Development of an Analytical Method using Gas Chromatography – Mass Spectrometry (GC-MS) to Quantify Hydrocarbons Released into the Environment during an Oil Spill

Scottish Marine and Freshwater Science Vol 11 No 6

S Cunningham, G Hermann, L Webster and K Yates

marine scotland

Development of an Analytical Method using Gas Chromatography – Mass Spectrometry (GC-MS) to Quantify Hydrocarbons Released into the Environment during an Oil Spill

Scottish Marine and Freshwater Science Vol 11 No 6

S Cunningham, G Hermann, L Webster and K Yates (Robert Gordon University)

Marine Scotland is the directorate of the Scottish Government responsible for the integrated management of Scotland's seas. Marine Scotland Science (formerly Fisheries Research Services) provides expert scientific and technical advice on marine and fisheries issues. Scottish Marine and freshwater Science is a series of reports that publishes results of research and monitoring carried out by Marine Scotland Science. It also publishes the results of marine and freshwater scientific work that has been carried out for Marine Scotland under external commission. These reports are not subject to formal external peer-review.

This report presents the results of work commissioned by Marine Scotland Science.

Development of an Analytical Method using Gas Chromatography – Mass Spectrometry (GC-MS) to Quantify Hydrocarbons Released into the Environment during an Oil Spill

S Cunningham, G Hermann, L Webster and K Yates (Robert Gordon University)

Marine Scotland Science, Marine Laboratory
375 Victoria Road, Aberdeen, AB11 9DB

Executive Summary

A gas chromatography – mass spectrometry (GC-MS) method was developed and validated for the determination of n-alkanes and isoprenoids (pristane and phytane) in fish (trout muscle), using seven deuterated internal standards. Reflux saponification coupled with liquid-liquid extraction was utilised for the extraction of n-alkanes. Clean up and fractionation was performed in order to minimise interferences and obtain the aliphatic fraction from the extract. Chromatographic determination by GC-MS was applied to the separation and identification of n-C_{10} to n-C_{35}, as well as pristane and phytane. The newly developed method was validated and compared to a pre-existing method used by Marine Scotland Science (MSS) based on a two-component internal standard containing squalane and heptamethylnonane (HMN).

Introduction

Petroleum, often also known as crude oil, is a liquid mixture composed of organic contaminants that can be released into the environment during an oil spill. Petroleum typically comprises of 60-75% aliphatic hydrocarbons by weight, however, this can differ between sources (Heimann, Karthikeyan and Muthu, 2016). A lower percentage of polycyclic aromatic hydrocarbons (PAHs) are usually present though these compounds have a significantly more toxic and persistent effect on the environment. Derived from petroleum and similar to unbranched normal alkanes (n-alkanes), pristane (C_{19}) is an acyclic saturated aliphatic hydrocarbon compound which is classed as a norterpene and is formed from the loss of a methyl group within phytane (C_{20}) (Stauffer, Dolan and Newman, 2008). Both pristane and phytane are natural constituents of crude oil and chromatographically elute as a doublet with n-heptadecane (n-C_{17}) and n-octadecane (n-C_{18}), respectively.

n-Alkanes are non-polar molecules that tend to be unreactive compounds (Stauffer, Dolan and Newman, 2008). Therefore, due to their chemical stability in the

environment, they exhibit low susceptibility to microbial degradation; hence their widespread use for oil spill source identification (Artifon, Zanardi-Lamardo and Fillmann, 2019). Each crude oil product contains a specific hydrocarbon composition. This can allow for the identification of the source and/or origin of the petroleum spill if the hydrocarbon concentration and profile is known, and also liability (Nwadinigwe and Alumona, 2018). However, a number of weathering processes for example, evaporation, dispersion, oxidation, biodegradation can considerably alter the fate including chemical and physical properties of oil spills. For example evaporation leads to the loss of the more volatile fractions present such as n-dodecane (n-C_{10}) and n-undecane (n-C_{11}), which may change the oil profile.

While n-alkanes can occur naturally, generated from the decomposition of marine and terrestrial plants and animals, hydrocarbon contamination in the environment is predominantly a result of anthropogenic activity. Anthropogenic sources include industrial effluents and municipal waste, urban runoff, automotive emissions and marine transportation (Adeniji, Okoh and Okoh, 2017). Atmospheric deposition of hydrocarbons into water sources often occurs as the result of combustion processes (Benson and Essien, 2009). Natural oil spillage can also come from underwater cracks in drilling wells, this can cause seepage that can result in the contamination of marine ecosystems. This also includes gas exploration and production offshore.

There are several different types of oil products that differ based on their viscosity, volatility and toxicity. Gasoline products are extremely volatile therefore evaporate shortly after release into the environment. Diesel products or light crude oils tend to form thin slicks on the water surface and are also subject to evaporation. Medium crude oil products are less volatile so the evaporation process is significantly slower and can, therefore, result in fur-bearing mammals being smothered due to persistent oil slicks. Heavy crude oil and residual products can cause a great deal of problems due to their thick and sticky properties on the water surface resulting in little evaporation or dissolution thus increased danger to marine life. Beyond the heavy crude oils are sinking oils which will sink in the water column making the clean-up process significantly more challenging and these types of oils are less effected by weathering in comparison to gasoline or diesel products (Wang, Fingas and Page, 1999).

Geochemical biomarkers such as steranes and triterpanes can also be used in conjunction with n-alkanes for oil spill fingerprinting (Webster *et al.,* 2011). PAHs are, however, of more concern due to their distribution and persistence in the environment, along with their potential to bioaccumulate in marine species and exhibit toxic effects including carcinogenic, mutagenic and teratogenic effects

(Chirwa and Bezza, 2015). On January 5th 1993, the oil tanker MV *Braer* grounded on Garths Ness, Shetland, releasing approximately 85,000 tons of light crude oil. As a result harmful naphthenic and aromatic crude was released into the marine environment consequently prohibiting the harvesting of farmed or wild fish and shellfish (Webster *et al.,* 1997). The ability to detect the presence of *n*-alkanes and biomarkers can help confirm oil spill occurrences and ultimately the presence of PAH contamination in marine life.

Due to their low hydrophobicity, hydrocarbons, including *n*-alkanes can bioaccumulate in marine organisms where they have a high affinity for the macromolecules present within the organism (El-Namr *et al.,* 2003). This can be by direct uptake across permeable membranes such as the gills or skin, ingestion of suspended particulates or by ingesting contaminated food. As a result of the MV *Braer* oil tanker releasing hydrocarbons into the marine environment, under The Food and Environment Protection Act (1985), a fisheries exclusion zone was set up to prevent contaminated seafood from reaching the market (Webster *et al.,* 1997).

Following an oil spill incident it is crucial that the extent of the contamination is ascertained with immediate effect. This includes determining the extent of contamination on marine life and also the risk to public health. If the oil spill is concluded to have a significant impact on the environment then a monitoring programme is introduced which will include the collection of marine samples including fish, shellfish, sediment and water. These samples will be analysed for hydrocarbons including toxic PAHs which can cause a great deal of harm to marine organisms but also have the potential to contaminate sea food for human consumption; and *n*-alkanes and geochemical biomarkers for their use in oil spill fingerprinting and source identification which ultimately can determine liability.

Currently PAHs and biomarkers are analysed for by GC-MS at MSS (Webster *et al.,* 2017) and *n*-alkane analysis was previously performed by gas chromatography – flame ionisation detection (GC-FID) using squalane and heptamethylnonane (HMN) as internal standards. MSS have since moved from GC-FID to GC-MS for the detection and quantification of *n*-alkanes, maintaining the use of squalane and HMN as internal standards. However, using GC-MS can allow for the use of deuterated internal standards which hold similar physical and chemical properties as the target analytes though do not interfere with the analysis. In comparison to GC-FID, GC-MS has the capability of distinguishing between deuterated and non-deuterated compounds based on their molecular weight though retention times remain the same. Therefore, this work will investigate the utilisation of deuterated internal standards using GC-MS for *n*-alkane analysis in fish (trout muscle).

The proposed method was performed with the use of seven deuterated internal standards, similar to the reported literature by Mazeas and Budzinski (2001). Although, the study was based around sediment samples, it is believed that a similar instrumental technique can be applied to trout muscle samples. At this time, there is limited literature on *n*-alkane analysis in biota by GC-MS however the method is often applied to PAH analysis in the same matrix (Tolosa *et al.,* 2005; Webster *et al.,* 2017). This report describes the method validation for GC-MS quantification of *n*-alkanes using deuterated internal standards. The two-component internal standard containing squalane and HMN was included in the validation so a comparison could be made between the newly proposed deuterated internal standard method and the current two-component internal standard method.

Materials and Methods

Biota Samples

Trout samples exposed to portions of Forties crude oil for the purposes of an oil spill response sensory panel, were utilised for the extraction validation process.

Materials

Dr Ehrenstorfer Alkanes-Mix 10 certified reference standard (500 µg ml^{-1} in Toluene) was obtained from QMX, United Kingdom. This standard contained 26 *n*-alkanes (*n*-Decane, *n*-Undecane, *n*-Dodecane, *n*-Tridecane, *n*-Tetradecane, *n*-Pentadecane, *n*-Hexadecane, *n*-Heptadecane, *n*-Octadecane, *n*-Nonadecane, *n*-Eicosane, *n*-Heneicosane, *n*-Docosane, *n*-Tricosane, *n*-Tetracosane, *n*-Pentacosane, *n*-Hexacosane, *n*-Heptacosane, *n*-Octacosane, *n*-Nonacosane, *n*-Triacontane, *n*-Hentriacontane, *n*-Dotriacontane, *n*-Tritriacontane, *n*-Tetratriacontane, *n*-Pentatriacontane). Pristane standard (Sigma-Aldrich, United Kingdom) and Phytane standard (QMX, United Kingdom) were also included in the calibration standards. A range of deuterated internal standards were employed for the analysis, these included *n*-Dodecane d26, *n*-Tetradecane d30, *n*-Pentadecane d32, *n*-Hexadecane d34, *n*-Eicosane d42, *n*-Tetracosane d50 and *n*-Triacontane d62 (Cambridge Isotope Laboratories, United Kingdom). In addition, a two-component internal standard containing Squalane and Heptamethylnonane (HMN) (Sigma-Aldrich, United Kingdom) were also used for comparison purposes.

All solvents used were of HPLC grade including dichloromethane (DCM), *iso*-hexane, methanol and water (Rathburn Chemicals Ltd Walkerburn, Scotland). Anhydrous sodium sulphate (Fisher Scientific, United Kingdom) and methanolic

sodium hydroxide (VMR Chemicals, United Kingdom) were also used during the extraction process.

From the alkane-mix certified reference standard containing the 26 n-alkanes (n-C$_{10}$ to n-C$_{35}$) a solution was prepared in *iso*-hexane to give a nominal concentration of 50 µg ml^{-1}. In addition, a solution of pristane and phytane was prepared in *iso*-hexane to give a nominal concentration of 50 µg ml^{-1}. Both standard working stock solutions (50 µg ml^{-1}) were used to prepare the calibration standard solutions.
A solution of the seven deuterated internal standards was prepared in *iso*-hexane to give a nominal concentration of 50 µg ml^{-1}, which was also included in the preparation of the calibration standards at 1 µg ml^{-1}.

Extraction of Hydrocarbons from Fish Muscle

In a round bottom flask, trout muscle tissue (~ 7 g) was spiked with the two-component internal standard mix (squalane and HMN) and the deuterated internal standard mix containing n-dodecane d26, n-tetradecane d30, n-pentadecane d32, n-hexadecane d34, n-eicosane d42, n-tetracosane d50 and n-triacontane d62. Methanolic sodium hydroxide (10%) in methanol/water (90:10 v/v, 40 ml) and anti-bumping granules were added to the flask. The mixture was refluxed for 3 hours 45 minutes, 10 ml of water was then added and refluxed for a further 15 minutes. Thereafter, the hot solution was extracted with *iso*-hexane (2 x 80 ml), the final combined extract was washed three times with water (40 ml). The extract was then passed through a glass column containing anhydrous sodium sulphate and collected in a flask. The extract was concentrated to approximately 1-2 ml by rotary evaporation and further by nitrogen blow down (approximately 500 µl). Clean up and fractionation was performed using normal phase high performance liquid chromatography (HPLC) to obtain the aliphatic fraction. The aliphatic fraction was collected and concentrated to approximately 50 µl to be analysed for n-alkanes by GC-MS.

Determination of the Aliphatic Hydrocarbons by GC-MS

The aliphatic fraction was analysed for n-alkanes by way of GC-MS using an HP6890 Series gas chromatography interfaced with an HP5973 MSD. Automated cool on-column injections were achieved using an auto injector. The chromatographic column used was a fused silica capillary column (HP5-MS, 30 m x 0.25 mm id, 0.25 µm film thickness). Analysis was performed with helium as the carrier gas, controlled using the constant flow mode at 0.7 ml min^{-1}. Injections were performed at 50°C and the oven temperature was held constant for three minutes.

