A Liquid Chromatography-High Resolution Mass Spectrometry Method (LC-HRMS) for the Comprehensive Determination of Fatty Acids

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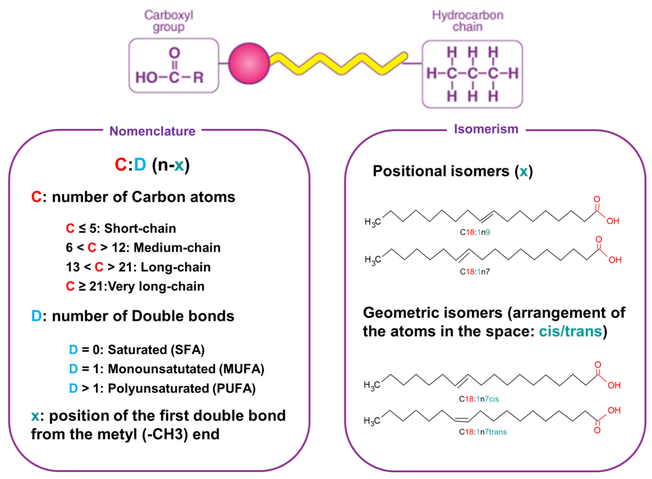
BACKGROUND

- Fatty acids (FA) are key metabolites that play a central role in cellular biology.
- Dysregulated FA metabolism has been associated with many diseases, including obesity, type 2 diabetes, non-alcoholic fatty liver disease or cancer.
- Despite LC-MS is the most widespread analytical approach for metabolomics and lipidomics, GC-MS determination after sample derivatization remains the most common platform for FA analysis.

AIM

- ✓ Development and validation of a derivatization-free liquid chromatography (LC)-High Resolution (HR)-MS-based method that covers the quantitation of 48 FA, from 12 to 24C, up to 6 unsaturations and up to 5 isomers of a given specie.
- ✓ Combination of this new LC-HRMS method with other available tools as FAMetA[1] for the identification of unknown FA in biological samples.



UPLC-HRMS

Equipment:  **UPLC-Q Exactive Plus**

UPLC CONDITIONS

Column: Cortecs C18 column (2.1mm × 150mm, 1.6µm)

Mobile phases:
A1: 2.5mM ammonium acetate in 60:40 water: methanol
B1: 2.5mM ammonium acetate in 95:5 acetonitrile: isopropanol
B2: 50:50 acetonitrile:isopropanol (clean-up between inyections)

Gradients

Separation method

Time (min)	%B
0.5	45
19	55
23	99
36	99

Equilibration method

Time (min)	%B
0.5	99
2	99
4	45
6	45

Flow rate: 300uL/min
Temperature: 45 ºC
Chromatogram elution time: 36 min + 6 min equilibration/conditionning

MS DETECTION

Source parameters
- Ionization mode: ESI-
- Spray Voltage (kV): 1.5
- Sheath gas flow rate (a.u): 60
- Capillary temperature (ºC): 300
- S-lens RF-level (a.u.): 75
- Auxiliary gas temperature (ºC): 300

Orbitrap adquisition
- Full scan
- Mass range: 100-450 m/z
- Scan type: Centroid
- Resolution: 140.000
- AGC target: 10^6
- Maximum IT: 100 ms

