Cracking the Code: advancing fatty acid analyses in Antarctic benthic species by Gas Chromatography-Mass Spectrometry

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Context & Objectives

In Antarctica, benthic primary consumers rely on a **variety of food sources** (e.g., phytoplankton, sea ice algae, benthic primary producers). Fatty acids (FAs) serve as key **trophic biomarkers** for food web studies, with chromatography commonly used to analyze the diversity of FAs in these consumers. However, the complex FA profiles of species found in Antarctica can complicate the identification of individual FAs, making accurate **dietary assessments more challenging**.

How to optimize chromatographic fatty acid analysis to improve identification of sample compounds?

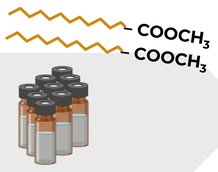
Methods

Nacella concinna

1 **Gas Chromatography** – Flame Ionization Detector (GC-FID)

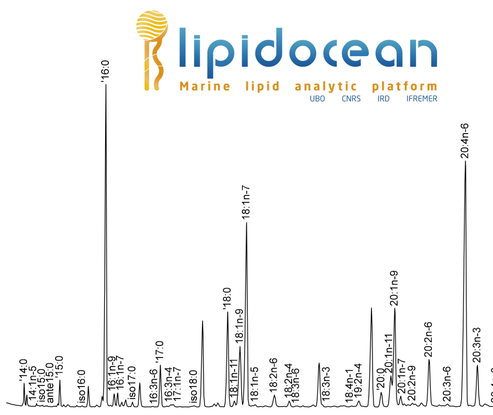
$CHCl_3$/MeOH (2:1, v:v)

Saponification & Transmethylation



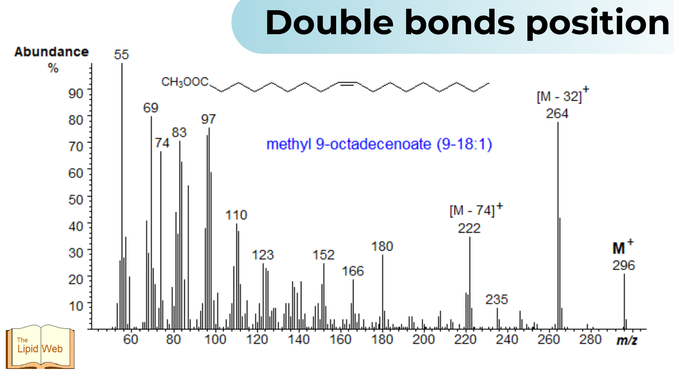
FAME (Fatty Acid Methyl Ester)

GC-FID



2 **Gas Chromatography** – Mass Spectrometry (GC-MS)

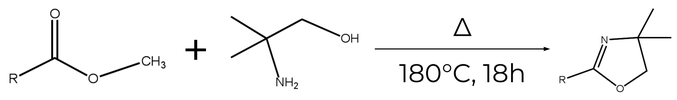
Double bonds position ?



Analysis of 4,4-dimethyloxazoline (DMOX) derivatives

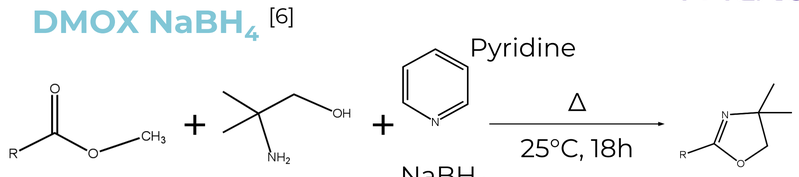
Protocol development
with Supelco 37 Component FAME Mix (S37)

DMOX 180°C [5]



FAME + **AMP** (2-amino-2-methyl-1-propanol) → (180°C, 18h) → **DMOX**

DMOX $NaBH_4$ [6]



FAME + **AMP** (2-amino-2-methyl-1-propanol) + $NaBH_4$ + Pyridine → (25°C, 18h) → **DMOX**

