
**Soil quality — Determination of content
of hydrocarbon in the range C₁₀ to C₄₀ by
gas chromatography**

*Qualité du sol — Dosage des hydrocarbures de C₁₀ à C₄₀ par
chromatographie en phase gazeuse*

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Contents

Page

Foreword.....	iv
1 Scope.....	1
2 Normative references	1
3 Terms and definitions.....	2
4 Interferences.....	2
5 Principle	2
6 Reagents	2
6.7 Hydrocarbon standard solution for calibration	3
6.8 Control solution	3
6.9 System-performance standard solution	3
7 Apparatus	3
8 Sampling, sample conservation and pretreatment	4
9 Procedure	5
9.1 Preparation of the clean-up column.....	5
9.2 Blank.....	5
9.3 Extraction and clean-up	5
9.4 Determination by gas chromatography.....	5
9.5 Quality control.....	7
10 Precision	8
11 Test report	8
Annex A (informative) Examples of gas chromatograms of mineral oil hydrocarbon standard and soil samples.....	10
Annex B (informative) Determination of the boiling range of mineral oil hydrocarbons from the gas chromatogram.....	16
Annex C (informative) Precision data.....	17
Bibliography	18

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16703 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

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Soil quality — Determination of content of hydrocarbon in the range C₁₀ to C₄₀ by gas chromatography

1 Scope

This International Standard specifies a method for the quantitative determination of the mineral oil (hydrocarbon) content in field-moist soil samples by gas chromatography.

The method is applicable to mineral oil contents (mass fraction) between 100 mg/kg and 10 000 mg/kg soil, expressed as dry matter, and can be adapted to lower limits of detection.

This International Standard is applicable to the determination of all hydrocarbons with a boiling range of 175 °C to 525 °C, of *n*-alkanes from C₁₀H₂₂ to C₄₀H₈₂, of isoalkanes, cycloalkanes, alkylbenzenes, alkylnaphthalenes and polycyclic aromatic compounds, provided that they are not absorbed on the specified column during the clean-up procedure.

This International Standard is not applicable to the quantitative determination of hydrocarbons < C₁₀ originating from gasolines.

On the basis of the peak pattern of the gas chromatogram obtained, and of the boiling points of the individual *n*-alkanes listed in Annex B, the approximate boiling range of the mineral oil and some qualitative information on the composition of the contamination can be obtained.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8466-1:1990, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO 10381-1, *Soil quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 11465:1993, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

hydrocarbon content

(by gas chromatography) sum of compounds extractable with acetone/*n*-heptane (2+1) that do not adsorb on a Florisil¹⁾ column and can be chromatographed on a non-polar capillary column with retention times between those of *n*-decane (C₁₀H₂₂) and *n*-tetracontane (C₄₀H₈₂).

NOTE Substances that comply with this definition are mainly long-chain or branched aliphatic, alicyclic, lower polycyclic- or alkyl-substituted aromatic hydrocarbons.

4 Interferences

Non-polar and weakly polar compounds (e.g. halogenated hydrocarbons) and high contents of polar compounds may interfere with the determination.

5 Principle

A known amount of the homogenized soil sample is extracted by mechanical shaking or sonication with acetone/*n*-heptane. The organic layer is separated and washed twice with water. Polar compounds are removed by adsorption on Florisil. An aliquot of the purified extract is analysed by capillary gas chromatography with flame ionization detection. The total peak area in the range delimited by the standards *n*-decane and *n*-tetracontane is measured, and the amount of hydrocarbons in the sample is quantified against an external standard consisting of equal amounts of two different types of mineral oil.

Instead of heptane, another non-polar solvent (e.g. petroleum ether, cyclohexane, *n*-hexane) may be used, however its suitability for the extraction of hydrocarbons from soil shall be proven.

NOTE If lower detection limits are required, petroleum ether can be used as extraction solvent in combination with large-volume injection or concentration of the final extract.

6 Reagents

In general, all reagents shall be reagent grade and suitable for their specific purposes.

6.1 Acetone, (CH₃)₂CO.

6.2 *n*-Heptane, C₇H₁₆.

6.3 Florisil¹⁾ for preparation of clean-up column, particle size 150 µm to 250 µm (60 mesh to 100 mesh), heated for at least 16 h at 140 °C and stored in a desiccator over a molecular sieve.

NOTE Commercially available cartridges containing 2 g of Florisil and 2 g of sodium sulfate are also applicable.

6.4 Anhydrous sodium sulfate (Na₂SO₄), heated for at least 2 h at 550 °C.

6.5 Test solution of stearyl stearate (C₃₆H₇₂O₂).

1) Florisil is a trade name for a prepared diatomaceous substance, mainly consisting of anhydrous magnesium silicate. Florisil an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Dissolve about 100 mg of *n*-octadecanoic acid octadecyl ester in 100 ml *n*-heptane (6.2).

6.6 Retention-time window (RTW) standard solution, containing *n*-tetracontane and *n*-decane.

Retention-time window (RTW) standard solution is the range-defining standard solution. Weigh (30 ± 1) mg of *n*-tetracontane into a 1 l volumetric flask, dissolve completely in an appropriate volume of *n*-heptane (6.2), add 30 μ l of *n*-decane (about 21 mg), mix well, fill up to volume with *n*-heptane and homogenize. This solution shall be used for all dilution steps of the hydrocarbon standard (6.7).

Store at room temperature.

NOTE *n*-Tetracontane is only moderately soluble in *n*-heptane. Slight warming and/or sonication accelerates the dissolution process.

