IAEA-TECDOC-1953
SUPPLEMENTARY FILES

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A GUIDE TO THE USE OF SEDIMENTS IN RECONSTRUCTING
THE POLLUTION HISTORY OF COASTAL AREAS
IAEA, VIENNA, 2023
IAEA-TECDOC-1953 (SUPPLEMENTARY FILES)
ISBN 978-92-0-143623-8 (PRINT), 978-92-0-143523-1 (PDF)
ISSN 1011-4289

Produced by the IAEA in Austria
December 2023

A GUIDE TO THE USE OF SEDIMENTS IN RECONSTRUCTING THE POLLUTION HISTORY OF COASTAL AREAS

ANNEXES

Contents

Annex I

SEDIMENT SAMPLE COLLECTION FORMS

This annex contains the forms for recording sampling information as used in project RLA/7/012 and described in the publication *A Guide to the Use of Sediments in Reconstructing the Pollution History of Coastal Areas*.

I

I-1. SURFACE SEDIMENT COLLECTION FORM

INTERNATIONAL ATOMIC ENERGY AGENCY
Project RLA/7/012

Country:_____ Institution:_____ Sampling site:_____

Date (dd/mm/yy):_____ Method of collection:_____Thickness of sample collected:_____

Station[a]	Latitude[a]	Longitude[a]	Depth (m)[a]	Time	Temperature	Salinity	pH	Dissolved oxygen	Tide	Wind

Person responsible for the campaign: _____

Name of the person responsible for taking the samples: _____

Remarks:_____

_____

[a]Essential information. Note: please attach a map with coordinates of the sampling stations.

I-2. SEDIMENT CORE COLLECTION FORM

INTERNATIONAL ATOMIC ENERGY AGENCY
Project RLA/7/012

Country:_____Institution:_____Location:_____Station:_____

Date (dd/mm/yy): _____Time (24h):_____Depth: _____Latitude:_____Longitude:_____

Climatic conditions: sea conditions (calm, heavy swell, etc.): _____Wind: Direction: _____Speed (m/s): _____

Cloud cover: _____Person responsible for the campaign: _____

	Temperature (ºC)	Salinity	pH	Dissolved oxygen (mg/L)
Surface				
Bottom				

Core code	Length (cm)	Person responsible for collection

Remarks:_____

_____

_____

_____

_____

_____

_____

I-3. CORE CHARACTERIZATION FORM

INTERNATIONAL ATOMIC ENERGY AGENCY
Project RLA/7/012

Country: _____Institution: _____Location: _____Station: _____Date (dd/mm/yy):_____

Person responsible for cutting: _____Core length (cm): _____Core code: _____

Section (cm)	Colour	Odour	Texture	Macroremains	$Weight_{wet}$ (g)	$Weight_{dry}$ (g)	DW_{init} (g)*	DW_{550} (g)*

*see Annex III.2, Loss on ignition.

Remarks:_____

_____

_____

Annex II

ANALYTICAL REPORTS

This annex contains guidance for reporting analytical results and an example of the report format used in project RLA/7/012 and described in the publication *A Guide to the Use of Sediment in Reconstructing the Pollution History of Coastal Areas*.

II.1. Reporting analytical results
II.2. Example of an analytical report

II.1. REPORTING ANALYTICAL RESULTS

The following describes the information to be provided by the analytical laboratories when reporting the results of the analyses assigned to them (Table II-2 in section II.1.5 lists the tests to be performed by each laboratory). A results report is to be provided for each core analysed.

II.1.1. General information

- Analysis: list of the analyses carried out
- Laboratory: name of the laboratory or unit that carried out the analyses (institution and country)
- Staff: person(s) responsible for the results

II.1.2. Description of methods

Each participating laboratory is to describe briefly the analysis methods used, including the following information:

a) Sample preparation: briefly describe the processing the sample undergoes before testing, e.g. drying, grinding, sieving, etc.
b) Testing: provide a description of the method including, at least, the main equipment, the calibration methods and the reference for the standard method used.
c) Quality controls: method and frequency, including a list of the reference materials used and a report on the values obtained for the certified reference materials.
d) Uncertainty: a brief description of the method used to calculate uncertainties and the sources considered.

II.1.3. Presentation of results

Results of the analyses are to be presented in the format set out in Table II-1:

TABLE II-1. GENERAL FORMAT FOR REPORTING ANALYTICAL RESULTS

Sample code	Element/Radionuclide/Mineral/Compound/Parameter	
	Activity/Concentration/UNIT	Absolute uncertainty UNIT
As per instructions in section 7 of this guide		

Several items from the same type of test may be reported in the same report, e.g. radionuclides by gamma ray spectrometry, elements by X-ray fluorescence, etc.
The units in which results are reported must be clearly stated in the heading of each column (as illustrated in Table II-2 in section II.1.5).

II.1.4. Sample identification codes

Each test report must retain the origin code of the core including the analysis (see coding instructions, section 6). Example: RLA7012CUB060208BI-XRF.

II.1.5. Reports on analytical results

The following describes the information to be provided by the analytical laboratories when reporting the results of the analyses assigned to them (Table II-2 lists the tests to be performed by each laboratory). A results report is to be provided for each core analysed.

General information

a) Analysis: list of the analyses carried out
b) Laboratory: name of the laboratory that carried out the analyses (institution and country)
c) Staff: person(s) responsible for the results

Description of methods

Each participating laboratory is to describe briefly the analysis methods used, including the following information:

a) Sample preparation: briefly describe the processing the sample undergoes before testing, e.g. drying, grinding, sieving, etc.
b) Testing: provide a description of the method including, at least, the main equipment, the calibration methods and the reference for the standard method used.
c) Quality controls: method and frequency, including a list of the reference materials used and a report on the values obtained for the certified reference materials.
d) Uncertainty: a brief description of the method used to calculate uncertainties and the sources considered.

Presentation of results

Results of the analyses are to be presented as described in section II.1.3.

