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HUMAN Harmonising and Unifying Blood Metabolomic Analysis Networks
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Volumetric Absorptive Microsampling in Biomarker Analysis: Insights from Targeted and Untargeted Metabolomics Approaches

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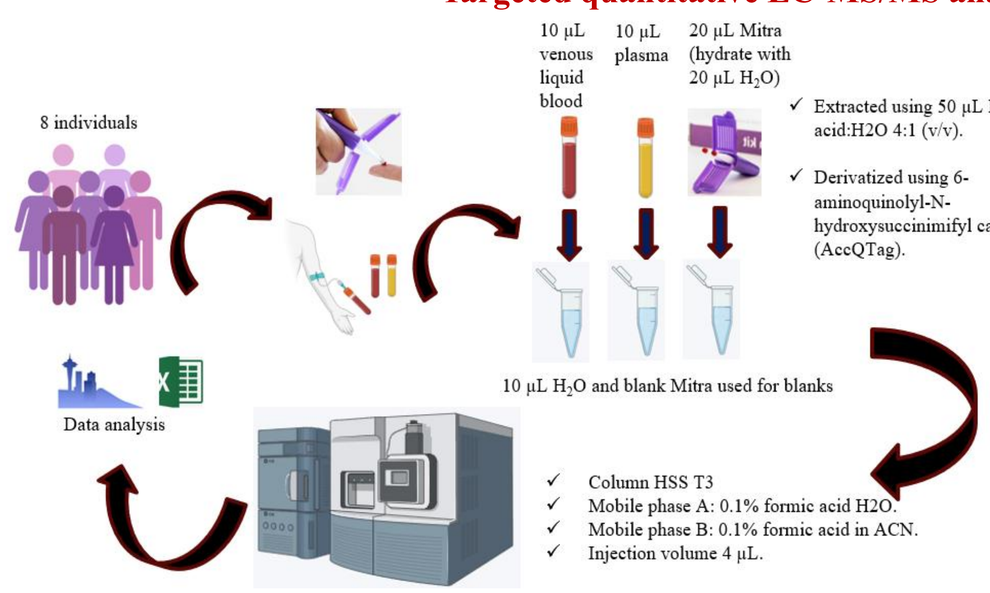
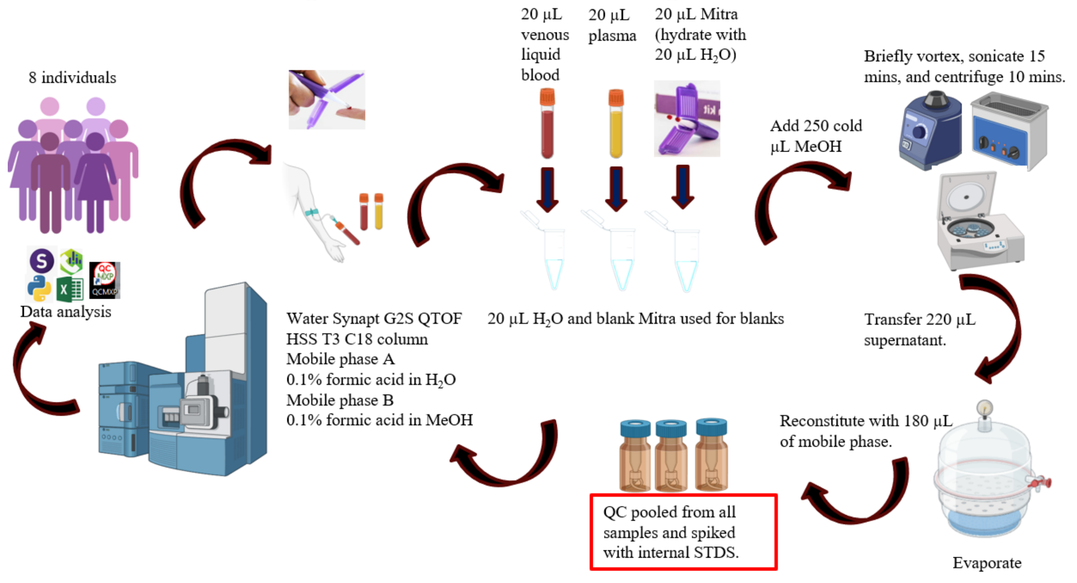
Introduction: The aim of the study was to assess the feasibility of **volumetric absorptive microsampling (VAMS)** using the **Mitra** device in biomarker analysis. Amino acids and biogenic amines were quantified in the **Mitra** blood microsampling (BμS) device, liquid venous blood (blood), and plasma using a LC-MS/MS assay and also profiled using LC-QTOF-MS.

