Effects of Ramp Rate and Starting Temperature on Gas Chromatography-Mass Spectrometric Analyses of Petroleum Hydrocarbons

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Received 30 July 2020; accepted 30 October 2020, published online 03 November 2020

Abstract

The quest for better GC-MS/FID starting temperature and ramping conditions for the quantification of hydrocarbons in our environment necessitated this study. A surrogate n-alkane standard was screened using nine GC-MS conditions involving the alteration of ramp rates and/or initial temperatures. There was observed increase in the TIC chromatogram or ionic mass unit as the ramp rate or initial temperatures were increased. The peak areas of the analytes were significantly affected. The R^2 and response factor values of the eight standard calibration curves (each for a modified method) varied from 0.9224 – 0.9971 and 0.0034 – 0.0045 respectively. Consequently, the quantification of the diesel concentration in diesel spiked water and soil by using the eight methods was different from each other and from the standard method. Average differences of 11.5 and 15 % from the theoretical values were observed for the water and soil analyses respectively. Increasing ramp rates or initial temperatures led to shorter throughput but less data accuracy. The use of 60 °C as starting temperature and ramp at 5 °C/minute was better for quantification of the diesel range organics.

Key words: Methods modification, Gas Chromatograph-Mass Spectrometric analyses, Petroleum Hydrocarbons

Introduction

The Gas Chromatography-Mass Spectrometer is a very sensitive and robust spectrometric instrument for the evaluation of the quality and quantity of various organic compounds including the petroleum hydrocarbons in the environment. The use of the instrument involves proper adjustment, control and monitoring of some basic parameters such as the ramp rate and initial temperature which could affect its analytical performance. The United State Environmental Protection Agency (USEPA 1994) recommended initial temperature of 40 °C and ramp rate of 5 °C/minute for the analyses of the diesel Range Organic (DRO). Recently, the discrepancy in analytical results method specified type 4 column for DRO analyses. The other screening conditions for this standard method are stated in Table 1. To determine both the total quantity of pollutants

on some oil polluted samples sent to three laboratories was reported [1]. The researchers presumed the differences observed to be due to different laboratory equipment and instrumental conditions which may include the screening conditions of the GC-MS/FID used by the analysts [1]. “Users, government laboratories and private laboratories sometimes disagree on the scope, range of application and quality of information obtained from using different methods” [2]. Analysis of DRO by United State Environmental Protection Agency (USEPA) methods, 8015, [3] or modified USEPA method 8015B [4] is a commonplace in most environmental analytical laboratories. The and the composition such as petroleum oils in the soil, an efficient instrumental procedure should be followed. This is more so when the allowable amount of a pollutant in an

environmental matrix is very small. For instance, the maximum allowable contamination level of BTEX (benzene, toluene, ethyl benzene and xylene) in drinking water (in ppm) is 0.005, 2.0, 0.66 and 0.44 respectively [5]. Thus, the importance of using appropriate GC-MS/FID screening condition for BTEX and indeed total hydrocarbon analyses cannot be overemphasised. Following the USEPA

specifications [3], it would take about 55 minutes to complete the GC-MS screening of samples for DRO analysis. In attempts to achieve shorter throughput periods, several laboratories have resorted to increasing ramp rate and/or starting temperature. The key to such aim is a method that is both fast and robust, allowing the laboratory to achieve shorter analysis time and at lowest cost.

Table 1: USEPA method 8015, GC-MS/FID screening conditions [3]

Column 4 type:	Column 4 (DROs)x
Carrier gas (Helium) flow rate:	5-7 mL/minute
Make up gas (Helium) flow rate:	30 mL/min
Injector temperature:	200 °C
Detector temperature:	340 °C
Temperature program	
Initial temperature:	45 °C, hold 3 minute
Program:	45 °C to 275 °C at 12 °C/min
Final temperature	275 °C, hold 12 min

Some studies have shown the effects of ramp rate and temperature on the throughput period of GC-MS/FID analyses [6]. Fernandez *et al* [6] carried out GC-FID analysis of diesel spiked soil at initial temperature and ramp rate of 60 °C and 5 °C/minute. They used about 50 minutes to complete analysis of a sample. In a similar analysis, Paula *et al* [13] used initial temperature of 40 °C and ramp rate of 5 °C/minute and got a throughput period of about 55 minutes per sample. In order to run a similar analysis at a shorter throughput, Corte *et al* [2] used starting temperature of 40 °C and increased ramp rate to 15 °C/minute and they completed the analysis in less than 30 minutes per sample. Similarly, Rauckyte, *et al* used modified USEPA method 8015B, using initial temperature of 45 °C and ramp rate of 12 °C/minute and they completed the analysis of DRO sample at less than 35 minutes [14]. Throughput periods of less than 20 minutes per sample using the oven technology have been advertised by GC-MS/FID manufacturers [7]. Ramp rate and initial temperature are important parameters in gas chromatographic - mass spectrometric (GC-MS/FID) analyses. Adjustment of these parameters could have analytical implication on the retention time indices and fragmentation pattern as well as quantification of organic

compounds. Retention time (t_R) relates to the column temperature by the equation:[8]

$$t_R = L(k+1)/\bar{u} \quad (1)$$

Where k is the solute capacity ratio (capacity factor, retention factor),

L is the column length and $\bar{u}$ is the average carrier gas velocity.