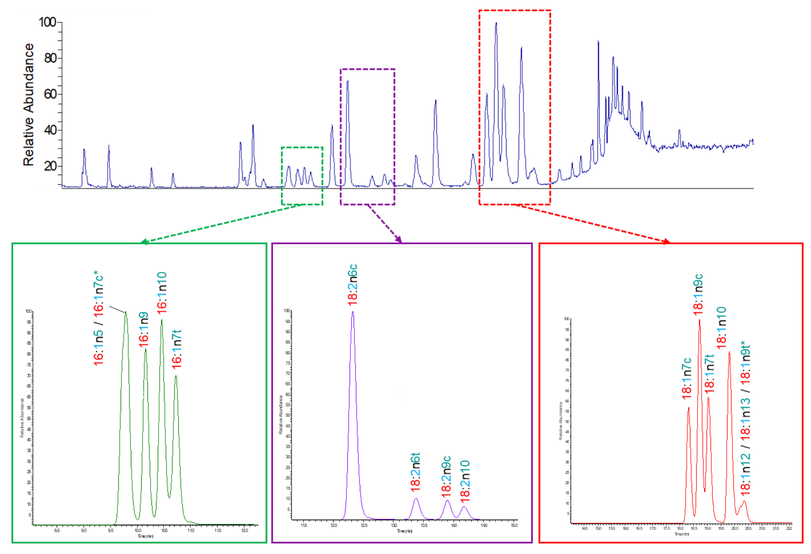
48 FATTY ACIDS

C	SFA (D=0)	MUFA + PUFA (D>1, n-x)							
		n3	n5	n6	n7	n9	n10	n12	n13
10	10:0								
12	12:0								
14	14:0		14:1n5						
15	15:0								
16	16:0		16:1n5		16:1n7c/t	16:1n9	16:1n10		
17	17:0								
18	18:0	18:3n3		18:2n6c/t 18:3n6	18:1n7c/t	18:1n9c/t 18:2n9	18:1n10 18:2n10	18:1n12	18:1n13
19	19:0								
20	20:0	20:3n3 20:4n3 20:5n3		20:2n6 20:3n6 20:4n6	20:1n7	20:1n9 20:3n9		20:1n12	
22	22:0	22:3n3 22:5n3 22:6n3		22:2n6 22:3n6 22:4n6 22:5n6		22:1n9 22:3n9			
24	24:0	24:5n3 24:6n3		24:4n6 24:5n6		24:1n9			

c=cis; t=trans

ELUTION PROFILE

TIC standard mix of 48 FA in solvent

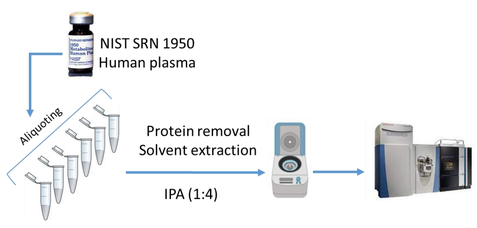


Chromatographic separation between positional (n-x) and geometric (cis/trans) isomers

*The method is not able of chromatographically separating FA 16:1n5 and 16:1n7cis, 18:1n12, 18:1n13 and 18:1n9trans, 22:3n3, 22:3n6 and 22:3n9.

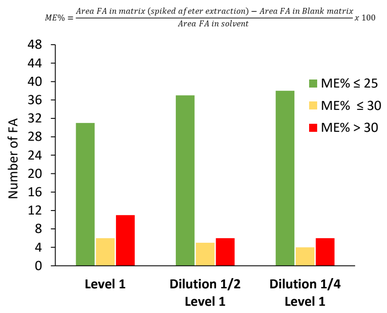
METHOD VALIDATION

SAMPLE PREPARATION FFA (Free fatty Acids)



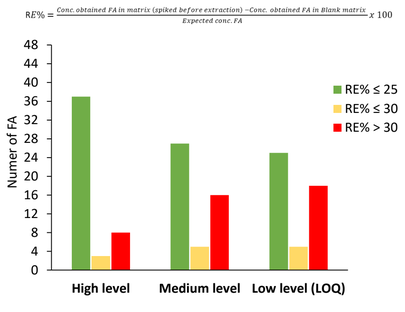
MATRIX EFFECT (ME%)

$$ME\% = \frac{Area\ FA\ in\ matrix\ (spiked\ after\ extraction) - Area\ FA\ in\ Blank\ matrix}{Area\ FA\ in\ solvent} \times 100$$



ACCURACY (Recovery, RE%)

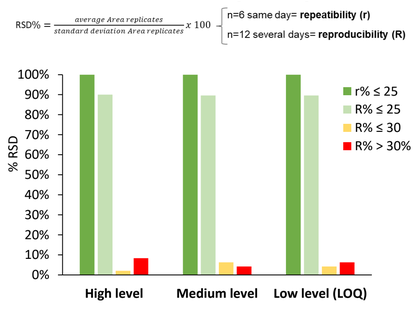
$$RE\% = \frac{Conc.\ obtained\ FA\ in\ matrix\ (spiked\ before\ extraction) - Conc.\ obtained\ FA\ in\ Blank\ matrix}{Expected\ conc.\ FA} \times 100$$