Thereafter, the temperature was raised at 20ºC/min up to 100ºC followed by a ramp of 4ºC/min up to 270ºC, 40ºC/min up to 290ºC and then another ramp of 40ºC/min up to a final temperature of 300ºC. The MS was set for selective ion monitoring (SIM) mode at 70 eV with a dwell time of 50 msec. A total of seven deuterated internal standards and 28 *n*-alkane compounds were analysed; the quantifier and qualifier ions are displayed in Table 1 and Table 2, respectively.

In order to obtain the relevant quantifier and qualifier ions seen in Table 1 and Table 2, a solution of 50 µg ml^{-1} deuterated internal standard mix, a solution of 10 µg ml^{-1} *n*-alkane standard mix and a solution of 100 µg ml^{-1} pristane and phytane mix was injected into the GC-MS to firstly determine retention times. Analysis was performed in full scan mode to obtain the most abundant and characteristic ions to use as qualifier and quantifier ions. This included the molecular ion for each compound as this is unique to a specific compound. Once these parameters had been established, they were inputted into the GC-MS method, from there analysis could then be performed in selective ion monitoring (SIM) mode where detection was based on the identified qualifier and quantifier ions.

Table 1
Quantifier and Qualifier ions measured using the GC-MS for the seven deuterated internal standards and their respective retention times.

Deuterated Internal Standard	Retention time (minutes)	Quantifier ion (m/z)	Qualifier ions (m/z)	
			Most abundant ions	Molecular ion
n-Dodecane d26	11.0	66.1	50.1, 82.1	196.3
n-Tetradecane d30	16.1	66.1	50.1, 82.1	228.4
n-Pentadecane d32	18.8	66.1	50.1, 82.1	244.5
n-Hexadecane d34	21.6	66.1	50.1, 82.1	260.5
n-Eicosane d42	31.8	66.1	50.1, 82.1	324.6
n-Tetracosane d50	40.4	66.1	50.1, 82.1	388.8
n-Triacontane d62	50.2	66.1	50.1, 82.1	485.0

Table 2

Quantifier and qualifier ions measured using the GC-MS for the *n*-alkane compounds including pristane and phytane, and their respective retention times.

Compound	Retention time (minutes)	Quantifier ion (m/z)	Qualifier ions (m/z)	
			Most abundant ions	Molecular ion
n-Decane (*n*-C$_{10}$)	7.5	57.1	71.1, 85.1	142.1
n-Undecane (*n*-C$_{11}$)	9.3	57.1	71.1, 85.1	156.2
n-Dodecane (*n*-C$_{12}$)	11.4	57.1	71.1, 85.1	170.2
n-Tridecane (*n*-C$_{13}$)	13.9	57.1	71.1, 85.1	184.2
n-Tetradecane (*n*-C$_{14}$)	16.6	57.1	71.1, 85.1	198.2
n-Pentadecane (*n*-C$_{15}$)	19.4	57.1	71.1, 85.1	212.2
n-Hexadecane (*n*-C$_{16}$)	22.2	57.1	71.1, 85.1	226.3
n-Heptadecane (*n*-C$_{17}$)	24.9	57.1	71.1, 85.1	240.3
Pristane	25.2	57.1	71.1, 85.1	268.3
n-Octadecane (*n*-C$_{18}$)	27.6	57.1	71.1, 85.1	254.3
Phytane	27.8	57.1	71.1, 85.1	282.4
n-Nonadecane (*n*-C$_{19}$)	30.1	57.1	71.1, 85.1	268.3
n-Eicosane (*n*-C$_{20}$)	32.5	57.1	71.1, 85.1	282.3
n-Heneicosane (*n*-C$_{21}$)	34.8	57.1	71.1, 85.1	296.4
n-Docosane (*n*-C$_{22}$)	37.0	57.1	71.1, 85.1	310.4
n-Tricosane (*n*-C$_{23}$)	39.1	57.1	71.1, 85.1	324.4
n-Tetracosane (*n*-C$_{24}$)	41.2	57.1	71.1, 85.1	338.4
n-Pentacosane (*n*-C$_{25}$)	43.1	57.1	71.1, 85.1	352.5
n-Hexacosane (*n*-C$_{26}$)	44.9	57.1	71.1, 85.1	366.5
n-Heptacosane (*n*-C$_{27}$)	46.8	57.1	71.1, 85.1	380.5
n-Octacosane (*n*-C$_{28}$)	48.4	57.1	71.1, 85.1	394.5
n-Nonacosane (*n*-C$_{29}$)	49.6	57.1	71.1, 85.1	408.5
n-Tricontane (*n*-C$_{30}$)	50.9	57.1	71.1, 85.1	422.5
n-Hentriacontane (*n*-C$_{31}$)	52.2	57.1	71.1, 85.1	436.5
n-Dotriacontane (*n*-C$_{32}$)	53.4	57.1	71.1, 85.1	450.5
n-Tritiracontane (*n*-C$_{33}$)	54.9	57.1	71.1, 85.1	464.6
n-Tetratriacontane (*n*-C$_{34}$)	56.6	57.1	71.1, 85.1	478.6
n-Pentatriacontane (*n*-C$_{35}$)	58.6	57.1	71.1, 85.1	492.6

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) values determined as standard deviation (SD) of the procedural blank x 4.65 and SD x 10, respectively (Cheeseman, Wilson and Gardner, 1989).

Quality Control

Presently, there are no certified reference materials (CRM) for *n*-alkane analysis in any biota matrices therefore spiking extractions were performed as an alternative. Procedural blanks and control samples (un-spiked trout muscle samples) were analysed alongside batches to check for contamination. In addition, solvent checks were also performed to determine if any contamination was present – this was an important step as the extraction method proposed involved a large volume of different solvents. Instrument suitability checks were run prior to analysing samples in order to check the performance of the GC-MS instrumentation.

Results and Discussion

Method Development

The selection of the seven deuterated internal standards; *n*-dodecane d26, *n* - tetradecane d30, *n*-pentadecane d32, *n*-hexadecane d34, *n*-eicosane d42, *n*-tetracosane d50, *n*-triacontane d62, was based on literature published by Mazeas and Budzinski (2001), and covered the range of *n*-alkanes currently analysed for by MSS. Based on the Mazeas and Budzinski (2001) report, the determined retention times of the deuterated internal standards and the analyte compounds were utilised to establish which deuterated internal standard would be best suited for the quantification of the target compounds (Table 3). If the selected deuterated internal standard is unsuitable for a specific compound then errors can occur in the data obtained for example optimum recovery could be jeopardised.

Table 3

Deuterated internal standards utilised for quantification of associated *n*-alkane, pristane and phytane compounds.

Deuterated Internal Standard	Compounds Quantified
n-Dodecane d26 (*n*-C$_{12}$)	*n*-C$_{10}$, *n*-C$_{11}$, *n*-C$_{12}$, *n*-C$_{13}$
n-Tetradecane d30 (*n*-C$_{14}$)	*n*-C$_{14}$
n-Pentadecane d32 (*n*-C$_{15}$)	*n*-C$_{15}$
n-Hexadecane d34 (*n*-C$_{16}$)	*n*-C$_{16}$, *n*-C$_{17}$, Pristane, *n*-C$_{18}$, Phytane, *n*-C$_{19}$
n-Eicosane d42 (*n*-C$_{20}$)	*n*-C$_{20}$, *n*-C$_{21}$, *n*-C$_{22}$, *n*-C$_{23}$,
n-Tetracosane d50 (*n*-C$_{24}$)	*n*-C$_{24}$, *n*-C$_{25}$, *n*-C$_{26}$, *n*-C$_{27}$, *n*-C$_{28}$, *n*-C$_{29}$
n-Triacontane d62 (*n*-C$_{30}$)	*n*-C$_{30}$, *n*-C$_{31}$, *n*-C$_{32}$, *n*-C$_{33}$, *n*-C$_{34}$, *n*-C$_{35}$

The process of obtaining retention times and quantifier and qualifier ions by full scan mode is described in the method section of this report. Consequently analysis could then be performed in SIM mode where detection was based on these specific masses of interest and retention times (Table 1 and Table 2). Full scan mode allows for a range of masses to be investigated; these masses are known as mass to charge ratios (m/z). The most abundant ions and the molecular ion for each compound were selected to aid identification of the compound as they eluted from the column. Figure 1 shows the full scan mass spectrum of *n*-dodecane d26, illustrating the desired quantifier ion (66.1 m/z) and qualifier ions (50.1 and 82.1 m/z) including the molecular ion for this compound (196.3 m/z). These ions were selected based on their high abundance and the molecular ion 196.3 m/z was chosen as this is specific to this particular compound therefore, will increase confidence of identification.
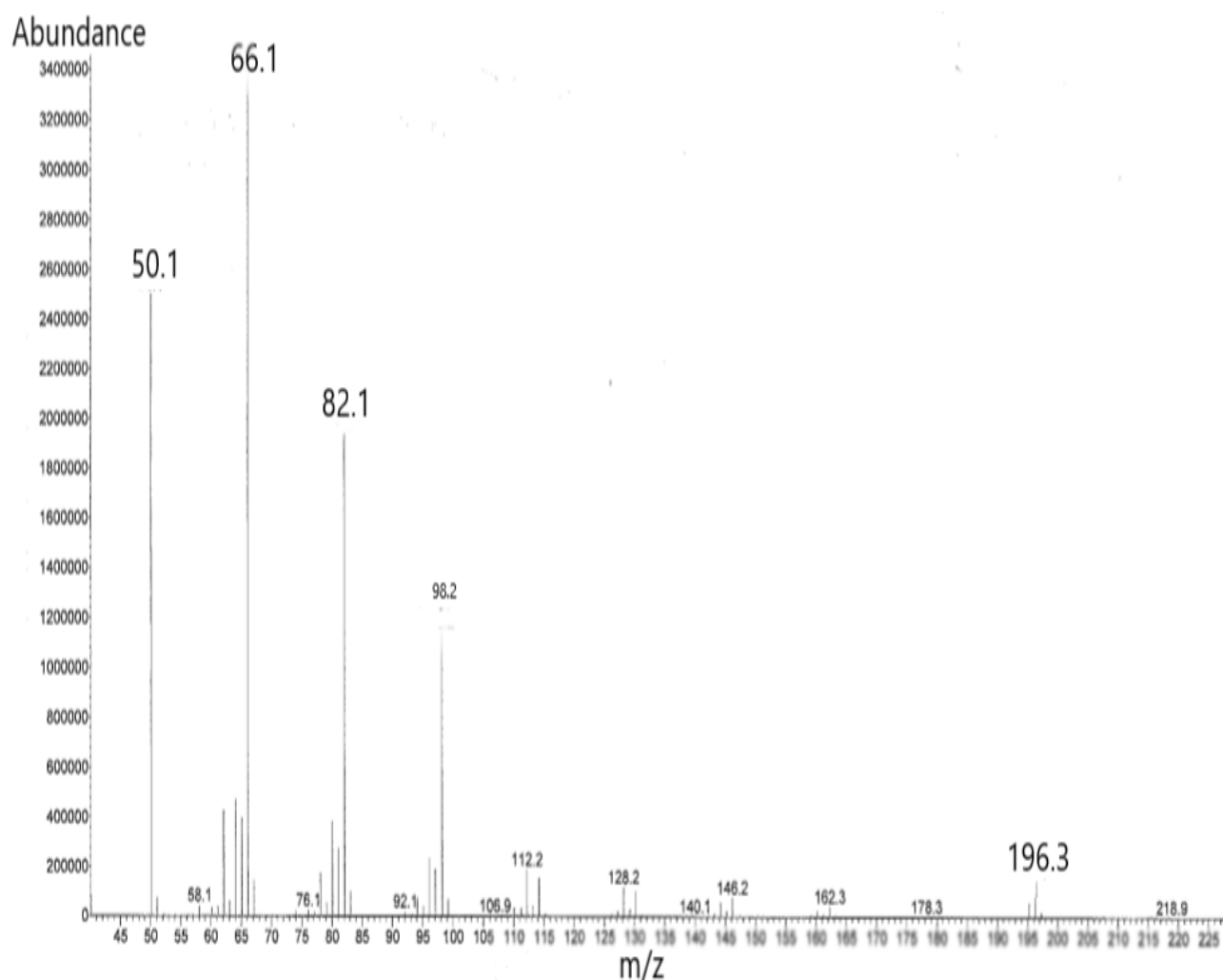
Figure 1: Full scan mass spectrum of *n*-dodecane d26 showing the selected quantifier (66.1 m/z) and qualifier ions (50.1, 82.1 and 196.3 m/z).

