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Development of method and validation for Assay in Cyclizine Hydrochloride Injection by Applying Stability Indicating HPLC Methodology by HPLC

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ABSTRACT

A validated HPLC method is developed to determine Cyclizine Hydrochloride (CYHC) in pharmaceutical formulation. Isocratic elution at a flow rate of 1.0 ml/min was employed on C18 5 µm (150 x 4.6 mm), 4µm or equivalent column, or similar is used for this chromatography analysis. Ammonimum acetate, acetonitrile, Glacial acetic acid, and triethylamine are used as a buffer solution. Mobile phase is 10mM ammonium acetate as 64v/v, acetonitrile as 35v/v, and triethylamine as 1v/v. The UV detection wavelength was 254nm, and 20.0µl sample was injected. The run time is 55 minutes for diluted standard and 30 minutes for sample, blank, placebo, system suitability, sensitivity solution. The retention time is ± 2.1min. The% R.S.D CYHC is identified. The mean Percentage recovery for Irinotecan is found within the specification limit. Thus, the proposed HPLC method can be successfully applied for routine quality control analysis formulations. The method developed is simple and is better than the methods reported in the literature.

Key words: RP-HPLC Refractive index detector, CYHC, flow rate, column, ICH Guidelines, USP reference.

Introduction

The molecular Formula for Cyclizine Hydrochloride (CYHC) is $C_{10}H_{16}ClNO$. This CYHC is a medication utilized for treating as well as preventing problems like nausea, vomiting as week as dizziness can be obtained as a result of neither motion sickness nor vertigo[2]. This CYHC should also be utilized for nausea latter common anesthesia because whatever is developed by opioid utilization.[1][2] This medicine is taken as oral i.e., by mouth, in rectum, nor passed as an injection into the body as a vein. [3] This CYHC consists of minor side problems which includes effecting sleepiness, mouth converting into dry, constipation, as well as trouble with vision.[4]

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Another name containing medicine, Diconal is in associated with cyclizine along with the opioid dipipanone.[5] This dipipanone was treated as a schedule I controlled substance/compound in the country US.[6] This drug is generally appearing as magazine hydrochloride, also initiated for selling in US under brand name Marezine. Selling began in France as Marzine in 1965. [7][8] Maha Mohamed Abdelrahman et.al.,[9] have described CYZ based on routes like oral or intravenous, to euphoric or hallucinatory variations. Neven M Habib et al. [10] proposed TLC and performed chromatographic separation by silica gel 60 F254 utilizing a developing system containing methylene chloride 7.0v/v acetone 1.0v/v methanol 0.50v/v scanning, which divided at 220nm. 1.00mL/min is the rate of flow—wavelength as 220nm. The linear relationship obtained among peak area of integrated as well as strength as 10µg/mL -50µg/mL to PYH, 10-50µg/mL to CYH, and 7-50µg/mL to MEH. GislaineKuminek Hellen K et al., [11] developed with the help of C18 column with methanol 80v/v water 20v/v at a pH range of 2.6 as mobile phase. The authors identified rate of flow s of 1.00 mL/min. Wavelength is identified at 230 nm. Amina M. Abass et al.,[12] characterized sensors revealed Nernstian gradients of 59.61 ± 0.50, 57.71 ± 0.23, 53.01 ± 0.14, also 53.20 ± 0.35 mV per decade by pH range as 2.5–5.5 in DBPH along with 3.5–5.0 in *O*-NPOE electrodes, and 4.0–5.5 to DOPH as well as DBP plasticized film-based sensors.