PRECISION (RSD%)

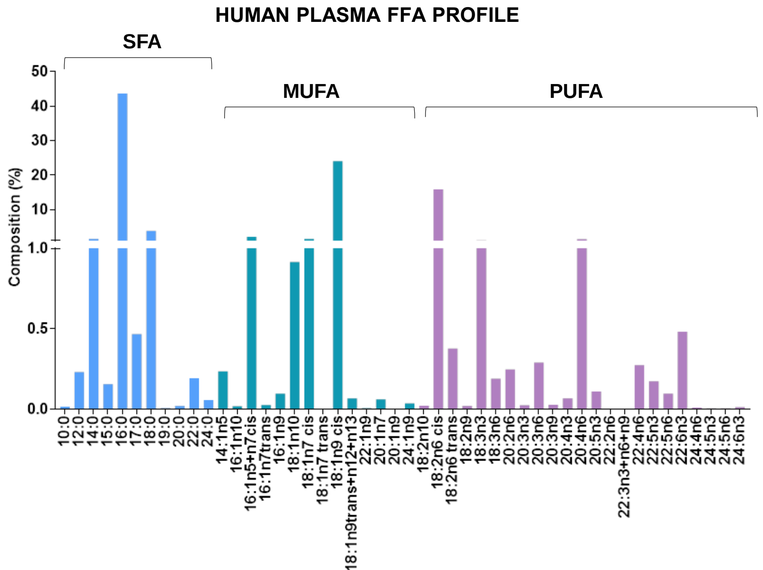
$$RSD\% = \frac{average\ Area\ replicates}{standard\ deviation\ Area\ replicates} \times 100$$

n=6 same day= **repeatibility (r)**
n=12 several days= **reproducibility (R)**



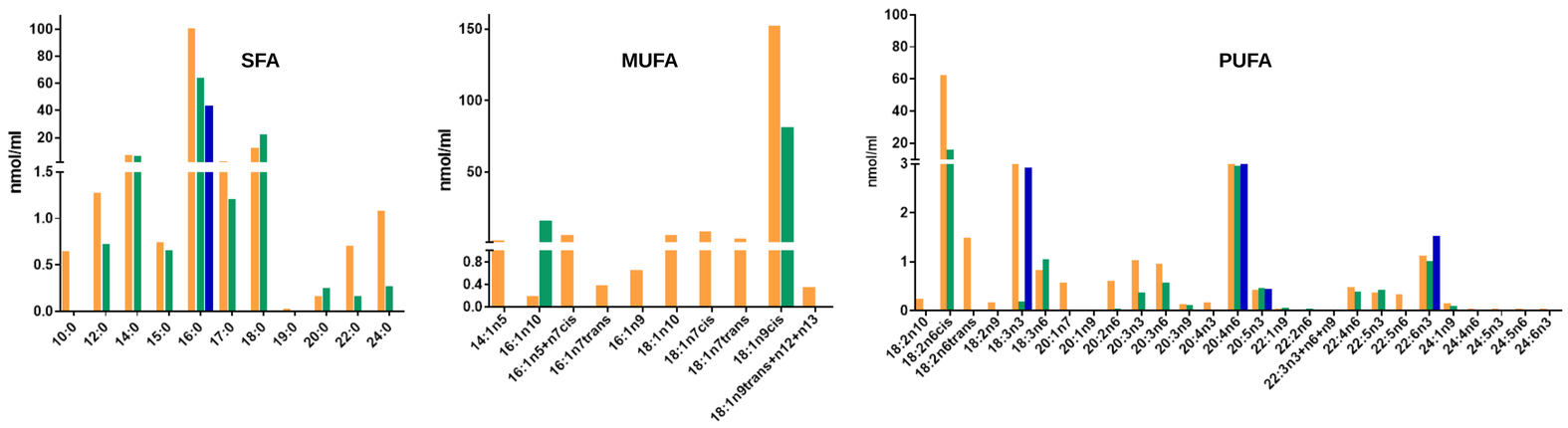
- ✓ Dilution of sample decrease ME%
- ✓ Most of FA present good or acceptable RE% in 3 levels
- ✓ Deuterated ISTD present good RE%
- ✓ Correction factors applied when RE%<30
- ✓ 100% of FA present good repeatibility in the 3 levels
- ✓ >90% of FA present good or acceptable reproducibility in 3 levels
- × Deuterated ISTD don´t present similar ME% as FA
- × Special attention in FA with ME% and R%>30

