

Investigation of Solid Phase Microextraction as an Alternative to Dried Blood Spot

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Introduction

- There has been a growing trend in bioanalysis toward the utilization of microextraction techniques for sample isolation and transportation. Not only is there an interest in cost reduction associated with sampling, but also ethical advantages and improving sample retention using microsampling techniques.
- Major focus has been on utilizing dried blood spot (DBS) media as an inexpensive alternative to terminal blood draws. Though DBS does offer benefits towards cost reduction, shipping and sample storage, it is not without limitation specifically with respect to blood hematocrit levels.
- The purpose of this study is to explore the utility of solid phase microextraction (SPME) as an alternative sampling device to DBS media cards. BioSPME, as described as functionalized particles bound to a core fiber substrate, enables direct micro sampling of biological matrices without the need for additional sample treatment

What is BioSPME?

Solid Phase Microextraction is a sample preparation technique based on an equilibrium process in which the analyte partitions between the SPME coating and the sample matrix.

- Silica particles modified with C18 phase chemistries are embedded in a biocompatible proprietary binder
- Particles are coated on a durable, flexible metal fiber with 45 μm coating thickness
- Binder does not impede extraction of small molecules
- The amount of analyte extracted by SPME is directly proportional to the unbound concentration of the drug present in a given system.

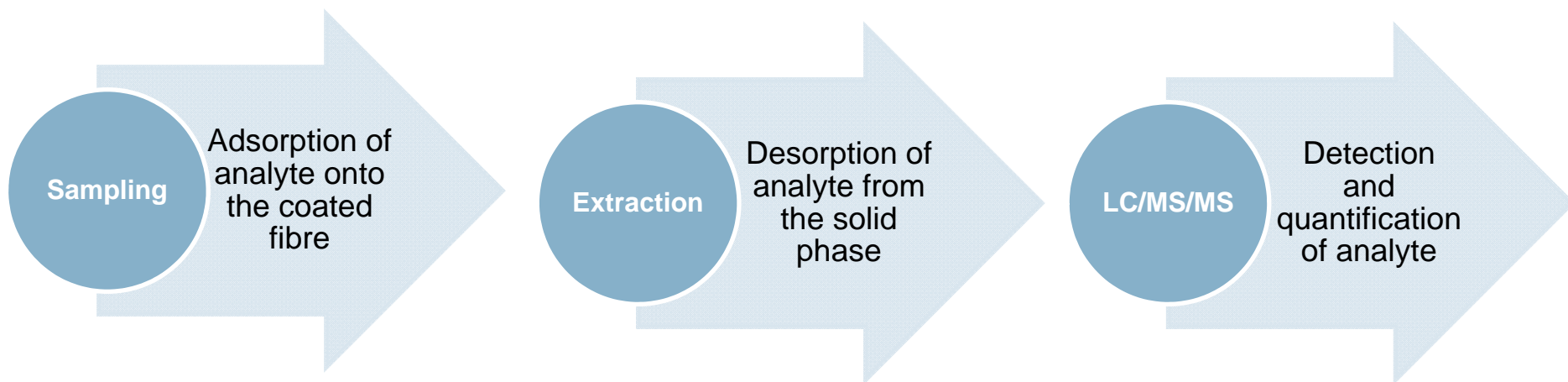
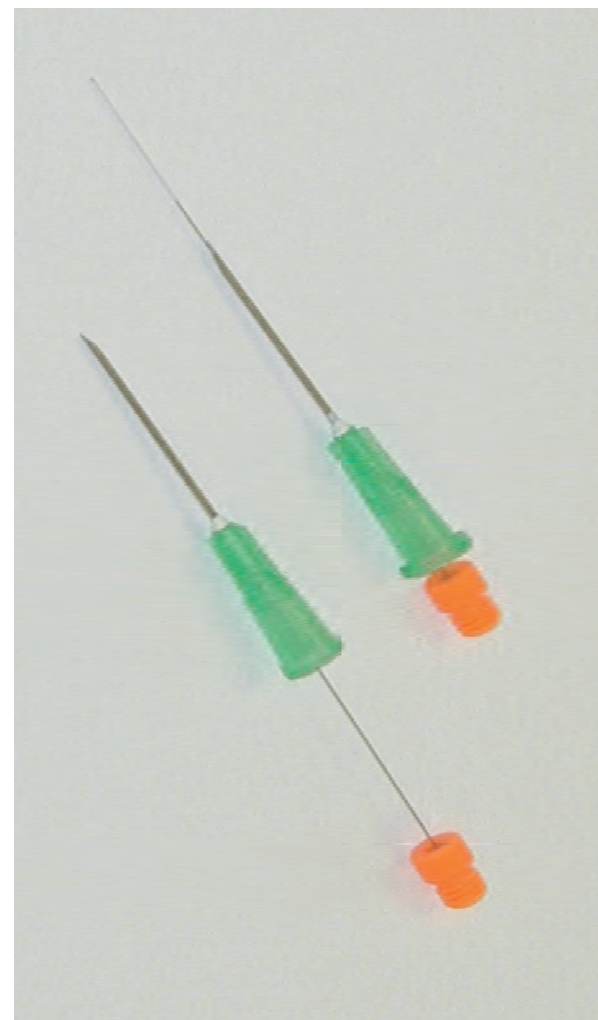
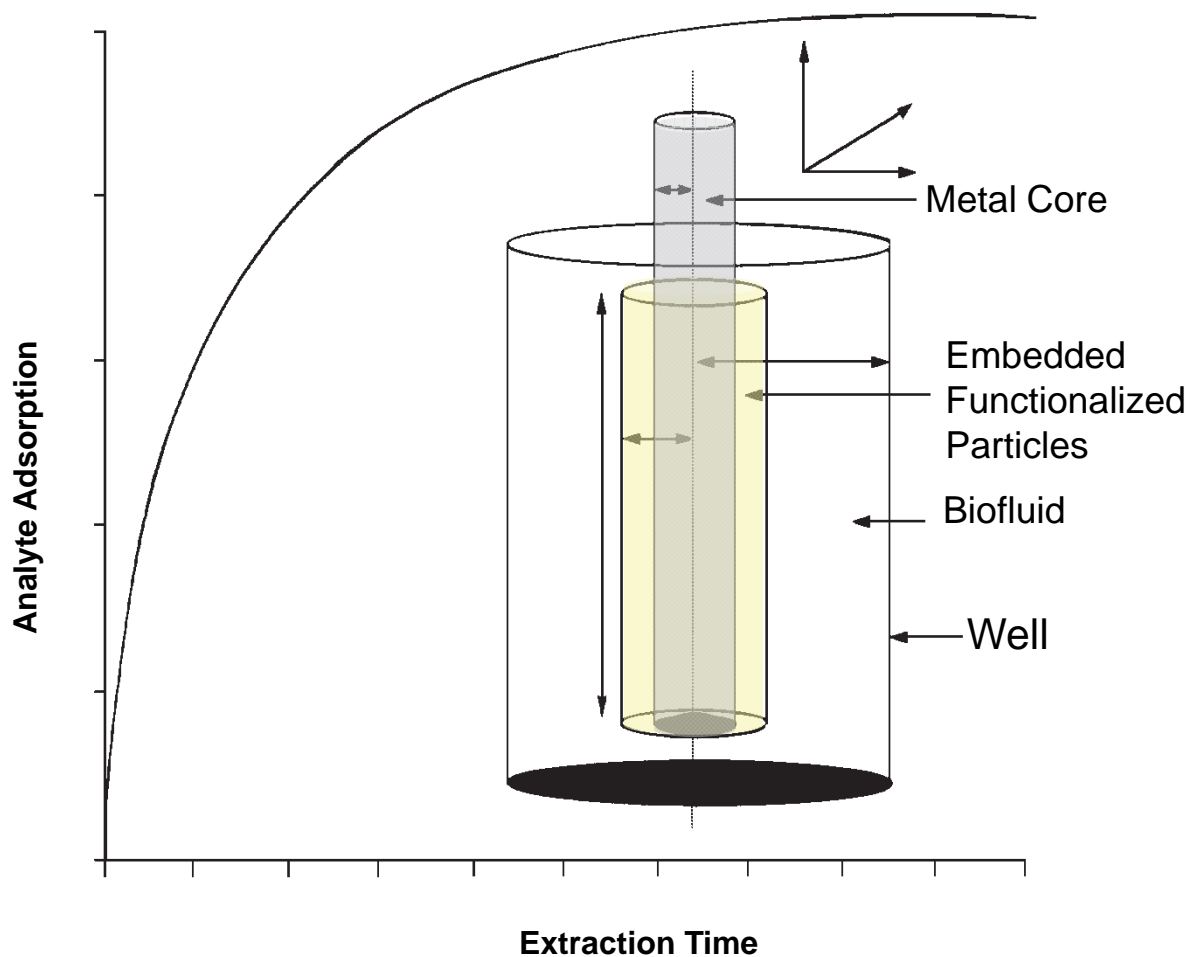