6.7 Hydrocarbon standard solution for calibration²⁾

Mix approximately equal masses of two different types of mineral oil. Weigh accurately this mixture and dissolve in the RTW standard solution (6.6) to give a hydrocarbon mass concentration of about 8 g/l.

The first oil type should show discrete peaks (e.g. a diesel fuel) in the gas chromatogram, as can be seen in Figure A.1 (left part of the chromatogram). The second type should have a boiling range higher than the first one, and should show a hump in the gas chromatogram, as can be seen in Figure A.1 (right part of the chromatogram). A suitable oil of this type is for example a lubricating oil without any additives.

The calibration solutions can be prepared by diluting an aliquot of this standard solution (6.8) with different volumes of the RTW standard solution (6.6).

6.8 Control solution

Prepare an independent control solution in accordance with (6.7) using a hydrocarbon concentration about in the middle of the working range of the system-performance standard solution (6.9).

6.9 System-performance standard solution

Prepare a mixture of equal amounts, on a mass basis, of the *n*-alkanes having even carbon numbers from C₁₀ to C₄₀, dissolved in *n*-heptane (6.2), to give mass concentrations of about 50 mg/l of each *n*-alkane. Store at room temperature.

NOTE 1 This solution is used to verify the suitability of the gas chromatographic system for the resolution of *n*-alkanes, as well as for the detector response.

NOTE 2 This solution is used to provide information on the retention times of the *n*-alkanes, in order to characterise the hydrocarbons in the samples.

7 Apparatus

7.1 **Standard laboratory glassware**, which shall be treated at high temperatures or rinsed with acetone (6.1) and dried before use.

2) General purpose hydrocarbon standards for calibration are available commercially. Calibration standards specific to this International Standard can be purchased from Bundesanstalt für Materialforschung und -prüfung, Fachgruppe I.2, Richard-Willstätter-Strasse 11 D-12489 Berlin, Germany. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

7.2 Mixing device.

A mechanical shaker with at least 120 horizontal shaking movements per minute, or alternatively an ultrasonic bath, can be used.

7.3 Laboratory centrifuge, capable of producing an acceleration of at least 1 500 g.

7.4 Gas chromatograph, equipped with a non-discriminating injection system [preferably on-column or programmable-temperature vaporization injection (PTV)], a capillary column and a flame ionization detector (FID).

NOTE The use of a large-volume injection system can improve the limit of detection considerably.

7.5 Capillary column, of fused silica, with the following properties:

- non-polar stationary phase: e.g. immobilized 100 % dimethyl polysiloxane, 95 %-dimethyl-5 %-diphenyl polysiloxane, or modified siloxane polymer;
- length: 10 m to 25 m;
- internal diameter : 0,1 mm to 0,32 mm;
- film thickness: 0,1 µm to 1,0 µm.

The column shall give a baseline separation for the *n*-alkanes in the system-performance standard solution (6.9).

Thermally stable low-bleed columns are preferred.

The use of a pre-column, e.g. wide-bore (0,53 mm internal diameter) deactivated fused silica of at least 2 m of length that suits the analytical column and is connected to it using a zero-volume connector, is recommended.

7.6 Data system, capable of integrating the total area of the chromatogram, compensating for column bleed and reintegrating after defining a new baseline.

7.7 Glass extraction vessel, of volume at least 100 ml, with screw caps provided with an inlay of PTFE.

7.8 Glass tube, of volume 25 ml, with a ground-glass stopper or with screw caps provided with an inlay of PTFE.

7.9 Separatory funnel, of capacity at least 500 ml, with a ground-glass stopper.

7.10 Chromatography column for clean-up.

Glass columns of about 10 mm internal diameter shall be used. The upper part of the column should be widened to use as a solvent reservoir and the lower part narrowed to form a tip.

8 Sampling, sample conservation and pretreatment

Sampling shall be carried out according to ISO 10381-1 and in coordination with the analytical laboratory.

The samples should be kept sealed in darkness at a temperature of about 4 °C, and extracted within a period of 1 week.

If this is not possible, samples shall be stored at –18 °C or lower. Before analysis the samples shall be homogenized.

9 Procedure

9.1 Preparation of the clean-up column

Push a plug of pre-washed glass wool or a PTFE frit down into the column (7.10). Then, successively add 2 g of Florisil (6.3) and 2 g of sodium sulfate (6.4). Prepare the column immediately before use.

9.2 Blank

With each series of samples, carry out a blank determination according to 9.3 using all reagents in identical amounts but without a sample. If blank values are unusually high (more than 10 % of the lowest value of interest), every step in the procedure shall be checked to determine the cause of these high blanks.

9.3 Extraction and clean-up

Weigh an exact amount (about 20 g) of the homogenized field-moist or pretreated soil sample according to ISO 14507 into a glass extraction vessel (7.7) and add (40 ± 1) ml of acetone (6.1). After briefly shaking by hand, add $(20 \pm 0,1)$ ml of the RTW-standard solution (6.6). Close the vessel and extract the sample for 1 h using mechanical shaking or sonication (7.2). After settling of the solid material, transfer as much as possible of the supernatant into a separatory funnel (7.9). To remove the acetone, wash the organic phase twice by shaking thoroughly (5 min) with 100 ml of water. Collect the organic layer in a glass tube (7.8). Add sufficient sodium sulfate so that no lumps are formed. Transfer 10 ml of the extract to a clean-up column filled with Florisil (9.1). Do not pre-wash the column with organic solvent. Collect the entire eluate. Transfer an aliquot of the purified extract to a GC-vial and analyse by gas chromatography.

