Journal of Analytical Science and Technology

RESEARCH ARTICLE

Open Access

Performance comparison between solid phase extraction and magnetic carbon nanotubes facilitated dispersive-micro solid phase extractions (Mag-CNTs/d-μSPE) of a cyanide metabolite in biological samples using GC–MS

Sun Yi Li[1], Ilona Petrikovics[2] and Jorn Yu[1*]

Abstract

Dispersive-micro solid phase extraction (d-μSPE) has gained increasing attention due to its convenience, effectiveness, and flexibility for sorbent selection. Among a various selection of materials, magnetic carbon nanotubes (Mag-CNTs) is a promising d-μSPE sorbent with excellent separation efficiency in addition to its high surface area and adsorption capability. In this work, two different surface-modified Mag-CNTs, Mag-CNTs-COOH and Mag-CNTs-SO_3H, were developed to facilitate d-μSPE (Mag-CNTs/d-μSPE). The cyanide metabolite, 2-aminothiazoline-4-carboxylic acid (ATCA), was selected to evaluate their extraction performance using gas chromatography–mass spectrometry (GC–MS) analysis. The Mag-CNTs-COOH enabled a one-step derivatization/desorption approach in the workflow; therefore, a better overall performance was achieved. Compared to the Mag-CNTs-SO_3H/d-μSPE and SPE workflow, the one-step desorption/derivatization approach improved the overall extraction efficiency and reduced solvent consumption and waste production. Both Mag-CNTs/d-μSPE workflows were validated according to ANSI/ASB 036 guidelines and showed excellent analytical performances. The limit of detection (LOD) and limit of quantitation (LOQ) of ATCA in synthetic urine were 5 and 10 ng/mL, respectively, and that in bovine blood were achieved at 10 and 60 ng/mL. The SPE method's LOD and LOQ were also determined at 1 and 25 ng/mL in bovine blood samples. The Mag-CNTs/d-μSPE methods demonstrated great potential to extract polar and ionic metabolites from biological matrices. The extraction processes of ATCA described in this work can provide an easier-to-adopt procedure for potential routine forensic testing of the stable biomarker in cyanide poisoning cases, particularly for those cases where the cyanide detection window has passed.

Keywords: Magnetic carbon nanotubes (Mag-CNTs), Dispersive-micro solid phase extraction, Cyanide metabolite, Surface modification, Functionalization, Solid phase extraction, 2-Aminothiazoline-4-carboxylic acid (ATCA), Derivatization, Death investigation

Introduction

Sample preparation plays a vital role in the successful and accurate determination of trace-level analytes, especially from complex biological matrices in analytical chemistry. Conventional sample preparation methods, including

*Correspondence: jornyu@shsu.edu
[1] Department of Forensic Science, Sam Houston State University, Huntsville, TX 77341, USA
Full list of author information is available at the end of the article

Springer Open

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

solid phase extraction (SPE) or liquid–liquid extraction (LLE), are widely utilized to isolate analytes in different biological samples (Coopman et al. 2016; Glicksberg et al. 2016; Palmquist and Swortwood 2019; Skillman and Kerrigan 2018; Smith et al. 2019; Truver et al. 2019). Despite the extensive and successful applications, SPE may suffer from drawbacks such as limited sorbent capacity and unequal flow rates. As for LLE, the process is usually time-consuming, labor-intensive, and consumes a more considerable amount of organic solvents. In the past decade, microextraction has increasingly been applied to different disciplines, aiming to overcome some of the disadvantages of conventional methods (Shi et al. 2019; Cruz Fernandes et al. 2020; Khezeli and Daneshfar 2017; Saito et al. 2014; Sun et al. 2020). The miniaturization of the extraction method dramatically reduces the use of organic solvents, making it a green approach in sample preparation. Among the different microextraction methods, dispersive-micro solid phase extraction (d-μSPE) is gaining popularity as an alternative approach due to its effectiveness, applicability for automation, and flexibility for sorbent selection (Ahmadi et al. 2017; Medina et al. 2018; Fikarová et al. 2019; Mafra et al. 2019; Płotka-Wasylka et al. 2016, 2015; Ghorbani et al. 2019). A wide variety of novel sorbents have been developed for d-μSPE, including molecular recognition sorbents, such as molecularly imprinted polymers (MIPs), immune-affinity sorbents, and nanoparticles (Jiang et al. 2006; Pichon et al. 1999; Wen et al. 2014). In recent years, magnetized carbon-based nanoparticles, for example, graphene and carbon nanotubes (CNTs), have become one of the most popular sorbents due to their high surface area, adsorption capability, and the increased convenience for separation of sorbents (Abd Wahib et al. 2018; Fernandes et al. 2018; Jalilian et al. 2017). The potential for surface enhancement and functionalization further enhances their flexibility, selectivity, and applicability for different biochemical analyses.

In the past few years, several studies have demonstrated the use of 2-aminothiazoline-4-carboxylic acid (ATCA) as a stable biomarker for the diagnosis of cyanide exposure (Li et al. 2019a; Logue et al. 2010; Rużycka et al. 2017; Vinnakota et al. 2012; Yu et al. 2012). Li et al. recently reviewed the potential use of ATCA as a forensic marker in a cyanide-related death investigation (Li et al. 2019b). Briefly, the detection window of cyanide in human biological samples is short ($t_{1/2}$ = 0.34–1.28 h) (Logue et al. 2010; Li et al. 2019b; Hartung et al. 1982; Ansell and Lewis 1970; Gyamfi et al. 2019), which could lead to possible false-negative results in a death investigation involving cyanide exposure. Alternative testing for stable biomarkers for cyanide exposure should be established in forensic analysis of biological evidence, especially when the cyanide detection window has passed. In the practice of forensic toxicology, when the "parent" molecule is rapidly metabolized in the body, the detection of stable metabolites of the parent molecules can be used as evidence to build a case. For example, the co-detection of morphine and 6-monoacetylmorphine are specific metabolites used for the confirmation of heroin use due to its rapid metabolism in the body (Gottas et al. 2013). In cyanide metabolism, ATCA was found to be specific to cyanide metabolism and was stable at a wide range of storage conditions, including post-mortem conditions (Logue et al. 2010, 2009; Rużycka et al. 2017; Vinnakota et al. 2012; Wood and Cooley 1956; Ballantyne 1977). Successful SPE and LLE methods have been developed to isolate ATCA from biological samples. However, the detection of ATCA was predominately achieved using liquid chromatography-tandem mass spectrometry (LC–MS/MS) due to its high polarity (Rużycka et al. 2017; Yu et al. 2012; Giebułtowicz et al. 2016). At present, such analytical workflow might not be quickly adopted in all forensic laboratories due to factors related to budget and human resources. Because gas chromatography-mass spectrometry (GC–MS) is the most common instrument in a forensic laboratory, an effective extraction protocol that allows the quantitative measurement of ATCA in biological samples using the GC–MS platform is desirable.

In 2019, the National Academies of Sciences, Engineering, Medicine published a research agenda calling for the transformation and advancement of separation science (The National Academies of Sciences, Engineering, Medicine 2019). The proposal urged for increased scientific research addressing gaps and challenges in selectivity, capacity, and throughput of separation methods. The research and development of using Mag-CNTs/d-μSPE for ATCA extraction closely reflected the proposed agenda. The extraction of ATCA from biological matrices has gradually advanced from conventional SPE and LLE methods to more selective techniques. With the use of LC–MS/MS, Jackson et al. proposed a highly selective molecularly imprinted polymer stir bar sorption extraction (MISBSE) method for ATCA extraction (Jackson et al. 2010). Giebułtowicz et al. compared the performance of different MIPs to a commercially available d-μSPE sorbent (Giebułtowicz et al. 2019). Satisfactory recoveries and sensitivities were obtained from the commercially available d-μSPE sorbent and in some MIPs. And with the rapid advancement in ambient ionization technique, ATCA was detected almost in real-time from sample collection to analysis with minimal sample preparation and consumption (Hisatsune et al. 2020). However, the limited access to tandem mass spectrometry instrumentation due to the high cost might restrict the

applications of these methods to most regional forensic laboratories.

