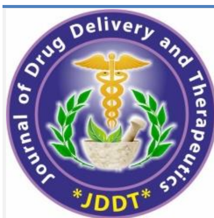

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Open Access Full Text Article Research Article

Development of a Validated RP-HPLC/UV Method for the Quantitative Determination of Tyrosine Kinase RET Inhibitor: Selpercatinib in Capsule Formulation

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Abstract

A validated RP-HPLC/UV method was developed to estimate the selpercatinib in capsules. The wavelength chosen for detection was at 248 nm. The RP-HPLC separation was carried out using a Hypersil ODS C_{18} column (250×4.6 mm, 5 µm) with a mobile phase consisting of 0.2% TFA (pH 6.5) and acetonitrile in a ratio of 70:30 v/v. The flow rate was set at 1 ml/min. The retention time for selpercatinib was determined to be 3.012 min. Linearity was detected within the concentration range of 2.5-15 µg/mL for selpercatinib. The approach has been confirmed to be linear, accurate, precise, robust, and has established limits of detection and quantitation. The established procedure was uncomplicated, cost-effective, and suitable for the routine analysis of selpercatinib in capsule dosage form.

Keywords: Selpercatinib, RP-HPLC and Validation.

INTRODUCTION

Selpercatinib (RETEVMO®) is a potent and effective inhibitor of the receptor tyrosine kinase RET (rearranged during transfection), which has been specifically designed by Loxo Oncology[1] to target and treat certain solid cancers, including those of the thyroid, lungs and other organs with RET alterations[2]. When the RET gene is excessively activated, it can function as a cancer-causing gene in different types of cancers. In particular, RET gene fusions that still have the kinase domain are responsible for driving the solid tumors. On the other hand, activating mutations in the RET gene are linked to various manifestations of "multiple endocrine neoplasia type 2 (MEN2)" and "sporadic medullary thyroid cancer (MTC)"[3]. Selpercatinib (SELP) received approval in the United States in May 2020 for the treatment of three specific conditions: "metastatic RET fusion-positive non-small cell lung cancer (NSCLC)" in adult patients, advanced or "metastatic RET-mutant medullary thyroid cancer (MTC)" in adult and pediatric patients aged 12 years and older who need systemic therapy, and advanced or "metastatic RET fusion-positive thyroid cancer" in adult and pediatric patients aged 12 years and older who need systemic therapy and are unresponsive to radioactive iodine[4]. The prescribed dosage of the medication is 120 mg for patients weighing less than 50 kg, or 160 mg for individuals weighing 50 kg or more. The medication should be taken orally twice a day, approximately every 12 hours, until there is either illness progression or the occurrence of intolerable side effects[5]. Selpercatinib is chemically known as "6-(2-hydroxy-2-methyl propoxy)-4-[6-[6-[(6-methoxy pyridin-3-yl)methyl]-3,6-diazabicyclo [3.1.1] heptan-3-yl]pyridin-3-yl] pyrazolo [1,5-a] pyridine-3-carbonitrile". The molecular formula of SELP is $C_{29}H_{31}N_7O_3$ with a molecular weight of 525.6 g/mol (see figure 1).

