Automated SPE Method for the Determination of Cotinine in Biological Fluid

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Abstract

Cotinine is a major metabolite of nicotine that may be used as a marker for both active smoking, and as an index to Environmental Tobacco Smoke (ETS) exposure, or “passive smoking”.

Cotinine is generally preferred over nicotine for such assessments because of its substantially longer half-life. The half-life of cotinine in plasma has been estimated to be about 15-20 hrs (1-3); whereas the half-life of nicotine is only 0.5-3 hrs (3-5).

Cotinine concentrations tend to be higher (3-8x) in urine than in serum; however, for studies requiring a quantitative assessment of exposure, plasma or serum is chosen.

Cotinine has been extracted via LLE (6). This procedure employs SPE, a new polymeric based cation exchange resin that resists drying, shows excellent flow characteristics, greater load capacity and resistance to aggressive washings.

System and Components

- GX-271 ASPEC/406 dilutor, 10 mL syringe
- HPLC binary gradient
- Atlantis® dC18, 3 um, 4.6 x 150 mm
- SPE: Strata-X-C 33 um Polymeric Strong Cation, 200 mg/mL/3 mL

SPE Procedure

- Pretreat: 50 uL 1 M Formic Acid per 1 mL plasma
  - Dilute 1 mL plasma with 3 mL 50 mM NH_4OAc, pH 6
  - Mix thoroughly and centrifuge

  Condition: 3 mL MeOH, 3 mL 50 mM NH_4OAc, pH 6

  Load: Plasma 2 mL x 2

  Wash: 2 mL 50 mM NH_4OAc, pH 6, 500 uL MeOH

  Elute: 95:5 (v/v) MeOH/NH_4OH 500 uL x 2

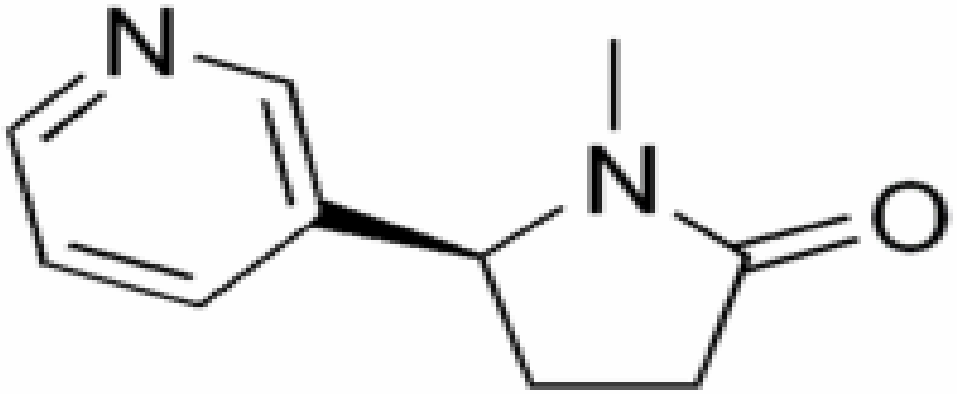
  Evaporate eluent, reconstitute in 1 mL 0.5% TFA/H_2O

HPLC Procedure

- Mobile Phase: A: 0.5% TFA/H_2O, B: 0.5% TFA/ACN
- Gradient: 0-1 min 5% ACN
  1-9 min 94% ACN
  9-11 min 94% ACN
  11-12 min 5% ACN
  12-16 min 5% ACN
  flow rate 0.75 mL/min