The SIM method allowed for satisfactory chromatographic separation of the deuterated internal standards and the *n*-alkane compounds. Figure 2 and Figure 3 demonstrates the chromatograms produced from the seven deuterated internal standard mix and the *n*-alkane mix, respectively.
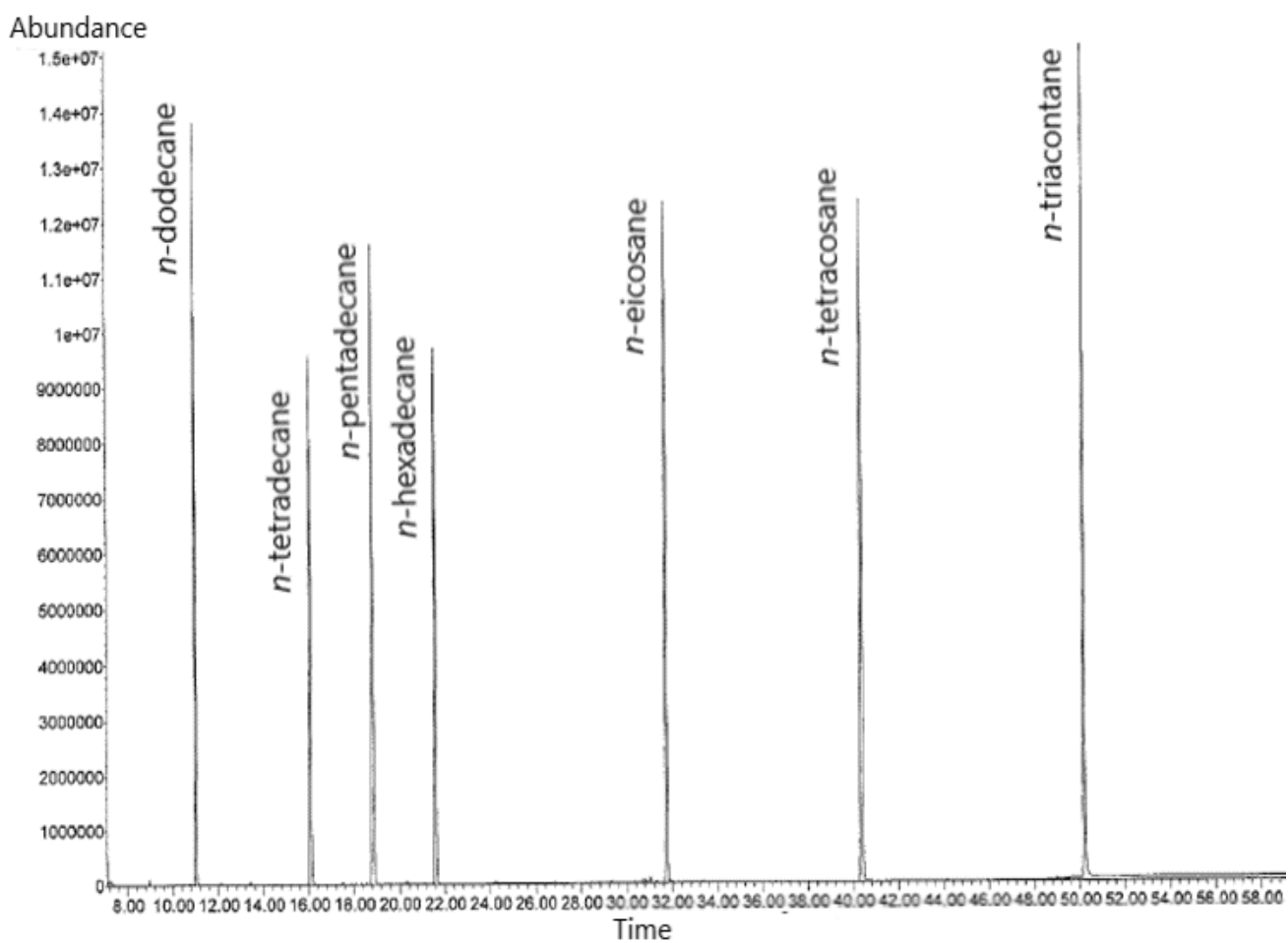
Figure 2:. Chromatogram produced from the seven deuterated internal standard mix (50 µg ml^{-1}) showing satisfactory chromatographic separation.
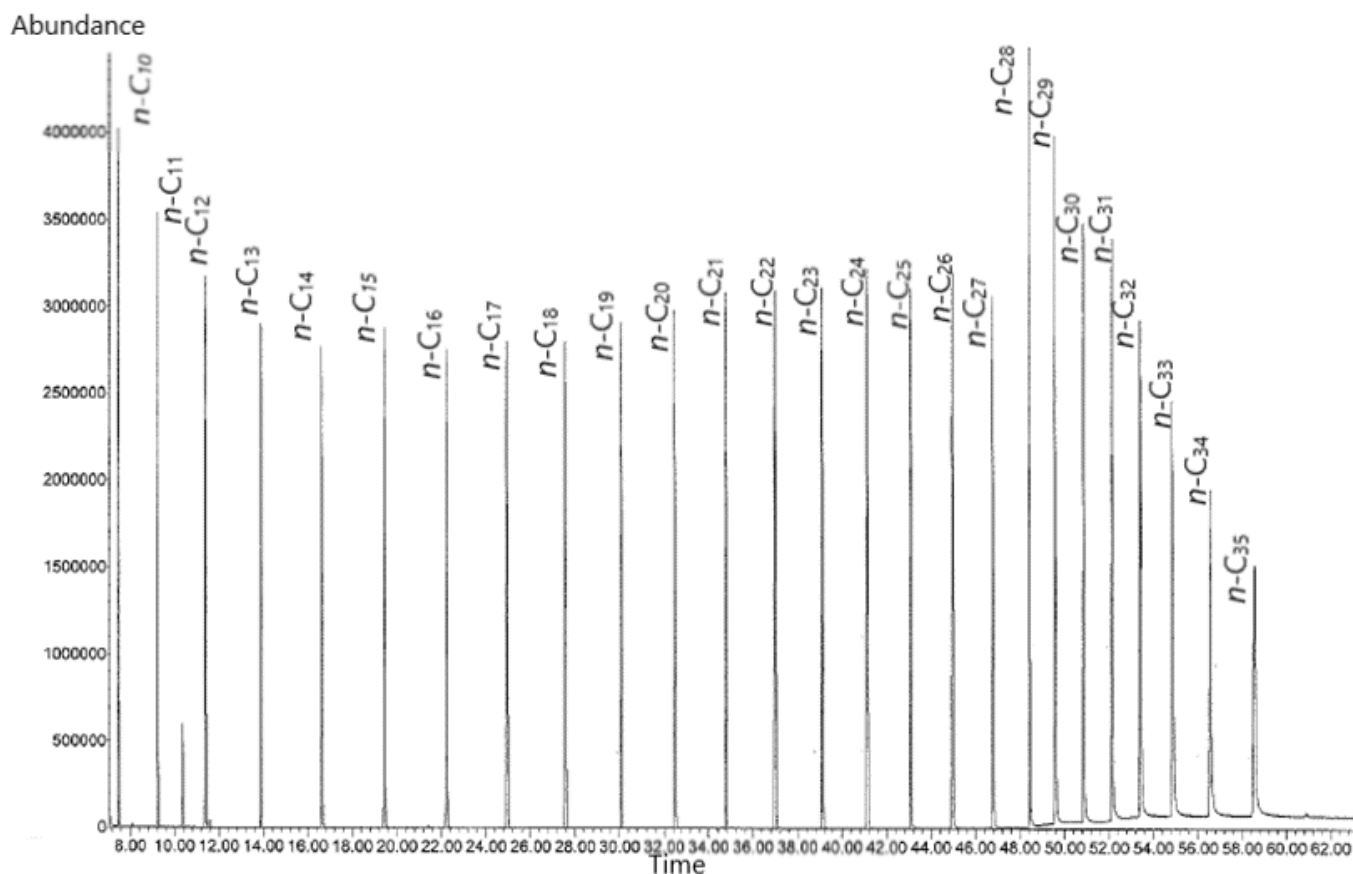
Figure 3: Chromatogram produced from the *n*-alkane mix (50 µg ml^{-1}) showing satisfactory chromatographic separation.

GC-MS Method Validation

The proposed GC-MS method was validated for linearity, precision, limit of detection (LOD) and limit of quantification (LOQ). Method validation checks were performed to determine whether the selected method was effective for the purpose of quantifying *n*-alkanes in trout muscle tissue.

Calibration and Linearity

The linear response range was evaluated by analysing a series of calibration standards (0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 10 µg ml^{-1}). Calibration curves were generated for each *n*-alkane compound including pristane and phytane, an example is given in Figure 4 (*n*-tetracosane). Linearity was established by assessing the correlation coefficient (R^2) values which ranged from 0.987 to 0.999. Acceptable linearity was established for all compounds with the exception of *n*-C$_{35}$ which produced an R^2 value of 0.987.
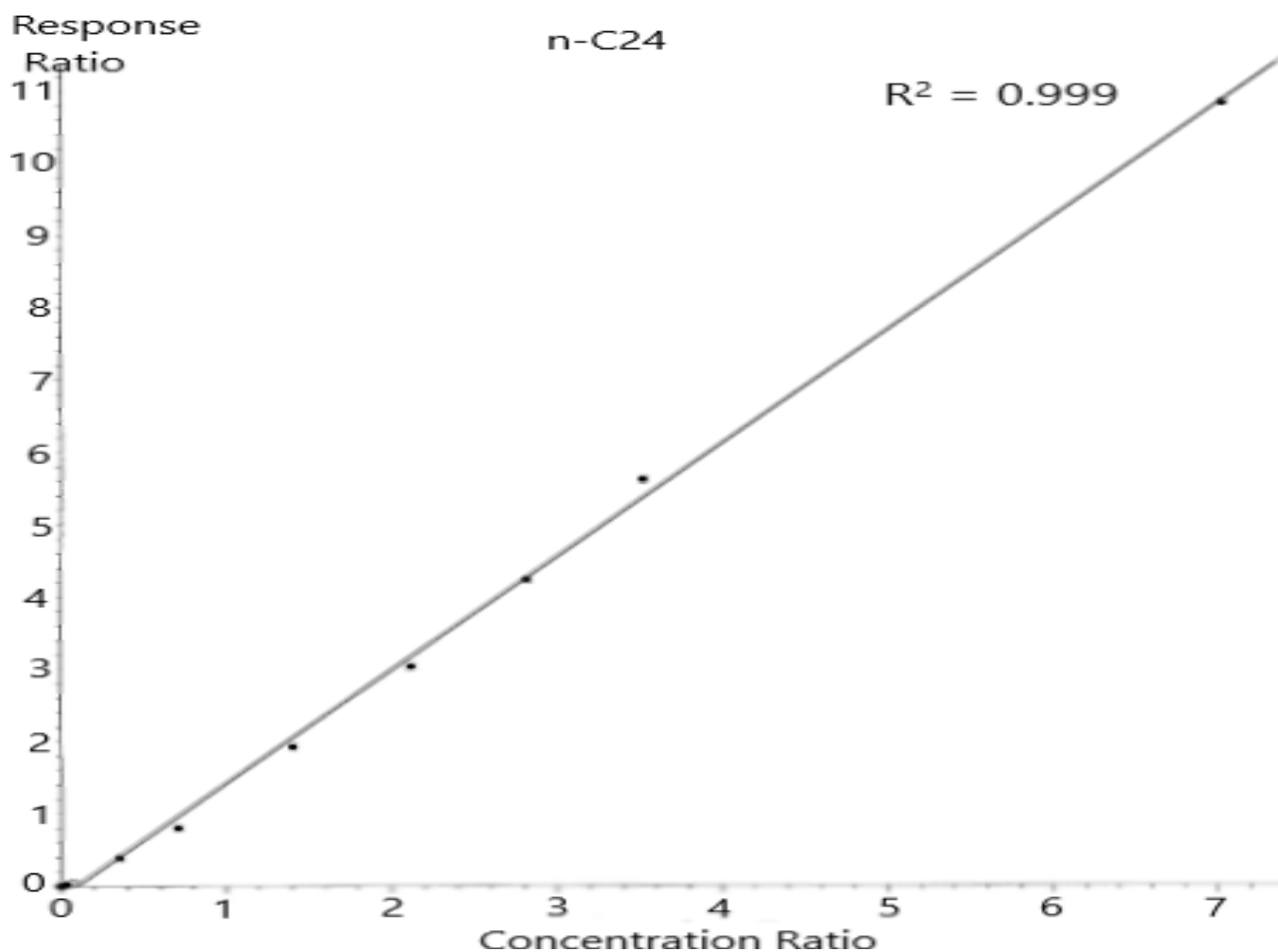
12

Figure 4: Linear calibration curve of *n*-Tetracosane (*n*-C₂₄) showing acceptable linearity (R^2 = 0.999).