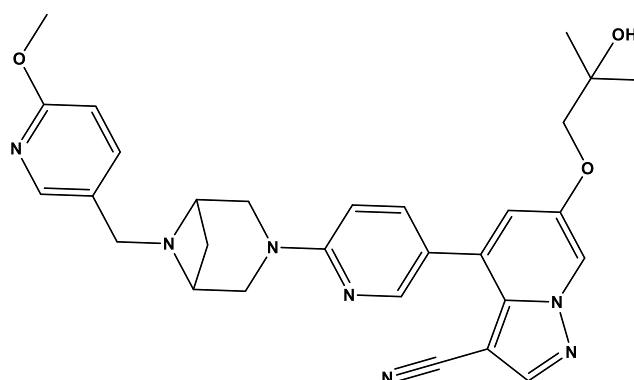

Figure 1: Structure of selpercatinib

The literature is poor in the reported methods for the analysis of SELP. Only two LC-MS/MS methods were reported for the analysis of selpercatinib in alone and with the combination of pralsetinib, brigatinib and lorlatinib[6-7]. Only one RP-HPLC method was reported on assay[8]. It is crucial to establish a rapid and simple HPLC analysis method for ETX. In recent years, it has been advised that any HPLC technique development for active pharmaceutical substances have a good peak separation between the drug and its degradation products[9-11]. However, these approaches are insensitive and inaccurate when measuring SELP at low concentrations. Furthermore, these techniques are costly, time-consuming, and un-suitable for routine analysis. As a result, we elected to use a low-cost UV-coupled HPLC technology that is widely available in laboratories with minimal financial resources and necessitates regular monitoring of SELP in the formulation. Given these considerations, the goal of this research is to develop an RP-HPLC method for quantifying SELP in capsule formulations using ICH recommendations under Q specification[12].

MATERIALS AND METHODS

Chemicals and reagents

SELP was procured from Ascentyo Biosciences (Hyderabad, India). Throughout the analysis, all HPLC-grade solvents were employed, including acetonitrile, methanol, and water Merck Ltd. (Mumbai, India).

Instruments

The study was conducted using the Shimadzu HPLC system (Model No. LC-20AD) coupled with a UV detector (Model No. SPD-M20A). Data acquisition was done using Empower software version 2. The experimental setup consisted of a Hypersil ODS C_{18} column (dimensions: 250 mm × 4.6 mm, 5 µm). The samples were introduced via a Rheodyne injection valve equipped with a 20 µL sample loop. Weighing was done using an analytical balance from Mettler Toledo.

Chromatographic conditions

An isocratic mode was employed using a mobile phase composed of a volumetric ratio of 70:30 v/v (0.2% TFA in water: ACN). The analysis was performed at room temperature (25°C) with a mobile phase flow rate of 1.0 mL/min. Each trial required the injection of 20 µL of the sample into the HPLC system. The UV detector was configured to detect SELP in the effluents from the column at a wavelength of 248 nm as shown in figure 2.

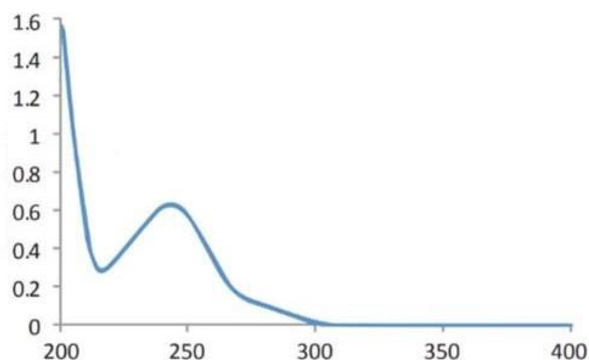

Figure 2: UV spectra of selpercatinib

Trifluoroacetic Acid Buffer (0.2% v/v) preparation:

In a volumetric flask, two milliliters of trifluoroacetic acid (TFA) were taken and diluted with 1000 milliliters of water (HPLC grade).

Mobile Phase preparation

After precisely measuring and mixing TFA (700 mL, pH = 6.5) and acetonitrile (ACN) (300 mL) in the v/v ratio of 70:30. The mixture was allowed to degas for 10 minutes in an ultrasonicator. The mixture was then filtered through a 0.45 µ membrane filter under vacuum.

Diluents preparation

Acetonitrile and HPLC grade water were mixed well in a ratio of 70:30 (% v/v).

Preparation of standard stock solution

A 20 mg of SELP powder with purity more than 99% was placed in a 100 mL volumetric flask (VF). Next, the flask was filled with the diluent and subjected to sonication for 20 minutes. The volume was set to 100 mL by adding the diluent. Then, 0.5 mL of the solution was moved to a 10 mL volumetric flask and topped up with 10 mL of diluent to reach a concentration of 10 µg/mL of SELP.

Preparation of sample solution from dosage form

After taking ten 40 mg RetevmoTM capsules, the contents were drained and placed into a dry watch glass. To obtain a standard stock solution of 200 µg/mL, capsule powder equivalent to 20 mg of SELP was weighed and then placed into a 100 ml volumetric flask with 50 ml of diluents. To make sure the drug was completely soluble; the mixture was sonicated intermittently for one hour. After that, it was filtered through a 0.45 µm membrane filter, and 100 ml of diluent was added to create a stock solution. To achieve a concentration of 10 µg/mL of SELP, 0.5 mL of the solution was then transferred to a 10 mL volumetric flask and topped off with 10 mL of diluent.

