Highlighting the effects of co-eluting interferences on compound specific stable isotope analysis of polycyclic aromatic hydrocarbons using comprehensive two-dimensional gas chromatography

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Accuracy is the most important issue when carrying out compound specific stable isotope analysis of polycyclic aromatic hydrocarbons extracted from complex samples. It depends on two main factors: the possible isotopic fractionation of the compounds during extraction and the potential co-elution with interfering compounds with different isotopic signatures. We present here a simplified pressurised liquid extraction method for compound specific stable isotope analysis of polycyclic aromatic hydrocarbons (PAHs) in non-aqueous phase liquids of coal tar. Samples extracted using the new method and using fractionation on silica gel column were analysed using comprehensive two-dimensional gas chromatography. We were able to evaluate the effect of co-elution on carbon and hydrogen stable isotope signatures of the 16 US EPA priority PAHs in the coal tars with various proportions of aromatic and aliphatic content. Even in samples that presented a good baseline resolution, the PAHs of interest co-eluted with other aromatic compounds with a notable effect on their stable isotope values; it demonstrated the necessity to check the quality of all extraction and clean-up methods (either the simplified pressurized liquid extraction or more traditional labour-intensive methods) for the more complex samples prior to data interpretation. Additionally, comprehensive two-dimensional gas chromatography enabled visualisation of the suspected co-elutions for the first time.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous global contaminant, produced by a number of sources, including vehicle emissions and fuel combustion1. Many PAHs are known to have toxic, mutagenic and carcinogenic properties2, making their source, fate and remediation within the environment a

highly studied topic. However, due to the great number (and similarity) of potential PAH sources it is often difficult to provide irrefutable source attribution. This is evident in the analysis of coal tars, a by-product produced by manufactured gas plants (MGPs), often found in high volumes at contaminated former MGP sites across Europe and North America3.

Isotope studies have gained much popularity within environmental forensic investigations, due to their ability to differentiate between chemically identical contaminants4. Compound-specific isotope analysis (CSIA) has the additional ability to measure the isotope ratios of individual compounds within a mixture by the coupling of a gas chromatograph to an online combustion or pyrolysis unit and an isotope ratio mass spectrometer5.

Nevertheless, this technique still presents a number of challenges, including the requirement for chromatographic baseline separation to ensure co-eluting components do not interfere with the accuracy of the isotope ratios5. Unfortunately, most environmental samples, like coal tars, are extremely complex, thus require labour-intensive chemical fractionation processes, such as column chromatography, to separate the initial sample extract into chemical classes (e.g. aliphatics and aromatics). The procedure is time-consuming and generally requires large solvent volumes. Buczyńska et al.6 recently published an excellent review on the extraction and clean-up methods for source identification and apportionment of PAHs using carbon CSIA demonstrating the variety of clean-up methods adopted for CSIA of PAHs. Therefore, it would be of great benefit to develop a fast and efficient method of sample fractionation.

Accuracy of compound specific stable isotope results will depend on two main factors. Firstly, it is possible that during extraction of the compounds of interest into the solvent phase the ratio of stable isotopes of carbon (13C/12C expressed in δ13C) and hydrogen (D/H expressed in δD) can be changed through isotopic fractionation6-8. Secondly, because in the combustion reactor, compounds are all converted quantitatively into CO2, (and to H2 in the pyrolysis reactor) if two or more compounds are in the reactor at the same time, the resulting stable isotope ratio value is a weighted average of the stable isotope ratio of the co-eluting compounds7,8. The two factors are conflicting, as while limiting extraction steps will limit the isotopic fractionation, it will also result in greater chromatographic interference.

The lack of reference material for stable isotope analysis of PAHs in complex environmental samples means that the influence of these factors is difficult to evaluate or quantify. In their review, Buczyńska et al.6 summarise the various approached adopted by scientists to deal with these issues. In general,

We present a one-step extraction method of PAHs from coal tar dense non-aqueous phase liquids (DNAPLs) using pressurised liquid extraction (PLE) with on-line clean-up. PAHs are the main chemical family in coal tar samples as coal tar is produced from the combustion of coal. The concentrations of PAHs in coal tar are significantly higher compared to other important families such as alkanes and olefins while in crude oil samples the opposite is true. Consequently, the extraction of PAHs from crude oil samples for stable isotope analysis requires extensive chemical fractionation, clean-up and concentrations9. We demonstrate here that because of the prevalence of PAHs in coal tar sample, simpler, less stringent extraction methods can be employed for compound specific stable isotope analysis with comparable precision and accuracy to other methods.

The repeatability, and isotopic fractionation associated with the methods were investigated. We uniquely used comprehensive two-dimensional chromatography coupled with time of flight mass spectrometry (GCxGC-TOFMS) to assess co-elution of PAHs with interfering compounds in the aromatic fraction and compared it to the aromatic fraction obtained through a silica gel column, providing the first ever visual evidence of co-elution.

