Stability Indicating RP-HPLC Method Development and Validation of Tolterodine Tartrate and its Degradant in its Capsule Dosage Form

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Abstract
Stability indicating reversed phase High Performance Liquid Chromatography (HPLC) method was developed to determine Tolterodine tartrate and its degradants in its capsule dosage form. The chromatographic conditions comprised Inertsil C18 column 3V (250 x 4.6mm), 5µm with a binary mobile phase consisting Buffer solution (3.85 g Ammonium acetate in 1 L of water, (pH 4.5±0.05 using Glacial acetic acid)) as mobile phase A and Acetonitrile (100% v/v) as mobile phase B at a flow rate of 1.0 ml/min. Detection carried out at 290 nm. The retention time of Tolterodine was 20 min. The resolution of Tolterodine tartrate and six impurities (Process and Degradant impurities) was greater than 2.0 for all components. The repeatability and intermediate precision, expressed by the RSD, were less than 2.0%. The calibration plot of standard showed linear relationship with coefficient of regression value r^2 0.99 in the concentration range 2.5-7.5 µg/ml, and for degradants known impurities showed coefficient of regression value r^2 0.99 in the concentration range 5-15 µg/ml. The method was validated as per ICH guideline. The specificity of the method was investigated under different stress conditions including hydrolytic, oxidative, photolytic and thermal. Degradation was found under oxidative condition. All these results provide that the method has stability indicating properties being fit for its intended purpose; it may find application for the analysis of the degradants of Tolterodine tartrate formulations according to ICH Q2 (R1).
Key words: Tolterodine tartrate, RP-HPLC, Stability indicating, Method Development, Degradation impurities.

INTRODUCTION

Tolterodine tartrate, chemically (R)-2[3-[Bis (1-methylethyl) amino]-1-phenylpropyl]-4-methylphenol (Fig. 1) is used in the treatment of urinary incontinence[1]. The drug is listed in Merck index. The empirical formula of Tolterodine tartrate is $C_{26}H_{37}NO_7$ and its molecular weight is 475.57. Tolterodine belongs to a family of drugs called antimuscarinic. The main effects of the Tolterodine are increase in residual urine, reflecting incomplete emptying of the bladder, and a decrease in detrusor pressure used in the treatment of the overactive bladder. The structural formula of Tolterodine tartrate is represented below[2]
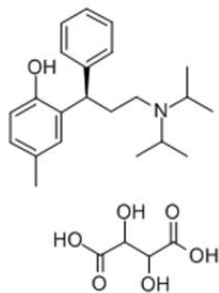

Figure 1- Chemical structure of Tolterodine tartrate

For the newly developed formulation there is no specific method available with a very low interference of placebo with principle peak. The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substance and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance[3]. The aim of the present work is to develop an accurate, specific, reproducible and stability indicating method for the determination of Tolterodine tartrate and related impurities as per ICH guideline[4]. Forced degradation studies were performed on the placebo and the drug product to show the stability indicating nature of the method.

The literature survey revealed various spectrophotometric methods[5, 6] stability-indicating HPLC methods for the quantification of Tolterodine[7-10] and in plasma[11-14] the dosage forms, an enantio-specific HPLC method for the determination of (S)-enantiomer impurities in (R)-Tolterodine tartrate[15], a validated chiral HPLC method for the separation of enantiomers[16], and HPLC methods for the determination of related substances of Tolterodine tartrate[17, 18] have been reported. The present study describes development of a stability indicating RP-HPLC method using USP-Related substance impurity mixture for Tolterodine tartrate and validated using known degradants (Monoisopropyl Tolterodine and Tolterodine Dimer impurity) from that USP-Related substance impurity mixture (Table 1). These studies were performed in accordance with established ICH guidelines[19, 20].

Table 1 Names and structure of known degradant impurities

Impurity name	Structure
Monoisopropyl Tolterodine	
Tolterodine Dimer	

MATERIALS AND METHODS

Chemicals and reagents

Tolterodine tartrate was supplied by Zydus Cadila and capsules (Label claim: 4mg/capsule). Ammonium acetate, Acetonitrile, water, methanol, glacial acetic acid and HPLC grade water was obtained by passage through a Milli-Q system.

