Small & Fast

How to improve bioanalytical throughput whilst maintaining/improving quality

Farjana Mahammed

EBF 10th Open Meeting:
10 - A New Journey begins
Today’s World…
‘Do More, With Less?’

The Bioanalytical World..

- Pre-clinical drug development is both a timely and costly process, leading to focused efforts on shrinking the budget and improving R&D efficiency
- Continuously evaluating productivity and process,
  - People and instrumentation
  - Streamline and simplify the process

The Social World..

- Are constantly embracing new people & experiences
- ‘Swiping right’ to find their true match..

How can we implement new technology to improve throughput?

Whilst maintaining a MATCH between QUALITY and THROUGHPUT
Our Process

Embracing New Technologies

Succeeding in high-throughput (HT) method development and sample analysis, with less people, reduces the time taken to move to the clinic.

Less Steps (Automation)
- Embed
- Review Bottlenecks
- Seek Automation Solution
- Evaluate

Less Time (RapidSep)
- Rapid discovery quantitation
- Gradients from minutes to seconds, whilst retaining proportionate peak separation

Less Surprises (Isobaric)
- Decrease attrition in the portfolio
- Understanding interfering signals can prevent over-quantification of the drug

Method Development
Wet lab sample preparation
LC-MS/MS Method
Data analysis
‘Swipe Right!’

HP D300e Digital Dispenser

Agilent Bravo Automated Liquid Handling Platform
HP D300e Digital Dispenser
New Picolitre Dispensing Technology

- Assay miniaturization
- Decreasing solvent consumption
- Pipette directly into dispense head cassettes
  - T8 : 20 uL limit
  - T4 : 200 uL limit
- Highly intuitive and easy-to-program software for simple to complex assays
- Minimise drug titration time
- Increase productivity
HP D300e Digital Dispenser

Workflow of manual line preparation Vs. D300e line preparation

**Manual Calibration Line**

- **2 mins**
  - Prepare working solutions in 50:50 ACN:H2O by serially diluting the stocks

- **15 mins**
  - Follow spiking scheme to create calibration line in Eppendorfs®

- **5 mins**
  - Sub aliquot 50µl from Eppendorfs® into tubes

- **3 mins**
  - Precipitate proteins with acetonitrile to remove impurities

- **5 mins**
  - Centrifuge at 3000g and remove clean supernatant

**D300e Calibration Line**

- **2 mins**
  - Pipette 50µl control matrix into tubes

- **30 secs**
  - Place stock solution on D300e and set to create calibration line

- **3 mins**
  - Precipitate proteins with acetonitrile to remove impurities

- **5 mins**
  - Centrifuge at 3000g and remove clean supernatant
Troubleshooting
Evaluating the dispensing capability of the D300e

D300e and Manual Calibration Line Comparison With Matrix

Decreasing Dispense Volumes

DMF Dispensing Capability
To Regulated Validation…

Benefits

D300e and Manual Calibration Line For A Validated Method

Peak Area Ratio

Analyte Concentration (ng/ml)

r = 0.9989

r = 0.9963

Capable of dispensing DMF stocks diluted into DMSO

→ Highly beneficial for practical and safety implications
Agilent Bravo
Workflow

‘Providing speed and precision on your benchtop’

- Pipette control matrix into tubes
- Prepare STDs/QCs
- Vortex
- Sub-Aliquot STDs, QCs & Samples
- Vortex
- Dispense I.S. Crash solution to all tubes
- Centrifuge
- Supernatant Transfer
- Inject on LC-MS/MS
Isobaric Interferences

Background

**What?** Why?

- Common interferences observed in LC-MS/MS analyses caused by the response of drug-related metabolites to the MRM channel of a given drug, as a result of in-source reactions or decomposition of either phase I or II metabolites.
- Can potentially interfere with the quantification of the parent molecule if they co-elute under the same chromatographic conditions

