

Abstract

Commonly referred to as 'humps', unresolved complex mixtures (UCMs) of hydrocarbons present in crude oils (obvious features of one-dimensional gas chromatograms of biodegraded oils) are probably the most complex mixtures on Earth. Specifically, the monoaromatic UCM appears to be of particular environmental relevance as it has been demonstrated to impair the feeding rate of mussels (*Mytilus edulis*). Knowledge of UCM composition is therefore crucial to an improved understanding of the environmental fate and toxicity of oil components. Comprehensive GCxGC-ToF-MS analysis of aromatic UCMs suggests that sub-fractionation of this bulk oil fraction can lead to a more focused assessment of aromatic UCM toxicity potential

1. Introduction

Previous studies^{1,2} have demonstrated that when monoaromatic hydrocarbon fractions obtained from health impaired mussels (*Mytilus edulis*, Sutton Harbour, UK), exhibited as reduced feeding rate (an important Scope for Growth, S/G, parameter in toxicological assays), and a crude oil (Gullfaks, North Sea) were exposed to healthy mussels a reduction of up to 65% in the mussel feeding rate was observed. One dimensional gas chromatograms of these monoaromatic fractions are dominated by hydrocarbon Unresolved Complex Mixtures (UCMs). GCxGC-ToF-MS analysis of aromatic fractions from a heavily biodegraded crude oil (Tia Juana Pesado, Venezuela; TJP) suggests that some prior fractionation of the whole aromatic fraction is judicious for developing a relatively rapid method for screening oils for toxic monoaromatic components³.

3. Results and Discussion

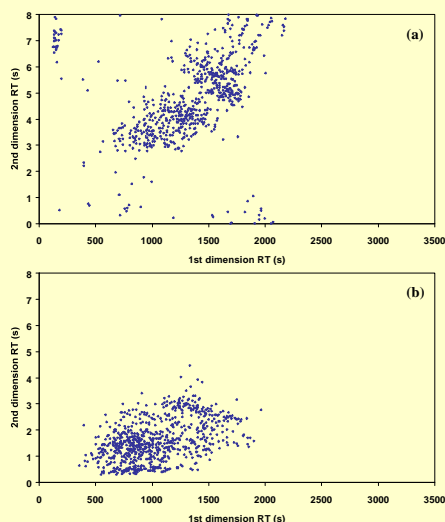


Figure 1. GCxGC-ToF-MS peak marker plots of TJP (a) aromatic fraction and (b) 4-6 DBE fraction

Methyl and dimethylnaphthalenes were identified in the GCxGC chromatogram of TJP aromatic fraction (Figure 2) based on comparison with standards, C₃-C₆ naphthalenes were tentatively identified by the position of base ions (*m/z* 141, 155, 169, 183) and molecular ions (*m/z* 170, 184, 198, 212) on the chromatogram. A base ion plot of *m/z* 183 from TJP 4-6 DBE fraction (Figure 2) indicates the presence of a series of unidentified compounds whose mass spectra (Figure 3b) are similar to those from the substituted naphthalenes (Figure 3a). In some cases distinct molecular

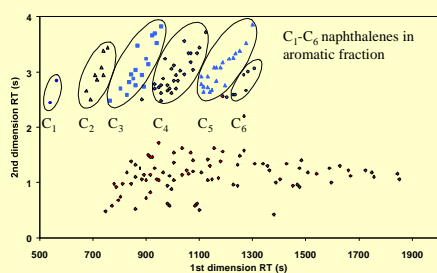


Figure 2. Overlay of base ion peak marker plots from GCxGC-ToF-MS analysis of TJP aromatic fraction (blue; *m/z* 141, 142, 155, 156, 169, 170, 183, 184, 197, 198, 212) and 4-6 DBE fractions (red; *m/z* 183)

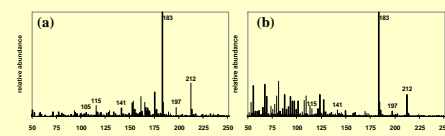


Figure 3. Mass spectrum of (a) C₃-naphthalene from TJP aromatic fraction (RT 1228, 2.58 s) and (b) unidentified compound from TJP 4-6 DBE fraction (RT 948, 1.72 s)

ions (*m/z* 212, 226, 240, 254, 282, 296 and 310) indicated loss of C₂-C₅ and C₇-C₉ alkyl chains. Analysis and processing of TJP aromatic and 4-6 DBE fractions under identical conditions (Figure 1) indicates that most of the 4-6 DBE fraction was 'overlooked' when only analysing the bulk fraction. This is probably a consequence of the relative concentration of individual components within the UCM, with preferential inclusion in the prescribed number of deconvoluted peaks to be detected biased towards those present in higher concentrations. Altering data processing parameters to include an increased number of detectable peaks and/or decreasing the signal:ratio for peak acceptance can lead to extended data processing time and poorer quality mass spectra.