In this work, a conventional SPE method and two magnetic carbon nanotubes (Mag-CNTs) facilitated d-μSPE (Mag-CNTs/d-μSPE) methods were developed for ATCA analysis using GC–MS. Two Mag-CNTs/d-μSPE workflows were developed using two types of Mag-CNTs with different surface modified functional groups, i.e., carboxyl Mag-CNTs (Mag-CNTs-COOH) and sulfonyl Mag-CNTs (Mag-CNTs-SO_3H). The Mag-CNTs-COOH/d-μSPE method was evolved based on the previously published method (Li et al. 2019a) by aiming to exclude the elution step and achieve a one-step desorption/derivatization approach for efficient extraction. The use of phenylsulfonic acid functionalized multi-walled carbon nanotubes (MWCNTs-Ph-SO_3H) has been demonstrated successful for the preconcentration of Ni(II) ions in petrochemical wastewater samples (Shirkhanloo et al. 2019). Another goal in this study was to develop the Mag-CNTs-SO_3H/d-μSPE workflow to facilitate ionic extraction between the negatively charged sulfonyl groups on the Mag-CNTs and the positively charged amino moieties on ATCA. The sensitivity and extraction efficiency of the new workflows were compared to the previously published model (Li et al. 2019a), and a SPE method was developed for comparison purposes. Ultimately, the successful improvement of the new Mag-CNTs/d-μSPE approaches could provide broader applicability in forensic laboratories, especially for polar and ionic targets that are usually presented to be challenging using the GC–MS platform. The repeatability and robustness of the Mag-CNTs/d-μSPE methods were further evaluated according to the guidelines suggested in the approved American National Standard Institute/AAFS Standards Board (ANSI/ASB) Standard 036 for method validation in forensic toxicology (ANSI, ASB 2019).

Methods and materials

Chemicals, reagents, and standards

2-aminothiazoline-4-carboxylic acid (ATCA) was purchased from Chem-Impex International (Wood Dale, IL), and the internal standard, 2-aminothiazoline-4-carboxylic acid-^{13}C, ^{15}N (ATCA-^{13}C, ^{15}N), was purchased from Toronto Research Chemical, Inc. (North York, Canada). Multi-walled carbon nanotubes (MWCNTs) (110–170 nm in diameter, 5–9 μm in length), iron (II) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (III) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA), and ammonium sulfate were obtained from Sigma-Aldrich (St. Louis, MO). All solvents used in the study were at least HPLC grade. Hydrochloric acid (12.1 M), nitric acid (15.9 M), methanol, acetone, dichloromethane (DCM), isopropyl alcohol (IPA), pyridine, ethanol, and ammonium hydroxide (NH_4OH, 14.5 M), were obtained from J.T. Baker (Avantor, Allentown, PA). Sulfuric acid was purchased from Macron Chemicals (Avantor, Allentown, PA), and acetonitrile and hexane were obtained from Fisher Scientific (Hampton, NH). Formic acid (23.6 M) was purchased from Millipore (Billerica, MA), and the deionized (DI) water was obtained through a Merck Millipore water purification system (Burlington, MA). The Cerex Polycrom Clin II SPE columns (3 mL, 35 mg) were obtained from Tecan (Baldwin Park, CA). The synthetic urine, Surine™, was obtained from Cerilliant (Round Rock, TX), and the defibrinated bovine blood was purchased from Quad Five (Ryegate, MT).

Synthesis of Mag-CNTs

Two surface-functionalized Mag-CNTs were developed in this study: Mag-CNTs-COOH and Mag-CNTs-SO_3H. The Mag-CNTs-COOH were prepared according to Li et al. with a slight modification (Li et al. 2019a). Briefly, MWCNTs were first purified with a 1:1 ratio of concentrated nitric acid and concentrated sulfuric acid overnight at 60 °C with stirring to produce carboxy CNTs (CNTs-COOH). Next, the CNTs-COOH were washed in a sequence of a copious amount of (1) DI water, (2) ethanol, and (3) DI water. The rinsed CNTs-COOH were dried at 100 °C overnight. Subsequently, 50 mg of the dried CNTs-COOH were suspended in 25 mL of DI water with 70 mg of $FeCl_2 \cdot 4H_2O$ and 135 mg of $FeCl_3 \cdot 6H_2O$. The suspension was sonicated for 30 min at 50 °C, followed by slow addition of 1 mL concentrated NH_4OH to reach the final pH value of 10–11. After addition, the system was raised to 80 °C and held at the temperature for 30 min. Finally, the suspension was allowed to cool to room temperature, and the Mag-CNTs-COOH were isolated using a strong magnet, followed by washing and drying steps as described previously.

The method for Mag-CNTs-SO_3H synthesis was based on that of the Mag-CNTs-COOH with additional steps. After the CNTs were purified with the concentrated acids and dried, the purified CNTs (~ 1.1 g) and ammonium sulfate (1 g) were added to 20 mL of DI water. The whole suspension was heated up to 235 °C for a maximum of 30 min. The system was then allowed to cool to room temperature before the CNTs-SO_3H were washed with DI water and ethanol and dried. The dried CNTs-SO_3H (100 mg) were suspended in 20 mL of DI water with 180 mg of $FeCl_2 \cdot 4H_2O$ and 350 mg of $FeCl_3 \cdot 6H_2O$ to perform the remaining magnetization process described in the Mag-CNTs-COOH method.

Preparation of internal standard, calibrators, and controls

The stock solutions of ATCA (100 mg/L) and ATCA-^{13}C, ^{15}N (1250 mg/L) were prepared by accurately weighing the appropriate amounts of the chemicals and dissolved in methanol with 2% (v/v) formic acid. The ATCA-^{13}C, ^{15}N working solution (3125 ng/mL) was prepared by dilution with methanol, and 40 µL of this solution was spiked in every sample to yield a concentration of 250 and 62.5 ng/mL for Mag-CNTs-COOH and Mag-CNTs-SO_3H, respectively. Working solutions of ATCA at intermediate concentrations were prepared from the stock solution by dilutions with methanol: 500 and 5000 ng/mL for Mag-CNTs-COOH in both matrices and SPE, 1000 and 10,000 ng/mL for Mag-CNTs-SO_3H in synthetic urine, and 400 and 4000 ng/mL for Mag-CNTs-SO_3H in bovine blood. The working solutions were used in both the development and validation phases. The lower concentration ATCA working solutions were used to prepare calibrators at concentrations of 10, 25, 30, 50, 60, and 100 ng/mL and the low concentration quality controls (QC) samples at 30 and 75 ng/mL. As for the higher concentration working solution, they were used to prepared calibrators at 200, 400, 800, and 1000 ng/mL and QC samples at both low (180 ng/mL), medium (500 ng/mL), and high (800 ng/mL) concentrations. The stock and the working solutions of ATCA and ATCA-^{13}C, ^{15}N were stored at 4 °C.

Optimized Mag-CNTs/d-µSPE procedures

The optimized Mag-CNTs/d-µSPE methods for the two Mag-CNTs followed the general experimental design with slightly different extraction parameters. The extraction processes for the biological matrices were described in the text with the detailed extraction parameters summarized in Table 1.