Selection of the detection wavelength

A SELP solution with a concentration of 10 µg/mL was used for the HPLC analysis. The solution was analysed using a UV spectrophotometer within the wavelength range of 190 to 400 nm, with the mobile phase serving as a reference. The scanning process aimed to determine the wavelength where the SELP absorbs UV light most effectively for its detection for subsequent HPLC studies. The drug showed maximum absorbance at 248 nm (see figure 2).

Analytical method development

In order to design a method for HPLC analysis of SELP, several parameters have to be optimised while maintaining a set of constants. Mobile phase composition, column selection, and flow rates were carefully modified to obtain optimal chromatographic separation. Nevertheless, in order to maintain uniformity and facilitate validation, several parameters were kept constant: detector type, injection volume (20 µL), oven temperature (25 ± 2 ºC), and elution mode. A spectrum has been recorded for each set of chromatographic conditions at the specified detection wavelength. Throughout the method's development, additional factors considered were peak height, column pressure, accuracy, resolution, analysis time, and solvent efficiency per run.

Validation

After acceptable chromatographic conditions were established, the method was validated following the ICH Q2 requirements[12]. Additionally, the stability of reagents and solvents was investigated as well.

Evaluation of system suitability

System suitability studies were performed to verify the dependability of the HPLC system. Column efficiency, plate count, and tailing factor were measured by making six injections at a dose of 10 µg/mL. The results confirmed that the system matched the established criteria and adhered to stated boundaries, demonstrating consistency.

Specificity and selectivity

The method's specificity and selectivity were validated by successfully detecting SELP in the sample without any interference. The chromatogram of the SELP reference standard yielded a positive result, whereas the blank, consisting just of the diluent, exhibited no reaction or interference. The chromatogram for the standard is displayed in figures 3, respectively.

Linearity

Linearity of the SELP was assessed via generating dilutions ranging from 2.5 to 15 µg/mL from the standard stock solution. Peak area responses were quantified for each concentration during the HPLC procedure. The linearity graph is shown in Figure 4.

Precision

Accuracy was evaluated by performing six repeated injections of the reference solution, yielding a low % RSD of 0.85, demonstrating great precision and consistent outcomes. Six injections of the test solution exhibited a % RSD of 0.46, meeting the accuracy requirement of 2.0% and confirming the method's reliability in sample analysis.

Intermediate precision (IP)

The intermediate precision was assessed through the utilisation of distinct HPLC instruments by two analysts on separate days and in different laboratories to analyse the standard solution. Both achieved essentially identical test findings, with a small difference of 0.18% and an RSD that was within the permissible limit of 2.0% on both days. This was the case despite the fact that time-based changes were associated with the results.

Accuracy or recovery studies

To validate the HPLC method, a triplicate recovery investigation was conducted. SELP was injected into pre-analyzed samples at doses of 5, 10, and 15 µg/mL. The average recovery % was calculated from these experiments to confirm precision.

Robustness

By manipulating the flow rate and wavelength on purpose, the HPLC method's robustness was evaluated. The method's robustness was maintained despite variations in flow rate and wavelength, as seen by insignificant changes in the chromatogram, tailing factor, and plate count, maintaining accuracy and precision.

Application of the developed method

The validated HPLC method was utilised to estimate and estimate the amount of SELP in commercially available capsules in an efficient manner. The sample solutions were produced according to the standard protocol outlined in the materials and methods section. Each sample underwent three injections into the HPLC equipment to guarantee the correctness and dependability of the data.

