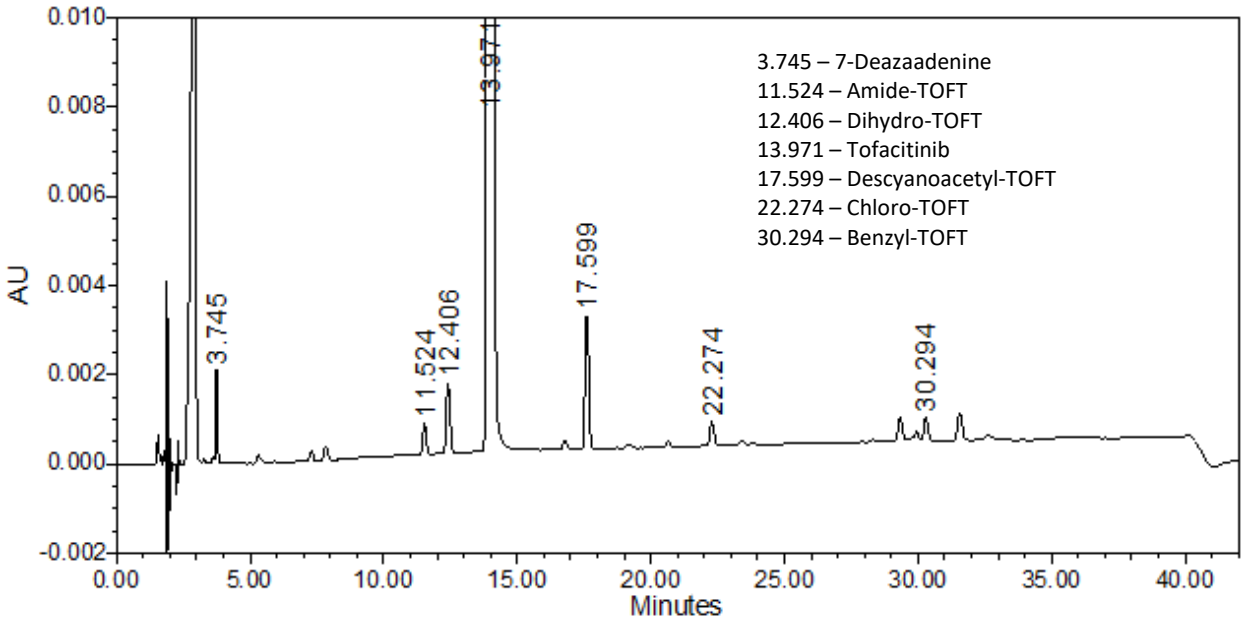

Figure 12. *Sample solution* spiked with impurities at LOQ level (0.10%).

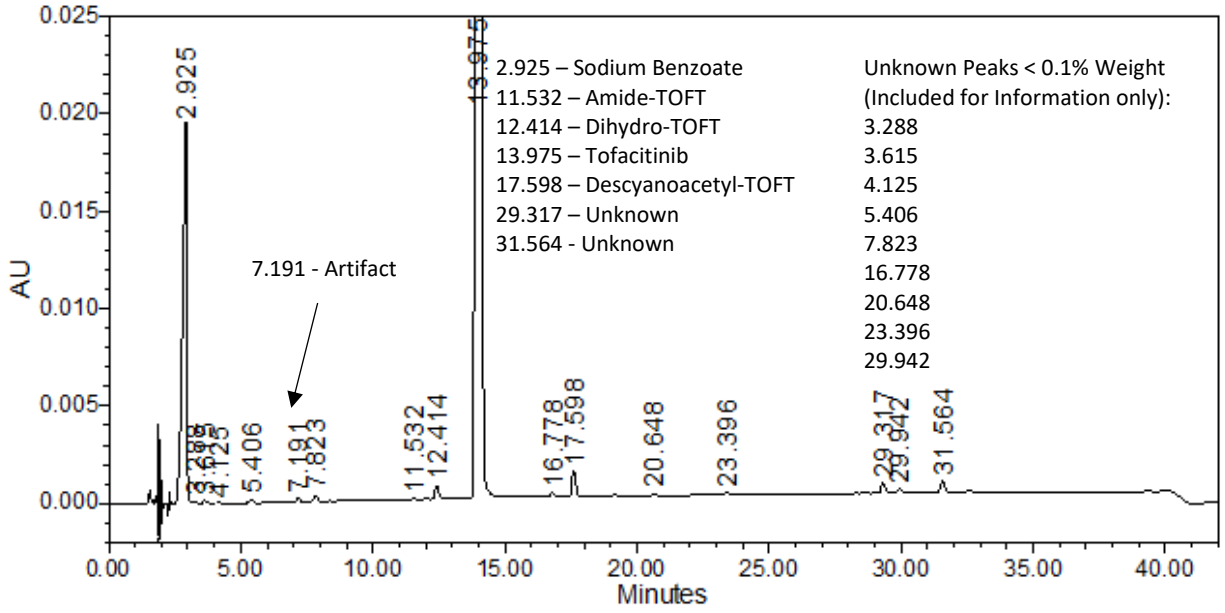

Figure 12. Chromatogram of *Sample solution*.

REFERENCE

1. National Library of Medicine. Tofacitinib MedlinePlus. February 15, 2022. https://medlineplus.gov/druginfo/meds/a613025.html
2. United States Pharmacopeia. Validation of Compendial Procedures <1225>. DOI: https://doi.org/10.31003/USPNF_M99945_04_01