Instrumental Limit of Detection (LOD) and Limit of Quantification (LOQ)

The instrumental LOD and LOQ were determined using a 0.025 µg ml⁻¹ standard, below the linear calibration range where replicate analysis (n=7) was performed consecutively on the same day. The LOD and LOQ values were determined as 4.65 x standard deviation (SD) and 10 x standard deviation of replicate (0.025 µg ml⁻¹) standard, respectively. The instrumental LOD values generated ranged from 0.004 (*n*-C₁₀, *n*-C₁₁ and *n*-C₂₆) to 0.076 µg ml⁻¹ (*n*-C₃₀). The instrumental LOQ values produced ranged from 0.008 to 0.164 µg ml⁻¹. The LOD and LOQ data generated is displayed in Figure 5. Damas *et al.* (2009) reported on the analysis of hydrocarbons in sediment samples by GC-FID; LOD values ranged from 2.4 to 3.3 µg ml⁻¹ for *n*-alkanes *n*-C₁₄ to *n*-C₃₂. The instrumental LOD values achieved by the GC-MS method in this report were significantly lower than those by GC-FID reported by Damas *et al.* (2009). Therefore, demonstrating that the GC-MS system operates at

lower detection limits in comparison to the GC-FID, consequently allowing trace concentrations of n-alkanes to be detected and quantified.
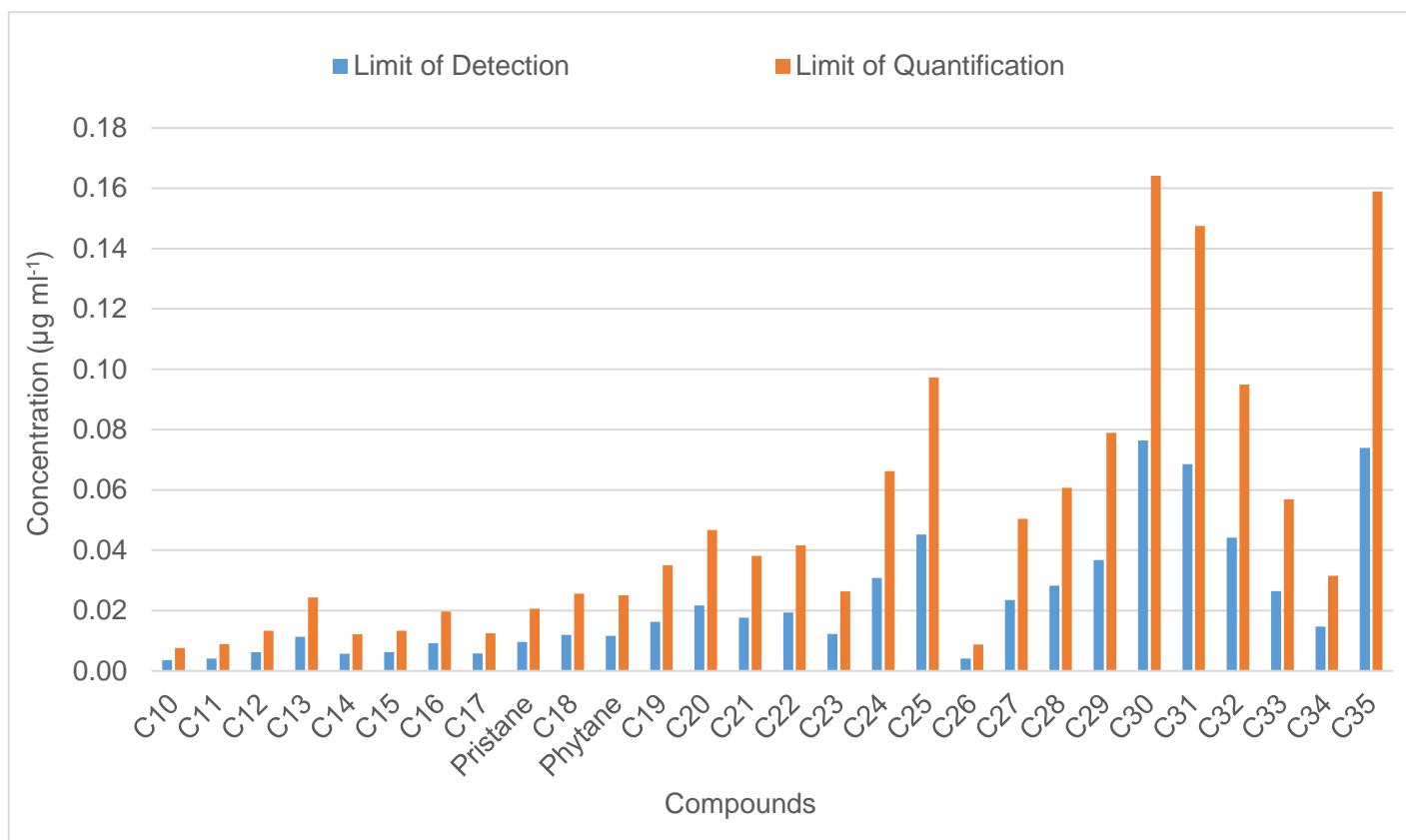

Figure 5: Instrumental LOD and LOQ for n-alkane (n-C_{10} to n-C_{35}), pristane and phytane compounds.

Precision

The precision of the GC-MS system was determined by performing intra- and inter-day injections of both 0.5 and 8.0 µg ml^{-1} and, thereby, evaluating the repeatability and reproducibility using the coefficient of variation (CV %) values. Each standard was analysed in replicates of seven within each day (intra-day) for five days (inter-day), the data generated is displayed in Figure 6. The CV % values obtained for all compounds within and between days were less than 5% for the low standard (0.5 µg ml^{-1}) and the high standard (8.0 µg ml^{-1}). The low CV % values indicate that within and between days the GC-MS system has a high level of precision at both low and high concentrations.

Single factor analysis of variance (ANOVA) was performed on all compounds to determine whether there was a significant difference in precision over the 5 days. It was determined that for all compounds at the low (0.5 µg ml^{-1}) and high (8.0 µg ml^{-1})

standard, there was no significant difference in precision between the five days (p-value > 0.05).
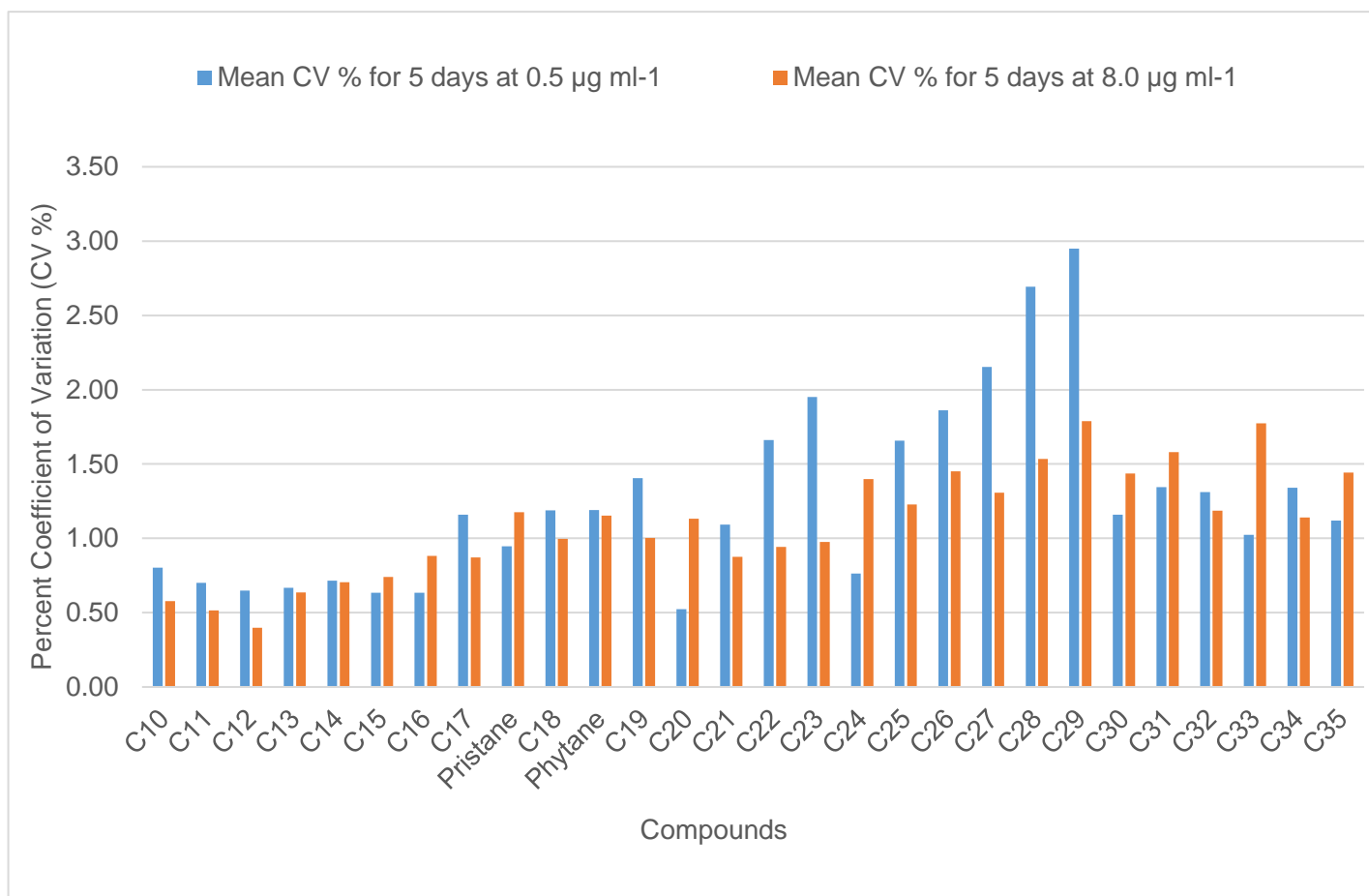

Figure 6: Instrumental precision (% coefficient of variation – CV) of the low standard (0.5 µg ml^{-1}) and the high standard (8.0 µg ml^{-1}) from intra- and inter- day injections.

Extraction Method Validation

Extraction Method Limit of Detection (LOD) and Limit of Quantification (LOQ)

The method LOD and LOQ was determined by replicate analysis (n=7) of procedural blanks which were analysed on different days. As this was based on the extraction method, the final LOD and LOQ concentrations were determined by dividing the LOD and LOQ (µg ml^{-1}) obtained by the standard deviation of the procedural blanks used in the extraction method, by the nominal weight of the trout muscle tissue (~ 7 g). The method LOD values ranged from 0.44 (n-C_{15}) to 9.66 µg kg^{-1} wet weight (n-C_{30}) and the LOQ values ranged from 0.94 to 20.8 µg kg^{-1} wet weight (Table 4).

15

Table 4

Method limit of detection and limit of quantification (based on 7 g sample size) values determined for *n*-alkanes (*n*-C_{12} to *n*-C_{35}), pristane and phytane.

Compounds	Procedural blank		LOD (µg kg^{-1})	LOQ (µg kg^{-1})	CV %
	Mean concentration (µg kg^{-1})	Standard deviation (SD)			
n-C_{12}	3.78	0.21	0.98	2.10	5.56
n-C_{13}	3.64	0.14	0.66	1.41	3.88
n-C_{14}	4.36	0.20	0.93	2.00	4.58
n-C_{15}	3.54	0.09	0.44	0.94	2.66
n-C_{16}	3.83	0.23	1.07	2.31	6.02
n-C_{17}	4.40	0.21	0.98	3.10	4.78
Pristane	4.20	0.16	0.73	1.56	3.71
n-C_{18}	4.80	0.34	1.59	3.43	7.13
Phytane	3.99	0.12	0.58	1.24	3.11
n-C_{19}	5.59	0.24	1.12	2.41	4.31
n-C_{20}	3.78	0.32	1.51	3.24	8.56
n-C_{21}	6.07	0.16	0.73	1.56	2.58
n-C_{22}	6.68	0.44	2.03	4.36	24.2
n-C_{23}	7.30	0.71	3.28	7.06	9.67
n-C_{24}	5.03	1.03	4.80	10.3	20.5
n-C_{25}	7.03	1.96	9.11	19.6	27.9
n-C_{26}	7.35	1.62	7.53	16.2	22.0
n-C_{27}	8.41	1.78	8.27	17.8	21.2
n-C_{28}	9.26	1.81	8.44	18.2	19.6
n-C_{29}	9.58	1.93	8.98	19.3	20.2
n-C_{30}	8.99	2.08	9.66	20.8	23.7
n-C_{31}	9.36	0.14	0.66	1.41	1.51
n-C_{32}	11.6	1.65	7.68	16.5	14.3
n-C_{33}	12.6	1.36	6.31	13.6	10.8
n-C_{34}	13.6	1.26	5.84	12.6	9.27
n-C_{35}	14.6	1.03	4.78	10.3	7.04

As there was high variation in the peak response between replicates for a number of compounds, demonstrated by the standard deviation (Table 4), there was consequently high LOD and LOQ values produced. This is illustrated in Figure 7 where LOD and LOQ values generally increase with increasing carbon number. As

the larger *n*-alkane compounds elute from the column, peaks become flatter and broader and this can have an adverse effect on the LOD and LOQ values.

