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Developing a highly validated and sensitive HPLC method for simultaneous estimation of cefotaxime and paracetamol in pure and pharmaceutical preparations

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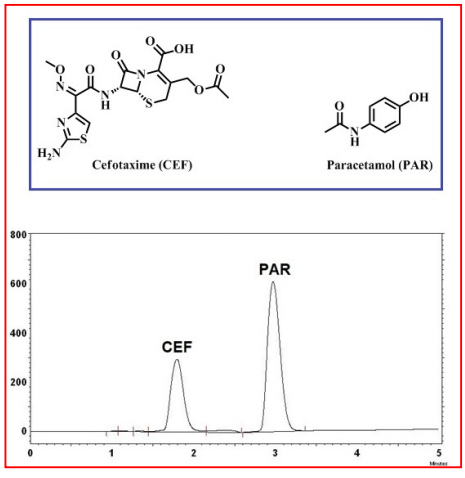
An isocratic HPLC technique was exploited and validated for the quick simultaneous separation and measurement of cefotaxime and paracetamol in vials dosage forms, with a total analysis time of 3 minutes. The process of separation was carried out on a Thermo Scientific® Venusil XBPC_{18} (L) (5µm, 4.6x250 mm) using a mobile phase of ACN: distilled water (70:30, v/v) at the ambient temperature. The flow rate used in the experiment was 1 mL/min, and the highest level of absorption was determined by high-performance liquid chromatography with photodiode array detection (HPLC-PDA) employing a PDA detector set at a wavelength of 255 nm. The established retention times for cefotaxime and paracetamol were 1.79 and 2.97 minutes, respectively, suggesting reduced analysis duration. The observed limits of detection for ceftaxime and paracetamol were 4.2×10^{-5} and 1.2×10^{-5} µg/mL, respectively, indicating a significant level of sensitivity in the approach. The approach was subsequently verified in accordance with the requirements set out by the Food and Drug Administration (FDA) for the quantification of medicines in vial dosage form.



Graphical Abstract

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1. Introduction

Many organic compounds are known to possess a wide range of biological and pharmacological activities as well as low toxicity toward mammals. Cefotaxime and paracetamol are considered important bioactive compounds due to their high activity in the medical field. Cefotaxime is chemically named as (6*R*,7*R*,*Z*)-3-(acetoxymethyl)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**Fig. 1**). Cefotaxime (CEF) belongs to the third generation of cephalosporin antibiotics. It is widely utilised in the formulation of recommended antibiotic medications as an effective treatment against both gram-positive and gram-negative pathogens. CEF is used for the therapeutic management of several medical conditions, such as gonorrhoea, meningitis, and severe infections including pyelonephritis and urinary tract infections. Morover, it is used before an operation to prevent infection after surgery.[1] The literature review revealed that several analytical approaches have been utilised in the determination of cefotaxime such as HPLC, UV-VIS spectrophotometric, potientiometry, electrophoresis and hydrophilic interaction chromatography.[2-9]

Paracetamol (PAR); *N*-(4-Hydroxyphenyl) acetamide (**Fig. 1**) is related to a non-steroidal anti-inflammatory drugs (NSAIDs) which acts centrally and peripherally for treatment of non-inflammatory conditions.[10] It is well known that PAR is recommended as a first-line treatment of pain and fever in paediatric patients. Intravenous (IV) infusions are recommended to be administered as a 15-minutes infusion to minimize local tissue trauma and related pain.[11]

Because CEF (cephalosporin antibiotic) and PAR (NSAID) are administrated together for treatment of patients with several types of infections,[12-15] consequently; the objective of the current research study is to find a highly sensitive method for rapid simultaneous separation and determination of cefotaxime and paracetamol in their pharmaceutical dosage forms. The literature study revealed that there are analytical methods utilized to determine paracetamol. These methods include spectrofluorimetric approaches,[16-18] voltammetric determination,[19] a micellar electrokinetic chromatography,[20] HPLC and GC-MS[21-22] methods.

