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RP-HPLC Method Development and Validation for the Estimation of Tivozanib in Bulk and Formulations

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ABSTRACT

The goal of the current study was to create and progressively validate a novel, concise, responsive, and stable RP-HPLC method for the evaluation of Tivozanib's active pharmaceutical ingredient. Chromatographic conditions were studied under the stationary phase (Zobrax 150 (150mm x 4.5mm, 5m), Mobile phase used is Acetonitrile: Ammonium Formate (pH is adjusted by adding Ammonium hydroxide –pH (3.5), the flow rate was maintained at 1.0ml/min, detection wavelength was 268nm, column temperature was set to 30℃, and diluent as mobile phase. Conditions were set as the best course of action. A 2.637-minute retention time was discovered. The standard was injected six times to study system suitability parameters, and the results fell far short of the threshold for acceptance. An analysis of linearity between levels of 25% and 150% revealed an R^2 value of 0.999. The results showed that the method precision was 0.4 and the intermediate precision was 0.4. The LOD and LOQ are, respectively, 0.06 and 0.17 g/ml. The above method was used to conduct a 99.86% presence assay on a commercial formulation. Studies on the degradation of tivozanib were conducted, and under every circumstance, the purity threshold was higher than the purity angle and within the acceptable range. Although the full-length method has not been tested, it can be used to analyze Tivozanib routinely.

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INTRODUCTION:

The most typical type of urinary system cancer is renal cell carcinoma (RCC)[1]. RCC can develop as a first 1^{o} cancer (first primary RCC, 1st RCC) or as a second primary cancer (second RCC). One of the most prevalent SPMs is the 2nd RCC, with an incidence of 10.9–28.9% [2,3]. The five most prevalent SPCs components were identical for leukemia, non-Hodgkin's lymphoma, and kidney cancer. The three most prevalent SPCs (lung, bladder, and colorectal cancers) as well as the majority of other primary cancers had the same rankings and proportions in both populations after prostate cancer. The remarkably consistent SPC patterns[4]. The kinase inhibitor tivozanib (Fotivda) was approved by the Food and Drug Administration for adult patients with advanced renal cell carcinoma (RCC) that had relapsed or refractory after two or more prior systemic therapies[5].it is a VEGF receptor tyrosine kinase[6] inhibitors primary pathway linked to the development of renal cell carcinoma is VHL mutation-HIF upregulation-VEGF transcription. Tyrosine kinase inhibitors, which stop tumor growth, have an important target in the form of vascular endothelial growth factor receptors (VEGFR receptors)[7,9]. The main effect is to prevent vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, and VEGFR-3 from becoming phosphorylated. It also inhibits other kinases like c-kit and platelet-derived growth factor beta (PDGFR)[7,11]. Serum-soluble VEGFR2 (sVEGFR2) levels decreased over time and became more pronounced with tivozanib exposure in clinical studies, suggesting that sVEGFR2 could be used as a pharmacodynamic indicator of VEGFR inhibition[8]. Demethylation, hydroxylation, N-oxidation, and glucuronides are the metabolites[10], To create a new RP-HPLC method for determining Tivozanib's stability and to create a validated method following ICH guidelines.

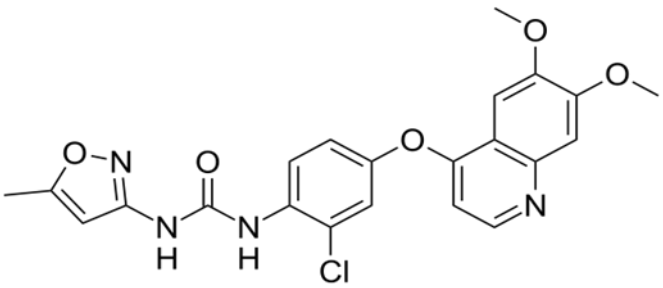


Figure no -1 shows the structure of Tivozanib.

