

O3 BIO1

DEVELOPMENT OF AN INNOVATIVE ANALYTICAL PLATFORM FOR ENRICHMENT AND IDENTIFICATION OF SHORT PEPTIDES IN BODY FLUIDS

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Body fluids are complex mixtures of metabolites of various chemical nature and thus significant sources of biomarkers for human diseases.[1] Despite being frequently described as potential biomarkers[2], short endogenous peptides still lack of proper strategies for their analysis, as they represent an analytical challenge due to their inhomogeneous chemical-physical properties, poor ionization efficiency using electrospray-mass spectrometry (MS) for their determination and the absence of automation in their detection. In order to overcome those issues, an innovative platform for enrichment, separation and identification has been developed. First, an enrichment strategy based on graphitized carbon black (GCB) was tested and developed on a mixture of analytical standard peptides which was representative of the naturally occurring peptides in body fluids. Short peptide enrichment was necessary due to the low abundance of those substances and the extreme complexity of body fluids samples. Ultra high performance liquid chromatography separation was carried out using two orthogonal chromatographic strategies, namely reversed phase (RP) C18 and Zic-HILIC columns, in order to maximize the number of identified peptides. A suspect screening approach was chosen for high resolution MS coupling.[3] In particular, a database of all the amino acid combinations for short peptides was compiled and MS/MS fragmentation was only performed on precursor ions matching with those in the database, resulting in a significant boost in sensitivity. Finally, MS/MS spectra were manually matched to spectra generated *in silico* to confirm the identity and the correct amino acid sequence.

The method was applied to the investigation of short endogenous peptides in human urine and plasma from healthy individuals resulting in the identification of 161 and 41 amino acid sequences, respectively. To the best of our knowledge, this is the first proposed method for enrichment and identification of short peptides in body fluids samples.

References

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[3] S. Piovesana, C. M. Montone, C. Cavaliere, C. Crescenzi, G. La Barbera, A. Laganà, A. L. Capriotti, *Journal of Chromatography A*, 2019, 1590, 73-79.