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METHOD DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF BRIVARACETAM

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ABSTRACT:

The objective of this experimental work is the development and validation of stability-indicating the HPTLC method for the determination of Brivaracetam. A simple, fast, specific, rugged, and reproducible analytical method was developed and validated on a Camag HPTLC machine programmed with Camag Linomat 5 sample applicator Camag TLC 3 detector. The stationary phase used was precoated aluminium plates of silica gel $60F_{254}$ plates (10 cm ×10cm) with 250µm thickness, mobile phase used in the ratio of Toluene: Acetone: Methanol (6:2:2), saturated for15 min at room temperature (25°C±2) followed by densitometric scanning at 222nm. The developed protocol was most accurate, reproducible, and detectable towards Brivaracetam without any unwanted interference. When evaluated on various parameters like system suitability, precision, accuracy, linearity, specificity, robustness, LOD, and LOQ, force degradation study, the method is found to be efficient.

Keywords: Method Validation, HPTLC method, Brivaracetam, System suitability.

1. INTRODUCTION

HPTLC is superior to other analytical techniques in terms of total cost and time for analysis. It is an offline process in which various stages are carried out independently **[1]**. Important features of HPTLC include the ability to analyse crude

samples containing multi-components, application of a large number of samples and a series of standards using the spray-on technique, and choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step **[2]**, processing of standards and samples identically on the same plate leading to better accuracy and precision of quantification, different and universal selective detection methods, and in situ spectra recording in sequence to obtain identification of fractions, storage of total sample on a layer without time constrains **[3]**. In addition, the HPTLC method may help to minimizes the exposure risk of toxic organic effluents and significantly reduces its disposal problems, consequently, reducing environmental pollution **[4]**. Method development demands primary knowledge about the physicochemical characteristics of the sample, and the nature of the sample, such as structure, polarity, volatility, stability, and solubility. It involves considerable trial and error procedures. Steps involved in HPTLC method development are a selection of stationary and mobile phases, application of sample, development, derivatization, documentation of plate, labelling, quantitative evaluation of chromatogram, and documentation of work performed **[5]**.

Brivaracetam, [(2S)-2-[(4R)-2-oxo-4-propylpyrrolidin1-yl] butanamide], consists of two types of structural units such as a pyrrolidinone ring and a butanamide, whereas each motif such as pyrrolidinone at 4-position and butanamide at the 2-position has chiral centers. Thus, three different stereoisomers apart from the desired molecule are possible to exist during the process of development of a drug molecule **[6]**. Brivaracetam is a 4-n-propyl analogue of levetiracetam and a third-generation antiepileptic racetam derivative. In February 2016, the Food and Drug Administration (FDA) approved it as an add-on treatment. Brivaracetam is a drug that binds to the glycoprotein 2A in synaptic vesicles (SV2A) **[7]**.

There is relatively little information in the literature on brivaracetam development and validation using HPTLC. Bansode et al. established a TLC-based stability-indicating analytical method for determining Brivarecetam from bulk drugs and used it in forced degradation experiments **[8]**. LC–HRMS technologies were used to conduct metabolism investigations to identify drug metabolites from serum and plasma. The degradation behaviour of brivaracetam was investigated using high-performance liquid chromatography (HPLC) **[9]**. Considering the scarcity of the analytical methods of Brivarecetam we have undertaken this work to develop a simple, robust, cost-effective, and precise analytical method for

estimation of Brivarecetam using the HPTLC technique.

2. MATERIALS AND METHODS:

Chemicals and Reagents:

Brivaracetam drug was obtained as a gift sample from Glenmark Life Sciences Ltd, Pune, Maharashtra. Brivahenz 50 mg tablet (Intas pharmaceuticals Ltd) was procured from the local market. Toluene, Acetone, and Methanol were procured from Loba Chemie, Mumbai India. All the chemicals used were analytical grade.

