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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR DETERMINATION OF LOXAPINE SUCCINATE IN CAPSULE DOSAGE FORM

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ABSTRACT: To develop and validate simple, selective, specific, sensitive and accurate stability indicating assay method by RP-HPLC for determination of Loxapine succinate in the capsule dosage form. Several trials are taken by changing mobile phase composition, and ratio and finally the drug was eluted on a C18 Purospher star (250 mm × 4.6 mm, 5 µm) column using mobile phase consisting of water: Methanol: Acetonitrile: TEA:: 40:10:50:1 ratio and pumped at flow rate of 1.0 ml/min using 10 µl injection volume at 254 nm. The Retention time of Loxapine succinate was found to be 10.783 ± 0.02 min. The calibration curve was linear over concentration range of 75-225 µg/ml (r^2 = 0.999). The accuracy of the method was estimated by recovery studies and % recovery was in a range of 98% to 102%. Stability of Loxapine succinate was studied under different stressed condition like acid, alkali, peroxide, thermal and photolytic conditions. The drug was degraded in all conditions except thermal and photolytic conditions. The developed method was validated as per ICH guidelines.

INTRODUCTION: Loxapine succinate is a typical antipsychotic drug, member of dibenzoxazepine class and used to treat certain mental or mood disorders (such as schizophrenia). It is a dopamine antagonist and also serotonin 5-HT2 blocker. The exact mode of action of Loxapine succinate has not known. This drug helps in reducing aggression and hallucinations (such as hearing/ seeing things that are not occurred). Loxapine is present in capsules as the succinate salt [1, 2, 3]. Its IUPAC name is 8-chloro-6-(4-methyl piperazine-1yl)benzo[b][1,4] benzoxazepine. Its molecular weight is 445.9 gm/mole [4, 5].

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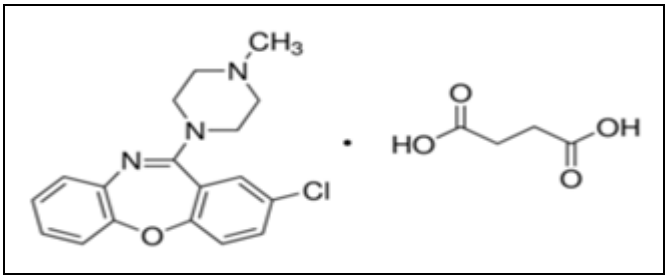

FIG. 1: CHEMICAL STRUCTURE OF LOXAPINE SUCCINATE [6, 7]

A literature search reveals that the following methods are reported *viz*. UV method, HPLC method, HPLC-MS/MS, GC-MS method [8, 9, 10, 11, 12, 13]. There is a need for developing stability indicating assay method in the capsule dosage form. So, the authors attempted to develop simple, sensitive, selective and specific stability indicating assay method for determination of Loxapine succinate in a capsule dosage form as per ICH guidelines [14, 15, 16, 17].

MATERIALS AND METHODS:

Chemicals and Reagents: Loxapine succinate was provided as a gift sample by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. Capsule dosage form was procured from the local market. All the chemicals and reagents like methanol, acetonitrile, water were of HPLC grade and were procured from Merck Specialties Pvt. Ltd., Mumbai, India.

Instrumentation: HPLC waters Alliance 2695 series, equipped with an autosampler, temperature control, and auto-injector with the capacity to inject 5 µl to 500 µl with PDA detector. Waters HPLC system is equipped with Empower 2 software.

METHODS:

Experimental Conditions: The HPLC system was operated isocratically at a flow rate of 1.0 ml/min at 30°C column temperature for 30 min. The mobile phase was found to be most suitable for analysis was water: Methanol: Acetonitrile: TEA:: 40:10:50:1 ml which was filtered through 0.45 µm PVDF filter. Detection was carried out at 254 nm. The mobile phase is used as a diluent.

Preparation of Standard Solution of Loxapine Succinate: Accurately weighed and Transfer 60 mg of Loxapine succinate into 200 ml volumetric flask. Add diluent about 70% of flask volume. Sonicate to dissolve and volume made up to mark with diluents. Pipette out 10 ml of the stock solution into 20 ml volumetric flask; volume made up to mark with diluents (150 µg/ml). Results were shown in **Fig. 3** and **Table 1**.

