OC19: Performance of a new HPLC-based method for 3-NT quantification in different biological matrices

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Introduction: 3-nitrotyrosine (3-NT) levels in biological samples have been associated with numerous physiological and pathological conditions. For this reason, throughout the last years, several attempts have been made in order to develop methods that accurately detect and quantify 3-NT in biological samples. In fact, a variety of works have been published on methods that allow the detection of 3-NT in several biological fluids and tissues. However, the accurate quantification of this molecule, which is present at very low concentrations both at physiological and pathological conditions, is always a complex task and a matter of concern.

Objectives: In order to overcome this problem, we aimed to evaluate the performance of an HPLC protocol for 3-NT quantification, previously developed and validated by our group, in a wide range of biological matrices.

Materials and Methods: An HPLC system and a RP-18 column were used. The biological matrices used were quality control samples (serum, whole blood, and urine), melanoma cell line B16, F-10 growth medium conditioned with the same cell line, gram-negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*) bacterial and yeast (*Saccharomyces cerevisiae*) suspensions. Selected samples were also spiked with three 3-NT concentration levels (50, 1000, and 25000 ng/mL).

Results and Discussion: The protocol used (0.5% CH_3COOH:MeOH (15:15:70) as the mobile phase, and detection at 356nm) allowed the successful detection and quantification of 3-NT in all biological matrices tested. Extraction efficiency was also calculated in serum, urine and whole blood samples, with mean recovery rates of 94.31±4.43%, 95.03±5.12% and 80.14±13.64%, respectively.

Conclusion: Unlike other previously described methods for 3-NT quantification, our HPLC-based method was successfully applied to a wide range of biological specimens, exhibiting a great performance in all of them.

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References

1. ICH Validation of analytical procedures: Text and Methodology Q2(r1) (2005).