Quality Control Principles in Sample Preparation

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Sample Preparation Issues are Multifaceted

- Points to consider
  - Method Robustness *(Before You Touch A Sample)*
    - QA measures must control for variance from sampling to quantification.
    - Consider Analyte Interactions / Solubility / Stability
  - Sample Collection *(Preventing Artifacts And Losses)*
  - Sub-sampling *(Accounting For Sample Heterogeneity)*

- Data quality depends on sample handling
Minimum Quality Control Requirements

- Robust and reproducible performance based methods
  - Procedural corrections for losses
    - Surrogates correct for analyte losses
    - Internal standards correct for instrumental variance
  - Assess precision and accuracy
    - Reference Material assess accuracy
    - Analytical Replicates assess precision
Method Optimization: Pitfalls in Published Methodology

• Validate published methods BEFORE running samples

  – Undisclosed variables can be critical
Method Optimization: Pitfalls in Published Methodology

e.g. Surrogate Spiking Procedures

- To correct for analyte losses, surrogates **must be added** **BEFORE** initial processing step.
Method Optimization: **Surrogate Selection**

- **Surrogates should behave** like analytes throughout the method
  - Stable isotopes are safest but …
  - Polarity & Solubility
    - A range of compounds to cover physical properties of targets may provide a viable suite of surrogates
Method Optimization: Internal Standard Selection

Correcting For Instrument Variance

Cyclohexyl-dodecyl-urea

- robust ESI-LC/MS ISTD
- moderately hydrophobic
- positive and negative mode
Method Optimization:
*Derivatization Issues*

- **Confirm time to completion** WITH SAMPLES
  - e.g. Hindered alcohols

- **Are underivatized targets stable?**
  - If fingerprinting, use gentle derivatizations (i.e. room temp, pH neutral) or none at all.

- **Know the chemistry!!!**
Method Optimization & Calibration Pitfalls: Poor Manufacturing Quality

• IF AVAILABLE, metabolites can be expensive, but this does not mean purchased materials can be trusted.

• Confirm purity BEFORE preparing calibrants

EXAMPLE

• Targeted profiles of ~90 oxylipids using dilute solutions w/o certificates of analysis from a handful of sources as primary calibrants.

• Major discrepancies between 2003 & 2007 analyte lots – 8% to 233% theoretical.
Calibration Pitfalls: Commercial Variability

Calibrants stored under inert gas at -80°C in ampuled 0.5mL aliquots until analysis.
Calibration Pitfalls: Addressing the Issue

• Always check the quality of new standards against old.

• Manufacturer Quality Control Guidelines
  – Certificate of analysis
  – Lot Purity
  – Request Sealed ampules

WE MUST DEMAND QUALITY MATERIALS
Calibration Pitfalls: “Quantifying” Unknowns

i.e. *Pseudo-quantitation*

- Calibration establishes the detector’s concentration-dependent response

- Unknowns can be “quantified” using response factors of known compounds

- Quantitative changes within a study

![Graph showing calibration and quantification of unknowns and knowns](graph.png)
Method Performance: Replicate Precision

Precision criteria should be set with respect to the analyte limit of quantitation (LOQ).

Criterion For this Assay

>80% analytes
>3xMDL with precision <20%

Remember the LOQ is a function of extracted mass !!!!
Method Performance: 
Reference Material Analysis


### Continuous Accuracy
Evaluations can provide actionable method Control Criterion

<table>
<thead>
<tr>
<th>Events</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; ± 3s</td>
<td>&gt; 1 consecutive</td>
</tr>
<tr>
<td>&gt; ± 2s</td>
<td>&gt; 2 consecutive</td>
</tr>
<tr>
<td>&gt; ± 1s</td>
<td>&gt; 3 consecutive</td>
</tr>
</tbody>
</table>
Sample Handling Considerations: *Randomizing to Control for Systematic Bias*

- Spread experimental samples / ages / treatments across extraction and analysis batches
  - Identify batch specific problems
  - Prevent bias due to systematic drift over time

- Blind analyst to:
  - Treatments
  - Replicates
Pitfall In Analysis: 
**Surrogate Purity**

Analyze surrogates and internal standard spiking solutions as controls in each batch.

