

Elucidation of MOAH profiles associated with predictive toxicity evaluated in DMSO extracts

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INTRODUCTION

Mineral oil hydrocarbons (MOH) are a complex mixture of hydrocarbons originating from petrogenic crude oil. They can serve various purposes based on their refining degree, such as spanning from engine oil to lubricant, but also food grade or pharmaceutical paraffins. During crude oil refining, a key objective is to minimize the presence of mineral oil aromatic hydrocarbons (MOAH) in the produced fractions of interest, as MOAH are associated with toxicological risks, particularly those with three or more aromatic rings (1).

The monitor the presence of these carcinogenic compounds, MOH producers employ the EU-mandated IP346 gravimetric test, which provides an indication of carcinogenic potential through its correlation with mouse skin painting assays (2). The IP346 method consists of extracting aromatic compounds dissolved in cyclohexane using DMSO, followed by re-extraction into cyclohexane, evaporation of the solvent, and gravimetric determination of the remaining residue. Interestingly, despite its correlation with the mouse skin painting assay, the precise composition of the extracted DMSO fraction has never been fully characterized, and particularly not with the perspective of analytical approaches used to determine MOH contamination in food.

In this work, a scaled down approach of the IP346 protocol is presented, resulting in an approximate 95% reduction in solvent consumption while maintaining a detailed characterization of MOH compounds within the fraction of interest. Detailed compositional insights are achieved through the combination of on-column large-volume injection (OC-LVI) and comprehensive two-dimensional gas chromatography (GC×GC-FID) whose structured chromatographic separation allows for differentiation of MOAH based on the number of aromatic rings and the degree of alkylation. In addition, this study also explores the use of UV analysis of the DMSO and hexane fractions, revealing a relationship between the concentration of the most hazardous compounds and their UV absorbance, with GC×GC-FID data providing additional support to enhance the interpretation of the UV results.

