Bioanalysis of Dried Blood Spot (DBS) by Mass Spectrometry for FDA Regulated Clinical Studies

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Regulated Bioanalysis Workshop: Current Requirements and Expectations

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Disclaimer

This presentation reflects the views of the author. It should not be construed to represent FDA's views or policies.

Learning Objectives

- Describe the current use of DBS sampling technique
- Identify the advantages and limitations of DBS in regulated bioanalysis
- Understand the specific considerations for DBS method validation and regulated bioanalysis

Outline

- Overview of DBS bioanalysis
- Advantages and limitations of DBS bioanalysis
- Bioanalysis considerations in DBS method validation and sample analysis
- Case studies
- Challenge questions

Overview of DBS Bioanalysis

1913

First introduced as an unconventional sampling method

1960s

First used as a semi-quantitative bacterial inhibition test to screen the newborns for phenylketonuria.

1970s

Coupled with mass spectrometry (MS) as a quantitative technique.

Post 1990s

Coupled with liquid chromatography tandem MS (LC-MS/MS) for improved specific and sensitive quantitation:

- **Clinical pharmacokinetic (PK) studies**
- Therapeutic drug monitoring (TDM)
- Clinical diagnostic of biomarkers, hormones, etc.
- Others (forensic toxicology, sports doping, environmental contamination, etc.)

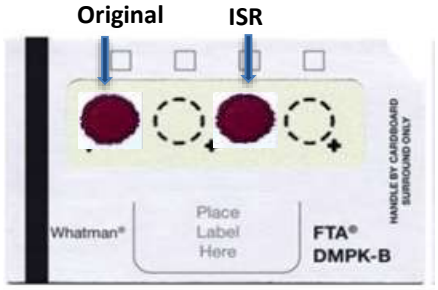
Bioanalysis of DBS in Clinical PK Studies

Advantages

- Reduced sample volume
- Ease of sample collection at clinical sites
- Convenience of sample storage and transportation

Limitations

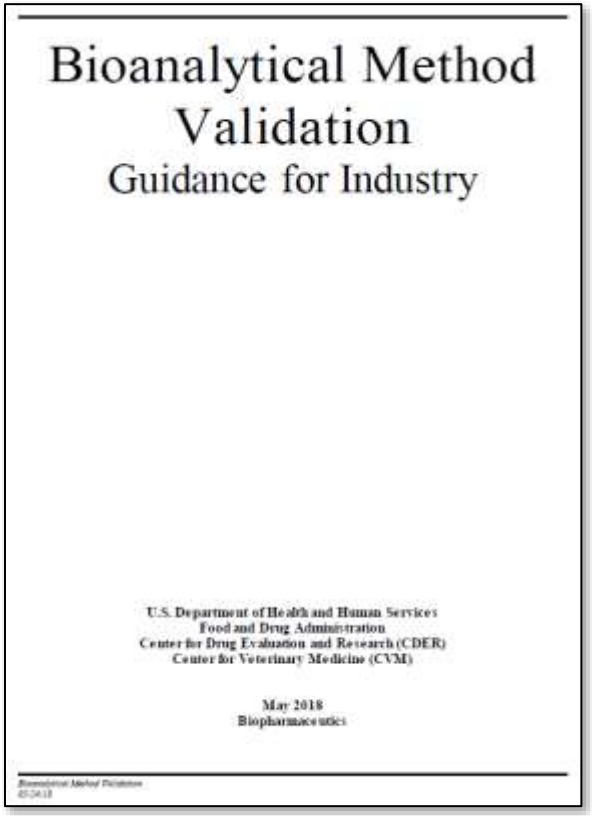
- Limited sample volume
- Consists of capillary blood
- Homogeneity and hematocrit variability
- Incurred Sample Reanalysis (ISR)