Results and Discussion

1. Chemical fractionation using the one-step pressurised liquid extraction.

We previously developed a one-step pressurised liquid extraction (PLE) for coal tar samples for the analysis of coal tar DNAPL through GCxGC-TOFMS using a single hexane fraction10-12. We propose here a modified version of this extraction producing two fractions: a hexane fraction (F1) and a (9:1) (v:v) (hexane:toluene) fraction (F2). DNAPL is mixed with equivalent amount of sodium sulphate and diatomaceous earth and silica gel deactivated at 10% is placed below the sample for in-cell clean-up. The first fraction was aimed at the alkanes and the olefins and the second fraction at the PAHs and other aromatics. When carrying out isotopic measurements, precision, repeatability and more importantly accuracy of the extraction are the crucial factors to be assessed. PLE has been previously shown to provide consistent and reproducible carbon isotope data when used as an extraction method, prior to silica column fractionation, for PAH contaminated soils13. The use of PLE for simultaneous sample extraction and fractionation, however, has not been reported in the literature. Two different coal tars were used to validate the extraction method: coal tar D7 and coal tar D12. These two coal tar DNAPLs are part of a coal tar "library" that was collected by the authors and more information can be found on both samples and their composition in previous publications10-12. Coal tar D7 came from a site where horizontal retorts were used for the gas manufacturing process while coal tar D12 originated from a carburetted water gas (CWG) sites and contained high levels of alkanes due to the use of oil during gas manufacture. After chemical fractionation, PAHs were found in both fractions. A third fraction (F3) was also extracted using 100% toluene. Gas chromatography coupled with mass spectrometry (GC-MS) analysis of F3 for the one-step PLE showed no measurable volatile or semi-volatile compounds, all compounds were extracted through F1 and F2. The difficulty with coal tar samples is the ease of dissolution of the components from the sample matrix. It was found that optimal separation of the aliphatic (F1) and aromatic (F2) classes could be obtained by restricting the duration of time in which the solvent resides in the accelerated solvent extractor cell. This was achieved by setting the static time in the cell to zero, meaning that the solvent flowed directly through the pressurised cell. The oven was also switched off for the initial extraction to limit the elution of PAHs within F1. A portion of the PAHs was still extracted into F1 with the aliphatic compounds; however, F2 presented a good baseline separation for PAHs for both D7 and D12 (see Figure 1 (a) and (b)).

2. Repeatability

The repeatability of both the carbon and hydrogen stable isotopic values of a series of well resolved PAHs in F2 was investigated by extracting coal tar D7 six times and establishing 95% confidence intervals for $\delta^{13}C$ and δD. The GC-IRMS procedure had to be modified slightly to account for exceptionally high levels of

naphthalene in this sample using a high split ratio to prevent overloading and damage to the reactor. The remaining PAHs were analysed in a separate run at a lower split ratio with the naphthalene peak sent to waste. The GC-c-IRMS chromatogram excluding the naphthalene (N) peak is presented in Figure 1(c). The isotopic values of naphthalene were analysed separately. The 95% confidence intervals for the δ13C were all found to be between 0.2 ‰ and 0.5 ‰, which are within the range of the instrumental precision and confirmed good precision and repeatability of the extraction method (Table 1). Similarly, the 95% confidence interval for the δD were found to be around 1 or 2 ‰ for the 14 PAHs that could be measured (the signal for the higher molecular weight PAHs (Indeno(123-cd)pyrene, dibenzo(a,h)anthracene, Benzo(ghi)perylene) were too low to be measurable for hydrogen isotopes) except for chrysene for which the 95% confidence interval was 13‰ (Table 1). In three out of six repetitions of the extraction, the δD of chrysene was measured around -28 ‰ and in the three others it was measured at around -59‰. Given the extraction conditions (pressure and temperature), it is unlikely that the chrysene would exchange its aromatic hydrogen atoms with the solvent, so this discrepancy in signature could be due either to non-reproducible isotopic fractionation, which does not seem likely as there is a clear cluster of two values or to differences in the integration of the peak. As it can be seen in Figure 1(c), the peak of chrysene in D7 was relatively small and eluted close to other compounds. With the exception of chrysene, which could potentially present more reliable values in other tar samples, the extraction method could also be deemed precise and repeatable for hydrogen stable isotopes.