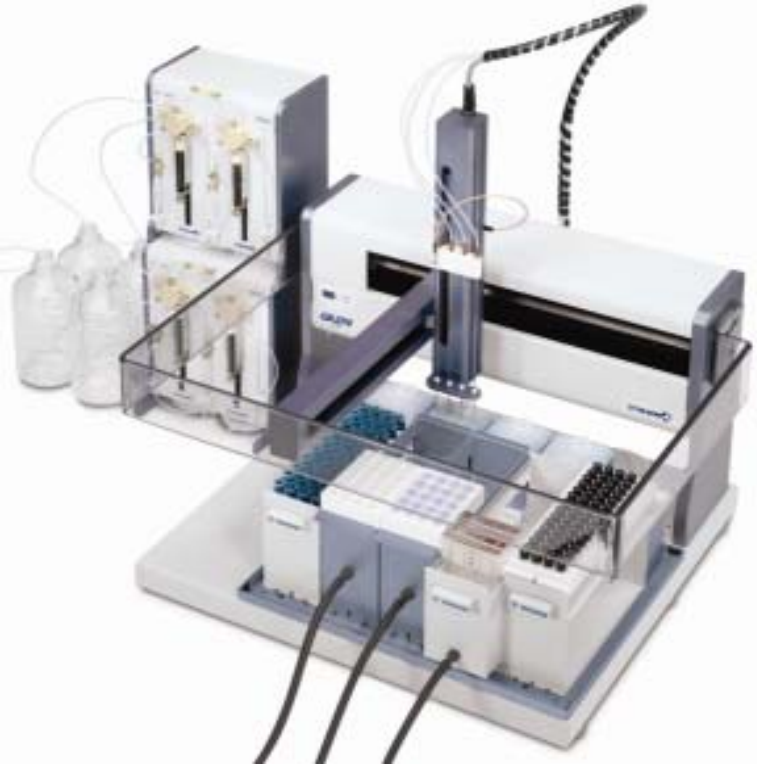
- UV detection dual wavelength: 254, 263 nm, 0.005 AUFS

Cotinine

Cotinine	
	
IUPAC name	(S)-1-Methyl-5-(3-pyridinyl)-2-pyrrolidinone
Other names	1-Methyl-5-(3-pyridinyl)-2-pyrrolidinone S-(-)-Cotinine

pKa ~8.8

Automated SPE: GX-274 ASPEC



Positive Pressure Extraction

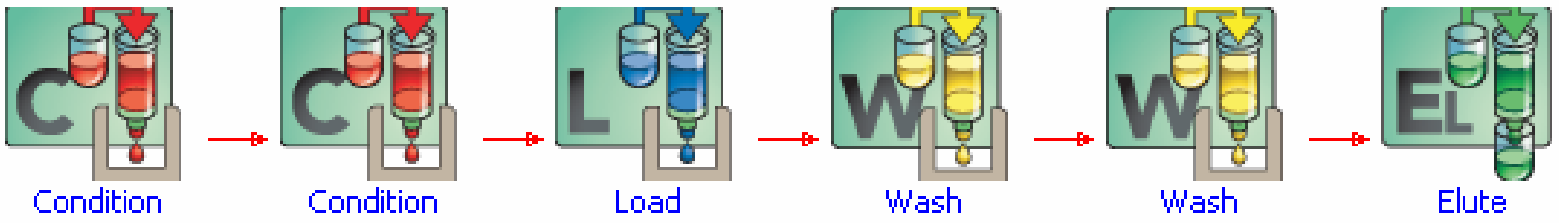
The probe introduces the sample or solvent to the SPE cartridge, once delivery is complete a gas valve is turned on by the software and Nitrogen is introduced to the cartridge which pushes the liquid through the cartridge. The time that the cartridge is exposed to the gas pressure is settable, hence DRYING or NOT DRYING the cartridge is not an issue and since every cartridge is treated exactly the same, consistency is optimized from the 1st cartridge to the last cartridge.

Mobile SPE Racks

- **The GX-274 ASPEC automated instruments have movable SPE racks that do not require additional electronics or gripper arms to move back and forth from the drain to the collection tubes. The probe itself moves the rack holding the SPE cartridges to its correct location. Individual steps are also available via the probe allowing a set of SPE cartridges to be placed over each row of collection tubes, in order to collect each step in the extraction process (optimization) or unique fractions based on classes of compounds.**

TLH:SPE Software

- TLH is a drag and drop icon driven software that allows you to run method development directly on the instrument through the use of variables or different methods within one application
- Various conditioning, washing and elution solvent can be chosen and the results evaluated for optimum sample cleanup methodology



