Trace level extraction of the cyanide biomarker, 2-amino-2-thiazoline-4-carboxylic acid (ATCA), from biological samples by applying magnetic nanomaterials in dispersive solid phase extraction

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INTRODUCTION

Cyanide is infamous with its rapid acting characteristic and is considered 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). Difficulties for cyanide exposure confirmation are also encountered in toxicological analyses. Although successful methods are developed to detect cyanide directly , limitations exist due to the high volatility and reactivity of cyanide. An alternative method that is proposed for confirming cyanide exposure is by the analysis of its minor metabolite: 2-amino-2-thiazoline-4-carboxylic acid (ATCA), which is suggested 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 the detection of gas chromatography/mass spectroscopy (GC/MS). The successful development of this method will provide a reliable alternative to confirm the exposure of cyanide and is envisioned to overcome some of the limitations associated with the traditional solid phase extraction and liquid-liquid extraction, such as the high cost extraction columns and labor intensive extraction procedures.

RESULTS & DISCUSSION

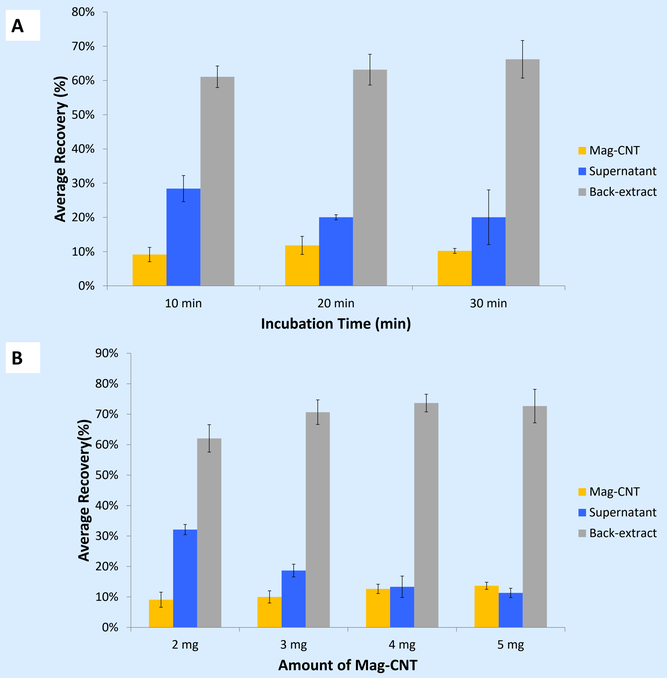

Figure 2: ATCA recoveries found in Mag-CNT, supernatant, and back extract with (A) extraction time of 10, 20, and 30 min, and (B) 2, 3, 4, and 5 mg of Mag-CNT at concentration of 1000 ng/mL. Error bars are expressed in terms of standard deviations

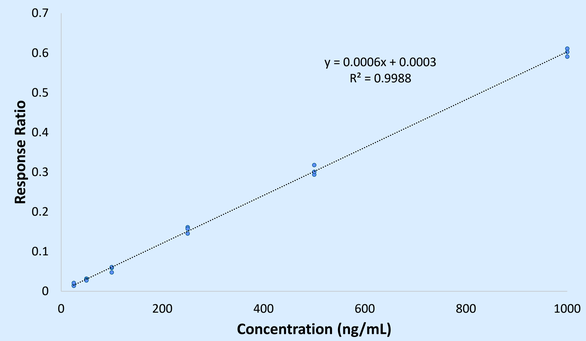

Figure 3: Linear model of calibration standards dynamic range at 25, 50, 100, 250, 500, and 1000 ng/mL.

Detection Limit	Concentration (ng/mL)
LOD	12.5
LOQ	25

Table 1: Detection limit of the linear model shown in Figure 2.

Extraction Portion	Average Recovery (%)
Mag-CNT	10.5 ± 0.03%
Supernatant	21.7 ± 0.09%
Back-extract	67.0 ± 0.07%

Table 2: Average recovery obtained of ATCA in each portion of the Mag-CNT/d-SPE from the optimization results.

- No significant difference in ATCA concentrations in back-extract was found among extraction time.
- No significant difference in ATCA concentration in back-extract was found among 2, 3, 4, and 5 mg of Mag-CNT.
  → A higher deviation was observed when using 2 mg Mag-CNT, which may due to errors in consistently weighing out small amount of Mag-CNT.
  → For more precise result, 3 mg of Mag-CNT is preferred for extraction.
- Dynamic range obtained from the linear model of concentration range from 25 – 1000 ng/mL, are sensitive to detect and quantitate endogenous level of ATCA in human biological samples.
- Average recovery of ATCA in back-extract was low.
  → Further optimization is needed for higher recovery.
  → Recovery issue may not affect accurate quantitation in extracting unknown concentration of biological samples if internal standard is added for normalization.

