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RP-HPLC Method Development and Validation for Cleaning Residue Determination of Tofacitinib Citrate in Tofacitinib Tablets

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

A novel, Specific, and precise RP-HPLC method was developed to determine the residue content of Tofacitinib citrate left on the surface of equipment used in the manufacturing process. The manufacturing equipment considered in assessment of cleaning has been verified and found the tools assembled to the equipment are made up of Stainless steel, Glass, Teflon and plastic. Hence, these surfaces of manufacturing equipment that come in contact with the drug product during manufacturing are considered for evaluation of the cleaning procedure. By developing and validating an analytical method for residue estimation, the manufacturing equipment can be evaluated for efficient cleaning and to release the manufacturing equipment for further intended use by minimizing the cross contaminations. The stationary phase suited for the well separation of components is CAPCELL PAK C18 150 x 4.6 mm, 3 µm; 0.4 % perchloric acid and acetonitrile in the ratio of 85:15 % v/v is the mobile phase pumped at a flow rate of 1.2 mL/min through the column at temperature of 40 ºC. Each run extended for 10 min as the Tofacitinib peak elutes at RT of 5.2 min. The method has been validated successfully for Specificity, Precision, Linearity, Accuracy, Ruggedness and Filter validation of both rinse and swab methods. The LOD, LOQ concentrations found to be 0.006, 0.019 µg/mL for swab method and 0.03 and 0.1 µg/mL for rinse method respectively. The correlation coefficient is 0.999 and method found linear from LOQ to

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500% for swab method and LOQ to 200% for rinse method. Solution stability has been established to ensure the test solution get tested within the stable time (4 Days). Based on the filter validation data, it is concluded that PVDF filter is not suitable for cleaning sample analysis and 2 mL sample should be discarded when 0.45 µm Nylon filter is used for cleaning sample analysis.

Keywords: RP-HPLC method; Tofacitinib; analytical method; rheumatoid arthritis; ulcerative colitis.

1. INTRODUCTION

Tofacitinib sold under the brand name "Xeljanz" is an inhibitor of the enzyme Janus kinase 1 (JAK1) and Janus kinase 3 (JAK 3), which means that it interferes with the JAK-STAT signaling pathway, which transmits extracellular information into the cell nucleus, influencing DNA transcription. Tofacitinib Tablets are used to treat rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis. Tofacitinib is chemically known as 3-((3R,4R)-4-methyl-3-(methyl (7H-pyrrolo [2,3-d]pyrimidin-4-yl) amino)piperidin-1-yl)-3-oxopropanenitrile [1,2,3,4]. The structure of Tofacitinib, as depicted below –.

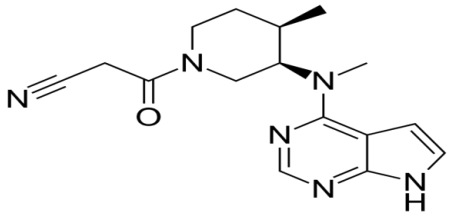

Fig. 1. Chemical structure of Tofacitinib citrate

Tofacitinib film coated tablets sold under the brand name "Xeljanz" were formulated as oral tablets having Tofacitinib citrate as an API, which is the target component to assess the particulate containment performance of manufacturing equipment in formulation area for cleaning validation and the residue acceptability limit (RAL), which are 0.38 ppm for swab method and 10 ppm for rinse method.

Solubility evaluation: Based on the solubility criterion mentioned below, Tofacitinib has been evaluated with 1gm of sample in 10000 mL of water and found that the material was dissolved and fall under category of very slightly soluble in water [5,6].

The manufacturing process of Tofacitinib API has been reviewed and found that the below solvents are part of process and the specification limits have been established in line with ICH guidelines (ICH Q3C) [7].

Based on the manufacturing process assessment solubility of the material has verified in Ethanol and found that the material is very slightly soluble in Ethanol. To identify the solubility of the material the other organic solvents has also verified and found that the material is soluble in N, N-Dimethylacetamide. Consideration of N, N-Dimethylacetamide as cleaning agent could be a challenge as it become an additional impurity to estimate the absence of the solvent in drug product and on equipment.

