
MAXIMIZING THE PEAK PRODUCTION RATE IN ONE- AND TWO-DIMENSIONAL LC-MS/MS USING MONOLITHIC COLUMNS

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Poly(styrene-*co*-divinylbenzene) monolithic columns have become an attractive alternative for packed silica columns for the separations of contemporary proteomic samples with LC-MS. The control that can be exerted over the preparation process facilitates optimization of the macropore and microglobule sizes of the monolith, which influences the chromatographic performance. In addition, columns of virtually any length and shape are easily accessible. However, to generate the maximum peak capacity in the shortest possible time, called peak production rate, the LC conditions need to be adjusted to work effectively with new column formats.

The present contribution discusses how to maximize the peak production rate in one-dimensional and two-dimensional liquid chromatography using monolithic column technology. The effects of gradient time, flow rate, and column length were studied in order to maximize the peak production rate in a one-dimensional RP gradient LC. An off-line two-dimensional LC approach was developed using the micro-fractionation option of the autosampler, which allows automatic fractionation of peptides after a first-dimension. To get the highest peak production rate in LC×LC, effects of sampling time and gradient times were investigated.

The potential of 1D-LC, using monolithic columns, up to 1 m in length, and off-line LC×LC is demonstrated by analyzing proteomics samples of various complexities. Finally a trade-off between peak production rate in 1D-LC and LC×LC is presented.