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Sensitive Spectrophotometric Determination of Atorvastatin in Pharmaceutical Formulation by Ion Pair Complexation with Pararosaniline Hydrochloride

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ABSTRACT

Objectives: A sensitive visible spectrophotometric method was developed for the determination of atorvastatin in tablet dosage form. **Methods**: Atorvastatin (ASN) has a free carboxylic moiety in its structure, which when being deprotonated in basic buffered medium facilitates its ion pairing with the cationic pararosaniline dye. **Results**: The red colored ion pair is easily extractable in organic solvent and exhibits maximum absorption at 547 nm. Different factors affecting the formation of the ion pair and its stability were studied and optimized in order to obtain the best conditions for the experiment. Method validation was done over a concentration range of 1 to 8 µg/mL. The method was proven to be sensitive with limit for quantitation of 0.93 µg/mL and limit of detection of 0.31 µg/mL. **Conclusion**: The proposed method was easily and successfully applied for the quantitative determination of atorvastatin in commercial tablets without interference from extractable tablet additives. The sensitivity and cheapness of the method will encourage its use in routine quality control of atorvastatin tablets.

Keywords: *Atorvastatin; Ion pair complexation; Pararosaniline; Pharmaceutical formulation; Spectrophotometry*

INTRODUCTION

Atorvastatin (**Figure 1**) is one of the most important cholesterol lowering drugs which acts by the competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme which catalyzes the rate-limiting step in cholesterol biosynthesis[1]. Different techniques have been reported for the determination of ASN in pharmaceutical formulations and body fluids. These techniques involve HPLC with UV detection[2-5], HPLC with fluorescence detection[6], LC-MS/MS[7-10], HPTLC[11-13], voltametry[14-16], capillary electrophoresis[17,18], spectrophotometry[19-21] and spectrofluorometry[22].

Ion pair complexes or ion association complexes are complexes in which the species of analytical interest associates with oppositely charged ions to form a neutral extractable species[23]. Ion pair complexation was utilized for the visible spectrophotometric determination of different drugs in the literature through their ion pairing with oppositely charged dyes to form colored ion pairs. Reported methods of determination of atorvastatin by ion pairing extractive spectrophotometry are very rare and mainly based on the cationic nature of its azole moiety in acidic medium which can be paired with anionic dyes like bromocresol green, alizarin red, or bromophenol blue[24]. Literature survey revealed that there is no single spectrophotometric method reported for determination of atorvastatin by ion pairing with cationic dyes and this was the motive for this work.

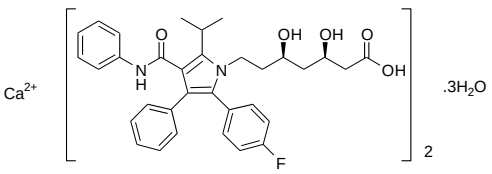

Figure 1. Chemical structure of atorvastatin calcium trihydrate.

MATERIALS AND METHODS

Pure drug

Atorvastatin calcium trihydrate was given as gift by Egyptian International Pharmaceutical Industry Co. (EIPICO) (10th of Ramadan City, Sharqia, Egypt), with a purity of 98.7% according to the USP method[25].

Dosage form

ATOR 10 tablets containing an amount of atorvastatin calcium trihydrate equivalent to 10 mg of atorvastatin free acid (Batch number 1102999), produced by EIPICO were purchased from the local market.

Chemicals and reagents

Deionized water (PureLab Flex) was used in the whole experiment. Pararosaniline HCl was purchased from S D Fine-Chem Limited (SDFC), Mumbai, Maharashtra, India. Boric acid, Sodium hydroxide and n-Hexane were purchased from El-NASR Company, Egypt. Chloroform AR and Dichloromethane AR were purchased from Central Drug House Company (CDH), New Delhi, India.

Apparatus

Jasco V-630 UV-VIS Spectrophotometer operated with Spectra Manager™ II software was used.

Preparation of Boric acid/NaOH buffer

Boric acid/NaOH buffer was prepared by dissolving 6.20 gm of boric acid in 500 mL of deionized water and the pH is adjusted to the desired value (8-9.4) with 1 M sodium hydroxide[26].

