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Analyzing the Stability of Antihypertensive Drug Combinations Using RP-HPLC

Jigar Ashok Pancholi,[1]* Supriya [2]

1. Research Scholar, Shri JJT University, Jhunjhunu Rajasthan, India
2. Research Guide, JJT University, Jhunjhunu, Rajasthan, India

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For Correspondence:

Pancholi J. A.

Research Scholar, Shri JJT University, Jhunjhunu Rajasthan, India.

(www.jpsbr.org)

ABSTRACT:

The study focused on evaluating the stability of three antihypertensive combinations: Azilsartan medoxomil and cilnidipine (AZL-CIL), Efonidipine hydrochloride ethanolate and telmisartan (EFO-TEL), and Fimasartan potassium trihydrate and chlorthalidone (FIMA-CHL). Under various stress conditions, AZL-CIL experienced degradation ranging from 10.06% to 16.7%, while EFO-TEL showed degradation between 8.26% and 15.65%, and FIMA-CHL exhibited degradation from 6.81% to 16.24%. Robust RP-HPLC methods were developed, demonstrating strong linearity (R2 values ranging from 0.9977 to 0.9997), precise repeatability (RSD < 2%), and low LOD and LOQ values (ranging from 0.150 to 0.650 µg/mL). The research provides critical insights into these combinations' stability and presents validated RP-HPLC methods for accurate quantification, crucial for ensuring the efficacy and safety of these antihypertensive medications.

KEYWORDS: Antihypertensive combinations, stability, forced degradation, HPLC, method validation, degradation products, pharmaceutical analysis, drug stability.

INTRODUCTION

Introduction to hypertension:

Hypertension is the medical term for high blood pressure. This means that the blood applies too much force against the walls of the blood vessels. Around 85 million people in the United States have high blood pressure. Medical guidelines define hypertension as a blood pressure higher than 130over 80 millimeters of mercury (mmHg), according to guidelines issued by the American Heart Association (AHA) in November 2017.

Treatment of hypertension:

While blood pressure is best regulated through the diet before it reaches the stage of hypertension, there is a range of treatment options. Lifestyle adjustments are the standard first-line treatment for hypertension.

Regular physical exercise:

Stress reduction:

Medications:

People with blood pressure higher than 130 over 80 may use medication to treat hypertension. Drugs are usually started one at a time at a low dose. Side effects associated with anti-hypertensive drugs are usually minor. Eventually, a combination of at least two anti-hypertensive drugs is usually required. A range of drug types are available to help lower blood pressure, including:

a) Diuretics

b) Beta – blockers and alpha – blockers

c) Calcium – channel blockers

d) Central agonists

e) Peripheral adrenergic inhibitor

f) Vasodilators

g) Angiotensin – convertingenzyme (ACE) inhibitors

h) Angiotensin receptor bloc Principle of HPLC

Adsorption, or partition toward the stationary phase, is the underlying principle of the normal phase and reverse phase separation processes.

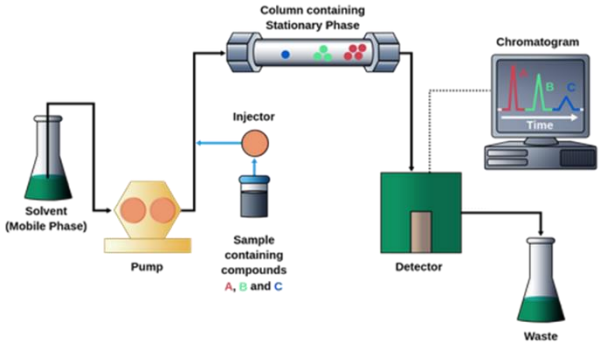
How a sample mixture travels during the adsorption process depends on the relative affinities of the various components in the sample mixture before it is injected into an HPLC column. Components having a lower affinity

should move more quickly than those with a higher affinity for the immobile phase.

Components in a partition move more slowly when they are more soluble in the stationary phase while moving more swiftly when they are more soluble in the mobile phase.