There are few RP-HPLC methods that have been reported in the literature for the determination of Tivozanib in bulk and pharmaceutical dosage form by Rp-HPLC,[12,13,14]. An effort has been made to create an RP-HPLC method for the quantitative determination of

tivozanib in a bulk and pharmaceutical dosage form that is straightforward, specific, quick, precise, and affordable. The International Conference on Harmonization's (ICHQ2 (R1) guidelines have been used to validate this method.[15]

MATERIALS AND METHODS

Chemicals and reagents

Tivozanib pure drugs (API) brought from Merck India limited (GOA), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, and Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: The instrument used in the study was HPLC (Waters 2695 with PDA detector 2996) was monitored and integrated using Empower 2 software. electronic balance, sonicator, hot air oven, digital pH meter, and UV-Visible chamber.

Buffer Preparation: -

(0.1N Ammonium Formate)-Weigh accurately 0.63gm of Ammonium Formate and add to 1000-mL graduated cylinder to measure 900 mL of filtered HPLC-grade water and mix well, Check the pH of the solution. As needed, adjust to pH 3.5 using the ammonium hydroxide solution, Cap the reservoir bottle and mix well and sonicate for 20 minutes.

Preparation of Standard stock solution:

Accurately weighed 6.7mg of Tivozanib transferred 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up of diluents and labeled as Standard stock solution (134µg/ml of Tivozanib).

Preparation of Standard working solution:): 1ml of Tivozanib from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (13.4µg/ml of Tivozanib).

Preparation of Sample stock solution:

10 capsules of reference standards were weighed and the average weight of each Capsule was calculated, then the weight equivalent to 1 capsule was transferred into a 10 ml volumetric flask, 5ml of diluents were added and sonicated for 25 min, further, the volume was made up with diluent and filtered by HPLC filters (134 µg/ml of Tivozanib).

Preparation of Sample working solution: 1ml of filtered sample stock solution was transferred to a 10ml volumetric flask and made up with a diluent. (13.4µg/ml of Tivozanib)

Chromatographic conditions:

Flow rate:	0.9ml/min
Column :	Zobrax 150 (150mm x4.5 mm, 5m)
Wavelength:	268.0 nm
Column temperature: 30°C	
Injection volume:	10.0µL
Run time:	6.0minutes
Diluent:	Same as the Mobile phase

Observation: Tivozanib eluted at 2.637 min respectively with good resolution(Fig_2). The plate count and tailing factor were very satisfactory, so this method was optimized and to be validated.

Degradation: According to ICH recommendations and standard industry practice, forced deterioration is typically carried out in conjunction with a control sample under various stress conditions, including acid, alkali, peroxide, heat, and UV. Although there are no established standards for industrial degradation, it is recommended that 5 to 30 percent of degradation be reached under any of the applied stress conditions. The goal of the degradation to be accomplished by stress testing is to replicate the stability circumstances of the control room temperature[16]. To conduct the forced degradation experiment, standard stock solutions of Tivozanibwas exposed to various stress conditions, including 1 mL of 20% H_2O_2 (for oxidative degradation), 1 mL of 2N HCl (for acidic degradation), and 1 mL of 2N NaOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes at 60^{o}C. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 13.4µg/ml of Tivozanib for degradation studies. To examine sample stability, 10µl samples were fed into the system, and chromatograms were obtained.

Method Validation: The method was validated following ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Tivozanib (13.4ppm) the solutions were injected six times and the parameters like peak tailing, resolution, and USP plate count were determined % RSD for the area of six standard injections results should not be more than 2%.

Specificity: checking for interference in the method that was optimized. At the retention times of these drugs using this method, we shouldn't observe interfering peaks in the placebo or blank groups. Thus, it was claimed that this method was specific.