Sample identification codes

Each test report must retain the origin code of the core including the analysis (see coding instructions in this guide). Example: RLA7012CUB060208BI-XRF.

TABLE II-2. INSTITUTIONS RESPONSIBLE FOR THE ANALYSES CARRIED OUT

Analysis (unit)	Institution
MOI (%)	All countries participating in project RLA/7/012
LOI (%)	All countries participating in project RLA/7/012
C-N (%)	Division of Chemistry, CIEMAT, SPAIN. Marine and Limnological Science Institute, Mazatlán, UNAM, MEXICO.
XRF (%, mg/kg)	Division of Chemistry, CIEMAT, SPAIN. Radiometrics Laboratory, Marine Environment Laboratories, IAEA. Environmental Testing Laboratory, CEAC, CUBA.
XRD	Division of Chemistry, CIEMAT, SPAIN.
GAM (Bq/kg)	Division of Chemistry, CIEMAT, SPAIN. Environmental Testing Laboratory, CEAC, CUBA. Radiometrics Laboratory, Marine Environment Laboratories, IAEA.
ALP (Bq/kg)	Marine and Limnological Science Institute, Mazatlán, UNAM, MEXICO. Radiometrics Laboratory, Marine Environment Laboratories, IAEA. Environmental Testing Laboratory, CEAC, CUBA.
GRA (%)	Division of Chemistry, CIEMAT, SPAIN. Radiometrics Laboratory, Marine Environment Laboratories, IAEA.
DMA (µg/kg)	Division of Chemistry, CIEMAT, SPAIN. CIRA, UNAN, NICARAGUA.
PAH (mg/kg)	CIMAB, CUBA INVEMAR, COLOMBIA CICA, COSTA RICA INDICASAT, PANAMA Universidad de Oriente, IOV, BOLIVARIAN REPUBLIC OF VENEZUELA
PES (mg/kg)	CIMAB, CUBA INVEMAR, COLOMBIA CICA, COSTA RICA INDICASAT, PANAMA Universidad de Oriente, IOV, BOLIVARIAN REPUBLIC OF VENEZUELA

II.2. EXAMPLE OF AN ANALYTICAL REPORT

This example includes fictitious data and is for illustrative purposes only.

NAME OF LABORATORY: INSTITUTION:

REF.: Core and analysis code
E.g.: RLA7012MEX010508AIII-CN

RESULTS REPORT
TOTAL CARBON, INORGANIC CARBON AND NITROGEN

Place, date: _____

Assigned laboratory: Division of Chemistry, CIEMAT
Technique used: analysis by combustion and specific total organic carbon analyser
Persons responsible for the analyses:
Perla Griselle Mellado Vazquez, IAEA Fellow
Cristina García Diego, Ionic and Elemental Analysis Laboratory Technician
Alberto José Quejido Cabezas, Head of the Division of Chemistry

II.2.1. Description of method

Preparation of samples

The samples received at the CIEMAT Division of Chemistry Division were brought by project fellow Ms Perla Griselle Mellado Vázquez personally. The quantities sent were sufficient for the required parameters to be measured. The samples were not ground to an extent that would enable immediate preparation for elemental analysis, meaning that the submitted material had to be reprocessed. The sample was homogenized and pulverized using an agate mortar to a grain size of approximately 63 μm.

Analysis of total carbon and total nitrogen

Total carbon (C_{tot}) and total nitrogen (N_{tot}) were determined using a LECO TruSpec elemental analyser. An amount on the order of 100–150 mg of dried, ground and homogenized sample is weighed — in duplicate — in an Sn container on an analytical balance. The container is sealed tightly and placed in the automatic sampler, and from there it is put into the combustion furnace. It is kept in the furnace for 2–3 min, in several stages, with different amounts of O_2 in the carrier gas stream (combustion profile) to ensure complete oxidation. All the gases generated during this process are collected in a 4.5 L container and, once mixed, a small aliquot of them is fed to the detectors where the CO_2 is determined using an infrared detector and, after the gases pass through a Cu reduction tube, the N_2 is determined using a thermal conductivity detector.

To create the calibration curves, two certified standards with different amounts of C and N are used to cover a wide calibration range: one soil standard (Soil LECO / 502-062 / Lot 1012) containing 0.130 ± 0.018 (k=2) % N and 1.30 ± 0.04 (k=2) % C, and another soil standard (Soil LECO / 502-309 / Lot 1002) containing 0.80 ± 0.02 (k=2) % N and 10.14 ± 0.10 (k=2) % C. Different quantities (on the order of 50, 100 and 150 mg) of these certified reference materials are weighed in the Sn container and put into the analyser following the same procedure as was used with the samples. By plotting the readings from the detectors against the absolute amounts in mg of C and N in the standards, one obtains the calibration curves for N and C.

Having gone through the process previously using a number of blanks (typically an empty Sn capsule), the mean area obtained from those runs is automatically subtracted from the readings obtained for the standards and the samples containing the elements of interest (C and N). Once the analysis is completed, the mean value for the blank is established and the calibration curves are produced using the results obtained for the standards. The calibration curve for N is obtained using a first-degree (linear) fit; the calibration curve for C is obtained using a quadratic fit. The C and N content of the samples is recalculated using the new calibration curves obtained.

Analysis of total inorganic carbon

Total inorganic carbon (TIC) is determined using the Shimadzu SSM-5000 solid sampling module along with pre-acidification with phosphoric acid in a furnace at 200°C. This module is a special accessory for the Shimadzu total organic carbon analyser series. The CO_2 produced is transported via air stream and measured in the high sensitivity non-dispersive infrared analyser (NDIR TOC-V). The analytical procedure described in European standard UNE-EN 13137:2002 is followed. A calibration line for $CaCO_3$ ($R^2 = 0.9991$) was used for quantification.

Total organic carbon (TOC) is determined by taking the difference between the results obtained for total C and inorganic C as per the UNE standard mentioned above.

