

Research question: How can a simulated-plasma model study identify the extraction and FTIR modelling strategy best suited to future quantitative plasma lipid profiling?

1 Rationale and simulated plasma model

Plasma fatty-acid composition is relevant to cardiometabolic research, while reference lipidomics requires specialist instrumentation and extended analytical workflows. This study used a controlled simulated plasma lipid model to test whether rapid ATR-FTIR can recover chemically meaningful and quantitative information after extraction. PA, OA and EA represent saturated, cis-unsaturated and trans-unsaturated chemistry.

PA C16:0
saturated

OA C18:1 cis-9
cis-unsaturated

EA C18:1 trans-9
trans-unsaturated

2 Controlled simulated plasma lipid model

1 Formulate | 30 simulated-plasma compositions: 9 single, 9 binary, 12 ternary; 100, 300 or 600 µM total

2 Prepare replicates | 3 independent preparations per composition: 90 per method

3 Compare extraction | Folch (chloroform-methanol) and MTBE (methyl tert-butyl ether-methanol)

4 Acquire spectra | ATR-FTIR, 4000–800 cm⁻¹; 6 technical spectra per sample, 1,080 in total

5 Process spectra | ALS baseline; spectral windows; derivative and SNV for multivariate models

6 Evaluate information | Univariate bands, then PCA, then PLS on the same samples

The simulated plasma matrix contains the full PA/OA/EA composition design: single formulations establish reference signatures, binary mixtures test whether one fatty acid can be resolved beside another, and ternary mixtures create the highest spectral overlap. Three independent preparations per condition support extraction-method comparison, while repeated spectra assess technical precision. This design challenges FTIR analysis in a plasma-relevant matrix while preserving known composition for rigorous evaluation.

3 Univariate FTIR: sensitivity versus selectivity

Band areas were calibrated on 108 pure-standard spectra (50–750 µM), frozen, and applied unchanged to both extracts. The CH₂ stretch at 2920 cm⁻¹ is shared by all three (R² 0.948–0.964; OA responds 47% more strongly than PA); only 3006 cm⁻¹ (cis =C–H, OA) and 966 cm⁻¹ (trans =C–H, EA) are selective (R² 0.968, 0.957).

Apparent recovery (100 × predicted ÷ nominal) reflects extraction, measurement and calibration transfer together; it is not a mass-based extraction efficiency.

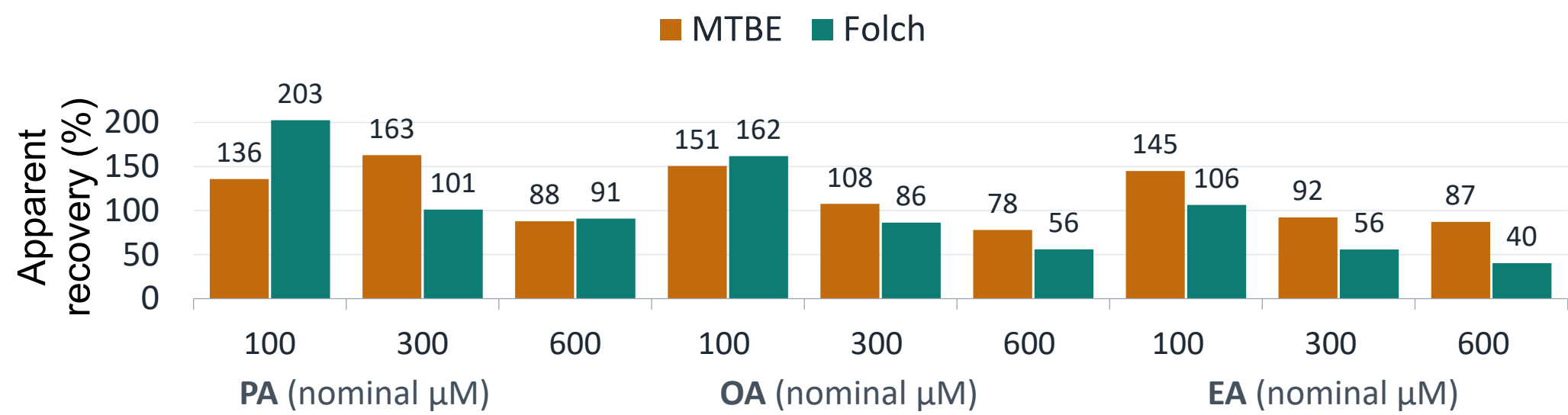


Fig 1. Mean apparent recovery of single-fatty-acid samples (n = 3 preparations; 2920 cm⁻¹ band). Recovery fell with concentration in both extracts.