Linearity: Inject 6 standard solutions containing Tivozanib at concentrations ranging from 3.35 ppm to 20.10 ppm to show the linearity of the assay method. Produce a graph that shows peak area versus concentration. The calculated slope was 55112x + 756.1, and the correlation coefficient was 0. 999. The results were shown in table 2 and fig 6.

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (13.4µg/mlTivozanib) were created. Each injection was given from each working sample solution, and the results are shown in Table 3. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.4% for Tivozanib. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 3 shows the information results.

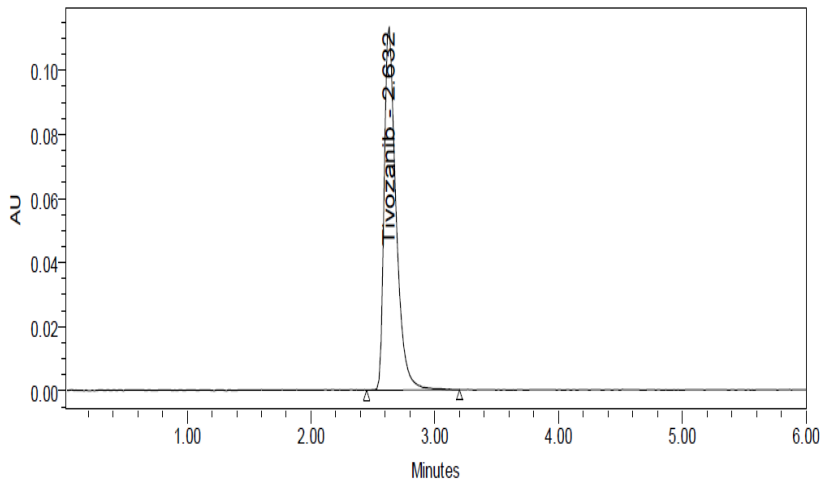
Intermediate Precision: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (13.4µg/ml of Tivozanib) were prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 4. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.4% for Tivozanib. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 4 shows the information results.

Accuracy: The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Tivozanib was found to be 99.53 %. Tables 5 show the outcomes. Because satisfactory recovery values were achieved, the accuracy of this approach was passed.

Robustness: Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C), and temperature plus (35°C) were maintained, and samples were injected in a duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 6 shows the data.

Assay: Tivozanib had a label claim of Tivozanib1.34mg per unit formulation. The mentioned formulation was used for the assay. The average % assay achieved for Tivozanib was 99.86%.



Assay Chromatogram of reference standard

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using the proposed method. The assay % of Tivozanibin in the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 7.

