PRODUCTION OF SEIZED DRUG ANALYSIS STANDARDS BY INKJET PRINTING

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Introduction

- The availability of standards of illicit/designer drugs to help confirm the identity of drugs of abuse poses a challenge for forensic laboratories[1].
- Limited number are available commercially, and even fewer can be obtained as certified reference materials (CRMs).
- Due to this, laboratories must often prepare in-house reference standards (by chemical synthesis or through isolation of the desired compound from seized materials)[2].
  - Each laboratory must confirm the identity of its in-house standard and demonstrate their suitability for the intended purpose
- Lack of readily available reference standards has lead to identification efforts based primarily on mass spectrometry; however, these approaches have limited applicability because of the potential for different compounds to yield nearly identical mass spectra[3].
- Currently, NIST does not offer any pure standards for seized drugs but does have a number of matrix-based Standard Reference Materials (SRMs) for drugs of abuse in hair, blood, and urine.

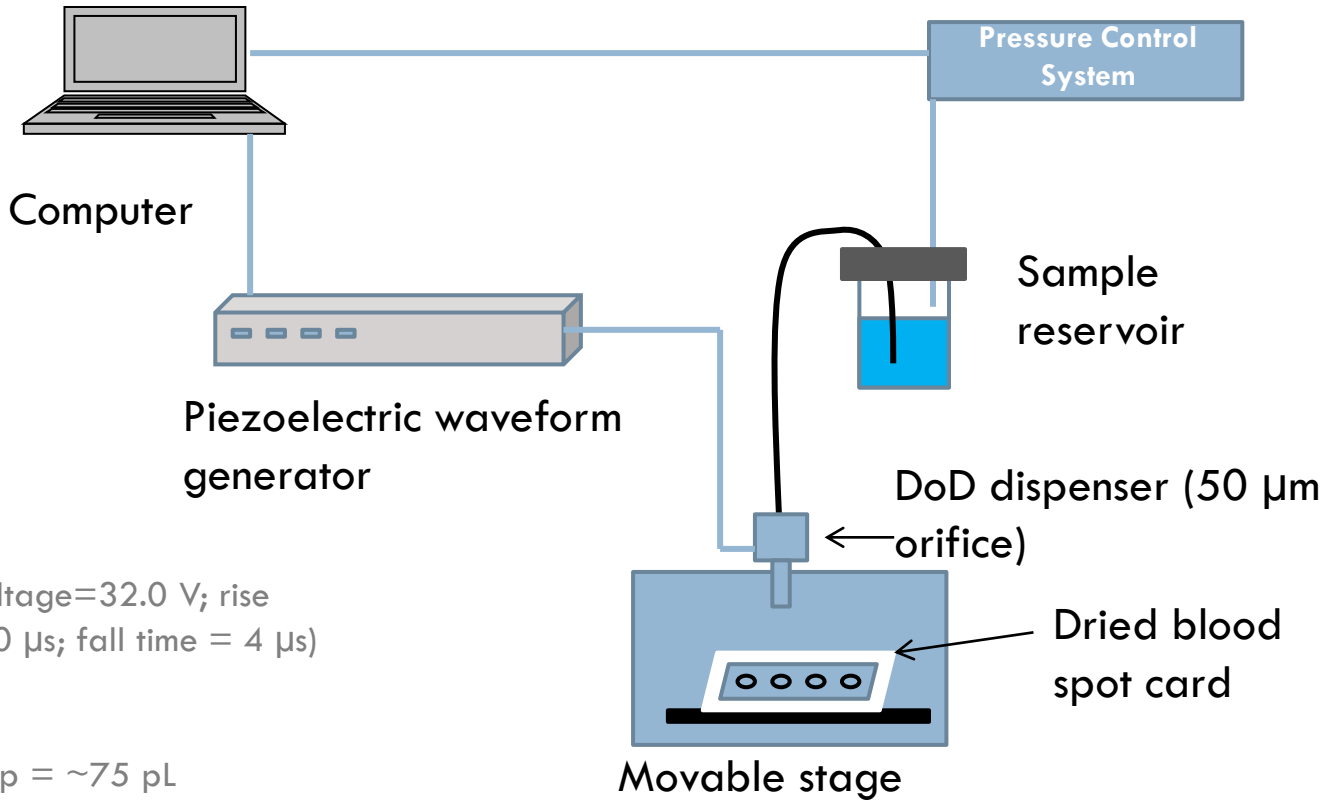
Objectives

- The goal of the proposed research is to produce cost effective, easy to use seized drug standards by inkjet printing technology for analysis by LC-MS(/MS) or GC-MS.
- If successful, this method could be expanded to the development of quantitative standards for drug analysis and to the production of materials for quality assurance programs.
- This work would also serve as a foundation for possible NIST reference material (RM or SRM) development in the future.