Instrumentation and conditions

The apparatus used were Waters (empower software) instrument equipped with an inbuilt solvent degasser, pump, photodiode array detector with variable injector and autosampler. The column Inertsil ODS 3V (250 x 4.6 mm), 5μm was used.

Chromatographic conditions

Chromatographic separation was achieved on reversed phase column using a mobile phase consisting of Buffer solution (3.85g Ammonium acetate in 1 L of water, pH adjusted to 4.5±0.05 using Glacial acetic acid) as mobile phase A and Acetonitrile (100% v/v) as mobile phase B at a flow rate of 1.0 ml/min. The optimized gradient programme (time (min)/%B) was set as 0/30, 25/35, 35/60, 40/70, 55/30, and 60/30. The mobile phase was filtered through 0.45μm membrane filters and degassed. The column temperature was maintained at 30ºC. Detection was carried out at 290 nm. A concentration of the standard is 5 μg/ml and for sample is 1000 μg/ml. The injection volume was 20 μl during whole related substance method development.

Preparation of solutions

Buffer: About 3.85 g of Ammonium acetate dissolved in 1000 ml of water, adjusted to pH 4.5 ± 0.05 with dilute glacial acetic acid solution was used as buffer (mobile phase A).

Diluent: A mixture of analytical solvent Water and Acetonitrile in the ratio of (30:70) %v/v was used as the diluent.

Standard solution: Working standard solution containing 5 μg/ml of Tolterodine tartrate was prepared by weighing accurately 25 mg of Tolterodine tartrate into a 100 ml volumetric flask and further diluted by withdrawing 2 ml from above solution into 100 ml volumetric flask. Solution was diluted up to mark using diluent.

Sample solution: Weighed and transferred the granules from five capsules containing Tolterodine tartrate equivalent to 20 mg in 20 ml volumetric flask. 10-12 ml of diluent was added and sonicated for 45 minutes at frequent time interval shaking until gets dissolved. Diluent was added to make volume up to mark. The solution was mixed well and sonicated at 5000 rpm for 5 minutes. Clear supernatant solution containing 1000 μg/ml of Tolterodine tartrate was injected into HPLC system.

Impurity stock solution: Weighed 5 mg of each impurity (Monoisopropyl Tolterodine and Tolterodine Dimer impurity) in 20 ml volumetric flask separately and make it up to mark with diluent. Take 5 ml from each individual impurity stock solution in 25 ml volumetric flask and make it up to mark with diluent (50 ppm).

Method Validation

Linearity

Impurity Stock solution: Weighed and transferred 5 mg of each individual impurity (Monoisopropyl Tolterodine,

Tolterodine dimer impurity) in 25.0 ml of volumetric flask. 10-12 ml of diluent was added and sonicated until it gets dissolved. Then diluent added to make volume up to mark and mixed well. Concentration of solution was 200μg/ml. Pipette out 5.0 ml from each of above individual impurity stock solution into 20.0 ml of volumetric flask. Diluent added to make it up to mark and mixed well. Final concentration of this linearity stock solution was 50 ppm.

Tolterodine tartrate standard stock solution:

Accurately weighed and transferred 50 mg of Tolterodine tartrate in 100 ml volumetric flask. After adding diluent sonicated for 5 minutes and diluent was added to make it up to mark. From this solution, withdraw 10.0 ml in 100 ml volumetric flask and diluted it up to 100 ml. Final concentration of the solution was 50 ppm.

Linearity test solutions were prepared by diluting the stock solutions to the required concentrations i.e. 2μg/ml, 5μg/ml, 7μg/ml, 10μg/ml, 12μg/ml, and 15μg/ml. The solutions of each impurity with diluted standard were prepared at five concentration levels from the LOQ to 150% of the specification level. Calibration curves were plotted between the responses of the peak versus the analyte concentrations. The coefficient correlation, slope and y- intercept of the calibration curve were reported.

