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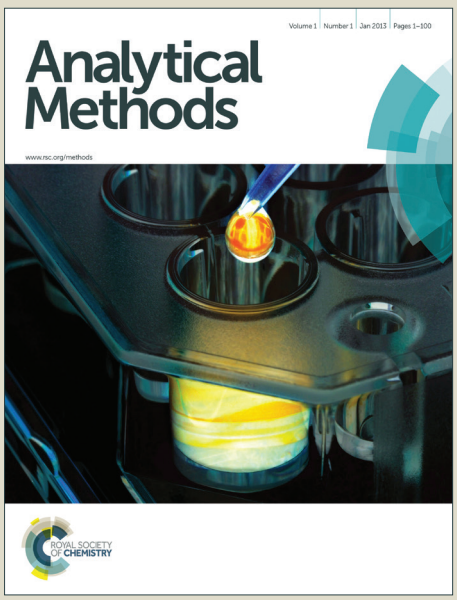

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An analytical method for quantifying petroleum hydrocarbon fractions in soils, and its associated uncertainties

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This paper proposes a method for measuring total petroleum hydrocarbons (TPH) and other hydrocarbon fractions in soils, and discusses its uncertainties. In this method, hydrocarbons are microwave-extracted from the soil and then subjected to solid phase extraction (SPE). The resulting fractions are analyzed by gas chromatography with flame ionization detection. In tests using reference soil samples of known TPH content, recoveries were on average 90% for all fractions. The limit of detection for TPH in the tested soils was below 0.2 mg kg^{-1}. Inter-repetition variation in the quantification of aliphatic hydrocarbons was <3%, and <4% for aromatic hydrocarbons. The expanded uncertainty associated with the method ranged from 9% to 15% for aliphatic hydrocarbons and from 19% to 30% for aromatic hydrocarbons. The possible contributors towards the overall uncertainty are discussed.

Keywords Petroleum hydrocarbons; Hydrocarbon fractions; Soil; Solid phase extraction; Uncertainty; Validation.

Introduction

Petroleum products, including a wide range of hydrocarbons from light gases to heavy residues, are common environmental contaminants [1, 2]. Once released into the environment, they have different fates. Lower molecular weight compounds are commonly subject to volatilization, but the heaviest may slowly enter into the soil. In fact, hydrocarbons from petroleum are among the most common soil pollutants [3]. The term total petroleum hydrocarbons (TPH) refers to the total concentration of non-polar petroleum hydrocarbons in a substrate. The most popular TPH identification methods are based on gas chromatography, infrared spectroscopy or gravimetric analytical techniques. Currently, gas chromatography-based methods are the most used since they can also quantify the TPH. These methods consist of an extraction stage during which TPH compounds are separated from the soil, a clean-up stage to remove any impurities and or to fractionate the TPH. Several works have examined the TPH in soils [1, 4-7] including studies for the remediation of contaminated soils by TPH [8-12].

As stated by the Total Petroleum Hydrocarbons Criteria Working Group (TPHCWG) [13, 14], the equivalent carbon (EC) of any given hydrocarbon is based on the comparison of its boiling point with that of the reference compound n-hexane. The variable EC is used to define hydrocarbon fractions.

International standard ISO 16703, which involves gas chromatography and flame ionization detection (GC-FID), is currently the most commonly employed for determining TPH in soils [15]. This standard can be used to identify and quantify all hydrocarbons with a boiling point range of 175°C to 525°C. However, this method is costly and environmentally unfriendly. The European Committee for Standardization has issued a draft European Standard for the quantitative determination of TPH in solid wastes that has also been used with contaminated soils [16-18]. However, the authors of these latter studies report that several compounds may be co-extracted, reducing the quality of the overall results. Thus, the earlier ISO 16703 would appear to provide the best starting point for the development of new methodologies without the stated drawbacks.

This paper presents a method based on microwave extraction followed by solid phase extraction (SPE) and GC-FID analysis for determining TPH and its fractions in soils contaminated by petroleum products. Microwave extraction uses less organic solvent, is quicker than Soxhlet extraction, and can be used with smaller samples. SPE allows the hydrocarbon fractions to be separated more easily than column separation. The proposed method was validated using samples of known hydrocarbon concentration. The uncertainties associated with the method are discussed.

Validating the measurement method to analyze TPH and the further fractions in contaminated soils and assessing the uncertainty associated to the measurement were the goals of this paper.

Experimental

Chemical and reagents

Aliphatic hydrocarbons (C_8 to C_{40}, phytane and pristane at 500 µg mL^{-1} in hexane) were purchased from AccuStandard (New

Haven, USA). Polyaromatic hydrocarbons (acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorine, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene and pyrene at 100 μg mL^{-1} in cyclohexane) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The above compounds were used to verify chromatographic separation quality.

The certified reference materials CRM357 (TPH–sandy loam), CRM359 (TPH–clay loam) and CRM372 (TPH–sand) were acquired from RT–Corp (Laramie, USA). These materials were used to determine the accuracy of the results provided by the proposed procedure.

Isolute Sorbent EPH (25 mL/5 g) extraction cartridges (Biotage, Uppsala, Sweden) and a vacuum manifold (Agilent, Santa Clara, USA) were used for sample extraction and elution. GC grade acetone, dichloromethane and hexane were purchased from Sigma Aldrich (St. Louis, USA) and used as extraction solvents.

