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Improved sensitivity of Selected Reaction Monitoring-based protein quantification by LC-MS/MS using methanol instead of acetonitrile as organic modifier.

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It is now commonly accepted that Selected Reaction Monitoring Mass Spectrometry (SRM-MS) coupled to Stable Isotope Dilution (SID) might be an alternative to conventional ELISA test during the clinical evaluation of protein putative biomarker. However a significant gain of sensitivity is still required to reach the limit of quantification of the classical immuno enzymatic assays, typically in the nanogram to sub nanogram range per mL of serum or plasma. Overall, the sensitivity of any SRM-based quantification method is influenced by the intensity of the precursor ion, which is governed for a large part by the yield of the desolvatation process of the electrospray source. In the proteomic community, acetonitrile is the common organic modifier for C18 reverse phase chromatographic coupling with mass spectrometry, though Giorganni et al. (Anal. Chem. 2004, 76, 7028-7038) evidenced that methanol-based procedure contributed to an increased number of identified protein. In this report, we evaluated the influence of methanol or acetonitrile organic modifier on the intensity of SRM signals tracking a panel of 80 proteotypic heavy peptide standards spiked into a whole trypsin hydrolysate of human plasma. Keeping identical chromatographic parameters, the use of methanol organic modifier improved both peak height and area for nearly 90% of the proteotypic peptides with an average gain of about 50 %. Moving from acetonitrile to methanol organic modifier also improved the sensitivity of SRM calibration curves of protein models spiked in human blank plasma. Thus, methanol proves a superior organic modifier than acetonitrile for quantification of peptides in complex matrices by the SRM methodology.