HPTLC method development:

Chromatographic Conditions and instrument:

Pre-coated aluminium plates of silica gel $60F_{254}$ plates (10 cm ×10cm) with 250 µm thickness (E. Merk, Darmstadt, Germany) were used as stationary phase. The standard solutions were spotted in the form of bands and were applied with the help of Camag 100 µl sample (Hamilton, Bonaduz, Switzerland) syringe, on the silica gel plates, using a Camag Linomat V (Switzerland) sample applicator. Prewashing of plates was done with methanol and activated at 110°C for 5min in the oven before chromatography. Bands were applied with a constant application rate of 0.1µl/s with a space of 6mm. The chromatographic development was carried out using toluene: acetone: methanol (6:2:2 v/v) as a mobile phase with a chamber saturation time of 15 minutes and a migration distance of 80 mm. Densitometric scanning was performed using Camag TLC scanners 3 at 222 nm. The dimension of the slit was kept at (5mm×0.45mm) and the scanning speed was 10mm/s. Baseline correction was done and each track was scanned three times.

Mobile phase optimization:

Various solvent systems at different concentrations were tried for the mobile phase to separate and resolve the spot of brivaracetam from its impurities and other excipients of the formulation. Based on the satisfactory Rf value of Brivaracetam, Toluene: Acetone: Methanol (6:2:2) composition was selected as Optimised mobile phase. Development of plates was carried out in the linear ascending direction in a (20cm×10cm) twin trough glass chamber with a saturation time of 15 min at room temperature (25°C±2).

Preparation of standard stock solution:

The stock solution was prepared by dissolving accurately weighed 50 mg of Brivaracetam in a 50 ml volumetric flask containing methanol to give a 1000 µg/ml solution.

Analytical method validation:

As per ICH guidelines, the developed HPTLC method was validated for various parameters such as linearity, accuracy, precision, the limit of detection, the limit of quantification, repeatability, specificity, and robustness **[10, 11]**.

Linearity and calibration curve:

The linearity of the method was evaluated between the standard working solution of 5000 to 25000 ng/spot of Brivaracetam spotted on the plate. The calibration curve was plotted between concentration and peak area.

Precision:

The intraday (morning, afternoon, and evening) and inter-day (Day 1, Day 2, and Day 3) precision were evaluated at three different concentrations of (10000, 15000, and 20000 ng /band). The % relative standard deviation was calculated.

Accuracy:

For determining the accuracy of the developed method recovery studies of the drug were carried out. Accuracy was assessed by mixing a known quantity of standard drug Brivahenz 50 mg tablet (Intas Pharmaceuticals Ltd) and the contents were analysed by the standard method. Recovery studies were carried out at 80-120% levels. Percentage recovery and percentage RSD were then calculated.

Limit of detection (LOD) and limit of quantitation (LOQ):

The LOD and LOQ are calculated by using the equations, LOD = 3.3 × σ/S and LOQ = 10 × σ/S which are based on the standard deviation of the regression lines (σ) and slope of the calibration curve (S).

Specificity:

Brivaracetam was identified on a TLC plate, developed, and scanned to validate the specificity. The spectrum of brivaracetam recovered from the tablet was compared to the normal UV spectra of brivaracetam. By comparing their relative spectra at different positions of the spot, the peak purity of brivaracetam was determined.

Robustness:

Various factors were chosen for the study of robustness, including mobile phase composition, saturation time change, and scanning wavelength variation. The influence of a slight adjustment in one element on the outcomes was investigated.

Forced Degradation Studies (FDS):

Acid and base-induced FDS:

Acid and base hydrolysis studies were carried out by exposing drug solution to 0.1N HCl and 0.1N NaOH solution at RT for 60 mins. These solutions were applied on silica plates and developed in the mobile phase. Changes were observed on the denisitogram.

H_2O_2 induced FDS:

The sample was treated with a 3% hydrogen peroxide solution for 1 hour at RT. This solution was applied on a silica plate and developed in the mobile phase. Changes were observed on the denisitogram.

Thermal FDS:

The sample solution was exposed to 40^0C for 30 min and the sample was applied on a silica plate and developed in the mobile phase. Changes were observed on the denisitogram.

UV light-induced FDS:

The stock solution was exposed to direct UV light (254 nm) for 24 hrs. This solution was applied on a silica plate and run during the mobile phase. Changes were observed on the denisitogram.

3. RESULTS AND DISCUSSION

Mobile phase optimisation:

The most suitable mobile phase was found to be Toluene: Acetone: Methanol (6:2:2) which gave excellent resolution as well as reproducibility in the migration of Brivaracetam **(Figure 1)**. Based on the satisfactory Rf value of Brivaracetam, Toluene: Acetone: Methanol (6:2:2) composition was selected as an Optimized mobile phase with a saturation time of 15 min at room temperature (25°C±2).

