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Analysis of VLC-PUFA: a method comparison study

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Background

Recently, the n-3 very-long-chain polyunsaturated fatty acids (n-3 VLC-PUFA) have received attention due to their abundance and highly specialized role in various mammalian tissues and their potential health promoting properties. They have a chain length equal to or greater than twenty-four carbons and three to nine double bonds. However, due to their low levels in mammalian tissue samples, analytical methods with high sensitivity and specificity are needed.

We have investigated three analytical methods, GC-FID and GC-EI/MS based on the fatty acid methylesters (FAME) of the VLC-PUFA and GC-NCI/MS based on the pentafluorobenzyl esters of the VLC-PUFA respectively.

Experimental

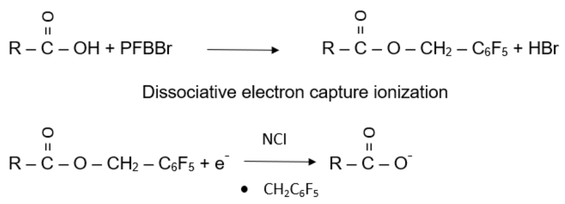
Samples and preparation

Refined anchovy fish oil (South America) converted to ethyl ester oil and enriched in VLC-PUFA after several distillation steps was used in this study.

Esterification

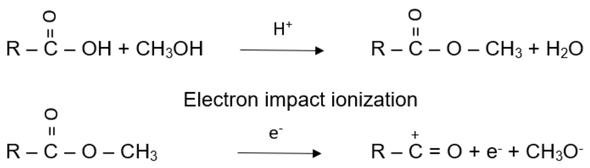
The EE oil was saponified, fatty acids were hexane extracted, followed by solvent evaporation and addition of PFBBr in acetontrile and diisopropylethylamine, kept 30 min at room temperature to make the BFB-esters of the fatty acids (**Figure 1A**). Water, HCl and hexane was added, and the hexane phase was used for GC-NCI-MS analysis.

Figure 1A



EE oil was extracted with Folch solution, dried and methylated by adding methanolic sulfuric acid and 2,2-dimethoxy propane to make the fatty acid methyl esters (FAME, **Figure 1B**).

Figure 1B



Extraction of mammalian kidney tissue

Fat was extracted from kidney tissue by using Folch solution, followed by the PFB esterification procedure earlier described.

Instrumental analysis

GC-MS

Agilent 7890A GC interfaced with an Agilent 5975C EI/CI MSD
HP5 GC column (Agilent J&W, L 30 m x 0.32 mm i.d., 0.25 µm film)
Temp. program: 90 °C (1 min) -45 °C/min – 200 °C – 2.5 °C/min - 280 °C – 10 °C/min – 340 °C (1 min)
NCI-MS: Scan mode and SIM mode of the respective VLC-PUFA M^{-1} carboxylate fragments
EI-MS: Scan mode and SIM mode of the characteristic n-3 and n-6 PUFA and VLC-PUFA EI fragments (m/z 67, 79, 91, 108, 150)

GC-FID

HP6890 GC-FID
BPX70 GC column (L 60 m, 0.25 mm i.d., 0.25 µm film)
Temp. program: 90 °C (2 min) -30 °C/min – 190 °C – 3 °C/min - 225 °C – 5 °C/min – 240 °C (30 min)

Two commercial VLC-PUFA standards, C25:3 and C27:3 (provided by Larodan, Sweden), were used to validate the instrumental performance (splitless and full scan MS) with regard to the limit of detection (LOD).

Results

Chromatograms of the pentafluorobenzyl and fatty methyl VLC-PUFA esters of the EE oil analyzed by respectively GC-NCI/MS (SIM), GC-EI/MS (SIM) and GC-FID with the identified VLC-PUFA are shown in **Figure 2**.

A chromatogram of kidney extract analyzed by GC-NCI/MS is shown in **Figure 3**.

The estimated instrumental limit of detection based on signal to noise ratio of 3 (S/N=3) for the respective methods is given in **Table 1**. Lowest LOD was found for the NCI/MS method, followed by EI/MS, 5 times higher and FID 10 times higher.

