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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ITRACONAZOLE AND TERBINAFINE IN COMBINE DOSAGE FORMS BY RP HPLC METHOD

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ABSTRACT:
This study introduces a novel RP-HPLC technique for simultaneous measurement of Itraconazole and Terbinafine. To get the best results, we ran a series of experiments C18 ODS Agilent make column with 4.6*150mm at a temperature of 5, a pumping flow of 1 ml/min, and a MP ratio of (70:30 v/v) to separate Itraconazole and Terbinafine. The detection wavelength was 254 nm and the medium was methanol: (KH2PO4and KH2PO4) pH 3. Both Itraconazole (101.27%) and Terbinafine (99.99%) passed their purity tests with comparable findings. The analysis method was validated in accordance with ICH standards (ICH, Q2 (R1)). Linearity study conducted on the medications Itraconazole and Terbinafine throughout the concentration ranges of 50 mg to 250 mg and 5 mg to 50 mg, respectively, revealed recoveries of 99.56 and 99.48%, respectively. Intermediate precision had an RSD of 0.1, while repeatability was 0.2. Reliability and repeatability of the research were not compromised in any way.

KEYWORDS: ODSC18, Itraconazole and Terbinafine, RP-HPLCmethod

INTRODUCTION:

Itraconazole is an antifungal agent that inhibits cytochrome P-450-dependent enzymes resulting in impairment of ergosterol synthesis. It has been used against histoplasmosis, blastomycosis, cryptococcal meningitis & aspergillosis. Itraconazole interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Itraconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the

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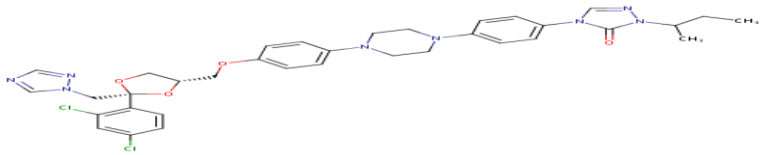
transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis.

Figure 1: Chemical structure of Itraconazole



Terbinafine hydrochloride (Lamisil) is a synthetic allylamine antifungal. It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues. Like other allylamines, terbinafine inhibits ergosterol synthesis by inhibiting the fungal squalene monooxygenase (squalene 2,3-epoxidase), an enzyme that is part of the fungal cell wall synthesis pathway. Terbinafine is hypothesized to act by inhibiting squalene monooxygenase, thus blocking the biosynthesis of ergosterol, an essential component of fungal cell membranes. This inhibition also results in an accumulation of squalene, which is a substrate catalyzed to 2,3-oxydo squalene by squalene monooxygenase. The resultant high concentration of squalene and decreased amount of ergosterol are both thought to contribute to terbinafine's antifungal activity

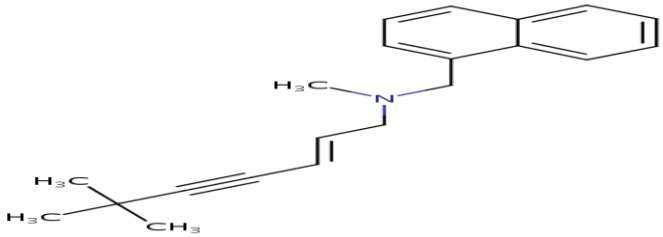


Figure 2: Chemical structure of Terbinafine

MATERIALS AND METHODS:

Equipment: Chromatographic separation was conceded on WATERS HPLC system which is outfitted with the 515 dual head reciprocating pump & a 2489 UV Visible detector. The software used is Empower-2 software and Phenomenex kinetex C_{18} (250mm×4.6mm i.d, 5µm) column is used for the investigation.

Chemicals and reagents: Itraconazole and Terbinafinedrugs were gifted by Aurobindo Pharmaceuticals, Hyderabad, Telangana, India. Acetonitrile, methanol, HPLC grade waterand mono sodium hydrogen orthophosphate and di sodium hydrogen ortho phosphatewere procuredfrom local manufacturers.

Selection of Detection wavelength:

10mg of Itraconazole and Terbinafine was dissolvedin mobilephase. The solution was scanned from200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Itraconazole and Terbinafine. The isobestic point was taken

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as detection wavelength.

Selection of column:

Column is selected basedon solubility, polarity and chemical differences among Analytes [Column: AgilentC18(4.6x250mm,5µm]

Selection of mobile phase:

Methanol:ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pHshould bebetween 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved.If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response,Area,Tailing factor,Resolution will be achieved.

