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Using variable ionisation energy time-of-flight mass spectrometry with comprehensive GC×GC to identify isomeric species

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1

26 **ABSTRACT**

27 Although GC×GC-ToF-MS allows the separation of thousands of peaks, many of these peaks are

28 not positively identified owing to the lack of mass spectral library data and/or standard materials,

29 leading to a substantial amount of information being inaccessible. The fragmentation patterns of

30 molecules in mass spectrometers using electron impact ionisation at 70 eV can be useful for

31 molecule identification, provided a match is available in a published EI MS library, but are

32 indistinguishable for many isomeric organic compounds (for example, aliphatic and branched

33 alkanes). Lower ionisation energies have been exploited leading to organic compounds being

34 ionised with lower excess internal energy and less fragmentation, retaining the molecular ion and

35 maximising its relative signal. This has enabled the identification of a large number of isomeric

36 organic compounds, both aliphatic and aromatic, between C_{12}-C_{36}, in the previously unresolved

37 complex mixture (UCM) of two motor oil samples.

38

39 This technique also demonstrates problems associated with separation of co-eluting isomers,

40 particularly for the *n*-alkanes, which are routinely measured by 1D GC/MS and may be

41 overestimated, due to co-elution. As a consequence retention times in 2 dimensions and mass

42 spectra at variable ionisation energies are shown to give unparalleled power to identify specific

43 isomers.

44

45 **Keywords:** GC×GC, gas chromatography, mass spectrometry, ionisation, n-alkanes, fragmentation

46

47 **INTRODUCTION**

48 There is a well-established need for the characterisation of organic matter in a range of media. The

49 study of organic compounds in the atmosphere has been an interest of scientists for many years

50 owing to the potential detrimental health effects.[1-4] The complexity of the atmosphere as well as the

51 drawbacks associated with current analytical techniques have limited studies in investigating

52 specific compound classes and/or target analytes. Thus, detailed knowledge of the identities and

53 chemistry of organic compounds as a whole remains elusive. Goldstein and Galbally[5] illustrated the

54 exponential increase of number of unique isomers possible as a function of the number of carbon

55 atoms in an organic molecule, with *ca*. 1×10^6 constitutional isomers possible for a C_{20} alkane.

56

57 The study of organic compounds within crude oils has also generated much interest as it plays an

58 important role in petroleum exploration. Crude oils have a limited number of compound classes, but

59 an enormous number of individual components within a class. The composition of crude oil is also

60 susceptible to physical, chemical, biological and weathering processes, leading to significant

61 differences between any two crude oil samples.[6] Sutton et al.[7] isolated individual hydrocarbon

62 fractions from an unresolved complex mixture (UCM) of hydrocarbons isolated from a biodegraded

63 crude oil and suggested that the entire oil UCM may contain *ca*. 250,000 unidentified compounds.

64 This suggests that a substantial fraction of chemical composition information is inaccessible and

65 therefore unexploited.

66

67 The most common method for the speciation of organic compounds has been gas chromatography–

68 mass spectrometry (GC/MS). This technique has been exploited to separate crude oils;[7-9] airborne

69 samples which encompass multiple emission sources containing multiple compound classes and

70 both polar and non-polar components;[10-13] and sediments which can contain fingerprints of both

71 crude oils and various other source emissions.[6,14] The peak capacity of conventional GC, however,

72 is insufficient to resolve the full complexity of these mixtures and a pronounced rising baseline or

73 series of rising baselines in the gas chromatogram are observed, often referred to as the unresolved

74 complex mixture (UCM).[15] For example, the UCM in conventional GC separation of oil-based

75 samples typically accounts for *ca.* 95 % of the area of the chromatogram and is usually unresolved

76 by analysis. It is thought to comprise a major fraction of constitutional linear, branched and cyclic

77 alkane isomers and a minor fraction of aromatic compounds.[16,17] Due to the difficulties in

78 identifying isomers, information regarding their molecular structures has generally been

79 unavailable. This knowledge, however, is crucial in understanding the environmental fates of these

80 compounds, as the degree and positioning of branching in these alkanes strongly affect their

81 oxidation mechanisms in the environment and secondary organic aerosol (SOA) formation yields in

82 the atmosphere.[18-20]

83

84 Multidimensional or comprehensive gas chromatography (GC×GC) provides enhanced peak

85 capacity and separating powers, due to an orthogonal separation. This technique utilises two

86 capillary columns of different stationary phases connected in series by a modulator, which delivers

87 discrete bundles of effluent from the primary column to the secondary column.[21,22] Compounds of

88 similar chemical structure are grouped into distinct patterns in ordered chromatograms, providing

89 useful information that aids identification.[23] GC×GC is often coupled with time-of-flight mass

90 spectrometry (ToF-MS) which provides extra information in the identification of compounds, due

91 to its fast data acquisition rate, full range mass sensitivity and, in some cases, high resolution. These

92 advantages make GC×GC-ToF-MS an attractive analytical technique for separating complex

93 mixtures, measuring a wide range of volatile and semi-volatile organic compounds in air and oils.[24-

94 27]

95

96 Although GC×GC-ToF-MS allows the separation of thousands of peaks, many of these peaks are

97 not positively identified owing to the lack of mass spectral library data and/or standard materials.[28]

98 This means that a substantial amount of information is still inaccessible. Traditional mass

4

99   spectrometers (MS) employ electron impact (EI) ionisation at 70 eV, where a molecule undergoes

100 ionisation by an electron (most organic molecules have ionisation energies <11 eV). The electron

101 imparts a large amount of excess energy causing extensive fragmentation. The fragmentation mass

102 spectra can in most cases be useful for molecule identification, provided a match is available in a

103 published EI MS library, but for many organic compounds, such as the isomeric alkane structures

104 found in the UCM, are indistinguishable. This is because the fragment ions found in the mass

105 spectra of aliphatic compounds, for example alkanes, are very similar *i.e. m/z* 43, 57, 71, 85, 99 *etc*

106 and the molecular ion is often barely visible or absent. Interpretation of EI-MS spectra of complex

107 mixtures can therefore be ambiguous. These limitations in EI have led to a continual demand for

108 more robust lower (or soft) ionisation techniques for MS. Using lower ionisation energies can lead

109 to organic compounds being ionised with minimal excess internal energy and thus less

110 fragmentation, hence retaining the identity of the molecule by retaining a much larger fraction of

111 the molecular ion. This has been the subject of rigorous research by Maccoll and co-workers,[29]

112 since their early work on ion enthalpies and their application in mass spectrometry.[30] A series of

113 literature has since been published, in seventeen parts over a decade, reporting low EI (12.1 eV),

114 low temperature (~350K) mass spectra of various compound classes.