The value of retention factor is related to column temperature (T_c) by

$$\ln K = \left(\frac{A}{T_c}\right) + B \quad (2)$$

Where constants A and B have unique values for every compound and every stationary phase type and phase volume ratio [8]

Diesel is a petroleum mixture containing about 18 hydrocarbons ranging mainly from decane (C10) to octacosane (C28) [4]. As a mixture, its boiling point varies 170 – 430 °C [4, 15, 16]. The DRO are prominent members of petro-oil contaminants in the environment [17].

The GC-MS/FID identification and quantification of organic substances including the DRO is common and have been very successful. Qualifications of the unknown DRO involve the study of the fragmentation pattern and identification of the M^+ fragments and calculation of retention time indices (e.g Kovats retention index) of individual member [8, 20, 21]. On the other hand, quantification analyses

involve the integration of the area under the individual compound peaks (i.e. peak area) [18]. Sometime, Internal standard (IS) is introduced during the GC-MS/FID analysis and the peak area ratio (peak area of the analyte/peak area of (IS) is calculated and applied for the standard calibration [18]. Some electronic programmes such as the Thermo X CaliburQual browser have been developed to achieve this [22]. The pattern in a chromatogram emerged as a decisive factor of qualitative analysis. Preliminary identifications were made by visual comparison of chromatogram profiles between the unknown and a set of standards prior to calculation of Kovats or relative retention index for each component. Increasing the starting temperature and ramp rate may affect the fragmentation pattern of DRO. Qualitatively, GC-MS analyses of DRO in all environmental matrices involve identification of the mass of the M^+ fractions from the mass spectra. Sometimes, the relative abundance of this fragment could be very small and may be overshadowed by background noise thereby making its identification somehow difficult visually. To ease the identification, the mass spectrum is exported to standard mass spectrometric data base such as by The National Institute of Standards and Technology (NIST) where it is automatically matched with existing similar mass spectra in the data base [19, 20]. The analyst is then left to consider the best match. The approach may give room for subjectivity and probability. It is often difficult to find exact match. This may be due to the different GC-MS/FID screening conditions from which the standard spectrometric data were generated. A better knowledge of how DRO fragmentation relates to GC-MS/FID screening conditions may ameliorate the difficulties researchers often encounter in the course of identifying DRO or organic compounds in environmental matrices.

At a starting temperature of 100 °C and ramp rate of 10 °C/minute, it would take about 25 minutes (as investigated in this study) to complete a similar analysis reported by Corte *et al* (mentioned earlier) [2]. This could save the life span of GC-MS machine and make such analyses time saving and more economical. However, shorter retention time would

obviously, be expected for every hydrocarbon present when compared to the method used by Ferná'ndez *et al* [6]. In this study, we investigated the extent to which the use of different ramp rates and starting temperatures could affect spectrometric qualification and quantification of the DRO. Eight GC-MS different screening conditions involving adjustment of the initial temperatures and ramp rates were evaluated with the view to assessing their effects on the fragmentation pattern of individual DRO. Also assessed, is the effects of the environmental matrices from which the DRO were extracted on their fragmentation pattern. The unique fragmentation of DRO compound may be a symbol of its identity. Every compound has specific GC-MS retention time or index but this depends on the nature of the GC machine and the status of the column as well as the screening conditions [8, 19, 20]. The fragmentation pattern is also unique for families of compounds [19, 20, 21]. Therefore, a good knowledge of the fragmentation of a compound in GC-MS analyses could be a useful aid in identifying the presence of such compound in a given environmental matrix by GC-MS/FID analyses. In this study, we specifically aimed at: 1) evaluating the effects of increasing starting temperature and/or ramp rate on the fragmentation pattern of petro-genic diesel compounds. 2) assess how starting temperature and ramp rate relate to quantification of DRO by GC-MS and 3) compare the accuracy of quantification of DRO (in water and soil) by the 8 modified methods to that of standard USEPA Method 8015B.

Materials and methods

Two Surrogate standards of DRO (containing 1000 µg/ml 16 or 21 DRO compounds were purchased. The first surrogate hereafter refers to as SStd 1 was supplied by Restek Plc Bellefonte, and the second surrogate hereafter refer to as SStd 2 was purchased from Sigma-Aldrich Plc. UK. The said standards have been certified by Restek and Supelco laboratories respectively. According to the certificate of analysis, SStd 1 and SStd2 contain 1000µg/ml each of saturated alkanes C10 – C25 and C7 – C30 respectively. Their GC-MS chromatograms as well as the conditions of analyses and certificate of analysis

were contained in the literatures that accompanied the standards. The SStd 1 was used to assess the effects of the different GC-MS screening conditions on the qualification and peak area of DRO compounds while SStd2 was used as calibration reference to evaluate the effects of the 8 GC-MS screening conditions and US ES EPA method 8015B on quantification of DRO in diesel spiked water and soil. Commercial diesel (branded ESSO) was procured from a local petrol station in Bangor Gwynedd, sharp sand soil (branded as washed sharp sand) was obtained from B and Q Plc. Bangor Gwynedd; de-ionized water was obtained locally from School of Chemistry, Bangor University, Bangor Gwynedd, all in the United Kingdom (UK) and 1,3,5 trichlorobenzene (TCB) and dichloromethane (DCM), HPLG grade were bought from Sigma-Aldrich plc.,UK. 1,3,5 trichlorobenzene was used as the suitable internal standard because its retention time does not overlap with those of the samples. The experimental work was carried out at the School of Chemistry laboratory, Bangor University, Bangor Gwynedd, North Wales, UK.