Calibration Curve of Loxapine Succinate: Transfer accurately weighed 30 mg of Loxapine into 50 ml volumetric flask. Add 5 ml of diluent. Sonicate to dissolve, volume made up to mark with diluents (600 µg/ml). from 600 µg/ml appropriate aliquots like 2.5 ml, 4 ml, 5 ml, 6 ml, and 7.5 ml taken into 20 ml volumetric flask and made up to mark with diluents, so the resulting solutions become 75, 120, 150, 180 and 225 µg/ml.

Preparation of Sample Solution of Loxapine Succinate: Open 20 capsules and mix and blend powder. Accurately weighed and transferred powder equivalent to 75 mg of loxapine succinate into 50 ml volumetric flask. Add diluent about 50% of flask volume. Sonicate for 30 min with intermitting shaking, volume made up to mark with diluent. Solution filter using 0.45 µm PVDF filter after 5 ml of filtrate discarded. Pipette out 5 ml of the stock solution into 50 ml volumetric flask; volume made up to mark with diluents (150 µg/ml). Results were shown in **Fig. 3** and **Table 2**.

Method Development and Optimization: During the process of method development, several trials were taken using different aqueous phase, organic phase, different column, good peak shape was observed when using the C18 Purospher star (250 mm × 4.6 mm, 5 µm) column and water: Methanol: Acetonitrile: TEA:: 40:10:50:1 as mobile phase at a flow rate of 1.0 ml/min. for the quantitative analytical purpose, the wavelength was selected as 254 nm.

Initially, standard solution 150 µg/ml was injected into column five times and the retention time of Loxapine succinate was found to be 10.783 min. Then the same concentration of sample solution was injected into the system and the resulting chromatogram was recorded.

Method Validation: The developed method was validated as per the USP and ICH guideline Q2(R1).

Specificity: The specificity of the method was evaluated to ensure there was no interference from the placebo components or from products resulting from forced degradation.

Forced Degradation Studies:

Acid Degradation Sample: Accurately weighed and transferred Loxapine succinate equivalent to 75 mg of loxapine into 50 ml volumetric flask. Add 10 ml 1N HCl solution. Keep solution at room temperature for 5 h. After 5 h neutralize with 10 ml of 1N NaOH. Add diluent about 50% of flask volume. Sonicate for 30 min with intermittent shaking, volume made up to mark with diluent. Solution filter using 0.45 µm PVDF filter after 5 ml of filtrate discarded. Pipette out 5 ml of the stock solution into 50 ml volumetric flask, volume made up to mark with diluent. Results are shown in **Fig. 4** and **Table 3**.

Base Degradation Sample: Accurately weighed and transferred Loxapine succinate equivalent to 75 mg of loxapine into 50 ml volumetric flask. Add 10 ml 0.1N NaOH solution. Keep solution at room

temperature for 5 h. After 5 h neutralize with 10 ml of 0.1N HCl. Add diluent about 50% of flask volume. Sonicate for 30 min with intermittent shaking, volume made up to mark with diluent. Solution filter using 0.45 µm PVDF filter after 5ml of filtrate discarded. Pipette out 5 ml of the stock solution into 50 ml volumetric flask; volume made up to mark with diluent. Results are shown in **Fig. 5** and **Table 4**.

Peroxide Degradation Sample: Accurately weighed and transferred Loxapine succinate equivalent to 75 mg of loxapine into 50 ml volumetric flask. Add 10 ml 30% H_2O_2 solution. Keep solution at room temperature for 5 h. Add diluent about 50% of flask volume. Sonicate for 30 min with intermittent shaking, volume made up to mark with diluent. Solution filter using 0.45 µm PVDF filter after 5 ml of filtrate discarded. Pipette out 5 ml of the stock solution into 50 ml volumetric flask; volume made up to mark with diluent. Results are shown in **Fig. 6** and **Table 5**.

