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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AZILSARTAN MEDOXOMIL AND CILNIDIPINE IN BULK PRODUCT AND MARKETED FORMULATION BY RP-HPLC

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ABSTRACT

For the simultaneous measurement of Azilsartan Medoxomil (AZIL) and Cilnidipine (CIL) in both bulk and tablet formulation, a novel, accurate, precise, and robust RP-HPLC technique has been devised, coupled with sensitive characteristics. The solutes were estimated using an Agilent C_{18} column with a size of 100 mm × 4.6 mm, 2.5 µm. AZIL and CIL were eluted with orthophosphoric acid (0.1 %) buffer: acetonitrile in a ratio of 82:18 v/v in a 10-minute gradient trial at a flow rate of 1.0 ml/min with an ambient column temperature of 25°C and monitored at a wavelength of 240 nm. AZIL and CIL were shown to have retention times of 4.253 min and 6.933 min, respectively. With r^2 of 0.999 in both instances, the Q2A and Q2B validation of the analytical technique showed excellent linearity across the concentration ranges of 20-100 µg/mL for AZIL and 5-25 µg/mL for CIL. The method also displayed high accuracy values, superb precision (inter-day and intra-day) values, and impressive robustness values. For regular analysis of the medication combination in bulk and tablet forms, the proposed analytical technique proved precise, accurate, and robust.

Keywords: Azilsartan Medoxomil, Cilnidipine, RP-HPLC, Simultaneous, Validation, Estimation

INTRODUCTION

Azilsartan medoxomil (AZIL) (**Figure 1A**) is a prodrug that is broken down to azilsartan, an angiotensin-converting enzyme inhibitor (ACEI). It is an angiotensin-II receptor antagonist of the AT_1 subtype. AZIL is an antihypertensive medication that was authorized by the FDA for the first time in February 2011. Many recommendations suggest that angiotensin receptor blockers (ARBs) be used as first-line therapy when starting antihypertensive medication, and that their clinical effectiveness is similar to that of ACE inhibitors, which are also used as first-line therapy for hypertension. The drug AZIL is sold under the brand name Edarbi. It may be used alone or in conjunction with other antihypertensive medications to treat hypertension. It is also available as a chlorthalidone-based combo product. Because hypertension is a significant risk factor for cardiovascular disease, treating it early has a number of consequences for patients' long-term survival and quality of life. Lowering blood pressure is linked to a lower risk of fatal and non-fatal cardiovascular events, such as strokes and heart attacks. As a result, AZIL is thought to reduce mortality and the development of cardiovascular disease. AZIL may have potential off-label applications in individuals with a history of myocardial infarction or heart failure, but no therapeutic relevance has yet been established **[1]**.

Cilnidipine (CIL) is a dihydropyridine calcium antagonist (**Figure 1B**). Fuji Viscera Pharmaceutical Company, Japan, and Ajinomoto, Japan, collaborated on the drug, which was authorized in 1995. In contrast to other calcium antagonists, CIL may act on the sympathetic nerve end's N-type calcium channel as well as the L-type calcium channel, as do other calcium antagonists. This medication has been authorized in China, Japan, Korea, India, and a number of European Union nations. CIL reduces blood pressure by blocking incoming calcium and inhibiting blood vessel contractions via the L-type calcium channels of blood vessels. CIL also inhibits the release of norepinephrine and suppresses the rise in stress blood pressure by acting on the N-type calcium channel at the end of the sympathetic nerve. *In vitro* and *in vivo*, CIL administration has been demonstrated to have an anti-sympathetic characteristic. It lowers blood pressure safely and efficiently without causing tachycardia or excessive blood pressure drop **[2]**.

Several reports have used validated analytical reverse phase-high performance liquid

chromatography (RP-HPLC), ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), fluorescence, and spectrophotometric methods for individual estimation of both AZIL and CIL and their metabolic products in plasma, bulk formulation, and pharmaceutical formulation (capsule, controlled-release formulations) for individual estimation of both AZIL and CIL and their metabolic products in plasma, bulk formulation, and pharmaceutical. However, no studies have been published that estimate both the medicine and the tablet formulation at the same time **[3,4]**.

