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SPECTROPHOTOMETRIC QUANTIFICATION OF VILAZODONE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM USING QUALITY BY DESIGN APPROACH

(Kuantifikasi Spektrofotometri Vilazodon Hidroklorida dalam Bentuk Dos Farmaseutikal Menggunakan Pendekatan Reka Bentuk Kualiti)

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Abstract

The present work deals with development and validation of a novel, robust, precise and accurate spectrophotometric method, for the estimation of vilazodone hydrochloride in tablets using the principle of Quality by Design (QbD). A fractional factorial design (FFD) was employed for initial parameter screening. Further the screened parameters were subjected to central composite design (CCD) for evaluating method robustness and method optimization. Different statistical parameters were evaluated to decide appropriateness of experimental data. Vilazodone shows absorption maximum at 285nm using methanol. Factor screening slit width and sampling interval were identified as critical method variables, which were further evaluated by a CCD. Good linearity was obtained for vilazodone in the range of 5-60 µg/mL with $R^2 > 0.999$. The method was found to be accurate with good average % recovery (more than 100%). Developed method was validated as per ICH guidelines. Based on QbD development of spectrophotometric method ensured that quality is built into the method. The method was robust and can be applied for determination of the vilazodone in pharmaceutical dosage form.

Keywords: vilazodone hydrochloride, spectrophotometric, quality by design, validation

Abstrak

Kajian ini melibatkan pembangunan dan validasi kaedah spektrofotometri yang asli, teguh, jitu dan tepat bagi anggaran kandungan vilazodon hidroklorida di dalam tablet dalam menggunakan prinsip reka bentuk kualiti (QbD). Satu reka bentuk faktor pecahan (FFD) telah digunakan untuk saringan awal parameter. Selanjutnya parameter yang diuji adalah tertakluk kepada analisis reka bentuk komposit berpusat (CCD) untuk menilai pengoptimunan dan keteguhan. Ujian statistik yang berbeza telah dinilai untuk menentukan kesesuaian data eksperimen. Vilazodon menunjukkan penyerapan maksimum pada 285nm menggunakan metanol. Saringan faktor lebar celah dan selang pensampelan telah dikenal pasti sebagai pembolehubah yang kritikal, yang kemudian dinilai lebih lanjut melalui CCD. Kelinearan baik telah diperolehi bagi vilazodon bagi julat di antara 5-60 µg/mL dengan $R^2 > 0.999$. Kaedah ini didapati tepat dengan % perolehan semula mencapai skor yang baik (lebih daripada 100 %). Kaedah telah ditentusahkan menurut peraturan ICH. Berdasarkan pembangunan QbD terhadap kaedah spektrofotometri, ia dipastikan bahawa kualiti analisis terbina ke dalam kaedah ini. Kaedah ini adalah teguh dan boleh digunakan untuk penentuan vilazodon dalam bentuk dos farmaseutikal.

Kata kunci: vilazodon hidroklorida, spektrofotometri, reka bentuk kualiti, validasi

Introduction

Vilazodone, 5-[4-[4-(5- cyano-1H-indol-3-yl) butyl]-1-piperazinyl]-2-benzofurancarboxamide, hydrochloride (1:1) (Figure 1) is a novel antidepressant agent used in treatment of major depressive disorders [1-3]. It acts as a serotonin partial agonist and reuptake inhibitor [4]. It is found to improve anxiety symptoms and exhibits sustained response in patients with major depressive disorder [5, 6].Vilazodone is also found to produce favorable sexual profile in male rats [7].

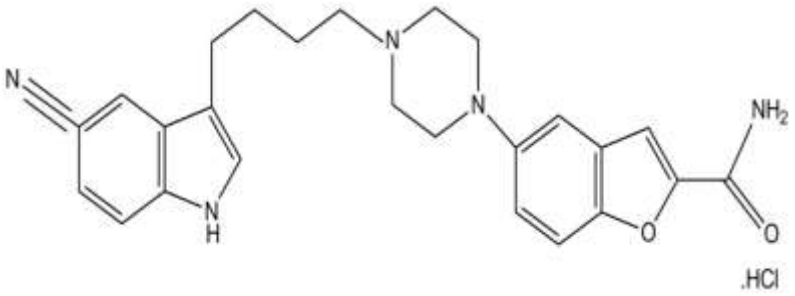

Figure 1. Chemical structure of vilazodone hydrochloride

Vilazodone hydrochloride in dosage forms and biological samples is estimated by UV-visible spectroscopy [8], HPLC [9, 10] and LC-MS [11] methods. However, the reported UV spectrophotometric method has many drawbacks like narrow linearity range, absence of sandells sensitivity and inability to present molar extinction coefficient etc. Thus, attempts were taken for developing improved and new UV spectrophotometric method for quantification of vilazodone in tablet formulation using Quality-by-Design (QbD) approach.