Description of the analytical method

In this proposed method, hydrocarbon-contaminated soil samples are air-dried over a 7-day period to a constant weight in an environmental chamber at 20ºC (humidity 50%). Extraction solvent (20 mL acetone and *n*-hexane at 1:1 v/v) is added to 1 g samples of the dried soil and this mixture subjected to microwave extraction using a 1000 W microwave oven (in the development of the proposed method a Milestone Ethos Sel model was used). The extraction temperature is 150ºC; when this is reached it is held for 20 min. The soil and solvent are then separated by filtration through a polytetraflourethylene membrane with a pore size of 20 μm. The filtered extracts are then evaporated with nitrogen to a volume of 1 mL.

The reduced extracts are fractionated following an optimised clean-up procedure involving *Isolute* EPH cartridges (25 mL/5 g). The cartridges are conditioned prior to sample loading with 30 mL of hexane without letting them become dry. After loading the sample, elution is performed using 12 mL of hexane, and then 20 mL of dichloromethane, both at a flow rate of 2-3 mL min^{-1}. The two fractions produced are evaporated under nitrogen to 1 mL and injected into a GC-FID apparatus. An Agilent 7820A gas chromatography apparatus equipped with an FID device plus an Agilent G4513A autosampler with a HP5-MS capillary column (30 m x 0.32 mm i.d.; nominal film thickness 0.25 μm). Splitless injection was used with a deactivated inlet liner (4 mm i.d.) with glass wool and a single taper. The injection temperature is 250ºC, the injection volume 3 μL. Helium is the carrier gas (74 kPa). The oven operating conditions are: initial temperature 80ºC, increasing to 200ºC at a rate of 7ºC min^{-1}, then increasing to 300ºC at rate of 11ºC min^{-1}, holding at this temperature for 17 min. FID is operated at 325ºC and 20 Hz.

The aliphatic and aromatic fractions detected are defined on the basis of their EC. The number of carbons and the EC values for each are those shown in Electronic Supplementary Information Tables I and II.[†] Quantification is always determined from baseline. Table 1 shows the aliphatic and aromatic fractions that can be examined.

Table 1: Definition of aliphatic and aromatic hydrocarbon fractions identifiable by the proposed methodology.

Aliphatic hydrocarbon fraction	Beginning marker	Ending marker	Aromatic hydrocarbon fraction	Beginning marker	Ending marker
$>C_{10}$ to C_{12}	0.1 min after decane	0.1 min before tridecane	$>EC_{10}$ to EC_{12}	0.1 min before naphthalene	0.1 min before acenaphthylene
$>C_{12}$ to C_{16}	0.1 min before tridecane	0.1 min before heptadecane	$>EC_{12}$ to EC_{16}	0.1 min before acenaphthylene	0.1 min before fluorene
$>C_{16}$ to C_{21}	0.1 min before heptadecane	0.1 min before docosane	$>EC_{16}$ to EC_{21}	0.1 min before fluorene	0.1 min before benzo(a)anthracene
$>C_{21}$ to C_{35}	0.1 min before docosane	0.1 min before hexatricontane	$>EC_{21}$ to EC_{35}	0.1 min before benzo(a)anthracene	0.1 min before Indeno(123cd)pyrene
$>C_{35}$	0.1 min before hexatricontane	0.1 min after tetracontane	$>EC_{35}$	0.1 min before Indeno(123cd)pyrene	0.1 min after benzo(ghi)perylene

Calibration of the GC-FID for soil testing

The GC-FID used in tests involving reference materials to validate the method was calibrated using a series of dilutions of six standards representing the above-defined fractions. These were introduced into the gas chromatograph (performed in triplicate). Aliphatic and aromatic compounds were injected separately. The calibration factors for the different fractions were calculated as the sum of the peak areas of all components in a corresponding fraction against the total concentration injected (full details are provided in Electronic Supplementary Information: Table III and Figure 1).[†]

Analysis of uncertainty

A bottom-up estimation of the uncertainty associated with the proposed method was performed following EURACHEM / CITAC Guide CG 4 [19] This approach divides the analytical method being evaluated into different steps and allows each source of uncertainty to be identified. Repeated measurements analysis was performed to determine the uncertainly associated

with each identified source. This approach has been used successfully with complex analytical methods [20-26].

Results and Discussion

Validation of the measurement procedure

The validation of the proposed method was undertaken according to International Union of Pure and Applied Chemistry (IUPAC) [27, 28]. The following variables were therefore determined: linearity, bias, accuracy, traceability, intermediate precision, repeatability, limit of detection (LOD) and limit of quantification (LOQ).

Linearity was confirmed after calculating the calibration factor (δ) from the results provided by the six GC-FID calibration standards (Table 2). The lowest concentration of each standard therefore reflects the lower limit of the linear range, while the highest concentration defines the upper limit. Table 2 shows the mean calibration factor values, the associated relative standard deviations (s_{rel}), and the working range for each hydrocarbon fraction.

The traceability of the proposed method and the accuracy of its measurements were evaluated in recovery experiments, quantifying the three certified reference materials (CRM-357, CRM-359 and CRM-372) eight times. This allowed the closeness of agreement between the measured value and the true concentration to be examined [29].

Table 2: Calibration factors for each hydrocarbon fraction.

