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CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION STABILITY-INDICATING TWO IMPURITIES AND ITS DEGRADATION PRODUCTS IN TOFACITINIB ORAL SOLUTION 1MG/ML

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ABSTRACT

A Novel RPHPLC Quantification method was developed for estimation of Tofacitinib known impurities like its Amine impurity and Metabolite-1which, were separated on Waters Sunfire®C18, 50 x 4.6 mm, 5 µm (Part No.: 186002560). Using a mixture of Mobile phase-A: Buffer Solution pH 5.5/Acetonitrile = 90/10 (v/v) and Mobile phase-B: Acetonitrile/Buffer Solution pH 5.5 = 70/30 (v/v) as a gradient mobile phase with a flow rate of 1.2 ml/min; λ max at 210 nm. The developed method was validated all the parameters like linearity, specificity, LOD, LOQ, accuracy, robustness, ruggedness, precision, filter variation, solution stability and forced degradation studies.

KEYWORDS: Method development and validation, Tofacitinib, Related substances, Stability-indicating, Oral solution.

INTRODUCTION

Tofacitinib is chemically 3-((3R,4R)-rel-4-Methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)-3-oxopropanenitrile (fig. 1). (tofacitinib) is indicated for patients who have had an inadequate response or intolerance to one or more TNF blockers in adults with moderately to severely active rheumatoid arthritis (RA), active psoriatic arthritis (PsA), active ankylosing spondylitis (AS), or moderately to severely active ulcerative colitis (UC), and patients 2 years of age and older with active polyarticular course juvenile idiopathic arthritis (pcJIA). Limitations of Use**:** XELJANZ in combination with biological therapies or with potent immunosuppressants

is not recommended. Many techniques have been reported quantitave estimation including Spectrophotometric[3-4], liquid chromatographic[5-6], UPLC[7], LC/MS method for human plasma.[8]

Since no method has been developed for the separation and estimation of impurities in Tofacitinib Oral solution and the drug is being marketed in domestic and international market the present study by the author describes a rapid, accurate and precise RP – HPLC method for the estimation of known related impurities, i.e., Amine impurity impurity(3R,4R)-4-Methyl-3-(methyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino)-1-piperidine,Metabolite-1(3-[(3R,4R)-4-Methyl-3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl]-3-oxopropanenitrile) and degrading products under stress conditions present in Tofacitinib Oral solution. The method was validated as per ICH guidelines.[9]

EXPERIMENTAL

MATERIAL AND METHODS

Chromatographic Conditions

Waters system with empower software & photo diode array detector, Waters Sunfire column compartment connected with Empower software connected with a Waters Sunfire®C18, 50 x 4.6 mm, 5 µm.

Chemicals and reagents

Tofacitinib pure drug and impurities, Potassium Phosphate Monobasic (HPLC Grade), Potassium Hydroxide (HPLC Grade), 1-Octane Sulfonic Acid Sodium Salt (HPLC Grade), Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Water USP Purified or Equivalent., was used in the present study. The oral solution formulations purchased from local market Hyderabad, India.

Mobile Phase

Buffer preparation: Dissolve about 2.72 g of Potassium Phosphate Monobasic and 1.0 g of 1-Octane Sulfonic Acid Sodium Salt into 1000 mL of water. Mix the solution well. Adjust the pH of the solution to 5.5 ± 0.05 with Diluted Potassium Hydroxide Solution. Filter the buffer solution through a 0.45 µm membrane filter.

Mobile phase-A Preparation: Mix 900 mL of Buffer Solution pH 5.5 with100 mL of Acetonitrile. Mix well and degas for at least 10minutes.

Mobile phase-B Preparation Mix 700 mL of Acetonitrile with 300 mL of Buffer Solution pH 5.5. Mix well and degas for at least 10minutes.

The flow rate of the mobile phase was maintained at 1.2ml/min. The column temperature was maintained at 30ºC and the detection was carried out at 210nm with an injection volume of 20µl.

Diluent

Mix 800 mL of Buffer Solution pH 5.5 with 200 mL of Acetonitrile, mix well and label as Diluent.

Standard solution preparation

Accurately weigh and transfer about 32.4 mg of Tofacitinib Citrate Standard into a 200-volumetric flask. Add diluent, sonicate (about 5 minutes) to dissolve, dilute to volume with diluent and mix well. The concentration is about 162 µg/mL of Tofacitinib Citrate and about 100 µg/mL of Tofacitinib.

Pipet 2.0 mL of Standard Stock Solution into a 200-mL Volumetric flask, diluted to the volume with diluent, mix well and label as Standard Solution. The concentration is about 1.62 µg/mL of Tofacitinib Citrate and about 1.00 µg/mL of Tofacitinib.

Placebo Preparation

Accurately weigh and transfer about 10.7 g of Tofacitinib Oral Solution placebo, 1 mg/mL into a 50-mL volumetric flask with aid of the diluent. Add diluent to about 70% volume of flask and sonicate for 5 minutes with intermittent shaking. Dilute to volume with diluent and mix well. Filter the sample solution through a 0.45 µm Nylon syringe filter, discarding the first 5 mL of the filtrate prior to collecting the sample solution in HPLC vial for analysis.

Sample Preparation

Accurately weigh and transfer about 10.7 g of Tofacitinib Oral Solution, 1 mg/mL into a 50-mL volumetric flask with aid of the diluent. Add diluent to about 70% volume of flask and sonicate for 5 minutes with intermittent shaking. Dilute to volume with diluent and mix well. The concentration is about 200 µg/mL of Tofacitinib.

Filter the sample solution through a 0.45 µm Nylon syringe filter, discarding the first 5 mL of the filtrate prior to collecting the sample solution in HPLC vial for analysis.

Impurities Calculation

$$\% \text{ of Impurity} = \frac{\text{Impurity Area x Standard weight x 1 x 100 x 1 x standard Potency}}{\text{Average Standard Area x 50 x 100 x sample weight x Label amount}} x\ RF$$

% of Total Impurities = Sum of % Individual impurities,

RF – Response Factor

RESULTS AND DISCUSSION

System Suitability

System suitability was evaluated from the standard solution preparation by injecting six times into the HPLC. The parameters measured were Theoretical plates, asymmetry, %RSD, the observed results asymmetry is about 1.2, theoretical plates about 50000, % RSD is 0.28 and the resolution between two peaks greater than 2.0 indicates the method suitable for related substances estimation.