Optimization of the SPE Method

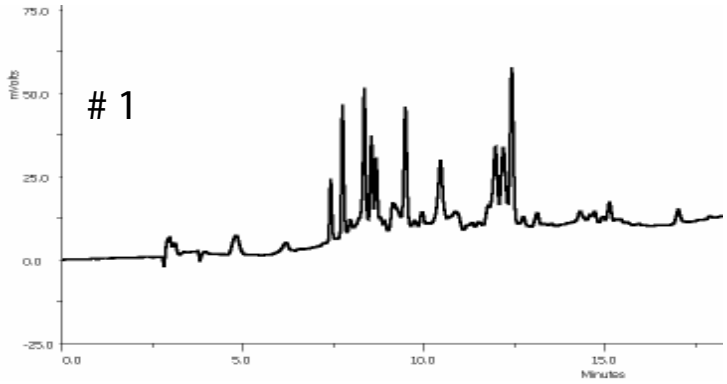
- Starting with the excellent work provided from the CDC; Cotinine in Serum, **NHANES 1999-2000**, *Organic Analytical Toxicants Branch, division of Laboratory Sciences, National Center for Environmental Health*
- Our goal was to investigate other choices in SPE sorbent including the new polymeric based material in order to avoid multiple manual manipulations and the use of chlorinated solvents

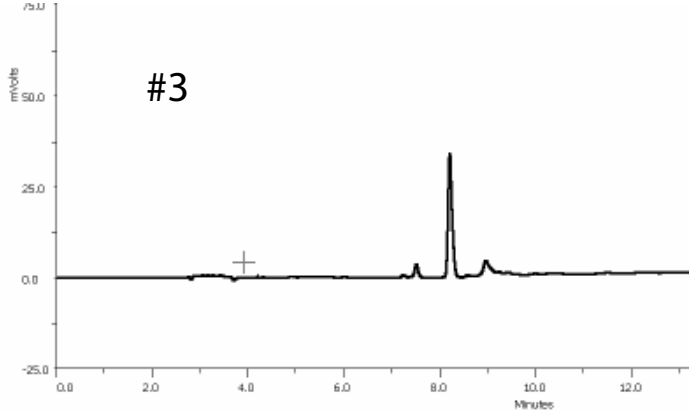
- A number of silica based SPE cartridges both RP and CX were investigated, from several manufacturers
- A number of polymeric based SPE cartridges both RP and CX were also investigated, from several manufacturers
- A head to head comparison was evaluated with the silica based SPE, RP and CX, since identical media amounts are available, e.g. 200 mg/3mL cartridge
- The polymeric based SPE cartridges, both RP and CX, however vary, and the CX is in many cases only available in 25-100 mg/3 mL
- Head to head comparison of the polymeric based material therefore was quite difficult