MATERIALS & METHODS

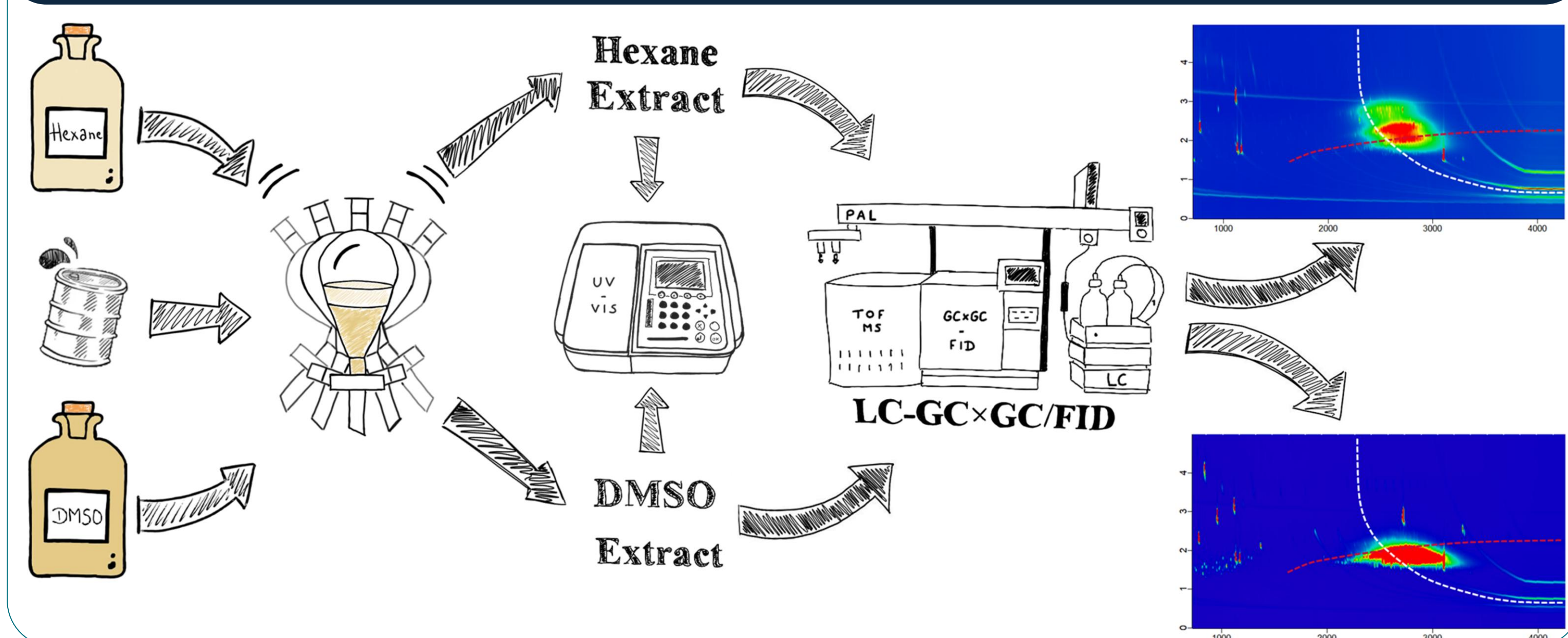


Fig 1: Schematic representation of the modified IP346 procedure applied to the samples.

The scaled-down IP346 procedure was applied to 11 samples, which differed substantially in aromatic ring distribution, degree of alkylation, and MOAH content in the oil.

The raw mineral oil samples were diluted and fractionated by LC to separate the MOSH and MOAH fractions, now called MOSH_{Tot} and MOAH_{Tot}. The two fractions were then analyzed by GC×GC-FID. In parallel, the same sample was subjected to the miniaturized IP346 method.

The first *n*-hexane fraction, MOAH_{Hex}, was fractionated by LC into MOSH and MOAH and the resulting fractions were also analyzed by GC×GC. The DMSO back-extract (MOAH_{DMSO}) was directly analyzed by GC×GC, as shown in Fig 1.

A comparison of the GC×GC profiles of MOAH_{Tot}, MOAH_{Hex} and MOAH_{DMSO} for sample MO-10 is presented in Fig 2.

