B204 An Advanced Extraction Method for Cyanide Metabolite Using Magnetic Carbon Nanotubes Facilitated Dispersive Micro Solid Phase Extraction (Mag-CNT/d-µSPE)

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Learning Overview: After attending this presentation, attendees will better understand an advanced extraction process for the extraction of a cyanide metabolite from biological samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by: (1) reviewing the potential use of the cyanide metabolite 2-Aminothiazoline-4-Carboxylic Acid (ATCA) as a forensic marker in cyanide exposure and in medicolegal death investigation; and (2) demonstrating advancement of Mag-CNT/d-µSPE in extracting ATCA from biological samples using a one-step derivatization approach.

Cyanide intoxication can occur due to accidental exposures in industrial settings, consumption of cyanide-containing foods, or by illegal uses of cyanide in suicide, homicide, or terrorist acts. Sensitive analytical techniques have been developed to detect cyanide from biological samples. However, due to the unstable nature and incomputable kinetics of cyanide in biological samples upon storage, the analysis of its metabolites is considered to be more feasible for the purpose of exposure confirmation. The detection of the minor metabolite of cyanide, ATCA, has been proposed as an alternative method to determine cyanide exposure because of its stability and specificity. Studies have been extensively published to extract ATCA using conventional Solid Phase Extraction (SPE) and Liquid-Liquid Extraction (LLE). This research of ATCA extraction based on d-µSPE has also been demonstrated to be promising for biological samples. In this work, a brief review of studies on the potential use of ATCA as a forensic marker will be discussed, and the performance of an advanced Mag-CNT/d-µSPE in extracting ATCA from biological samples will be presented.

The Mag-CNT used in this study were synthesized in the Sam Houston State University research laboratory. The application of Mag-CNT/d-µSPE to extract ATCA from biological samples was found promising and has been published. In this work, the combination of desorption and derivatization steps of ATCA from the Mag-CNT surface into a one-step process was investigated. Deionized water was used in method development and synthetic urine was used in optimization steps. In a typical extraction process, 5mg of Mag-CNT was used to extract ATCA from 500uL of biological sample in a microcentrifuge tube, which was spiked with known amount of ATCA and its isotopic internal standard, ATCA-^{13}C, ^{15}N. The samples were then acidified with hydrochloric acid, vortexed, and sonicated for d-µSPE for 10min. After d-µSPE, the Mag-CNT were isolated with the aid of a strong magnet and were dried at 65^{o}C under vacuum. N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added to the dried Mag-CNT to derivatize and desorb ATCA, and the derivatized extracts were subjected to Gas Chromatography/Mass Spectroscopy (GC/MS) analysis. Optimization of the extraction parameters, including the amount of Mag-CNT, sample and derivatization volume, extraction and derivatization time, and dilution solvent systems was performed in triplicates.

The advanced Mag-CNT/d-µSPE with the one-step derivatization approach was capable of reaching the detection limit of 5ng/mL and has a linear dynamic range between 10–1,000ng/mL, which is capable of detecting human endogenous level of ATCA in urine samples. The average extraction efficiency of 97.0% was obtained with a satisfactory bias and precision of ±20% at the low, medium, and high level of quality control samples. It is envisioned that this advanced extraction platform will provide an additional tool for the quantitation of ATCA from biological samples with GC/MS analysis in forensic settings.

Cyanide Metabolite, 2-Aminothiazoline-4-Carboxylic Acid, Magnetic Carbon Nanotubes

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