115

116 Several soft ionisation techniques have been developed such as chemical ionisation (CI),[31,32] field

117 ionisation (FI)[33,34] and photoionisation (PI).[35,36] CI typically utilises reagent ions produced by EI of

118 a reagent gas (such as methane, isobutane or ammonia) at high pressure (*ca.* 1×10^{-4} mbar). The

119 reagent ions are only slightly reactive with the reagent gas itself, but readily react to ionise the

120 sample via ion-molecule reactions. Although this ionisation technique often gives molecular mass

121 information with reduced fragmentation compared to EI, results are highly dependent upon reagent

122 gas type, pressure, reaction time and the nature of the sample. In addition, CI requires a different

123 ion-source configuration and thus it can be time consuming and resource intensive if a single MS

124 instrument is being used for both EI and CI. FI has been exploited by a number of studies.[37-39] FI

125 produces dominant molecular ions with little or no fragmentation. Hejazi et al.[38] developed a

126 method that utilises GC with parallel hard (EI) and soft ionisation (FI) mass spectrometry. This

127 instrument consisted of two mass spectrometers, with differing ionisation methods, connected to a

128 single GC. Wang et al.[39] investigated the separation of diesel fuel using a two dimensional

129 separation approach whereby FI-MS was coupled to a GC. FI, however, produces weak ion currents

130 with significantly more fluctuation on the one second time scale of a typical scan for a scanning

131 MS, resulting in poor repeatability of the relative abundances of measured ions.[37]

132

133 An increasingly popular soft ionisation technique is PI, where principally two different PI

134 techniques have been investigated, namely resonance-enhanced multi-photon ionisation (REMPI)

135 and single photon ionisation (SPI).[35] REMPI utilises intense ultraviolet (UV) light laser pulses for a

136 two photon absorption process, whereas SPI uses vacuum ultraviolet (VUV) photons for a single

137 photon absorption process. REMPI is highly sensitive and selective for specific compounds such as

138 aromatics and polyaromatic compounds,[36,40,41] which prevents this soft ionisation technique from

139 being applicable to crude oil samples that are rich in aliphatic compounds. SPI, however, with VUV

140 is capable of soft ionisation of all organic compound classes including the aliphatic alkanes.[35,42]

141 Isaacman et al.[43] used VUV ionisation at 10 – 10.5 eV to elucidate some of the structures within the

142 UCM of atmospheric particles, by separating components using GC. Chan et al.[44] also exploited

143 this technique in order to characterise the UCM and to provide insights into atmospheric processing

144 of these components. Their results showed that the most abundant isomer for each carbon number

145 was the linear alkane accounting for >30% of the total alkane mass. Drawbacks of SPI VUV,

146 however, are the relative experimental complexity of the technique and the reduced stability of the

147 molecular ions that are formed (radical cations) in comparison to other techniques such as MALDI

148 and atmospheric pressure CI (even-electron ions). It is therefore, important to develop and

149 investigate reliable, inexpensive and simple ionisation techniques that can help identify the highly

150 numerous unknown compounds that are present in samples such as aerosols and petroleum samples.

151 In this study, we use variable ionisation time-of-flight mass spectrometry, ranging from 10 to 70 eV

152 coupled to GC×GC, to determine the structures of aliphatic and aromatic compounds within the

153 UCM. By using soft EI and conventional 70 eV EI, both molecular ions and fragment ions can be

154 obtained. EI provides high ion yield and sensitivity, generating highly reproducible spectra, for

155 which there is a large database of spectra of known compounds (NIST). Soft ionisation provides the

156 molecular ion and reduced fragmentation, for which there is no database available. Both mass

157 spectral data at 14 and 70 eV, as well as retention times from the GC×GC, give insights into

158 structural positioning and extent of alkyl branching of aliphatic (linear) alkanes and aromatics.

159

160 **EXPERIMENTAL**

161 **Instrumentation**

162 Sample analyses were conducted by a gas chromatograph (7890B, Agilent Technologies,

163 Wilmington, DE, USA) equipped with a Zoex ZX2 cryogenic modulator (Houston, TX, USA). The

164 first dimension was equipped with a SGE DBX5, non-polar capillary column (30 m, 0.25 mm ID,

165 0.25 μm – 5% phenyl polysilphenylene-siloxane), and the second dimension column was SGE

166 DBX50 (4.0 m, 0.1 mm ID, 0.1 μm – 50% phenyl polysilphenylene-siloxane). The GC×GC was

167 interfaced with a BenchTOF-Select, time-of-flight mass spectrometer (Markes International,

168 Llantrisant, UK). The acquisition speed was 50 Hz with a mass resolution of >1200 FWHM at 70

169 eV and >800 FWHM at 14eV over 100 – 1000 *m/z*.[45] The mass range was 35 to 600 *m/z*. The GC

170 sensitivity at 70 eV has been calculated by the injection of 1 pg octafluoronaphthalene (*m/z* 272)

171 giving a signal-to-noise ratio (S/N) of >2000:1 rms, while for 14eV, the injection of 1 pg of

172 benzophenone (*m/z* 182) gave a S/N of >80:1 rms.[45] Although ionisation energies can be varied

173 from anywhere between 10 and 70 eV, explored energies in this work were 14 eV and 70 eV, by

174 means of repeat injection runs. All data produced were processed using GC Image v2.3 (Zoex

175 Corporation, Houston, US).

176

7

Principle of soft ionisation technique

For the conventional EI ion source, electrons are accelerated from the surface of a negatively charged filament towards a positively charged ion chamber, owing to a potential difference of 70 V, thus efficiently ionising compounds from the GC. Reducing this potential difference (for soft ionisation) results in a dramatic loss of sensitivity due to the inefficiency of drawing electrons into the ion chamber and clustering of electrons around the filament. The variable ionisation source uses ion optics in order to retain the high potential difference, thus accelerating the electrons from the filament and reducing their energy prior to arriving at the ion chamber.[46] This allows the ionisation energy to be tuned between 10 and 70 eV. Unlike CI and FI, no reagent gases, adjustments in pressure or switching of sources are required.

Methodology

Two motor/lubricating oil samples (synthetic 5W30 and pure base oil analogous to mineral oil, see Supporting Information for more details) were analysed, utilising GC×GC-ToF-MS to identify aliphatic isomeric compounds. The protocol applied to the motor oil analytical sample was to inject 1 µL of diluted (1:1000) sample in a split ratio 100:1 at 300 °C. The initial temperature of the primary oven (175°C) was held for 5 min and then increased at 1°C/min to 325°C. The secondary oven temperature program was the same as the primary oven but was increased at 1°C/min to 330°C to ensure all species passed through the column. The transfer line temperature was 325°C and the ion source temperature was 280°C. Helium was used as the carrier gas at a constant flow rate of 0.8 mL/min. A modulation time of 7 seconds was used, while a total run time of each sample was 120 minutes.

RESULTS & DISCUSSION

A typical two-dimensional separation is presented in Figure 1(A) and (B) for the base lubricating oil. The white line in Figure 1(A) illustrates the separation achievable using traditional 1D GC/MS.

8

203 Each coloured spot represents an individual compound with a corresponding full mass spectrum.

204 The intense dark blue spots are major peaks, while the lighter blue spots represent small peaks.

205 Compounds were separated in the first dimension (X-axis) on a volatility basis and polarity in the

206 second (Y-axis).