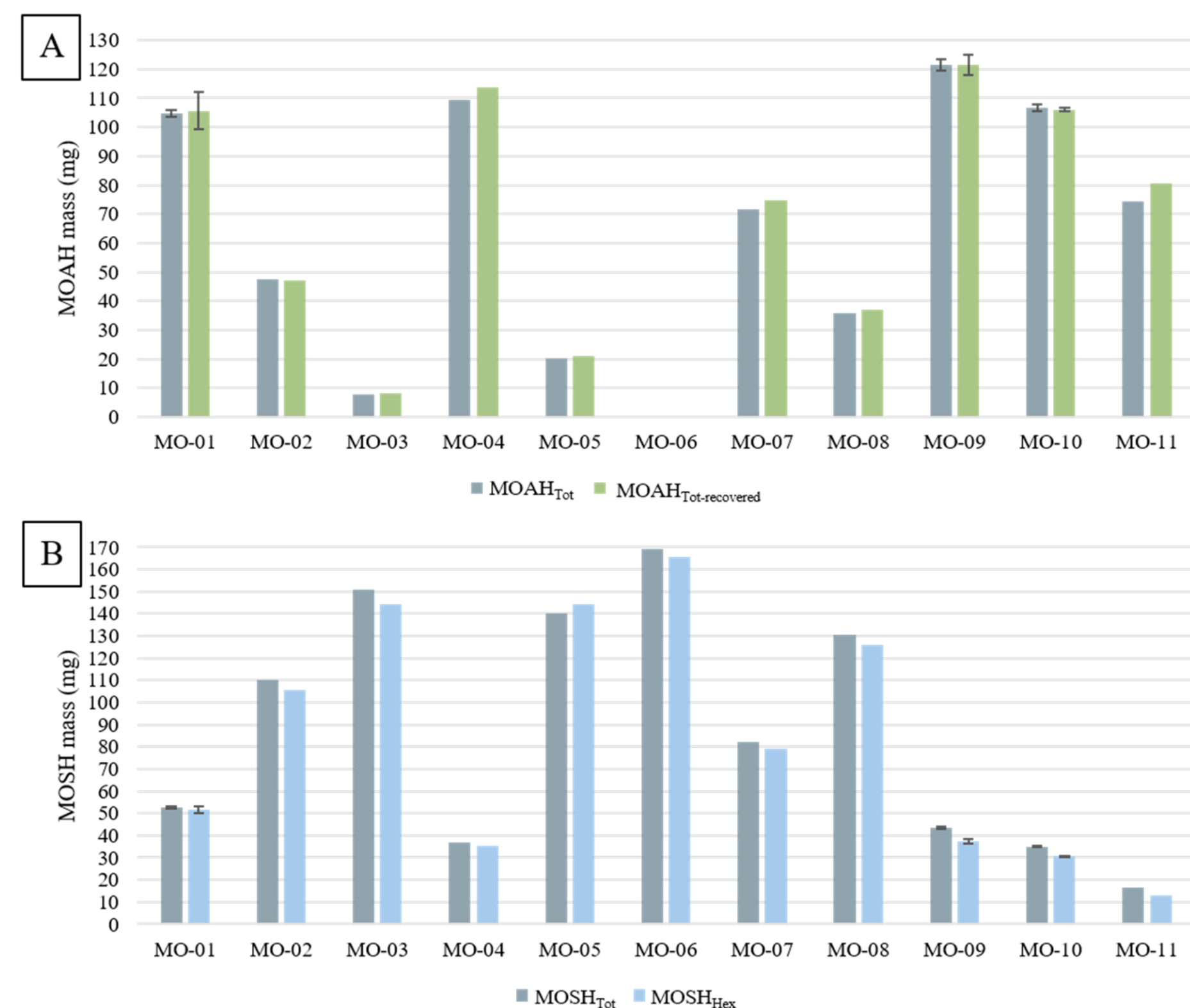


Fig 3: (A) Comparison of the mass (mg) of MOAH_{Tot} with the sum of MOAH_{Hex} + MOAH_{DMSO} (MOAH_{Tot}-recovered) of each sample. (B) Comparison of the mass (mg) of MOSH_{Tot} with MOSH_{Hex} in each sample.