If appropriate, test portions of 5 g to 30 g can be used (e.g. smaller test portions should be used if samples adsorb the major portion of the extraction solvent added; sample intake should be increased if high sensitivity is required).

Alternative extraction procedures, e.g. accelerated solvent extraction (ASE), may be used provided they give comparable extraction performances.

It is very important that the clean-up column be freshly prepared and active, and that the extract be free of acetone [less than 0,1 % (volume fraction)], especially when the sample contains polycyclic aromatic hydrocarbons (PAH) in addition to mineral oil hydrocarbons. Make sure that PAHs are adsorbed on the clean-up column. If the distinct peaks of PAHs are observed in the GC-FID chromatogram (see Annex A), this should be mentioned in the test report.

NOTE To improve and accelerate phase separation, centrifugation can be applied provided that the necessary safety precautions, especially with regard to flammable solvents, are taken into account.

9.4 Determination by gas chromatography

9.4.1 Test of the performance of the gas chromatographic system

Use a capillary column with one of the specified stationary phases (7.5) for gas chromatographic analysis. Adjust the gas chromatograph (7.4) to provide an optimal separation. The *n*-alkanes in the system-performance standard solution (6.9) shall be baseline-separated. The relative response of the *n*-tetracontane (C_{40}) shall be at least 0,8, with respect to *n*-eicosane (C_{20}).

For an example of gas chromatographic conditions, see Annex A.

9.4.2 Repeatability test

Record a gas chromatogram of the column bleed by injection of an appropriate volume of *n*-heptane. Then inject the same volume of a suitable concentration of the control solution (6.8) three times, and record the chromatogram for each injection. Integrate the chromatograms according to 9.4.5, and calculate the mean of

the measured peak areas and the corresponding standard deviation. The relative standard deviation shall not be greater than 5 %.

9.4.3 Calibration

When the method is used for the first time and/or when the apparatus or operator is changed, carry out a basic calibration according to ISO 8466-1 including the determination of the limit of detection and limit of determination.

Perform an external calibration by analysing a minimum of 5 dilutions of hydrocarbon standard solution (6.7) which shall cover the working range. Calculate a calibration function by linear regression analysis of the corrected peak areas. Use a chromatogram of *n*-heptane to correct the peak area of the chromatograms of the hydrocarbon standard solutions for the column bleed. From the calculated regression line, determine the actual sensitivity of the method.

9.4.4 Validity check of the calibration function

Check the validity of the calibration function within each batch of samples by analysis of one independent control solution (6.8). The validity check identifies problems of calibration before real samples are run. Check whether the result is within ± 10 % of the reference value of the control solution. If this is the case, the actual calibration function is assumed to be valid. If not, perform a new calibration according to 9.4.3.

NOTE It is good analytical practice to perform both a calibration check and analytical quality control using an independent solution randomly placed during the analysis of the batch of samples. This independent solution can perform both functions.

9.4.5 Measurement

Analyse the blank (9.2) and the sample extracts (9.3), calibration standards (6.7) and control solutions (6.8) under identical gas chromatographic conditions.

n-Heptane shall be analysed in each sample batch. The resulting chromatogram is used to correct chromatograms of blanks (9.2), sample extracts (9.3), calibration standards (6.7) and control solutions (6.8) for column bleed prior to integration.

9.4.6 Integration

Integrate the total area between the *n*-decane C_{10} and *n*-tetracontane C_{40} peaks of the chromatogram. Start the integration at the retention time just after the end of the *n*-decane-peak at the signal level in front of the solvent peak. End the integration of the total area at the retention time just before the beginning of the *n*-tetracontane peak at the same signal level (see Annex A). Integrate *n*-tetracontane (C_{40}) as a separate peak for the recovery check.

The presence of peaks on the tail of the solvent peak with retention times less than that of *n*-decane indicates that the sample contains low-boiling volatile hydrocarbons. This should be mentioned in the test report.

A non-horizontal baseline at the end of the chromatogram (retention time greater than that of *n*-tetracontane), with a signal level greater than the bleed, indicates that the sample contains high-boiling hydrocarbons with more than 40 carbon atoms. This should be mentioned in the test report. It should be ensured that these compounds elute completely from the column, otherwise they can cause interferences with the subsequent sample analysis.

All chromatograms should be checked visually for correct integration. The start and stop times of the integration should be visible on the chromatogram.

NOTE 1 The range of the carbon numbers of *n*-alkanes present in the sample is determined by comparing the gas chromatogram of the sample extract with that of the system-performance standard solution (6.9). The corresponding boiling range can be derived from Annex B.

NOTE 2 The peak shape and signal intensity of *n*-tetracontane are sensitive to changes in the surface properties of the injector and/or the pre-column due to contamination by sample constituents. Therefore, they can be used as a good indication of the need for replacing pre-column and/or liner.

9.4.7 Calculation

Calculate the mineral oil content of the soil sample using Equation (1):

$$w_h = \rho \cdot \frac{V_h}{m} \cdot f \cdot \frac{100}{w_s} \quad (1)$$

where

$$\rho = \frac{A_s - b}{a} \quad (2)$$

and where

w_h is the hydrocarbon mass fraction of the soil sample, in milligrams per kilogram dry matter;

ρ is the hydrocarbon mass concentration of the extract calculated from the calibration function, in milligrams per litre;

V_h is the volume of the *n*-heptane extract, in millilitre;

f is the dilution factor (if applicable);

m is the mass of the sample taken for analysis, in grams;

w_s is the dry matter content of the soil sample, expressed as a percentage (mass fraction), determined according to ISO 11465;

A_s is the integrated peak area of the sample extract, expressed in instrument-dependent units;

b is the intercept of the Y-axis, expressed in instrument-dependent units;

a is the slope of the calibration function, expressed in litres per milligram (l/mg);

Round the result to two significant figures.