Placebo and impurities interference

Interference from placebo and impurities was carried out by preparing the following specificity samples. Performed related substances on Placebo equivalent to the amount present in test preparation and injected into the chromatography. By preparing and inject impurities at 1.0 % of test concentration, by preparing active sample as per test concentration, by spiking the active sample with individual known impurities at 1.0% of test concentration. The above samples were injected and observed for any interference from blank and placebo at the retention time of analyte and known impurity peaks. This was further demonstrated by determining the peak purity of analyte and known impurity peaks. Since no interference of blank, placebo and known impurities was observed at the retention time of analyte. Individual impurity peaks are separated from the analyte peak. Peak purity of analyte peak and known impurity peaks are purity angle less than purity threshold, so the method is specific for Tofacitinib Oral solution.

Limit of Quantitation and Detection

The limit of quantitation (LOQ) and detection (LOD) were conducted on the basis of signal to noise ratio method. Different concentrations of impurities with sample solution were injected, LOQ established the values which give the signal to noise ratio about 10.0, for LOD

of impurities were established which give the signal noise ratio about 3.0; the results of both LOQ & LOD values were tabulated in Table-2.

Linearity and Detector Response

The linearity of detector response for impurities was demonstrated by prepared solutions of Lacosamide and its impurities over the range of LOQ to 200% level and the detector response was found to be linear and the correlation coefficient was more than 0.998, proves Tofacitinib and its impurities are linear, the results were tabulated in Table-3.

Establishment of RRT's and RF Values for Impurities

The RRT's and RF values were calculated from the linearity levels of 0.05%, 0.20%, 0.50% 0.75% and 1% i.e., 0.5%, 1.0% and 0.2.0% of test concentration. The RRT's and RF values were calculated and the results were tabulated in Table-4.

Precision

Six sample preparations representing a single batch were injected, the each impurity area were determined and the precision was evaluated, the %RSD of each impurity results was less than 10.0 indicates the method is precise, the results are tabulated in Table-4.

Intermediate Precision

The ruggedness of the method was injected six preparations of a single batch sample by different analyst (analyst-2), different column (column-2) and different instrument (instrument-2). The %RSD of each impurity was calculated; the results were less than 10.0. consider the precision results for analyst-1, column-1 and system-1, the mean %RSD values of both precision and intermediate calculated, the results were less than 15.0 shows the method is rugged and the results were tabulated in Table-4.

Accuracy

The accuracy of the test method was prepared recovery samples (i.e. test sample with known quantities of Amino hydroxide Impurity, Metabolite-1 and Keto Impurity) at the level of LOQ, 50%, 100%, 150% and 200% of target concentration, as the recovery results were found between 90 to 110% the method is accurate for the estimation of Tofacitinib Oral solution and its impurities over the range of LOQ to 200% level of target concentration and the results were tabulated in Table-5.

Robustness

The solution stability & mobile phase stability

The standard and sample solution kept for bench top, under refrigerator were injected initially, after 24 hours and 48 hours. The difference between initial, 24hrs and 48hrs of individual impurity less than 0.03% and total impurities less than 0.1% and the similarity factor after 24 hours and after 48 hours is between 0.95 to 1.05 indicates the solution is stable up to 48hrs and the results were tabulated in Table 8. for mobile phase stability the standard and sample solutions injected initially, after 24 hours and after 48 hours, a slight variation of parameters like theoretical plates, asymmetry and % RSD indicates the mobile phase is stable up to 48 hours.

Extraction time of analyte

The difference between as such condition and different extraction samples for % of individual impurity less than 0.03% and % of total impurities 0.1% found within the limits.

Filter variation

The filter variation was injected the test solution of centrifuged and filtered through 0.22µ nylon filter 0.45µnylon filter and 0.22µ PVDF, 0.45µ PVDF filter and the difference between filtered portions of individual impurity less than 0.03% and total impurities were less than 0.1% with respect to centrifuged sample shows no effect of filter variation.

Effect of Column Temperature and Flow Variation

The standard preparation was injected under normal condition (i.e. as such condition) and of the altered conditions column temperature 30±5°C and flow rate 1.2±0.1ml the difference between as such for all changed conditions parameters like theoretical plates, asymmetry and % RSD within the limits proves the method is robust.

FORCED DEGRADATION CONDITIONS

Sample Name	Conditions
Control	Not Stressed
Acid Hydrolysis	2.5 mL of 0.5 N HCl, 60°C for 24 hours
Base Hydrolysis	2.5 mL of 0.1 N NaOH, Room Temperature for 2 hours
Water Hydrolysis	5.0 mL of Water, 60°C for 24 hours
Oxidation	5.0 mL of 3% H2O2, Room Temperature for 24 hours
UV/White Light	SUNTEST CPS+, Room Temperature for 8 hours
Elevated Temperature	60°C for 5 days

Table 1: HPLC Gradient Program.

Time (min)	Mobile phase A	Mobile phase B
0	82	18
22	82	18
32	65	35
42	65	35
45	82	18
50	82	18

Table 2: LOD & LOQ results.

S. No	Name of the Component	LOD RESULTS		LOQ RESULTS	
		S/N Ratio	% level of component w.r.t to sample concentration	S/N Ratio	% level of component w.r.t to sample concentration
1	Tofacitinib	2.94	0.0032	9.90	0.0115
2	Amine impurity	2.82	0.0031	9.98	0.0113
3	Metabolite-1	2.95	0.0035	9.99	0.0095

Table 3: Linearity Results.

Compound Name	Correlation coefficient	Slope	Y-Intercept	Residual sum square	Residual standard deviation
Tofacitinib	0.9999	387590.17	2982.93	1.2910×10^{9}	18965
Amine impurity	1.0000	398692.33	-258.60	1.0861×10^{9}	17478
Metabolite-1	1.0000	365985.48	522.11	9.7337×10^{8}	15599

Table 4: Precision, Intermediate Precision, RF and RRT Results.

Parameter	Amine impurity	Metabolite-1
Precision(n=6)		
Tofacitinib	**0.52**	**0.62**
Intermediate Precision(n=6)		
Tofacitinib	**0.75**	**0.95**
RRT&RF Values		
RRT values	**0.80**	**0.92**
RF values	**0.46**	**0.42**

Table 5: Accuracy results.