Synthetic urine samples

ATCA spiked known samples were prepared by adding appropriate concentrations of ATCA and the internal standard (IS), ATCA-^{13}C, ^{15}N, to separate Eppendorf tubes. The mixture was then vortexed, dried at 65 °C, and reconstituted in synthetic urine. The ATCA spiked synthetic urine samples were then vortexed, and 0.1 M HCl and Mag-CNTs were added. The samples were thoroughly vortexed and were subjected to Mag-CNTs/d-µSPE by sonication at room temperature for the corresponding extraction time. After Mag-CNTs/d-µSPE, the samples were briefly centrifuged to acceleration the subsequent separation of Mag-CNTs with the aid of a strong neodymium magnet. For Mag-CNTs-COOH, after the

Table 1 Extraction parameters for the two Mag-CNTs/d-µSPE methods and the SPE methods

	Mag-CNTs-COOH/d-µSPE (Li et al. 2019a)[a]		Mag-CNTs-COOH/d-µSPE		Mag-CNTs-SO_3H/d-µSPE		SPE
Extraction Parameters	**Synthetic urine**	**Bovine blood**	**Synthetic urine**	**Bovine blood**	**Synthetic urine**	**Bovine blood**	**Bovine blood**
Sample Volume (µL)	100	100	500	500	1000	200	500
Amount of 0.1 M HCl	2% (v/v)[b]	2% (v/v)[b]	5% (v/v)	5% (v/v)	5% (v/v)	2.5% (v/v)	1 mL
Amount of sorbent (mg)	2	2	5	5	10	5	35
Extraction Time (min)	10	10	20	20	10	20	N/A
Elution Solvent	5% (v/v) NH_4OH in water	5% (v/v) NH_4OH in water	N/A	N/A	DCM/IPA/NH_4OH (78/20/2)	DCM/IPA/NH_4OH (78/20/2)	5% (v/v) NH_4OH in MeOH
Elution volume	150 µL	150 µL	N/A	N/A	1 mL	1 mL	1 mL
Derivatization solvent	30% (v/v) MSTFA in hexane	30% (v/v) MSTFA in hexane	30% (v/v) MSTFA in acetone	30% (v/v) MSTFA in acetone	30% (v/v) MSTFA in hexane	30% (v/v) MSTFA in hexane	30% (v/v) MSTFA in hexane
Derivatization Volume (µL)	150	150	50	50	50	50	50
Derivatization Time (min)	10	10	10	10	10	10	10
Solvent used[c] (µL)	152	352	25	1150	1050	1440	6100

[a] Mag-CNTs-COOH/d-µSPE method published by Li et al. (2019a)

[b] In this method, 2% (v/v) of 0.1 M formic acid was used instead of HCl

[c] The amount of solvent used did not include derivatization volume

Mag-CNTs were dried at 65 °C under centrifugal evaporation, the ATCA retained on the Mag-CNTs was desorbed and derivatized with 30% (v/v) MSTFA in acetone at 50 °C. After brief centrifugation, the Mag-CNTs were separated by a permanent magnet, and the supernatant was transferred to GC–MS vials fitted with glass inserts for analysis. As for Mag-CNTs-SO_3H, the retained ATCA was first desorbed in an elution system consisting of DCM/IPA/NH_4OH (78/20/2). The eluates were then dried at 50 °C under centrifugal evaporation and derivatized with 30% (v/v) MSTFA in hexane. The derivatized samples were then transferred to separate vials fitted with inserts for GC–MS analysis.

Bovine blood samples

For bovine blood samples, additional protein precipitation was performed before Mag-CNTs/d-μSPE. Briefly, the appropriate concentrations of ATCA and IS were added in conical tubes and dried at 50 °C with a nitrogen gas stream. Bovine blood was then added for reconstitution and to make known ATCA spiked samples. Afterward, methanol (100 μL for Mag-CNTs-COOH and 40 μL for Mag-CNTs-SO_3H) was added and vortexed, followed by ice-cold acetonitrile (1 mL for Mag-CNTs-COOH and 400 μL for Mag-CNTs-SO_3H) with vortexing, the samples were then centrifuged. The supernatant portions were transferred to separate Eppendorf tubes, followed by the addition of 0.1 M HCl and the Mag-CNTs. The remaining workflow of the Mag-CNTs/d-μSPE was as described in the synthetic urine samples. For Mag-CNTs-COOH, the one-step derivation/desorption approach was performed. For Mag-CNTs-SO_3H, the multi-stepped elution and derivatization process was performed as described in the previous section.

SPE procedures

The SPE method was performed in bovine blood samples. In this method, appropriate concentrations of ATCA were dried with the internal standard (200 ng/mL) in conical tubes and dried at 50 °C under a stream of nitrogen gas. The samples were then reconstituted in bovine blood with vortexing. Methanol (100 μL) was added to the blood, vortexed, and 1 mL of ice-cold acetonitrile was added as the samples were vortexing. The samples were centrifuged, and 1 mL of the supernatant layers were transferred to separate disposable culture tubes, and 1 mL of 0.1 M HCl was added and vortexed thoroughly. The samples were transferred to the Cerex Polycrom Clin II SPE columns seated on the SPEware System 48 Positive Pressure Processor. The samples were passed through the column with positive pressure and were then washed with 1 mL of 0.1 M HCl, methanol, and hexane. The columns were then dried with the flow of nitrogen under positive pressure for 5 min and were then eluted with 1 mL 5% (v/v) NH_4OH in methanol. The eluates were dried under a nitrogen stream and derivatized with 30% (v/v) MSTFA in hexane. The derivatized samples were transferred to vials fitted with inserts and subjected to GC–MS analysis.

Method performance

The optimized methods were evaluated following the ANSI/ASB standard for method validation in forensic toxicology (ANSI, ASB 2019). The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the signal-to-noise (S/N) ratio of samples with descending concentrations. The concentrations with a minimum S/N value of 3 and 10 were selected as the LOD and LOQ, respectively. The LOQ concentration was used as the lowest calibrator concentration. Once established, the LOD and LOQ were analyzed in triplicates over three separate runs. The calibration models were evaluated over five runs for both methods. Linearity was obtained using the least square model with an acceptable R^2 value of > 0.99.

The bias (percentage, %) and precision (percent coefficient of variance, %CV) of the method between runs were determined using QC samples at low, medium, and high concentrations (30, 500, and 800 ng/mL for synthetic urine; 180, 500, and 800 ng/mL for bovine blood) in triplicates over five days. Additional within-run precision was also evaluated based on the same QC samples. Bias and precision were considered acceptable within ± 20%. Carryover was assessed over three days by analyzing extracted blank samples in triplicates immediately after testing the highest concentration calibrator. Carryover was deemed to be negligible when the S/N ratio of the analyte was below LOD.

Interference from 34 common drugs of abuse was evaluated in both matrices by spiking the drug mixes to low concentration QC samples to yield a 200 ng/mL concentration. The list of common drugs of abuse was showed in Table 2. Endogenous matrix interference from the bovine blood samples was evaluated by extracting ten different sources of bovine blood spiked with low concentration QC levels. These results were dimmed negligible with the % bias fell within ± 20%. Interference between ATCA and IS was also evaluated by extracting blank matrices only with the addition of the IS and extracting a high concentration QC sample without the addition of the IS. The effect was considered negligible if the ATCA or the IS peaks were below the LOD value with the absence of the other molecule. Extraction efficiency was not a requirement in the validation parameters, but it was determined for comparison purposes. It was determined by

Table 2 A34 Common drugs of abuse used in drug interference study

Basic drug	Barbiturates	Cannabinoids	Neutral drugs	Endogenous compounds
Alprazolam	Phenobarbital	Δ^9-tetrahydrocannabinol	Carbamazepine	β-Hydroxybutyric acid
Amitriptyline	Butalbital	Tetrahydrocannabinolic acid	Carisoprodol	
Caffeine	Secobarbital		Meprobamate	
Cocaine	Amobarbital			
Codeine	Pentobarbital			
Cotinine				
Cyclobenzaprine				
Dextromethorphan				
Diazepam				
Diphenhydramine				
Hydrocodone				
Ketamine				
Methadone				
Morphine				
Nicotine				
Nordiazepam				
Oxazepam				
Oxycodone				
Phencyclidine				
Propoxyphene				
Tramadol				
Zolpidem				
Lorazepam				

the percentage of the experimental value divided by the expected value by:

$$\text{Extraction efficiency (\%)} = \frac{\textit{experimental value}}{\textit{expected value}} \times 100\%$$

A fit-for-purpose validation for the SPE workflow was performed for comparison purposes. The LOD and LOQ values were determined based on the same criteria as described above. The calibration model was evaluated at 25, 50, 100, 200, 400, 800, and 1000 ng/mL over three days alongside the bias and precision study at low (75 ng/mL), medium (500 ng/mL), and high concentrations (800 ng/mL). Extraction efficiency was also evaluated.