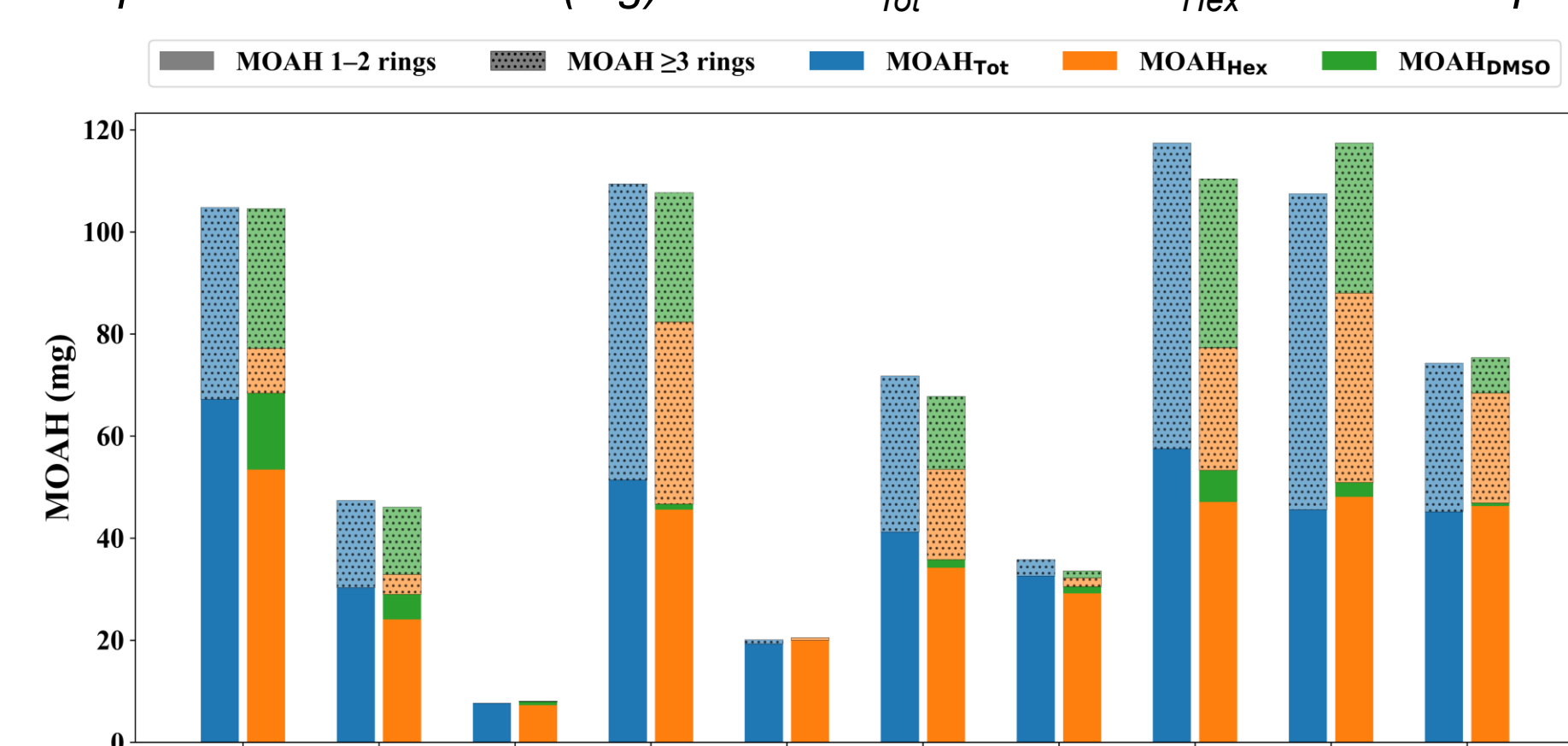


Fig 4: Comparison of the distribution of 1-2 rings and ≥3 rings (solid vs. dotted) MOAH content in MOAH_{Tot}, MOAH_{Hex} and MOAH_{DMSO}

Three samples, representing light, medium and heavy aromatic profiles with different degrees of alkylation, were analyzed in triplicate, whereas all other samples were analyzed only once.

No significant differences were observed between MOAH_{Tot} and the sum of MOAH_{Hex} and MOAH_{DMSO} for any of the samples. Similarly, no significant differences were observed between MOSH_{Tot} and MOSH_{Hex} (Fig 3).

The DMSO extractable fraction of each oil and the corresponding remaining profile in MOAH_{Hex} are shown in Fig 4, together with the distribution of 1-2 rings and ≥3 rings MOAH distribution in each fraction.

DMSO selectively extracts non- or weakly alkylated ≥3 rings MOAH compounds. However, a notable proportion of ≥3 rings MOAH remains in the hexane phase, where it is associated with more highly alkylated compounds that preferentially partition in the non-polar phase.

RESULTS & DISCUSSION

An HVGO sample spiked with several standard compounds was used to create a mask to separate zones of the chromatogram. Compounds with 1-2 aromatic rings are separated from those with 3+ rings, as indicated by a red dashed line in all figures. This mask also enables visualization of the carbon distribution along the chromatogram. Notably, the C25 threshold is marked by a white dashed line.

