Rapid Metabolomics in Blood 🩸 Microsamples: Optimization and Biomarker Discovery Feasibility

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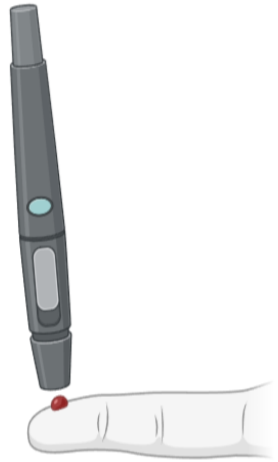
Authors declare no competing financial interest.

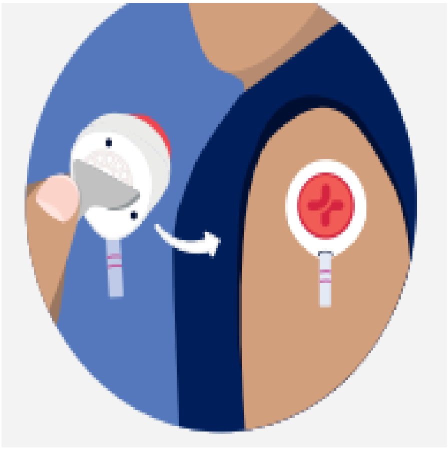
Outline

- ✓ Background and significance:
  - ✓ Rapid microbore metabolic profiling (RAMMP).
  - ✓ Blood microsampling/microsamples (BμS).
- ✓ Optimization and evaluation of a RAMMP assay.
- ✓ Feasibility of RAMMP to identify biomarkers discriminating BμS and traditional blood matrices (liquid venous blood and plasma).
- ✓ Summary and conclusions.

Background and significance

- ✓ **Microbore** (≤ 1 mm column internal diameter) analysis is a high throughput LC-MS technique.
  - ✓ A common **Microbore** application is rapid microbore metabolic profiling (**RAMMP**):
    - ➢ Reduced solvent and sample consumption.
    - ➢ Fast chromatography (**RAMMP** 1.5 min – 3.8 min vs **conventional LC-MS** 7.5 min – 30 min).
- ✓ Blood microsampling (BμS):





Collection of < 100 μl of capillary blood.

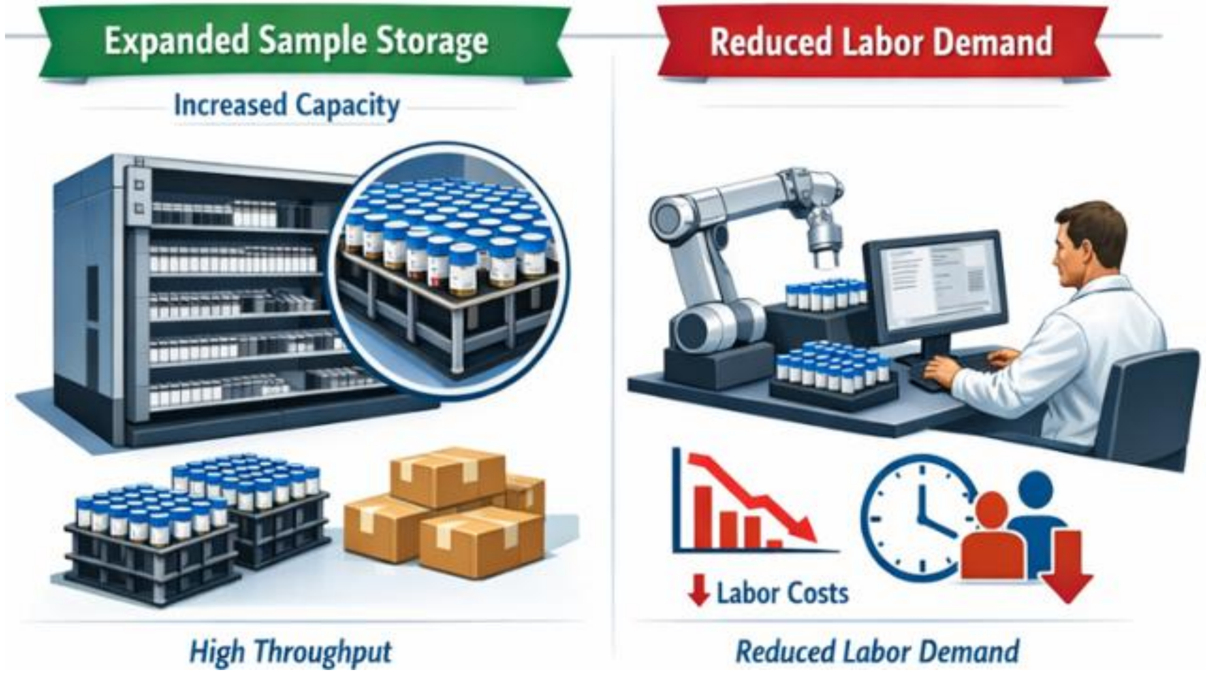
Background and significance

RAMMP

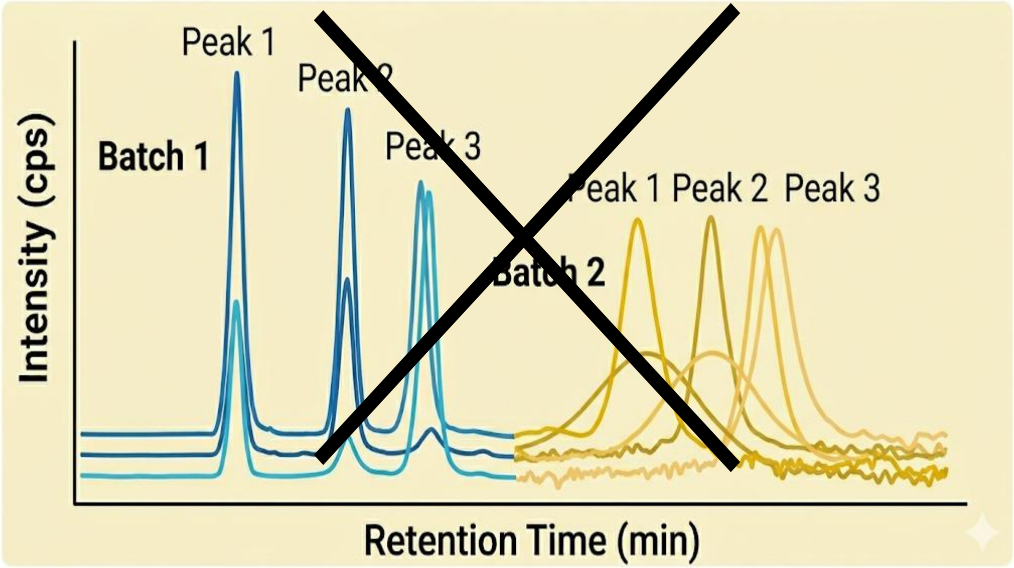
Low reagent and sample consumption

$$

Improved laboratory logistics.



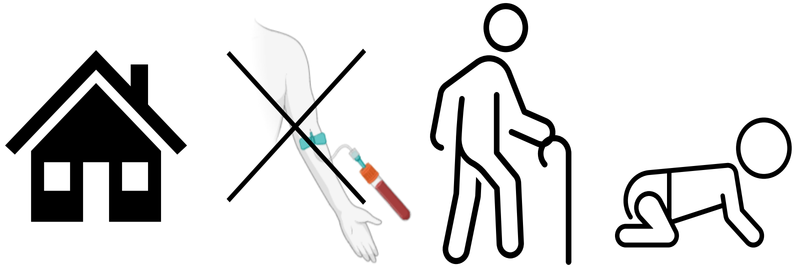
Mitigation of batch effects



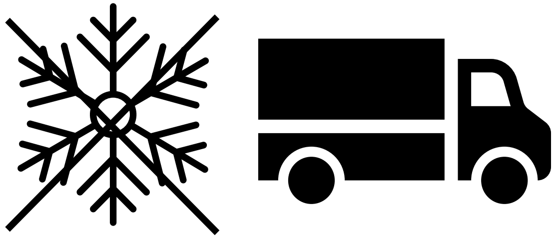
Some AI generated images

Background and significance

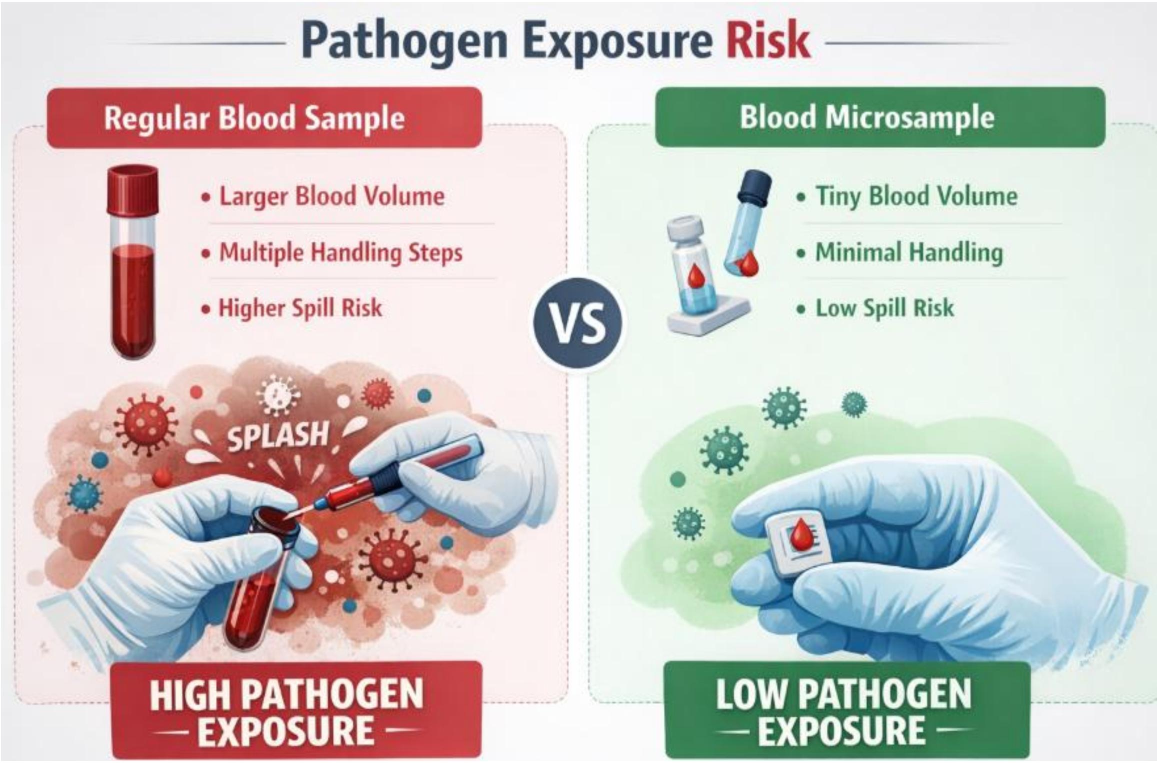
BµS



"Patient centric" (ease and convenience).
Feasibility for longitudinal research.



✓ Improved transportation logistics



Low risk of pathogen exposure.

Enhanced stability for certain metabolites e.g., up to 10 years.

Some AI generated images

Background and significance

RAMMP + BμS

Can provide a paradigm shift in the analysis of

LARGE PHENOTYPIC STUDIES.

Some images generated by AI.

Optimization and evaluation of a RAMMP assay

Conventional LC-QTOF

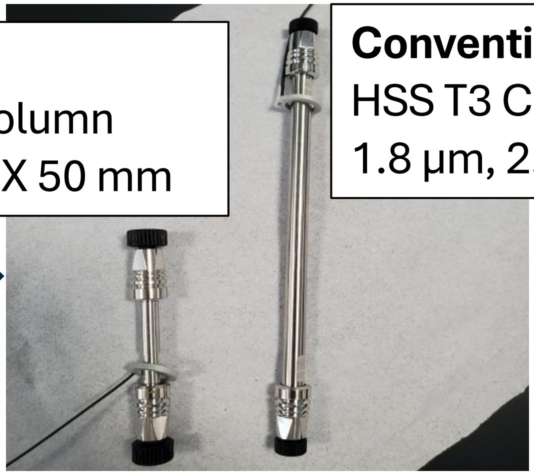
Waters Acquity UPLC-Synapt G2S QTOF

Department of Metabolism, Digestion, and Reproduction
Imperial College London.

