Logistical Considerations and Experiences in Utilising Dried Blood Spots in Quantitative Bioanalysis

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Introduction

- Sample shipping and storage
- Utilisation in clinical studies
- Considerations for bioanalytical method validation
- Other issues arising from the DBS format

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Sample Shipping and Storage

- Samples classified as “non-hazardous” for shipping
- CDC "Guidelines for the Shipment of Dried Blood Spot Specimens" state that dried spots can be transported via normal postal systems without special mailing cartons.
- Transportation and storage at room temperature
  - No dry ice for shipping
  - No requirement for freeze/thaw stability in validation
  - No freezers needed

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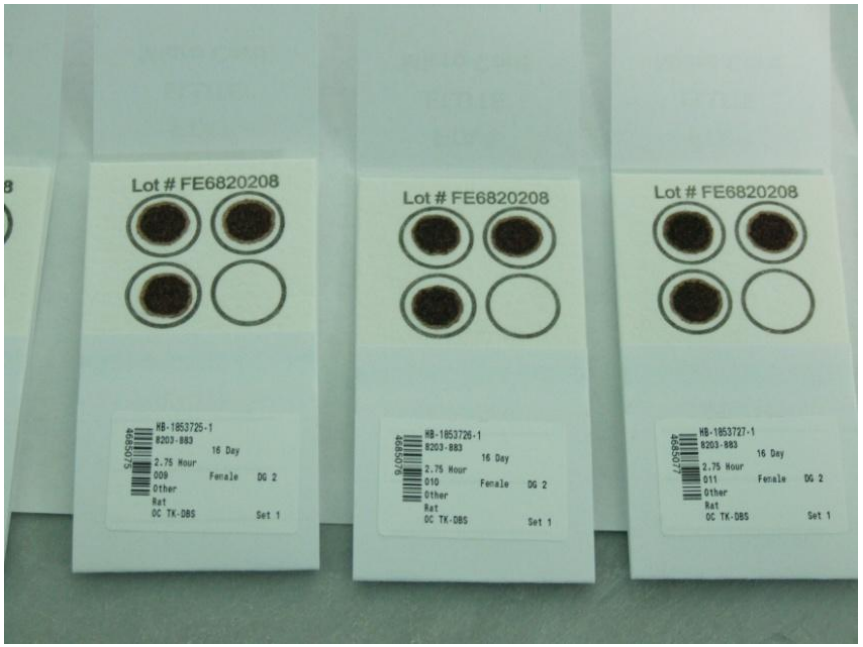
Sample Shipping and Storage

- Each card stored individually in a sealable, polypropylene bag with a desiccant pouch
- Each card has a barcode label
  - Sample tracking in LIMS and blood spot robot



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Sample Shipping and Storage

- Presence of moisture can influence extraction
- Potential to cause problems when investigating long-term stability
- Ensure sufficient desiccant is present
- Storage bags with double seals

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Sample Shipping and Storage

- Validation in dog whole blood
- Quality control samples at each level stored together in one sealable bag with desiccant
- After one month storage, desiccant pouches had become saturated with moisture
- Compared to freshly spiked QCs, the one month storage QCs have low accuracy

Sample Name	Number Of Values Used	Mean	Standard Deviation	%CV	Accuracy
QC 3 (15 Jan 10)	2 of 2	2.763229	0.010011	0.362308	92.107638
QC 3 (10 Dec 09)	6 of 6	2.191045	0.109801	5.011347	73.034829
QC 500 (15 Jan 10)	2 of 2	482.790367	6.527785	1.352095	96.558073
QC 800 (15 Jan 10)	6 of 6	750.217774	37.256340	4.966070	93.777222
QC 800 (11 Dec 09)	6 of 6	615.107702	43.281429	7.036399	76.888463

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Utilisation in Clinical Trials

- DBS technology applicable to clinical studies
- Already widely used
  - HIV testing in Africa
- Finger prick sampling
  - Non medical sample collectors rather than phlebotomists

