Advanced extraction method for cyanide metabolite using magnetic carbon nanotubes facilitated dispersive micro solid phase extraction (Mag-CNT/d-µSPE)

Sun Yi Li, B.S.*; Jorn Yu, Ph.D., D-ABC

Department of Forensic Science
Sam Houston State University
Huntsville, TX, USA

FSF Emerging Forensic Scientist Award
Paper Presentation

Disclaimer

- The authors have no conflict of interests to disclose

Introduction

Cyanides (CN)

- AAPCC 2017 Annual Report (Gummin et al. 2018)
  - 154 single-substance CN exposure cases
    - 72% accidental, ~8% intentional
  - 71 cases confirmed with other substances

- Sources of exposure:
  - Fire smoke
  - Industrial: ≥ 300,000 tons produced in US yearly
  - Pesticides
  - Natural: seeds or root of plants containing amygdalin
  - E-cigarette cartridge?

Difficulties in Exposure Confirmation

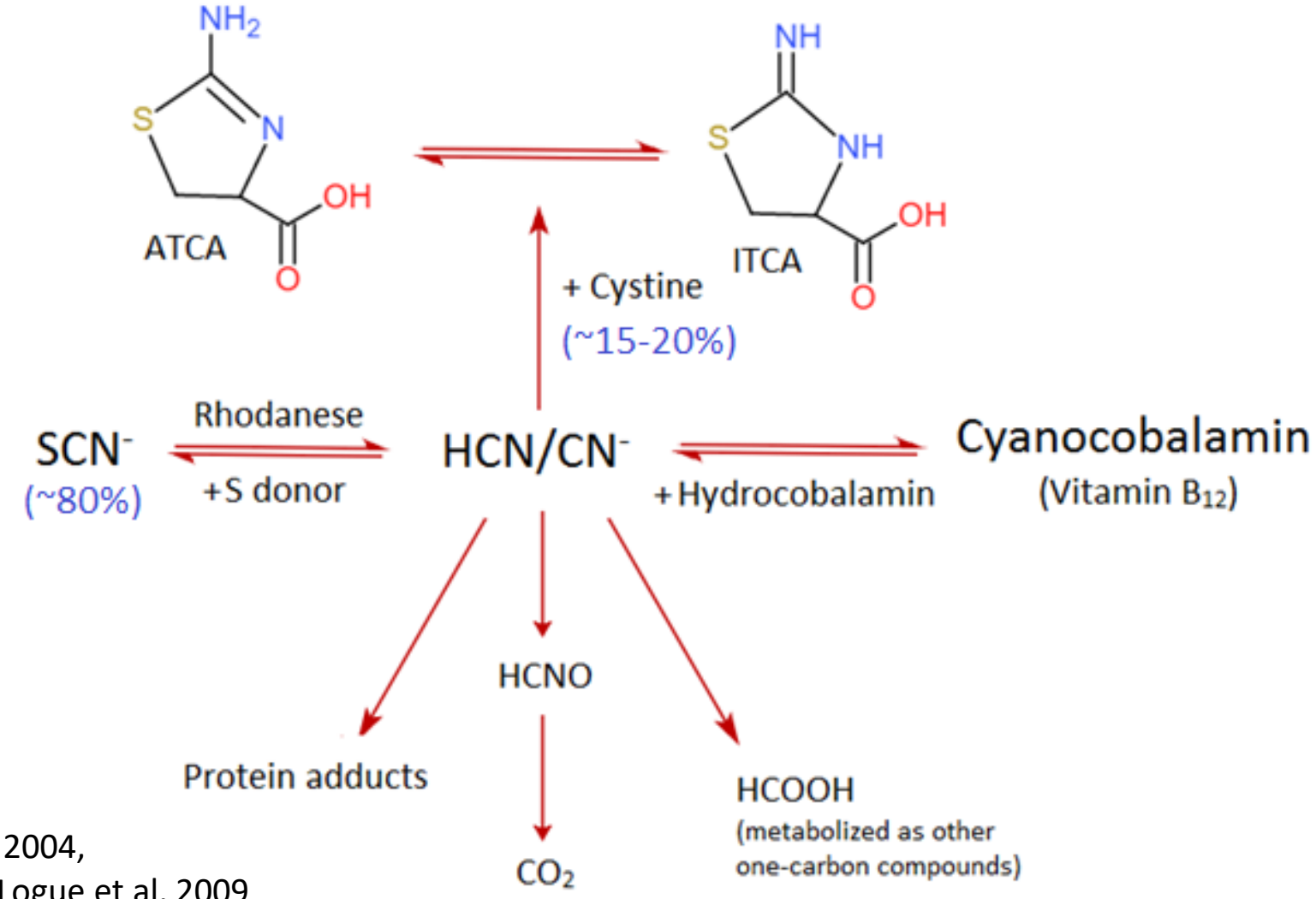
- Lack of conclusive autopsy findings (Gill et al. 2014)
  - "bitter-almond" odor, "pink" lividity

- Subjected to interpretation errors
  - CN^- → HCN (pKa = 9.2) in body
    - Highly volatile and short $t_{1/2}$ (0.34 – 1.28 h) (Logue et al. 2010)

  - Unstable upon storage (Ružycka et al. 2004)
    - ~40% loss in -80°C after one day
    - Post-mortem production of CN
      - ~1.5X increase in later stage of storage

Alternative Approaches

- Minor Metabolite: 2-aminothiazoline-4-carboxylic acid (ATCA)