3. Stable isotopic fractionation through chemical fractionation.

Because a portion of the PAHs was extracted into the first fraction, isotopic fractionation could potentially have occurred with heavy or light molecules being preferentially selected into a particular fraction. A standard solution containing the 16 US EPA priority PAHs in equal concentration was extracted three times through the one-step PLE method and the stable isotope values for both hydrogen and carbon were measured: before extraction, in F1 and in F2 to evaluate possible isotopic fractionation associated with the extraction steps alone (Figure 2). In F1, only the PAHs eluting between naphthalene and pyrene (see Table 1) were measurable for carbon stable isotope analysis and only those eluting between naphthalene and anthracene were measurable for hydrogen stable isotope analysis. In F2, the carbon and hydrogen stable isotopic signatures of PAHs between acenaphthylene and benzo(ghi)perylene could be determined. The measured δ13C and δD values for each PAH in each fraction were compared by two-tailed homoscedastic Student's t test to the δ13C and δD measured in the stock solution before extraction. For carbon stable isotopic values, only one out of 16 PAHs failed the Student's t test in F1 with a 95% confidence (anthracene) while four failed in F2 (phenanthrene, anthracene, benzo(k)fluoranthene, benzo(a)pyrene). The differences between the δ13C of these PAHs in the stock solution and the fraction ranged between 0.3 and 1.26 ‰, with only two above 1‰ (anthracene in both fractions). Although statistically significant these differences are negligible for the purpose of source apportionment, since any difference in δ13C ≤ 1‰ is not considered conclusive by environmental isotopic scientists14. Authors have used isotopic profiles, where the x-axis present the

compounds and the y-axis their δ value as a visual representation of the isotopic signature of complex samples15. As it can be seen in Figure 1, the isotopic profile of the stock solution is conserved through the extraction process. For the hydrogen stable isotopic values, only the δD of fluorene failed the t test in F1 and in F2 the values for fluorene, fluoranthene and chrysene were statistically different. For fluorene and chrysene, the differences between the δD values from the stock solution and the fraction are minimal (between 3 and 4‰). With the difference in values for fluoranthene in F2 being more significant (43‰), due to two out of the three F2 extracts providing values considerably depleted compared to the stock. As with the carbon values, however, the isotopic profile of the stock solution is preserved after extraction, which is the most crucial factor in source apportionment.

4. Effect of co-elution with interferences

Because the clean-up phase of the proposed extraction is much less stringent than extraction methods usually employed for compound specific analysis of PAHs, it was necessary to evaluate the effect of co-elution of PAHs with interfering compounds from the matrix. To evaluate the extent and the effect of co-elution on the carbon and hydrogen stable isotope signatures, GCxGC-TOFMS was employed. F1 and F2 of two different tars: D7 and D12 were analysed by GCxGC-TOFMS using in the first dimension the same capillary column that was used in the GC-IRMS for isotope measurements. A mid-polarity column (Crossbond® diphenyl dimethyl polysiloxane) was placed in the second dimension and any further separation of constituents in the second dimension showed the number of co-eluting components, which may contribute to the isotopic signature.

As mentioned above, D7 is a tar that was likely produced in a horizontal retort while D12 originated from a CWG site. D12 is expected to contain much more aliphatic compounds susceptible to co-elute with the PAHs. Figure 3 presents the contour plots for the F1 and F2 of D7 and D12, it also shows the three-dimensional surface plots for the two D12 fractions. The PAHs are the family of compounds eluting as an almost straight line across the chromatographic plan on the contour plot of all fractions and the aliphatic elutes below this line. By visual inspection of the total ion two-dimensional chromatogram only, it was apparent that D7 did not contain aliphatic compounds in amount susceptible to affect the stable isotopic signatures of the PAHs. The F1 of D12, on the other hand, showed a very high aliphatic content, that was absent from F2. Because the same column used for the GC-IRMS analysis was used in the first dimension of the GCxGC and the oven temperature and pressure programmes were identical, it is possible to directly conclude for the first time on the possible matrix interferences and their influences on the isotopic values. Therefore, when choosing F2 for both samples, visual inspection was enough to demonstrate that aliphatic compounds did not contribute to the interferences to the δ13C and δD values of the PAHs. Within the aromatic compounds, it is possible that co-elution with the analysed PAHs might occur. To study the effect of aromatic co-elution on the stable isotopic signatures, D7 and D12 were also extracted using a silica gel column after dissolution of the tar in hexane. Three fractions were also produced this way for both coal tars, with a first elution in 100% hexane (F'1), a second elution for aromatics in a mixture of 50% hexane and 50% toluene (F'2)