Gas chromatography-mass spectrometry

Instrumental analysis was performed using the Agilent GC–MS system that consists of a 7890A series gas chromatograph, a 5975C series mass spectrometer, and a 7683B series auto-sampler (Agilent, Santa Clara, CA). Chromatographic separation was achieved with a DB-5 bonded phase column (30 m × 0.25 mm × 0.25 µm, Agilent, Santa Clara, CA) with helium as a carrier gas at a flow rate of 1 mL/min. The autosampler was set for 1 µL injection, and the injection port was held at 290 °C, lined with a Cyclosplitter liner (Restek, Bellefonte, PA). The total flow was set to 54 mL/min with a septum purge flow of 3 mL/min at a 10:1 split ratio. The GC oven was programmed as follows: initial held at 100 °C for 1 min, elevated at 15 °C/min to 230 °C, then at 30 °C/min to 300 °C, and finally held at 300 °C for 1 min. Using the described method, ATCA-$(TMS)_3$ and ATCA-^{13}C, ^{15}N-$(TMS)_3$ eluted at approximately 8.76 min, with a total run time of 13 min. The mass-selective detector was programmed under selected ion monitoring (SIM) mode for the detection of ATCA-$(TMS)_3$ (245, 347, 362 m/z) and ATCA-^{13}C, ^{15}N-$(TMS)_3$ (248, 350, 365 m/z).

Results

Performance of the optimized Mag-CNTs/d-µSPE method

The optimized Mag-CNTs/d-µSPE methods were evaluated according to the ANSI/ASB guidelines (ANSI, ASB 2019), and the extraction performances for each method were summarized in Table 3. After the residual plots of the calibration models were reviewed and analyzed, the Mag-CNTs-COOH/d-µSPE process followed a non-weighted simple linear regression while the Mag-CNTs-SO_3H/d-µSPE process followed a quadratic model. During the optimization phase and the later validation study, the quadratic calibration model was evaluated to produce minimal impacts on precision and accuracy,

Table 3 Extraction performance of the two Mag-CNTs/d-µSPE methods and the SPE methods

	Mag-CNTs-COOH/d-µSPE (multi-stepped)[a]		Mag-CNTs-COOH/d-µSPE (one-step)		Mag-CNTs-SO_3H/d-µSPE		SPE
Performance	**Synthetic urine**	**Bovine blood**	**Synthetic urine**	**Bovine blood**	**Synthetic urine**	**Bovine blood**	**Bovine blood**
LOD (ng/mL)	15	25	5	10	5	10	1
LOQ (ng/mL)	30	30	10	60	10	60	25
Level of Calibrators	7	6	7	6	7	6	7
Regression Model	Non-weighted linear	Non-weighted linear	Non-weighted linear	Non-weighted linear	Quadratic	Quadratic	Non-weighted linear
R^2 value	0.9985	0.9919	0.9982	0.9983	0.9982	0.9982	0.9980
Linear range	30–1000	30–1000	10–1000	60–1000	10–1000	60–1000	25–1000

[a] Data was obtained from Li et al. (2019a)

especially at the low concentration range. The LOD and LOQ values for both methods were determined to be 5 and 10 ng/mL for synthetic urine and 10 and 60 ng/mL for bovine blood, respectively. The working ranges for the respective matrices were established at 10–1000 ng/mL and 60–1000 ng/mL, with the R^2 values all greater than 0.998. The mean percent bias and the between-run and within-run precision were determined at low, medium, and high concentrations and were reported in Table 4. The % bias values were within the acceptable ± 20% range, and the %CV of the between- and within-run precision were lower than 20% as per the ANSI/ASB guidelines. The extraction efficiency was all above 91%. For method development and validation, the use of bovine blood samples is an acceptable animal equivalent to human blood specimens in forensic toxicology research.

Endogenous interference from the bovine blood was evaluated from ten different bovine blood samples, and the % bias results fell within the required ± 20% range. The interference from 34 common drugs of abuse was considered negligible based on the acceptable bias of ± 20% when the drug mixes were spiked at low concentration QC samples. The interference between ATCA and its stable isotopic IS was also determined to be

Table 4 For the two Mag-CNTs/d-µSPE methods, the mean bias (%), and the between-run and within-run precision (%CV), and extraction efficiency (%) of the QC samples were determined at low (30 ng/mL for synthetic urine, 180 ng/mL for bovine blood), medium (500 ng/mL), and high concentration (800 ng/mL) of synthetic urine and bovine blood samples over five separate sets ($n = 3$)

Methods	Matrices	QC samples	Mean Bias (%, $n = 15$; $n = 9$ for SPE)	Between-run precision (%CV, $n = 15$; $n = 9$ for SPE)	Max within-run precision (%CV, $n = 3$)	Extraction efficiency (%, $n = 15$; $n = 9$ for SPE)
Mag-CNTs-COOH/d-µSPE	Synthetic Urine	Low	− 8.30	10.63	12.7	91.70
		Medium	3.06	4.60	4.18	103.06
		High	1.72	2.58	3.53	101.72
	Bovine Blood	Low	3.68	3.32	4.20	103.70
		Medium	1.79	6.33	9.44	101.79
		High	0.72	2.95	5.47	100.73
Mag-CNTs-SO_3H/d-µSPE	Synthetic Urine	Low	− 8.55	9.17	7.45	91.45
		Medium	− 4.61	7.50	7.80	95.39
		High	− 2.63	10.21	10.44	97.37
	Bovine Blood	Low	2.55	8.04	16.47	102.55
		Medium	2.95	8.32	10.29	102.95
		High	2.09	10.39	9.22	102.09
SPE	Bovine Blood	Low	− 5.92	3.14	4.26	94.08
		Medium	5.24	7.73	9.35	105.23
		High	0.91	2.95	5.44	100.91

For SPE method, the parameters were determined at low (75 ng/mL), medium (500 ng/mL), and high (800 ng/mL) concentration in bovine blood samples over three sets ($n = 3$)

insignificant. No peaks of ATCA higher than the LOD value were detected when blank samples were extracted only with the IS. Similar results for IS peaks were obtained when extracting at a high concentration QC sample without the addition of the IS.

Performance of the SPE method

The calibration model, the LOD and LOQ values, and the bias and precision study were conducted in bovine blood samples only for the SPE method. Seven non-zero calibrators were involved in constructing the calibration model with a non-weighted simple linear regression with an average R^2 value of 0.998. The LOD and LOQ values were 1 and 25 ng/mL, respectively, and the working range of 25–1000 ng/mL was established for the SPE method. The bias, precision, and extraction efficiency studies were conducted at low, medium, and high concentrations (three sets, $n = 3$), and the results were shown in Table 4. The mean % bias was within the ± 20% range, and the within-run and between-run %CV were less than 20%.