EXPERIMENTAL

LC 20AT pump and UV-Visible detector with flexible wavelength program and injector named Rheodyne utilized in a present developmental method by the author. C18 5 µm (150mm x 4.6mm) or equivalent was used for this chromatography analysis. With the help of Loba ultrasonic bath sonicator mobile phase containing the gas was separated. The common balance to measure the drugs is employed. From the local market sample, CYHC is procured. In this present method Ammonium acetate, Acetonitrile, triethylamine, acetic acid, Trifluoroacetic acid, and O-phosphoric acid were used as AR grade; Acetonitrile and water collected as HPLC grade are procured and utilized in this process by the author. Exactly 10.00g ammonium acetate is transferred in a 100.00mL standard flask. To this, 50.00mL Acetonitrile is added after that is subjected to dissolution. Again, for this Solution, 10.00mL glacial acetic acid and 2.00mL of triethylamine is added. After that subjected to dilution to a suitable volume by the required diluent. The mobile phase is prepared by dissolving 10mM ammonium acetate as 64v/v, Acetonitrile as 35v/v and triethylamine as 1v/v. Whose pH is adjusted to 3.7 by using approx. 90.00ml acetic acid per liter. 10 mM Ammonium Acetate was prepared by taking 0.77gm Ammonium Acetate is dissolved in suitable solvent latter, it is diluted up to mark in 1000ml of water. Standard Solution is prepared by taking 80mg CYHC standard into a 100ml classic flask. Dissolve in and makeup to suitable volume by suitable solvent. It is finally filtered by 0.45µm filter, discarding the first 5.00ml filtrate. A sample solution is prepared by taking 20 tablets after determining the mass average. Grinded these 20 tablets until the fine powder was obtained. Latter weighed 200mg of powdered sample in 100.00ml standard flask. Added 80.00ml solvent after that sonicated to a time interval of 5 minutes. In this case, the precaution is taken as the product doesn't allow get warm. It diluted the volume with a suitable solvent and mixed thoroughly. It was filtered the same by 0.45µm filter paper and discarded the first 5ml of filtrate.

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METHOD DEVELOPMENT

To 10ppm solution of CYHC with the help of UV spectrophotometer spectrum in Acetonitrile is recorded individually. 254nm is the maximum absorbance wavelength. Maximum absorbance showed spectra for CYHC. Essential dissolution and peak performance were identified with the help of C18 5 µm (150mm x 4.6mm), 4µm, or equivalent columns. 10mM ammonium acetate as 64v/v, Acetonitrile as 35v/v with triethylamine as 1v/v was consumed as eluent. To identify optimum separation, measuring reaction course between 0.50mL/min – 1.50mL/min is enforced. Using a reaction 1.00mL/min rate of flow is adequate for the analyte separation as successful.

Proposed Method Validation and requirements

System Suitability

% RSD of peak responses as per CYHC to six replicate injections is 2.0 %. The tailing factor for the peak is mainly CYHC should not be more than that 2.0. The theoretical plate count is less than 2000. A total of six replicate injections to working standard solution were passed as per the analysis method. % RSD to peak responses is measured. Analytical system complies with requirements that are specified by system suitability. System suitability results are tabulated in Table 1.

Sample	CYHC Area	CYHC Tailing	CYHC Tangent
1	1228700	0.9	5552
2	1225786	0.9	5583
3	1224860	0.9	5574
4	1224390	0.9	5581
5	1221977	0.9	5609
6	1223832	0.9	5589
Mean	1223754	0.9	5581
% RSD	0.2		

Table 1:system suitability results

Specificity

Carried out this experiment by passing Blank injecting Blank as the diluent, placebo preparation, diluted standard solution, Monohydroxy, Dihydroxy, isopropyl ester, the dimer of CYHC, prepared sample, spiked sample by known foreign substances which documented retention times are as different. Peaks of blank as diluents, impurity, and Placebo should not interfere with the peak of CYHC. Specificity criteria is performed by introducing Blank as the diluent, preparation of Placebo, regular solution, Monohydroxy CYHC, Dihydroxy CYHC, CYHC isopropyl ester, CYHC Dimer, Sample, sample which is spiked by known foreign substances in the chromatographic system and recorded retention times. From the below results, it can be concluded that there is no retardation

