European Bioanalysis Forum

DBS Dilution Sub-team

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Team members (alphabetical order)

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Aim and scope:

Aim: To investigate and summarise ways to perform dilutions for DBS samples

1. Off-line approaches:
   a) Matrix match dilution
   b) Solvent dilution
   c) MS based approaches

2. On-line approaches
   a) Internal std based
   b) MS based approaches
1. Off-line approaches

a) Matrix match dilution
   - Ensures matrix effects relatively constant

• Matrix blank + IS
  - Most common approach used by team members
  - Drawback: labor intensive, not easy to automate

• Variant: Dilute with extracted blank matrix (no IS)
  - Extract sample with a more concentrated IS
  - Dilute with the blank extract
1. Off-line approaches

a) Matrix match dilution, cntd

**Halo (doughnut) dilution**

- Dilution of a smaller punch with blank extraction
- Less commonly used by team members but routinely used in some laboratories
- Can be semi-automated on certain instruments
- Dilution factors possible (up to x80 tested)
- Hematocrit values might alter halo results?
- Example of process
Halo (doughnut) dilution

» 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.

\[
\text{dilution factor} = \frac{(\text{core diameter } B)^2}{(\text{core diameter } A)^2}
\]
1. Off-line approaches

b) Dilution with solvent

elements for 10-fold dilution

- Extract with x10 more concentrated IS, dilute x10 with solvent
  Same injection volume used for entire batch.
- As above, inject x10 less for dilution samples.
- Extract all samples with the same concentrated IS solvent, dilute
  the extract x10 after, and track the dilution by the response of the IS

All 3 approaches may be biased by matrix effects
(less of an issue for stable isotope labelled IS)
Dilutions up to x100 have been reported.
1. Off-line approaches

c) LC-MS approaches:

• Multiple assay ranges
  ➢ Used to support preclinical and clinical studies where dose escalation requires quantification across a broad range of concentrations.
  ➢ Not a preferred approach as need to perform two validations.

• Isotope peak detection
  ➢ Selection of a less abundant isotope peak for dilution samples generates a reduction in signal
1. Off-line approaches

c) LC-MS approaches, cntd:

• Alternative mass transition and MS condition
  ➢ Use less sensitive mass transition or MS condition for the dilution samples
  ➢ Results suggest up to a 100,000 fold linear range can be achieved on a MS/MS instrument.

• Injection of different volumes
  ➢ Only possible if the assay does not use an IS
2. On-line approaches to DBS dilution

a) Instruments still being developed/ tested
   - Too early to fully explore
   - Some instruments use more or less IS in the loop
   - IS applied to the spots

b) LC-MS approaches: Isotope peak detection and alternative MS conditions can be applied in the same manner as discussed for off-line work
Next steps

- Off-line DBS dilution experiments planned for the next couple of months...
  - Isotope peak detection
  - Alternative mass transition
- Publication of ideas and findings related to DBS dilutions
Conclusions

- Many different approaches to DBS dilution possible
- Different companies have different preferences
- Regardless of which approach is used for a specific assay, that procedure should be confirmed as suitable during validation (precision and accuracy within usual 15% limits)