**REVIEW 453-1339-1-RV**

The work is novel and presents important contributions in terms of the calculation of % inhibition of angiotensin converting enzyme (ACE) with the use of several new compounds grouped as LQM300 to be compared with the effectiveness found with Captopril to reduce high blood pressure. However, it is recommended to complete the calculations with the detailed description of the use of the areas obtained from the HA with each inhibitor to obtain HA concentrations. The authors indicated that the measurements were made in triplicate, but they do not show the average with their dispersion. This weakens the discussion of the differences between the new inhibitors studied with the known Captopril, since it cannot be demonstrated if the differences are significant. It is suggested to strengthen the analysis of results with better statistical management.

The authors do not comply with several format conditions and other details defined in the submission Checklist. No graphical abstract was received, nor figures in files separately.

On the other hand, even though, the authors indicate that they have used the services of a professional translator in the section of acknowledgments, the document is not written clearly and simply. It is urgent to improve the grammar and syntax. I suggest that the manuscript be reviewed and corrected by a native speaker with knowledge of chemistry.

List of suggestions and comments in balloons of the manuscript:

**Title, authors and adscription**

I suggest 2 possible best titles for this job:

1) Evaluation of new thiomorpholine compounds as inhibitors of angiotensin-converting enzyme by capillary zone electrophoresis

2) Evaluation of the inhibition of angiotensin-converting enzyme by new thiomorpholine compounds using capillary zone electrophoresis.

Done, Selected the second tittle for this paper.

The manuscript has many drafting errors. This makes some paragraphs difficult to understand. I suggest that the article should be corrected by a native speaker.

The surname should be "Valadez" Corrected

Add point to abbreviated surnames missing points added

It should be "S.A. de C.V." instead "SA de CV" missing points added

Use lowercase letters for "s/n". Corrected

it should be "María" Corrected

Use: Estado de México Corrected

**Abstract in English and Spanish**

Captopril is the name of an active ingredient and is not a commercial name, so it cannot be registered. Correct this throughout the document Corrected through all the document

Could it be:?

The study was done using the technique of capillary electrophoresis with an in-line reaction using hippuryl-histidyl-leucine (HHL) as substrate to produce hippuric acid, which was detected at 254 nm Added this fragment to the document

Are you referring to the detection wavelength? specify it. Specified in both paragraphs.

The wording is extremely confusing. Clarify the ideas you want to express here Modified and simplified

Please, use the same abbreviation than in the English version Corrected

Please, use the same abbreviation than in the English version Corrected

I suggest:

“…en línea utilizando una longitud de onda de detección de 254 nm” Added this fragment to the document

The sentences you want to express are very confusing (even in Spanish). I suggest you complete and clarify each idea you want to express. Modified and simplified

The authors do not provide the graphical abstract, please add it. The indications for its elaboration can be seen in the Journal web page, in section: Submission Checklist.Added

**Introduction**

The body of the manuscript should be inserted in simple format, thus, avoid using double columns. See: Submission Checklist. Corrected

Eliminate additional space Corrected

I suggest using: "are" Corrected

I suggest using: "diagnosis" Corrected

techniques Corrected

spectrophotometry, fluorometry or radioisotopic methods Corrected

Clarify the idea Corrected

Enzymatic reaction of what? Be more precise and specify whether the references apply to which substrate for the activity of which enzyme. Corrected

I propose to write as follows:

The quantification of ACE activity by capillary zone electrophoresis has been reported [3], and the in-line reaction conditions are well established, where ACE activity is quantified using hippuryl histidyl leucine (HHL) as tripeptide substrate. Added this fragment to the document

Eliminate "at length of" and replace for: “with a UV detector at 254 nm” Corrected

Eliminate: “with the following reaction” Corrected

Eliminate additional space Corrected

I suggest:

…short analysis time and using small sample volumes (usually nL). Added this fragment to the document

The wording of this paragraph is too complicated. Simplify it please. Or

As the paragraph is not strictly necessary to understand the rest of the manuscript, it is better to eliminate it.

makes Removed

Please, as far as possible, try to write clearly and simply. Corrected

The idea is confused and repeated before. Corrected

Repetitive Corrected

Use the appropriate reference format. See: Submission Checklist. Corrected

Repetitive words. Use synonyms Corrected

Eliminate additional space Corrected

I suggest: "molecular docking" Corrected

I suggest: "its" used as possessive adjective Corrected

Abbreviation not yet defined Corrected

Repetitive idea and already raised in P1 (Introduction) L38 to L48. Indicate in what differs or eliminates its use. Modified