Spike Level	***Amount added(ppm)***	***Mean Amount recovered(ppm)***	***% Mean Recovery***	***%RSD***
Recovery of Amine impurity impurity				
LOQ level	0.045	0.04109	100.25	0.60
50%	2.45	2.4163	99.48	0.35
100%	5.07	5.0745	100.19	0.45
150%	7.68	7.611	99.19	0.47
200%	10.51	10.539	100.73	0.52
Recovery of Metabolite-1				

LOQ level	0.047	0.0462	98.12	0.52
50%	2.55	2.5485	100.52	0.33
100%	5.06	5.0234	99.78	0.35
150%	7.55	7.5127	99.74	0.81
200%	10.06	10.1251	100.65	0.30
Recovery of Tofacitinib				
LOQ level	0.052	0.0504	98.57	1.52
50%	2.55	2.5493	100.71	0.22
100%	5.06	4.9975	98.97	0.17
150%	7.59	7.4817	98.78	1.52
200%	10.21	10.1335	100.33	0.32

Table 6: Degradation Results.

TOFACITINIB ORAL SOLUTION

S.No:	Stress conditions	Duration	% of Total imp's	% of Amine impurity imp	% of Metabolite-1	% of major unknown imp
1	Normal	NA	0.11	0.01	ND	0.02
2	Thermalat60°C	5th day	0.11	0.01	ND	0.02(2.17)
3	75% RH	5th day	0.12	0.01	ND	0.02(2.17)
4	UV	200 watt hours/m^2	0.11	0.01	ND	0.02(2.17)
5	Sunlight	1.2 million Lux hours	0.11	0.01	ND	0.02(2.17)
6	0.5N HCl at	5 hours	9.40	0.60	ND	2.21(0.77)
7	0.1N NaOH	48 hours	0.17	0.06	ND	0.02(2.17)
8	3% H2O2 at	5 hours	6.90	0.19	ND	1.01(0.73)
9	Water at 60°C	5th day	0.12	0.01	ND	0.02(2.16)

Figure Captions

Fig 1). Tofacitinib chemical structure

Fig 2). Representative Chromatogram of Sample Solution

Fig 3). Representative Chromatogram of Acid Hydrolysis Sample Solution

Fig 4). Representative Chromatogram of Base Hydrolysis Sample Solution

Fig 5). Representative Chromatogram of Oxidation Sample Solution

Fig 6). Representative Chromatogram of Water Hydrolysis Sample Solution

Fig 7). Representative Chromatogram of UV/White Light Sample Solution

Fig 8). Representative Chromatogram of Elevated Temperature Sample Solution

Fig 9). Representative Chromatogram of Aged Standard Solution

Fig 10). Representative Chromatogram of Diluent

Fig 11). Representative Chromatogram of Placebo solution

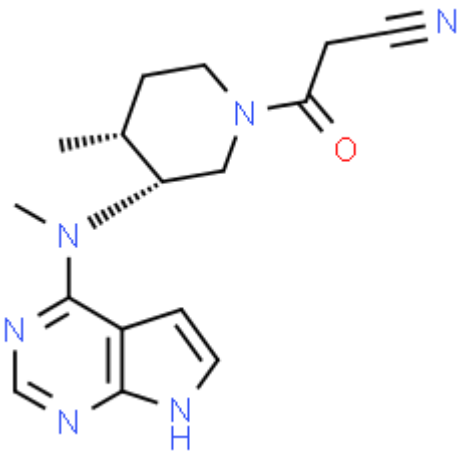
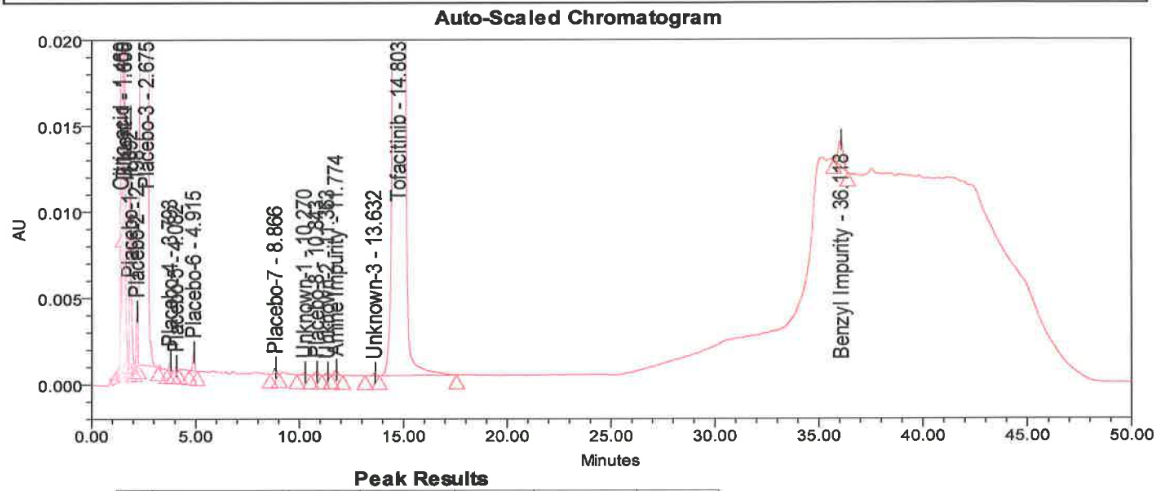


Fig 1: Tofacitinib chemical structure.

SAMPLE INFORMATION

Sample Name:	Repeatability Prep-1	Acquired By:	MansiG
Sample Type:	Unknown	Sample Set Name:	052521_TOFA_IMP_SS_RP
Vial:	10	Acq. Method Set:	TOFA IMP ARD041 MS RP
Injection #:	1	Processing Method:	TOFA_IMP_PM_RP
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 210nm
		Result Id 5341	
Date Acquired:	5/25/2021 11:12:34 PM EDT -04:00		
Date Processed:	6/1/2021 10:15:10 AM EDT -04:00		
	Sample Set Id 1703	Result Set Id 5279	



