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A NOVEL CHROMATOGRAPHIC METHOD ALLOWS ONLINE REANALYSES IN PROTEOMIC INVESTIGATIONS AND ACQUIRING MORE INFORMATION FROM BIOLOGICAL SAMPLES

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Liquid chromatography combined with electrospray ionization is the standard for the analysis of polar molecules by mass spectrometry (LCMS). The online coupling in LCMS is a major strength, but has the limitation that the mass spectrometer is not able to analyze all co eluting compounds immediately. Multiple injections or fraction collection can overcome this problem but time, sample limitation and the difficulty to fraction collect low volumes from a nanoLC system makes this unpractical. A new chromatographic strategy which enables to analyze a LCMS run twice with a single injection is described. After column separation the flow from a 75 µm column at a typical flow rate of 250 nL/min is split, so that part is directed to the mass spectrometer for analysis whilst the remainder flows to a capillary tube where it is stored. After the direct LCMS run, the flow is switched and the portion stored in the capillary is analyzed (‘replay run’). Since electrospray is a concentration dependent process the splitting system maintains full signal at decreased flow rates. An additional short column between the storage capillary and the mass spectrometer refocused the stored peaks in the second analyses so that width and intensity is identical to the initial run. To qualify the set up, the chromatographically performance and MS intensity was compared with a standard nanoLC set up using BSA tryptic digest for typically 60 min separations and mouse liver homogenate for 120-180 min LCMS runs. The chromatography performance and peak intensity for the normal setup are identical with the first and second run of the RePlay setup. Furthermore to explore the ability to analyze a LCMS run twice, examples of **c**ombining exploratory and targeted analysis are shown including the quantitation, using different fragmentation techniques, combining MSMS and MSn analyses or different mass spectrometers are shown.