BMV Guidance on DBS

E. Dried Blood Spots

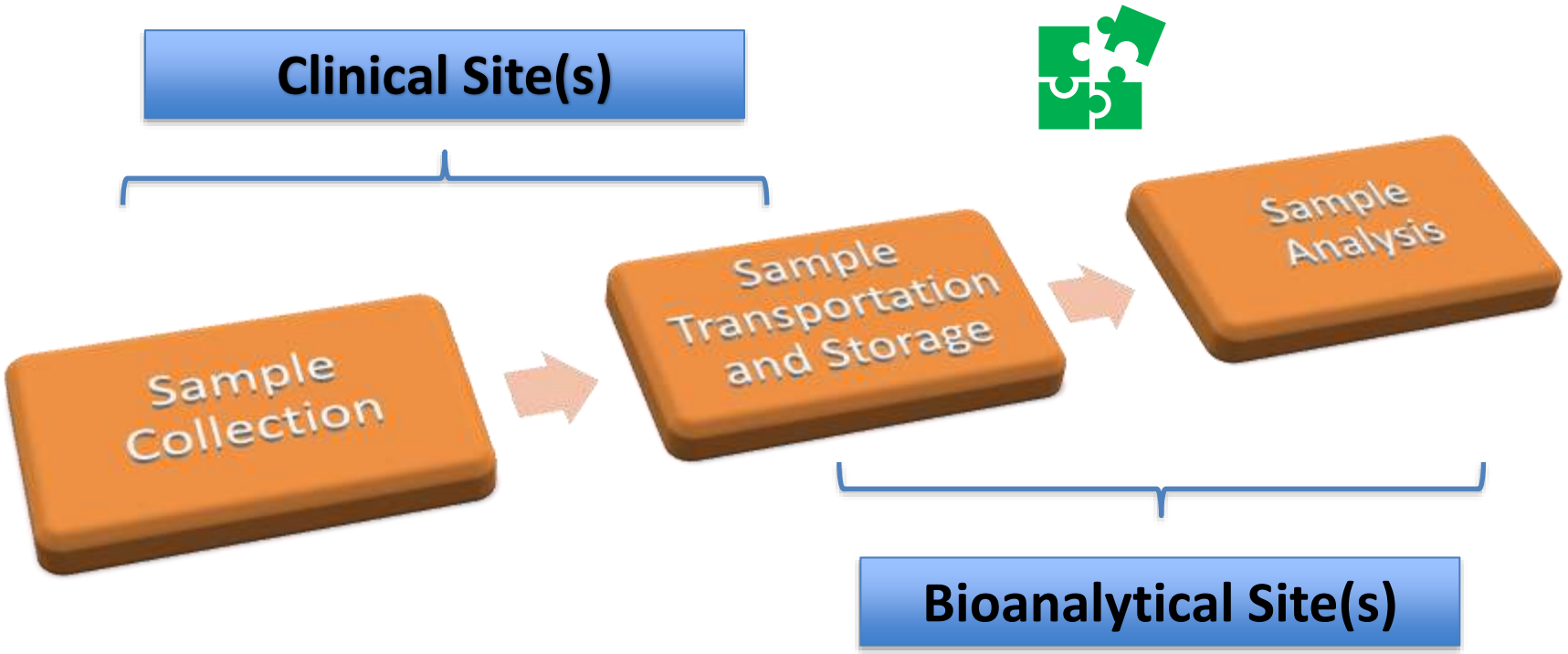
…… Additional validation of this sampling approach is essential before using DBS in regulatory studies. This validation should address, at a minimum, the effects of the following issues: storage and handling temperatures, homogeneity of sample spotting, hematocrit, stability, carryover, and reproducibility, including ISR. Correlative studies with traditional sampling should be conducted during drug development……


Bioanalytical Method Validation
Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

