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Metabolic Profiling in Breast Cancer Applying Micro-Sampling of Biological Fluids and Analysis by Gas Chromatography – Mass Spectrometry

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Abstract : Recently, collection of biological fluids on special filter papers has become a popular micro-sampling technique. Especially, the dried blood spot (DBS) micro-sampling technique has gained much attention and is momently applied in various life sciences reserach areas. As a result of this popularity, DBS are not only intensively competing with the venous blood sampling method but are at this moment widely applied in numerous bioanalytical assays. In particular, in the screening of inherited metabolic diseases, pharmacokinetic modeling and in therapeutic drug monitoring. Recently, microsampling techniques were also introduced in “omics” areas, whereunder metabolomics. For a metabolic profiling study we applied micro-sampling of biological fluids (blood and plasma) from healthy controls and from women with breast cancer. From blood samples, dried blood and plasma samples were prepared by spotting 8uL sample onto pre-cutted 5-mm paper disks followed by drying of the disks for 100 minutes. Dried disks were then extracted by 100 uL of methanol. From liquid blood and plasma samples 40 uL were deproteinized with methanol followed by centrifugation and collection of supernatants. Supernatants and extracts were evaporated until dryness by nitrogen gas and residues derivated by O-methyxyamine and MSTFA. As internal standard C17:0-methylester in heptane (10 ppm) was used. Deconvolution and alignment of and full scan (m/z 50-500) MS data were done by AMDIS and SpectConnect (http://spectconnect.mit.edu) software, respectively. Statistical Data analysis was done by Principal Component Analysis (PCA) using R software. The results obtained from our preliminary study indicate that the use of dried blood/plasma on paper disks could be a powerful new tool in metabolic profiling. Many of the metabolites observed in plasma (liquid/dried) were also positively identified in whole blood samples (liquid/dried). Whole blood could be a potential substitute matrix for plasma in Metabolomic profiling studies as well also micro-sampling techniques for the collection of samples in clinical studies. It was concluded that the separation of the different sample methodologies (liquid vs. dried) as observed by PCA was due to different sample treatment protocols applied. More experiments need to be done to confirm obtained observations as well also a more rigorous validation .of these micro-sampling techniques is needed. The novelty of our approach can be found in the application of different biological fluid micro-sampling techniques for metabolic profiling.

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