Figure 1. Adsorption Mechanism on BioSPME Extraction



Isolation of target analytes on BioSPME fibers occurs through differential migration of analytes in solution into fiber stationary phase. The distribution of analyte absorbed onto the fibers is described through the following distribution constant equation. Concentration of analyte in stationary phase compared to concentration of analyte in solution:

$$K = n_s / V_1 C_2^{\circ}$$

K = Distribution constant

n_s = Moles of analyte in stationary phase

V_1 = Volume of stationary phase

C_2° = Final analyte concentration in solution

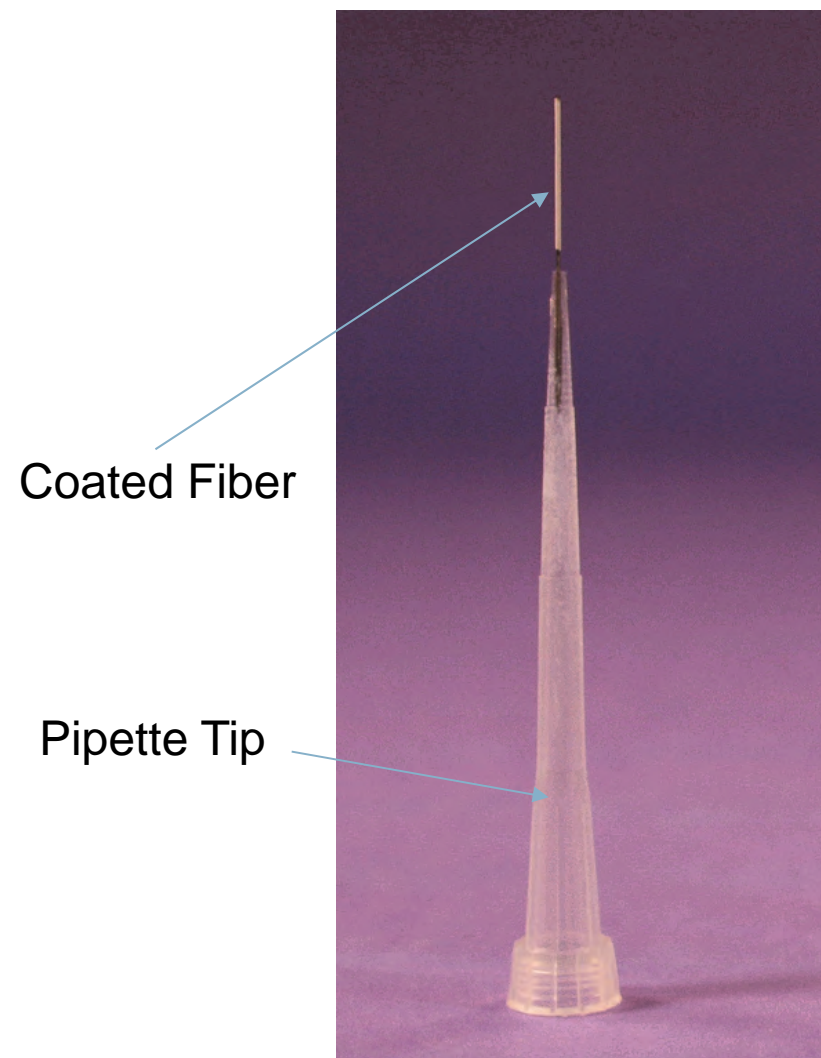
Advantages for SPME

- SPME combines **sampling, sample preparation** and **extraction** in one step
- Reduced overall number of sample processing steps
 - No sub-aliquoting
 - No centrifugation
 - No sample freezing/thawing
- Increased speed and improved efficiency
- Simplified sample preparation, reduced blood handling by analytical personnel
- Allows effective sample cleanup achieving high sensitivity