207

208 Samples were run using two ionisation energies, 70eV and 14eV to gain insight on fragmentation

209 patterns and molecular ions, respectively. The procedure for peak identification is outlined in detail

210 elsewhere.[23] Briefly, peaks were identified based primarily on mass spectral data from the NIST

211 library (for 70eV), where two match factors (MF>750 and RMF>800) were used to assess the

212 similarity between the library and measured mass spectra. For samples analysed with lower

213 ionisation energy (14eV), the identification of species was determined by interpretation of mass

214 spectral data and comparing their retention indices and mass spectra at 70eV, together with the

215 presence of the species molecular ion (possessing higher relative intensity than in a 70eV mass

216 spectrum). The samples analysed at both ionisation energies therefore provided greater insight into

217 a compound's identity, indicating the power of soft EI. Many differences were observed between

218 the two oils (total ion chromatogram for 5W30 oil not shown in Figure 1A), the most notable being

219 the presence of more high molecular weight (HMW) (less volatile) compounds in the 5W30

220 synthetic oil.

221

222 *Monomethylalkanes*

223 Monomethylalkanes are present in many natural and synthetic organic materials, including crude

224 oil, coal and sediments;[47-50] and have been proposed as endogenous biomarkers in exhaled breath.[51]

225 The separation and identification of monomethylalkanes in a broad range of carbon atoms is

226 difficult owing to the number of isomeric possibilities and close retention times Recently, Szabó et

227 al[52] utilised GC×GC-FID and GC/MS/MS with 100m columns to separate 63 C_9-C_{19}

228 monomethylalkanes, reporting up to 8 isomers for the monomethyl-nonadecane (C_{19}) in exhaled

229 breath. Krkošová et al.[47] reported the linear retention indices of 196 C_4-C_{30} monomethylalkanes

230 using GC/MS equipped with a 100m column and used these retention indices to identify these

231 species in diesel fuel. All these studies have considered a targeted approach in their analyses and

232 not considered the presence of other compounds in complex mixtures.

233

234 The use of variable ionisation energy coupled to mass spectrometry, as well as retention time

235 information enables a non-targeted approach to be conducted. For example, 2-methyl-eicosane and

236 3-methyl-eicosane are $C_{21}H_{44}$ isomers which have different retention indices and are largely

237 experimentally indistinguishable from other aliphatic isomers using their fragmentation mass

238 spectra at 70eV (see Figure 2A and 2D). The absence of the molecular ion (*m/z* 296) means that it is

239 difficult to deduce, without retention time information, which isomeric aliphatic (with

240 corresponding C number) it belongs to; as all aliphatic compounds with $\geq C_{22}$ show relatively small

241 peaks at *m/z* 253 and *m/z* 267. At 14eV however, both these compounds are distinguishable,

242 allowing the identification of the positioning of branching on an aliphatic alkane (see Figure 2C and

243 2F). The presence of the molecular ion at *m/z* 296 allows the identification of these compounds

244 without the use of retention indices. The 14eV mass spectrum of 2-methyl-eicosane shows a

245 relatively large peak at *m/z* 253, signifying a loss of a C_3H_7 fragment (see Figure 2C), while for 3-

246 methyl-eicosane the presence of a significant peak at *m/z* 267 signifies the loss of C_2H_5 indicating

247 the most likely point of cleavage (see Figure 2F). This information together with their identical

248 retention indices in both 14eV chromatography and 70eV chromatography allows the identification

249 of 7 further C_{21} monomethylalkane isomers. For example, the 14eV mass spectrum of 4-methyl-

250 eicosane showed the presence of peaks *m/z* 225 signifying the loss of a C_5H_{11} fragment and *m/z* 253,

251 signifying a loss of a C_3H_7 fragment (see Figure 2J). 2-Methyl and 4-methyl-eicosane mass spectra

252 were distinguishable from one another, due to the presence of the *m/z* 225 fragment, together with

253 their positioning in the chromatography which was confirmed using Kovats retention indices. The

254 mass spectra of 5-methyl-eicosane shows relatively large peaks at *m/z* 239 and *m/z* 211 signifying

255 the loss of mass fragments C_4H_9 and C_6H_{13}, respectively (see Figure 2K). 6-Methyl-eicosane mass

256 spectra exhibited peaks at m/z 225 and m/z 197, where the m/z 71 and m/z 99 peaks showed similar

257 relative intensities (see Figure 2L). From these peaks together with the m/z 197, 6-methyl-eicosane

258 was distinguishable from 4-methyl-eicosane (which has the same m/z 225 fragment). Peaks at m/z

259 225 and 197 corresponded to C_5H_{11} and C_7H_{15} fragment losses. This analogy was completed for 7,

260 8, 9 and 10-methyl-eicosanes; however, there is significant co-elution for 9-methyleicosane and 10-

261 methyleicosane compounds (Figure 3(A)). All methyl-eicosane mass spectra retained the presence

262 of the molecular ion at m/z 296 (see Figure 2A-2L).

263

264 As compounds with similar chemical structure are grouped into distinct patterns in ordered

265 chromatograms, the order of branching with increasing retention time is consistent throughout the

266 chromatogram with increasing carbon number (up to C_{28}). The faster eluting monomethylalkanes

267 are those methylated alkanes with methyl groups furthest away from the first carbon position (with

268 the exception of 2-methylmonoalkanes, determined by assessing each monomethylalkane's mass

269 spectrum at 14eV and previously published literature.[47] Thus, for C_{21} isomers the order of branching

270 with increasing retention time would be 10-, 9-, 8-, 7-, 6-, 5-, 4-, 2- and then 3-methyl-eicosane.

271 The majority of these isomeric monomethylalkanes are present in the synthetic oil and are not all

272 present within the base oil. For example, for C_{21} isomers, there is no 5-methyl-eicosane present in

273 the base oil (see Figure 3A). Signal intensities of the monomethyl $C_{24} - C_{28}$ alkanes in the base oil

274 are also 20-30% lower than the synthetic oil, which may reflect the differences in the production

275 and refining of the two oils (the base oil being an API group 1 oil and having ~77% saturates).

276 These compounds were easily identifiable using 14eV spectra throughout the UCM for the base oil

277 but not for the 5W30 oil, most probably due to the coelution of the larger number of isomers present

278 in the latter. The base oil also consists of a number of di- tri- tetra- and penta-methylalkanes

279 extending beyond each n-alkane species as shown in Figure 3(B), identified tentatively by their

280 mass spectra at both ionisation energies (for <C22) and published retention times (where available).

281 The tri- and tetra-methyl alkanes (belonging to the C_{23} carbon number) were not present in

282 abundance in the 5W30 lubricating oil (see Figure 3) and elute faster than the C_{21} *n*-alkane. It must

283 be noted that the first dimension is separated by volatility and is not a carbon number distribution.

