

Stationary phase screening in supercritical fluid chromatography and high-performance liquid chromatography, new insights for mineral oil hydrocarbons analysis

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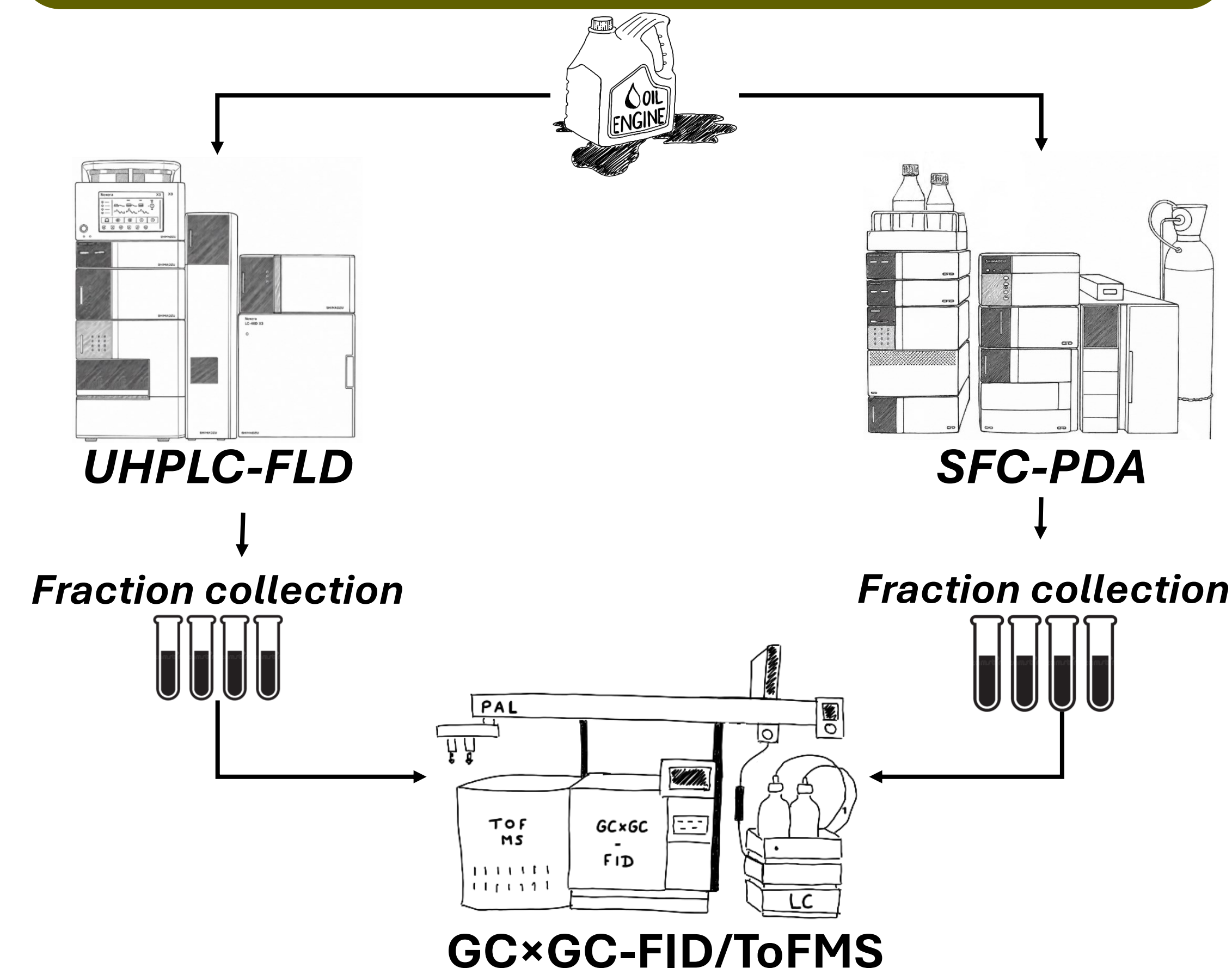
INTRODUCTION

Mineral oil hydrocarbons (MOHs) are **food contaminants**, mainly derived from petroleum-based products. They are generally classified into two groups: mineral oil saturated hydrocarbons (**MOSH**) and aromatic hydrocarbons (**MOAH**). MOAH contain one or several aromatic rings with varying alkylation degree, from weak to strong. Their analysis is usually carried out by online LC-GC-FID; unfortunately, this method does not provide a full characterization of the various subclasses of compounds present in each fraction. As a result, the use of comprehensive two-dimensional gas chromatography (GC×GC) has grown significantly as an alternative to traditional one-dimensional GC.