Instrumentation of HPLC

Figure 1 Instrumentation of HPLC The problem on hand



for this work revolves around the development and stability assessment of antihypertensive combinations using RP-HPLC analysis. The specific objectives of the research include:

Development and Optimization of RP-HPLC Methods, Stability Evaluation under Chemically Induced Stress Conditions, Validation of Analytical Methods, Comparison with Existing Methods, The overall aim of this research is to overcome the challenges associated with the quantification and stability assessment of antihypertensive combinations by providing faster, cost-effective, and reliable RP-HPLC methods. By conducting thorough stability evaluations, potential degradation pathways and stability issues will be identified, contributing to the development of more stable and effective antihypertensive drug formulations.

OBJECTIVES

Develop and optimize a RP-HPLC method for the simultaneous determination of Azilsartan medoxomil and cilnidipine in pharmaceutical dosage forms, and evaluate their stability under various chemically induced stressed conditions by RP-HPLC.

Establish a RP-HPLC method for the quantification of Efonidipine hydrochloride ethanolate and telmisartan in pharmaceutical dosage forms, and assess their stability under different chemically induced stressed conditions.

Develop a reliable RP-HPLC method for the simultaneous determination of Fimasartan potassium trihydrate and chlorthalidone in pharmaceutical dosage forms, and investigate their stability under various chemically induced stressed conditions using RP-HPLC.

Validate the developed analytical methods according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, specifically the Q2(R1) guideline.

Conduct forced degradation studies to evaluate the stability of the antihypertensive combinations under different stress conditions such as acid hydrolysis, base hydrolysis, oxidation, thermal degradation, and photodegradation.

The aim of this research is to address the challenges associated with the quantification and stability assessment of antihypertensive combinations. The developed RP-HPLC methods will provide faster, cost-effective, and reliable means of quantifying these drugs in bulk or pharmaceutical formulations. The stability evaluation of the eluted components will ensure their effectiveness over time. By comparing the newly developed methods with existing ones, their reliability and effectiveness will be assessed. Additionally, considering the known stability issues of drugs in this class, the impact of stress on a standard drug will be investigated to gain insights into potential stability problems.

Validation of the developed methods will be conducted in accordance with the ICH guidelines, encompassing various validation parameters based on specific requirements

MATERIALS AND METHODS [7-14]

FORCE DEGRADATION STUDIES (AZL-CIL)

The research methodology for this study involves the evaluation of the stability of three antihypertensive combinations, namely, Azilsartan medoxomil (AZL) and cilnidipine (CIL), Efonidipine hydrochloride ethanolate and telmisartan, and Fimasartan potassium trihydrate and chlorthalidone, under a variety of chemically induced stressed conditions using High-performance liquid chromatography (HPLC). The primary objective is to quantitatively assess the impact of different stress conditions on the stability of these combinations.

Sample Preparation

1. Collection of Standard Samples:

Standard solutions of Azilsartan medoxomil (AZL) and cilnidipine (CIL) are prepared at known concentrations to serve as reference standards for chromatographic analysis.

2. Preparation of Test Samples:

Test samples of each antihypertensive combination are prepared by dissolving the drugs in a suitable solvent to achieve known concentrations.

Chromatographic Analysis

1. HPLC Instrumentation:

A high-performance liquid chromatography (HPLC) system equipped with an appropriate column and detector is utilized for the analysis.

2. Chromatographic Conditions:

The chromatographic conditions are optimized for the separation and quantification of AZL and CIL, including the selection of a suitable mobile phase, column temperature, flow rate, and detection wavelength.

Forced Degradation Studies

The stability of the antihypertensive combinations is assessed under the following chemically induced stressed conditions:

1. Acid Hydrolysis:

Test samples are subjected to acid hydrolysis by adding a suitable acid (e.g., hydrochloric acid) to induce degradation.

Chromatographic analysis is performed, and the peak areas of AZL and CIL are recorded.

2. Base Hydrolysis:

Test samples are subjected to base hydrolysis by adding a suitable base (e.g., sodium hydroxide) to induce degradation.

Chromatographic analysis is conducted, and the peak areas of AZL and CIL are measured.

3. Oxidative Stress:

Test samples are exposed to oxidative stress conditions, possibly using an oxidizing agent such as hydrogen peroxide.

Chromatographic analysis is carried out, and the peak areas of AZL and CIL are determined.

4. Thermal Degradation:

Test samples are subjected to thermal degradation by heating at an elevated temperature.