284 The mass spectra for many substituted methylalkanes with >C22 were indistinguishable at 70 eV.

285 Figure 4 illustrates the experimentally determined mass spectra for 2,6,10,14-tetramethyl-

286 nonadecane ($C_{23}H_{48}$; upper panel) and 2,6,10,14,18-pentamethyl-nonadecane ($C_{24}H_{50}$; lower panel)

287 at 70 eV (4A), 14 eV (4B) and their respective NIST reference spectra (4C). Although these

288 compounds, in both motor oils, elute at their specific published retention times, they are

289 indistinguishable at 70 eV. The mass spectral peaks are those found in all aliphatic compounds with

290 several CH_2 (*m/z* 14) cleaved mass fragments. At 14 eV, however, the presence of the molecular ion

291 and relatively larger intensities of ions that signify likelihood of the cleavage of a C-C bond, allows

292 the positive identification of these compounds within complex mixtures. For example, for

293 2,6,10,14-tetramethyl-nonadecane, the loss of fragments C_5H_{11} + C_6H_{13}, $C_{10}H_{21}$ + $C_{11}H_{23}$ and

294 $C_{15}H_{31}$ + $C_{16}H_{33}$ signify bond breaking either side of the methyl group, indicating the positioning of

295 the methyl carbon bond and give rise to *m/z* 253+239, 183+169, and 113+99, respectively. While

296 for 2,6,10,14,18-pentamethyl-nonadecane the loss of $C_{11}H_{23}$ and C_8H_{17} fragments results in

297 relatively large peaks at *m/z* 183 and 113, respectively. This information as well as the presence of

298 the molecular ion enables confident structural assignment of these compounds. It is interesting that

299 the NIST (70eV) reference spectrum looks significantly softer that the experimental 70eV spectrum

300 and remarkably similar to the 14eV ionisation mass spectrum. The NIST library at 70 eV also

301 signifies the presence of the molecular ion (see Figure 4C); however, this is not easily observed for

302 many aliphatics within complex mixtures, particularly for high molecular weights.[53]

303

304 ***n-Alkanes***

305 *n*-Alkanes are ubiquitous in the environment. They are biosynthesized by several organisms such as

306 terrestrial and aquatic plants, algae and insects and are used as biomarkers in biogeochemistry.[54] *n*-

307   Alkanes are a major constituent of crude oil and are routinely and easily measured by GC/MS. In

308   petroleum polluted sediments the *n*-alkanes generally range between C_{12} and C_{40}, whereas in marine

309   organisms the range is normally <C21.[55]

310

311   Diagnostic ratios such as, natural *n*-alkane ratio (NAR), carbon preference index (CPI),

312   terrigenous/aquatic ratio (TAR) and low molecular weight (LMW)/HMW ratios are exploited to

313   assess possible sources of *n*-alkanes. For example, NAR estimates the proportion of natural and

314   petroleum *n*-alkanes, where ratios close to zero signify petroleum hydrocarbons and crude oil

315   sources, while ratios close to one suggest terrestrial or marine plant sources.[56] CPI are ratios

316   between odd and even numbered *n*-alkanes, where values in the range $n\text{-}C_{24}$ and $n\text{-}C_{32}$ close to one

317   are found for crude oils and other petroleum sources, while a predominance of odd numbered *n*-

318   alkanes is characteristic of terrestrial plant debris.[55,57,58] TAR is used as a crude indicator of relative

319   terrigenous versus aquatic organic matter input, where the ratio between the concentrations of long

320   chain *n*-alkanes ($n\text{-}C_{27}$ + $n\text{-}C_{29}$ + $n\text{-}C_{31}$) and short chain *n*-alkanes ($n\text{-}C_{15}$ + $n\text{-}C_{17}$ + $n\text{-}C_{19}$) is

321   evaluated.[59] All these ratios are crude indicators of the sources of *n*-alkanes where concentrations

322   are generally measured by conventional GC/MS.

323

324   When these hydrocarbons are measured from within a complex mixture using conventional GC/MS,

325   large distinct peaks are observed above a rising baseline (or UCM). Although the retention times of

326   *n*-alkanes are very different from one another, their retention times may be similar to other

327   structural isomers, particularly for larger carbon numbers, owing to the increased probability of

328   possible isomers. If co-elution occurs, however, then the MS used in conjunction with GC could

329   potentially distinguish between the two co-eluting peaks, unless they have similar mass spectra at

330   70eV.

331

13

332 Figure 5A and 5B shows the contour plots of two portions of the motor oils, focusing on the *n*-

333 alkanes between C_{18} and C_{22} (see Figure S1 for contour plot for 5W30 for *n*-alkanes C_{23} to C_{30}).

334 Figure 5A (synthetic 5W30) shows the presence of an unknown species (compound X) eluting at

335 the same retention time as the *n*-alkane (in the X-axis), and is separated by polarity in the Y-axis.

336 The MS of compound X (see Figure 5A) possesses the molecular ion of the C_n *n*-alkane + CH_2 (*m/z*

337 14) at 14eV, suggesting that it is a branched alkane belonging to the C_{n+1} aliphatic alkane. This is

338 illustrated by Figure 5A where the black lines signify the overlap between the isomers of two *n*-

339 alkanes. The mass spectrum of compound X at 70eV, however, cannot be used to distinguish

340 between the C_n *n*-alkane and the C_{n+1}-alkane isomer, as there is no molecular ion (particularly for

341 the larger *n*-alkanes, >C19), see Figure 5(A). This unknown species is not distinguishable from the

342 *n*-alkane (for >C_{19}) when analysing using conventional GC (or by GC×GC with shorter secondary

343 columns), as the retention time (volatility axis) of compound X is that of the *n*-alkane and therefore

344 it will co-elute. This co-elution cannot be separated by its MS at 70eV as the primary and secondary

345 ions that would be used to identify both compound X and the *n*-alkane would be the same (*i.e. m/z*

346 43, 57, 71, 85) see embedded MS in Figure 5A. In Figure 5A, the volume of compound X co-

347 eluting with the *n*-alkane is approximately 40% of the total volume of the two together.

348 Furthermore, the separation of these two compounds by polarity (y-axis) is enhanced when

349 increasing the length of the secondary column (to 4m), as when using a shorter length column, GC

350 Imagev2.3 software was unable to automatically deconvolve the two peaks. This suggests that in

351 studies utilising GC/MS, *n*-alkane concentrations may be overestimated (particularly for >C_{19}) and

352 thus may affect the calculated/reported ratios. According to previously published literature, one

353 possibility of compound X could be a dimethyl isomer belonging to the C_{n+1}-alkane.[43,60] Dimethyl

354 isomers can be shifted by ~100 delta-Kovats (~1 carbon number),[43] whereas tri-methyl and tetra-

355 methyl have been reported to be shifted by ~150 and ~200 delta-Kovats.[60] The shift in delta-Kovats

356 for tetra-methyl alkanes identified in this study for example, 2,6,10,14-tetramethylhexadecane (C_{20}),

357 2,6,10,14-tetramethylheptadecane (C_{21}), 2,6,10,14-tetramethyloctadecane (C_{22}), 2,6,10,14-

14

358   tetramethylnonadecane (C_{23}, see Figure 4) etc., are in agreement with previous studies (~2 carbon

359   numbers)[60] and therefore are not likely candidates for compound X, due to the presence of the C_{n+1}-

360   alkane molecular ion in the mass spectra of the latter (see Figure 5).