Discussion

Assay efficiency

The development of the Mag-CNTs-COOH/d-µSPE method in this study aimed to enhance the overall throughput and efficiency of the previously published method (Li et al. 2019a) by testing the possibility of performing derivatization and desorption in a single step. The choice of desorption solvent was critical in achieving the one-step derivatization/desorption approach to improve the workflow's efficiency. Since the derivatization was performed by MSTFA, the coupling desorption solvent cannot contain any hydroxyl, carboxyl, or amino functional groups. Otherwise, the desorption solvent could be preferentially derivatized due to the high abundance compared to the target analyte. Different solvents were tested during a preliminary study to determine a suitable solvent to pair with the derivatization agent. The desorption/derivatization system using MFTFA/acetone yielded reproducible signals with sufficient polarity to desorbed the ATCA from Mag-CNTs-COOH and demonstrated suitable sensitivity. Since the desorption/derivatization process was conducted in a closed Eppendorf tube, no significantly high volumetric loss of the acetone/MSTFA system was observed when the process was conducted at 50 °C, which is close to the boiling point of acetone (56 °C) (O'Neil 2013). It is worth mentioning that the polarity of the coupling solvent played an essential role in the proposed desorption/derivatization approach. Hexane was previously used in the multi-stepped approach to couple with MSTFA for derivatization (Li et al. 2019a), and it was tested as a possible desorption solvent. As expected, no ATCA and the internal standard above the detectable level were obtained. This result might provide additional support to Li et al. that the significant interaction between ATCA and Mag-CNTs-COOH was through hydrogen bonding (Fig. 1A) (Li et al. 2019a). Hexane is too non-polar to interrupt the interact and desorb the charged ATCA or the IS from the Mag-CNTs. The one-step desorption/derivatization approach was not successful with the Mag-CNT-SO_3H/d-µSPE method with the tested solvent systems. It is hypothesized that the mechanism for the ATCA extraction by Mag-CNTs-SO_3H was based on the ionic interactions between the $–SO_3^-$ groups on the Mag-CNTs-SO_3H and the charged amino moieties on ATCA (Fig. 1b). The charged ATCA could not be desorbed merely by the difference in polarity of the desorption solvent. As a result, the optimized Mag-CNTs-SO_3H/d-µSPE method followed a multi-stepped extraction approach similar to the one previously published using Mag-CNTs-COOH by Li et al. (Li et al. 2019a).

The optimized Mag-CNTs-COOH/d-µSPE eliminated the elution step required before derivatization. This "shortcut" improved the efficiency of the Mag-CNTs/d-µSPE process and reduced solvent consumption and waste simultaneously. In the previously published multi-step approach with Mag-CNTs-COOH, 5% (v/v)

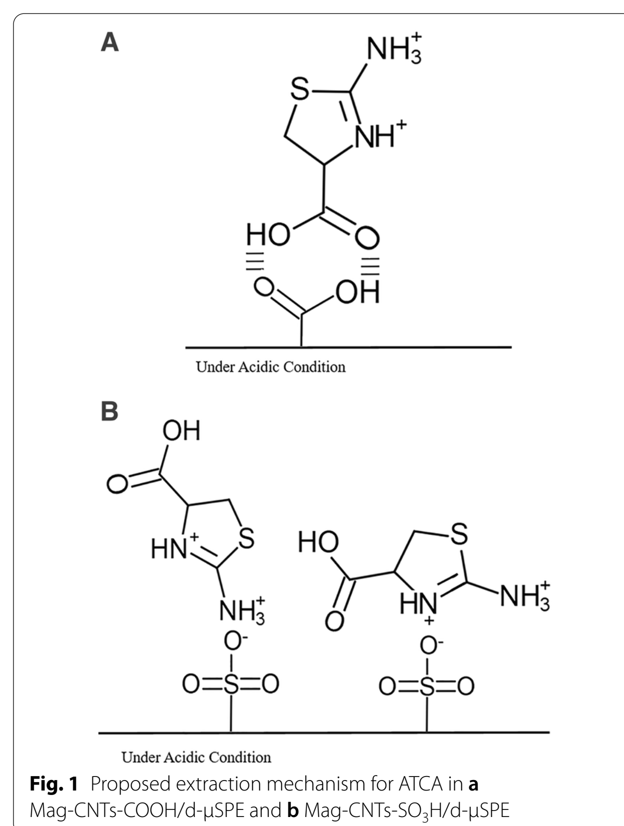

Fig. 1 Proposed extraction mechanism for ATCA in **a** Mag-CNTs-COOH/d-µSPE and **b** Mag-CNTs-SO_3H/d-µSPE

NH_4OH in DI water was used to first desorb the ATCA from Mag-CNTs, followed by derivatization. Besides, to prevent potential ring-opening of ATCA under heat and basic conditions (Lundquist et al. 1995; Bradham et al. 1965), the desorbed extracts were evaporated at a relatively low temperature (65 °C) with centrifugal evaporation before derivatization. The high boiling point of DI water required an average of 120 min of evaporation time for the 150 µL desorbed extracts due to the relatively low-temperature setting. To improve efficiency in the multi-stepped desorption approach, an organic solvent system consisting of DCM/IPA/NH_4OH was used in the Mag-CNTs-SO_3H/d-µSPE method to reduce evaporation time to about 25–30 min for both matrices. As for the advanced Mag-CNTs-COOH/d-µSPE, the evaporation time was also significantly reduced. It took approximately 30 min in bovine blood samples and 50 min in synthetic urine samples to dry the Mag-CNTs-COOH. The main reason for such drastic reduction was that most supernatants were removed from the Mag-CNTs before the derivatization/desorption step. The evaporation was especially efficient in bovine blood samples because the supernatant portion mainly consists of organic solvent (acetonitrile) after protein precipitation. It has a lower boiling point than synthetic urine, which consists primarily of water. The combination of derivatization and desorption steps eliminates the need for additional elution solvents and shortens the time required for solvent evaporation in a multi-stepped extraction process. The total time for performing the multi-stepped Mag-CNTs-COOH/d-µSPE procedure could take up to 8 h to analyze a sample batch of 30, excluding the time of preparing calibrators and controls. With the improved one-step Mag-CNTs-COOH/d-µSPE and Mag-CNTs-SO_3H/d-µSPE approach, the extraction time was reduced to 5–6 h for the same amount of sample. Although a slightly higher amount of Mag-CNTs-COOH and ten extra minutes of extraction time were needed in the one-step derivatization/desorption approach compared to the previously published procedure, the new approach provided an improved extraction efficiency.

However, since the Mag-CNTs/d-µSPE processes were not automated, efforts had to be invested in ensuring well dispersion of the Mag-CNTs in all extraction, desorption, and derivatization steps. Taking a sample size of 30 as an example, each dispersion process for the Mag-CNTs took 25–30 min in addition to the extraction or desorption time. With the aid of the automated SPE platform, the extraction time from loading samples to the columns to elute the analytes was under 30 min with the same sample size. This might show why conventional SPE methods are preferred and widely applied in forensic laboratories. Although the use of solvents in the SPE method was at least four times higher than the two other blood methods using Mag-CNTs (Table 1), the high throughput of sample extraction might offset the drawbacks regarding the high solvent consumption and waste handling in most forensic laboratories.