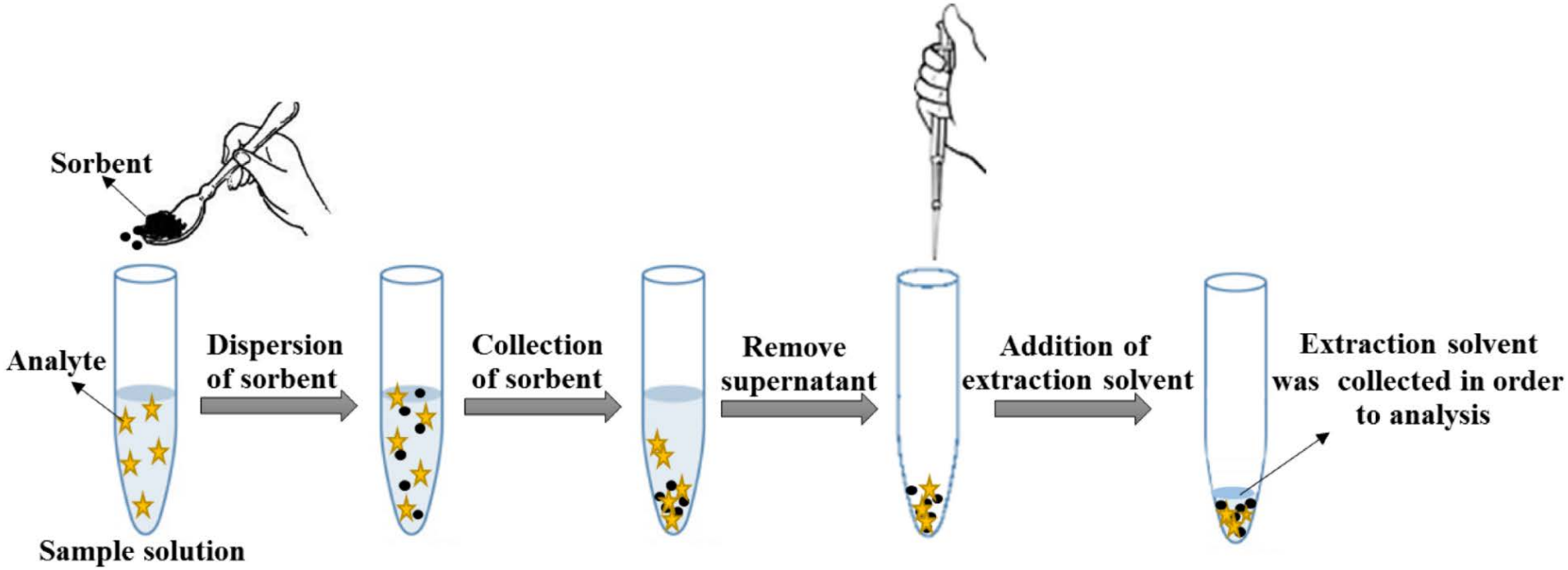
Adopted from Baskin et al. 2004, Nagasawa et al. 2004, and Logue et al. 2009

Previous Studies of ATCA

- Animal model (mice) (Yu et al. 2012)
  - Proportional increase of ATCA with KCN injection
- Fire and CN exposed victims (Ružycka et al. 2016, Giebułtowicz et al. 2015)
  - Elevated ATCA levels
  - Excellent thermal stability
  - Putrefaction of matrices does not affect ATCA level
- Stability studies (Logue et al. 2009 & 2010, Ružycka et al. 2016)
  - Excellent short- and long-term stability
  - Room temperature, refrigeration, freeze-thaw cycles, and derivatized extracts

Emerging Extraction Method

- Dispersive micro solid phase extraction (d-µSPE)
  - Performed in “micro” scale (µL range)
  - Clean-up (2nd stage in QuEChERS)
  - Target analytes extraction, e.g. Molecularly Imprinted Polymer (MIP)

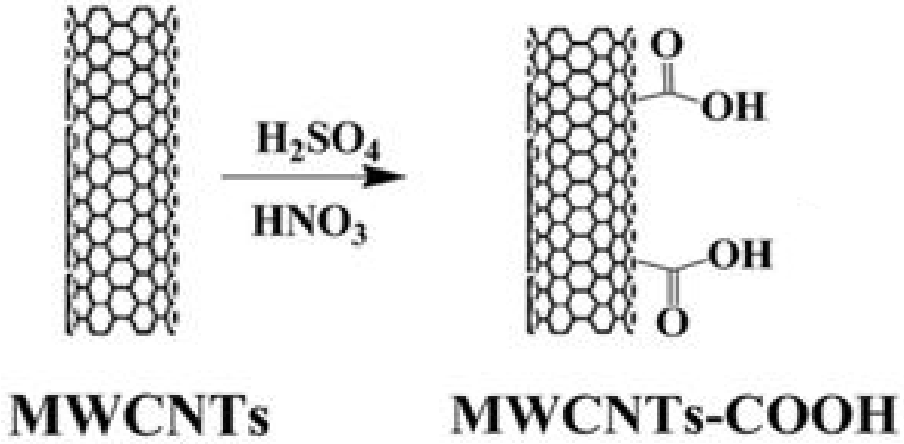


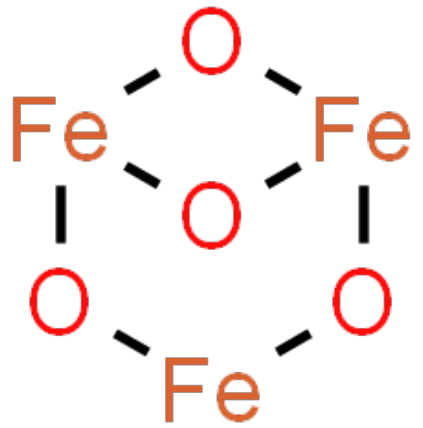
Adopted from Moyakao et al. 2018

Magnetic Carbon Nanotubes (Mag-CNT)

