G7 Monolithic Substrate Assisted Micro Liquid-Liquid Extraction of Alprazolam From Urine Samples

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The goal of this presentation is to provide information regarding a new monolithic substrate which was developed for Micro Liquid-Liquid Extraction (MLLE) of alprazolam from urine samples. Under the optimized extraction condition, the new MLLE achieved an extraction recovery of 37% for a 100ng/mL alprazolam-spiked urine sample.

This presentation will impact the forensic science community by discussing microextraction techniques that benefit the chemical analysis by consolidating analytical steps and streamlining analysis. This project presents a newly developed technique that combines the advantages of liquid-phase and solid phase microextraction, while minimizing their common disadvantages.

Even with rapid advances in analytical instrumentation, sample preparation represents the majority of the analysis time and has a significant impact on the analysis outcome. One of the oldest and most prevalent forms of sample preparation is Liquid-Liquid Extraction (LLE). LLE utilizes the properties of solubility and solvent polarity to sequester analytes into an extraction solvent. In traditional LLE, extraction solvent is added to a pH-adjusted sample and mechanically stirred to encourage the analyte to partition into the extraction phase and separate from the matrix components. The extraction phase containing the analyte is isolated, dried, and reconstituted. This technique is time consuming, labor intensive, and relies heavily on the skill of the analyst. In an effort to reduce these factors, MLLE techniques were developed. MLLE techniques use a small amount of extraction solvent (2 to 20µL) to concentrate the analyte from the sample. In MLLE, the analyte is concentrated in microliters of extraction phase, and then the extraction phase is removed and injected directly onto the analytical instrument. The aim of this work was to make a Monolithic Substrate to assist Micro Liquid-Liquid Extraction (MSA-MLLE). The main approach is to synthesize an inert and porous material that is capable of holding microliters of organic extraction solvent in an aqueous environment.

In this work, the monolithic substrate was prepared by sol-gel chemistry. Methyltriethoxysilane (MTES) was found to increase the pore size of resulting siloxane. The addition of bulky alkoxysilanes, such as Phenyltrimethoxysilane (Ph-TMS), to the reaction mixture was shown to contribute to hydrophobicity. The addition of a bulky alkoxysilane increased hydrophobicity by reacting with open hydroxyl groups present on the alkoxysilane. MTES and varying levels of Ph-TMS were chosen as the siloxane precursors. The sol-gel reaction was optimized to a molar ratio of 1:13:4.8:0.1 Methyltriethoxysilane:Methanol:Water:Phenyltrimethoxysilane with the reaction adjusted to pH 10 with ammonium hydroxide. The selected sol-gel produced a large-pored, hydrophobic, and viscous siloxane that could be adhered to a metal surface (a pyrolyzer hook) for MLLE.

The Monolithic Substrate-Coated Hook (MSCH) was used for MLLE. The MSCH appeared porous under magnification and were able to hold organic extraction solvent from 0.3 to 1.4µL of toluene prior to exposure to water. After 20 minutes of exposure to stirred water, the retained solvent dropped to 0.1 to 0.2µL depending on the molar ratio of Ph-TMS. Alprazolam is used as a test drug in this experiment because it is commonly encountered in toxicological analysis. It is also readily available and has been studied by using other microextraction techniques. One of the MSCHs was able to concentrate alprazolam from a spiked urine sample using both toluene and octanol as extraction solvents. The best condition for MSA-MLLE was extraction with octanol for 20 minutes with 600rpm stirring. This condition achieved an extraction recovery of 37% for the low spike (100 ng/mL) and 7% for the high spike (500ng/mL). Unfortunately, the MSCH could be thermally degraded after 5 – 10 runs using thermal desorption. Increase the thermo-stability of the MSCH should be addressed for future work.

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