RAMMP
HSS T3 C18 column
1.8 µm, 1 mm X 50 mm

Conventional
HSS T3 C18 column
1.8 µm, 2.1 mm X 150 mm

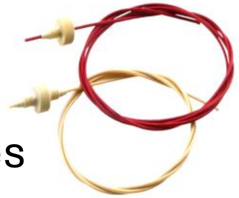
RAMMP SETUP



For RAMMP

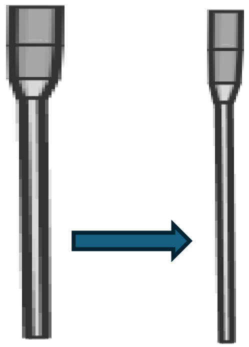
Shorter
Narrower peek tubing

0.004 inches
0.0025 inches



https://uhplcs.com/peek-fittings-for-hplc/

Narrower MS probe



125 µm to 50 µm

Some AI generated images

Optimization and evaluation of a RAMMP assay

Geometrically scaled down conventional LC method (by **Gray et al. 2015)**.

$$\textbf{RAMMP flowrate} = Conventional\ flowrate\ x\ \frac{RAMMP\ ID^2}{Conventional\ ID^2}$$

$$\textbf{RAMMP Inj Vol} = Conventional\ flowrate\ x\ \frac{(RAMMP\ ID^2 x\ RAMMP\ Column\ Length)}{(Coventional\ ID^2\ x\ Conventional\ Column\ length)}$$

$$\textbf{Difference in RAMMP gradient timepoints} = Difference\ in\ conventional\ gradient\ timepoints\ x\ \frac{(RAMMP\ Column\ vol)}{(Conventional\ Column\ vol)}\ x\ \frac{Conventional\ flowrate}{RAMMP\ flowrate}$$

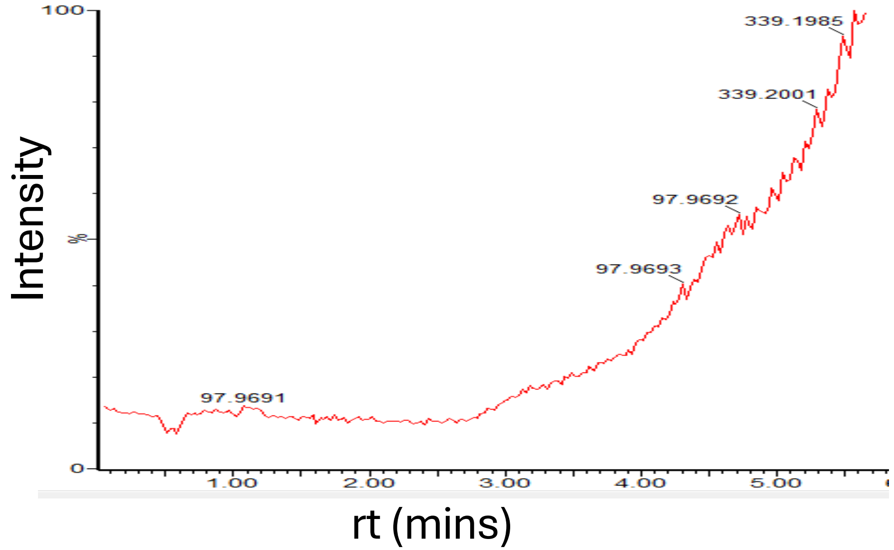
$$\textbf{RAMMP gradient timepoints} = Previous\ timepoint + Difference\ in\ RAMMP\ gradient\ timepoints$$

Conventional
Flow rate 0.35 mL/min
Injection vol 10 µL
Gradient 17 min

Scaled RAMMP
Flowrate 0.1 mL/min
Injection vol 0.8 µL
Gradient 5.7 min

Same **MS parameters** used for both assays.

No **signal** observed using the scaled **RAMMP** assay

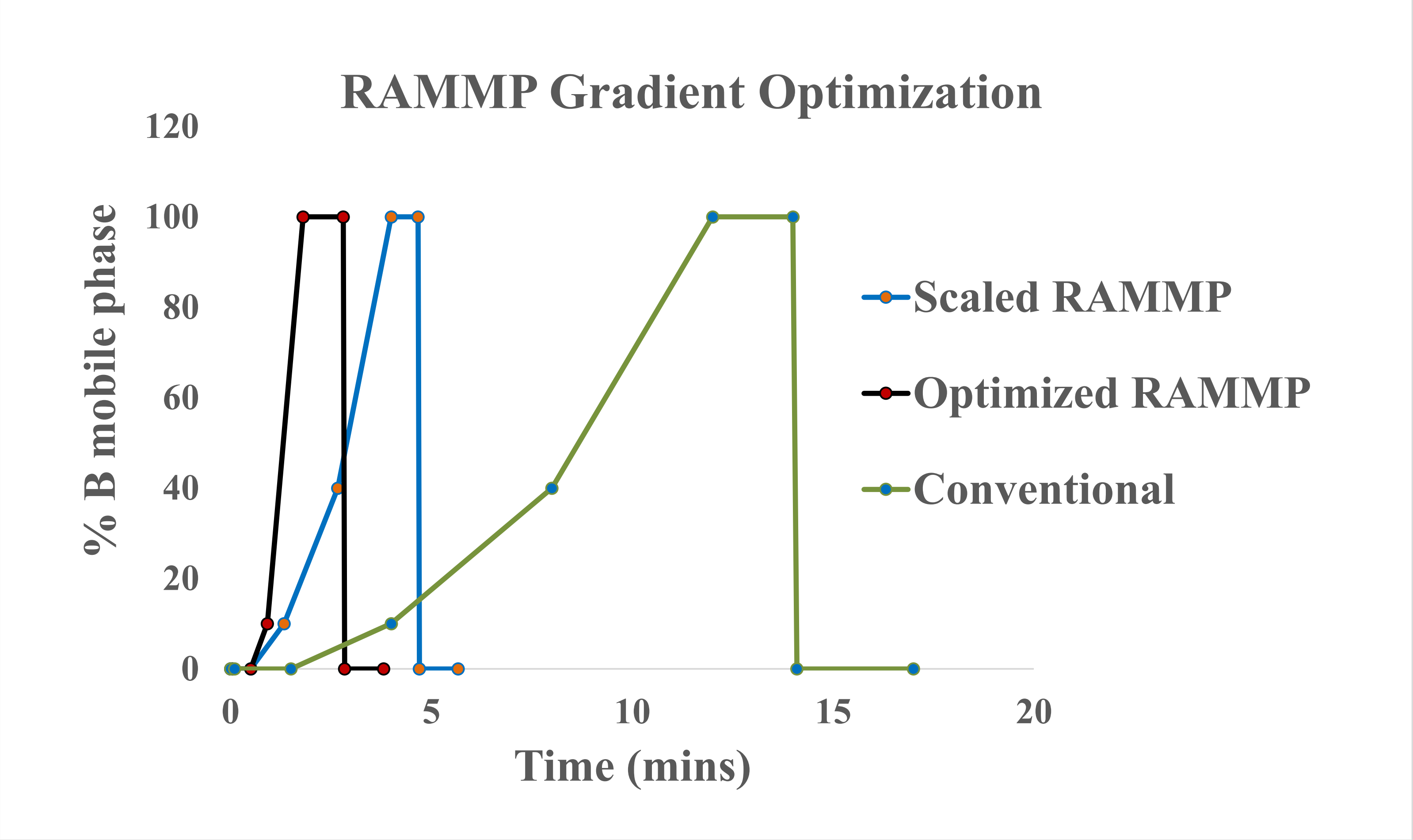


Optimization and evaluation of a RAMMP assay

Scaled RAMMP
Flowrate 0.1 mL/min
Injection vol 0.76 μL
Gradient 5.7 min

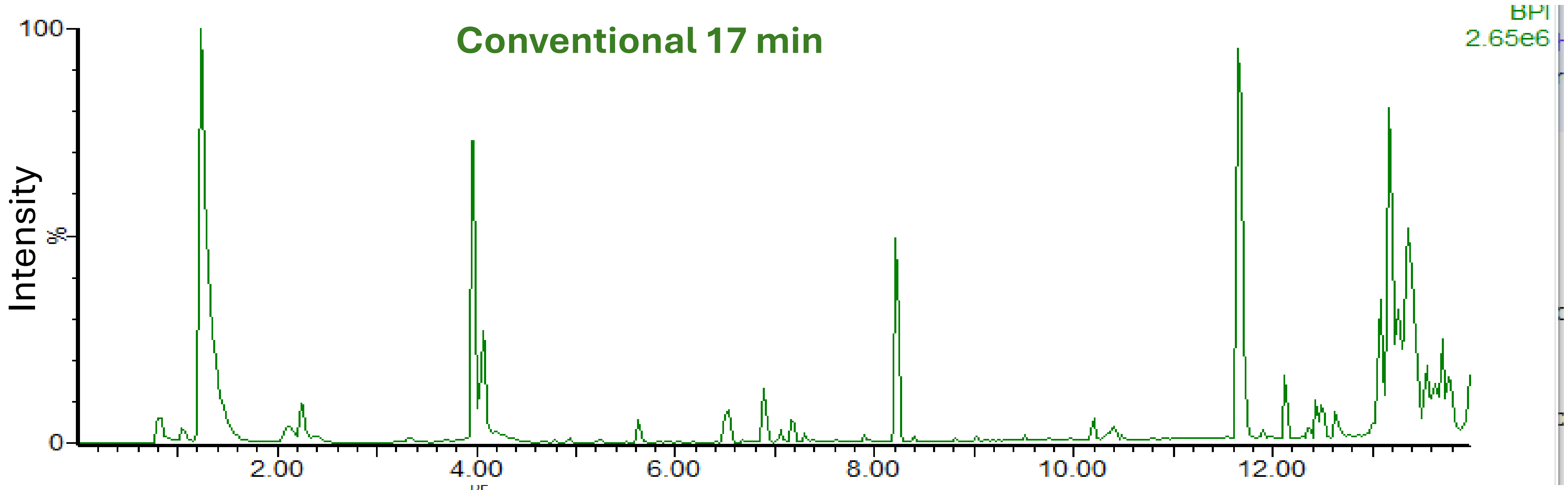
Optimized RAMMP
Flowrate 0.2 mL/min
Injection vol 5 μL
Gradient 3.8min

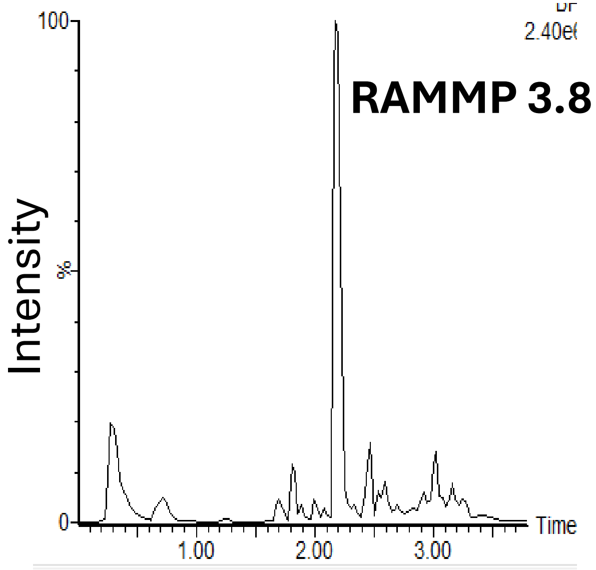
Conventional
Flow rate 0.35 mL/min
Injection vol 10 μL
Gradient 17 min



Optimization and evaluation of a RAMMP assay

Comparison of a base peak chromatogram (BPC)



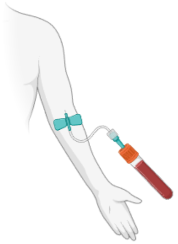


Optimization and evaluation of a RAMMP assay

Evaluate **RAMMP's performance** against a **conventional** assay.