RESULT AND DISCUSSION

Method development and optimization

Mobile phase

In order to identify the best mobile phase, several mobile phases, including phosphate buffer (0.025 M) pH 6.5, acetate buffer (0.025 M) pH 4.5, 0.1% OPA in water, and 0.2% TFA, were investigated using organic modifiers like MeOH and ACN. Since methanol is less expensive than ACN, studies were first conducted with it as the organic phase. Considering SELP has a pKa of 6.28 (www.drugbank.ca), the pH of the mobile phase was maintained at 6.5 ± 1.0 to prevent peak splitting. Since silanol activity is reduced at lower pH values (pH 2.5 to 6.5), lower pH was chosen. We experimented with varying ratios of methanol to phosphate buffer (pH 6.5). The tailing factor was greater than 2.0 and the peak owing to SELP was broad at lower methanol concentrations. Similar to this, peaks with acetate buffer pH 4.5 and varied methanol ratios were broad, whereas those with 0.1% OPA in water and varying methanol ratios were somewhat better but had a higher tailing factor. Without any influence from placebo or diluent, a strong peak was obtained using 0.2% TFA with different ratios of methanol/ACN (Figure 3). Using ACN as the organic phase also resulted in less baseline noise and a better theoretical plate count than methanol. It was discovered that 30% of the organic ratio produced the best peak shape for SELP. 0.2 TFA and ACN (70:30% v/v) was determined to be the ideal mobile phase for the measurement of SELP in capsules based on the findings.

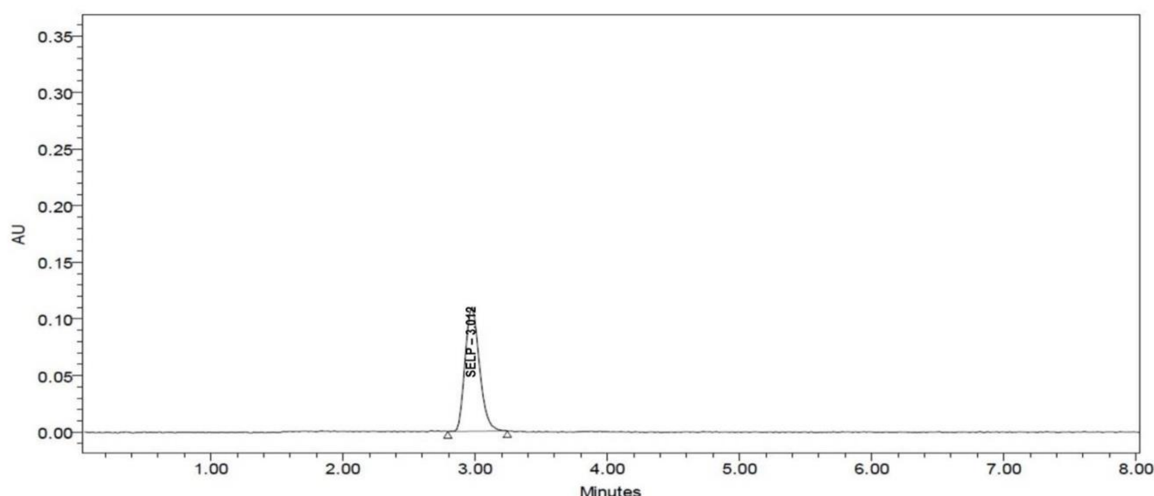

Figure 3: Optimized chromatogram of selpercatinib

Effect of column

Several columns were tested for the elution of SELP, including C4 and C18. A peak form that was adequate could not be achieved when the C4 column was tested. SELP's strong affinity for the stationary phase may be the cause of its wider peak and tailing in the C4 column even at greater organic ratios in the mobile phase. Less retention time and an excellent peak shape were displayed by the C18 column. When contrasted with the Luna (Phenomenex) chemistry, the C18 column using Hypersil ODS column chemistry displayed superior peak form and peak separation.

Effect of flow rate

When the flow rate was adjusted from 1 mL/min to 1.2 mL/min, there was no discernible change in the form of the SELP peak. Thus, a 1 mL/min flow rate was maintained.

Column oven temperature

The temperature of the column oven had no apparent impact on peak shape. Peak form altered only slightly when the temperature was raised from 25° to 40°. Because a greater temperature could shorten the column's lifespan, the column oven temperature was maintained at 25°.

System suitability parameters

The HPLC system's precision was evaluated by calculating the % RSD (relative standard deviation) based on six repeated injections of the standard solution. The condition was that the Relative Standard Deviation (RSD) should not surpass 2% to be deemed acceptable. The procedure demonstrated reliability for accurately quantifying SELP in samples as the RSD of the standard solution was within the prescribed limit, showing precision within the defined criterion. The results are outlined in table 1.