Chart 1. Solubility criteria as per USP

Descriptive term	Part of solvent required per part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	10,000 and over

Chart 2. Solvent specifications

Solvent Name	Specification
Ethanol	Not more than 5000
Acetaldehyde	Not more than 25

Cleaning validation Approach: Cleaning validation is performed for entire equipment line used in manufacturing of Tofacitinib Tablets. Swab and rinse sampling methods are considered for Cleaning Effectiveness. Based on the solubility criteria, toxicity, potency of the drug, the MACO values are established. This determines the acceptance criteria limits for product residue [8,9,10,11].

$$MACO = \frac{ADEorPDEprevious \times MBS\ (next)}{TDD}$$

Where, MACO [12,13] = Maximum allowable carryover i.e. Acceptable transferred amount in the next product (mg)
ADE = Acceptable Daily Exposure (mg/day)
PDE = Permitted Daily Exposure(mg/day)
MBS= Minimum Batch size for next product (mg)
TDD= Therapeutic Daily Dose for next product (mg/day)

For Swab samples, the residual limits are established by below calculation:

$$\frac{MACO \times RF \times 1000 \times SA}{TSA \times DV}$$

Where, Rf = Recovery factor
1000 is conversion factor into ppm
SA = Swabbed area of the total equipment in cm²
TSA= Total surface area of the equipment in cm²
DV= Disorbent volume of solvent used for dipping and squeezed of swab sample in mL

For Rinse sample, the acceptance criteria are calculated by

$$\frac{\mathrm{MACO} \times \mathrm{Rf} \times 1000}{V}$$

Where, V= volume of the solvent used in final rinse in mL.

A cleaning method has been adopted to clean the residues of Tofacitinib from manufacturing contact surfaces of equipment to meet the established residual limits. To analyze the cleaning samples for determination of residue content the particular analytical method has been developed and validated.

Selection of Cleaning Solvent: Based on the solubility profile of Tofacitinib, cleaning solvent has been selected. Tofacitinib is very slightly soluble in water (0.01g in 10mL of water). As the equipment is to be cleaned of drug product to eliminate the residue of previous product manufactured in the same equipment, water have been selected to clean the equipment [14].

2. SWABBING AND RINSING

2.1 Swabbing

Lint free swabs shall be used for performing the swab sampling. Swab stick shall allow to extract the compound from the surface and shall not release fibres or any contamination.

Swab the 5 cm x 5 cm (25 cm^2) surface of the equipment for chemical residue as per the following swabbing pattern in different locations as per the sampling plan.

2.2 Rinse

Rinse sampling shall be performed for where surfaces are inaccessible, swabbing is impractical, closed equipment/part and residues are soluble in the rinse solvent.

3. METHODS AND MATERIALS

3.1 Methods

The analytical method development and validation has been performed using Liquid chromatography equipped with UV and PDA detectors operated through software Empower 3. CAPCELL PAK C18 4.6 x 150 mm, 3µm HPLC column has been used as suitable stationary phase. The flow rate was kept at 1.2 mL/min to reduce the runtime of method to 10 minutes. The column temperature was maintained at 40ºC to get early elution of analyte and sample cooler temperature at 10 ºC. Texwipe swabs with part number TX761D have been selected as swab sticks for sample collection as the risk of fibers interference is absent.

Table 1. Based on the above formulae, the MACO value has been calculated and is as follows

Sampling Method	MACO Value	Concentration
Swab	161.35 mg	0.38 ppm
Rinse		10 ppm

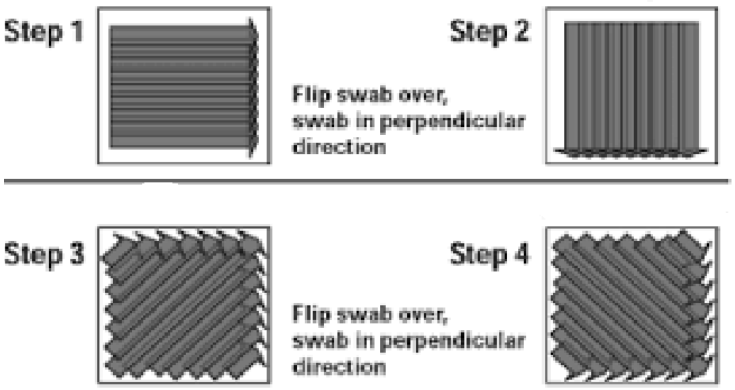

Fig. 2. Swabbing technique

Mobile phase included 0.4% Perchloric acid and Acetonitrile in the ratio of 85: 15 (%, v/v). The injection volume is 40 µL, the method was optimized at 289 nm as the maximum absorbance for Tofacitinib is at 289nm [15].

3.2 Materials

Tofacitinib standard and Placebo.

Sample Extraction Solvent for Swab Method: Same as mobile phase (0.4% Perchloric acid: Acetonitrile = 85: 15 (%, v/v)).

Swab Solvent: Mixed Ethanol and water (50/50; v/v).