Preparation of stock and working solutions

Stock standard solution of atorvastatin was prepared by dissolving atorvastatin calcium trihydrate in methanol to yield a concentration equivalent to 1 mg/mL of atorvastatin free acid and stored at -20 ºC. 10 mL aliquot was transferred to a 100mL volumetric flask and the volume was completed with methanol to yield a 0.1 mg/mL working standard solution which is then stored at 4 ºC. Working solution of pararosaniline was prepared directly by dissolving pararosaniline in water to yield a 1mg/mL aqueous solution and stored at 4 ºC.

Determination of the spectral characteristics of the ion pair complex

0.5mL aliquot of the working solution was transferred to a 125mL separating funnel followed by 3mL pH 8.6 borate buffer, 4 mL of the working pararosaniline solution and 1.4 mL of water to bring the final volume of the aqueous layer to 10 mL which is mixed by swirling for 3 min at room temperature. Extraction of aqueous layer was done by addition of 9 mL dichloromethane and shaking for 1 min. The organic layer was then transferred to a 10 mL volumetric flask and the volume was brought to 10 mL with dichloromethane. The absorption spectrum was plotted against a reagent blank.

Preparation of calibration standards

Different aliquots of the working solution of atorvastatin (0.1-0.8mL) were transferred into a series of 125 mL separating funnels, followed by 3mL pH 8.6 borate buffer, 4 mL of the working pararosaniline solution and water to adjust the aqueous layer to 10 mL. The final concentrations of the calibration series will be 1-8 µg/mL. Then the aqueous layers were mixed by swirling for 3 min at room temperature. The aqueous layers were then extracted with 9 mL of dichloromethane for 1 min. The organic layers were then transferred to a series of 10 mL volumetric flasks and the volumes were brought to 10 mL with dichloromethane and scanned spectrophotometrically against a reagent blank which was prepared in the same way.

Application

Ten tablets were weighed then crushed; powdered and homogenized. An accurate weight equivalent to 10 mg of the drug was extracted with 50 mL methanol, filtered through a dry funnel and filter paper. The filtrate was then filtered through 0.2 µm Millipore filter, transferred to 100 mL volumetric flask and the volume was completed with methanol and stored at 4 ºC. A 0.2 mL aliquot was taken to yield a 2 µg/mL solution of the drug, then this solution was analyzed three times as described under Determination of the spectral characteristics of the ion pair complex section.

RESULTS AND DISCUSSION

Ion Pair formation

The acidic nature of atorvastatin due to the presence of a carboxylic group was used as a basis to form an ion pair with a basic cationic dye. The pKa of atorvastatin is 4.33 and in order to obtain maximum ionization; a basic buffer should be used. Boric acid/NaOH buffer was found to be a suitable buffer. In this method pararosaniline (**Figure 2**) was used as the basic cationic dye to form an ion pair with deprotonated atorvastatin.

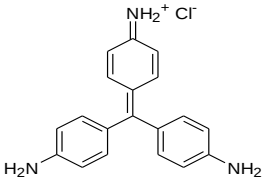

Figure 2.Chemical structure of pararosaniline HCl

The ASN-pararosaniline ion pair formation is based on the electrostatic interaction between the deprotonated anionic carboxyl moiety of atorvastatin in basic buffer and cationic amino group of pararosaniline (**Figure 3**). This ion pair can be easily extracted from the aqueous phase into organic phase and it was found to have maximum absorbance at λ 547 nm (**Figure 4**) when scanned in the UV-visible spectrophotometer.

Optimization of method parameters

Effect of pararosaniline volume

The same procedures described in Determination of the spectral characteristics of the ion pair complex section were done using different volumes of pararosaniline solution (1-5 mL). It was found that maximum absorbance was obtained upon using 4mL of pararosaniline (**Figure 5**).

Effect of the extraction solvent

The extraction was carried out using dichloromethane, chloroform and n-hexane each in a time and was found that maximum absorbance was achieved by using dichloromethane for extraction of the ion pair (**Figure 6**).

Effect of borate buffer pH

To study the effect of pH on complex formation, the experiment was carried out using varying pH values of borate buffer (8-9.4). It was found that borate buffer of pH 8.6 is optimal for complex formation (**Figure 7**).