According to a comprehensive survey, few analytical techniques have been employed like RP-HPLC and LC-MS for determination of CEF and PAR,[23,24] simultaneously. Elhassan *et al.* developed a RP-HPLC method using 1% formic acid in methanol as a mobile phase was optimal with a lower flow rate of 0.8 mL/min. However, this method was less sensitive according to the limit of the detection and the limit of the quantification.[23] Saranya *et al.*, reported isocratic LC-MS method for using acetonitrile: 20 mM ammonium acetate buffer in the ratio of 40: 60. Our developed method does not require buffer solution.[24]

Our study presents a chromatographic approach that is characterized by its simplicity, speed, reproducibility and sensitivity. The method was developed for the accurate detection of CEF and PAR in a synthetic mixture. The flow rate of our designed method is faster than the reported method.[23] The approach was ultimately subjected to a statistical comparison with a reference method, which demonstrated comparable levels of accuracy, repeatability, and no statistically significant deviation from the reported method.

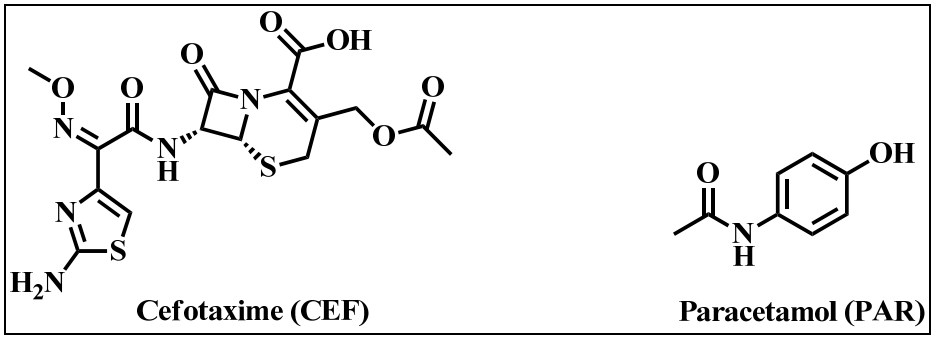

Fig. 1. Chemical structures of paracetamol (PAR) and cefotaxime (CEF).

2. Experimental

2.1. Apparatuss

Finnigan surveyor PDA plus detector® HPLC-PDA instrument (Germany) with a Thermo Scientific® Venusil XBP C 18 (L) (5µm, 4.6 x 250 mm), PDA absorbance detector, HPLC QUAT pumps and connected to PC computer loaded with Chromquest software.

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Serial No.:019166

2.2. Materials and reagents

All solvents and reagents were of an HPLC analytical grade (acetonitrile; ACN, was supported from Sigma Aldrich).

Cefotaxime and paracetamol were kindly provided as a gift from Egyptian International Pharmaceutical Industries Co. (EIPICO), located in 10th of Ramadan city, Egypt. Their purity was reported to be 99.70% and 99.80% respectively. Standard solutions of 200 µg/mL were prepared by dissolving 20 mg of each pure drug in 100 mL of the mobile phase.

Mobile phase consisted of a freshly prepared binary mixture of acetonitrile (ACN) and distilled water in a volumetric ratio of 70:30. Prior to use, the mobile phase was filtered and degassed using a 0.45 µm membrane filter from Millipore, USA.

Cefotax® vial (Epico Pharm, Egypt) and **Perfalgane®** vial (UPSA Labs): The vials had been labelled as having a cefotaxime content of 1000 mg and 1000 mg paracetamol, respectively.

2.3. Procedures

2.3.1. Establishment of standard calibration curves

The standard stock solutions of CEF and PAR were appropriately diluted in 10 mL volumetric flasks to achieve final concentrations of 2.50, 5, 10, 12.50, 25 and 50 µg/mL for both drugs. A volume of ten microliters from each combination was introduced into the column, and the resulting chromatogram was acquired using wavelengths of 210 nm, 220 nm and 255 nm. A concentration-response graph was constructed to depict the relationship between drug concentration and reaction, specifically peak area. In relation to the validation of quality control (QC) samples, values of 2.50, 12.50 and 50 µg/mL were chosen to represent low (LQC), medium (MQC), and high (HQC) levels, respectively.