- High surface area and adsorption capability

- Fe_3O_4 (Ferrosoferric oxide/Magnetite/"super oxide")
  - Fe^{2+} & Fe^{3+}





Materials & Methods

Mag-CNT & GC/MS

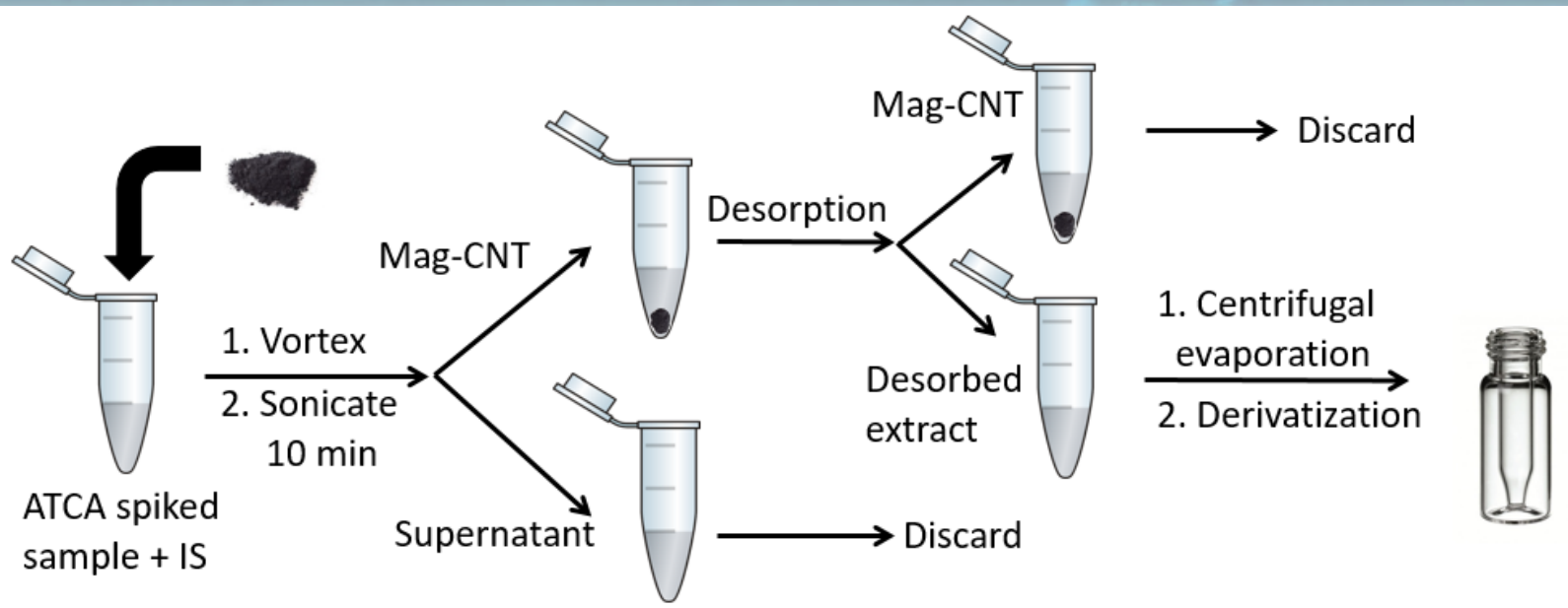
- Mag-CNT synthesis
  - Method by Li et al. (2018)*

- Agilent 7890A Series GC/ 5975C Series MS
  - DB-5 Column (30m x 0.25 mm x 0.25 µm)
    - Split mode (10:1)
    - SIM mode
      - ATCA-$(TMS)_3$: 245, 347, 362 m/z
      - ATCA-^{13}C,^{15}N-$(TMS)_3$: 248, 350, 365 m/z

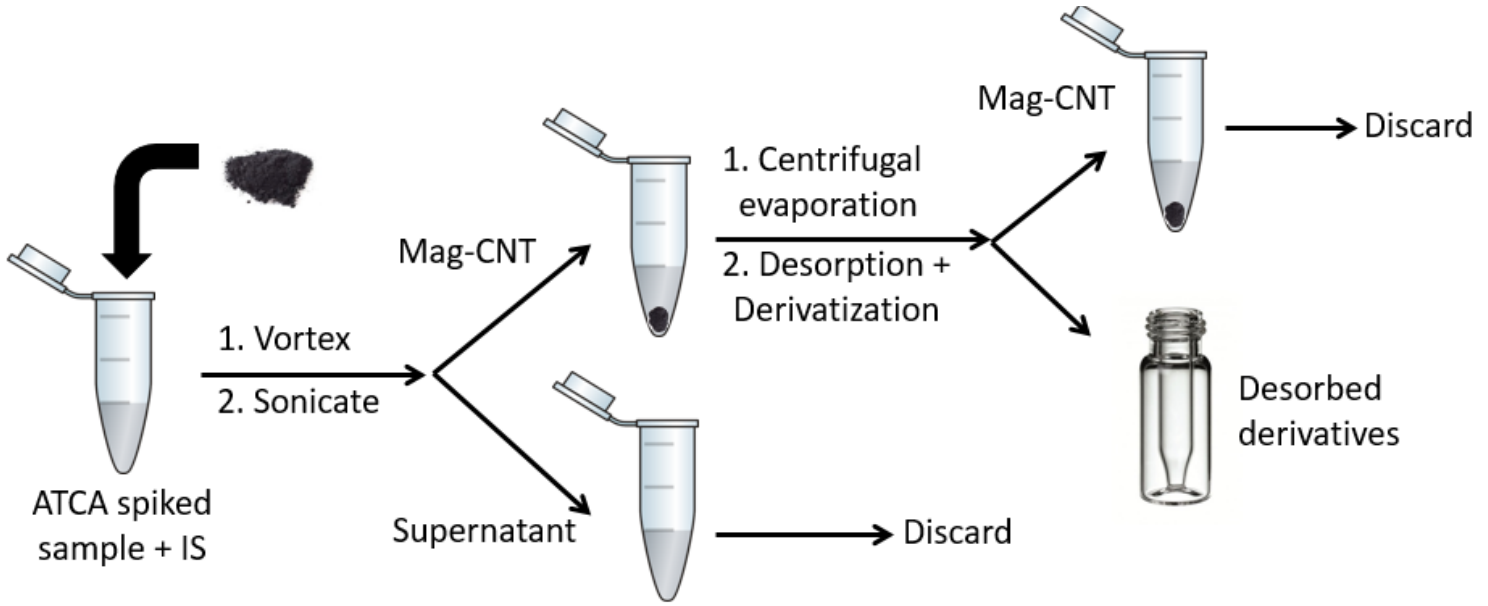
*Li, SY, Petrikovics, I, & Yu, J. (2019). Development of magnetic carbon nanotubes for dispersive micro solid phase extraction of the cyanide metabolite, 2-aminothiazoline-4-carboxylic acid, in biological samples. *J. Chrom B.* 1109:67-75.