Quality by Design (QbD) is a collective approach which ensures quality is built into the process to get the intended result. According to ICH-Q8-(R2) QbD is "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"[12]. QbD found its birth after the implementation of "Pharmaceutical Current Good Manufacturing Practices (cGMPs) for the 21st Century" by US Food and Drug Administration in 2002[13]. Analytical QbD involves six steps for holistic development of an analytical method with enhanced performance coupled with high robustness [14].

Employing QbD approach not only decreases the time required for development of an efficient analytical method but also is considered to be a cost-effective way to ensure quality right from the beginning of method development view point. Design of Experiments (DoE) being an indispensable part of QbD provides a robust design space for optimum method performance. The present research, in this regard, is specifically based on the appliance of rational experimental designs for reducing variability associated while spectrophotometric measurement of vilazodone to identify the optimal solutions. At first the factor screening study was performed using fractional factorial design (FFD) for identifying the critical method parameters influencing the method performance followed by method optimization with the help of central composite design (CCD) for assuring the robustness, with in the predefined objectives. The objective of this study was to develop a new, precise and accurate UV spectrophotometric method for quantification of vilazodone in tablet dosage form using the QbD-based method development and validating the developed method as per ICH guidance [15].

Materials and Methods

Reagents and Standards

Pure standard drug of vilazodone hydrochloride (purity > 99.5%) was procured as gift sample from Glenmark Pharmaceuticals Ltd., India. Methanol was purchased from Merck Ltd., Mumbai, India and was used for preparing drug and reagent solutions. As the marketed tablet formulation of vilazodone hydrochloride (40mg) was not available in the local market, in-house tablets were prepared and analyzed by the current developed method.

Instrumentation and Optical Characteristics

A Shimadzu 1800 UV spectrophotometer (Kyoto, Japan) with 10mm matched quartz cuvettes were used for spectral measurements. A high precision analytical balance was used for weighing the reagents. Ultrasonicatior (Enertech,India) was used to affect dissolution of tablet formulation.

Establishment of Analytical Target Profile

A thorough survey of available literature reports and drug profile (physical and chemical properties) was carried out for establishment of analytical target profile containing a dynamic summary of quality characteristics of an analytical method. This primarily encompassed development of a fast, reliable and cost-effective analytical method for estimation of vilazodone in pharmaceutical dosage form. Therefore, based on the prime intent of this study, an UV spectrophotometric method was adopted for rapid analysis of vilazodone. The rationale justification of selection of UV spectrophotometric method was owing to simple and rapid analysis of drug as compared to other sophisticated analytical methods.

Establishment of cause-effect relationship and Risk Management

Ishikawa fish-bone diagram being one of the simplest tools is helpful in understanding the cause-and-effect relationship among the potential method factors, which may affect the method performance. In this regard, the fish-bone diagram was drawn (not shown in figure) by highlighting diverse method variables plausibly influencing the method attributes of UV spectrophotometric method of vilazodone. In the present studies, a Cause-Effect Risk Assessment Matrix with CNX (Control-Noise-Experimentation) approach was utilized for identifying the high risk variable affecting the analytical attributes. Critical method variables (CMVs) viz. variation in solvent used, detection wavelength, scan speed, sampling interval, sample integrity and slit width were found to be associated with high final scores indicating high risk variables. Further, the CMVs were evaluated using a screening design to identify the critical method parameters (CMPs) and then subjected to response surface optimization using suitable experimental design.