Hydrocarbon fraction	Lower limit ($\mu g\ mL^{-1}$)	Upper limit ($\mu g\ mL^{-1}$)	δ (10^4 pA s mL μg^{-1})	s_{rel} (%)
TPH	32	1600	36.6	3
Aliphatic				
$>C_{10}$ - C_{12}	20	400	53.8	7
$>C_{12}$ - C_{16}	40	800	52.2	6
$>C_{16}$ - C_{21}	70	1400	50.2	10
$>C_{21}$ - C_{35}	140	2800	53.6	12
$>C_{35}$	50	1000	49.4	17
Aromatic				
$>EC_{10}$ - EC_{12}	10	100	58.6	6
$>EC_{12}$ - EC_{16}	20	200	58.0	5
$>EC_{16}$ - EC_{21}	50	500	56.0	5
$>EC_{21}$ - EC_{35}	50	500	54.7	7
$>EC_{35}$	30	300	37.2	4

Table 3 shows recovery values of >90%, confirming the procedure as suitable for quantifying the studied hydrocarbon fractions in soils. The high recovery (157%) shown by the $>EC_{10}$ - EC_{12} aromatic fraction can be attributed to problematic blank values. Even though the vessels used in the microwave device were thoroughly cleaned, they still returned high blank values. The standard deviation for the aliphatic fractions was up to 10%, while for the aromatic fractions it was around 20%. These results agree with the certificated values provided.

The results obtained for all three reference samples agreed with their certified values for TPH. The concentrations detected for the different hydrocarbon fractions in CRM-372 also agreed with the certified values, except for the fraction $>EC_{21} - EC_{35}$ (Table 3). Table 3 shows the relative error for the TPH readings to be <10%. The errors for the hydrocarbon fractions were even better, especially those for the aromatic hydrocarbon fractions, except for the $>EC_{10}$-EC_{12} fraction (50%). This could be due to the confidence interval for the certified value being large (40%). Even so, the present error is very similar to this interval.

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Table 3: Results of recovery test using the certified reference materials.

Hydrocarbon fraction	Certified value (mg kg^{-1})*	Measured value (mg kg^{-1})	Mean recovery (%)	**s_{rel} (%)	Relative error (%)
		CRM-372			
TPH	2020 ± 163	2042 ± 295	101	21	1
Aliphatic					
>C_{10} - C_{12}	70.9 ± 5.88	72.7 ± 4.0	102	8	2
>C_{12} - C_{16}	314 ± 6.25	288.6 ± 19.7	92	10	8
>C_{16} - C_{21}	209 ± 14.0	191.9 ± 6.7	92	5	8
>C_{21} - C_{35}	460 ± 8.17	558.0 ± 16.2	121	4	21
Aromatic					
>EC_{10} - EC_{12}	17.1 ± 7.52	26.8 ± 4.3	157	23	52
>EC_{12} - EC_{16}	112 ± 40.7	113.3 ± 19.4	101	25	1
>EC_{16} - EC_{21}	96.2 ± 15.2	96.1 ± 8.1	100	12	0.1
>EC_{21} - EC_{35}	39.4 ± 7.21	40.4 ± 7.8	103	28	2
		CRM-359			
TPH	1100 ± 110	1015 ± 123	91	11	9
		CRM-357			
TPH	3220 ± 310	2978 ± 63	92	2	8

*Confidence interval 95%.

**s_{rel}. Standard deviation of recovery.

Several measures are available for validating an analytical procedure, including repeatability and intermediate precision. A repeatability study was undertaken by repeating the whole analytical procedure on the same day. Repeatability was calculated from the coefficient of variation for four injections of extracted CRM-372. The intermediate precision was calculated from the relative standard deviation of eight injections of extracted CRM-372 on different days. Table 4 shows the values of the variables recorded.

Table 4: Repeatability and intermediate precision of the CRM372.

Hydrocarbon fraction	Repeatability	Intermediate precision
	s_{rel} (%) CRM-372 (n=4)	s_{rel} (%) CRM-372 (n=8)
TPH	0.4	1.8
Aliphatic		
>C_{10} - C_{12}	0.4	1
>C_{12} - C_{16}	0.3	1
>C_{16} - C_{21}	0.6	0.8
>C_{21} - C_{35}	0.4	1
>C_{35}	2.0	3
Aromatic		
>EC_{10} - EC_{12}	3	3
>EC_{12} - EC_{16}	2	4
>EC_{16} - EC_{21}	2	4
>EC_{21} - EC_{35}	1	4
>EC_{35}	2	3

Journal Name

RSCPublishing

ARTICLE

Table 4 shows the repeatability and intermediate precision values for the aliphatic hydrocarbon fractions to be slightly lower than those recorded for the aromatic hydrocarbon fractions.

LODs and LOQs were determined using spiked blanks. Six blank samples were spiked with hydrocarbons (0.1 μg mL^{-1}) and analysed, and the LOD and LOQ values calculated according to the recommendations of the IUPAC [30]. In this case, the LOD was calculated as three times the standard deviation and the LOQ as ten times the standard deviation. The results are summarized in Table 5. The LOD values were below 0.5 mg kg^{-1}, considerably lower than those measured in soils contaminated by hydrocarbons described by other authors [4, 5, 7, 31-34].

Table 5: Limits of detection and quantification.