The CV % values displayed in Table 4 were significantly higher (above 20%) for a number of compounds (*n*-C$_{22}$, *n*-C$_{24}$, *n*-C$_{25}$, *n*-C$_{26}$, *n*-C$_{27}$, *n*-C$_{29}$ and *n*-C$_{30}$). This is primarily due to several factors which can influence the precision of the extraction method such as the glassware, solvents used and the handling of those solvents. In addition, procedural blanks were analysed on different days therefore variability will be greater but remain acceptable.
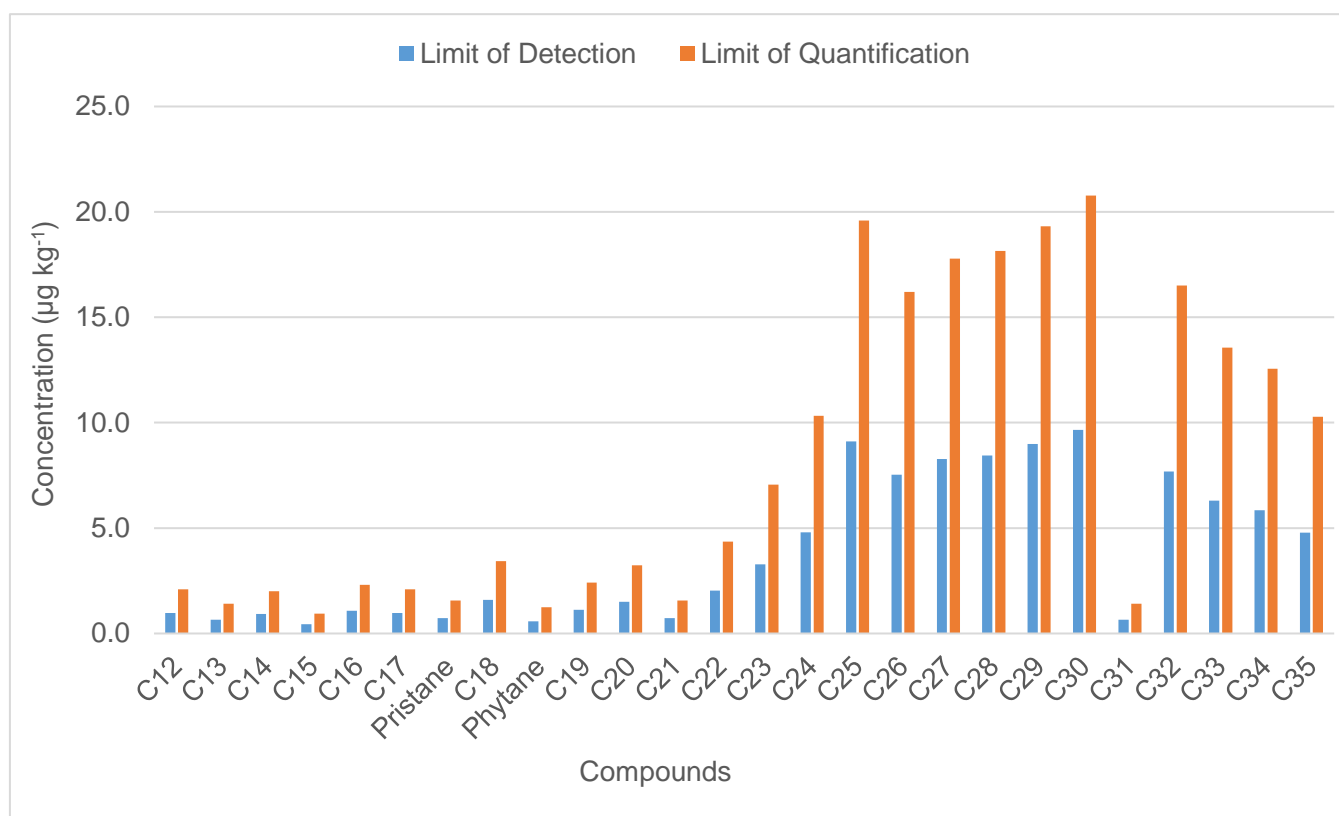

Figure 7: Method limit of detection and limit of quantification for *n*-alkanes (*n*-C$_{12}$ to *n*-C$_{35}$), pristane and phytane.

Recovery

Trout samples sourced from a local fish farm are typically kept in the MSS aquarium for up to a week prior to the sensory assessment for the detection of petrogenic taint (Webster *et al.,* 2016), where they are in clean water and have the chance to acclimate to the water temperature conditions. These trout samples will be free from hydrocarbon taint and will therefore, be classed as control samples. Procedural blanks (no trout) and control samples (7 g of trout muscle tissue) were spiked with *n*-alkane standard and internal standards, extracted and analysed by GC-MS. Seven

17

replicates for both the procedural blank samples and the control samples were analysed consecutively on the same day. The spiking solutions used to determine the extraction recovery were n-alkane solution (n-C_{10} to n-C_{35}, including pristane and phytane) (400 µl of 1 µg ml^{-1}), deuterated internal standard solution (200 µl of 1 µg ml^{-1}) and two-component solution containing squalane and HMN (200 µl of 1 µg ml^{-1}).

The recovery values (%) obtained from the spiked procedural blank extraction (no trout), were determined by subtracting the un-spiked procedural blank concentration from the spiked procedural blank concentration generated by the GC-MS. Generally, acceptable recoveries were achieved ranging from 71 to 116%, with the exception of n-C_{32}, n-C_{33}, n-C_{34} and n-C_{35} as recoveries were out with the MSS acceptable limits of 70 – 120%. As seen in Figure 8, recovery values (%) begin to decrease from n-C_{32} to n-C_{35}, this coincides with the increase of retention time compared to the last deuterated internal standard, n-triacontane d62. The deuterated internal standard n-triacontane d62 may not be ideal for quantification of the n-alkane compounds beyond n-C_{31} however no other deuterated internal standard was available.
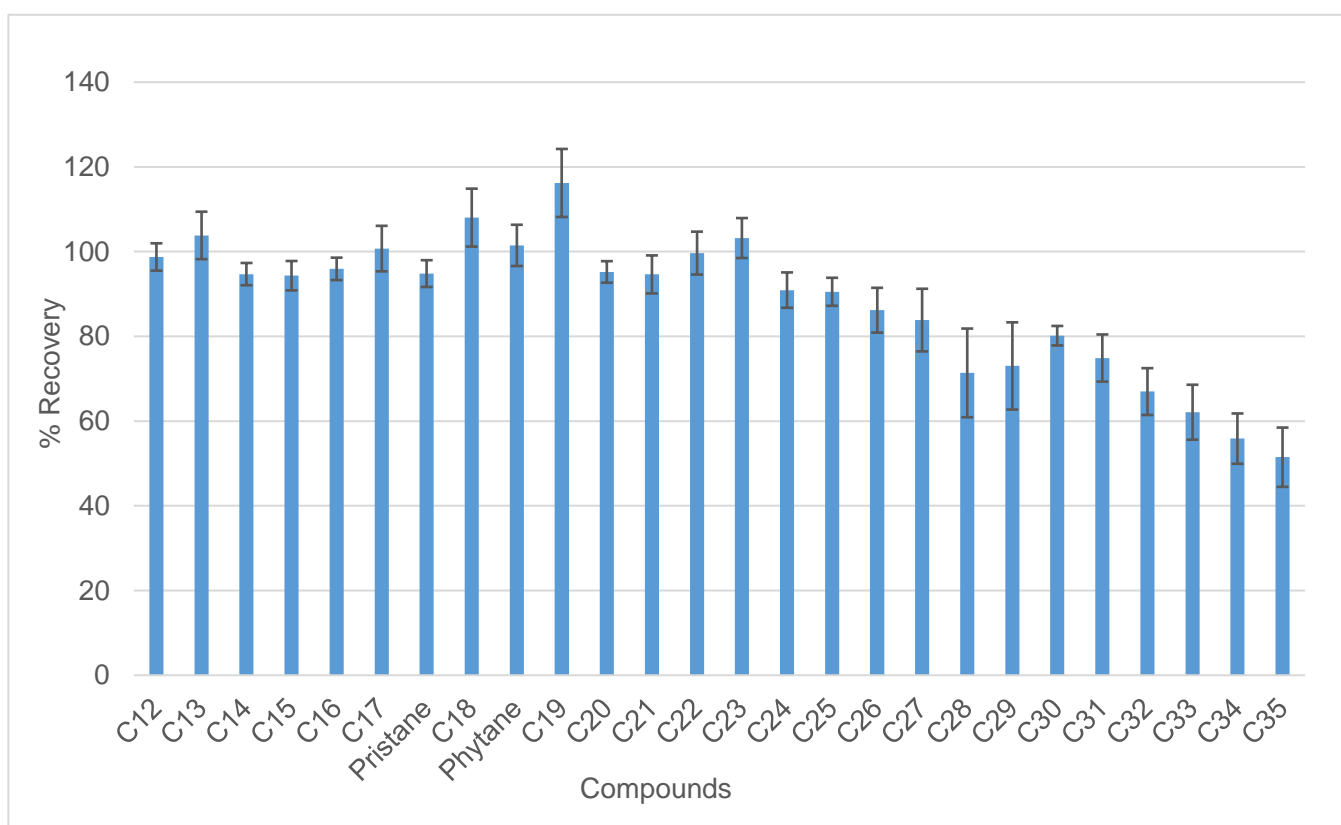

Figure 8: Mean recovery data for the spiked blank samples (n=7) using the deuterated internal standards.

Similarly, acceptable recoveries were obtained for the spiked trout muscle tissue control samples. Recovery values were generated by subtracting the un-spiked trout control concentrations from the spiked control concentrations generated by the GC-MS. As seen in Figure 9, the recovery values achieved ranged from 70 to 116% however the % recovery value for n-C$_{35}$ was below the acceptable limit (70%) set by MSS. The low values can be associated with the deuterated internal standards that were used to quantify this compound. This is evident as there was a similar trend with the spiked blank samples in Figure 8 where the % recovery values decreased after the last deuterated internal standard (n-triacontane d62).
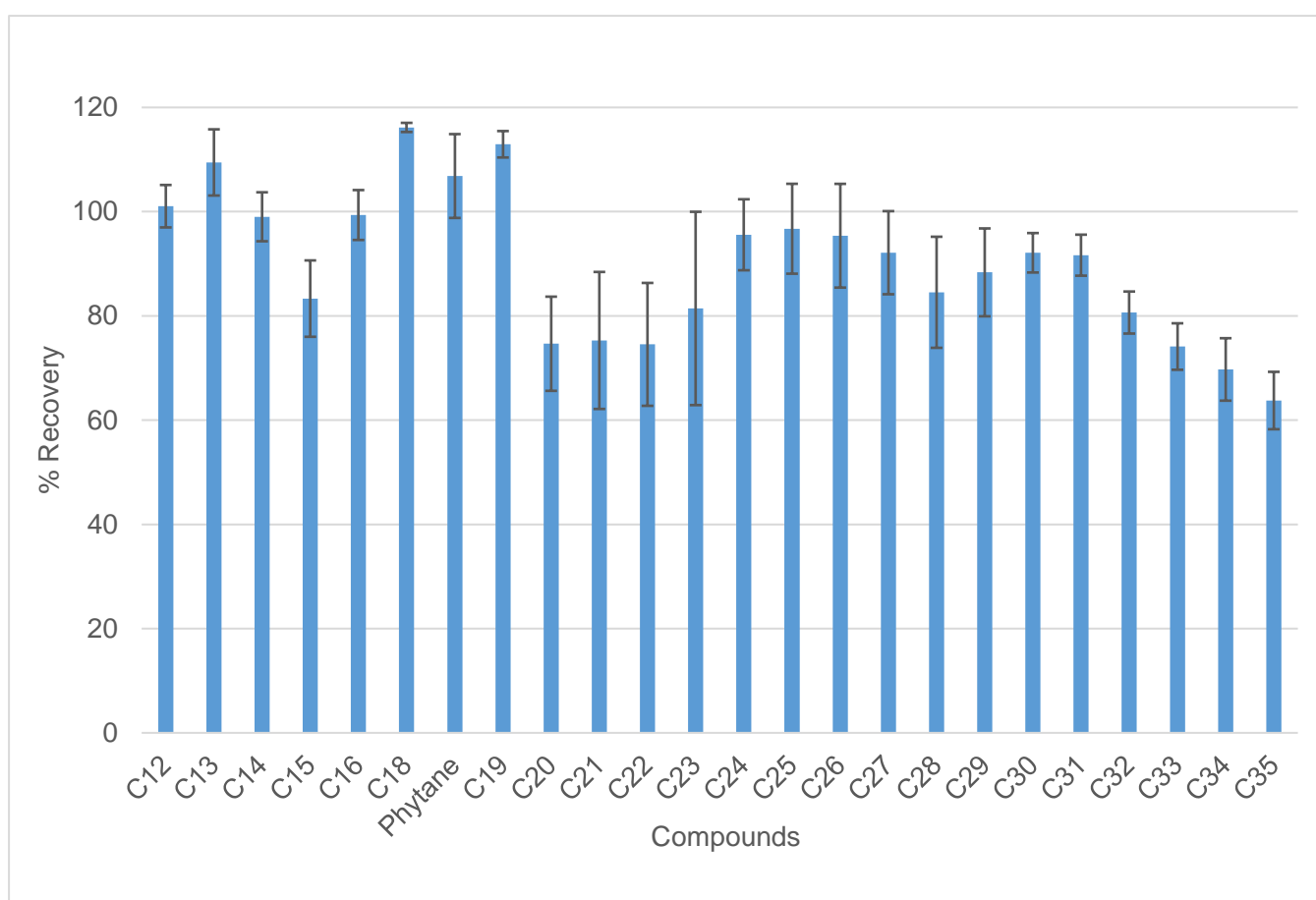

Figure 9: Mean recovery data for the spiked trout control samples (n=7) using the deuterated internal standards. Compounds n-C$_{17}$ and pristane have been removed.