The use of Internal Standard

1,3,5 trichlorobenzene was used as the suitable internal standard because its retention time does not overlap with those of the DRO. The internal standard (25 g) was dissolved in 250 ml of n-hexane or DCM in a 1 dm³ volumetric flask and made up to mark with the solvent. The n-hexane form was used in the preparation of the SStd 1 standard solutions while the DCM form is used

in the preparation of the SStd 2 standard calibration solutions [9].

Samples treatment and extraction

Soil:

The soil was air dried under ambient laboratory conditions and passed through 2 mm sieve. Then 10 g (12,246 µl) diesel (of density 0.81 g/cm³) was added to 250 ml of petroleum spirit in a 1 litre volumetric flask and made up to mark as the spiking solution. 100 ml of the spiking solution was then added to 10 g of soil in a 250 ml beaker. A blank sample was also made by measuring same volume into a pre-weighed beaker. Both samples were prepared in triplicates and were kept in a fume cupboard overnight for the petroleum spirit to evaporate completely. The blank samples were re-weighed as a control to ascertain the actual diesel left in the soil after the evaporation of the spirit. Both the soil and the blank samples were then gently extracted by Vortex method [13] for 1 hour each using 30 ml DCM as the extracting solvent. The mixtures were passed through Fisher brand (150 mm) filter paper and each residue was washed twice with additional 10 ml (each time) of the extracting solvent. The extracts were combined accordingly in a 50 ml volumetric flask and made up to mark with the extracting solvent. The extracts were then filled into 1 ml GC-MS vials for analyses by the nine selected GC-MS screening conditions including the USEPA method 8015B (Table 2) after adding 0.65 g of TCB as internal standard.

Table 2: The eight modified GC-MS screening (Used in this study) and USEPA 8015B Methods [4]

GC-MS conditions (SC)	Screening	Starting Temp.(°C)	Ramp rate (°C/min)	Final Temp.(°C)
1 (SC 1)		40	5	300
2 (SC 2)		40	10	300
3 (SC 3)		60	5	300
4 (SC 4)		60	10	300
5 (SC 5)		80	5	300
6 (SC 6)		80	10	300
7 (SC 7)		100	5	300
8 (SC 8)		100	10	300
US EPA Method 8015B [4]		45	12	275

Water samples

100 ml of de-ionized water was measured into 250 ml conical flask. 1 g (1,235 µl) diesel (of density 0.81 g/cm) was added. The samples were prepared in 3 triplicates and left on the bench top for 1 hour to stabilize. At the end of the period, they were poured into different separating funnels. To each funnel was added 40 ml of the extracting solvent and shaken vigorously for 5 minutes and clamped to rest. After 15 minutes rest, the aqueous layer was separated and re-extracted twice with 10 ml each of the extracting solvent. The three portions (DCM) extract were added up and 5 g of sodium sulphate was added to the extract; shaken vigorously for about 3 minutes and passed through a Fisher Scientific 150 mm brand filter paper and washed twice with additional 10 ml (each time) of DCM. The combined extract was then made up to mark in 100 ml volumetric flask with the extracting solvent. All the extracts were then screened (with GC-MS by the nine screening conditions (Table 2) after the addition of 1.25 g of TCB to each of the flask as internal standard.

Analyses of samples

Qualification analyses:

Qualification analyses of DRO by GC-MS involve the identification of individual hydrocarbon in the series [19, 20, 21]. It includes examining the GC chromatograms and the mass spectra for compounds fragmentation patterns and calculation of retention indices. In this work, the surrogate standard (SStd 1) was given 100% dilution with n-hexane and screened by the 8 GCMS screening conditions. The observed changes in the compounds fragmentation were shown in Tables 4.

