

ANALYTICAL CAPABILITY AND VALIDATION OF A METHOD FOR TOTAL PETROLEUM HYDROCARBON DETERMINATION IN SOIL USING GC-FID

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ABSTRACT. The paper presents the analytical capability and validation of a method for quantitative determination of total petroleum hydrocarbons in soil by gas chromatography with flame ionization detector (GC-FID). In order to validate the method, the main figures of merit such as limit of detection and limit of quantification, working range, precision and recovery were studied and the measurement uncertainty was estimated based on the bottom-up approach according to the international guidelines of ISO/IEC 17025. Limit of detection, estimated from chromatograms measured for spiked blank at low level mass concentration, was 8.3 mg/kg, while limit of quantification was 25 mg/kg. Precision was studied in terms of repeatability and reproducibility for the concentration range of 25 – 1000 mg/kg. Standard deviation of repeatability (s_r) was 6.3% ($n=10$ parallel samples), while standard deviation of reproducibility (s_R) was 9.9 % ($n=10$ parallel samples). Recovery (%) estimated using a certified reference material (CRM), was 93 ± 7.0 %, while the estimated expanded relative uncertainty was 17.2 %. This paper offers all the steps necessary to validate the determination method for total petroleum hydrocarbons in soil applied according to the standard ISO 16703 and to evaluate the measurement uncertainty for this method. The obtained figures of merit fulfil the requirements of the standardized method and also of the Romanian legislation, and demonstrate that the laboratory can properly apply the method in order to achieve accurate results. The paper represents a model for the method validation in analytical laboratories in order to check the fit for purpose of analytical methods.

Keywords: GC-FID, total petroleum hydrocarbons, validation, measurement uncertainty, soil, fit for purpose

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INTRODUCTION

All over the world there are numerous sites polluted with petroleum products that may have adverse effects on living organisms' health [1, 2]. Petroleum products results from crude oil by fractional distillation. In a simplified scheme of petroleum refining, crude oil is first distilled into different boiling range fractions. A processed petroleum product contains a complex mixture of many different organic substances counting paraffinic, naphthenic, olefinic, aromatic and polycyclic aromatic hydrocarbons, as well as heteroatoms (N, O, S) containing organic compounds. Also, it may contain traces of metals and organometallic compounds [3, 4].

The persistence and toxicity of different pollutants, between them petroleum hydrocarbons [5-7], created an imperative need for developing reliable methods for their qualitative and quantitative determination in environmental samples. The reliability of a measurement can be expressed by method validation as well as by stating the uncertainty of the measurement result [8-11]. However, the evaluation of measurement uncertainty pose a great challenge for analytical chemists due to the need for a complete understanding of the whole analysis steps and of the method performance parameters.

There are several techniques used for the determination of petroleum hydrocarbon content in soils [12]. Among them, the most known are those based on gravimetry, infrared spectroscopy and gas chromatography with different detection modes (FID, MS), applied subsequent to extractions in different organic solvents [12, 13].

Even if there are numerous instrumental techniques that can be used for petroleum hydrocarbon quantification, the consistent analysis of petroleum products contaminated soil is a complicated task, both due to the complex composition of petroleum products and also due to the complex matrix of soil [3]. Prior to the instrumental determination, the extraction of analyte can be an important uncertainty source. Consequently, the identification and quantification of major sources of uncertainty in the petroleum hydrocarbon determination sequence is necessary. By finding the main sources of uncertainty and critical steps of the determination, the decrease of the uncertainty related to petroleum hydrocarbon determination is possible.

The standardized GC-FID method [14] can be applied for samples with total petroleum hydrocarbon (TPH) (mass fraction) between 100 mg/kg and 10 000 mg/kg soil, expressed as dry matter, but can be adapted to lower concentrations. The method allows the determination of hydrocarbons with a boiling range of 175 °C to 525 °C (n-alkanes from C10 to C40, isoalkanes, cycloalkanes, alkylbenzenes, alkylnaphthalenes and polycyclic aromatic

compounds) [14]. Comparing to the gravimetric or IR methods, gas chromatographic determination offers qualitative information about the components of the sample.

When presenting the measurement results it is necessary to evaluate their confidence intervals [15, 16]. The International Organization for Standardization (ISO) guide [17] recommends the calculation of uncertainty using a model equation, based on evaluation of its uncertainty components, and by using the law of propagation of uncertainty.