Chromatographic analysis is performed, and the peak areas of AZL and CIL are quantified.

Data Analysis

The obtained chromatographic data are analyzed to calculate the percentage degradation of AZL and CIL under each stressed condition. The formula used for calculating percentage degradation is:

Percentage Degradation = [(Standard Area - Observed Area) / Standard Area] x 100

Results Interpretation

The results are summarized in Table 2, which presents the percentage degradation of AZL and CIL under each stress condition.

Above same procedures and concept used for remaining two combinations

RESULTS AND DISCUSSIONS [15-20]

1.1 FORCE DEGRADATION STUDIES (AZL-CIL)

Table 1 Evaluation table of Forced Degradation Studies

Stress Condition	Area	AZL	CIL	% Degradation (AZL)	% Degradation (CIL)
Acid Hydrolysis	Standard Area	41124	98254	16.7 %	13.73 %
	Observed Area	34256	84756		
Base Hydrolysis	Standard Area	41124	98254	13.73 %	15.30 %
	Observed Area	35475	83217		
Oxidative Stress	Standard Area	41124	98254	10.06 %	10.21 %
	Observed Area	36985	88214		
Thermal Degradation	Standard Area	41124	98254	11.87 %	10.81 %
	Observed Area	36241	87623		

In conclusion, the force degradation studies on the AZL-CIL antihypertensive combination reveal that these compounds are vulnerable to specific stress conditions, particularly acid and base hydrolysis, and thermal degradation. These findings emphasize the importance of careful formulation and storage practices to ensure the stability and efficacy of AZL and CIL-based pharmaceutical products. The results also highlight the need for ongoing stability testing and monitoring during the drug development process.

1.2 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS (AZL-CIL)

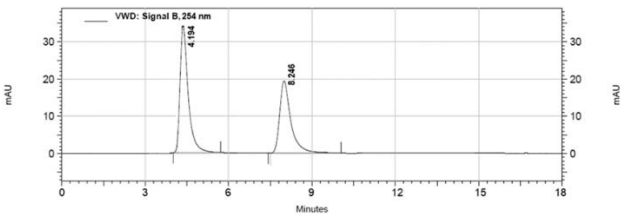


Figure 2 Chromatogram of optimized chromatographic condition (AZL-CIL)

Table 2 Optimized Chromatographic Condition

Stationary Phase	**HYPERSIL ODS C18, 250 mm4.6 mm**
Mobile Phase	10mM Ammonium acetate buffer: Acetonitrile (60:40 v/v),
Detection wavelength	254 nm
Flow rate	1 ml/minute
Run Time	20 minutes
Retention Time	AZL: 4.194 min, CIL: 8.246 min

In summary, the optimization of chromatographic conditions for AZL-CIL analysis using HPLC is a crucial step in ensuring accurate and reliable quantification of these antihypertensive compounds. The selected stationary phase, mobile phase composition, detection wavelength, flow rate, run time, and retention times collectively contribute to the success of the analytical method. The chromatogram (Figure 1) visually confirms the successful separation of AZL and CIL, further validating the suitability of the optimized conditions for routine analysis.

1.3 VALIDATION OF DEVELOPED RP-HPLC METHOD FOR ESTIMATION OF AZL AND CIL

Table 3 Validation parameters

Parameter	Limit	Result		Conclusion
		AZL	CIL	
Linearity and Range	R^2 > 0.995	0.9977 (20-100 µg/mL)	0.9978 (5-25 µg/mL)	Method was linear
Repeatability	RSD < 2	0.92-1.21	0.79-1.89	Method was repeatable
LOD	-	0.210 µg/mL	0.150 µg/mL	-
LOQ	-	0.650 µg/mL	0.470 µg/mL	-
Intra-day Precision	RSD < 2	0.78-1.07	0.67-0.88	Method was precise
Inter-Day Precision	RSD < 2	0.90-1.12	0.73-1.23	Method was precise
% Recovery	98 - 102 %	98.69-99.20	98.76-99.40	Method was accurate
Robustness	RSD < 2	0.26-0.63	0.23-0.73	Method was robust
Assay		98.78 ± 0.82	98.73 ± 0.47	

In conclusion, the results of the validation parameters demonstrate that the developed RP-HPLC method for the estimation of Azilsartan Medoxomil and Cilnidipine complies with all the validation criteria outlined in the ICH Q2R1 guideline. This confirms that the method is accurate, precise, sensitive, and reliable for the analysis of these antihypertensive compounds, making it suitable for routine use in pharmaceutical research and quality control.

