Development And Validation of Stability Indicating Assay Method For Estimation of Tofacitinib Citrate In Its Extended Release Tablets

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Abstract- *The present study was focused to develop a simple, precise, accurate and cost effective RP-HPLC method for estimation of Tofacitinib from Tofacitinib Citrate extended release tablet dosage form. The chromatographic method was carried out using Inertsil ODS-3V (150 mm × 4.6 mm, 5 µm) column with mobile phase Phosphate buffer (pH 5.5) and Acetonitrile in ratio of 65:35 %v/v. The flow rate was set 1.0 ml/min with 5 µL injection volume. Total run time 7 min. Detection was carried out at the wavelength of 287 nm. The developed analytical method was validated according to the ICH guideline. The Develop method was also subjected to various stress condition like acid and alkali hydrolysis, oxidation, photolysis, humidity and thermal degradation. The detector response was linear in the concentration range of 21.86– 174.94 µg/ml. The developed method is successfully applied for estimation of Tofacitinib from Tofacitinib Citrateextended release tablet dosage form.*

Keywords- Tofacitinib, RP-HPLC, Stability Indicating, Validation

I. INTRODUCTION

Tofacitinib Chemically known as 3-[(3R,4R)-4-methyl-3-[methyl({7H-pyrrolo[2,3-d]pyrimidin-4yl})amino] piperidin-1-yl]-3-oxopropanenitrile. It is an oral Janus Kinase inhibitor for the treatment of rheumatoid arthritis.

Tofacitinib is not official in Indian, United States and European Pharmacopeia. Literature survey reveals that few methods have been reported for the quantification of tofacitinib by using HPLC. There is no Stability indicating analytical method was reported for estimation of Tofacitinib from Tofacitinib Citrate Extended Release tablet dosage form. The aim of the present research work to develop a linear, accurate, precise, robust and cost effective method for estimation of Tofacitinib from Tofacitinib Citrate Extended release tablets accordance with ICH guidelines (Q2R1). The Develop method was also subjected to various stress condition like acid and alkali hydrolysis, oxidation, photolysis, humidity and thermal degradation.

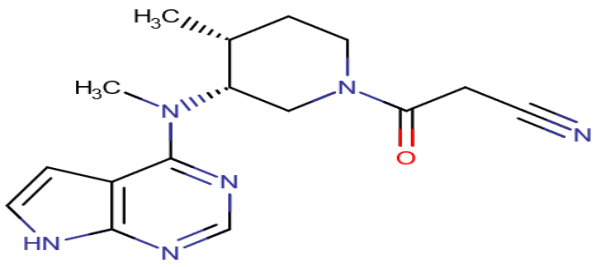

Figure 1: Chemical Structure of Tofacitinib

II. MATERIALS AND METHODS

In the present research work Acetonitrile and Potassium dihydrogen phosphate monohydrate were used of Merck Life science, Methanol and 1- Octane sulphonic acid sodium salt monohydrate were used of Spectrochem Pvt. Ltd. Potassium Hydroxide was used of Sigma Aldrich. The Sample of Tofacitinib API and Tablets were kindly gifted by ZYDUS CADILA HEATHCARE, Moraiya, Ahmedabad.

III. EQUIPMENT

The analysis was performed on HPLC Agilent technologies 1200 series, Isocratic mode, Photodiode array detector, injector of 100 µL loop volume. Inertsil ODS-3V (150 mm × 4.6 mm, 5 µm) column. Chromeleon software was used for data collecting processing.

IV. PREPARATION OF BUFFER SOLUTION

Transfer accurately 2.72 gm of Potassium Dihydrogen Phosphate and 1 gm of 1-Octane sulphonic acid sodium salt monohydrate in 1000 mL with water (Milli-Q) and

mix. Adjust the pH to 5.5 ± 0.05 with dilute (10%) potassium hydroxide solution. Filter the solution through 0.45 µm.

PREPARATION OF MOBILE PHASE:

Prepare a filtered and degassed mixture of buffer solution and acetonitrile in the ratio of (65:35) %v/v.

DILUENT:

Prepare a mixture of purified water and acetonitrile in ratio of (50:50) %v/v.

BLANK PREPARATION:

Prepare a mixture of methanol and diluent in ratio of (20:80) %v/v.