Results

1 **GC-FID + GC-MS of FAME**

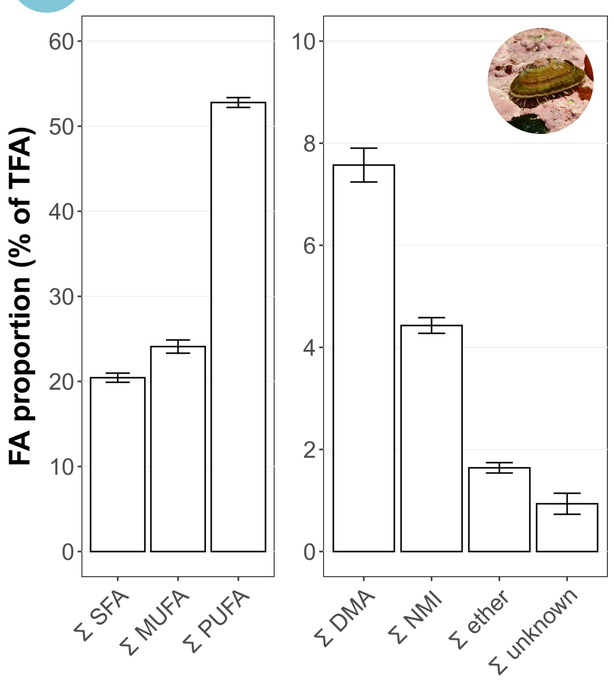
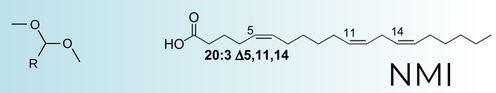


Fig. 1: Principal fatty acid class proportion of the limpet *N. concinna*, expressed by mean and standard error.

GC-MS ⇨ compound diversity identification.

DMA (dimethyl acetal)



NMI (non-methylene-interrupted)

⇨ Specific lipid membrane association[7]

⇨ Function in stress resistance[7;8]

Uncertain molecular spectra distinction according to double bond position[5;9] ⇨ unknown.

2 **Conversion efficiency**

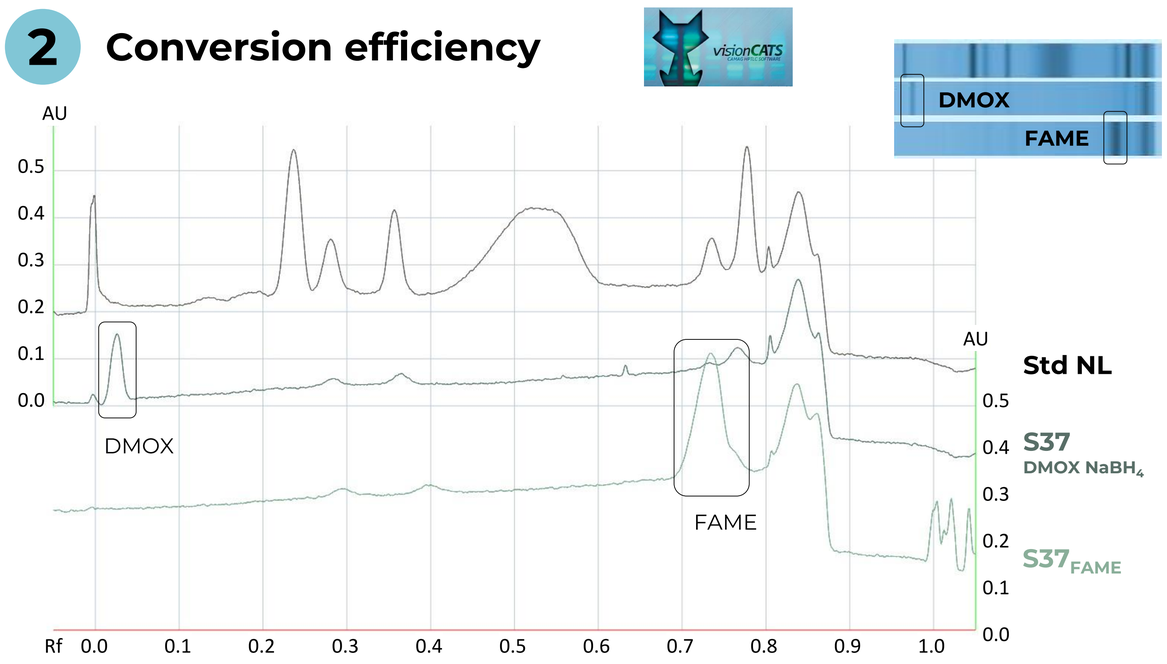
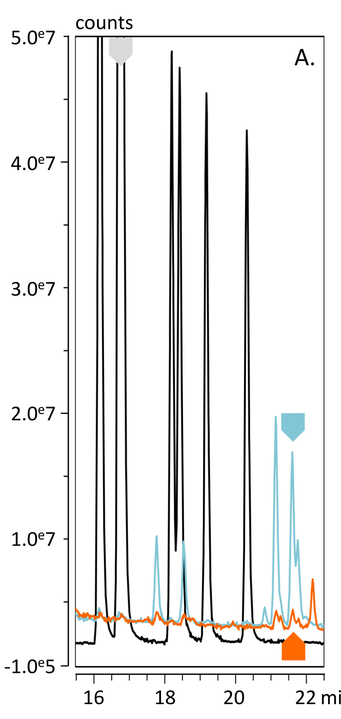