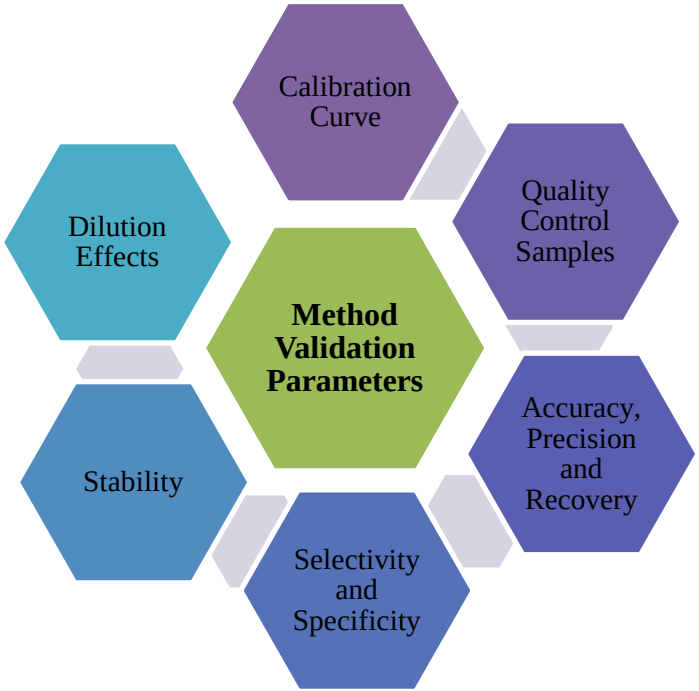
May 2018
Biopharmaceutics

Bioanalysis Considerations of DBS

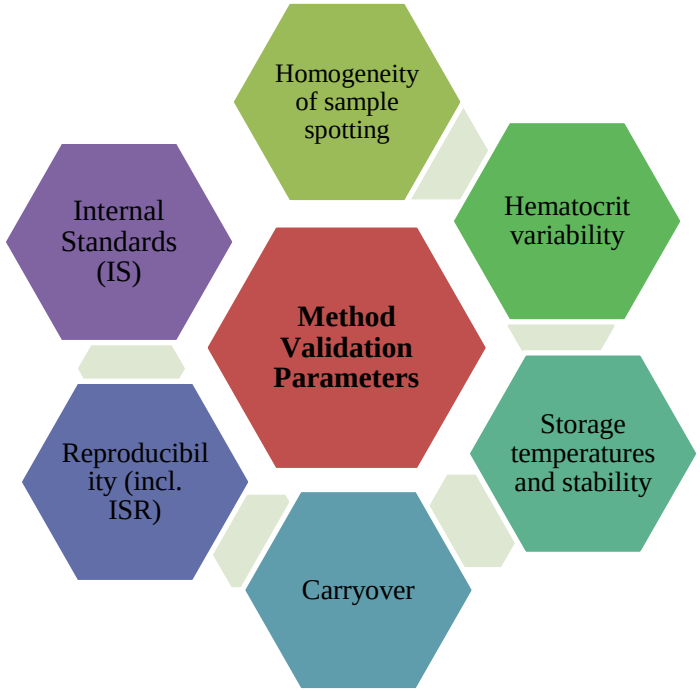


Bioanalysis Considerations of DBS

Routine Bioanalytical Method Validation



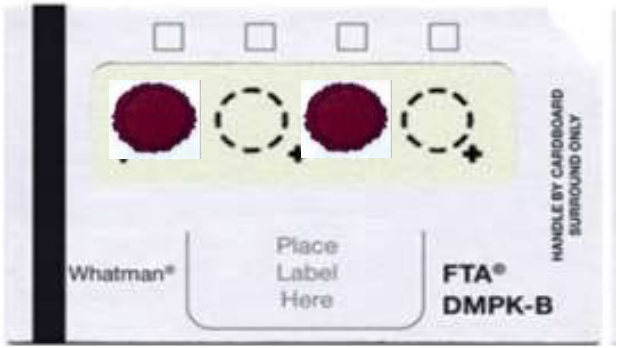
Additional DBS-Specific Method Validation

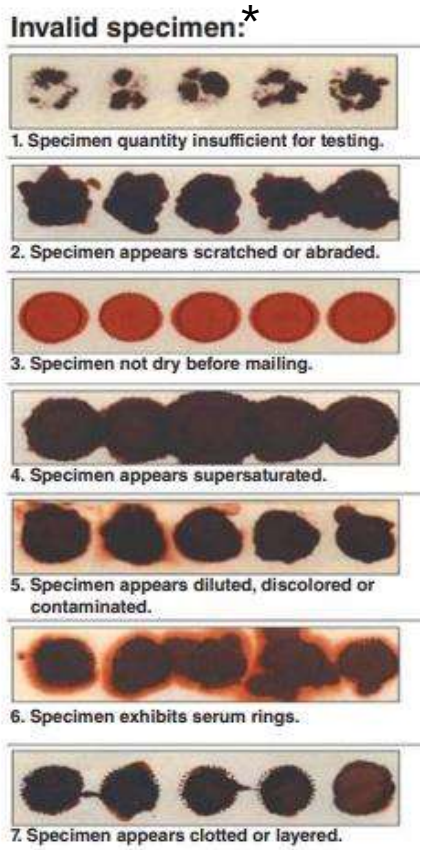


DBS Sample Collection

Homogeneity of sample spotting

- Blood collection method (pipette or capillary tube)
- Sample collection card
- Shape and size of the blood spots
- Drying conditions of the blood spots

