Implementing Dried Blood Spot Technology: Practical Considerations from Discovery to Development

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Implementing Dried Blood Spot Technology: Practical Considerations from Discovery to Development

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Goals of DBS Research at BMS

- Implement DBS technology from Drug Discovery through Development for appropriate projects
- Implement DBS technology for programs where it enables critical studies that otherwise would be challenging to conduct
- Explore DBS technology in other applications

BMS DBS Strategy

Discovery Studies

- Gather information on DBS
  - Non-regulated space
  - Flexibility in integrating DBS into the Discovery workflow
    - Advantages in Discovery?
  - Evaluate both plasma/serum and DBS
  - Identify “gaps”, potential issues

BMS DBS Strategy

Discovery Studies → Non GLP Studies

- Enable the addition of DBS collection in Non-GLP Early Development Studies
  - Form studies, dose range finding studies
  - Less impact on filing timelines
  - Optimize Development workflow
  - Establish procedures and validation protocols for DBS methods

BMS DBS Strategy

- Add DBS to GLP studies
  - Open dialogue with regulatory agencies
  - Transition to DBS only collection
- Conduct technically challenging studies
  - Reproductive toxicology
  - Juvenile animal and pediatric studies
  - Remote sites

Early Discovery Applications

- Improved Sample Stability with DBS
  - Compounds in Discovery dosed at risk
    - No a priori assessment of stability
  - Expedite in-vivo screening of prodrugs
    - DBS collection can reduce/eliminate the need for esterase inhibitors [1]

- Simplify and improve Globally integrated support
  - Evaluating shipment of DBS samples between Discovery Bioanalytical groups in US and India

- Early Discovery program with an interest in exposures obtained from whole blood
  - All studies DBS collection only

[1] D'Arienzo et al, Bioanalysis 2010 2(8)

Late Discovery Applications

- Multispecies *in-vivo* and *in-vitro* data available for Lead compounds in late Discovery
  - Sampling, handling, bioanalytical methods established

- In 2010 DBS collection was added to the protocols of nine Discovery Toxicology studies
  - Six dosed compounds, one metabolite
  - Three different species
  - Plasma and DBS samples collected, analyzed, and the data was compared
  - Plasma data was the “official” data in each Toxicokinetic report

Late Discovery Applications

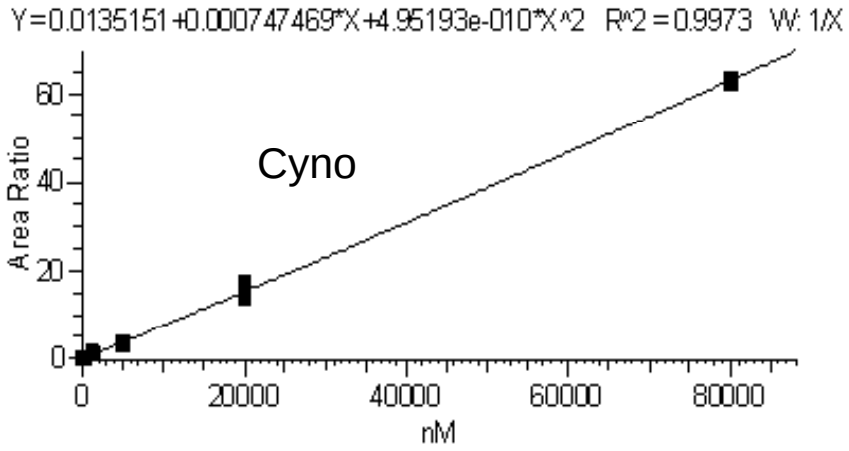
- DBS and plasma data provided "*in-vivo* RBC" partitioning data which was used to confirm in the *in-vitro* data
  - DBS being evaluated to replace the current *in-vitro* RBC experimental design