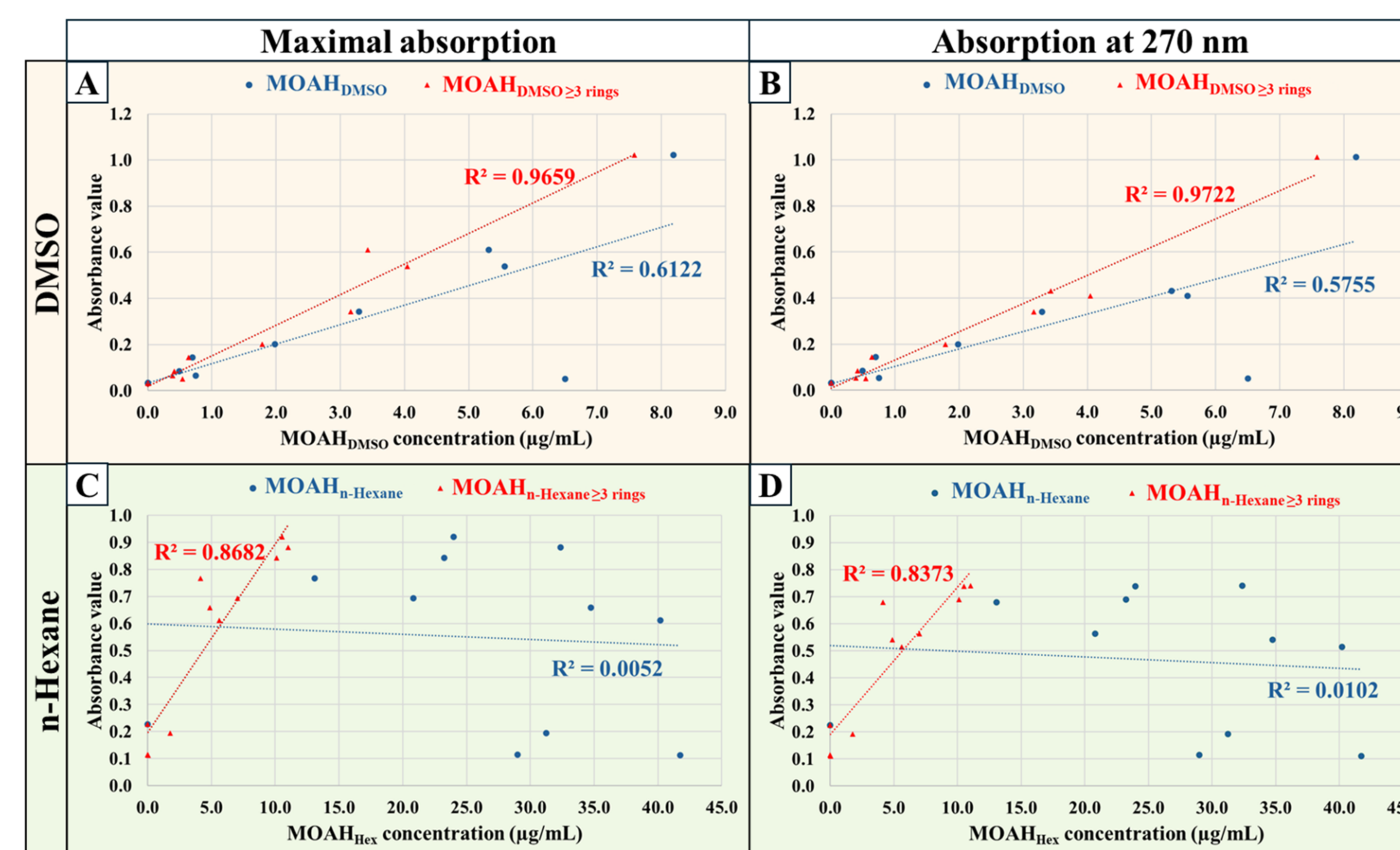