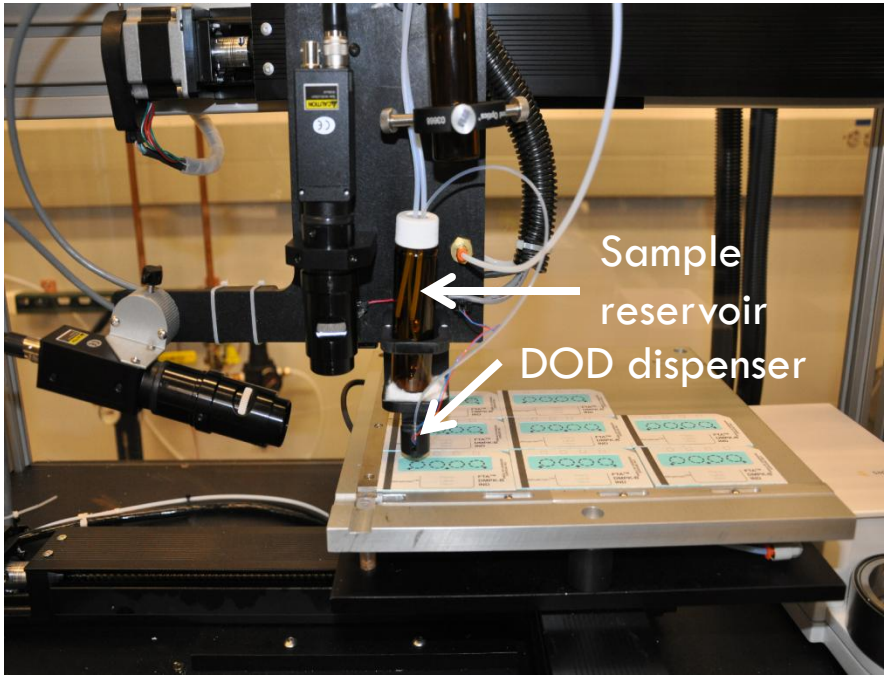
Inkjet Printing Technology

- Piezoelectric drop-on-demand (DoD) inkjet printing is a versatile method for the quantitative delivery of micro volumes of solution[4].
- Inkjet printing technology will be used to deposit known amounts (nanoliter-picoliter volumes) of pure substances (illicit drugs), or mixtures of substances onto inert substrates.
- At the time of use, the compounds will be desorbed by a small amount of solvent, then used in analysis.

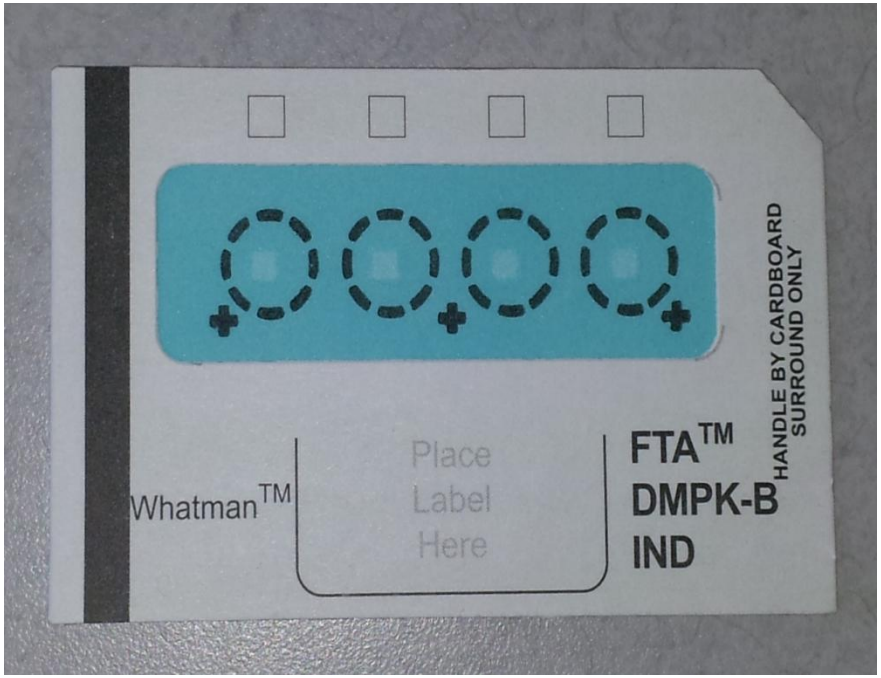
Inkjet Printing Schematic



Optimized parameters:
Standard singular wave
•Voltage pulses (dwell voltage=32.0 V; rise time=4 µs; dwell time=30 µs; fall time = 4 µs)
•Frequency: 500 Hz
•Average mass : ~60 ng
•Average volume per drop = ~75 pL
•Average ejection velocity ~2.0 m/s
•Uncertainties of drop dispensed: 1 % RSD (by mass) which corresponds to1% RSD by volume and 0.3% RSD by diameter



Inkjet printing apparatus



4 single use samples printed on a dried blood spot card
(Dimensions per spot: 2.4 mm x 2.6 mm)

Benefits of Inkjet Printed Standards

- Noncontact, high-throughput deposition of precise quantities (nano- to picoliter deposits)[5].
- Reproducibility of optimized inkjet printers have been reported better than 1% relative standard deviation for day-to-day measurements[6].
- Dynamic ranges in deposited analyte concentrations (10^5) can be achieved by varying the number of drops printed[5].
- Very small amount of compound needed for each printed standard (cost effective).
- Single use materials reduce potential contamination from multiple uses of the same batch of material.

Application of Inkjet Printing Technology

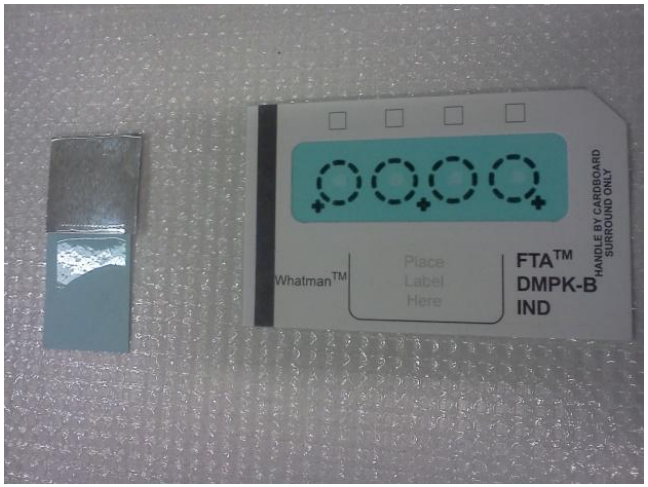
- Has been applied to diverse range of applications
  - Printing of DNA microarrays [7]
  - Dispensing metallic solder for interconnects in the electronic industry[8]
  - Production of RDX (1,3,5-trinitro-13,5 triazcyclohexane) test materials for trace level explosive analysis to evaluate ion mobility spectrometers [5].

