Development of magnetic carbon nanotubes for dispersive solid phase extraction of cyanide metabolite, 2-amino-2-thiazoline-4-carboxylic acid (ATCA), in biological samples

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INTRODUCTION

Cyanide has been known as one of the deadliest toxins in the world. In 2016 alone, 198 cyanide exposure cases were reported in the United States, of which, nearly 70% were unintentionally exposed and more than 8% were due to intentional poisoning (1). Due to its rapid onset and non-specific symptoms, cyanide exposure is usually difficult to diagnose and detect. One of the biggest challenges to confirm cyanide exposure in forensic settings is the lack of conclusive and consistent autopsy findings (2). Although successful methods are developed to detect cyanide directly, limitations exist due to the high volatility and reactivity of cyanide. An alternative method proposed for confirming cyanide exposure is the analysis of its minor metabolite: 2-amino-2thiazoline-4carboxylic acid (ATCA), which has been found to be a stable and specific biomarker for cyanide metabolism over time (3-5).

The goal of this project is to develop a novel extraction method using magnetic carbon nanotubes facilitated dispersive solid phase extraction (Mag-CNT/d-SPE) to extract ATCA with detection by gas chromatography/mass spectroscopy (GC/MS). The successful development of this method might provide a reliable alternative to confirm cyanide exposure and is envisioned to overcome some of the limitations associated with the traditional solid phase extraction and liquid-liquid extraction.

RESULTS AND DISCUSSION

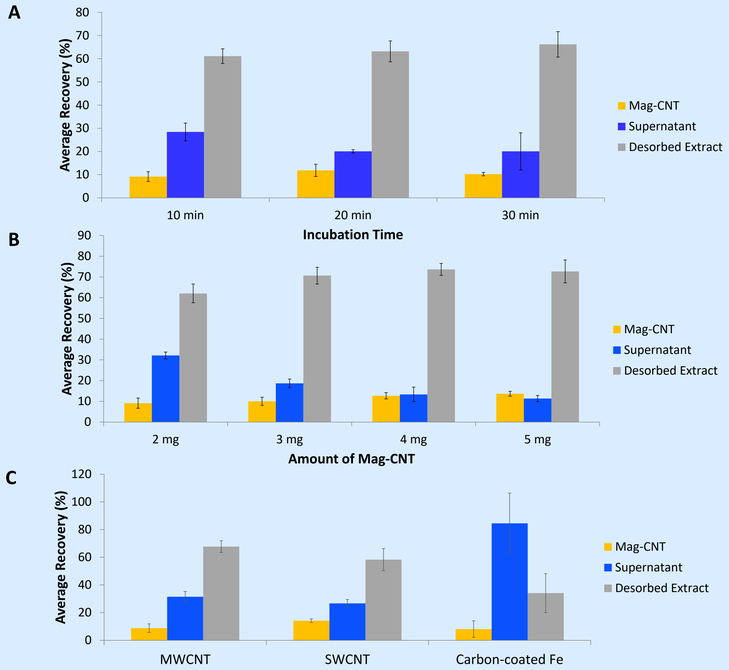

Figure 2: ATCA concentrations found in Mag-CNT, supernatant, and back extract with different (A) extraction time (10, 20, and 30 min),(B) amount of Mag-CNT (2, 3, 4, and 5 mg), and (C) types of magnetic nano-particles (multi-walled and single-walled Mag-CNT, and commercial carbon-coated iron nano-particles) at concentration of 1000 ng/mL. Error bars are expressed in terms of standard deviations

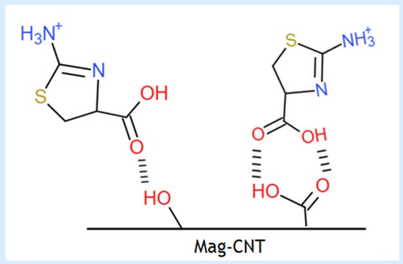

Figure 3: Proposed hydrogen bonding interaction between Mag-CNT and ATCA under acidic condition.

Detection Limit	Synthetic Urine (ng/mL)	Bovine Blood (ng/mL)
LOD	15	25
LOQ	30	30

Table 1: Detection limit of the linear model in synthetic urine and bovine blood

QC (ng/mL)	Synthetic Urine (%)	Bovine Blood (%)
90	96.5 ± 10.4	84.9 ± 2.8
500	97.9 ± 4.4	97.0 ± 7.0
800	98.6 ± 4.0	107.7 ± 8.5

Table 2: Average recovery of ATCA in synthetic urine (n=9) and bovine blood (n=9) using the optimized Mag-CNT/d-SPE method.