HUMAN PLASMA FFA PROFILE



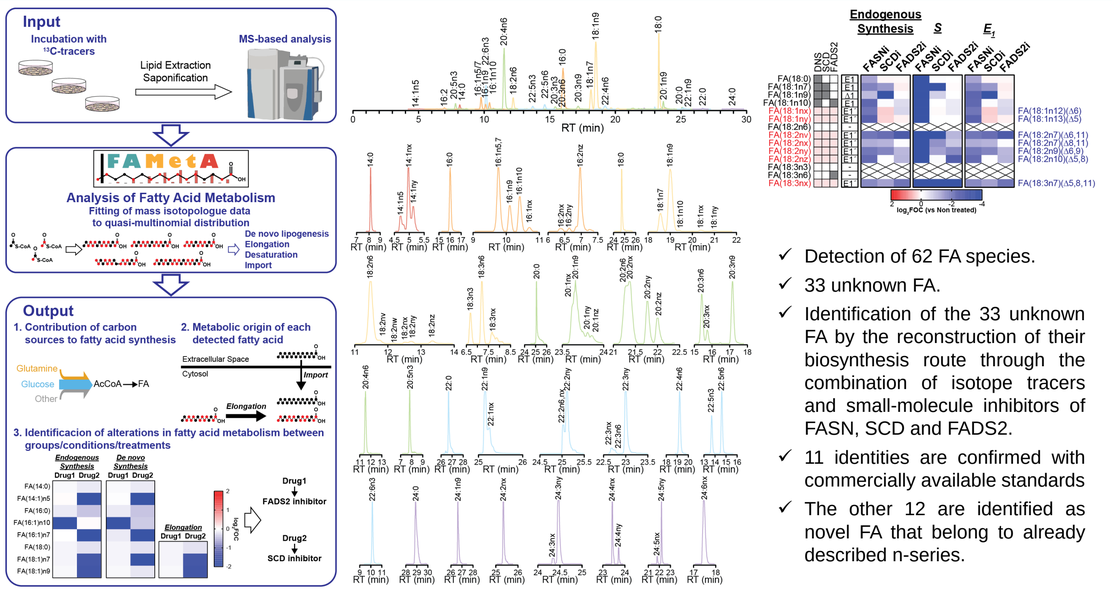
FFA LEVELS

Roca et al., 2023 (LC-HRMS) · Quehenberger et al., 2010. (GC/MS) · Bowden et al., 2017. CONSENSUS



- ✓ We have improved the number of FFA detected
- ✓ Comparable levels with other studies and methods

COMBINED USE WITH FAMetA[1]



- ✓ Detection of 62 FA species.
- ✓ 33 unknown FA.
- ✓ Identification of the 33 unknown FA by the reconstruction of their biosynthesis route through the combination of isotope tracers and small-molecule inhibitors of FASN, SCD and FADS2.
- ✓ 11 identities are confirmed with commercially available standards
- ✓ The other 12 are identified as novel FA that belong to already described n-series.

CONCLUSIONS

- ✓ A derivatization-free LC-HRMS has been developed for the quantitation of 48 FA.
- ✓ The method has been validated for the analysis of FFA in plasma NIST SRN 1950.
- ✓ Calculated FFA levels are comparable to FFA levels obtained by other authors and methodologies.
- ✓ The combination of this new LC-HRMS method with other available tools as FAMetA[1] enables the identification of unknown FA in biological samples.
- ✓ This method will be validated for determination of total FA composition, after sample saponification, in different biological samples.
- ✓ This method can be used in different research fields (pharma, foods, plants, cosmetics, etc.).

ACKNOWLEDGMENTS

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[1]María I Alcoriza-Balaguer et al. , FAMetA: a mass isotopologue-based tool for the comprehensive analysis of fatty acid metabolism, ***Briefings in Bioinformatics***, Volume 24, Issue 2, March 2023. https://doi.org/10.1093/bib/bbad064