2.3.2. Pharmaceutical procedure

Cefotax® and perflagan® vials formulations were utilized for the analysis. A precise quantity of the solution, corresponding to 20 mg of each medication, was dissolved in the mobile phase. The resulting solution was then filtered into 100 mL measuring flasks and brought to the desired volume using the mobile phase. The procedure was then completed as mentioned above through standard addition techniques.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Table 1 presents a comprehensive depiction of the various chromatographic conditions. The chromatographic detection was performed at 210 nm, 220 nm and 255 nm as the appropriate wave lengths using a PDA detector (**Fig. 2**) to detect the best optimal wavelength. The method was performed on a Thermo Scientific® Venusil XBP C_{18} column (L) (5 µm, 4.6 x 250 mm). Moreover, after several experimental trials, the optimisation of the mobile phase was conducted to determine the ideal composition ratio and pH; it was observed that the optimised mobile phase was determined as a mixture of ACN:distilled water (70:30, v/v) at a flow rate of 1 mL/min. Under these conditions, cefotaxime and paracetamol in both pure form and vials formulations can be separated and eluted at 1.79 and 2.97 minutes respectively as illustrated in **Fig. 3**, respectively. However, in all cases, the optimum mobile phase showed symmetrical peaks ($1.5< T < 3.5$), capacity factor ($1 < k < 10$), resolution >2 and theoretical plates >2000. **Table 1** presents the whole set of system suitability characteristics for the proposed (HPLC-PDA) approach, which enables the simultaneous analysis of the two medicines in both pure form and synthetic mixtures.

Table 1. Chromatographic conditions for the proposed HPLC method.

Parameters	Conditions
Column	Thermo Scientific® Venusil XBP C 18 column (L) (5µm, 4.6 x 250 mm).
Mobile phase	a mobile phase of ACN : distilled water (70 : 30, v/v) at ambient temperature degassed using 0.45µm membrane filter
UV detection, nm	301 nm
Flow rate, ml/min	1 mL/min.
Injected volume, µL	10 µL
Temperature	Ambient
Retention time, min	
Cefotaxime	1.79 min.
Paracetamol	2.97 min.

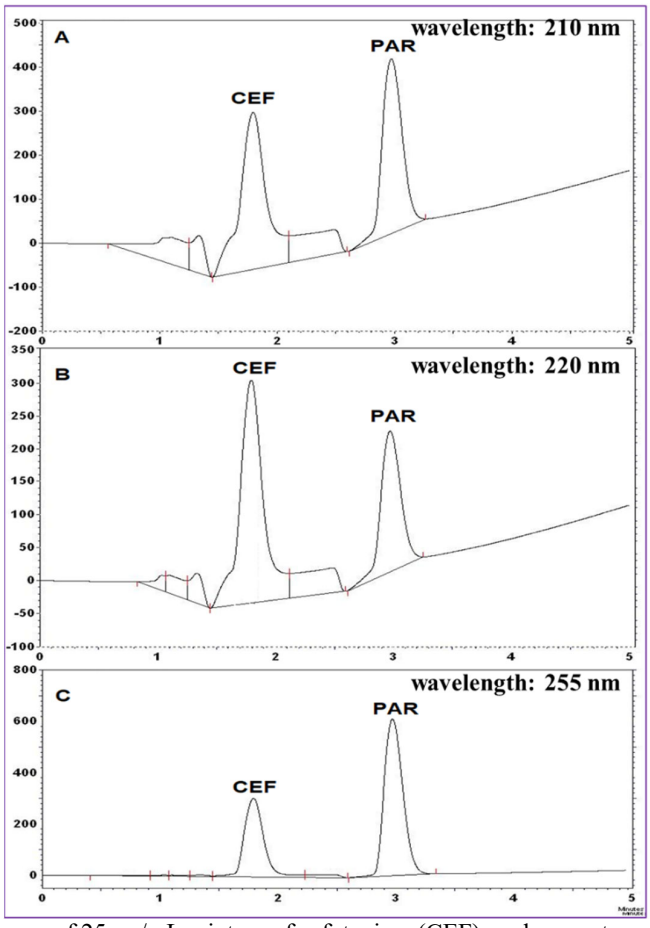

Fig. 2. HPLC Chromatogram of 25 µg/mL mixture of cefotaxime (CEF), and paracetamol (PAR) using Vensuil XBP C_{18} column (L) (5 µm, 4.6 x 250 mm) column and an isocratic mobile phase of ACN:Distilled Water (70:30, v/v) at different wave lengths (A) 210 nm, (B) 220 nm and (C) 255 nm, respectively. Other chromatographic conditions are stated in **Table 1**.

