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Determination of Lisinopril in Bulk and Pharmaceutical Formulations by Cloud Point Extraction—A Green Method

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Abstract

A sensitive and eco-friendly method was developed for the spectrophotometric determination of Lisinopril (LSP) in bulk and pharmaceutical formulations by cloud point extraction technique. The method was based on the formation of a blue-colored coordination complex between Lisinopril (LSP) and Cobalt Thiocyanate (CTC) at a suitable pH. The Complex in aqueous medium was extracted into surfactant layer by cloud point extraction using a non-ionic surfactant Triton X-114 and then the surfactant layer was dissolved in a suitable volume of ethanol and the amount of Lisinopril was determined spectrophotometrically at a wavelength of 625 nm. The conditions like concentration of the drug, concentration of CTC and of Triton X-114, P^H, etc. were optimized by OFAT (One Factor At a Time) method. The linear range of calibration curve was 1 - 6 µg/ml and the linear regression equation with a correlation coefficient of 0.99996 was y = 0.0021 + 0.084x. Preconcentration and enrichment factors were found to be 100 and 3.12 respectively, achieving the detection limit of 0.0588 µg/ml. The proposed method was successfully applied for the determination of LSP in the drug formulations. The obtained values were in agreement with the values as quoted by the manufacturers.

Keywords

Lisinopril (LSP), Cobalt Thiocyanate (CTC), Coordination Complex Formation, Cloud Pint Extraction (CPE), Spectrophotometry

1. Introduction

For the body's production of angiotensin II, Angiotensin converting enzyme inhibitors (ACE inhibitors) drugs are applied. It is a hormone that circulates and

constricts blood vessels and has many effects on the cardiovascular system and sometimes causes heart attack or heart failure. Blocking production of angiotensin II with ACE inhibitors prevents constriction of blood vessels, lowers blood pressure, and weakens the energy the heart has to expend from beat to beat [1]. Lisinopril (LSP) is a drug of angiotensin converting enzyme inhibitors class used primarily in the treatment of high blood pressure and heart failures and after heart attacks. Lisinopril is chemically known as 1-{N^2-[(1S)-1-Carboxy 3-Phenyl Propyl]-L-Lysyl} L-Proline [2] and the structure is shown in **Figure 1**.

Few methods reported so far includes spectrofluorimetric methods [3] [4] [5], polarographic method [6], high performance liquid chromatography (HPLC) [7], liquid chromatography-mass spectrometry (LC-MS) [8], UV spectroscopy [9], and spectrophotometry [10]-[17]. Determination of drugs by spectrophotometry using Cobalt Thiocyanate (CTC) [18]-[23] was carried out by extracting the drug-CTC complex into an organic solvent like nitrobenzene which is a toxic solvent. Hence a sensitive, low-cost and green method was developed for the spectrophotometric determination of Lisinopril using CTC by cloud point extraction technique. Moreover, there are a few reports available for the determination of drugs by CPE coupled with spectrophotometry [24] [25], spectrofluorometry and RP-HPLC [26] [27] [28] [29] [30]. In the present work, a new, sensitive and green method was developed for the determination of Lisinopril in bulk and pharmaceutical formulations by CPE in combination with UV-Vis spectrophotometry. The method was based on the formation of coordination complex between LSP and CTC at a suitable pH followed the extraction of the complex by cloud point extraction using Triton X-114 under the optimum conditions. The extracted surfactant layer was dissolved in a little volume of ethanol and the drug was determined by UV-Vis spectrophotometry at λ_{max} of 625 nm. The proposed method was applied for the determination of LSP in pharmaceutical formulations.

2. Materials and Method

2.1. Instrumentation

The absorption spectra and absorbance values of the selected drug were scanned by using a Systronics-119 double-beam UV-Vis Spectrophotometer with 10-mm superior quality quartz cuvettes. Thermostatic water bath (SISCO, Maharashtra,

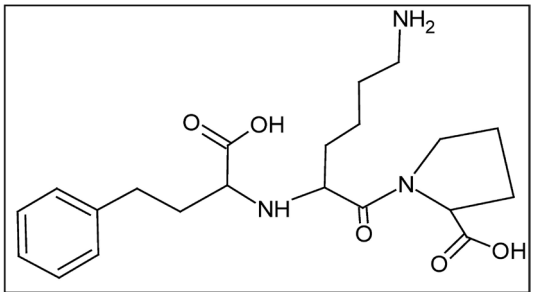

Figure 1. Structure of Lisinopril.