Mag-CNT/d-µSPE

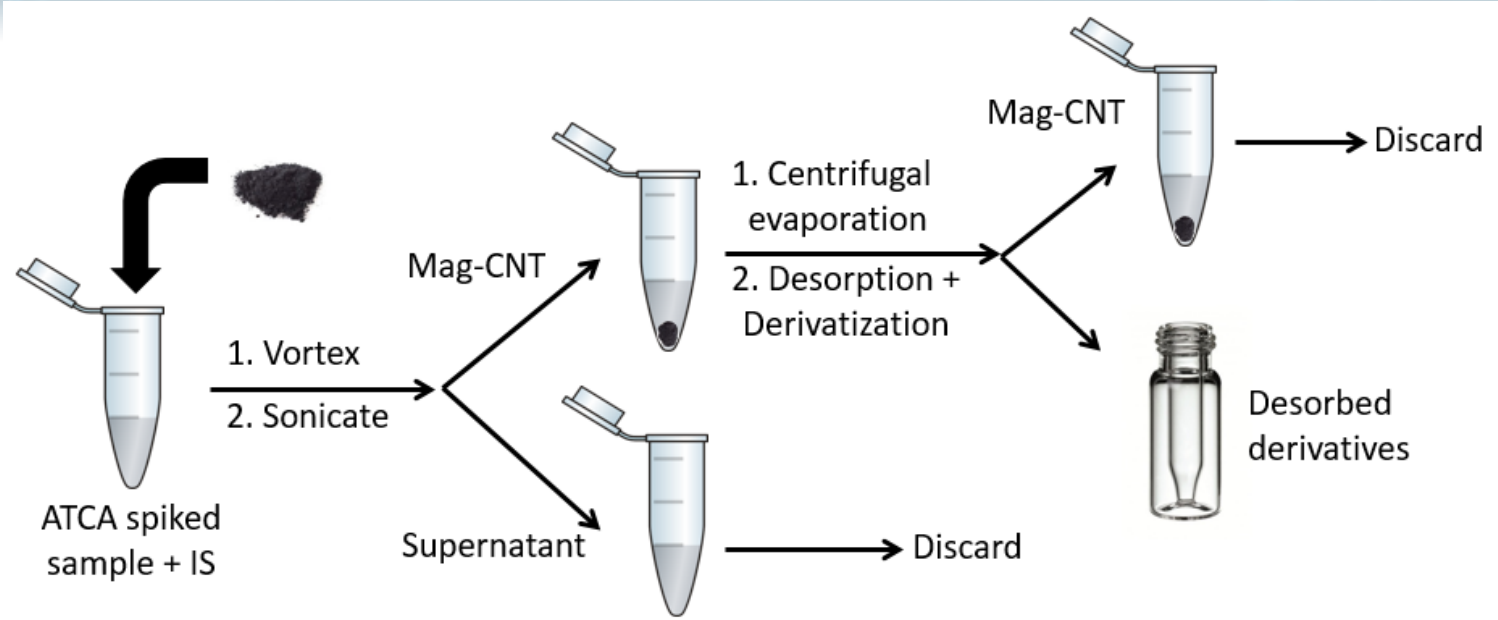
Published Method:
Desorption → Derivatization
(Li et al. 2018)



Advanced Method:
Desorption & Derivatization in one step



Optimization Parameters



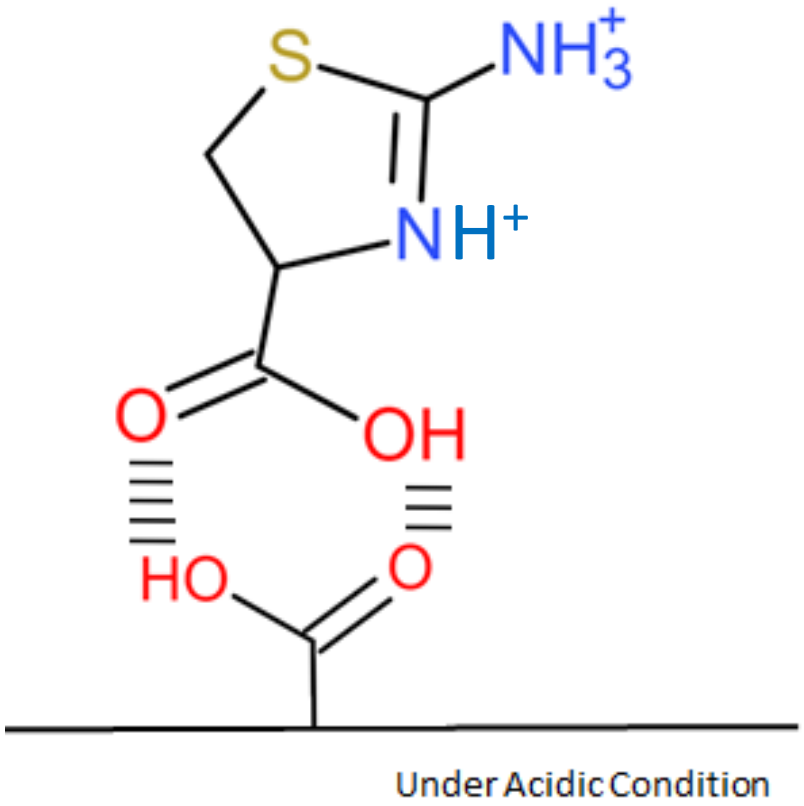
- Amount of Mag-CNT
  - 2, 3, 4, 5, 10 mg
- Extraction & Deriv. Time
  - 10, 20, 30, 60 min
- Sample Volume
  - 100, 200, 500, 750, 1000 µL
- Derivatization Volume
  - 50 & 100 µL
- Desorption + Deriv. Solvent
  - Hexane, Chloroform, Acetone, Acetonitrile, Pyridine
  - Coupled w/ 30% (v/v)MSTFA

Results & Discussion

Method Optimization

- 5 solvent systems with 30% (v/v) MSTFA:
  - Hexane
  - Chloroform
  - Acetone
  - Acetonitrile
  - Pyridine

