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Research Article

Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Irbesartan And Amlodipine Besylate In Pharmaceutical Dosage Form

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ABSTRACT

The simultaneous quantification of irbesartan and amlodipine besylate in bulk and pharmaceutical formulation has been achieved by the development of a reverse phase high performance liquid chromatographic technique employing an RP-C18 column. At a flow rate of 1.0 mL/min, the mobile phase (0.2% Triethylamine: Acetonitrile) PH 5.0 adjusted with orthophosphoric acid was pumped in a 50:50% v/v ratio, and its wavelength was measured at 236 nm. The suggested HPLC method's high degree of accuracy and precision was demonstrated by the statistical validation of the procedure, which revealed an RSD of less than 2%. Irbesartan and amlodipine besylate have retention times of 4.4 and 4.1, respectively. Additionally, there is no diluent interference with the standard and sample chromatograms. In accordance with ICH requirements, its accuracy, precision, specificity, and robustness were statistically validated. We are able to state that this method can be used for additional analysis after analyzing the validation data. Because routine analysis can be conducted using this suggested strategy.

INTRODUCTION

Based on anhydrous calculation, irbesartan has a C25H28N6O content of at least 99.0 percent and up to 101.0 percent. The drug irbesartan is used to treat high blood pressure and shield the kidneys against diabetic kidney disease. It belongs to the group of drugs known as angiotensin II receptor blockers (ARBs). Irbesartan relaxes blood arteries to facilitate easier blood flow, which lowers blood pressure1,2. Irbesartan prevents the body's natural production of angiotensin II, which narrows blood vessels and lowers blood flow. Irbesartan causes

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blood arteries to dilate and reduce blood pressure by blocking the actions of angiotensin I3,4.

The IUPAC name of this compound is :

2-butyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]methyl][4.4] -1,3-diazaspironon-1-en-4-one

Formula for a molecule:

C25H28N6O

Weight in molecules:

428.5 g/mol

Type:

Medicine for hypertension

Solubility:

Almost insoluble in water, soluble in methanol, ethanol, and isopropyl alcohol.

Melting point:

180–181 degrees Celsius;

pKa:

4.12–7.40

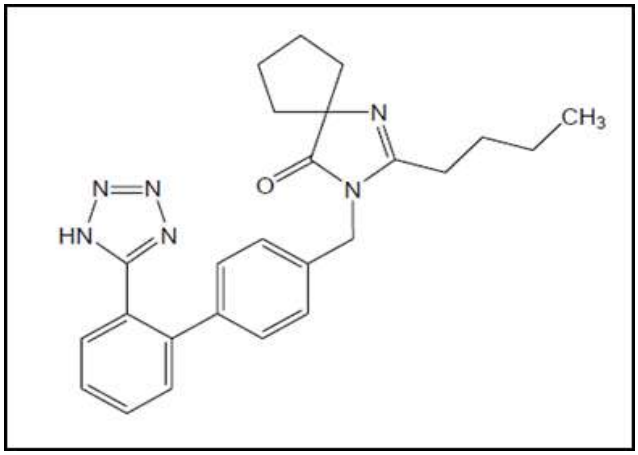

Fig. No:1 Irbesartan

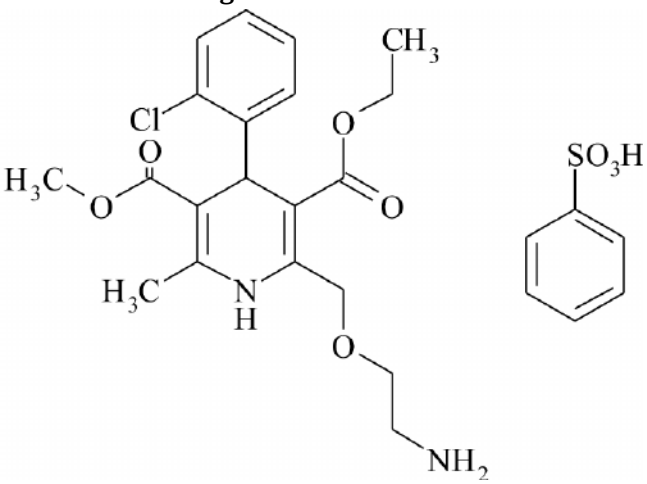

Fig. No: 2 Amlodipine Besylate

Based on anhydrous calculation, Amlodipine Besylate has a minimum of 97.0 percent and a maximum of 102.0 percent C26H31Cl N2O8S. Amlodipine Besylate is a dihydropyridine calcium antagonist, also known as a slow-channel blocker or calcium ion antagonist, which prevents calcium ions from entering cardiac and vascular smooth muscle5,6. Additionally, some forms of coronary artery disease and angina (chest pain) are treated with it (narrowing of the blood vessels that provide blood to the heart)7,8.