India), Microprocessor based Laboratory Centrifuge (Laby, India) were used for CPE procedure. For calibrating the pH measurements of solutions, digital pH-meter (Analab, India) was used.

2.2. Reagents and Materials

A. R. grade chemicals were used in the present work. An aqueous solution 0.5 M of Triton X-114 (obtained from Sigma Aldrich, India) was prepared by dissolving 25.4 ml of Triton X-114 in distilled water to get a final volume of 100 ml solution. A pure grade LSP was supplied by Dr. Reddy's Laboratories, Hyderabad, India. A stock solution of 1 mg/ml of LSP was prepared by dissolving 100 mg of the drug in distilled water and made up to 100 ml. A working solution of 100 μg/ml was further prepared for analysis. A stock solution of CTC (2.5×10^{-1} M) was prepared by dissolving 7.25 g of cobalt nitrate and 3.8 g of ammonium thiocyanate in 100 ml of distilled water. Buffer solutions of pH ranging from 2 to 10 were prepared and calibrated with a digital pH meter.

2.3. General Procedure for CPE

Aliquot of working standard solution of LSP was transferred into a 15 ml graduated centrifuge tube and 3.0 ml of CTC, 1.0 ml of buffer (pH = 2.0) and 1.0 ml of 0.5 M Triton X-114 were added and diluted with distilled water up to 10 ml. The solution was shaken thoroughly and then kept for 30 min in the thermostatic bath at 40˚C. The separation of the phases was further carried out by centrifugation at 4000 rpm for 8 min. The phases were cooled down in an ice water bath for 10 minutes. The layers were separated by inverting the tube. The surfactant-rich layer containing the complex was dissolved with suitable volume of ethanol and the absorbance of the complex measured at 625 nm against a reagent blank prepared under similar conditions.

2.4. Procedure for the Tablets

Four tablets of Listril (Manufactured by Torrent Pharmaceuticals Ltd., India) each containing 2.5 milligrams of LSP were initially crushed, powdered, weighed out and the average weight of one tablet was determined. An accurate weight equivalent to 2.5 mg of LSP was dissolved in 25 ml distilled water and then filtered. Aliquot of this solution was taken within the calibration range and then analyzed as described under the general procedure. The drug content of the tablet was assayed from the calibration curve.

3. Results and Discussion

3.1. Absorption Spectra

The absorption spectrum of LSP-CTC Complex after cloud point extraction with Triton X-114 was scanned between 500 and 800 nm. The blue colored complex shows that the absorption maximum at λ_{max} of 625 nm in visible region as shown

in Figure 2. Thus the wavelength of maximum absorbance at 625 nm was chosen for the present study.

3.2. Optimization of Parameters that Affect CPE

All the important factors that affect the CPE efficiency of the drug LSP were sequentially investigated by OVAT method via changing one factor while keeping other factors constant. In this respect, the effect of pH, concentration of CTC, of non-ionic surfactant Triton X-114, temperature and centrifugation speed and time were optimized.

3.3. Effect of CTC Concentration

The effect of concentration of CTC on the absorbance of LSP-CTC complex in the presence of Triton X-114 was studied by recording the absorbance of the complex at λ_{max} (625 nm) over the range of 1.0 - 6.0 ml of CTC (2.5×10^{-1} M) while keeping the concentrations of LSP, buffer (pH = 2.0) and Triton X-114 constant. The results showed that the absorbance of complex in the surfactant layer increased as shown in Figure 3 with increasing CTC concentration with subsequent increase of absorbance in the aqueous layer also. It indicates that the CTC itself interferes with the absorbance of the complex as its concentration increases in the aqueous layer. 3.0 ml of CTC (2.5×10^{-1} M) was selected as its optimum concentration.

3.4. Effect of pH

In order to study the effect of pH on the extraction efficiency of the complex, the solutions containing LSP, CTC, and Triton X-114 were subjected to cloud point extraction by varying the pH of the solutions in the range of 2 - 10. The study showed that the absorbance and extraction efficiency of the complex decreased as shown in Figure 4 from pH 2.0 to pH 7.0 and then increased from pH 7.0 to pH 10.0. Since maximum efficiency was achieved at pH 2.0, this pH was selected for CPE procedure.