- Only EA differed significantly by extraction method: MTBE apparent recovery was 40.6 percentage points higher than Folch (Holm-adjusted p = 0.017). PA and OA showed no significant method difference.

3 continued | Selectivity across mixtures

- Summing the three shared-band equations over-counted total lipid (~300–350% in single-component samples and ~480% in ternary mixtures). Selective OA and EA bands reduced false-positive signal by about four-fold and two-fold, respectively.

KEY INSIGHT | Recovery and spectral selectivity are distinct criteria; both must inform extraction choices.

4 PCA reveals fatty-acid spectral structure

PCA summarises correlated spectral features without using analyte labels. Sample scores reveal spectral similarities and differences; loading peaks identify the wavenumbers driving them. We interpret separation together with concentration associations and plausible spectral chemistry.

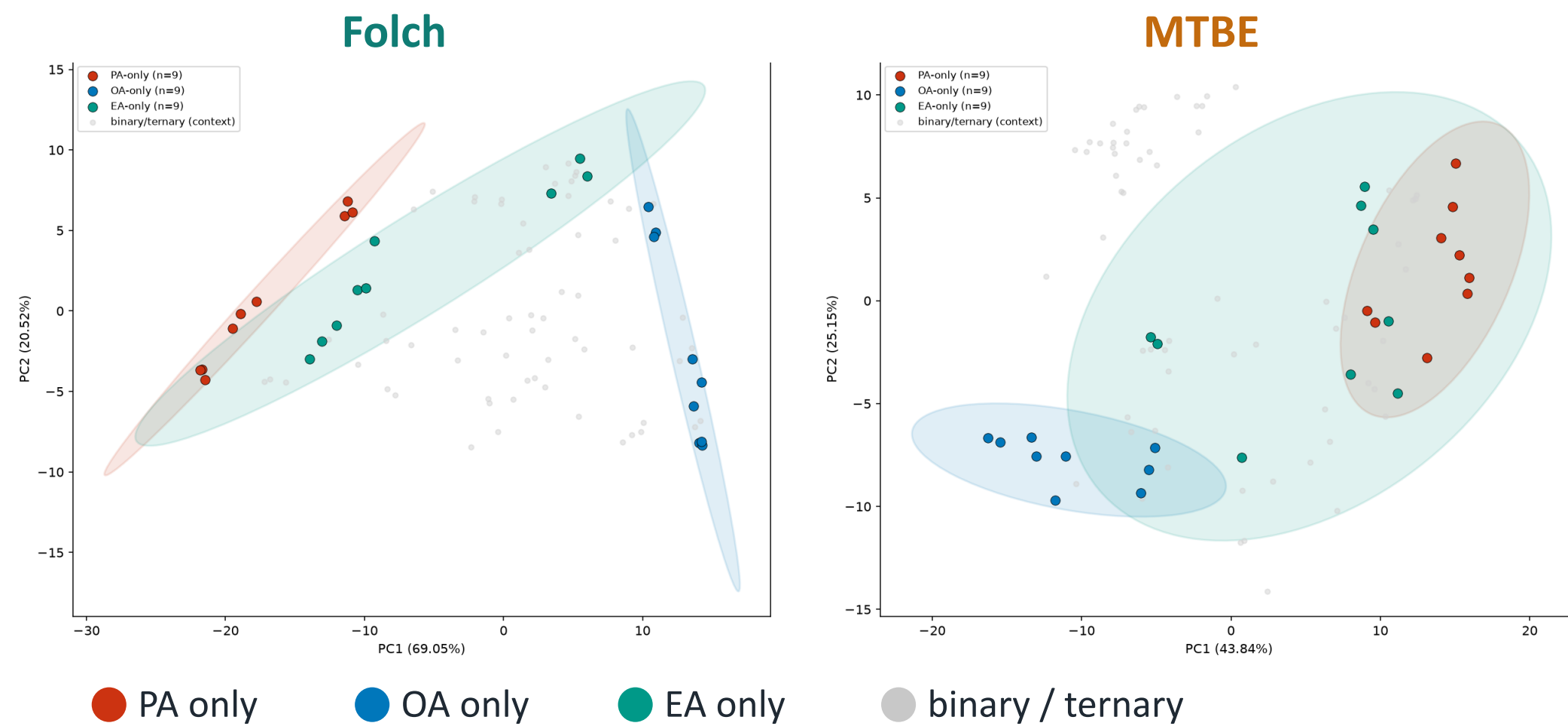


Fig 2. PC1–PC2 scores of single-fatty-acid samples (n = 9 each; 95% ellipses; mixtures grey). Axes: PC1 vs PC2 (Folch 69.1% / 20.5%, MTBE 43.8% / 25.2% of variance). PA and OA separate strongly (separation ratio 5.56 Folch, 7.29 MTBE); EA overlaps on these axes.