Assay sensitivity

Another objective of our study was to determine if the one-step derivatization/desorption approach and surface modification of the Mag-CNTs could enhance the detection limits of the overall assays. In the two proposed Mag-CNTs/d-µSPE methods, the end derivatization volume was reduced to 50 µL to improve the pre-concentration factor. The pre-concentration factor is the analyte's concentration ratio in the initial solution to the final extract that is ready to be analyzed. Among factors affecting the pre-concentration, initial sample volume and the final extract volume are the most critical parameters to consider since the assay's sensitivity will be directly impacted. The higher the folds of difference between the initial and final extract volume, the higher the pre-concentration factor. Different sample and derivatization volumes were among the optimized extraction parameters in the preliminary study, and the LOD values were improved with the final derivatization volume of 50 µL (Table 3). The LOD and LOQ values for synthetic urine samples improved from 15 and 30 ng/mL in the multi-step approach (Li et al. 2019a) to 5 and 10 ng/mL, respectively. Note that the lower LOD and LOQ were achieved because a larger sample volume could be adopted in the optimized new method. As for the bovine blood samples, only the LOD value was improved from 25 ng/mL to 10 ng/mL, and a slightly higher LOQ value (60 ng/mL) was obtained when compared to the 30 ng/mL in the previously published method. The one-step derivatization/desorption approach using Mag-CNTs-COOH and Mag-CNTs-SO_3H/d-µSPE method provided a simpler and more time-efficient way for ATCA analysis without losing the sensitivity of Mag-CNTs/d-µSPE.

It is interesting to note that the LOD and LOQ values of the Mag-CNTs-SO_3H/d-µSPE method were identical to the Mag-CNTs-COOH/d-µSPE method despite the differences in some optimized extraction parameters (Table 1). At first glance, it is believed that the equivalent sensitivity might be due to instrumental limitation from the GC–MS; however, much lower LOD and LOQ values were obtained after the sensitivity of the SPE method was tested (Table 3). Upon closer examination of the extraction parameters, it was worth noticing that the sorbent to extraction volume ratios in the Mag-CNTs/d-µSPE methods (0.5–1.25%) were much lower than that in the SPE method (3.5%). In this study, the highest amount of Mag-CNTs tested during optimization was 10 mg. The

optimization results showed no significant difference to that conducted with 5 mg of Mag-CNTs. Therefore, it is possible that the sensitivity of the Mag-CNTs/d-μSPE methods could further be enhanced when performed at a higher sorbent to extraction volume ratio similar to the SPE method.

It is most ideal for developing assays to cover the analyte's concentration range when applied to authentic samples. From previously published studies, the endogenous ATCA level in human urine (non-smokers) and blood samples ranged from 38–251.4 ng/mL and 55–213 ng/mL, respectively (Vinnakota et al. 2012; Li et al. 2019b; Logue et al. 2005). Elevated ATCA concentration, ranging from 790 to 2070 ng/mL, was found in the blood samples of cyanide-intoxicated victims (Rużycka et al. 2017; Li et al. 2019b; Giebułtowicz et al. 2016). No ATCA concentration associated with cyanide-intoxicated in urine samples were published, but an elevated ATCA concentration (233.8–935.4 ng/mL) was found in people consuming a high cyanide exposure diet (Li et al. 2019b; Lundquist et al. 1995). The SPE method developed in this comparison study provided accurate and precise detection and quantitation of ATCA at the endogenous and slightly elevated level. As for the proposed Mag-CNTs/d-μSPE assays, the working concentration range of ATCA for synthetic urine (10–1000 ng/mL) covers both the endogenous and elevated levels of ATCA. The improved approach for analyzing blood samples (60–1000 ng/mL) might not provide accurate and precise quantitation at the lower end of the endogenous ATCA level; however, the limit of detection of 10 ng/mL is sufficient for the detection of endogenous ATCA to avoid false-negative scenarios. As for elevated concentrations above the upper limit of the calibration model, samples could be diluted within the calibration range for accurate quantitation.

The optimized one-step derivatization/desorption method using Mag-CNTs-COOH and Mag-CNTs-SO_3H/d-μSPE method were evaluated according to the ANSI/ASB guidelines. The analytical performances of the Mag-CNTs/d-μSPE/GC–MS methods for ATCA analysis showed excellent accuracy, precision, extraction efficiency, and sensitivity. For the interference study, the effects from the IS, 34 other common drugs of abuse, and different sources of bovine blood matrix were found to be insignificant. Synthetic urine samples were used in the optimization and validation studies because endogenous ATCA is present in human urine and blood sample and can vary based on smoking habits and dietary consumption (Vinnakota et al. 2012; Li et al. 2019b; Logue et al. 2005). The endogenous ATCA might significantly affect the determination of LOD, LOQ, and potential interference with the bias and precision of the assay. Thus, human urine was not considered to be a suitable matrix in this study. And since the variations in enzyme and protein levels are accounted for in synthetic urine, different sources of matrix interference were not examined. The fit-for-purpose validation for the SPE method also demonstrated good precision, accuracy, sensitivity, and extraction efficiency on the limited tested parameters. These successful results from the SPE method provide an easier-to-adapt tool for forensic laboratories to establish ATCA analysis as potential routine testing for cyanide intoxication in post-mortem blood samples.

Regarding ATCA studies from post-mortem forensic samples, one research team has reported an elevated ATCA concentration in confirmed cyanide poisoned victims (Rużycka et al. 2017; Giebułtowicz et al. 2016; Luliński et al. 2015). The majority of the post-mortem ATCA analyses focused on measuring endogenous ATCA levels in autopsy blood samples collected from non-cyanide-related cases and fire victims. These data confirmed an elevated ATCA concentration could be detected from the autopsy blood due to the potential exposure of cyanide produced in fire scenes (Rużycka et al. 2017; Hisatsune et al. 2020; Luliński et al. 2015). The promising results of the Mag-CNTs/d-μSPE/GC–MS assay in synthetic urine and bovine blood samples could encourage inter-agency research to accelerate the groundwork for the use of ATCA as forensic evidence in death investigation related to cyanide exposure. In addition, the development of Mag-CNTs-SO_3H/d-μSPE further expanded the application of Mag-CNTs/d-μSPE to facilitate ionic extraction in forensic toxicology analysis. Method development and validation for the ionic extraction of common basic controlled substances using Mag-CNTs-SO_3H/d-μSPE are underway when this article is prepared.

Conclusions

The one-step derivatization/desorption approach of the Mag-CNTs-COOH/d-μSPE significantly decreased the solvent requirement, waste, and overall extraction time from the previous multi-step procedure. A higher sensitivity was also obtained through the one-step derivatization/desorption workflow. The Mag-CNTs-SO_3H/d-μSPE method showed comparable sensitivity and performance to the Mag-CNTs-COOH/d-μSPE and expanded the possibilities for ionic extraction in forensic toxicology analysis. Excellent robustness and repeatability were demonstrated by both Mag-CNTs/d-μSPE methods by fulfilling the ANSI/ASB validation requirements. The Mag-CNTs/d-μSPE methods coupled with GC–MS analysis have shown excellent potential as an alternative sample preparation technique applied to forensic toxicology and drug chemistry for polar and ionic analytes analysis using standard GC–MS

instrumentation. The partially validated SPE method provided an additional tool for a higher throughput adaptation for ATCA analysis. ATCA has not yet been accepted as forensic evidence in medical-legal settings in the United States. The novel workflow reported in this work will assist accredited forensic laboratories in quantifying ATCA in biological samples. More inter-laboratory studies of measuring ATCA levels in forensic samples of urine and blood will provide more data to verify the use of ATCA as forensic evidence in cyanide death investigation when the cyanide detection window has passed. Moreover, the Mag-CNTs/d-μSPE methods showed great potential to extract polar or ionic metabolites from biological matrices. Applications of magnetic nanomaterials for in vivo and in vitro studies in analytical chemistry and medicinal science are expected. Further research of using the new extraction process to other ionic analytes will further extend the application of Mag-CNTs-SO_3H/d-μSPE to the ionic extraction system.