II.2.2. Quality control

Analysis of total carbon and total nitrogen

For every ten samples, at least the two standards (in duplicate) and one pair of blanks are analysed. A total of 25 measurements were performed using each of the standards over the course of the analysis. The certified reference materials Soil LECO / 502-062 / Lot 1012 and Soil LECO / 502-309 / Lot 1002 were measured. The following results were obtained:

Soil LECO / 502-062 / Lot 1012:

Element	Obtained	Uncertainty	Certified	Uncertainty	Diff (%)
C_{tot} (%)	1.315	0.078	1.30	0.02	1.2
N_{tot} (%)	0.1317	0.0044	0.130	0.009	1.3

Soil LECO / 502-309 / Lot 1002:

Element	Obtained	Uncertainty	Certified	Uncertainty	Diff (%)
C_{tot} (%)	10.08	0.018	10.14	0.05	-0.64
N_{tot} (%)	0.7859	0.0008	0.80	0.01	-1.8

II.2.3. Calculating uncertainty

The uncertainty was calculated as the standard deviation for the analysis of at least two replicates of each core sample. As the masses taken from the samples are different, the relative standard deviation (RSD) is also reported in order to facilitate comparison between results. This example includes fictitious data and is for illustrative purposes only.

In addition, F-tests were used to compare the uncertainties for the samples with the uncertainties in the measurements made on the reference materials, and they were generally found to be comparable, although the uncertainty was higher in samples with very low total N content.

Reference	C_{tot} (%)		N_{tot} (%)	
	U_C	RSD (%)	U_N	RSD (%)
RLA7012MEX010508AIII0-1	0.02	1.19	0.021	14.09
RLA7012MEX010508AIII1-2	0.06	3.54	0.007	5.24
RLA7012MEX010508AIII2-3	0.00	0.00	0.007	5.66
RLA7012MEX010508AIII3-4	0.01	0.90	0.021	13.69
RLA7012MEX010508AIII4-5	0.01	0.47	0.014	8.84
RLA7012MEX010508AIII5-6	0.01	0.42	0.000	0.00
RLA7012MEX010508AIII6-7	0.02	1.25	0.007	4.56
RLA7012MEX010508AIII7-8	0.03	1.82	0.007	4.88
RLA7012MEX010508AIII8-9	0.04	2.40	0.007	5.66
RLA7012MEX010508AIII9-10	0.04	3.14	0.007	7.44
RLA7012MEX010508AIII10-11	0.08	5.62	0.007	7.44
RLA7012MEX010508AIII11-12	0.06	4.71	0.007	7.44
RLA7012MEX010508AIII12-13	0.08	7.04	0.015	21.37
RLA7012MEX010508AIII13-14	0.00	0.00	0.023	31.87
RLA7012MEX010508AIII14-15	0.02	2.05	0.017	24.96
RLA7012MEX010508AIII15-16	0.06	5.34	0.010	16.46
RLA7012MEX010508AIII16-17	0.04	4.12	0.004	5.48
RLA7012MEX010508AIII17-18	0.03	2.95	—	—
RLA7012MEX010508AIII18-19	0.02	2.27	—	—
RLA7012MEX010508AIII19-20	0.06	6.40	—	—
RLA7012MEX010508AIII20-21	0.01	0.84	—	—
RLA7012MEX010508AIII21-22	0.01	0.83	—	—
RLA7012MEX010508AIII22-23	0.06	6.22	—	—
RLA7012MEX010508AIII23-24	0.05	5.47	—	—
RLA7012MEX010508AIII24-25	0.04	3.86	—	—
RLA7012MEX010508AIII25-26	0.01	1.46	—	—
Soil LECO / 502-062 / Lot 1012	0.078	5.91	0.0044	3.33
Soil LECO / 502-309 / Lot 1002	0.018	0.18	0.0008	0.10

U_C, U_N = uncertainty in measurements of C_{tot} and N_{tot} respectively.
RSD (%) = relative standard deviation

II.2.4. Results

The following example includes fictitious data and is for illustrative purposes only.

Total carbon (C_{tot}) and total nitrogen (N_{tot})

Reference	C_{tot} (%)		N_{tot} (%)	
	Conc	U_C	Conc	U_N
RLA7012MEX010508AIII0-1	1.785	0.021	0.146	0.021
RLA7012MEX010508AIII1-2	1.600	0.057	0.135	0.007
RLA7012MEX010508AIII2-3	1.520	0.000	0.125	0.007
RLA7012MEX010508AIII3-4	1.570	0.014	0.155	0.021
RLA7012MEX010508AIII4-5	1.505	0.007	0.160	0.014
RLA7012MEX010508AIII5-6	1.695	0.007	0.140	0.000
RLA7012MEX010508AIII6-7	1.695	0.021	0.155	0.007
RLA7012MEX010508AIII7-8	1.550	0.028	0.145	0.007
RLA7012MEX010508AIII8-9	1.475	0.035	0.125	0.007
RLA7012MEX010508AIII9-10	1.125	0.035	0.095	0.007
RLA7012MEX010508AIII10-11	1.385	0.078	0.095	0.007
RLA7012MEX010508AIII11-12	1.200	0.057	0.095	0.007
RLA7012MEX010508AIII12-13	1.105	0.078	0.070	0.015
RLA7012MEX010508AIII13-14	1.110	0.000	0.071	0.023
RLA7012MEX010508AIII14-15	1.035	0.021	0.068	0.017
RLA7012MEX010508AIII15-16	1.060	0.057	0.059	0.010
RLA7012MEX010508AIII16-17	1.030	0.042	0.065	0.004
RLA7012MEX010508AIII17-18	0.960	0.028	<0.05	-
RLA7012MEX010508AIII18-19	0.935	0.021	<0.05	-
RLA7012MEX010508AIII19-20	0.995	0.064	<0.05	-
RLA7012MEX010508AIII20-21	0.845	0.007	<0.05	-
RLA7012MEX010508AIII21-22	0.855	0.007	<0.05	-
RLA7012MEX010508AIII22-23	0.910	0.057	<0.05	-
RLA7012MEX010508AIII23-24	0.905	0.049	<0.05	-
RLA7012MEX010508AIII24-25	0.915	0.035	<0.05	-
RLA7012MEX010508AIII25-26	0.970	0.014	<0.05	-

U_C, U_N = uncertainty in measurements of C_{tot} and N_{tot} respectively.