Linearity and calibration curve:

The calibration curve was plotted as the peak area of the compound against the concentration over the range of 5000 to 25000 ng/spot of Brivaracetam. The amount of fraction applied on the plate and the peak area obtained are presented in **Table 1**. **Figure 2** exhibits a good correlation between the regression coefficient and the concentration of the drug.

Precision:

The intra-day and inter-day precision results are presented in **Tables 2 and 3** respectively. The relative standard derivations were found in the range of 0.40-1.3687% for intraday precision while 0.22-1.01 for interday precision. Smaller values of intra-day and inter-day variation in the analysis indicated that the method was precise.

Accuracy:

The % recovery of brivaracetam was found to be 98.49, 101.04, and 99.4 % (at 80%, 100% and 120% respectively). The recovery studies results indicated that the given method was accurate for estimation of the drug in a tablet dosage form. The results of the recovery study are presented in **Table 4**.

LOD and LOQ:

The LOD of 4350 ng /mL was observed for the Brivaracetam while LOQ was observed to be 13190 ng /mL. The approach demonstrated good LOD and LOQ sensitivity; nevertheless, because the goal of this developed method was to quantify Brivaracetam, these values should be regarded as the method's sensitivity limit.

Specificity:

Specificity is the method for analysing the Rf value and spectra pattern of a drug **[12]**. The good correlation among spectra gives the peaks at the start (s), apex (m), and end (e) indicating the peak purity of

Brivaracetam. Therefore, it can be assured that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drug. It was observed that excipients present in the formulation did not interfere with the peak of the drug (Rf 0.57±002). Hence, the above HPTLC method was found to be specific. Brivaracetam was identified on a TLC plate, developed, and scanned to validate the specificity. The spectrum of brivaracetam recovered from the tablet was compared to the normal UV spectra of brivaracetam. By comparing their relative spectra at different positions of the spot, the peak purity of brivaracetam was determined **(Figure 3)**.

Robustness:

The robustness of the developed method was confirmed by changing the mobile phase composition, saturation time, and scanning wavelength. In the case of change in the composition of the mobile phase, there was no significant change was observed in the peak area of the Brivaracetam. The % RSD was found between 0.440 to 1.061 which is quite within the acceptable limit of NMT 2% **(Table 5)**. Even variation in saturation time also did not affect the peak areas and % RSD (0.267 to 1.299) was also found between the acceptable limit **(Table 6)**. Moreover, variation in scanning wavelength also showed satisfactory results with a % RSD of 0.246 to 1.009 **(Table 7)**. These observations confirmed the robustness of the developed method.

Brivaracetam was tested with the addition of HCl and NaOH at the same concentration. On the chromatogram obtained from a sample after acidic hydrolysis reaction **(Figure 4A)** a satisfactory separation of Brivaracetam and the degradation products is observed. Separation of the basic hydrolysis degradation products of Brivaracetam was also achieved with a satisfactory result **(Figure 4B)**. Complete degradation of Brivaracetam was observed in samples containing hydrogen peroxide **(Figures 5A)**. Chromatographic separation of degradation products was achieved. During thermal degradation of Brivaracetam, three degradation products were produced **(Figure 5B)**. 24-h exposure to UV radiation caused complete decomposition of Brivaracetam in solution **(Figure 6)** as well as reduction of the intense of the peak. The details of different forced degradation conditions with relative peaks, peak area, and % degradation are presented in **Table 8**.