IUPAC Name:

3-O-ethyl benzenesulfonic acid 5-O-methyl 2-(2-chlorophenyl)-4-(2-aminoethoxymethyl)-6-methylDihydropyridine-1,4-d-3,5-dicarboxylate

The Molecular Formula:

C26H31ClN2O8S

Molecular Weight:

567.1 g/mol;

Category:

Antihypertensive, Antianginal;

Solubility:

slight solubility in water and sparingly soluble in ethanol and soluble in Iso propyl alcohol

Melting point:

200 °C

pKa:

8.6

MATERIALS AND METHOD

Instrumentation

HPLC equipment was an Agilent 1260 Infinity. For chromatographic separation and quantification, an Agilent 5TC C18 (250 mm × 4.6 mm) analytical column was utilized, and it was maintained at room temperature the entire time. Tablet samples, solution samples, and mobile phases were filtered using Whatman filter paper before being injected into the HPLC apparatus.

Chemicals, solvents, and drugs

We bought free gift samples of Amlodipine Besylate and Irbesartan from Aurochem Pharmaceuticals in Palghar, India. Acetonitrile of HPLC quality was provided by Research Laboratories Ltd. (Mumbai, India). tablets called AMIX R that contain 10 mg of amlodipine besylate and 100 mg of irbesartan. Water of HPLC quality was utilized throughout the entire analysis..

Conditions for chromatography

Mobile phase :

TEA(PH-5.0) : Acetonitrile (50:50 v/v)

Flow rate :

1mL/min

Wavelength of detection:

236 nm

Column temperature :

Room temperature

Injection volume :

20 µL

Run time :

7 min

Detector:

PDA detector

Standard drug solution

Weigh out 100 milligrams of irbesartan and 13.9 milligrams of amlodipine besylate precisely, and then transfer each amount into a separate 100 milliliter volumetric flask. Next, fill the volumetric flask with irbesartan and amlodipine besylate. Add 55 ml of isopropyl alcohol and 45 ml of water (acting as a diluent). Filter the mixture using filter paper No. 41. Using the distill water, adjust the final volume to the mark on the volumetric flask. 5 ml of each of the previously prepared solutions should be pipetted out and transferred to a second 100 ml volumetric flask. Distill water should be added to the volumetric flask until the desired volume is reached.(Figure 1)

Tablet sample solution

Calculate the average weight of the ten tablets by weighing each one. Make a fine powder out of the crushed tablets. Weighed out of the powdered tablet, 100 mg of irbesartan and 13.9 mg of amlodipine besylate were added to a 100 ml volumetric flask. To get rid of any contaminants or solid particles, strain the mixture containing the tablet powder through filter paper No. 41. 10 milliliters of the filtered solution were pipetted into a second 100 milliliter volumetric flask. Distill water should be added to the volumetric flask until the desired volume is reached. After the solution is ready, add it to the HPLC apparatus for examination.(Figure 2)

Preparation of mobile phase

After dissolving 1 milliliter of triethylamine in 500 milliliters of water, adjust the pH to 5.0 with the aid of orthophosphoric acid.