Preparations and procedures:

Preparation of mobile phase*:*

A mixture of Methanol 700ml (70%),300 mL of ACN (30%) are taken and degassed in ultrasonic waterbath for 5minutes. Then this solution is filtered through 0.45µ filter under vacuum filtration.

DiluentPreparation*:*

Mobile phase is used as Diluent**.**

Preparation of the individual Itraconazole standard preparation:

10mg of Itraconazole working standard was accurately weighed and transferred in to a10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent.(Stock solution). Further 10.0 ml from the above stock solution is pipette into a100ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Terbinafine standard preparation:

10mg of Terbinafine working standard was accurately weighed and transferred in to a10ml clean dry volumetric flask and about 2ml of DMF is added. Then it issonicated to dissolve it completely and made volumeup to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette in to a100ml volumetric flask and was diluted upto the mark with diluent.

Preparation of Sample Solution:(Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to10mg of Terbinafine and Itraconazole (marketed formulation) sample in to a10mLclean dry volumetric flask and about 7mLof diluents is added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stocksolution)Further 3ml of above stock solution was pipetted in to a10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for Terbinafine and Itraconazole peaks are measured and the %Assayare calculated by using the formulae.

System Suitability:

Tailing factor for the peaks due to Terbinafine and Itraconazole in Standard solution should

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not be more than 2.0.

Theoretical plates for the Terbinafine and Itraconazole p e a k s in Standard solution should not be less than 2000

Preparation of standard stock solution:

Accurately 10mg of Terbinafine and 10mg of Itraconazole working standard were weighed and transferred in to a10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further this Stock was pipette(3mland0.3ml) in to a10ml volumetric flask and dilute upto the mark with diluents.

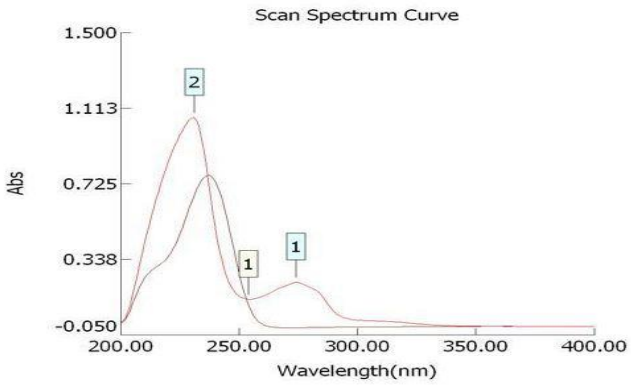
Procedure

The standard solution was injected for five times and the area for all five injections measured in HPLC.The % RSD for the area of five replicate injections was found to be with in the specified limits.

RESULTS AND DISCUSSION

Selection of detection wavelength: The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/m lfor individual and mixed standards. The resulting solution was scannedin U.V range from 200-400nm. The overlay spectrum of Itraconazole and Terbinafine was obtained and the isobestic point of Itraconazole and Terbinafine showed absorbance's maxima at 238nm shown in figure 3.

Figure 3: Overlay spectrum of Itraconazole and Terbinafine



METHOD DEVELOPMENT [4-6]

The chromatographic method development for the simultaneous estimation of Itraconazole and Terbinafine were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for these paration and quantification of Itraconazole and Terbinafine in API and pharmaceutical dosage form by RP-HPLCmethod.

Optimized Chromatographic conditions:

Column: Phenomenex kinetex C_{18} (250mm×4.6mm i.d, 5µm) column

Mobile phase: Methanol: Mono and disodium Hydrogen orthophosphate buffer of pH 6.8: acetonitrile (47:23:30 %V/V)

Flow rate: 1ml/min

Injection volume: 20µl

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Detection wavelength: 287nm
Mode of elution: Isocratic
Column temperature: Ambient

VALIDATION OF THE METHOD [7-10]

System suitability test: Solution for system suitability test was all set by moving 1ml of standard stock arrangement (1000µg/ml) into 10ml volumetric flagon, weakening to check with diluent and sonicated. This preparation was injected six times into the HPLC system for assessing parameters like number of hypothetical plates (N), peak area and tailing factor. The results were shown in table 1 and the overlain chromatogram for system suitability was shown in figure 3.