Thermal Degradation Sample: Accurately weighed and transferred Loxapine succinate equivalent to 75 mg of loxapine into 50 ml volumetric flask. Keep at 85 ºC for 7 days in vaccum oven. Add diluent about 50% of flask volume. Sonicate for 30 min with intermittent shaking, volume made up to mark with diluent. Solution filter using 0.45 µm PVDF filter after 5ml of filtrate discarded. Pipette out 5 ml of the stock solution into 50 ml volumetric flask; volume made up to mark with diluent. Results are shown in **Fig. 7** and **Table 6**.

Photolytic Degradation Sample: Put Capsule Sample into UV Chamber for 7 days to complete one ICH cycle accurately weighed and transferred Loxapine succinate equivalent to 75 mg of loxapine into 50 ml volumetric flasks. Add diluent about 50% of flask volume. Sonicate for 30 min with intermittent shaking, volume made up to mark with diluent. Solution filter using 0.45 µm PVDF filter after 5 ml of filtrate discarded. Pipette out 5 ml of the stock solution into 50 ml volumetric flask; volume made up to mark with diluent. Results are shown in **Fig. 8** and **Table 7**.

Results for % degradation and % assay after degradation were shown in **Table 8.**

Linearity: The concentration range 75, 120, 150, 180 and 225 µg/ml was injected into the system. Linearity was evaluated by linear regression analysis which was obtained by plotting the Loxapine succinate against peak area. The regression coefficient was found to be 0.999. linearity data were shown in **Table 9**.

Accuracy: The accuracy of the method was determined at three different concentration levels 50%, 100% and 150% of target concentration (150 µg/ml) by recovery studies. The recovery studies were carried out in triplicate on composite blend collected from 20 capsules of Loxapine succinate and analyzed as per the proposed method.

Individual recovery was found to be in the range of 97.0% to 103% and mean recovery was found to be in a range of 98% to 102%. The results of accuracy revealed that the method was accurate. Results for accuracy were shown in **Table 10**.

Precision: Repeatability study was carried out by estimating six determination of Loxapine succinate with 150 µg/ml. The intraday precision study of loxapine succinate was carried out by estimating the peak response for three determination on the same day with 120 µg/ml, 150 µg/ml and 180 µg/ml concentration and interday precision study of Loxapine succinate was carried out by estimating the peak response for three determination on different days using 120 µg/ml, 150 µg/ml and 180 µg/ml.

SD and % RSD was calculated and found to be within limits. The results of the precision study revealed that the method was precise as shown in **Table 11, 12, 13**.

Robustness: The Robustness of the analytical method was determined by analysis of aliquots from the homogenous sample by varying the chromato-graphic parameters like change in flow rate, change in column oven temperature, change in mobile phase ratio, change in wavelength, change in HPLC column and their impact on retention time and peak area were studied.

The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters. Results are shown in **Table 14**.

RESULTS AND DISCUSSION:

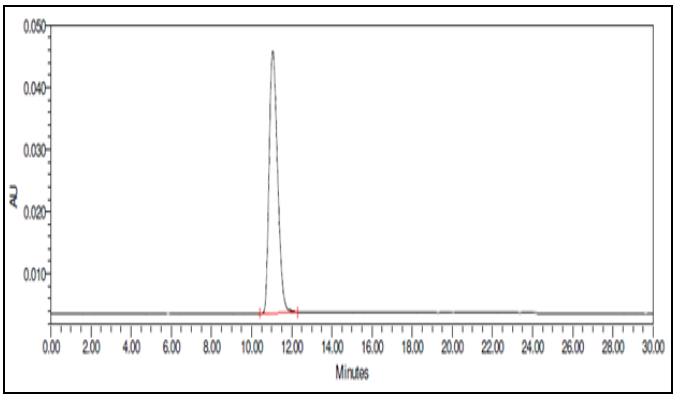

FIG. 2: STANDARD CHROMATOGRAM FOR LOXAPINE SUCCINATE 150 µg/ml

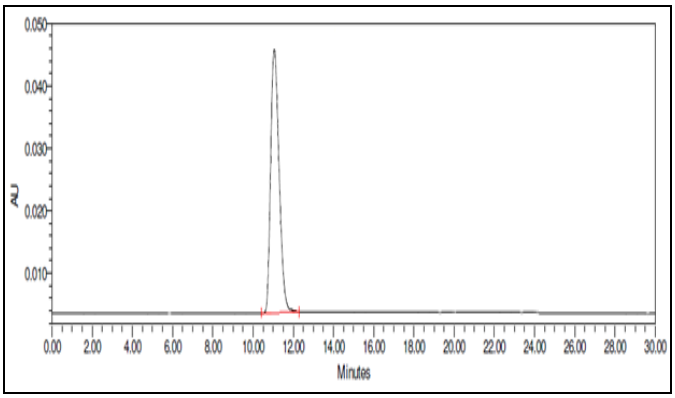

FIG. 3: SAMPLE CHROMATOGRAM FOR LOXAPINE SUCCINATE 150 µg/ml

TABLE 1: STANDARD LOXAPINE SUCCINATE

Peak Name	RT	Area	USP Tailing	USP Plate count	Purity Angle	Purity Threshold
Loxapine	10.783	552387	1.12	23495	1.276	1.735

TABLE 2: FOR SAMPLE LOXAPINE SUCCINATE

Peak Name	RT	Area	USP Tailing	USP Plate count	Purity Angle	Purity Threshold
Loxapine	10.985	542976	1.12	23478	1.324	1.712

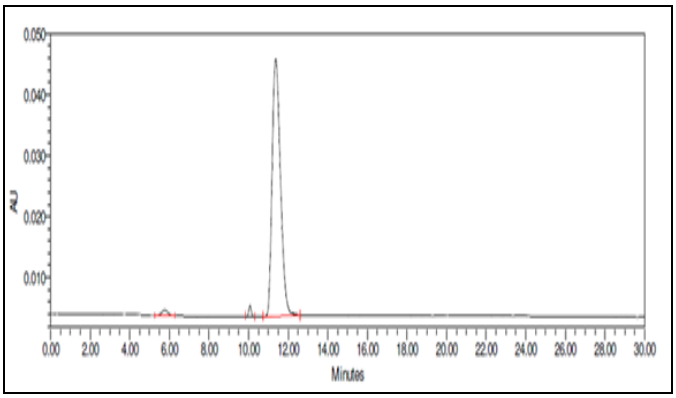

FIG. 4: CHROMATOGRAM FOR LOXAPINE SUCCINATE - ACID DEGRADATION

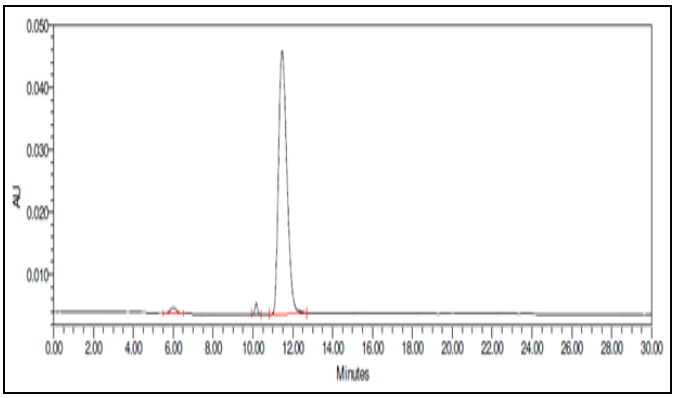

FIG. 5: CHROMATOGRAM FOR LOXAPINE SUCCINATE - BASE DEGRADATION

TABLE 3: FOR ACID DEGRADATION

Peak Name	RT	Area	USP Tailing	USP Plate count	Purity Angle	Purity Threshold
UnkImp	5.872	20487	1.45	4236	2.875	3.986
UnkImp	9.985	38125	1.01	3478	1.798	1.143
Loxapine	10.823	501254	1.20	21870	0.998	1.572

TABLE 4: FOR BASE DEGRADATION

Peak Name	RT	Area	USP Tailing	USP Plate count	Purity Angle	Purity Threshold
UnkImp	5.932	18765	1.54	4187	1.246	2.467
UnkImp	9.879	35398	1.05	3562	5.986	4.651
Loxapine	10.756	511659	1.21	22870	1.876	2.246

TABLE 5: FOR PEROXIDE DEGRADATION

Peak Name	RT	Area	USP Tailing	USP Plate count	Purity Angle	Purity Threshold
Loxapine	10.752	522541	1.21	22212	1.684	1.709