Tofacitinib Standard Stock Solution: Weighed about 7.6 mg of Tofacitinib working standard (or reference standard) into a 200mL volumetric flask, added 140 mL of 90% Acetonitrile, sonicated with shaking for 5 minutes and diluted to volume with Acetonitrile. (The concentration is about 38 µg/mL of Tofacitinib).

Tofacitinib Standard Solution: Pipetted 1 mL of Tofacitinib Standard Stock Solution (38 µg/mL) into a 100-mL volumetric flask and diluted to volume with extraction solvent, mixed well. Filtered a portion of the solution through a 0.45 µm Nylon filter, discarded the first 2 mL of filtrate and collected. (The concentration is about 0.38 µg/mL of Tofacitinib).

Placebo stock solution for Swab Method: Weighed about 79.4 mg of Common placebo powder of Tofacitinib film coated tablets, 5 mg and 10 mg into a 100-mL volumetric flask. Added 50 mL of 90% Acetonitrile and sonicated for 5 minutes, mixed well and diluted to the volume with 90% Acetonitrile. Mixed well. (P1 solution). (Equivalent to 794 µg/mL of placebo).

Placebo solution for Swab Method: Pipetted 10 mL of P1 solution into a 100-mL volumetric flask and diluted to volume with extraction solvent and mixed well. Filtered a portion of the solution through a 0.45 µm Nylon filter, discarded the first 2 mL of filtrate and collected. (P2 solution) (Equivalent to 79.4 µg/mL of placebo).

Blank solution for swab: Rinsed a clean swab with swab solvent (Ethanol and water (50/50; % v/v) and put the swab into a 15-mL centrifuge tube. Pipetted 10 mL of sample extraction solvent (0.4% Perchloric acid: Acetonitrile = 85: 15 (%, v/v)) into the centrifuge tube. Vortexed for 15 seconds. Filtered a portion of the solution through a 0.45 µm Nylon filter, discarded the first 2 mL of filtrate and collected as Solution C.

Blank solutions for swab templates: Rinsed a clean swab with 0.5 mL of swab solvent (Ethanol and water (50/50; % v/v)) and swabbed the surface of stainless-steel template on the area size of 5 cm × 5 cm template plate. Then put the swab back to the centrifuge tube, Pipetted 10 mL of sample extraction solvent (0.4% Perchloric acid: Acetonitrile = 85: 15 (%, v/v)) into the tube and vortex for 15 seconds. Filtered a portion of the solution through a 0.45 µm Nylon filter, discarded the first 2 mL of filtrate and collected as Stainless steel swab blank.

Similarly, swab blanks for Plastic, Glass and Teflon have been prepared using the respective surfaces.

3.3 Solution Preparations for Rinse Methods

Sample Extraction Solvent for Rinse Method: Purified Water

Tofacitinib Standard Stock Solution: Weighed about 20.0 mg of Tofacitinib working standard into a 20mL volumetric flask, added 14 mL of 90% Acetonitrile, sonicated and shook for 5 minutes and diluted to volume with 90% Acetonitrile. (The concentration is about 1000 µg/mL of Tofacitinib).

Tofacitinib standard solution: Pipetted 1 mL of Tofacitinib Standard Stock Solution (1000 µg/mL) into a 100-mL volumetric flask and diluted to volume with purified water, mixed well. Filtered a portion of the solution through a 0.45 µm Nylon filter, discarded the first 2 mL of filtrate and collected (The concentration is about 10 µg/mL of Tofacitinib).

Placebo stock solution for Rinse Method: Weighed about 100.0 mg of Common placebo powder of Tofacitinib tablet into a 100-mL volumetric flask. Added 50 mL of 90% Acetonitrile and sonicated for 5 minutes, mixed well and diluted to the volume with 90% Acetonitrile. Mixed well. (P3 solution). (Equivalent to 1 mg/mL of placebo).

Placebo solution for rinse Method: Pipetted 10 mL of P3 solution into a 100-mL volumetric flask and diluted to volume with purified water and mixed well. Filtered a portion of the solution through a 0.45 µm Nylon filter, discarded the first 2 mL of filtrate and collected. (Equivalent to 100 µg/mL of placebo) (P4 solution).

Blank solution for rinse templates: Rinsed the stainless-steel template (5 cm × 5 cm) by 20 mL of purified water and collected the rinse solution into a 50-mL centrifuge tube. Made up to 20 mL with purified water and mixed well. Filtered a portion of the solution through a 0.45 µm Nylon filter, discarded the first 2 mL of filtrate and collected as Solution E1.
Similarly, rinse blanks for Plastic, Glass, Silicon and Teflon have been prepared using the respective surfaces.