Quantification analyses:

Unlike qualification analyses, GC-MS quantification of organic compounds (including

the DRO) aims at specifying the quantity or concentration of an analyte in a given sample. It involves the integration of the peak area of individual compound in the GC-Mass spectra [18, 19]. Several peak area integrating program such as the Thermo X caliburQual Browser have been developed in recent years [22]. The peak area of a compound is a direct function of its concentration in a given sample. The two variables obey linear regression relationship such that:

$$PA = mx + c \quad (3)$$

Where PA and x = peak area and concentration of the analyte respectively, m and c are the slope and intercept of the curve respectively. To minimise instrumental difference on triplicated analyses, a known compound refer to as internal standard is introduced into the extracts prior to filling the extracts into the GC-MS vials [18]. The peak area of each analyte is divided by that of the internal standard during data processing to give peak area ratio (PAR) which could be substituted into equation 3:

$$PAR = mx = c \quad (4)$$

In this study, the effects of 8 different GC-MS screening conditions (Table 3).on the PAR of DRO in SStd 1 were investigated

Standard calibrations:

Quantification of DRO in environmental matrices (by GC-MS analyses) includes the making of standard calibration curves from known DRO standards. From the standard curve, the values of m and c (equation 1 or 2) are calculated hence the amount of the analyte in a given sample [18 – 22]. From equation 2, it is obvious that any defect or change on the PAR, could equally affect the standard calibration curve and hence the quantification of the analyte. In this study, standard calibration curves were made from GC-MS screening of the same concentrations of the second surrogate standard (SStd 2) with the 8 different selected conditions and USEPA Method 8015B (Table 2). The

summations of the PAR of the DRO were plotted against the concentrations of the corresponding standard solution. (SStd 2).

In a perfect correlation between PAR and concentration of an analyte, the correlation coefficient (R^2 value) should be 1. However the PAR of DRO responds to concentration of DRO in the standard solution is found from the percentage of linearity as:

$$\% \text{ linearity} = 1 - (1 - R^2) \times 100 \quad (5)$$

Examination of the R^2 (correlation coefficient), the slopes (or response factor) and percentage linearity values could show the best GC-MS running condition among the eight in comparison to the USEPA Method 8015B.

Fragmentation pattern of DRO from different sources and concentration

To assess the influence of sources of DRO on the fragmentation pattern of the of individual

compound, extracts from diesel polluted water and soil, commercial diesel and crude oil were GC-MS screened using GC-MS condition 8 (SC 8). Different standard solutions of the commercial diesel were also made in DCM and screened with same GC-MS to assess the effects of concentration on the fragmentation of the DRO compounds. In this study, we report the results from diesel spiked soils and water

Samples programming in the GC-MS used for this study

The mode and spectrometric quality of the GC-MS used in this study is enlisted in table 2. The samples were fed in the GC-MS machine and programmed for the nine screening conditions in such a way that one sample is completely screened on the nine methods before the next one. The samples were screened in one batch. The other conditions of the GCMS as stated (Table 3) was same for all the nine variable conditions.

Table 3: Other conditions of the GC-MS machine used for the analyses

GC-MS mode	Trace 1300 GC/ITQ900 Ion Trap Mass Spectrometer
Column type	DB 5
Column size	30 m x 0.25 mm with 0.25 μ m film
Electron volts	130 eV
Carrier gas	Helium
Gas flow rate	1.5 ml/min.
Inlet temp.	250 °C
Injection mode	Split
Split flow	10.0 ml/min.
Septum purge flow	5.0 ml/min.:
Solvent delay	3 mins.
Initial temp. holding time	3 mins.
Final temp. holding time	2 mins.
Transfer line temp.	300 °C
Ion source temp.	200 °C
Mass range	35 – 500 amu.

Quality control and safety

Analytical precautions were taken to ensure high quality and safety of the analyses. The standards

and samples were prepared in triplicates of 3 each. The analyses were carried out in the same

batch. The spiked soil samples were kept in the fume cupboard for evaporation of the petroleum spirit. Extraction of the soil samples were also carried out in the fume cupboard with the beakers covered with washing glasses. Gravimetric (weight) and volumetric measurement were made using four digital electronic balances and well calibrated measuring cylinders respectively. Micro-pipette (Eppendorf UK Model) was used to fill the samples extracts into the GC-MS vials with new pipette tip being used per sample. The extraction efficiency was 98.7 and 99.4 for the soil and water samples respectively

Data analyses and statistics

The spectrometric data were processed using Thermo X CaliburQual Browser. The processed data were then exported to Microsoft Excel version 2010 for statistical analysis. The peak area ratio was calculated as:

$$PAR = PAs/PAI \quad (6)$$

Where PAs is the peak area of individual diesel hydrocarbon and PAI is the peak area of the internal standard.

The effect of the screening conditions on each DRO member was evaluated statistically using SSPS (one way Anova). Mean Values, Standard deviation, Standard error, relative standard deviation (RSD) and correlation coefficient for the analytical results were evaluated and calibration done using Excel program version 2010.

Results and Discussion

The aim of this analysis is to assess the effect of changing ramp rate or initial temperature on quantification of DRO in environmental matrices. It is mainly on comparing the screening conditions on the qualification and quantification of DRO. As a result, the physico-chemical properties of the soil and solvent extraction efficiency of n-hexane were not reported. It is obvious that whatever quantity of DRO in the soil and water samples should be quantified similarly if the nine screening conditions were with no significant difference

Retention time and Fragmentation pattern of DRO

Retention time is a good preliminary identification property of every organic compound but it varies according to type and status of the GC machine used as well as the screening conditions. Normally, temperature affects the retention time as shown by equation 1. However, the retention time of an analyt screened under the same condition and with the same GC machine would be relatively same irrespective of the environmental sources of the compound as was the case in this study too. We observed that the retention time of each compound of the DRO in the standards and those extracted from water and soil; when screen under the same condition only varied infinitesimally. But increase in the starting temperature decreased the compounds retention time at the average of 0.20 minute per 1 °C rise in starting temperature.