While searching the standard worldwide databases for literature on any analytical RP-HPLC technique for the routine simultaneous determination of AZIL and CIL medication combinations in bulk and pharmaceutical formulations, no single publication was identified. To address the problem, a simple, durable, precise, cheap, and accurate solution was created. The goal of this study is to establish a validated RP-HPLC technique for estimating AZIL and CIL in bulk and tablet formulations.

MATERIAL AND METHODS

Materials

SL Drugs & Pharmaceuticals Ltd., Hyderabad, sent a large gift sample of AZIL and CIL. JB Chemicals and Pharmaceuticals Ltd., Mumbai, provided the Myotan® CN 40/10 Tablet, which included 40 mg of AZIL and 10 mg of CIL. The research used analytical quality chemicals and HPLC grade solvents from HiMedia Ltd., Mumbai.

Instruments

The weighing was carried out using a Shimadzu® AUW220D balance (Kyoto, Japan). A VSI® VSI-1B digital pH meter (Mohali, India) was used to determine the pH. Transonic Digital S sonicator was used for sonication (Mumbai, India). The technique was developed using a reverse-phase Denali C_{18} column with a particle size of 2.5 µm and a dimension of 100 mm × 4.6 mm, coupled to an Agilent® 1100 Gradient HPLC system with a PDA detector 2996 and a manual rheodyne injector (20 L loop), all managed by Chemstation v.2 software.

Selection of the mobile phase

For the elution of the solutes, the mobile phase must be carefully chosen. On the basis of theoretical plates, peak purity index, and peak symmetry, the mobile phase was chosen. The experiment began with the buffer systems and an eluant such as methanol, acetonitrile, or other solvents. The elution using an equal mixture of buffer KH_2PO4 and methanol generated low-intensity peaks with a lot of tailing. The use

of KH_2PO4 buffer (pH 4.8) with acetonitrile resulted in the development of a wide peak with tailing, although this was an improvement over the prior experiment. When the buffer was substituted with orthophosphoric acid (OPA) (0.1%), the peak symmetry improved significantly and tailing was decreased when used in an equal ratio with methanol, but it was still insufficient to elute the solutes. Methanol was used in conjunction with OPA to get a sharp peak with an excellent Gaussian peak. The 82:18 v/v ratio produced the highest peak purity index as well as the most theoretical plates. A vacuum was used to degas the mobile phase, which was then filtered through a 0.45 µm membrane filter. The mobile phase was allowed to equilibrate until it reached a stable baseline.

Chromatographic conditions

AZIL and CIL were eluted in a 10-minute gradient trial at a flow rate of 1.0 ml/min with an ambient column temperature of 25°C and monitored at a wavelength of 240 nm using Methanol: OPA (0.1 %) buffer in the ratio of 82:18 v/v.

Preparation of analytical solutions

Preparation orthophosphoric acid (0.1%) buffer

1 mL orthophosphoric acid was precisely weighed and diluted with 1000 mL HPLC grade water before being sonicated to remove gas.

Preparation of mobile phase

In a ratio of 82:18 v/v, the above-prepared buffer was thoroughly mixed with methanol. After that, the solution was degassed for 5 minutes using sonication and filtered through a 0.45 µm membrane filter under vacuum.

Diluent preparation

The diluent used in the formulation of the standard and sample solutions was an 82:18 v/v ratio of water and methanol.

Standard preparation

In a 25 ml dry volumetric flask, a precise quantity of 20 mg AZIL and 5 mg CIL were introduced, along with 5 ml of diluent. To make 2000 µg/ml of AZIL and 500 µg/ml of CIL, the aforementioned content was sonicated for 10 minutes and the volume was increased to 10 ml. The material was then pipetted into a 25 ml volumetric flask and diluted to 20 ml to achieve 100 µg/ml and 25 µg/ml concentrations.

Sample preparation

The average weight of 20 tablets was determined after they were properly weighed. A weight equal to a pill was transferred to a 100 mL volumetric flask and half-filled with the diluent. The contents were sonicated for 20 minutes and then filtered to provide 40 mg/ml of AZIL and 10 mg/ml of CIL. Then,

1 ml of the solution was transferred to a volumetric flask with a capacity of 25 ml, and 20 ml of diluent was added to create 2000 µg/ml and 500 µg/ml of content, respectively.