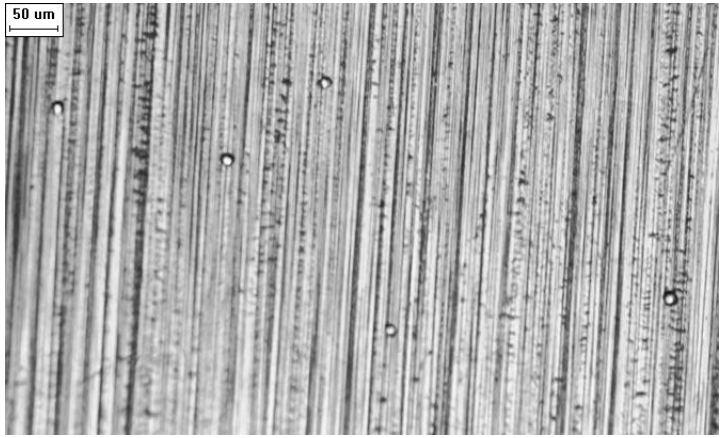
Factors to consider

1. Identification of suitable substrates and solvents for printing
2. Determination of quantities of drug compounds that need to be deposited for extraction and use with LC-MS and/or GC-MS
3. Efficiency of release of the printed substances from the substrate
4. Stability of printed compounds on different substrates and under varying storage conditions

Preliminary work will focus on Methamphetamine (MAMP), a common drug of abuse, to access feasibility before investigating other substances

Substrates, solvents, and extraction methods





Transparent teflon film with Al foil backing

- Substrates
  - Teflon
  - Whatman cards
    - DMPK-A, B, C
    - DMPK-A, B, C-IND
- Deposition solvents
  - Isobutanol
    - Isobutanol: Methanol (9:1) (solubility issues)
    - Stock solution (100 ng/µL)
      - Nominal masses of 10 ng, 50ng, and 500 ng were first tested.
- Extraction solvents
  - MeOH
  - IPA
  - 0.2 N H_2SO_4

Desorption Methods

Cotton swab (spike in IS)

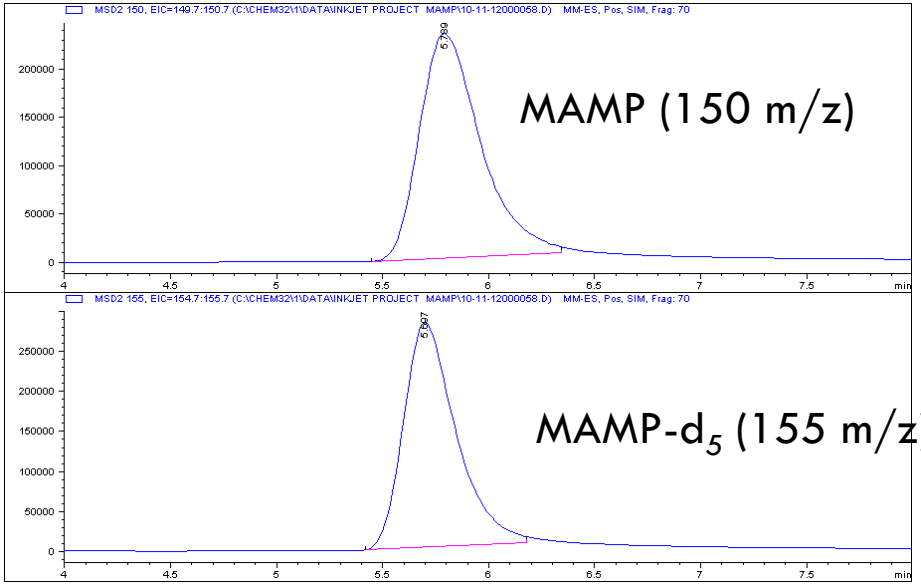
Peel and submerge (spike in IS)

Cut and submerge (solvent contains IS)

- Vortex-mix (1hr)[9]
- Remove (cotton swab, teflon, or card)
- Evaporate solvent to dryness under N_2 at 40 °C (add 50 µL MeOH: conc HCl (9:1))
- Transfer to clean centrifuge tube with ethyl acetate (precipitate excess cellulose fibers)
- Centrifuge; filter supernatant; evaporate to dryness.
- LC-MS: reconstitute in 100 µL Solvent A→Analyze in SIM mode (150 and 155 m/z monitored)
- GC-MS-derivatize with HFAA (30 min at 65 °C→ Analyze in SIM mode (254 and 258 m/z monitored).

LC-MS parameters for MAMP analysis

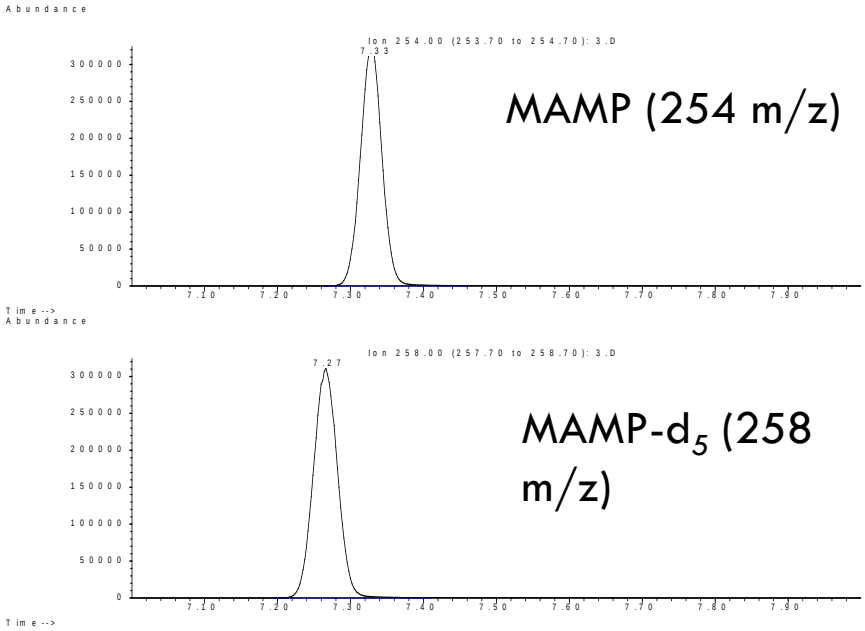
- LC/MSD with multimode electrospray ionization in the positive mode.
- Column: Phenomenex Luna C_{18} 15 cm x 2.0 mm; 5µm); 20 °C; 0.25 mL/min flow rate
- Gradient : Initially 0.1% acetic acid in H_2O: MeOH (92:8) for 3 min, ramped to 90:10 at 10 min, then ramped back to 92:8 at 15 min, and equilibrated for 5 min.
- MSD Parameters:
  - Fragmentor: 70 V
  - Drying gas: 12 L/min; 350 °C
  - Nebulizer Pressure: 25 psig
  - Vaporizer temperature: 150 oC
  - Capillary Voltage: 3500 V



Reconstructed selected ion chromatograms for MAMP calibrant (1:1)

GC-MS parameters for MAMP analysis

- HP 5973 Mass Selective Detector
- Column: DB-5MS capillary column (0.25 mm x 30 m x 0.25 µm); splitless injection
- Temperature program: 120 °C (initial), 1 min (hold time), 5° C/min (heating rate) to 150 °C, 8 min (hold time) for a total run time of 15 min.
- Monitoring (m/z 254 and 258) began at 7 min.