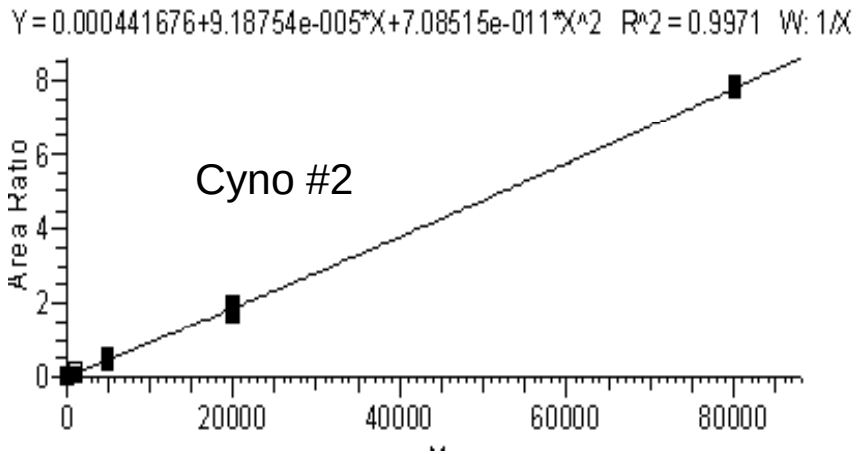
- DBS data for BMS-1 enabled an Exploratory Toxicology study for the back up program
  - DBS only
  - Serial bleeds
  - Less compound required
  - Resulted in quick scheduling/dosing

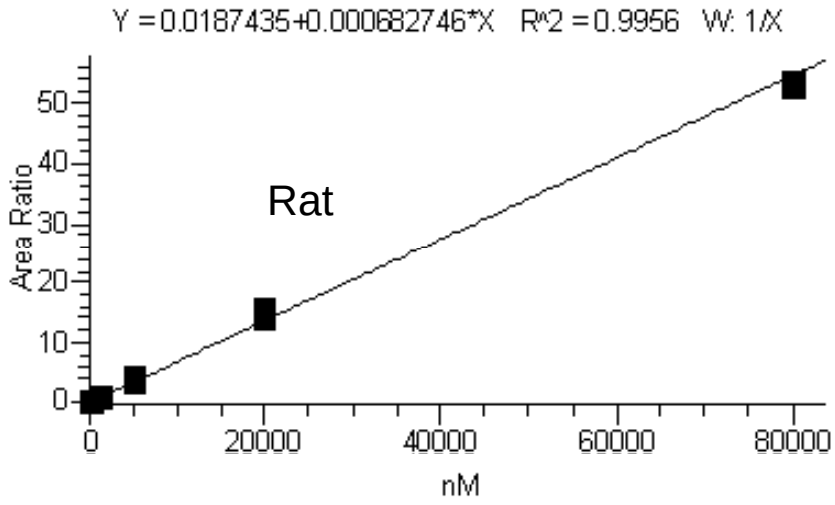
Late Discovery Applications

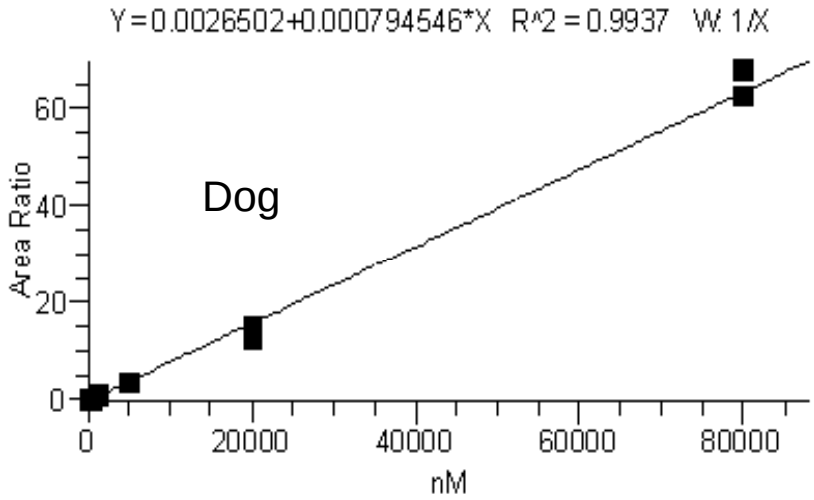
- DBS enabled bioanalysis without sample dilution in Discovery Toxicology studies
  - Dilutions of 50-fold or more are common
  - uHPLC-MS/MS analysis
  - DBS small sample size an advantage
  - Less analyte injected
  - “Right shifted” linear dynamic range
    - 10 fold higher ULQ possible
  - %Difference between individual plasma and DBS data was acceptable
  - Good linear correlation for TK parameters