Method validation

The method was validated using the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2A and Q2B guidelines, as well as USFDA advice.

Linearity and Range

The method's linearity was determined by using five different concentrations of the solutes, ranging from 20 to 100 µg/mL for AZIL and 5 to 25 µg/mL for CIL. To determine the peak area, the solutions were made with the diluent and an equivalent amount was injected into the HPLC apparatus. Each solute's concentration and average area were plotted on a linearity graph. The value of the regression coefficient (r^2) was also calculated **[5]**.

Accuracy

Spiking the reference drug solutions at concentrations of 80 %, 100 %, and 120 % in the HPLC system evaluated the accuracy (recovery). The experiment was carried out in triplicate, with results reported as percent recovery % relative error on the basis of specified concentrations **[6]**.

Precision

By spiking concentrations of 40%, 60%, and 80% six times in a single day (intra-day) and on three separate days, the accuracy of the proposed technique was evaluated in terms of inter-day and intra-day variability (inter-day). The data were represented in % relative error precision **[7]**.

Robustness

The method's robustness was assessed by changing the mobile phase composition by 1% v/v (i.e. 81:19 percent v/v and 83.17 percent v/v), flow rate by 0.1 mL/min (i.e. 0.9 mL/min and 1.1 mL/min), and wavelength by 1 nm (i.e. 239 nm and 241 nm), while keeping all other chromatographic parameters constant **[8]**.

Systems suitability parameters

By injecting five times the standard solution and monitoring data including retention duration, peak area, theoretical plates, and tailing factor, the analytical method's repeatability profile was calculated **[9]**.

Limit of detection and quantification

The limit of detection (LOD) is the lowest concentration that any analytical technique can detect, although it is not essential to quantify the precise quantity **[10]**.

The limit of detection (LOD) was determined by the formula:

LOD = 3.3 (σ / S)

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

The lowest measurable quantity by any analytical technique with a certain degree of accuracy and precision is known as the limit of quantification (LOQ) **[11]**.

The limit of quantification (LOQ) is determined by the formula:

LOQ = 10 (σ / S)

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Because no prior comparable techniques had been established, the new technique was completely dependent on trial and error. However, when choosing the stationary phase, some inspiration was taken from the previously existing reports. The Agilent® reverse phase C_{18} stationary phase with a particle size of 2.5 µm and a diameter of 100 mm × 4.6 mm i.d. was used. After many continuous trials, the mobile phase Methanol: OPA (0.1 %) in the ratio 82:18 v/v was used for the elution. The mobile phase was kept at a low pH to guarantee that peak tailing was reduced and the analytical method's robustness was substantially improved. Because high basic pH causes dissolution in silica-based reverse-phase columns, the use of acidic pH was justified to a larger degree. It was also determined that the pH of the mobile phase and the pKa of the solute are in close agreement, allowing them to stay in the unionized form. As a result, the pH value based on two units was selected. The elution was carried out on an Agilent® C_{18} column in gradient mode for 10 minutes with a mobile phase of Methanol: OPA (0.1 %) at a ratio of 82:18 v/v. The flow rate was kept constant at 1.0 ml/min, the column temperature was kept at 25°C, and the detection wavelength was 240 nm. AZIL and CIL had retention times of 4.253 minutes and 6.927 minutes, respectively (**Figure 2A**). AZIL had a retention time of 4.253 minutes and CIL had a retention time of 6.933 minutes in the tablet sample solution (**Figure 2B**). This clearly demonstrated that the proposed analytical technique was precise, accurate, and robust for regular medication combination analysis in bulk and tablet forms.

Method validation

Linearity and range

Extremely high linearity was detected between the dosage and peak area throughout the range of 20-100 µg/mL for AZIL and 5-

25 µg/mL for CIL, with linear regression equations of $y = 36.57x + 16.28$ and $y = 127.9x + 13.41$, respectively (Table 1). In both instances, the regression coefficient values were 0.999, indicating that there was a high degree of linearity (**Figure 3**).