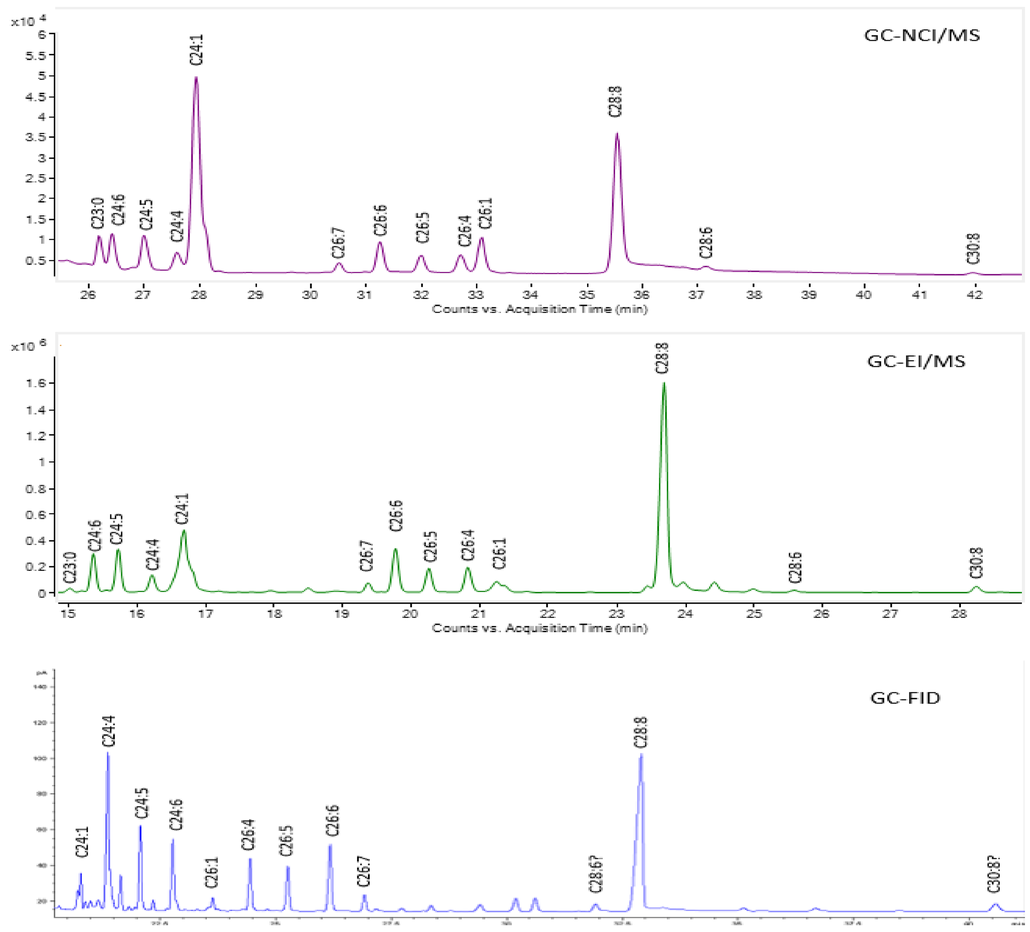


Figure 2. *Chromatograms of esterified ethyl ester oil of the three respective instrumental methods: GC-NCI/MS (SIM), GC-EI/MS (SIM) and GC-FID.*

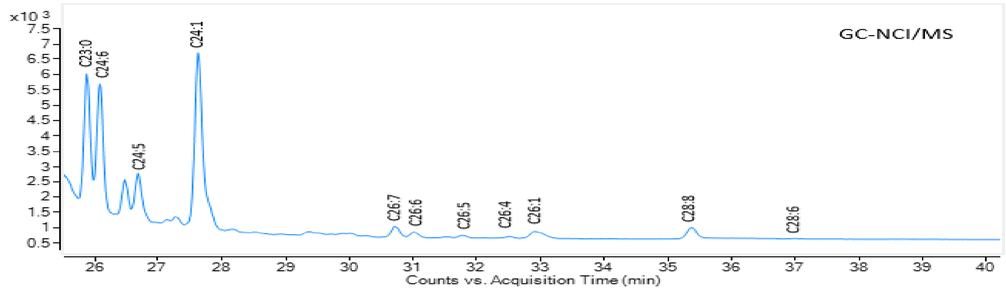


Figure 3. *GC-NCI/MS (SIM) chromatogram of kidney extract.*

Method	NCI/MS	EI/MS	FID
C25:3	0.08±0.10	0.35±0.12	0.74±0.13
C27:3	0.07±0.04	0.38±0.15	0.70±0.06

Table 1. *Estimated LOD (ng) based on S/N=3 for the three instrumental methods*

Conclusions

- The three instrumental methods seem to have similar ability to analyze VLC-PUFA
- NCI/MS has the advantage that identification of VLC-PUFA is far easier compared to the other two methods since the carboxylate (M^{-1}) fragment of the VLC-PUFA are measured
- NCI/MS has the lowest LOD, followed by EI/MS and FID
- NCI/MS has about 5 times higher sensitivity than EI/MS
- FID has the limitation that no structural information can be obtained and requires standard compounds
- It remains to validate the performance of the entire method protocol including extraction of biological tissues with regard to sensitivity and specificity

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