Internal Standard Use With DBS

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Introduction

- Best way to use internal standards (IS) with DBS is an open topic
- IS mostly added in extraction solvent
  - Simple and convenient to handle
  - Does not compensate for extraction recovery
  - Does compensate for matrix effects
- Goal of team
  - Evaluate alternative ways of IS addition
  - IS and analyte should be similarly extracted from blood spot

Agreements / Standardization

- DBS methods: At least fit-for-purpose of these experiments
- Per company test several analytes and their IS
- Use same IS concentration as for plasma or liquid blood method.
- Analytes: To cover a range of different protein binding, blood/plasma distribution, water solubility etc.
  - Prepare List of compounds to be tested giving their pKa and log P values
- Untreated Ahlstrom 226 cards, same batch for all companies and all experiments
- Extraction solvent and volume per punch according to established method
- Dry spots for 2 h at RT, followed by 22 h - 7 days storage in plastic bags with desiccant at RT until extraction.
- Serve some cards for re-analysis after several months

Agreements / Standardization

- Extent of testing and what to compare:
  - QCmed, n= 6
  - Bias % on single results, % CV of analyte and IS
  - Recovery of analyte and IS calculated separately and from area ratio analyte/IS
  - Intra-day evaluation: one run per compound
  - Spot a blood volume of 15 µL (or depending on existing assay),
  - Punch 3 mm and whole spot
- Take fresh whole blood
  - Stored at about 5ºC up to 10 days, document storage time
  - From one source (one subject, clinical) or pool from several animals
  - Use same pool of blood for all DBS methods (analytes) and experiments

Experimental Part

- Prepare pools from blood:
  - One spiked with analyte
  - One spiked with IS
  - Mix both pools: analyte and IS together in blood
  - IS in blood serving as theoretical best case scenario (reference), which in routine DBS use will mostly not be done for logistics reasons

Five Experiments

1. **Spot individual spots either with analyte or IS blood**
   Punch analyte and IS spots, extract them together.
2. **Spike analyte and IS into same blood pool, spot card**
   Punch spots, extract them
3. **Spot analyte containing blood (mimicking real samples)**
   - Extract spot with IS containing solution (common practice at the moment)
4. **Spot analyte containing blood (mimicking real samples)**
   - Spike IS on dried analyte blood spots, dry and extract
   - Spiking by pipette, by spraying or other technique
5. **Spike IS on neat untreated DBS cards, dry**
   - Spot analyte blood onto IS spot, dry, extract
   - Spiking by pipette, by spraying or other technique

Evaluation

1. Which of the 5 experiments shows the best agreement between recovery of analyte and IS?
2. What about the precision in the experiments?
3. Which experimental setup would be most suitable for routine DBS use?
   - Do we have to make a compromise with the „best suitable“ setup regarding 1) and 2)?

Next Steps

- Perform agreed experiments, and depending on outcome decide upon additional experiments if needed (Q2-2012)
- Present and discuss results at next DBS topic team meeting
- Prepare a publication (by end 2012)

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