As already mentioned in introduction section, fragmentation pattern of organic compounds in Mass spectroscopy is a major preliminary identification parameter [18 – 20]. Generally aliphatic alkanes show common fragmentation of 39, 41, 43, 57, 71, 85 and 99 [10] corresponding to positive ions of cyclopropene, cyclopropyl, propyl, butyl, pentyl, hexyl and heptyl respectively. However, the abundance of a fragment may vary from one alkane compound to another. The GC-MS analyses of n-alkane surrogate, commercial diesel, and extract from diesel spiked soil and water samples were carried out at initial temperature of 40°C and ramp rate of 10°C (results not shown). Other GC-MS screening conditions for this experiment were as stated (Table 3) and the fragmentation pattern of the DRO hydrocarbons were carefully evaluated. A similar exercise was also carried out with different concentration of commercial diesel. In both cases, there was no significant difference observed in the type and relative abundance of the basic fragments formed which showed that sources and concentrations of DRO have no significant effect on the fragmentation nature of the DRO hydrocarbons present. But when the surrogate standard was screened under the eight modified conditions, obvious differences were noticed among the various fragments formed. For instance, in tetradecane,

the fragment, 41 has the highest abundance (intensity) of 100 % when screened under condition 1 (SC 1) but this changed to fragment

57 when screening condition was changed to condition eight (SC 8, Figures 1 and 2, Table 4).

Figure 1: Mass spectrum of Tetradecane screened at ramp rate of 5 °C/min and starting temperature of 40 °C (SC 1, Table 2)

Figure 2: Mass spectrum of Tetradecane screened at ramp rate of 10 °C/min and starting temperature of 100 °C (SC 8, Table 2)

In a similar way, Eicosane has fragment 71 as second in abundance when screened at lower temperature but this fragment became third in abundance when high starting temperature were applied.

Table 4: Fragmentation pattern of DRO by 8 GC-MS screening conditions (SC 1 –SC 8)

DRO	SC 1 (St = 40 °C, Rr = 5 °C/min)	SC 2 (St = 40 °C, Rr = 10 °C/min)	SC 3 (St = 60 °C, Rr = 5 °C/min)	SC 4 (St = 60 °C, Rr = 10 °C/min)	SC 5 (St = 80 °C, Rr = 5 °C/min)	SC 6 (St = 80 °C, Rr = 10 °C/min)	SC 7 (St = 100 °C, Rr = 5 °C/min)	SC 8 (St = 100 °C, Rr = 10 °C/min)
C10	41,57,39,4 3,71,85,99	41,57, 43,39,71,8 5,99	41,57, 43,39,71,8 5,99	41,57,43,39, 71,85,99	41,57,43,3 9,71,85,99	41,57,43,3 9,71,85,99	41,57,43,39, 71,85,99	ND
C11	41,57,43,3 9,71,85,99	41,57,43,7 1,39,85,99	41,57, 43,39,71,8 5,99	41,57,43,(39 ,71),85,99	41,57,43,3 9,71,85,99	41,57,43,(3 9,71),85,99	41,57,43, 71, 39,85,99	41,57,43,39, 71,85,99
C12	41,57,43,7 1,39,85,99	41,57,43,7 1,39,85,99	41,57, 43,(39,71), 85,99	41,57,43,71, 39,85,99	41,57,43,7 1,39,85,99	41,57,43,(3 9,71),85,99	41,57,43, 71, 39,85,99	41,57,43,39, 71,85,99
C13	41,57,43,7 1,39,85,99	41,57,43,7 1,39,85,99	41,57,43,7 1,39,85,99	41,57,43,71, 39,85,99	41,57,43,7 1,39,85,99	41,57,43, 71,39, 85,99	41,57,43, 71, 39,85,99	41,57,43, 71, 39,85,99
C14	41,57,43,7 1,39,85,99	41,57,43,7 1,39,85,99	41,57,43,7 1,39,85,99	41,57,43,71, (39,85),99	41,57,43,7 1,39,85,99	41,57,43, 71,39, 85,99	57,41, 43, 71, (39,85),99	57,41,43,71, 39,85, 99
C15	41,57 (43,71),85, 39,99	57,41,71,4 3,85,39,99	41,57,43, 71, (39,85),99	57,41,(43,71 , 85,39,99	57,41,43,7 1,39,85,99	57,41,43,7 1, 85, 39,99	57,41,(43, 71), 39,85,99	57,41,71,43, 85, 39, 99
C16	57,41,71,4 3,85,39,99	57,41,71,4 3,85,39,99	57,41,43, 71,85, 39,99	(41,57),71,4 3,85,39,99	57,41,(43,7 1),(39,85), 99	57,41, 71, 43, 85, 39,99	57,41, 71, 43, 85,39, 99	57,41,71,43, 85, 39, 99
C17	57,41,71,4 3,85,39,99	57,41,71,4 3,85,39,99	57,41,71,4 3,85, 39,99	57,41,71,43, 85,39,99	57,41, 71,43, 85,39, 99	57,41, 71, 43, 85, 39,99	57,41,71,43, 85,39,99	57,41,71,43, 85, 39, 99
C18	57,41,71,4 3,85,39,99	57,71,41, 43,85,39,9 9	57,41,71,4 3,85, 39,99	57,41,71,43, 85,39,99	57,41, 71,43, 85,39, 99	57,41, 71, 43, 85, 39,99	57,41,71,43, 85,39,99	57,41,71,43, 85, 39, 99
C19	57,41,71,4 3,85,(39,99)	57,71,41,(4 3,85),(39,9 9)	57,41,71,4 3,85, 39,99	57,41,71,43, 85,39,99	57,41, 71,43, 85,39, 99	57,41, 71, 43, 85, 39,99	57,41,71,43, 85,39,99	57,41,71,43, 85, 39, 99
C20	57,71,41,(4 3,85),(39,9 9)	57,71,41, 85,43,99,3 9	57,41,71,4 3,85, 39,99	57,41,71,43, 85,39,99	57,41, 71,43, 85,39, 99	57,41, 71, 43, 85, 39,99	57,41,71,43, 85,39,99	57,41,71,43, 85, 39, 99
C21	57,71,41,8 5,43,99,39	57,71,41, 85,43,99,3 9	57,41,71,4 3,85, 39,99	57,41,71,43, 85,39,99	57,41, 71,43, 85,39, 99	57,41, 71, 43, 85, 39,99	57,(41,71),4 3,85,39,99	57,41,71,43, 85, 39, 99
C22	57,71,41,8 5,43,99,39	57, 71,41, 85,43,99,3 9	57,41,71,4 3,85, (39,99)	57, 71,41, 43,85,39,99	57,41, 71,43, 85,39, 99	57,41, 71, 43, 85, 39,99	57, 71,41, 43,85,39,99	57,41,71,43, 85, 39, 99
C23	57,71,41,8 5,43,99,39	57,71,85,4 1,43,99,39	57, 71,41, 43,85, (39,99)	57, 71,41, 43,85,39,99	57, 71,41, 43, 85,(39, 99)	57, 71, 41, 43, 85, 39,99	57, 71,41, 43,85,39, 99	57,41,71,43, 85, 39, 99
C24	57,71,85,4 1, 43,99,39	57,71,85,4 1,43,99,39	57,71,41, (43,85,) (39,99)	57,71,41, 43,85,(39,99)	57,71,41, 43, 85,(39, 99)	57,(41,71), 43, 85, 39,99	57,71,41, 43,85, 99,39	57,(41,71),4 3,85, (39, 99)

