

Basics of Chromatography, Miniaturization and Multidimensional Separation for Proteome Analysis

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With the substantial progress in the high-throughput identification and structural elucidation of compounds by means of mass spectrometry, the adequate separation of highly complex mixtures of analytes in various biological matrices (urine, plasma, tissue, cell culture, etc) becomes more and more important. Miniaturization, high peak-capacity separations as well as multidimensional separations have become key components in the concept of comprehensive biological analysis, mainly enforced by the availability of only very low amounts of precious sample. In this workshop, we therefore focus on single- and multidimensional separations of biomolecules and their application to proteomic analysis. An overview of the different chromatographic separation modes is given, followed by information about the state of the art in instrumentation and column technology developed to operate the single- and multidimensional analytical systems. We present the new trends of ultrahigh- pressure chromatography, monolithic separation media, and chip-based separation devices, which have facilitated a significant increase in separation performance and speed of analysis. Finally, some selected examples of application including top-down or bottom-up proteome analyses are presented in order to shed light on the potential and the limitations of (multidimensional) separations in combination with mass spectrometry.

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