and a final elution in 100% toluene (F'3), which as for F3 did not show any detectable compounds. The aromatics were present in both F'1 and F'2 but for D12 in F'2 only were their concentrations high enough for isotope analysis. δ13C and δD values of the PAHs were compared in F2 and F'2 in both samples. The measurable Δδ13C (δ13C(F2)-δ13C(F'2)) were all calculated between -2.1‰ and 1.6‰ (Figure 4a) . Notably, the δ13C of the 5 PAHs measured in the F'2 of D12 were consistently enriched (between 1.2 and 2.1‰) compared to that of F2. In D7, the F'2 values were generally depleted (up to 1.6‰ for 1-MeN) but only Δδ13C(1-MeN) was greater than 1‰. When it was possible to measure them, the ΔδD (δD(F2)-δD(F'2)) were all between -1.4 and 6.7‰, which fall within a range that would be considered not significant for hydrogen values, except for ΔδD(N) in D7 (-12.5‰) and ΔδH(BaA) in D12 (52.4‰) (Figure 4b) . The value for benzo(a)anthracene can be explained by a poor separation with chrysene.
The GCxGC chromatography for these fractions was studied to estimate co-elution. We have demonstrated previously12 that in GCxGC chromatography configuration, aromatic compounds with similar number of carbon atoms elute in similar times in the first dimension and can be separated in the second dimension. This is particularly relevant to CSIA because compounds with the same number of carbon atoms theoretically have the same response factor in a GC-c-IRMS system (because each carbon atom produces a CO2 molecule). Although the response factor in the TOF-MS in total ion mode of two aromatic compounds with the same number of carbon atoms might be marginally different, comparing the peak heights of a measured PAH and a co-eluting compound will be useful to estimate the effects on the carbon isotopic signature. Figure 5 presents various zooms on the GCxGC chromatograms of F2 and F'2. The intensity gradient was set so that the maximum is around the intensity of the measured PAH (or the intensity of the least concentrated PAH when more than one are presented together) and that all compounds with peak heights within 25% of this value will appear red. The lowest intensity was set to a one thousandth of the maximum. This aimed to only highlight the peaks that might affect the stable isotopic signatures. For D7, it appeared that F'2 generally presented cleaner chromatograms than F2 with less potential co-elutants. For instance, Figure 5a shows how the values for naphthalene and its two methyl-substituted daughter compounds are likely to be more accurate in F'2 over F2. As stated previously, the mean δ13C of most measured PAHs is enriched in F2 compared to F'2 with only the carbon signature of 1-MeN appeared significantly enriched (>1‰). The methylnaphthalene appeared to co-elute with substituted heterocycles, C3 benzofurans with 11 carbon atoms and C1 benzothiophene with 9 carbon atoms. There are very little reported stable isotope values for heterocyclic PAHs in coal tar. Steinbach et al.16 reported values for δ13C of methylbenzofuran from an aquifer situated at a former manufactured gas plants. The values at various site locations were all around -20‰, and the authors suggested that either there was no biodegradation of the compound occurring or that there was no isotopic effect associated with the degradation. If benzofurans had indeed more enriched values compared to other PAHs, the slightly more enriched values for the naphthalenes in F2 could be explained. The hydrogen signature of naphthalene is also significantly affected but depleted. In the case of the D12 sample, both F2 and F'2 presented high concentrations of interfering compounds with significant intensities. F'2 appeared this time more affected

than F2, leading to significantly enriched δ13C values in F'2 (the δH seemingly less affected).
Generally, even when the one-dimensional chromatogram displayed good baseline separation, the GCxGC chromatogram enabled to demonstrate co-elution and it appeared that more interferences lead to more enriched values but only in the case of severe co-elution was the isotopic effect significant. Results for D7 also helped confirmed that there is no isotopic effect associated with the PLE since F2 and F'2, which had not been subjected to PLE, presented similar results. While the accuracy of the values for D7 was demonstrated, it is hypothesised that accurate values for D12 would actually be more depleted than measured in F2 because of interferences. D12 is an extreme example of coal tar non aqueous phase liquid, because of its CWG origin, it is closer in composition to a petroleum oil sample17. When its carbon and hydrogen isotopic profiles are compared to these of D7 (Figure 4c and d), it can be seen that they are still carrying information on the origin of the sample. In fact, while the hydrogen values were closely related, the δ13C values, notably of the higher molecular weight PAHs, were significantly depleted. Petroleum oils exhibit lower δ13C values (in the range -28 to -31‰) than parent coal (c.a. -25‰)17,18. The carbon stable isotopic signatures of D12 are therefore expected to fall between these two ranges of values.

Conclusions

We developed a fast method for CSIA of PAHs in coal tar DNAPLs for both carbon and hydrogen values. The method produced reproducible and precise results. The accuracy of the methods was deemed appropriate for samples with high PAHs contents while the δ13C values are possibly enriched in lower PAHs contents samples. The accuracy of the δD was always good for the more concentrated PAHs in a sample. The automated PLE procedure minimises solvent consumption to less than 100 mL per sample and two fractions are produced within 30 minutes. It is more efficient for high throughput analysis of coal tars, for example to evaluate several samples from a same site for source apportionment, compared to more lengthy and complicated routine extraction methods.