The silica stationary phases traditionally employed in HPLC allows for the separation of MOSH and MOAH fractions from one another, while also removing most triglycerides and other polar matrix constituents that could interfere with accurate quantification. Although commonly applied, this traditional method often encounters limitations in removing natural interferences like terpenes, carotenoids, and olefins from the MOAH fraction, making accurate quantification difficult for some samples. Gorska et al.¹ succeeded in separating carotenoids and olefins from the MOAH elution zone using the regular silica column, although terpenes could still not be effectively resolved.

In the present study, several HPLC columns were evaluated in order to explore different selectivities, while supercritical fluid chromatography (SFC) was also investigated as a potential alternative approach. GC×GC analysis was subsequently applied to fractions obtained from HPLC and SFC in order to obtain a more detailed characterization of the elution profiles. Preliminary results obtained with HPLC and SFC are presented, revealing an unusual elution profile in SFC that is generally not observed in HPLC. Further work is ongoing to complete the comparative analysis, optimize the MOSH and MOAH separation, and strengthen the removal of interferences.

MATERIALS & METHODS



RESULTS & DISCUSSION

HPLC column screening

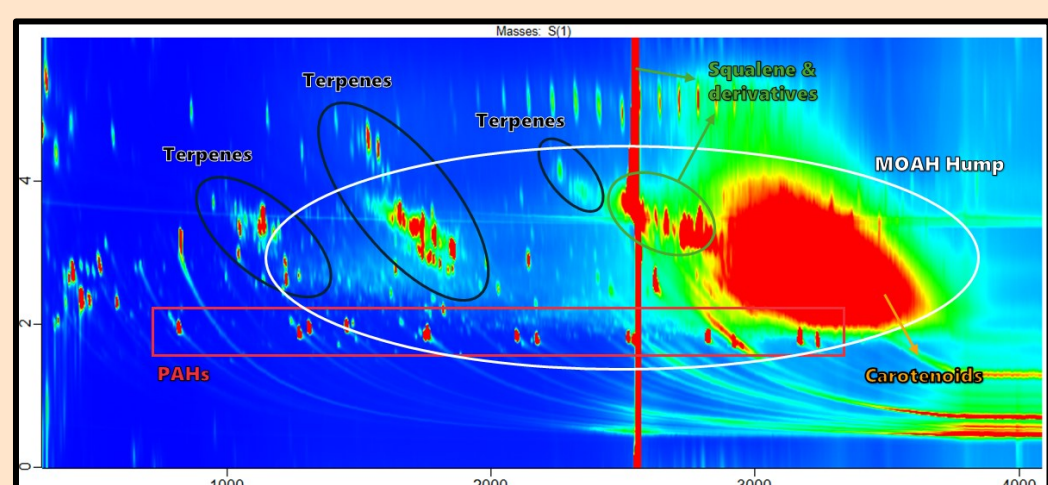


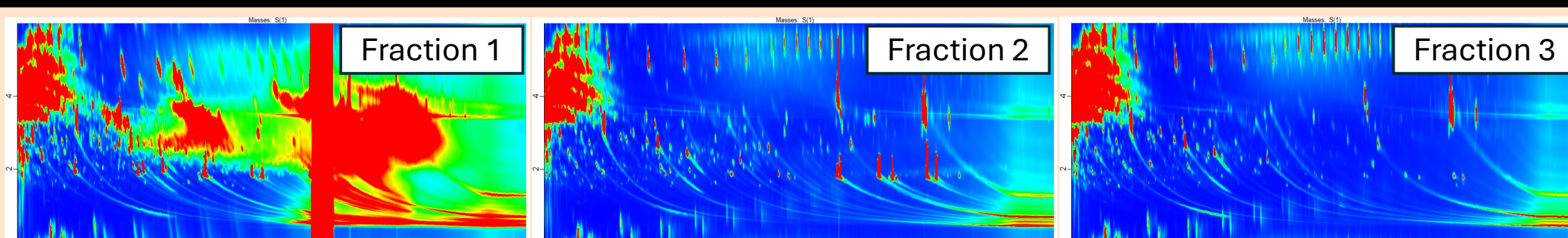
Fig 1. GC×GC chromatogram of the mixture used to assess the selectivity of HPLC columns.