PREPARATION OF STANDARD SOLUTION [110ppm]:

Transfer an accurately weighed quantity of about 88.8 mg of Tofacitinib citrate working standard (Eq. to 55mg of Tofacitinib) into 50 mL volumetric flask. Add 30 mL of blank preparation and sonicate to dissolve. Make volume up to mark with blank preparation and mix. Further dilute 5.0 mL of this solution to 50 mL with blank preparation and mix.

SAMPLE PREPARATION [110ppm]:

[Label claim: 11mg]

Weigh accurately 20 tablets and calculate the average weight. Weigh and transfer 10 intact tablets in to a 1000ml volumetric flask, add 200.0 ml of methanol and sonicate with shaking for 1 minutes at each interval of 5minutes till tablets disperses completely. Add about 500ml of diluent and sonicate with shaking for 1minute at every interval of 5minutes for 45minutes. Make volume up to mark with diluent and mix. Filter the solution through Millipore PVDF 0.45 µm filter. Collect the filtrate by discarding first 5ml of the filtrate.

CHROMATOGRAPHIC CONDITION:

Column: Inertsil ODS-3V (150 mm × 4.6 mm, 5 µm)
Detector: 287 nm
Flow rate: 1.0 ml/min
Injection volume: 5 µL
Column oven temperature: 40^0C
Run time: 7 minutes
Wash vial: Purified Water : ACN (50:50) %v/v
Sampler temperature: 25^0C

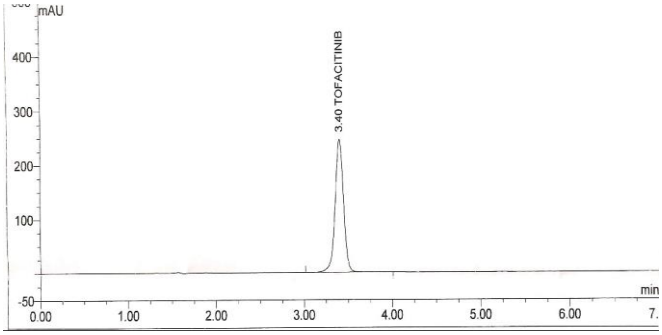

Figure 2: Chromatogram of Tofacitinib

V. RESULT AND DISCUSSION

ACID DEGRADATION:

Sample preparation:

Weigh accurately 20 tablets and calculate the average weight. Crush the tablet into fine powder and mix well. Weight and transfer crushed tablet powder equivalent to 25 mg of tofacitinib and transfer into 50 ml volumetric flask. Add 2.0 ml of ACN and sonicate with shaking to disperse completely. Add accurately 10 ml of diluent (0.1 N HCl : ACN (80 : 20) % v/v) and sonicate with shaking for 60 second at every 10 min for 60 min. Add 5 ml 1 N HCl solution and heated on boiling water bath for 90 min. Neutralize with 5.0 ml of 1 N NAOH. Cool the sample at room temperature and make volume up to the mark with diluent (0.1 N HCl : ACN (80 : 20) % v/v) and mix. Dilute 5.0 ml of above solution to 25 ml with diluent (Water : ACN (50:50) %v/v) and mix. Filter the solution through 0.45 µm Millipore PVDF filter by discarding first 5 ml of the filtrate.

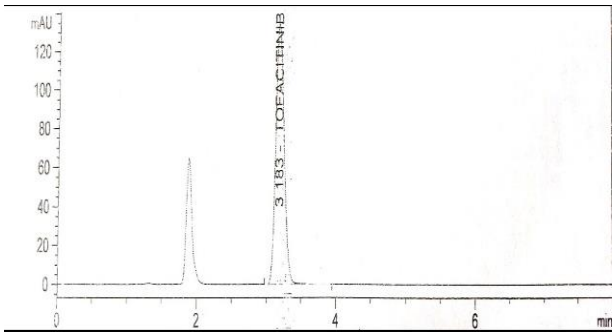

Figure 3: Acid degradation of Tofacitinib

ALKALI DEGRADATION:

Sample preparation:

Weigh accurately 20 tablets and calculate the average weight. Crush the tablet into fine powder and 0mix well. Weight and transfer crushed tablet powder equivalent to 25

mg of tofacitinib and transfer into 50 ml volumetric flask. Add 2.0 ml of ACN and sonicate with shaking to disperse completely. Add accurately 10 ml of diluent (0.1 N HCl : ACN (80 : 20) % v/v) and sonicate with shaking for 60 second at every 10 min for 60 min. Add 3 ml 1 N NAOH solution and keep at room temperature for 5 min. Neutralize with 3.0 ml of 1 N HCl. Make volume up to the mark with diluent (0.1 N HCl:ACN (80 : 20) % v/v) and mix. Dilute 5.0 ml of above solution to 25 ml with diluent (Water : ACN (50:50) %v/v) and mix. Filter the solution through 0.45 µm Millipore PVDF filter by discarding first 5 ml of the filtrate.

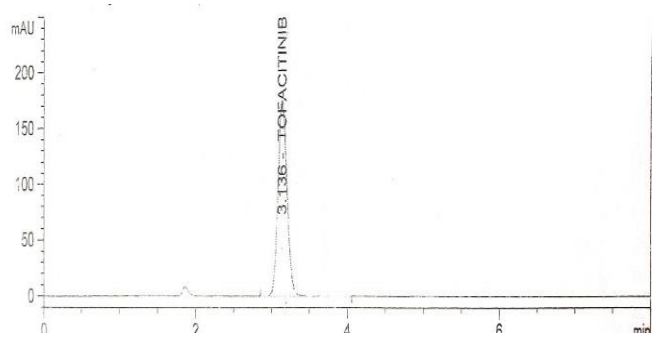

Figure 4: Alkali Degradation of Tofacitinib

OXIDATIVE DEGRADATION:

Sample preparation:

Weigh accurately 20 tablets and calculate the average weight. Crush the tablet into fine powder and mix well. Weight and transfer crushed tablet powder equivalent to 25 mg of tofacitinib and transfer into 50 ml volumetric flask. Add 2.0 ml of ACN and sonicate with shaking to disperse completely. Add accurately 10 ml of diluent (0.1 N HCl : ACN (80 : 20) % v/v) and sonicate with shaking for 60 second at every 10 min for 60 min. Add 5.0 ml 5% H_2O_2 solution and keep at room temperature for 30 min. Make volume up to the mark with diluent (0.1 N HCl : ACN (80 : 20) % v/v) and mix. Dilute 5.0 ml of above solution to 25 ml with diluent (Water : ACN (50:50) %v/v) and mix. Filter the solution through 0.45 µm Millipore PVDF filter by discarding first 5 ml of the filtrate.

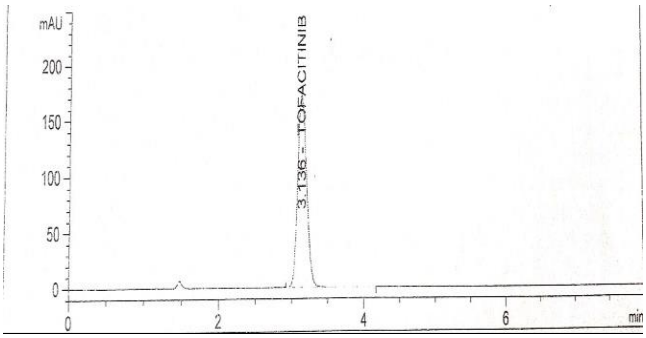

Figure 5: Oxidative Degradation of Tofacitinib

THERMAL DEGRADATION:

Sample preparation:

Weigh accurately 20 tablets and calculate the average weight. Crush the tablet into fine powder and mix well. Weight and transfer crushed tablet powder equivalent to 25 mg of tofacitinib and transfer into 50 ml volumetric flask. Kept the sample at 100^0C in oven for 5 days. Remove and kept a flask until room temperature achieved. Make volume up to the mark with diluent (0.1 N HCl : ACN (80 : 20) % v/v) and mix. Dilute 5.0 ml of above solution to 25 ml with diluent (water : ACN (50:50) %v/v) and mix. Filter the solution through 0.45 µm Millipore PVDF filter by discarding first 5 ml of the filtrate.