![Graph showing EKODE Plasma Concentrations by Extraction Method/Matrix]

- **Deuterated Surrogate Contaminated With Endogenous Analyte**
Sample Handling Considerations: Sample Heterogeneity

- When reducing sample size, you should evaluate how small can you go and ...
  - Minimize transfer steps
  - Increase rinses at transfers
  - Compare replicate precision with larger samples

- Homogenizing before sampling vs. after?
  - Homogenizing first can reduce sampling bias
  - Can loose information in heterogeneity
    - Kidney Cortex vs. Medula
    - High local metabolite densities
Sample Handling Considerations: 
*Ex vivo* Metabolite Destruction/Production

- Biological and chemical factors can destroy analytes during all sample handling steps.

Targeted Analyses = Targeted Preventive Measures

Fingerprinting = Broad Preventive Measures
Sample Handling Considerations: *Ex vivo* Metabolite Destruction/Production

*Examples of Issues and Actions*

- **Freeze/Thaw Stability**
  - Fluids: Sub-aliquot on initial collection
  - Tissues: Sub-sample frozen (cut sample on a block of dry ice)

- **Enzymatic Action - Lipase, protease, cyclooxygenase**
  - Add inhibitors to sub-aliquots
    - Orlistat – carboxy esterase inhibitor
    - Phenyl methyl sulfonyl fluoride – serine proteinase inhibitor
    - Naproxen – COX1 and COX2 inhibitor

- **Preventing Autooxidation**
  - Butylated hydroxy toluene
  - Triphenylphosphene - reducing agent to quench peroxidation
Sample Handling Considerations: Blood Handling and Plasma Preparation

- **Serum Coagulation:**
  - Time and Temperature make a difference

- **Controlling for Plasma Hemolysis**
  - Collect with large bore needle and slow draw
  - Slow spin (1000G, 20 min @ °4C)
  - Record plasma color and look for correlating metabolites
    - e.g. Thromboxane B2 & 12-HETE ↑ with hemolysis
Sample Handling Considerations: *Urine Samples*

- **Frozen urine samples form precipitates when thawed**
  - Return to body temp (37°C) … THEN ALIQUOT

- **Disease can effect solvent compatibility.**
  - *e.g.* Insoluble components in ACN
    - Healthy rat OK
    - Renal failure … Bad Idea!!!!! Insoluble precipitate

- **Elimination of Conjugates**
  - Glucuronides / sulfate / glycine/ etc. …
    - Consider analysis w/ & w/o Glucuronidase / sulfatase
    - Run boiled enzyme reaction control

  - Species specific
Sample Preparation: Reducing Matrix Interference

- Ionization Interferences (e.g. Phospholipids)
- Adduct formation (e.g. Salts, acids)
- Co-eluting impurities (e.g. pthalates, siloxanes)
- pH Effects (e.g. chromatographic tailing, chemical shift instability)

Solutions
  - Dilution
    - noise vs. signal reduction
  - Change chromatography (phase, pH, derivative, etc)
  - Cleanup
    - Analyte / interferant differential solubility
    - Fractionation
Metabolomics-based Investigations Can Address Questions in Many Matrices

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Goal</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Identifying Pathology</td>
<td><img src="image1" alt="Chart" /></td>
</tr>
<tr>
<td>Plasma</td>
<td>Quantifying Responsiveness</td>
<td><img src="image2" alt="Chart" /></td>
</tr>
<tr>
<td>Tissue</td>
<td>Comparative Biochemistry</td>
<td><img src="image3" alt="Chart" /></td>
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- The quality of the data is only as good as the sample handling and preparation