

Exploring Ultra High Pressure LC and Prefractionation Strategies to Improve Compound Identification in Lipidomics and Metabolomics

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Abstract

Metabolomics, including lipidomics, has become an important approach for fundamental studies of biology and biomarker discovery. LC-MS is a popular approach to analyzing the metabolome. It is not uncommon to detect thousands of “features” in a given analysis; however, only a small fraction of these features can be identified. Further, many compounds are not detected at all. We have investigated two chromatography-centric approaches to increasing identifications. Reasons for not detecting compounds is co-elution of isomers and low signal due to ionization suppression. Both of these issues can be resolved by improving the chromatographic efficiency and peak capacity. It is well-known that smaller particles and longer columns provide higher resolution; but also, require higher pressure for flow in LC. We have experimented with systems that operate up to 50 kpsi for lipid and other metabolite separations. We demonstrate that these systems allow higher peak capacity, especially with relatively long 50 cm columns packed with 1.7 μm particles. When used to analyze plasma lipid extracts, we find that the improved resolution increases the number of lipids that can be identified. Indeed a linear relationship between peak capacity and identifications is found. Columns with peak capacity of 400 allow over 1000 potential identifications. Improvements are attributed to reduced ionization suppression among other factors. Recent work with smaller particles shows comparable results in < 60 min. Another reason for inability to identify compounds is that signals may be too low to acquire sufficiently high quality MS/MS spectra. We have evaluated use of prefractionation (essentially 2D LC) and extensive preconcentration to improve signal to noise ratio for MS/MS. Using these approaches, we have increased the number of compounds identified by 2-fold in plasma samples.