There are several options to evaluate the measurement uncertainty existing in the literature [18]. The main ways to assess uncertainty are based on the top-down or bottom-up approaches. In the top-down approach the major sources of uncertainty are considered and estimated, while in the bottom-up approach all the uncertainty sources are thoroughly estimated and only those with significant contributions are used to calculate the measurement uncertainty. The top-down approach is time-consuming and requires a very good understanding of the analytical procedure, but it enables identification of major uncertainty sources and consequently reduction of total measurement uncertainty [16, 19].

The aim of this study was to perform a detailed validation for TPH determination in soil by GC-FID applied according to the standard ISO 16703 and to evaluate the measurement uncertainty for this method. The validation steps taken into account the guidelines of the international standard ISO/IEC 17025 [20]. The measurement uncertainty was calculated using modelling approach following the estimation of combined uncertainty. This paper represents a model for the method validation in analytical laboratories in order to check the fit for purpose of analytical methods.

RESULTS AND DISCUSSION

Method validation

The validation of the analytical procedure for quantitative determination of TPH in soil was performed by evaluating the main figures of merit: limit of detection (LoD), limit of quantification (LoQ), working and linear range, trueness/accuracy and precision (both repeatability and reproducibility) according to the EURACHEM guide requirements [21].

A specific chromatogram for a standard solution used in TPH determination is presented in Figure 1. In the chromatogram, the total peak area is that delimited by the retention times of n-decane and n-tetracontane. Therefore, only semi-volatile (>C₁₀-C₁₆) and non-volatile hydrocarbons (>C₁₆-C₄₀) [22] are included in the TPH parameter measured by this method.

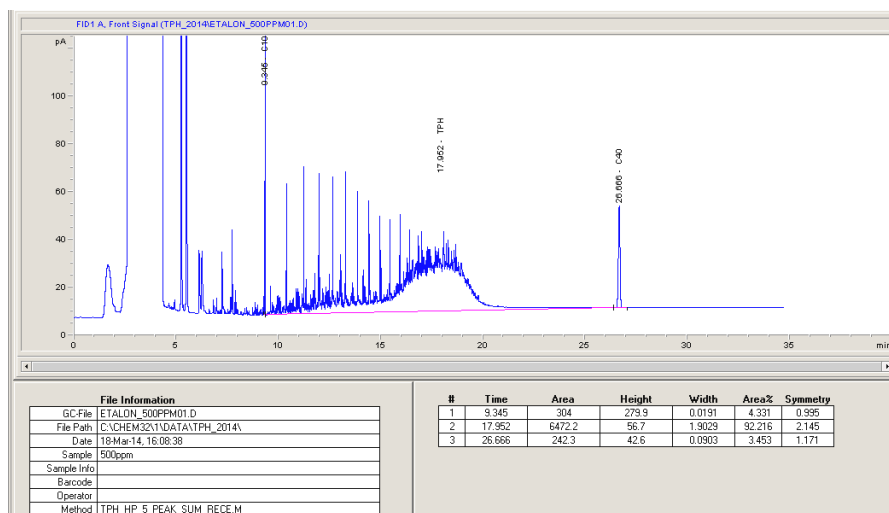


Figure 1. GC-FID chromatogram of TPH standard (500 mg/kg). The first and the last identified peaks (C10 and C40, respectively) delimitate the retention time window (RTW)

LoD and LoQ

In order to estimate LoD and LoQ, chromatograms of 10 independent spiked blank solutions at low level mass concentration (10 mg/L) were measured and the standard deviation of signal area was calculated. LoD was estimated for an area equal to the net signal of spiked blank and three times its standard deviation, while LoQ was estimated for a signal equal to the area signal of spiked blank and nine times its standard deviation [20]. The target was to obtain a value for LoQ of at least 25% from alert level for TPH in soil (200 mg/kg) established by Ministerial Order 756 [23] which means a value for LoQ of maximum 50 mg/kg. Data in Table 1 showed that the performance target was achieved by our method.

Table 1. LoD and LoQ for the determination of TPH in soil

Stdev (s) blank signal (signal area)	LoD (3s/b) (mg/kg)	LoQ (9s/b) (mg/kg)
39.5	8.3	25

Ten standard solutions at the calculated LoQ concentration (25 mg/L) were prepared and analysed for its confirmation by evaluation of precision (repeatability) and trueness (recovery). The targeted repeatability expressed as relative standard deviation (RSD) was 20 %, while targeted recovery was 90-115 %.