9.5 Quality control

9.5.1 Suitability check of the clean-up procedure

The clean-up efficiency of each batch of Florisil column packing shall be checked (if Florisil cartridges are used, their suitability for the clean-up procedure shall be checked in the same way) by the following procedure:

Add 10 ml of the *n*-octadecanoic acid octadecyl ester solution (6.5) to the clean-up column (9.1) filled with 2.0 g of Florisil and 2 g of sodium sulfate, and collect the entire eluate. Analyse a portion of the resulting solution by gas chromatography. Analyse a 1+19 dilution of the untreated *n*-octadecanoic acid octadecyl ester test solution (6.5) as reference. Determine the recovery of the *n*-octadecanoic acid octadecyl ester after clean-up on the base of the peak area in respect to the untreated *n*-octadecanoic acid octadecyl ester [see Equation (3)].

$$R_{oo} = \frac{A_{foo}}{A_{uoo}} \times 5 \quad (3)$$

where

R_{oo} is the recovery of *n*-octadecanoic acid octadecyl ester (1+19), as a percent (%);

A_{foo} is the peak area of *n*-octadecanoic acid octadecyl ester after clean-up on the Florisil column, in instrument-dependent units;

A_{uoo} is the peak area of the (1+19) dilution of untreated *n*-octadecanoic acid octadecyl ester, in instrument-dependent units.

The recovery shall not exceed 5 %. If the recovery of *n*-octadecanoic acid octadecyl ester acid is above 5 %, activate the Florisil according to (6.3) and repeat the test.

9.5.2 Recovery of the hydrocarbon standard solution

The recovery of the hydrocarbon standard solution shall be checked with each batch of Florisil (if Florisil cartridges are used, recovery shall be checked in the same way) by the following procedure:

Add 10 ml of the hydrocarbon standard solution (6.7) to the clean-up column (9.1) filled with 2,0 g of Florisil and 2 g of sodium sulfate, and collect the entire eluate. Analyse a portion of the purified solution by gas chromatography. Analyse the untreated hydrocarbon standard solution as reference. Determine the recovery of the hydrocarbons on the base of the peak area of the purified and untreated standard solutions [see Equation (4)].

$$R_{HC} = \frac{A_{fHC}}{A_{uHC}} \times 100 \quad (4)$$

where

R_{HC} is the recovery of the hydrocarbon standard;

A_{fHC} is the peak area of the hydrocarbon standard after clean-up on the Florisil column, in instrument-dependent units;

A_{uHC} is the peak area of untreated hydrocarbon standard, in instrument-dependent units.

The recovery shall be more than 80 %.

10 Precision

The performance characteristics of the method as determined by interlaboratory studies are given in Annex C.

11 Test report

The test report shall contain at least the following information:

- a) a reference to this International Standard (ISO 16703:2004);
- b) a reference to the method used for extraction (shaking or sonication or other) and clean-up;
- c) a complete identification of the sample;

- d) the results of the determination;
- e) a reference to the occurrence of low- ($< C_{10}$) and/or high-boiling ($> C_{40}$) compounds in the chromatogram;
- f) any details not specified in this International Standard or which are optional, as well as any other factor that might have affected the results.

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Annex A (informative)

Examples of gas chromatograms of mineral oil hydrocarbon standard and soil samples

Figure A.1 shows the gas chromatogram of the calibration mixture of mineral oil consisting of equal parts of a diesel fuel and a lubricating oil. Figure A.2 shows the gas chromatogram of the “column bleed” after injection of *n*-heptane, and Figure A.3 shows the integrated gas chromatogram of the calibration mixture of mineral oil corrected for the “column bleed”. The total peak area between *n*-decane (C₁₀) and *n*-tetracontane (C₄₀) used for quantification is indicated as hatched area.

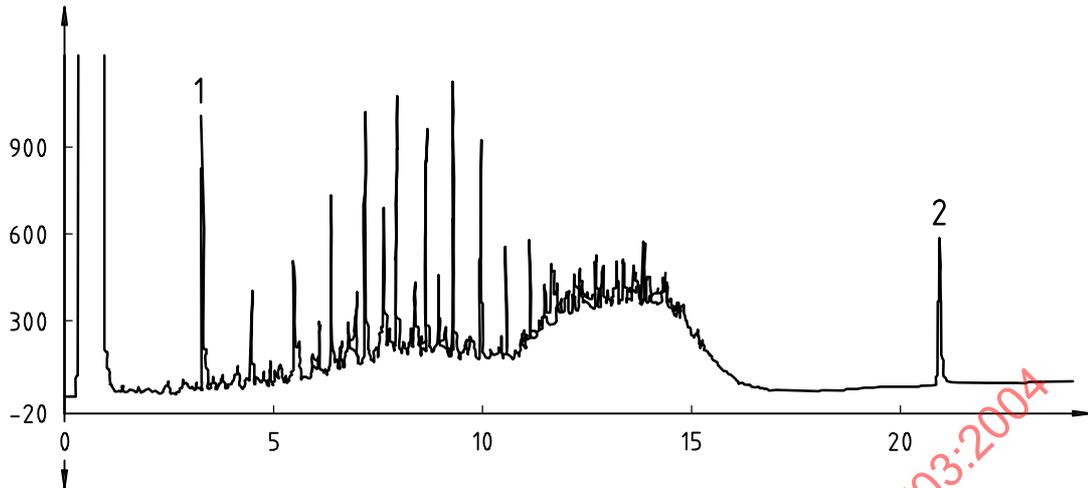
The Figures A.4 and A.5 show integrated gas chromatograms corrected for the “column bleed” of low and highly contaminated soil samples, respectively.