BioSPME Fiber Formats

- Two configurations of the BioSPME allow for direct sampling of a range of biological matrices such as whole blood, urine, saliva, tissue.
- Both BioSPME probe and tippet assemblies are designed for convenient on-site sample collection. Fibers can be packaged for shipment to testing facility.
- BioSPME probes designed for insertion into sample, i.e. septa vial, fruit, tissue. Not approved for human testing
- The BioSPME tippets are designed for sampling of isolated samples and for batch processing/ automation. Tip format allows for use with robotics automation, or can be manipulated manually by transferring tip rack.
- Fibers are functionalized with C18 modified silica particles.

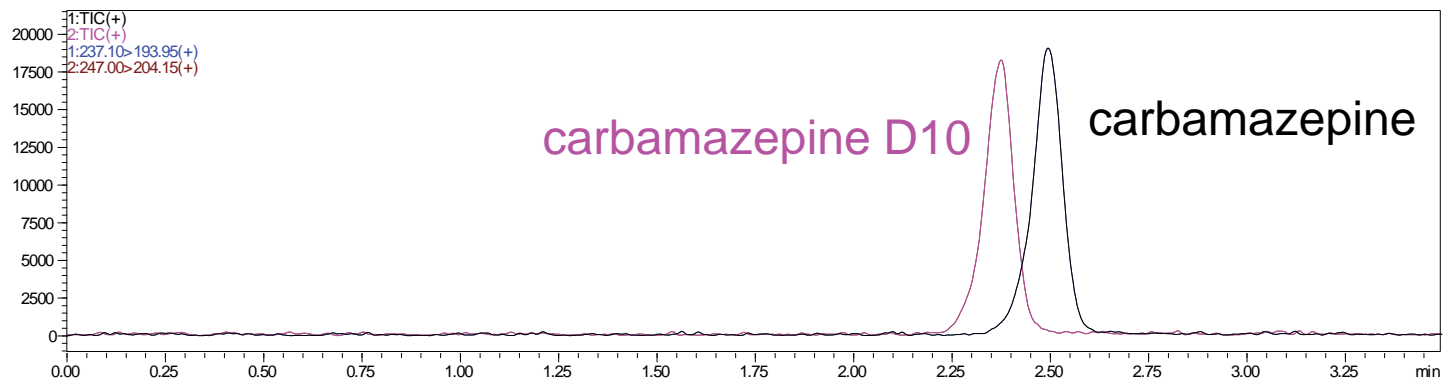
Figure 2. Single Use BioSPME Tippet Format



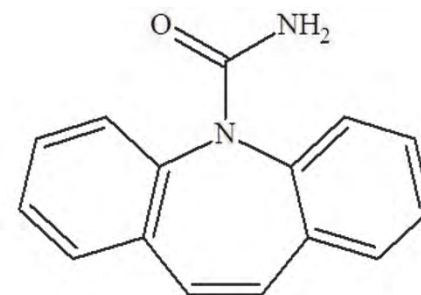
Experimental Conditions

- The purpose of this study is to explore the utility of solid phase micro-extraction (SPME) as an alternative sampling device to DBS media cards. Goal of the study is to demonstrate the ability to isolate target compounds from whole blood samples and compare response to DBS sampling media.
- A simple model compound was used to explore extraction efficiencies, detection limits, binding issues, and also hematocrit impact differences between DBS and BioSPME sampling techniques.
- Blood samples with ranging hematocrit levels are extracted using both techniques for comparison of analyte detection. These preliminary experiments utilize a larger volume of whole blood for the BioSPME extractions to demonstrate proof of concept that fibers could be used to isolate target molecules.

Figure 3. Chromatographic Conditions on Ascentis® Express C18



column: Ascentis® Express C18, 5 cm x 2.1 mm, 2.7 μ m
mobile phase: (A) 5 mM ammonium formate water
(B) 5 mM ammonium formate (85:15 acetonitrile:water)
ratio: A:B 70:30
flow rate: 0.3 mL/min
column temp.: 35 °C
injection: 2.0 μ L
MS det.: ESI+, MRM
system: Shimadzu® LSC-30, LCMS8030



Carbamazepine
Monoisotopic Mass = 236.094963 Da

DBS Extraction Conditions

Sample:

Human blood samples with hematocrit level ranging from 20%, 45% and 70% HCT (Bioreclamation), spiked at 50 ng/mL with mixed compounds

DBS Cards:

Whatman® FTA DMPK-A Cellulose Based media Sigma Part# WB12941

Agilent® Bond Elute DMS Glass Fiber Based media Agilent Part# A400150

Sample Extraction:

A 20 µL aliquot of sample was added onto each DBS media card. Cards were allowed to dry overnight, then stored in a ziplock bag for 3 weeks prior to desorption.

Desorption:

Solvent: carbamazepine D10, 20 ng/mL in methanol

A 3 mm punch from each spot was placed into a 100 µL conical vial and desorbed with 50 µL of solvent for 18 hours. The remaining punch was removed from the vial prior to analysis.