Study size – 8 individuals

LVB



- 3-day Conventional analysis
- 3-day RAMMP analysis

(n = 3)

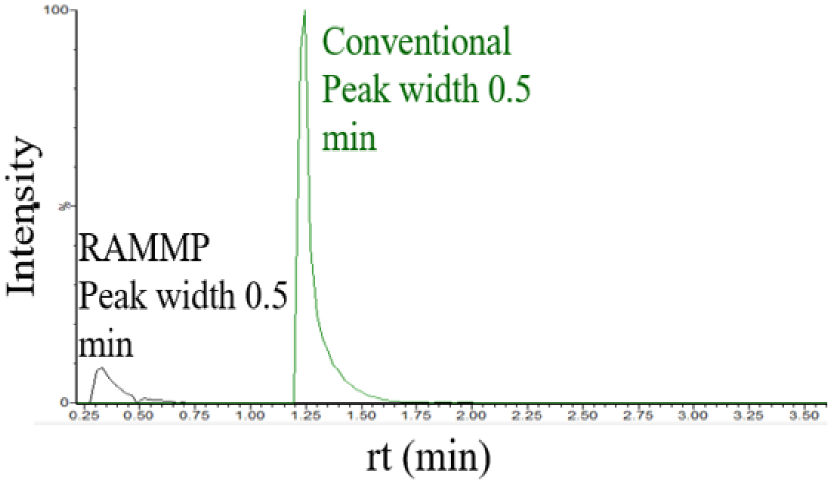
Evaluation

- Chromatography
- Metabolic coverage and abundance
- Precision

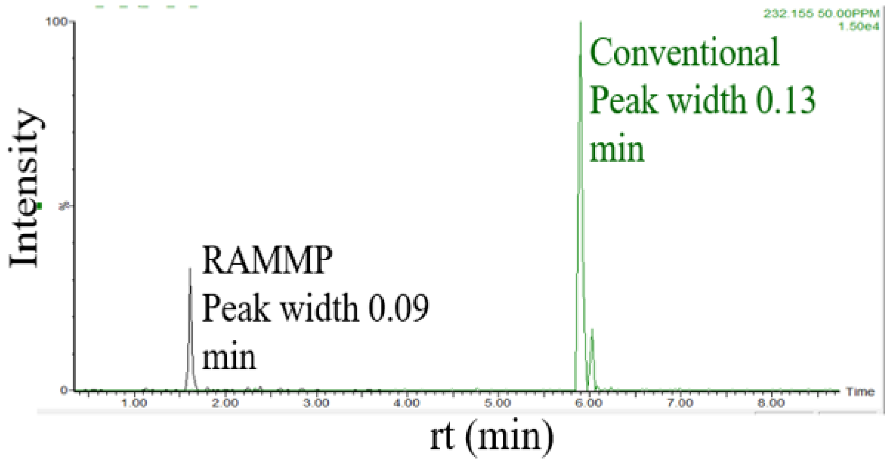
Optimization and evaluation of a RAMMP assay

Chromatography comparison: Four metabolites representing different classes and spanning the gradient compared.

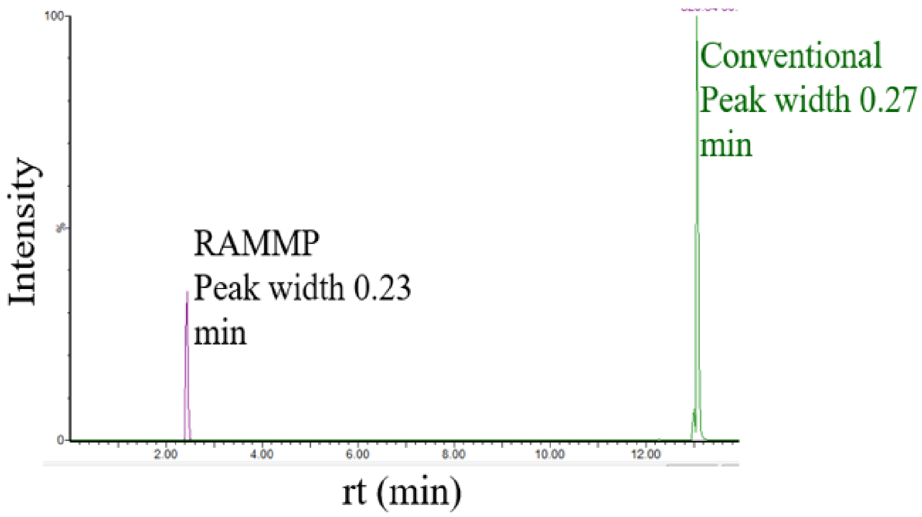
4-Hydroxyproline



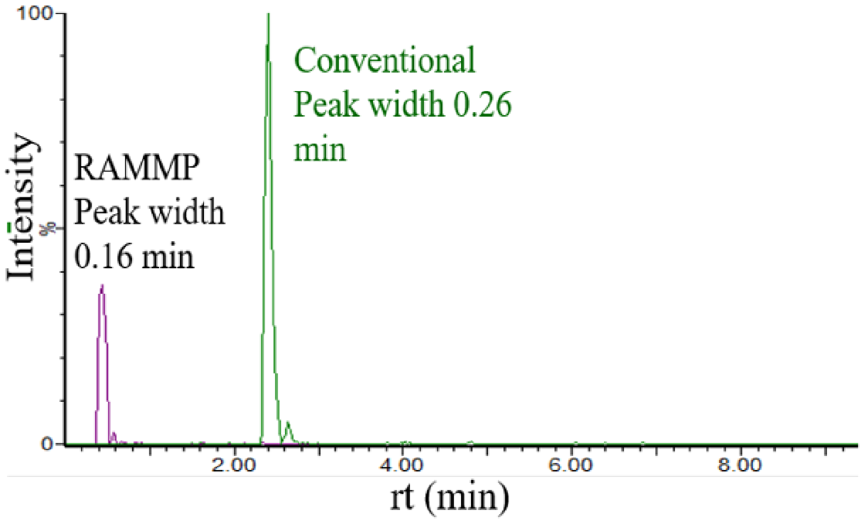
Butyrylcarnitine



Lysophosphatidylcholine 18:2



Hypoxanthine

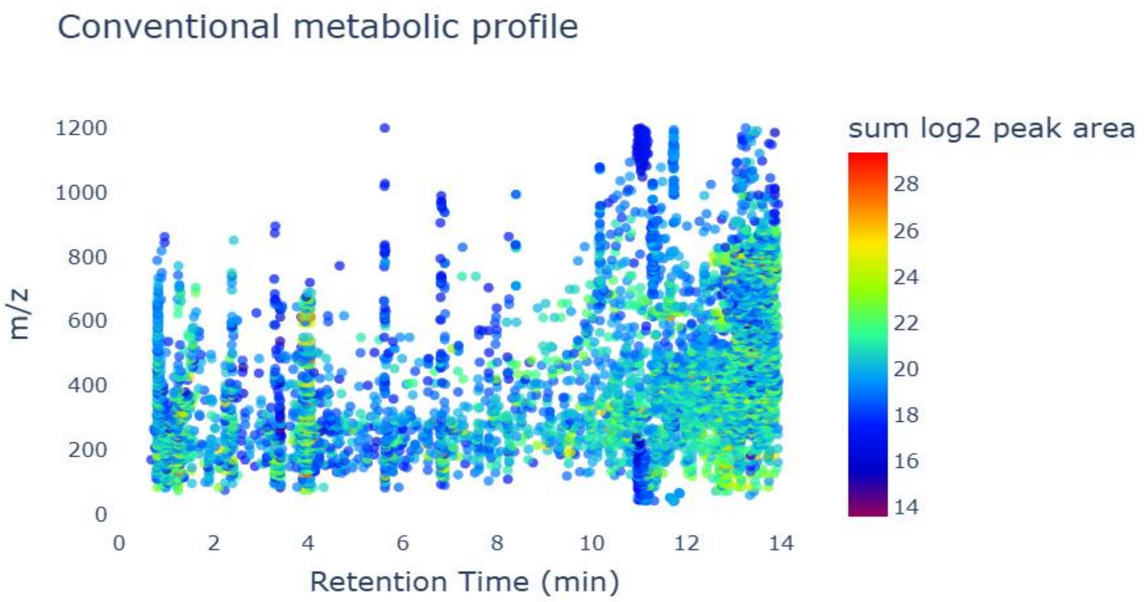


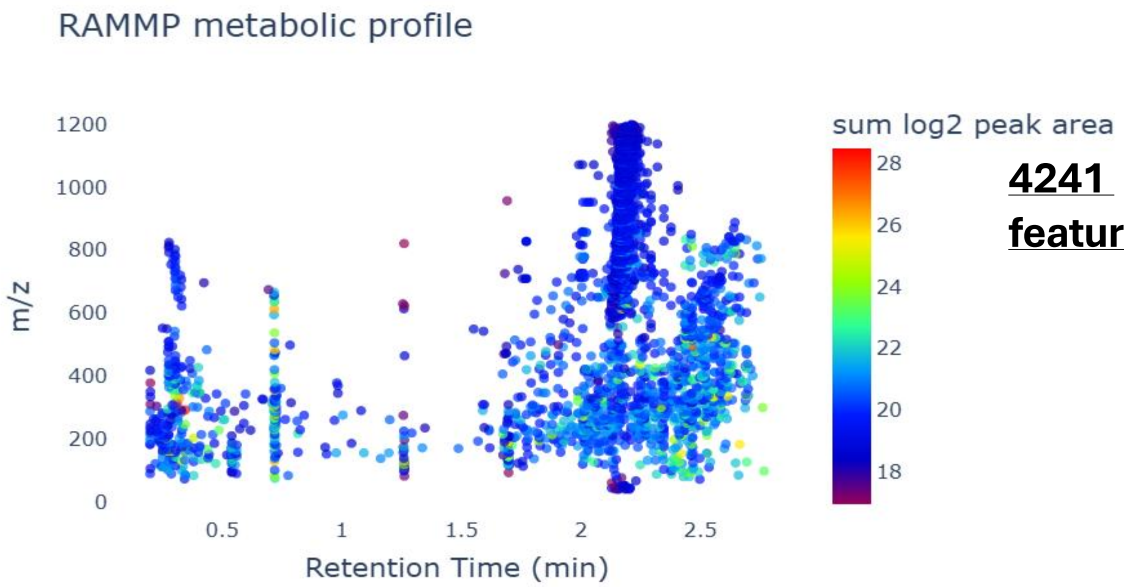
- ✓ Slightly narrower peaks in **RAMMP**; no isomeric resolution.
- ✓ Shorter gradient and column = lower peak capacity and loss of isomeric resolution.