Using GCxGC, we demonstrated that even in samples that presented a good baseline resolution, the PAHs of interest co-eluted with other aromatic compounds; it demonstrated the necessity to check the quality of the extraction and clean-up processes (either PLE or more traditional silica gel column) for the more complex samples prior to data interpretation.

Samples from the "library" of coal tar DNAPLs we recently characterised through a newly developed GCxGC-TOFMS method for environmental forensic investigations10-12 are currently being analysed for stable carbon and hydrogen isotope values so that the efficacy of both methods for source identification and apportionment can be compared.

Experimental Section

1. Samples and Standards

Coal tar samples were obtained from FMGP sites within the UK. All samples were obtained as free phase coal tar DNAPLs, sealed and stored at 4 °C prior to analysis. Coal tar D7 was obtained from a site known to have used high

temperature horizontal coal retorts for gas manufacture, which produced a very viscose, dark tar with high aromatic content. Coal tar D12 was obtained from a carburetted water gas (CWG) plant, where additional oil sprays were used to enrich the gas stream, thus producing coal tar with a high aliphatic content and broader range of components. All solvents were of analytical grade, purchased from Fisher Scientific (Loughborough, UK). All standards were supplied by Sigma Aldrich (Gillingham, UK).

2. Fractionation by Accelerated Solvent Extraction

Accelerated solvent extraction was performed using an ASE 350 system (Dionex, Camberley, UK) equipped with 10 mL the extraction solvents.

The extraction cells were lined with 2 filter papers (to ensure unwanted particulate matter did not collect in the extract) and packed with 3 g silica gel 60 (10% deactivated w/w using deionised water) to provide simultaneous sample extraction, cleanup and fractionation. Approximately 2 g of a homogenised DNAPL/diatomaceous earth (D.E.) mixture was added to the extraction cell and the remaining cell volume was packed with D.E.

To allow sample fractionation, three separate ASE methods were employed to sequentially extract the same cell using solvents of increasing polarity. To obtain the first fraction, hexane (50 % cell volume) was used to extract the cell. The oven and static times were switched off to allow the solvent to flow straight through the cell and encourage only the aliphatic portion to elute. The second fraction was eluted with hexane:toluene in a 9:1 ratio (70 % cell volume). The oven temperature was maintained at 50 °C with the cells heated for 5 minutes prior to extraction. The final fraction was extracted using toluene (70% cell volume) at 100 °C (with 5 minute heating time). All extracts were concentrated to 1 mL prior to analysis using a Büchi Syncore® Analyst (Oldham, UK).

3. GC-C-IRMS Analyses

The system used for compound specific carbon isotope analysis comprised of a Trace GC, GC Isolink and Conflo IV interfaces and a Delta V advantage isotope ratio mass spectrometer (all Thermo Fisher Scientific, MA, USA). The gas chromatograph was fitted with a 30 m DB-5 capillary column (0.25mm ID, 0.25µm film thickness) supplied by J&W Scientific. The GC Isolink interface consists of both a high temperature conversion unit (1420 °C) for δD measurements and a combustion/oxidation furnace (Ni/Cu reactor; 1020 °C) for δ13C measurements.

The helium flow was kept constant at 1 mL/min. The initial oven temperature was set to 55 °C and held for 2 minutes before the temperature was ramped at 5 °C/min to 320 °C, with a final temperature hold time of 12 minutes. One microlitre of sample was injected using a Triplus (Thermo Scientific) autosampler. The split ratio varied between 10 and 100 for different extracts in order to obtain a signal size higher than 0.3 V for each of the PAHs investigated. All δ13C and δD values were calculated against references gases of CO2 (-35.4 ‰) and H2 (-246 ‰ VSMOW) respectively. Unless otherwise stated, isotopic values (δ) represent means of triplicate analysis, where variations were generally <0.3 ‰ for δ13C and <2 ‰ for δD.

4. GCxGC-TOFMS Analyses

All GCxGC TOFMS analyses were performed using a Leco (St. Joseph, Michigan) time of flight mass spectrometer, model Pegasus 4D, connected to an Agilent 7890A gas chromatograph equipped with a Leco thermal modulator. The TOF ion source was fixed at 200 ºC with a detector voltage of 1700 V, applied electron ionization voltage of 70 eV and a scan rate of 200 spectra/second.
The column set comprised of a Rxi5Sil MS 30 m x 0.25 mm i.d. x 0.25 µm film thickness) as the primary column and an Rix17 (1.2 m x 0.10 mm i.d. x 0.2 µm film thickness) both supplied by Thames Restek (Buckinghamshire, UK) as the secondary column, connected via a Thames Restek Press-tight® connector.
All extracts were analysed using the same primary oven temperature programme as that of the GC-C-IRMS analyses, with a secondary oven and modulator temperatures offset of 20 °C. The modulation period was 6 seconds with a 1.3 second hot pulse time. Helium was used as the carrier gas, with a flow rate of 1.0 mL/min. An MPS2 twister autosampler (Gerstel, GmbH & Co., Germany) was used to inject one microlitre of sample per run at a split ratio of 1:50 and injection port temperature of 250 °C.

