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## **SPE-POPLC: WHAT MAKES THIS COUPLE SO ATTRACTIVE FOR BIOANALYTICAL MS/MS?**

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Solid Phase Extraction (SPE) is the dominant technique in bioanalytical HPLC. During the last decade on-line SPE-LC became increasingly popular due to the fact that several manufacturers have implemented the required hard- and software into their LC-instruments. In addition, special SPE-column packings have been developed for the injection of preprocessed samples e.g. supernatants of precipitated biofluids or even native biofluids (e.g. plasma) and successfully applied in numerous bioanalytical applications. Most often, reversed phase chromatography (RPC) is coupled to such an on-line SPE clean-up step. Established LC-UV(FD)-protocols preferably use phosphate buffers to achieve optimal resolution and peak geometry. Phosphate salts, however, are obsolete in MS/MS. Therefore, scientists select and check numerous RPC-columns and / or modify the mobile phase composition (pH, MS compatible salts, ionic strength) and temperature, in order to find out the optimal separation.

Often, the desired separation only is achieved by applying a (complex) gradient. This elution mode however, not only requires a re-equilibration of the LC-column, but results in varying ionization conditions for the target analyte(s) and thus reduces detection sensitivity significantly. An isocratic elution mode, on the other hand, often results in a relatively long analytical run time, a situation which is not adequate for MS/MS detection. Thus, ideal separation conditions for LC-MS/MS would be 1.) a mobile phase composed of salts and organic liquids which results in the highest ionization yield for the target analyte(s), 2.) an isocratic elution mode which (fully) separates the analyte(s) within a (very) short run time. In addition, the elution window of the analyte(s) should be adjustable in such a way that no co-elution of matrix components occurs which otherwise would disturb the ionization process. This also holds for so called system peaks which appear in chromatograms when applying SPE-LC column switching.

To achieve these goals we investigated the newly developed Phase Optimized Liquid Chromatography (POPLC<sup>TM</sup>) kit. In POPLC LC-column segments with different length and packed with different stationary phases are combined and coupled together, respectively, to create a tailor-made analytical LC-column for a given separation and MS detection problem.