- **BD Microtainer**® Contact-Activated Lancet
- Range of blade depths and needle gauges
- Rapid, precise incision that is consistent in depth and width
- Automatic and permanent lancet retraction minimizes possible injury and eliminates accidental reuse

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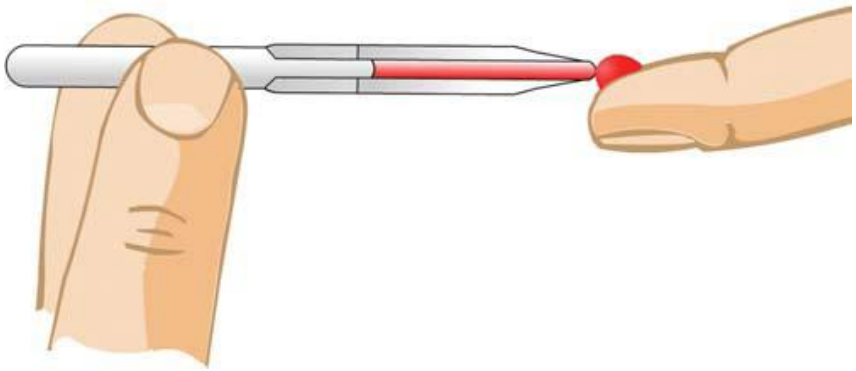
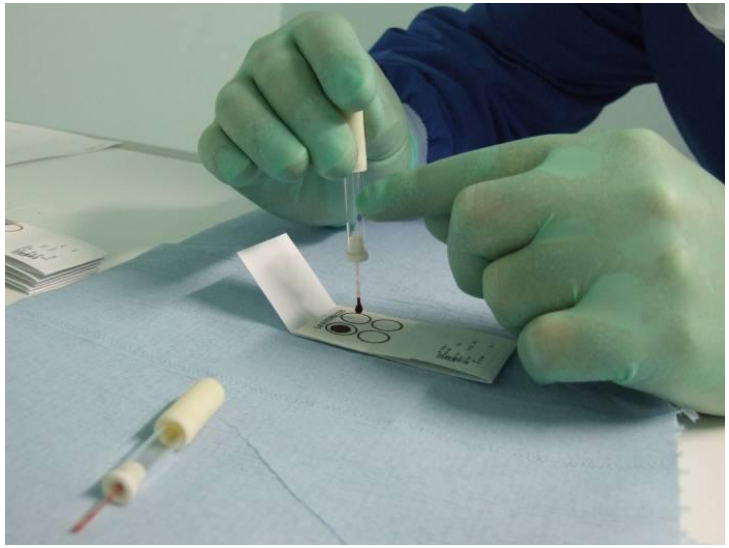
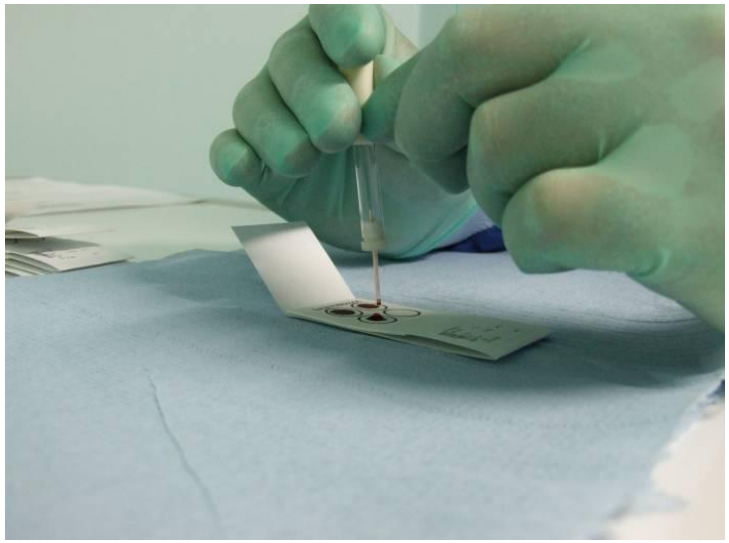
Clinical Testing – How to Spot Cards

- **Microsafe® Pipettes or Minicaps**
- Fill using capilliary action
- Squeeze to spot on card