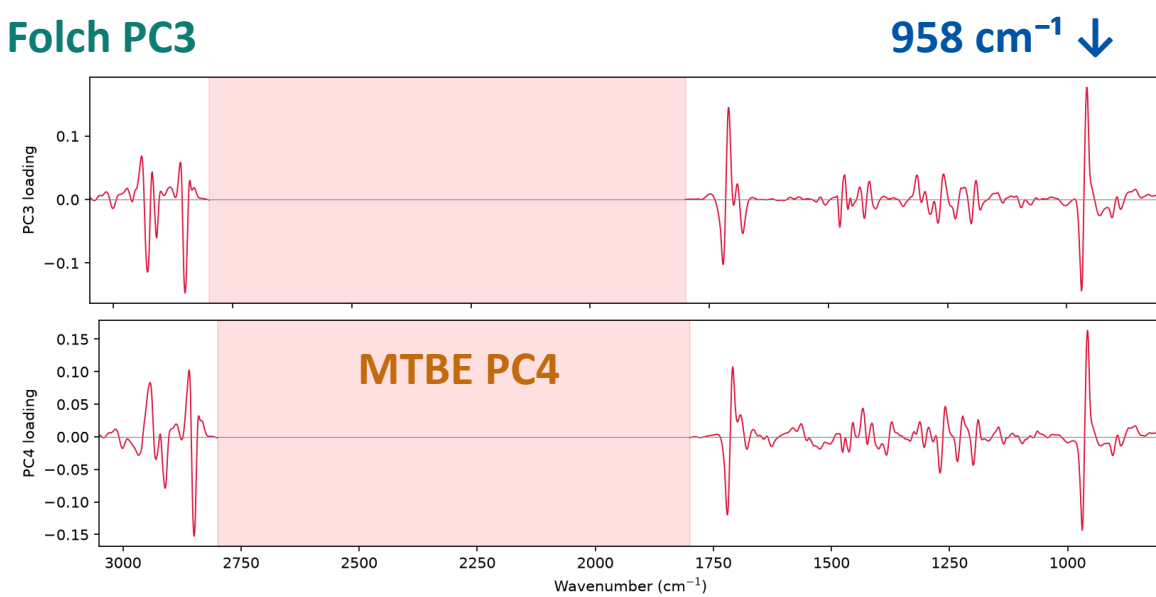


Fig 3. Loadings of the EA-associated component peak at 958 cm⁻¹ (isolated trans =C–H) in both extracts; its scores track EA concentration (|r| = 0.935). Folch packs 88.8% of variance into PC1–PC2 (4 PCs reach 95%); MTBE spreads it (68.0%; 6 PCs). Concentration lies on Folch PC2 but MTBE PC3.

Implication: both extractions retained related composition, concentration and EA-linked spectral features, but organised them differently. Folch concentrated information in fewer components, while MTBE distributed it across more dimensions.

Why the simulated plasma model matters

- Known composition: Defined fatty acid concentrations enable meaningful evaluation of prediction accuracy.
- Controlled complexity: Single, binary and ternary mixtures reveal the impact of spectral overlap.
- Matrix relevance: Albumin introduces protein-associated interference relevant to plasma analysis.
- Generalisation: Held-out formulations test prediction performance on previously unseen compositions.

5 PLS: predicting all three at once

The decisive quantitative question is whether overlapping FTIR spectra contain enough information to estimate PA, OA and EA simultaneously in simulated plasma. PLS learns shared spectral patterns that predict all three concentrations. Here, 90 independently prepared simulated-plasma sample-mean spectra per extraction method enter models built using selected spectral regions and nested cross-validation.

Validation addresses two practical questions. Held-out samples test a new preparation of a composition already represented. Formulation-held-out validation withholds every preparation of a composition, testing transfer to an unseen simulated-plasma formulation. RMSECV is the typical prediction error in µM.