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Figure Legends

Figure 1. (a) and (b) GC-MS chromatograms of F1 (red trace) and F2 (black trace) of samples D7 and D12 respectively. (c) Chromatogram obtained by GC-c-IRMS for sample D7 with naphthalene peak cut-off.

Figure 2. Carbon (top) and Hydrogen (bottom) stable isotopic profiles for 16 EPA PAHs standard solution.

Figure 3. Total ion extract contour plot chromatograms for sample D7, F1 (a) and F2 (b). Total ion extract contour plot chromatograms for sample D12, F1 (c) and F2 (d). (c') and (d') are the equivalent surface plot chromatograms for sample D12.

Figure 4. $\Delta\delta^{13}C$ (a) and $\Delta\delta D$ (b) measuring the difference in isotopic values between F2 and F'2 in samples D7 and D12, error bars are the 95% confidence intervals calculated through error propagation. $\delta^{13}C$ (c) and δD (d) isotopic profiles of samples D7 and D12 for F2.

Figure 5. Aromatic co-elutions in various fractions. Comparison of GCxGC chromatograms between the F2 and F'2 of D7 for (a) the separation of naphthalene (N) and the two methylnaphathalene (2-MeN and 1-MeN), (b) acenaphthylene (ACY) and (c) fluorene (FLU), phenanthrene (PHE) and anthracene (ANT). Comparison of GCxGC chromatograms between the F2 and F'2 of D12 for (d) a series of priority PAHs (between fluorene and pyrene)

Table 1. Mean $\delta^{13}C$ and δH values for 18 PAHs in 6 extractions of sample D7 (F2 only).

PAHs		#	$\delta^{13}C$ (‰)	95% CI[a] (‰)	δH (‰)	95% CI[a] (‰)
Naphthalene	N		-25	0.1	-65.7	0.8
2-methylnaphtahlene	2-MeN	1	-25.4	0.4	-80.8	0.6
1-methylnaphthalene	1-MeN	2	-24.5	0.3	-59.8	0.4
Acenaphthylene	Acy	3	-23.3	0.3	-52.2	0.8
Acenaphthene	Ace	4	-24.6	0.2	-62.7	0.8
Fluorene	Flu	5	-25.2	0.3	-41.7	0.3
Phenanthrene	Phe	6	-26.0	0.3	-53.8	0.8
Anthracene	Ant	7	-24.9	0.2	-79.4	2.3
Fluoranthene	Flt	8	-26.1	0.3	-48.9	0.4
Pyrene	Pyr	9	-25.8	0.2	-51.1	0.4
Benzo(a)anthracene	BaA	10	-25.3	0.5	-36.3	1.1
Chrysene	Chr	11	-25.1	0.3	-43.2	12.8
Benzo(b)fluoranthene+ Benzo(k)fluoranthene[b]	BbF+ BkF	12	-25.4	0.3	-46.6	0.5
Benzo(a)pyrene	BaP	13	-25.4	0.3	-51.7	0.6
Indeno(123-cd)pyrene +dibenzo(a,h)anthracene[b]	IP+ DBA	14	-24.9	0.3	--	--
Benzo(ghi)perylene	BP	15	-23.4	0.3	--	--

[a] CI= confidence interval; [b] these compounds co-elute.

Figure 1. (a) and (b) GC-MS chromatograms of F1 (red trace) and F2 (black trace) of samples D7 and D12 respectively. (c) Chromatogram obtained by GC-c-IRMS for sample D7 with naphthalene peak cut-off.