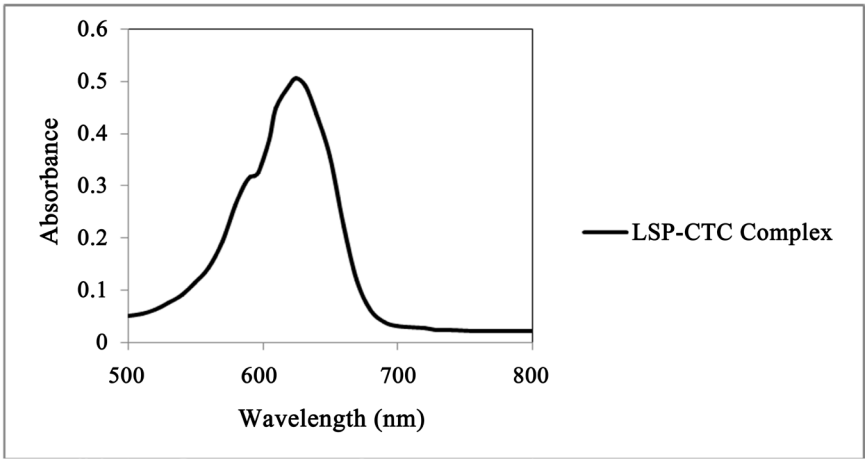

Figure 2. Absorption spectra of LSP-CTC complex.

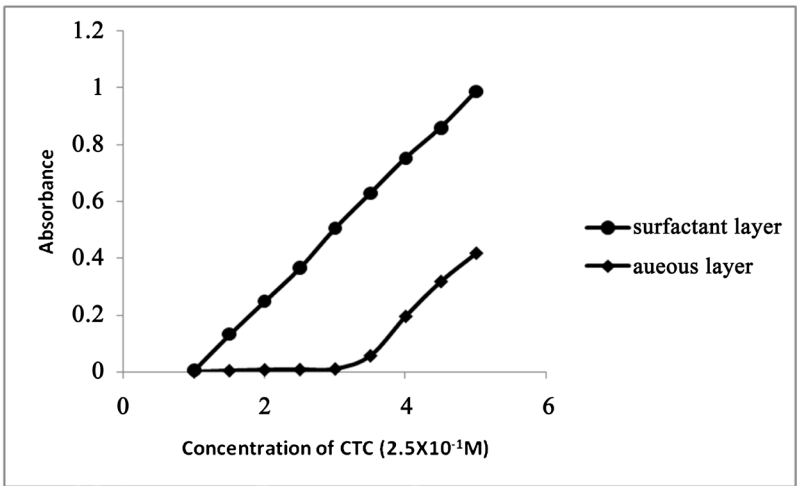

Figure 3. Effect of CTC concentration on the absorbance of the complex.

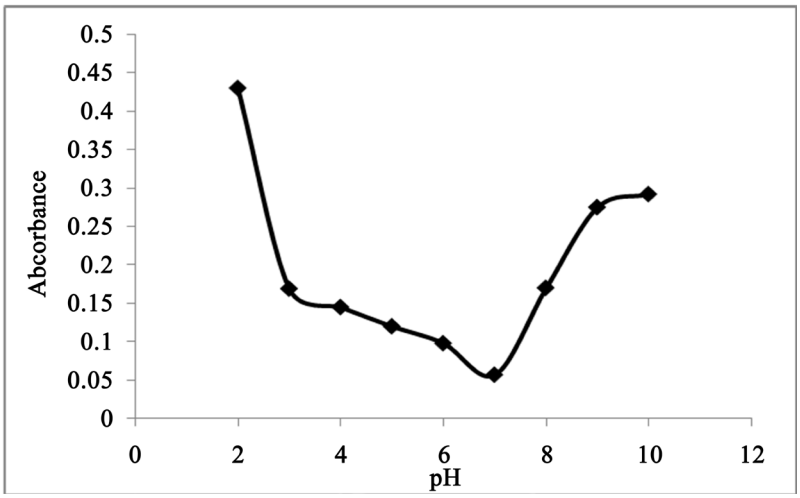

Figure 4. Effect of pH on the absorbance of the complex.