BioSPME Extraction Conditions

SPME Fibers:

C18 lot# 1881-48

SPME Conditions:

Condition C18 fibers in 50:50 acetonitrile:water prior to extraction (minimum of 20 minutes)

Sample Extraction:

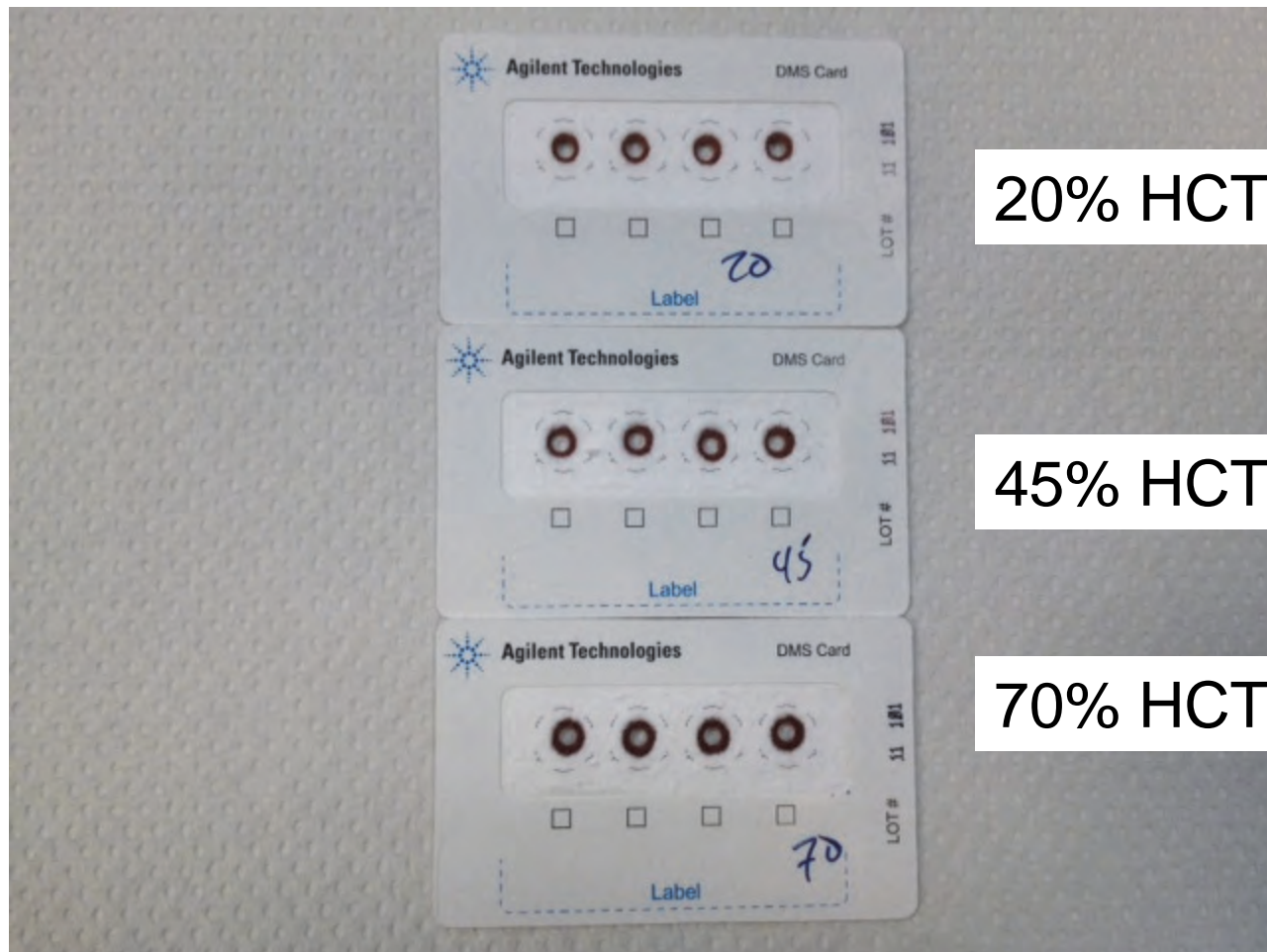
A 300 μL aliquot of sample was added into a conical 96-well plate. SPME fibers were added directly and extractions occurred for 15 minutes with vortex agitation at 500 rpm on an IKA MS3 shaker. Fibers were then dipped into water for 30 seconds to wash off excess fluid, then wiped prior to desorption

Desorption:

Solvent: carbamazepine, 20 ng/mL in methanol

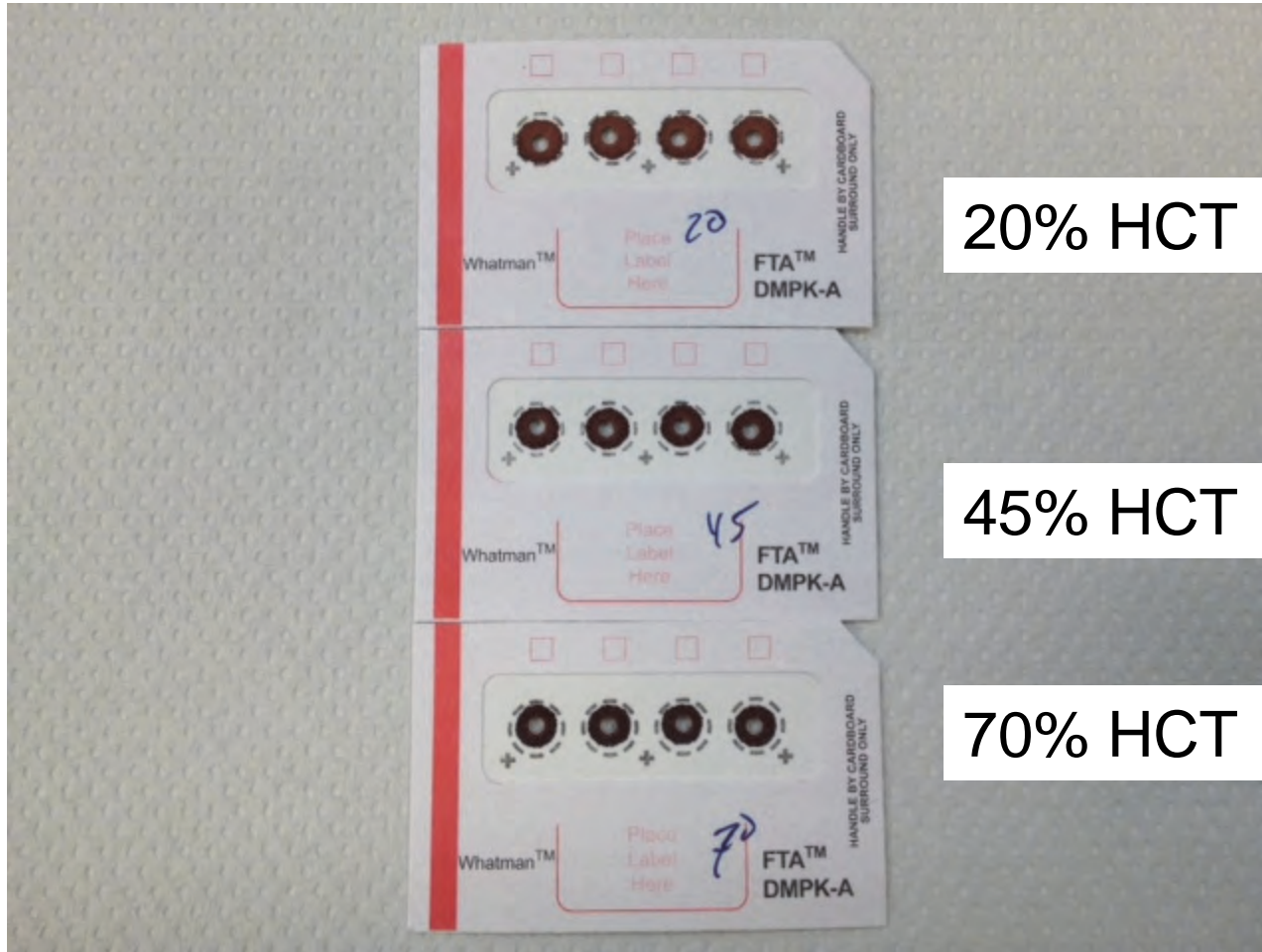
When extraction was complete, fibers were then desorbed for 30 minutes in a conical HPLC vial with 50 μL of desorption solvent. Desorption conducted on IKA shaker for 30 minutes at 500 rpm. Samples were then analyzed directly by LC MS/MS.