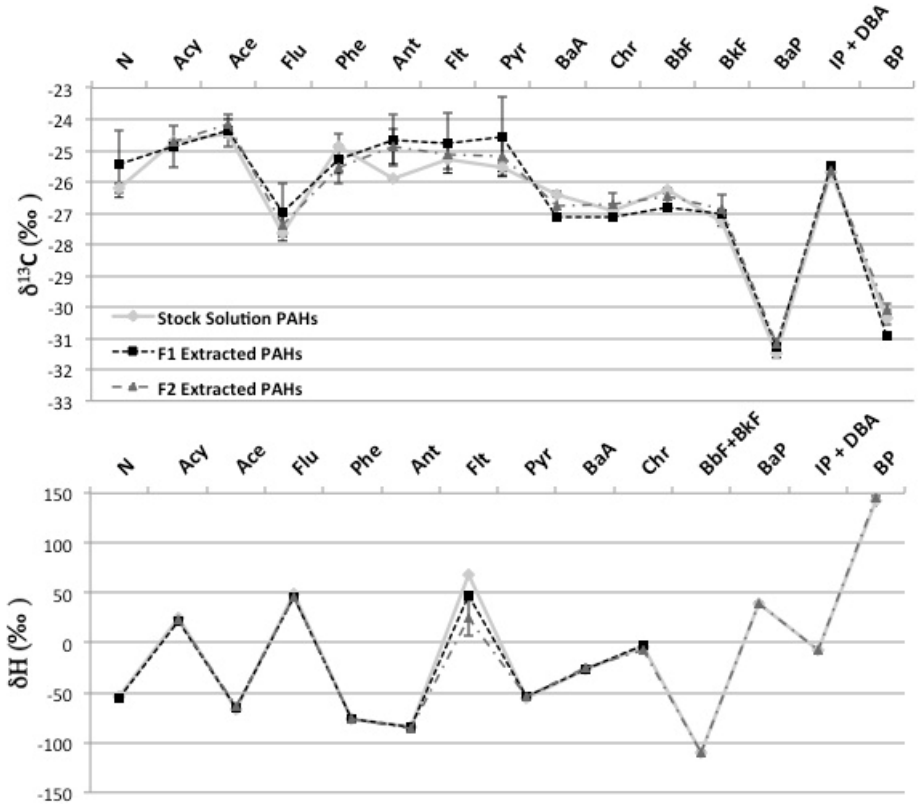

Figure 2. Carbon (top) and Hydrogen (bottom) stable isotopic profiles for 16 EPA PAHs standard solution.