Summary of recovery from Teflon

~Amount Deposited	MeOH Cotton Swab (ng/sample)	Recovery %	~Amount Deposited	IPA Cotton Swab (ng/sample)	Recovery %	~Amount Deposited	Submerged in MeOH (ng/sample)	% Recovery
10 ng	0.83	8.29	10 ng	0.13	1.26	10 ng	ND	
50 ng	6.64	13.29	50 ng	7.83	15.66	50 ng	ND	
500 ng	210.61	42.12	500 ng	179.09	35.82	500 ng	308.60	61.72

Important notes:

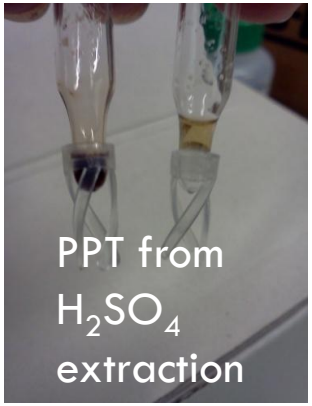
1. Recoveries from the 10 ng and 50 ng samples are very low; decided not to proceed with low concentration samples (All subsequent printing was done to yield 500 ng deposits).
2. While submersion of teflon into solvent provided higher recoveries compared to the cotton swab method, the adhesive backing lead to undesirable, inconsistent chromatograms.
3. MeOH, has slightly higher recovery than IPA (similar to literature).

Recovery from Whatman cards

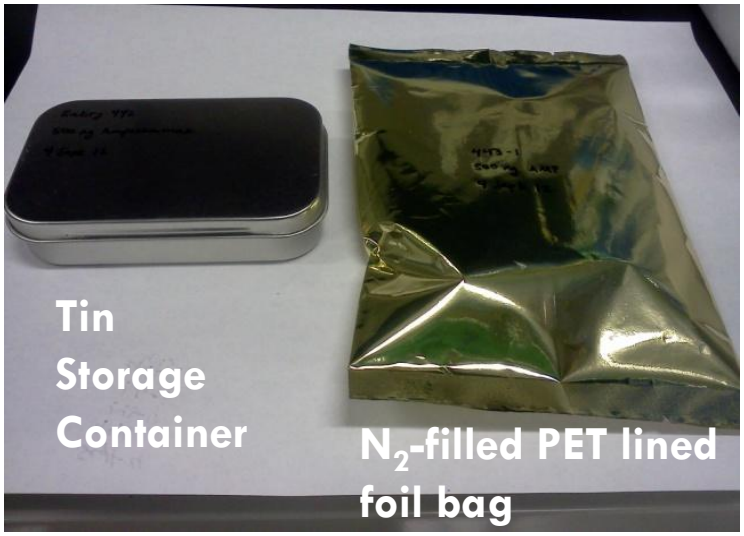
Card Stocks	3 mL H_2SO_4 desorption (ng/sample)	Recovery %	Card Stocks	3 mL MeOH Desorption (ng/sample)	Recovery %
DMPK-A	275.6694502	55.13389005	DMPK-A	277.643265	55.52865299
DMPK-B	PPT	NA	DMPK-B	378.4267106	75.68534211
DMPK-C	362.8086883	72.56173766	DMPK-C	353.7828365	70.75656729
DMPK-A-IND	PPT	NA	DMPK-A-IND	257.6502374	51.53004748
DMPK-B-IND	PPT	NA	DMPK-B-IND	406.4521899	81.29043797
DMPK-C-IND	PPT	NA	DMPK-C-IND	366.6126089	73.32252178

•500 ng DMPK-B-IND deposits was chosen because it yielded the highest recoveries and permitted visualization of the spots.

•Intraday and Interday precisions are both better than 4% RSD.