Effect of the volume of pH 8.6 borate buffer

Different volumes of pH 8.6 borate buffer have been tried (1-5 mL) and it was found that 3 mL is the optimal volume (**Figure 8**).

Effect of the time of extraction

Different times were applied for the extraction of the formed ion pair complex (1-5 minutes) using dichloromethane. One minute was enough for extraction to obtain maximal absorbance (**Figure 9**).

Complex color stability in the organic phase

Complex stability in the organic phase was assessed within a one hour period and it was found to be stable during this period which is satisfactory for measurements (**Figure 10**).

Assessment of the stoichiometry of the reaction

The stoichiometry of the reaction was assessed using Job's method [27] and Molar ratio method[28]. In Job's method the concentration of atorvastatin and pararosaniline was 0.001 M of each. The mole fraction of atorvastatin was ranging from 0.1-0.9. In Molar ratio method the concentration of atorvastatin and pararosaniline was 0.0025 M. The volume of atorvastatin used was 0.1 mL in all cases while the volume of pararosaniline was ranging from 0.05-0.5mL. The stoichiometric ratio of the reaction was found to be 1:2 atorvastatin to pararosaniline by Job's method (**Figure 11**) and Molar ratio method (**Figure 12**).

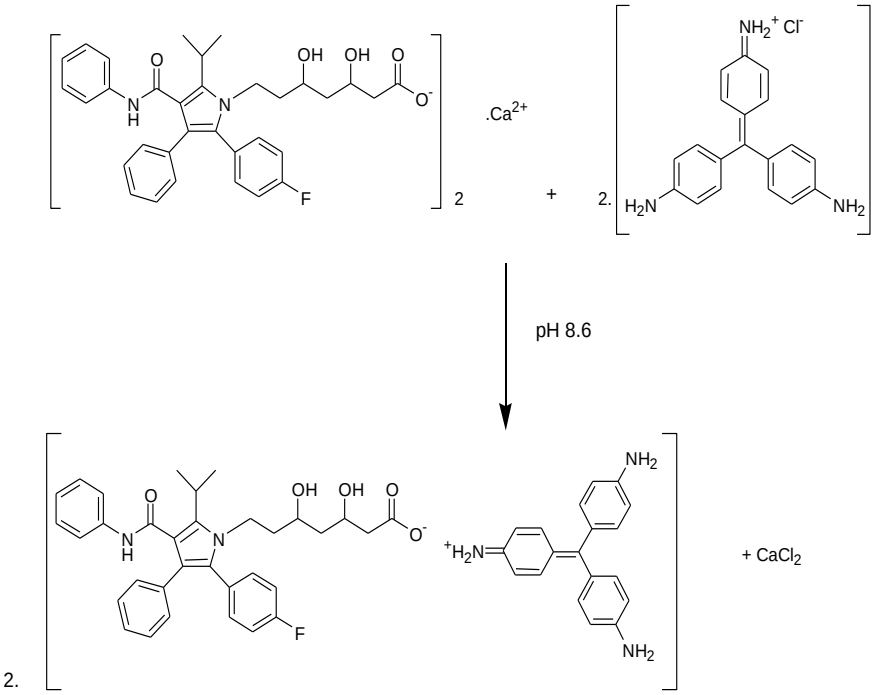

Figure 3.Structural representation of atorvastatin-pararosaniline ion-pair complex.

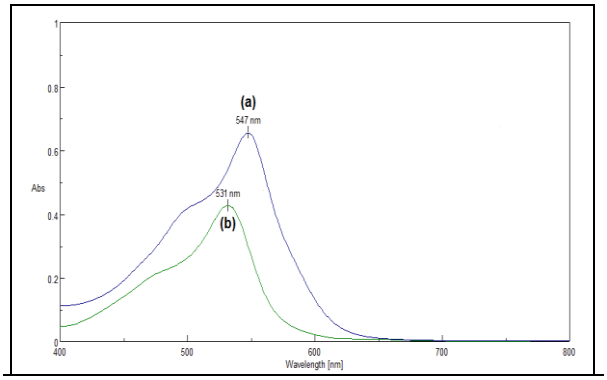

Figure 4. (a) Absorption spectrum of atorvastatin (5µg/mL) with pararosaniline dye at λ max 547 nm and (b) absorption spectrum of pararosaniline in borate buffer pH 8.6.