Optimization and evaluation of a RAMMP assay

Metabolic coverage comparison

<u>6001 features</u>



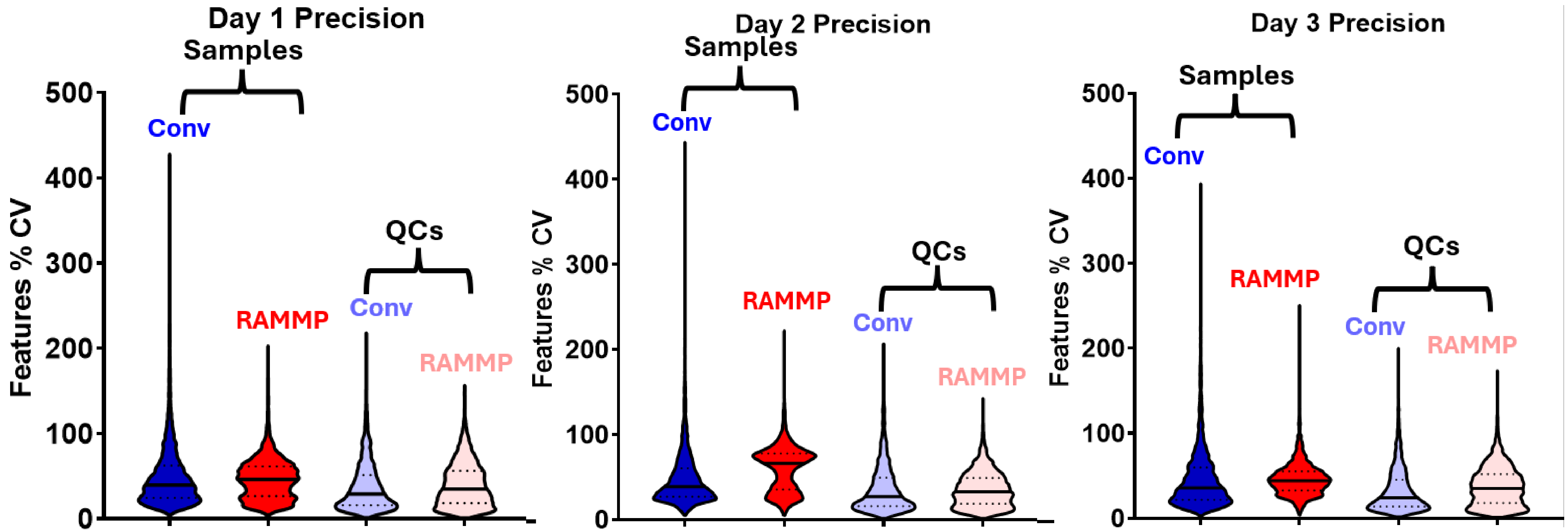


<u>4241 features</u>

- ✓ Significant loss (30%) of metabolic coverage using **RAMMP:**
  - ➢ Low abundant features in the **conventional** assay are undetected in **RAMMP**.
  - ➢ Loss of metabolic coverage and abundance are attributed to the scaling down of the chromatography.
- ✓ However, **RAMMP** had similar level of annotation:
  - ➢ **Conventional = 51** and **RAMMP = 49** (**butyrylcarnitine isomer** and **3-methylxanthine** were undetected).

Optimization and evaluation of a RAMMP assay

Precision comparison: Intra (within-day) and inter day (day-to-day) comparison**.**



✓ **RAMMP** demonstrated similar precision relative to the **conventional** assay.

RAMMP's Biomarker Discovery Feasibility

- ✓ **RAMMP** performed similar relative to the conventional assay except for loss of metabolic coverage.
- ✓ Biomarker feasibility study:
  - can **RAMMP** capture discriminating features between **Mitra BµS** and **traditional blood matrices** despite the loss of metabolic coverage?

8 individuals

Mitra BµS

LVB and plasma

20 µL Sample volume

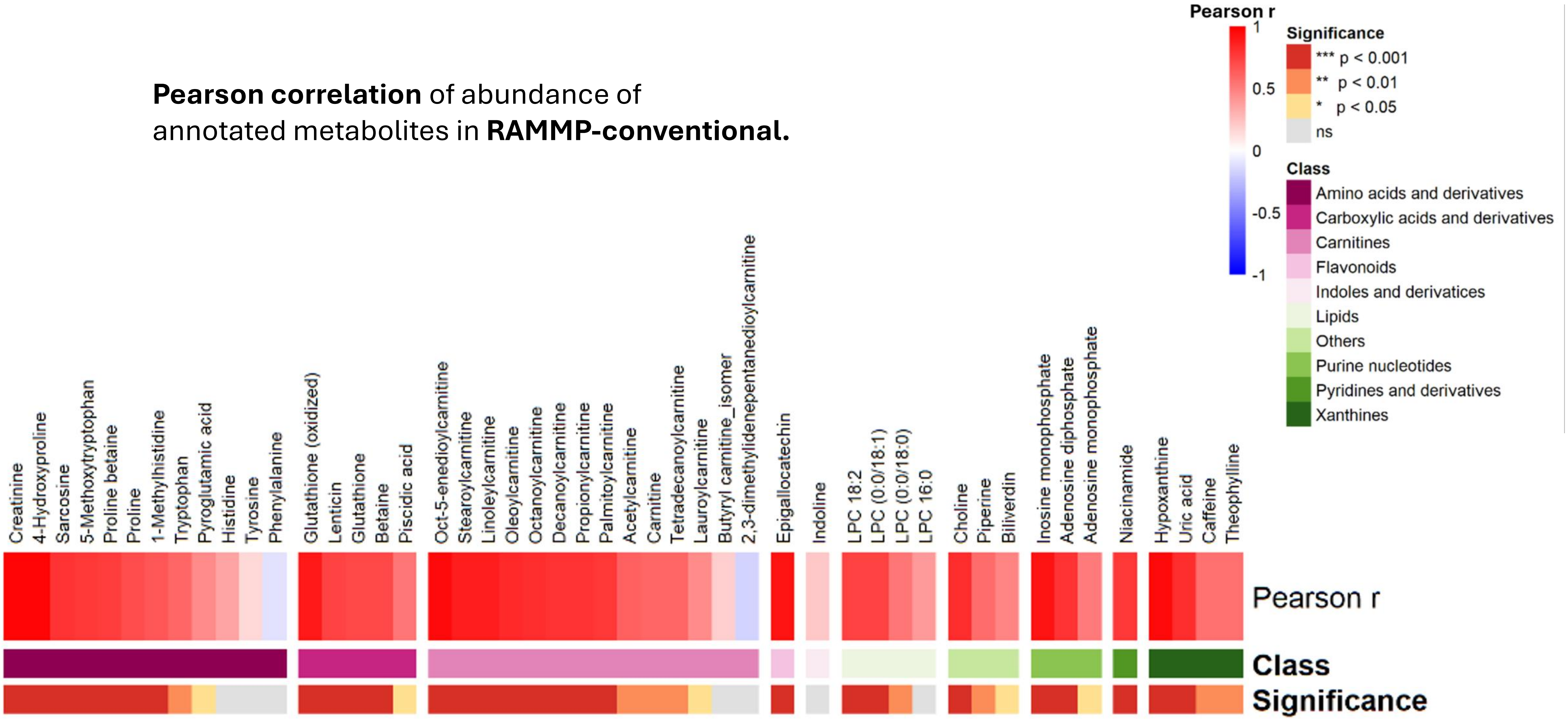
- Conventional analysis
- RAMMP analysis

Evaluation

- Metabolite correlation
- Multivariate analysis

RAMMP's Biomarker Discovery Feasibility

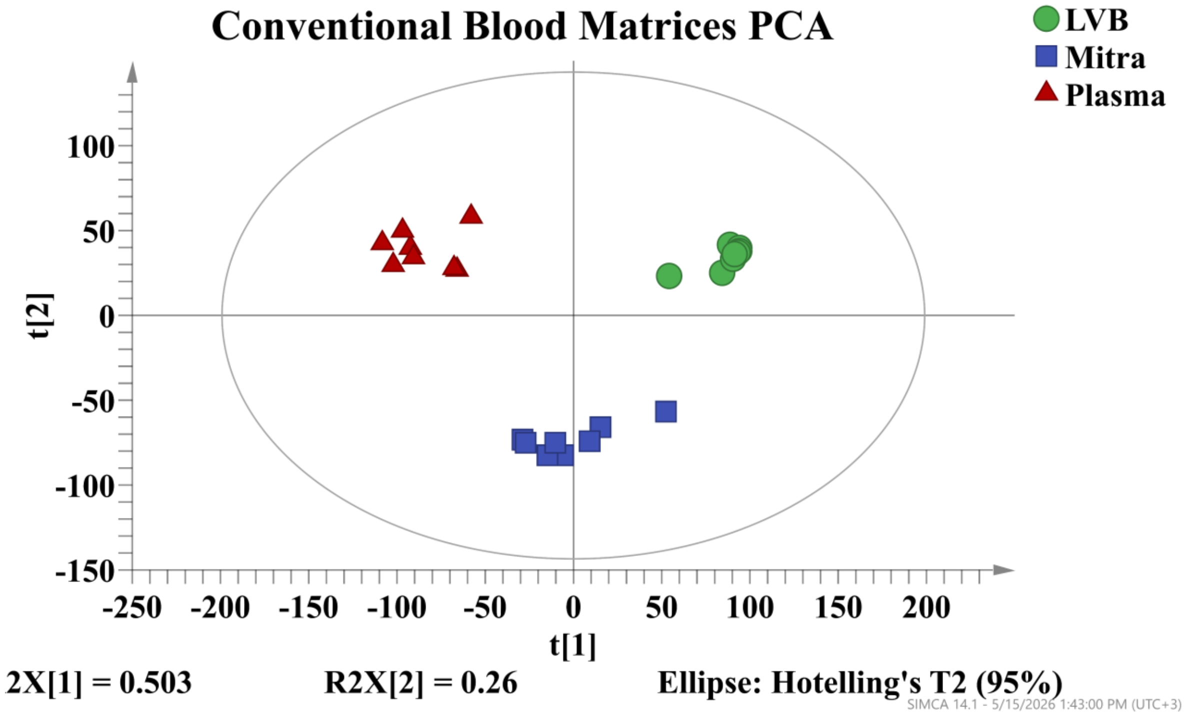
Pearson correlation of abundance of annotated metabolites in **RAMMP-conventional.**

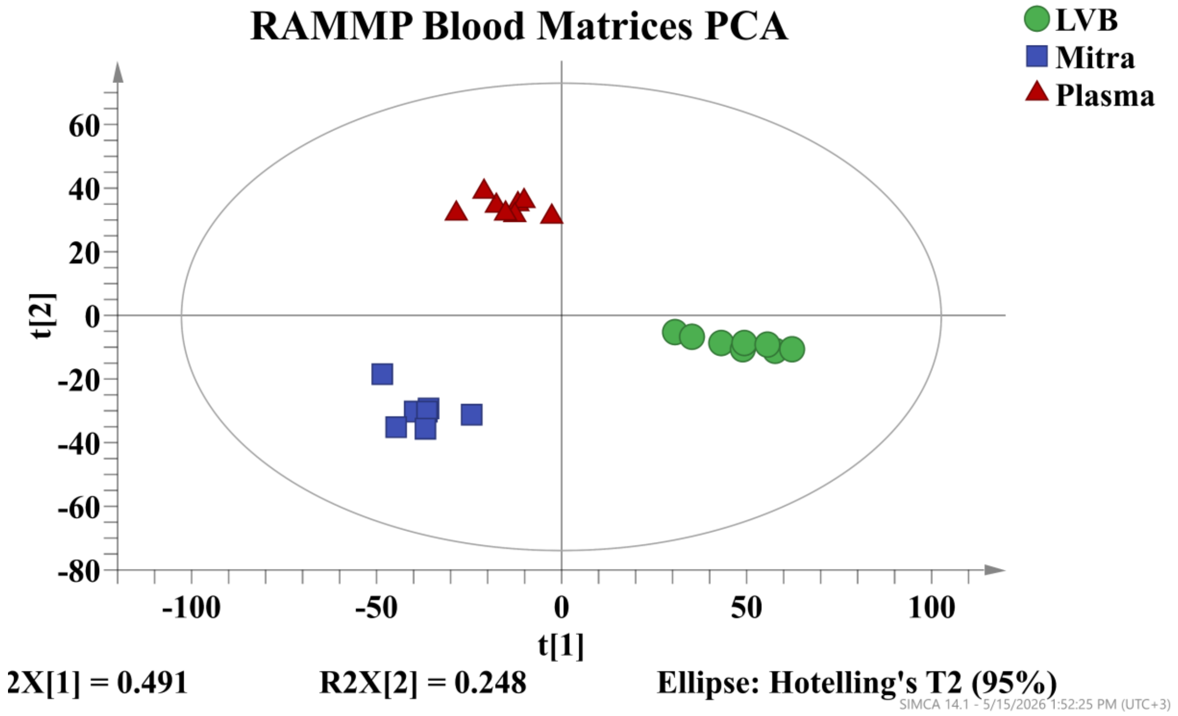


✓ **RAMMP** assay can qualitatively capture similar information.