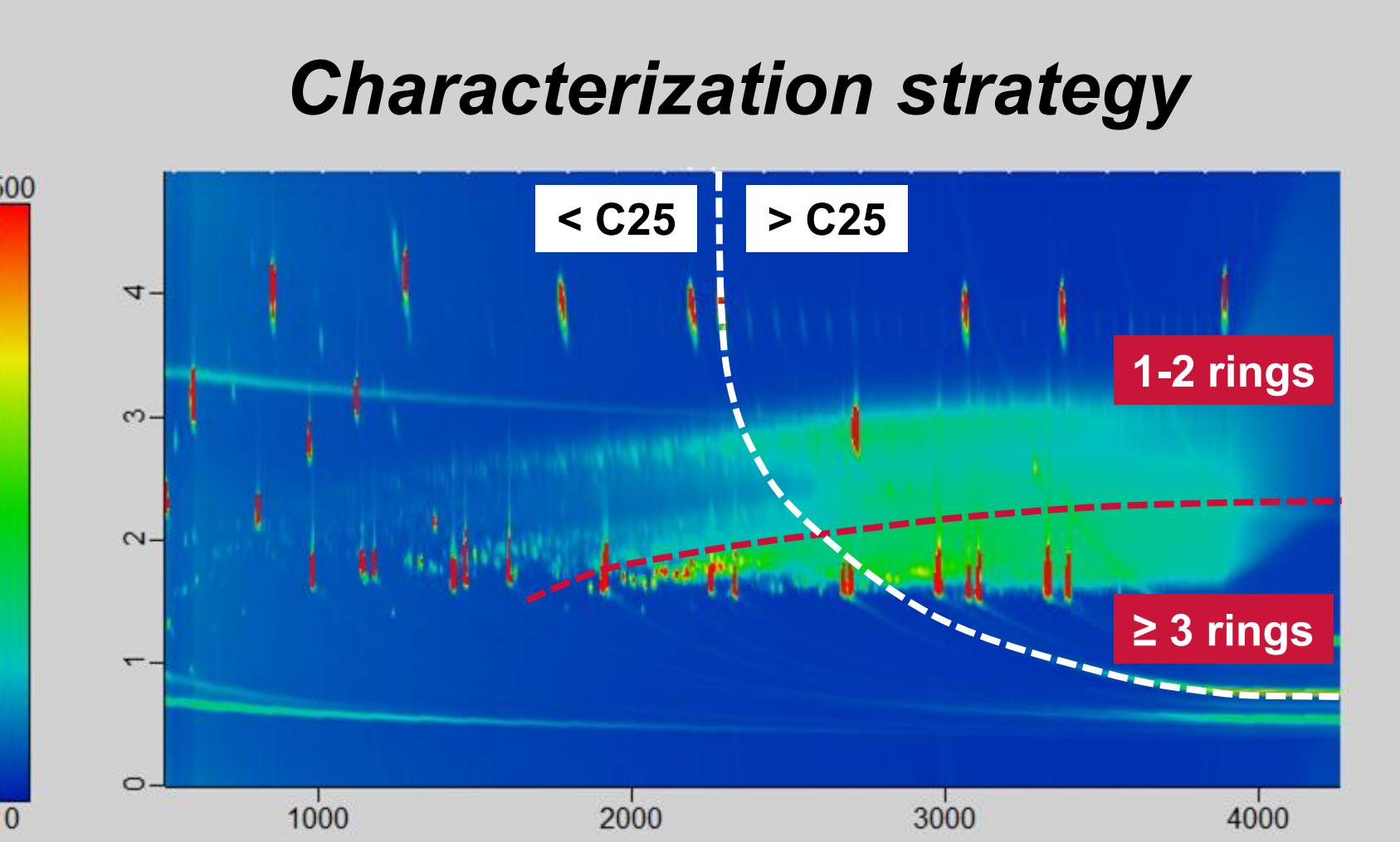