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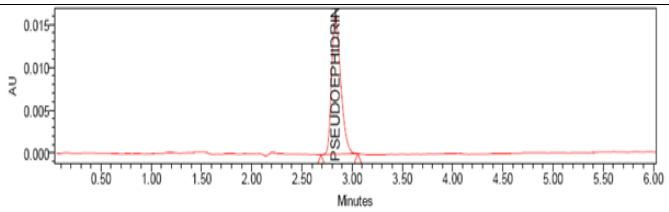
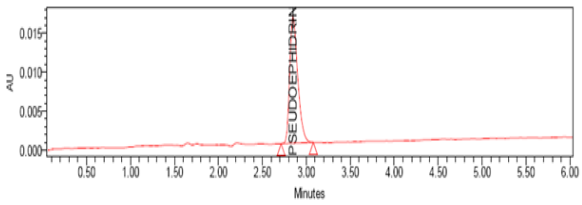
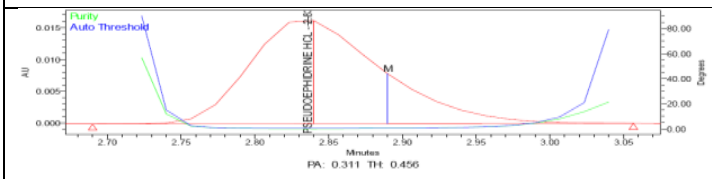
of other peaks of placebo & impurity peaks, including the CYHC peak. Chromatograms to solvent, drug active, product, Drug Active andproduct UV-stressed, and placebo peaks are denoted in Figure 1.



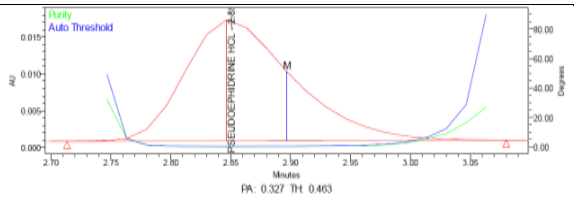
Drug active – Peak due to Cyclizine HCl



Product – UV stress: Peak due to Cyclizine HCl



Drug active Purity angle < Threshold = 0.311< 0.456



Purity angle < Threshold = 0.285 < 0.42

Figure:1

STRESSED CONDITION STUDIES:

A Stressed condition of CYHC injection should be performed to finalize either information of stability through its shelf life or any non-persistent compound that may be assessed that should not combine with the help of peak obtained by injecting CYHC. Along with the above, a stressed condition study should be helpful to know high about degradation type pathways other than different parameters. These include oxidants, alkali solution, acidic, neutral, heat, or in the presence of light to every degradant. For these studies authors are used Preparedness of Sample, Preparedness of Placebo, Acid Stressed sample (1.0N HCl), Alkali Stressed sample (1.0N NaOH), 3.0% w/v Peroxides of Hydrogen Stressed (3.0%w/v H2O2), Neutral Stressed, UV light exposed, Photo stability exposed, Sunlight exposed, Thermal Stressed (Dry heat) sample, Alkali Stressed sample (1.0N NaOH) at 25°C for 5 minutes, Neutral Stressed sample for 2 hours. Sample is found to be degrading in both circumstances of Alkali as well as neutral conditions. They were slightly degrading in Hydrogen peroxide, state of sunlight. No other degradation is identified in various conditions like Photo stability, acidic, thermal and UV light stressed conditions. Anyhow, unknown foreign substances, related substances and degradation foreign substances are scattered by CYHC peak. CYHC peak is purer, which is finalized by Chrome Leon software. Hence these related substance circumstances are completed as product release ad shelf-life.

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Intermediate Precision

% RSD due toCYHCconcentration to six samples are 2.0 %. Mean results obtained in the repeatability; also, intermediate precision must not vary by more than 3.0%. %RSD of impurities 0.05% latter above total 6 measurements were NMT 15.0%RSD of total impurities to total 6 measurements is NMT 10.%RSD of e related compounds 0.05% as well as above to12 preparation NMT is 15.0. %RSD to total impurities is NMT 10.0. Therefore, finally obtained results by this procedure is rugged.% RSD for intermediate precision is 1.5%. Intermediate precision as well as repeatability comply as they differ by 2.0 %. Finally obtained values are tabulated in table: 4.3.% RSD due toCYHCstrengthto total six samples must be less than or equal to 2.0%. Six separate sample preparations of batch 240598 are analyzed according to the method of analysis. The % RSD due toCYHCstrengthto assay meets requirements to reproducibility as 0.7 % respectively. Finally, obtained values are tabulated in the table: 2.