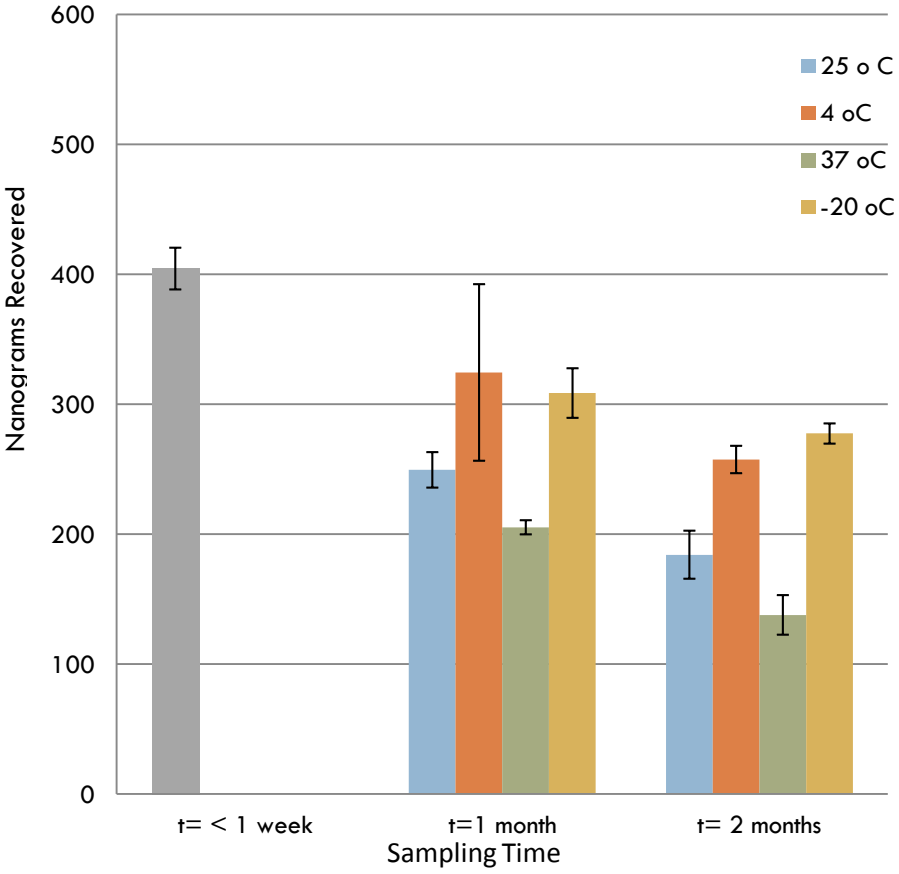
Storage Conditions



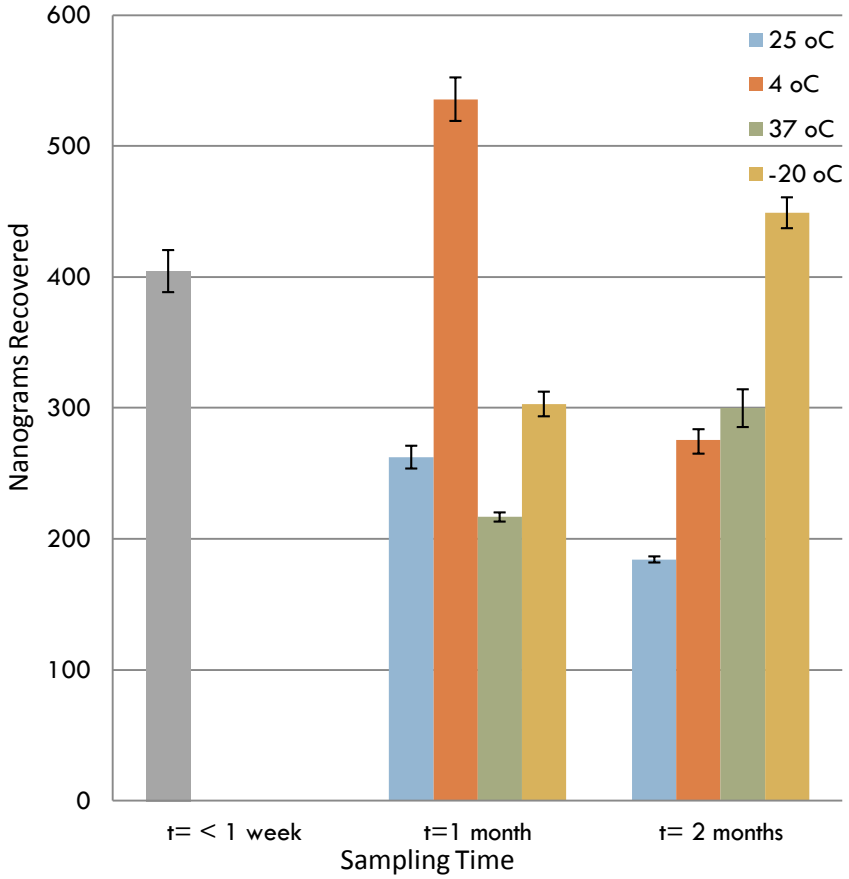
- Immediately after printing, cards were placed in either tin storage containers or N_2-filled storage bags
- Stored at -20 °C (freezer), 4 °C (refrigerator), 25°C (room temperature), and 37 °C (incubator)
- Samples were analyzed at various time points
  - (ie < 1week after printing, 1 month, 2 months etc…)

MAMP stability results by LC-MS

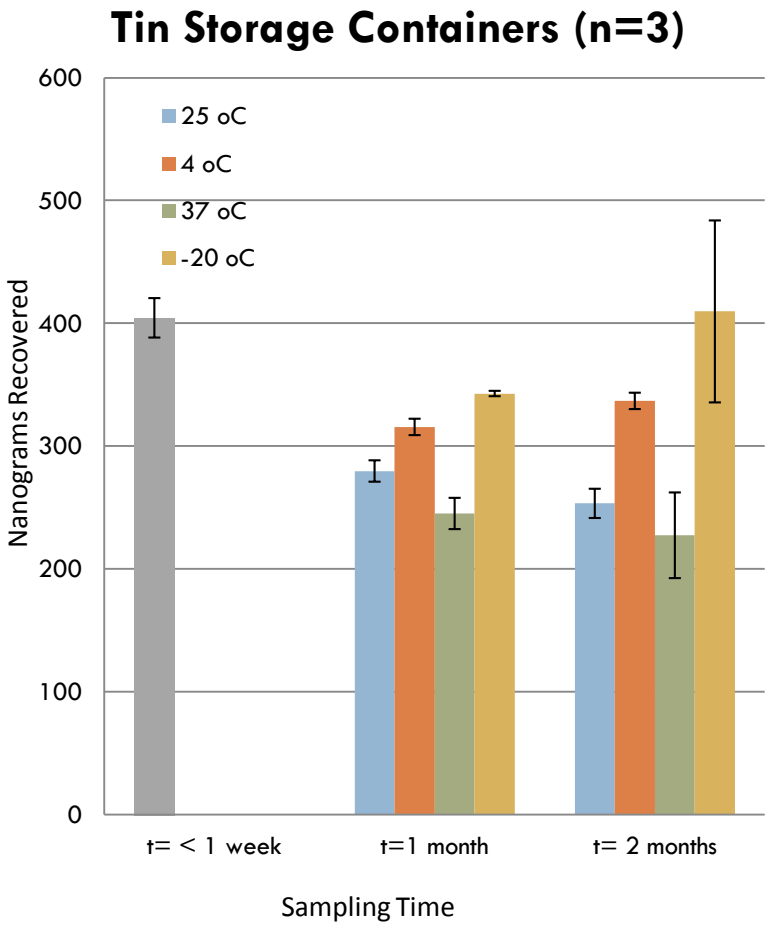
Tin Storage Containers (n=3)