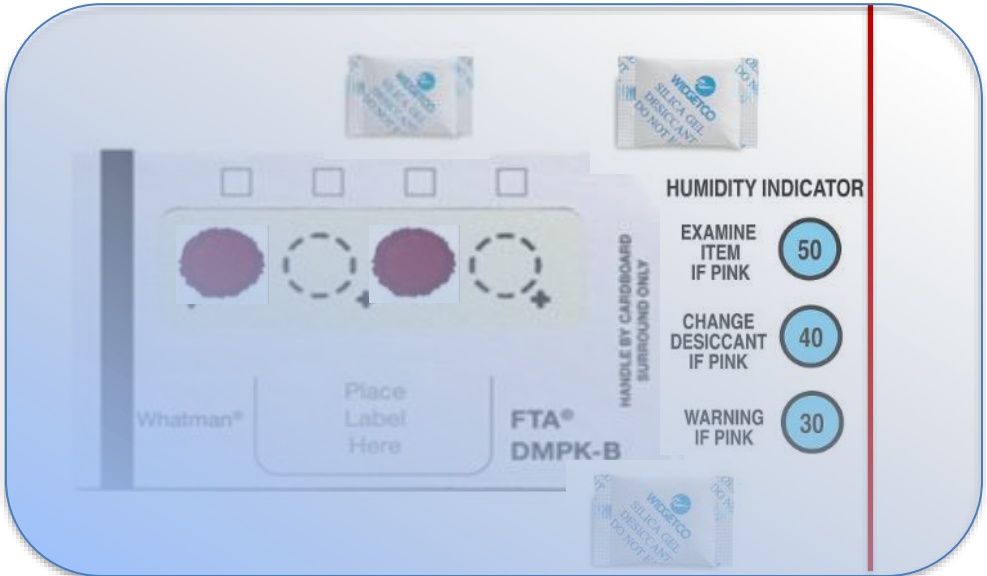




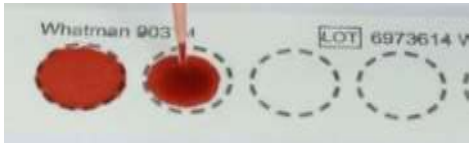
** Dried Blood Spots: Applications and Techniques, Li 2014*

Sample Handling, Transportation and Storage

- Humidity
  - Monitored right after collection and during transportation (using humidity indicator cards, desiccant packets)
- Temperature
  - Room temperature
  - -20°C or -70°C
- Analyte stability



DBS Sample Processing and Analysis



DBS Sample Cards

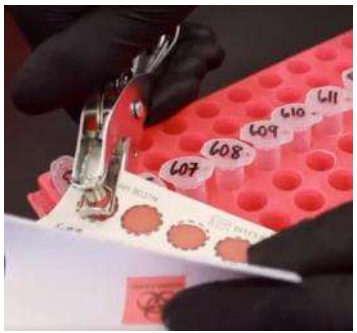
"Punch"
(size of 3 - 6 mm, either from the center or close to the outer edge)

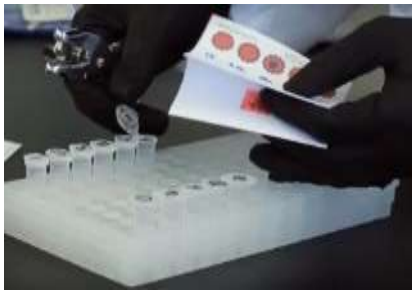
Sample Extraction & Protein Precipitation

Liquid-liquid extraction (LLE)	Solid phase extraction (SPE)	Supported liquid extraction (SLE)

Sample Analysis

LC/MS	GC/MS	Desorption electrospray ionization (DESI)/MS





** Screenshots from Jova.com, Shokati et al., 2015*

Case Study 1

A pediatric PK study submitted to an Investigational New Drug (IND) application

Analyte: a small molecule drug **Matrix**: DBS **Methodology**: LC-MS/MS

Method Validation:

- Routine parameters:
  - Calibration standards, QCs, accuracy and precision, selectivity, dilution, recovery, matrix effect, carryover, stability, and reproducibility
- DBS-specific parameters:
  - Storage temperatures, sample homogeneity variability, sample volume variability, collection media variability
  - Sample hematocrit (hct) variability:
    - Used CSs and QCs at 45% hct level to evaluate samples at 30% and 45% hct levels
    - Used CSs and QCs at 25% hct level to evaluate samples at 15%, 20%, 25%, 30% and 35% hct levels

