

3rd International Conference and Exhibition on
Advances in Chromatography & HPLC Techniques
July 13-14, 2017 Berlin, Germany

Hyphenation of on-line sample pretreatment to HPLC for bioanalysis

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Analysis of drugs and metabolites in biological fluids is essential for bioanalysis. An optimal and effective sample preparation method plays the most important role as the depletion of the matrix in biological fluids is the biggest issue for a trouble-free analysis. It is impossible to inject the biofluid directly to the chromatographic system with traditional methods due to possible matrix effect and clogging issues. Liquid-liquid extraction (LLE), protein precipitation and solid-phase extraction (SPE) are the most common and offline/manual sample preparation methods to deplete macromolecules (i.e., proteins) present in the biological fluid prior to liquid chromatographic analysis. To speed-up the clean-up process, fully automated on-line techniques that combine sample preparation with separation could be a remarkable alternative. This could be achieved by the hyphenation of SPE with LC via a switching valve resulting online SPE-LC. This method allows direct repetitive injection of biological sample to a single SPE column. Use of restricted access materials (RAM) as SPE-column packing materials enables the depletion of high molecular weight matrix while the small analyte molecules are retaining; this fractionation is mostly based on 2D chromatography combination of size exclusion chromatography with reversed phase chromatography. Coupling SPE column with LC leads to complete automation improving the analytical quality due to enhanced reproducibility, elimination of human errors and the possibility of multiple step elutions for clean-up of complex samples, reducing the cost and analysis time required.

Biography

Sena Caglar Andac has completed her PhD from Istanbul University and Post-doctoral studies from Medical Center of Munich University, Laboratory of BioSeparation. She is now working as an Associate Professor at Istanbul University, Faculty of Pharmacy. She has her research works published in liquid chromatography mass spectrometry for biological fluid analysis of drugs; on-line solid phase extraction coupled liquid chromatography, determination of drugs and degradation studies by high performance liquid chromatography, spectrophotometry and spectrofluorimetry.

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