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What about nitrogen? Using nitrogen as a carrier gas during the analysis of petroleum biomarkers by gas chromatography mass spectrometry

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A B S T R A C T

Gas chromatography mass spectrometry (GC–MS) is a commonly used method for organic geochemistry for both academic research and applications such as petroleum analysis. Gas chromatography requires a carrier gas, which needs to be both volatile and stable and in most organic geochemical applications helium or hydrogen have been used, with helium predominating for gas chromatography mass spectrometry. Helium, however, is becoming an increasingly scarce resource and is not sustainable. Hydrogen is the most commonly considered alternative carrier gas to helium but has characteristics that in certain respects make its use less practical, foremost is that hydrogen is flammable and explosive. But as hydrogen is increasingly used as a fuel, higher demand may also make its use less desirable. Here we show that nitrogen can be used for the GC–MS analysis of fossil lipid biomarkers. Using nitrogen, chromatographic separation of isomers and homologues can be achieved, but sensitivity is orders of magnitude less than for helium. It is reasonable to use nitrogen as a carrier gas in applications where low levels of detection are not needed, such as the characterization of samples of crude oil or foodstuffs, or potentially as part of a gas-mixture seeking to reduce helium-demand but maintain a level of chromatographic separation sufficient to support proxy-based characterizations of petroleum.

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1. Introduction

Because of its volatility, chemical inertness and minimal interference with electron impact ionization mass spectrometry helium is the most commonly used carrier gas for the analysis of fossil fuels by gas chromatography mass spectrometry (GC–MS) [1]. GC–MS can be applied in varied modes but in simultaneous ion monitoring entire mass spectra are not acquired, instead only specific ions indicative of homologous series are monitored. This has the benefit that time that would have been used to collect full mass spectra is instead focused to dwell on only a few ions and thereby greatly increase signal over noise (an example of application to terpane analysis is presented in Seifert et al. [2]). As it improved analysis of trace amounts of analyte within complex sample matrices GC–MS was transformative for applications such as characterizing lipids within food and microbial cultures [3] and the identification of hydrocarbons in fossil fuels [1].

The first chemical fossils (biomarkers) to be identified in fossil fuels were the petroporphyrins [4], but at the present day the most commonly considered chemical fossils are lipid-derivatives in the form of homologous series of hydrocarbon compounds [5]. GC–MS methods that use helium as a carrier gas to analyse hydrocarbon fossil lipid-derivatives or petroleum biomarkers originate from methods used for GC-FID analysis [6,7] which use hydrogen as a carrier gas. However, helium and hydrogen were not the only carrier gases considered for gas chromatography and at onepoint other carrier gases including nitrogen were considered [8]. It is not the case nitrogen is not used for the chromatographic separation of petroleum-components, but that its use has been limited to hyphenated gas chromatographic methods such as GC-ECD. Examples of GC-ECD methods for petroleum include determining proportions of volatile fractions of petroleum [9] or lubricating oils [10], as well to measuring halogenated and volatile organic compounds [11]. However, it is notable that these methods typically assay bulk fractions or offer high levels of specificity only for volatile organic components. These methods do not seek to achieve the relatively high chromatographic resolution needed to resolve individual isomers within the multiple homologous series that character-

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Table 1
GC–MS method comparison
Table 1 instrument parameters.

	Agilent 6890GC	Agilent 8890 GC
Carrier Gas	Nitrogen (N5.5)	Helium (N4.6)
Injector	S/SL	MMI
Injector Temp (°C)	300	300
Initial Oven temp (°C)	90	60
Initial time (min)	2.0	2.0
Rate 1 (°C/Min)	20.0	20.0
Final temp (°C)	120	120
Rate 2 (°C/Min)	4.0	4.0
Final temp (°C)	290	290
Mode	Constant Pressure (7 psi)	Constant Flow (1.2 ml/min)
Column Manufacturer / type	Agilent DB-5	Agilent HP5-MS
Dimensions	30.0 m × 250 µm × 0.25µm	30.0 m × 250 µm × 0.25µm
	Agilent 5975 MSD	Agilent 5977 MSD
EM Voltages (Ave)	2104	1360
Number of ions	27	38
Dwell time (ms)	40	40
MS Quad temp °C	150	150
MS Source temp °C	230	230

ize fossil lipid-derivatives, suggesting the potential of nitrogen as a carrier gas for analysing petroleum biomarkers has been overlooked.