Screening of Critical Method Variables by Fractional Factorial Design

Critical parameters were screened using FFD (Trial Version JMP software v.11, SAS Institute, Inc., NC, USA) for identifying the high risk variables. By comparing the spectral shape, sharpness and absorbance, few parameters were selected as critical method variables. Discerned from the prioritization studies based on the prior knowledge and Ishikawa fish-bone diagram, solvent type, detection wavelength and sample integrity were evaluated based on physical observation. However, the method variables i.e. scan speed (X1), slit width (X2) and sampling interval (X3) were screened by a FFD with minimal 5 runs (1 center point) using JMP software. The variables were experimented at their high and low levels. Then the script was run to obtain the critical method variables affecting the variable response absorbance (Y). Screening for identifying CMVs was carried out by evaluation of actual vs. predicted plot, summary of fit plot, Pareto chart and prediction expression.

Method optimization and robustness study using Central Composite Design

Central Composite Design was applied to assure the method robustness for identifying optimized method conditions. Ten experimental runs were obtained with minimal two center points according to the CCD to optimize CMVs such as slit width (A) and sampling interval (B), as obtained from the screening studies. The obtained experimental runs were evaluated for absorbance at 285nm as response variable. A standard vilazodone 10 µg/mL was used for all the experimental runs.

Data obtained from the experiment was fitted to a suitable mathematical model by multiple linear regression analysis (MLRA) using JMP software. The developed model was allowed to study both the main effects and interaction effects. Only the coefficients of model terms found to be significant ($p<0.05$) as per ANOVA analyses were considered in framing the polynomial equation and analyzing the model for parameters like comparing the actual versus predicted plot, summary of fit, analysis of variance (ANOVA) followed by analysis of parameters like coefficient of correlation (R^2), adjusted and predicted R^2, predicted residual sum of squares (PRESS), respectively. Besides, other vital tools like prediction profiler, interaction profiler, 2-D contour plot and 3-D response surface

profiler were used to decide aptness of the model. Optimal solution was identified by numerical desirability function by trading-off the studied factors for the responses followed by demarcation of the same in the design space region.

Method Control Strategy
Method control strategies were developed based on the design space generated by the DoE approach, within which the slight variations in method performance was allowed to maintain the method robustness.

Preparation of Standard Stock Solution
Standard stock solution of vilazodone (1000µg/mL) was prepared by dissolving accurately weighed 25mg of vilazodone in methanol up to 25mL. From this standard stock, 2.5mL of the solution was taken into a 25mL volumetric flask and was diluted upto 100mL to produce working standard solutions of concentration 100 µg/mL.

Analysis of Tablet Dosage Forms
Twenty tablets were weighed and equivalent weight was calculated from the average weight. The tablets were then finely ground to powder. A quantity of tablet powder equivalent to 25 mg of vilazodone was accurately weighed and transferred into a 25 mL volumetric flask, 10 mL of methanol was added and content was ultrasonicated for 30 min. Final volume was made up with methanol and mixed well. This solution was further filtered by using Whattmann filter paper to remove particulate matter, if any. The filtered solution was further diluted with methanol for analysis. Drug present in the sample solution was determined using the calibration curve of standard vilazodone.

Method Validation: Specificity
Specificity of the UV spectrophotometric method was determined based on the determination of drug in presence of its formulation excipients. Spectrums were evaluated for the possible interference due to the excipients.

Linearity
Different aliquots were taken from the working standard of vilazodone in separate 10mL volumetric flasks and finally diluted with methanol to prepare a series of concentrations ranging from 5-60 µg/mL. UV absorbance was measured at 285 nm. Calibration curve was plotted to evaluate the linearity by taking the absorbance on the y-axis and the concentration (µg /mL) on the x-axis.

Accuracy and Precision
To find out accuracy of the method, recovery studies were carried out at 80,100 and 120% of the test concentration (20µg/mL) of vilazodone by standard addition method. The recovery study was performed triplicate at each level. Vilazodone standard drug added to the recovery solutions was calculated using calibration curve. To assess the intraday and interday precision, six replicates of a fixed concentration of vilazodone (30µg/mL) were scanned on the same day and a different day and percent RSD values were calculated.