GCxGC-ToF-MS analysis of aqueous soluble material from TJP 4-6 DBE fraction (Figure 4) suggests that: (1) relatively polar but minor constituents of the 4-6 DBE oil fraction (i.e. 2nd dimension RT > 3 s, above pink line) may be preferentially solubilised, and (2) that there may be a molecular weight solubility cut-off (red line in Figure 4) in biodegraded oils.

The GCxGC-ToF-MS peak marker plot of the organic extract from health impaired mussels (Figure 5) was dominated by compounds with base ions at *m/z* 105 and 119 indicative of alkyltoluenes and alkylxylenes. Series with base ions at *m/z* 91, 133 and 147 indicative of alkylbenzenes, trimethylbenzenes and tetramethylbenzenes were also present but less prevalent. Comparison of the ToF mass spectrum of one of the components (Figure 6a) with that of the electron impact mass spectrum of a synthetic *n*-decylxylene (Figure 6b) showed good agreement.

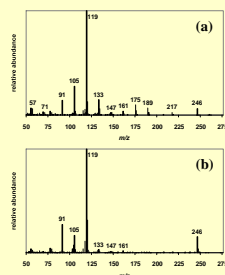


Figure 6. Comparison between (a) ToF mass spectrum of compound from organic extract of health impaired mussel and (b) EI mass spectrum of synthetic decylxylene

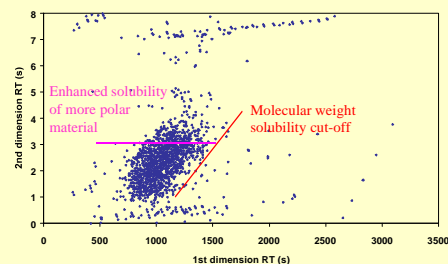


Figure 4. GCxGC-ToF-MS peak marker plot of the aqueous soluble components of TJP 4-6 DBE fraction

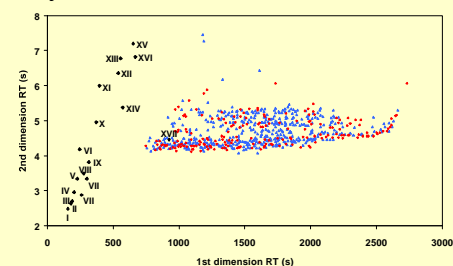


Figure 5. Distribution of peak markers with base ions of *m/z* 105 (red) and 119 (blue) from organic extract of health impaired mussels (Sutton Harbour, UK). Standards: I isopropylbenzene, II propylbenzene, III 1,3,5-trimethylbenzene, IV 1,2,4-trimethylbenzene, V 1,2,3-trimethylbenzene, VI indene, VII decahydronaphthalene (*cis*, *trans*), VIII adamantane, IX 1,2,4,5-tetramethylbenzene, X 1,2,3,4-tetrahydronaphthalene, XI naphthalene, XII 2-methylnaphthalene, XIII 1-methylnaphthalene, XIV cyclohexylbenzene, XV 1,1'-biphenyl, XVI 2-vinylnaphthalene, XVII nonylbenzene

4. Conclusions

The use of GCxGC-ToF-MS enables a more thorough comparison between the monoaromatic fraction isolated from mussels with impaired and similar fractions obtained from crude oils. This monoaromatic fraction appears to be dominated by alkyl substituted xylenes. Some oil components are still unresolved.

References

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5. Further Work

We are currently investigating whether the unidentified compounds with base ions *m/z* 183 are alkylmethyl-ethyl-naphthalenes⁴, and synthesising and toxicity testing a suite of alkylxylenes in order to characterise more fully the monoaromatic fractions isolated from crude oils, health impaired mussels and aqueous soluble fractions of oils.

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