Method validation

Linearity and range

The proposed method was successfully validated under the optimized experimental conditions according to ICH[29] guidelines regarding the linearity, limits of detection and quantitation, accuracy and precision. To construct the calibration graph, the absorbance values of calibration peaks were plotted against the corresponding concentrations (µg/mL). Calibration graph was found to be linear over the concentration range of 1-8µg/mL. The computed regression equation was the following:

$$Y = 0.12915*X - 0.01391 \qquad r = 0.999$$

Where X is the concentration in µg/mL, Y is the absorbance and r is the regression coefficient. The linearity data are listed in (**Table 1**).

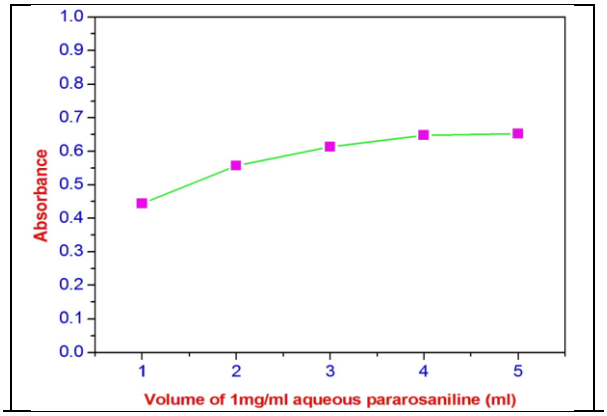

Figure 5. Effect of the volume of 1mg/mL pararosaniline on the absorbance of atorvastatin (6µg/mL) with pararosaniline at λ max 547 nm.

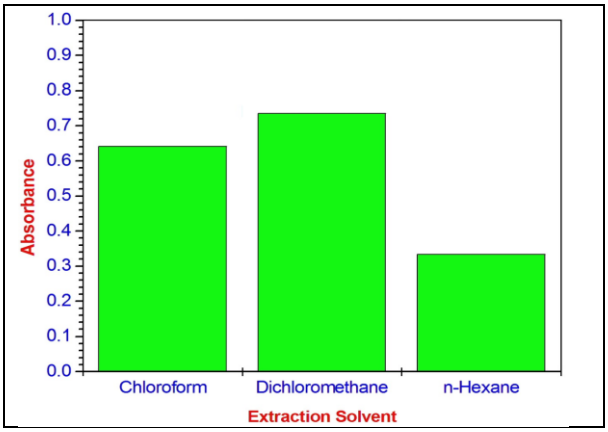

Figure 6. Effect of different extraction solvents on the absorbance of atorvastatin (6µg/mL) with pararosaniline at λ max 547 nm.

LOD and LOQ

Limit of detection (LOD) and limit of quantitation (LOQ) are the lowest concentrations of the analyte that can be readily detected and quantified respectively by the proposed analytical method[29]. LOD and LOQ were calculated based on the residual standard deviation of the regression line and were found to be 0.31 and 0.93 µg/mL respectively

Table 1. Validation parameters for determination of atorvastatin by pararosaniline reagent method.

Linearity range	1-8 µg/mL
Slope	0.129
Intercept	-0.014
Correlation coefficient	0.999
r^2	0.999
S.E of slope	1.973×10^{-3}
Confidence limit of slope	$0.129 \pm 5.05 \times 10^{-3}$
S.E of intercept	9.285×10^{-3}
Confidence limit of intercept	-0.014 ± 0.024
Residual S.D ($S_{y/x}$)	0.012
LOD	0.31 µg/mL
LOQ	0.93 µg/mL
Accuracy	99.36 ± 0.92 (%)
Intraday precision(%RSD)	0.66%
Inter-day precision(%RSD)	0.83%

Accuracy and precision

Accuracy of the method was evaluated analyzing five concentrations from the working standard solution within the linearity range of the drug, each three times. The method was found to have acceptableaccuracy (**Table 1**). The results of the proposed method were compared with those of the USP method[24]. It was found that there were no significant differences in their accuracy and precision as indicated by student's t-test and F-test results respectively (**Table 2**). Evaluation of the intraday precision was made by replicate assay of three concentrations of the working standard solution of the drug within the linearity range on the same day, while the inter-day precision was evaluated through the replicate assay of three concentrations of the working standard solution of the drug within the linearity range on three successive days. The method showed satisfactory precision (**Table 1**).

