Automated sequential derivatization for human blood metabolites profiling: GC-HRMS metabolomics

Akrem JBEBLI[1], Kateřina Coufalíková[1,2], Moira Zanaboni[3], Manuela Bergna[3], Renzo Picenoni[4], Jana Klánová[1,2] & Elliott J. Price[1,2]

1. RECETOX, Masaryk University, Brno, Czechia; 2. EIRENE-CZ, Brno, Czechia ; 3. Thermo Fisher Scientific, Milan, Italy; 4. CTC Analytics AG, Zwingen, Switzerland

BACKGROUND

- GC-MS metabolite profiling of human bio-samples often employs a two step-derivatisation to increase analyte volatility and amenability for GC[1]
  - Formation of different derivatives with different reaction times[2]
- Sample preparation is time consuming and manual handling contributes to sample-to-sample variation[3]
  - Different times from end of reaction to injection means batch processing impacts reproducibility
- Sequential online sample preparation offers a solution[4], but throughput is lacking in routine application
- QC materials are limited for long-term human blood analysis
  - Standard reference blood materials are expensive
  - Venous and capillary blood are only partially concordant
  - Pooled capillary samples difficult to generate at scale

AIM

- Development of robust automated method for human blood micro-sample metabolite profiling using GC-HRMS
- Implement affordable quality control (QC) strategy using synthetic culture cell media

CONCLUSION

- Sequential online two-step derivatization sample preparation was implemented for GC-HRMS metabolite profiling of various human blood matrices with ~30 min sample to sample analysis time
- SOPs openly available:
  https://doi.org/10.5281/zenodo.10612856
  https://doi.org/10.5281/zenodo.10612909
- HPLM demonstrated as affordable material with value for long-term QA/QC control
- Method will be adopted for routine application within EIRENE-CZ

REFERENCES

1. Koek et al., 2006, doi:10.1007/s11306-010-0254-3
2. Kanani and Klapa, 2007, doi:10.1016/j.ymben.2006.08.001
3. Hübschmann, 2022, doi:10.1002/9783527817511
4. Miyagawa and Bamba, 2019, doi:10.1016/j.jbiosc.2018.07.015

ACKNOWLEDGMENT

A.J, K.C, J.K. & E.J.P. thank the RECETOX Research Infrastructure (No LM2023069) financed by the Czech Ministry of Education, Youth and Sports for supportive background.

METHOD

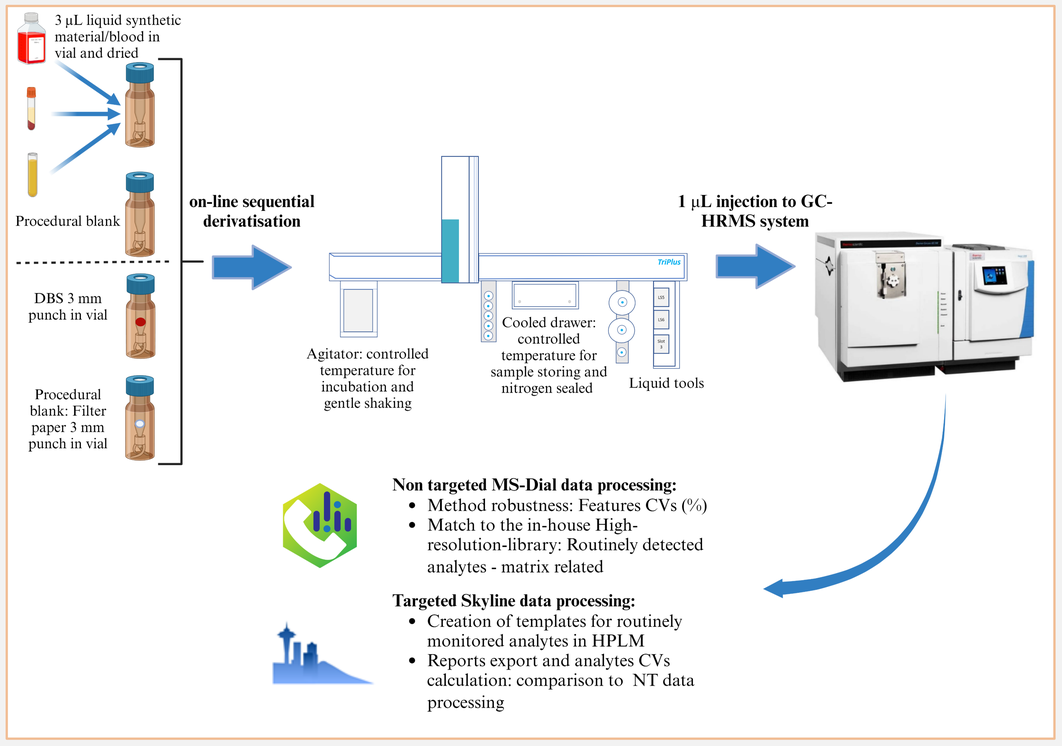

Figure 1. Schematic of automated, sequential sample preparation developed on Thermo Scientific™ TriPlus™ RSH. Following derivatization, extracts undergo GC-Orbitrap MS analysis, with data analysed via open-source software tools.