361

362   *Monocyclic Alkanes and Aromatics*

363   *n*-Alkylcycloalkanes have been recognised as major constituents in sediments and crude oils.[61]

364   Worton et al.[17] recently reported that primary organic aerosol (POA) emissions were dominated by

365   branched cycloalkanes with one or more rings and one or more branched alkyl side chain (>80% by

366   mass). The authors investigated the chemical composition of POA and its similarity to lubricating

367   oil. It was shown that cyclic and branched alkanes dominated the mass of POA (>60%), while

368   aromatics and *n*-alkanes accounted for <2 and <5%, respectively. The components of the base oil in

369   this study were predominantly cyclic and branched alkanes (based on TIC signal, see Supporting

370   Information), in agreement with Worton et al.[17], but were not for the 5W30 motor oil, where

371   straight and branched chain alkanes were dominant.[17] This is associated with the differences in

372   physical properties of the oils (*e.g.* viscosity and viscosity index) see Table S1. It has been

373   suggested that cycloalkanes are predominant compounds found in lubricating oils, owing to their

374   desirable properties making them efficient lubricants across a wide range of operating

375   temperatures.[17]

376

377   *n*-Alkylcyclopentanes and *n*-alkylcyclohexanes were identified by retention times of known

378   standard materials, mass spectral fragmentation at both ionisation energies and by comparison with

379   published data.[17] *n*-Alkylcycloheptanes have received considerably less attention, probably due to

380   the difficulties associated in measuring these compounds with various sensitive detectors or owing

381   to them being present in trace amounts. Although *n*-alkylcycloheptanes were tentatively identified

382   they were represented by very small chromatographic peaks in comparison to *n*-alkylcyclohexanes

383   and *n*-alkylcyclopentanes. The selected mass fragment ions for *n*-alkylcyclopentanes, *n*-

384 alkylcyclohexanes and *n*-alkylcycloheptanes were *m/z* 68, *m/z* 82 and *m/z* 97 respectively. The

385 identified monocyclic alkanes ranged from C_{12} to C_{26} and their selected ion chromatograms are

386 shown in Figure 6. These could be readily identified by 70 eV ionisation.

387

388 Using 14eV fragmentation energy mass spectra, alkyl-methylcyclohexanes (*m/z* 82+97), alkyl-

389 dimethylcyclohexanes (*m/z* 82+111) and alkyl-trimethylcyclohexanes (*m/z* 82+125) were also

390 tentatively identified, (and confirmed by published retention indices for alkyl-methylcyclohexane)[62]

391 using the summed selected ion chromatogram with their respective mass fragments as is illustrated

392 in Figure 6. The wealth of peaks identified signifies the complexity of the base motor oil with only

393 the monomethyl-alkylcycloalkanes being positively identified. Although it is not possible to

394 determine the positioning of the methyl group on the cyclohexane ring (*i.e.* ortho-, para- or meta-

395 position), it is possible to determine the positioning of branching on the alkyl chain as demonstrated

396 for the monomethyl alkanes. Three $C_{22}H_{44}$ isomers were differentiated by their mass spectral

397 differences (at 14eV), according to the positioning of the methyl group on the alkyl chain

398 (analogous to the monomethylalkanes) and the presence of a methyl group on the cyclohexane ring.

399 All three compounds, 1-methyl-4-pentadecylcyclohexane, (3-methylpentadecyl)cyclohexane and 1-

400 methyl-4-(3-methyltetradecyl)cyclohexane possessed their molecular ion of *m/z* 308, see Figure 7A

401 – 7C. 1-methyl-4-pentadecylcyclohexane and 1-methyl-4-(3-methyltetradecyl)cyclohexane showed

402 mass fragments of *m/z* 82+97, signifying a methyl-cyclohexane fragment. A relatively smaller peak

403 at *m/z* 97 was observed for (3-methylpentadecyl)cyclohexane signifying no methyl group on the

404 cyclohexane ring, and peaks *m/z* 111, 139, 169 and 197 were relatively large, corresponding to the

405 C-C bond breakage on either side of the methyl group on the alkyl chain, leading to mass fragments

406 C_8H_{15}, $C_{10}H_{19}$, $C_{12}H_{25}$ and $C_{14}H_{29}$, respectively, see Figure 7B. For compound 1-methyl-4-

407 pentadecylcyclohexane a relatively large peak at *m/z* 211 corresponding to the breaking for the alkyl

408 chain from the cyclohexane ring was observed, as well as peaks at *m/z* 111, 197, which most likely

409 signify a C_8H_{15} ring fragment and a $C_{14}H_{29}$ aliphatic fragment, see Figure 7A. The compound 1-

410   methyl-4-(3-methyltetradecyl)cyclohexane, however, possessed relatively large peaks at *m/z* 125,

411   153, 155 and 183 which were significantly different to those of the other two isomers. These peaks

412   correspond to mass fragments C_9H_{17}, $C_{11}H_{21}$, $C_{11}H_{23}$ and $C_{13}H_{27}$, respectively, see Figure 7C.

413

414   Not all (methyl-alkyl)-cyclohexanes were positively identifiable in the motor oil, owing to the

415   intensities of the mass fragments *m/z* 82 and 97 being relatively large in comparison to the

416   individual bond cleavages along the alkyl chain. However, the methyl-alkylated cyclohexanes are

417   analogous in their positioning in the chromatograph to that of the *n*-alkanes and monomethylalkanes

418   (with higher polarity).[17] Similarly to the alkyl-cycloalkanes, a series of alkyl-benzenes (*m/z* 92)

419   were positively identified ranging from tridecyl-benzene ($C_{19}H_{32}$) to eicosyl-benzene ($C_{26}H_{46}$).

420   However, surprisingly, no methyl-alkyl-benzenes were observed in either of the lubricating oils.

421   Alkyl-benzenes are often used as refrigeration lubricants and although they are present in mineral

422   oils, are found in small quantities. This is in agreement with the observed relative intensities of this

423   compound class in the chromatography, with respect to the cycloalkane derivatives which are more

424   abundant.[17]

425

426   *Bicyclic Alkanes*

427   Bicyclic aliphatics occupy the same section of the chromatogram as the monocyclic aliphatics,

428   albeit with higher polarity (larger in the y-axis of the chromatography).   Alkyl-

429   decahydronaphthalene (or alkyl-decalin) isomers have received increasing interest as potential

430   petroleum biomarkers. Tran et al.[63] tentatively observed this compound class using GC×GC-ToF-

431   MS and stressed that the assignments were necessarily tentative owing to the lack of molecular

432   specificity in the interpretation of the mass spectra. Avila et al.[64] utilised GC×GC-ToF-MS and EI

433   coupled to Fourier transform cyclotron resonance mass spectrometry to analyse compounds found

434   in heavy gas oils, tentatively identifying alkyl-decalins. More recently, however, Wang et al.[65]

435   characterised these compounds by synthesizing and investigating their mass spectra using GC×GC-

436 ToF-MS, reporting the mass spectra and retention times for decalins ranging from C_1 to C_{11} in alkyl

437 chain length. Worton et al.[17] reported that bicyclic alkanes with various branched alkyl chains

438 contributed about 30% of the observed mass in POA and lubricating oil. Up to 11 % of the observed

439 mass fraction of diesel fuel has also been attributed to bicyclic alkanes.[43] In this study, we identify