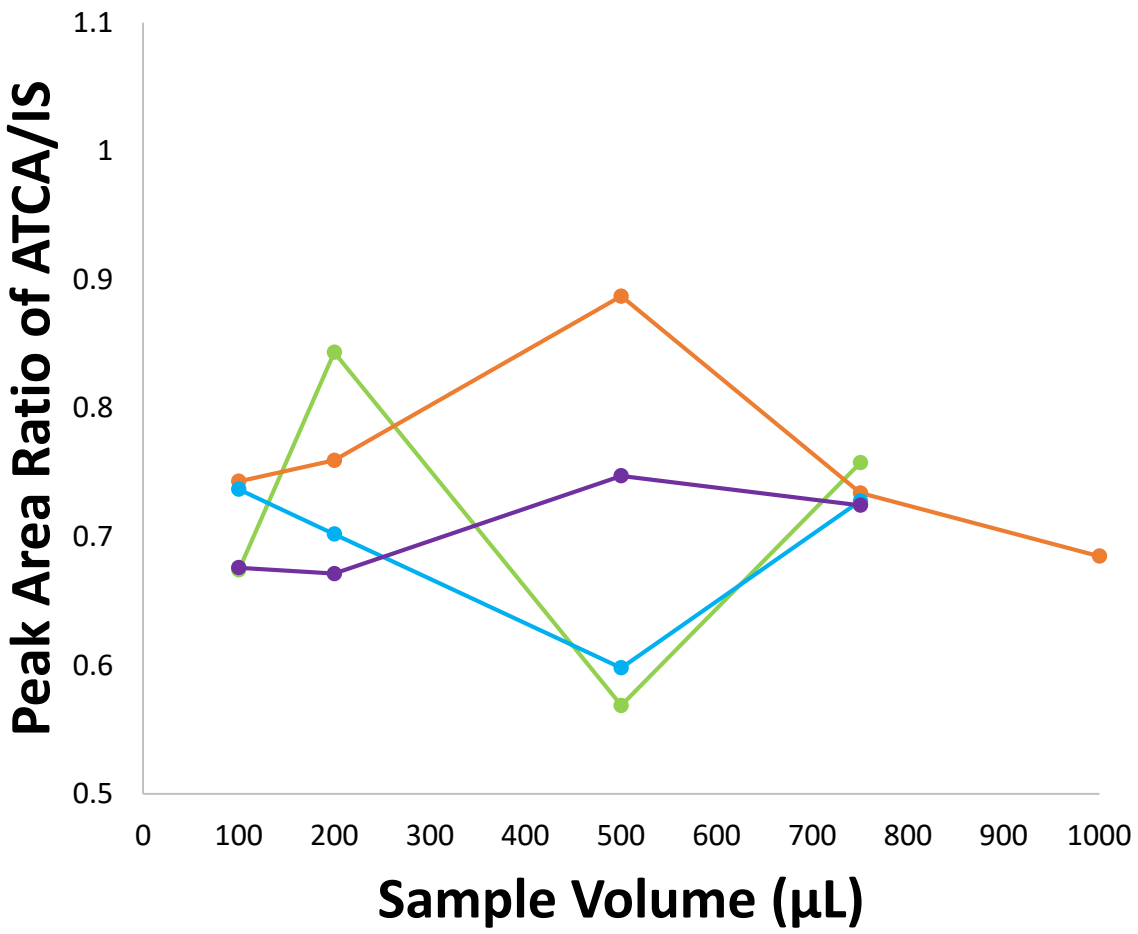
Increase Polarity



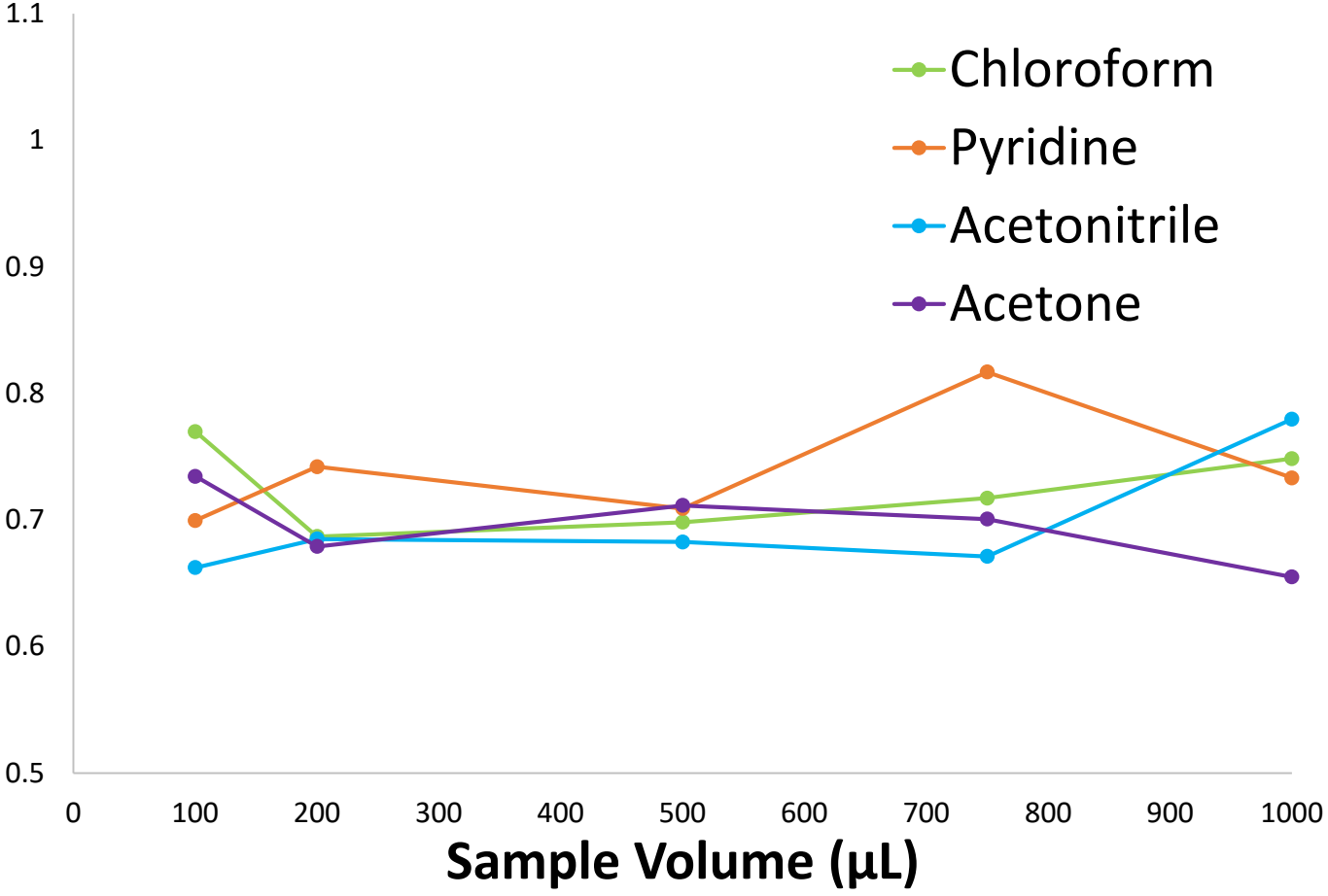
Under Acidic Condition

Method Optimization (cont.)

