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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF TRAZODONE IN BULK FORM AND MARKETED TABLET DOSAGE FORM

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ABSTRACT

The present work includes a simple, economic, rapid, accurate and precise isocratic RP-HPLC method development for estimation of Trazodone in bulk form and its marketed formulation. Estimation was done at 286nm which was found to be λmax of Trazodone. The simple, selective, isocratic RP-HPLC method for Trazodone was developed on Phenomenex Luna (C_{18}) RP Column; 250 mm x 4.6 mm, 5µm with a mobile phase of Phosphate Buffer (pH-4.6) and Methanol were taken in the ratio of 65:35% v/v at a flow rate of 1.0 ml/min and detection wavelength 286nm. The developed method was validated successfully according to ICH Q2 (R1) guidelines. The chromatographic methods showed a good linear response with r2 values of 0.9995. The percentage relative standard deviation for method was found to be less than two, indicating that the methods were precise. The mean percentage recovery was for RP-HPLC method was 100.437%. From the results it could be concluded that both the developed method was specific, selective and robust. The method could be successfully applied for analysis of Bulk form and Marketed formulation of Trazodone.

KEYWORDS: Trazodone, RP-HPLC, Method Development, Validation, ICH Guidelines.

INTRODUCTION

Trazodone[1] is triazolopyridine derivative from the serotonin receptor antagonists and reuptake inhibitors (SARIs) class of antidepressants. It is used in adults and has been shown to be comparable in efficacy to other drugs such as tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine receptor inhibitor (SNRIs) in the treatment of depression.20 A unique feature of this drug is that it does not promote the anxiety symptoms, sexual symptoms, or insomnia, which are commonly associated with SSRI and SNRI therapy. Trazodone acts on various receptors, including certain histamine, serotonin, and adrenergic receptors, distinguishing it from other antidepressants that cover a narrow range of neurotransmitters. Trazodone[2] treats depressed mood and other depression-related symptoms and shows benefit in the treatment of insomnia due to its sedating effects. It is known to prolong the cardiac QT-interval. Memory, alertness, and cognition may be decreased by Trazodone, especially in elderly patients due to its central nervous system depressant effects. Trazodone is an antidepressant medicine. It's used to treat depression, anxiety, or a combination of depression and anxiety. Trazodone[3] works by increasing your levels of serotonin and noradrenaline so you feel better. It can help if you're having problems like low mood, not sleeping (insomnia) and poor concentration. The IUPAC name of Trazodone is 2-[3-[4-(3-chloro phenyl) piperazin-1-yl] propyl]-[1, 2, 4] triazole [4, 3-a] pyridin-3-one. The Chemical Structure of Trazodone is shown in follows.

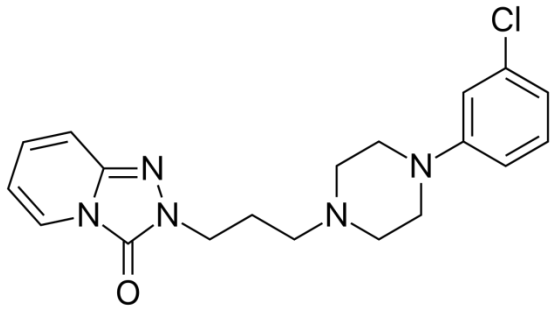

Fig-1: Chemical Structure of Trazodone.

MATERIALS AND METHODS

Instruments Used

Table 1: List of Instrument used.

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Symmetry ODS RP C_{18}, 5mm, 15mm x 4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Chemicals / Reagents Used

Table 2: List of Chemicals used.

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai
7.	Ethanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai
8.	Octanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Method Development and its Validation for Trazodone by RP-HPLC

Method Development

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase[4] diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Trazodone, so that the same wave number can be utilized in HPLC UV detector for estimating the Trazodone. The scanned UV spectrum is attached in the following page.

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Trazodone standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. The UV spectrum[5] of Trazodone was shown in fig-2.

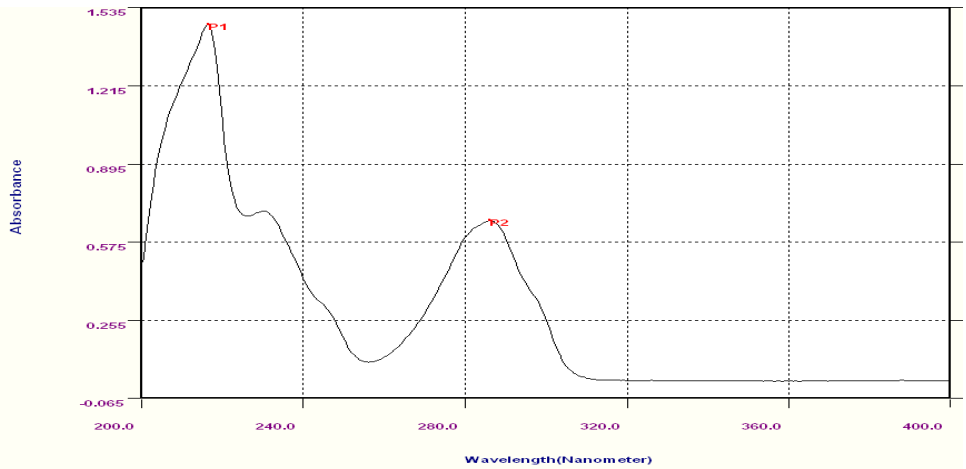

Fig-2: UV Spectrum for Trazodone.