Total inorganic carbon (TIC)

Every fifth core sample was analysed to determine the core's carbonate background, as the total carbon levels are very similar throughout the profile. Total organic carbon was calculated as the difference between C_{tot} and C_{tic}. The following example includes fictitious data and is for illustrative purposes only.

Reference	Inorganic carbon		Organic carbon	
	Conc **(%)**	U_{TIC}	Conc **(%)**	U_{TOC}
RLA7012MEX010508AIII0-1	0.398	0.002	1.388	0.021
RLA7012MEX010508AIII5-6	<0.30	–	1.695	0.007
RLA7012MEX010508AIII10-11	<0.30	–	1.385	0.078
RLA7012MEX010508AIII15-16	0.469	0.001	0.591	0.057
RLA7012MEX010508AIII20-21	0.625	0.016	0.220	0.017
RLA7012MEX010508AIII25-26	0.746	0.003	0.224	0.014

Annex III

METHODS OF ANALYSIS

This annex describes the methods for analysing sediment-related variables that were used in project RLA/7/012 and described in the publication *A Guide to the Use of Sediments in Reconstructing the Pollution History of Coastal Areas*.

III.1. MOISTURE CONTENT IN SEDIMENT

The moisture content of sediments is a very useful variable in studying sediment cores, as it can be used to estimate sediment porosity and density.

III.1.1. Materials

- Wet sediment (core section);
- Oven (45°C);
- Balance (±0.001 g);
- Plastic bags with hermetic seal;
- Spatulas.

III.1.2. Procedure

1. Determine the weight of the plastic bag (tare weight).
2. Use a spatula to put the wet sediment inside the plastic bag.
3. Record the total weight of the wet sediment (bag + sediment).
4. Dry the sediment in the oven at a maximum temperature of 45°C for as long as required to achieve a constant weight.
   Constant weight has been achieved when the difference between weighings of the same sample is less than 1%.
5. Remove the sample from the oven and place it in a desiccator until it is at room temperature.
6. Weigh and record the total weight of the dry sediment (bag + sediment).
7. Calculate the relative moisture content (percentage, %) as follows:

$$\text{Moisture (\%)} = \left(\frac{\text{Weight}_{\text{wet sediment}} - \text{Weight}_{\text{dry sediment}}}{\text{Weight}_{\text{wet sediment}}}\right) \times 100 \qquad \text{(III-1)}$$

III.2. LOSS ON IGNITION

Estimating the percentage of organic matter in sediments by the loss-on-ignition method (LOI_{550}) is based on the weight lost through combustion of the sediments at 550°C to ash and carbon dioxide [1].

The sample must be perfectly dry (check that it has reached a constant weight) and hence the result is expressed as a percentage of the dry weight.

III.2.1. Materials

- Porcelain crucibles;
- Muffle furnace capable of heating at 550°C and 950°C (constant temperature);
- Spatulas;
- Balance (±0.001 g).

III.2.2. Procedure

1. Dry the porcelain crucibles for at least 12 hours in a 105°C oven.
   Allow them to cool to room temperature in a desiccator and record the weight.
2. Homogenize the dried and ground sediment sample (check that it has reached a constant weight) and weigh approximately 0.5 g; record the weight.
3. Combust at 550°C for 4 hours [2].
4. Remove from the muffle furnace when the temperature has dropped to 100°C.
5. Allow to cool to room temperature in a desiccator and record the weight.

The LOI_{550} is calculated using Eq. (III-2):

$$LOI_{550}(\%) = \frac{DW_{init} - DW_{550}}{DW_{init}} \times 100 \quad \text{(III-2)}$$

where:
LOI_{550} is the loss on ignition at 550°C (%), DW_{init} is the dry weight of the sample before combustion and DW_{550} is the weight of the sample after combustion at 550°C (both weights in grams). The LOI_{550} value should be proportional to the quantity of organic matter contained in the sample and can be converted to percentage of organic carbon by multiplying the LOI_{550} value by a factor of 0.5 [3].

Next, in a second combustion phase (following steps 3 to 5), this time at 950°C for 2 hours, the loss on ignition at 950°C (LOI_{950}) can be used to estimate the carbonate concentration in the samples using equation Eq. (III-3):

$$LOI_{950}(\%) = \frac{DW_{550} - DW_{950}}{DW_{init}} \times 100 \quad \text{(III-3)}$$

where:
LOI_{950} is the loss on ignition at 950°C (%), DW_{550} is the weight of the sample after combustion at 550°C, DW_{950} is the weight of the sample after combustion at 950°C and DW_{init} is the dry weight of the sample before combustion (weights in grams). Assuming a ratio of 1.36 between the weight of carbonate (CO_3^{2-}, 60 g/mol) and the weight of carbon dioxide (44 g/mol) molecules, the LOI_{950} multiplied by 1.36 yields the weight of carbonates in the original sample [4].

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III.3. PREPARING SAMPLES FOR ANALYSIS OF ^{210}Po ACTIVITY IN SEDIMENTS

This section describes the radiochemical processing of sediment samples in order to isolate polonium isotopes by spontaneous deposition onto silver discs (as described in [1], adapted from [2]) in preparation for activity analysis by alpha particle spectrometry.

III.3.1. Reagents

- HCl, concentrated and 0.5N;
- HNO_3, concentrated;
- HF, concentrated;
- ^{209}Po as a recovery tracer;
- Ascorbic acid;
- MilliQ deionized H_2O;
- Methyl alcohol;
- Silver cleaner;
- Acrylic spray paint;
- Phosphate-free soap.