Solution for Limit of Detection and Quantification

The LOD and LOQ for the Tolterodine tartrate and its impurities were determined at a signal to noise ratio of 3:1 and 10:1, respectively by injecting the series of dilute solutions with known concentrations. The recovery study was also carried out at the LOQ level by injecting three replicates spiked sample of Tolterodine tartrate with its impurities and calculating the %RSD of the area.

Precision

The precision of the method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of Tolterodine tartrate drug product spiked with that two degradant impurities; Monoisopropyl Tolterodine and Tolterodine Dimer impurity at the 1.0% specification level (1.0% of impurity with respect to 1000 μg/ml Tolterodine tartrate sample). The RSD (%) of the area for each impurity was calculated. The intermediate precision of the method was also evaluated using a different instrument and performing the analysis on the different days.

Specificity

All the known impurities spiked in the sample preparation were checked for their optimum resolution as well as interference from placebo checked to confirm the specificity of the method.

Accuracy

By spiking the impurity stock solution at three different levels in the sample separately, recovery experiments were conducted to determine the accuracy of the related substance method. The accuracy of the method for Tolterodine tartrate, Monoisopropyl Tolterodine and Tolterodine Dimer impurity was evaluated in triplicate injections using three concentration levels of the 50% (i.e. at LOQ level), 100%, and 150% of the target concentration level resulting in solutions of 5μg/ml, 10μg/ml, 15μg/ml respectively. By spiking impurity stock solution in the

sample recovery study was carried out. The percentage recoveries for each impurity were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the system suitability parameters for the Tolterodine tartrate standard were recorded. The variables evaluated in the study were the pH of the mobile phase (± 0.2 units), column temperature (± 5ºC), and flow rate (± 0.02 ml/min).

Solution stability and mobile phase stability

The solution stability of the Tolterodine tartrate and its impurities were determined by keeping the test and standard solutions in tightly capped volumetric flask at the room temperature for up to 48 hrs and measuring the amount of the known impurities at every 24 hr interval against the freshly prepared standard solution.

Forced degradation studies/Stress studies

Forced degradation studies were performed to evaluate the stability indicating properties and the specificity of the method.

Acid hydrolysis

Weighed and transferred the granules from five capsules containing Tolterodine tartrate equivalent to 20 mg in 20 ml volumetric flask. 10-12 ml of diluent was added and sonicated for 45 minutes at frequent time interval shaking until gets dissolved. 3 ml of 0.5 N and 5 N HCl to the sample separately added and kept it at 80ºC for 2 hr. Cool that sample at room temperature and neutralized by adding respective ml of NaOH. Diluent was added to make it up to mark and centrifuged for 5 minutes. The supernatant was injected in the HPLC system.

Base hydrolysis

Sample was prepared in same way as mentioned above. 0.5 N and 5 N of NaOH was used for the degradation of the sample that is neutralised with respective ml of HCl.

Hydrogen peroxide induced degradation

Sample was prepared in same way as mentioned above. 3 ml of 3%, 10% and 1 ml of 30% H_2O_2 was used for the degradation of the sample separately.

Photochemical degradation

Weighed the sample accurately that is equivalent to 20 mg and kept in UV light for 2 days.

Thermal degradation

Sample was prepared in same way as mentioned above and kept at 100ºC for 3 days.

Note: A peak purity test was carried out for the Tolterodine peak using the PDA detector in the stress samples or peak purity can be showed from purity angle less than that of purity threshold. Placebo interference was evaluated by analysing as per the test method.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The USP-Related substance impurity mixture (Imp-A, Imp-B, Imp-C, Imp-D, Monoisopropyl Tolterodine, Tolterodine Dimer, Tolterodine tartrate) 1 mg/ml was used as system suitability solution for separation during method development.

Inertsil C8 (150 x 4.6 mm) 5µ column and Inertsil C18 (250 x 4.6 mm) 5µ column were tried in which Inertsil C8 150mm column not showing proper separation of all impurities from analyte, so by shifting to Inertsil C18 250 mm column proved to be more effective with optimum resolution among all impurities as well as from the analyte peak.