Table1. System suitability results For Terbinafine (Flowrate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	3536	1.7
2	1.0	2931	1.7
3	1.2	2713	1.7

*****Resultsforactualflow (1.0ml/min) have been considered from Assay standard.

Table2. System suitability results for Itraconazole (Flowrate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	2158	1.8
2	1.0	2114	1.7
3	1.2	2069	1.7

*****Results for actual flow(1.0ml/min) have been considered from Assay standard

MobilePhase:

The Organic composition in the Mobile phase was varied from 70% to 60%. Standard solution 300 µg/ml of Terbinafine & 3µg/ml of Itraconazole was prepared and analyzed using the varied Mobilephase composition along with the actual mobile phase compositionin the method.

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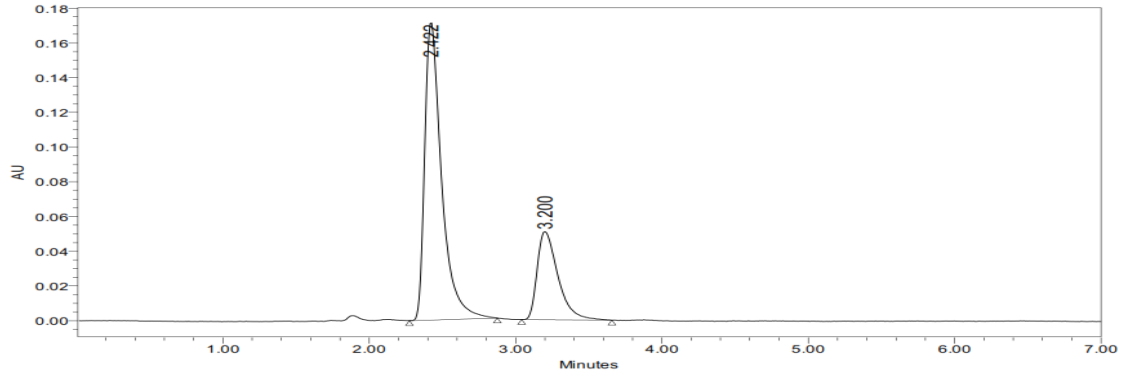

Fig.4.Chromatogram for Robustness more organic

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.422	1378798	171546	2358.0	1.7
2	Terbinafine	3.200	499679	50843	2616.1	1.6

Table3 Details of Robustness more organic

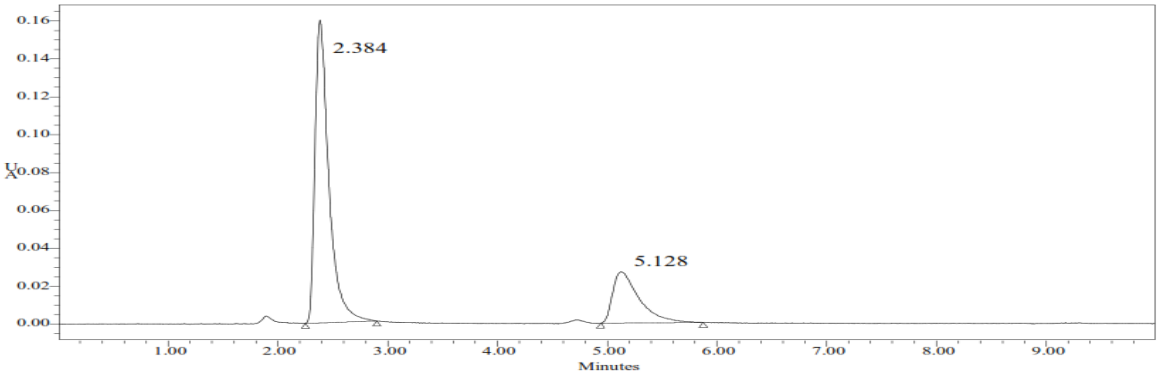

Fig5 Chromatogram for Robustness less organic

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.384	1404976	159808	2910.4	1.8
2	Terbinafine	5.128	453297	27049	2840.1	1.7

Table4. Details of Robustness les organic

The results are summarized. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobilephase±10

Table5. System suitability results for Terbinafine (Mobilephase)

S.No	Changein Organic Compositionin the MobilePhase	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	10%Less	2910	1.8

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2	Actual	2860	1.7
3	10%More	2358	1.7

*****Results for actual Mobile phase composition (55:45Buffer:Methanol)have been considered from Accuracy standard

Table6.System suitability results for Itraconazole (Mobilephase)