SELP had a retention time (RT) of 3.012 minutes, indicating successful separation and prompt identification. The HPLC method for SELP analysis was customized to achieve fast analysis and good resolution by carefully choosing the mobile phase composition, appropriate wavelength, and fine-tuning parameters. This thorough optimization guarantees accurate and efficient measurement of SELP, meeting strict analytical requirements.

Table 1: System suitability parameters for selpercatinib

S.No.	Parameter	Selpercatinib	Acceptance criteria
1.	Retention time (RT)	3.012	--
2.	Theoretical plates (N)	8845	NLT 2000
3.	Tailing factor (T)	1.49	NMT 2.0
4.	Linearity range (μg/mL)	2.5-15	--
5.	Detection Limit (μg/mL)	0.31	--
6.	Quantification limit (μg/mL)	0.94	--
7.	Regression data: Slope	51859	--
8.	Regression data: Intercept	602.99	--
9.	Regression data: Correlation coefficient	0.9993	--

Linearity

A linearity graph was created for SELP, displaying concentration in μg/mL on the x-axis and area under the curve (AUC) on the y-axis. A linear association was found between drug concentrations and peak area responses within the range of 2.5–15 μg/mL. The findings, shown in Figure 4 and outlined in table 2, emphasize the significance of linearity in analytical techniques. This feature guarantees accurate assessment of drug levels over a broad concentration range by analyzing peak areas. The HPLC approach demonstrates exceptional linearity in the designated concentration range, providing a robust correlation between concentration and peak area response, which enables precise SELP analysis.

Table 2: Linearity of selpercatinib

S.No.	Drug	Values of X and Y variables							Correlation coefficient
1.	SELP	Variable	1	2	3	4	5	6	0.999
		X	2.5	5	7.5	10	12.5	15	
		Y	120573.1	267940.3	392706.1	521118.4	651750.8	772141.9	

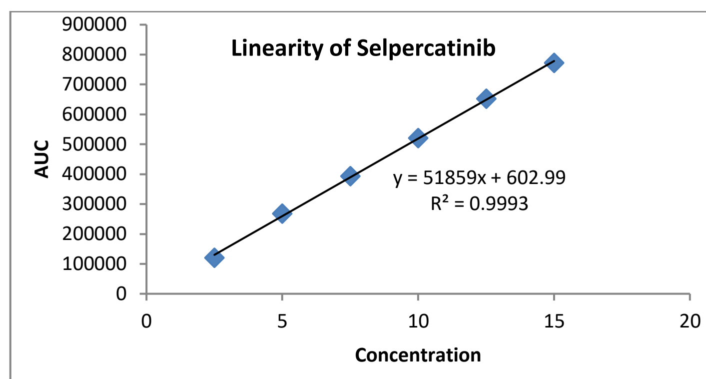

Figure 4: Linearity graph of selpercatinib

Precision

The method's precision, which includes the repeatability of sample and standard preparations, was deemed good. The summary of validation parameters is given in table 3. The results demonstrate the consistency and dependability of the HPLC approach in SELP analysis. This validation enables the method to be consistently used for precise quantification in a wide range of sample types.

Table 3: Precision study

S.No.	System Precision		Method Precision	
	Rt	AUC	Rt	AUC
1	3.024	517344.7	3.022	524271.5
2	3.021	518617.9	3.024	522951.7
3	3.024	527476.3	3.023	521411.9
4	3.001	517703.1	3.021	525239.2
5	3.021	526163.4	3.022	523845
6	3.024	522575.6	3.021	528617.9
Mean	**3.0**	521646.8	**3.0**	524390
SD	0.01	4438.035	0.0	2443.16
% RSD	**0.29**	0.850	**0.039**	0.466

Intermediate precision

The HPLC method's consistent performance across many laboratories, apparatus, and analyzers, even on different days, highlights its robustness. Its intermediate precision confirms its appropriateness for regular use, guaranteeing consistently precise and dependable outcomes across different experimental settings. Table 4 shows that the percentage RSD values for intermediate precision are below 2.0%, indicating the method and instrument's precision.

