

## **High-throughput Comprehensive Coverage of Hydrophilic and Hydrophobic Metabolites in Beer Utilizing a Dual Separation/High Resolution Accurate Mass Spectrometry System**

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Untargeted metabolomics studies aim to detect, measure, and identify as many metabolites as possible in a sample without bias. However, the diversity in structural, chemical, and physical properties of small molecules, renders complete coverage of the metabolome of a given sample challenging. Diverse separation strategies have been developed to improve metabolome coverage, but unavoidably have introduced bias toward hydrophilic or hydrophobic metabolites. Here, we explored the integration of reverse phase (RP) and hydrophilic interaction chromatography (HILIC) to maximize metabolites detected in a single sample, with high reproducibility and increased throughput. This dual UHPLC separation system was coupled to a Thermo Scientific™ Orbitrap™ mass spectrometer and used for untargeted metabolomic analysis of beer samples, identifying compounds important in discriminating flavor and indicating spoilage.

We developed a dual liquid chromatography / mass spectrometry (LC/MS) system that combines RP and HILIC separation and we evaluated it for its reproducibility and separation capabilities of complex samples. Two independent UHPLC pumps were incorporated into the system to allow independent control of the two columns (RP and HILIC). This enabled the use of different solvents, additives and pH ranges and, as a result, a broader metabolite coverage. Having two independent fluidic paths resulted in a 25% decrease in analysis time and increased the overall throughput of the method. This was accomplished by equilibrating one column, while separation was carried out on the other.