Performance of DBS standard curves from four Discovery toxicology studies for BMS-1, 78-80,000 nM

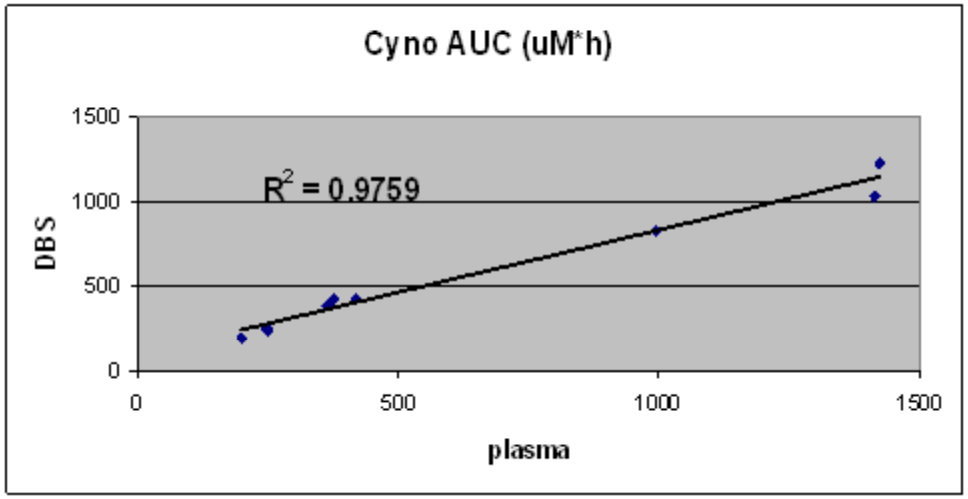


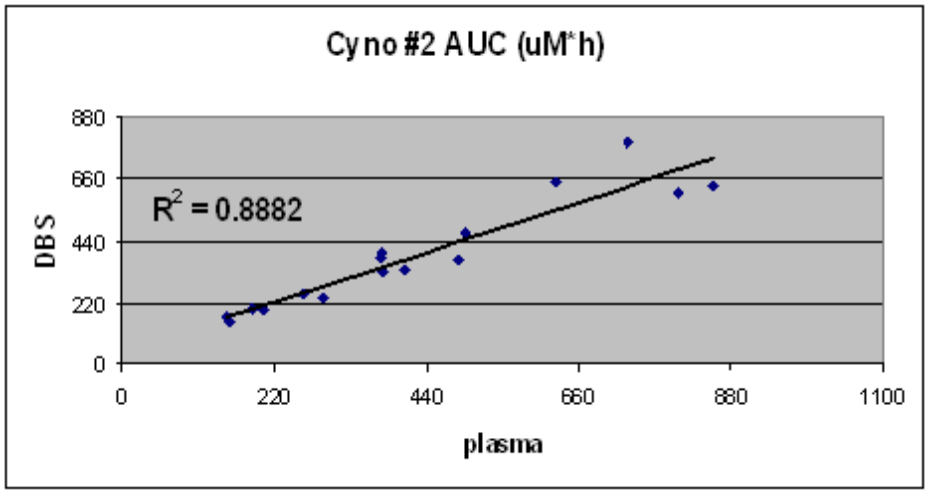


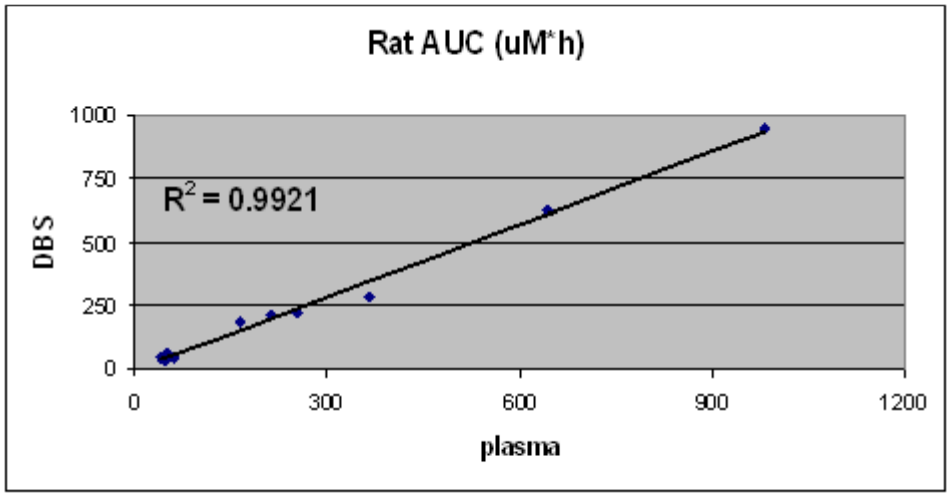


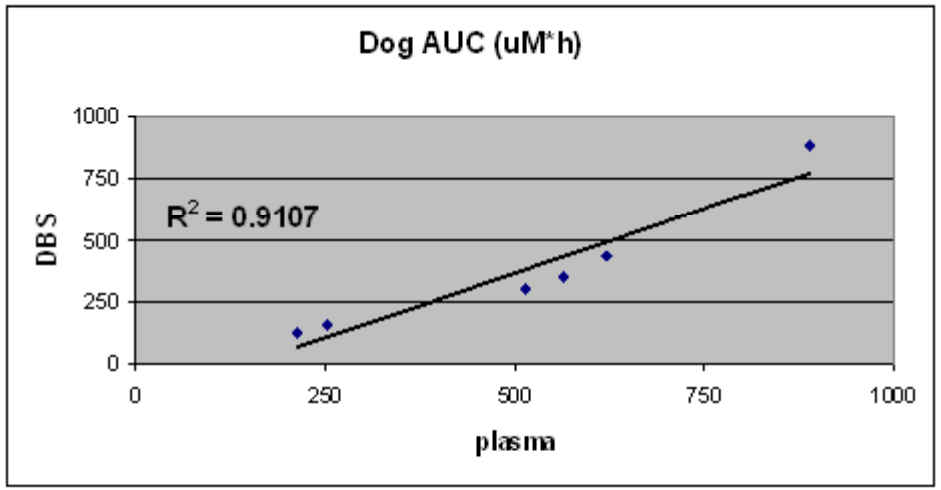


Comparison of AUC (µM*h) obtained from DBS and Plasma samples from four Discovery toxicology studies for BMS-1









Discovery DBS Procedures: Sample Collection

- Untreated cards
  - DMPK C, Ahlstrom 226
- DBS only studies
  - Accurately pipette two spots (20 µL mouse, 25 µL rat)
- Studies where both DBS and plasma collected
  - Routine blood draw
  - Accurately pipette four 15 µL spots/timepoint
    - Room temperature storage
  - Generate plasma
    - Freeze at -20°C

Discovery DBS Procedures: Sample Preparation and Bioanalysis

- BSD600 semi-automated puncher
  - 3.2 and 6 mm dual punch head
  - Center punch from spot into a 96-well plate
  - 96-well format enables automated liquid handling for processing

- Filtration assisted “protein precipitation”
  - Need some aqueous to recover compound from the card
  - Inject supernate

- uHPLC-MS/MS detection
  - Same chromatography as for liquid samples

Sample Preparation Comparison for Toxicology Studies for BMS-1

Biological Matrices	Plasma	DBS
Internal standard solution (IS)	Acetonitrile only	Acetonitrile:water (3:1)
Preparation Procedure	25 µL 50 µL IS 1:2	3.2 mm (~3 µL) 50 µL IS 1:16
Standard Curve (nM)	7.8-8,000	78-80,000
% Samples Diluted	100	0

Potential Gaps/Issues

- Resources
  - Collection of both plasma and DBS samples doubles the workflow
    - May not need double collection at all timepoints
  - When, how to bridge/switch?
    - Only one of our programs has done DBS from the beginning

- DBS may not be appropriate for all compounds
  - What criteria should be used to select DBS for Development studies?