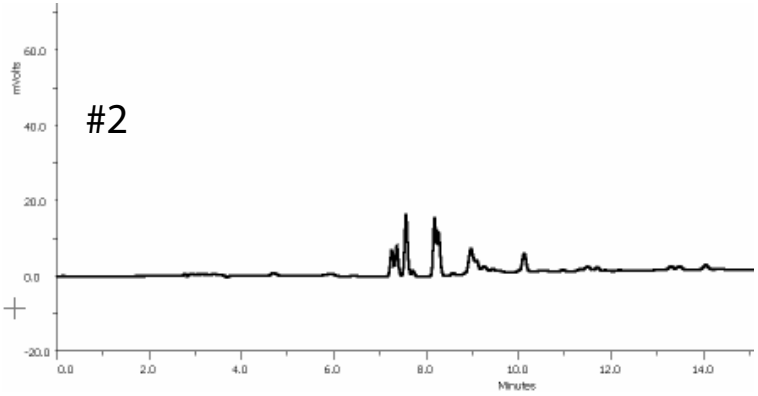
Comparison of SPE Media

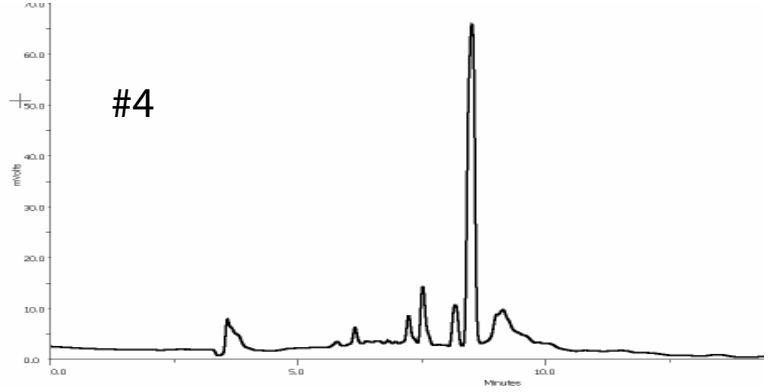
- In general all silica based SPE media (RP and CX) did not clean the plasma sample as well as the polymeric CX
- Polymeric CX 200 mg/3ml was the support that gave clean and best recovery on 1 mL plasma samples
- It was determined that approximately 60% of the cotinine was protein bound so the addition of a protein binding interrupter was required, 1 M Formic acid was employed

Blank Plasma Data From Various SPE Media









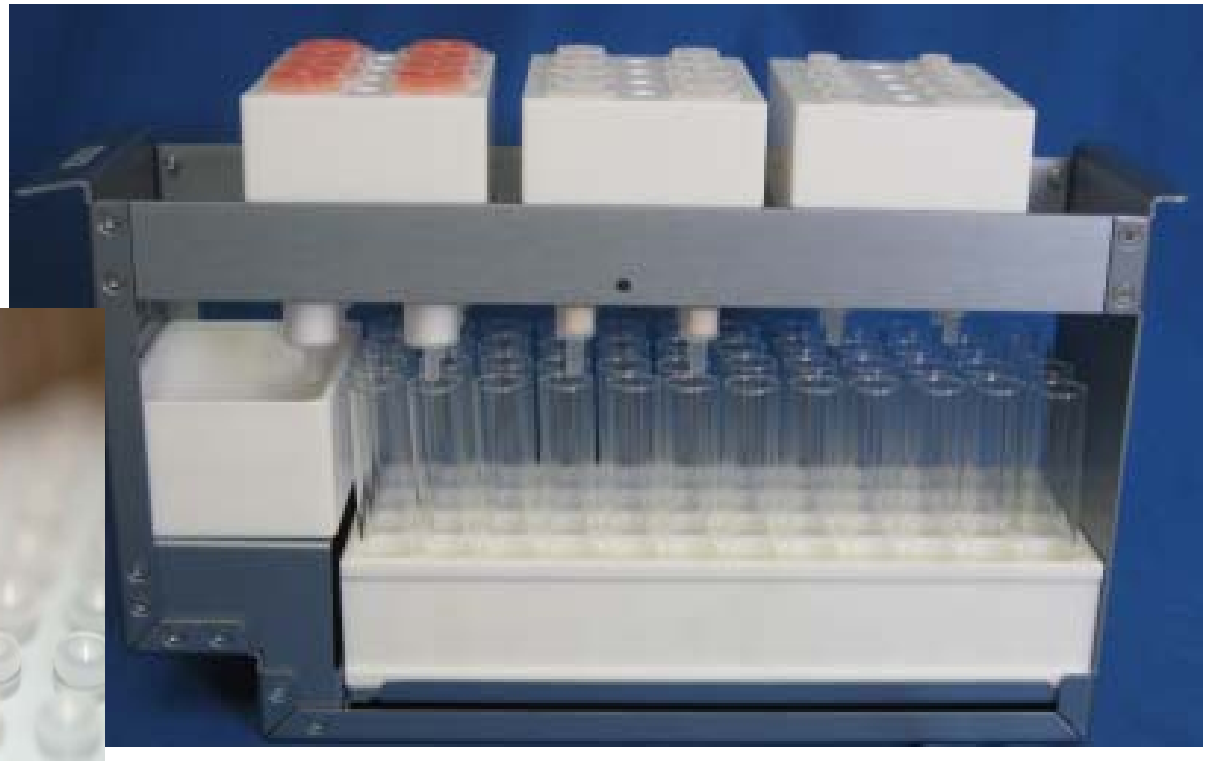
Examples of the various polymeric cation exchange resins evaluated chromatograms show a blank plasma sample after SPE. Please note that although #3 polymeric CX cartridge is cleaner than #4 StrataX-C the cartridge only contained 60 mg of material and therefore did not give adequate recovery with a 1 mL of plasma, which is why #4 StrataX-C was chosen.