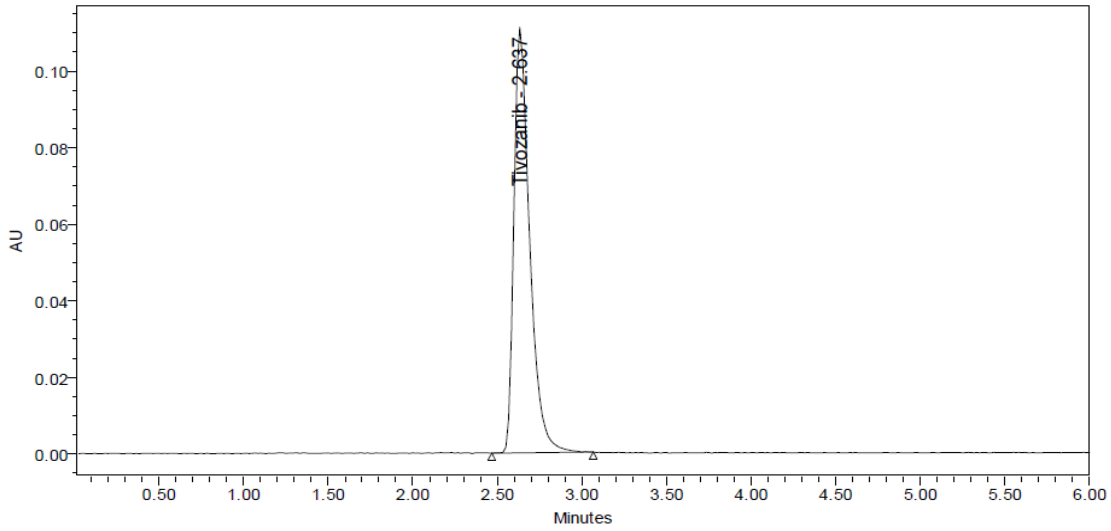


Figure No.2: Optimised Chromatogram

Table No.1: System suitability parameters

S no	Tivozanib		
Inj	RT(min)	USP Plate Count	Tailing
1	2.623	3852	1.52
2	2.626	3802	1.32
3	2.629	3848	1.48
4	2.632	3830	1.30
5	2.637	3841	1.49
6	2.640	3847	1.50