As helium becomes a scarce material and demand for hydrogen rises [12], there is a need to find alternatives, both out of practical and ethical considerations. While problems in supply issues are most obvious for helium, hydrogen because of its flammable nature may not be used in all instances and while not scarce its use as a fuel may place a high demand on supply. Therefore, here are presented methods and results for nitrogen as a carrier gas, to demonstrate that nitrogen can be used for the GC–MS analysis of hydrocarbons and can provide sufficiently high chromatographic resolution to analyse structurally related compounds such as petroleum biomarkers.

2. Methods

Analyses were performed on saturate hydrocarbon fractions of petroleum, prepared by elution with hexane during silica-gel column chromatography [1]. This separates GC-amenable hydrocarbons from non-hydrocarbon fractions not amenable to GC–MS. No data are presented for whole oil analyses in this study. Methods used for both helium and nitrogen carrier gases are summarized in Table 1.

Research grade nitrogen (N5.5) was used as carrier gas for an Agilent 6890 GC connected to a 5975 MS electron impact ionization quadrupole mass spectrometer, with a split/splitless injector operating in splitless mode. The column used was an Agilent DB-5 (30 m length, 250 μm ID, and 0.25 μm film thickness). For use with nitrogen the oven temperature programme was 90 °C (2 min) rising to 120 °at 20 °C /min, then rising to 290 °C at 4 °C /min and hold at 290 °C for 23 min. For use with nitrogen as a carrier gas the instrument was operated in constant pressure mode with a column head pressure of 7 psi. By way of comparison, prior to this when the same instrument was used with helium and a similar column, the column head pressure was initially 9.98 psi and the starting temperature 60 °C. For use with nitrogen the mass spectrometer was operated at same temperatures used for helium, but the Electron multiplier voltage was approximately twice as high when using nitrogen, and the scan duration reduced by monitoring fewer ions in SIM mode (27 ions each with a dwell time of 40 ms, approximately half the cycle-time when the instrument was used with helium).

Analyses using helium as carrier gas were performed on an Agilent Technologies 8890 N GC fitted with a 5977 MSD, an Agilent HP-5-MS column (30 m length, 250 μm ID, and 0.25 μm film thickness) and multimode inlet injector. Oven temperature was programmed at 60 °C (2 min) to 120 °C at 4 °C /min then to 290 °C at 4 °C /min and held at 290 °C for 23 min. The SIM mode had 38 ions each with a dwell time of 40 ms.

The mass spectrometers of both instruments were calibrated using a pure standard of Perfluorotributylamine (supplied by Agilent) and the instruments inbuilt tuning procedure (same tuning procedure was used for helium and nitrogen). A quantification standard of 20R-5α,14α,17α(H)-[2,2,4,4-d_4] cholestane was obtained from Chiron AS, Norway and used to evaluate sensitivity. Compounds within oils were identified by their relative retention times and by reference to published chromatograms [1]. Oil samples used are listed in Table 2. These include a set of lighter oil samples that are genetically related (they were generated from the

Table 2
List of samples and descriptions
Table 2 sample description.

Sample Code in Manuscript	Description
Oils from three different reservoirs in same region suffixed by letters A, B and G:	Light crude oils (∼30° API) from a range of genetically related oil fields in the Middle East
Group A: A03S, A04S, A09S	These oil fields have oil generated from similar source rocks, but found in different reservoirs and fields, and have slightly different compositions.
Group B:BH40S, BH42S, BH140S	Data for sample GS46S shown in Figs. 1 and 2
Group G: GS46S	
GR02S	Intermediate crude oil (∼25° API), from shallow northern European field
ND0004S	Intermediate crude oil (∼25° API) from West Africa, with distinctive petroleum source rock