III.3.2. Materials

- Analytical balance;
- Teflon containers (50 mL capacity) with screw cap, tightly sealable;
- Variable-volume micropipettes (maximum capacity 5, 1 and 0.1 mL);
- Hotplate with temperature control;
- Centrifuge tubes (50 mL);
- Centrifuge;
- Glass beakers (250 mL);
- Silver discs, 2.5 cm in diameter;
- Orbital shaker;
- Alpha spectrometer.

III.3.3. Procedure

1. Weigh 0.1–0.5 g dried ground sediment in a Teflon container (with a tight-sealing lid) using a balance having a precision of 0.001 g or better.
2. Add a known amount of ^{209}Po tracer and record the weight.
3. Add 4 mL HCl_{conc} + 5 mL HNO_{3conc} + 1 mL HF_{conc}.
4. Seal the container tightly and heat at 150°C for 8 hours (or overnight).
5. Remove from the hotplate and wait for the container to cool to room temperature.
6. Open the container, rinse the lid with HCl_{conc} and pour the rinse into the container.
7. Evaporate the acid mixture at controlled temperature (<90°C) until dry. Note: This is a good time to start preparing the silver discs for Po isotope deposition (see step 16).
8. Add more HCl_{conc} and allow to evaporate. Repeat this step twice.
9. Resuspend the residue using 0.5 N HCl, taking care to rinse the sides of the container thoroughly, and transfer the sample solution to a 50 mL centrifuge tube.
10. Centrifuge at 4000 rpm for 10 min and recover the supernatant solution in a 250 mL beaker. Resuspend the residue with 0.5 N HCl, centrifuge again for 10 min and add the supernatant to the beaker with the sample from the previous centrifugation.
11. Add approximately 200 mg ascorbic acid (or until the solution becomes colourless).

12. Place a silver disc at the bottom of the beaker with the polished side up and place the beaker on an orbital shaker at room temperature for 8 hours.
13. Remove the silver disc from the beaker, rinse with deionized H_2O and then with methyl alcohol and allow to dry.
14. Once the disc is dry, store it in a plastic or cardboard box (Note: a paper envelope or plastic bag will work equally well), marking the sample identification code clearly.
15. The silver disc (2 cm in diameter, 0.25 cm thick) must be pre-cleaned with silver-cleaning solution to remove any traces of oxidation. These discs normally have a polished side and an unpolished side. If so, be sure to identify the polished side and paint the unpolished side with acrylic spray paint to prevent Po isotope deposition on the side that will not be exposed to the detectors. Allow the paint to dry for at least 2 hours before using the discs.
16. Place the silver disc in the alpha detector and wait until both isotopes (^{209}Po tracer and ^{210}Po in the sample) reach at least 400 net counts (equivalent to 5% counting uncertainty). See [3] for more information on ^{210}Po determination using alpha particle spectrometry and ^{210}Pb dating models.

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[2] FLYNN, W.W., The determination of low levels of polonium-210 in environmental materials, Analytical Chimica Acta **43** (1968) 221–227.

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III.4. DETERMINING RADIONUCLIDE ACTIVITY USING GAMMA RAY SPECTROMETRY

III.4.1. Objective

To establish the requirements and workflow for gamma spectrometry testing.

III.4.2. Scope

The methodology is applicable to gamma spectrometry measurements in high-purity germanium (HPGe) semiconductor detector systems.

III.4.3. Definitions

- Activity: Number of radioactive decays in a unit of time. The SI unit for this quantity is the becquerel (1 Bq = 1 s^{-1});
- Area: Region of the peak above the upper boundary of the pedestal, containing the counts corresponding to the gamma quanta of the radionuclide in the sample;
- Absolute peak efficiency: Number of counts recorded under the total energy absorption peak divided by the total number of gamma quanta with that energy emitted by the sample during the measurement time. Depends on the measurement geometry;
- Count rate: Number of counts recorded in a given region of the spectrum divided by the recording time;
- Critical limit: Net count rate value that must be exceeded before the sample can be considered to contain any measurable radioactive material above background;
- Detection limit: Smallest amount of radioactive material detectable with a specified degree of confidence;
- Determination limit: Lowest net count rate detectable with a specified relative uncertainty;
- Integral: Region of the peak containing the background counts plus the counts from the radionuclide in the sample, i.e., integral = area + pedestal;
- 'Less-than' level: Amount of radioactive material that could be present but remain undetected. It is defined as the maximum net count rate that a sample could have and is based on the net count rate measured which is less than the critical limit;
- Measurement geometry: Spatial configuration in the sample–detector assembly. The shape of the container holding the sample and the position of the container relative to the detector greatly influence the measurement results. Various measurement geometries exist;
- Pedestal: Region of the peak delimited by the boundaries in the zone containing the background counts;
- Specific activity: Activity normalized using the values for the sample tested and the sampling process;
- Spectrum: Discrete function relating the count number and the channel number according to the energy that the gamma quanta deposit in the detector.

III.4.4. Supplementary material

Methodology for calculating the photopeak area and estimating the measurement time.
Formulas for calculating specific activity according to the type of sample.

III.4.5. Responsibilities

It is the responsibility of the physical measurements specialist to comply with this procedure.

III.4.6. Preparing the equipment

Spectrometry systems make it possible to determine the relationship between the number of gamma quanta incident on the detector and their energy. The presence of a radionuclide in the sample is indicated by an increase in the counts at the energies of its characteristic gamma quanta. Once the system has been calibrated and the information for each radionuclide has been recorded, its activity can be determined.