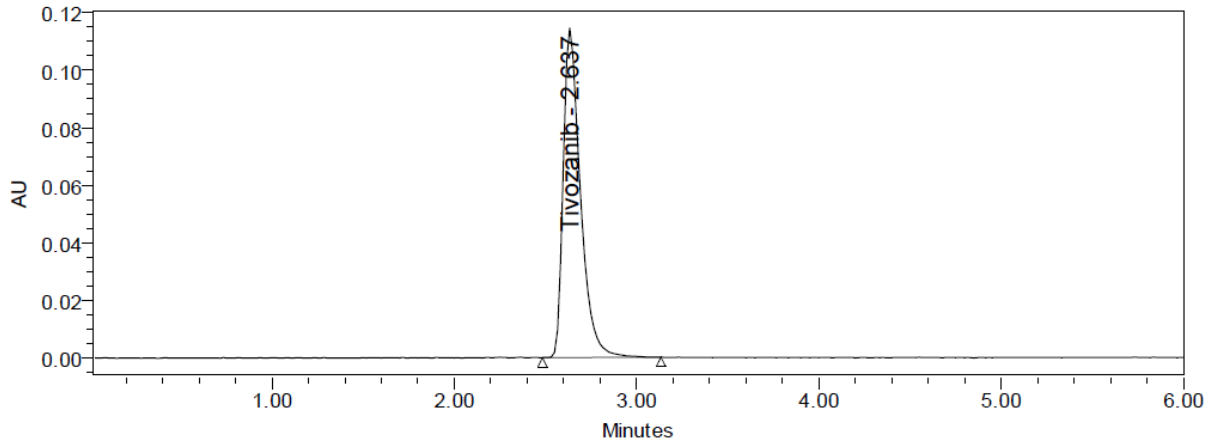


Fig No.3: Standard solution chromatogram

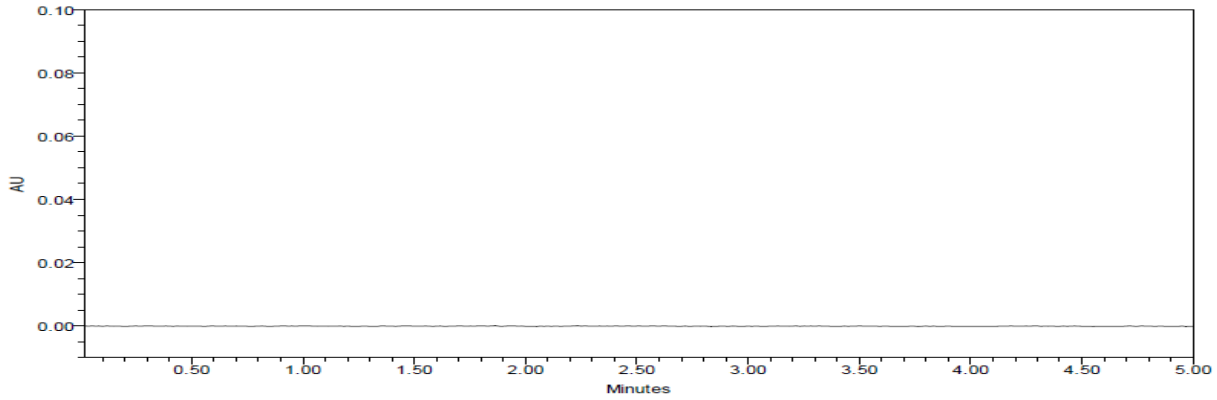


Figure No.4: Blank chromatogram

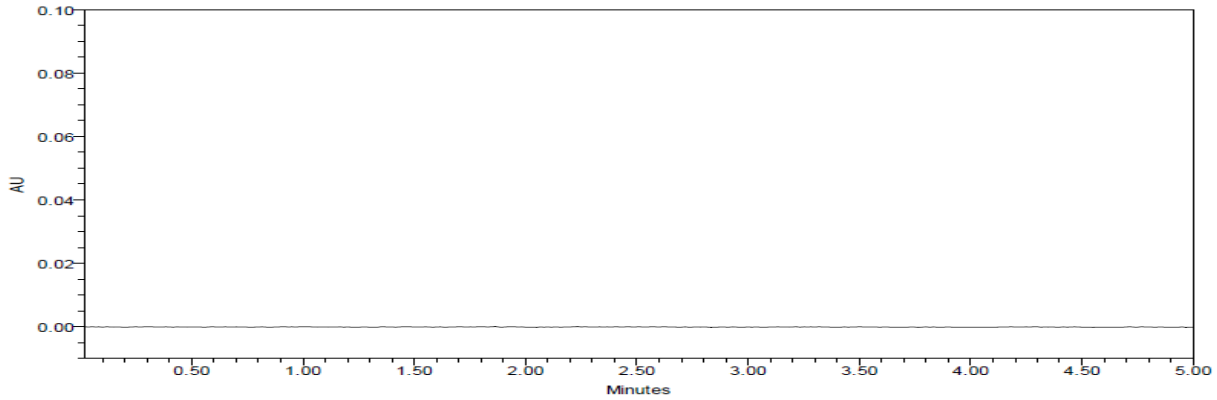


Fig No.5: Placebo chromatogram

Table No.2: Linearity table for Tivozanib,

Tivozanib	
Conc (µg/mL)	**Peak area**
3.35	188391
6.7	368609
10.05	551595
13.4	746770
16.75	913047
20.10	1114012

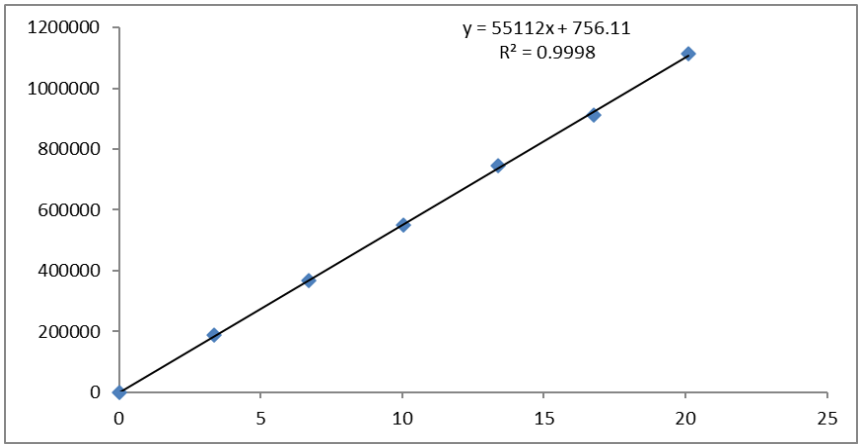


Fig No 6: Calibration curve of Tivozanib

Table No.3: Repeatability for Tivozanib

S.no.	**Tivozanib**
1	749548
2	749698
3	748865
4	747866
5	748723
6	742255
Mean	747826
S.D	2806.8
%RSD	0.4