Potential Gaps/Issues

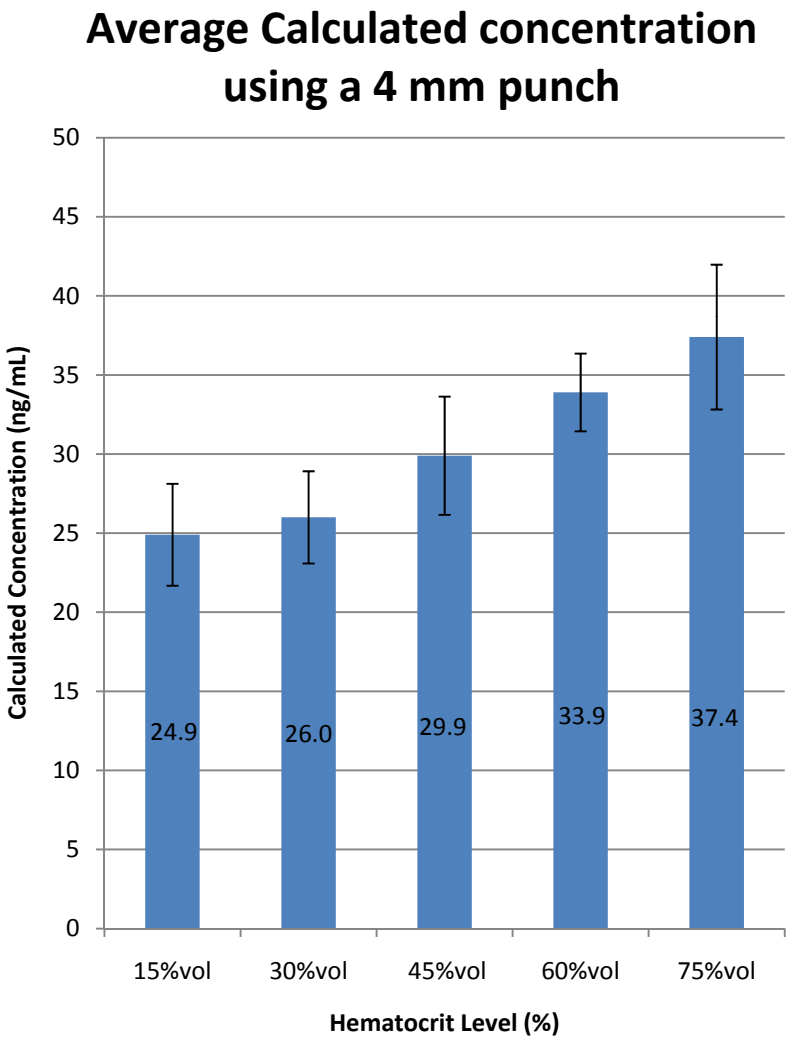
- Parameters to consider in compound selection [2]
  - Free fraction
  - Blood to plasma ratio (B/P)
  - Hematocrit

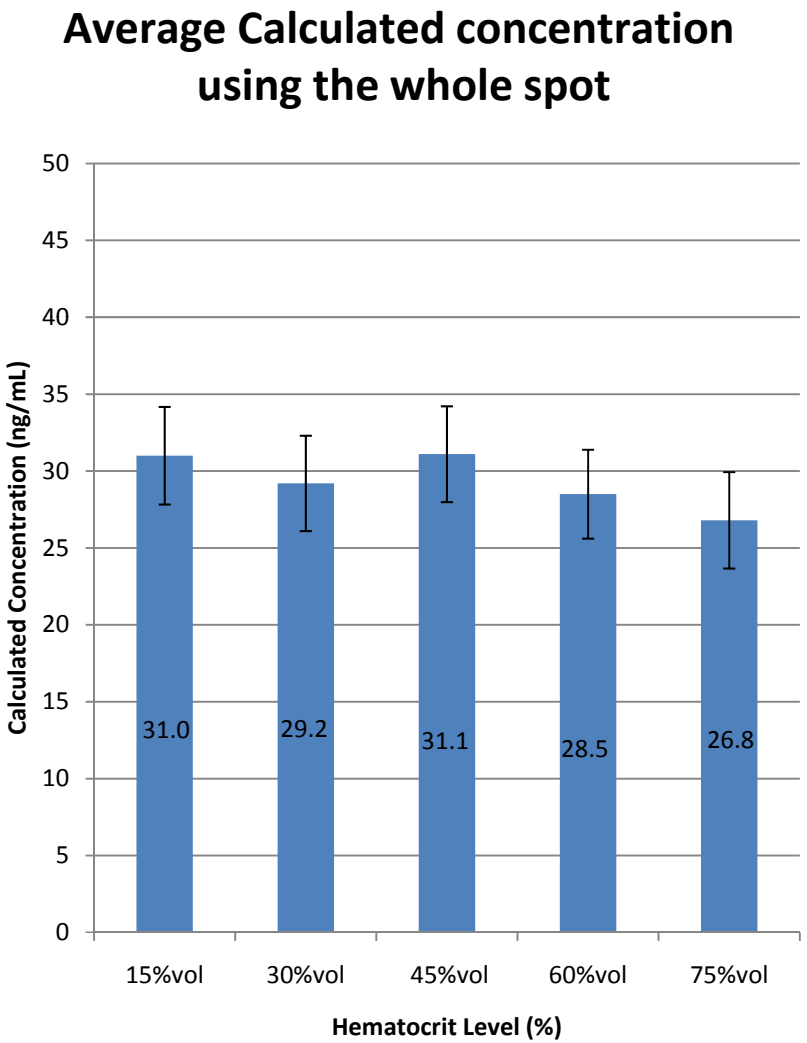
- These data available for compounds in Discovery toxicology studies
  - Plasma/DBS comparison, only considering B/P