Different composition of mobile phase were tried and the final optimized gradient is reported in below,

Optimized conditions

Water:Acetonitrile = (30:70) %v/v (Optimized diluent)
Mobile Phase A: Buffer
Mobile Phase B: Acetonitrile (100%v/v)
Oven Temp: 30ºC
Flow Rate: 1.0 ml/min
Detection Wavelength: 290 nm
Column: Inertsil ODS 3V, 250 X 4.6mm, 5µ
Injection Volume: 20 µl
Gradient:

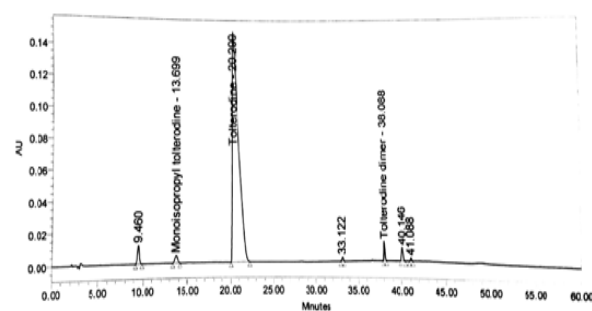**Table 2: Final optimized gradient**

Time(min)	Flow (ml/min)	Solvent (%A)	Solvent (%B)
0	1.0	70	30
25	1.0	65	35
35	1.0	40	60
40	1.0	30	70
55	1.0	70	30
60	1.0	70	30

(Solvent A: Ammonium acetate buffer (pH 4.5); solvent B: Acetonitrile)

Chromatogram for the optimized chromatographic conditions in which all the known impurities were well separated from main drug peak with optimum resolution showed below (figure-2)

Figure-2 chromatogram for optimized conditions

Specificity/ Force degradation study:

The peak purity indices for the analytes in stressed solutions were determined with PDA detector under optimized chromatographic conditions found to be better (purity angle < purity threshold) indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference. Baseline resolution was achieved for all investigated compounds. Degradation was not observed in a Tolterodine tartrate sample during photolytic,

Table 3: Forced Degradation Studies of Tolterodine tartrate & degradants

Sr. No.	Condition	Purity angle	Purity threshold	%Related impurities observed after degradation	%Mass balance
1	5 N HCl, 2Hr, 80ºC	0.689	0.753	0.2	99.4019
2	5 N NaOH, 2Hr, 80ºC	0.879	0.923	0.1	99.4017
3	3%H_2O_2, 2Hr, 80ºC	0.902	1.005	2.76	99.9901
4	10%H_2O_2, 2Hr, 80ºC	0.937	1.028	7.05	100.0251
5	30%H_2O_2, 2Hr, 80ºC	0.907	1.102	13.55	101.5344
6	Thermal	0.526	0.699	0.01	101.01
7	Photolytic	0.652	0.789	0.02	101.03
8	Humidity	0.852	0.958	0.01	101.01

hydrolytic, thermal and humidity stress. About 14% of degradation was observed in oxidative stress. The method is linear in the tested range. Mass balance was checked with respect to different stress conditions and it was also found within the acceptance limits.

Results for mass balance during optimized stress conditions given in the following table- 3
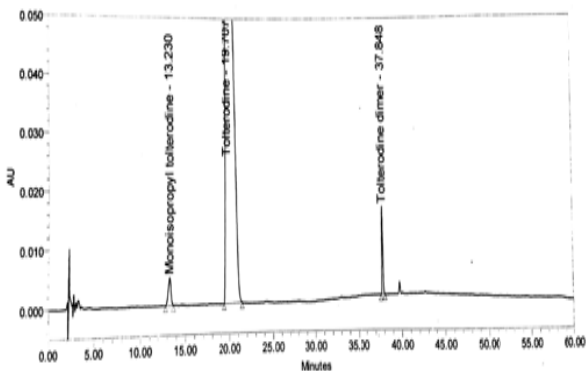
Method validation