C25	57,71, 85,41, 43,99,39	57,71,85,4 1,43,99,39	57, 71,41, (43,85), (39,99)	57, 71,41, (43,85),(39, 99)	57, 71,41, 85,43, 99,39	57, 71,41,(43, 85), (39,99)	57, 71,41, (43,85),(39, 43,85, 99,39	57, 71,41, (43,85),(39, 43,85, 99,39
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NB The first fragment in each group is of 100% abundance.

Another effect of starting temperature was the non-detection of decane in the surrogate standard when starting temperature of 100 °C was used (Figures 3 and 4).

Figure 3: GC-MS chromatogram of 16 n- alkane surrogate (SStd 1) screened at ramp rate of 5 °C/min. and starting temperature of 40 °C (Nb. The peaks at 3.32 and 14.32 are for solvent and internal standard respectively)

Figure 4: GC-MS chromatogram of the same n- alkane surrogate (SStd 1) screened at ramp rate of 10 °C/min. and starting temperature of 100 °C (Nb. The peak at 4.16 is for internal standard)

It was observed that in figure 4, decane (C10) was not captured by the mass spectrometer which could be attributed to the too high starting temperature. Therefore, this starting temperature may be too high for the analysis of DRO in

environmental matrices if decane is one of the compounds in mind of the analysts to be qualified or quantified. It is pertinent to note that SStd 1 was certified to contain 16 DRO (i.e., C10 – C25) by Restek Plc, Bellefonte and the

company's Mass spectrometric chromatogram for this surrogate standard solution contained exactly 16 peaks.

Quantification of organic compounds by GC-MS methods

The quantification of organic compounds in environmental matrices is based on the integration of the peak area or the peak ratio [18] since this quantity is in linear regression relationship with the concentration of the analytes. The procedure requires making a calibration curve (s) from known standard(s). Therefore, a poor or inefficient standard calibration has analytical implication on the quantification of the analyte. In this study, the effect of GC-MS screening conditions on the peak area and peak area ratio of DRO in the surrogate standard (SStd 1) was examined

(Table 5). It was observed that as the ramp rate or starting temperature changed, changes were observed in the peak areas for all the compounds analysed by the 8 GC-MS conditions. For instance, Dodecane (C12) has the PAR of 2.40 and 1.99 when screened by condition 1 (SC 1) and condition 2 (SC 2) respectively. The difference between these two screening conditions is the ramp rate. In both methods, the starting temperature was 40 °C but the ramp rate was changed from 5 °C/min. in (SC 1) to 10 °C/min. in condition 2 (SC 2 Table 2). Similarly, Cosane (C20) has PAR of 5.46 and 4.65 when screened at the same ramp rate of 5 °C/minute but different starting temperature of 60 °C and 100 °C respectively. In general, relative standard deviation of $\pm 6 - 11\%$ was observed across the DRO from decane to pentacosane.