Materials and methods:

Targeted quantitative LC-MS/MS analysis



Untargeted metabolomics using LC-QTOF



Results and Discussions

Targeted quantitative LC-MS/MS analysis

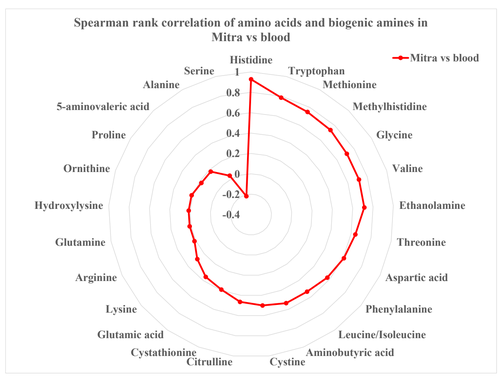


Fig 1. Spearman rank correlation of metabolites in Mitra Bμs versus blood.

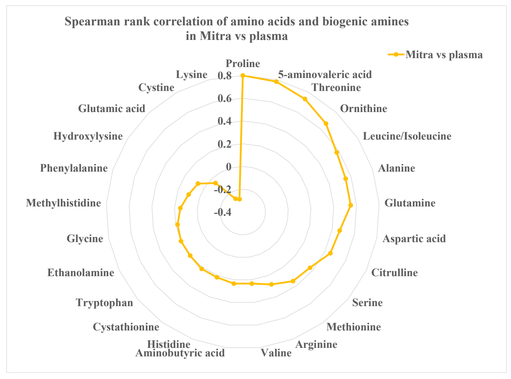


Fig 2. Spearman rank correlation of metabolites in Mitra BμS versus plasma.

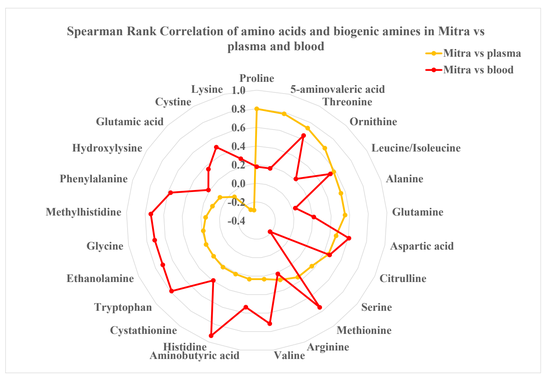


Fig 3. Spearman rank correlation of metabolites in Mitra BμS versus plasma and blood.

Table. 1 showing Mitra vs blood/plasma concentration bias (%) comparison

Metabolite	Mitra vs blood bias (%)	Mitra vs plasma bias (%)
Alanine	12.3	11.6
Arginine	NA	21.3
Aspartic acid	> 30	NA
Glutamic acid	3.7	> 30
Glutamine	18.2	7.5
Glycine	11.1	> 30
Histidine	> 30	> 30
Leucine/isoleucine	11.8	14.2
Lysine	15.9	> 30
Methionine	3.7	> 30
Phenylalanine	11.3	14
Serine	16.9	> 30
Threonine	12.5	> 30
Tryptophan	> 30	10.1
Valine	16.6	13.8
Ethanolamine	> 30	> 30
Hydroxylysine	> 30	> 30
Aminobutyric acid	6.3	> 30
Cystathionine	> 30	NA
Proline	> 30	13.1
5-aminovaleric acid	> 30	21.8
Cystine	> - 30	> - 30
Ornithine	18.5	> 30
Methylhistidine	25	> 30
Citrulline	> 30	> 30

Discussion

- ✓ Thirteen metabolites had a strong correlation (Spearman rank (r_s) > 0.6) between the three blood matrices.
- ✓ Fifteen metabolties had an accurate concentration comparison (bias < 15% mean).