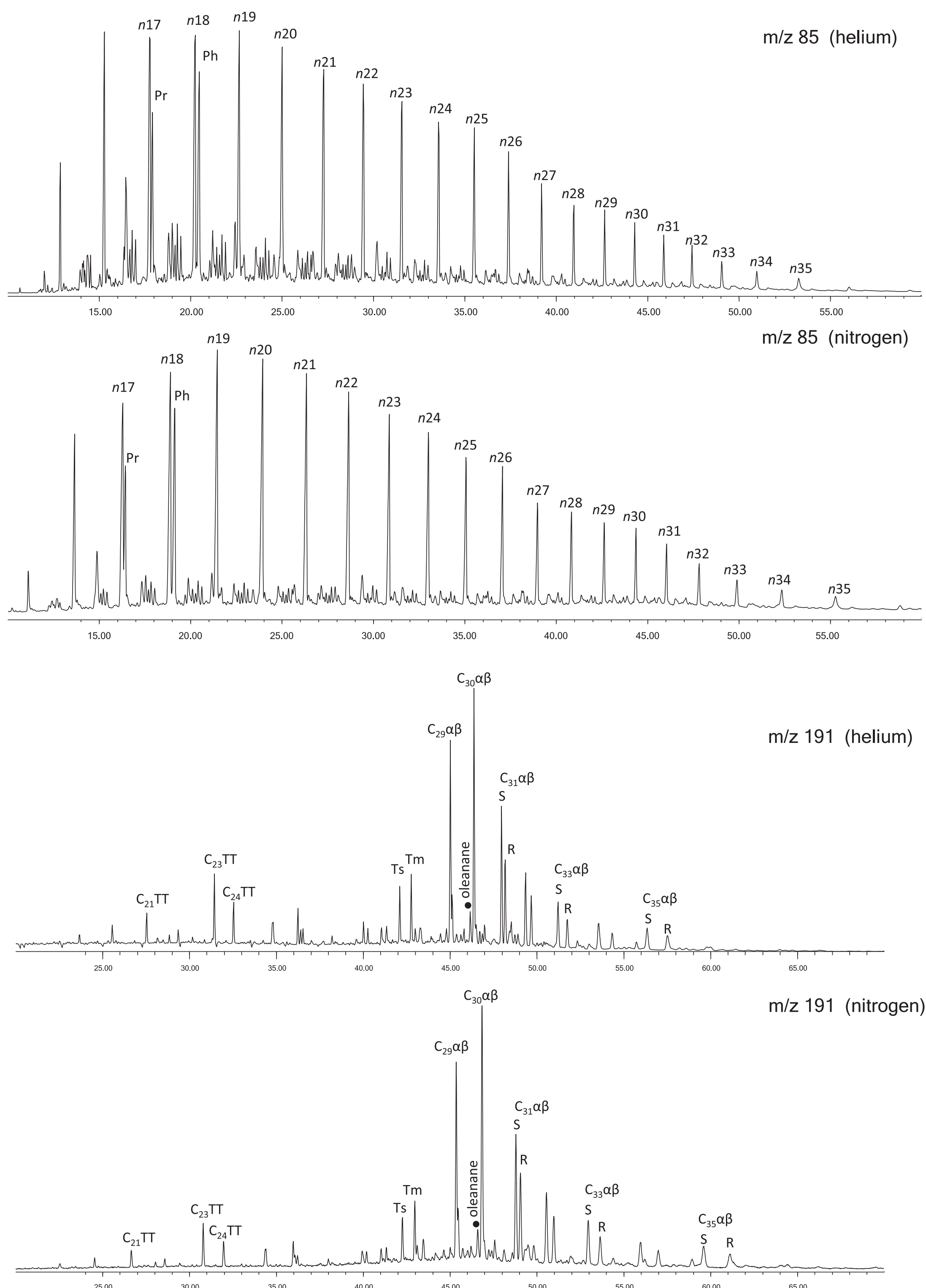

Fig. 1. Ion Chromatograms for the saturate fraction of sample GS46S. Note that chromatograms have not been aligned along the x-axis and thus differences in absolute retention time have been preserved. For *m/z* 85 ion chromatograms; *n*17 = C17 *n*-alkane, Pr = pristane (2,6,10,14 tetramethylpentadecane) Ph = phytane (3,7,11,15 tetramethylhexadecane). For *m/z* 191 ion chromatograms; C_{23}TT = C23 Tricyclic Terpane, Ts = C_{27} 18α(H)–22,29,30-Trisnorneohopane, Tm = C_{27} = 17α(H)–22,29,30 trisnorhopane, C_{29} $\alpha\beta$ = C_{29} 17α(H),21β(H) homohopane; C_{31} $\alpha\beta$ S = C_{31} 17 α(H),21β(H) 22S homohopane; C_{31} $\alpha\beta$ R = C_{31} 17 α(H),21β(H) 22R homohopane.

same source rocks, but were produced from different oil fields and reservoirs), and intermediate oils with varied biomarker characteristics from different petroleum systems.