*****Results for actual Mobile phase composition (55:45Buffer: Methanol) have been considered

S.No	**Changein Organic Compositionin the MobilePhase**	**Systemsuitabilityresults**	
		USPPlatecount	**USPTailing**
1	10%Less	2540	1.7
2	Actual	2458	1.7
3	10%More	2616	1.7

from Accuracy standard

Linearity: Working standard solution was prepared according to the procedure and after filtering and sonicating the solution for 5mins further dilutions were madeto getdifferent concentration levels ranging from 20 to 300µg/ml. Every solution was injected into HPLC system as well as linearity was appraised. The calibration curve was designed taking concentration on X-axis along withpeak area on Y-axis. The linearity plots were shown in figure6 and 7

Table 7. Linearity Results

	Name	**RT**	**Area**	**Height (µv)**
1	Itraconazole	2.297	869216	109198
2	Itraconazole	2.264	1148093	145069
3	Itraconazole	2.308	1398858	164962
4	Itraconazole	2.370	1676584	193291
5	Itraconazole	2.322	1936686	238262
6	Terbinafine	3.458	296156	30269
7	Terbinafine	3.351	371946	39434
8	Terbinafine	3.488	452984	45638
9	Terbinafine	3.712	537383	50538
10	Terbinafine	3.535	617463	65483

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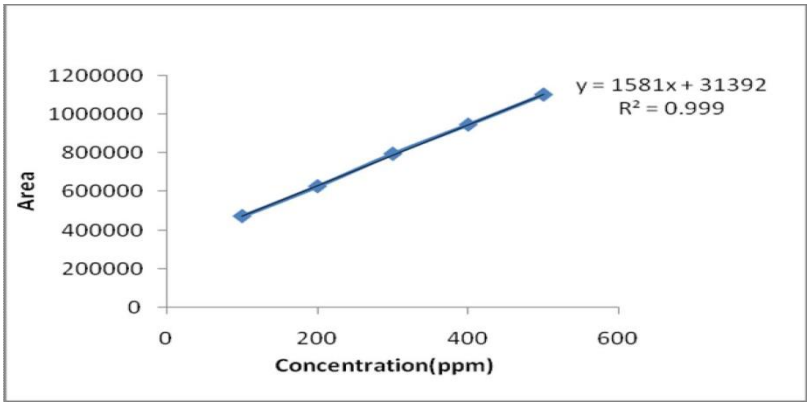

Fig.6 Calibration curve of Terbinafine

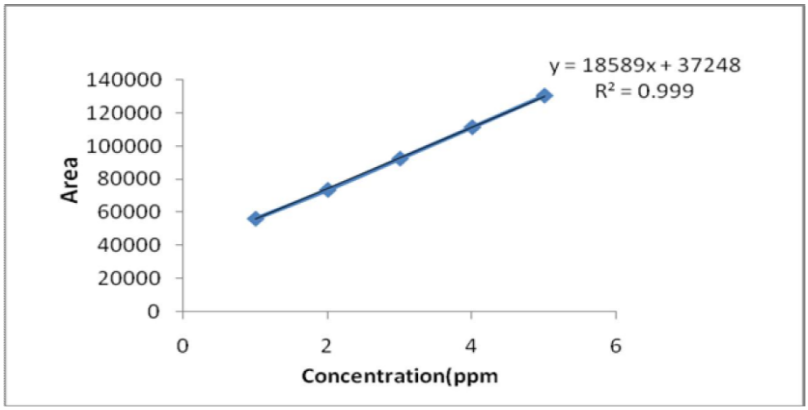

Fig.7 Calibration curve of Itraconazole

Robustness

Aspartof the Robustness, deliberate change in the Flowrate, MobilePhase composition, Temperature Variation was made to evaluate the impacton the method.

