Dried Blood Spot Analysis – From The Clinic To The Laboratory

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From The Clinic To The Lab

Blood Spot Sampling First Used In Neonatal Screening
Dried Blood Spots – Timeline of Implementation

Dried Blood Spots have been around for over 40 years

>10 yrs ago
DBS were exclusively used for neonatal, infectious disease screening, therapeutic drug monitoring

5 Years Ago
2 manuscripts – DBS to discovery-stage PK and metabolite ID (Merck Frost)

Present Day
Potential application to routine drug development was realized within GlaxoSmithKline in 2006
Quickly saw the broadly applicable advantages offered by DBS
Other pharmaceutical companies (sanofi-aventis et. al.) becoming heavily involved with DBS (as well as CROs and vendors)
To date GSK has developed >150 validated DBS methods for nearly 75 compounds >200 studies supported using DBS >10 compounds have reached phase 1 pre-clin GLP studies
Issues and Advantages of Dried Blood Spot Analysis

- [Card based sample collection]
  - Reduced animal usage/ cost/ 3 R’s
  - Lower shipping costs
  - Reduced compound needed for tox studies
  - Improved data- PK data from toxicology animals

- [Small sample volumes 10-20µL]
  - Assay sensitivity

- [Background card interference]
  - More complicated methods development

- [Resolution from metabolites and matrix needed]
  - High LC separation efficiency
Analytical Issues

- Non – liquid format

- Current work flow will not work with this format

- Current approach requires punching, either manual or automated

- Card background may cause issues

- Matrix effects from blood components
  - Resolution from metabolites and matrix needed for quality
Analytical Issues

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- Matrix effects from blood components
Blood Spotting

- Aliquot 15μL blood per spot
  - 3 spots per sample plus ‘spare’
  - Using a pipette or capillary
    - Do NOT allow tip to touch card surface!

- Dry for ≥ 2 hours at room temperature

- Ship & store in sealable bags containing desiccant
Sample Prep & Analysis

- Analytical sample obtained by punching small circular disc (typically 3 mm) from centre of DBS
  - Manually
  - Automation – BSD1000

- Extract disc in organic solvent (typically methanol) containing internal standard

- Quantification by validated LC-MS/MS assay
Chromatography Requirements

- **Long separation**
  - Resolution of Metabolites
  - Method Development

- **Short Separations**
  - Sensitivity
  - Speed of analysis
  - Fast Screening
Analysis with LC/MS/MS

- Quantification by validated LC-MS/MS assay

Use of UPLC:
- Increased sensitivity
- Increased resolution
- Increased speed of analysis
Rapid Mode Switching
Precursor Ion Confirmation Scan

PIC Scan Acquisition

Confirm peak identity in the presence of a complex matrix in one analytical run.
Analytical Issues

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- Matrix effects from blood components
Dried blood spot microvolume sampling for DMPK

- Three types are available (Whatman, GE), which have different chemistries.
  - FTA DMPK-A (treated)
  - FTA DMPK-B (treated)
  - FTA DMPK-C (untreated)

- FTA DMPK A & B cards contain proprietary chemical mixtures that lyse cells, inactivate pathogens and denature degradative enzymes and other proteins.

- FTA DMPK-C cards contain only cellulose.

Rapid MS to MRM Switching
Using RADAR to Evaluate Card Type To Be Used

Full scan blank card -background signal

DMPK A

DMPK B

DMPK C

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Using RADAR to Evaluate Card Type To Be Used

Analyte Response

DMPK A

DMPK B

DMPK C

1: MRM of 3 Channels ES+
309.2 > 281 (Alprazolam)
9.61e5

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Matrix – The Challenge

- Matrix effects from blood components
- Gain adequate resolution from matrix interferences and other analytes/metabolites
- Maximize sensitivity
- Maintain or improve productivity
- Removal of matrix components
Resolved more effectively from the endogenous blood and card matrix peaks
Alternate Approach for Calculating Matrix Factors

1) Determine Analyte Profile
   - AN and IS injected through the column

2) Inject Solvent Blank
   - Solvent Blank injected with post column infusion of AN and IS

3) Inject Extracted Matrix Blank
   - Extracted Blank injected with post column infusion of AN and IS

Analyte = AN
Internal Standard = IS
Automating Matrix Factor Calculations: Integrated Intellistart Fluidics
TargetLynx™ automatically calculates matrix effects from 6 injections

Results Presented in Final Report
Removal of Phospholipids

9-OH Risperidone
MW 426.48
pKa 7.86

Basic Protocol
1. Place blood spot in well
2. Add extraction solvent
3. Vortex to mix
4. Apply vacuum
5. Analyze
Removal of Phospholipids
Removal of Phospholipids

![Retention Time vs. Area Graph]

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Alprazolam (benzodiazepine)
100pg/mL - 500ng/mL

Published - Journal Bioanalysis Feb 2011, Vol 3:4 p411-420
Rapid Analysis of DBS samples with sub 2µm LC/MS/MS  Mather J et al.
Sitamaquine (WR-6026) is an orally active 8-aminoquinoline analog for the potential treatment of visceral leishmaniasis (also known as kala-azar, black fever, and Dumdum fever).

LLOQ of 50pg/mL with a linear dynamic range of 4 orders of magnitude.

LLOQ of 50pg/mL
Heart Cut Configuration – 2D UPLC/MS/MS

**Trapping** – Decrease Matrix Effects and Increase Sensitivity

**At-Column Dilution** – Inject Large Volume Samples in Strong Solvent

No evaporation & reconstitution
2 Dimensional Dried Blood Spot Analysis of Rosuvastatin

- Direct injection of methanol extract from DBS sample
- 80uL injection of methanol
- At column dilution
2 Dimensional UPLC/MS/MS Bioanalysis

100pg/mL

Blank

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Conclusions

- Dried Blood Spots offer a significant scientific and expense advantage.

- The lower sample volume derived from the dried blood spots and the background card matrix poses a significant analytical challenge.

- Information rich LC/MS/MS (RADAR) can simplify the process of DBS analysis/method development.

- Analysis times as low as 3 minutes possible.

- 2D LC to remove Matrix components (trap) and allow injection of large volume samples in high organic solvent.
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