

Abstract

This work is focused on urinary sample preparation before their proteomic analysis by liquid chromatography with mass spectrometric detection. Urinary proteomics is in many cases inefficient due to low urine protein concentration, variations in pH and the presence of high amount of salts, pigments, and other metabolites. These components can interfere with proteomic protocol and with subsequent mass spectrometric analysis. Therefore, there is the need to develop new effective sample preparation method.

The main benefit of this work is the optimization of the method for the effective preconcentration of proteins from urine samples using carboxylate-modified paramagnetic microparticles and the principles of HILIC chromatography. This innovative process was compared with a routine method using centrifugal filters (FASP) and the method based on proteins isolation by divinyl sulfone activated cellulose magnetic particles.

Using carboxylate paramagnetic microparticles, a total of 856 proteins was identified from only 0,5 mL of healthy human urine by LC-MS/MS. This optimized protocol provided comparable results to the standard FASP method in a much shorter time and with a lower cost per analysis.