The aim of gamma spectrometry testing is to determine the specific activity of radionuclides in the test sample. To that end, the steps of the procedure must be performed in a certain order:

1. In the main logbook, monitor and record the environmental parameters, which must be in the following range: temperature 18–26°C and relative humidity 48–60%.
2. Check the control parameters.
    a. This consists in periodically checking the value of detector parameters that may vary for some reason. The values to check are 1) detector efficiency for a given energy and a given geometry and 2) the number of background counts for a given energy range.
    b. Place a suitable standard in position for measurement, adjust the position of the peak in the corresponding channel, collect the spectrum and calculate the peak area, making sure the relative uncertainty is less than 5%. Use the method explained in section III.4.11, or use an already validated spectrum processing program, and then calculate the absolute peak efficiency using the following formula:

$$\varepsilon = \frac{R_t - R_b}{A_{std}\, P_\gamma} \tag{III-4}$$

where:

R_t is the standard-plus-background radionuclide count rate;
R_b is the background radionuclide count rate;
A_{std} is the activity of the standard;
P_γ is the probability of gamma emission.

Note: Update the activity of the standard on the day of measurement, adjusting for the decay of the radionuclide between the reference date and the day of measurement. Use the following formula:

$$A_{st} = A_0 \, e^{-0.693 \, t/T_{½}} \quad \text{(III-5)}$$

where:

A_0 is the reported activity of the standard on the reference date;
t is the time elapsed between the reference date and the day of measurement;
$T_{½}$ is the half-life of the radionuclide in the standard.

c. Plot the value obtained on a control chart and analyse the behaviour of the parameter.
d. If it is concluded from the control chart analysis that efficiency is under control, the first requirement for conducting the test is met. If it is not under control, do not perform the test; check the environmental parameters, the detector's operating voltage and the measurement geometry or investigate the performance of all system components to identify which one is causing the problem.
e. Collect the background spectrum for 2000 seconds and determine the integral for the interval (300–1400) keV.
f. Repeat step (c), this time using the count rate corresponding to the integral determined.
g. If the background is under control, the second requirement for conducting the test is met.
h. If it is not under control and possible contamination is suspected, decontaminate the detector with 1% citric acid, a binding solution (EDTA) and distilled water. Wearing waterproof gloves, dampen cloths with each of these substances separately and clean the detector surface with each cloth in turn. Change the cloths several times, using a single substance on each, to ensure complete removal of any contamination.
i. Repeat steps (e) to (g) until the parameter is under control.
j. Save the results of the control parameter check as evidence that the system is under control for measurement.
k. Simultaneous control of both parameters is essential for the next step of the test.

III.4.7. Measuring the samples

This consists in collecting the spectrum of the sample for a length of time that guarantees a certain relative uncertainty in the calculation of specific activity specified in the accompanying model.

1. Samples for gamma spectrometry measurements are delivered packaged, with an accompanying model. Check that the sample complies with the characteristics of one of the geometries for which the system was calibrated, and that the accompanying model has all the necessary data for the calculations.
2. If, on inspection, the sample that was delivered meets the requirements, accept it by signing the delivery receipt for samples and models presented by the person delivering it; if it does not meet the requirements, reject it on those grounds.

3. After checking the control parameters and making sure that the spectrometry system is operating correctly, cover the sample with some thin material to prevent accidental contamination of the detector, place it in position for measurement and collect its spectrum. Note:

   a. Sealed samples for measuring ^{226}Ra and ^{232}Th under equilibrium conditions with their progeny species must be left for at least 20 days before being measured.
   b. Measure the samples for a length of time that guarantees a certain relative uncertainty in the calculation of specific activity specified in the accompanying model. Use the formula in section III.4.11 to estimate this measurement time.
   c. Samples that have undergone prior radiochemical separation are to be measured first for a length of time that allows the chemical yield of the process to be calculated. If the value obtained meets the requirement of the procedure, continue measuring the sample. Otherwise, stop measuring, return the sample to the technician who performed the separation and note the incident in the accompanying model. This is considered to be an instance of non-conformance.

4. Save the collected spectra with the same code as the sample's and keep them as evidence of the test.

III.4.8. Processing the spectrum

This consists in identifying the peaks in the spectrum using knowledge of the energy–channel function, identifying the radionuclides present in the sample and determining the data needed in order to calculate activities. The results are saved as evidence of the testing process.

Determine the energy corresponding to each peak in the spectrum and, using a nuclear data table, identify the radionuclides having gamma quanta with an energy very close to a peak. An uncertainty of ±0.5 keV is acceptable for HPGe. From the radionuclides identified, identify the radionuclide actually present in the sample, taking into account the percentage of gamma emission, the presence or absence of other photopeaks that radionuclide might have, its half-life and the likelihood of it being present in the type of sample being measured. Develop these skills.

III.4.9. Calculating activities

Determine the area and the relative uncertainty of the peaks from which one has decided to calculate activities. With the above data, calculate the net count rate R_{net} for each peak using the following formula:

$$R_{net} = R_t - R_b \tag{III-6}$$

and the uncertainty

$$u(R_{net}) = [u^2(R_t) + u^2(R_b)]^{1/2} \tag{III-7}$$

where:

R_t is the total sample-plus-background count rate due to the radionuclide;
R_b is the background count rate due to the radionuclide;
$u(R_{net})$ is the uncertainty in the net count rate;
$u(R_t)$ is the uncertainty in the total count rate;
$u(R_b)$ is the uncertainty in the background count rate.

Calculate the critical limit L_c during the measurement time as follows:

$$L_c = k\left[\frac{R_b}{T_b}\left(1+\frac{T_b}{T}\right)\right]^{1/2} \tag{III-8}$$

where:

T is the sample measurement time;
T_b is the background measurement time;
k is the 95% confidence factor for one-tailed tests (1.65).