Hydrocarbon range	w_{LOD} (mg kg^{-1})	w_{LOQ} (mg kg^{-1})
TPH	1.21	4.03
Aliphatic		
$>C_{10}$ - C_{12}	0.14	0.46
$>C_{12}$ - C_{16}	0.12	0.40
$>C_{16}$ - C_{21}	0.34	1.13
$>C_{21}$ - C_{35}	0.52	1.73
$>C_{35}$	0.19	0.64
Aromatic		
$>EC_{10}$ - EC_{12}	0.03	0.10
$>EC_{12}$ - EC_{16}	0.06	0.19
$>EC_{16}$ - EC_{21}	0.11	0.38
$>EC_{21}$ - EC_{35}	0.14	0.48
$>EC_{35}$	0.18	0.61

Assessment of uncertainty associated with the proposed methodology

The first step in measuring uncertainty is to specify the measurand. The mass fraction of hydrocarbon fractions in a soil sample (w_{HC}), expressed in mg kg^{-1}, is obtained from Equation (1).

$$w_{HC} = \frac{A \times V}{\delta \times m \times R} \qquad (1)$$

where A is the accumulated peak area of all components in the fraction considered, V is the final volume (1 mL) before injection into the gas chromatograph, δ is the calculated calibration factor, m is the weight (1 g or 9.8 mN) of the analysed soil sample, and R is the percentage recovery.

The second step is to identify each source of uncertainty. Figure 1 shows the main sources in a "*cause and effect diagram*". The inhomogeneity of the sample (I) was here included as source of uncertainty. The diagram facilitates the identification of the main contributors to the uncertainty, and helps prevent an uncertainty contribution being incorporated more than once.

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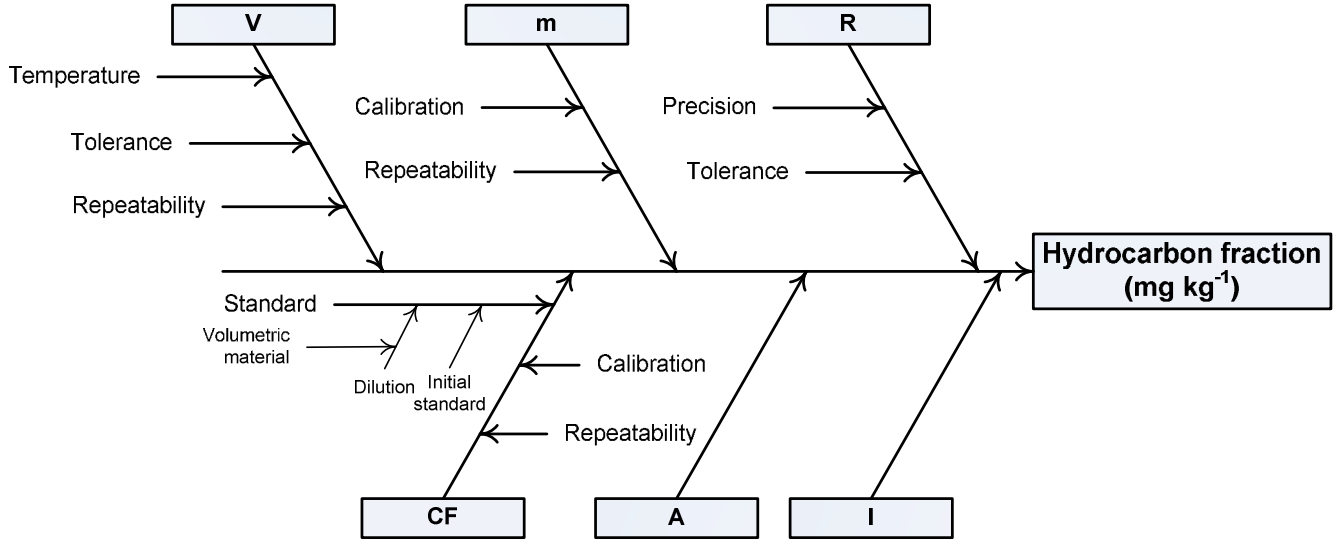

Figure 1: Cause and effect diagram of uncertainty associated with the proposed method.

UNCERTAINTY DERIVED FROM THE CHROMATOGRAPHIC AREA. In the development of the proposed method, the data acquisition system of the GC used returned the FID signal in picoamperes (pA). The chromatographic peak area was therefore measured in pA s. According to the manufacturer, the data acquisition system of this equipment has a resolution of 1 pA s; the uncertainty associated with the peak area was therefore calculated assuming a rectangular distribution according to Equation (2).

$$u(A)_r = \frac{u(A)}{A} = \frac{a/\sqrt{3}}{A} \qquad (2)$$

where *a* is the resolution, and *A* the chromatographic area. To calculate the standard uncertainty *u(A)*, the hydrocarbon fraction areas obtained in the repeatability study were employed. This uncertainty encompasses the resolution, noise and baseline drifts. The uncertainty derived for the chromatographic area (Electronic Supplementary Information Table IV) was negligible since the hydrocarbon fractions areas were large.