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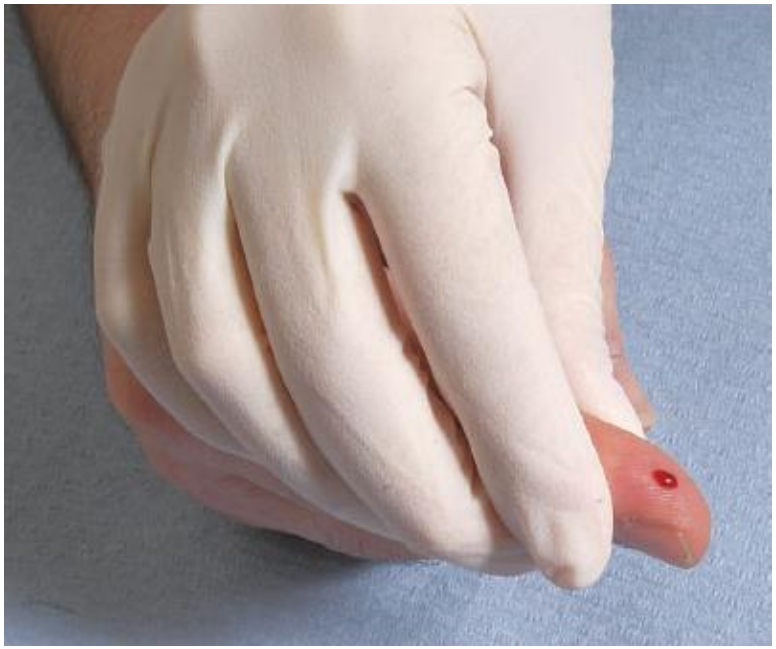
Benefits for Clinical Trials

- Procedure less stressful for patients
- Reduced blood volumes taken – potential for more sample occasions, especially for neonatal/ paediatric studies
- Simplified sample processing in the clinic – no centrifugation to generate plasma
- Reduced shipping costs – no ice
- No freezers required
- DBS card handling less hazardous than plasma. Cards offer bacterial lysis and viral inactivation.
- Potentially greater compound/metabolite stability, especially for enzyme-sensitive compounds.
- Patients could generate own samples at home



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Considerations for Method Validation

- Variation of blood spot size
  - Blood sample may be applied to card using capillary
  - Produces variability in blood drop volume and blood spot size
  - No impact if blood spreads evenly across the spotting zone
    - » At medium QC level
    - » Spot approximately 85 % and 115 % of intended assay spot volume
    - » Analyse six replicates
    - » 15 % acceptance criteria for accuracy and precision
  - A study sample spot may be much larger or smaller than other typical blood spot samples from that study
    - » Study Director assesses validity of analysing that particular spot

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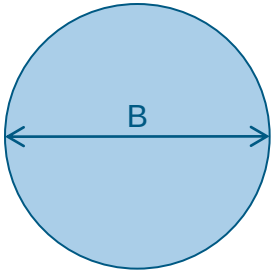
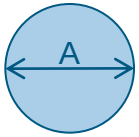
Considerations for Method Validation

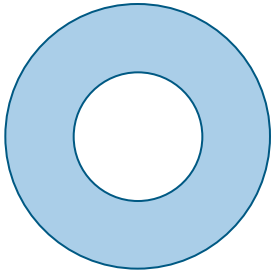
- Sample dilutions
  - Method 1 (preferred)
    - » Assay punch (Diameter B)
    - » Punch blood spots requiring dilution with a smaller core (Diameter A)
    - » To maintain a consistent level of matrix in each sample, prior to extraction add a core (diameter B) from a control matrix sample which has had a core (diameter A) removed.



Assay punch



Diluted sample punched with smaller diameter



Control matrix sample with core removed

$$\text{dilution factor} = \frac{(\text{core diameter B})^2}{(\text{core diameter A})^2}$$

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Considerations for Method Validation

- Sample dilutions
  - Method 2 (required for larger dilution factors)
    - » Extract the DBS sample requiring dilution in an Eppendorf tube (the addition of internal standard and solvent extraction steps must be performed)
    - » Extract a blank blood spot in an Eppendorf tube as above (the addition of internal standard and solvent extraction steps must be performed)
    - » Transfer aliquots of the resultant solutions to a well plate in appropriate proportions to achieve the required dilution and mix. Perform the remaining extraction steps, if required.