440 alkyl-decalins without the use of authentic standards owing to the characteristic mass spectra

441 obtained for these compounds, with the presence of their corresponding molecular ion; the mass

442 fragments of the respective observed isomers are in good agreement with the results published by

443 Wang et al.[65]. Alkyl-decalins were identified by their characteristic mass fragment m/z 137 together

444 with the molecular ion (using 14eV spectra). The two closely eluting peaks correspond to the alkyl

445 chain at the 1- and 2- positions of the decahydronaphthalene structure but cannot be distinguished

446 from one another. No (methyl-alkyl)-decalins, however, were observed in either of the lubricating

447 oils, which is in contrast to other studies.[17]

448

Tricyclic Aromatics and Alkanes

450 Tricyclic aromatic compounds occupy the bottom half of the chromatographic section of the

451 contour plot illustrated in Figure 1B. In the base oil, these compounds were more abundant

452 (according to TIC signal, see Supporting Information) than the bicyclic aromatics/alicyclics but less

453 than the monocyclic aromatics/alicyclics. Selecting mass fragment m/z 191, two groups of tricyclic

454 aromatic compounds were identified; alkyl-anthracenes (or alkyl-phenanthrenes) and

455 alkyl-perhydroanthracenes (or alkyl-perhydrophenanthrenes). The mass spectra of alkyl-anthracenes

456 showed little fragmentation, possessing a dominant molecular ion (M^+) and the m/z 191, signifying

457 the 3 fused benzene rings; whereas the observed mass fragments for alkyl-perhydroanthracenes

458 were m/z 55, 67, 81, 95, 109, 135 with the two most intensive peaks belonging to the M^+ molecular

459 ion and m/z 191. These mass spectra were confirmed using the interpretation of Kiselev et al.[66], who

460 investigated the mass spectrometry of the stereoisomers of perhydroanthracene and

461 perhydrophenanthrene. Compounds from both tricyclic groups ranged from C_1 (methyl-) to C_{12}

18

462   (dodecyl-) in alkyl chain length, corresponding to molecular masses (M^+) of *m/z* 192 to *m/z* 360,

463   respectively. Very limited tricyclic aliphatics were found in either of the lubricating oils in contrast

464   with other studies.[17,67] We attribute this to the differences in formulation of different oils, or to the

465   different methods used as highlighted in a recent study.[68]

466

CONCLUSION

468   The primary reason why very little quantitative analysis has been conducted on hydrocarbons of

469   greater than C_{10} is that as a consequence of their huge diversity conventional GC is unable to

470   provide a separation and these compounds appear in the chromatogram as a large hump referred to

471   as UCM. The advent of two dimensional gas chromatography techniques in recent years has

472   provided a means to disaggregate the UCM hump providing the separation of thousands of

473   compounds which can be characterised on the basis of their mass spectra. This however has its

474   limitations as each organic compound, particularly in petroleum or environmental samples deriving

475   from petroleum, has a large number of isomers with rather similar mass spectra. The 70eV mass

476   spectra show minor differences in the fragmentation patterns which offer opportunities for

477   distinguishing isomeric species; however, a substantial limitation is that the mass spectra rarely

478   contain a signal from the molecular ion hence greatly complicating the identification process as

479   rather similar mass-spectra at 70eV can result from compounds of differing carbon chain length, as

480   demonstrated by Figure 4(A). Using a variable energy EI source operating at 14 eV has allowed the

481   generation of mass-spectra with clearly visible molecular ions. The combination of retention times

482   in two dimensions and mass-spectra at low and high ionisation energies confers unparalleled power

483   to identify specific isomers within the chromatograms.

484

ACKNOWLEDGEMENTS

491

Conflict of Interest Disclosure

493   The authors declare no competing financial interest.

494

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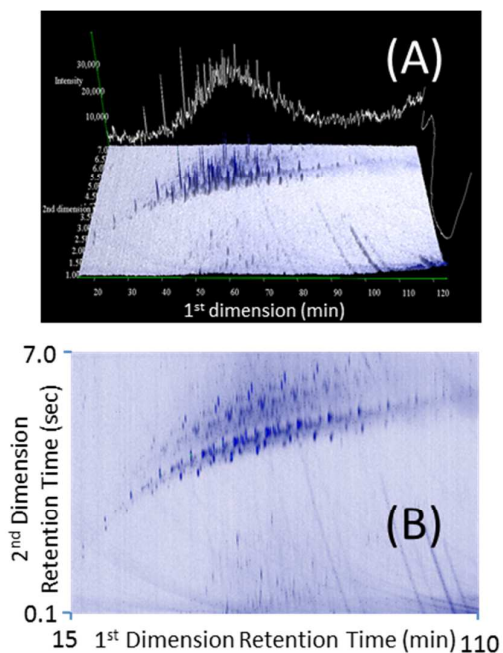589 *Sci. Technol.* **2015**, *49*, 13130-13138.