Figure A.6 shows a chromatogram of a system-performance standard solution.

Examples of chromatograms of soil samples with representative characteristics are given in Figures A.7 to A.10.

The gas chromatograms were recorded under the following conditions:

Injection technique:	on-column
Injection volume:	1 µl to 3 µl
Column type:	WCOT fused silica
Column length:	12 m
Internal diameter:	0,32 mm
Liquid phase:	BPX-5
Film thickness:	1,0 µm
Pre-column:	deactivated fused silica capillary, 2 m x 0,53 mm
Carrier gas:	helium
Pressure:	100 kPa
Detector:	flame ionization detector
Detector temperature:	360 °C
Temperature programme:	80 °C for 1 min; 20°C/min to 360 °C; 360 °C for 15 min

**Key**

- 1 *n*-decane
- 2 *n*-tetracontane

Figure A.1 — Gas chromatogram of the calibration mixture consisting of equal parts of diesel fuel and lubricating oil

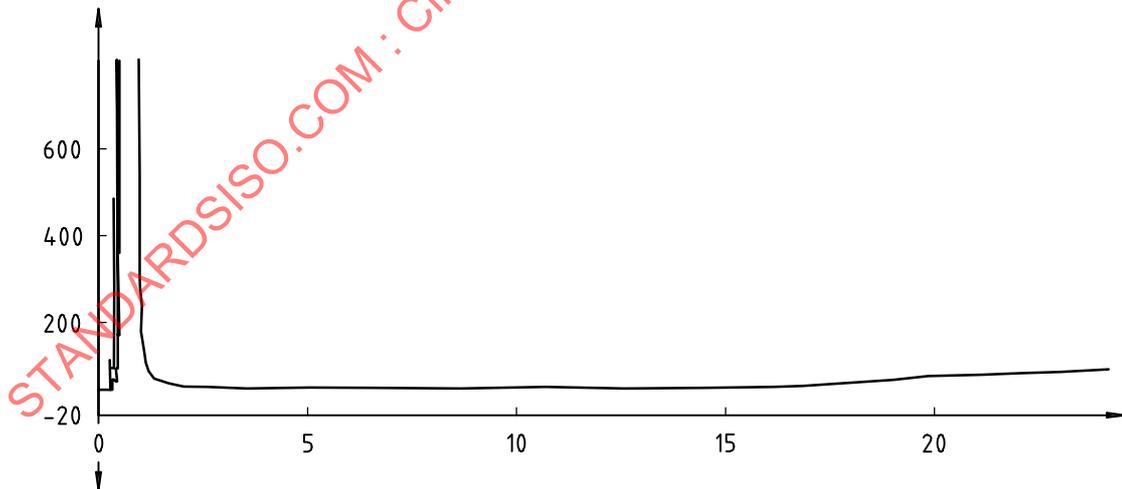
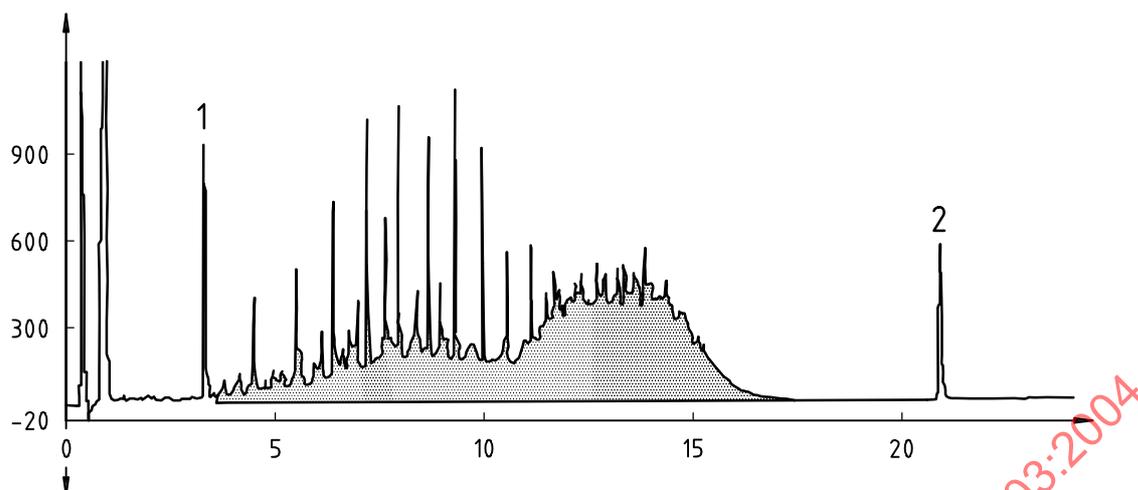