Fig8 Chromatogram for Robustness more flow

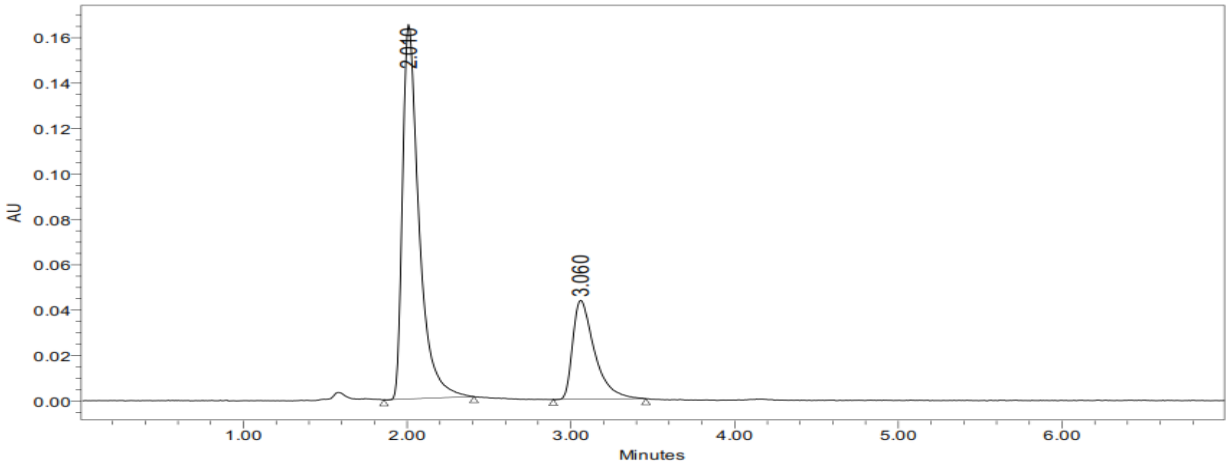

Table8 Details of Robustness more flow

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.010	1150303	165118	2069.9	1.7
2	Terbinafine	3.060	402322	43574	2713.8	1.7

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Fig.9 Chromatogram for Robustness less flow

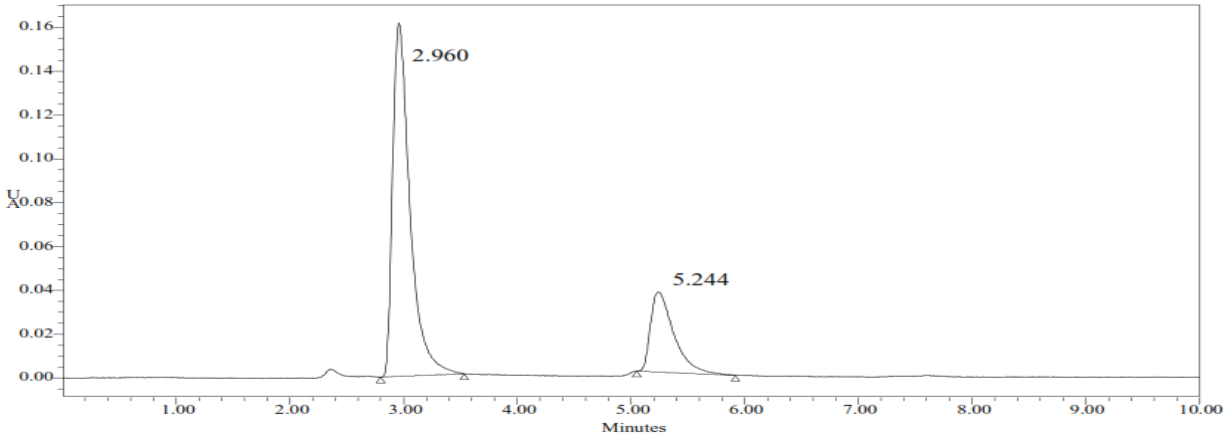

Table9 Details of Robutness less flow

	Name	RT	Area	Height (µv)	Usp plate count	Usptaililng
1	Itraconazole	2.960	1690740	161204	2158.1	1.8
2	Terbinafine	5.244	519208	36602	3536.2	1.7

CONCLUSION: In order to determine Itraconazole and Terbinafine simultaneously, a unique approach based on RP-HPLC was devised. To separate Itraconazole, an Agilent C18 column (4.6 x 150 mm, 5), and a detection wavelength of 254 nm were determined to be optimal. The calculated retention times were 2.34 and 3.28 hours. Terbinafine and Itraconazole were both found to be 99.97% pure, whereas Itraconazole was found to be 101.27 percent pure. In a linearity investigation spanning 50–250 mg of Itraconazole and 5–50 mg of Terbinafine, respectively, we found recoveries of 99.56 and 99.48%, respectively. Intermediate precision had an RSD of 0.1, while repeatability was 0.2. Reliability and repeatability of the research were not compromised in any way. The LOQ values varied from 0.0172 to 0.2125, whereas the LOD values were 3.17 and 5.68.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interests.

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