Equipment number	LC 5
HPLC column	C18 5 µm (4.6 x 150 mm)
Sample	Results (mg/tab)
	Cyclizine Hydrochloride
1	47.6
2	48.5
3	48.5
4	48.7
5	49.5
6	49.6
Mean	48.7
% RSD	1.5
Sample	Mean Results (mg/tab)
	CYHC
Repeatability	50.1
Intermediate Precision	48.7
Mean	49.4
% RSD	2.0

Table 2: Intermediate precision Results

LINEARITY:

The correlation coefficient of the regression line to CYHC is within range, i.e., 0.99. The Y-intercept of the line should not be significantly different from zero, i.e., z falls within specified limits only when +2 > z > -2. Total five solutions containing 50, 75, 100, 125, and 150 % of CYHC, relative to working strengths of 0.8000 mg/ml, are prepared later passed as per rules and regulations in the analysis method. A linear regression curve was constructed, and finally, R2and assessment values were measured. R^2 for CYHC is 1.000. The obtained plot is a straight line shown in figure 2.

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Noted z is 0 for CYHC.

CALIBRATION CURVE: y = Bx + A, R^2 = coeff. of determination

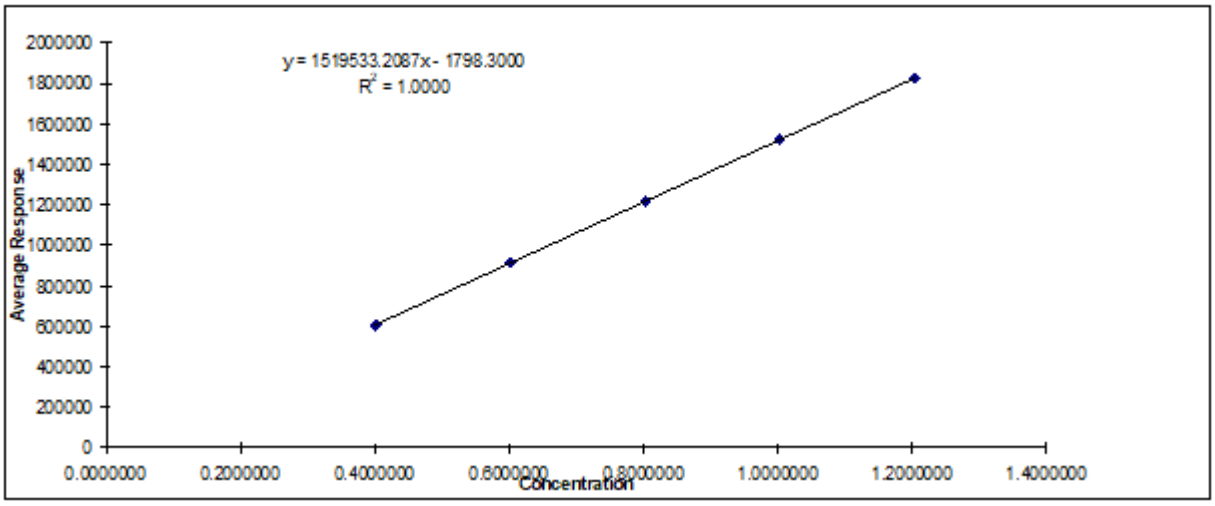

Figure2: Linearity Graph for CYHC

ACCURACY

A spiked known quantity of Monohydroxy CYHC, standard in a sample. Spike known quantity of CYHC into placebo at specification level of unknown foreign substances. The percentage recovery of active compounds/substances for each solution prepared is 98.0 – 102.0 % of the actual amount. Sample solutions are spiked by known strengths of CYHC to result in strength of 0.4000mg/ml, 0.6000mg/ml, 0.8000mg/ml, 1.0000mg/ml, and 1.2000mg/ml representing respectively 50, 75, 100, 125, as well as 150 % of CYHC relative to working strength as 0.8000mg/ml. These samples were passed in duplicate according to the method of analysis. By accuracy results below, percentage recovery values to CYHC satisfy acceptance criteria to accuracy across 50 % - 150 %. % of individual and mean recovery at every stage is 95 to 105 to the known foreign substance to Monohydroxy CYHC. % individual and mean recovery at every stage varied between 80 to 120 to Known impurity. % individual and mean recovery at every stage varied between 80 to 120 for Unknown foreign substances. Results were represented in table 3.