Figure 4. Blood Spot Size Variation: Agilent DMS Media



There was a noticeable increase in blood spot diameter with increasing hematocrit level on the glass fiber base media of the Agilent card. The Agilent cards utilize glass fiber materials as the absorbent for the blood spots.

Figure 5. Blood Spot Size Variation: Whatman DMS Media



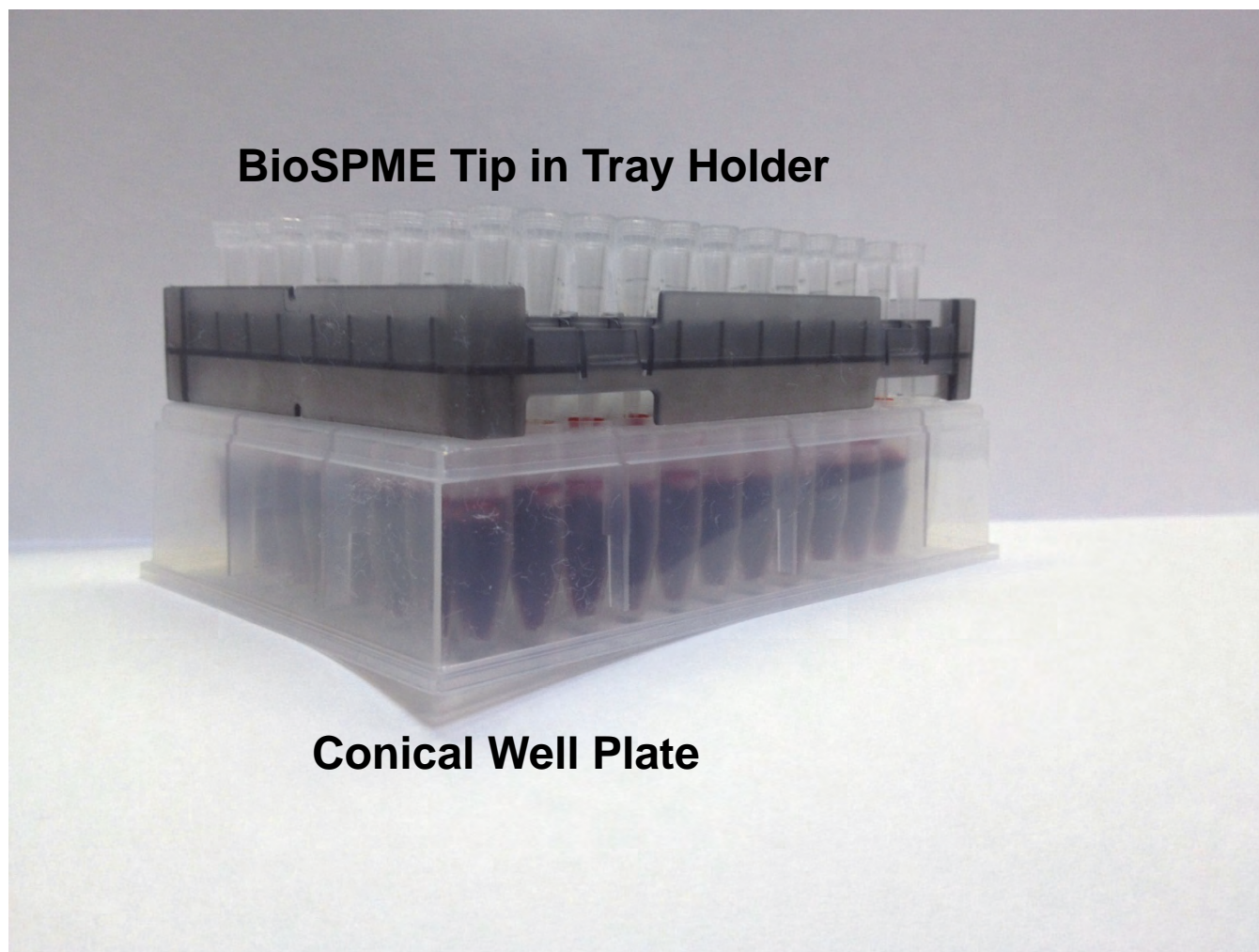
20% HCT

45% HCT

70% HCT

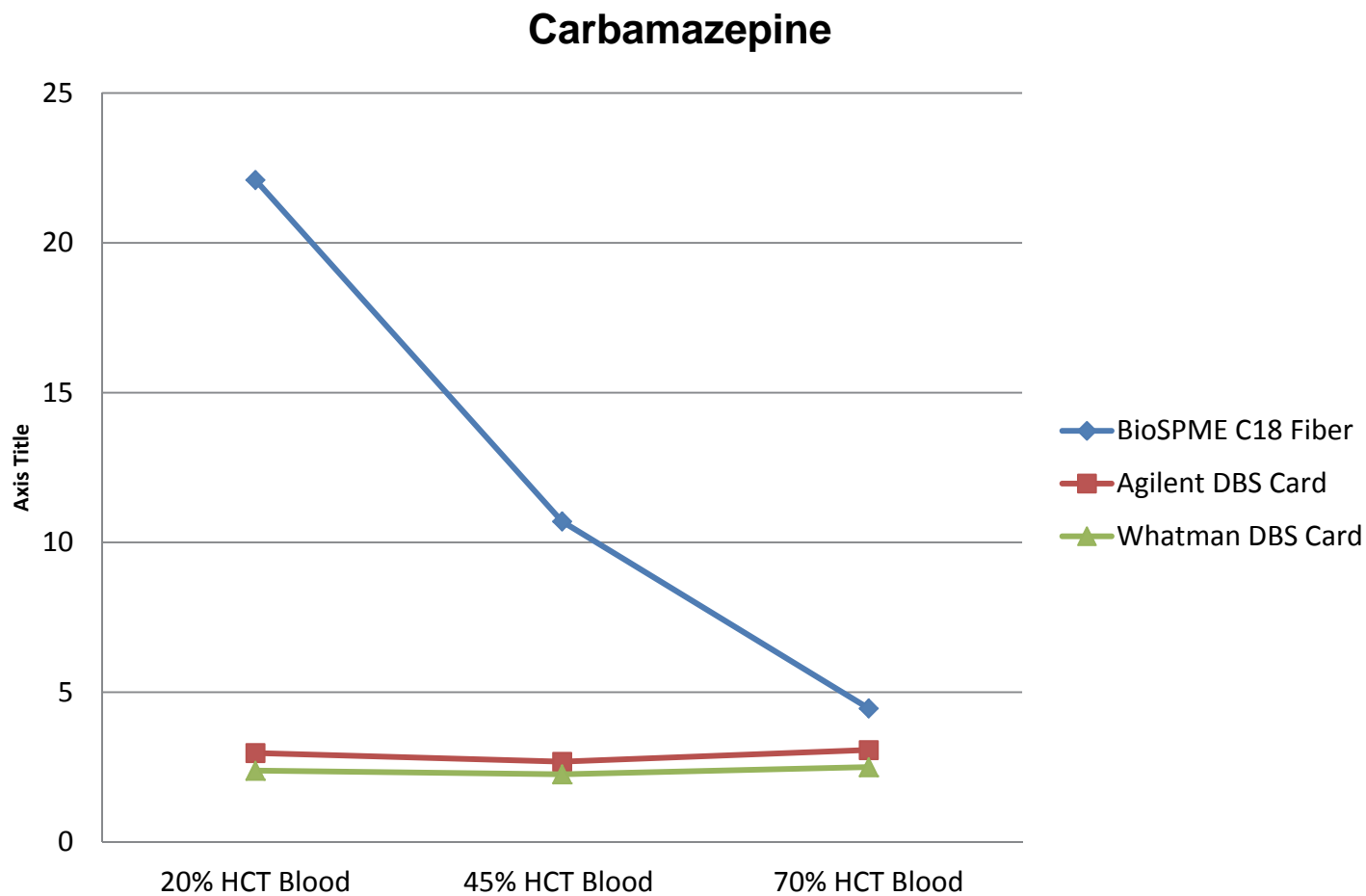
There was a noticeable decrease in blood spot diameter with increasing hematocrit level on the cellulose base media of the Whatman card. This is counter to observations with the glass fiber media. Whatman cards utilize cellulose fibers as the absorbent for the blood spots.

Figure 6. BioSPME Extraction Setup



Whole blood samples were placed into a conical well plate, and BioSPME fibers were inserted for isolation of target analytes. Fibers were then dipped into a water wash after extraction to displace remnants of blood from the fibers

Figure 7. Hematocrit impact on Carbamazepine



Results

- The BioSPME fibers exhibited significantly higher levels of detected carbamazepine as compared to the DMS media. At 45% HCT levels (typical for normal blood) the BioSPME technique demonstrate 5X increase in sensitivity as compared to the DMS media.
- For the BioSPME technique, there does appear to be a correlation of recovery of carbamazepine to hematocrit level.
- Detected carbamazepine levels did decrease with increasing hematocrit level using the BioSPME technique.
- Carbamazepine levels were fairly consistent with both DMS media, but response was very low as compared to BioSPME.
- Could reduced extraction efficiency with increasing HCT be due to competitive binding on the fiber?
- To further investigate this phenomenon, aliquots of whole blood samples were centrifuged to isolate plasma samples. A comparison of BioSPME extraction efficiencies was then conducted between whole blood and derived plasma samples. This experiment should help determine if hematocrit is impacting analyte extraction.