Peak Results

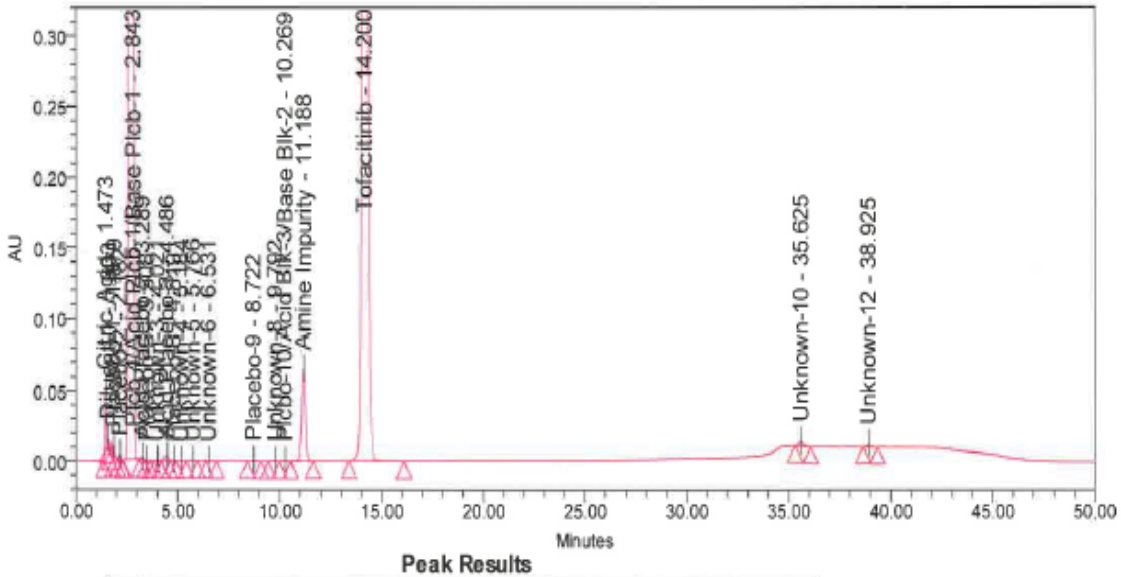
	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Citric acid	1.469	213059	BB	1.1	0.099
2	Diluent-1	1.606	90172	BB	1.8	0.109
3	Placebo-1	1.892	77056	BB	1.6	0.128
4	Placebo-2	2.198	13225	BB	1.0	0.148
5	Placebo-3	2.675	12695525	BB	0.6	0.181
6	Placebo-4	3.793	2562	BB	1.1	0.256
7	Placebo-5	4.082	1090	BB	1.2	0.276
8	Placebo-6	4.915	7440	BB	1.1	0.332
9	Placebo-7	8.866	3204	BB	1.0	0.599
10	Unknow n-1	10.270	1961	BB	1.0	0.694
11	Placebo-8	10.843	2437	BB	1.0	0.732

Fig 2: Representative Chromatogram of Sample Solution.

SAMPLE INFORMATION

Sample Name:	Acid_0.5N _HCL_24hr_60C_Sample	Acquired By:	MansiG
Sample Type:	Unknown	Sample Set Name:	060221_TOFA_IMP_SS_FD
Vial:	62	Acq. Method Set:	TOFA IMP ARD003 MS FD
Injection #:	1	Processing Method:	TOFA_IMP_PM_FD
Injection Volume:	20.00 ul	Channel Name:	210 nm
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 210.0 nm (2998
		Result Id 8190	
Date Acquired:	6/3/2021 3:44:30 AM EDT -04:00		
Date Processed:	6/7/2021 12:18:29 PM EDT -04:00		
	Sample Set Id 6915	Result Set Id 8058	

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Citric Acid	1.473	191755	BB	1.0	0.104
2	Diluent-1	1.603	27009	BB	1.3	0.113
3	Placebo-1	1.879	53662	BB	1.8	0.132
4	Placebo-2	2.182	24334	BB	1.1	0.154
5	Plcb-4/Acid Plcb-1/Base Plcb-1	2.843	12125914	BB	0.6	0.200
6	Acid Placebo-2	3.289	17700	BB	0.9	0.232
7	Placebo-5	3.508	5004	BB	1.3	0.247
8	Unknow n-3	4.021	3341	BB	1.0	0.283
9	Acid Placebo-3	4.486	33900	BB	0.7	0.316
10	Placebo-8	4.819	4010	BB	0.9	0.339

Fig 3: Representative Chromatogram of Acid Hydrolysis Sample Solution.

SAMPLE INFORMATION

Sample Name:	Base_0.1N NaOH_2hr_RT_Sample	Acquired By:	MansiG
Sample Type:	Unknown	Sample Set Name:	060221_TOFA_IMP_SS_FD
Vial:	60	Acq. Method Set:	TOFA IMP ARD003 MS FD
Injection #:	1	Processing Method:	TOFA_IMP_PM_FD
Injection Volume:	20.00 ul	Channel Name:	210 nm
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 210.0 nm (2998
		Result Id 8188	
Date Acquired:	6/3/2021 2:02:42 AM EDT -04:00		
Date Processed:	6/7/2021 12:18:28 PM EDT -04:00		
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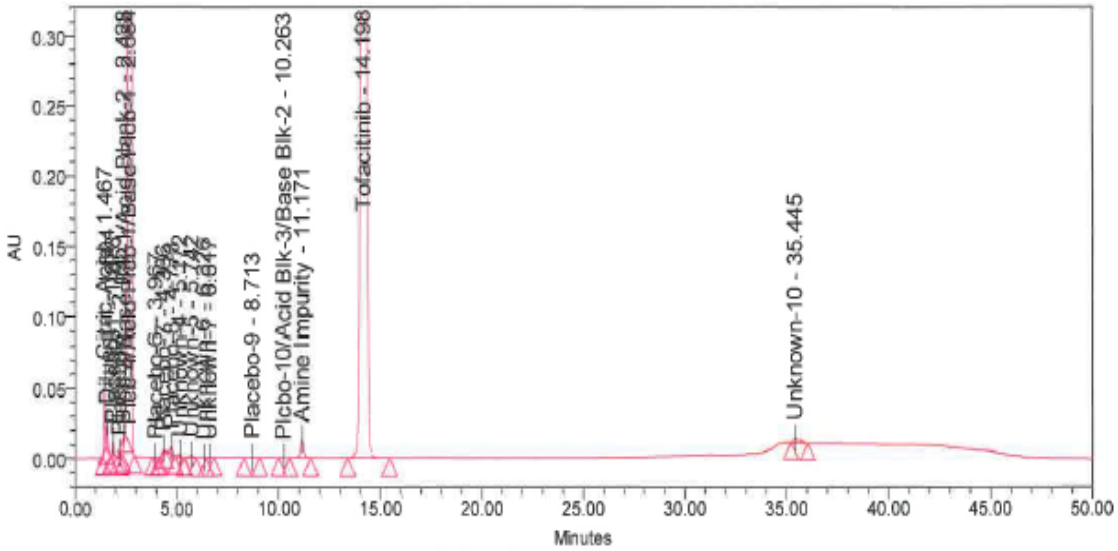
Auto-Scaled Chromatogram