Results and Discussion

In this study, an UV spectrophotometric method has been developed to determine the amount of vilazodone present in tablet formulation. QbD approach was utilised to obtain the variable parameters for developing final spectrophotometric conditions. A typical Ishikawa fish-bone diagram was developed for identifying the method variables. Based on the C-E Risk Assessment matrix (Table 1) employing C-N-X approach total scores were calculated for different method variables and were prioritized. Physical evaluation of the method variables was carried out. The drug was found to be insoluble in 0.1N HCl, 0.1N NaOH and water. However, vilazodone was clearly soluble in methanol. Hence, methanol was selected as suitable solvent system for further studies. Standard vilazodone solution shows absorption maximum (λmax) at 285nm in methanol (Figure 2) and it was selected as detection wavelength.

Table 1. Cause-Effect based risk assessment

Cause	Effect of Risk Level on Absorbance	Total Score	C,N,X	Strategy
Scan Speed	10	100	X	DoE
Slit Width	10	100	X	DoE
Sampling Interval	10	100	X	DoE
Solvent	5	50	C	Controlled
Detection Wavelength	4	40	C	285nm
Sample Integrity	4	40	N	Quality Assessed
Sample Preparation	4	40	C	Controlled
Detector Equilibration	3	30	C	Controlled

C, N, X- Controlled, Noise, Experimental, Total Score = (Risk Level of CMA×10), Score: 1-Negligible Risk, 5-Low Risk, 10 – High Risk

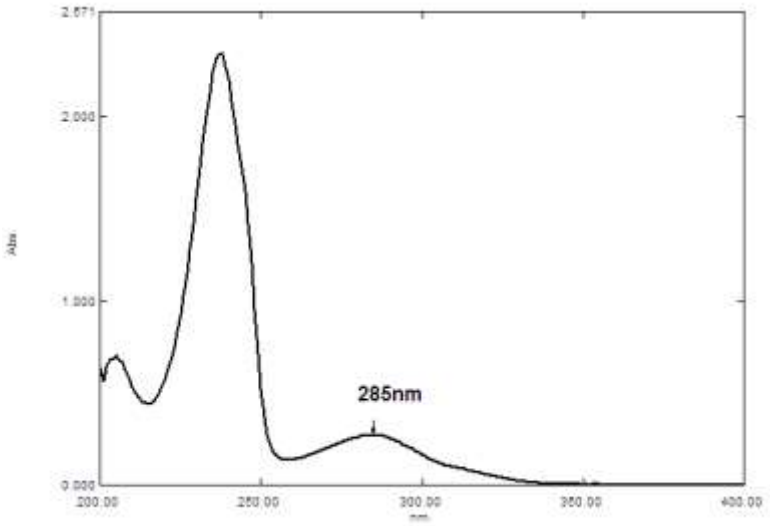

Figure 2. Typical UV absorption spectrum of vilazodone hydrochloride

The sample integrity was found satisfactory as per the melting point test. However, the method variables like scan speed, sampling interval and slit width needed a systematic study to determine their effect on method robustness. The use of FFD approach helped in screening the CMVs out of scan speed, sampling interval and slit width. Evaluation model using actual *vs.* predicted plot revealed apt fitness of the adopted model. The model p-value (0.0959), R^2 (0.99) and RMSE (0.0002) also suggested model aptness. Evaluation of summary of fit revealed satisfactory R^2 (0.9943) and adjusted R^2 (0.9772) values. The Pareto plot (not shown) suggested that slit width (X_2) and sampling interval (X_3) were the two CMVs with significant influence on the absorbance (Y) as the response variable. An analogous interpretation was noticed in the prediction expression of the selected model (Equation 1), confirming the report of Pareto plot.

$$Absorbance\ (Y) = 0.2708 + 0.00025X_1 + 0.00125X_2 + 0.00075X_3 \quad (1)$$

where, X_1=Scanning speed, X_2=Slit width and X_3=Sampling interval.

The CCD was used to evaluate the effect of CMVs on response absorbance. Ten experiments were performed on a UV spectrophotometer in a randomized order to obtain bias free response with minimal two center points. Response obtained for each of the experiments and the spectrophotometric range studied is listed in Table 2.