- Evaluate variation in hematocrit on measured concentration
  - Ketoconazole as the test compound
  - Standard curves prepared in 45% hematocrit blood
  - Five different hematocrit levels for QCs, 15-75%
  - Center punch *vs.* whole spot punch

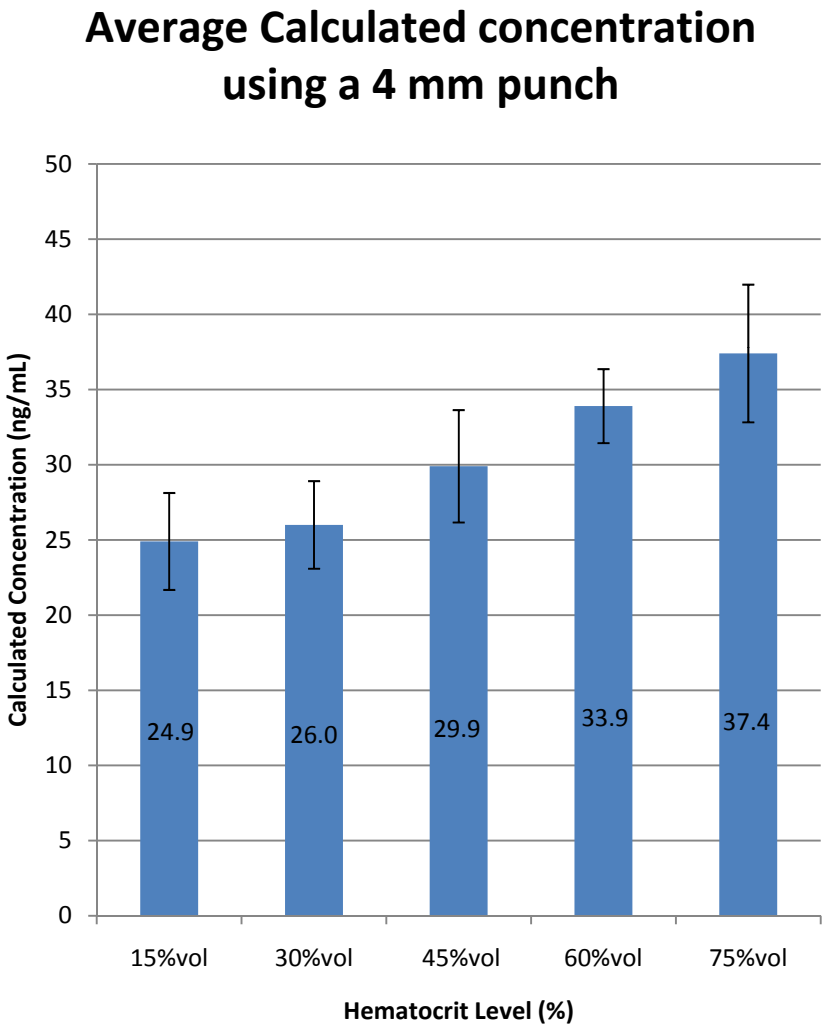
[2] Emmons and Rowland Bioanalysis 2010 2(11)