Optimization of SPE Method for Cotinine

- Since a method was not available using the polymeric cation exchange columns one was developed
- Parameters are available through the SPE manufacturer
- We needed to not only develop an SPE method but optimize the method
- We incorporated a method/optimization development rack which allowed us to not only choose from several solvent but collect loads, washes and elution for optimization within the automated system
- Once determined the parameters could easily be used in the method on the automated system to prep the plasma samples for cotinine determination via SPE

Automated SPE Optimization Kit:

- Simple Liquid Handler and SPE racks
- The SPE method development rack allow for multiple elutions or fractionation with various solvents to optimize each and every step of an SPE method without manual intervention
- 1, 3, or 6 mL SPE method racks are available
- Methods easily transferred since it was developed on the automated liquid handler



Multi-Collect to Optimize SPE Procedure:

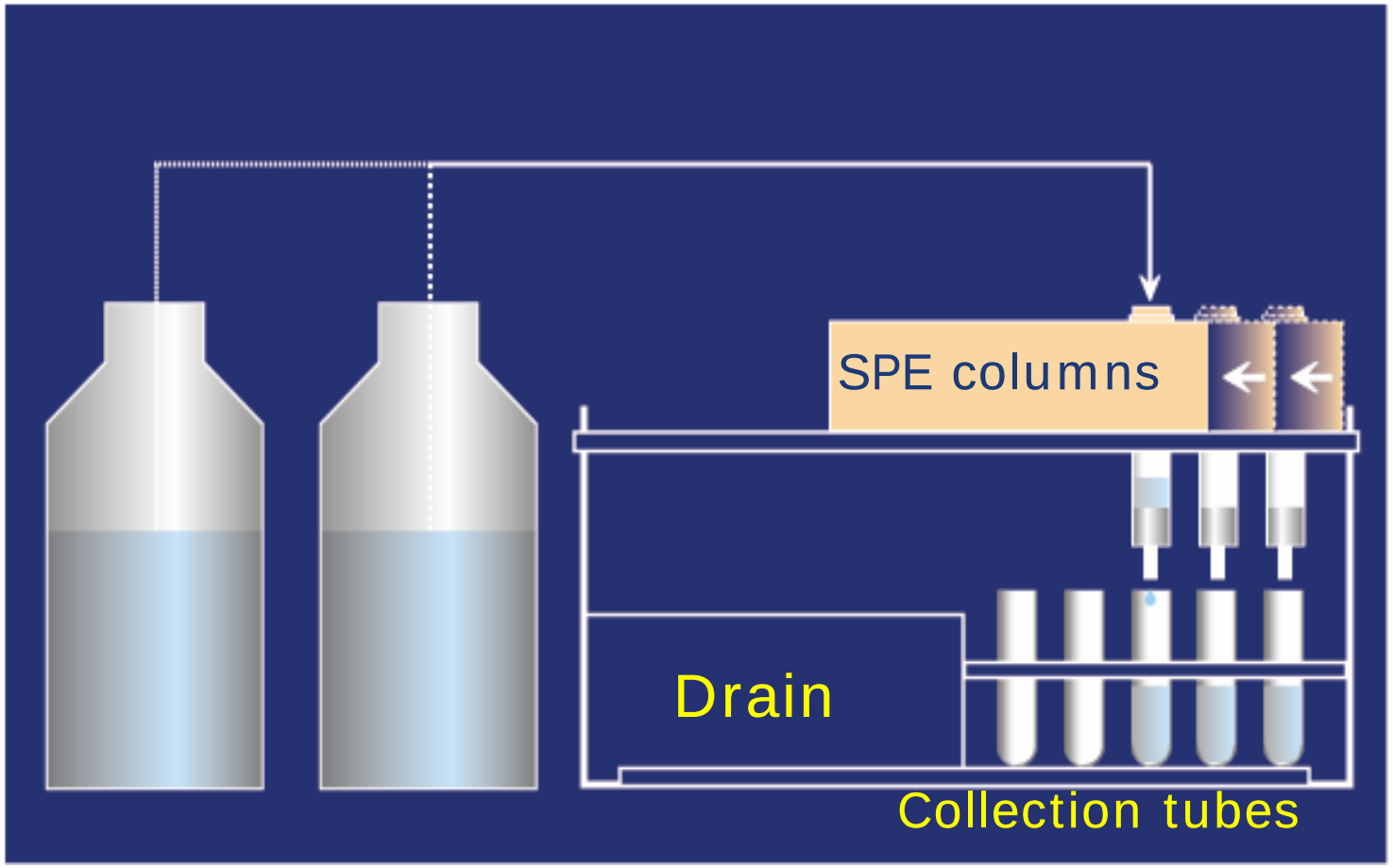
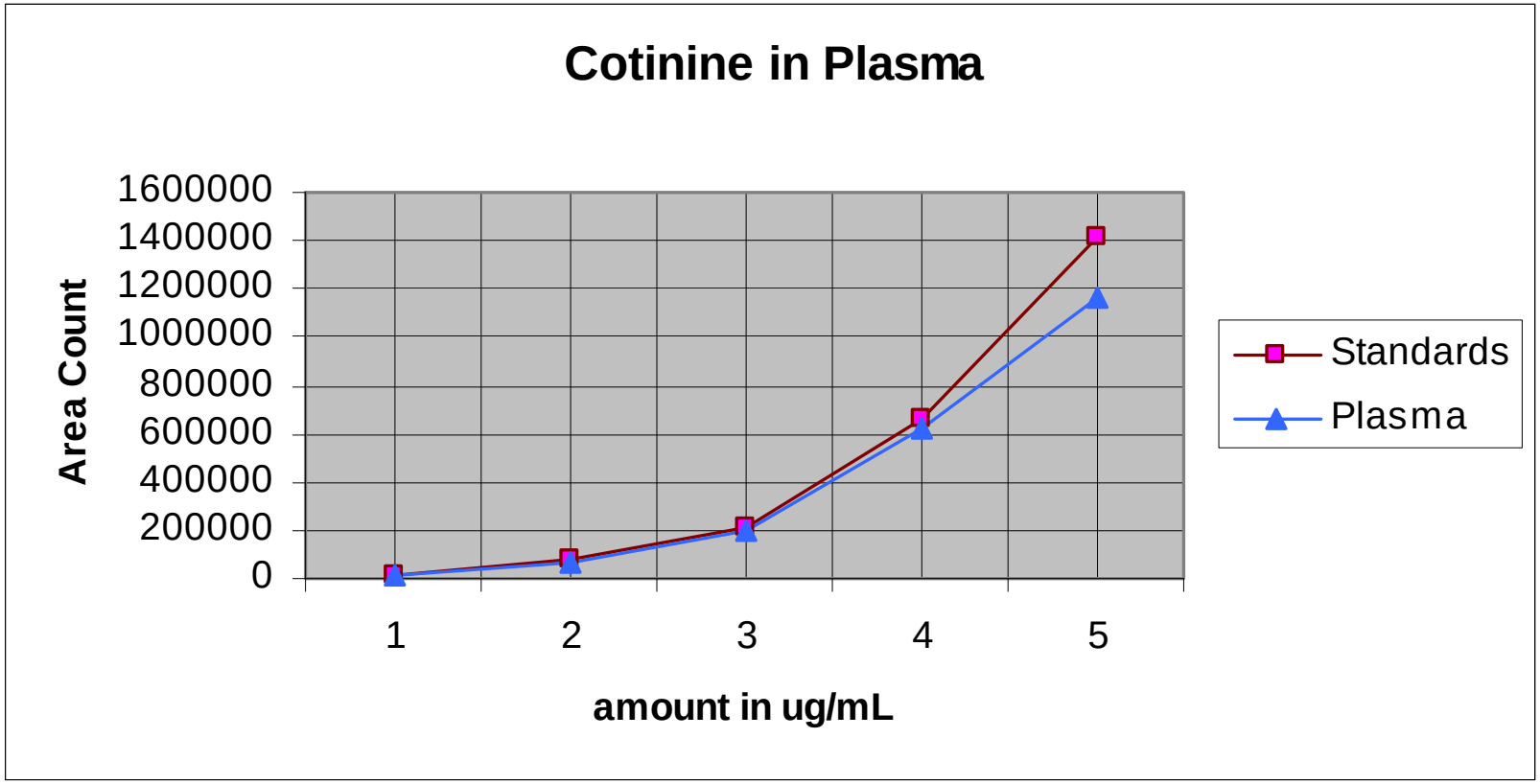
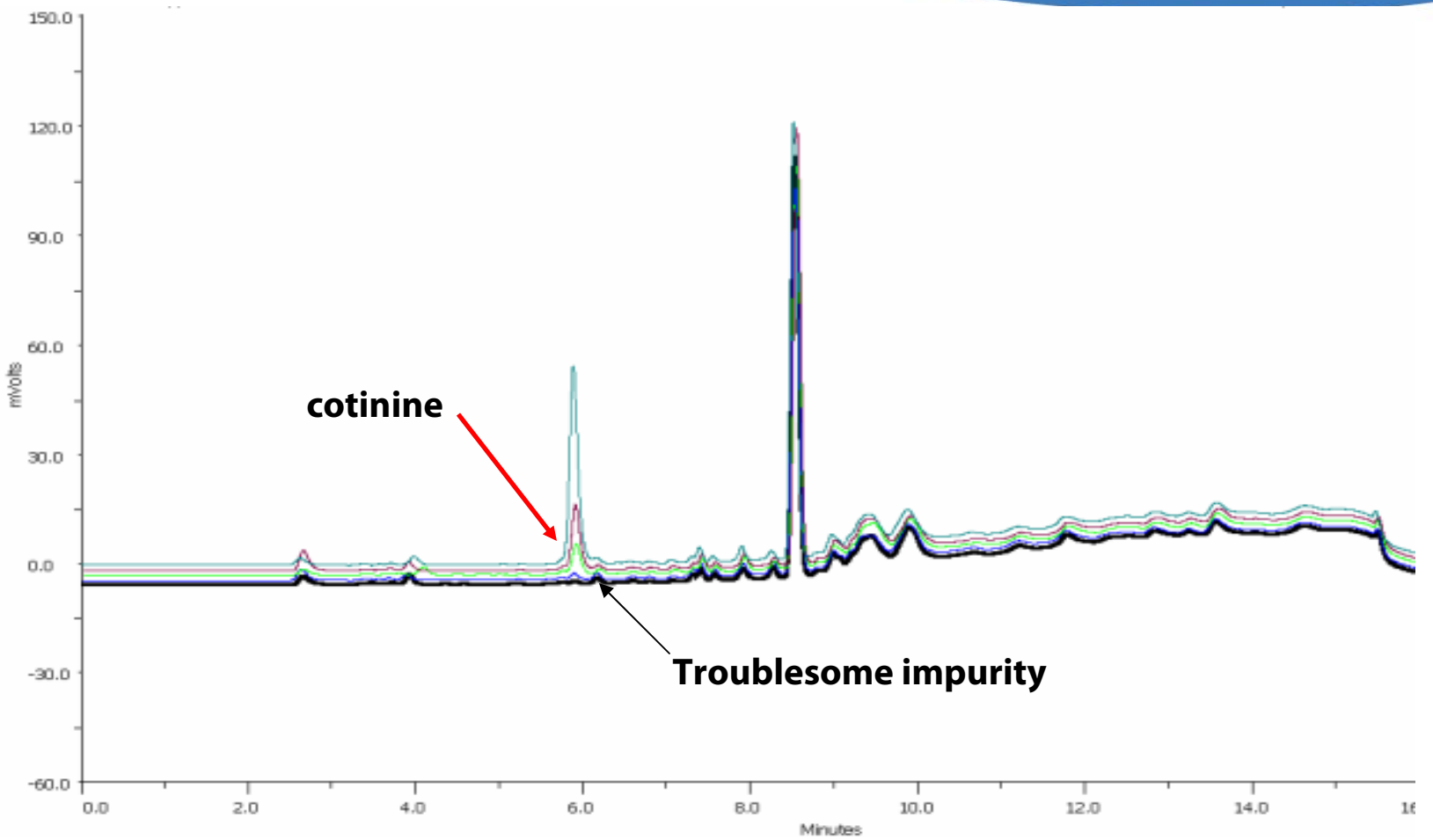