Observation: While scanning the Trazodone solution we observed the maxima at 286nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.

Optimization of Chromatographic Conditions: The chromatographic conditions[6] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection

wavelength & different diluents for sample preparation etc.

Preparation of Mobile Phase: 650ml of prepared phosphate buffer and 350ml of HPLC Grade Methanol were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Method Validation

Method validation[7-12] is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

1. Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity[13] of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

2. Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

3. Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.[14] Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

3.1. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability[15] is also termed intra-assay precision.

3.2. Intermediate precision: Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

3.3. Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

4. Detection Limit: 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.

5. Quantitation Limit: 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.

6. Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte[16] in the sample.

7. Range: The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure[17] has a suitable level of precision, accuracy and linearity.

8. Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters[18] and provides an indication of its reliability during normal usage.

9. System Suitability Parameters: The theory of chromatography[19] has been used as the basis for system-suitability tests, which are set of quantitative criteria that test the suitability of the chromatographic system to identify and quantify drug related samples by HPLC at any step of the pharmaceutical analysis.

RESULTS AND DISCUSSION

Method Development

Summary of Optimized Chromatographic Conditions: The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table 3: Summary of optimized Chromatographic Conditions.

Mobile phase	Phosphate Buffer (pH-4.6) : Methanol = 65:35%
Column	Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	286 nm
Flow rate	1.0 ml/ min.
Run time	10 min.

Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic
Retention time	4.862 minutes

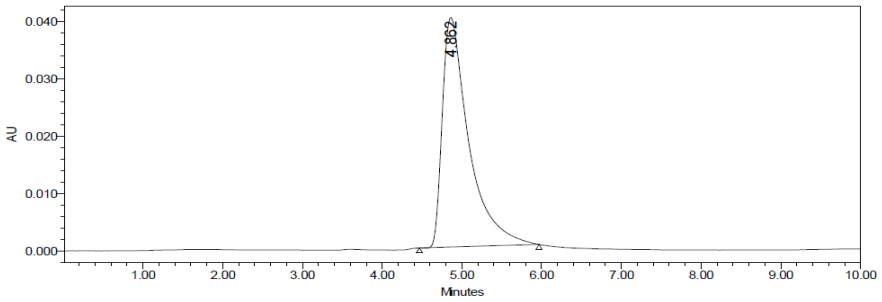

Fig-3: Chromatogram of Trazodone in Optimized Condition.

Observation: The selected and optimized mobile phase was Phosphate Buffer (pH-4.6): Methanol = 65:35% and conditions optimized were flow rate (1.0 ml/minute), wavelength (286nm), Run time was 10 mins. Here the peaks were separated and showed better resolution[20], theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

Validation of Method

The optimized method for determination of Trazodone has been validated as per International Conference of Harmonisation (ICH) guidelines Q2 (R1)[27,32] for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

1. System Suitability Test

System suitability testing is an integral part of many analytical procedures.[21] The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-4.

Table 4: Data of System Suitability Parameter.

S.No.	Parameter	Limit	Result
1	Retention Time	RT > 2	Trazodone= 4.778
2	Asymmetry	T ≤ 2	Trazodone= 1.35
3	Theoretical plate	N > 2000	Trazodone= 6859
4	Tailing Factor	T<2	Trazodone= 1.37

2. Linearity

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 60-140µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve[22] was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

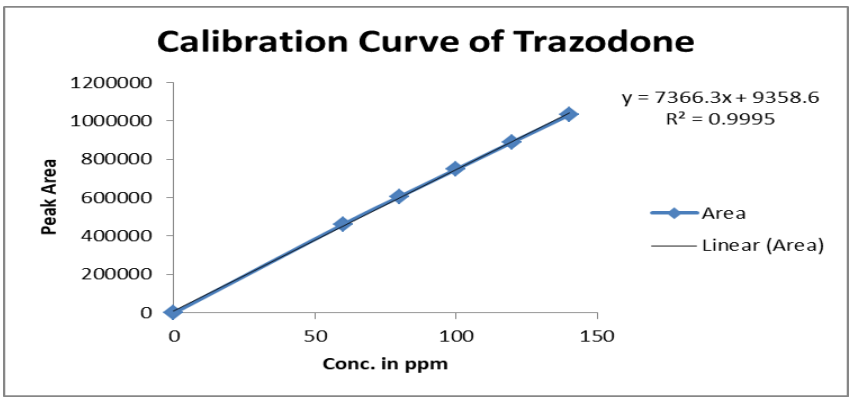

Fig-4: Calibration Curve of Trazodone.

Table 5: Linearity Data for Trazodone.