Peak Results

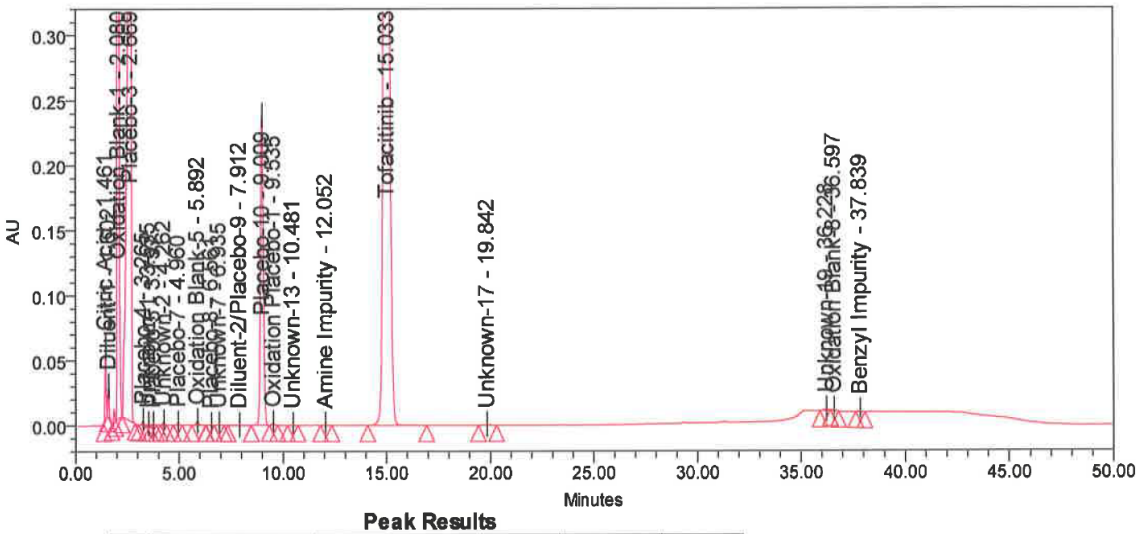
	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Citric Acid	1.467	204140	BB	1.0	0.103
2	Diluent-1	1.604	86476	BB	1.8	0.113
3	Placebo-1	1.878	57456	BB	1.7	0.132
4	Placebo-2	2.195	13675	BB	1.1	0.155
5	Picb-3/Base Blk-1/Acid Blank-2	2.428	42673	BB	0.9	0.171
6	Picb-4/Acid Picb-1/Base Picb-1	2.684	11819862	BB	1.2	0.189
7	Placebo-6	3.967	4687	BB	0.9	0.279
8	Placebo-7	4.366	28308	BB	0.9	0.308
9	Placebo-8	4.773	63366	BB	0.8	0.336
10	Unknow n-4	5.172	26031	BB	0.8	0.364

Fig 4: Representative Chromatogram of Base Hydrolysis Sample Solution.

SAMPLE INFORMATION

Sample Name:	Oxidation_3%H2O2_RT_24hrs_Sample	Acquired By:	MansiG
Sample Type:	Unknown	Sample Set Name:	052721_TOFA_IMP_SS_FD
Vial:	12	Acq. Method Set:	TOFA IMP ARD003 MS FD
Injection #:	1	Processing Method:	TOFA_IMP_PM_FD
Injection Volume:	20.00 ul	Channel Name:	210 nm
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 210.0 nm (2998
		Result Id 6788	
Date Acquired:	5/27/2021 9:34:39 PM EDT -04:00		
Date Processed:	6/2/2021 9:52:49 AM EDT -04:00		
	Sample Set Id 3249	Result Set Id 6623	

Auto-Scaled Chromatogram



Peak Results

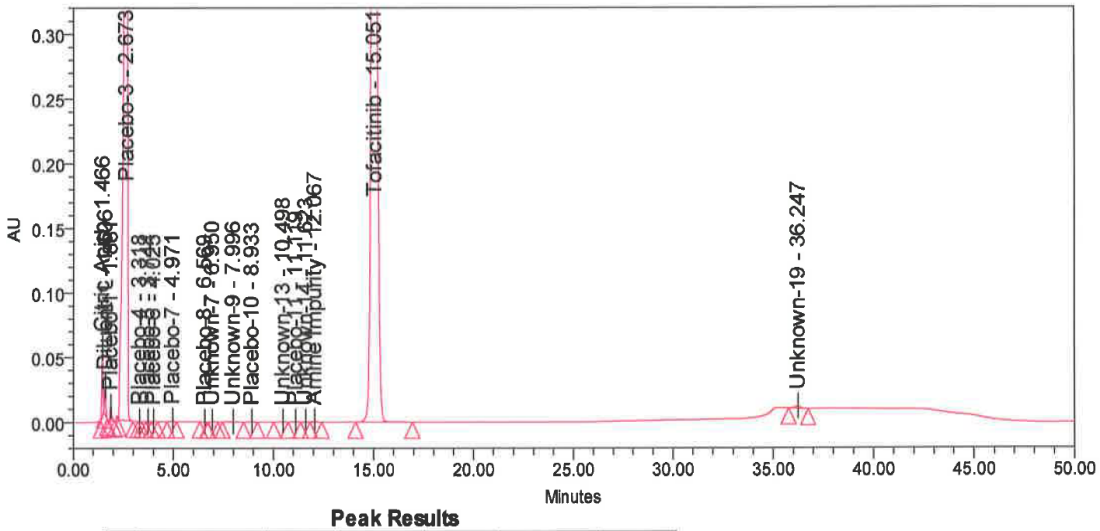
	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Citric Acid	1.461	216955	BB	1.0	0.097
2	Diluent-1	1.602	106991	BB	1.7	0.107
3	Oxidation Blank-1	2.080	11808022	BB	1.1	0.138
4	Placebo-3	2.669	12104115	BB	0.6	0.178
5	Placebo-4	3.265	16199	BB	1.2	0.217
6	Unknow n-1	3.535	2328	BB	1.0	0.235
7	Placebo-5	3.757	4584	BB	1.3	0.250
8	Unknow n-2	4.262	12199	BB	1.1	0.283
9	Placebo-7	4.960	8291	BB	1.1	0.330
10	Oxidation Blank-5	5.892	23182	BB	1.3	0.392

Fig 5: Representative Chromatogram of Oxidation Sample Solution.