RAMMP’s Biomarker Discovery Feasibility

Variance in the metabolic profiles.





✓ Similar clustering captured using RAMMP.

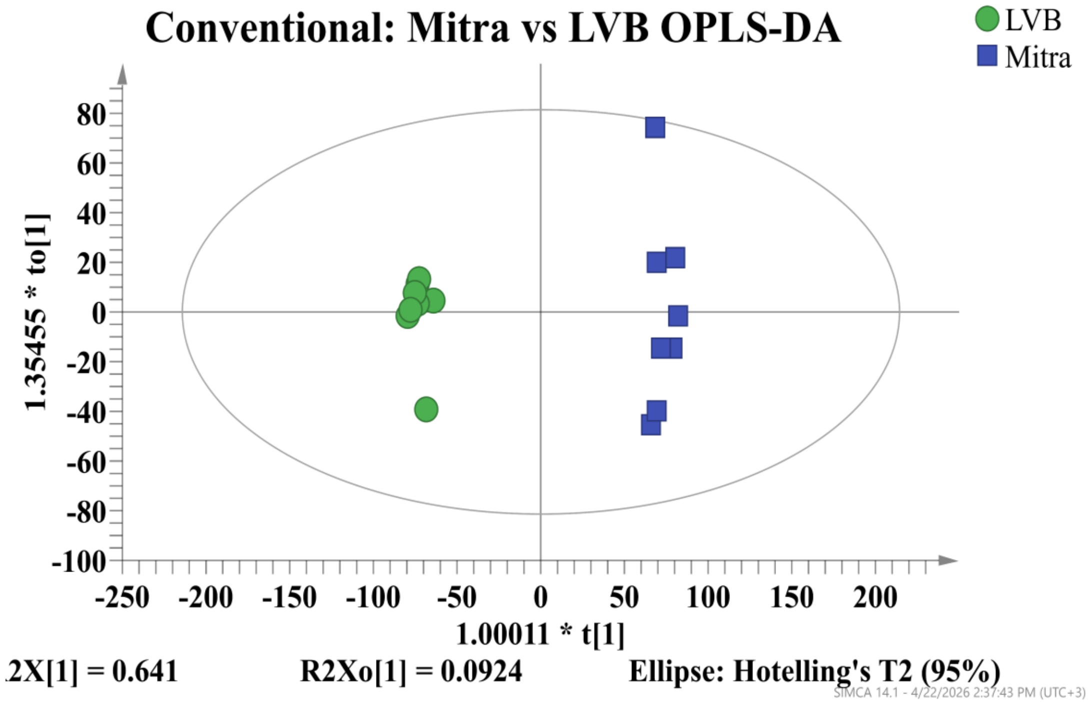
RAMMP's Biomarker Discovery Feasibility

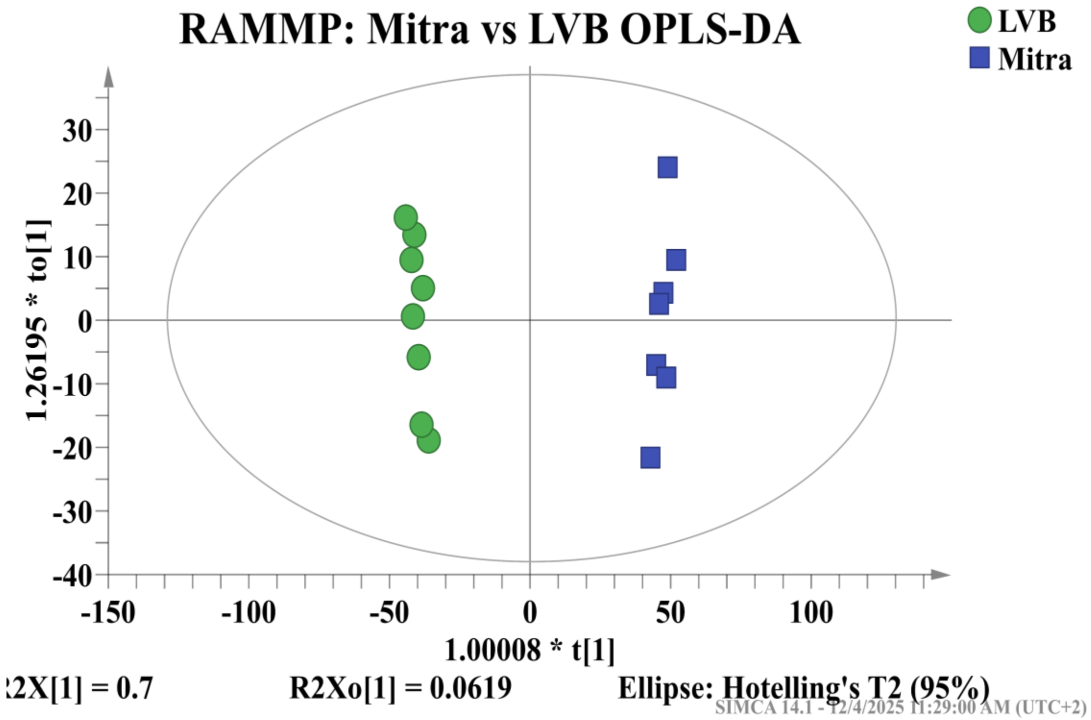
Mitra BµS

vs

LVB

Significance of discrimination analysis using OPLS-DA.





- ✓ Reliable models: CV ANOVA $p < 0.05$.
- ✓ Valid and predictive: Permutations R2 and Q2 < original models R2 and Q2 .

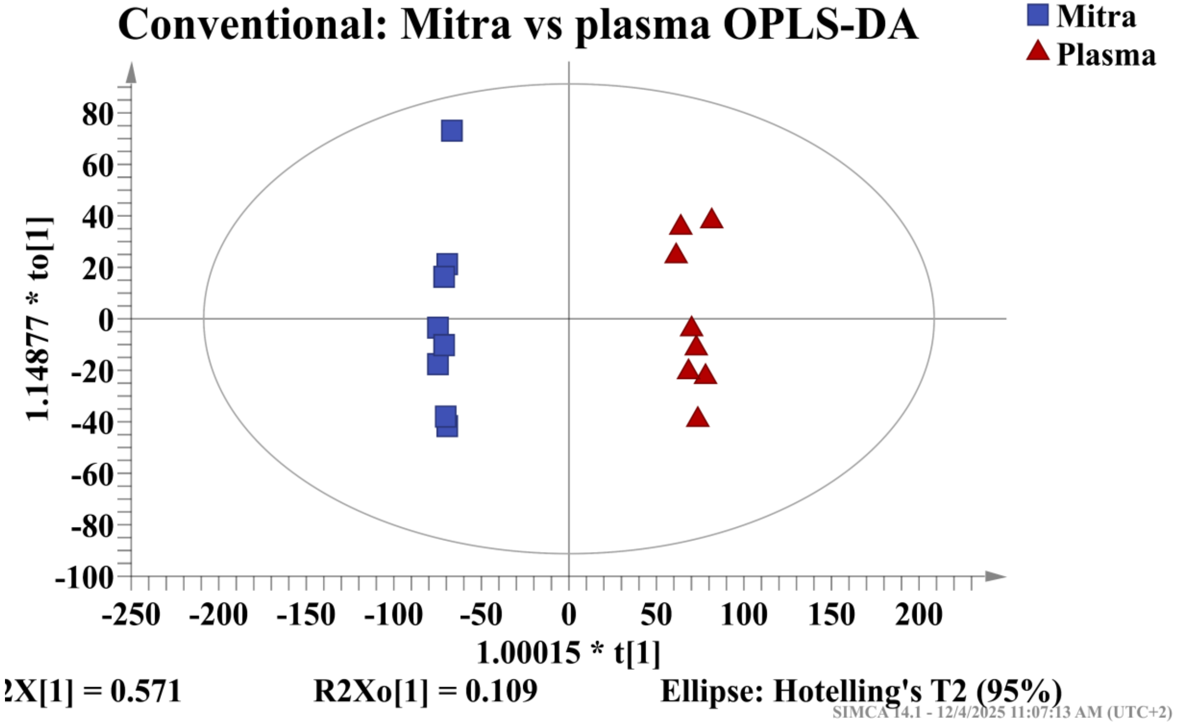
RAMMP's Biomarker Discovery Feasibility

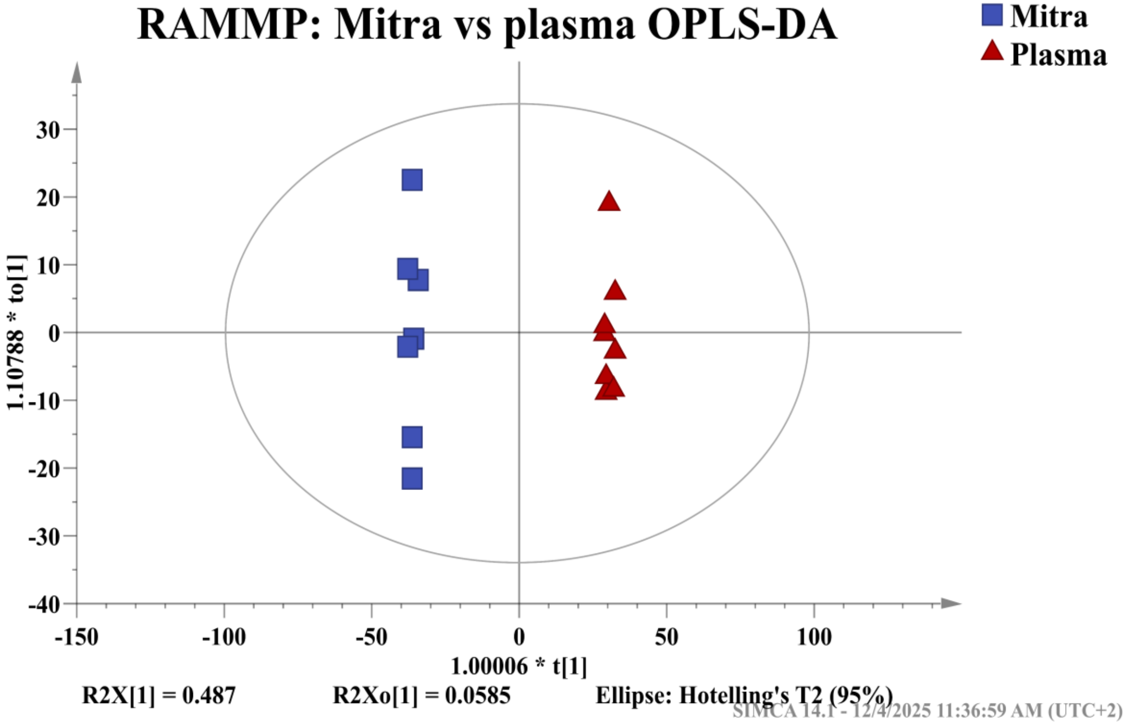
Mitra BµS

vs

Plasma

Significance of discrimination analysis using OPLS-DA.