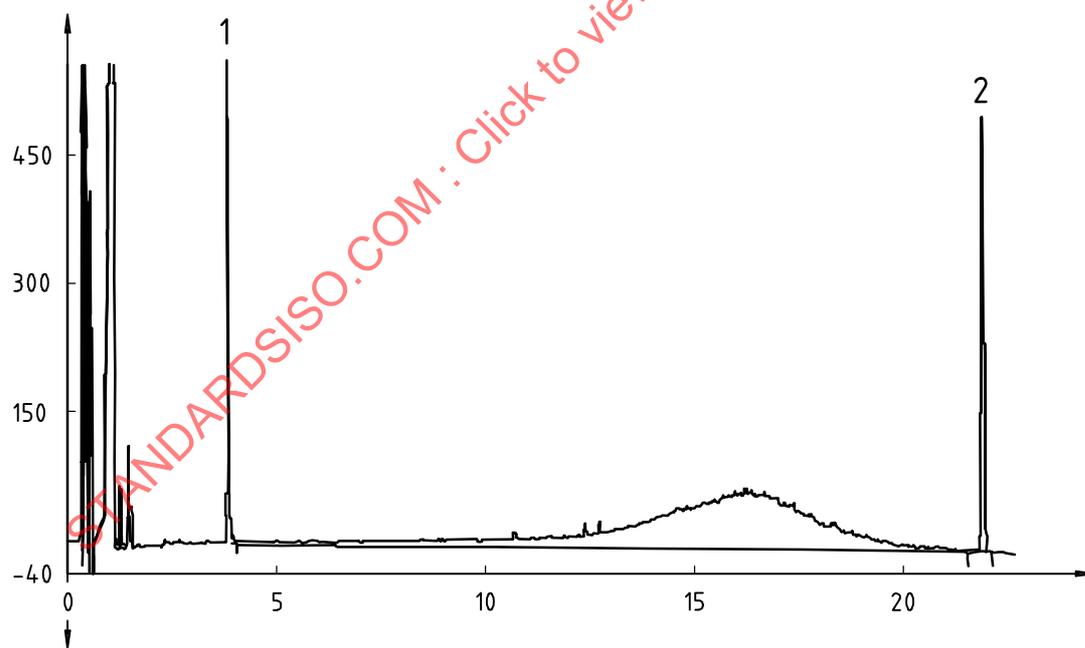
Figure A.2 — Gas chromatogram of the “column bleed”



Key

- 1 *n*-decane
- 2 *n*-tetracontane

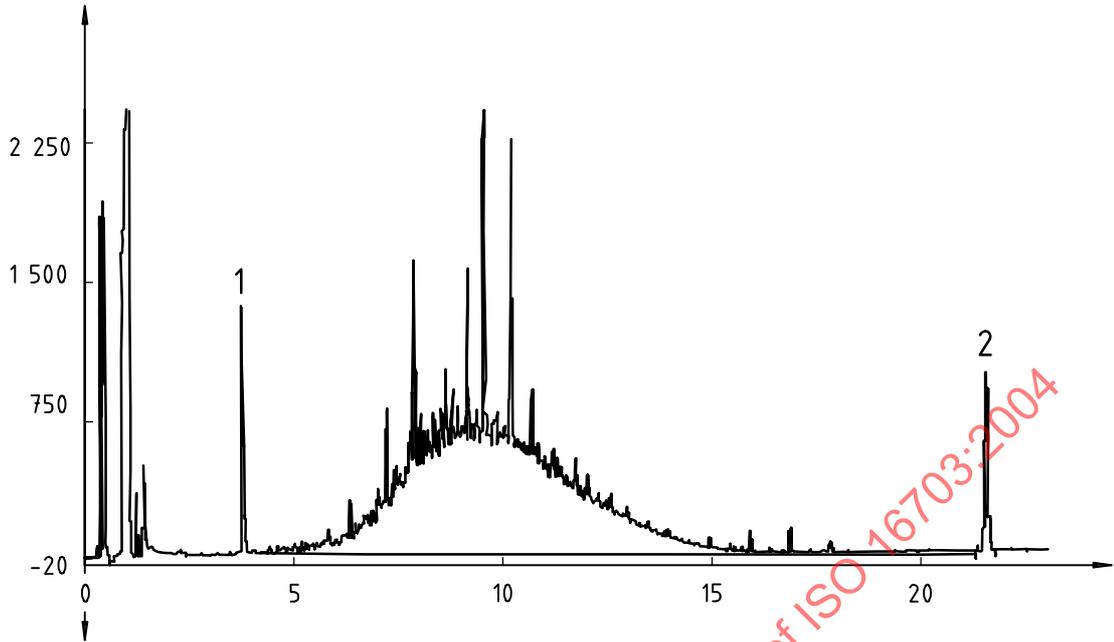
Figure A.3 — Integrated gas chromatogram of the calibration mixture of mineral oil corrected for the “column bleed”



Key

- 1 *n*-decane
- 2 *n*-tetracontane

Figure A.4 — Integrated gas chromatogram of a low-contaminated soil sample corrected for the “column bleed”



Key

- 1 *n*-decane
- 2 *n*-tetracontane

Figure A.5 — Integrated gas chromatogram of a highly contaminated soil sample corrected for the “column bleed”