Table 2. Experimental design matrix showing spectrophotometric range studied for robustness study and obtained responses

Run No	**Slit Width (A)**	**Sampling Interval (B)**	**Absorbance (Y)**
1	-1	1	0.268
2	-1	-1	0.269
3	0	0	0.271
4	1	0	0.273
5	0	0	0.271
6	1	-1	0.272
7	1	1	0.269
8	0	1	0.268
9	0	-1	0.27
10	-1	0	0.27
Coded Level	**-1 (Low)**	**0 (Nominal)**	**+1 (High)**
Variable A	0.5	1.0	2.0
Variable B	0.5	1.0	2.0

P-value below 0.05 was set as a criterion for accepting the null hypothesis (H_0). A thorough analysis of the CCD model using different statistical analysis tools was carried out and inferences were drawn through ANOVA, parameter estimates, Pareto plot and prediction profiler. Figure 3 illustrates the baseline model (blue line) in actual versus predicted plot, where the obtained line for the experimental data was found to be well within the limit or boundaries of confidence intervals. This rejects the null hypothesis, as the model effectively describes variation in data where the predicted and observed data were found to be quite analogous. Further, ANOVA suggested the P-value is less than 0.0008, indicating the model appropriateness to address the variability and suggests rejecting the null hypothesis. Besides, the lower values for predicted residual sum of squares (PRESS) also ratified the model suitability.

For assessing the variability risk from different variables evaluation of parameter estimates is crucial. An observed p-value less than 0.05 suggest a non-zero value of slope. Sampling interval × sampling interval (B^2) and slit width (A) were found to be the most influencing method variables. The Pareto plot revealed that the earlier one has more significant effect on absorbance (Figure 4). The prediction expression revealed an analogous result to Pareto plot. The expression for adopted model is given below:

$$Absorbance\ (Y) = 0.271 + 0.0011667A - 0.001B + 0.0005AB + 0.0005A^2 - 0.002B^2 \quad (2)$$

where, A=Slit Width, B=Sampling Interval.

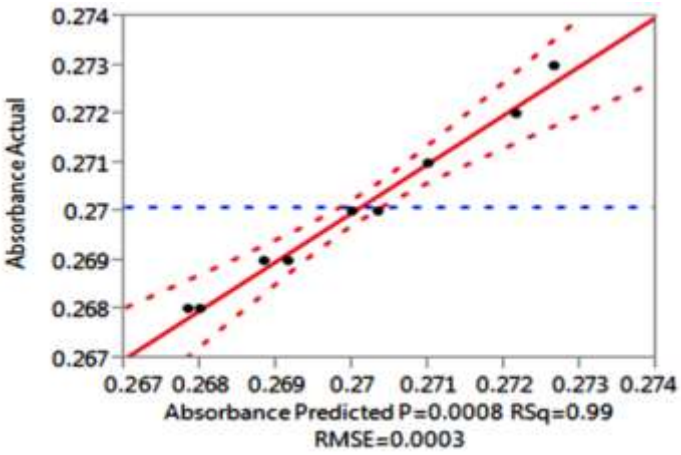

Figure 3. Actual *vs.* predicted plot for robustness study

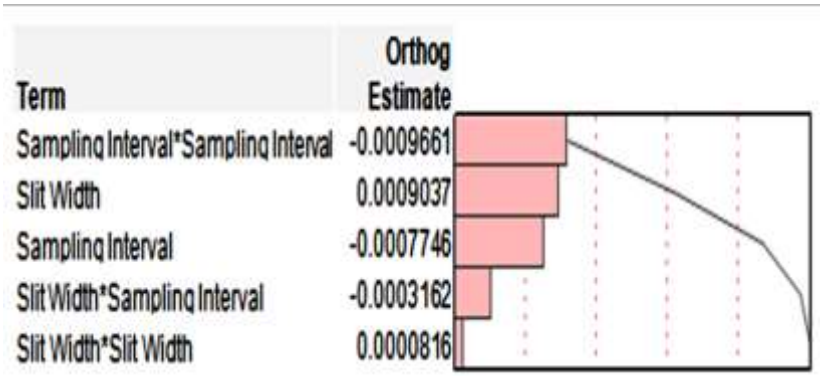

Figure 4. Pareto plot showing effect of slit width and sampling interval on absorbance

The interaction effect analysis was carried out employing interaction plots (not shown in figures), which explains a nonlinear pattern among all the studied factors on the response variable. The response surface analysis was carried out employing 3D response surface plot, which showed higher values for the response (i.e., absorbance) at higher levels of slit width and lower levels of sampling interval, as illustrated in Figure 4. However, a typical "saddle point" is noticed at lower levels of slit width and intermediate levels of sampling interval. Further, the data analysis employing the prediction profiler plot (not shown in figure) revealed a nonlinear type trend for both the factors, i.e. slit width and sampling interval, suggested significantly influential nature of with prominent effect of sample interval. Further, the desirability plot for optimized solutions suggested to proceed the method with selected nominal values (slit width=1.0 and sampling interval=1.0) for both the factors. Hence, the method was established at the selected nominal values for the two CMVs.