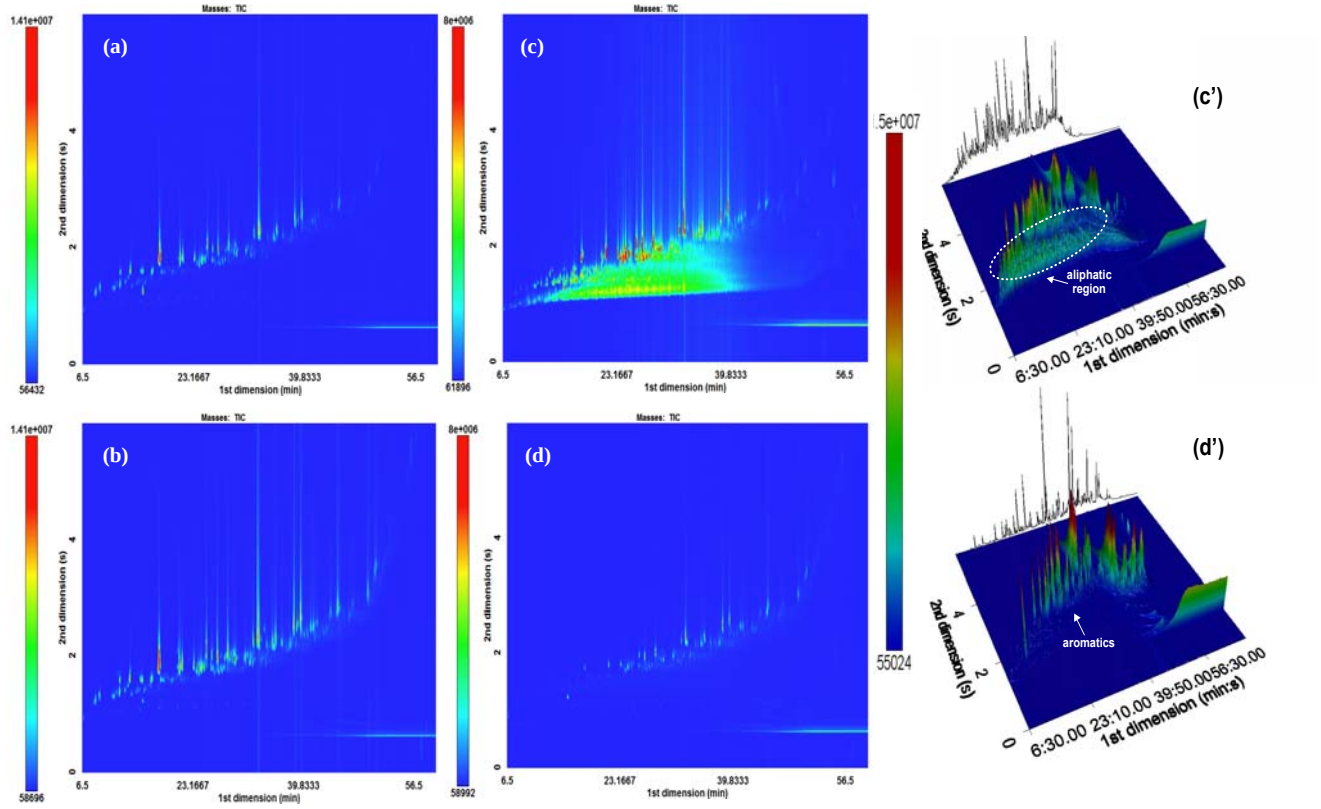

Figure 3. Total ion extract contour plot chromatograms for sample D7, F1 (a) and F2 (b). Total ion extract contour plot chromatograms for sample D12, F1 (c) and F2 (d). (c') and (d') are the equivalent surface plot chromatograms for sample D12.

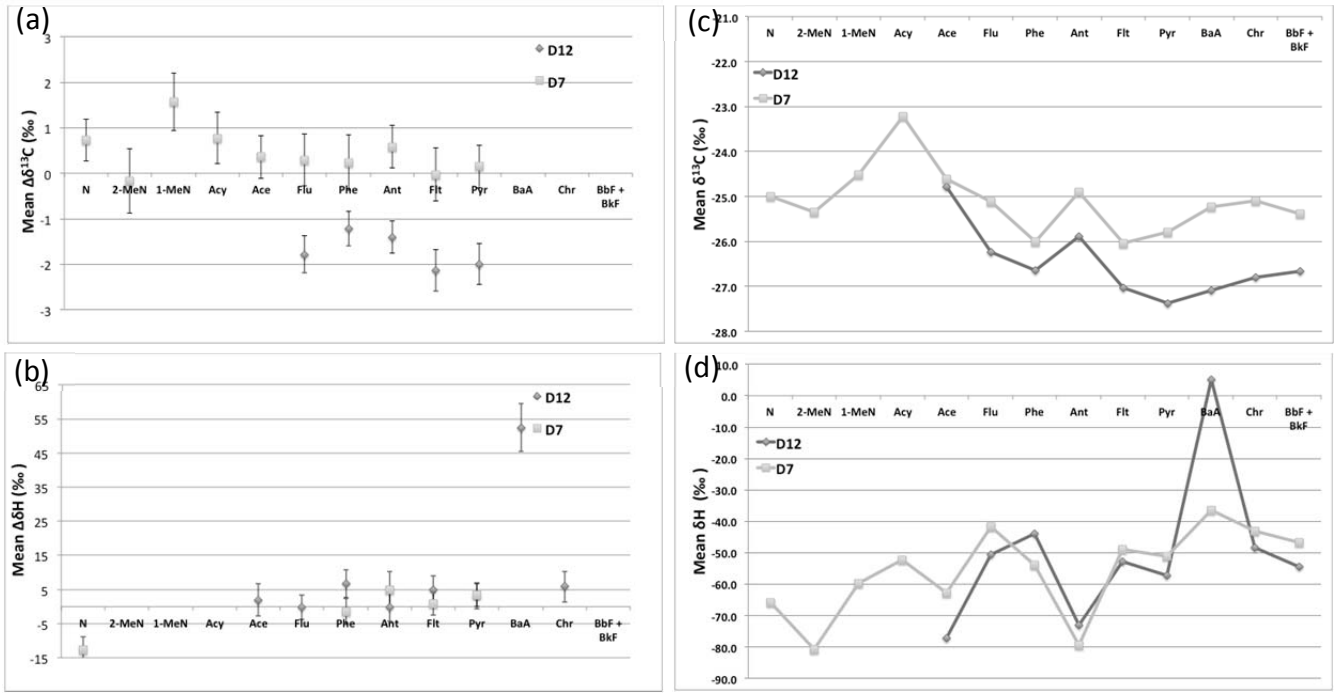

Figure 4. $\Delta\delta^{13}C$ (a) and $\Delta\delta D$ (b) measuring the difference in isotopic values between F2 and F'2 in samples D7 and D12, error bars are the 95% confidence intervals calculated through error propagation. $\delta^{13}C$ (c) and δD (d) isotopic profiles of samples D7 and D12 for F2.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

Is it really clean? A novel extraction method for polycyclic aromatic hydrocarbons in coal tare non-aqueous phase liquids using pressurized liquid extraction is described. Comprehensive two-dimensional gas chromatography was used to evaluate possible interferences in the sample that could affect compound specific stable isotope analysis of the polycyclic aromatic hydrocarbons.

Keywords: Comprehensive Two-Dimensional Gas Chromatography • Compound Specific Stable Isotope Analysis • Polycyclic Aromatic Hydrocarbons • Environmental Forensics• Pressurised Liquid Extraction