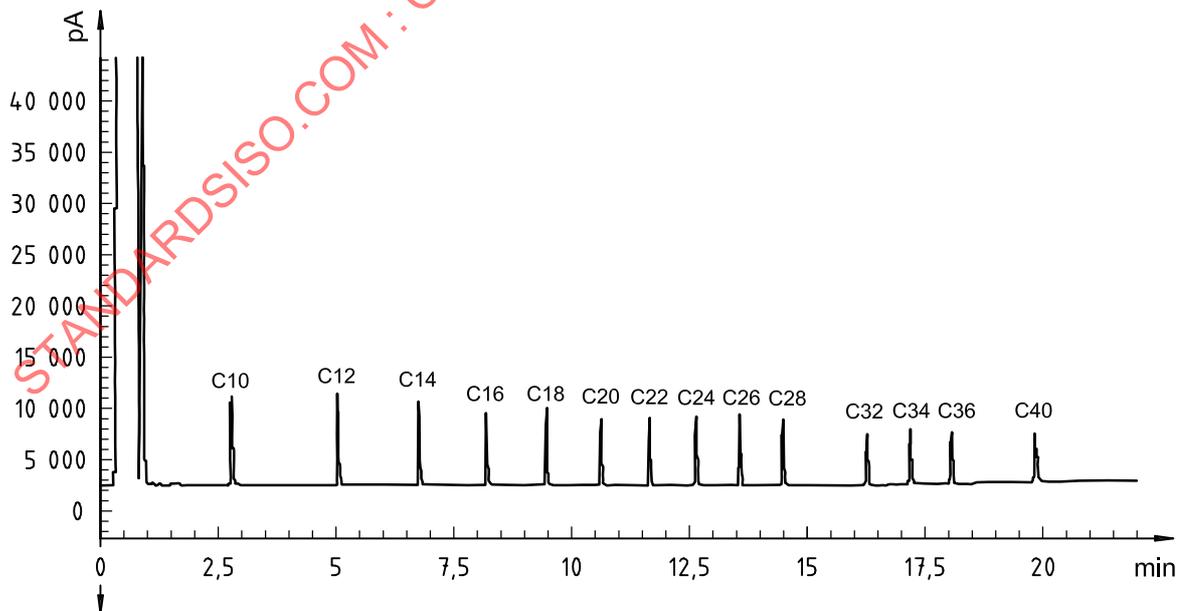


Figure A.6 — Gas chromatogram of a system-performance standard solution

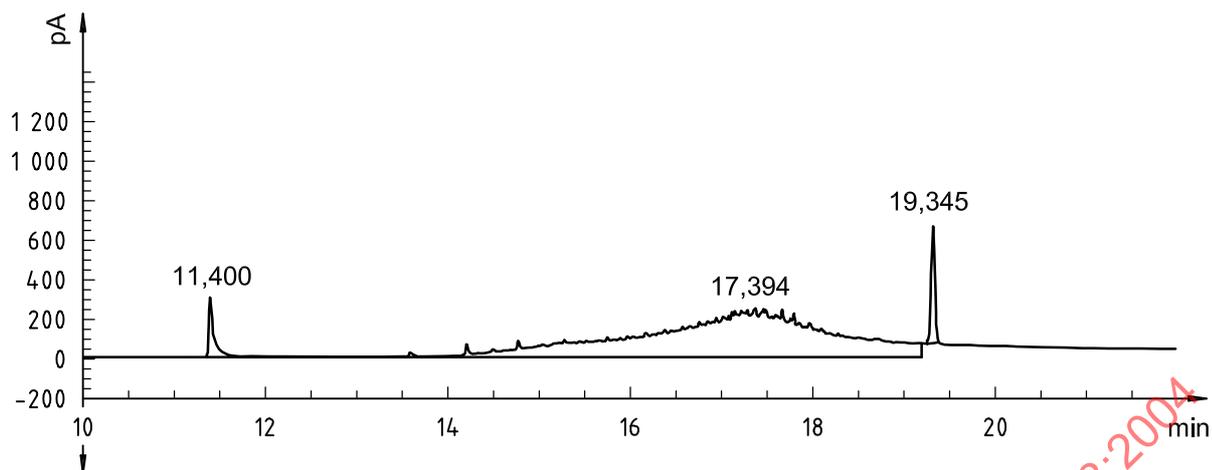


Figure A.7 — Gas chromatogram — Sample 3 GC Soil 1 (sufficient clean-up for PAHs)

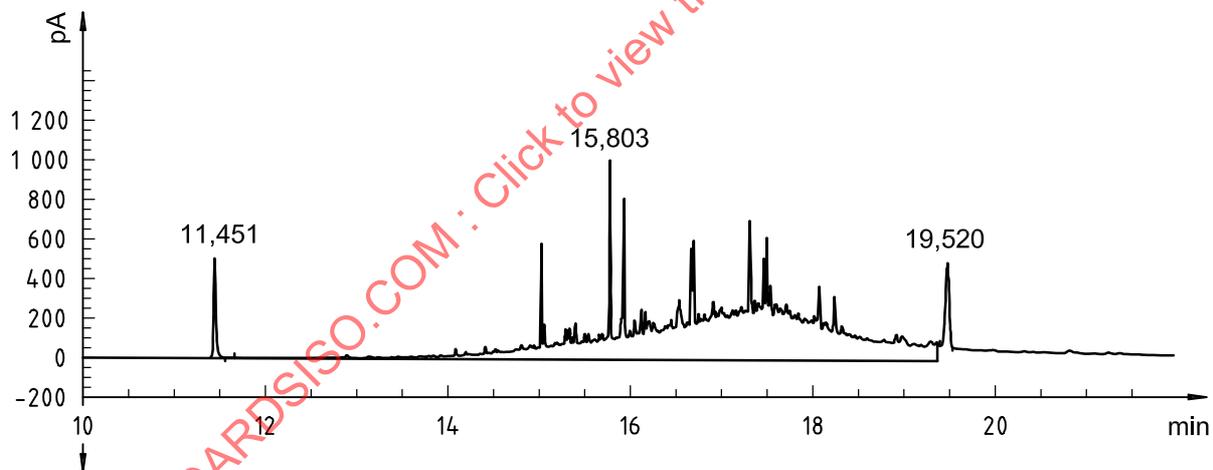


Figure A.8 — Gas chromatogram — Sample 3 GC Soil 1 (insufficient clean-up for PAHs)

