In proteomics, more than 100,000 peptides are generated from the digestion of human cell lysates. Proteome samples have a broad dynamic range in protein abundance; therefore, it is critical to optimize various parameters of LC–ESI–MS/MS to comprehensively identify these peptides. However, there are many parameters for LC–ESI–MS/MS analysis. In this study, we applied definitive screening design to simultaneously optimize 14 parameters in the operation of monolithic capillary LC–ESI–MS/MS to increase the number of identified proteins and/or the average peak area of MS1. The simultaneous optimization enabled the determination of two-factor interactions between LC and MS. Finally, we found two parameter sets of monolithic capillary LC–ESI–MS/MS that increased the number of identified proteins by 8.1% or the average peak area of MS1 by 67%. The definitive screening design would be highly useful for high-throughput analysis of the best parameter set in LC–ESI–MS/MS systems.

Definitive screening design enables simultaneous optimization of LC-ESI-MS/MS parameters in proteomics