UNCERTAINTY DERIVED FROM THE PRE-INJECTION SAMPLE VOLUME. After performing the extraction and fractionation steps, samples were dissolved in 1 mL of hexane before being injected into GC/MS. Three uncertainty components were taken into account to calculate the uncertainty associated with this volume u(V): the influence of temperature, the tolerance of the volumetric material, and random error. The uncertainty associated with the variation in temperature was calculated via the coefficient of expansion (α) of n-hexane assuming a rectangular distribution for a temperature variation of 20 ± 5ºC. The volumetric accuracy of the syringe was calculated assuming a triangular distribution (v) for the smallest marked divisions - 10 µL – of the 1000 µL syringe used. The random error was deemed that due to variations in filling the syringe to the mark, and was calculated via the standard deviation obtained from eight weighings of a syringe full of n-hexane. Consequently, the uncertainty due to volume is given in Equation (3). A constant value of 5.65 x 10^{-3} was obtained for all hydrocarbon fractions.

$$u(V)_R = \frac{u(V)}{V} = \sqrt{\left(\frac{V \times \Delta T \times A}{\sqrt{3}}\right)^2 + \left(\frac{v/\sqrt{6}}{V}\right)^2 + \left(\frac{s}{M}\right)^2} \qquad (3)$$

UNCERTAINTY DERIVED FROM THE ESTIMATION OF THE CALIBRATION FACTOR. Six standard solutions (in triplicate, concentration range 1-50 µg mL^{-1} for the aliphatic hydrocarbon fractions and 1-100 µg mL^{-1} for the aromatic hydrocarbon fractions) were analysed. Equation (4) shows how the calibration factor (δ) is determined for each fraction, where *A* is the total area of the fraction components, and *C* the total concentration injected.

$$\delta = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{A}{C}\right) \qquad (4)$$

Thus, the uncertainty associated with the estimation of the calibration factor is a combination of two uncertainties (see Equation (5)): the contribution of the chromatographic area *u(A)* and of the concentration of the standard solutions $u(C_{std})$. These sources are, in turn, combinations of several other uncertainties.

$$u(\delta)_r = \sqrt{\left(\frac{u(A_{chr})}{A_{chr}}\right)^2 + \left(\frac{u(C_{std})}{C_{std}}\right)^2} \qquad (5)$$

Uncertainty due to chromatographic area. The uncertainties associated with the areas for the standard solution of each hydrocarbon fraction has two components: the uncertainty associated with the resolution of the system *u(res)* (assuming a rectangular distribution), and the uncertainty due to repeatability, *u(rep)*. A spiked sample, at a concentration within the corresponding calibration range was injected six times, and the uncertainty due to chromatographic area determined as follows:

$$u(A_{chr})_r = \frac{u(A_{chr})}{A_{chr}} = \sqrt{\left(\frac{a/\sqrt{3}}{A}\right)^2 + \left(\frac{s}{A}\right)^2} \qquad (6)$$

The first term of Equation 6 is associated with the resolution of the data acquisition system, and, as established above can be eliminated since the repeatability makes the greatest contribution to uncertainty. Table 6 shows the results for this uncertainty for each hydrocarbon fraction.

Table 6: Uncertainty derived from the chromatographic area.

Hydrocarbon range	A_{chr} (x 10^6 pA s)	*s* (n=8) (x 10^6 pA s)	$u(A_{chr})_r$ (x 10^{-2})
TPH	855	15.2	1.77
Aliphatic			
>C_{10} - C_{12}	45	0.6	1.25
>C_{12} - C_{16}	165	2.2	1.35
>C_{16} - C_{21}	104	0.9	0.83
>C_{21} - C_{35}	324	4.3	1.34
>C_{35}	26	0.7	2.62
Aromatic			
>EC_{10} - EC_{12}	17	0.4	2.54
>EC_{12} - EC_{16}	62	2.7	4.43
>EC_{16} - EC_{21}	56	2.4	4.28
>EC_{21} - EC_{35}	29	1.3	4.46
>EC_{35}	27	0.8	2.80

The uncertainty associated with the chromatographic area for the aromatic hydrocarbon fraction was twice that of the aliphatic hydrocarbon fraction.

Uncertainty derived from the preparation of standard solutions: To estimate the calibration factor for each hydrocarbon fraction, six standard solutions at different concentrations (in triplicate) were prepared by dilution of standards according to Equation (7):

$$C_c = C_{std} \times \frac{V_0}{V_f} \qquad (7)$$

where C_c is the concentration of the calibrate solution, C_{std} is the concentration of the initial standard solution, V_0 is the volume of the standard solution, and V_f is the final volume of the calibrate solution. The uncertainty derived from the preparation of each standard solution is calculated according to Equation (8):

$$\left(\frac{u(C_c)}{C_c}\right) = \sqrt{\left(\frac{u(C_{std})}{C_{std}}\right)^2 + \left(\frac{u(V_f)}{V_f}\right)^2 + \left(\frac{u(V_0)}{V_0}\right)^2} \qquad (8)$$

The uncertainty of the analytical standards was also provided by the manufacturer. Table 7 summarizes the uncertainties associated with the preparation of the standard solutions.

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Table 7: Uncertainty associated with preparation of the standard solutions.