I suggest: "molecular docking" Removed

Repetitive ideas and already expressed in P1: L38 to L48 and P2: L12 to L15 and L19 to L27 Removed

I suggest using only the abbreviations of the compounds, already defined before. Corrected

**Experimental**

Use only abbreviation Corrected

The percentage symbol must be separated with space from the number that precedes it. Corrected

Add a space Corrected

Please add a space between the number and its units Corrected

Please add a space Corrected

Please add a space Corrected

Very repetitive phrase. I suggest use of synonyms Corrected

Correct name "watt" or use the "W" symbol of S.I. Corrected

**Instrumentation of CE**

Add: Fullerton, CA, USA Corrected

P/ACE MDQ software (version 1.5) Corrected

USA Corrected

**CE Conditions and solutions**

Add a space following the criteria S.I. Corrected

It must be "Milli-Q®"or Type 1 water. Corrected

Type 1 water (or deionized water) means: resistivity of typically 18.2 MΩ•cm at 25 °C. Corrected

It must be "Milli-Q® water" Corrected

Add a space Corrected

USA Corrected

Be consistent The name of the company and the country has been written in parentheses. Corrected

Modify: "to pH 8.0" Corrected

Add a space Corrected

I suggest "0.1 M NaOH" Corrected

It must be "psi" Corrected

Add a space Corrected

I suggest "0.1 M NaOH" Corrected

 "0.1 M NaOH" Corrected

I suggest: "with water for 2 min" Corrected

Eliminate additional space Corrected

Explain clearly what hydrodynamic effect refers removed from the text

Change to: "ACE of rabbit lung" Corrected

Add a space between the number and its units Corrected

Add a space Corrected

Add a space Corrected

Eliminate the additional space Corrected

**Calibration curve of HA in different concentrations**

Replace "in" for "at” Corrected

The number must be sub-index Corrected

Decide if the name of the company, city and country will be written in parentheses. If the country will only be written in parentheses, then homogenize this throughout the manuscript Corrected

Add a space Corrected

**In-line Reaction**

Should be: “psi” Corrected

psi Corrected

Add a space Corrected

Use in lower case

In my opinion Fig. 3 and Table 1 contain similar information Figure 3 eliminated and substituted by another image

Add a space Corrected

Add a space Corrected

Improve your writing and syntax Corrected

Add a space Corrected

I suggest: “”with 10 mM Buffer HEPES, pH 8.0” Corrected

I suggest: “150 mM NaCl” Corrected

Remove registered trademark Corrected

The number must be sub-index Corrected

Add a space Corrected

**Calibration for the HA concentration** Corrected and eliminated because the information is duplicated

Add a space

Space

I suggest: “”with 10 mM Buffer HEPES, pH 8.0 + 150 mM NaCl”

Add a space

“to a pH 8.0”

This information is the same in sections 2.4 and 2.6. Eliminate duplicities.

**Results and discussion**

**In-line reaction through capillary electrophoresis.**

I suggest:

…”to the HHL with the procedure reported previously [3].” Corrected

Remove this text that only causes confusion. Corrected

Use the appropriate reference format. See: Submission Checklist. Corrected

Use brackets, not parentheses Corrected

Add a space Corrected

This paragraph is not understandable. Please, improve the writing. Corrected and simplified

Clarify this idea by improving the wording Corrected

Add point. Corrected

Add a space Corrected

**Calibration Curve of HA**

Add a figure of the calibration curve of HA (measured in triplicate) indicating the equation obtained.

Clarify which property you used as proportional to the concentration of HA Corrected and specified that the AUC was used to measure the HA concentration.

Repetitive information I suggest: The calibration curve was measured as described in the experimental section 2.4 Corrected

Take care of your writing and write simple ideas. You want to say this ?: Corrected and modified

Due to the low solubility of the inhibitors in water, they were dissolved in a 50:50 water / DMSO mixture. It should be mentioned that under these conditions the mixture is stable only on the same day so it must be prepared fresh.

Accuracy is not measured with %CV. Maybe, you want to talk about data precision. Corrected and clarified that we talk about precision

Based on which guide does you indicate that a% CV of 6% is acceptable? Didn’t mean that, the complete idea is now specified in the document in section 3.2

Refer this in the manuscript.