Sample %	Theoretical	Actual	% Recovery	Average % Recovery
50	39.39	38.74	98.3	98.2
50		38.62	98.0	
75	59.09	58.50	99.0	99.1
75		58.60	99.2	

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100	78.78	78.51	99.7	99.7
100		78.49	99.6	
125	98.48	98.91	100.4	100.4
125		98.81	100.3	
150	118.17	118.03	99.9	100.0
150		118.31	100.1	

Table 3: Accuracy results for CYHC

RANGE:

Derived specified range from linearity and accuracy studies. %RSD obtained for all accuracy range measurement is NMT 10.0. Correlation and regression coefficient is NLT 0.995 to Linearity as well as accuracy range parameter. With the help of obtained values finally,concluded this method proposed is varying LOQ Level as 300% of target strength to monohydroxy CYHC, dihydroxy CYHC, Isopropyl ester as well asCYHC. Range of method is varying LOQ to 400% of target strength to CYHC dimer.Based on accuracy values, range to assay of used Emitex tablets is 25 – 75mg/tab of CYHC, this denoted 50 % - 150 % of working strength. From these obtained values are represented in Table: 4&5 and graphs obtained are represented in Figure 3.

Level (in %)	**Peak area counts from Linearity**
50	964332.559
100	2004482.159
200	4273985.563
300	6444178.663
Coefficient of Correlation	**1.000**
Coefficient of Regression	**1.000**

Table 4: Coefficient of correlation and Regression

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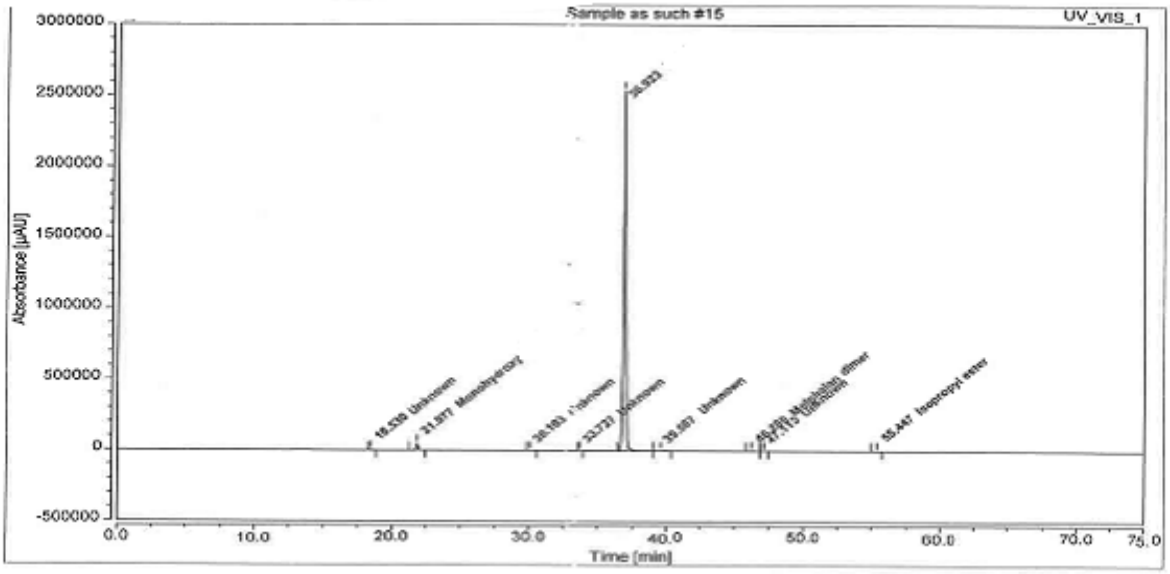