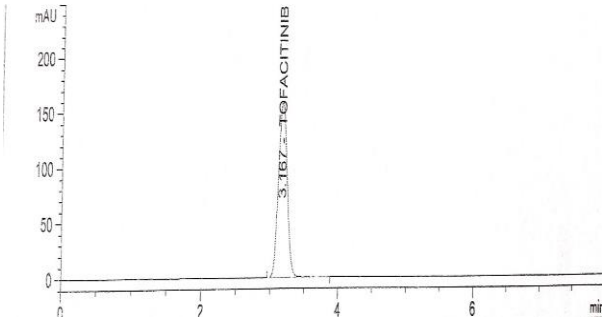

Figure 6: Thermal Degradation of Tofacitinib

UV LIGHT DEGRADATION:

Sample preparation:

Weigh accurately 20 tablets and calculate the average weight. Crush the tablet into fine powder and mix well. Weight and transfer crushed tablet powder equivalent to 25 mg of tofacitinib and transfer into 50 ml volumetric flask. Expose under UV light in a photo stability chamber for exposure up 1.2 million lux hours. Make volume up to the mark with diluent (0.1 N HCl : ACN (80 : 20) % v/v) and mix. Dilute 5.0 ml of above solution to 25 ml with diluent (Water : ACN (50:50) %v/v) and mix. Filter the solution through 0.45 µm Millipore PVDF filter by discarding first 5 ml of the filtrate.

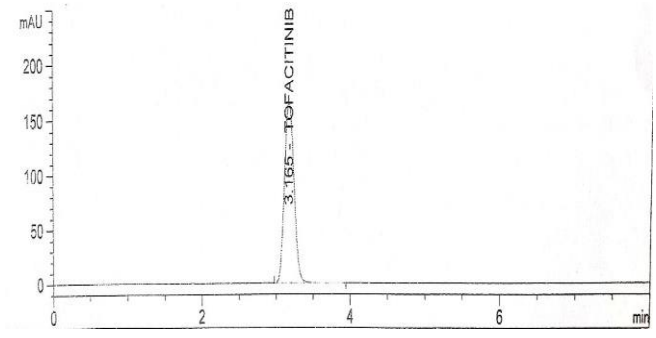

Figure 7: Photolytic Degradation of Tofacitinib

HUMIDITY DEGRADATION:

Sample preparation:

Weigh accurately 20 tablets and calculate the average weight. Crush the tablet into fine powder and mix well. Weight and transfer crushed tablet powder equivalent to 25 mg of tofacitinib and transfer into 50 ml volumetric flask. Kept the sample at 40^0C/75% RH for 5 days. Make volume up to the mark with diluent (0.1 N HCl : ACN (80 : 20) % v/v) and mix. Dilute 5.0 ml of above solution to 25 ml with diluent (Water : ACN (50:50) %v/v) and mix. Filter the solution through 0.45 µm Millipore PVDF filter by discarding first 5 ml of the filtrate.

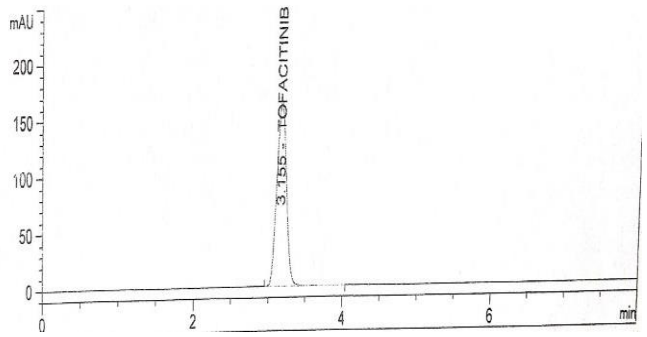

Figure 8: Humidity Degradation of Tofacitinib

Table 1: Degradation Summary

Sr No.	Stress Condition	% As Such Assay	% Degradation	% Assay After Degradation	% Mass Balance	Peak Purity
1	Acid Hydrolysis	100.5	23.1	77.3	100.4	999
2	Alkali Hydrolysis		2.4	98.1	100.5	999
3	Peroxide Degradation		0.0	102.3	102.3	999
4	Thermal Degradation		2.8	97.7	100.5	999
5	Photolytic Degradation		0.0	100.9	100.9	999
6	Humidity Degradation		0.2	100.3	100.5	999

VI. METHOD VALIDATION

SYSTEM SUITABILITY:

System suitability and precision were demonstrated by injecting five replicate injections of standard solution prepared as per the test method. The peak area of analyte of replicate standard injection was recorded. The theoretical plate and tailing factor for analyte peak were evaluated from standard solution. The precision was evaluated by computing the relative standard deviation for the peak area of these replicate injections.