4. RESULTS AND DISCUSSION

4.1 System Suitability

4.1.1 For swab method

System suitability test was performed by injecting the standard solution at target concentration 0.38µg/mL. The results are tabulated in below table.

4.1.2 For Rinse method

System suitability test was performed by injecting the standard solution at target concentration 0.38µg/mL. The results are tabulated in below table.

Table 2. System suitability results

Area % RSD (≤10.0)	USP Tailing (NMT 2.0)	USP Column efficiency (NMT 3000)
5.5	1.1	6665

Table 3. System suitability results

USP Tailing (NMT 2.0)	USP Column efficiency (NMT 3000)
1.0	8424

Table 4. % RSD results

S.No.	Injection No.	Area
1.	Injection No-1	558990
2.	Injection No-2	560444
3.	Injection No-3	561788
4.	Injection No-4	560124
5.	Injection No-5	558612
6.	Injection No-6	565955
Average		560986
% RSD		0.5

Table 5. Swab method

Name of solution	RT (min)	Purity Angle	Purity Threshold
Blank	ND	NA	NA
Standard Solution	5.30	0.525	3.253
Mobile Phase	ND	NA	NA
Placebo solution	ND	NA	NA
Swab Blank	ND	NA	NA
Solution D1	ND	NA	NA
Solution D2	ND	NA	NA
Solution D3	ND	NA	NA
Solution D4	ND	NA	NA

Table 6. Rinse method

Name of solution	RT(min)	Purity Angle	Purity Threshold
Purified Water	ND	NA	NA
Standard	5.23	0.077	0.259
Placebo solution	ND	NA	NA
Solution E1	ND	NA	NA
Solution E2	ND	NA	NA
Solution E3	ND	NA	NA
Solution E4	ND	NA	NA
Solution E5	ND	NA	NA

4.2 Specificity

4.2.1 For swab method

Specificity of the method has been established by injecting the placebo solution for swab method, mobile phase, Solution C, Solution D1, Solution D2, Solution D3 and Solution D4 and Standard solution (0.38 ppm for swab method) into a chromatographic system [16-21]. Evaluated the Blank interference, Swab interference at Tofacitinib peak and peak purity. Data has been reported in below table.

4.2.2 For rinse method

Specificity of the method has been established by injecting the Placebo solution for rinse method, purified water, Solution E1, Solution E2, Solution E3, Solution E4, Solution E5 and Standard solution (10 ppm) into a chromatographic system. Evaluated the Blank interference, Swab interference of Tofacitinib peak and peak purity. Data has been reported in below table.

4.2.3 Acceptance criteria

1. The chromatograms of Placebo solution, Mobile phase and cleaning surface blanks should have no interference occurred at the retention time of Tofacitinib peak.
2. The purity angle should be less than purity threshold for Tofacitinib peak.

4.3 Limit of quantification (LOQ) and Limit of detection (LOD) [22]

Limit of detection and limit of quantification has been established as per the S/N ratio method by injecting the known concentrated solutions and reported its S/N ratio values. Upon establishment, precision has been proved. Results are tabulated below.

4.4 Linearity

4.4.1 For swab method

The linearity of the detector response for Tofacitinib in swab method has been established by injecting the linearity solutions ranging from LOQ (0.019 µg/mL) to 500% level (1.9 µg/mL) of the nominal concentration of Tofacitinib. These triplicate solutions were injected into the HPLC system and the response of the same was recorded. A plot of concentration vs average analyte peak area was done. The correlation coefficient between concentration and response was evaluated. The results are tabulated below.

Table 7. For swab method

Swab method				
Injection	**Conc (µg/mL)**	**Report level**	**Peak area**	**S/N**
LOD	0.006	1.65%	404	6.59
LOQ				
1	0.019	5%	1198	13.98
2			1186	19.95
3			1238	19.44
4			1247	20.16
5			1136	23.47
6			1218	20.02
% RSD (NMT 15.0%)			3.3	NA
Rinse method				
Injection	**Conc (µg/mL)**	**Report level**	**Peak area**	**S/N**
LOD	0.03	0.3%	2236	33.67
LOQ				
1	0.1	1%	6277	125.06
2			6168	119.77
3			6361	134.17
4			6161	113.80
5			6200	122.73
6			6199	89.51
% RSD (NMT 15.0%)			1.21	NA

Table 8. The correlation coefficient between concentration and response was evaluated