Table No.4: Intermediate Precision for Tivozanib

S.no.	**Tivozanib**
1	742591
2	744936
3	741813
4	746493
5	744990
6	749648
Mean	745079
S.D	2819.9
%RSD	0.4

Table No.5: Accuracy for Tivozanib

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	6.7	6.76	100.87	99.89%
	6.7	6.69	99.79	
	6.7	6.66	99.38	
100%	13.4	13.34	99.57	
	13.4	13.32	99.39	
	13.4	13.47	100.55	
150%	20.1	19.94	99.21	
	20.1	20.03	99.66	
	20.1	20.23	100.63	

Table No.6: Robustness Data

S.no	Condition	%RSD of Tivozanib
1	Flow rate (-) 0.9ml/min	0.3
2	Flow rate (+) 1.1ml/min	0.4
3	Mobile phase (-) 65B:35A	0.3
4	Mobile phase (+) 75B:25A	0.6
5	Temperature (-) 27°C	0.5
6	Temperature (+) 33°C	0.4

Table No.7: Degradation Data

S.No.	Condition	%Undegraded	%Degraded
1	**Acid**	91.50	8.50
2	**Base**	92.32	7.68
3	**Oxidation**	91.04	8.96
4	**Dry heat**	96.39	3.61
5	**UV Light**	98.35	1.65

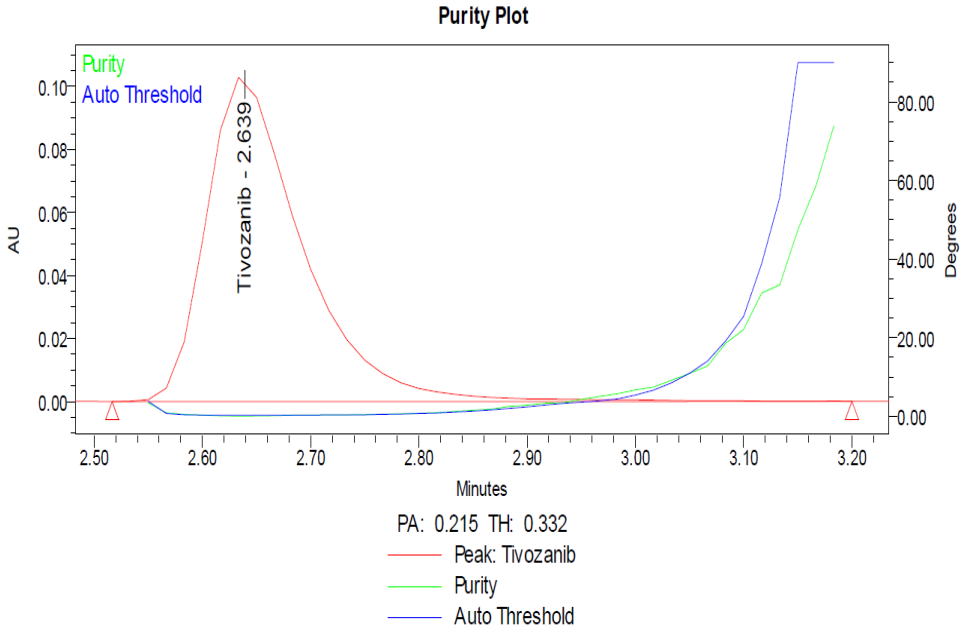


Fig No 7- purity plots

CONCLUSION:

An isocratic RP-HPLC technique with UV detection was developed for the identification and quantification of tivozanib in pure and pharmaceutical formulations. The method's selectivity, linearity, sensitivity, precision, and accuracy were statistically confirmed after it was validated following ICH guidelines. The stability of analytical solutions can be investigated using the suggested method. Given that regular drug analysis requires a low retention time for Tivozanib, the new method seems to be applicable as a quality control tool in the pharmaceutical industry.

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