In addition to issues arising with the deuterated internal standard (n-triacontane d62) utilised for the higher carbon number n-alkanes, a prominent problem when analysing trout muscle tissue is that some of the target compounds, can be found naturally in the organism. This was evident for n-C$_{17}$ and pristane which ultimately meant recovery values determined were poor. When examining the chromatograms produced for the un-spiked control sample, a high response for n-C$_{17}$ and pristane was detected, this indicated that these compounds were naturally present in the trout

muscle tissue samples. When spiking control samples with the alkane mix (400 µl, 1 µg ml^{-1}), the resulting response was similar to that of the control. Therefore, because of this small variation, % recovery values were adversely affected. By analysing procedural blank samples alongside the trout samples it was confirmed that no contamination was present.

Predominantly, hydrocarbon contamination in the environment is the result of anthropogenic activity, however, they can occur naturally in plants and animals. High levels of pristane and phytane are often found naturally in marine organisms and, therefore, this can be seen in the control trout sample background. Kemis, Nelson and Sreenivasan (2012) reported that pristane can be generated by copepods in the marine environment, and it is likely that consumption of these copepods results in the accumulation of pristane in other organisms. Mironov, Shchekaturina and Tsimbal (1981) also investigated the presence of n-alkanes and isoprenoids in various fish species recovered from different locations. The presence of pristane dominated in almost all cases and often exceeded that of the n-alkane compounds. They also explored the presence of n-alkanes in algae species from clean and populated areas. It is difficult to obtain samples from "clean" areas, therefore, algae from polluted areas were washed thoroughly with solvents until luminescence was no longer visible in order to represent a clean sample. Chromatograms obtained showed high levels of n-C$_{17}$ in clean water samples, similar responses were observed in the polluted samples. The study conducted by Mironov, Shchekaturina and Tsimbal (1981) showed similarities with the data obtained in this study, high concentrations of n-C$_{17}$ were observed in the control and spiked control samples resulting in unreliable recovery data when conducting the spiking experiments. In addition, Linko and Kaitaranta (1976) investigated the presence of hydrocarbons in Baltic herring lipids, it was reported that pristane was found to be a major component in herring. Both studies show that hydrocarbons such as n-C$_{17}$ and pristane can be found naturally in biota therefore illustrating the difficulties when performing spiking experiments. As n-C$_{17}$ and pristane are of similar carbon chain length and elute as a doublet, it was expected that they would have similar characteristics such as recovery (Stauffer, Dolan and Newman, 2008). Unfortunately, as a consequence of the lack of CRMs and laboratory reference materials (LRM), spiking experiments was the only alternative to obtain recovery data.

Compounds n-C$_{10}$ and n-C$_{11}$ produced significantly higher LOD and LOQ values in comparison to other target compounds. In addition recovery values were also poor for both compounds, therefore, it was decided to remove these from the analysis. The deuterated internal standard, n-dodecane d26 was potentially not suitable for these compounds which would give rise to quantification errors. Furthermore, lower

n-alkane compounds are the most volatile therefore can be difficult to analyse. As a result of the highlighted problems with n-C_{10} and n-C_{11}, the extraction method validation data for these are not reported. Currently, n-C_{10} and n-C_{11} are not included in the current MSS method for n-alkane analysis due to these issues. Both compounds were originally included in the analysis as they came included in the alkane-mix standard (containing n-alkanes n-C_{10} to n-C_{35}). It is not essential to be able to analyse for the more volatile compounds as these will most likely be lost by evaporation during the early stages of an oil spill.

Revised Analysis of n-C_{17} and Pristane

To allow for the quantification of n-C_{17} and pristane, the established calibration range was extended to investigate whether linearity could be achieved at higher concentrations. The calibration concentrations were prepared in a ratio mix which is detailed in Table 5. Although, these solutions were run a number of weeks after the original calibration standards; the use of internal standards should account for changes in responses of the GC-MS.

Table 5
Extended calibration points for pristane and n-C_{17}.

Concentration of calibration standard pristane:n-C_{17} (μg ml^{-1})	Concentration of deuterated internal standard (μg ml^{-1})
25:2.5	1.0
50:5	1.0
100:10	1.0
150:15	1.0
200:20	1.0
400:40	1.0

Acceptable linearity was not achieved for n-C_{17} and pristane as the R^2 values produced were 0.988 and 0.973, respectively. The loss of linearity was a result of an increase in concentration, however, it could also potentially be a result of running calibration standards on different weeks and combining them. In this case, the internal standards may not have fully accounted for the instrumental change. Therefore, in future work it would be beneficial to have a wider calibration range that

could account for compounds such as n-C_{17} and pristane, which evidently are highly abundant in some fish species for example trout.

In addition, a second spiking extraction was performed, increasing the spiking solution so that there was a tenfold increase and 100 fold increase for n-C_{17} (400 µl at 10 µg ml^{-1}) and pristane (100 µl at 500 µg ml^{-1}), respectively. As a result, a greater difference between the un-spiked trout control and the spiked trout control was observed for n-C_{17} and pristane, recovery values were 95 and 177%, respectively.

Although 177% recovery for pristane was significantly out with the acceptable recovery limits, spiking with a larger amount for these specific compounds allows for a larger variation between the un-spiked trout control and the spiked trout control. Additionally, the internal standard used to quantify pristane was n-hexadecane d34 which is a deuterated n-alkane standard, therefore, because this internal standard is not specific to pristane, it may, therefore, not have been ideal.

However, due to being unable to establish linearity for both n-C_{17} and pristane, the investigation performed here is simply an indication and highlights the difficulties that can arise when spiking biota that has naturally high concentrations of some n-alkane compounds. Unfortunately, only semi-quantitative analysis can therefore be performed over the studied range.

Comparison with the Two-Component Internal Standard Approach

Previously MSS have been using a two-component internal standard containing squalane and heptamethylnonane (HMN) for n-alkane determination in biota by GC-MS. The use of this two-component internal standard was carried over when the analysis was moved from GC-FID to GC-MS. The two-component internal standard was included in the extraction method for comparison purposes.

Extraction Method Limit of Detection (LOD) and Limit of Quantificaion (LOQ)

The method LOD and LOQ was determined by replicate analysis (n=7) on procedural blanks which were analysed on different days. Calculation of method LOD and LOQ is outlined previously for analysis with the deuterated internal standards. The method LOD and LOQ generated with the use of the two-component internal standard mix ranged from 0.30 (phytane) to 8.76 µg kg^{-1} wet weight (n-C_{27}) and 0.64 to 18.8 µg kg^{-1} wet weight, respectively (Table 6).

Table 6

Method limit of detection (LOD) and limit of quantification (LOQ) (based on 7 g sample size) values using the two-component internal standard.

Compounds	Procedural blank		LOD (µg kg^{-1})	LOQ (µg kg^{-1})	CV %
	Mean concentration (µg kg^{-1})	**Standard deviation (SD)**			
n-C$_{12}$	4.02	0.20	0.91	1.95	4.85
n-C$_{13}$	4.02	0.09	0.43	0.92	2.30
n-C$_{14}$	4.52	0.25	1.17	2.52	5.58
n-C$_{15}$	3.50	0.27	1.27	2.74	7.82
n-C$_{16}$	4.36	0.34	1.59	3.43	7.86
n-C$_{17}$	4.50	0.08	0.37	0.80	1.78
Pristane	4.28	0.11	0.52	1.12	2.62
n-C$_{18}$	4.89	0.12	0.57	1.22	2.50
Phytane	4.12	0.06	0.30	0.64	1.56
n-C$_{19}$	5.63	0.12	0.56	1.21	2.15
n-C$_{20}$	4.66	0.13	0.60	1.29	2.76
n-C$_{21}$	6.88	0.15	0.69	1.49	2.17
n-C$_{22}$	7.55	0.32	1.48	3.19	4.23
n-C$_{23}$	8.02	0.55	2.55	5.48	6.83
n-C$_{24}$	7.51	0.98	4.54	9.77	13.0
n-C$_{25}$	9.43	1.69	7.84	16.9	17.9
n-C$_{26}$	9.61	1.44	6.68	14.4	15.0
n-C$_{27}$	10.7	1.88	8.76	18.8	17.6
n-C$_{28}$	11.4	1.69	7.86	16.9	14.8
n-C$_{29}$	11.7	1.87	8.67	18.7	16.0
n-C$_{30}$	11.3	1.73	8.06	17.3	15.3
n-C$_{31}$	12.8	1.69	7.86	16.9	13.2
n-C$_{32}$	13.8	1.48	6.88	14.8	10.7
n-C$_{33}$	14.8	1.38	6.42	13.8	9.31
n-C$_{34}$	15.7	1.13	5.27	11.3	7.25
n-C$_{35}$	16.7	0.96	4.45	9.56	5.74

Single factor ANOVA was performed to determine whether the LOD and LOQ values significantly differ between the deuterated internal standard method and the two-component internal standard method. It was determined that the method LOD and LOQ values obtained differ significantly for compounds *n*-C$_{13}$, *n*-C$_{16}$, Phytane, *n*-C$_{20}$,

n-C_{21}, *n*-C_{22}, *n*-C_{24}, *n*-C_{25}, *n*-C_{26}, *n*-C_{27}, *n*-C_{28}, *n*-C_{30}, *n*-C_{31}, *n*-C_{32}, *n*-C_{33}, *n*-C_{34}, *n*-C_{35} (p-value < 0.05).

Assessing the specific LOD and LOQ values for these compounds using the deuterated internal standard (Table 4) and the two-component internal standard method (Table 6), it was evident that of the compounds that were identified as being significantly different, only *n*-C_{27}, *n*-C_{31} and *n*-C_{33} produced lower LOD and LOQ values using the deuterated internal standard method. A visual comparison of the LOD values, for both the deuterated internal standard and two-component method is shown in Figure 10. Generally, the LOD and LOQ values are lower using the two-component internal standard method, however, values remain low using the deuterated internal standard method. In addition, other factors contribute to the benefits of using the deuterated internal standards for quantification such as recovery.
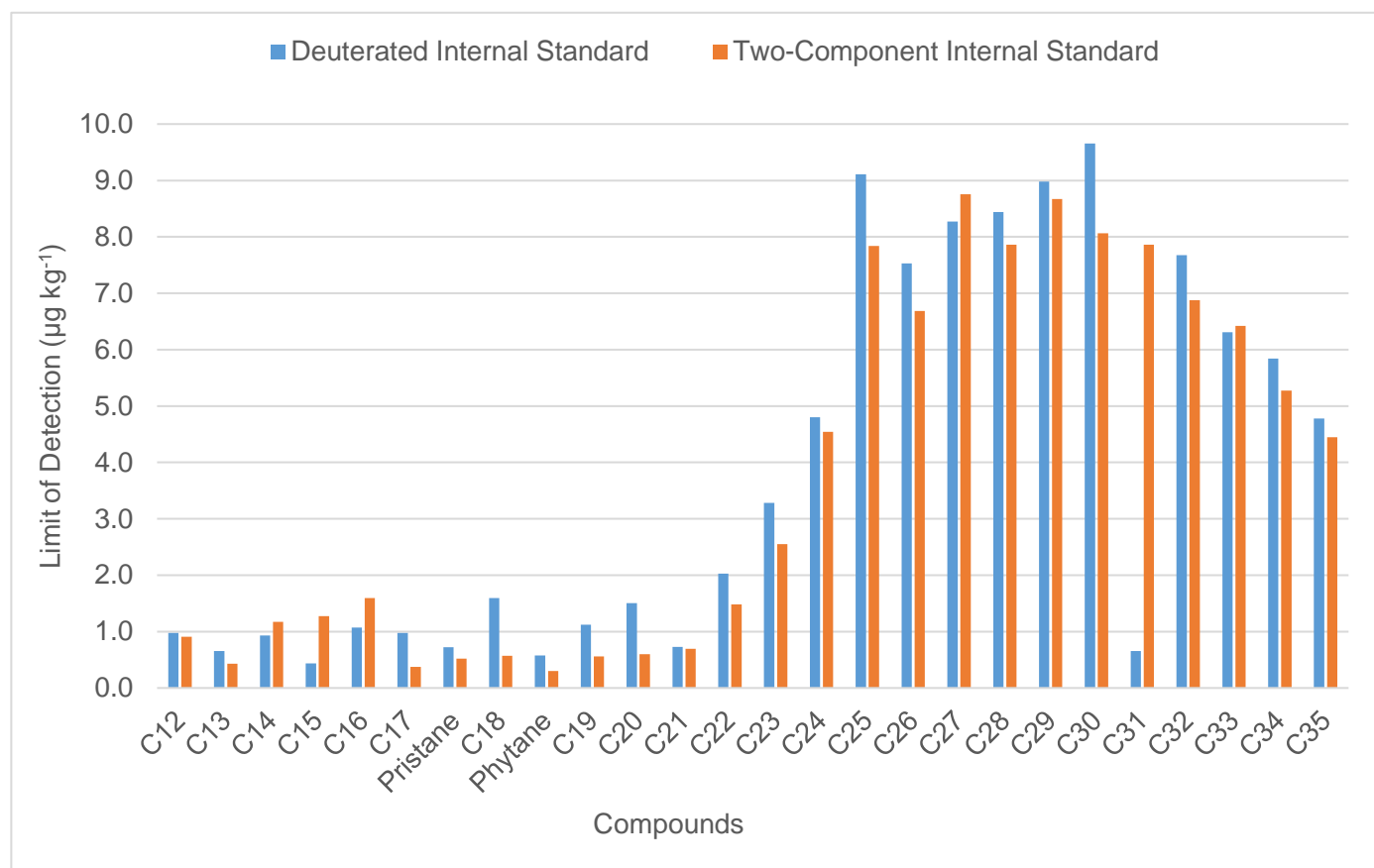

Figure 10: A comparison of the limit of detection determined by the use of deuterated internal standards and the two-component internal standard.