Working and linear range

At the lower end of the range, the restrictive factor is LoQ, while, at the upper end, limitations are imposed by various effects depending on the instrument response. Linearity was evaluated from the regression function of calibration using 7 standards (25, 50, 100, 250, 500, 750, and 1000 mg/L TPH). The fit for purpose linear range was selected to be between LoQ and 1000 mg/L.

Ten replicates of the 25 mg/L and ten of the 1000 mg/L calibration standards were measured. To check homogeneity of variances, the standard deviations (s_1) and (s_7) of the lowest and the highest concentrations from calibration curves, and the PG ratios (s_1^2/s_7^2 or s_7^2/s_1^2) were calculated and compared with the Fischer value $F_{9;9;0.99} = 5.35$. The values for intercept (a), slope (b), determination coefficient (r^2), and PG ratio are presented in Table 2.

Table 2. Calibration curves for working range LoQ – 1000 mg/L

Parameter	a	b	r^2	PG
TPH	-197.78	14.34	0.9994	4.96

a – intercept; b – slope; r^2 – determination coefficient, PG – test value factor for significant differences of variances at the limits of the linear range

The experimental data showed that variances are homogenous, therefore linear regression curve can be used [24].

Trueness / accuracy

Trueness was studied by evaluating the recovery of a soil CRM (Sandy Loam CRM). Thus, 6 parallel samples of soil CRMs were analysed in order to determine the methods trueness, and the results are presented in Table 3. Average recovery for soil TPH Sandy Loam CRM was 93% with relative expanded uncertainty of 7.0% ($n = 6$ parallel samples). In addition, trueness was evaluated using the recovery for real soil samples spiked with known content of TPH. To the each 100 g soil sample (6 parallel samples) amounts of 27.8 mg BAM K008 standard were added. Hence, the added THP concentrations were 278 mg/kg. The recovery rate was calculated by taking into account the found concentrations in the enriched samples and the added concentration.

The average recovery for spiked soil samples was 88% with a relative expanded uncertainty of 7.5% ($n = 6$ parallel samples), which conforms satisfactory performance according to the requirements of ISO 16703 standard (the recovery shall be more than 80% [14]). The possible factors that contribute to the recoveries below 100% may be the loss of parts of analyte during the sample preparation step, due to the volatility of some compounds from TPH class. Thus, when calculate the final result, the average recovery for spiked samples should be considered.

Table 3. Results of analysis of TPH Sandy Loam CRM (mean \pm expanded uncertainty, $n = 6$ parallel samples) and certified value \pm expanded uncertainty

Parameter	Measured value (mg/kg)	Certified value (mg/kg)
TPH	3395 \pm 238	3650 \pm 270

The results showed that the confidence interval of the measured value was within the confidence interval of the certified value.

Precision

Commonly, the precision is estimated in terms of repeatability and reproducibility, and, in our case, were estimated considering within and between days variation, respectively. For the repeatability study the results were obtained by analysing 10 parallel samples by a single operator using the same equipment. The target was to obtain a limit of repeatability (r) below 8.3% (according to the precision data given in ISO 16703). For the reproducibility study, a real soil sample was measured in 10 different days by different operators using the same equipment. The target was to obtain a limit of reproducibility (R) below 28.5% (according to the precision data given in ISO 16703), which mean a standard deviation of reproducibility (s_R) below 10.2 %. According to the obtained results, r was 6.3 %, while s_R was 9.9 % ($R = 27$ %), which conforms satisfactory performance.

In Table 4 is presented a summary of the results of method validation.

Table 4. Results of method validation for the measurement of TPH in soil by using GC-FID method

Validation parameter	Results
<i>Limit of detection</i>	8.3 mg/L
<i>Limit of quantification</i>	25 mg/L
<i>Linear range</i>	25 – 1000 mg/L
<i>Trueness (recovery)</i>	93% for CRM; 88% for spiked samples
<i>Precision (limit of repeatability, r)</i>	6.3% ($n=10$ parallel samples)
<i>Precision (limit of reproducibility, R)</i>	27% ($n=10$ parallel samples)

Measurement uncertainty evaluation

Measurement uncertainty was estimated based on the bottom-up approach [18]. All the contributions were obtained from calibration certificates (volumetric flasks, pipettes, reference materials, etc.) and from statistical analysis of repeated measurements (CRM analysis, precision experiments) through the method validation study performed in the laboratory. In brief, the steps of the method are as shown in Figure 2.