Fig. 2: High Performance Thin Layer Chromatography (HPTLC) plate by densitometric detection at 371nm – from $NaBH_4$ protocol.

Complete transformation of FAME into DMOX with $NaBH_4$.

Increase of molecular polarity due to the DMOX ring[6] ⇨ earlier migration front.

3 **FAME *vs* DMOX 180°C *vs* DMOX $NaBH_4$**



GC-MS detection:
FAME > DMOX $NaBH_4$ > DMOX 180°C

Delay between FAME and DMOX due to molecular structural differences[6].

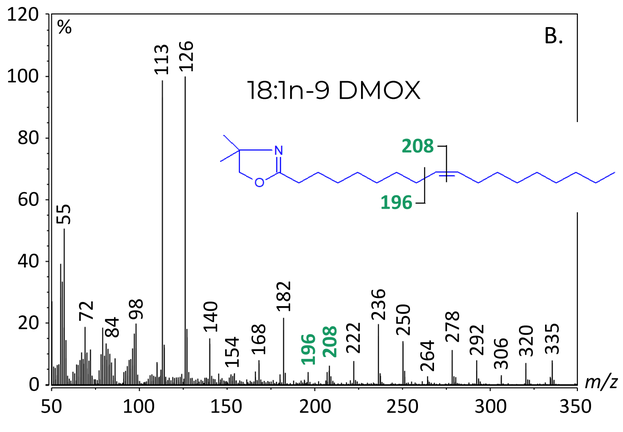


Fig. 3: **A.** GC-MS chromatogram zoom on the 18:1n-9 peak for FAME (black line) and DMOX (180°C – orange line & $NaBH_4$ – blue line). **B.** DMOX $NaBH_4$ 18:1n-9 molecular spectra.

Table 1: Fatty acid assignment probability (%) based on its molecular spectrum (libraries: mainlib and replib).

Methods	18:1n-9	18:1n-7
FAME	14.63	11.88
DMOX 180°C	32.47	3.53
DMOX $NaBH_4$	42.34	3.37

DMOX: increase in the % of molecular spectrum assignment[9].

DMOX $NaBH_4$: Distinction for SFA, MUFA and PUFA.

Conclusions

This work demonstrates the **effective conversion of derivative products** (i.e., FAME into DMOX) with the S37 standard.

$NaBH_4$ protocol avoids heating samples (i.e., 180°C) and gave **more accurate** results.

DMOX analysis **facilitates** the production of **libraries** representing the **FA diversity** within a sample.

Protocol requires further optimization (e.g., solvent quality or GC column parameters[5;6]).

References

[1] Dalsgaard et al., 2003. *Mar Biol*, 46: 225-340.
[2] Kelly and Scheibling, 2012. *Mar Ecol Prog Ser*, 446: 1-22.
[3] Duclow et al., 2013. *Oceanography*, 26, 190-203.
[4] Henley et al., 2019. *Prog Oceanogr*, 173, 208-237.
[5] Hocart et al., 2021. *In Mass Spectrometry-Based Lipidomics*, 105-121.
[6] Santalova and Svetashev, 2022. Nat Prod Commun, 17, 8pp.
[7] Mathieu-Resuge, 2020. *Conserv Physiol*, 8: 14pp.
[8] Munro and Blier, 2012. *Aging Cell*, 11: 845-855.
[9] Christie and Han, 2010. *Lipids Analysis*, 4th, 446pp.

Acknowledgments

Authors would like to thank the LipidOcean facility for its efficient analysis. Thanks to the funds of the TANGO project by the Belgian Science Policy Office (BELSPO) and ISblue, this project is possible through the cotutelle agreement between UBO and Liege universities.