Abbreviations
ATCA: 2-Aminothiazoline-4-carboxylic acid; ATCA-^{13}C, ^{15}N: 2-Aminothiazoline-4-carboxylic acid-^{13}C, ^{15}N; ANSI/ASB: American National Standard Institute/AAFS Standards Board; CNT: Carbon nanotubes; Mag-CNTs-COOH: Carboxyl magnetic carbon nanotubes; DI: Deionized; d-μSPE: Dispersive-micro solid phase extraction; GC–MS: Gas chromatography-mass spectrometry; LOD: Limit of detection; LOQ: Limit of quantitation; LLE: Liquid–liquid extraction; LC–MS/MS: Liquid chromatography-tandem mass spectrometry; Mag-CNTs: Magnetic carbon nanotubes; Mag-CNTs/d-μSPE: Mag-CNT facilitated d-μSPE; MISBSE: Molecularly imprinted polymer stir bar sorption extraction; MIP: Molecularly imprinted polymers; MWCNTs: Multi-walled carbon nanotubes; MSTFA: *N*-methyl-*N*-trimethylsilyl-trifluoroacetoamide; %CV: Percent coefficient of variance; MWCNTs-Ph-SO_3H: Phenylsulfonic acid functionalized multi-walled carbon nanotubes; S/N: Signal to noise ratio; SIM: Selected ion monitoring; SPE: Solid phase extraction; CNTs-SO_3H: Sulfonyl carbon nanotubes; Mag-CNTs-SO_3H: Sulfonyl magnetic carbon nanotubes; QC: Quality control.

Acknowledgements
We gratefully acknowledge the Department of Forensic Science at Sam Houston State University for the support in conducting this research.

Authors' contributions
SYL performed the experiment in method development and optimization, analyzed and interpret the data, and was the major contributor in writing the manuscript. IP provided consultations for ATCA analysis. JY oversaw and monitored the experimental and data interpretation processes and edited and formatted the manuscript. All authors read and approved the final manuscript.

Funding
The author received funding from the Department of Forensic Science at Sam Houston State University in conducting the research.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests
The authors declare that they have no competing interest.

Author details
[1]Department of Forensic Science, Sam Houston State University, Huntsville, TX 77341, USA. [2]Department of Chemistry, Sam Houston State University, Huntsville, TX 77341, USA.

Received: 20 April 2021 Accepted: 17 September 2021
Published online: 24 September 2021

References
Abd Wahib SM, Wan Ibrahim WA, Sanagi MM, Kamboh MA, Abdul Keyon AS. Magnetic sporopollenin-cyanopropyltriethoxysilane-dispersive micro-solid phase extraction coupled with high performance liquid chromatography for the determination of selected non-steroidal anti-inflammatory drugs in water samples. J Chromatogr A. 2018;1532:50–7. https://doi.org/10.1016/j.chroma.2017.11.059.

The National Academies of Sciences, Engineering, Medicine (2019) A research agenda for transforming separation science. Washington, DC. https://doi.org/10.17226/25421.

Ahmadi M, Elmongy H, Madrakian T, Abdel-Rehim M. Nanomaterials as sorbents for sample preparation in bioanalysis: a review. Anal Chim Acta. 2017;958:1–21. https://doi.org/10.1016/j.aca.2016.11.062.

Ansell M, Lewis FAS. Review of cyanide concentrations found in human organs. Survey of literature concenrning cyanide metabolism, "normal", nonfatal, and fatal body cyanide levels. J Forensic Med. 1970;17:148–55.

ANSI/ASB, Standard Practices for Method Validation in Forensic Toxicology, ANSI/ASB Standard 036, First Edition, AAFS Standards Board, Colorado Springs, CO, 2019.

Ballantyne B. In vitro production of cyanide in normal human blood and the influence of thiocyanate and storage temperature. Clin Toxicol. 1977;11(2):173–93. https://doi.org/10.3109/15563657708989832.

Bradham LS, Catsimpoolas N, Wood JL. Determination of 2-iminothiazolidine-4-carboxylic acid. Anal Biochem. 1965;11:230–7. https://doi.org/10.1016/0003-2697(65)90010-2.

Coopman V, Van Geert P, Jan C, Peter B, Van Serge C. A case of acute intoxication due to combined use of fentanyl and 3,4-dichloro-N-(2-(dimethylamino)cyclohexyl)-N-methylbenzamide (U-47700). Forensic Sci Int. 2016;266:68–72. https://doi.org/10.1016/j.forsciint.2016.05.001.

Cruz Fernandes V, Pacheco FM, JG, Fernandes Domingues V, Delerue-Matos C, . Evaluation of the QuEChERS and magnetic micro dispersive solid-phase extraction of brominated flame retardants in red fruits with determination by GC/MS. Food Chem. 2020;309: 125572. https://doi.org/10.1016/j.foodchem.2019.125572.

Fernandes VC, Freitas M, Pacheco JPG, Oliveira JM, Domingues VF, Delerue-Matos C. Magnetic dispersive micro solid-phase extraction and gas chromatography determination of organophosphorus pesticides in strawberries. J Chromatogr A. 2018;1566:1–12. https://doi.org/10.1016/j.chroma.2018.06.045.

Fikarová K, Horstkotte B, Sklenářová H, Švec F, Solich P. Automated continuous-flow in-syringe dispersive liquid-liquid microextraction of mono-nitrophenols from large sample volumes using a novel approach to multivariate spectral analysis. Talanta. 2019;202:11–20. https://doi.org/10.1016/j.talanta.2019.04.044.

Ghorbani M, Aghamohammadhassan M, Chamsaz M, Akhlaghi H, Pedramrad T. Dispersive solid phase microextraction. TrAC Trends Anal Chem. 2019;118:793–809. https://doi.org/10.1016/j.trac.2019.07.012.

Giebułtowicz J, Rużycka M, Fudalej M, Krajewski P, Wroczyński P. LC-MS/MS method development and validation for quantitative analyses of 2-aminothiazoline-4-carboxylic acid—a new cyanide exposure marker in post mortem blood. Talanta. 2016;150:586–92. https://doi.org/10.1016/j.talanta.2015.12.076.

Giebułtowicz J, Sobiech M, Rużycka M, Luliński P. Theoretical and experimental approach to hydrophilic interaction dispersive solid-phase extraction of 2-aminothiazoline-4-carboxylic acid from human post-mortem blood. J Chromgr A. 2019;1587:61–72. https://doi.org/10.1016/j.chroma.2018.12.028.

Glicksberg L, Bryand K, Kerrigan S. Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. J Chromatogr B. 2016;1035:91–103. https://doi.org/10.1016/j.jchromb.2016.09.027.

Gottas A, Oiestad EL, Boix F, Vindenes V, Ripel A. C.H. Thaulow, J. Morland, Levels of heroin and its metabolites in blood and brain extracellular fluid after i.v. heroin administration to freely moving rats. Br J Pharmacol. 2013;170:546–56. https://doi.org/10.1111/bph.12305.

Gyamfi OA, Bortey-Sam N, Mahon SB, Brenner M, Rockwood GA, Logue BA. Metabolism of cyanide by glutathione to produce the novel cyanide metabolite 2-aminothiazoline-4-oxoaminoethanoic acid. Chem Res Toxicol. 2019;32(4):718–26. https://doi.org/10.1021/acs.chemrestox.8b00384.

Hartung R. Cyanides and nitriles. In: Clayton GD, Clayton E, editors. Patty's industrial hygiene and toxicology, vol. 2C. New York: Wiley; 1982. p. 4845–900.

Hisatsune K, Murata T, Ogata K, Hida M, Ishii A, Tsuchihashi H, Hayashi Y, Zaitsu K. RECiQ: a rapid and easy method for determining cyanide intoxication by cyanide and 2-aminothiazoline-4-carboxylic acid quantification in the human blood using probe electrospray ionization tandem mass spectrometry. ACS Omega. 2020;5:23351–7. https://doi.org/10.1021/acsomega.0c03229.

Jackson R, Petrikovics I, Lai EPC, Yu JCC. Molecularly imprinted polymer stir bar sorption extraction and electrospray ionization tandem mass spectrometry for determination of 2-aminothiazoline-4-carboxylic acid as a marker for cyanide exposure in forensic urine analysis. Anal Methods. 2010;2:552–7. https://doi.org/10.1039/b9ay00251k.