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Figure 1. (A) Total ion current chromatogram of a base lubricating oil examined by GC×GC-ToF-MS illustrating improved chromatographic resolution by GC×GC compared with GC (white line in background). **(B)** Contour plot of total ion current chromatogram of a base lubricating oil examined by GC×GC-ToF-MS. Colours indicate the intensities of response (dark blue > blue > white).
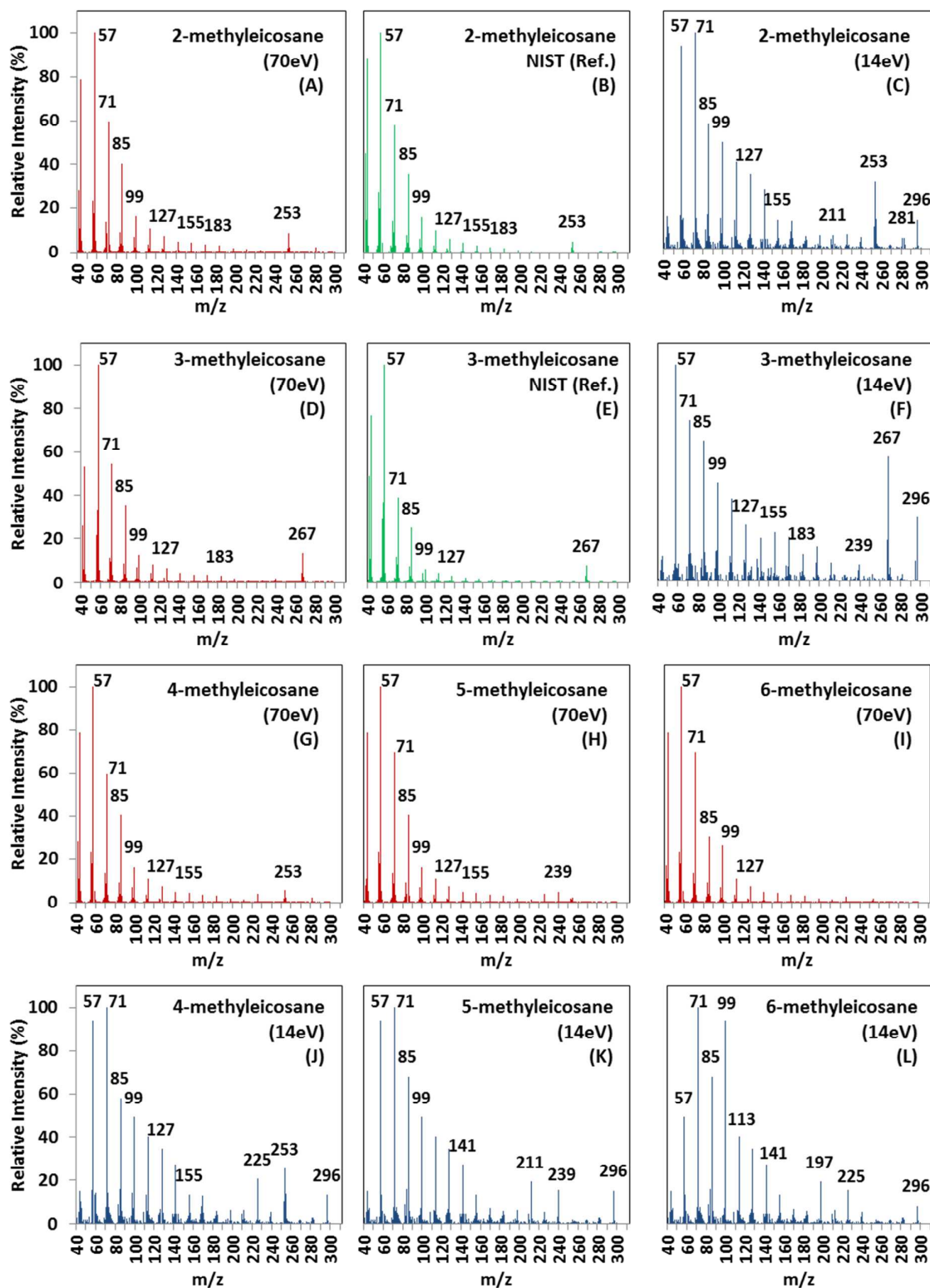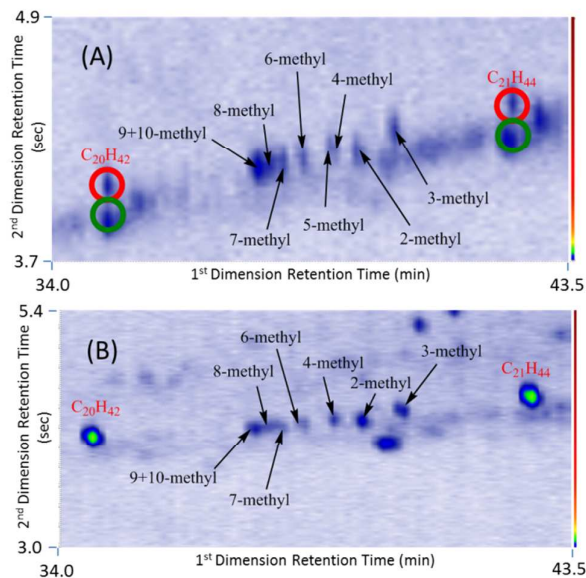
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Figure 2. Experimentally determined mass spectra of 2-methyleicosane (at 14eV, 70eV and NIST reference), 3-methyleicosane (at 14eV, 70eV and NIST reference) and 4-, 5- and 6-methyleicosanes (at 14eV and 70eV)

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618 **Figure 3.** Contour plots of **(A)** 5W30 lubricating oil; and **(B)** mineral base oil; illustrating species
619 identified within two *n*-alkane species ($C_{20} - C_{21}$).

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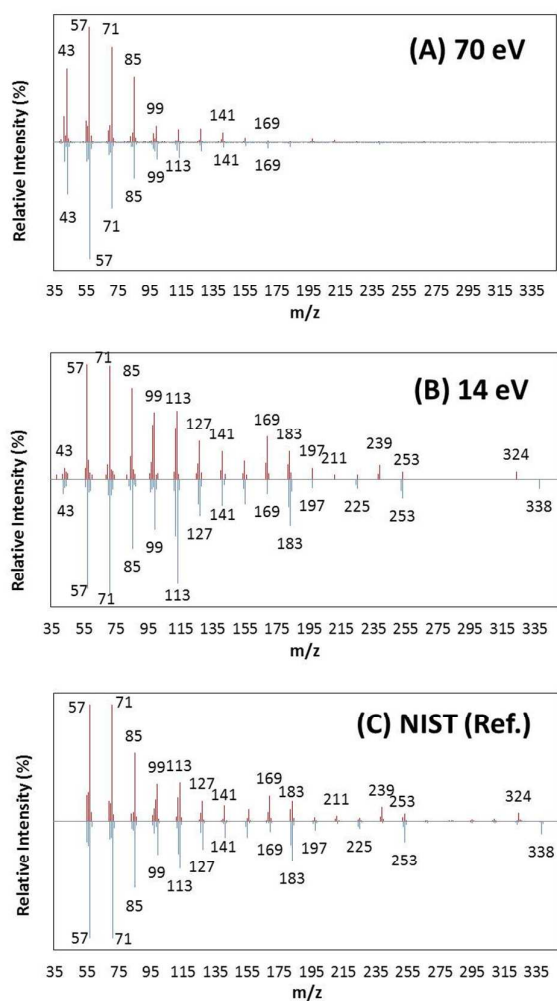
Figure 4. (A) Experimentally determined mass spectra from base lubricating oil for **(A)** 2,6,10,14-tetramethyl-nonadecane (upper panel) and 2,6,10,14,18-pentamethyl-nonadecane (lower panel) at 70 eV; **(B)** 2,6,10,14-tetramethyl-nonadecane (upper panel) and 2,6,10,14,18-pentamethyl-nonadecane (lower panel) at 14 eV; and **(C)** reference spectra of 2,6,10,14-tetramethyl-nonadecane (upper panel) and 2,6,10,14,18-pentamethyl-nonadecane (lower panel) from NIST library.

