V24. AMINO ACID ANALYSIS BY LC-MS/MS USING PRECOLUMN DERIVATIZATION WITH aTRAQ™ REAGENT: A PRELIMINARY STUDY.

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INTRODUCTION Brazilian meningococcal conjugate vaccine developed by Bio-Manguinhos initially involves a selective chemical activation in carrier protein aspartic (Asp) and glutamic (Glu) residues generating an hydrazide activated monomeric tetanus toxoid (MATT). To characterize the modified protein, liquid chromatography and multiple reaction monitoring mass spectrometry (LC-MRM-MS) method using a stable isotope-labeled internal standard has been proposed. This analytical process, consists of three steps: acid hydrolysis of the protein; isotopic labeling of amino acid released by hydrolysis and analysis by LC-MRMMS, using a scheduled MRM algorithm. Here we describe the analytical procedures required to amino acids quantitation using a standard amino acid solution (Asp/Glu) and myoglobin as models, to set up the experimental parameters.

OBJECTIVE Evaluate the feasibility of using amino acid analysis by LC–MS/MS and isotopic labelling aiming to further MATT structural characterization.

METHODOLOGY Myoglobin (0.01 mg) and standard amino acid solution: Asp/Glu (0.018 mg / 0.010 mg) were submitted to hydrolysis condition: 5.8 N HCl, 110°C, 24 h using Eldex H/D Workstation (Pico Tag®). Residual HCl was removed by freeze drying. Hydrolysis products and standard amino acid solution (non-submitted to hydrolysis condition) were labeled according to "Amino Acid Analysis for Physiological Samples" protocol (SCIEX). Labeled samples were analyzed by LC using following conditions: 2 μL injection volume; C18 column (4.6 i.d × 150 mm); 0.8 mL min- 1 flow rate and water/methanol gradient, both containing 0.1% formic and 0.01% heptafluorobutyric acids. Fractions were detected by API 3200 (SCIEX) mass spectrometer, using a schedule MRM acquisition method.

RESULTS Recoveries of Asp and Glu in solution nonsubjected to hydrolysis condition were > 80% for Asp and 100% for Glu. This same sample showed, when submitted to hydrolysis condition, only 10% recovery (Asp and Glu) – without freeze drying step (pH about 3.0). On the other hand, it was achieved 90% recovery for both amino acids with the additional freeze drying stage (pH about 5.0). Myoglobin hydrolysis efficiency was monitored by MALDI-TOF and the absence of peptide peaks in the range of m/z 300 – 24,000 was used as a reference to show complete hydrolysis of protein. Recovery of Asp and Glu in myoglobin was higher than 75%.

CONCLUSION Three freeze drying cycles were necessary for complete removal of HCl, consequently to improve the amino acid (Asp e Glu) labelling and recovery. By using myoglobin as protein model and taking into account the absence of peptide peaks (m/z ≥ 300) after hydrolysis, it was considered the amino acid analysis as satisfactory (recoveries > 75 % for Asp and Glu). However further improvement in this methodology must be performed to reach amino acids recoveries above 90 %. This is essential to begin the study related to MATT characterization.

KEYWORDS amino acid analysis, meningococcal conjugate vaccine, LC-MS / MS.