Small Molecule Extraction Optimization and Analysis from River Water Using Liquid Chromatography Coupled to Mass Spectrometry

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The purpose of this study was to improve protocols for small molecule isolation and concentration from environmental water samples to maximize the identification of unknown compounds. The primary objectives were to optimize evaporation of organic solvents and the subsequent evaporation of solubilized analytes in aqueous solution. The EPA Method 537.1, which utilizes solid-phase extraction (SPE), was the foundation of our protocol. A Speedvac was implemented to replace a slower nitrogen blowdown system for organic solvent evaporation. Samples containing organic solvents were evaporated at room temperature under varying pressures, and recovery ratios were calculated. To replicate SPE elution solvents, water was added to samples prior to evaporation. A segmented evaporation approach was developed to first remove organic solvents, followed by aqueous content. Surface water samples collected from the Rutgers University golf course were analyzed using the modified Method 537.1 for an untargeted assay. Liquid chromatography-mass spectrometry, combined with untargeted data analysis using Mzmine and Compound Discoverer were used. Recovery of standard spikes in organic solvents decreased at lower pressures, likely due to rapid evaporation leading to analyte loss. The optimized single-step method of organic solvents, 25℃ at 30 torr for 30 minutes, yielded 63-73% recovery of internal standards. The segmented evaporation method in which organic solvents were first evaporated at 30 torr for 30 minutes followed by aqueous solutions at 5.1 torr for 2 hours, improved recovery ratios to 63-84%. Preliminary field data analysis revealed several compounds, including zeranols, tramadol, and PFBS. Future work will expand this method to profile small molecules across multiple New Jersey surface waters. Supported by NIH R25ES020721