Sample Preparation Conditions

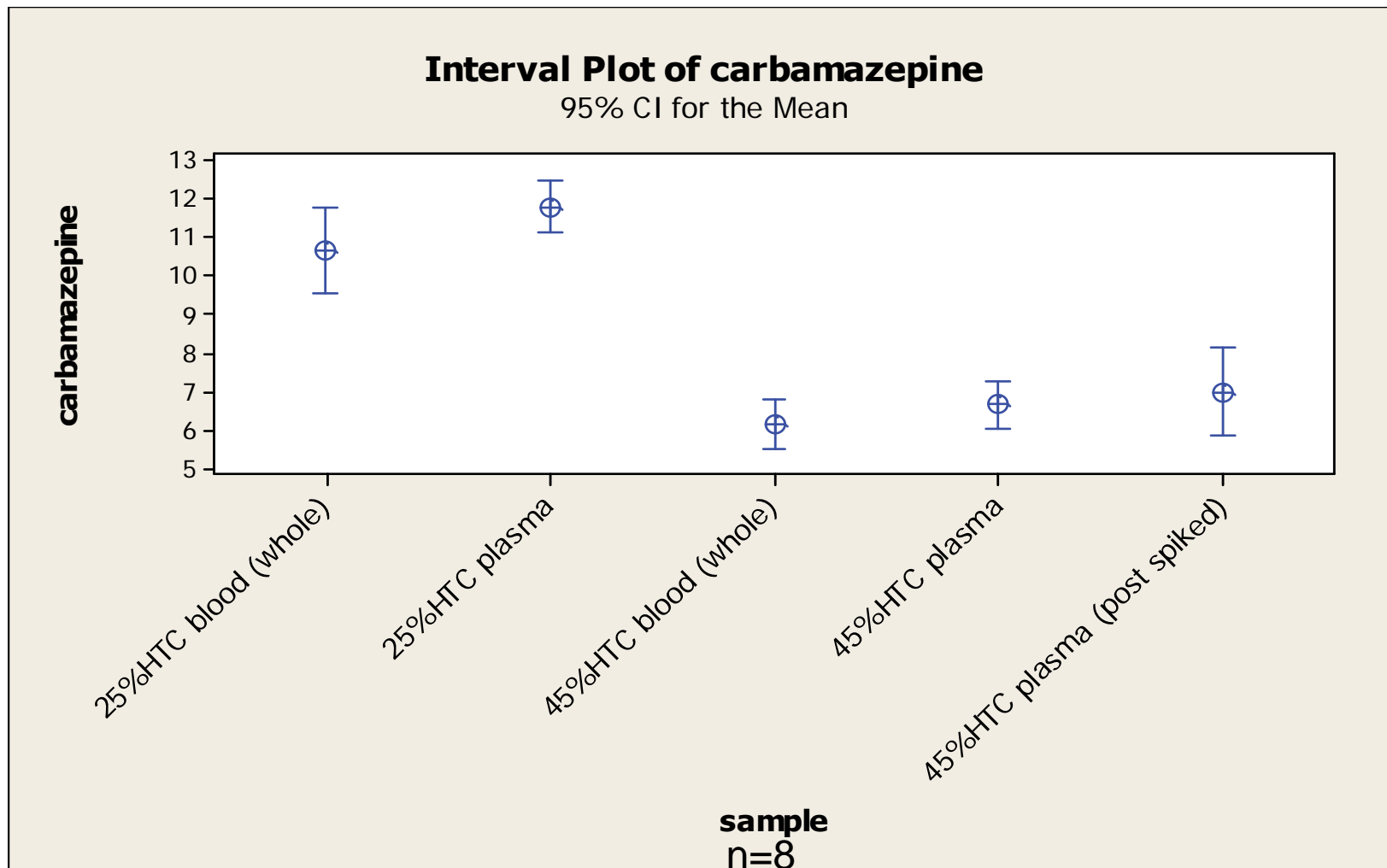
Sample:

Set 1. Human whole blood samples with hematocrit levels of 25% and 45% (Bioreclamation), spiked at 50 ng/mL with mixed compounds. Blood samples were equilibrated for 1 hour prior to SPME extraction. A 300 μ L aliquot of each sample was added into a 96 conical well plate for extraction.

Set 2. Human whole blood samples with hematocrit levels of 25% and 45% (Bioreclamation), spiked at 50 ng/mL with mixed compounds. Blood samples were equilibrated for 1 hour prior to SPME extraction. After equilibration, whole blood samples were centrifuged for 14 minutes at 4000 rpm. The resultant plasma was collected for analysis. A 300 μ L aliquot of each plasma sample was added into a 96 conical well plate for extraction.

Set 3. Human whole blood samples with hematocrit levels of 45% (Bioreclamation), were centrifuged for 14 minutes at 4000 rpm. The resultant plasma was collected and spiked at 50 ng/mL with mixed compounds. The plasma samples were equilibrated for 1 hour prior to SPME extraction. After equilibration, a 300 μ L aliquot of each plasma sample was added into a 96 conical well plate for extraction.

Figure 8. Carbamazepine Levels in Whole Blood vs. Plasma BioSPME Extraction



Results

- As observed in previous experiments, there was a dramatic difference in extraction efficiency between 25% HTC and 45% HTC blood samples. The 25% HTC blood samples had nearly 2X response to the 45% HTC blood samples.
- Carbamazepine demonstrated decrease extraction efficiencies with the 45% HTC samples.
- There does not appear to be a significant difference in extracted analytes between the whole blood sample and the plasma sample, when compared to the same starting hematocrit level.
- Plasma that had been spiked after centrifugation of red blood cells gave similar response to spiked whole blood/plasma. This would indicate minimal analyte binding to the red blood cells.
- Noted that isolated plasma from whole blood samples had significant red discoloration indicating hemolysis of red blood cells. Is this impacting extraction efficiencies?
- Will continue experiments to determine if sample viscosity impacts extraction efficiency on whole blood samples.

Results (contd.)

- A third series of experiments was conducted to evaluate the impact that sample viscosity has to the extraction efficiency of the BioSPME fibers, will conduct additional experiments where the whole blood is diluted prior to extraction.
- Target is to dilute whole blood samples to a calculated 25% HTC value. Blood samples were diluted 2:1.6 with saline solution. ($45\% \times 0.5555 = 25\%$)
- A comparison of extraction efficiencies to 25% HTC and 45% HTC whole blood samples was conducted.

Sample Preparation Conditions

Sample:

Set 1. Human whole blood samples with hematocrit levels of 45% (Bioreclamation), spiked at 50 ng/mL with mixed compounds. Blood was then diluted 2:1.6 with saline to give a relative HTC level of 25%. Blood samples were equilibrated for 1 hour prior to SPME extraction. A 300 μ L aliquot of each sample was added into a 96 conical well plate for extraction.