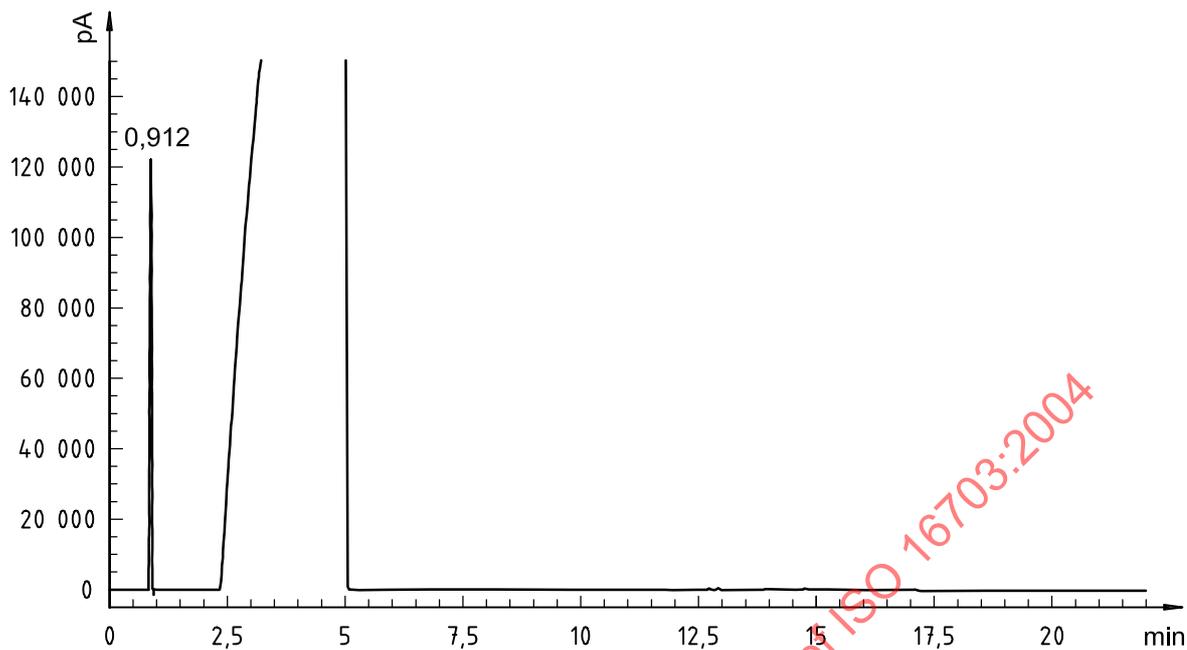


Figure A.9 — Sample 3 GC Soil 1 (acetone content about 2,5 % in extract) with insufficient clean-up of PAHs)

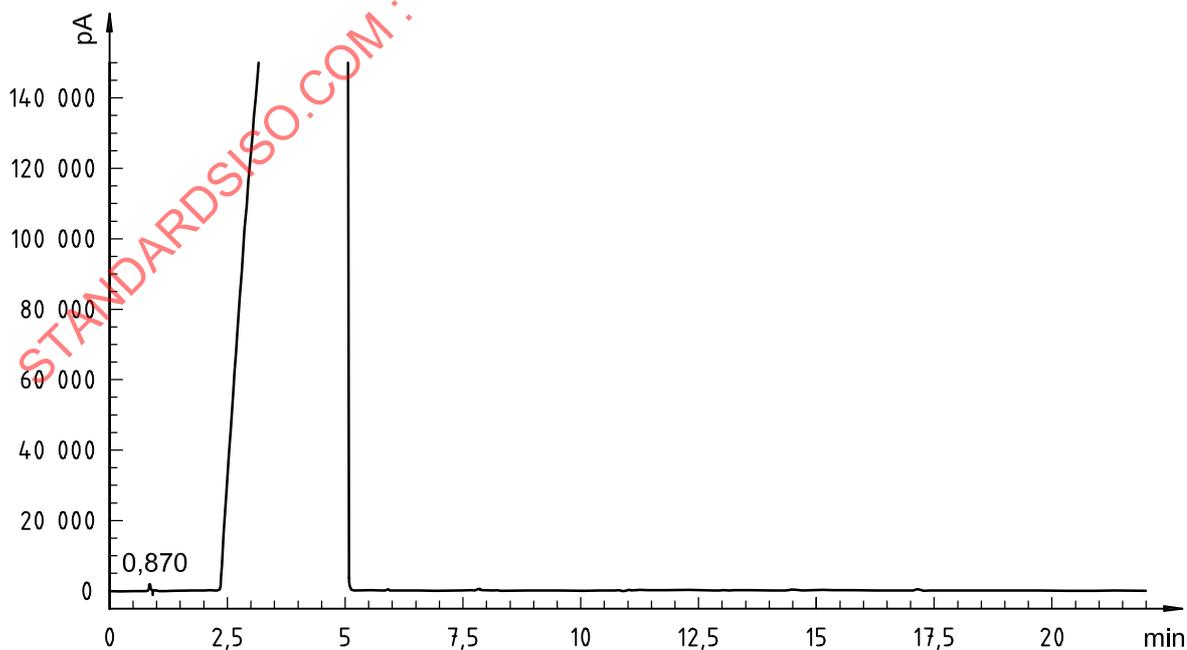


Figure A.10 — Sample 3 GC Soil 1 (acetone content less than 0,1 % in extract) with sufficient clean-up of PAHs