Considerations for Method Validation

- Recovery
  - Only a portion of the DBS sample is extracted
    - » Peak area of the test article recovery pure standard is reduced by a correction factor
    - » Measure diameter of six DBS samples to nearest mm and calculate the mean diameter
    - » Not required for internal standard (added after punch is taken)

$$\text{correction factor} = \frac{(\text{core diameter})^2}{(\text{mean blood spot diameter})^2}$$

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Experiences with Whole Blood

- Fresh blood obtained from suppliers and stored refrigerated
- Acceptable length of storage can vary from species to species
- Standardised on 2 week maximum storage
- Once haemolysis has occurred
  - » Viscosity of blood increases
  - » Blood does not completely permeate the card
  - » Produces blood spots with larger diameters compared to fresh blood
  - » Effectively punching a smaller sample volume

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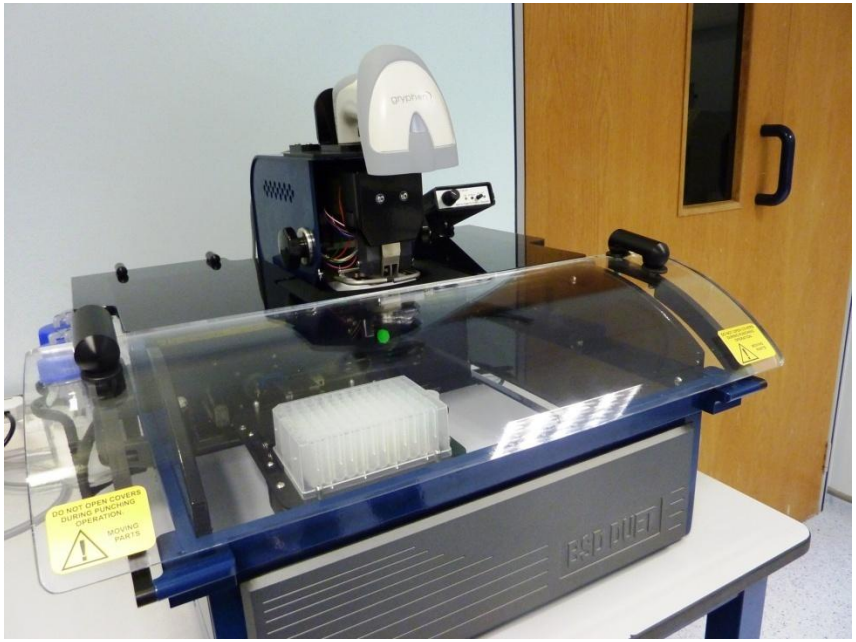
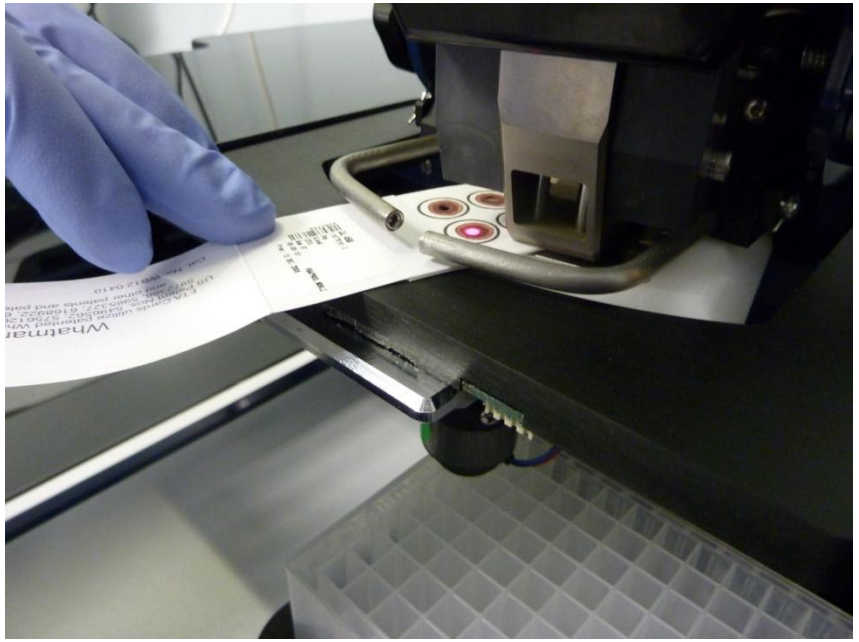
Punch Automation – Carry Over?