Table 4: Intermediate precision or Ruggedness study

Analyst Name	**Analyst I**			**Analyst II**		
Area of Std.	505484.8			512259.4		
S.No.	**Concentration (μg/mL)**	**AUC**	**Assay (%)**	**Concentration**	**AUC**	**Assay (%)**
1	10	514130.97	101.71	10	512321.60	100.01
2	10	502113.71	99.33	10	508367.77	99.24
3	10	513848.10	101.65	10	511767.18	99.90
4	10	514766.96	101.84	10	513568.13	100.26
5	10	504688.84	99.84	10	513415.02	100.23
6	10	502764.82	99.46	10	523871.94	99.49
	Mean	**508719**	100.64	**Mean**	**513885**	99.85
	SD	6123.71	1.21	**SD**	5242.70	0.41
	% RSD	1.204	1.20	**% RSD**	1.020	0.41
Difference between mean assay of two different analysts = 0.79 %						

Accuracy

The assay resulted in a mean recovery percentage of 100.59%, indicating the accuracy of the HPLC method in measuring the SELP concentration within the anticipated range. The results from table 5, showing spiking concentrations and mean recovery percentages, confirm the method's reliability for quantitative analysis by demonstrating successful recovery of SELP from different spiked samples. The method's satisfactory performance in measuring the SELP content is confirmed by the accuracy of the recovery falling within the permissible range.

Table 5: Accuracy study

S.No.	Level	Amount added (µg/mL)	Mean Amount recovered (µg/mL)	Mean % Recovery
1.	50%	5	5.54	100.54
2.	100%	10	10.21	100.5
3.	150%	15	15.26	100.75

Robustness

Table 6 displays the outcomes of rigorous experiments, outlining the several variables examined and how they affected the method's performance. The established reliability of the HPLC method confirms its appropriateness for regular use, assuring consistent and dependable result even when operating parameters are significantly modified. The approach demonstrates its capability to produce consistent results under many settings, as evidenced by minor fluctuations in peak areas and retention times. Compared to existing analytical procedures, this HPLC approach stands out for its shorter retention periods, increased theoretical plates (suggesting better resolution), and a mobile phase that facilitates improved separation of SELP from other components. Therefore, its enhanced efficiency and accuracy make it better suited for the regular measurement of SELP in various sample types.

Table 6: Robustness study

Parameters	Variation	Mean Peak area	%RSD	Tailing factor	No of Theoretical Plates
Wavelength minus	246 nm	505525.6	0.912	1.649	6682.3
Wavelength plus	250 nm	501624.1	0.7776	1.6975	6901.75
Flow rate minus	0.8 min/mL	504978.4	0.6528	1.2222	6566.4
Flow rate plus	1.2 min/mL	506755.5	0.7968	1.2222	7420.45
Organic phase ratio change (less)	0.2%TFA: Acetonitrile (80:20)	506604.4	0.4128	1.6587	6577.8
Organic phase ratio change (more)	0.2%TFA: Acetonitrile (60: 40)	502883.3	0.4896	1.6587	7612.35
Column change	Merck C_{18} column (250 mm × 4.6 mm × 5 µm)	571243.8	0.2784	1.649	7558.2
Temperature minus	20 °C	486575.4	0.2976	1.1252	6641.45
Temperature plus	30 °C	489829.1	0.6624	1.2804	7142.1

Analysis of marketed formulation

During the assay of marketed formulation, the mean of six determinations yielded 99.88% for SELP. The results revealed that the drug's %RSD was within acceptable levels. As a result, it is possible to conclude that the excipients do not interfere, as shown in table 7.

Table 7: Analysis of marketed formulation

Commercial Formulation	Ingredients	Labeled Amount (mg)	Amount Found (mg)	Found %
RETEVMO®	Selpercatinib	40 mg	39.95 mg	99.88

CONCLUSION

An RP-HPLC technique was developed and confirmed according to the ICH criteria. A Shimadzu HPLC system and a UV detector (Model No. SPD-M20A) were used in the process and detection was achieved at a wavelength of 248 nm. An injection volume of 20 µl was employed using a Hypersil ODS C_{18} column (250 x 4.6 mm, 5 µm) with isocratic elution. The final technique is sensitive, economical, rapid, reliable, simple, and accurate. Its short duration (less than 7 minutes) and great resolution are only two of its many features. The % RSD values of all validation parameters met the required standards, demonstrating the method's appropriateness for routine laboratory analysis of selpercatinib and quality control.

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Conflicts of Interest

The authors declare no conflict of interest.

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