Method Validation

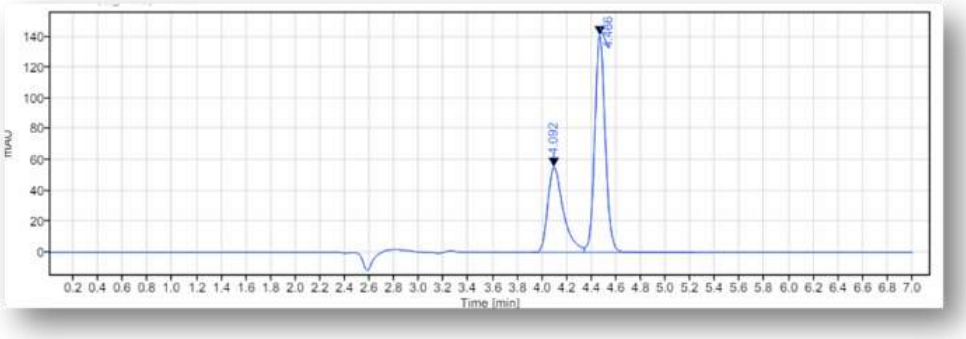

Fig.1: chromatogram of standard

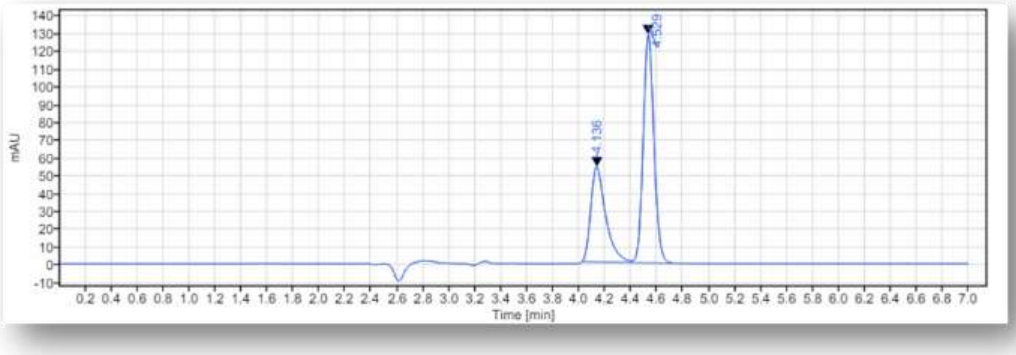

Fig.2:chromatogram of tablet sample

System suitability

The production of irbesartan and amlodipine besylate followed protocol, and the HPLC system received five injections of the working standard solution. Standard chromatograms were used to assess the appropriateness of the system by computing the percentage RSD of the retention period, tailing factor, theoretical plates, and peak regions from five replicate injections.

Table1:System suitability of Amlodipine Besylate and Irbesartan

Sr.no	Surface area of Amlodipine Besylate	Retention time Amlodipine besylate	Surface area of Irbesartan	Retention time Irbesartan
1	421.379	4.137	185.734	4.543
2	410.965	4.126	186.891	4.539
3	425.998	4.132	186.852	4.538
4	418.322	4.091	187.052	4.464
5	409.051	4.091	190.093	4.458
Average	417.143		187.32	
SD	7.09564849		1.6339	
%RSD	1.701011042		0.8722	