Untargeted metabolomics using LC-QTOF

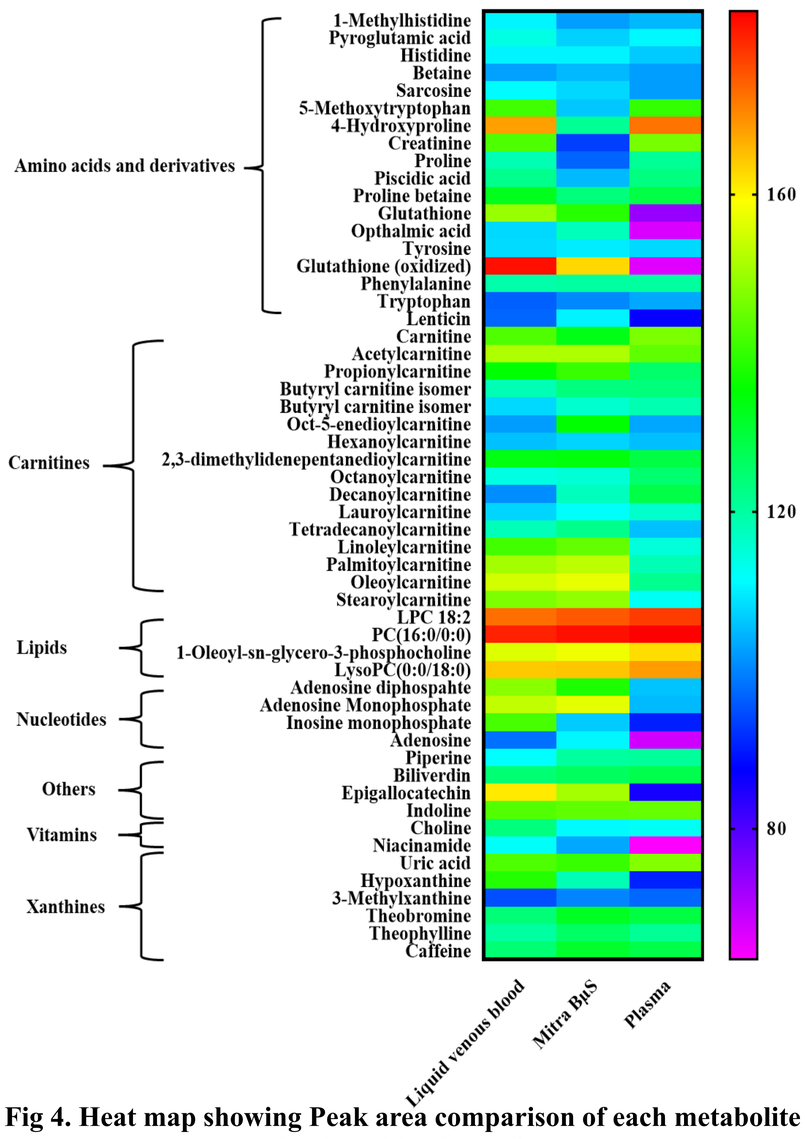


Fig 4. Heat map showing Peak area comparison of each metabolite in the three blood matrices.