Level	**Conc (µg/mL)**	**Area**	**Average area**
LOQ	0.019	1057	1055
		1053	
		1055	
50%	0.193	10516	10568
		10577	
		10612	
80%	0.309	17070	17110
		17169	
		17092	
100%	0.386	21444	21417
		21398	
		21409	
120%	0.579	32009	32029
		31999	
		32080	
150%	0.773	42984	42981
		42963	
		42995	
200%	1.159	64331	64481
		64652	
		64459	
150%	1.932	108170	108222
		108195	
		108300	
Correlation coefficient (NLT 0.995)			0.999
intercept			-236.86
slope			56029.51
% intercept			-0.73

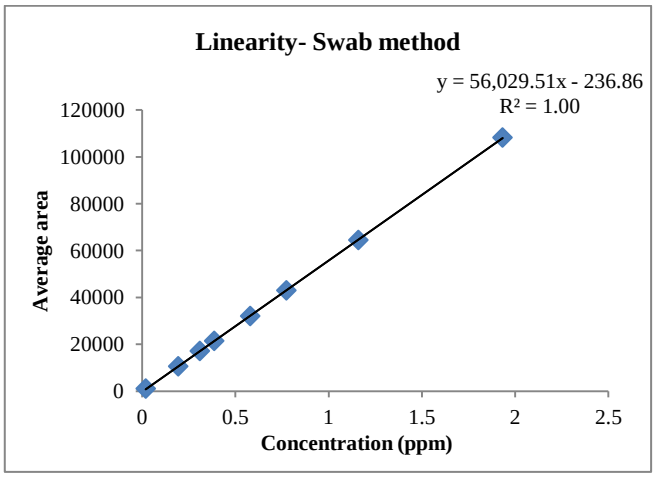

Fig. 3. Linearity Graph for Swab method

Table 9. Response for Tofacitinib in swab method

Level	Conc (mg/mL)	Area	Average area
LOQ	0.1	1152	1133
		1134	
		1113	
20%	2.007	110262	109916
		110222	
		109263	
40%	4.015	225995	226268
		225743	
		227066	
80%	5.019	278129	278315
		278445	
		278370	
100%	10.038	560555	560312
		560254	
		560127	
120%	12.046	670299	673658
		676940	
		673734	
150%	15.057	835726	837246
		838073	
		837938	
200%	20.076	1122351	1122203
		1121392	
		1122867	
Correlation coefficient (NLT 0.995)			0.999
intercept			-2091.08
slope			55966.80
% intercept			-0.37

For Rinse method: The linearity of the detector response for Tofacitinib in swab method has been established by injecting the linearity solutions ranging from LOQ (0.1 µg/mL) to 200% level (20 µg/mL) of the nominal concentration of Tofacitinib. These triplicate solutions were injected into the HPLC system and the response of the same was recorded. A plot of concentration vs average analyte peak area was done. The correlation coefficient between concentration and response was evaluated. The results are tabulated below.

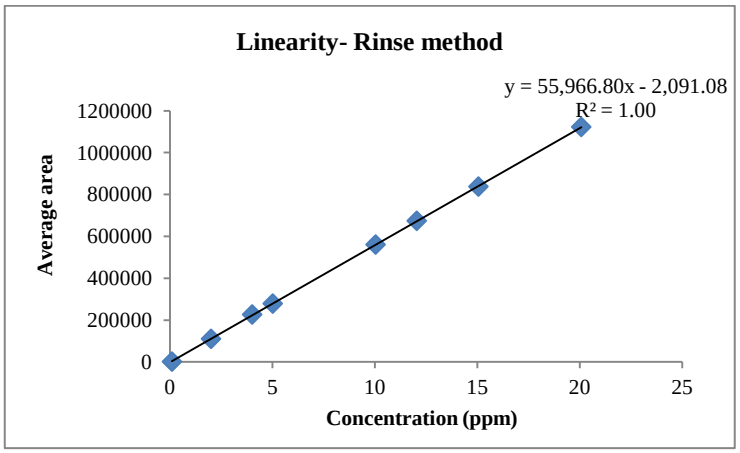

Fig. 4. Linearity Graph for Rinse method

4.5 Method repeatability

4.5.1 For swab method

Method Precision has been established by injecting six spiked solution in each plate (stainless template 316 L, plastic, glass and Teflon) for swab method at the RAL concentration about (0.38 ppm) and calculated the recovery for six spiked samples in all swab templates. Results are tabulated below.

4.5.2 For rinse method

Method Precision has been established by injecting six spiked solutions on each plate (stainless template 316 L, plastic, glass, Teflon and Silicone) for rinse method at the concentration about (10 ppm) and calculated the recovery for six spiked samples in all templates. Results are tabulated below.