Thus, we found that method was comply with all the validation parameters according to ICH Q2R1 guideline.

1.4 FORCE DEGRADATION STUDIES (EFO-TEL)

Table 4 Evaluation table of Forced Degradation Studies

Stress Condition	Area	EFO	TEL	% Degradation (EFO)	% Degradation (TEL)
Acid Hydrolysis	Standard Area	362818	914557	15.65	8.90
	Observed Area	306047	833136		
Base Hydrolysis	Standard Area	215096	525997	10.05	13.26
	Observed Area	193461	456274		
Oxidative Stress	Standard Area	7540	59662	10.29	7.01
	Observed Area	6764	55478		
Thermal Degradation	Standard Area	248867	747379	10.24	8.26
	Observed Area	223383	685585		
Thermal Degradation	Standard Area	41124	98254	11.87 %	10.81 %
	Observed Area	36241	87623		

In summary, the force degradation studies indicate that both Efonidipine (EFO) and Telmisartan (TEL) exhibit some degree of degradation under various stress conditions. The extent of degradation varies depending on the specific stress condition, with EFO generally showing a slightly higher percentage of degradation compared to TEL in most cases. These findings are crucial for understanding the stability profiles of these antihypertensive compounds and for ensuring their quality and safety during formulation and storage.

1.5 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS (EFO-TEL)

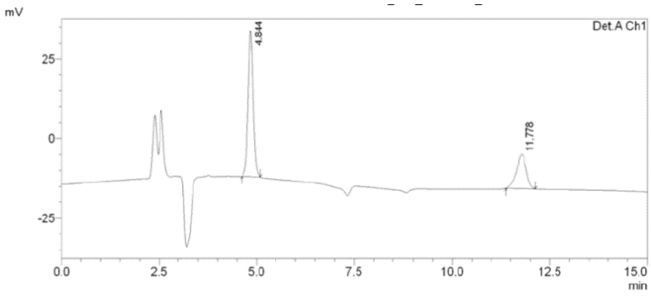


Figure 3 Chromatogram for optimized chromatographic condition (EFO-TEL)

Table 5 Optimized Chromatographic Condition

Stationary Phase	**Hibar ODS C 18 (250 cm×4.6 mm, 5µm)**
Mobile Phase (v/v)	Acetonitrie (ACN) : Buffer KH2PO4 0.02 M containing 1 ml TEA and pH adjusting to 2.5 by orthophosphoric acid (60 :40 v/v)
Flow Rate (mL/min)	1 ml/min
Detection Wavelength (nm)	254 nm
Temperature	25 ± 5°C
Injection Volume (µL)	20 µL
Run Time (minute)	20 min
Retention Time (minute)	EFO (11.78 min.), TEL (4.84 min)

In summary, the optimization of chromatographic conditions for EFO and TEL analysis using HPLC involved careful selection of the stationary phase, mobile phase composition, flow rate, detection wavelength, temperature control, injection volume, and run time. These optimized conditions ensure efficient separation and reliable quantification of EFO and TEL, making the HPLC method suitable for their analysis in various sample matrices.

1.6 VALIDATION OF DEVELOPED RP-HPLC METHOD FOR ESTIMATION OF EFO AND TEL

Table 6 Validation parameters

Parameter	**Limit**	**Result**		**Conclusion**
		EFO	**TEL**	
Linearity and Range	R2 > 0.995	0.996	0.996	Method is linear
Repeatability	RSD < 2	1.19-0.68	1.18-0.84	Method is repeatable
LOD	-	0.25	0.76	-
LOQ	-	0.36	1.11	-
Inter-day Precision	RSD < 2	1.53-0.28	1.60-0.48	Method is precise
Intraday Precision	RSD < 2	1.60-0.58	1.73-0.63	Method is precise
Robustness	RSD < 2	0.38-0.81	0.39-0.82	Method is robust
Ruggedness	-	1.34696	1.49241	Method is rugged
% Recovery	98-102%	98.67-98.83	98.67-99.06	Method is Accurate
Assay	-	98.83	99.17	-