As I can see, you did not use the migration time as a parameter of your measurements. It seems that you used corrected migration times. Explain this in the manuscript. Apparently, you selected water as a neutral marker (Table 2). Indicate if this is true and explain why you selected it, if DMSO gives a positive signal at 254 nm and can be used as a neutral marker. We can use both (Water and DMSO), because both compounds move along with the electroosmotic flow. Independently the chemical molecular size, the EOF moves at the same velocity all neutral compounds. In conclusion the EOF marker could be both, the water and DMSO

You do not show migration times in the electropherogram, but signals (peaks) of compounds. Corrected and modified

Add a space Corrected

Add a space Corrected

Indicate the concentration of ACE used. Corrected

Eliminate the additional space Corrected

I suggest not to modify the electropherograms and present them as they were measured. The electropherograms were not modified, they were only modified on resolution due the lack of clarity from the software.

The correct term for "r2" is coefficient of determination Corrected

**Comparison of different inhibitors**

Eliminate the additional space Corrected

you should talk about: "the calibration curve", not "the process". Corrected

Eliminate trademark symbol Corrected

Add a space Corrected

Indicate what is the HA concentration in the blank. Corrected

Explain how did you calculate the HA concentrations in the experiments with each inhibitor. Corrected

At this point did you use the calibration curve of HA to interpolate HA areas with each inhibitor? You must carefully explain this to the reader. Corrected and specified in the text

Eliminate trademark symbol Corrected

Please round to 2 decimal places Corrected

Add a space Corrected

Indicate the concentration of ACE and LQM used. Corrected

Add a space Corrected

I suggest not to modify the electropherograms and present them as they were measured. The electropherograms were not modified, they were only modified on resolution due the lack of clarity from the software.

You shows signals (or peaks), not migration times.

**Electrophoretic Mobility**

Mobility does not depend on the applied voltage.

 Cheked, M, the electrophoretic mobility of a particle population (or subpopulation), follows from the translation rate compared with the translation rate of a neutral dopant, which moves from the inlet past the UV detector at the electroosmotic velocity of the buffer M = (LtotLd/V) (1/tp − 1/tref), where Ld is the distance from the inlet reservoir to the detector, V is the voltage difference applied across the length of the capillary (Ltot), and tref and tp are the mean migration times of the reference marker and the particle population (or subpopulation), respectively.

Capillary Electrophoresis Measurements of Electrophoretic Mobility for Colloidal Particles of Biological Interest

J. R. Glynn, Jr., B. M. Belongia, R. G. Arnold, K. L. Ogden, and J. C. Baygents

Remove P6, L13 Corrected

There is no power source that can apply an electric field. What is applied is a potential difference between 2 electrodes that are separated by a conductive medium (buffer), in the solution is where the electric field is generated. Agreed and Corrected

In your case you can not assume (Ltot) 2, since in your capillar L total is 30 cm and L effective is 20 cm. Its not an assumption, L tot is the complete length of the capillary and Ld or Leff is the distance from the injection point to the detector.

Very complicated syntax. I suggest:

…as the EOF, therefore, the negative peak of the water (due to its lower absorption compared with the buffer) was used as a neutral marker. Corrected

I suggest:

Effective mobilities of EOF and HA obtained with Ec. 2 y 3 are show in Table 3. Corrected

I suggest:

The effective migration times are shown to give an idea of the precision on the identification of the HA peaks. Corrected

Correct to:

 Effective mobility. Corrected

HA instead of AH Corrected

Please round to 2 decimal places. Corrected

possess a lower inhibition compared to Captopril Corrected

Eliminate trademark symbol Corrected

Eliminate trademark symbol Corrected

Compare the averages of% inhibition (hypothesis test), since it has measurements in triplicate. Conclude if there are significant differences or not.

Eliminate trademark symbol Corrected

Add a space Corrected

change the point to a comma Corrected

Add a space between the number and the percentage symbol. Corrected

Add a space Corrected

Eliminate Trademark symbol Corrected

This trend is not necessarily true since the LQM329 should then also have high% inhibition. The LQM329 also have two tiomorpholine rings and two hydroxyl groups, however this compound does not have those substituents at the same positions, this slight modification drastically modify the biological activity. This behavior could be labeled as an activity cliff.

**Concluding remarks**

This work does not present any validation Corrected

Eliminate trademark symbol Corrected

Add a space Corrected

Eliminate trademark symbol Corrected

Add a space Corrected