CONCLUSIONS

- Mag-CNT were able to extract ATCA from aqueous matrix.
- Recovery issue of Mag-CNT/d-SPE will be resolved in extracting biological sample with the addition of internal standard at the beginning of the extraction to compensate lost during the process.
- Further optimization steps are needed to maximize recovery if 2 mg of Mag-CNT were used in extraction in biological samples.

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. Then 50 mg of purified CNT were suspended in water with 70 mg of iron (II) chloride tetrahydrate and 135 mg of iron (III) chloride hexahydrate at 50°C with a slow addition of 1 mL concentration ammonium hydroxide to a pH range of 10 - 11. The suspension was then heated to 80°C for 30 min. After the mixture was cooled to room temperature and the magnetized CNT were washed with copious amount of water and followed by ethanol. The Mag-CNT were re-suspended 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 was added to a microcentrifuge tube containing 100 ng of ATCA in 100 µL of deionized water in triplicates. The samples were vortexed and d-SPE at 50°C with sonication for 10 min. Then, the Mag-CNT were separated using a strong magnet and the supernatant was transferred to separate tubes. Back extraction was preformed by adding 150 µL of deionized water with 5% (v/v) ammonium hydroxide. The samples were vortexed and ATCA was back extracted under the same condition as described as above. The Mag-CNT were separated using a strong magnet and the back-extract was transferred to separate tubes. All samples were then added with ATCA – ^{13}C, ^{15}N as internal standard (8.33 ng/ µL). The extracts were dried at 100°C under vacuum and ATCA was derivatized. The back-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 extraction time were tested. Different amount of Mag-CNT (2, 3, 4, and 5 mg) were added to the spiked solution to perform d-SPE. Different extraction time (10, 20, and 30 minutes) was also evaluated for optimal recovery. Optimization was performed in triplicates. Significant difference was determined by conducting one-way ANOVA. Calibration standards with ATCA concentrations of 5, 10, 25, 50, 100, 250, 500, 1000 ng/mL were prepared from a stock solution of 60 µg/mL in 5% (v/v) formic acid in methanol 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. Response ratios of the derivatized products were plotted against the concentration to obtain a calibration equation.

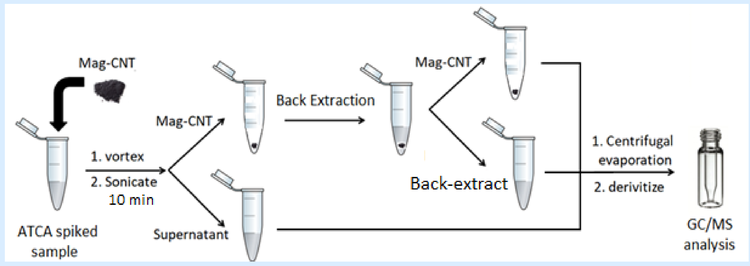

Figure 1: Simplified Mag-CNT/d-SPE-GC/MS procedures.

REFERENCES

1. Gummin DD, Mowry JB, Spyker DA, Brooks DE, Fraser MO, Banner W. 2016 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 34th Annual Report. Clinical Toxicology. 2017 2017/11/26;55(10):1072-254.
2. Gill JR, Marker E, Stajic M. Suicide by cyanide: 17 deaths. J Forensic Sci. 2004;49(4):826-8.
3. Logue BA, Kirschten NP, Petrikovics I, Moser MA, Rockwood GA, Baskin SI. Determination of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine and plasma by gas chromatography–mass spectrometry. Journal of Chromatography B. 2005;819(2):237-44.
4. Logue BA, Maserek WK, Rockwood GA, Keebaugh MW, Baskin SI. The analysis of 2-amino-2-thiazoline-4-carboxylic acid in the plasma of smokers and non-smokers. Toxicology Mechanisms And Methods. 2009;19(3):202-8.
5. Ružycka M, Giebułtowicz J, Fudalej M, Krajewski P, Wroczyński P. Application of 2-Aminothiazoline-4-carboxylic Acid as a Forensic Marker of Cyanide Exposure. Chemical Research in Toxicology. 2017 2017/02/20;30(2):516-23.
6. Pardasani D, Kanaujia PK, Purohit AK, Shrivastava AR, Dubey DK. Magnetic multi-walled carbon nanotubes assisted dispersive solid phase extraction of nerve agents and their markers from muddy water. Talanta. 2011;86:248-55.