3.5. Effect of Triton X-114 Concentration

The influence of Triton X-114 concentration on the absorbance was studied by varying the concentration of it in the range of 0.01M to 0.13 M in the CPE procedure. The absorbance of the complex gradually increased as shown in **Figure 5** with the increase in the concentration of Triton X-114 up to 0.05 M and then decreased (**Figure 5** & **Figure 6**). 0.05 M concentration of the surfactant was chosen for the study.

3.6. Effect of Triton X-114 Concentration on Extraction Efficiency, Preconcentration Factor and Phase Volume Ratio

The effect of Triton X-114 on the % extraction efficiency was studied. It was observed that the % extraction efficiency gradually increased with the increase of Triton X-114 concentration up to 0.05 M and then decreased as shown in the **Figure 6**. The effect of the surfactant concentration on the Preconcentration factor and phase-volume ratio was also studied and the results were shown the

Figure 7.

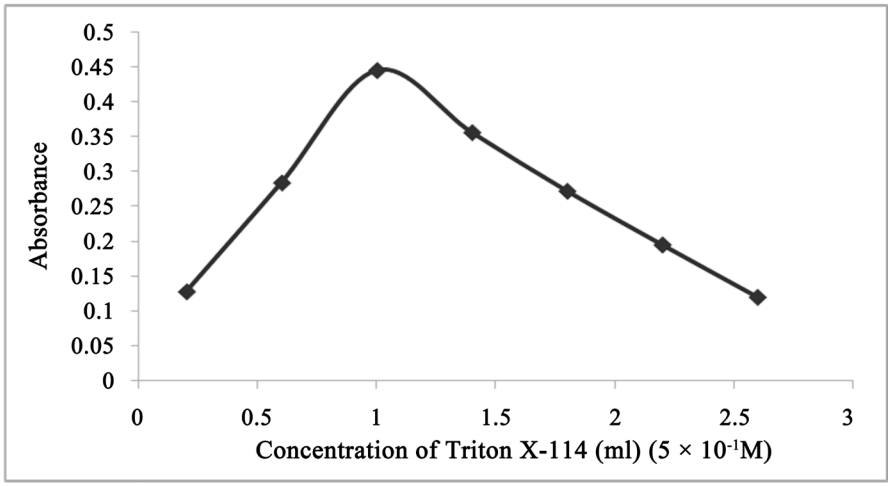

Figure 5. Effect of Triton X-114 on the absorbance of the complex.

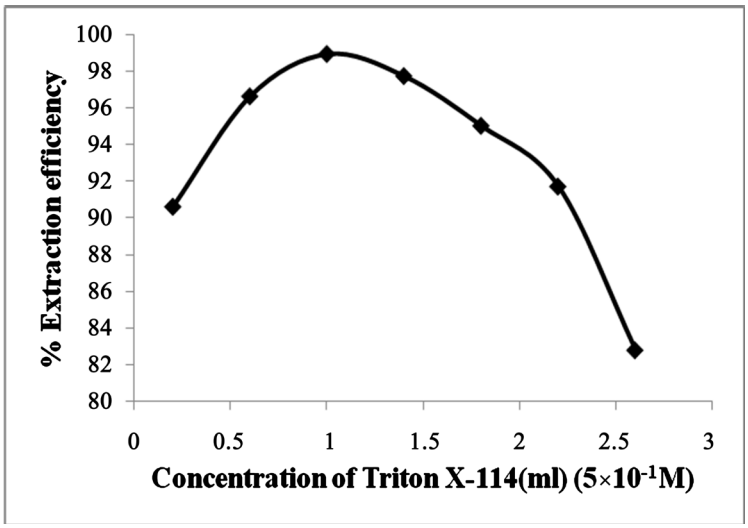

Figure 6. Effect of Triton X-114 on the % extraction efficiency.