Hydrocarbon fraction	Standard concentration ($\mu g\ mL^{-1}$)	$u(C_{std})_r$ x 10^{-3}
TPH	16000	2.25
Aliphatic		
$>C_{10}$ - C_{12}	1000	3.02
$>C_{12}$ - C_{16}	2000	5.13
$>C_{16}$ - C_{21}	3500	2.09
$>C_{21}$ - E_{35}	7000	1.70
$>C_{35}$	2500	1.46
Aromatic		
$>EC_{10}$ - EC_{12}	100	1.16
$>EC_{12}$ - EC_{16}	200	7.31
$>EC_{16}$ - EC_{21}	500	4.07
$>EC_{21}$ - EC_{35}	500	5.83
$>EC_{35}$	300	4.85

Equation (3) involves the three contributions taken into account to calculate the uncertainty of volume. Two syringes of 50 and 100 µL were employed to prepare the calibration solutions. The standard uncertainty associated with these syringes was 5.525 x 10^{-3} and 6.071 x 10^{-3} respectively. Thus, the uncertainty associated with the preparation of the standard solution for each hydrocarbon fraction is the sum of the uncertainties associated with each calibration solution. This uncertainty can be determined according to Equation (9):

$$\left(\frac{u(C)}{C}\right)^2_{Total} = \sum_{i=1}^{n=6}\left(\frac{u(C_i)}{C_i}\right)^2 \quad (9)$$

Table 8 summarizes the uncertainty for each calibration solution (full details can see in section 5 of the Electronic Supplementary Information).

Table 9 summarizes the contribution of each uncertainty associated with the calibration factor, calculated according to Equation (5).

Journal Name

RSCPublishing

ARTICLE

Table 8: Uncertainties associated with the preparation of each calibration solution.

Hydrocarbon range	$u(C_1)_\tau$ (x 10^{-3})	$u(C_2)_\tau$ (x 10^{-3})	$u(C_3)_\tau$ (x 10^{-3})	$u(C_4)_\tau$ (x 10^{-3})	$u(C_5)_\tau$ (x 10^{-3})	$u(C_6)_\tau$ (x 10^{-3})	$u(C_{total})_\tau$ (x 10^{-2})
TPH	6.48	8.88	8.88	8.51	8.51	8.51	2.04
Aliphatic							
$>C_{10}$ - C_{12}	6.78	9.10	9.10	8.75	8.75	8.75	2.10
$>C_{12}$ - C_{16}	7.95	10.0	10.0	9.68	9.68	9.68	2.33
$>C_{16}$ - C_{21}	6.42	8.84	8.84	8.47	8.47	8.47	2.03
$>C_{21}$ - C_{35}	6.30	8.75	8.75	8.38	8.38	8.38	2.01
$>C_{35}$	6.24	8.71	8.71	8.34	8.34	8.34	2.00
Aromatic							
$>EC_{10}$ - EC_{12}	6.18	8.66	8.66	8.29	8.29	8.29	1.99
$>EC_{12}$ - EC_{16}	9.50	11.3	11.3	11.0	11.0	11.0	2.66
$>EC_{16}$ - EC_{21}	7.31	9.50	9.50	9.16	9.16	9.16	2.20
$>EC_{21}$ - EC_{35}	8.42	10.4	10.4	10.1	10.1	10.1	2.43
$>EC_{35}$	7.77	9.86	9.86	9.53	9.53	9.53	2.30

Table 9: Uncertainties associated with the calibration factor.

Hydrocarbon fraction	$u(C_{total})_\tau$ (x 10^{-2})	$u(A_{std})_\tau$ (x 10^{-2})	$u(\delta)_\tau$ (x 10^{-2})
TPH	2.04	1.77	2.71
Aliphatic			
$>C_{10}$ - C_{12}	2.10	1.25	2.44
$>C_{12}$ - C_{16}	2.33	1.35	2.70
$>C_{16}$ - C_{21}	2.03	8.26	2.19
$>C_{21}$ - C_{35}	2.01	1.34	2.42
$>C_{35}$	1.20	2.62	2.88
Aromatic			
$>EC_{10}$ - EC_{12}	1.99	2.54	3.23
$>EC_{12}$ - EC_{16}	2.66	4.43	5.17
$>EC_{16}$ - EC_{21}	2.20	4.28	4.81
$>EC_{21}$ - EC_{35}	2.43	4.46	5.07
$>EC_{35}$	2.30	2.80	3.63

UNCERTAINTY DERIVED FROM THE SAMPLE MASS. The soil sample was weighed in an analytical balance; the main uncertainty components associated with this weighing are the calibration of the balance and the repeatability of measurements. The uncertainty associated with the calibration of the balance is obtained from the certificate given by the manufacturer. For the balance used in the work, u_{cal} was 3.1 x 10^{-5} g (k=2) for weights under 10 g. The repeatability was calculated from the data recorded in repeated weighing experiment (eight weighings of an aliquot of soil performed at 5 min intervals). This uncertainty was calculated assuming a rectangular distribution (u_{rep} 4.022 x 10^{-4} g). Finally the uncertainty associated with the mass of the sample was determined according to Equation (10) (1.38 x 10^{-4}). Full details regarding the uncertainty associated with the sample

mass can be seen in Electronic Supplementary Information Tables IX and X.

$$u(m)_r = \frac{u(m)}{m} = \sqrt{\left(\frac{u_{cal}}{m}\right)^2 + \left(\frac{s/\sqrt{3}}{m}\right)^2} \quad (10)$$

UNCERTAINTY ASSOCIATED WITH RECOVERY VALUES. The recovery study was performed using reference material CMR-372. Eight replicates were used. The percentage recovery was calculated as in Equation (11):

$$R = \left(\frac{C_{found}}{C_{spiked}}\right) \quad (11)$$

The uncertainty associated with the recovery value covers the contribution made by the actual spiking of the reference material, incomplete microwave extraction, losses through evaporation, losses on SPE, and the stability of the GC-FID instrument. This uncertainty is calculated according to Equation (12).

$$u(R)_r = \left(\frac{u(R)}{R}\right) = \left(\frac{s/\sqrt{n}}{R}\right) \quad (12)$$

Table 10 provides the uncertainty associated with the recovery value for each hydrocarbon fraction.