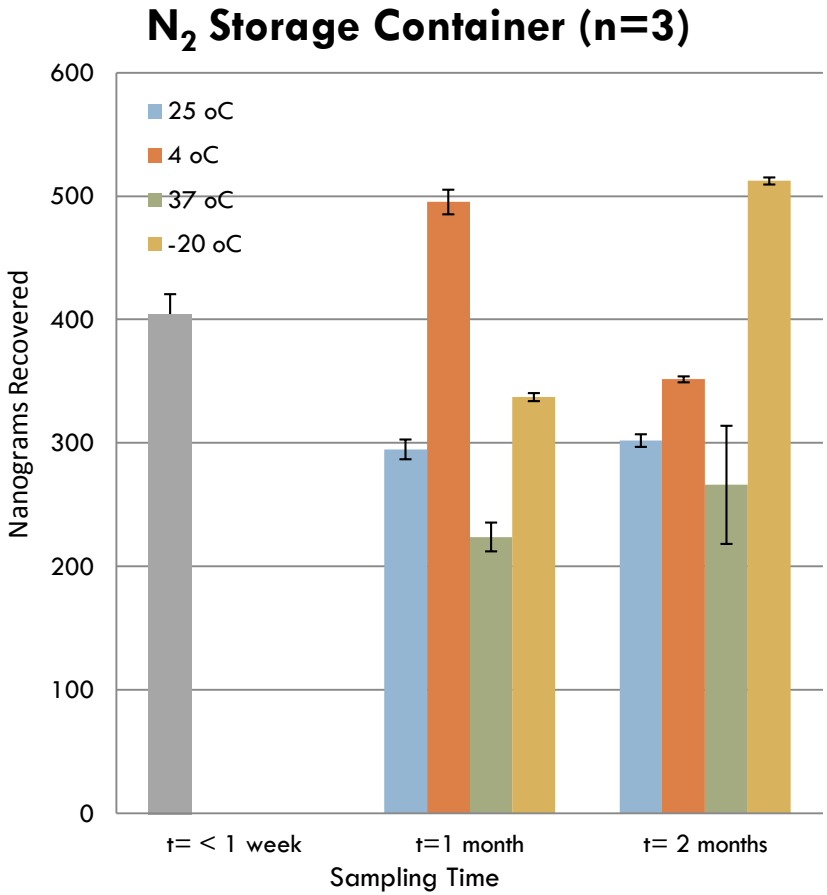


N_2 Storage Containers; (n=3)



MAMP stability results by GC-MS





Stability of MAMP at various storage conditions

Stability was assessed in triplicate at all storage conditions; the results are expressed as % of initial concentration

Tin (LC-MS)	1 month	2 months
25 °C	61.7	45.6
4 °C	80.2	63.7
37 °C	50.8	34.1
-20 °C	76.3	68.6

Tin (GC-MS)	1 month	2 months
25 °C	69.2	62.6
4 °C	78.0	83.3
37 °C	60.6	56.2
-20 °C	84.8	109.2

N_2 (LC-MS)	1 month	2 months
25 °C	64.9	59.5
4 °C	132.5	68.1
37 °C	53.6	74.1
-20 °C	74.9	111.1

N_2 (GC-MS)	1 month	2 months
25 °C	72.9	74.7
4 °C	122.4	86.9
37 °C	55.3	65.9
-20 °C	83.5	126.6

MAMP stability discussion

- Appears that storage at 4°C and -20°C mitigates the degree of degradation of MAMP on the DMPK-B-IND cards.
  - Reduction trend similar to Saussereau et. al. report that observed~50-59% (4 °C) and ~85-87% (-20°C) of MAMP remained in dried blood spots on filter paper after 6 months compared to the initial measurement[10].
- The data is too preliminary to determine if there is benefit to sealing in the N_2-filled storage bag.
  - Sample analysis will continue to determine if a more definitive trend exists.

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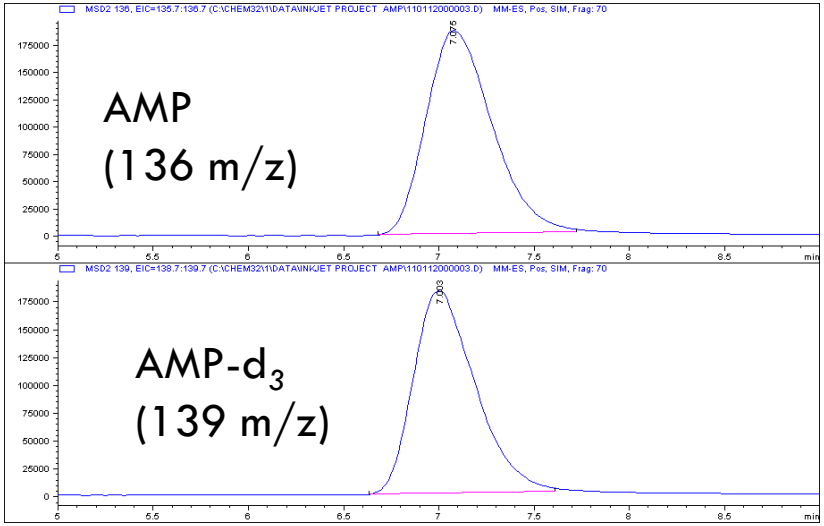
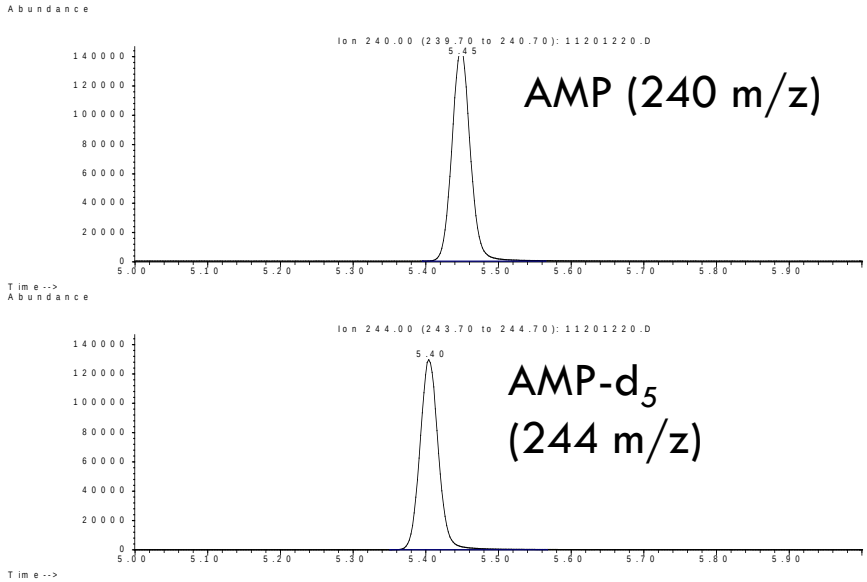
Amphetamine on DMPK cards

- Proceeded to determine if amphetamine would be a suitable analyte for this methodology.
  - Deposited, desorbed, and analyze using the same procedure as MAMP.
- LC-MS and GC-MS parameters were identical as those used for MAMP except for the SIM ions.