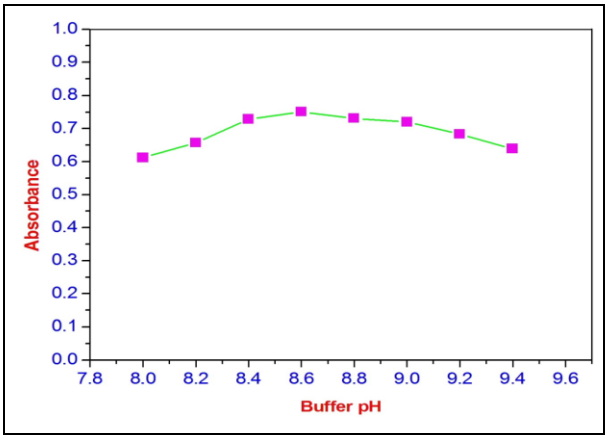

Figure 7.Effect of the pH of the borate buffer on the absorbance of atorvastatin (6 µg/mL) with pararosaniline at λ max 547 nm.

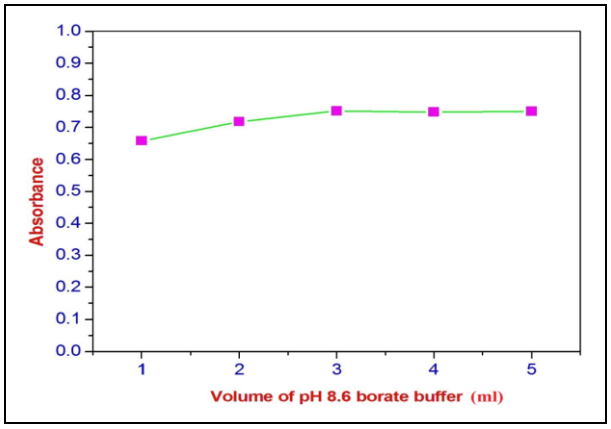

Figure 8. Effect of the volume of borate buffer pH 8.6 on the absorbance of atorvastatin (6 µg/mL) with pararosaniline at λ max 547 nm.

Table 2. Statistical comparison of the results of the pararosaniline reagent method with those of the USP method for the analysis of atorvastatin in pure form.

Item	Proposed	USP method*
Mean accuracy ± SD	99.36±0.92	99.74±0.73
%RSD	0.93%	0.73%
%RSE	0.41%	0.33%
N	5	5
Variance	0.85	0.53
t- test (2.31)	0.71	
F- test (6.39)	1.60	

Values in parenthesis represent the tabulated values of t and F at p = 0.05[30].

**HPLC method and was performed using C8 Column. Programmed elution was performed using two solutions, solution A: acetonitrile, tetrahydrofuran and pH 5 ammonium acetate buffer (21:12:67) and solution B: acetonitrile, tetrahydrofuran and pH 5 ammonium acetate buffer (61:12:27). The detection was carried out at 244 nm.*

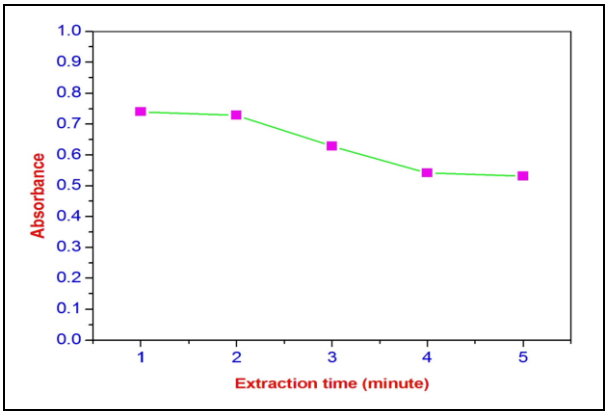

Figure 9. Effect of the extraction time on the absorbance of atorvastatin (6µg/mL) with pararosaniline at λ max 547 nm.