Silica is the most commonly used stationary phase for MOSH and MOAH separation. However, amino² and donor-acceptor complex chromatography (DACC)³ columns have also been reported for MOAH separation based on the number of aromatic rings. A more in-depth investigation of these phases, as well as of a diol phase, was conducted to evaluate their potential for separating the MOAH fraction from naturally occurring interferences.

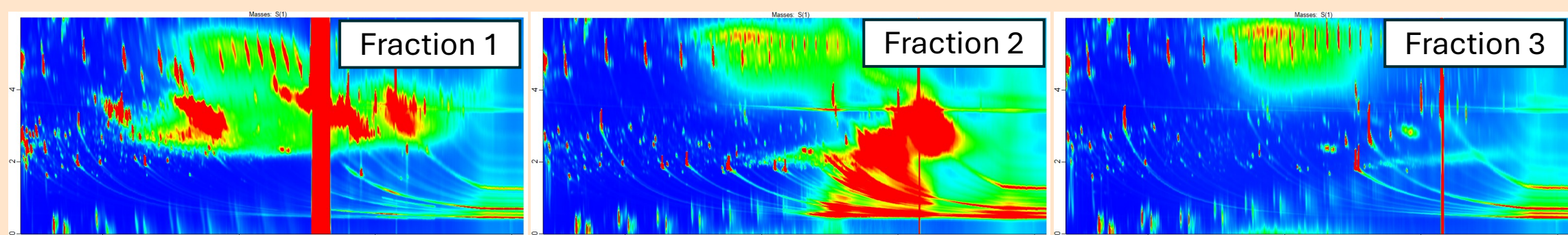
To assess the selectivity of each HPLC column, a mixture containing MOAH and the major interferences found in food (squalene, carotenoids, and terpenes) was prepared, and the corresponding chromatogram is shown in Fig. 1. The same elution gradient was applied for all tested column for screening purposes.

Diol column

Any separation of the interferences from the MOAH was achieved with the diol phase.