4.6 Intermediate Precision

4.6.1 For swab method

Intermediate Precision has been established by injecting six spiked solutions on each plate (stainless template 316 L, plastic, glass and Teflon) for swab method at the RAL concentration about (0.38 ppm) by different analyst, different instrument on different day and calculated the recovery for six spiked samples on all swab templates. Intermediate precision results are tabulated below.

For Rinse Method: Intermediate precision has been established by injecting six spiked solutions on each plate (stainless template 316 L, plastic, glass, Teflon and Silicone) for rinse method at the concentration about (10 ppm) by different analyst, different instrument on different day and calculated the recovery for six spiked samples in all templates. Intermediate precision results are tabulated below.

Accuracy: The method accuracy was validated by a recovery study on spiked sample solutions. The spiked sample solutions were prepared by spiking the Tofacitinib drug substances stock solution and placebo stock solution into the suitable volumetric flask. The spiked sample solutions containing Tofacitinib at 5% (LOQ) 50%, 100%, 150, 200%, 500% levels of the nominal concentration for swab method and 1% (LOQ),50%, 100%,150%,200% levels of nominal concentration of rinse method were analyzed to demonstrate the accuracy. Triplicate sample preparation at each level have been prepared and injected as per the procedure. Calculated the swab recovery results and reported in below table.

4.6.2 Acceptance Criteria

1. % recovery at LOQ level should be in between 80.0 and 120.0.
2. % recovery at other levels should be in between 95.0 and 105.0.

4.6.3 Solution stability

Prepared the standard and spiked sample solutions for both rinse and swab method as per the procedure. Established the solution stability at room temperature for 4 days.

Table 10. Method repeatability

Material	Plate No	% Recovery (NLT 50%)	Average	%RSD (NMT 15.0)
Stainless steel	1	84.07	83.2	1.4
	2	82.24		
	3	83.28		
	4	81.22		
	5	84.07		
	6	84.07		
Plastic	1	74.41	72.2	2.4
	2	71.8		
	3	74.41		
	4	70.75		
	5	71.54		
	6	70.49		
Glass	1	77.54	79.2	1.9
	2	77.8		
	3	79.63		
	4	81.46		
	5	78.59		
	6	80.15		
Teflon	1	79.37	78.4	1.6
	2	77.54		
	3	80.41		
	4	78.06		
	5	77.54		
	6	77.28		

Table 11. Method Precision has been established by injecting six spiked solution

Material	Plate No	% Recovery (NLT 50%)	Average	%RSD (NMT 15.0)
Stainless steel	1	92.55	93.1	1.9
	2	89.46		
	3	90.75		
	4	92.14		
	5	88.88		
	6	93.10		
Plastic	1	84.42	83.5	2.9
	2	79.48		
	3	83.45		
	4	84.50		
	5	82.55		
	6	86.80		
Glass	1	84.08	83.1	2.6
	2	83.93		
	3	86.05		
	4	80.41		
	5	83.31		
	6	80.88		
Teflon	1	91.50	89.2	2.0
	2	87.79		
	3	86.67		
	4	88.82		

Material	Plate No	% Recovery (NLT 50%)	Average	%RSD (NMT 15.0)
	5	90.04		
	6	90.65		
Silicone	1	92.94	91.4	1.7
	2	90.12		
	3	91.28		
	4	92.05		
	5	92.91		
	6	88.98		

Table 12. Intermediate Precision has been established by injecting six spiked solution

Material	Plate No	% Recovery (NLT 50%)	Average	%RSD (NMT 15.0)
Stainless steel	1	78.06	79.1	1.7
	2	80.41		
	3	78.32		
	4	77.54		
	5	80.93		
	6	79.63		
Plastic	1	71.80	72.9	2.3
	2	71.01		
	3	74.93		
	4	72.58		
	5	72.06		
	6	74.93		
Glass	1	81.20	79.9	1.8
	2	78.59		
	3	79.11		
	4	80.15		
	5	78.32		
	6	81.98		
Teflon	1	78.32	79.1	1.9
	2	80.41		
	3	78.32		
	4	77.28		
	5	81.46		
	6	78.85		

Table 13. Worst % recoveries between two different analysts

Material	Set No	%Mean Recovery	Worst % recovery
Stainless steel	1	83.2	79.1
	2	79.1	
Plastic	1	72.2	72.2
	2	72.9	
Glass	1	79.2	79.2
	2	79.9	
Teflon	1	78.4	78.4
	2	79.1	

