Increased sensitivity of MS – based proteomics methods obtained by sample fractionation technique.

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The Chromosome – centric Human Proteome Project (C – HPP) aims to find high-stringency evidence for all proteins encoded by the human genome, the major splice forms of each protein. The Russian part of this project consists of detecting proteins encoded by the 18th human chromosome. In this work HT-29 human cell line was used as a biological object of interest. Whole-cell protein extract was prepared and digested by trypsin protease. Fifteen and sixty – three proteins encoded by the 18th human chromosome could be detected in complex peptide sample by shotgun mass – spectrometry (MS) and Multiple Reaction Monitoring with stable isotope – labelled standards (MRM SIS) respectively. MRM SIS is 6 folds more sensitive compared to shotgun MS, but due to the high complexity of peptide sample (4359 unique peptides identified by shotgun MS, related to 1200 proteins), some of the identifications detected by MRM SIS were doubtful (low signal, signal interference). To decrease peptide sample complexity and increase the sensitivity of shotgun mass – spectrometry and MRM SIS methods peptide sample fractionation technique was used. It was shown that reversed – phase liquid chromatography (RP – LC) in alkaline conditions performed prior to MS analysis allowed to confirm all the doubtful identifications detected by MRM SIS and to detect 11 additional proteins encoded by the 18th human chromosome with shotgun MS (11750 unique peptides identified, related to 2837 proteins). Thus, using RP – LC in alkaline conditions allowed to detect 63 proteins by MRM SIS and 34 proteins by shotgun MS (18 protein identifications were common for both methods), 45 proteins were detected by MRM SIS only, 16 proteins were detected by shotgun MS only. In summary 79 proteins encoded by the 18th human chromosome were detected. Applying RP – LC in alkaline conditions in combination with shotgun MS and MRM SIS allows deeper proteome coverage in chromosome - centric way.