Accuracy

The Y-intercept and slope of the graph played a critical part in determining the % recovery characteristic of the suggested technique for simultaneous estimation by using the calibration curve. The established % RSD values for AZIL were 1.02, 0.99, and 1.01, respectively, and for CIL were 0.98, 1.00, and 1.03, all of which were under the US Pharmacopeia acceptability standard of < 2% (**Table 2**). Overall, the technique indicated that the recovered data was accurate.

Precision

The technique was shown to be very accurate throughout the tested ranges of 20-100 µg/mL for AZIL and 5-25 µg/mL for CIL in both intra-day and inter-day variability tests for precision data. In both instances, the peak area of the sample solution matched that of the standard solution, with a % RSD of less than 2%. The % RSDs for AZIL and CIL in intra-day studies (**Table 3**) were 0.02 % - 0.04 % and 0.01 % - 0.09 %, respectively, whereas the % RSDs for AZIL and CIL in inter-day studies (**Table 4**) were 0.06 % - 0.35 % and 0.17 % - 1.07 %, respectively, indicating high precision and minimal variation.

Robustness

A significant shift in the chromatogram for both medications was found with the deliberate modification of certain important chromatographic parameters such as mobile phase composition, flow rate, and wavelength by 1%, 0.1 ml/min, and 1 nm, respectively. The % RSD was found to be 2% when the mobile phase combination was changed to 81:19 v/v (0.15 for AZIL and 0.05 for CIL). Similarly, when the composition was changed by 83:17 v/v, the % RSD was found to be less than 2%. (0.12 for AZIL and 0.56 for CIL). The % RSD was found to be 2% when the flow rate was increased by 0.1 ml/min (0.09 for AZIL and 0.07 for CIL). In comparison, a comparable decrease in flow rate resulted in a % RSD of less than 2%. (0.08 for AZIL and 0.18 for CIL). The difference in wavelength of 1 nm resulted in a % RSD of 2%. (0.13 and 0.15, respectively for AZIL and 0.03 and 0.04, respectively for CIL). The intentional modification in the parameters in all of the experiments showed that the proposed technique had resilient properties.

Systems suitability parameters

The proposed method's system suitability characteristics showed a high degree of repeatability and may be used for regular medication combination analysis. The proposed technique for AZIL resulted in an average retention time (Rt) of 4.255 minutes and a mean theoretical plate (TP) of 3687. In the instance of CIL, the Rt and TP were 6.922 and 5711 minutes, respectively (**Table 5**). In all instances, a tailing factor of less than 2% indicated no particular tailing. An ideal Gaussian peak with good peak symmetry (asymmetric factor = 1) has both symmetric and asymmetric factors of equal size. The fact that the proposed technique fulfilled the minimal criteria of US Pharmacopoeia monographs (minimum theoretical plates of 2000 and tailing factor of < 2%) indicates that it has a notable resolution, substantial separation, high column effectiveness, and improved repeatability. The separation factor (α) and resolution factor (Rs) were found to be substantially higher than the minimal ICH limits and required recommendations of 1 and 1.5, indicating that the suggested analytical technique produces a greater separation of both peaks with less tailing and greater resolution. As a result of its great precision, repeatability, and accuracy, the technique may be used for routine analysis.

Limit of detection and quantification

The LOD and LOQ of AZIL were 0.2037 µg/mL and 0.6173 µg/mL, respectively, whereas the LOD and LOQ of CIL were 0.4524 µg/mL and 1.371 µg/mL, respectively, indicating the method's tremendous detection ability for the lowest possible concentration of the solute simultaneously from the combination or formulation.

