

P41: Development of a new method for the detection and quantification of 3-nitrotyrosine

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Introduction: The nitration of Tyrosine residues in proteins is associated with nitrosative stress, resulting in the formation of 3-nitrotyrosine (3-NT). Different methods have been described for the quantification of this molecule. Regarding chromatographic methods, they seem to be very accurate, showing very good sensibility and specificity. Gas chromatography-based methods exhibit the highest sensibility, however a derivatization step prior analysis is required, which ends up being time-consuming for the analyst. Conversely, High Performance Liquid Chromatography (HPLC) may not require such derivatization.

Objectives: We aimed to develop a simple, rapid, low-cost and sensitive 3-NT quantification method for use in medical laboratories as an additional tool for diagnosis and/or treatment monitoring of a wide range of pathologies.

Materials and Methods: All experiments were performed on a HPLC system and separation as carried out using a RP-18 column. Throughout the experiments, the influence of the following parameters was evaluated: 1) mobile phase composition, 2) wavelength settings, 3) temperature, and 4) flow rate.

Results and Discussion: From all the protocols tested, the best results were obtained using 0.5% CH₃COOH:MeOH as the mobile phase, at wavelengths 215, 276 and 356 nm, at 25°C, and flow rate of 1mL/min. Using this protocol, the calibration curve was linear (correlation coefficient=1), the limit of detection and the limit of quantification were in the order of ng/mL, and the time required for analysis did not exceed 15 minutes *per* sample.

Conclusion: The proposed method, which was successfully developed and validated for 3-NT quantification, is simple, cheap and fast. It would be interesting to assess whether this method is suitable for the quantification of 3-NT in several biological specimens. References:

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References

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