- ✓ Reliable models: CV ANOVA $p < 0.05$.
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RAMMP's Biomarker Discovery Feasibility

Mitra BμS

vs

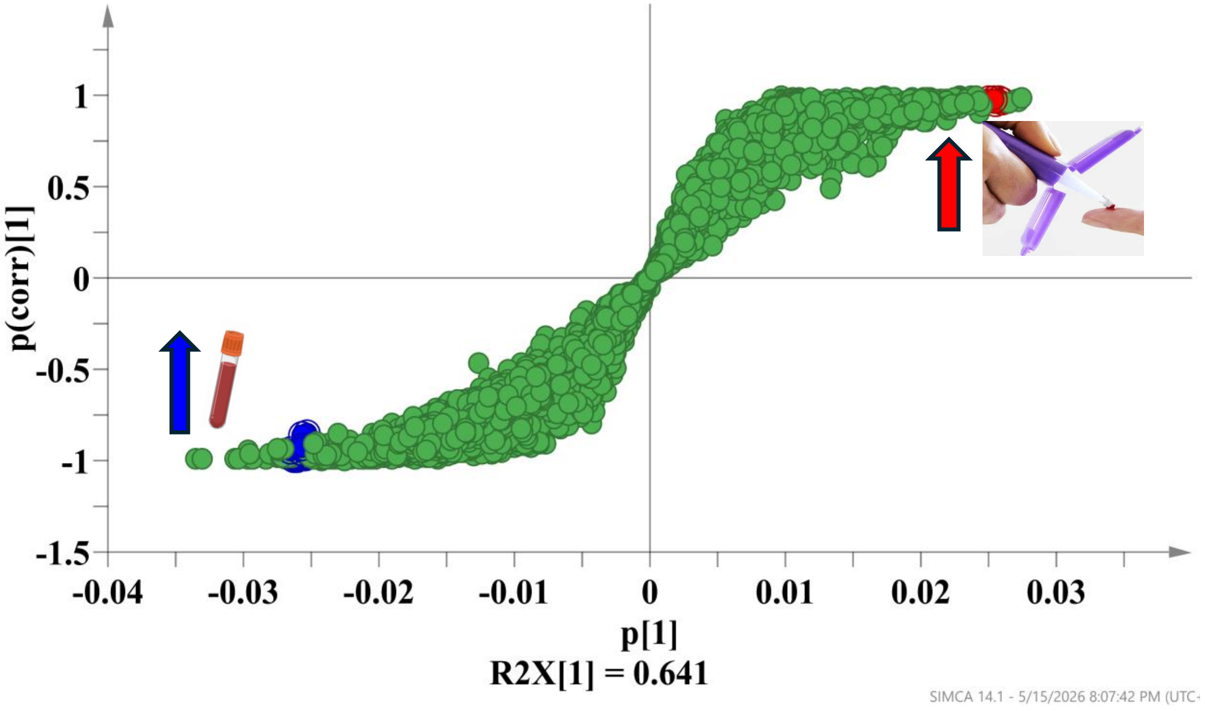
LVB

Statistically significant discriminating features:

- ✓ VIP > 1.5, p(1) > |0.02|, p(corr) > |0.5|.
- ✓ T-TEST $p < 0.05$; adjusted for false discovery rate (5%) (Benjamini, Krieger, & Yekutieli).

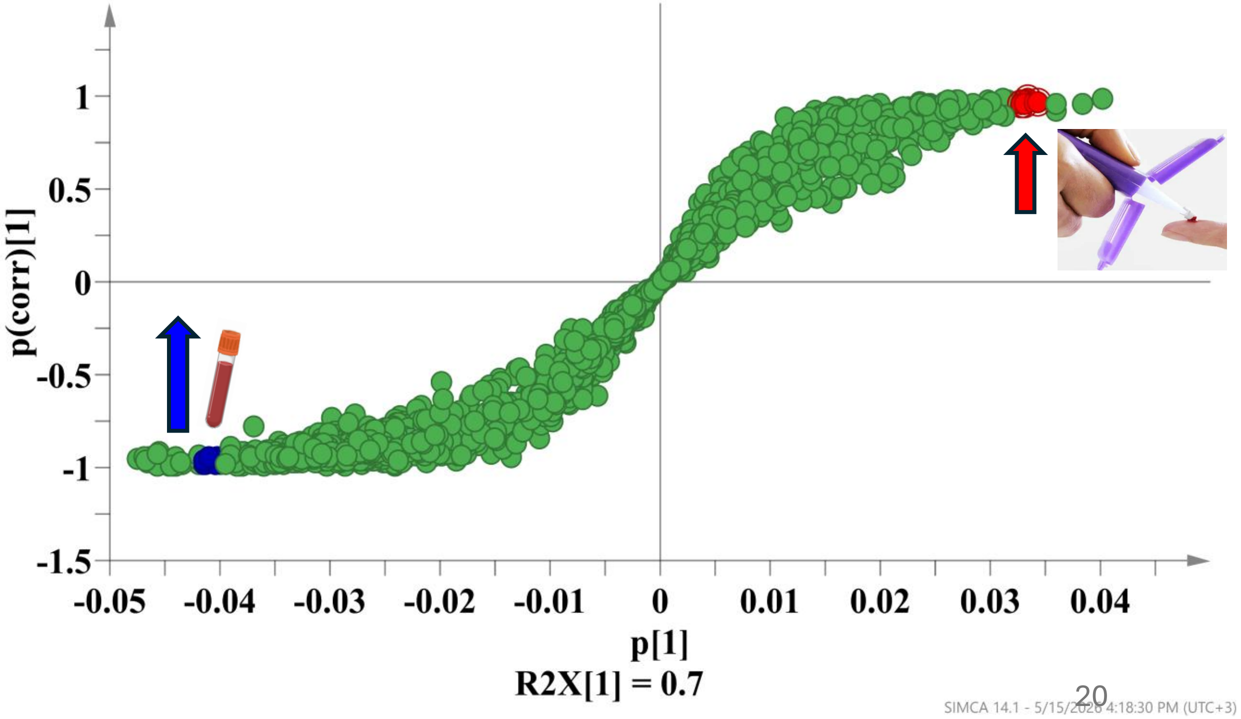
240 **(<u>3.9%</u>)** statistically significant features

S Plot: Conventional Mitra vs LVB



132 **(<u>6.4%</u>)** statistically significant features

S Plot: RAMMP Mitra vs LVB



RAMMP’s Biomarker Discovery Feasibility

Mitra BµS

vs

Plasma

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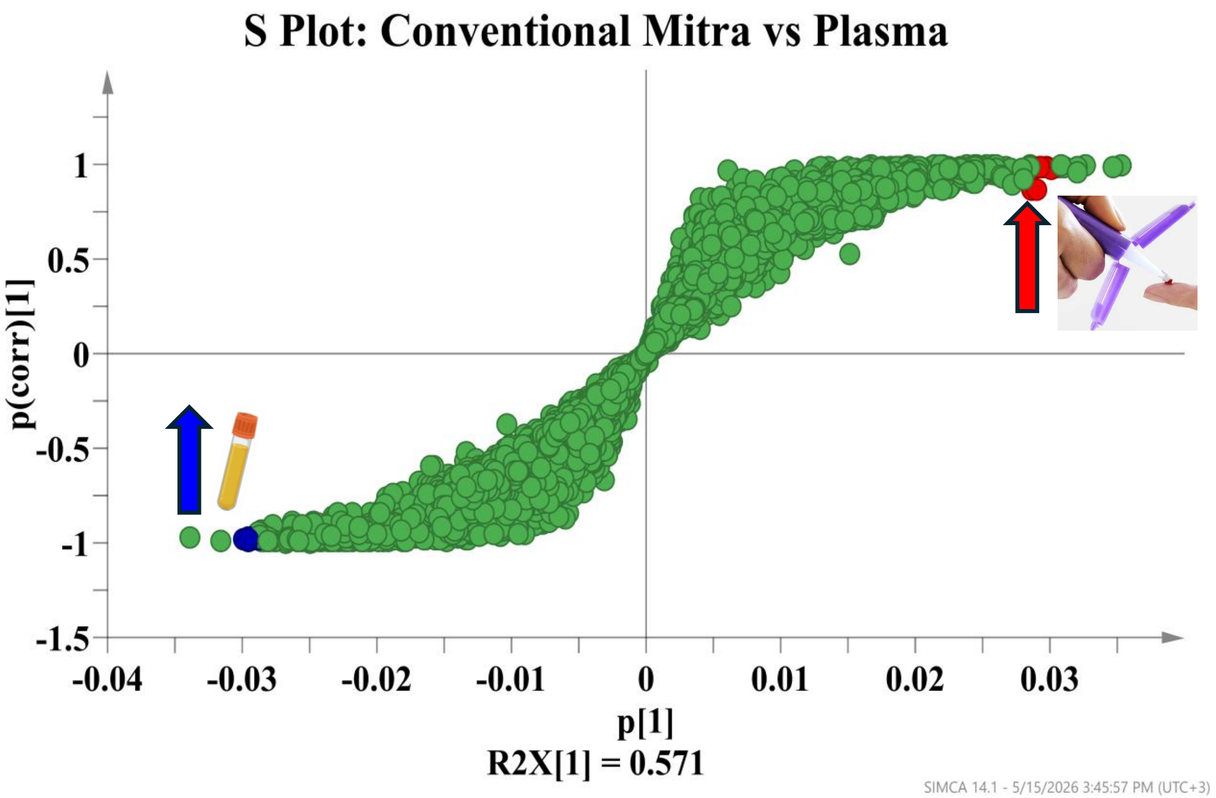
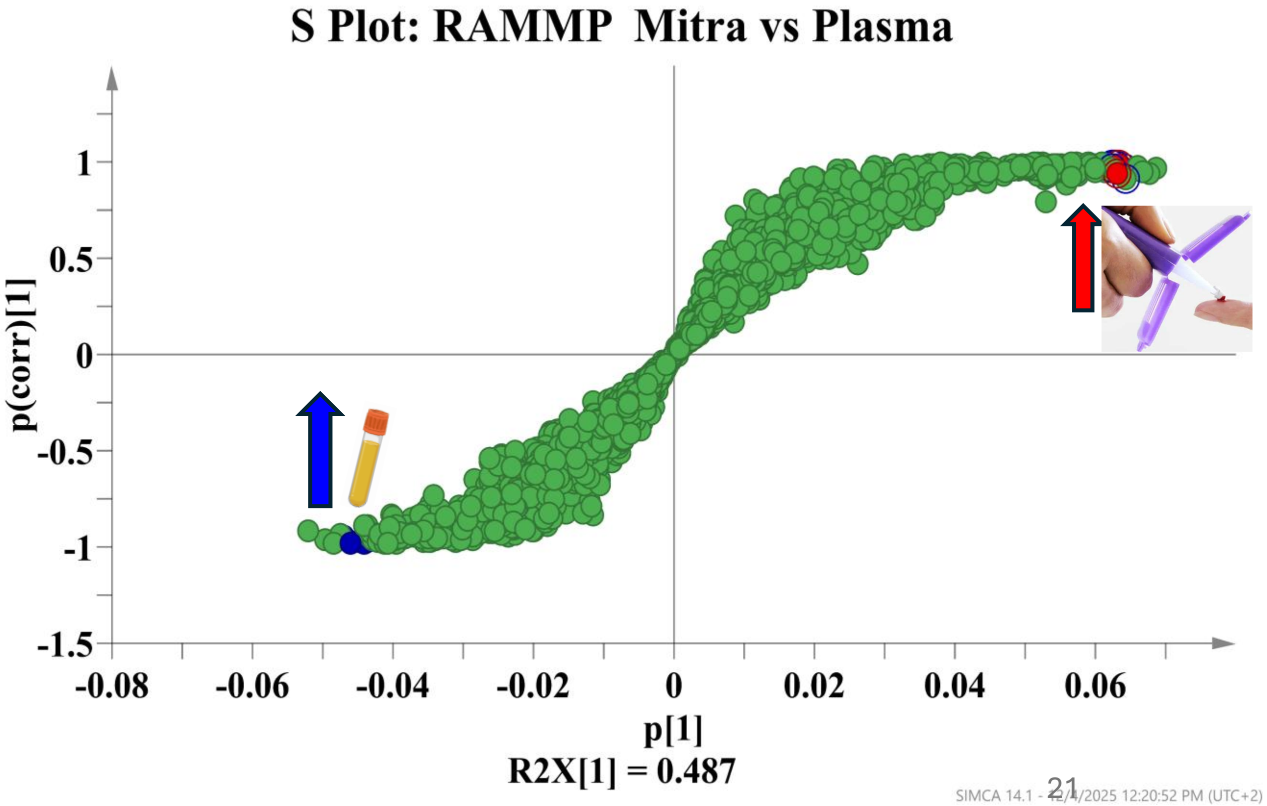
- ✓ T-TEST $p < 0.05$; adjusted for false discovery rate (5%) (Benjamini, Krieger, & Yekutieli).