SAMPLE INFORMATION

Sample Name:	Water hydro_60C_24hrs_Sample	Acquired By:	MansiG
Sample Type:	Unknown	Sample Set Name:	052721_TOFA_IMP_SS_FD
Vial:	15	Acq. Method Set:	TOFA IMP ARD003 MS FD
Injection #:	1	Processing Method:	TOFA_IMP_PM_FD
Injection Volume:	20.00 ul	Channel Name:	210 nm
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 210.0 nm (2998
		Result Id 6791	
Date Acquired:	5/28/2021 12:07:19 AM EDT -04:00		
Date Processed:	6/2/2021 9:52:51 AM EDT -04:00		
	Sample Set Id 3249	Result Set Id 6623	

Auto-Scaled Chromatogram



Peak Results

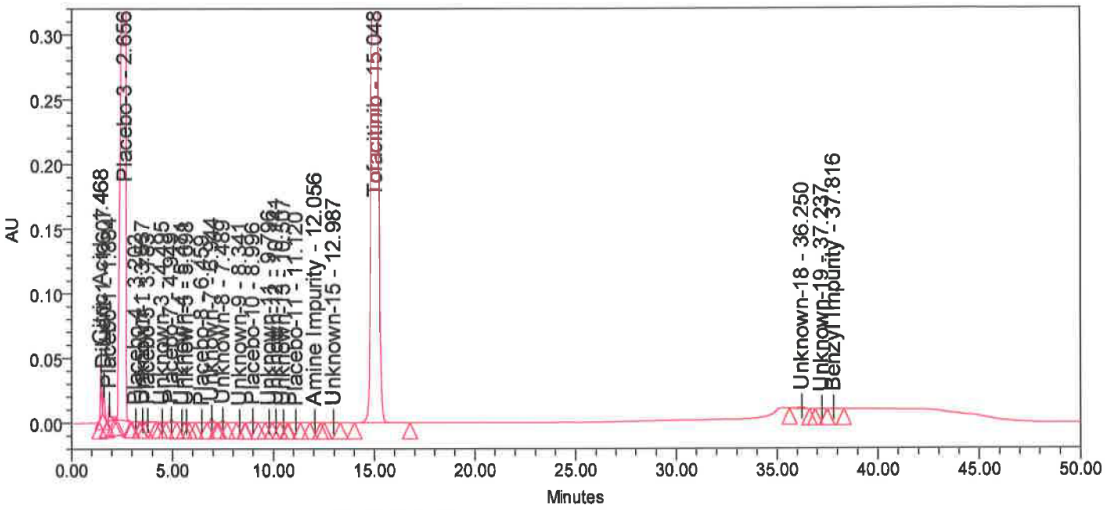
	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Citric Acid	1.466	201885	BB	1.1	0.097
2	Diluent-1	1.606	95018	BB	1.3	0.107
3	Placebo-1	1.881	58906	BB	1.8	0.125
4	Placebo-3	2.673	12201059	BB	0.6	0.178
5	Placebo-4	3.318	893	BB	1.2	0.220
6	Placebo-5	3.744	2151	BB	1.1	0.249
7	Placebo-6	4.025	1017	BB	1.4	0.267
8	Placebo-7	4.971	6571	BB	1.1	0.330
9	Placebo-8	6.569	761	BB	1.3	0.436
10	Unknow n-7	6.950	1307	BB	1.2	0.462

Fig 6: Representative Chromatogram of Water Hydrolysis Sample Solution.

SAMPLE INFORMATION

Sample Name:	UV/White Light_RT_8hrs_Sample	Acquired By:	MansiG
Sample Type:	Unknown	Sample Set Name:	052721_TOFA_IMP_SS_FD
Vial:	16	Acq. Method Set:	TOFA IMP ARD003 MS FD
Injection #:	1	Processing Method:	TOFA_IMP_PM_FD
Injection Volume:	20.00 ul	Channel Name:	210 nm
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 210.0 nm (2998
		Result Id 6792	
Date Acquired:	5/28/2021 12:58:12 AM EDT -04:00		
Date Processed:	6/2/2021 9:52:51 AM EDT -04:00		
	Sample Set Id 3249	Result Set Id 6623	

Auto-Scaled Chromatogram



Peak Results

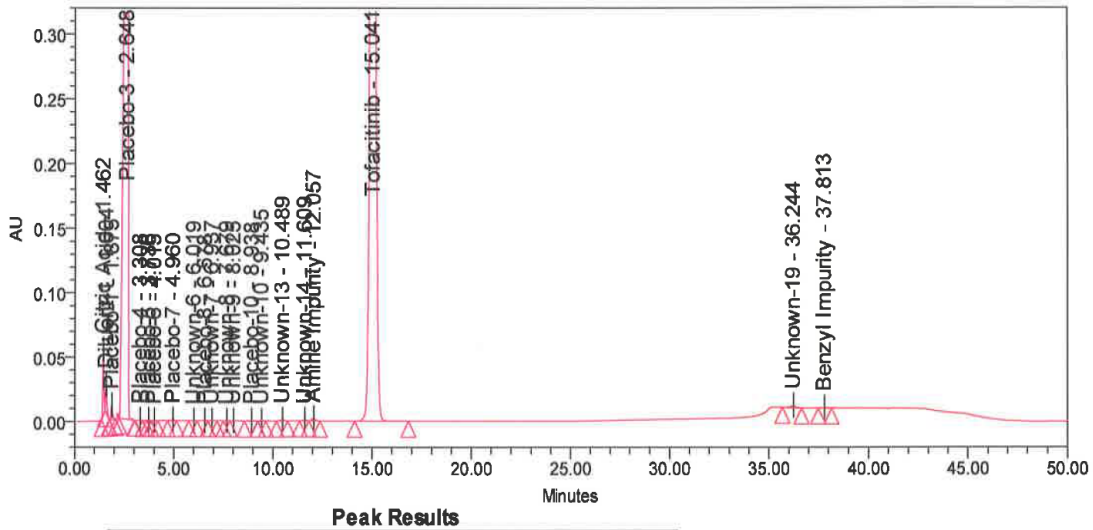
	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Citric Acid	1.468	168692	BB	1.0	0.098
2	Diluent-1	1.607	104863	BB	1.7	0.107
3	Placebo-1	1.884	74585	BB	2.2	0.125
4	Placebo-3	2.656	12147131	BB	0.6	0.176
5	Placebo-4	3.202	4607	BB	1.2	0.213
6	Unknow n-1	3.537	5376	BB	1.1	0.235
7	Placebo-5	3.783	11608	BB	2.3	0.251
8	Unknow n-3	4.495	2633	BB	1.3	0.299
9	Placebo-7	4.949	17637	BB	1.3	0.329
10	Unknow n-4	5.471	2333	BB	0.8	0.364

Fig 7: Fig Representative Chromatogram of UV/White Light Sample Solution.

