Application of magnetic carbon nanotubes facilitated dispersive micro solid phase extraction of the cyanide metabolite (2-aminothiazoline-4-carboxylic acid) in biological samples

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INTRODUCTION

Due to the high volatility and reactivity of cyanide, its concentration in post-mortem biological samples is subjected to change depending on the storage conditions, time elapsed between death and sample analysis, sample preparation methods, etc. Such instability might cause difficulties in the confirmation of cyanide exposure due to misinterpretation of the cyanide concentration. An alternative method that is proposed for confirming cyanide exposure is by the analysis of its minor metabolite: ATCA, which is suggested to be a stable and specific biomarker for cyanide metabolism over time (1-3).

The goal of this project is to apply a novel extraction method: magnetic carbon nanotubes facilitated dispersive micro solid phase extraction (Mag-CNT/d-µSPE) to extract ATCA from urine and blood samples with the detection of gas chromatography/mass spectroscopy (GC/MS). In conventional solid phase extraction and liquid-liquid extraction, samples volume is typically in mL range, which may not be always available in forensic toxicology analysis. The novel Mag-CNT/d-µSPE method miniaturizes the extraction scale to µL range for the both samples and reagents to facilitate a more practical and cost-effective analysis. The successful application of this method in biological samples 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, such as the high cost extraction columns and labor intensive extraction procedures.

RESULTS & DISCUSSION

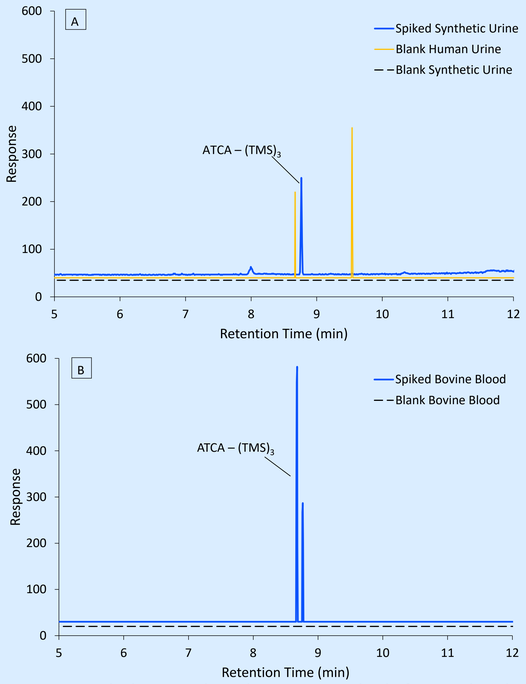

Figure 2: Overlaid extracted ion chromatograms (EIC) of ATCA – $(TMS)_3$ at 245, 347, and 362 m/z in (A) a blank synthetic urine, synthetic urine spiked with 1000 ng/mL ATCA only, and a human urine sample, and (B) a blank bovine blood and bovine blood sample spiked with 1000 ng/mL ATCA only.

	Synthetic Urine			Bovine Blood		
QC (ng/mL)	Recovery (%)	Bias (%)	Precision (%)	Recovery (%)	Bias (%)	Precision (%)
90	96.5 ± 10.4	-0.72	8.00	84.9 ± 2.8	-12.01	1.23
500	97.9 ± 4.4	2.08	2.78	97.0 ± 7.0	-2.24	6.93
800	98.6 ± 4.0	-0.40	3.74	107.7 ± 8.5	-5.37	7.98

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

- Recovery of ATCA in synthetic urine was more precise and accurate than that of synthetic urine.
  → complexity of the matrix may contribute to the difference.
- 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.
- Quantitation range obtained from the linear model of concentration range from 30 – 1000 ng/mL for both synthetic urine and bovine blood are sensitive to detect and quantitate endogenous level of ATCA in human biological samples.

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

- No carryover was observed in all blank-extracted samples.
- No interfering peaks from the matrix were observed in both biological samples.

MATERIALS AND METHODS

Magnetic carbon nanotubes facilitated dispersive micro solid phase extraction (Mag-CNT/d-µSPE)

A simplified extraction outline is illustrated in Figure 1. Briefly, different concentrations of ATCA and 833 ng/mL of ATCA – ^{13}C, ^{15}N (internal standard) was added to a microcentrifuge tube and was dried at 65°C under vacuum. Then, 100 µL of biological sample was added in the tubes in triplicates with vortexing and 2 mg of Mag-CNT was added. The samples were acidified with 2% (v/v) 0.1 M formic acid, vortexed, and extracted for 10 min at 50°C with sonication. The Mag-CNT were then separated using a strong magnet and the supernatant was discarded. Desorption was preformed by adding 150 µL of deionized water with 5% (v/v) ammonium hydroxide. The samples were vortexed and sonicated under the same condition as described as above. The Mag-CNT were separated using a strong magnet and the desorbed extracts were transferred to separate tubes. All samples were dried at 65°C under vacuum and then derivatized with 150 µL MSTFA/hexane, followed by GC/MS analysis under SIM mode.

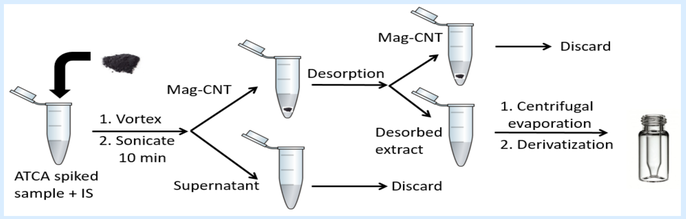

Figure 1: Simplified optimized Mag-CNT/d-µSPE-GC/MS procedures.

Sample Preparation

Synthetic urine and bovine blood samples were used in this experiment as the biological samples. Synthetic urine (100 µL) was added directly to the microcentrifuge tubes with the dried ATCA standard and internal standard without further sample preparation. As for bovine blood, protein precipitation was performed before the extraction. Briefly, 200 µL of ice-cold acetonitrile was added to 100 µL of the bovine blood samples while vortexing in a microcentrifuge tube. The samples were then centrifuged and the supernatants were separated and added to the microcentrifuge tube with the dried ATCA standard and internal standard. Then the samples were extracted and analyzed with the same procedures described in the "Mag-CNT/d- µSPE" section.

Testing Parameters

For both biological samples, 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 standard was added at three different concentration levels (90, 500, and 800 ng/mL) to evaluate the bias and precision of the method in triplicates. Carryover studies were performed by analyzing a blank-extracted sample (without the addition of ATCA nor internal standard) in the GC/MS subsequent to the analysis of the highest concentration calibrator to determine whether the ATCA peak will be observed in the blank-extracted chromatogram. Assay selectivity was also evaluated by determining the recovery of ATCA in the three concentration levels and the analysis of chromatographic profile in the biological samples. GC/MS analysis was performed under SIM mode (ATCA-$(TMS)_3$: 245, 347, and 362 m/z and ATCA-^{13}C, ^{15}N-$(TMS)_3$: 248, 350, and 365 m/z).

CONCLUSIONS

- Mag-CNT are able to extract ATCA successfully 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 the 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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