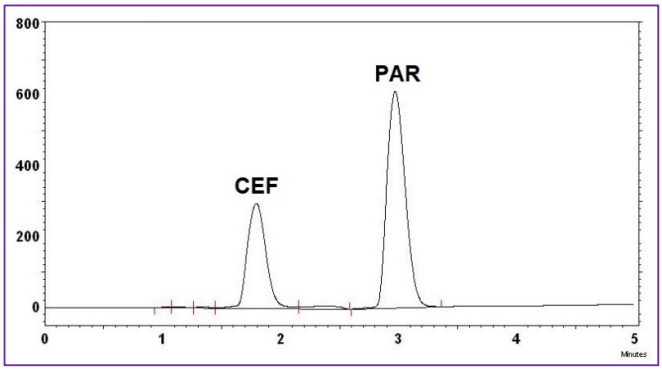

Fig. 3. HPLC Chromatogram of 25 µg/mL mixture of cefotaxime (CEF), and paracetamol (PAR) in their pharmaceutical formulations using the same conditions in **Fig. 2**.

3.2. Method Validation

The developed methods were validated according to the international conference on harmonisation guidelines ICH [24].

3.2.1. Linearity

For linearity studies, five various concentrations of the drug mixture were specified. The calibration curves obtained by plotting peak area against concentration showed linearity in the concentration range of 2.50-100 µg/mL for both drugs (**Table 2**). The linear regression equations for the CEF and PAR variables were determined to be y = 119547x + 258894 and y = 235699x + 484655, respectively. The estimated regression coefficient values (R^2) for both equations were found to be 0.9998, showing a significant degree of linearity for both medicines (**Fig. 4**).

Table 2. Results and characteristic parameters for the simultaneous determination of cefotaxime and paracetamol by the proposed method.

Parameters	Cefotaxime			Paracetamol		
	Conc. taken (µg/mL)	Conc. Found (µg/mL)	% Recovery	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Recovery
	5	5.14	102.85	5	4.97	99.41
	10	9.74	97.40	10	10.01	100.07
	12.5	12.54	100.32	12.5	12.25	98.05
	20	19.82	99.08	20	20.05	100.25
	25	25.31	101.26	25	25.35	101.42
	50	49.94	99.88	50	49.86	99.73
Mean recovery*			100.14			99.81
N			6			6
±SD			1.697			1.005
±RSD			1.694			1.007
Regression Equation Slope (b)**			119546.9			235699
Intercept (a)			258894.3			484654
LOD			4.2×10^{-5}			1.2×10^{-5}
LOQ			1.4×10^{-5}			4.2×10^{-5}
Correlation coefficient			0.9999			0.9999
Accuracy (Mean ± SD)			100.50±1.34			101.56± 0.66
Precision (±%RSD) Repeatability			100.64±1.6			101.25±1.08
Intermediate precision			100.69±1.66			101.62±0.89

* Average of three independent procedures.