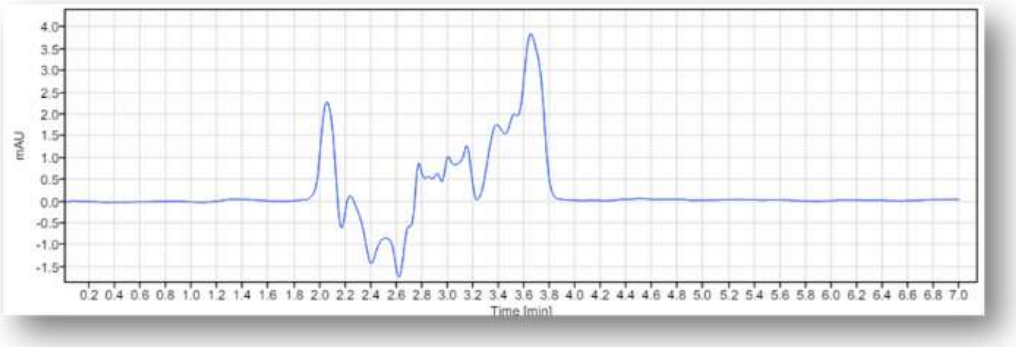

Fig.3: Specificity blank chromatogram

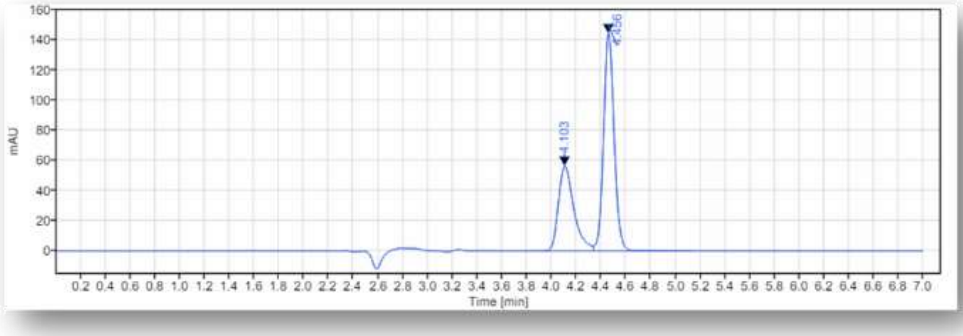

Fig.4:Specifity sample chromatogram

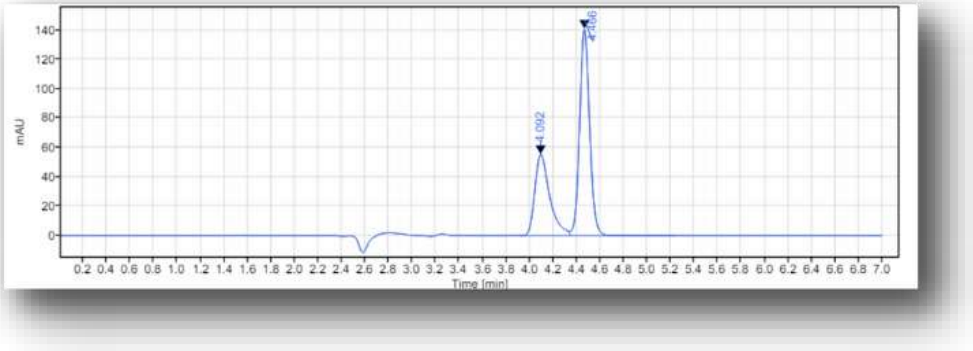

Fig.5:specificity standard chromatogram

Specificity

Specificity is the ability to isolate the target analyte from other sample constituents. The relationship between the chromatograms for the standard, sample, blank, and placebo ensured the procedure's specificity.

Table 2:Specificity data for sample

Name	Area	Retention time
Amlodipine besylate	488.62	4.103
Irbesartan	855.20	4.456

Table 3:Specificity data for standard

Name	Area	Retention time
Amlodipine besylate	488.08	4.092
Irbesartan	836.52	4.466

Linearity and Range

To demonstrate the assay's linearity, inject six reference solutions containing irbesartan and amlodipine besylate. A 0.99 correlation coefficient was discovered. There was a linearity plot created.

Table 4:Data sheet for linearity of Amlodipine Besylate

Concentration Amlodipine Besylate	Area of Amlodipine Besylate
0	0
3	246.28
4	379.273
5	435.431
6	496.527
7	612.385

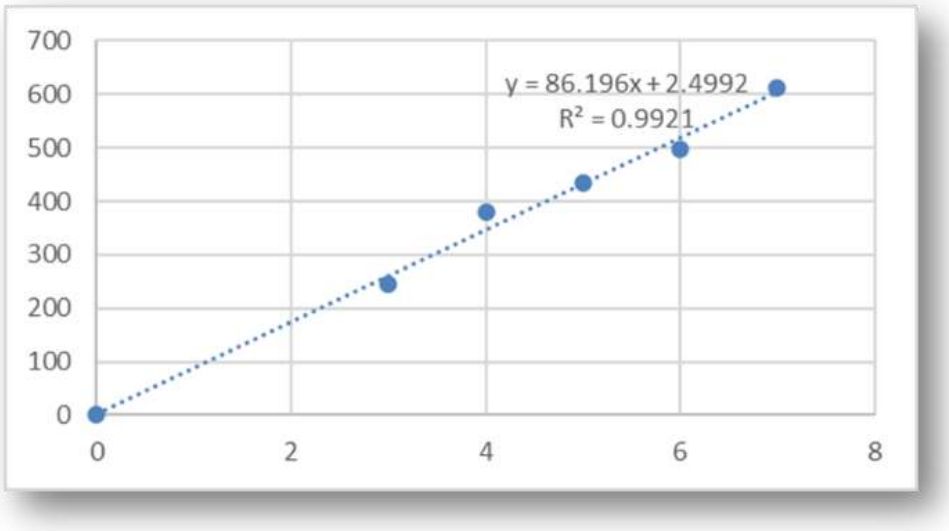

Fig.6: linearity graph of Amlodipine Besylate

Table 5:Data sheet for linearity of Irbesartan

Concentration of Irbesartan	Area of Irbesartan
0	0
30	424.162
40	633.869
50	749.57
60	865.02
70	1060.811