Derivatization Volume: 100 µL

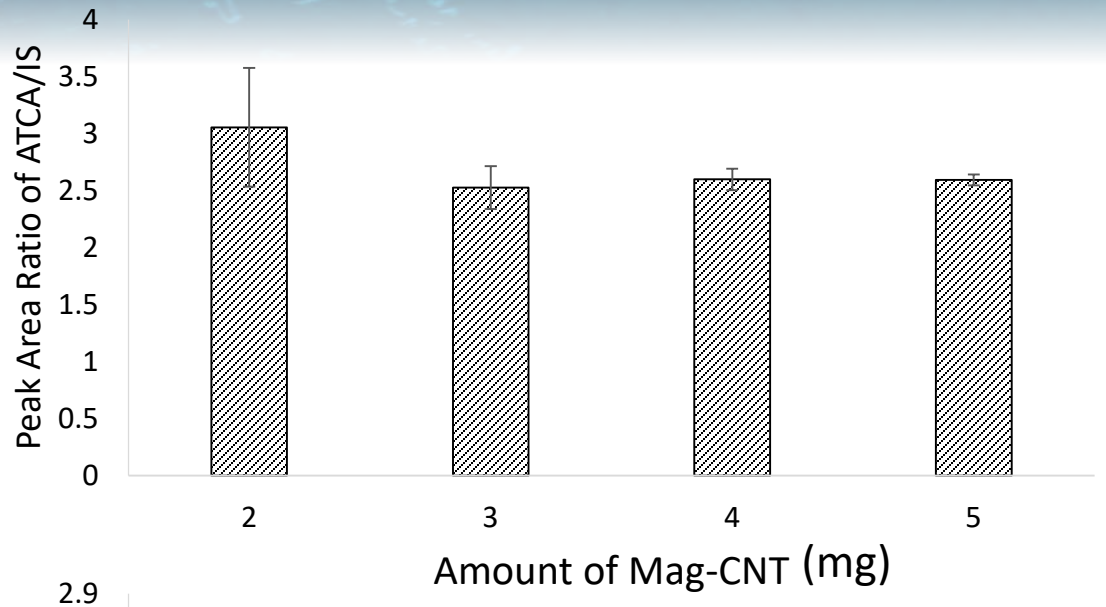


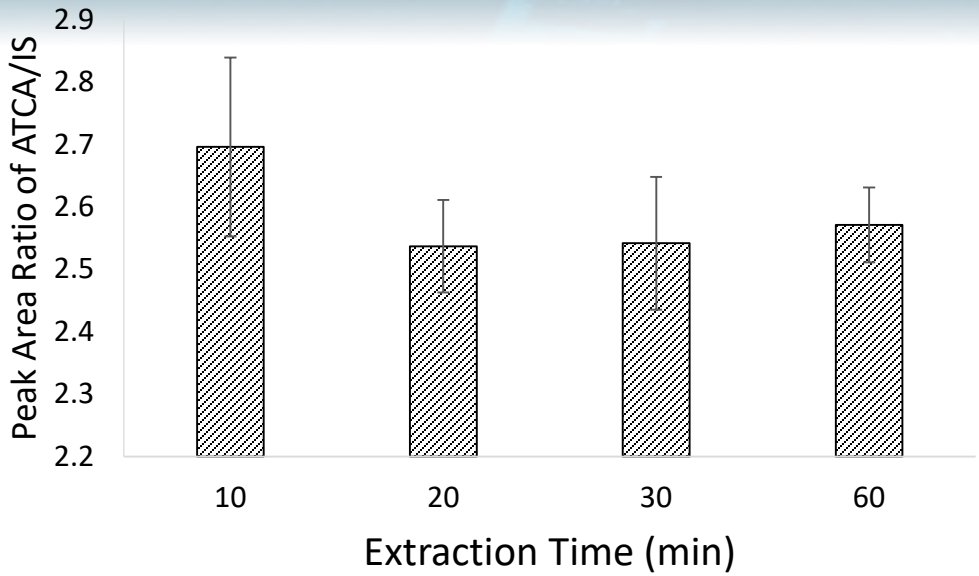
Derivatization Volume: 50 µL

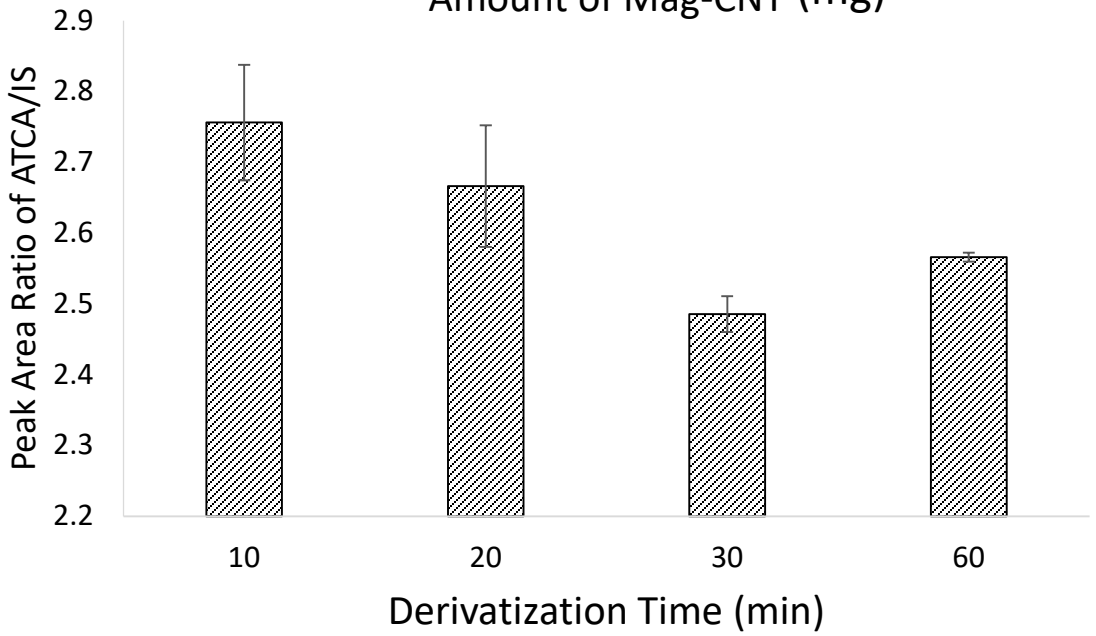


Polarity: Chloroform < Acetone < Acetonitrile < Pyridine

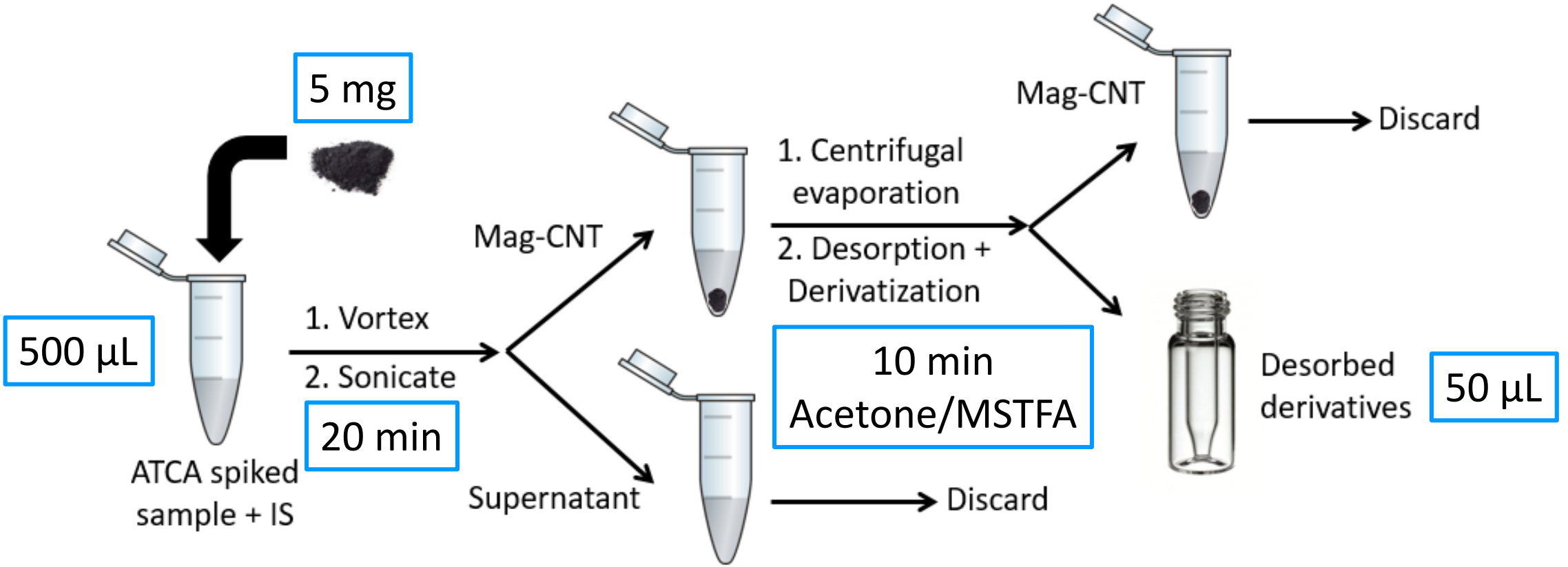
Method Optimization (cont.)







Optimized Mag-CNT/d-µSPE

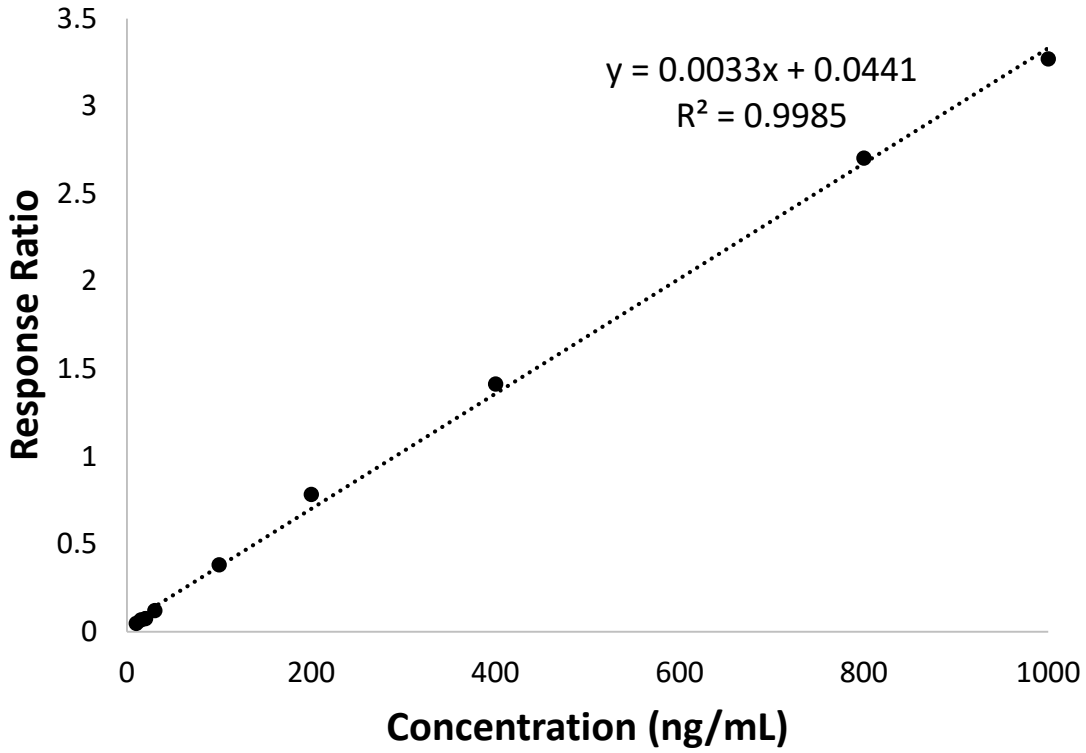


Academy Standards Board (ASB) Guidelines

- Calibration model
  - Minimum of 6 non-zero calibrators
  - 5 runs
- Bias
  - 5 runs
  - 3 concentrations in triplicates
  - Requirement: ±20%
- Precision
  - 5 runs
  - 3 concentrations in triplicates
  - Requirement: <20%
- Carryover
  - Triplicate
  - Requirement: no detectable analyte in blank matrix
- Interference studies
  - 34 Common drugs of abuse spiked at low QC samples
  - Stable isotopic IS
  - High QC w/o IS
- LOD
  - 3 runs
  - 3 matrix sources in duplicates
  - Requirement: S/N > 3
- LOQ
  - 3 runs
  - 3 matrix sources in duplicates
  - Requirement: S/N > 10