The operating conditions in Table 1 do not represent those needed to create an equivalency but are used for demonstration purposes; e.g. to show what might be achievable using only nitrogen for GC–MS analysis for hydrocarbon biomarkers. Note also that by using these conditions and nitrogen as a carrier gas the instrument/product is not being operated within OEM (original equipment manufacturer) specifications.

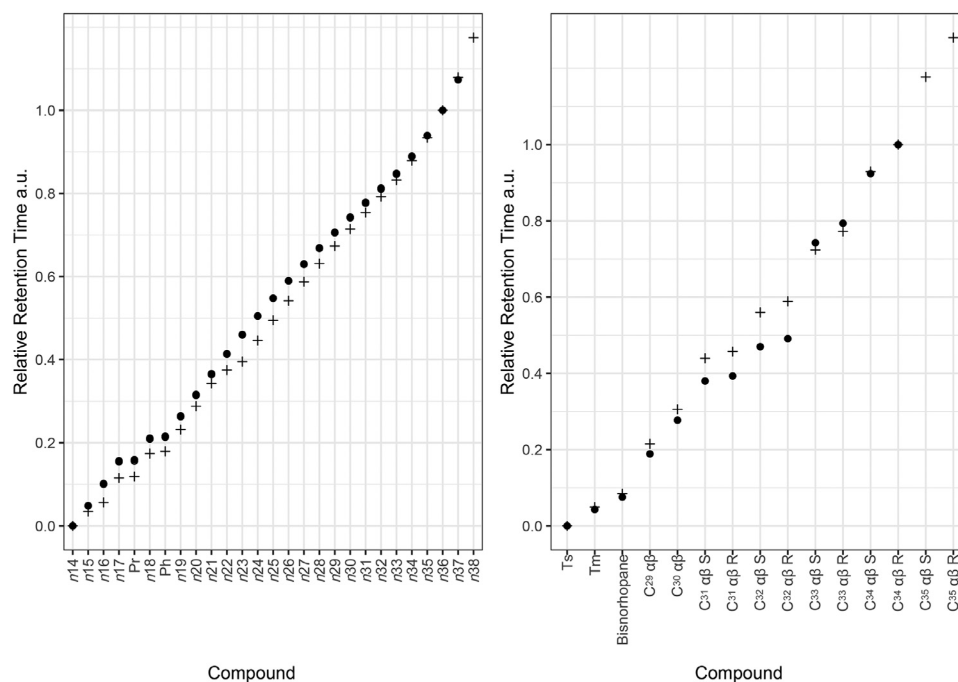

Fig. 2. Relative retention times for compounds labelled in Fig. 1 (sample GS46S); *n*17 = C17 *n*-alkane, Pr = pristane (2,6,10,14 tetramethylpentadecane) Ph = phytane (3,7,11,15 tetramethylhexadecane), Ts = C_{27} 17α(H)−22,29,30-Trisnorhopane, Tm = C_{27} Tm = 17α(H)−22,29,30 trisnorhopane, C_{29} $\alpha\beta$ = C_{29} 17α(H),21β(H) homohopane; C_{31} $\alpha\beta$ S = C_{31} 17 α(H),21β(H) 22S homohopane; C_{31} $\alpha\beta$ R = C_{31} 17 α(H),21β(H) 22R homohopane.