The optical characteristics for the spectrophotometric method are shown in Table 3. The developed method was found specific as well as selective as the commonly used formulation excipients present in the tablet dosage form were found noninterfering in the estimation process. The drug was linear over a concentration range of 5-60µg/ml. Regression analysis of linearity data showed overall goodness of fit. The values obtained for statistical parameters such as multiple R, R^2, adjusted R^2 and standard error were 0.9996, 0.9992, 0.9992 and 0.0154, respectively. ANOVA suggested appropriateness (P-value < 0.05) of the linearity data. The % recovery from tablet dosage form was found to be 100.58% (S.D. = ±0.97, n=3). The average recovery for accuracy study ranged from 98.17-

100.16%. The %RSD was well below 2%, for both intra-day and inter-day determinations showing high degree of precision of the proposed method. The results of the method lie within the prescribed limit, showing that method is free from interference from excipients.

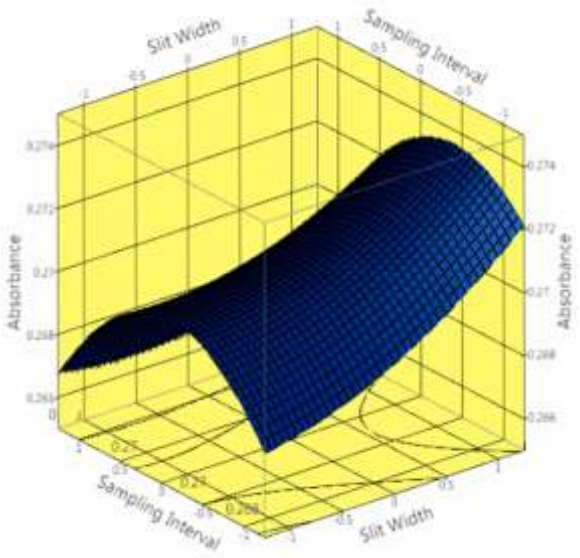

Figure 5. 3-D Response surface plot for absorbance against slit width Vs. sampling interval

Table 3. Optical Characteristics and summary of validation parameters

Parameter	**Obtained Values**
Wavelength(nm)	285
Linearity range((µg/ml)	5-60
Sandell's sensitivity(µg/cm^2/0.001AU)	0.037
Molar extinction coefficient (ltr/ mol.cm)	1.29×10^4
Regression equation(Y=ax+b)*	0.0246x+0.0216
Correlation coefficient(R^2)	0.9993
Precision (% R.S.D., n=6)	
Intra-day	0.16
Inter-day	0.099
Accuracy (% Recovery † ± S.D.)	
80%	98.93±0.62
100%	99.94±0.96
120%	100.10±0.07
% Range of error	
0.05 confidence limits	±0.13
0.01 confidence limits	±0.17

R.S.D. – Relative Standard Deviation; S.D. – Standard Deviation; A.U. - Absorbance Units,* is Y= ax+b, where Y = absorbance, a = slope, b = intercept and x is the concentration, † is average of three determinations at each level

Conclusion

A QbD approach was implemented for developing a robust UV spectrophotometric method for estimation of vilazodone. Employing the QbD workflow ensured quality of the analytical method. Sampling interval and slit width were the two influential CMVs which requires special attention by the analyst while setting up the method control strategies and future experimentations for continual improvement in method performance. The results suggest the method is novel, simple, accurate and precise. Statistical studies of the method validation results suggest appropriateness of the developed methods for use in quality control laboratories. This method is suitable for determining vilazodone in tablet dosage form without interference from commonly used excipients. Therefore, this method can be used for routine analysis purpose.

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