A Systematic Approach for Improving the Recovery of Hydrophobic Peptides during LC-MS Analyses

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Successful large molecule quantitation using LC-MS requires continuous attention to detail in each step of the workflow, from sample preparation to chromatographic separation and mass spectrometry detection. One frequently overlooked fact is that the analytes can be lost before LC-MS injections, especially in the sample container. Some peptides in solution tend to stick to the surfaces of the container, and may be permanently lost. This non-specific binding (NSB) can lead to inaccurate quantitative results, and thus can limit the LC-MS assay capability. Using a blocking agent has been the most common workaround to mitigate NSB problems but it may not be compatible with the downstream LC-MS conditions.

Peptide losses on the surface of polypropylene containers were mostly due to hydrophobic attraction, and the more hydrophobic peptides were more vulnerable. Increasing the percentage of organic solvent in the sample matrix helped to reduce the losses. While effective, this method may impact the subsequent LC-MS analysis. Highly organic sample matrices disrupted the retention of early eluting peptides on the chromatographic column. The types and concentrations of the acidic additives in the sample matrix also influenced the peptide recoveries but some additives induced peak tailing. Peptide losses depended on the storage time, at a given sample matrix and storage condition. Overall it was not trivial to find a perfect condition that ensured minimal peptide losses and, at the same time, was compatible with downstream LC-MS analyses.

Using a container with the high-performance surface proved to be a useful complementary option. Unlike standard polypropylene containers, the inert surface of low bind containers protects against peptide loss. The use of low bind containers and an LC-MS friendly sample matrix greatly simplifies the process of selecting the optimal storage conditions without compromising peptide recovery.