From Lipid Extraction to Analysis: The Clinical Potential of Vibrational Spectroscopy-Based Lipidomics of Human Plasma

RESEARCH WEEK
20-24 OCTOBER 2025
INSPIRED RESEARCHERS
Northern Health

Satnam Kaur[1,2,3], Shekhar Kumta[1,2,3]

[1]RMIT University, Melbourne, Victoria Australia, [2]Melbourne University, Melbourne, Victoria Australia, [3] Northern Health, Melbourne, Victoria Australia

BACKGROUND

Lipid profiling plays a vital role in assessing cardiovascular and metabolic disorders, where changes in lipid structure and composition can serve as important disease biomarkers.[1,2] Although mass spectrometry is the current gold standard, it requires expensive instrumentation, lengthy sample preparation, and skilled operation.[3,4] This study investigates a rapid, label-free alternative using vibrational spectroscopy (FTIR, Raman, and NIR)[1,5-6] to detect clinically relevant lipid structural features, including unsaturation, esterification, and trans-fat content, directly from plasma.

Goal

Develop and validate a new diagnostic workflow that uses FTIR and Raman spectroscopy for rapid lipid analysis, improving throughput and accuracy in clinical lipid profiling.

METHODOLOGY

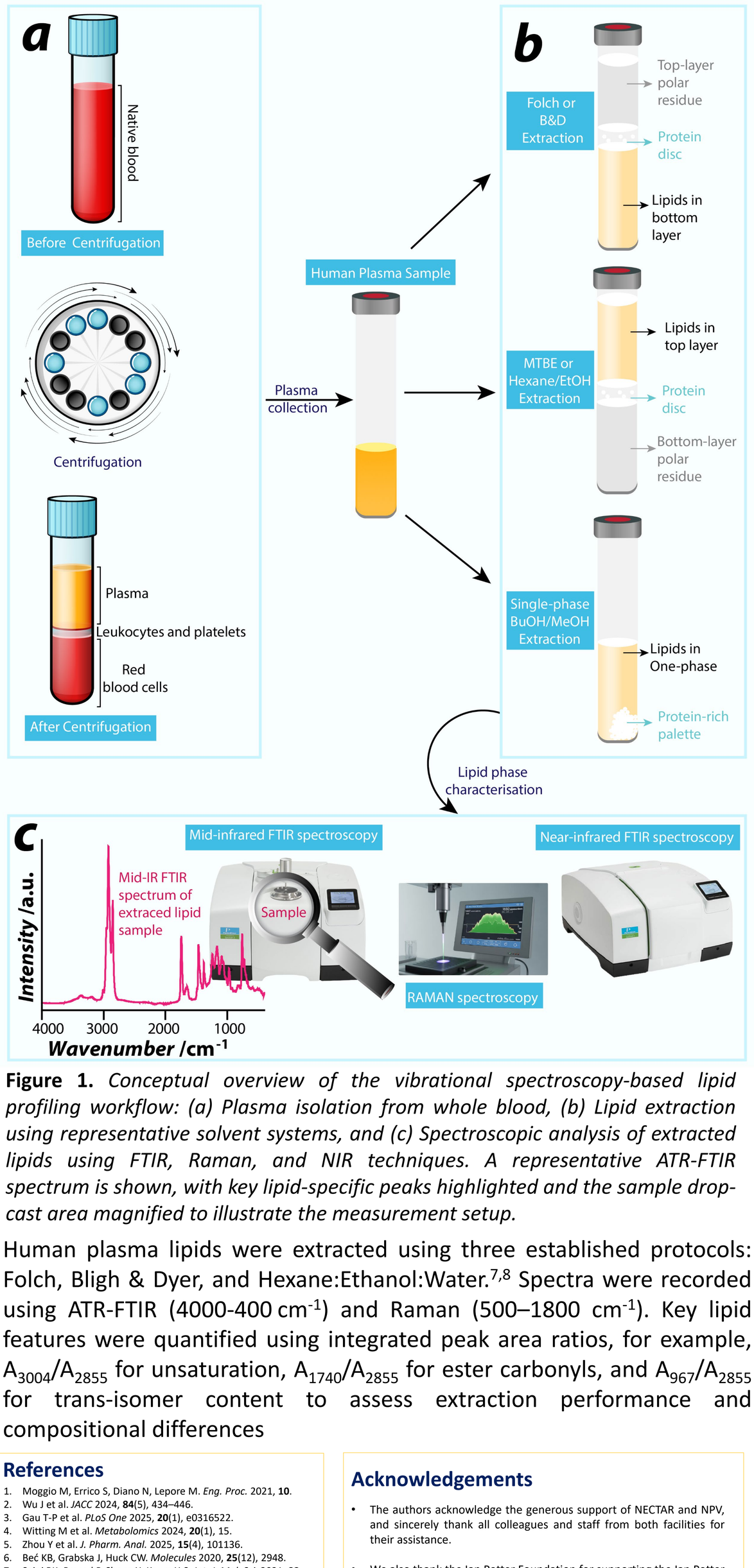

Figure 1. *Conceptual overview of the vibrational spectroscopy-based lipid profiling workflow: (a) Plasma isolation from whole blood, (b) Lipid extraction using representative solvent systems, and (c) Spectroscopic analysis of extracted lipids using FTIR, Raman, and NIR techniques. A representative ATR-FTIR spectrum is shown, with key lipid-specific peaks highlighted and the sample drop-cast area magnified to illustrate the measurement setup.*