SAMPLE INFORMATION

Sample Name:	Elevated Temp_60C_5Days_Sample	Acquired By:	MansiG
Sample Type:	Unknown	Sample Set Name:	052721_TOFA_IMP_SS_FD
Vial:	17	Acq. Method Set:	TOFA IMP ARD003 MS FD
Injection #:	1	Processing Method:	TOFA_IMP_PM_FD
Injection Volume:	20.00 ul	Channel Name:	210 nm
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 210.0 nm (2998
		Result Id 6793	
Date Acquired:	5/28/2021 1:49:04 AM EDT -04:00		
Date Processed:	6/2/2021 9:52:52 AM EDT -04:00		
	Sample Set Id 3249	Result Set Id 6623	

Auto-Scaled Chromatogram



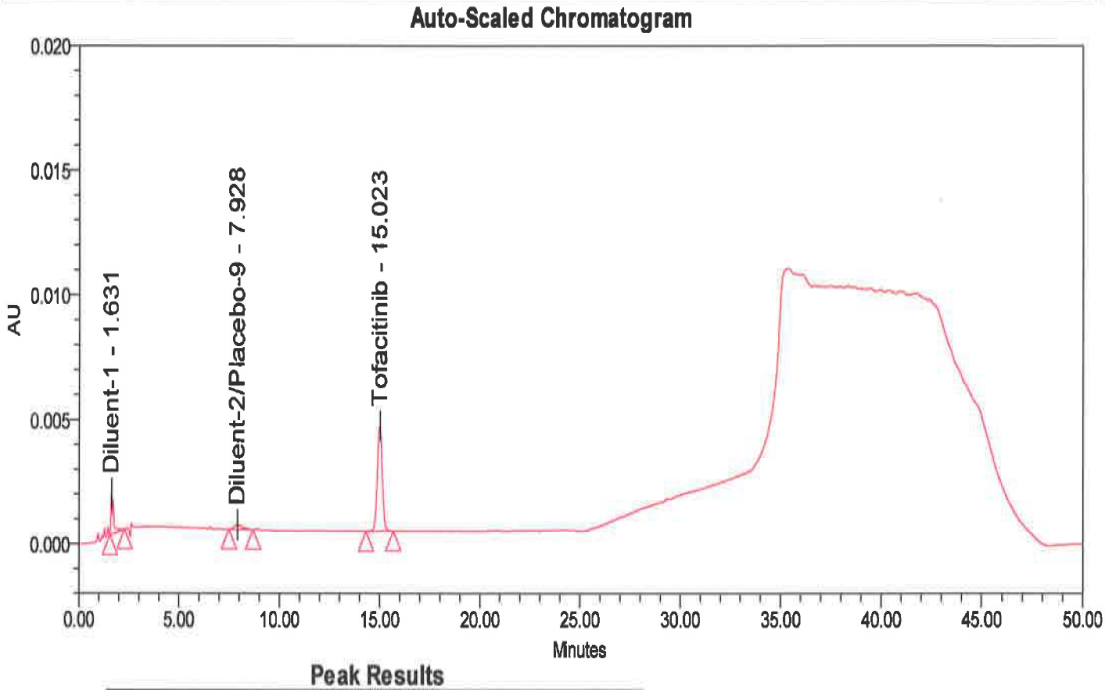
Peak Results

	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Citric Acid	1.462	199166	BB	1.0	0.097
2	Diluent-1	1.604	102475	BB	1.6	0.107
3	Placebo-1	1.875	59279	BB	2.0	0.125
4	Placebo-3	2.648	12051353	BB	0.6	0.176
5	Placebo-4	3.308	3407	BB	0.7	0.220
6	Placebo-5	3.736	2064	BB	1.2	0.248
7	Placebo-6	4.015	1324	BB	1.1	0.267
8	Placebo-7	4.960	7140	BB	0.8	0.330
9	Unknow n-6	6.019	1583	BB	0.8	0.400
10	Placebo-8	6.578	1087	BB	1.3	0.437

Fig 8: Representative Chromatogram of Elevated Temperature Sample Solution.

SAMPLE INFORMATION

Sample Name:	Std Stability_6Days	Acquired By:	MansiG
Sample Type:	Control	Sample Set Name:	052721_TOFA_IMP_SS_FD
Vial:	10	Acq. Method Set:	TOFA IMP ARD003 MS FD
Injection #:	1	Processing Method:	TOFA_IMP_PM_FD
Injection Volume:	20.00 ul	Channel Name:	210 nm
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 210.0 nm (2998
		Result Id 6787	
Date Acquired:	5/27/2021 7:52:54 PM EDT -04:00		
Date Processed:	6/2/2021 9:52:49 AM EDT -04:00		
	Sample Set Id 3249	Result Set Id 6623	



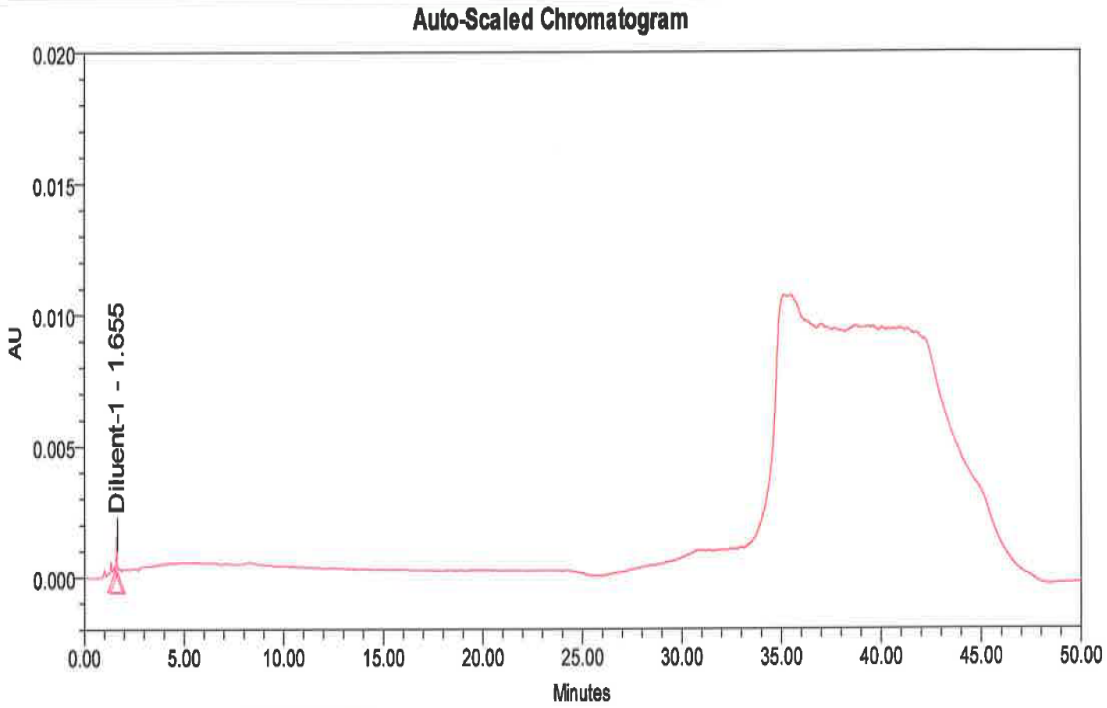
Peak Results

	Name	RT	Area	Int Type	USP Tailing	RT Ratio
1	Diluent-1	1.631	14543	BB	1.8	0.109
2	Diluent-2/Placebo-9	7.928	5609	BB	1.4	0.528
3	Tofacitinib	15.023	73758	BB	1.0	1.000

Fig 9: Chromatogram of Tofacitinib Standard solution.