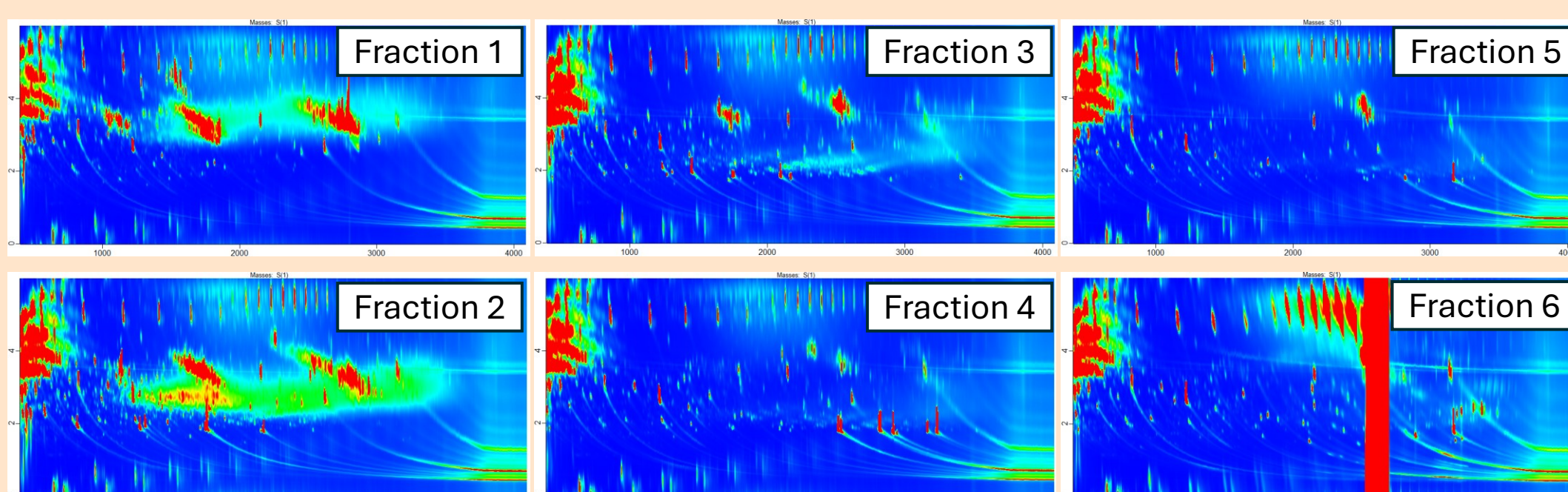
Amino column



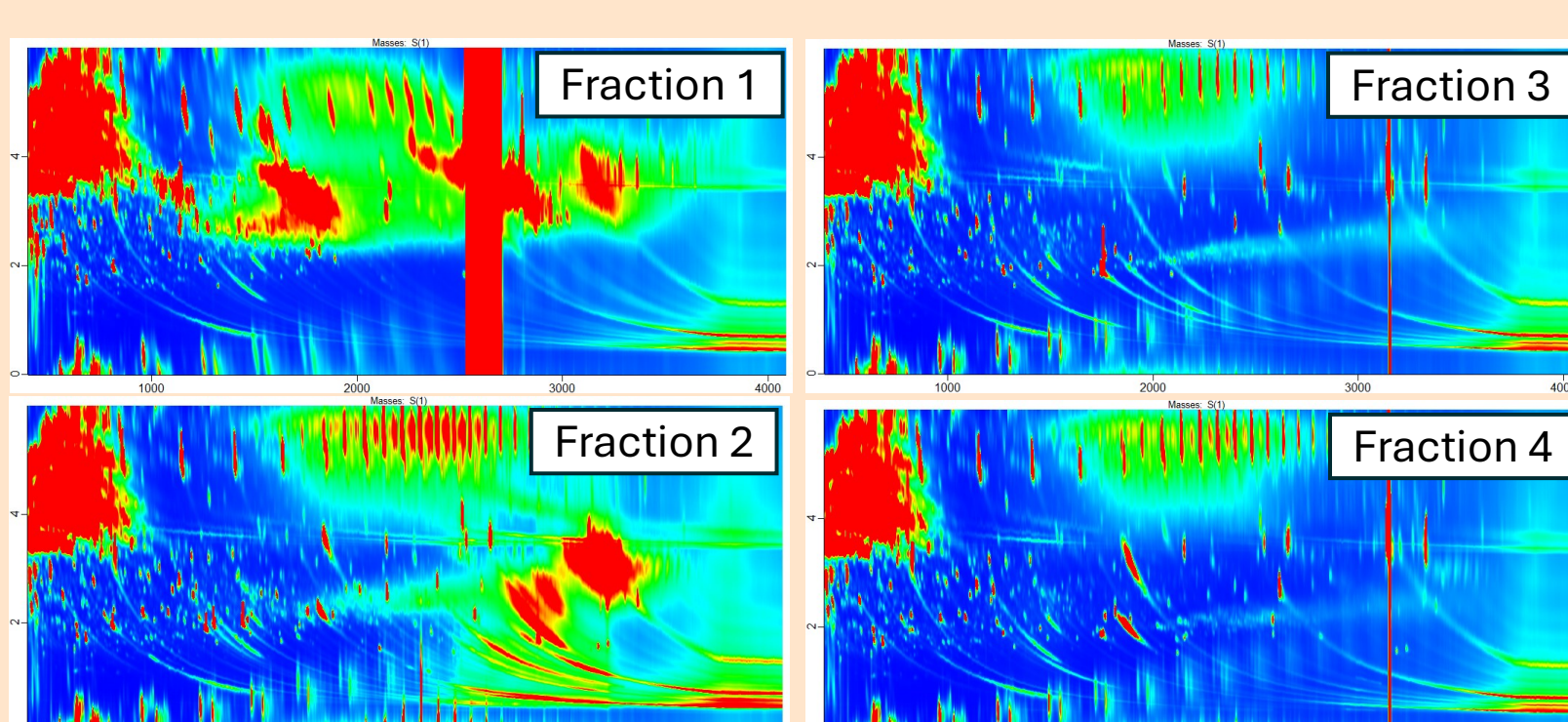
In the amino phase terpenes and squalene eluted in the first fraction along with mainly 1 and 2 ring MOAH, while carotenoids eluted with the MOAH with more than 3 rings.