Human plasma lipids were extracted using three established protocols: Folch, Bligh & Dyer, and Hexane:Ethanol:Water.[7,8] Spectra were recorded using ATR-FTIR (4000-400 cm^{-1}) and Raman (500–1800 cm^{-1}). Key lipid features were quantified using integrated peak area ratios, for example, A_{3004}/A_{2855} for unsaturation, A_{1740}/A_{2855} for ester carbonyls, and A_{967}/A_{2855} for trans-isomer content to assess extraction performance and compositional differences

RESULTS

Quantitative analysis of integrated peak areas in FTIR spectra revealed distinct differences in lipid composition across extraction methods. The **Hexane:Ethanol:Water method**[7] yielded the highest recovery of unsaturated and esterified lipids, reflected by an **A_{3004}/A_{2855} ratio of 0.13** and **A_{1740}/A_{2855} ratio of 0.52**, suggesting efficient extraction of long-chain unsaturated fatty acids and triglyceride-rich lipids.

In contrast, the **Folch method**[7,8] exhibited the strongest trans-fat signal (**A_{967}/A_{2855} = 0.17**), indicating higher extraction of isomerized or oxidized lipids. The **Bligh & Dyer method**[7] showed moderate values across all markers, reflecting a balanced recovery of both polar and non-polar lipid species.

These findings were further supported by **Raman spectroscopy**, which confirmed comparable spectral trends in the CH-stretching (2800–3100 cm^{-1}) and C=C stretching (~1650 cm^{-1}) regions. Raman spectra corroborated the higher unsaturation content in Hexane:Ethanol:Water extracts and the trans-isomer enrichment in Folch extracts, providing orthogonal validation of lipid structural differences

To enable accurate quantification, **Gaussian peak fitting** was applied to overlapping bands in FTIR spectra. Key vibrational regions deconvoluted include:

1) **CH_2/CH_3 stretching (2800–3050 cm^{-1})**: lipid chain order and saturation
2) **Ester C=O stretching (1700–1800 cm^{-1})**: esterified lipid content
3) **C=C trans-deformation (~960 cm^{-1})**: trans-fat isomer markers