2 mL blood spiked at 50 ng/mL + 1.6 mL saline

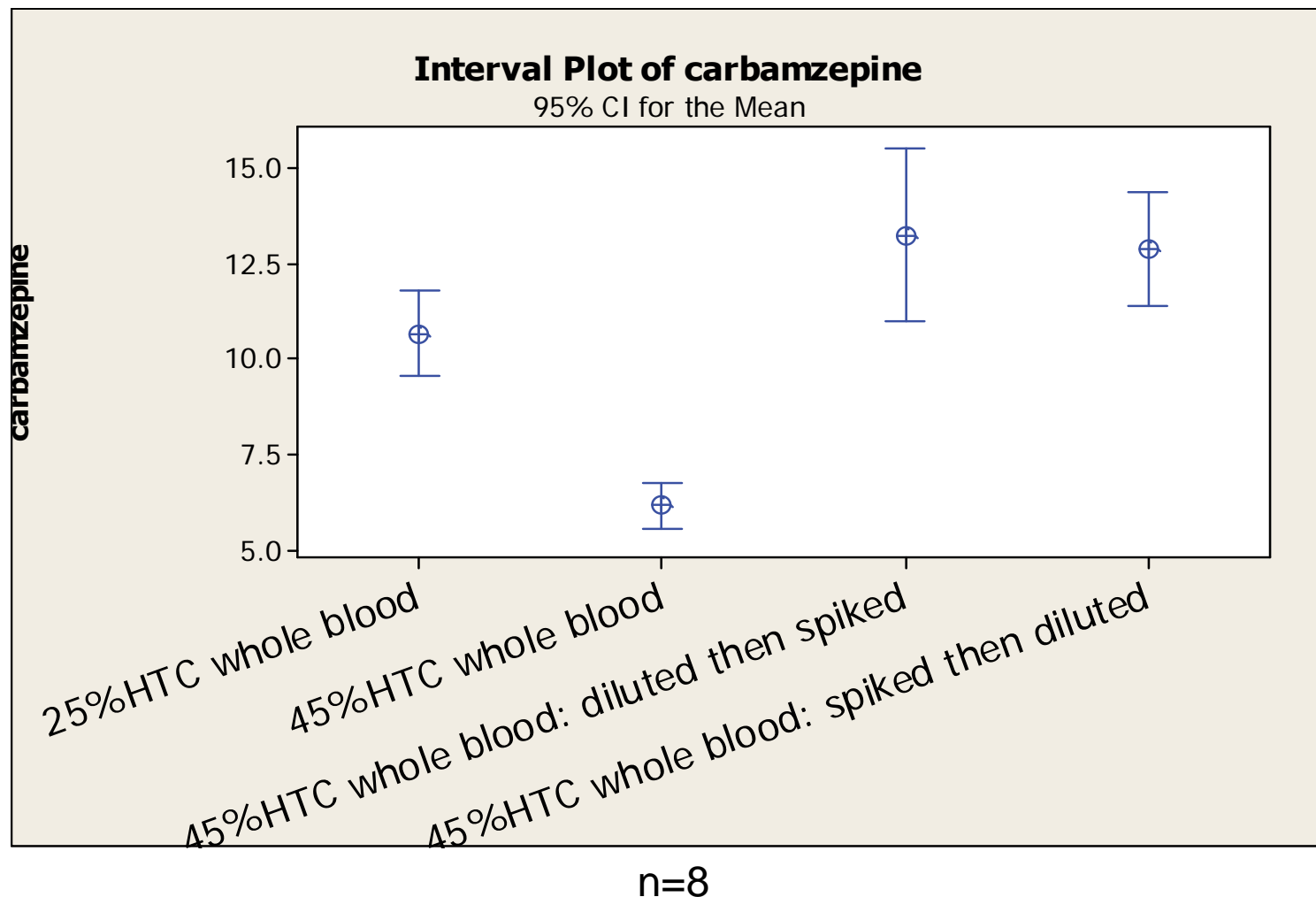
Mathematical correction

Set 2. Human whole blood samples with hematocrit levels of 45% (Bioreclamation), was diluted 2:1.6 with saline to give a relative HTC level of 25%. This diluted blood sample was then spiked at 50 ng/mL with mixed compounds. Blood samples were equilibrated for 1 hour prior to SPME extraction. A 300 μ L aliquot of each sample was added into a 96 conical well plate for extraction.

2 mL blood + 1.6 mL saline, spiked at 50 ng/mL

The difference between the two samples represents the dilution of matrix versus reduction in sample viscosity.

Figure 9. Impact of Sample Dilution on BioSPME Extraction of Carbamazepine



Summary

- From the dilution studies, it does appear that analyte diffusion is a major role in the extraction efficiency of the BioSPME fiber.
- Dilute 45% HCT blood samples demonstrated extraction efficiencies similar to 25% HCT levels.
- Dilute 45% HCT blood samples exhibited extraction efficiencies similar to that of 25% HCT. This trend was consistent in both 45% HCT diluted sample thus supporting minimal analyte binding to whole blood cells
- Blood hematocrit levels appear to be less of a contributing factor in extraction efficiency, more emphasis is placed upon fluid viscosity.
- There was a definite sign of hemolysis in the isolation plasma from the whole blood samples, this is a likely source of increased sample viscosity in the plasma samples. Need to determine if this is a major source of extraction variation in the plasma/blood samples.

Summary (contd.)

- Continuing work on freshly prepared hematocrit blood sample instead of commercially prepared samples, concerned that hemolysis is causing variation issue not hematocrit level.
- Continue to investigate the impact of BioSPME extraction time with regards to varied blood hematocrit levels. Are short extraction times impacted as much by blood hematocrit levels.
- The BioSPME technique does appear to have some benefits over dried blood spot sampling. Ability to concentrate analytes from sample matrix is an attractive approach for low level compounds.
- Device design is highly amenable for remote sample and shipping, making it a possible candidate for point-of-care and bedside sampling.
- Further work is continuing to better understand how to leverage the utility of this device.

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