Silica column

Changing the elution conditions compared with those used for MOSH/MOAH separation enabled greater retention of carotenoids and squalenes than of the MOAH fraction, while terpenes eluted in the first and second fractions together with most of the MOAH compounds.



DACC column



The DACC column provided an elution profile similar to that of the amino stationary phase. Squalene and terpenoids eluted at the very beginning of the LC chromatogram, preventing their separation from the MOAH. Carotenoids were mainly eluted in the second fraction, along with the majority of the MOAH with 3 and more rings.

SFC column screening

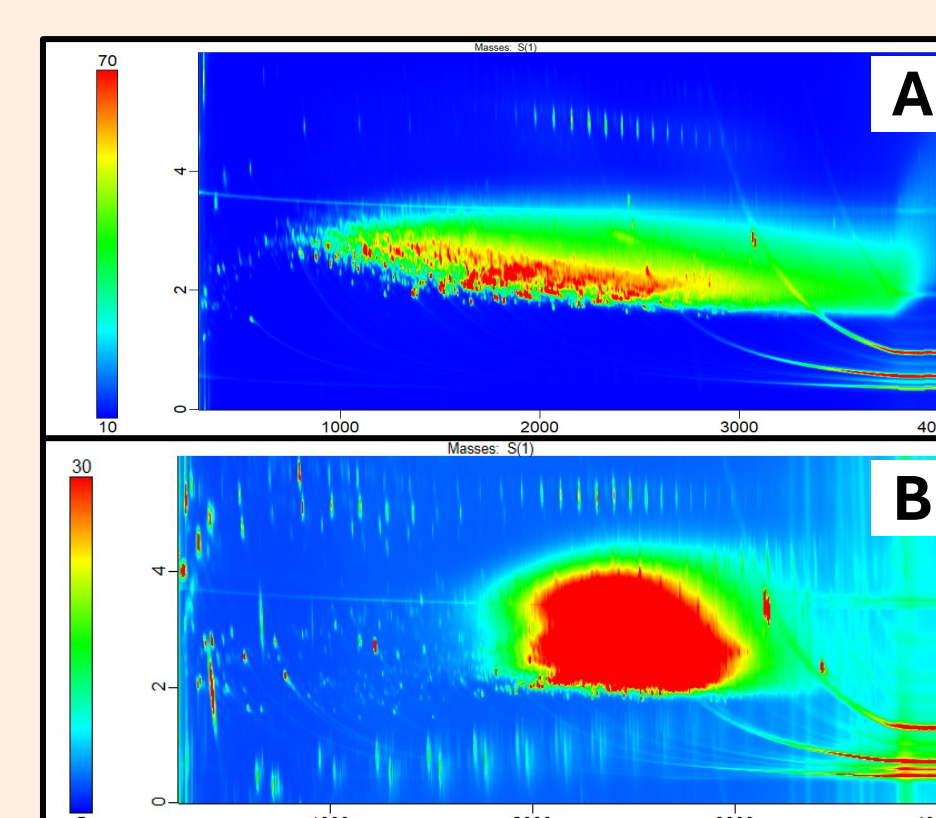


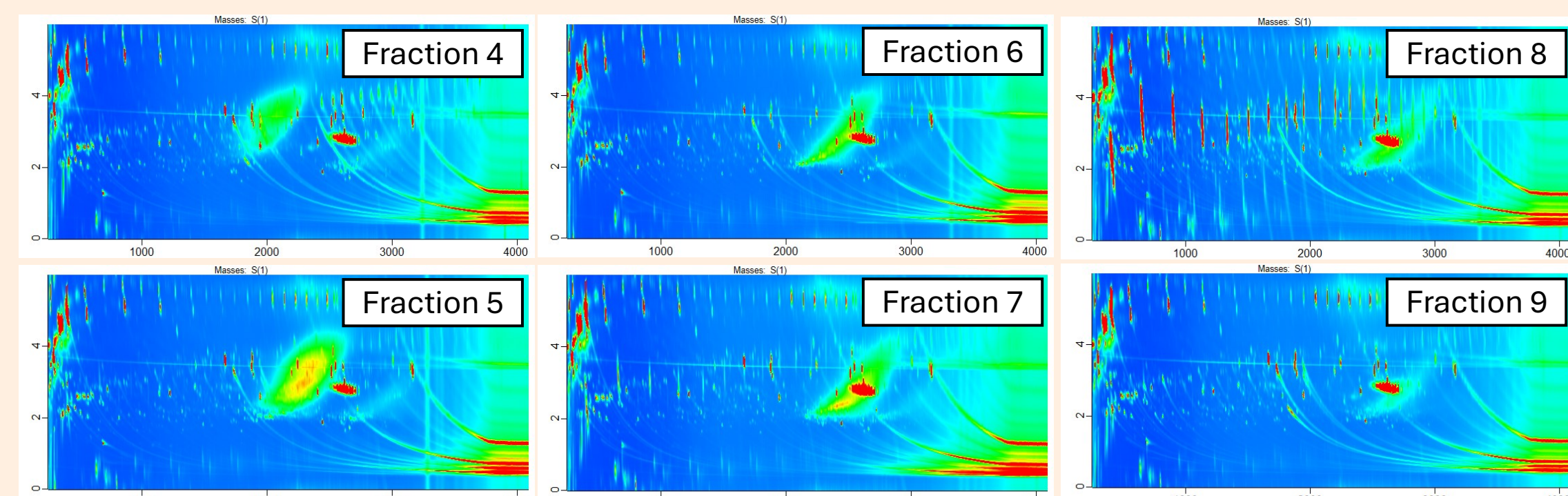
Fig 2. GC×GC chromatograms of (A) Nefti oil and (B) sternal motor oil, used to assess the selectivity of SFC columns.

In SFC, the selectivity of a stationary phase can differ significantly from that observed for the same stationary phase in HPLC. For some columns, this SFC-specific behaviour can be attributed to the mixed-mode elution mechanism provided by SFC⁴.

As an initial step toward progressively increasing the complexity of the study, pure mineral oils were first injected in SFC to evaluate the separation of MOSH and MOAH in this system. The MOAH fractions of the two MOHs used are shown in Fig. 2A and 2B.