- Hydrogen bonding was proposed to be the major contributing factor for the extraction of ATCA by Mag-CNT.
- No significant difference of average ATCA recovery in desorbed extract was found among different extraction times, amount of Mag-CNT, and between multi-walled (MWCNT) and single-walled Mag-CNT (SWCNT).
  - → A higher deviation was observed when using 2 mg Mag-CNT, which may be due to errors in consistently weighing out small amount of Mag-CNT.
  - → MWCNT was chosen for lower cost.
- Quantitation range obtained from the linear model of concentration range from 30 – 1000 ng/mL is sensitive enough to detect and quantitate endogenous level of ATCA in human biological samples.
- Bias and precision of the Mag-CNT/d-SPE method in synthetic urine and bovine blood is acceptable and within ±20% range for all three QC levels.
- Further study is needed to investigate the possibility of performing one-step derivatization of ATCA from the Mag-CNT.

MATERIALS AND METHODS

Mag-CNT Synthesis

The method of Mag-CNT synthesis was adopted from Padasani *et al.* with modifications (6). In short, the CNT were purified in a 3:1 concentrated nitric acid and sulfuric acid mixture at 60°C overnight. Fivety mg of purified CNT were suspended in 25 mL of water with 70 mg of iron (II) chloride tetrahydrate and 135 mg of iron (III) chloride hexahydrate at 50°C. Then, 1 mL of 28% ammonium hydroxide was added and the solution and heated to 80°C for 30 min. After the mixture was cooled to room temperature, the magnetized CNT was washed with copious amount of water followed by an ethanol wash. The Mag-CNT were resuspended in water and dried under vacuum at 100°C.

Mag-CNT facilitated d-SPE (Mag-CNT/d-SPE)

A simplified extraction outline is illustrated in Figure 1. To test the capability for Mag-CNT to extract ATCA, 2 mg of Mag-CNT were added to a microcentrifuge tube containing 1000 ng/mL of ATCA and 100 µL of deionized water in triplicates. The samples were acidified with 2% (v/v) 0.1 M formic acid, vortexed, and extracted for 10 min at 50°C with sonication. Then the Mag-CNT were separated using a strong magnet and the supernatant was transferred to separate tubes. Desorption was performed by adding 150 µL of deionized water with 5% (v/v) ammonium hydroxide. The samples were vortexed and incubated under the same condition as described as above. The Mag-CNT were separated using a strong magnet and the desorbed extract was transferred to separate tubes. All extracts were then added with internal standard, ATCA – ^{13}C, ^{15}N and dried at 100°C under vacuum and derivatized with MSTFA. The desorbed extract of the Mag-CNT portions were separated and all derivatized samples were then subject to GC/MS analysis.

Optimization of Mag-CNT/d-SPE

Extraction parameters, such as amount of Mag-CNT and incubation time were tested. Different extraction time (10, 20, and 30 minutes), amount of Mag-CNT (2, 3, 4, and 5 mg), and types of Mag-CNT (multi-walled vs single-walled CNT and commercial carbon-coated iron nano-particles) were evaluated for optimal recovery in the back-extract only. Optimization was performed in triplicate. Significant differences were determined by conducting one-way ANOVA or two-tailed *t*-test. The optimized method was applied to synthetic urine and bovine blood and the precision and bias of the method was determined from three quality control levels (90, 500, and 800 ng/mL) in triplicate. Calibration standards with ATCA concentrations of 5, 10, 30, 50, 100, 250, 500, 1000 ng/mL were prepared from a stock solution of 60 µg/mL in methanol/5% (v/v) formic acid to determine the limit of detection (LOD) and limit of quantitation (LOQ). ATCA – ^{13}C, ^{15}N (833 ng/mL) was used as the internal standard and added at the beginning of the extraction. Response ratios of the derivatized products were plotted against the concentration to obtain a linear model.

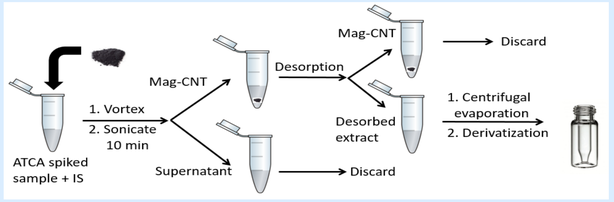

Figure 1: Simplified optimized Mag-CNT/d-SPE-GC/MS procedures.

CONCLUSIONS

- Mag-CNT was able to extract ATCA from synthetic urine and bovine blood.
- Average recovery, precision, and bias of extracting ATCA from biological samples using the Mag-CNT/d-SPE method was satisfactory with quantitative analysis using GC/MS.
- Mag-CNT/d-SPE may serve as an alternative extraction method to SPE and LLE in forensic analysis.

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