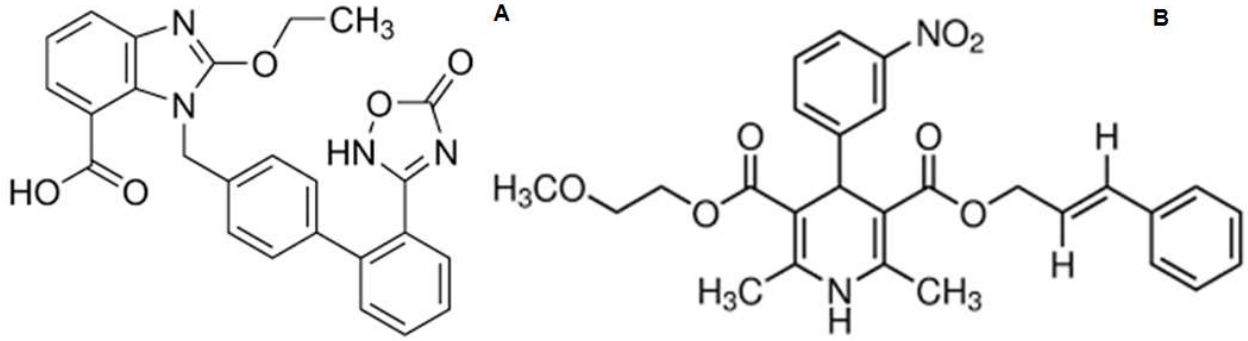

Figure 1: Structure of (a) Azilsartan and (b) Cilnidipine

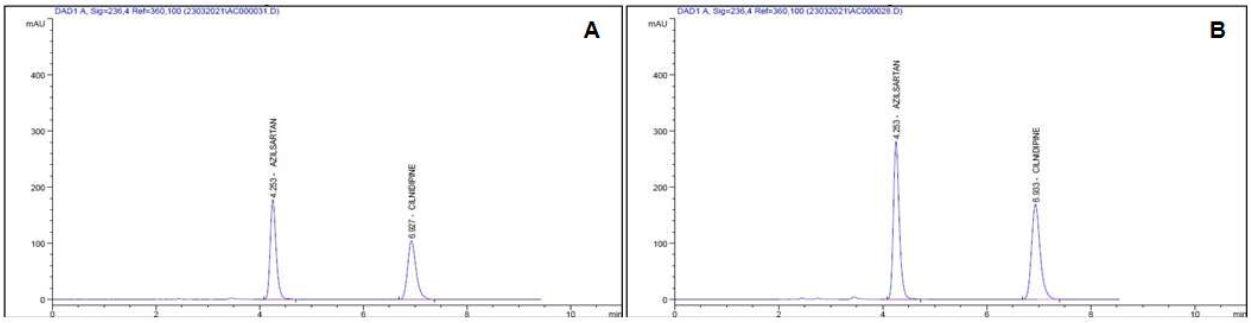

Figure 2: Chromatogram for Azilsartan Medoxomil and Cilnidipine (A) after method optimization and (B) after tablet sample solution