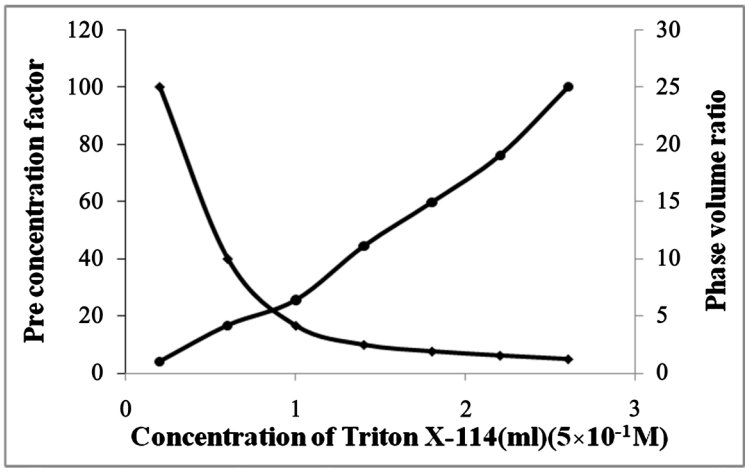

Figure 7. Effect of Triton X-114on the Preconcentration factor and phase volume ratio.

3.7. Effect of Incubation Temperature and Time

It is desirable to employ the incubation temperature and time as low as possible to achieve complete separation of phases and to improve the efficiency of CPE. The effect of incubation temperature was studied in the range of 30˚C - 60˚C and 40˚C was found to be sufficient for complete extraction. Similarly the incubation time was also evaluated in the range of 10 - 50 min. and for the completion of extraction. 30 min. time was found enough.

The influence of centrifuge time and speed on CPE were also studied in the range of 2 - 10 min. and 2000 - 5000 rpm respectively. It was found that 8 min. time and 4000 rpm speed were sufficient to achieve complete extraction.

3.8. Principle of the Method

Cobalt Thiocyanate (CTC) has been proved to be a valuable chromogen for the determination of amino compounds. Lisinopril has a secondary amino group and hence it was believed that the 2˚ amino group of the drug was coordinated to the Co^{+2} of CTC as shown in Figure 8 in the following scheme.

3.9. Calibration Curve of the Proposed Method

A series of standard of LSP solutions ranging from 1 - 6 µg/ml were taken and subjected to the general CPE and the absorbance of each solution was measured at λ_{max} of 625 nm, in order to construct the calibration curve as shown in Figure 9 from which the amount of LSP was determined. The calibration curve of the proposed method was given below.

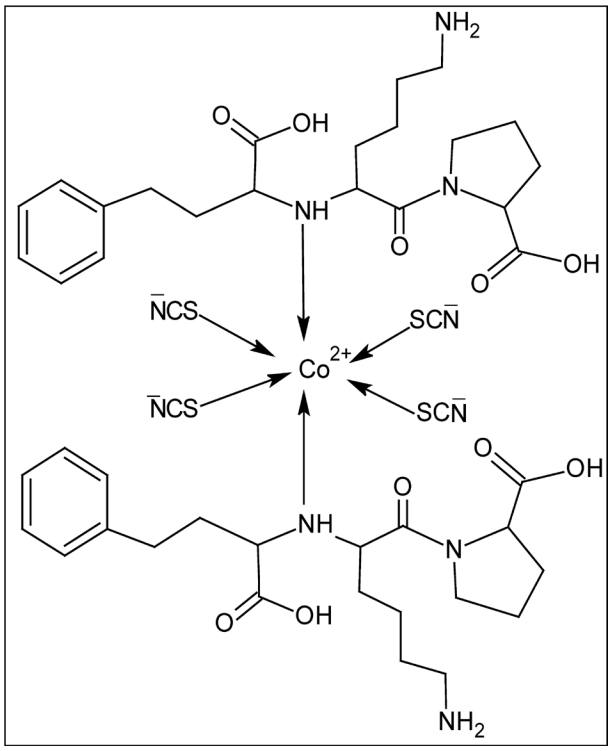

Figure 8. Coordination complex between LSP and CTC.

3.10. Evaluation of Calibration Curve

The statistical data obtained from the calibration curve were tabulated in Table 1.

3.11. Accuracy and Precision

Intra-day and inter-day was considered for the calculation of precision. Three concentrations of the drug were analyzed in six replicates during the same day (intra-day precision) and for three consecutive days (inter-day precision). Table 2 and Table 3 given below illustrates the analytical results. For the quality control analysis of the studied drug, the reported precision was adequate and relative standard deviation percentage (RSD%) was satisfactory.