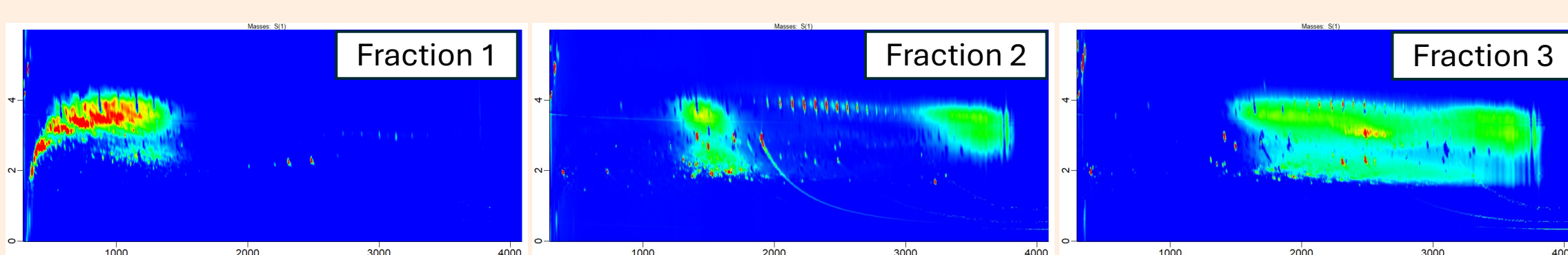
PyE column

The PyE stationary phase exhibits a very peculiar elution profile for the MOAH hump. Its enhanced π - π interactions in SFC may be of interest for improving the separation of common food interferences from the MOAH fraction.



Silica column

MOHs separation on the silica stationary phase in SFC



exhibited a markedly different separation mechanism from that observed in HPLC, which, under these conditions, did not allow separation of MOSH from MOAH.

CONCLUSION

The comparison of different stationary phases in HPLC revealed that the silica column showed the most promising selectivity to separate MOAH and naturally occurring interferences. Although carotenoids and squalene were separated, terpenes still co-elute within the MOAH elution zone.

Preliminary tests in SFC, suggested that the peculiar PyE stationary phase could be promising for separation of the interferences from the MOAH fraction. Further studies are required to complete the selectivity study and complete a proper comparison of the elution behaviour in HPLC and SFC.

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