Recovery

The recovery values obtained when using the two-component internal standard ranged from 30 to 130%. Compounds n-C$_{15}$, n-C$_{16}$, phytane, n-C$_{31}$, n-C$_{32}$, n-C$_{33}$, n-C$_{34}$ and n-C$_{35}$ were out with the recovery limits (70-120%). Similar to what occurred with the deuterated standard recovery data, pristane produced a significantly higher recovery out with the MSS recovery limits, therefore, was excluded. When comparing the recovery results obtained using both methods (Figure 11), recoveries improved when using deuterated internal standards as the majority of compounds were between the 70-120% limit with the exception of pristane and n-C$_{17}$ which has been explained previously. This was expected as the seven deuterated internal standards utilised were more specific to the compounds being analysed in comparison to the two-component internal standard. The seven deuterated internal standards were more chemically and physically similar to the target compounds (Mazeas and Budzinski 2001).
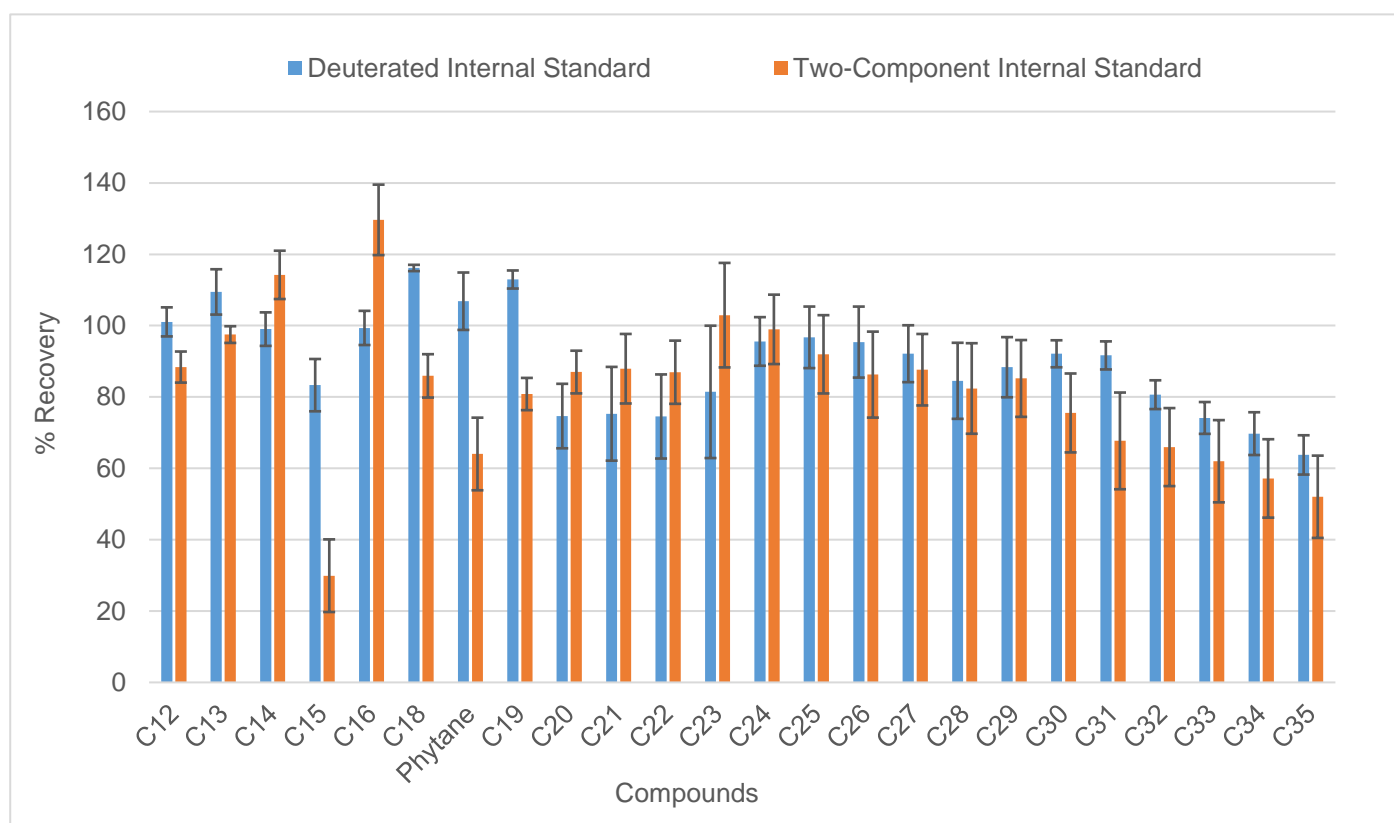

Figure 11: A comparison of the recoveries obtained from the deuterated internal standard method and two-component internal standard method.

The recovery values obtained from the spiking experiments were improved using the deuterated internal standards. The main downfall to using deuterated internal standards are the higher costs associated with purchasing them for analysis in comparison to the two-component internal standards – squalane and HMN. However, deuterated internal standards are more similar to the original non-deuterated n-alkanes thus allowing for representative analysis to be performed.

Application of the Method to Trout Samples

The method using deuterated internal standards and the two-component standard was applied to trout samples that had been exposed to crude oil for four hours in order to determine the concentration of n-alkanes in the samples (Table 7). With the exception of n-C$_{17}$ and pristane and with regards to the deuterated internal standard method, concentrations ranged from 3.60 (n-C$_{15}$) to 19.7 µg kg^{-1} (n-C$_{19}$). Similarly when quantifying with the two-component internal standard method, concentrations ranged from 3.44 (n-C$_{15}$) to 18.9 µg kg^{-1} (n-C$_{16}$).

It can be observed in Figure 12 and 13 that there is no significant difference between methods for compounds n-C$_{12}$ to n-C$_{19}$, pristane and phytane. However, for compounds n-C$_{21}$ to n-C$_{35}$ concentrations are higher using the two-component method for quantification. When comparing the recovery values determined by both methods (Figure 11), improved recoveries were established using the deuterated internal standard method for compounds n-C$_{12}$ to n-C$_{19}$. For compounds n-C$_{20}$ to n-C$_{35}$, there was no significant difference in recovery when using both methods. Therefore, for the more volatile n-alkanes (n-C$_{12}$ to n-C$_{19}$), the recovery experiment shows overall sounder results with recovery values being closer to 100%, using deuterated internal standards; however no significant difference in concentration between the two methods was observed when analysing trout samples exposed to crude oil for four hours. For heavier n-alkane compounds (n-C$_{20}$ to n-C$_{35}$), the recovery experiment showed no significant difference between methods although the concentration was found to be significantly higher using the two-component method for quantification.

Table 7

Mean (n=7) *n*-alkane concentrations in trout samples exposed to crude oil for four hours, determined by both the deuterated internal standard and the two-component internal standard methods.

Compounds	Deuterated Internal Standard		Two-Component Internal Standard	
	Concentration ($\mu g\ kg^{-1}$)	CV %	Concentration ($\mu g\ kg^{-1}$)	CV %
n-C$_{12}$	7.21	59.0	5.79	27.4
n-C$_{13}$	19.3	41.7	13.7	34.9
n-C$_{14}$	8.81	24.8	10.6	34.7
n-C$_{15}$	3.60	2.29	3.44	4.64
n-C$_{16}$	9.33	49.0	18.9	50.9
n-C$_{17}$	190	44.8	136	54.0
Pristane	2085	23.1	1070	79.3
n-C$_{18}$	4.73	0.92	4.89	0.75
Phytane	5.26	6.19	5.03	5.14
n-C$_{19}$	19.7	22.5	13.5	41.0
n-C$_{20}$	4.85	5.43	5.54	19.5
n-C$_{21}$	7.78	6.87	9.33	8.86
n-C$_{22}$	7.38	2.15	8.69	4.35
n-C$_{23}$	7.90	5.43	8.79	3.72
n-C$_{24}$	5.25	6.43	7.60	4.84
n-C$_{25}$	10.7	70.4	13.4	48.4
n-C$_{26}$	6.95	2.41	9.13	3.76
n-C$_{27}$	8.33	2.41	10.8	5.29
n-C$_{28}$	8.67	1.45	10.8	1.60
n-C$_{29}$	14.4	11.5	16.9	8.95
n-C$_{30}$	8.13	3.13	10.7	1.67
n-C$_{31}$	9.48	0.58	12.1	1.49
n-C$_{32}$	11.0	2.01	13.2	0.75
n-C$_{33}$	12.1	1.40	14.3	0.61
n-C$_{34}$	13.1	1.27	15.2	0.57
n-C$_{35}$	14.3	1.25	16.3	0.49

It should be taken into account that the recovery experiment performed and the analysis of trout exposed to crude oil for four hours have significant differences. During the recovery experiment the trout muscle is spiked with an *n*-alkane mix prior to extraction whereas the results obtained in Table 7 are based on trout being exposed to crude oil in a tank for four hours before being filleted and extracted. The data obtained from both the recovery and concentration experiments were not as expected however this potentially could have been a result of the different conditions in which the experiments took place. For the recovery experiment, all standards including the deuterated internal standard mix, two-component internal standard mix and the alkane mix were injected directly onto the fish muscle prior to being extracted. While the trout in the tank were absorbing alkanes for four hours prior to interacting with the deuterated internal standard and the two-component internal standard before the extraction process. This difference may have been a contributing factor as to why the results obtained for the recovery and concentration experiments were different for the range of compounds studied using the deuterated internal standard method and the two-component internal standard method.