Fig 6: Correlation between UV absorbance and MOAH concentration. Blue lines represent the total MOAH while the red lines represent only the ≥3 rings MOAH.

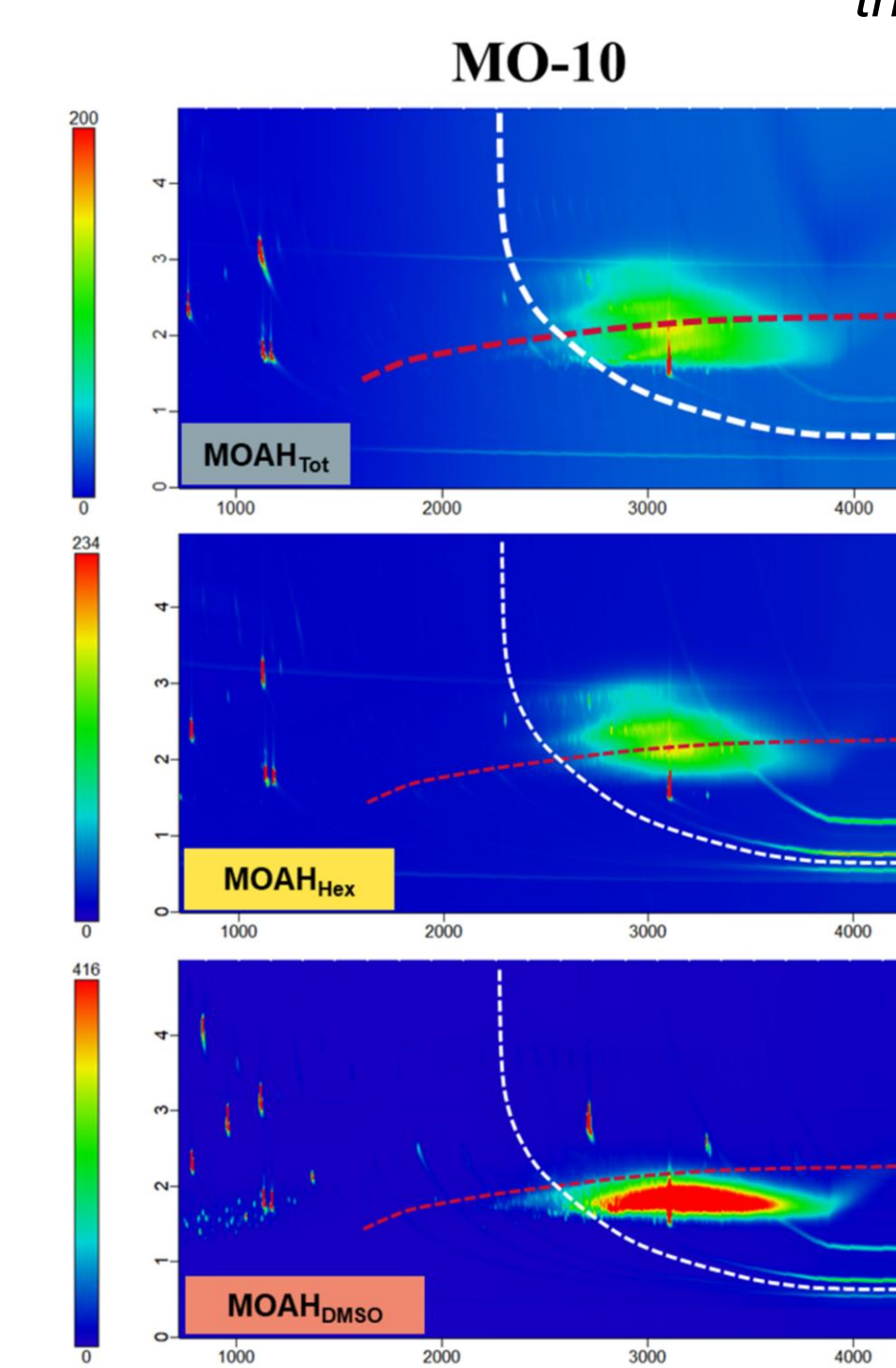


Fig 2: GC×GC-FID chromatograms of MOAH_{Tot}, MOAH_{Hex} and MOAH_{DMSO} of sample MO-10.

correlated with the outcomes of toxicological tests (e.g., mouse skin painting assay and the Ames test). Furthermore, they support the use of UV absorbance, as for the Pharmacopoeia procedure, which clearly correlates with the content of toxicological relevant MOAH (i.e., the ≥3 MOAH).

CONCLUSION

These results demonstrate that the IP346 sample preparation protocol can be successfully miniaturized and combined with GC×GC to provide detailed characterisation of the DMSO extracted fraction, which is



SCAN ME

Further information is available in the recently published paper :
Pierret et al., Food Res Int, 2026 242 119843
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