The optimized RP-HPLC method validated according to ICH guideline Q2 (R1), with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range, robustness and ruggedness. System suitability features were also assessed.
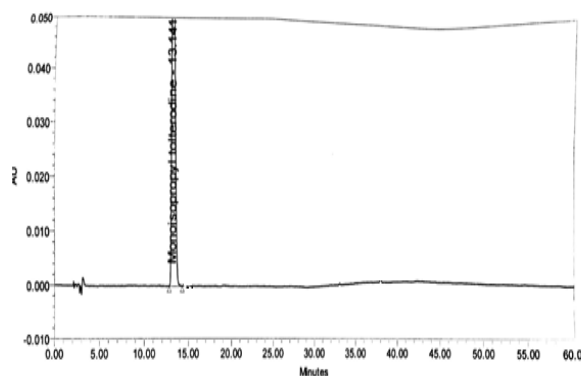
Method precision

ICH (International Conference on Harmonization of technical Requirements for Registration of Pharmaceuticals for Human Use) considers ruggedness as the method reproducibility and intermediate precision. The intermediate precision was determined from the difference in the %RSD of the areas of peaks by performing study on different day. The RSD of the area of Tolterodine tartrate related compounds were within 2.0% showing good intermediate precision.
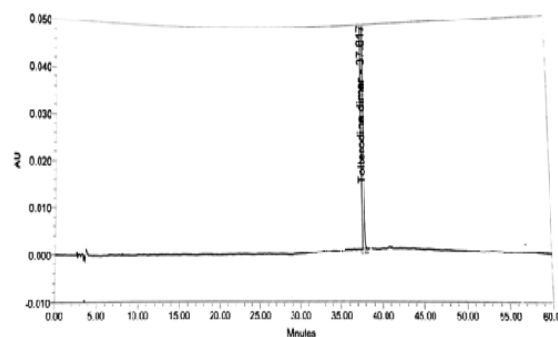
Specificity

The results for impurity spiked sample and individual chromatogram given in the figure 3-5 and its system suitability is given in the Table 4

Figure 3: Chromatogram for sample spiked with impurity

Figure 4: Chromatogram for Monoisopropyl Tolterodine impurity

Figure 5: Chromatogram for Tolterodine Dimer impurity

Table 4: Results of Specificity

Sr. no.	Peak name	RT	Area	Plate count	Resolution
1	Monoisopropyl Tolterodine Impurity	13.60	93378	7357	9.18
2	Tolterodine tartrate	20.20	7559091	25524	6.87
3	Tolterodine dimer Impurity	37.8	104266	9940	19.71

System suitability solution showing the resolution between all peaks found to be optimum. There was no interference of the blank at the retention time of known imp and Tolterodine tartrate.

Table: 5 Summary of Validation parameter

Parameters			Tolterodine tartrate	Monoisopropyl Tolterodine Impurity*	Tolterodine Dimer Impurity*
Linearity(y = mx + c)	(R^2 value)		0.997	0.998	0.999
	Linearity range		2.5-7.5µg/ml	5-15 µg/ml	5-15 µg/ml
	Slope		5827.36	8873	12001.74
	Intercept		1199.95	-3155	-814.43
	LOD		1 µg/ml	2 µg/ml	2 µg/ml
	LOQ		2.5µg/ml	5 µg/ml	5 µg/ml
Precision(%RSD)	Repeatability		0.44	1.83	0.51
	Interday precision	50%	1.87	1.21	1.30
		100%	0.69	0.65	0.26
		150%	0.86	0.87	0.29
	Intraday precision	50%	0.89	0.65	1.01
		100%	0.04	0.31	0.48
		150%	0.03	0.32	0.26
Accuracy(%Recovery)	50%		-	99.10	103.87
	100%		-	99.27	98.31
	150%		-	100.64	99.62
Robustness(%RSD)	Change in flow rate (±0.2ml/min)	0.8	1.81	1.68	0.77
		1.0			
		1.2			
	Change in column tem. (±5ºC)	25	1.57	0.97	1.09
		30			
		35			
	Change in pH of mobile phase	4.3	1.52	1.83	0.90
		4.5			
		4.7			

Note: * indicates known degradant impurities used for validation.