It is also worth mentioning that the concentration varies between *n*-alkane compounds as each individual compound will be absorbed differently and the trout may metabolise some faster than others. In addition natural *n*-alkane compounds may already be present giving rise to the higher concentrations as seen with n-C$_{17}$ and pristane (Figure 14). The trout samples that had been exposed to crude oil for four hours are more representative of samples that may be recovered after an oil spill incident.
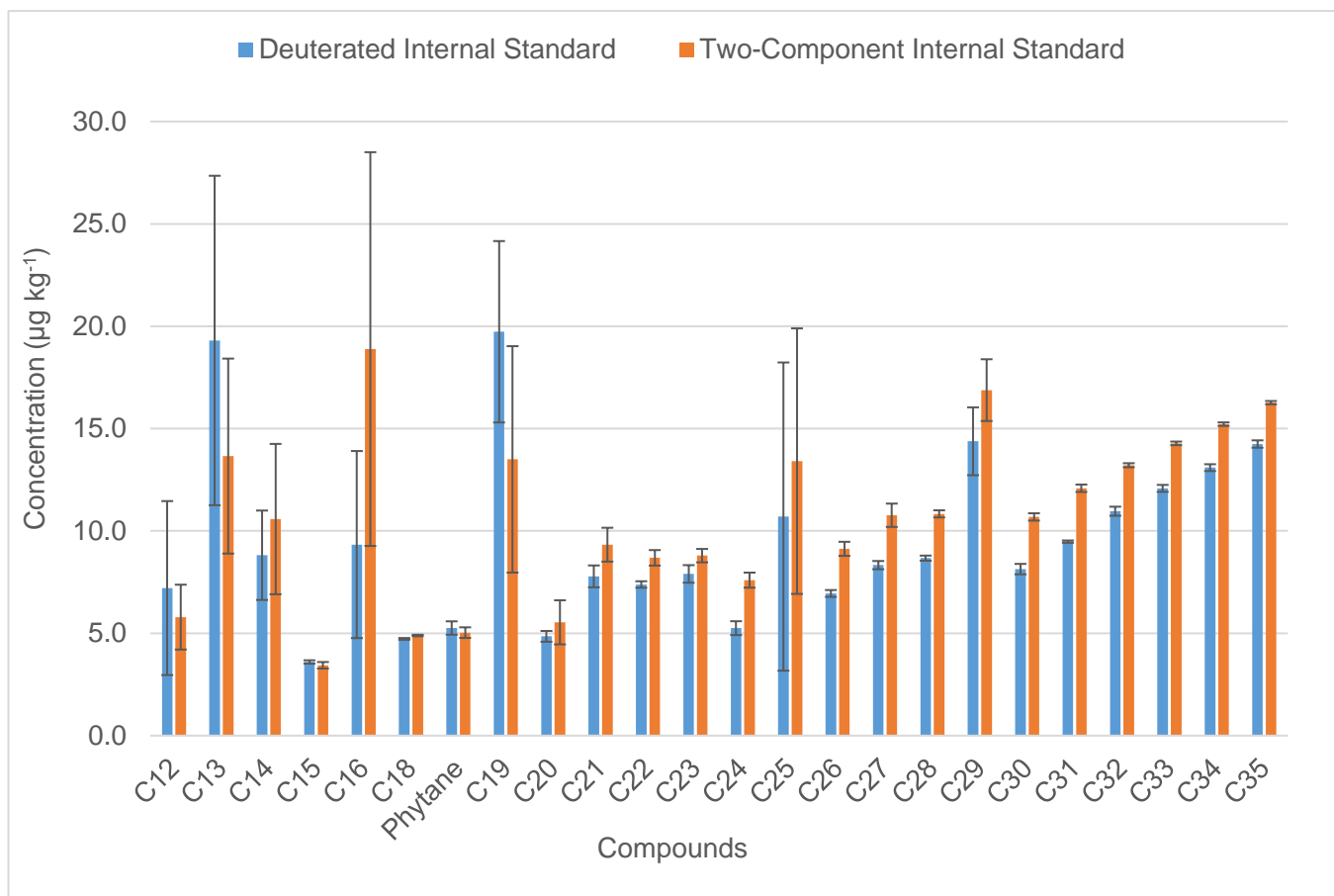
Figure 12: Concentration comparison in trout samples exposed to crude oil for four hours, between the deuterated internal standard method and the two-component internal standard method.
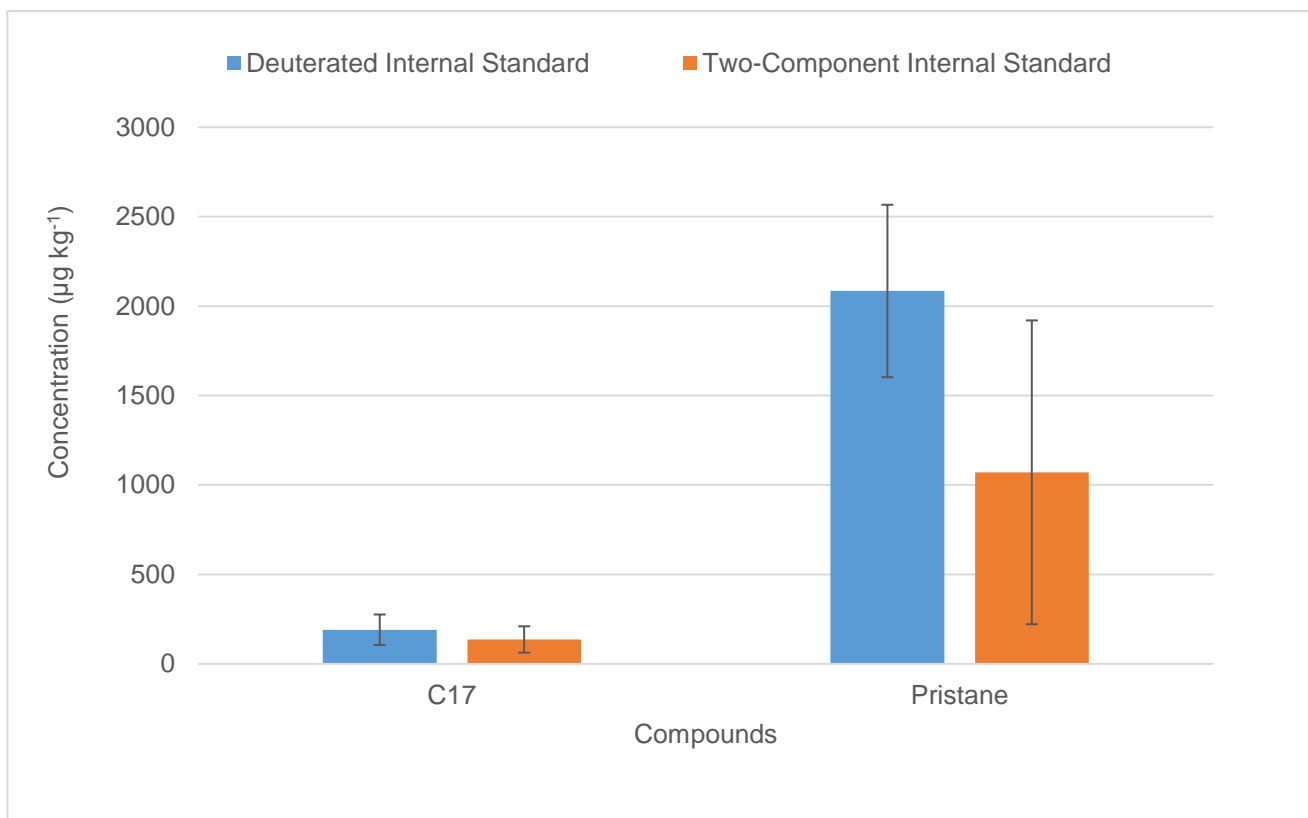
Figure 13: Concentration comparison for n-C_{17} and pristane in trout samples exposed to crude oil for four hours, between the deuterated internal standard method and the two-component internal standard method.

Conclusion

The work described within this study involved the development of a GC-MS method for the determination of n-alkanes in biota using seven deuterated internal standards. Consequently, validation was performed for the instrumental and extraction method. Calibration data was established over a linear range of 0.05 to 10 µg ml^{-1}, valid linearity was determined as the R^2 value was above 0.990 (except for n-C_{35}). Acceptable instrumental precision was observed by the analysis of seven replicates of a high and low standard over a five day period. The method LOD and LOQ established ranged from 0.44 to 9.66 µg kg^{-1} and 0.94 to 20.8 µg kg^{-1}, generally the highest LODs were for n-C_{27} and above. Recoveries of spiked samples ranged from 70 to 116% with the exception of n-C_{17}, pristane and n-C_{35}, whose response ratios were out with the calibration range. Therefore, an extended calibration was prepared however linearity could not be established at higher concentrations. A second spiking experiment was performed in order to obtain recovery values for n-C_{17} and pristane, resulting in recovery values of 95 and 177%, respectively. Subsequently, only semi-quantitative analysis could be performed for pristane and n-C_{35}. The deuterated internal standard method was compared with the existing two-component

internal standard method, where generally similar LOD and LOQ values were recognised and improved % recovery data was observed when quantifying with the deuterated internal standards.

The validated extraction method was effectively applied to the analysis of *n*-alkanes in trout muscle tissue; the GC-MS method provided adequate separation and identification of the target *n*-alkane compounds present in trout samples that were exposed to crude oil for four hours. The trout samples used were sourced locally from a fish farm and kept in the MSS aquarium for use during the sensory assessment for the detection of petrogenic taint in fish (Webster *et al.,* 2016). With regards to the two-component internal standard method, the determined concentrations were higher for heavier *n*-alkane compounds although no significant difference between the methods for the more volatile compounds. Whereas the recovery values obtained using the deuterated internal standard method showed a significant improvement in comparison to those established using the two-component internal standard method, with recoveries closer to 100%

Although the two-component method currently utilised by MSS was adequate for the analysis of *n*-alkanes, the proposed GC-MS method using the deuterated internal standards has lower detection and quantification limits and will improve the accuracy of the determination of the majority of *n*-alkanes in biota samples in comparison to the two-component internal standard method. Ultimately, this method is an improvement on the GC-MS method using the squalane and HMN internal standards and will be suitable for *n*-alkane quantification of contaminated fish (and potentially other matrices such as sediment and water). Unfortunately, recovery experiments will also be required for water and sediment analysis as no certified reference materials are available. In future this method will be applied to the determination of *n*-alkanes in any environmental impact assessment following an oil spill.

References

ADENIJI, A.O., OKOH, O.O. and OKOH, A.I., 2017. Analytical Methods for the Determination of the Distribution of Total Petroleum Hydrocarbons in the Water and Sediment of Aquatic Systems: A Review. *Journal of Chemistry, 1*(1), pp.1-13.

ARTIFON, V., ZANARDI-LAMARDO, E. and FILLMANN, G., 2019. Aquatic Organic Matter: Classification and Interaction with Organic Microcontaminants. *Science of The Total Environment,* 649(1), pp.1620-1635.

BENSON, N.U. and ESSIEN, J.P., 2009. Petroleum Hydrocarbons Contamination of Sediments and Accumulation in *Tympanotonus fuscatus* var. *radula* from the Qua Iboe Mangrove Ecosystem, Nigeria. *Current Science,* 96(2), pp.238-244.

CHEESEMAN, R.V., WILSON, A.L. and GARDNER, M.J., 1989. A Manual on Analytical Quality Control for the Water Industry. NS30, Water Research Centre, UK, ISBN 0902156853.

CHIRWA, E.M.N. and BEZZA, F.A., 2015. Petroleum Hydrocarbon Spills in the Environment and Abundance of Microbial Community Capable of Biosurfactant Production. *Journal of Petroleum and Environmental Biotechnology,* 6(5), pp.1-5.

DAMAS, E.Y.C., MEDINA, M.O.C., CLEMENTE, A.C.N. and DÍAZ, M.A.D., 2009. Validation of an Analytical Methodology for the Quantitative Analysis of Petroleum Hydrocarbons in Marine Sediment Samples. *Químíca Nova,* 32(4), pp.855-860.

EL-NEMR, A., MONEER, A.A., RAGAB, S. and SIKAILY, E., 2016. Distribution and Sources of N-alkanes and Polycyclic Aromatic Hydrocarbons in Shellfish of the Egyptian Red Sea Coast. *Egyptian Journal of Aquatic Research,* 42(1), pp.121-131.

HEIMANN, K., KARTHIKEYAN, O.P. and MUTHU, S.S., eds., 2016. *Biodegradation and Bioconversion of Hydrocarbons - The Assessment of Hydrocarbon Contamination in Contrasting Sedimentary Environments.* Singapore: Springer.

KEMIS, B., NELSON, B. and SREENIVASAN, A., 2012. Effects of Pristane on Growth of Pink Salmon (Oncorhynchus gorbuscha) [online]. United States, California: STAR (STEM Teacher and Researcher). Available from: https://digitalcommons.calpoly.edu/star/133 [Accesssed 28/07/19].

LINKO, R.R. and KAITARANTA, J., 1976. Hydrocarbons of Baltic Herring Lipids. *Rivista Italiana delle Sostanze Grasse,* 3(3), pp.37-39.

MAZEAS, L. and BUDZINSKI, H., 2001. Improved Accuracy of GC-MS Quantification of Aliphatic and Aromatic Hydrocarbons in Marine Sediments and Petroleum's. Validation on Reference Matrices and Application to the Erika Oil Spill. *International Journal of Environmental Analytical Chemistry,* 82(3), pp.157-173.

MIRONOV, O.G., SHCHEKATURINA, T.L. and TSIMBAL, I.M., 1981. Saturated Hydrocarbons in Marine Organisms. *Marine Ecology,* 5(1), pp.303-309.

NWADINIGWE, C.A. and ALUMONA, T.N., 2017. Assessment of N-alkanes and Acyclic Isoprenoids (Geochemical Markers) in Crudes: A Case Study of Iraq and Niger Delta, Nigeria. *Egyptian Journal of Petroleum*, 27(1), pp.111-116.

STAUFFER, E., DOLAN, J.A. and NEWMAN, R., 2008. *Fire Debris Analysis.* London: Academic Press.

TOLOSA, I., DE MORA, S.J., FOWLER, S.W., VILLENEUVE, J-P., BARTOCCI, J. and CATTINI, C., 2005. Aliphatic and Aromatic Hydrocarbons in Marine Biota and Coastal Sediments from the Gulf and the Gulf of Oman. *Marine Pollution Bulletin,* 50(1), pp.1619-1633.

WANG, Z., FINGAS, M. and PAGE, D.S., 1999. Oil Spill Identification. *Journal of Chromatography A,* 843(1-2), pp.369-411.

WEBSTER, L., ANGUS, L., TOPPING, G., DALGARNO, E.J. and MOFFAT, C.F., 1997. Long-term Monitoring of Polycyclic Aromatic Hydrocarbons in Mussels (*Mytilus edulis)* Following the *Braer* Oil Spill. *Analyst,* 122(1), pp.1491-1495.

WEBSTER, L., HUSSY, I., CRAIG., RUSSELL, M., PACKER, G., DALGARNO, E.J., MOORE, C. AND MOFFAR, C.F., 2011. Determination and environmental assessment of hydrocarbons in water, fish and sediment following an oil spill at the Gannet oil field. *Scottish Marine and Freshwater Science Report*, 2(12).

WEBSTER, L., SHEPHERD, N., RUSSELL, M., PACKER, G., DALGARNO, E.J. and NEAT, F., 2017. Monitoring of Polycyclic Aromatic Hydrocarbons (PAHs) in Scottish Deepwater Environments (MoreDeep). *Scottish Marine and Freshwater Science,* 8(9), pp.1-18.

WEBSTER, L., SHEPHERD, N., RUSSELL, M., WALSHAM, P., PACKER, G., HAYES, P. and TURRELL, W.R., 2016. Taint, Polycyclic Aromatic Hydrocarbon (PAHS) and N-Alkane Analysis of Farmed Mussels and Salmon Samples Following the Transocean Winner Rig Incident. *Scottish Marine and Freshwater Science,* 7(22), pp.1-21.