Table 2: System Suitability Parameter

Injection No.	Peak area of Tofacitinib
1	1674.050
2	1674.578
3	1678.252
4	1676.179
5	1671.163
Average	1674.844
% RSD	0.2

Theoretical plates of Tofacitinib peak: 6603
Tailing factor of the Tofacitinib peak: 1.1
The relative standard deviation for five replicates standard injections is not more than 2.0%

METHOD PRECISION:

Method precision was demonstrated by preparing six samples as per the test method representing a single batch. The assay of these samples was determined and the precision and the precision of method was evaluated by computing the percentage relative standard deviation of assay results.

Table 3: Method Precision Parameter

Method Precision	% Assay (Tablet)
1	98.9
2	99.8
3	99.5
4	100.1
5	98.8
6	100.9
Average	99.8
% RSD	0.7

Acceptance criteria: % RSD for assay of six preparations should not more than 2.0.

SONICATION TIME OPTIMIZATION:

The time required to extract the active ingredient (i.e. Tofacitinib) completely from the formulation matrix was optimized as follows:

Three sample preparation were done and sonicate for different time period 30 minutes and 45 minutes and 60 minutes with shaking at every 5min for a period of 1 minute after adding suitable quantity of diluent. After completion of sonication they were suitably diluted and the assay of these samples were determined.

Table 4: Sonication Time Optimization Parameter

Sonication time	% Assay
30 min	100.5
45 min	101.4
60 min	101.4

LINEARITY:

The linearity of detector response for Tofacitinib was demonstrated by preparing solutions of Tofacitinib working standard over the range of 20- 160 % of standard concentrations. These solutions were injected into the HPLC system and the area of analyte peak was recorded. A graph of concentration vs. analyte peak response was plotted. The concentration co efficient between concentration & analyte peak response and Y- intercept of the correlation plot was evaluated. The observations are tabulated in bellow table:

Table 5: Linearity Parameter

Linearity Level (%)	Conc. Of Tofacitinib (µg/ml)	Peak area of analyte
20	21.8679	327.856
50	54.6697	832.083
80	87.4716	1322.519
100	109.3395	1679.307
120	131.2074	1975.344
160	174.9432	2683.069

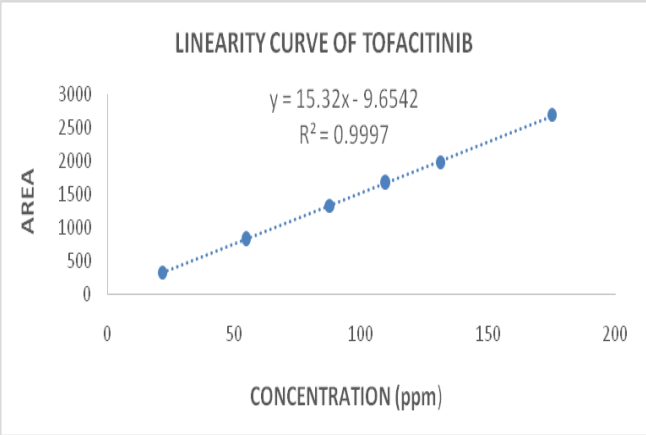

Figure 8: A graph of concentration vs. analyte peak response

The plot was found to be linear with a correlation co efficient 0.999 with respect to 100% linearity level response.

ACCURACY

The accuracy of the test method was demonstrated by preparing recovery samples (i.e. spiking of placebo with known quantities of API at the level of 50%, 100%, 150% of target concentration. The recovery samples were prepared in duplicate. The above samples were chromatograph and the percentage recovery for amount added was estimated. The precision of the recovery was determined by computing the relative standard deviation of duplicate recovery results.