Five automated assays validated to date. Carry-over observed for one compound.

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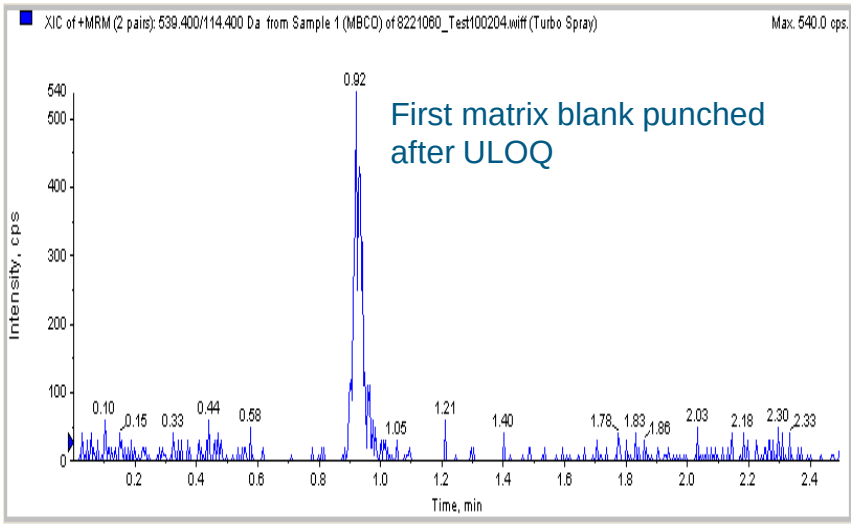
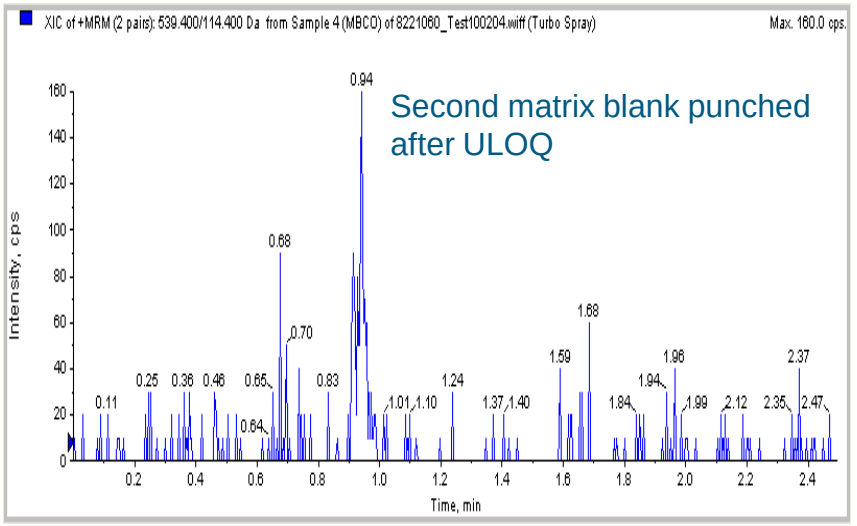
Punch Automation – Carry Over?