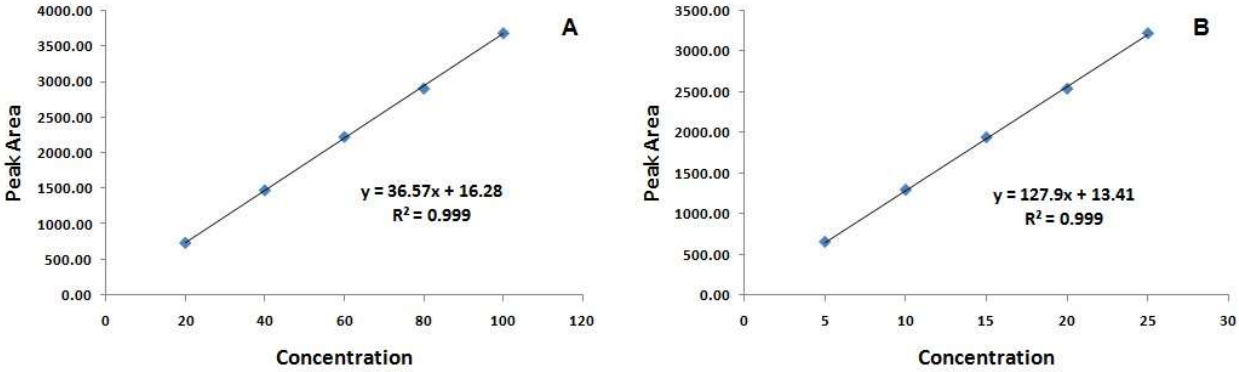

Figure 3: Linearity plot of (a) Azilsartan Medoxomil and (b) Cilnidipine

Table 1: Linearity study of Azilsartan Medoxomil and Cilnidipine

AZILSARTAN MEDOXOMIL		CILNIDIPINE	
Concentration (µg/mL)	Peak Area (mV)	Concentration (µg/mL)	Peak Area (mV)
20	741.5579	5	654.9105
40	1482.43	10	1294.321
60	2229.093	15	1941.159
80	2909.911	20	2544.032
100	3704.885	25	3241.098

Table 2: Recovery for accuracy studies for the combination

Spiked level %	Conc. of drug added (µg/mL)	Conc. of drug found (µg/mL)	Recovery %	Mean %	% RSD
AZILSARTAN MEDOXOMIL					
80	16	16.24386	101.52	101.68	0.22
	16	16.29407	101.84		
100	20	20.24659	101.23	100.80	0.62
	20	20.0717	100.36		
120	24	24.3347	101.39	101.35	0.06
	24	24.31274	101.30		
CILNIDIPINE					
80	4	4.030493	100.76	100.77	0.01
	4	4.031257	100.78		
100	5	4.979701	99.59	99.80	0.30
	5	5.000646	100.01		
120	6	6.10447	101.74	101.76	0.02
	6	6.106469	101.77		

Conc., Concentration; RSD, relative standard deviation

Table 3: Precision data of intra-day variability

Drug	Conc. (µg/mL)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
AZIL	40	1486.364	1486.83	100.53	0.04
	60	2225.869	2226.15	100.71	0.02
	80	2905.418	2905.97	98.77	0.03
CIL	10	1293.943	2265.54	100.09	0.04
	15	1944.162	3325.25	100.65	0.01
	20	2548.579	4464.77	99.17	0.09

Conc., Concentration; RSD, relative standard deviation

Table 4: Precision data of inter-day variability

Drug	Conc. (µg/mL)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
AZIL	40	2266.545	2265.54	153.76	0.06
	60	3333.542	3325.25	150.81	0.35
	80	4460.325	4464.77	152.05	0.14
CIL	10	2102.517	2118.54	164.59	1.07
	15	3170.365	3174.79	164.78	0.20
	20	4255.317	4260.33	166.03	0.17

Conc., Concentration; RSD, relative standard deviation

Table 5: Systems suitability parameters

AZILSARTAN MEDOXOMIL						CILNIDIPINE					
Rt (min)	Area (mV)	Theoretical Plates (TP)	Separation Factor	Resolution Factor	Tailing Factor	Rt (min)	Area (mV)	Theoretical Plates (TP)	Separation Factor	Resolution Factor	Tailing Factor
4.253	379967	3504	1.531	1.936	1.46	6.927	2521645	5583	1.534	1.898	1.39
4.259	375014	3764	1.537	1.933	1.48	6.923	2517961	5555	1.529	1.889	1.30
4.255	378053	3881	1.539	1.921	1.59	6.924	2516517	5656	1.533	1.890	1.36
4.258	378516	3505	1.532	1.930	1.55	6.927	2517892	5893	1.532	1.896	1.31
4.251	377337	3641	1.541	1.926	1.49	6.926	2512491	5885	1.544	1.881	1.29
4.255	377499	3687	1.539	1.924	1.513	6.922	2519297	5711	1.531	1.882	1.337
% RSD		0.17							0.46		

CONCLUSION

The proposed analytical technique may be used to estimate AZIL and CIL simultaneously in both bulk and tablet formulations. The technique has linearity across the range, accuracy, precision, and robustness, according to the ICH criteria for validation. The % RSD, theoretical plates, and tailing values were all within the US Pharmacopoeia's minimal standards. The verified stress degradation tests under thermal, oxidative, alkali, and acid conditions revealed the potentially damaged components, which will be very useful to chemists for quality control and assurance. Because of its great precision, repeatability, and accuracy, the technique may be used for regular analysis.

CONFLICT OF INTEREST

No conflict of interest is declared.

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None

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