TABLE 6: FOR THERMAL DEGRADATION

Peak Name	RT	Area	USP Tailing	USP Plate count	Purity Angle	Purity Threshold
Loxapine	10.687	542121	1.21	25687	1.342	1.673

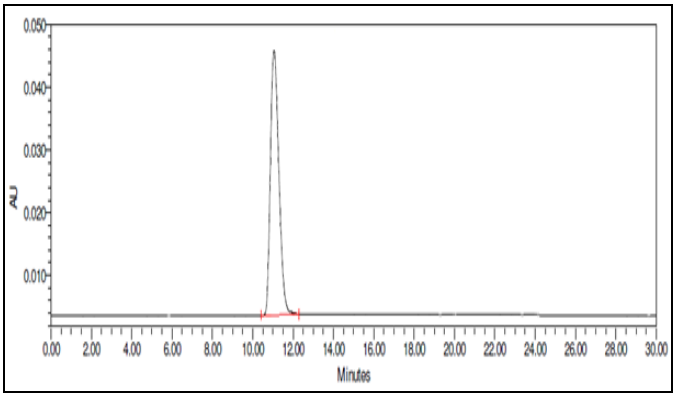

FIG. 6: CHROMATOGRAM FOR LOXAPINE SUCCINATE- PEROXIDE DEGRADATION

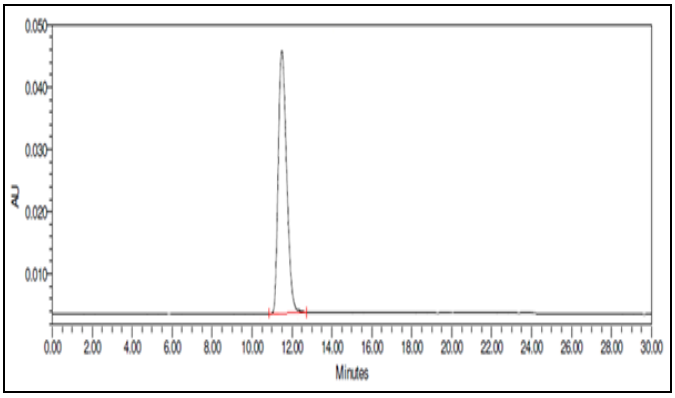

FIG. 7: CHROMATOGRAM FOR LOXAPINE SUCCINATE- THERMAL DEGRADATION

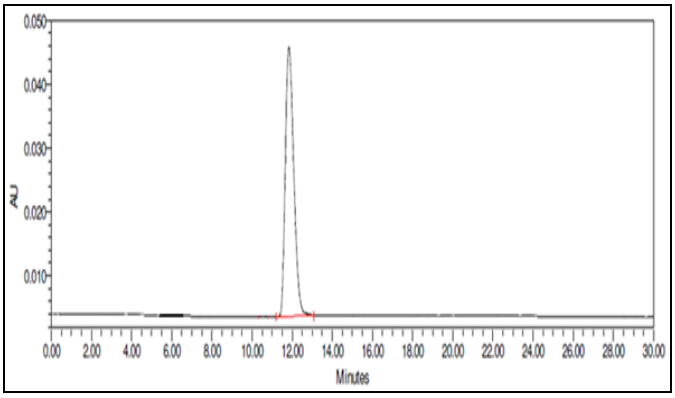

FIG. 8: CHROMATOGRAM FOR LOXAPINE SUCCINATE- PHOTOLYTIC DEGRADATION

TABLE 7: FOR PHOTOLYTIC DEGRADATION

Peak Name	RT	Area	USP Tailing	USP Plate count	Purity Angle	Purity Threshold
Loxapine	10.821	539921	1.17	26009	2.286	3.245

TABLE 8: FORCED DEGRADATION STUDIES FOR LOXAPINE SUCCINATE

Stressed condition	Retention time (min)	Peak area	% Purity	% of degradation
Standard drug	10.547	545432	100	-
Acid degradation	10.823	501254	90.95	9.0
Alkaline degradation	10.756	511659	92.83	7.2
Oxidative Degradation	10.752	522541	94.81	5.2
Thermal degradation	10.687	542121	98.36	1.6
Photolytic degradation	10.821	539921	97.96	2.0

