453-JMCS-2018 REFEREE B COMMENTS:

This is an interesting application of CE to study the inhibiting power of synthesized compounds against ACE activity; however, the script presents many shortcomings and the clarity of presentation is poor. The authors state that all compound used in this work had been included in patent application (Ref 33). The novel contribution as compared to the previous studies should be clearly defined. It should also be stated consistently throughout the text what was determined: ACE activity of ACE inhibition?? The text needs to be revised and modified to eliminate multiple repetitions. The authors often use the word “better”; however, it remains uncertain with what system or condition the comparison is made. The structure of English text and grammar needs to be improved. Some comments and suggestions and presented directly in the text.

The text was mainly corrected at the points where there was confusion about the ACE activity, we evaluated the inhibition potential of the proposed compounds through the direct measure of the ace activity to produce HA. The “better” word were modified and instead using the word an explanation are provided in the text.

Experimental: the details on synthesis and characterization of compounds LQM318, 319, 322, 328, 329 must be eliminated. As informed in the Introduction, these compounds were synthesized in 2007 and the authors cite their patent application (reference 33) Corrected and removed

Experimental: application of nylon filter for the samples is unacceptable. Nylon filters are used for filtration of mobile phases and certainly, regenerated cellulose filters would allow much better protein recovery. Only the HA, HHL, Buffer and LQM+HHL solutions were filtered through nylon filters. The enzyme was resuspended with type 1 water and used directly. Also the protein recovery is not the aim of the study. The aim is to quantify the HA produced from the enzymatic reaction.

Caption for Figure 3: please, provide the description for W(1), E(2), E(4), W(5) this figure was remover because it was repetitive with the info in the table 1.

Table 1: pressure instead of pression Corrected

3.2 Calibration Curve of HA: the calibration parameters are missing (you may consider to include a new table). In Figure 4, electropherograms for few calibration solutions should be given. Since the parameters are in 2.3 and the preparation of the solutions for the calibration curve are in 2.4, we did not mention them again, to avoid being repetitive. we only mention the section where are the detailed perocedure.

Figure 4, 5, 6: What was the migration time of the protein? Why the entire electropherograms are not presented? The quality of figures and the captions must be improved. We did not measured the protein’s migration time due three main reason: that was not the aim of the study, our scope was to measure the ACE activity resulting compound (HA). Second: the isoelectric point of the ACE is between 4.3 and 4.6 (Spontaneous change of human plasma angiotensin I converting enzyme isoelectric point, Joseph J. Lanzillo, Joan M. Stevens, John A Tumas, Barry L. Fanburg). Our working pH was 8.0, this means that the ACE is negatively charged. And taking into account this situation the HA measurements were performed at normal conditions with the CZE. This means that all the chemical species migrate to the anode thanks to the EOF. Third the normal wavelength used to detect proteins is 280nm and we worked at 254nm. In conclusion the migration time of the ACE must be too high and the analysis time must be too expensive. And since the aim is to quantify the HA; the calibration curve, the conditions for the enzymatic reactions are the main point in this study.

Table 2: the caption should be more specific, too many digits, why the mobility and effective migration time is not provided for ACE?? Even though the protein migration is not measured, its elution from the capillary must be observed. The elution of the protein is not necessary since the reports indicate that the elution order are Water, then HHL and HA, the main analite is the HA. once this compound elutes through the detector the ACE activity (aim of the study) can be measured.

Conclusions: The first sentence must be re-written Corrected and clarified and typical calibration parameters must be evaluated and provided. The calibration parameters ware modified an clarified in section 2.3 and 2.4.