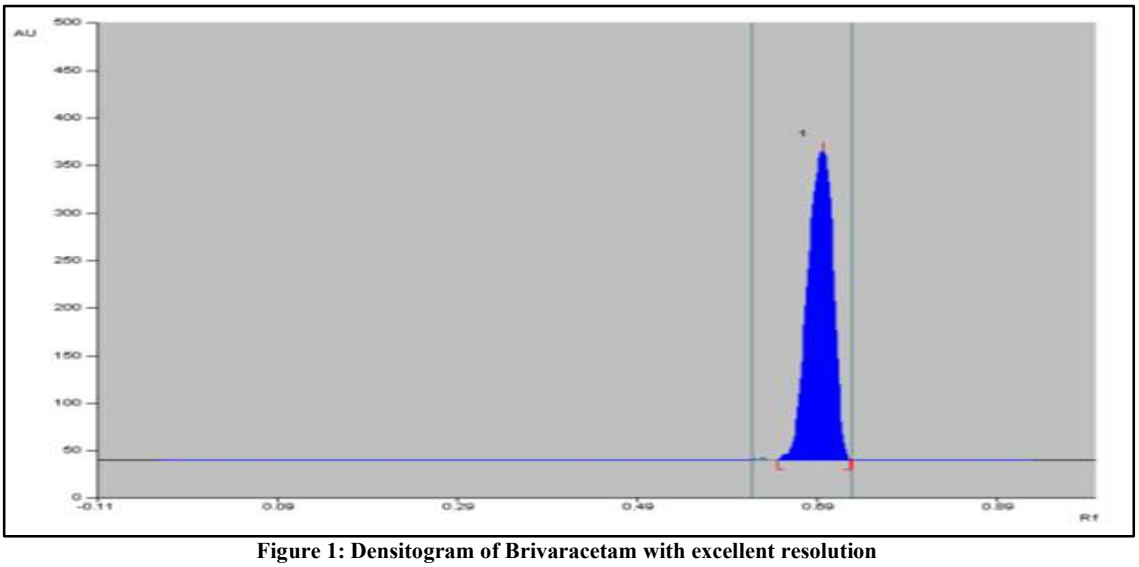

Figure 1: Densitogram of Brivaracetam with excellent resolution

Table 1: Concentration and peak area of Brivaracetam

Application volume (µl)	Amount fraction (ng)	Peak Area
5	5000	3699.60
10	10000	5725.69
15	15000	7606.75
20	20000	9842.43
25	25000	11255.29

The value of the regression coefficient (R^2) was found to be 0.9962 with linearity equation y=384.56x +1857.5

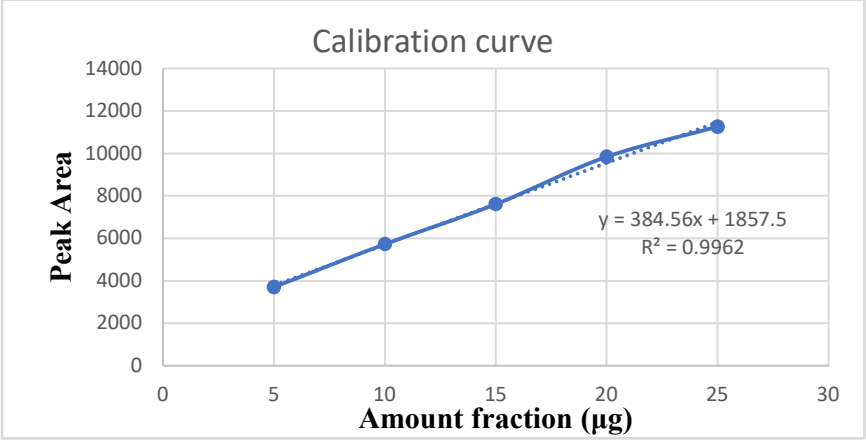

Figure 2: Densitogram of Brivaracetam with excellent resolution

Table 2: Intraday precision study results

Concentration (ng /band)	Mean area*	% RSD	Area	% RSD	Area	% RSD
	Morning		Afternoon		Evening	
10000	5763.45	0.76	5738.34	0.34	5826.72	0.74
15000	7591.24	0.68	7658.24	0.73	7658.24	0.97
20000	9809.64	0.40	9791.45	0.63	9791.45	1.36

***n=3**

Table 3: Interday precision study results

Concentration (ng /band)	Mean area*	% RSD	Area	% RSD	Area	% RSD
	Day 1		Day 2		Day 3	
10000	5788.95	0.61	5802.10	0.35	5785.75	1.01
15000	7648.21	0.25	7591.24	0.68	7721.00	0.73
20000	9796.33	0.61	9864.74	0.22	9763.82	0.33

***n=3**

Table 4: Recovery studies at different levels (n=3)