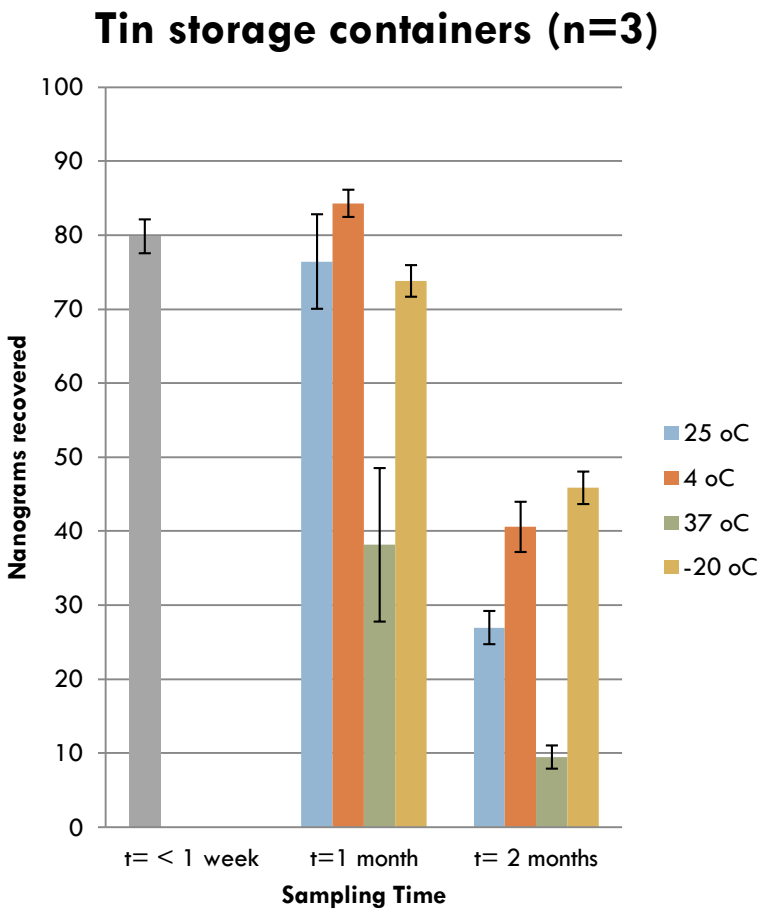


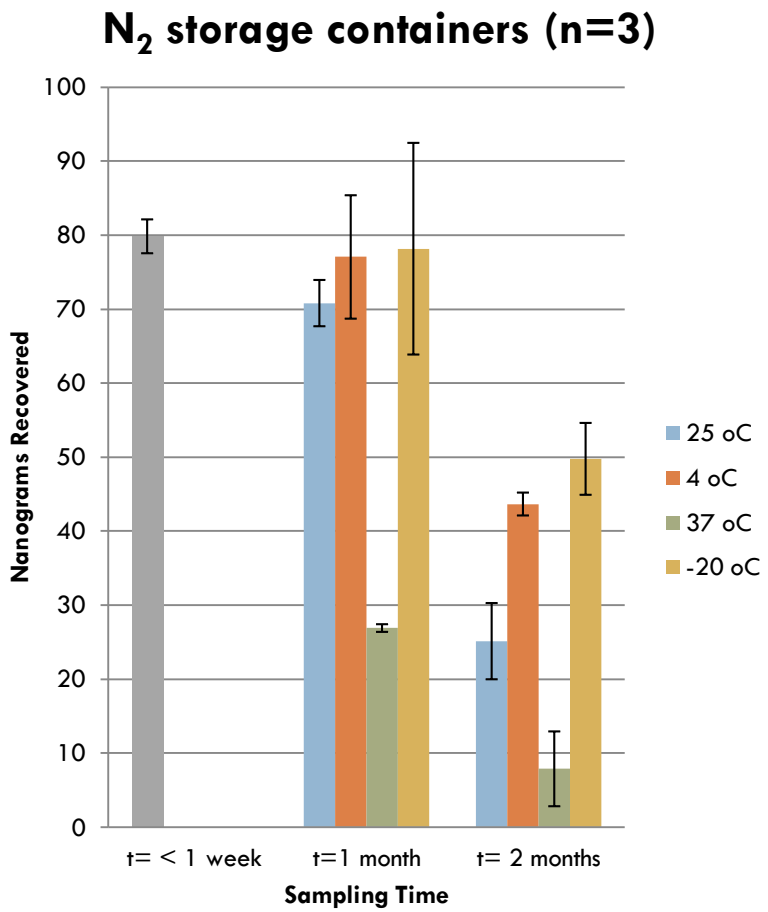


Reconstructed selected ion chromatograms for AMP calibrant (1:1) by LC-MS

Reconstructed selected ion chromatograms for AMP calibrant (1:1) by GC-MS

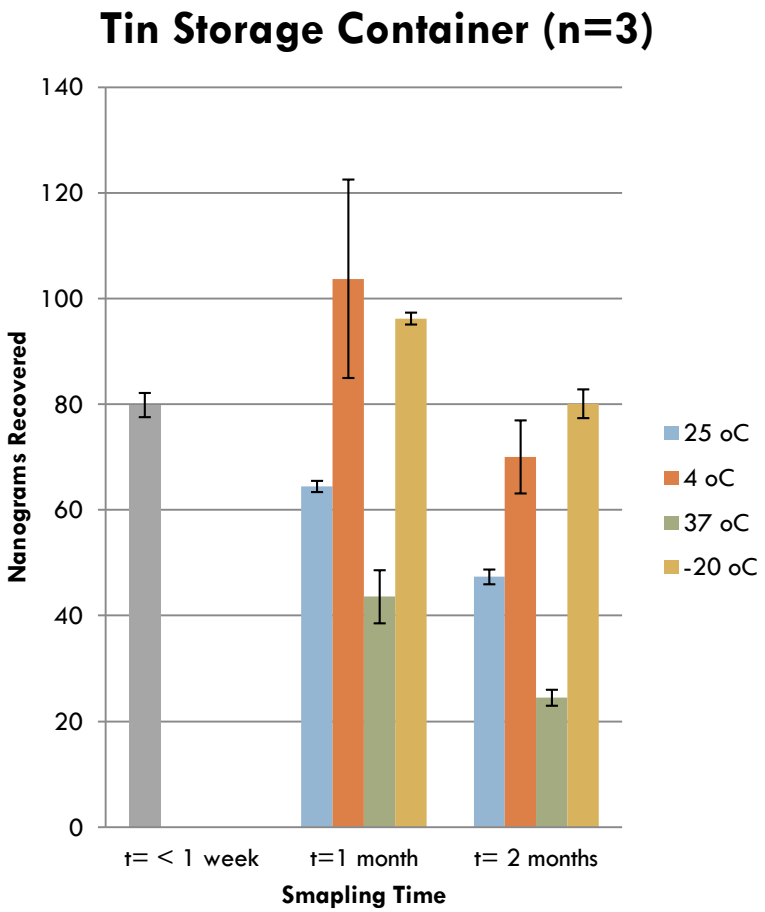
AMP stability results by LC-MS

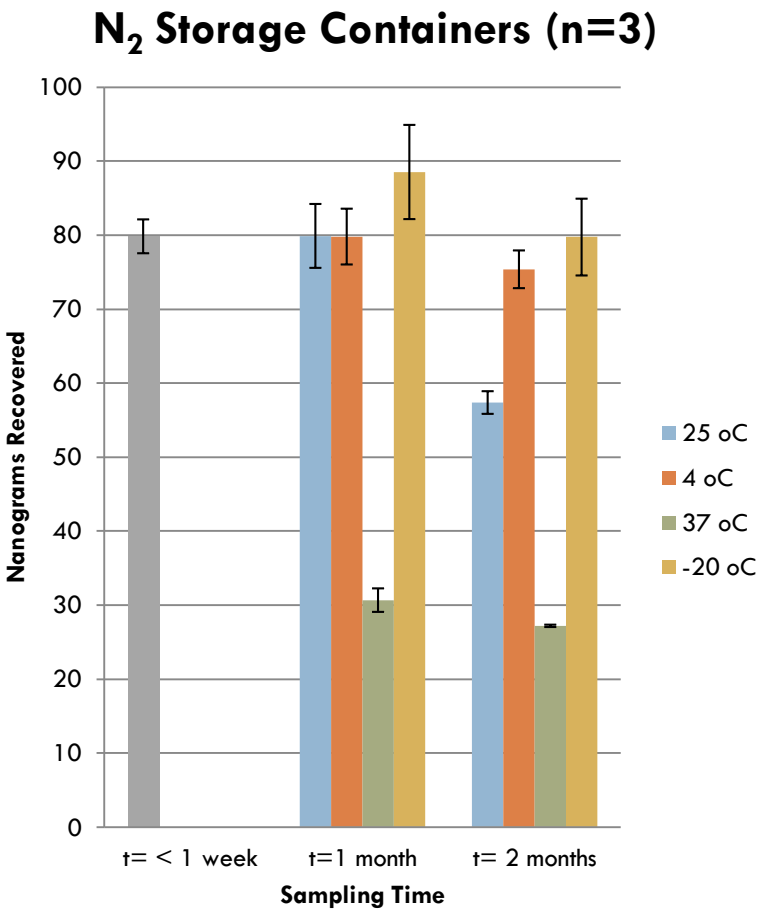




Recoveries from the initial measurement are much lower than MAMP (~15% vs ~80%)

AMP stability results by GC-MS





Stability of AMP at various storage conditions

Stability was assessed in triplicate at all storage conditions; the results are expressed as % of initial concentration

Tin (LC-MS)	1 month	2 months
25 °C	95.8	33.8
4 °C	105.8	50.8
37 °C	47.8	11.8
-20 °C	92.4	57.4

Tin (GC-MS)	1 month	2 months
25 °C	80.7	59.27
4 °C	129.9	87.7
37 °C	54.6	30.6
-20 °C	120.5	100.3

N_2 (LC-MS)	1 month	2 months
25 °C	88.7	31
4 °C	96.5	54.7
37 °C	33.7	9.9
-20 °C	97.9	62.3

N_2 (GC-MS)	1 month	2 months
25 °C	100.1	71.9
4 °C	100.0	94.4
37 °C	38.4	34.1
-20 °C	110.9	99.9

AMP stability discussion summary

- Recovery of AMP from the cards appeared to be extremely low.
  - Attributed this to crystallization in the print solution, which lead to a lower amount actually being deposited
- Despite this, when looking at relative amounts recovered over time, AMP appears to be more stable at 4°C and -20°C
  - Reduction trend similar to Saussereau et. al. report that observed~56-60% (4 °C) and ~86-96% (-20°C) of AMP remained in dried blood spots on filter paper after 6 months compared to the initial measurement[10].
- A new stock solution of AMP was prepared and used for printing immediately after preparation.
  - Recovery was improved; however, it was still less than desired (>80%).
  - Other extraction solvents will be explored to improve release efficacy of AMP from the DMPK-B-IND cards.

Summary/ Future Work

- This work has demonstrated the feasibility of utilizing inkjet printing technologies to produce single use, illicit drug testing material.
- Further characterization of the test materials will continue to better understand the stability of these compounds over time as well as efficacy of release from the printing substrates.
- In the future, this study will be expanded to include other illicit drugs (ie cocaine) and mixtures of drugs to serve as a foundation for potential NIST reference materials for seized drug analysis.

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