3. Results and discussion

Ion chromatograms for the saturate fraction of a light crude oil (samples GS46S in Table 1) are shown in Fig. 1, illustrating that the same range of components can be separated and identified using molecular nitrogen as with helium. Notable is the resolution of pristine and phytane from *n*-alkanes that elute at a similar time, as well as different tricyclic terpane and hopane homologues and isomers. Particularly noticeable are the separation of a compound called oleanane from C_{30} 14α, 17β (H) hopane. Both of these compounds have the same molecular mass and base fragments (*m/z* 191), thus their separation requires chromatography to be operating with a high degree of efficiency and carefully selected column phases and operating conditions. The separation of these kinds of structurally similar but genetically distinctive compounds is essential for organic geochemistry; oleanane is a unique biomarker for angiosperms and derives from oleanene and “appears” suddenly in the geological record [13], whereas hopanes and their potential precursor compounds in plants [14] and prokaryotes [15] are ubiquitous. Relative retention times for important members and isomers of two homologous series are plotted in Fig. 2, and while there are differences, it is not to the extent that elution order is altered to the degree that a reference standard or sample could no longer be used. Thus, if chromatographic separation alone is considered than nitrogen can be a suitable carrier gas for biomarker measurement in crude oil.

Chromatographic separation is only part of a GC–MS analysis as the sensitivity of the mass spectrometer is also crucial. In difference to helium, nitrogen is both denser and more easily ionized and thus would be expected to greatly attenuate analytical signals during electron impact ionization mass spectrometry. This effect can be seen in Fig. 3 for D4 cholestane, an analytical standard commonly used for quantifying petroleum biomarkers by GC–MS. When using nitrogen as a carrier gas, sensitivity is two orders of magnitude less than for helium. Orders of more noise is also observed when nitrogen is used as carrier gas. The low response of an analyte can be dealt with by concentrating procedures applied prior to analysis (e.g., isolation of hydrocarbon fractions as was done in this case), although for many of the chemical fossils within crude oil this may not be practical due to low absolute quantities of the original samples. For example, when analysing fluid inclusions (fluid inclusions are micron-dimensioned inclusions of oil present in individual grains and crystals in rocks) [16] the total amount of analyte available is very low, thus a concentration stage may not be analytically feasible. Furthermore, the relative concentration of a biomarker within a fraction of oil can also be proportionally low, and in such a case urea adduction can be used to isolate more abundant components such as *n*-alkanes and selectively enrich terpane-biomarkers [17].

Rather than measuring and quantifying absolute quantities, it is common to report measurements of hydrocarbon biomarkers and fossil-lipids in the form of proxies or chemometric parameters. These dimensionless numbers are calculated by measures of relative abundance taken directly from an ion chromatogram (e.g. peak height or area). These proxies find use in assessing such things as the depositional environments of the sedimentary organic matter that generated the fossil organic matter, and subsurface geological processes related to heating and biodegradation [1]. For some of these proxies comparisons are made within a dataset (e.g. measurements within a batch are compared to each other), but in other cases reference is made to an external point of reference. Examples of proxies making use of external points of reference include thermal maturity parameters (proxies that relate to the heating of an oil on a geological timescale) [18] but also parameters that reflect the type of fossil organic matter from which the oil was generated, such as the ratio of the abundances of pristane and phytane [19]. Thus, it is necessary to have sufficient similarity and reproducibility between data acquired with helium and nitrogen to compare within a dataset acquired on a single instrument, and also between different laboratories using different instruments. Fig. 4 plots data for two examples of thermal maturity parameters abbreviated to “Ts/Ts+Tm” and “Hopane 22S” for