Table 10: Uncertainty associated with the recovery value.

Hydrocarbon fraction	Recovery (%)								Average (%)	*s* (%)	$u(R)_r$ (x 10^{-2})
TPH	120	-	91	91	87	138	103	78	101	21	7.38
Aliphatic											
$>C_{10}$ - C_{12}	96	113	106	97	109	105	88	106	102	8	5.14
$>C_{12}$ - C_{16}	106	92	87	77	89	88	95	102	92	9	3.55
$>C_{16}$ - C_{21}	102	89	94	87	90	88	91	93	92	5	1.96
$>C_{21}$ - C_{35}	124	113	127	125	120	114	122	124	121	5	1.13
Aromatic											
$>EC_{10}$ - EC_{12}	148	151	116	174	179	109	154	223	157	36	8.17
$>EC_{12}$ - EC_{16}	109	97	155	69	98	104	87	90	101	25	8.72
$>EC_{16}$ - EC_{21}	108	100	122	80	98	103	92	96	100	12	4.31
$>EC_{21}$ - EC_{35}	123	66	106	53	99	127	121	126	103	29	9.89

UNCERTAINTY DERIVED FROM SOIL INHOMOGENEITY. Real-life soils contaminated by hydrocarbons can be inhomogeneous, a consequence of their physico-chemical properties. The contribution of inhomogeneity to overall uncertainty must therefore be taken into account. A collected soil (from the "La Carraca" arsenal ground in San Fernando, Cádiz, Spain), known to be contaminated by hydrocarbons, was analysed in triplicate The collection area was used as a petrol station during the 1970s, and shows a high degree of oil pollution. The uncertainty derived from the inhomogeneity of the soil can be calculated according to Equation (13), where s is the standard deviation of the analysis (performed in triplicate), and Cs the concentration of each range of hydrocarbons in the soil. Table 11 shows the results obtained.

$$u(I)_r = \left(\frac{s/\sqrt{n}}{C_s}\right) \quad (13)$$

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Table 11: Uncertainty associated with soil inhomogeneity.

Hydrocarbon fraction	Mass of hydrocarbons (mg kg^{-1})			Mean (mg kg^{-1})	s (mg kg^{-1})	$u(I)_r$
TPH	824	856	801	827.0	27.62	0.019
Aliphatic						
>C_{10} - C_{12}	6	6	5	5.7	0.58	0.059
>C_{12} - C_{16}	27	29	31	29.0	2.00	0.040
>C_{16} - C_{21}	112	124	115	117.0	6.24	0.031
>C_{21} - C_{35}	316	301	309	308.7	7.51	0.014
>C_{35}	110	100	107	105.7	5.13	0.028
Aromatic						
>EC_{10} - EC_{12}	23	21	24	22.7	1.53	0.039
>EC_{12} - EC_{16}	24	24	22	23.3	1.15	0.029
>EC_{16} - EC_{21}	46	51	41	46.0	5.00	0.063
>EC_{21} - EC_{35}	106	127	121	118.0	10.82	0.053
>C_{35}	28	25	20	24.3	4.04	0.096

Estimation of combined and expanded uncertainty

The last step in determining the overall uncertainty is to calculate the combined and expanded uncertainty. Figures 2 and 3 show the standard uncertainty for the six sources identified [$u(A)_r$, $u(V)_r$, $u(\delta)_r$, $u(m)_r$, $u(R)_r$ and $u(I)_r$] for both the aliphatic and aromatic hydrocarbons fractions.

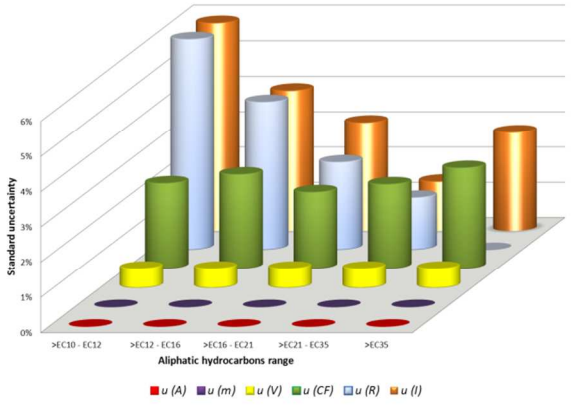

Figure 2: Standard uncertainty for the aliphatic hydrocarbon fractions.

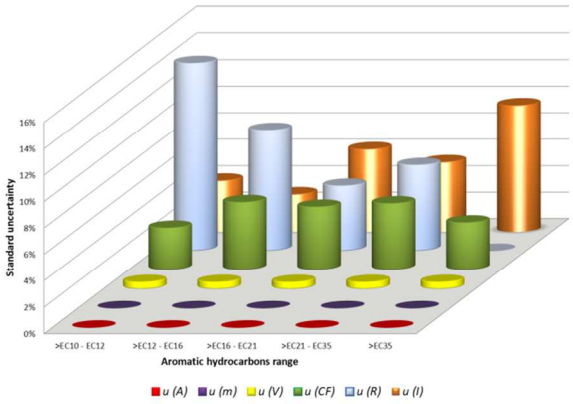

Figure 3: Standard uncertainty for the aromatic hydrocarbon fractions.