Table 6: Accuracy Parameter

Recovery at 50 % level:

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	89.16	89.53	100.4
2	89.36	91.01	101.3
Average			100.9
% RSD			0.6

Recovery at 100 % level:

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	179.52	182.96	101.3
2	177.13	173.86	98.8
Average			100.1
% RSD			1.8

Recovery at 150 % level:

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	268.28	269.93	100.6
2	267.28	271.83	101.5
Average			101.1
% RSD			0.6

Acceptance criteria: The recovery is 98.0- 102.0 % and the RSD is NMT 2.0 %.

FILTER COMPATIBILITY:

The filter saturation was verified by preparing the assay samples with optimized samples preparation and analyzed the samples by discarding different volume of analyte. The assay of these samples was determined.

Table 7: Filter Compatibility Parameter

Millipore Nylon (0.45 µm)

Discarded volume	% of Tofacitinib	% Difference
Unfiltered	99.8	-NA-
After 1 ml	100.2	-0.4
After 3 ml	100.0	-0.2
After 5 ml	100.1	-0.3
After 7 ml	99.6	0.2
After 9 ml	99.8	0.0

Millipore PVDF (0.45 µm)

Discarded volume	% of Tofacitinib	% Difference
Unfiltered	99.8	-NA-
After 1 ml	97.0	2.8
After 3 ml	98.8	1.0
After 5 ml	100.0	-0.2
After 7 ml	100.1	-0.3
After 9 ml	100.3	-0.5

Millipore PVDF (0.22 µm)

Discarded volume	% of Tofacitinib	% Difference
Unfiltered	99.8	-NA-
After 1 ml	99.7	0.1
After 3 ml	100.1	-0.3
After 5 ml	100.1	-0.3
After 7 ml	100.2	-0.4
After 9 ml	100.3	-0.5

Acceptance criteria: The difference between the unfiltered and filtered samples is NMT 2.0 %.

ROBUSTNESS:

According to robustness there is minor but deliberate change made in chromatographic parameter. To observe robustness, 100% level solution is used.

Table 8: Robustness Parameter

Condition	Theoretical plate	Tailing factor	Retention time(min)	%RSD
Normal	8330	1.1	3.21	0.2
Column temp (35ºC)	7819	1.1	3.25	0.2
Column temp (45ºC)	8605	1.0	3.17	0.3
Organic mobile phase ratio [buffer : ACN (67:33) %v/v]	8552	1.1	3.53	0.6
Organic mobile phase ratio [buffer : ACN (63:37) %v/v]	8056	1.1	2.91	0.2
Flow rate 0.9 ml/min	8810	1.1	3.57	0.3
Flow rate 1.1 ml/min	7790	1.1	2.92	0.2
Mobile phase buffer pH : 5.3	8244	1.1	3.14	0.2
Mobile phase buffer pH : 5.7	8196	1.1	3.27	0.4

STABILITY OF ANALYTICAL SOLUTION:

Stability of standard solution and sample solution were established at room temperature (about 25^0C) as mention below. Standard solution and sample solution were prepared as per test method.

Table 9: Stability of Analytical Solution

For Standard Solution:

Time (hrs.)	Peak area	% deviation from initial area
Initial	1674.050	-
4 HR	1691.800	1.1
7 HR	1681.316	0.4
17 HR	1690.953	1.0
22 HR	1693.093	1.1
27 HR	1703.817	1.8

For Sample Solution:

Time (hrs.)	Peak area	% deviation from initial area
Initial	1687.555	-
3 HR	1692.479	0.3
18 HR	1707.993	1.2
23 HR	1712.451	1.5
27 HR	1716.449	1.7

Acceptance criteria: The response of standard and sample solution should not differ by more than 2.0% from initial response for the accepted storage time.

VII. CONCLUSION

Stability Indicating RP-HPLC method has been developed and validated for the estimation of Tofacitinib from Tofacitinib Citrate Extended Release Tablets. The method was found to be specific for detection of all possible degradation in the dosage form under various stress condition. The present method has been found to be adequately robust and cost effective. The method was validated as per ICH guidelines. All parameter and results are found within the acceptance limit.

So, we can conclude that Developed Stability indicating RP-HPLC method is found to be linear, specific, accurate, robust and cost effective. Thus, the proposed method can be used in routine quality control analysis for estimation of Tofacitinib from Tofacitinib Citrate Extended Release Tablet Dosage Form.

VIII. ACKNOWLEDGEMENT

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