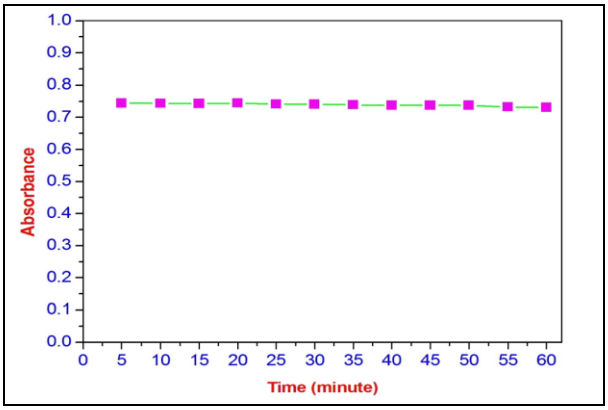

Figure 10. Effect of time on the stability of the absorbance of atorvastatin (6 µg/mL) with pararosaniline at λ max 547 nm.

Table 3. Assay of atorvastatin it its tablets by pararosaniline reagent method using standard addition technique.

Item	Taken concentration (µg/mL)	Added concentration (µg/mL)	Recovery percentage *
	2	1	98.30%
	2	2	98.30%
	2	3	100.37%
	2	4	98.90%
	2	5	99.40%
Mean ± SD	97.02±0.61		99.05±0.87
% RSD	0.63%		0.88%
% RSE	0.36%		0.39%
Variance	0.37		0.76

* *Average of three different determinations.*

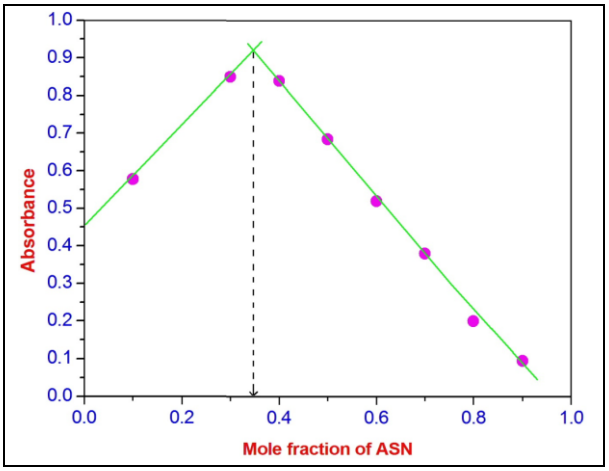

Figure 11. Stoichiometry of the reaction of atorvastatin and pararosaniline by Job's method.

Method application to pharmaceutical dosage form

The proposed spectrophotometric method was applied easily to the assay of ASN in Ator 10 mg. ASN concentration was calculated using the computed regression equation. Furthermore, standard addition technique was applied in order to validate method application on pharmaceutical dosage form (**Table 3**).

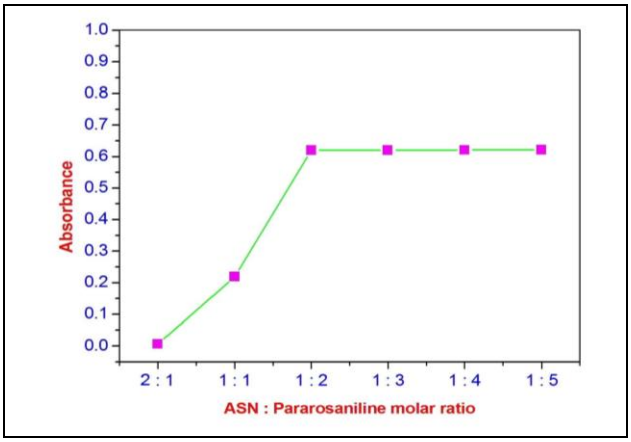

Figure 12.Stoichiometry of the reaction of atorvastatin and pararosaniline by Molar ratio method.

CONCLUSION

A sensitive spectrophotometric method has been developed for determination of ASN in pharmaceutical preparation based on a novel idea that to our knowledge was not reported before for the determination of ASN. This idea was the utilization of the anionic carboxylic moiety to combine ionically with a basic cationic dye.

Despite being cheap and simple, the method successfully quantitated ASN in a fair low concentration what proves its advantage of being sensitive. This method was proved to be easy and successful in the analysis of ASN in bulk powder and pharmaceutical dosage from.

Acknowledgement

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Conflict of Interest

The authors declare that they don't have any conflict of interest.

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