The standard uncertainties shown in Figure 2 confirm that the uncertainty associated with the chromatographic area and the sample mass can be dismissed. Thus, to reduce the uncertainty associated with the analysis of the aliphatic hydrocarbon fractions, the uncertainty associated with the calibration factor and the recovery need to be reduced. The uncertainty associated with the calibration factor is due to the uncertainty associated with the repeatability of the GC-FID results and to that of the calibration standards; it would therefore be very hard to make any practical improvement. However, the uncertainty related to the recovery values can be reduced somewhat. The recovery values recorded were all close 100%, but the standard deviation

of the more lightweight hydrocarbon fractions could be reduced. The high standard deviations were a consequence of the blanks used; strenuous cleaning would help avoid contamination problems, which might diminish the spread. In addition, we should mention that loss of the more volatile petroleum hydrocarbons may occur during sampling and on air-drying of the soils. Thus, in order to analyse only these compounds, the sampling and the analytical method must be different.

The uncertainty associated with inhomogeneity is related to the characteristics of an examined soil, but it might be reduced slightly by analysing larger samples (only 1 g samples were used in the present work).

The overall uncertainties associated with the aromatic hydrocarbons were similar to those affecting the aliphatic hydrocarbons. Uncertainty due to the calibration factor was higher because the analyses of the aromatic hydrocarbons were less accurate. Cleaning the blanks should be improved to reduce this problem.

The combined uncertainty requires the generation of partial differentials. Fortunately, the numerical method of Kragten [35] makes effective use of spreadsheet software to provide the combined standard uncertainty from input uncertainties. The following example shows the combined standard uncertainty for the method (Fig. 4). Table 12 shows the combined uncertainty u_c and expanded uncertainty *U (k=2)* for the hydrocarbon fractions.

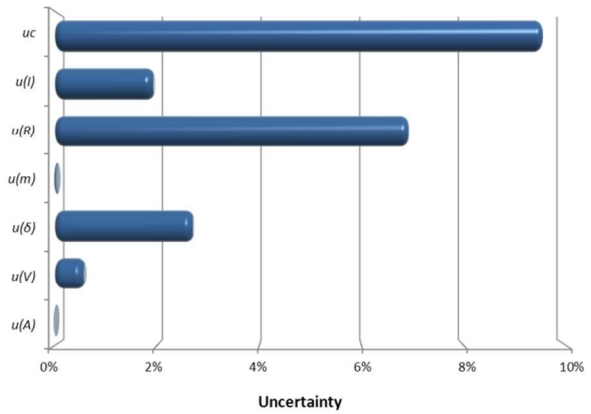

Figure 4: Contributions of the different uncertainty sources.

Table 12: Combined and expanded uncertainty.

Aliphatic hydrocarbons	u_c (%)	*U (k=2)* (%)	Aromatic hydrocarbons	u_c (%)	*U (k=2)* (%)
TPH	10	19	-	-	-
$>C_{10} - C_{12}$	10	19	$>EC_{10} - EC_{12}$	12	23
$>C_{12} - C_{16}$	8	15	$>EC_{12} - EC_{16}$	14	27
$>C_{16} - C_{21}$	6	11	$>EC_{16} - EC_{21}$	12	23
$>C_{21} - C_{35}$	5	9	$>EC_{21} - EC_{35}$	16	32
$>C_{35}$	6	11	$>EC_{35}$	16	32

Conclusions

This work provides a new method for quantifying the TPH and the aliphatic and aromatic hydrocarbon fractions in contaminated soils. The method involves microwave extraction with hexane acetone (1:1) followed by a clean-up procedure using *Isolute* Sorbent EPH cartridges, and then GC-FID analysis. The method is faster, cheaper and more environmentally friendly than the conventional methods currently in use.

The proposed method shows adequate linearity, bias, accuracy, traceability, intermediate precision, and repeatability, and has appropriate limits of detection and quantification. Recoveries of >90% for all hydrocarbon fractions.

The quantification of the aromatic hydrocarbon fractions is somewhat less reliable than that of the aliphatic hydrocarbon fractions. The traceability for TPH was confirmed.

An easy-to-follow guide for calculating the uncertainty associated with this procedure is described. Six uncertainty sources were identified. A detailed analysis of their contributions in this respect was performed, and the combined and expanded uncertainty established. The uncertainties associated with calibration, recovery and inhomogeneity were the most important; the contributions of the chromatographic area and sample mass were unimportant. The uncertainty associated with the recovery of the lighter hydrocarbon fractions was greater due to problems caused by the blanks used in their estimation. The expanded uncertainty ranged between 9% and 15% for aliphatic hydrocarbons, and between 19% and 30% for aromatic hydrocarbons.

In conclusion, the proposed method provides a rapid, cheap and accurate method for identifying and quantifying the hydrocarbons contaminating soils.

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Notes and references

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† Electronic Supplementary Information (ESI): This document summarises the uncertainty assessment and is divided into 6 sections, including 13 figures and 24 tables.

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