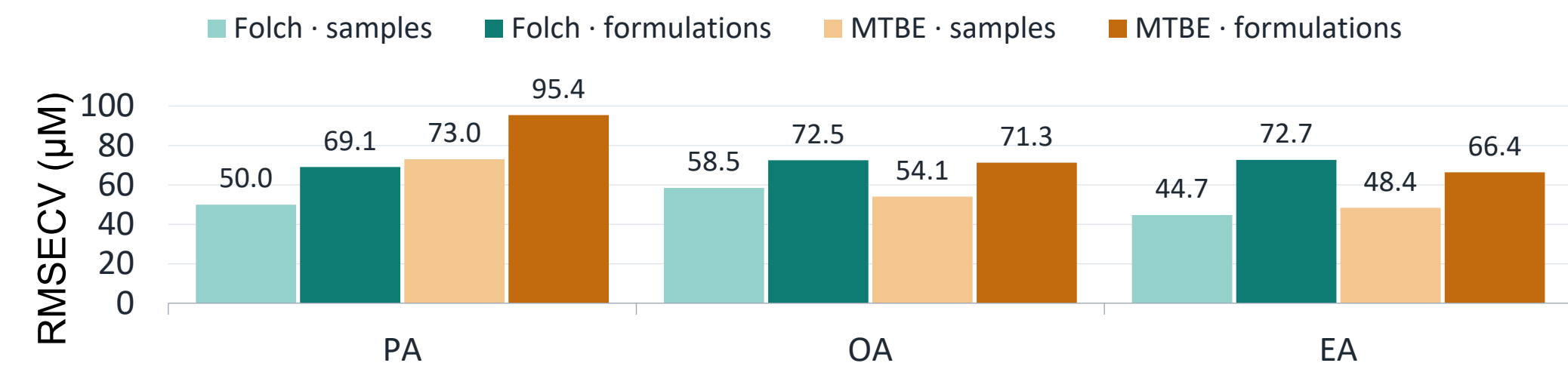
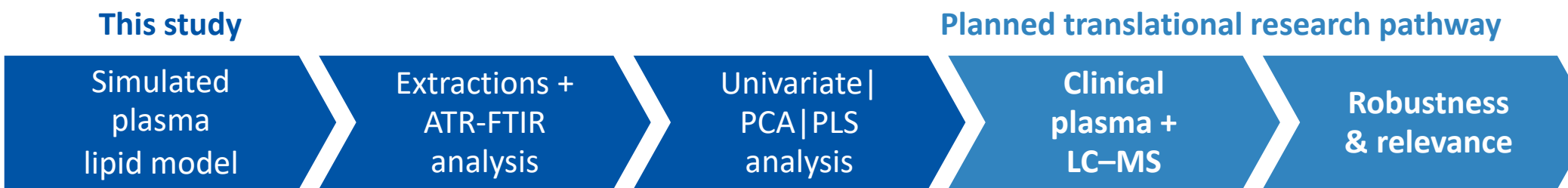


Fig 4. PLS cross-validated error (lower is better). Withholding complete formulations increased error, providing the stricter assessment of generalisation to unseen simulated-plasma compositions.

- Extraction effect: Folch predicted PA better (69.1 vs 95.4 µM), MTBE predicted EA better (66.4 vs 72.7 µM), and OA was similar. For unseen formulations every RMSECV increased.
- INTERPRETATION | Predictive performance depends on the fatty acid and the new composition being tested. This demonstrates quantitative potential in a plasma-relevant matrix and informs the next stage of translational validation.

6 From simulated plasma to clinical translation

These controlled fatty acid mixtures in albumin-containing simulated plasma enable systematic evaluation of extraction effects and FTIR-based quantification across different lipid compositions. Building on this foundation, the next stage will translate the optimised methodology to clinical plasma, addressing biological variability and matrix complexity while establishing quantitative accuracy, reproducibility and robustness through independent LC–MS lipidomics validation.



7 Synthesis and conclusion

Extraction effects by endpoint	Folch	MTBE
Recovery: EA	lower	+40.6 pp
Recovery: PA and OA	no difference	no difference
Unseen-formulation PA RMSECV	69.1 µM	95.4 µM
Unseen-formulation OA RMSECV	72.5 µM	71.3 µM
Unseen-formulation EA RMSECV	72.7 µM	66.4 µM

This study demonstrates the potential of ATR-FTIR for quantitative fatty acid profiling in albumin-containing simulated plasma. Univariate calibration revealed spectral sensitivity but limited selectivity; PCA identified composition-dependent spectral patterns; and PLS enabled simultaneous PA/OA/EA prediction, including for unseen formulations. Higher apparent recovery did not necessarily improve prediction accuracy, highlighting the importance of optimising extraction for quantitative performance. These findings provide a foundation for clinical plasma analysis, where accuracy, reproducibility and robustness will be evaluated against LC-MS lipidomics, supporting future cardiometabolic research applications.