529 **<u>(8.7%)</u>** statistically significant features



246 **<u>(12%)</u>** statistically significant features



Summary and conclusions

✓ RAMMP and BμS can provide a paradigm shift in the analysis of large phenotypic studies.

✓ RAMMP can capture discriminating features (biomarkers) between BμS and traditional blood matrices.

✓ Although loss of metabolic coverage is expected in RAMMP, results suggest it maybe insignificant.

✓ For RAMMP adoption, consider: the goals, budget, target metabolites, lab capacity, and time.

Cons
Loss of metabolic coverage and sensitivity.

Pros
Environmentally friendly
Low costs
Improved logistics
Mitigation of batch effects.

Acknowledgements

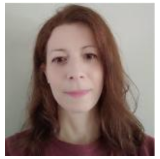
Imperial College London

Ass Prof. Elizabeth Want

Anna Sanchez Lorenzo

THETA BIOMARKERS

ARISTOTLE UNIVERSITY OF THESSALONIKI



Ass Prof. Helen Gika

Prof. Georgios Theodoridis

HUMAN Harmonising and Unifying Blood Metabolomic Analysis Networks

Marie Skłodowska-Curie Actions Doctoral Networks 2021

MARIE CURIE ACTIONS

European Commission | Horizon 2020 European Union funding for Research & Innovation

Questions?

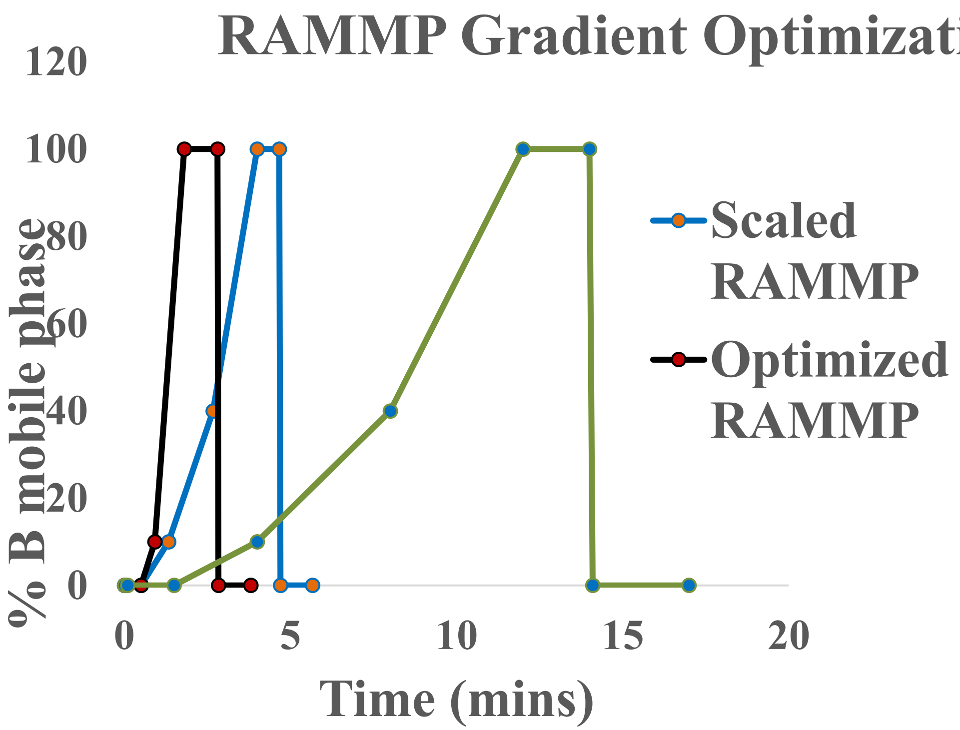
Let Connect

Marlene Thaitumu

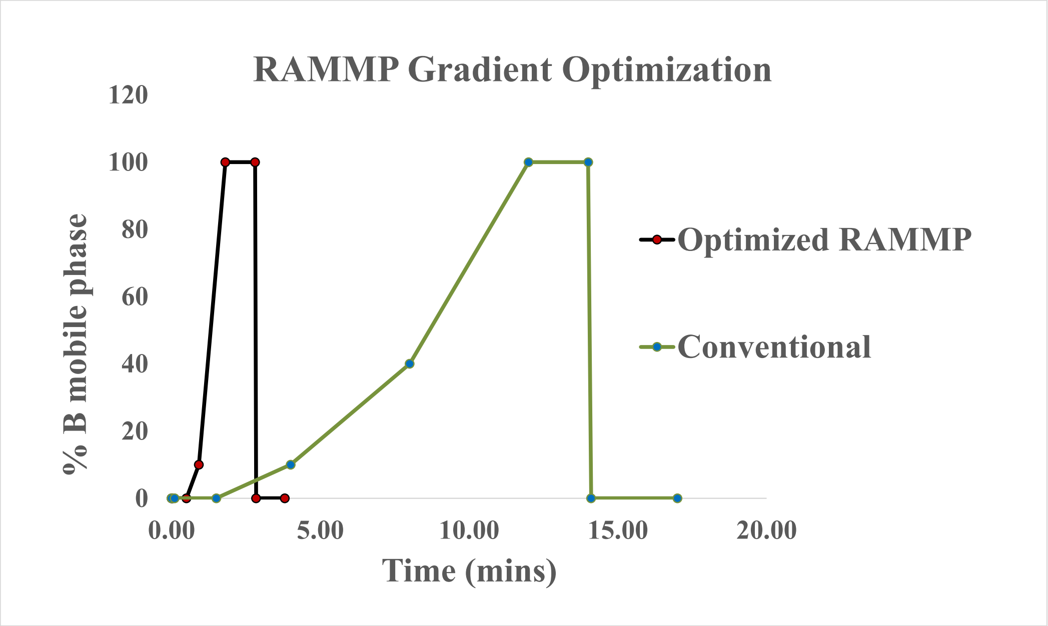
Doctoral Researcher

HUMAN Doctoral Network: Marie Sklo…

Extra slides



RAMMP Gradient Optimization
120
100
80
60
40
20
0
% B mobile phase
0
5
10
15
20
Time (mins)
Scaled RAMMP
Optimized RAMMP



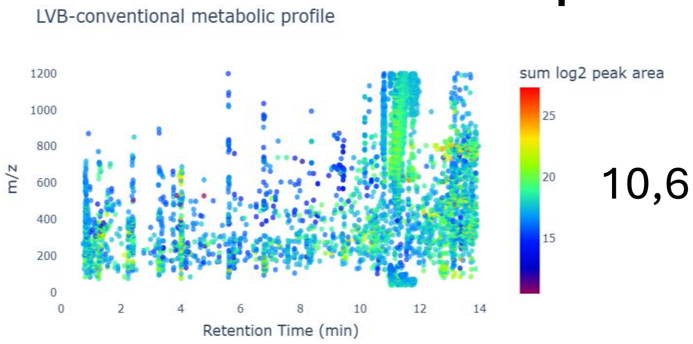
RAMMP Gradient Optimization
120
100
80
60
40
20
0
% B mobile phase
0.00
5.00
10.00
15.00
20.00
Time (mins)
Optimized RAMMP
Conventional

Biomarker discovery feasibility of RAMMP: Mitra BµS compared to LVB and plasma

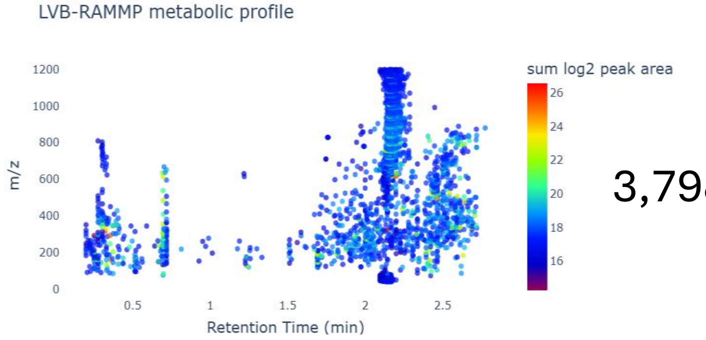
Conventional metabolic profiles

RAMMP metabolic profiles

LVB

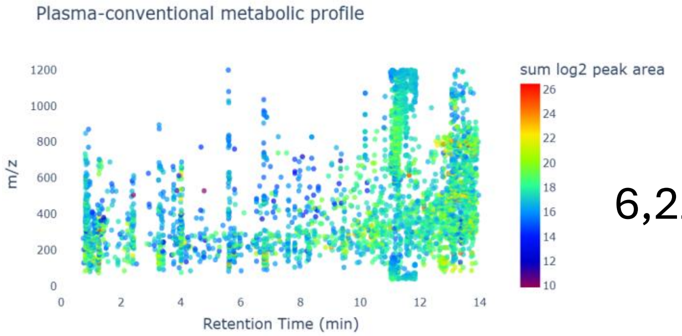


10,618 features

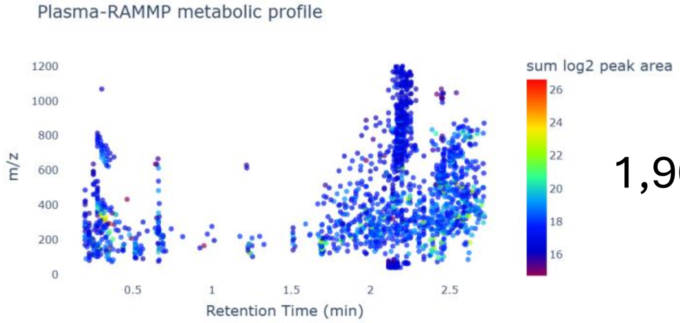


3,798 features

Plasma

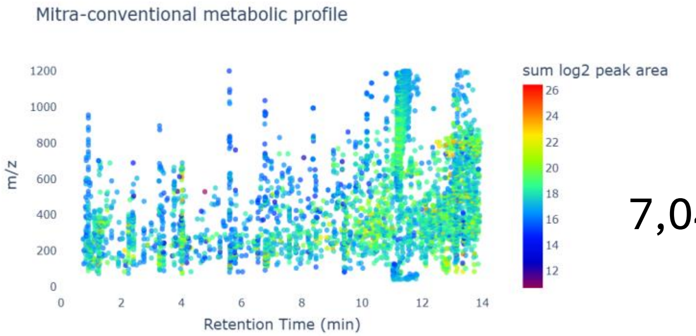


6,228 features

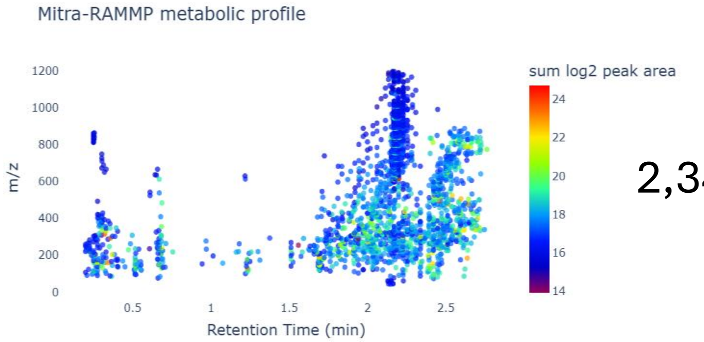


1,906 features

Mitra



7,040 features



2,342 features

✓ **RAMMP** showed loss in metabolic coverage as previously discussed.

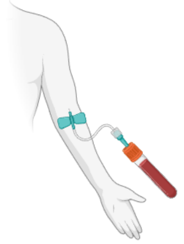
✓ Despite loss in metabolic coverage, **RAMMP** can capture important information compared to the conventional.

RAMMP vs conventional assay in LVB metabolic profiling

Validate **RAMMP's performance** against a **conventional** assay.