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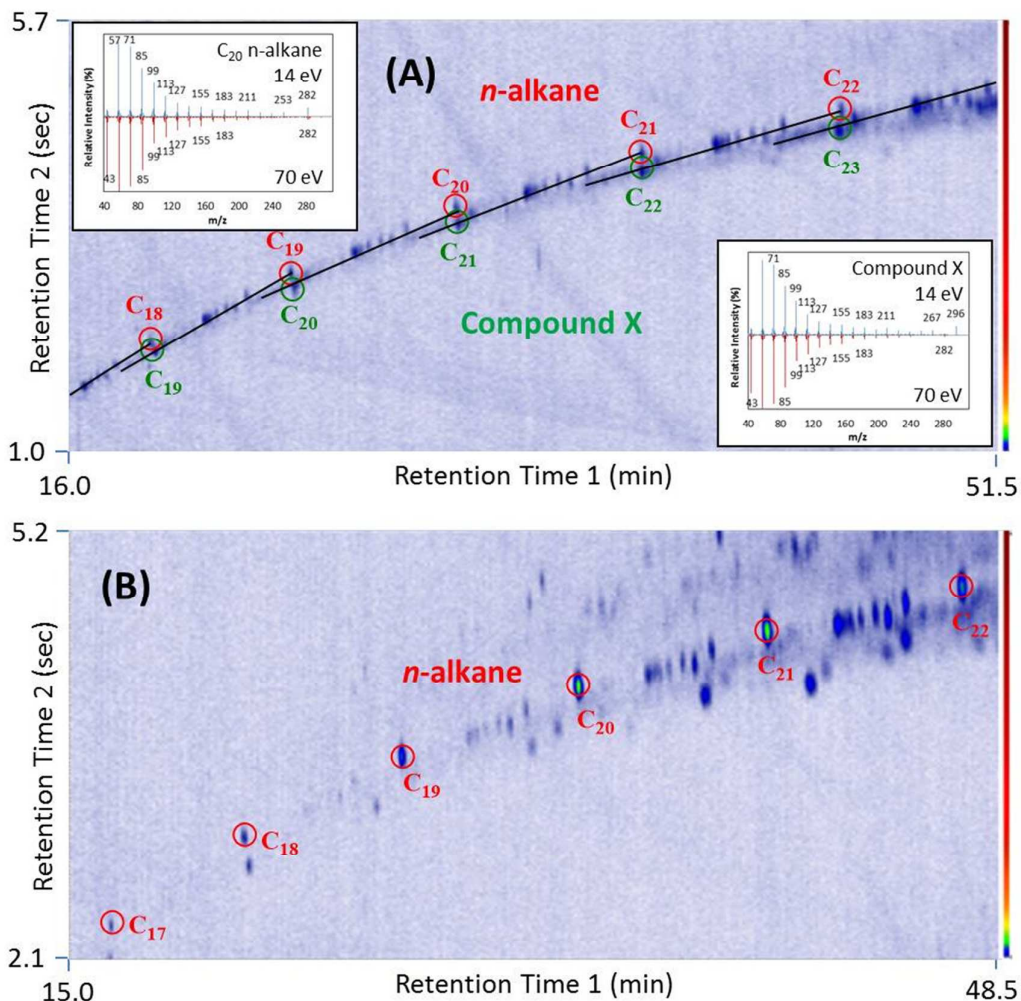
Figure 5. (A) Contour plot of synthetic motor oil 5W30 focusing on C_{18} to C_{22}. Red circles indicate *n*-alkanes, green circles indicate co-eluting species belonging to the isomer of the (*n+1*)-alkane. Embedded mass spectra of C_{20} *n*-alkane at 14eV and 70eV in top left and compound X in the bottom right, **(B)** Contour plot of mineral base oil signifying no co-eluting interfering species.
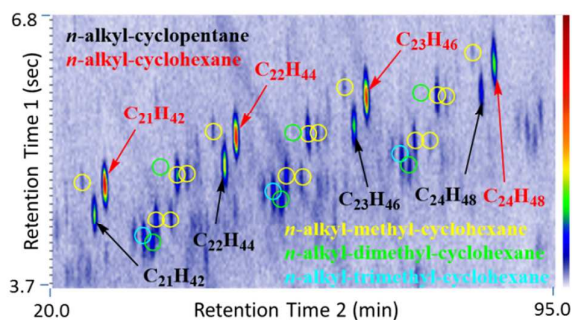
Figure 6. Selected ion chromatogram for summed mass fragments *m/z* 68+82+97+111, illustrating peaks identified for *n*-alkylcyclopentanes *m/z* 68 (black font); *n*-alkylcyclohexane *m/z* 82 (red font); alkyl-methyl-cyclohexanes *m/z* 82+97 (yellow circles); alkyl-dimethyl-cyclohexanes *m/z* 82+111 (green circles); and alkyl-trimethyl-cyclohexanes *m/z* 82+125 (light blue circles).

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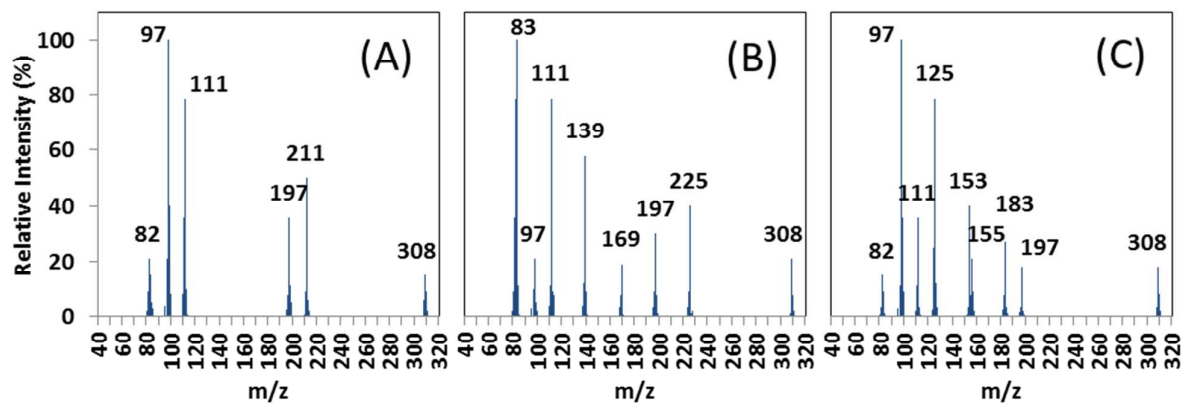
Figure 7. 14eV ionisation energy mass spectra of **(A)** 1-methyl-4-pentadecylcyclohexane, **(B)** (3-methylpentadecyl)cyclohexane, and **(C)** 1-methyl-4-(3-methyltetradecyl)cyclohexane

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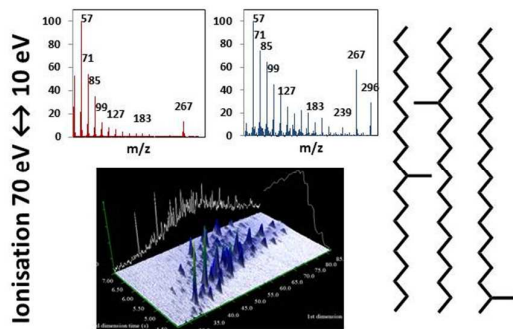699
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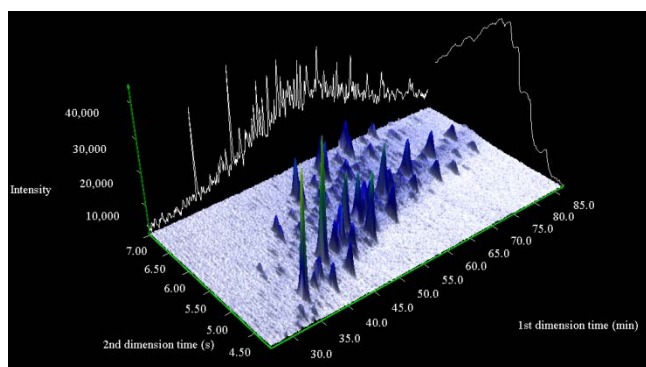709
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