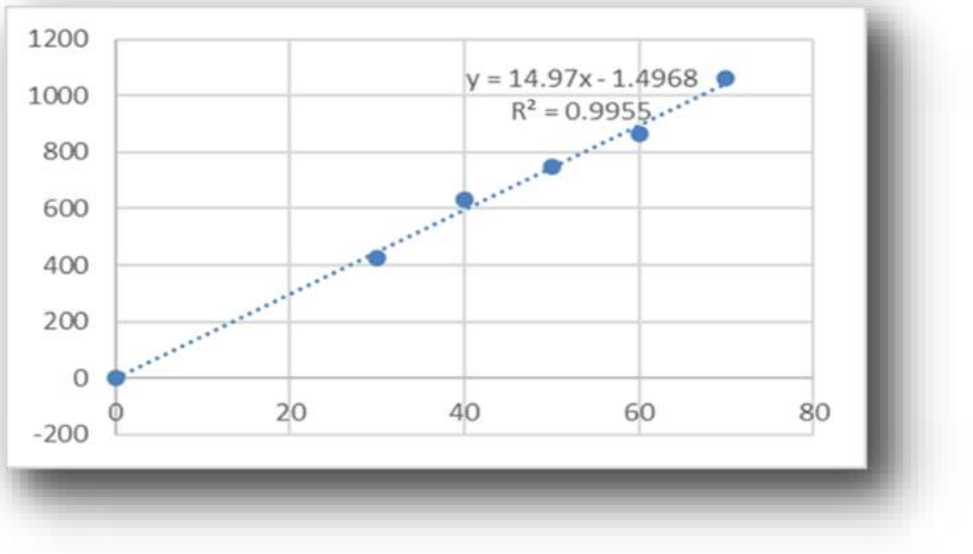

Fig.7:linearity graph of Irbesartan

Accuracy

The classic addition recovery approach was used to gauge the procedure's accuracy. The previously analyzed sample solutions were mixed with a standard stock solution at three distinct concentrations: 80%, 100%, and 120%. The standard stock solution contained known levels of irbesartan and amlodipine besylate. While going over these options again, three analyses of the answers at every level were carried out. The accuracy was caSlculated and reported as a percentage of the recovery.

Table 6:Data for Accuracy of Amlodipine Besylate

% Level	Area	Amount mg added	Mg found	% recovery	Average	SD	Relative standard deviation
80%	330.68	4	3.963664738	99.09161846	99.8	1.2	1.2
	330.74	4	3.964335971	99.10839928			
	337.72	4	4.048036285	101.2009071			
100%	403.79	4.8	4.839934986	100.8319789	99.2	1.5	1.5
	403.93	4.9	4.841649027	98.80916381			
	400.25	4.9	4.797491508	97.90798995			
120%	486.75	6	5.960821589	99.34702648	99.2	0.3	0.3
	497.30	5.9	5.834354166	98.88735875			
	497.01	6	5.957285631	99.28809385			

Table 7:Data for Accuracy of Irbesartan

% Level	Area	Amount mg added	Mg found	% recovery	Average	Stdev	Relative standard deviation
80%	547.53	40	39.83552861	99.58882153	101.5	1.7	1.7
	570.16	40.5	41.48264342	102.42628			
	578.12	41	42.06126897	102.5884609			
100%	646.94	48	47.06845723	98.0592859	98.6	1.8	1.8
	627.41	47	45.64775796	97.12288928			
	690.84	50	50.26417571	100.5283514			
120%	821.00	61	62.57653893	102.58449	101.1	1.5	1.5
	860.09	60	59.73244843	99.55408072			
	833.36	60	60.63192634	101.0532106			

Precision

The degree of agreement between several measurements made from repeated samplings of the same homogenous material under specified conditions is known as the analytical process' precision. System and method implementation both exhibit precision

.

