MUNI | RECETOX SCI

Novel QC sample for dried blood spots metabolomics analysis

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BACKGROUND

- DBS provides:
  - Minimally invasive- sample collection[1]
  - Low volume of blood sampled
  - Affordable sample transport & long-term storage
  - Suitable for infants and newborns
- Measuring small molecules in DBS is influenced by:
  - Extraction efficiency
  - Chromatographic separation
  - Mass spectrometry ionization effects[2]
- Challenges for long-term DBS QC materials[3,4]:
  - Standard reference blood materials are expensive
  - Venous blood is not representative of capillary blood
  - Pooled capillary samples difficult to generate at scale

AIM

- Simple and robust method for the profiling of metabolites in dried blood spots (DBS)
- Implement affordable quality control (QC) strategy using synthetic culture cell media

REFERENCES

1. Drolet and al., 2017 doi:10.3390/metabo7030035
2. Petrick and al., 2017, DOI 10.1007/s11306-016-1153-z
3. Moat and al., 2020 doi:10.3390/ijns6020026
4. Tobin and al., 2021 /doi.org/10.1007/s11306-021-01813-3

ACKNOWLEDGMENT

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RESULTS

- **Optimized derivatization reaction enables 15-minute analysis time sample-to-sample (*Figure 1*)**
- **Spectral matching enables confirmed identification of ~60 analytes in DBS and ~30 in HPLM (*Figure 2*)**
- **Detection of metabolites in DBS covering 14 chemical superclasses (*Figure 3*)**
- **Linearity ranging 0.01-100 ng on column for the 30 analytes co-present in HPLM and DBS (*Figure 4*)**
- **Average coefficient of variation < 10% for peak intensity of analytes with confirmed identification (*Figure 5*)**

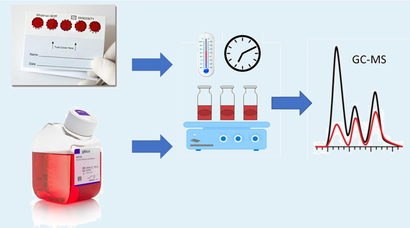

Figure 1. GC-MS based metabolomics method for the analysis of DBS and HPLM.

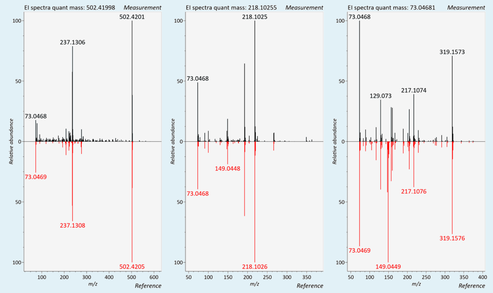

Figure 2. Example identifications of clinically relevant metabolites via matching to RECETOX Metabolome HR-[EI+]-MS mass spectral library.

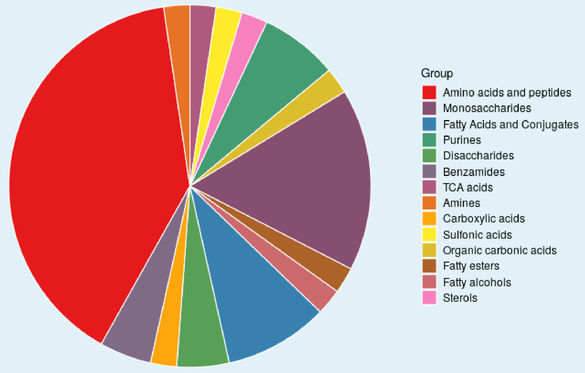

Figure 3. Pie chart of chemical class enrichment of compounds identified in DBS. Generated by MetaboAnalyst 5.0.

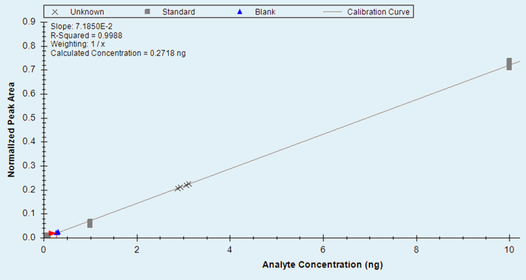

Figure 4. Calibration curve of phenylalanine enabling absolute quantification in DBS.

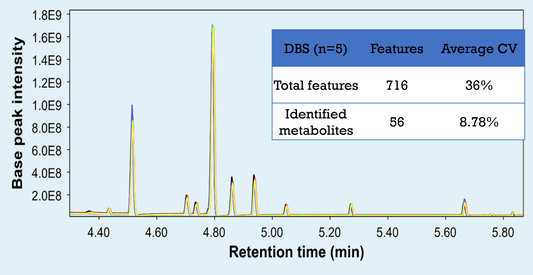

Figure 5. Total ion chromatogram of DBS extracts analysed in 5 replicates with inset table showing reproducibility.

METHOD

- Direct in-drop extraction/2 stage-derivatization of:
  - 3 mm punches from DBS cards fortified with deuterated internal standards (dIS)
  - 5 µL human-like plasma media (HPLM) fortified with dIS & including 3 mm blank matrix (Whatman 903 filter paper) punch
- Optimization of derivatization reaction parameters:
  - Temperature
  - Time
  - Reagent
- Analysis of derivatized extracts via gas chromatography electron ionization mass spectrometry (GC-[EI]-MS) (*Figure 1*)

CONCLUSION

- Developed simple method to detect ~60 metabolites in DBS with novel use of HPLM to provide cost effective long-term QC

FUTURE WORK

- Build calibration curve for absolute quantification in DBS of analytes contained in HPLM
- Expand HPLM usage for LC-MS methods to expand chemical space coverage
- Apply method in pilot studies to assess scalability:
  - Central European Longitudinal Study for Pregnancy and Childhood: The Next Generation (CELSPAC:TNG) cohort
  - Case-control children panel for autism of Ljubljana University Medical Centre
- Fully automate sample preparation via online derivatization using TriPlus RSH liquid sample handler