If $0 < R_{net} \leq L_c$ is satisfied, calculate the 'less than' value L_t as follows:

$$L_t = R_{net} + k\,u(R_{net}) \tag{III-9}$$

The above values are count rates that are converted into absolute activity by dividing by the probability of gamma emission P_γ and the efficiency ε, and are converted into specific activity by in addition normalizing them for the sample characteristics and the sampling process using the formulas in section III.4.12. The absolute activity A corresponding to a net count rate above the critical limit is calculated as follows:

$$A = \frac{R_{net}}{P_\gamma\,\varepsilon} \tag{III-10}$$

and the associated uncertainty u(A) as follows:

$$u(A) = A\left\{\left(\frac{u(R_{net})}{R_{net}}\right)^2 + \left(\frac{u(\varepsilon)}{\varepsilon}\right)^2 + \left(\frac{u(P_\gamma)}{P_\gamma}\right)^2\right\}^{1/2} \tag{III-11}$$

where $u(P_\gamma)/P_\gamma$ is the relative uncertainty in the probability of gamma emission.

The uncertainty in the determination of the specific activity is found by applying the law of uncertainty propagation to the equations in section III.4.12 and assessing the contribution of the uncertainty in each quantity to the total uncertainty.

The general formula is:

$$u(A_{sp}) = A_{sp}\left\{\left(\frac{u(A)}{A}\right)^2 + \sum_{i=1}^{n}\left(\frac{u(X_i)}{X_i}\right)^2\right\}^{1/2} \tag{III-12}$$

where:

$u(A_{sp})$ is the uncertainty in the specific activity;
A_{sp} is the specific activity;
$u(A)/A$ is the relative uncertainty in the absolute activity;
$u(X_i)/X_i$ are the relative uncertainties in the characteristic quantities for the sample and the sampling process.

When analysing radionuclides with a short half-life that may decay during sampling ($t_{sam} > 0.01T_{½}$) — for example, in aerosol and atmospheric precipitation sampling — the activity value is multiplied by a coefficient representing the decay that occurs during sampling:

$$\frac{0.693\ \frac{t_{sam}}{T_{1/2}}}{1 - e^{-0.693\ \frac{t_{sam}}{T_{1/2}}}} \tag{III-13}$$

where:

t_{sam} is the sampling time;
$T_{½}$ is the half-life of the radionuclide.

When analysing radionuclides with a short half-life that may decay during measurement ($t_{meas} > 0.01T_{½}$), the activity value is multiplied by a coefficient representing the decay that occurs during measurement:

$$\frac{0.693\ \frac{t_{meas}}{T_{1/2}}}{1 - e^{-0.693\ \frac{t_{meas}}{T_{1/2}}}} \tag{III-14}$$

where:

t_{meas} is the measurement time;
$T_{½}$ is the half-life of the radionuclide.

When correcting the activity to adjust for the sampling date, the activity value is multiplied by a decay correction coefficient:

$$e^{\frac{0.693\ t_{dec}}{T_{1/2}}} \tag{III-15}$$

where:

t_{dec} is the time elapsed from sampling to measurement;
$T_{½}$ is the half-life of the radionuclide.

When analysing radionuclides having gamma energies below 100 keV, such as ^{210}Pb, ^{241}Am and ^{234}Th, and the density of the standard differs from the density of the sample to be measured, the activity value is multiplied by a coefficient of attenuation by self-absorption:

$$\frac{\mu_{sam}\,\rho_{sam}\,x_{sam}\left[1-e^{(-\mu_{std}\,\rho_{std}\,x_{std})}\right]}{\mu_{std}\,\rho_{std}\,x_{std}\left[1-e^{(-\mu_{sam}\,\rho_{sam}\,x_{sam})}\right]} \tag{III-16}$$

where:

μ_{sam}, μ_{std} are the mass attenuation coefficients of the sample and the standard;
ρ_{sam}, ρ_{std} are the densities of the sample and the standard;
x_{sam}, x_{std} is the effective attenuation thickness for the sample and the standard.

Provided that the sample and the standard do not contain any macro amounts of elements with Z > 20, the mass attenuation coefficient is calculated using the equation:

$$\mu(cm^2 g^{-1}) = 1.287\,E^{-0.435} \tag{III-17}$$

where E is the gamma energy expressed in keV.

The effective attenuation thickness of a vial is calculated using the equation:

$$X = 0.2\,r + 0.8\,(h^2 + d^2)^{1/2} \tag{III-18}$$

where r, h and d are the radius, height and diameter of the vial, respectively.

For cylindrical containers placed on top of the detector, the effective attenuation thickness is considered to be the height of the sample in the cylinder.

III.4.10. Reporting results

Results are reported as follows:

a) If the net count rate is greater than the critical limit, report the corresponding specific activity and its uncertainty.
b) If the net count rate is less than or equal to the critical limit and greater than zero, report that the specific activity is less than the specific activity corresponding to the 'less-than' value.
c) If the net count rate is less than the critical limit and less than or equal to zero, report that the specific activity is less than the specific activity corresponding to the critical limit.

For practical purposes, calculate the detection limit using:

$$L_d = k^2/T + 2L_c \tag{III-19}$$

and the determination limit using:

$$L_q = \frac{f_q^2}{2\,T}\left\{1+\left[1+\frac{4\,T\,R_b(T+T_b)}{f_q^2\,T_b}\right]^{1/2}\right\} \tag{III-20}$$

where f_q is the reciprocal of the specified relative uncertainty. These values are converted into activity by dividing by the probability of gamma emission and the efficiency, and are converted into specific activity using the formulas in section III.4.12.

Process the spectrum using validated programs. At the end of the test, wash the containers with plenty of water and then clean them with one of the following decontaminants:

Mixture 1:

— Detergent, 5 g;
— Sodium hexametaphosphate, 10 g;
— HCl, 50–80 g;
— H_2O, up to 1 L.

Mixture 2:

— Detergent, 3 g;
— HCl, 35–100 mL;
— H_2O, up to 1 L.

Prepare the above substances in a suitable receptacle, immerse the containers and scrub with a brush for 1–3 min. Remove the containers, rinse them with distilled water and dry them. Analyse the background radiation of the containers using the spectrometry system. If they are not clean, repeat the cleaning steps. Keep the clean containers in a suitable place and provide them to the preprocessing technician when needed.