Amount of drug spiked (%)	Amount spiked (ng)	Amount recovered (ng)	% Recovery	% Mean recovery	Mean Peak Area	% RSD
80	12000	11818.8	98.49	99.65	13874.63	0.8307
100	15000	15156.0	101.04		15301.43	0.9637
120	18000	17899.2	99.44		16693.86	0.8770

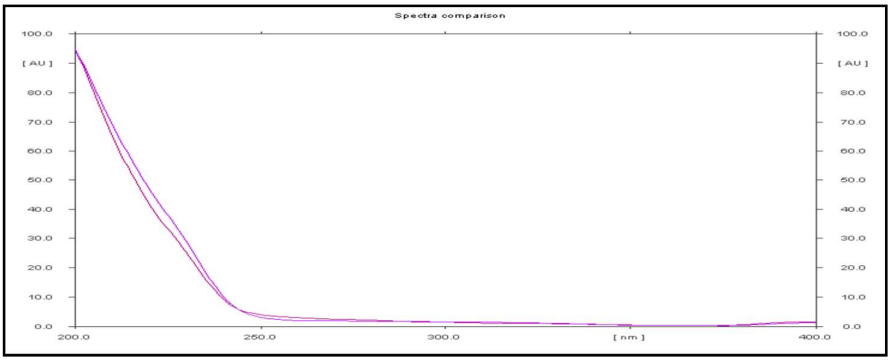

Figure 3: UV spectra of standard and formulation

Table 5: Variation in composition of mobile phase

Composition	Mean area**	%RSD
5.9: 2.1: 2	7668.613	0.495
6.1: 1.9: 2	7738.107	0.889
6: 1.9:2.1	7801.67	0.639
6: 2.1:1.9	7680.817	0.440
5.9: 2:2.1	7771.44	1.061
6.1: 1.9: 2	7748.703	0.837

****n=3**

Table 6: Variation in saturation time

Saturation time (±Min)	Mean area**	%RSD
10	7735.28	1.299
15	7630.107	0.267
20	7701.67	0.772

****n=3**

Table 7: Variation in scanning wavelength

Wavelength (nm)	Mean area**	%RSD
221	7632.593	0.841
222	7665.213	0.246
223	7678.703	1.009

****n=3**

Forced degradation studies:

Table 8: Forced degradation study results in different conditions

Force Degradation condition	Rf Values of degradation product		% Degradation	% Drug
Acid, 0.1 N HCl, RT for 60 min	Peak 1-	0.04		
	Peak 2-	0.50		
	Peak 3-	0.64	18.18421	81.81579
Alkaline 0.1 N NaOH, RT for 60 min	Peak 1-	0.08		
	Peak 2-	0.12		
	Peak 3-	0.26		
	Peak 4-	0.59	16.43936	83.56064
	Peak 5-	0.63		
	Peak 6-	0.71		
	Peak 7-	0.78		
	Peak 8-	0.83		
H_2O_2,3% w/v for 60 min	Peak 1-	0.12		
	Peak 2-	0.23		
	Peak 3-	0.38		
	Peak 4-	0.42		
	Peak 5-	0.47		

	Peak 6-	**0.63**	**13.79845**	**86.20155**
	Peak 7-	**0.70**		
Thermal degradation at 40°C for 30 min	**Peak 1-**	**0.37**		
	Peak 2-	**0.46**		
	Peak 3-	**0.52**		
	Peak 4-	**0.63**	**15.28314**	**84.71686**
UV (254 nm for 24 Hr)	**Peak 1-**	**0.38**		
	Peak 2-	**0.51**		
	Peak 3-	**0.64**	**16.14505**	**83.85495**

