

TEAR PROTEOMIC PROFILING WITH MALDI-TOF MASS SPECTROMETRY COUPLED WITH MAGNETIC-BEADS HIC-18 SEPARATION AS A DIAGNOSTIC TOOL IN BLEPHARITIS DISEASE.

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The protein and peptide components in tear are reported as useful indicators of the ocular health state, on the basis of the important role of those compounds in preserving the integrity and stability of the ocular surface. Tear samples are also easily attainable and manipulated, making them good substrates for biomarker research. Coupled with Solid Phase Extraction (SPE) procedures, MALDI-TOF is a powerful technique for this kind of peptide profiling studies because of its high resolution, high mass accuracy, high sensitivity and the selective retention capacity of SPE. There are several approaches in the SPE selection, so in order to choose the most suitable SPE method, we have analyzed four SPE methods, all of them reverse-phase based: ZipTip (Millipore) C18, Empore® (3M) disks-based microcolumns (both C8 and C18) and Magnetic Beads HIC18 (Bruker Daltonics). According to the results obtained, Magnetic Beads are better suited for the subsequent tear profiling study, where we have analyzed the mass profile of 15 healthy (with no evidence of ocular disease) and 6 blepharitis diagnosed volunteers in the range of medium to low molecular mass (1000 – 20000 Da.). With the use of MALDI-TOF MS coupled with Magnetic-Beads HIC-18 SPE, we were able to detect distinctive tear mass patterns for each ocular condition, showing that this approach has a high potential as an efficient, rapid and capable technique in the study of human tear peptidome profiling with further applications in the clinical diagnosis of several eye diseases.