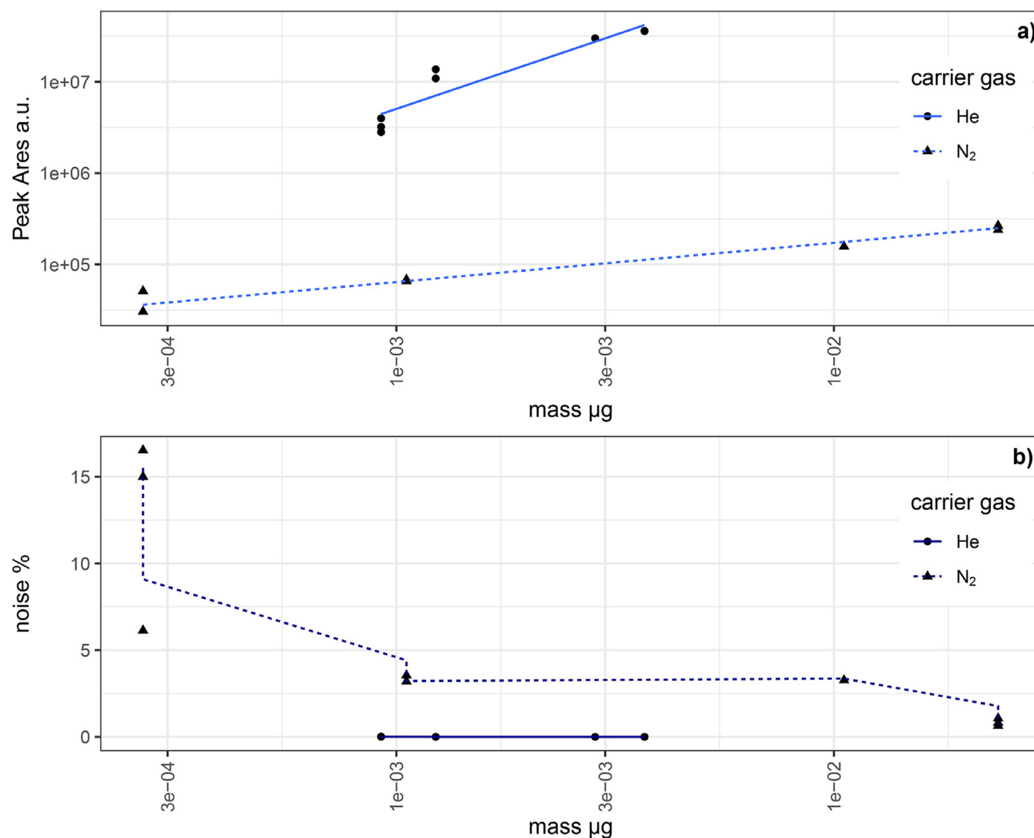

Fig. 3. Sensitivity analysis using D4 cholestane and its main ion *m/z* 221. Data shown for a) peak area per gram of sample and b) the% noise determined nearest eluting noise peak. Lines connecting data are visual aids only.