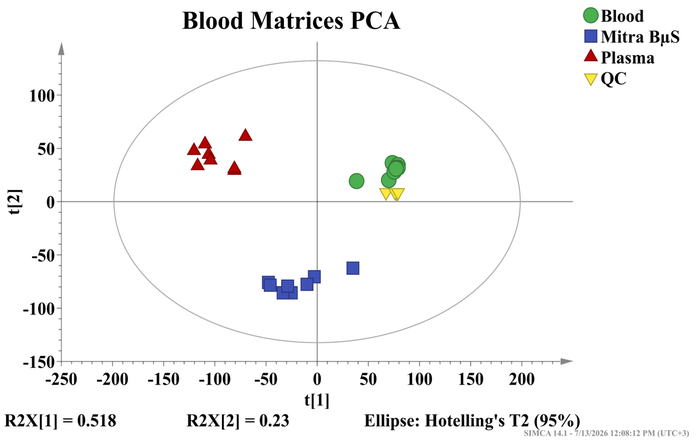


Fig 5. PCA - Separated clusters of the three blood matrices.

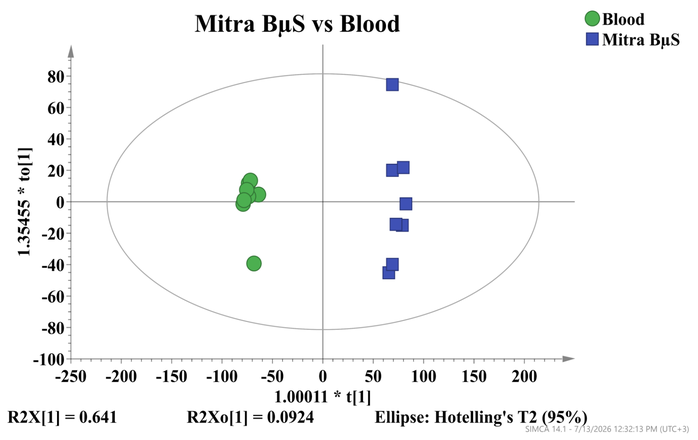


Fig 6. OPLS-DA of Mitra BμS vs liquid blood (CV ANOVA p < 0.05).

Discussion

- ✓ Mitra BμS blood profile is significantly different from blood and plasma.
- ✓ Abundance in the annotated metabolites was similar in Mitra and blood compared to plasma.
- ✓ Statistically significant discriminating features:
  - ❖ Mitra BμS vs blood: 240 (1.9% of features):
    - ➢ 168 upregulated in blood and 72 in Mitra BμS.
  - ❖ Mitra BμS vs plasma: 240 (4.3%):
    - ➢ 146 upregulated in plasma and 383 in Mitra BμS.

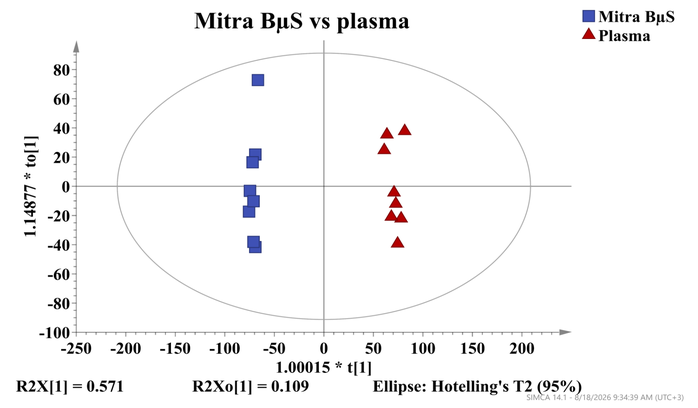


Fig 7. OPLS-DA of Mitra BμS vs plasma (CV ANOVA p < 0.05).

Conclusions: 1) **VAMS (Mitra)** has the capability of providing accurate and precise quantification of certain amino acids and biogenic amines in dried capillary blood compared to blood and plasma. 2) Concentrations of certain metabolites greatly differ between the matrices which may necessitate the establishment of their reference ranges in capillary blood. 3) Adoption of VAMS in biomarker research may be dictated by the target metabolites of a research study since specific metabolites are captured in different blood matrices.

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Conflicts of interest's disclosure: Authors declare no competing financial interests.

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