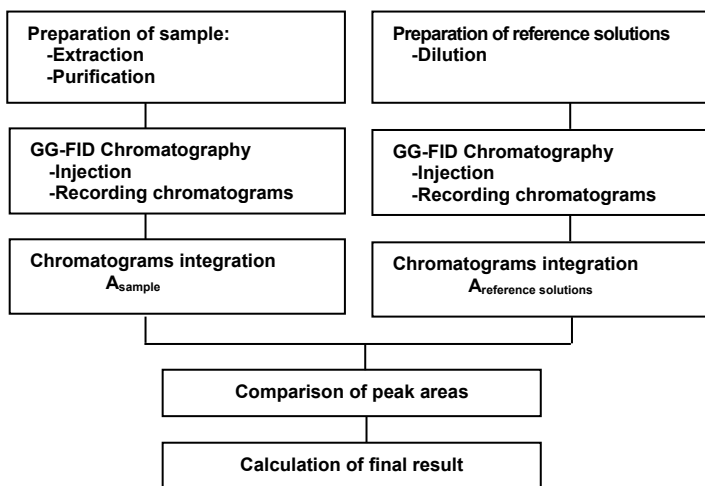


Figure 2. Experimental procedure for the measurement of mass concentration of TPH in soil by GC-FID

The identified main sources of measurement uncertainty were uncertainty of calibration reference materials (C_i), uncertainty of delivered volumes, uncertainty of signal area of the reference solutions, and recovery of the method, as presented in Figure 3 – cause and effects diagram.

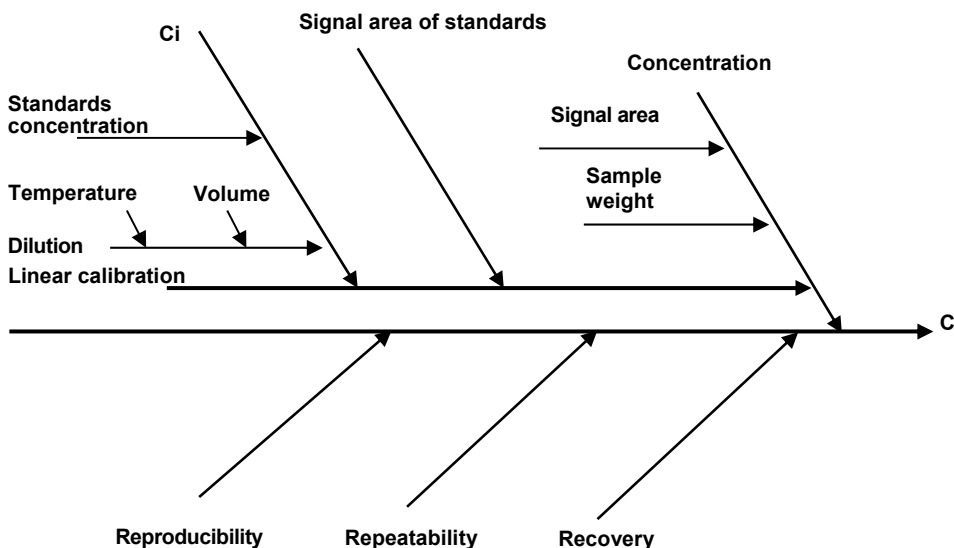


Figure 3. Cause and effects diagram of uncertainties in measurement of mass concentration of TPH by using GC-FID

Uncertainties of GC-FID and analytical balances were calculated from data obtained from calibration certificates (declared uncertainty). After estimation, all sources of uncertainty were combined according to the law of propagation of uncertainties, obtaining the combined standard uncertainty (U_c). The final result was reported as expanded uncertainty (U_E), calculated as $U_E = k \times U_c$, where k is the coverage factor, corresponding to a 95 % confidence level.