Table 5: The PAR of individual DRO in the surrogate standard (SStd 1) by the 8 different GC-MS screening conditions

DRO	SC 1 PAR	SC 2 PAR	SC 3 PAR	SC 4 PAR	SC 5 PAR	SC 6 PAR	SC 7 PAR	SC 8 PAR	AVE PAR	STDEV	RSD
C10	2.14	1.99	2.33	2.14	2.31	2.08	ND	ND	2.16	0.13	6.00
C11	2.35	2.25	2.67	2.36	2.63	2.35	2.66	2.52	2.47	0.17	7.00
C12	2.35	2.25	2.67	2.36	2.63	2.35	2.66	2.52	2.47	0.17	7.00
C13	2.70	2.58	3.05	2.70	3.03	2.31	3.01	2.79	2.77	0.26	9.00
C14	3.01	2.89	3.40	2.98	3.41	2.93	3.42	3.07	3.14	0.23	7.00
C15	3.30	3.20	3.71	3.22	3.70	3.19	3.74	3.28	3.42	0.25	7.00
C16	3.56	3.46	4.06	3.50	4.01	3.42	4.07	3.51	3.70	0.29	8.00
C17	3.90	3.79	4.43	3.82	4.36	3.71	4.42	3.75	4.02	0.32	8.00
C18	4.25	4.13	4.80	4.17	4.72	4.01	4.78	4.03	4.36	0.35	8.00
C19	4.65	4.51	5.25	4.66	5.10	4.36	5.18	4.39	4.76	0.36	8.00
C20	5.00	4.74	5.46	4.77	5.50	4.66	5.56	4.65	5.04	0.40	8.00
C21	5.41	5.05	5.66	5.08	5.89	4.99	5.97	4.91	5.37	0.42	8.00
C22	5.91	5.51	5.94	5.28	6.21	5.26	6.29	5.25	5.71	0.43	8.00
C23	6.59	5.94	6.36	5.63	6.59	5.59	6.75	5.63	6.13	0.49	8.00
C24	7.18	6.47	6.77	5.84	6.99	5.77	7.22	6.03	6.53	0.60	9.00
C25	7.73	6.89	7.17	6.12	7.35	5.97	7.60	6.30	6.89	0.69	10.00

Effect of different GC-MS screening methods on the quantification of DRO in water and soil

Standard calibration curves

To quantify the amount of diesel in the polluted soil and water by the 8 GC-MS methods,

standard solutions of the surrogate SStd 2 were made and 8 standard calibrations were drawn using Excel program version 2010. Table 6

present the correlation coefficients (R^2 values) and response factor and percentage of linearity of the standard curves.

Table 6 Correlation coefficient and percentage response of 8 GC-MS screening conditions on surrogate standard (SStd 2)

GC-MS Method	R^2 values	Response factor	% of correlation
1 (SC 1)	0.9932	0.0045	99.32
2 (SC 2)	0.9870	0.0045	98.70
3 (SC 3)	0.9971	0.0040	99.71
4 (SC 4)	0.9810	0.0040	98.10
5 (SC 5)	0.9805	0.0039	98.05
6 (SC 6)	0.9722	0.0044	97.22
7 (SC 7)	0.9305	0.0034	93.05
8 (SC 8)	0.9224	0.0036	92.24

In a perfect correlation between the PAR and concentration of the DRO, R^2 value should be 1. The percentage correlation or linearity was calculated from equation 5. The standard samples have been screened in the same batch at eight different screening conditions. The correlation coefficient (R^2) or percentage of linearity of the various standard calibration have indicated that GC-MS method 3 (SC 3) was the best condition for GC-MS screening of the DRO by this study. However the R^2 values are in a

narrow range of 0.922 – 0.997 with average difference of 0.01

Effect of different GC-MS screening Methods on the quantification of DRO in water

The quantity of the selected DRO in diesel spiked de-ionized water was calculated by using the different calibration (linear) equations and compared to the theoretical value (Table 7).