**Y = a + bC where Y is peak area, C is the concentration of the drug inµg/ml.

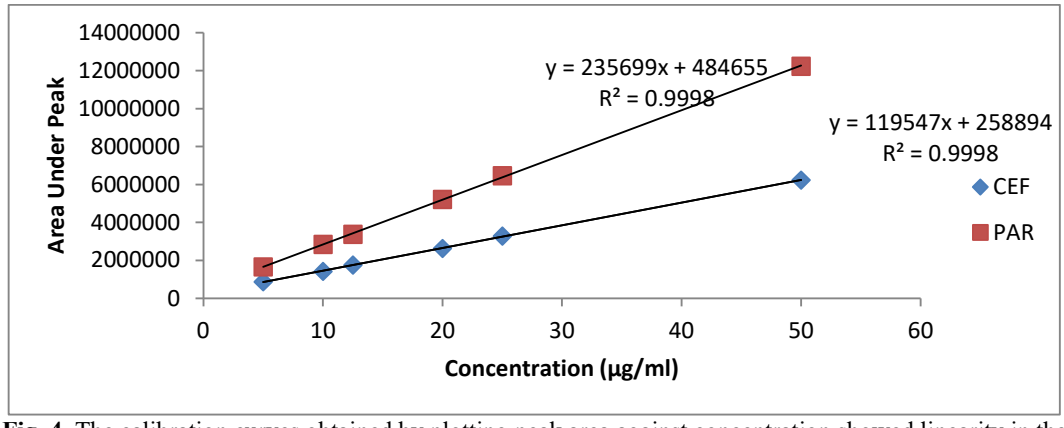

Fig. 4. The calibration curves obtained by plotting peak area against concentration showed linearity in the concentration range of 2.50-100 µg/mL for both drugs. Linear regression equations of cefotaxime and paracetamol.

3.2.2. Accuracy

The method's accuracy was assessed by examining the recoveries of commercially available CEF and PAR at various concentration levels within the designated range. This was done through using three duplicates of each concentration and applying the standard addition methodology. The experiment involved the addition of a predetermined concentration of each medication at various levels, in accordance with the specified methodology. The percentage of the recovery was estimated based on the determined quantity of the medication, and the data presented in **Table 3** demonstrate excellent recovery rates for both pharmaceuticals under investigation.

Table 3. Application of standard addition technique for the determination of cefotaxime and paracetamol in vials.

Items	Cefotaxime			Paracetamol		
	Conc. added form pure drug (µg/mL)	Conc. taken from vials (µg/mL)	% Recovery*	Conc. added form pure drug (µg/mL)	Conc. taken from vials (µg/mL)	% Recovery*
	10	0	97.75	10	0	98.98
	10	12.5	100.45	10	12.5	96.52
	10	30	102.79	10	30	102.71
	10	40	99.51	10	40	101.02
	10	50	99.45	10	50	98.63
Mean*			100.55			99.72
N			5			5
S.D.			2.53			2.36
R.S.D.			2.52			2.37
V			2.45			7.33
S.E.			1.26			1.18

*Mean of three different experiments.

3.2.3. Precision

The evaluation of the method's precision was conducted by assessing its intra-day and inter-day precision. This was achieved by employing validation quality control (QC) samples with concentrations of 12.5, 25, and 50 µg/mL. The evaluation of intra-day precision was conducted by assessing the standard deviation (SD) of three duplicate measurements using a solution containing pure drugs. The SD values (which ranged from 1.08 to 1.6) showed the method's excellent level of accuracy. The day-to-day SD values were similarly within the permissible range of 0.89-1.66 for inter-day repeatability (**Table 2**). The outcomes of this study demonstrate that the suggested methodology exhibits a satisfactory level of precision in the simultaneous determination of both drugs inside pharmaceutical formulations.

3.2.4. Selectivity and Specificity

The selectivity of the approach was assessed by individually injecting solutions of CEF and PAR into the column. This resulted in the appearance of two distinct peaks at retention durations of 1.79 and 2.97 minutes, respectively. These peaks were not seen in the blank solution. Furthermore, the experiments on specificity demonstrated that the presence of excipients in the vial formulations did not result in any impurity interference with the distinct and well-defined peaks of CEF and PAR (**Fig. 3**).

3.2.5. Limits of detection and limits of quantification

The approach employed for establishing the limits of detection (LOD) and quantitation (LOQ) involved applying a signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ. The limits of detection for CEF and PAR were 4.2 x 10^{-5} and 1.2 x 10^{-5} µg/mL, respectively. Similarly, the limits of quantification were determined to be 1.4 x 10^{-5} and 4.2 x 10^{-5} µg/mL for CEF and PAR, respectively (**Table 2**). On the other side, Elhassan *et al.* reported that the limits of detection for CEF and PAR were 0.316 and 0.248 µg, and the limits of quantification were 0.959 and 0.7515 µg.[23] Consequently, our results indicate that the proposed approach exhibits a high level of sensitivity.