**Common Phase I & II Drug Metabolites**

<table>
<thead>
<tr>
<th></th>
<th>Formula change</th>
<th>Mass Shift (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxylation + Dehydration, Primary/secondary alcohol to aldehyde/Ketone, desaturation</td>
<td>- H₂</td>
<td>-2</td>
</tr>
<tr>
<td>Oxidative defluorination</td>
<td>-F + OH</td>
<td>-2</td>
</tr>
<tr>
<td>Oxidative deamination to ketone</td>
<td>-NH₃+ -1</td>
<td>-1</td>
</tr>
<tr>
<td>Demethylation + oxidation to carboxylic acid</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Phase II (possible in source dissociation back to parent)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucuronidation</td>
<td>+C₆H₈O₇</td>
<td>+176</td>
</tr>
<tr>
<td>Sulphation</td>
<td>+SO₄</td>
<td>+80</td>
</tr>
</tbody>
</table>
What To Monitor?

Key Scans

Precursor Ion Scan

- Spectrum of all parent ions which have the same product ion in their spectrum
- Are the precursors identified the same mass as your parent or your metabolite?

Neutral Loss Scan

- Spectrum of all parent ions which lose a selected neutral loss fragment

From Column → SOURCE SCAN → Q1 → Q2 → Q3 → m/z SET

From Column → SOURCE SCAN → Q1 → Q2 → Q3 → OFFSET BY CHOSEN NL
Our Process

*In Silico* predictions to *In Vivo* samples

To Know Beforehand:
- Molecule structure
- MW
- Product ion mass
- Potential metabolites & masses

- Request *in silico* predictions - Assess results for potential isobaric concerns
- Set up appropriate MRM, precursor and neutral loss scans on MS
- Inject high dosed sample on a generic 2 minute Non-GLP gradient
- Check that parent is observed in the relevant precursor and neutral loss scans
- Extract chromatograms to check for isobaric peaks
- If isobaric peaks are detected, ensure they are separated from parent or investigate impact of co-elution contribution i.e. < 1%
‘It’s A Match!’
RapidSep

‘Minutes To Seconds’

RapidSep offers the potential for sub-30 second run times
All things being equal, a faster run time is always going to be advantageous for Bioanalysis

Short (1x10mm, 5µm) column attached directly to MS source
- Enables fast gradients (30secs cycle times) producing ultra sharp/narrow peaks whilst retaining proportionate peak separation.

Quicker Runs
→ Increase efficiency

Retain acceptable performance
→ Chromatographic separation
→ Assay sensitivity
→ A & P

Coupled to suitable automation
→ Better quality workflows
To Regulated Validation…

Comparison

**Generic C18 Column**
(Std 300 ng/mL)

- **Nominal Conc (ng/mL)**: 3, 9, 80, 800, 1000
- **Mean (ng/mL)**: 2.7, 8.6, 81.0, 756.3, 914.5
- **SD**: 0.2, 0.3, 5.5, 42.4, 29.4
- **%CV**: 7.22, 3.93, 6.74, 5.61, 3.22
- **Accuracy (%)**: 91.4, 95.7, 101.3, 94.6, 91.5

(2.52mins / 151secs)

**RapidSep Column**
(Std 300 ng/mL)

- **Nominal Conc (ng/mL)**: 3, 9, 80, 800, 1000
- **Mean (ng/mL)**: 2.8, 8.6, 79.9, 771.4, 898.0
- **SD**: 0.2, 0.5, 5.5, 36.4, 24.9
- **%CV**: 8.63, 6.00, 6.92, 4.71, 2.77
- **Accuracy (%)**: 92.9, 95.2, 99.9, 96.4, 89.8

17secs
Summary & Conclusion

‘Do More, With Less?’

There is importance for the industry in starting from improving the non-GLP pre-clinical area

- Unlocking New Technologies
  - Embedding Automation
  - Screening more samples in less time
  - Fit for purpose methods

Preliminary in vivo animal safety assessments aimed at Go/No-Go decisions, all determine the plausibility of selecting one compound in becoming a safe and efficacious drug

  - Performing isobaric checks as early as possible

Our process implements new technology, whilst maintaining a MATCH between QUALITY and THROUGHPUT
Thank you for listening

Acknowledgements
Scott Summerfield, Matthew Barfield, Adam Hughes, Teresa Heslop, Gabrielle Turvey, Arun Sen