Table 7 Concentrations of DRO in diesel spiked water by 9 GC-MS screening methods

GC-MS methods (SC)	R^2 vaues	TV of DRO (mg/l)	EV of (DRO (mg/)	% diff.of EV from TV
1 (SC 1)	0.9932	927.52	878.73	5.26 (<)
2 (SC 2)	0.9870	927.52	1026.95	10.72 (>)
3 (SC 3)	0.9971	927.52	916.39	1.20 (<)
4 (SC 4)	0.9810	927.52	959.89	3.49 (>)
5 (SC 5)	0.9805	927.52	1006.73	8.54 (>)
6 (SC 6)	0.9722	927.52	1016.19	9.56 (>)
7 (SC 7)	0.9305	927.52	1185.42	27.81 (>)
8 (SC 8)	0.9224	927.52	1212.36	30.71 (>)
USEPA Method 8015B	0.9987	927.52	937.26	1.05 (>)

Nb TV and EV = theoretical and experimental values of the DRO in the water respectively, < or > indicates the EV is less than or greater than the theoretical values respectively

The result obtained showed that no one of the GC-MS screening conditions (including the USEPA Method 8015B) could exactly quantify the amount of DRO in the diesel spiked water. In chemical analyses for pollutants in environmental matrices it is not usually possible to achieve 100% extraction hence in some cases; a less values may be got. In other cases, the presence of impurity may lead to higher amount. This is the case with several analytical reports. In any case TNRCC1005 (11) recommended a difference of not greater than 25% in method verification. The result in Table 7, showed that all the GC-MS screening conditions were within the limit of TNCC 1005(11) except condition SC 8 which gave verification difference far greater than the aforementioned limit. The difference could be trace to the calibration quality of this GC-MS screening method which as well gave the lowest R^2 and percentage of linearity values. When the results accuracy were

compared with regards to the theoretical values of the DRO in the spiked water, the USEPA method 8015B gave the best result with a minor 1.05% differences between the theoretical and experimental DRO values. And as for the 8 developed methods, method 3 (SC 3) gave the closest result to the USEPA method 8015B with a differences of 1.20% between the theoretical and experimental DRO values in the water samples (Table 7)

Effect of different GC-MS screening methods on the quantification of DRO in soil

Like the water analyses, similar quantification of the amount of the DRO in the diesel spiked soil samples was made. The results presented in table 8 shows that GC-MS screening methods have similar quantification of DRO in the soils as in water.

Table 8 Concentrations of the DRO in diesel spiked soil by 9 GC-MS screening methods

GC-MS methods	R^2 values	TV of DRO (mg/g)	EV of DRO (mg/g)	% diff.of EV from TV
1 (SC 1)	0.9932	862.02	783.92	9.06 (<)
2 (SC 2)	0.987	862.02	996.41	15.59 (>)
3 (SC 3)	0.9971	862.02	829.18	3.81 (<)
4 (SC 4)	0.981	862.02	899.26	4.32 (>)
5 (SC 5)	0.9805	862.02	957.36	11.06 (>)
6 (SC 6)	0.9722	862.02	943.48	9.45 (>)
7 (SC 7)	0.9305	862.02	1150.28	33.44 (>)
8 (SC 8)	0.9224	862.02	1161.66	34.76 (>)
USEPA Method 8015B	0.9984	862.02	871.07	1.87 (>)

Again, the results showed that the GC-MS screening methods gave quantification of the DRO in the diesel spiked soil with differences from the theoretical values. However, method 1 – 6 (SC 1 – SC 6), have quantification differences within the TNRCC 1005 recommendation [11]. Methods 7 and 8 (SC7 & SC 8) gave higher quantification and their percentage differences from the theoretical values were far greater than the recommended limit [11]. The reason for this may also be attributed to their low response factors as shown in Table 6. The high ramp rate or initial

temperature of these methods may be the reasons for the low responsive calibration curves for the standard samples. Again, the USEPA method 8015B and method 3 (SC 3) gave the best results with differences of 1.87 and 3.81 % respectively between the theoretical and experimental values of DRO in the diesel spiked soils

Conclusion

Variation in the use of ramp rates and initial temperatures has been observed in the usage of GC-MS/FID for petroleum hydrocarbons analyses by analysts. The use of various starting temperatures and/or ramp rates could lead to significant difference in the results from a given laboratory analysis. Discrepancies in results on the same sample sent to different laboratories have been reported. The quest for better GC-MS screening conditions for the qualification and quantification of petroleum hydrocarbons in our environment called for this study. The results have shown that increase in ramp rate and/or initial temperature has analytical effects on GC-MS analyses of the diesel range organics in particular and by extension hydrocarbons in general. Shorter throughput time in the analyses was obtained by increasing ramp rate or initial temperature but less accuracy for the quantification of DRO in soil and water. For the purpose of monitoring and regulating the concentration of DRO in the environment, initial temperature of 60 °C and ramp rate of 5 °C/min is by this study buttressed. Furthermore, temperature had noticeable effects on the fragmentation patterns of compounds but concentration or sources of contaminants showed no significant effect on the fragmentation profile or on the retention time of DRO analysed at a specified initial temperature and ramp rate.

Acknowledgement

The correspondence author, Oghoje U. S wishes to acknowledge the entire staff of School of Chemistry, Bangor University, Bangor Gwynned, North Wales, United Kingdom, where this study was carried out particularly Dr Vera Thoss and the GC-MS Technicians, Tobby Pactrick, Mr Gwyver Glynne and Mr Nicholas. Furthermore, the authors are grateful to the Petroleum Technology Development Fund (PTDF) for partial funding of the Ph.D. project from which this publication is sought.

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