SAMPLE INFORMATION

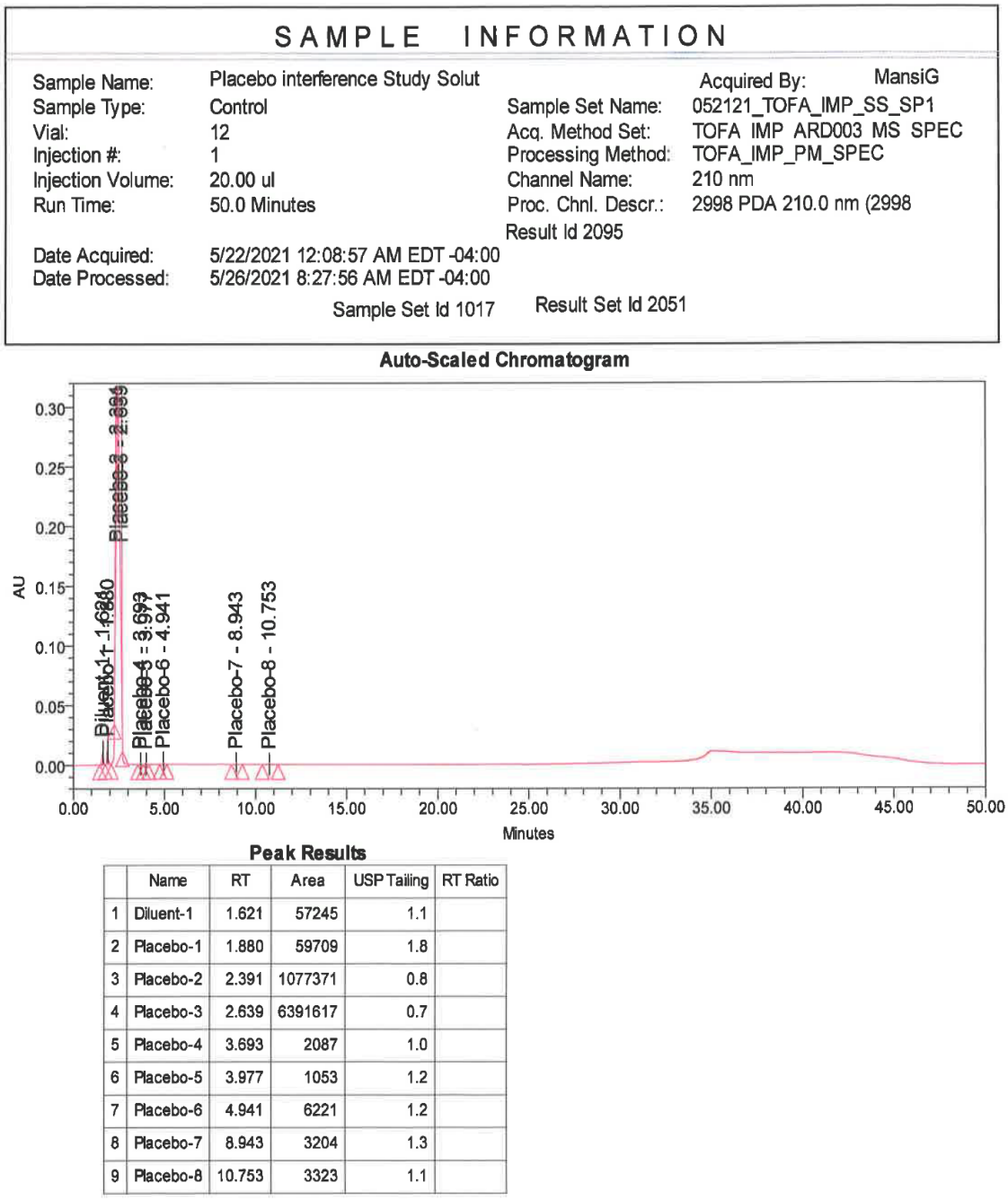
Sample Name:	Diluent	Acquired By:	MansiG
Sample Type:	Control	Sample Set Name:	052421_TOFA_IMP_SS_LIN
Vial:	2	Acq. Method Set:	TOFA IMP ARD042 MS LIN
Injection #:	1	Processing Method:	TOFA_IMP_PM_LIN
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	50.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 210nm
		Result Id 3071	
Date Acquired:	5/24/2021 4:38:20 PM EDT -04:00		
Date Processed:	5/27/2021 8:55:25 AM EDT -04:00		
	Sample Set Id 1306	Result Set Id 3065	



Peak Results

	Name	RT	Area	USP Tailing	RT Ratio
1	Diluent-1	1.655	4133	0.9	

Fig 10: Chromatogram of Tofacitinib diluent solution.



Peak Results

	Name	RT	Area	USP Tailing	RT Ratio
1	Diluent-1	1.621	57245	1.1	
2	Placebo-1	1.880	59709	1.8	
3	Placebo-2	2.391	1077371	0.8	
4	Placebo-3	2.639	6391617	0.7	
5	Placebo-4	3.693	2087	1.0	
6	Placebo-5	3.977	1053	1.2	
7	Placebo-6	4.941	6221	1.2	
8	Placebo-7	8.943	3204	1.3	
9	Placebo-8	10.753	3323	1.1	

Fig 11: Chromatogram of Tofacitinib Placebo solution

CONCLUSIONS

The proposed RP-HPLC method satisfies the parameters like system suitability, specificity, precision, accuracy, linearity, and robustness, ruggedness. The obtained results from the validation as per the ICH guidelines and drug stability were indicates this method is accurate, sensitive and best suitable Method for determination of known and unknown impurities in Tofacitinib regular laboratory analysis.

ACKNOWLEDGEMENTS

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