Figure 3: Chromatogram for CYHC

S.No	Name of Peak	Rt. In min.	Area µAU*Sec	Height µAU	Area %	Match Factor	Purity Limit	Results
1	Unknown	17.690	33806.555	3587	0.11	1000	990	Pure
2	Monohydrate CYHC	19.788	40439.496	47631	1.31	1000	990	Pure
3	Unknown	29.201	8169.863	749	0.03	990	990	Impure
4	Unknown	37.633	3927.433	480	0.01	998	990	Pure
5	CYHC	41.896	30270960.812	2537533	99.11	1000	990	Pure
6	Unknown	40.600	33059.100	919	0.22	995	990	Pure
7	CYHC dimer	49.300	48421.804	3499	0.09	999	990	Pure
8	Unknown	48.009	6678.950	511	0.05	982	990	Impure
9	Isopropyl ester	55.399	15947.630	1262	0.07	997	990	Pure
Total					100.00			

Table 5: Results of purity

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Robustness:

This parameter Robustness is an analytical procedure by a tiny portion of capability for remain those are not affected by very minute also calculates differentiation in this method parameters also provides a note of its reliability course of regular utilization. We changed the oven temperature to ±5°C. System suitability criteria compulsory obey total experimental processes. Total known impurities were separated by each other, and impurities spiked a peak of CYHC in the sample. This process is finalized as robust with minimal variation in process parameters. The Robustness obtained values were represented in table 6.

			Theoretical Plates NLT 2000	**Tailing factor is 2.0**	**%RSD is 5.0**
	Actual parameters		278389	1.13	0.6
	Abnormality in flow rate	-0.2 ml/min	298988	1.14	0.6
		+0.2 ml/min	264186	1.09	0.7
	Temperature	-5°C	254612	1.20	0.6
		+5°C	285999	1.11	0.6
	Wavelength	-5nm	258939	1.19	2.7
		+5nm	258988	1.29	1.2
	Difference in organic solvent as Methanol	+2%	296782	1.09	3.7
		-2%	296028	1.09	1.1
	Different in organic solvent as Acetonitrile	+2%	267958	1.22	0.4
		-2%	267982	1.22	0.4

Table 6: Robustness Results for CYHC

RESULTS AND DISCUSSION

For this procedure author has used mobile phase as 10 mM ammonium acetate as 64v/v acetonitrile as 35v/v along with triethylamine as 1v/v for this pH is adjusted as 3.7 with approx.90 ml acetic acid per liter gradient elution with 1.00ml/min flow rate is with peak shape. The column used in this measurement is C18 5 µm (150 x 4.6 mm). Run time is 55 minutes for diluted standard and 30 minutes for sample, blank, placebo, system suitability, and sensitivity solution. % RSD of the peak responses as per CYHC to six replicate injections should be less than nor equal to 2.0 %. The tailing factor for the peak is mainly CYHC should not be more than 2.0. The theoretical plate count should not be less than 2000. No components were identified to co-elute with the CYHC peak, and the peak purity results indicate that the CYHC peak can therefore be considered spectrally pure. %RSD of impurities 0.05% latter above six measurements are NMT 15.0. %RSD of total impurities to the total of 6 measurements is NMT 10. Impurity is more minor than LOQ, which may not be considered an

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entirely foreign substance. Therefore, the final results obtained by this procedure are rugged. % RSD for intermediate precision is 1.5%. % RSD due to CYHC strength to assay meets requirements to reproducibility as 0.7 % respectively. A linear regression curve is constructed. By accuracy results below, percentage recovery values to CYHC satisfied acceptance criteria to accuracy across 50 % - 150 %. % of individual and mean recovery at every stage lie as 95 to 105 to known impurity to Monohydroxy CYHC. % individual and mean recovery at each stage varied between 80 to 120 to Known contaminant. The correlation and regression coefficient are NLT 0.995 for Linearity and accuracy range parameters. This proposed method is linear in the specified range for CYHC and its related foreign substances. Accuracy, LOD & LOQ of this method is established to CYHC for 50mg/ml injection. Hence, by using this process, it is concluded that this method stands validated and may be utilized in routine with stability analysis.

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Conflicts of interest:
There are no conflicts of interest among the authors who were done this present work.

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