- Amphoteric compound
- 1000-fold range
- Punch carry-over approximately 30 % of LLOQ
- Three cleaning strikes introduced
- Punch carry-over gradually reduced during course of study
- New stainless steel punch coated in thin film of oil (slowly removed over time)





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Experiences with Treated Cards

- Impregnated with a chemical treatment
  - FTA Elute cards
    - » Sodium dodecyl sulphate (surfactant)
    - » Tris buffer
- Solvent extraction
  - These chemicals are present in the final extract
- Significant ion suppression observed for one compound compared to untreated cards
- Potential chromatographic influence
  - Modifies composition of the injection solvent
    - » HILIC chromatography most affected

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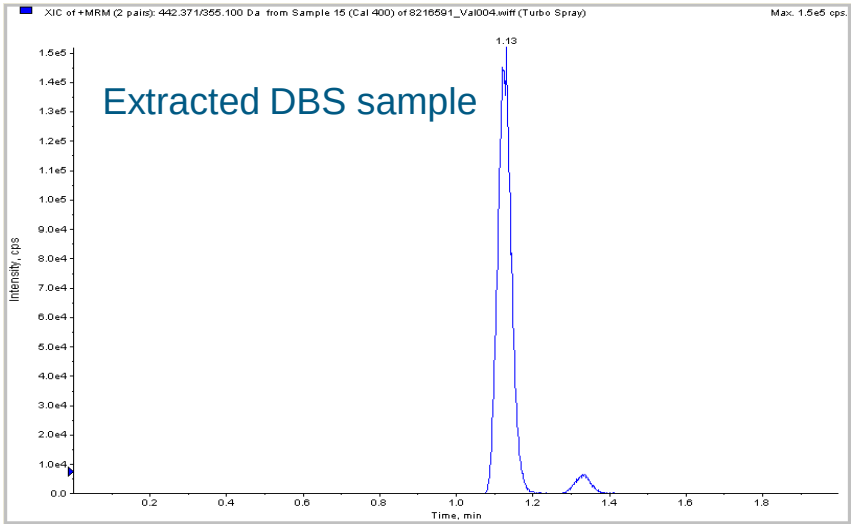
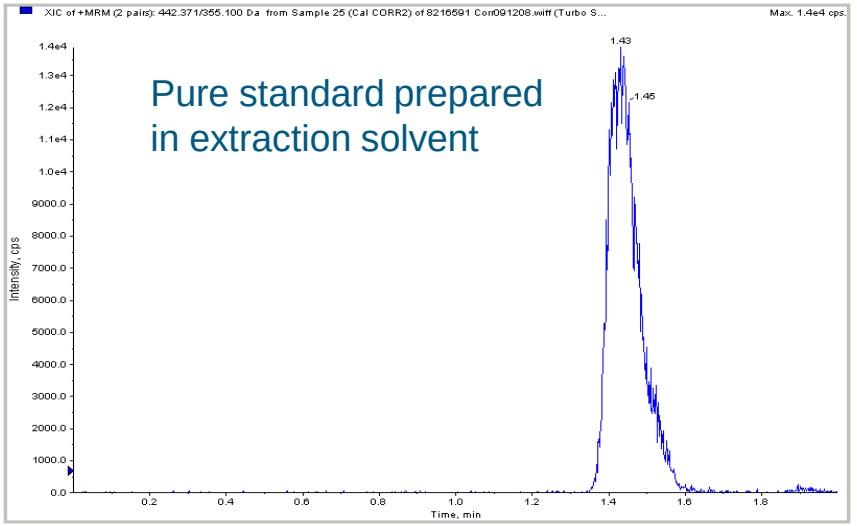
Experiences with Treated Cards

- **ZIC-HILIC**, 3.5 µm, 100 mm x 2.1 mm
- Mobile phase A: 50 mM ammonium formate + 0.5 % formic acid
- Mobile phase B: Acetonitrile
- Gradient elution
- 8% MPA ⟶ 15% MPA over 2 minutes
- Flow rate 0.6 mL/min
- Injection volume 10 µL
- Rat blood spotted on FTA DMPK-B card
- Solvent extraction with (10:90, v/v) water: acetonitrile





- **Chemicals on the DBS card modify the chromatographic system**
- **Perform chromatographic optimisation using extracted samples rather than pure standards**

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Acknowledgments

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