Table 8:Method Precision of Amlodipine Besylate

Sample no	Area	% Assay
1	426.043	100.00
2	400.884	97.05
3	431.475	99.97
4	416.993	99.61
5	422.946	99.71
6	441.916	102.52
	Average	99.8
	SD	1.7
	%RSD	1.7

Table 9:Method Precision of Irbesartan

Sample no	Area	% Assay
1	722.344	102.91
2	705.28	103.64
3	730.824	102.78
4	703.479	102.00
5	717.401	102.66
6	701.338	98.76
	Average	102.1
	SD	1.7
	%RSD	1.7

Robustness

An intended change to the process is the addition of mobile phases minus and plus. Additionally, the proportion of RSD is calculated.

Table 10:Robustness data for Amlodipine Besylate and Irbesartan (for flow rate 0.8ml/min)

Sr.no	Area Amlodipine Besylate	Retention time Amlodipine Besylate	Area Irbesartan	Retention time Irbesartan
1	1229.60	5.153	935.787	5.533
2	1223.37	5.153	943.178	5.533
3	1246.56	5.256	944.138	5.656
Average	534.4		941.0	
SD	2.6		4.6	
%RSD	0.5		0.5	

Table 11:Robustness data for Amlodipine Besylate and Irbesartan (for flow rate 1.1ml/min)

Sr.no	Area Amlodipine Besylate	Retention time Amlodipine Besylate	Area Irbesartan	Retention time Irbesartan
1	368.127	3.765	670.265	4.051
2	373.628	3.771	695.857	4.057
3	382.172	3.839	688.875	4.126
Average	374.6		685.0	
SD	7.1		13.2	
%RSD	1.9		1.9	

Solution stability

For a whole day at room temperature, standard and sample solutions were tested to demonstrate the stability of the solutions during analysis.

Table 12:Standard Solution Stability data for Amlodipine Besylate and Irbesartan

Name	Area Amlodipine Besylate	Relative difference Amlodipine Besylate	Area Irbesartan	Relative difference Irbesartan
Standard solution 0hr	454.88	0.47	705.28	1.01
Standard solution 24 hr	457.012		692.965	

Table 13:Sample Solution Stability data for Amlodipine Besylate and Irbesartan

Name	Area of Amlodipine Besylate	Assay of Amlodipine Besylate	Area of Irbesartan	Assay of Irbesartan
Sample solution 0 hr	400.884	97.05	762.773	103.64
Sample solution 24 hr	408.235	98.83	770.466	101.83

RESULT AND DISCUSSION

The measures for system appropriateness satisfy the requirements for approval. Verify the apparatus is appropriate before beginning any analytical tests, including HPLC analysis. (Reference Table 1). Irbesartan and amlodipine besylate-containing sample solution and reference solution's chromatograms are similar.(Figures 4, 5, and 3, 4) The gap has no effect on the retention time of the primary peak.(Tables 2 and 3). It is possible to establish a positive correlation with a correlation coefficient of 0.99 by modifying the area between the drug and its concentration.(Tables 4 and 5) (Figures 6 and 7).

The results of multiple tests showed that the cure rate ranged from 95% to 103%. This approach was selected, as evidenced by the fact that excipients do not interfere with the analyte measurement (Table 6&7). The precision percentage RSD values of the method are less than 2%, indicating that it has good precision for the simultaneous analysis of irbesartan and amlodipine besylate. (Tables 8 and 9). The results demonstrate the applicability of the method by demonstrating that minute changes in the mobile phase's composition and temperature have no bearing whatsoever on the drug's peak area.(Tables 10 and 11). At room temperature, samples and reference solutions remain stable for a whole day.(Tables 12 and 13).

In conclusion, we can state that the established, validated RP HPLC method provides accurate, precise, cost-effective, and user-friendly quantitative determination of irbesartan and amlodipine besylate in formulation. It was determined that the procedure complies with ICH criteria. Consequently, the method can be effectively used to the routine assessment of irbesartan and amlodipine besylate in tablet form.

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CONFLICT OF INTEREST

The authors claim to have no competing interests.

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