Determination of limit of quantification and detection (LOQ and LOD)
As per the guideline ICH Q2 (R1), LOD is defined as the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The linearity performed, used for the determination of limit of quantification and detection. S/N method was applied and the LOQ and LOD values were predicted and established the precision at these predicted levels. Overall summary on all the validation parameter shown in the table-5

Accuracy
Accuracy was carried out by adding known amount of each related impurities corresponding to different concentration levels of LOQ/50 %, 100 % and 150 % of the specification level in sample solution. The samples were prepared in triplicate at each level. The experimental results revealed that approximately 80–120% recoveries were obtained for all the investigated degradants.

Linearity and range
The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of the analyte in the sample. The linearity of

the test method was established from the LOQ to 150% of the test concentration for Tolterodine tartrate and its degradants. The plots of area under the curve (AUC) of the peak responses of the analytes against their corresponding concentrations, they fitted straight lines responding to equations. The correlation coefficient (r) exceeds 0.98, the acceptance threshold suggested for linearity of procedures for the determination of impurity content in bulk drug and it is found to be 0.999 in all the cases. Calibration curves were represented in following figure,
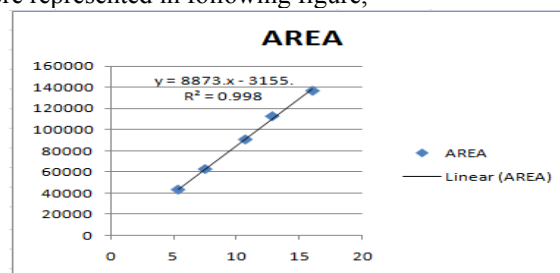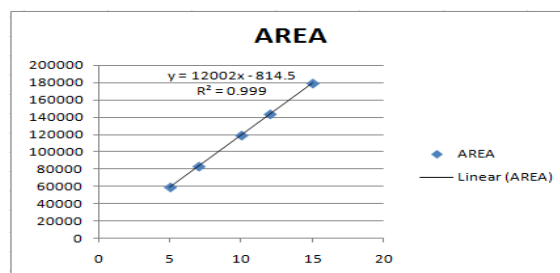

Figure 6: Curve for Monoisopropyl Tolterodine

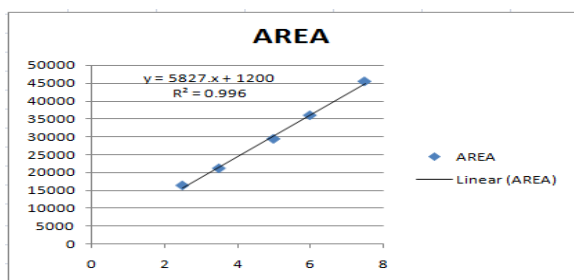**Figure 7: Curve for Tolterodine Dimer impurity**

Figure 8: Curve for Tolterodine standard

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate change in the chromatographic conditions, i.e., change in the flow rate by ±0.2ml/min, change in column oven temperature by ±5°C and change in pH of mobile phase by ±0.2 units. The sample spiked with all known impurities at impurity specification level was injected and the resolution among the impurities was monitored. The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions.

Solution stability

The RSD (%) values of two main degradant impurities (Monoisopropyl Tolterodine and Tolterodine Dimer) during solution stability experiments were within 1.0%. No significant change was observed in the area of impurities during solution stability experiment confirms that sample solutions used during the study were stable up to 48 hours.

CONCLUSION

A stability study was carried out and an efficient HPLC method for the separation of related Substances of Tolterodine tartrate drug product formulation was developed and validated. The results of the stress testing of the drug, undertaken according to the ICH guidelines, revealed the stability indicating nature of the Method. Validation experiments provided proof that the HPLC analytical method is linear in the proposed working range as well as accurate, precise (repeatability and intermediate precision levels) and specific, being able to separate the main drug from its degradation products. The proposed method was also found to be robust with respect to flow rate, column oven temperature and pH of mobile phase. Due to these characteristics, the method has stability indicating properties being fit for its intended purpose; it may find application for the routine analysis of the degradants of Tolterodine tartrate formulations.

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