COMPARISON BETWEEN DIFFERENT MULTIDIMENSIONAL ANALYTICAL SYSTEMS FOR PROTEIN IDENTIFICATION.

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Analysis of complex samples is usually performed in proteomic laboratories. To increase the number of analysed peptides it is a common practice to combine several separation techniques in series. Although the most frequent approach was on-line bidimensional chromatography (2DLC), the resolution capacity of this methodology is not good enough for high-complexity samples. Then, nowadays the current methodologies usually dissociate the different separation steps (off-line) to increase the flexibility and resolution for each sample type. While Reverse Phase chromatography is most often used in the last step due to its convenient coupling with the mass spectrometer, several approaches are available for the first separation stage and different electrophoretic and chromatographic systems have been described. The aim of this study is to analyse and compare three of the most frequently used systems: Strong Cation-Exchange chromatography (SCX), Reverse-Phase chromatography (RP) at basic pH, and peptide Isoelectrofocusing (IEF). Parameters such as resolution, yield, etc. as well as the main factors determining peptide behaviour in the different analytic systems have been established.