DBS for Metabolite Identification: Can we? Should we?

- Overview of metabolite identification in drug discovery/development
- Preliminary feasibility study
- Considerations for met id using dried blood spots
- Conclusions
- Future work
Metabolite identification in drug discovery/development

CANDIDATE SELECTION

IND

PoC  EoP2

CTD

Discovery  Expl. Dev.  Phase 1  Phase 2a  Phase 2b  Phase 3

In Vitro Human + Animal Circulating Animal
  • Semi-Quantitative
  • Non-Comprehensive

Circulating Human in MD study
  • Semi-Quantitative
  • Non-Comprehensive

Radiolabel ADME Human + Animal
  • Quantitative
  • Comprehensive

• Clearance

• Possible active metabolites

• Predict human metabolites:
  Any major human metabolites that will be minor or absent in animals?

• Identify major and/or pharmacologically active human metabolites

May prompt further analysis of tox samples

Definitive, comprehensive cross-species metabolic profile
Current approach to Met ID

- Residual plasma sample after quantitative assays

- Pooling– single representative sample
  - Preferably time-normalised (Hamilton)* pooling
    e.g. 1H - 10μL, 2H - 10μL, 5H - 30μL, 10H - 50μL (total 100μL)

- Protein precipitated with 3 x acetonitrile

- LC/MS analysis on Waters QTOF or Thermo Orbitrap (UV in-line)

Preliminary feasibility study:
Dried blood spots for Met ID
Compounds in contrived plasma/blood sample

CP-88,059

CP-374,269

CP-164,579

CP-251,573

CP-426,052
Compounds in contrived plasma sample/blood sample

UK-427,857

UK-463,977

UK-453,465

UK-408,027
Compounds in contrived plasma sample/blood sample

UK-343664

UK-343334
Compounds in contrived plasma sample

UK-453061

PF-03139905

ABS

PF-04580552

PF-04580552

UK-533713
Experimental

- Compared
  - **Rat plasma (current practice)**
    - 200µL precipitated with 3 volumes acetonitrile
  - **Protein precipitated whole rat blood**
    - 200µL precipitated with 3 volumes acetonitrile
  - **Dried blood spots**
    - Punches from 20µL spot extracted with 200µL solvent (50/50 acetonitrile/water)*

(5µl injection from 200µL)

Limit of Detection

- **Full scan QTOF**
  - Plasma = whole blood
    - Concentration: 100ng/mL
    - Absolute: 2.5ng on column
  - DBS
    - Concentration: 10μg/mL
    - Absolute: 2ng for two punches

100 x difference concentrations

High recovery
Observations

- DBS gave cleaner background (different volumes)
- More visible particulates
- Increasing extraction volume does not increase recovery
- Methanol and acetonitrile gave similar recovery
- Standard and chemical-free paper equivalent
DBS: Considerations for Met ID

- Pooling?

- Stability / binding/ partitioning – whole blood*

- Losing unknowns

- Limited by sample concentrations
  - need μg/mL in blood
  - Can take >sample

DBS Conclusions

- Preliminary data -> high recovery
- Suitable for unknowns?
  - Considerations also apply plasma
- Consider
  - structures individually
  - experimental design
- Efficient use of resources

Nothing to stop us trying DBS for met id
Further work

- Direct analysis
  - DART (Direct Analysis in Real Time)
  - DESI (Desorption Electrospray Ionization)

> Even more limited by amount?