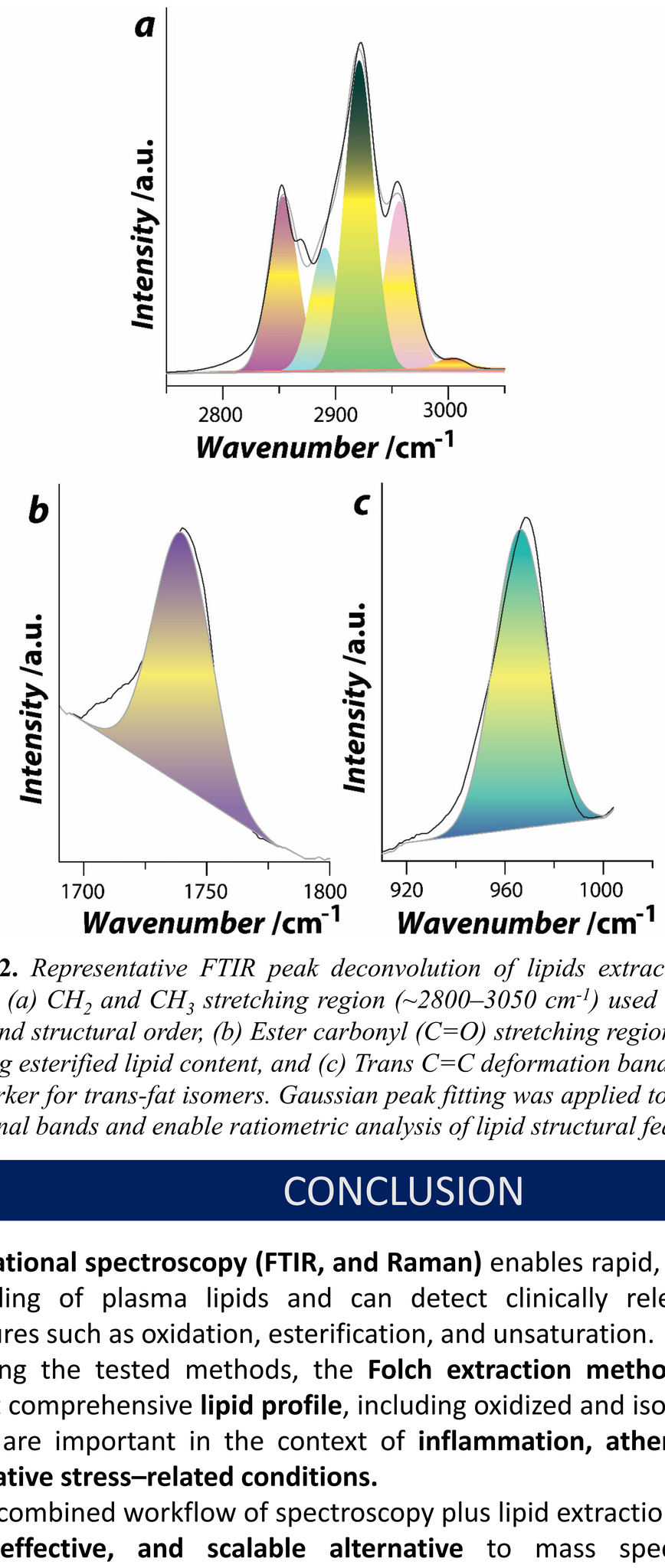

Figure 2. *Representative FTIR peak deconvolution of lipids extracted using the Folch method: (a) CH_2 and CH_3 stretching region (~2800–3050 cm^{-1}) used to assess lipid chain length and structural order, (b) Ester carbonyl (C=O) stretching region (~1700–1800 cm^{-1}) reflecting esterified lipid content, and (c) Trans C=C deformation band (~960 cm^{-1}) serving as a marker for trans-fat isomers. Gaussian peak fitting was applied to resolve overlapping vibrational bands and enable ratiometric analysis of lipid structural features.*

CONCLUSION

- **Vibrational spectroscopy (FTIR, and Raman)** enables rapid, non-destructive profiling of plasma lipids and can detect clinically relevant structural features such as oxidation, esterification, and unsaturation.
- Among the tested methods, the **Folch extraction method** provided the most comprehensive **lipid profile**, including oxidized and isomerized species that are important in the context of **inflammation, atherosclerosis, and oxidative stress–related conditions.**
- This combined workflow of spectroscopy plus lipid extraction offers a **faster, cost-effective, and scalable alternative** to mass spectrometry. This technique can support **lipid monitoring in cardiovascular risk screening and metabolic disease management**, **enabling early disease detection and clinical decision-making.**

References

1. Moggio M, Errico S, Diano N, Lepore M. *Eng. Proc.* 2021, **10**.
2. Wu J et al. *JACC* 2024, **84**(5), 434–446.
3. Gau T-P et al. *PLoS One* 2025, **20**(1), e0316522.
4. Witting M et al. *Metabolomics* 2024, **20**(1), 15.
5. Zhou Y et al. *J. Pharm. Anal.* 2025, **15**(4), 101136.
6. Beć KB, Grabska J, Huck CW. *Molecules* 2020, **25**(12), 2948.
7. Saini RK, Prasad P, Shang X, Keum Y-S. *Int. J. Mol. Sci.* 2021, **22**, 13643.
8. Folch J, Lees M, Stanley G. *J. Biol. Chem.* 1957, **226**(1), 497–509.

Acknowledgements

- The authors acknowledge the generous support of NECTAR and NPV, and sincerely thank all colleagues and staff from both facilities for their assistance.
- We also thank the Ian Potter Foundation for supporting the Ian Potter NanoBioSensing Facility at RMIT University.