Table 5. Uncertainty components of mass concentration of TPH in soil by using GC-FID method

Source	Unit	Value	Standard uncertainty	Interventions	Total standard uncertainty	Relative uncertainty
Concentration of standard	g/g	0.967	0.009	1	0.009	0.0090
Weight of standard	g	0.10	0.0001	1	0.0001	0.0010
Volumetric flask	mL	10	0.033	7	0.231	0.0230
Pipette	μ L	1000	4.60	1	4.60	0.0046
Pipette	μ L	750	4.20	1	4.20	0.0056
Pipette	μ L	500	3.90	1	3.90	0.0078
Pipette	μ L	250	3.70	1	3.70	0.0015
Pipette	μ L	100	0.590	1	0.590	0.0059
Pipette	μ L	50	0.540	1	0.590	0.0110
Pipette	μ L	25	0.520	1	0.540	0.0210
Weight of sample	g	10	0.001	1	0.0001	0.00001
Equipment	g/g	1	0.01	1	0.010	0.0100
Calibration	mg/L	382	14.2	1	14.2	0.0370
Reproducibility	mg/L	404	25.4	1	25.4	0.0630
Dry mass	%	70.0	1.50	1	1.50	0.0210

The combined relative uncertainty was calculated to be 8.6 %. The biggest contribution to combined uncertainty was represented by method reproducibility (28% of the total uncertainty), but also the calibration curve (16% of the total uncertainty) and the use of low volume pipettes (e.g. 9% of the total uncertainty is given by the delivered volume of 25 μ L). To calculate the expanded uncertainty of the result of a measurement at the 95 % confidence level, the result for the combined uncertainty was multiplied by a coverage factor of 2. Thus the expanded uncertainty of the TPH determination in soil by FT-IR method is 17.2 %.

Relative uncertainty contributions are used to illustrate the relative impact of different uncertainty components. As presented in Table 5, method reproducibility has the highest contribution to the combined uncertainty, while the weighting of sample has an insignificant contribution to the method uncertainty.

CONCLUSIONS

The paper presents all the steps necessary to evaluate the measurement uncertainty and validate the standardized method for TPH determination in soil according to the ISO 16703 standard in a laboratory in order to demonstrate its

fit for purpose. The studied figures of merit fulfil the requests in terms of LoD and LoQ, accuracy, and precision set out in the ISO 16703 standard. Gas chromatographic determination provides low LoQ, which make it suitable to measure the TPH concentrations at the limits imposed by environmental legislation. Trueness was studied by evaluating the recovery of a soil CRM and also by evaluating the recovery for spiked soil samples. The recovery for spiked samples was in the target imposed by ISO 16703 (higher than 80%). However, since it was well below 100% (only 88%) it should be taken into account when calculate the final result. Also, particular attention should be paid to sample preparation in order to avoid analyte loss during this step. It was demonstrated that that the method can be applied in the testing laboratory for the designed purpose, determination of TPH in soil by GC-FID.

EXPERIMENTAL SECTION

All reagents were of p.a. quality, purchased from Merck (Darmstadt, Germany). Sandy Loam soil (CRM358, Sigma-Aldrich, USA), diesel oil BAM-K008, Federal Institute for Materials Research and Testing, Germany) and 1/1 diesel oil/ lubricating oil mixture (BAM-K010e, Federal Institute for Materials Research and Testing, Germany) certified reference materials (CRMs) were used for the validation procedure. The volumes were measured using calibrated glassware (Hirschmann, Germany).

The method consists in the mixing of 20 g of well homogenized soil with 40 mL of acetone and 20 mL heptane containing n-decane and n-tetracontane to establish the Retention Time Window (RTW), removal of acetone by extraction with water followed by removal of polar substances using Florisil columns and recording of the GC chromatogram.

Standard solutions (25, 50, 100, 250, 500, 750, and 1000 mg/L TPH) prepared from a mixture of 1/1 diesel oil/lubricating oil CRM (BAM-K010e) were used for the external calibration of the instrument. Measurements were carried out using an Agilent Technologies 6890N gas chromatograph (GC) with flame ionization detector (FID). The used capillary column was a 30 m L×0.32 mm ID×0.25 µm, HP-5 95% dimethylpolysiloxane (Agilent J&W). High purity helium (Linde Gas, Romania) was used as carrier gas.

The GC-FID was operated in split mode and the oven temperature was: initial temperature 40°C, held for 6 min, then ramped to 315°C at 20°C/min and held for 15 min. Detector temperature was set at 330°C and the injector temperature was set at 300°C.

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