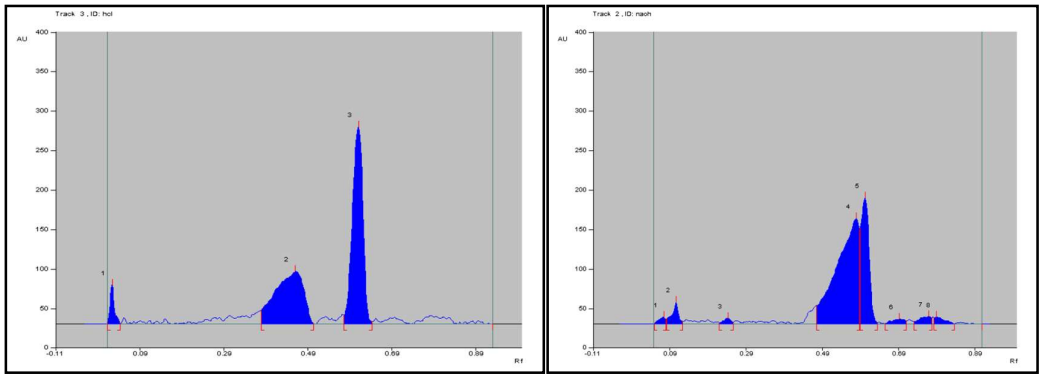

Figure 4: A: Densitogram of acid degradation and B: Densitogram of base degradation

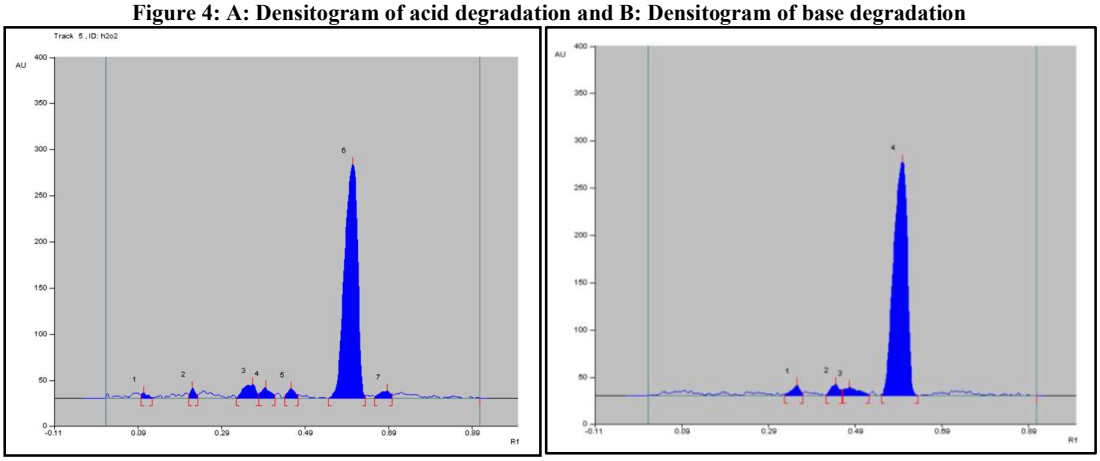

Figure 5: A: Densitogram of H2O2 degradation and B: Densitogram of thermal degradation

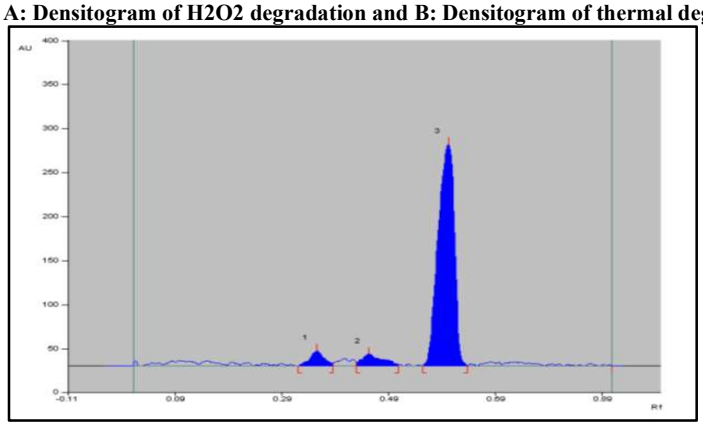

Figure 6: Densitogram of UV degradation

4. CONCLUSION:

The current research provides positive results for method validation and the development of a stability-indicating HPTLC method for brivaracetam quantification. For regular analysis of

brivaracetam bulk and tablet dosage forms, the HPTLC approach is straightforward, quick, affordable, and more adaptable. As a result, the suggested approach for quality control and analysis of brivaracetam from bulk drugs and formulations is significant and has strong industrial applications.

CONFLICT OF INTEREST:

The authors do not report any conflict of interest with respect to this manuscript and research work

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