Table 1: Relative Recoveries of Cotinine from Plasma versus Aqueous Solutions

Amount of Cotinine	Standards Area Count	Plasma Area Count	% Recovery	% CV
100 ng/mL	12369	15538	125%	5%
500 ng/mL	76584	71942	94%	7%
1 ug/mL	209053	199821	96%	5%
5 ug/mL	665194	621807	94%	2%
10 ug/mL	1418725	1163884	82%	3%


Cotinine in Plasma
Area Count
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
1
2
3
4
5
amount in ug/mL
Standards
Plasma



Chromatogram of the plasma samples containing cotinine, blank, 0.1 -10 ug/mL of cotinine

Conclusion

- The initial SPE method developed to determine cotinine in biological fluids, plasma has shown promise
- Future optimization and possibly using another polymer cation exchange resin (#3) may be in order
- The SPE method showed excellent recovery in the 94% range between 500 ng/mL and 5 ug/mL and CV between 2-7%
- Automating the method development and optimization process on the instrument was found to be very useful, without manual intervention

- This SPE method has removed the use of chlorinated solvents, drying columns and a large amount of manual intervention
- A troublesome impurity that chromatographs very close to the cotinine peak and sometimes found underneath the cotinine peak, and interferes with detection, LOQ >500 ng/mL, has been an issue and will need further investigation and elimination
- Future studies will be conducted with the Arkansas Public Health Laboratory, involving LC/MS detection, and cotinine-d^3

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- Jeffery H. Moran, Ph.D.
  - Section director and lead chemist, Arkansas Public Health laboratory, 201 South Monroe Street, Little Rock, AR 72205
  - jeffery.moran@arkansas.gov

References

1. Jarvis MJ, Russell, MAH, Benowitz NL, Feyerabend c. Elimination of cotinine from body fluids: **Implications for noninvasive measurement of tobacco smoke exposure**. *Am J Public Health*. 1988; 78: 696-8.
2. Benowitz NL, Kuyt F, Jacob P, Jones RT, Osman A-L. **Cotinine disposition and effects**. *Clin Pharmacol Ther*. 1983; 34: 604-11
3. Kyerematen GA, Morgan ML, Chattopadhyay B, beBethizy JD, Vesell ES. **Disposition of nicotine and eight methabolites in smokers and nonsmokers: Indentification of two methabolites that are longer lived than cotinine**. *Clin. Pharmacol Ther*. 1990; 46:641-51.
4. Jacob P, Yu L, Wilson M, Benowitz NL. **Selected ion monitoring method for determination of nicotine, cotinine and deuterium-labeled analogs: Absence of an isotope effect the clearance of (S)-nicotine-3', 3'-d^2 in humans. *Biol Mass Spec*. 1991; 20:247-52**.
5. Armiage AK, Dollery CT, George CF, Houseman TH, Lewis PJ, turner DM. **Absorption and metabolism of nicotine from cigarettes**. *Br Med* 1975; 4; 313-16.
6. Bernert T, Laboratory procedure manual, Cotinine in Serum, ID HPLC-APCI MS/MS, NHANES, 1999-2000.