TABLE 9: FOR LOXAPINE SUCCINATE- LINEARITY

Level %	Concentration(µg/ml)	Mean peak area
50	75	272175
80	120	437832
100	150	543456
120	180	657752
150	225	815184

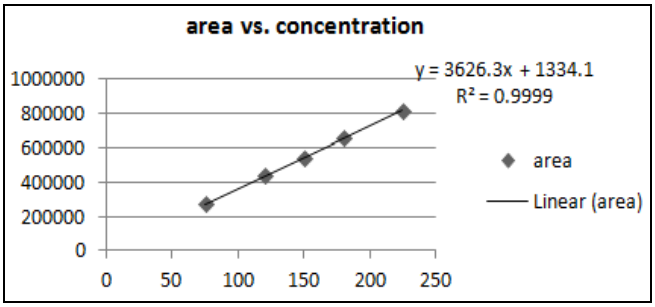

TABLE 10: RECOVERY STUDIES FOR LOXAPINE SUCCINATE

Level of recovery	Amt added (µg/ml)	Peak area	Amt recovered	% recovery	Mean %recovery	% RSD
50	75	275645	76.23	101.63	100.20	1.29
50	75	270789	74.90	99.86		
50	75	268709	74.33	99.10		
100	150	544560	149.75	99.83	99.04	0.74
100	150	539481	148.37	98.91		
100	150	536480	147.54	98.36		
150	225	814545	223.58	99.37	100.05	0.59
150	225	823989	226.16	100.51		
150	225	821883	225.59	100.26		

TABLE 11: REPEATABILITY STUDY

Concentration	Peak area
150 µg/ml	546756
	538917
	558789
	545587
	548483
	556480
Average	549169
% SD	7351.50
% RSD	1.34

TABLE 12: INTRADAY PRECISION

Conc. µg/ml	Peak area			Mean area	SD	% RSD
	Set-I	Set-II	Set-III			
120	437832	436711	425690	433411	6710.033	1.55
150	543456	542478	537812	541248.7	3016.145	0.56
180	657752	658321	641356	652476.3	9634.693	1.48

TABLE 13: INTERDAY PRECISION

Conc. µg/ml	Peak area			Mean area	SD	% RSD
	Set-I	Set-II	Set-III			
120	437832	426930	425421	430061	6772.046	1.57
150	543456	531546	529874	534958.7	7406.241	1.38
180	657752	646853	644370	649658.3	7118.42	1.1

TABLE 14: ROBUSTNESS

Change in parameter	Values	Retention time	USP plates	USP tailing	% RSD of standard area	% assay	% RSD
Control	As per method	10.78	23495	1.12	1.34	99.53	1.35
Flow rate	0.8 ml/min	10.54	22764	1.10	1.30	99.41	1.29
	1.2 ml/min	10.81	22542	1.10	1.31	98.88	1.29
Wavelength	252 nm	10.23	23985	1.20	1.33	99.34	1.36
	256 nm	10.89	22563	1.10	1.31	100.87	1.34
Column temperature	25 °C	10.78	23495	1.12	1.30	99.09	1.35
	35 °C	10.78	23494	1.11	1.34	99.12	1.28
Mobile phase composition	Water: MeOH: ACN: TEA 50:10:40:1ml	10.91	23652	1.12	1.35	99.32	1.38
	Water: MeOH: ACN: TEA 30:10:60:1ml	10.87	23127	1.11	1.30	99.50	1.28

TABLE 15: ASSAY OF CAPSULE DOSAGE FORM (LOXAPINE SUCCINATE)

Label claim	Amount found	% Assay	Average	SD	% RSD
34 mg	33.84	99.55	99.55	0.0252	0.0253
	33.86	99.58			
	33.84	99.53			

CONCLUSION: This study presents simple and validated stability-indicating HPLC method for the determination of Loxapine succinate in the presence of its degradation products. The developed method is specific, accurate, precise and robust. All the peaks of the degradation products formed during forced degradation studies were well separated from the analyte peak. Hence, the developed method can be used for routine analysis for the determination of Loxapine succinate in capsule dosage forms.

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CONFLICT OF INTEREST: The authors reveal that there are no conflicts of interest.

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