Case Study 1

Sample analysis

- Homogeneity of sample spotting: Visually checked prior to sample extraction
- Method performance:
  - Subject samples were analyzed using CSs and QCs at 45% and 25% hct levels
  - All the analytical runs met the acceptance criteria
  - The results from the same subject samples showed an average of 18% difference between two different hct levels
- Re-assayed sample: None
- ISR: met the acceptance criteria

Case Study 2

A pediatric PK study submitted to a New Drug Application (NDA)

Analyte: a small molecule drug **Matrix**: DBS **Methodology**: LC-MS/MS

Method Validation:

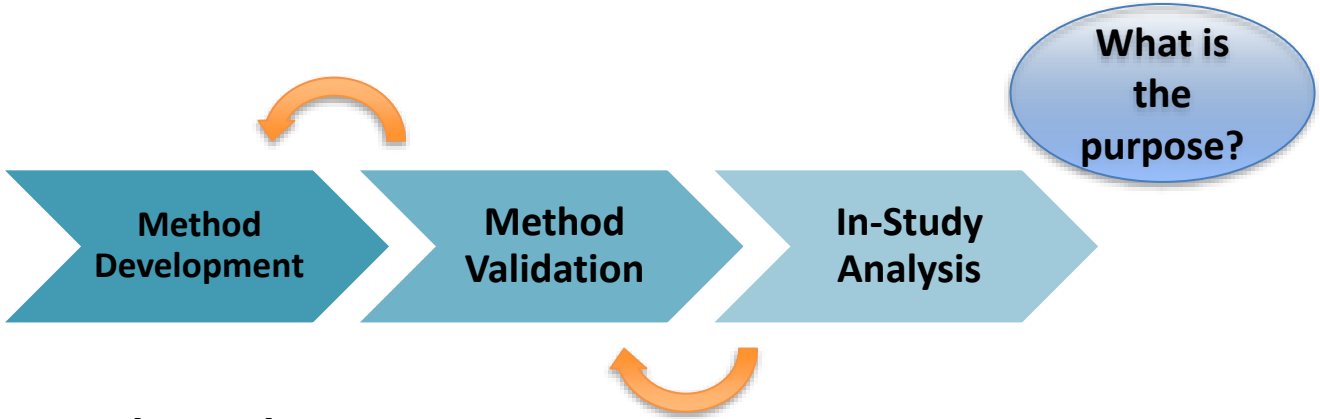
- Routine parameters:
  - Calibration standards, QCs, accuracy and precision, specificity, selectivity, carryover, matrix effect, stability, robustness
  - Dilution factor was established at 10
- DBS-specific parameters:
  - Storage temperatures, homogeneity of sample spotting (position of punching, blood spot size, and volume variability)
  - Hematocrit (hct) variability: Used CSs and QCs at 30% hct level to evaluate samples at 15%, 30% and 45% hct levels

Case Study 2

Sample analysis

- Homogeneity of sample spotting: Visually checked prior to sample extraction
- Method performance
  - 10% of the analytical runs did not meet the acceptance criteria
- Re-assayed sample:
  - 15% of total subject samples were re-assayed for being “False BLOQ” (BLOQ after dilution)
  - 85% of re-assayed samples yielded reportable results (above BLOQ)
- ISR: met the acceptance criteria

Future Directions



BMV Guidance (2018):

- **Correlative studies with traditional sampling should be conducted during drug development.**
- **Sponsors are encouraged to seek feedback from the appropriate FDA review division early in drug development.**

Summary

- Acknowledge the advantages and limitations of regulated DBS bioanalysis used in the clinical PK studies.
- Recognize the importance of close collaboration between the clinical sites and the bioanalytical sites for quality sample collection and handling.
- Understand and evaluate the DBS-specific method validation parameters as needed
- Customize and optimize the DBS bioanalytical methods based on the drugs, study subjects, study design, and sample handling conditions

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Chongwoo, Yu

Challenge Question #1

True or False?

There is no limitation to use bioanalysis of the dried blood spot (DBS) in the clinical PK studies regulated by FDA.

A. True

B. False

C. Depends

Challenge Question #2

Which of the following method validation parameters should be considered for DBS-specific bioanalytic method validation?

A. Storage and handling temperatures

B. Homogeneity of sample spotting

C. Hematocrit variability

D. Stability

E. All of the above


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