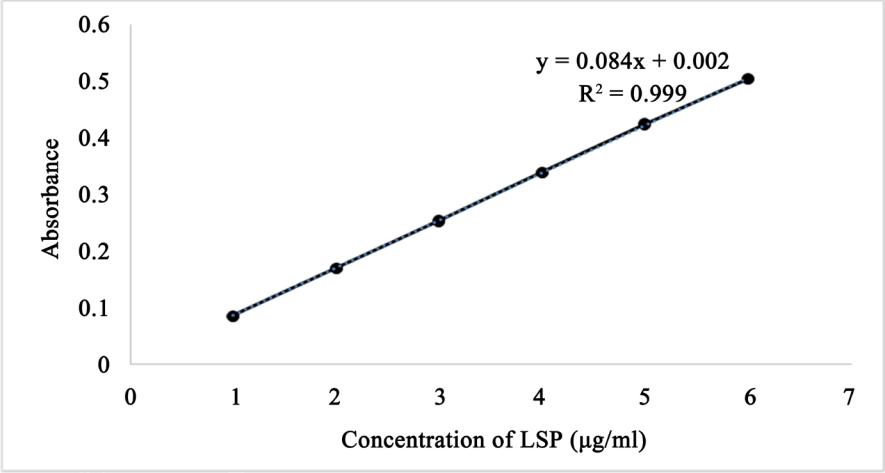

Figure 9. Calibration curve of the proposed method.

Table 1. Statistical data.

Parameter	value
λ_{max}	625 nm
Beer's Law limits	1 - 6 µg/ml
Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	3.53×10^5
Sandell's sensitivity ($\mu g \cdot cm^{-2}$/0.001A.U)	0.0115
Regression equation	y = 0.0842x + 0.0021
Correlation coefficient (r)	0.9999
Coefficient of determination (R^2)	0.9998
Std.error of regression (s_e)	0.00165
Std.error of slope (s_b)	0.000395
Limit of Detection (µg/ml)	0.0588
Limit of Quantization (µg/ml)	0.1964
Preconcentration factor	100
RSD% (n = 6) at 5 µg/ml	0.51

Table 2. Analytical results (Intraday).

Drug	Taken (μg·ml^{-1})	Intraday accuracy and precision			
		Found (μg·ml^{-1})	Recovery %	% RSD	RE %
LSP	5.0	4.996	99.92	0.51	−0.078
	5.0	4.992	99.8	0.69	−0.0667
	5.0	4.978	99.57	0.43	−0.044

Table 3. Analytical results (Interday).

Drug	Taken (μg·ml^{-1})	Interday accuracy and precision			
		Found (μg·ml^{-1})	Recovery %	% RSD	RE %
LSP	5.0	4.996	99.92	0.51	−0.078
	5.0	5.0	100	0.66	0.00
	5.0	5.03	100.67	0.34	0.67

Table 4. Assay of Lisinopril drug in pharmaceutical formulations.

Formulation/Tablet	Labeled amount (mg/Tablet)	Amount found (mg)	% recovery	% RSD
LISTRIL	2.5	2.495	99.8	0.45
CIPRIL 5	5	5.02	100.3	0.90

3.12. Determination of LSP in Pharmaceutical Formulations by Proposed Method

The proposed method was also employed for the determination of LSP content in two selected tablets containing LSP such as Listril (Torrent Pharmaceuticals Ltd. India) and Cipril 5 (Cipla, India) with stated values of 2.5 mg and 5 mg respectively. The results are tabulated in Table 4. The results obtained are satisfactorily accurate and precise as indicated by the excellent% recovery. It was found that the excipients and other active ingredients present in pharmaceutical formulations did not interfere with the proposed method.

4. Conclusion

The new spectrophotometric method developed for the determination of LSP is more sensitive, reproducible and less expensive when compared to other spectrophotometric methods reported. The developed CPE—spectrophotometric method was characterized with simplicity, good sensitivity, and low detection limit and reliable for the determination of LSP. In comparison with the existing visible spectrophotometric methods for the determination of LSP, the present modified method can be considered green as it makes use of spectrophotometry without the usage of organic solvent.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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