Study size – 8 individuals

LVB



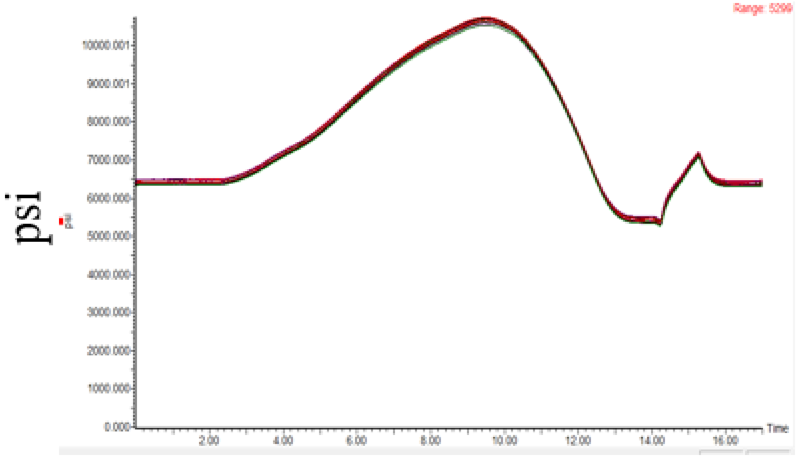
3-day analysis using:
- Conventional
- RAMMP

In triplicate (n = 3)

- Robustness
- Chromatography
- Metabolic coverage
- Precision
- Abundance

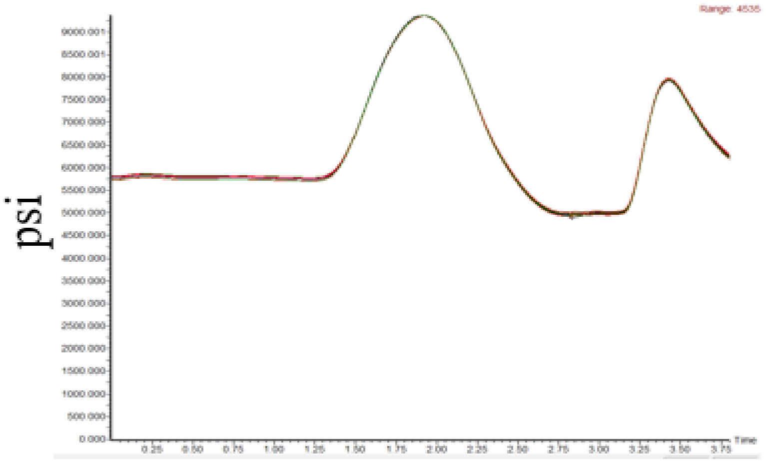
Robustness

Conventional pressure trace



rt (min)

RAMMP pressure trace



rt (min)

✓ **RAMMP** assay demonstrated comparable robustness despite scale down (1 mm internal diameter).

RAMMP's Biomarker Discovery Feasibility

Mitra BµS

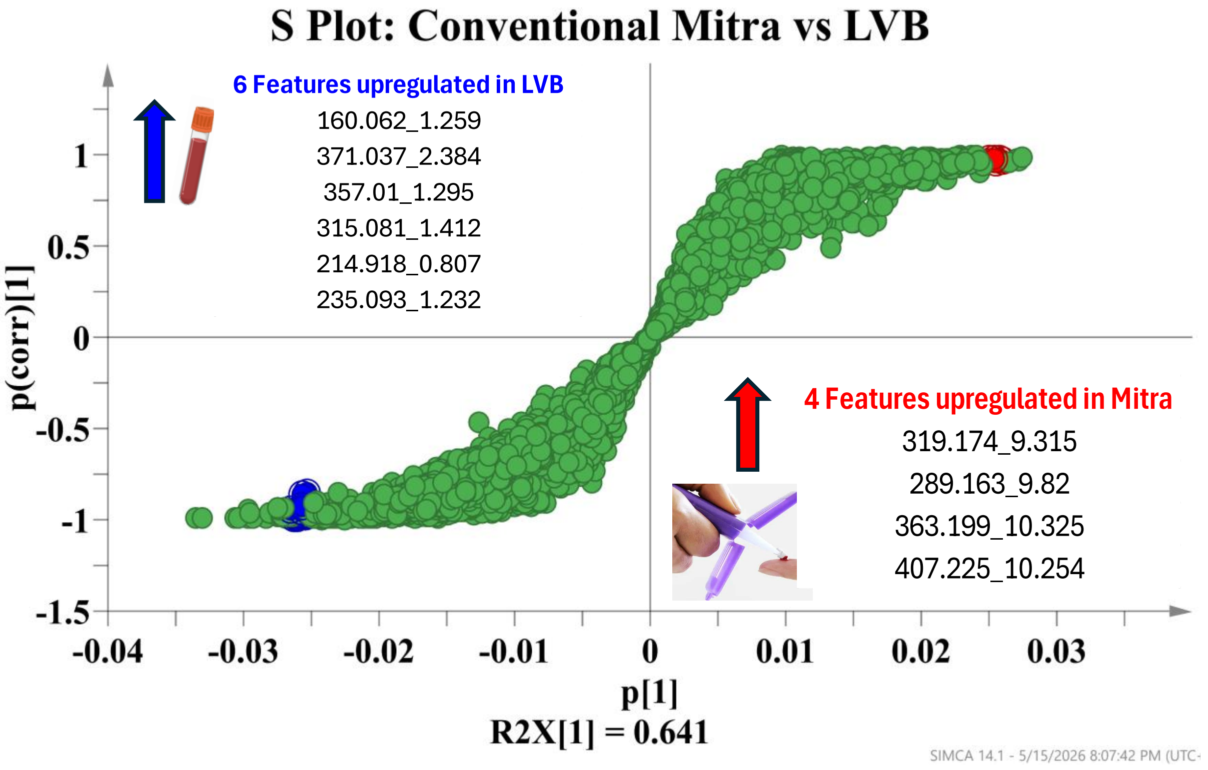
vs

LVB

Statistically significant discriminating features in the two assays:

- ✓ VIP > 1.5, p(1) > |0.02|, p(corr) > |0.5|.
- ✓ T-TEST p < 0.05; adjusted for false discovery rate (Benjamini, Krieger, & Yekutieli).

240 (3.9%) statistically significant features



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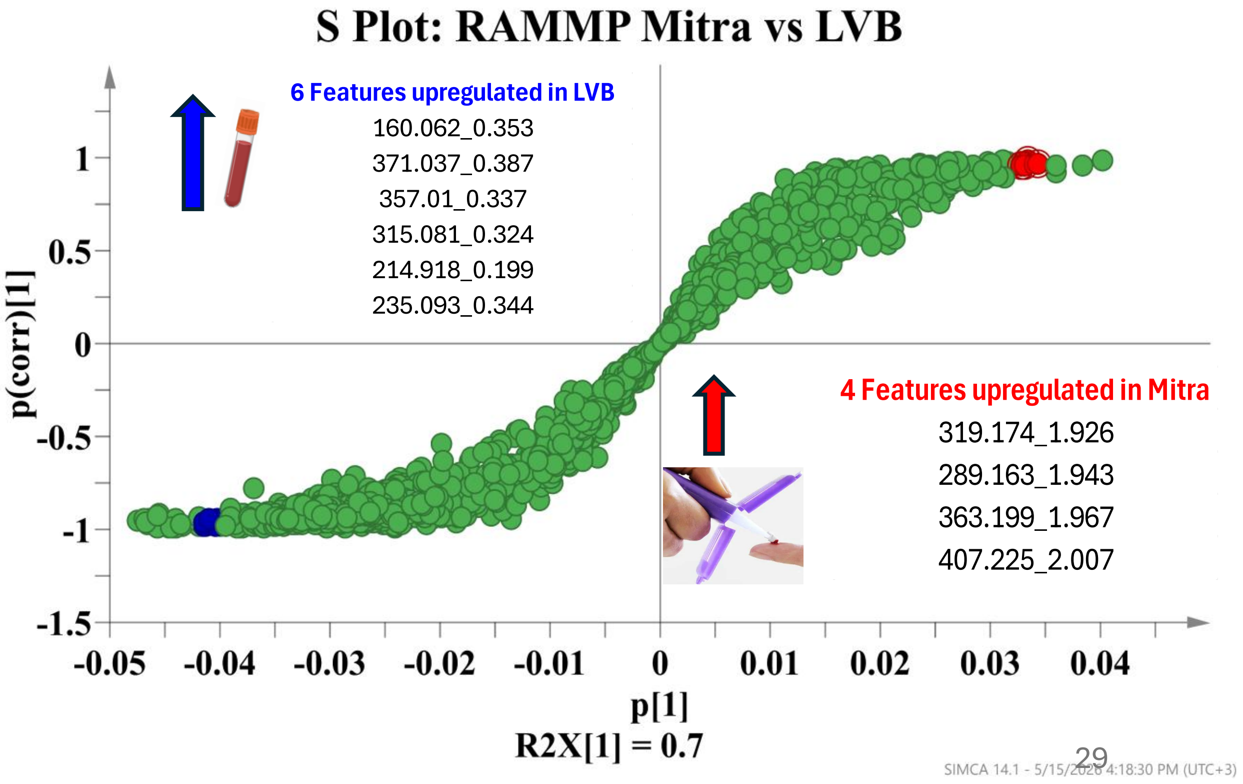

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vs

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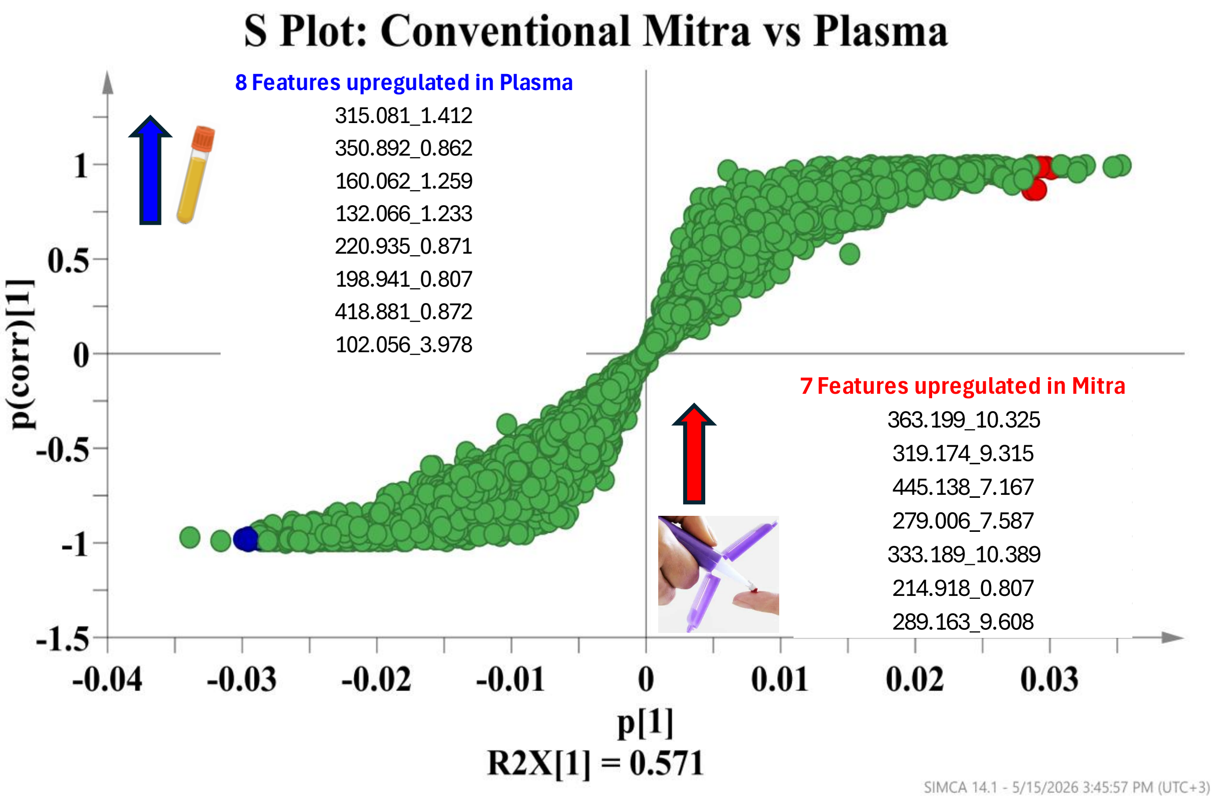

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