3.2.6. Robustness

The evaluation of the techniques' robustness involved purposeful and modest alterations (± 0.05) in the flow rate and mobile phase composition ratio, while maintaining the other chromatographic settings constant. The impact of the adjustments was examined by analysing the percentage of drug recovery and the standard deviation for both drugs. **Table 4** demonstrates that the alterations had little effects on the outcomes, as indicated by the minor standard deviation (SD) values of 1.58 and 0.96 for CEF and PAR, respectively.

Table 4. Results and characteristic parameters of Robustness 25 mcg at different temperatures T24, T25, T26 and flow rate 0.95, flow rate 1.05 keeping the other chromatographic conditions constant for the simultaneous determination of cefotaxime and paracetamol by the proposed method.

parameters	Cefotaxime			Paracetamol		
	Parameter at conc. 25 µcg	**Conc. Found (µg/mL)**	**% Recovery**	**Parameter at conc. 25µcg**	**Conc. found (µg/mL)**	**% Recovery**
	Normal condition	25.32	101.03	Normal condition	24.93	99.27
	T 24	25.69	102.7	T 24	25.43	101.71
	T 25	25.42	101.7	T 25	25.18	100.75
	T 26	24.63	98.53	T 26	25.56	102.27
	F.R 0.95	25.44	101.1	F.R 0.95	25.29	101.18
	F.R 1.05	25.69	102.8	F.R 1.05	25.62	102.46
Mean recovery*			101.2			101.12
±SD			1.587			0.966
±RSD			1.568			0.966
Regression Equation ** **Slope (b)**			119546.9			235699
Intercept (a)			258894.3			484654

3.3. Applications

3.3.1. Analysis of vial formulations

F test and T test were performed using GraphPad Prism version 5.01 for Windows, GraphPad Software. Cefotax® and perflagan® pharmaceutical formulation containing CEF and PAR had been successfully analyzed by the proposed method.[25-26] The absence of interference from excipients and contaminants demonstrates the method's excellent specificity. The acquired results were compared to those obtained by the reference techniques, in which statistical tests such as Student's t-test and F-test were employed for the purpose of comparison. Results shown in **Table 5** indicated that calculated t and F values were less than tabulated ones for CEF and PAR which in turn indicate that there is no significant difference between proposed method and reference ones relative to precision and accuracy. There is interest in these types of compounds, hence

the development of a method for their determination is so important to confirm the importance of synthetic compounds in various areas of life as reflected in many papers published before.[27-68]

Table 5. Statistical analysis of results obtained by the proposed method applied on cefotaxime and paracetamol tablets compared with reference methods.

Test	T test			F test			
	P value	t	df	F	DFn	Dfd	P value
Paracetamol	0.7154	0.3915	4	2.16	2	2	0.6328
Cefotaxime	0.8473	0.2053	4	22.85	2	2	0.0839

The results showed that $p > 0.05$, there is no significant difference. Calculated t-test and f-test are less than tabulated one.

4. Conclusion

An isocratic RP-HPLC method has been exploited for the simultaneous estimation of mixture of CEF and PAR. The approach that was introduced and verified in this study was designed to efficiently and accurately estimate the concentrations of CEF and PAR in a simultaneous manner, with a total analysis time of only 3 minutes. The obtained results demonstrate that the suggested technique exhibits characteristics of rapidity, accuracy, selectivity, robustness, and reproducibility. The linearity of the examined medicines (CEF and PAR) was found within a concentration range of 2.50 to 50 µg/mL. The technology has demonstrated successful application in the analysis of marketed vials cefotax® and perflagan® for the purpose of quality control, where the need for cost-effectiveness and expeditious analysis is essential. The proposed method can be used in quality control laboratories for routine analysis of mixture of CEF and PAR.

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Authors' contributions

Mahmoud M. Sebaiy, Sobhy M. El-Adl, and Alaa Nafie: supervision, conceptualization, designed the study, paper preparation, writing original draft, visualization, and revised the manuscript. Samar S. Elbaramawi: performed the searches, extracted the data, paper preparation, and writing original draft. Shaban A. A. Abdel-Raheem: revising and adjusting the paper linguistically and spelling and adjusting the paper according to the style of the journal.

Conflict of interest

The authors declare that there is no conflict of interest in the manuscript.

Ethical approval

This manuscript does not include any studies on human or animals.

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