Table 14. Intermediate precision results

Material	Plate No	% Recovery (NLT 50%)	Average	%RSD (NMT 15.0)
Stainless steel	1	86.75	88.8	2.3
	2	89.93		
	3	89.74		
	4	91.88		
	5	86.82		
	6	87.97		
Plastic	1	80.82	81.6	3.3
	2	81.87		
	3	85.08		
	4	76.99		
	5	82.70		
	6	82.29		
Glass	1	83.51	81.2	2.2
	2	78.58		
	3	82.04		
	4	82.06		
	5	79.65		
	6	81.34		
Teflon	1	86.8	87.1	1.9
	2	85.84		
	3	87.51		
	4	84.83		
	5	87.91		
	6	89.61		
Silicone	1	90.71	89.3	1.6
	2	90.71		
	3	87.02		
	4	88.82		
	5	90.05		
	6	86.75		

Table 15. Worst % recoveries between two different analysts

Material	Set No	%Mean Recovery	Worst % recovery
Stainless steel	1	93.1	88.8
	2	88.8	
Plastic	1	83.5	81.6
	2	81.6	
Glass	1	83.1	81.2
	2	81.2	
Teflon	1	89.2	87.1
	2	87.1	
Silicon	1	91.4	89.3
	2	89.3	

Filter validation: Prepared the standard and spiked sample solutions at specified concentration. Evaluated the recovery for centrifuge solution, Nylon and PVDF filtered solution for all 3 filtrate solutions. Nylon 0.45 µm and PVDF 0.45 µm filters were selected for this study. Each 1mL of filtered solution was collected individually from 1st to 3rd mL to evaluate the discard. Calculated % recovery and reported the results below.

Table 16. Swab recovery results

Level	Added Conc (ppm)	Found Conc (ppm)	Recovery	Avg	%RSD
LOQ	0.019	0.018	94.73	96.48	3.2
		0.018	94.73		
		0.019	100.0		
50%	0.191	0.188	98.42	98.59	0.31
		0.188	98.42		
		0.189	98.95		
100%	0.383	0.385	100.52	100.52	0.0
		0.385	100.52		
		0.385	100.52		
150%	0.575	0.575	100.0	99.99	0.34
		0.573	99.65		
		0.577	100.34		
200%	0.767	0.768	100.13	99.56	0.5
		0.761	99.21		
		0.762	99.34		
500%	1.918	1.895	98.80	99.39	0.53
		1.909	99.53		
		1.915	99.84		

Table 17. Rinse recovery results

Level	Added Conc (ppm)	Found Conc (ppm)	Recovery	Avg	%RSD
LOQ	0.101	0.103	101.98	103.0	0.96
		0.105	103.96		
		0.104	102.97		
50%	5.085	5.129	100.86	100.9	0.32
		5.141	101.10		
		5.108	100.45		
100%	10.17	10.236	100.64	100.8	0.14
		10.253	100.81		
		10.265	100.93		
150%	15.256	15.616	102.35	102.3	0.1
		15.586	102.16		
		15.583	102.14		
200%	20.341	20.605	101.29	101.7	0.3
		20.681	101.67		
		20.729	101.9		

Table 18. Results for swab method

Interval	Std Area	% Diff (NMT 5.0)	Spiked spl area	% Diff (NMT 5.0)
Day-0	22007	NA	22373	NA
Day-1	21977	0.13	22251	0.54
Day-2	21108	4.08	22555	0.81
Day-3	21181	3.75	22628	1.13
Day-4	22146	0.63	21831	2.42

Table 19. Results for Rinse method

Results for rinse material

Interval	Std Area	% Diff (NMT 5.0)	Spiked spl area	% Diff (NMT 5.0)
Day-0	564503	NA	616920	NA
Day-1	586806	3.95	617412	0.07
Day-2	581832	3.06	616617	0.04
Day-3	581758	3.06	616721	0.03
Day-4	588012	4.16	619515	0.42

Table 20. Results for swab method

Standard solution			
Centrifuged solution (%)	**Filtrate solution**	**Nylon filter (%)**	**PVDF filter (%)**
100.08	1st mL	99.92	99.87
	2nd mL	100.05	100.38
	3rd mL	99.73	100.13
Spiked solution			
Centrifuged solution (%)	**Filtrate solution**	**Nylon filter (%)**	**PVDF filter (%)**
99.97	1st mL	98.78	62.35
	2nd mL	79.03	104.15
	3rd mL	95.4	105.17