Jalilian N, Ebrahimzadeh H, Asgharinezhad AA. Dispersive micro-solid phase extraction of aromatic amines based on an efficient sorbent made from poly(1,8-diaminonaphtalen) and magnetic multiwalled carbon nanotubes composite. J Chromatogr A. 2017;1499:38–47. https://doi.org/10.1016/j.chroma.2017.03.087.

Jiang M, Zhang J, Mei S, Shi Y, Zou L, Zhu Y, Dai K, Lu B. Direct enrichment and high performance liquid chromatography analysis of ultra-trace bisphenol A in water samples with narrowly dispersible bisphenol A imprinted polymeric microspheres column. J Chromatogr A. 2006;1110(1):27–34. https://doi.org/10.1016/j.chroma.2006.01.051.

Khezeli T, Daneshfar A. Development of dispersive micro-solid phase extraction based on micro and nano sorbents. TrAC Trends Anal Chem. 2017;89:99–118. https://doi.org/10.1016/j.trac.2017.01.004.

Li SY, Petrikovics I, Yu J. Development of magnetic carbon nanotubes for dispersive micro solid phase extraction of the cyanide metabolite, 2-aminothiazoline-4-carboxylic acid, in biological samples. J Chromgr B. 2019a;1109:67–75. https://doi.org/10.1016/j.jchromb.2019.01.020.

Li SY, Petrikovics I, Yu J. The potential use of 2-aminothiazoline-4-carboxylic acid (ATCA) as a forensic marker for cyanide exposure in medicolegal death investigation: a review. Forensic Sci Rev. 2019b;31:45–58.

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. J Chromatogr B. 2005;819(2):237–44. https://doi.org/10.1016/j.jchromb.2005.01.045.

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. Toxicol Mech Methods. 2009;19(3):202–8. https://doi.org/10.1080/15376510802488165.

Logue BA, Hinkens DM, Baskin SI, Rockwood GA. The analysis of cyanide and its breakdown products in biological samples. Crit Rev Anal Chem. 2010;40(2):122–47. https://doi.org/10.1080/10408340903535315.

Luliński P, Giebułtowicz J, Wroczyński P, Maciejewska D. A highly selective molecularly imprinted sorbent for extraction of 2-aminothiazoline-4-carboxylic acid—synthesis, characterization and application in post-mortem whole blood analysis. J Chromatogr A. 2015;1420:16–25. https://doi.org/10.1016/j.chroma.2015.09.083.

Lundquist P, Kagedal B, Nilsson L, Rosling H. Analysis of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine by high-performance liquid chromatography. Anal Biochem. 1995;228:27–34. https://doi.org/10.1006/abio.1995.1310.

Mafra G, Vieira AA, Merib J, Anderson JL, Carasek E. Single drop microextraction in a 96-well plate format: a step toward automated and high-throughput analysis. Anal Chim Acta. 2019;1063:159–66. https://doi.org/10.1016/j.aca.2019.02.013.

Medina DAV, Santos-Neto ÁJ, Cerdà V, Maya F. Automated dispersive liquid-liquid microextraction based on the solidification of the organic phase. Talanta. 2018;189:241–8. https://doi.org/10.1016/j.talanta.2018.06.081.

O'Neil M (eds) (2013) The merck index—an encyclopedia of chemicals, drugs, and biologicals, London, UK

Palmquist KB, Swortwood MJ. Data-independent screening method for 14 fentanyl analogs in whole blood and oral fluid using LC-QTOF-MS. Forensic Sci Int. 2019;297:189–97. https://doi.org/10.1016/j.forsciint.2019.02.006.

Pichon V, Bouzige M, Miège C, Hennion M-C. Immunosorbents: natural molecular recognition materials for sample preparation of complex environmental matrices. TrAC Trends Anal Chem. 1999;18(3):219–35. https://doi.org/10.1016/S0165-9936(98)00120-4.

Płotka-Wasylka J, Szczepańska N, de la Guardia M, Namieśnik J. Miniaturized solid-phase extraction techniques. TrAC Trends in Anal Chem. 2015;73:19–38. https://doi.org/10.1016/j.trac.2015.04.026.

Płotka-Wasylka J, Szczepańska N, de la Guardia M, Namieśnik J. Modern trends in solid phase extraction: new sorbent media. TrAC Trends Anal Chem. 2016;77:23–43. https://doi.org/10.1016/j.trac.2015.10.010.

Rużycka M, Giebułtowicz J, Fudalej M, Krajewski P, Wroczyński P. Application of 2-aminothiazoline-4-carboxylic acid as a forensic marker of cyanide exposure. Chem Res Toxicol. 2017;30(2):516–23. https://doi.org/10.1021/acs.chemrestox.6b00219.

Saito K, Kikuchi Y, Saito R. Solid-phase dispersive extraction method for analysis of benzodiazepine drugs in serum and urine samples. J Pharm Biomed Anal. 2014;100:28–32. https://doi.org/10.1016/j.jpba.2014.07.020.

Shi Z, Jiang J, Pang W, Ma H, Chu X, Zhou C, Zhang H. Dispersive micro-solid phase extraction using cotton based carbon fiber sorbent for the determination of three polycyclic aromatic hydrocarbons in tea infusion by gas chromatography-quadrupole mass spectrometry. Microchem J. 2019;151:104209. https://doi.org/10.1016/j.microc.2019.104209.

Shirkhanloo H, Merchant K, Mobarake MD. Ultrasound-assisted solid-liquid trap phase extraction based on functionalized multi wall carbon nanotubes for preconcentration and separation of nickel in petrochemical waste water. J Anal Chem. 2019;74(9):865–76. https://doi.org/10.1134/S1061934819090090.

Skillman B, Kerrigan S. Quantification of suvorexant in blood using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. J Chromatogr B. 2018;1091:87–95. https://doi.org/10.1016/j.jchromb.2018.05.006.

Smith CR, Truver MT, Swortwood MJ. Quantification of U-47700 and its metabolites in plasma by LC-MS/MS. J Chromatogr B. 2019;1112:41–7. https://doi.org/10.1016/j.jchromb.2019.02.026.

Sun T, Ali MM, Wang D, Du Z. On-site rapid screening of benzodiazepines in dietary supplements using pipette-tip micro-solid phase extraction coupled to ion mobility spectrometry. J Chromatogr A. 2020;1610: 460547. https://doi.org/10.1016/j.chroma.2019.460547.

Truver MT, Palmquist KB, Swortwood MJ. Oral fluid and drug impairment: pairing toxicology with drug recognition expert observations. J Anal Toxicol. 2019;43:637–43. https://doi.org/10.1093/jat/bkz075.

Vinnakota CV, Peetha NS, Perrizo MG, Ferris DG, Oda RP, Rockwood GA, Logue BA. Comparison of cyanide exposure markers in the biofluids of smokers and non-smokers. Biomarkers. 2012;17(7):625–33. https://doi.org/10.3109/1354750x.2012.709880.

Wen Y, Chen L, Li J, Liu D, Chen L. Recent advances in solid-phase sorbents for sample preparation prior to chromatographic analysis. TrAC Trends Anal Chem. 2014;59:26–41. https://doi.org/10.1016/j.trac.2014.03.011.

Wood JL, Cooley SL. Detoxication of cyanide by cystine. J Biol Chem. 1956;218(1):449–57.

Yu JCC, Martin S, Nasr J, Stafford K, Thompson D, Petrikovics I. LC-MS/MS analysis of 2-aminothiazoline-4-carboxylic acid as a forensic biomarker for cyanide poisoning. World J Methodol. 2012;2(5):33–41. https://doi.org/10.5662/wjm.v2.i5.33.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.