Conc. (µg/ml)	Area
0	0
60	461404
80	606157
100	748506
120	891041
140	1032196

3. Accuracy: The accuracy of the method was determined by recovery studies[23] and the percentage recovery[24] was calculated. The recoveries of Trazodone were found to be in the range of 99-102%. The proposed Liquid Chromatographic method was applied to the determination of Trazodone. The results for Trazodone comparable with the corresponding labeled amounts.

Table 6: Shown Accuracy Observation of Trazodone.

Accuracy	Amount Added	Amount Recovered	Peak Area	% Recovery	Mean Recovery
80%	80	80.798	604517	100.997	100.437%
	80	80.673	603598	100.841	
	80	80.756	604213	100.945	
100%	100	99.933	745471	99.933	
	100	100.083	746574	100.083	
	100	100.365	748652	100.365	
120%	120	120.290	895415	100.241	
	120	120.201	894762	100.167	
	120	120.442	896541	100.368	

4. Precision

Repeatability: The precision of each method was ascertained separately from the peak areas & retention times[25] obtained by actual determination of six replicates of a fixed amount of drug. Trazodone (API). The percent relative standard deviation was calculated for Trazodone are presented in the table-7.

Table 7: Repeatability Data for Trazodone.

S. No.	INJECTION	PEAK AREA
1	Injection 1	743826
2	Injection 2	745277
3	Injection 3	742506
4	Injection 4	747576
5	Injection 5	746715
6	Injection 6	741278
7	**Average**	**744529.6667**
8	**SD**	**2440.4116**
9	**% RSD**	**0.32777**

Intermediate Precision

The Intermediate Precision[26] consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table 8: Results of Intra-Assay & Inter-Assay.

Conc. of Trazodone (API) (µg/ml)	Observed Conc. of Trazodone (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
80	80.096	0.487	79.685	0.688
100	100.074	0.968	100.057	0.789
120	120.056	0.847	120.016	0.698

Observations: The intra & inter day variation[27] of the method was carried out for standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Trazodone revealed that the proposed method is precise.

5. Specificity

Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.

6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ[28] parameter was evaluated by mistreatment the slope of line and variance obtained from accuracy studies.

The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.469 & 4.454µg/ml respectively.

7. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate 1.0 ml (± 0.1ml/min), Wavelength of detection 286 (±2nm) & organic phase content in mobile phase (±5%) studied to determine the robustness[29] of the method are also in favour of (Table-9, % RSD < 2%) the developed RP-HPLC method for the analysis of Trazodone (API).

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead of 35:65, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Table 9: Results for Robustness.

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	742946	4.778	1.37	2896
Less Flow rate of 0.9 mL/min	698965	4.783	1.39	2986
More Flow rate of 1.1 mL/min	786598	4.817	1.42	2985
Less organic phase	732642	4.842	1.29	3102
More organic phase	702546	4.773	1.37	3247

8. Estimation of Trazodone in Pharmaceutical Dosage Form

Label Claim: 16mg

Each tablet contains: 16mg

Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system[30] and the observations were recorded. The data are shown in Table-10.

ASSAY

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg Wt.} = \text{mg}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Table 10: Recovery Data for estimation Trazodone in Trazonil-50 Tablets.

Brand name of Trazodone	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Trazonil-50 Tab (Intas Pharmaceuticals)	50mg	49.558 (± 0.468)	99.825 (± 0.418)

Result & Discussion: The amount of drug[31] in Trazonil-50 Tablets was found to be 49.558 (± 0.468) mg/tab for Trazodone & % Purity was 99.825 %.

Stability Studies

Results of Degradation Studies: The results of the strain studies indicated the specificity of the tactic that has been developed. Trazodone was stable in oxidation and thermal stress conditions. The results of forced degradation studies[32] are given in the following table-11.

Table 11: Results of Forced Degradation Studies of Trazodone API.

Stress Condition	Time in hrs	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	92.406	7.594	100.0
Basic Hydrolysis (0.1 M NaOH)	24Hrs.	95.314	4.686	100.0
Wet heat	24Hrs.	93.241	6.759	100.0
UV (254nm)	24Hrs.	89.342	10.658	100.0
3 % Hydrogen peroxide	24Hrs.	90.355	9.645	100.0

SUMMARY

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Trazodone, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Phenomenex Luna (C18) RP Column, 250 mm x 4.6 mm, 5µm Column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, water, 0.1N NaOH, 0.1NHCl). Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Trazodone it is evident that most of the HPLC work can be accomplished in the wavelength range of 286 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay and stability related impurity studies which can help in the analysis of Trazodone in different formulations.

CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Trazodone in bulk and pharmaceutical dosage form. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Trazodone indicated that the developed method is specific for the simultaneous estimation of Trazodone in the bulk and pharmaceutical dosage forms. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The specific Retention time for Trazodone are found to be 4.778min. The tailing factor was found to be 1.37 with theoretical plates 6859 for Trazodone. The %Recoveries was determined as 100.437% for Trazodone in Accuracy. The %RSD in Repeatability is 0.327 with Intermediate Precision (Intra & Inter Day) are 0.767 & 0.725 for Trazodone in Precision respectively. In Linearity, the correlation coefficient was found to be 0.9995 for Trazodone. The LOD for Trazodone was 1.469 and LOQ for Trazodone are 4.454 respectively.

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