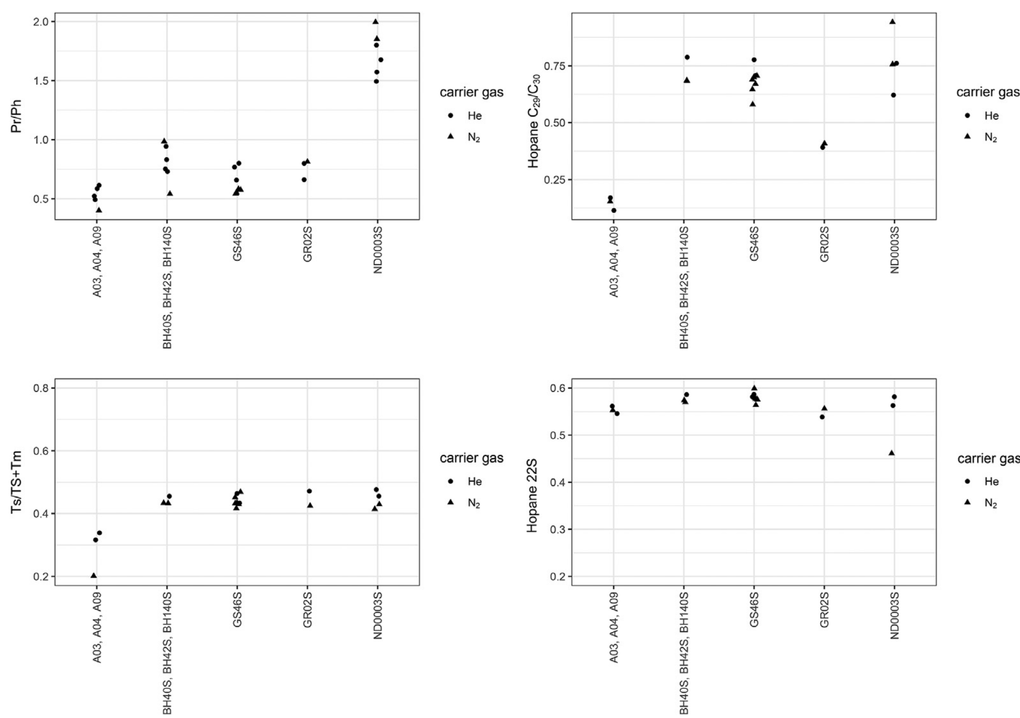

Fig. 4. Biomarker parameters calculated from peak areas, units are dimensionless numbers. Parameters on y-axis calculated as; Pr/Ph = pristane/phytane; Hopane C_{29}/C_{30} = C_{29} 17α(H),21β(H) homohopane / C_{30} 17α(H),21β(H) homohopane; Ts/Ts+Tm = C_{27} 18α(H)–22,29,30-Trisnorneohopane/ C_{27} 18α(H)–22,29,30-Trisnorneohopane + 17α(H)–22,29,30 Trisnorhopane; Hopane 22S = C_{31} 17 α(H),21β(H) 22S homohopane/ C_{31} 17 α(H),21β(H) 22S homohopane + C_{31} 17 α(H),21β(H) 22R homohopane.

which an external point of reference is essential, and also the ratio of pristane to phytane (abbreviated to Pr/Ph) which is commonly interpreted by use of an external reference. Data are also shown for the C_{29}/C_{30} $\alpha\beta$ hopane parameter, which is more typically interpreted in a qualitative way (difference within a dataset). Significant differences in value of the Pr/Ph parameter would typically be values < 1.0, values from 1 to 1.5 and values greater than 2.0 [17], and from figure it can been seen that differences between samples from different oil families are much greater than those with different carrier gases. A similar clustering of values by sample and not by sampling method is seen for the C29/C30 hopane parameter [1]. Indicating that it would be reasonable to substitute measurements made using nitrogen as a carriers gas for those using helium as a carrier gas for these parameters. For thermal maturity parameters the key point of reference is external to the dataset; for the Ts/Ts+Tm parameter values less than 0.3 indicate low thermal maturity, whereas values of 0.7 correspond to peak oil generation [16]. Thus, for thermal maturity parameters its necessary that values measured with nitrogen as a carrier gas correspond with those measured with helium and this can be seen to be the case in Fig. 4. Therefore, parameters used for non-quantitative analysis of biomarkers and fossil-lipids within oils can be shown to be reliably measured with nitrogen.

4. Conclusion

At present GC–MS instruments are not built and designed to use nitrogen as a carrier gas. Despite this it can be shown that petroleum biomarkers can be analysed using a nitrogen carrier and the resultant elution order is the same as that obtained for helium with small differences of less than 5% in relative retention time. While nitrogen supports good chromatographic separation, sensitivity for terpane petroleum biomarkers is likely much less, with signal to noise ratios becoming significant for peaks corresponding to masses less than 1 nanogram (1000 less sensitivity than when helium is used). Chemometric parameters based on biomarkers were also found to be comparable; for example significant differences in the Pr/Ph ratio of <1, 1–1.5 and >2 were conserved for both nitrogen and helium. The lower sensitivity for a nitrogen carrier gas in GC–MS is only likely to be significant in applications when the total mass of oil is small and concentration procedures can not be used. Therefore, when pressures exist on helium supply, it is reasonable to consider nitrogen or mixtures including nitrogen for GC–MS analysis of hydrocarbons and fossil-lipid petroleum biomarkers.

CRediT authorship contribution statement

Colin W. Taylor; Conceived idea, performed laboratory work, wrote manuscript.

Stephen A. Bowden; data visualisation, performed laboratory work and wrote manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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