Table 21. Results for Rinse material

Standard solution			
Centrifuged solution (%)	**Filtrate solution**	**Nylon filter (%)**	**PVDF filter (%)**
99.47	1st mL	100.0	53.28
	2nd mL	100.26	41.46
	3rd mL	99.47	25.45
Spiked solution			
Centrifuged solution (%)	**Filtrate solution**	**Nylon filter (%)**	**PVDF filter (%)**
100.0	1st mL	100.78	60.67
	2nd mL	100.52	80.20
	3rd mL	101.04	86.45

5. CONCLUSION

The cleaning analytical method for determination of Tofacitinib cleaning residue for Tofacitinib tablets was validated. The validation parameters, system suitability, specificity, precision, LOD & LOQ, Recovery, Accuracy, Linearity, solution stability and filter validation has been established and found all the results are well within the acceptable limit. Thus, it is concluding that the method is suitable for testing of cleaning residue samples of Tofacitinib tablets to estimate the acceptable residue of the manufacturing equipment for further intended use. The same method can be used for evaluation of Tofacitinib API residue content in bulk manufacturing.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tofacitinib 10 mg film-coated tablets - Summary of Product Characteristics (SmPC)". (emc); 2020.
   Retrieved 3 November2020.
2. "Xeljanz- Tofacitinib tablet, film coated Xeljanz XR- Tofacitinib tablet, film coated, extended release Xeljanz- Tofacitinib solution". DailyMed; 2020.
   Retrieved 3 November2020.
3. Lisa A. Raedler, “Xeljanz XR (Tofacitinib) First Once-Daily Oral JAK Inhibitor Approved for Patients with Rheumatoid Arthritis”; Eighth Annual Payers' Select Drug Profiles, Payers. 2017;10.
4. Material safety data sheet of Tofacitinib citrate, version 2.1, revision date; 2017.
5. Genereal notices, USP38 NF33; 5.30.0 Description and solubility.
6. Palak Bansal, Anoop Verma, Steffi Talwar “Detoxfication of Pharmaceuticals”, Chemical engineering Journal. 20187;349.
7. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use “Guideline for Residual solvents” ICH-Q3C, April, 2021.
8. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for

Human Use "Validation of Analytical Procedures" ICH-Q2A, Geneva; 1994.
9. Food and Drug Administration "Guide to Inspections, Validation of Cleaning Processes" Office of Regulatory Affairs, FDA, Rockville, MD; 1993.
10. Kirsch RB. "Validation of Analytical Methods Used in Pharmaceutical Cleaning Assessment and Validation" Pharmaceutical Technology (Supplement). 1998;40-46.
11. Sampling for cleaning validation – Analytical considerations by Sandeep Kalelkar and Jay Postlewaite.
12. Validation of analytical methods used in cleaning validation, By Herbert J. Kaiser, Ph.D. & Bruce Ritts, M.S. In institute of Validation technology. "Journal of validation technology", Published in; 2004.
13. Guidelines for Single-Laboratory Validation of Analytical Methods for Trace-Level Concentrations of Organic Chemicals" Special Publication - Royal Society of Chemistry: Principles and Practices of Method Validation. 2000;256: 179- 252.
14. Guidance on aspects of cleaning validation in active pharmaceutical ingredient plants by Active pharmaceutical ingredients committee (APIC), revision; 2016.
15. Sankar ASK, Datchayani B, Balakumaran N, Mohammed Rilwan R. Subaranjani Development of a Validated Reverse Phase Liquid Chromatographic Assay-Method for determination of Tofacitinib in pure form and in Physical Admixtures", Research Journal of Pharmacy and Technology. 2017;10:1.
16. Chowdary KPR, Rao GD, Himabindu G. "Validation of Analytical Methods" Eastern Pharmacist, 1999, 42(497), 39- 41.
17. Hsu H, Chien C. "Validation of Analytical Methods: A Simple Model for HPLC Assay Methods" Yaowu Shipin Fenxi. 1994;2(3): 161-76
18. Clarke GS. The Validation of Analytical Methods for Drug Substances and Drug Products in UK Pharmaceutical Laboratories" Journal of Pharmaceutical and Biomedical Analysis. 1994;12(5):643-52.
19. Validation of Compendial Methods" United States Pharmacopoeia. 2013;XXVI.
20. Food and Drug Administration "International Conference on Harmonization; Guideline on Validation of Analytical Procedures: Definition and Terminology; Availability" Federal Register. 1995;60(40):11260-11262.
21. Kirsch, R. "Validation of Methods Used in Pharmaceutical Cleaning Validation" Pharmaceutical Technology, 1998 (Supplement); 40-46.
22. Krull I, Swartz M. "Determining Limits of Detection and Quantitation" LC-GC. 1998; 16(10):922-924.

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