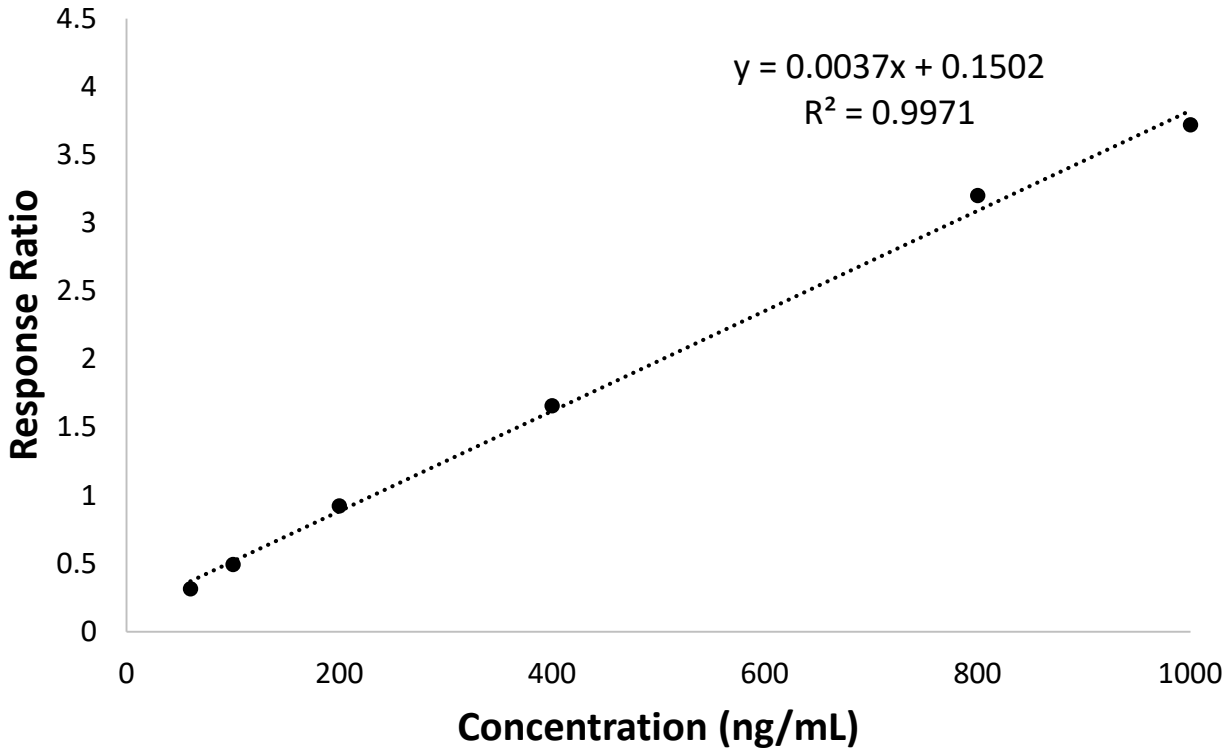
Calibration Model

Synthetic Urine



- Dynamic range: 10 – 1000 ng/mL
- LOD: 5 ng/mL
- LOQ: 10 ng/mL

Bovine Blood



- Dynamic range: 60 – 1000 ng/mL
- LOD: 10 ng/mL
- LOQ: 60 ng/mL

Extraction Efficiency, Bias, & Precision

	QC Samples	Extraction Efficiency (%)	Mean Bias (%, n=15)	Between-Run Precision (%CV, n=15)	Max Within-Run Precision (%CV, n=3)
Synthetic Urine	Low	91.70%	-8.30%	10.63%	12.70%
	Medium	103.06%	3.06%	4.60%	4.18%
	High	101.72%	1.72%	2.58%	3.53%
Bovine Blood	Low	103.68%	3.68%	3.32%	4.20%
	Medium	101.79%	1.79%	6.33%	9.44%
	High	100.72%	0.72%	2.95%	5.47%

Interferences and Recovery

	Interference		Recovery	
	Isotopic ISTD (n=3)	Common drugs of abuse (n=34)	Low Concentration (n=5)	High Concentration (n=5)
Synthetic Urine	No significant peak of interest	Bias ± 20%	116.3%	99.1%
Bovine Blood	No significant peak of interest	Bias ± 20%	100.5%	99.0%

Recovery Comparisons

	Recovery	
	Low Concentration (n=5)	High Concentration (n=5)
Synthetic Urine	116.3%	99.1%
Bovine Blood	100.5%	99.0%

Extraction Method	Recovery	Matrices	Citations
SPE (Oasis MCX)	84%	Synthetic Urine	Logue et al. 2009
SPE (Oasis MCX)	100%	Swine Plasma	Logue et al. 2009
LLE	81 – 89%	Post-mortem blood* & organ#	Giebułtowicz et al. 2015* & Rużycka et al. 2017#
Molecularly Imprinted Polymer Stir Bar Sorption Extraction	62%	Human Urine	Jackson et al. 2010

Conclusions

- Validated Mag-CNT/d-µSPE method showed excellent accuracy, precision, sensitivity, and recovery
- Comparable to other published and/or validated methods
- Promising alternative as an emerging sample preparation method in forensic settings

Acknowledgements

- Sam Houston State University
  - Department of Forensic Science

References

- Li, SY, Petrikovics, I, & Yu, J. (2019). Development of magnetic carbon nanotubes for dispersive micro solid phase extraction of the cyanide metabolite, 2-aminothiazoline-4-carboxylic acid, in biological samples. *J. Chrom B.* 1109:67-75.
- David D. Gummin, James B. Mowry, Daniel A. Spyker, Daniel E. Brooks, Michael C. Beuhler, Laura J. Rivers, Heba A. Hashem & Mark L. Ryan (2019): 2018 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 36th Annual Report, Clinical Toxicology, DOI: 10.1080/15563650.2019.1677022
- Gill JR, Marker E, Stajic M. Suicide by cyanide: 17 deaths. J Forensic Sci. 2004;49(4):826-8.
- Logue BA, Hinkens DM, Baskin SI, Rockwood GA. The Analysis of Cyanide and its Breakdown Products in Biological Samples. Critical Reviews in Analytical Chemistry. [Article]. 2010;40(2):122-47.
- Rużycka 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.
- Baskin SI, Petrikovics I, Kurche JS, Nicholson, JD, Logue BA, Maliner BJ, Rockwood GA, in: S.J.S. Flora, J.A. Romano Jr. Baskin SI, Sekhar K (Eds.), Pharmacological Perspectives of Toxic Chemicals and Their Antidotes, Narosa Publishing House, New Delhi, India, 2004, p.105
- Nagasawa HT, Cummings SE, Baskin SI. Org. Prep. Proc. Int. Brief. 36. 2004;36:178.
- 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.
- 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. doi:10.1016/j.talanta.2015.12.076.
- Moyakao, K., Santaladchaiyakit, Y., Srijaranai, S., & Vichapong, J. (n.d.). Preconcentration of Trace Neonicotinoid Insecticide Residues Using Vortex-Assisted Dispersive Micro Solid-Phase Extraction with Montmorillonite as an Efficient Sorbent. *MOLECULES*, *23*(4). https://doi-org.ezproxy.shsu.edu/10.3390/molecules23040883
- Jackson R, Logue BA. A review of rapid and field-portable analytical techniques for the diagnosis of cyanide exposure. Analytica Chimica Acta. 2017 2017/04/01/;960:18-39.

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Questions

Sun Yi Li, BS

sxl062@shsu.edu