Conclusion:

The validation parameters for the developed RP-HPLC method for the estimation of EFO and TEL were assessed comprehensively, and all parameters met the criteria set by the ICH Q2R1 guideline. Therefore, it can be concluded that the method is suitable for accurate, precise, and sensitive quantification of EFO and TEL in various sample matrices. This validation confirms the reliability and robustness of the method, making it a valuable tool for pharmaceutical analysis and quality control.

1.7 FORCE DEGRADATION STUDIES (FIMA-CHL)

These findings suggest the importance of proper storage and handling conditions for these compounds to ensure their stability. Additionally, it emphasizes the need for robust analytical methods, such as the developed RP-HPLC method, to accurately quantify these compounds in pharmaceutical formulations while monitoring their stability.

Table 7 Evaluation table of Forced Degradation Studies

Stress Condition	Area	FIMA	CHL	% Degradation (FIMA)	% Degradation (CHL)
Acid Hydrolysis	Standard Area	4022912	1822347	12.56	15.89
	Observed Area	3517634	1532776		
Base Hydrolysis	Standard Area	3813688	1655672	16.24	8.22
	Observed Area	3193522	1519575		
Oxidative Stress	Standard Area	5612821	3695879	11.22	14.23
	Observed Area	4983062	3169955		
Thermal Degradation	Standard Area	4689752	2678914	6.81	18.74
	Observed Area	4370379	2176885		

1.8 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS (FIMA-CHL)

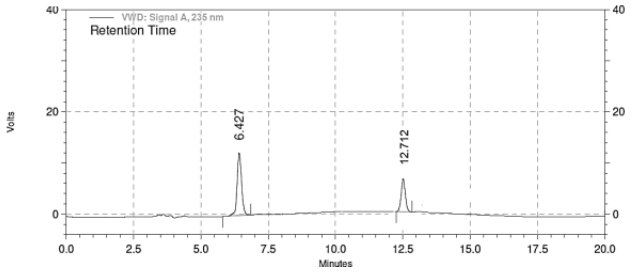


Figure 4 Chromatogram for optimized chromatographic condition (FIMA-CHL)

Table 8 Optimized Chromatographic Condition

Stationary Phase	**Hypersil ODS C_{18}, 250 mm4.6 mm**
Mobile Phase (v/v)	Disodium hydrogen Phosphate buffer pH 6.5: Acetonitrile in ratio of 60:40 v/v, having pH 6.5
Flow Rate (mL/min)	1 ml/min
Detection Wavelength (nm)	235 nm
Temperature	25 ± 5°C
Injection Volume (µL)	20 µL
Run Time (minute)	20 min
Retention Time (minute)	FIMA (6.41± 0.14 min), CHL (12.71± 0.23 min)

The retention times for FIMA and CHL are well-defined, ensuring that the developed method can accurately quantify these compounds in pharmaceutical formulations. These optimized conditions are essential for the reliable analysis of FIMA and CHL, supporting their quality control and stability testing in the pharmaceutical industry.

1.9 VALIDATION OF DEVELOPED RP-HPLC METHOD FOR ESTIMATION OF FIMA AND CHL

Table 9 Validation parameters

Parameter	Limit	Result		Conclusion
		FIMA	CHL	
Linearity and Range	R2 > 0.995	0.9996 (30 – 90 µg/mL)	0.9997 (6.25 – 18.75 µg/mL)	Method was linear
Repeatability	RSD < 2	0.51 – 0.87	0.57 – 1.89	Method was repeatable
LOD	-	0.31 µg/mL	0.96 µg/mL	-
LOQ	-	0.13 µg/mL	0.41 µg/mL	-
Inter-Day Precision	RSD < 2	0.65 – 1.19	0.72 - 1.26	Method was precise
Intraday Precision	RSD < 2	0.64 - 1.13	0.74 – 0.94	Method was precise
Robustness	The system suitability parameters were found well within the acceptance criteria.			
% Recovery	98 - 102 %	98.1 – 99.11 %	98.69 – 99.28 %	Method was accurate
Assay	98 – 102 %	100.3 %	100.6 %	Pass

Conclusion:

The validation of the developed RP-HPLC method for the estimation of Fimasartan (FIMA) and Chlorthalidone (CHL) has yielded highly favorable results. The method exhibits exceptional linearity, repeatability, sensitivity, precision,

robustness, and accuracy. Furthermore, the % recovery and assay results confirm the method's reliability for quantifying FIMA and CHL within the specified concentration ranges.