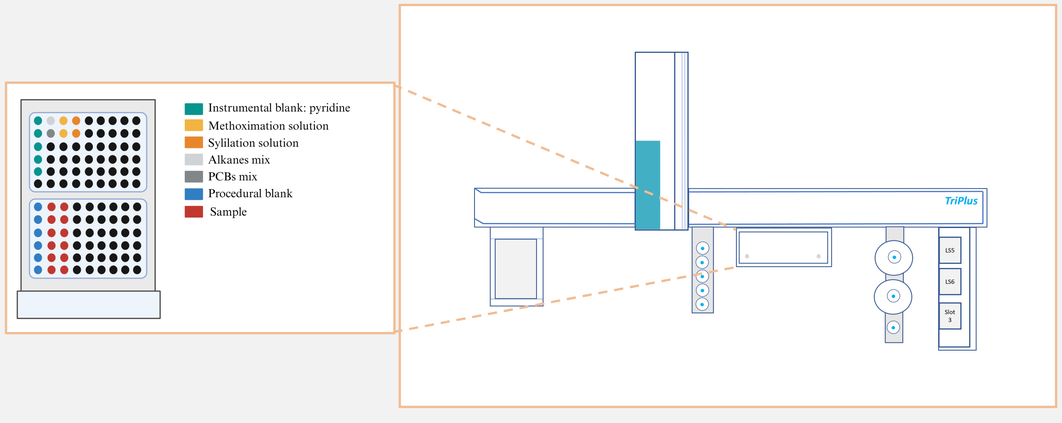

Figure 2. Layout of cooled drawer tray. Samples, derivatization reagents and QC materials kept at controlled temperature (here fixed at 5 °C with variation not going higher than 8 °C during analysis due to opening closing the door during sample preparation) sealed under nitrogen. System suitability checks of retention time, mass accuracy and ion intensity based upon certified reference materials of alkanes & PCBs mixtures. Alkanes also used to establish retention indices supporting analyte annotation.

RESULTS

- **Over 75 features with confirmed identification in each blood matrix**
- **Over 200+ features with RSD <15% per each blood matrix**

Liquid blood: Pooled serum/Plasma

Sample	Serum	Plasma
Feature number	966	969
Average CV	34%	29%
Feature number (CV≤30 %)	549	625
Feature number (CV≤15 %)	234	417
Annotated features	83	83

Dried blood: capillary and venous

Sample	Venous dried blood	Capillary dried blood
Feature number	985	976
Average CV	36%	33%
Feature number (CV≤30 %)	511	550
Feature number (CV≤15 %)	213	279
Annotated features	78	78

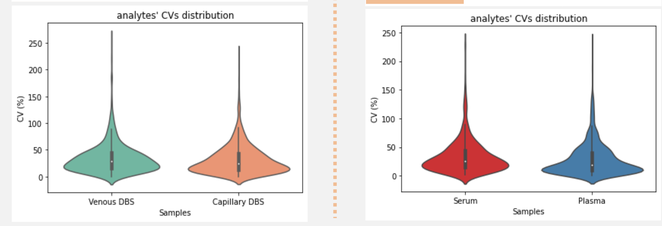

Figure 3. Summary statistics of developed method applied to various blood matrices. Features were annotated to the RECETOX Metabolome HR-[EI+]-MS spectra library (https://zenodo.org/records/5483565).

- **Human like plasma medium (HPLM) enables monitoring of 34 metabolites common to blood for long-term QA/QC**

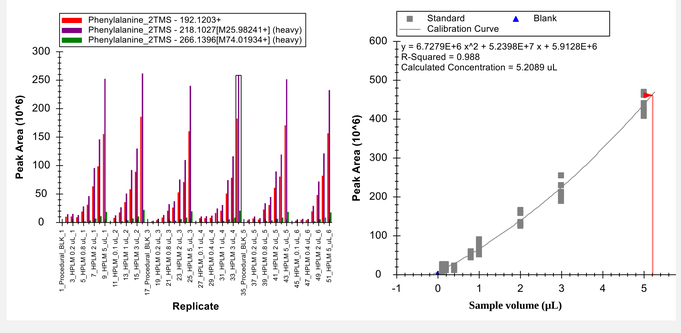

Figure 4. Example dilution series for HPLM matrix. Calibration curves built for each analyte with average R^2 0.934

.

- **HPLM has certified formulation & will be benchmarked to NIST 1950, NIST 1957 & NIST 1958 SRM material.**
- **Enables quantitative estimation of major metabolites at reduced cost i.e. HPLM = 40 EUR/L vs NIST 1950 = 0.5M EUR/L**