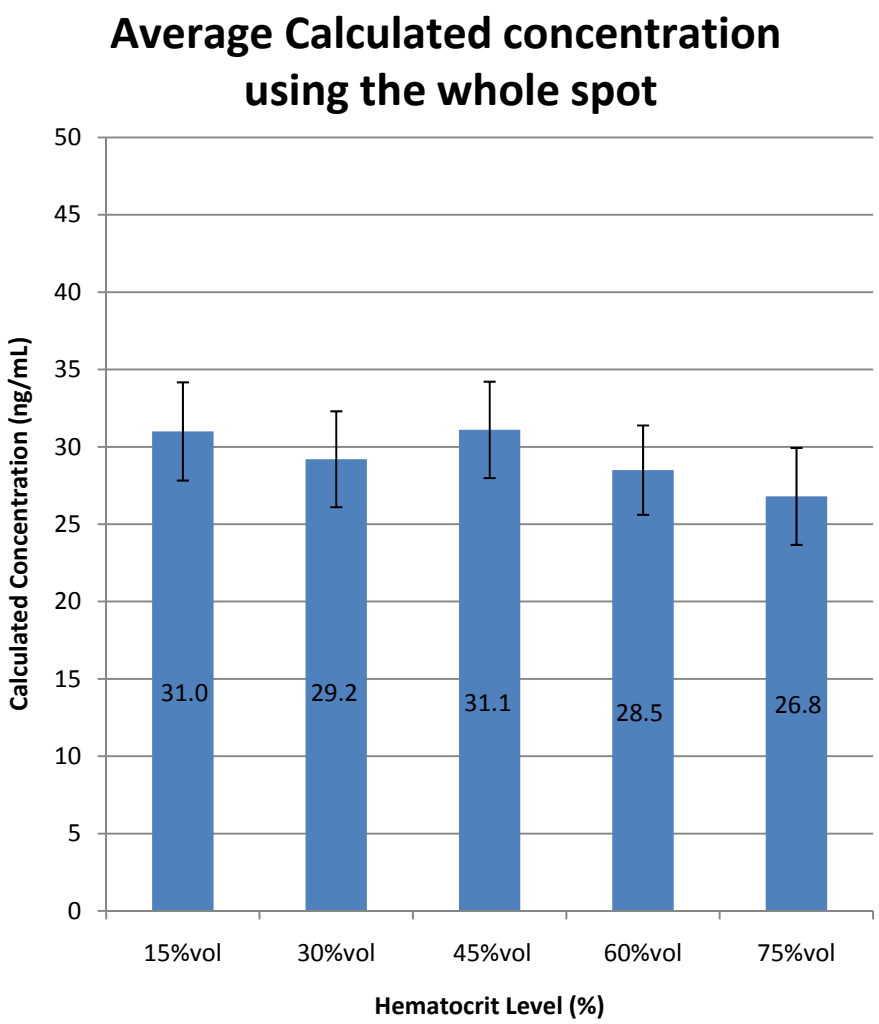
Data comparing analysis of punch *vs.* whole spot in QC samples of varying hematocrit, 30 ng/mL Ketoconazole





Data comparing analysis of punch *vs.* whole spot in QC samples of varying hematocrit, 4000 ng/mL Ketoconazole





Further Considerations

- Do we need to revise spotting and punch techniques for accurate bioanalysis?
  - 15-25 µL spots currently used
  - Need 10 µL or less to enable whole spot punching

- More experiments planned
  - Determine effect of serial sampling on hematocrit levels
  - Examine compounds with extremes in B/P
  - Effect of storage of whole blood used for standard curves

Summary and Conclusions

Discovery Studies

- DBS has utility in Early and Late Discovery
  - Investigate parameters that can affect accuracy
  - Data generated can inform selection of compounds for DBS in Development

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- DBS added to Early Development studies
  - Establish procedures, optimize workflow without impacting timelines

Summary and Conclusions

- DBS has utility in Early and Late Discovery
  - Investigate parameters that can affect accuracy
  - Data generated can inform selection of compounds for DBS in Development
- DBS added to Early Development studies
  - Establish procedures, optimize workflow without impacting timelines
- Plan for GLP and specialized studies

Collaborators

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