UNIVERSITÀ DEGLI STUDI DI SALERNO

DIFARMA

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Abstract of PhD Project

Development and Evaluation of Innovative Stationary Phases for Separation of Pharmaceuticals, Metabolites and Biocompounds

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Biofluids are typical complex matrices representing a source of potential biomarkers, but often require high demands in sample preparation prior to analysis. With this purpose, several techniques have been developed including solid phase extraction and methods based on the molecular recognition, such as immuno-affinity sorbents and molecularly imprinted polymers. Lipidomic is a research field in which it is needed to develop efficient techniques for sample pre-treatment in order to obtain reliable and reproducible results. Lipids are involved in many processes, as cellular structures, signaling, within the cell and between cells, and energy storage. Because of the role of lipids and all their functions, the field of Lipidomic is emerging with the focus on identifying alterations in lipids metabolism and lipid-mediated signalling processes that regulate cellular homeostasis and trying to understand the relationship between these processes in health and disease (Han & Gross, 2003). Following from that, when there is a disruption of lipid metabolism there is connection with the onset and progression of various metabolically linked diseases, including cancer (Santos & Schulze, 2012). The complexity of Lipidomic, expressed in lipids classes and subclasses present in human plasma, brings to highlight that the concentration range is quite wide, going from few pmol/L to mmol/L (Burla *et al*., 2018). Among other phospholipids, in this project we focused on Sphingosine 1-Phosphate (S1P), whose levels in circulatory stream have been recently determined below the nmol (Yatomi *et al*., 1997). S1P is a bioactive sphingolipid with broad range of activities coupled to its role in G-protein coupled receptor signalling. It is also an emerging biomarker for a variety of conditions comprising cancer, multiple sclerosis, rheumatoid arthritis and sepsis (Maceyka *et al*, 2012). In view of the dynamic nature of cell signaling, the time- and space-resolved quantification of S1P is crucial for both fundamental understanding and for developing improved diagnostic tests. Robust means of real-time lipid quantification *in-situ* are still to be realized. This requires the development of affinity techniques or probes (e.g. immunosensors) capable of continuously reporting the lipid levels in biofluids. Such methods would be particularly beneficial for monitoring S1P in blood or in living cells, but of equal urgency are sensors for Fingolimod (FTY720, Gilenya™, Novartis), a sphingosine analogue and S1P-receptor antagonist, approved by Food and Drug Administration (FDA) for the treatment of relapsing-remitting multiple sclerosis (Aktas *et al.,* 2010). We here report on three approaches to achieve these purposes. The *first* is based on LC-MS analysis to deep profiling selected classes of lipids in biofluids. It combines the use of solid phospholipid capture phases, specific for phosphomonoesters, with MS/MS, and allows extremely sensitive detection of phosphosphingolipids. The *second* approach concerns the use of fluorescent sensory core-shell molecularly imprinted polymer (MIP) particles responsive to near physiologically relevant levels of S1P and the S1P-receptor antagonist Fingolimod Phosphate (FP) in spiked human serum samples. Imprinting was achieved using FP-TBA salt or 1,2-dipalmitoyl-*sn*-glycero-3-phosphate sodium salt (DPPA(Na)) as templates, in combination with a polymerizable nitrobenzoxadiazole (NBD)-urea monomer with the dual role of capturing the phosphorus-anion and signaling its presence. In order to extend the capacity and ability of stationary phases to retain phosphorus-molecules, such as phospholipids, target molecules of our studies, the *third* approach developed was focused on the synthesis of materials created by the combination of two functional monomers (FM), 1,3-diarylurea based FM (neutral) and bis-imidazolium FM (cationic), using two different templates, phenyl phosphonic acid (PPA) and DPPA(Na), both mimicking the common phosphorus-moiety of phospholipids and probing the affinity towards the phosphate group. Finally, we demonstrated the potential use of the above techniques for monitoring S1P and FTY720-P in human plasma and human serum. Nevertheless, we considered this work as a first step towards a general sensory platform for phospholipids detection.