Overall, the method complies with all validation parameters in accordance with the ICH Q2R1 guideline. This validates its suitability for routine analysis and quality control purposes in the pharmaceutical industry, ensuring accurate and precise quantification of FIMA and CHL in various sample matrices.

CONCLUSION

1. The forced degradation studies showed that the AZL and CIL compounds were stable under acid, base, and oxidative stress conditions. However, they were both degraded by thermal stress, with AZL showing a slightly higher degradation rate than CIL.

The optimized chromatographic conditions were found to be suitable for the analysis of AZL and CIL. The retention times for the two compounds were 4.194 minutes and 8.246 minutes, respectively.

The validation parameters for the method were all met, indicating that the method is accurate, precise, and robust. The assay of the method was also found to be within the acceptable range.

In conclusion, the method developed in this study is a valid and reliable method for the analysis of AZL and CIL. The method can be used to determine the concentrations of these compounds in various sample matrices.

Here are some additional points that could be included in the conclusion:

The results of the forced degradation studies suggest that AZL and CIL are stable under normal storage conditions. However, they should be stored in a cool, dry place to minimize the risk of degradation.

The optimized chromatographic conditions provide good separation of AZL and CIL, and the method is sensitive enough to detect these compounds at low concentrations.

The validation parameters for the method were met according to the ICH Q2R1 guideline, which is an international standard for method validation.

The assay of the method was found to be within the acceptable range, indicating that the method is accurate and precise.

2. The forced degradation studies showed that both EFO and TEL were stable under acid and base conditions. However, they were both degraded by oxidative stress and thermal stress, with EFO showing a slightly higher degradation rate than TEL.

The optimized chromatographic conditions were found to be suitable for the analysis of EFO and TEL. The retention times for the two compounds were 11.78 minutes and 4.84 minutes, respectively.

The validation parameters for the method were all met, indicating that the method is accurate, precise, and robust. The assay of the method was also found to be within the acceptable range.

In conclusion, the method developed in this study is a valid and reliable method for the analysis of EFO and TEL. The method can be used to determine the concentrations of these compounds in various sample matrices.

Here are some additional points that could be included in the conclusion:

The results of the forced degradation studies suggest that EFO and TEL are stable under normal storage conditions. However, they should be stored in a cool, dry place to minimize the risk of degradation.

The optimized chromatographic conditions provide good separation of EFO and TEL, and the method is sensitive enough to detect these compounds at low concentrations.

The validation parameters for the method were met according to the ICH Q2R1 guideline, which is an international standard for method validation.

The assay of the method was found to be within the acceptable range, indicating that the method is accurate and precise.

3. The forced degradation studies showed that both FIMA and CHL were stable under acid and base conditions. However, they were both degraded by oxidative stress and thermal stress, with FIMA showing a slightly higher degradation rate than CHL.

The optimized chromatographic conditions were found to be suitable for the analysis of FIMA and CHL. The retention times for the two compounds were 11.78 minutes and 4.84 minutes, respectively.

The validation parameters for the method were all met, indicating that the method is accurate, precise, and robust. The assay of the method was also found to be within the acceptable range.

In conclusion, the method developed in this study is a valid and reliable method for the analysis of FIMA and CHL. The method can be used to determine the concentrations of these compounds in various sample matrices.

Here are some additional points that could be included in the conclusion:

The results of the forced degradation studies suggest that FIMA and CHL are stable under normal storage conditions. However, they should be stored in a cool, dry place to minimize the risk of degradation.

The optimized chromatographic conditions provide good separation of FIMA and CHL, and the method is sensitive enough to detect these compounds at low concentrations.

The validation parameters for the method were met according to the ICH Q2R1 guideline, which is an international standard for method validation.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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