III.4.11. Methodology for calculating the photopeak area and estimating measurement time.

Calculating the area or number of counts in the photopeak

This calculation is performed by hand. The distribution of the counts by channel is known, and every peak has three basic characteristics: the integral (N_t), the pedestal or background (N_b) and the area or net counts (N). These quantities are determined using the following three equations:

$$N = N_t - N_b \tag{III-21}$$

$$N_t = \sum_{i=a_2}^{b_1} N_i \tag{III-22}$$

$$N_b = \left(\sum_{i=a_1}^{a_2-1} N_i + \sum_{i=b_1+1}^{b_2} N_i \right) \frac{b_1 - a_2 + 1}{a_2 - a_1 + b_2 - b_1} \tag{III-23}$$

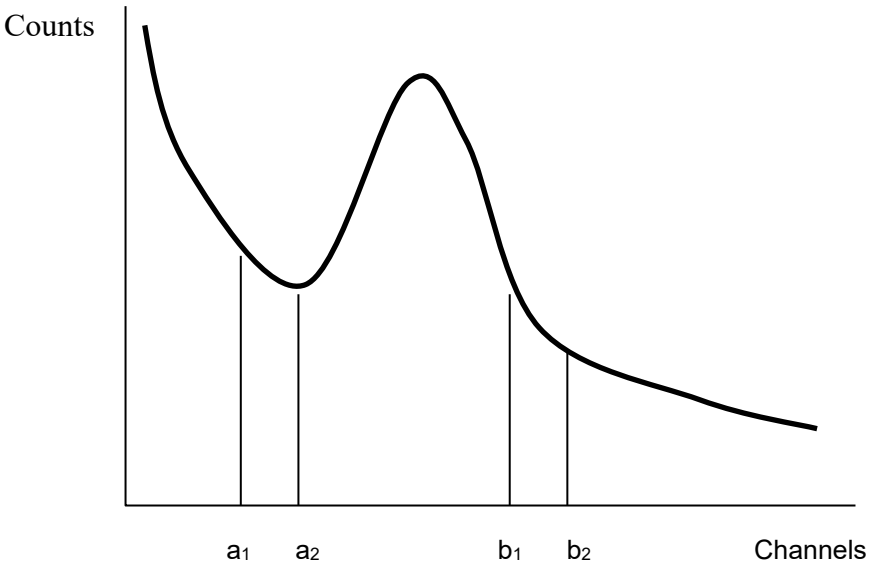
where:

N_i are the counts in channel i;
a_1, a_2, b_1, b_2 numbers of the respective channels or associated energy values.

Uncertainties in the photopeak area calculation

Applying the law of propagation of uncertainty to the expression for calculating the area, the uncertainty is determined as:

$$u(N) = \left[N_t + N_b \frac{b_1 - a_1 + 1}{a_2 - a_1 + b_2 - b_1}\right]^{1/2} \quad \text{(III-24)}$$



Using a coverage factor of 1.645, corresponding to the 90% confidence level in the normal distribution, the relative uncertainty is obtained using:

$$\frac{1.645\, u(N)}{N} \quad \text{(III-25)}$$

This uncertainty, expressed as a percentage, coincides with the uncertainty reported by the system.

Estimating measurement time

The measurement time to achieve a relative uncertainty established a priori in the calculation of the specific activity is estimated using the following formula:

$$T = \frac{R_t}{\left\{\left(\frac{u(A_{sp})}{A_{sp}}\right)^2 - \left(\frac{u(\varepsilon)}{\varepsilon}\right)^2 - \sum_{i=1}^{n}\left(\frac{u(X_i)}{X_i}\right)^2\right\}(R_t - R_b)^2 - u^2(R_b)} \quad \text{(III-26)}$$

where:

T	is the measurement time;
R_t	is the total count rate (sample plus background);
$u(A_{sp})/A_{sp}$	is the relative uncertainty in the calculation of the specific activity, taken from the accompanying model;
$u(\varepsilon)/\varepsilon$	is the relative uncertainty in the efficiency determination;
$u(X_i)/X_i$	are the relative uncertainties of the n characteristic quantities for the sample and the sampling process;
R_b	background count rate;
$u(R_b)$	is the uncertainty in the background count rate.

III.4.12. Formulas for calculating specific activity for various types of samples

In all cases, the absolute activity, or simply activity (A), expressed in becquerels, is assumed.

For determination in soil, sediment, sand and substrates

If measured in ash weight, it is reported in dry weight according to the formula

$$A_{sp} = \frac{1000\ AW}{w\ DWc} A \qquad \text{(III-27)}$$

where:

w is the measured sample ash weight (g);
DW_c is the dry weight to be combusted (g);
AW is the ash weight corresponding to DW_c (g);
A_{sp} is the specific activity (Bq/kg dry weight).

If measured in dry weight, it is reported in dry weight: AW and DW_c are taken as equal to 1 in the above formula.

For determination in food and grass

If measured in ash weight, it is reported in fresh weight according to the formula:

$$A_{sp} = \frac{1000\ DW\ AW}{w\ WW\ DWc} A \qquad \text{(III-28)}$$

where:

w is the measured sample ash weight (g);
WW is the wet or fresh weight (g);
DW is the dry weight (g);
DW_c is the dry weight to be combusted (g);
AW is the ash weight corresponding to DW_c (g);
A_{sp} is the specific activity (Bq/kg fresh weight).

If measured in dry weight, it is reported in fresh weight;

$$A_{sp} = \frac{1000\ DW}{w\ WW} A \qquad \text{(III-29)}$$

where w is the measured dry weight (g).

If measured in wet or fresh weight, it is reported in fresh weight:

$$A_{sp} = \frac{1000}{w} A \qquad \text{(III-30)}$$

where w is the wet or fresh weight (g).

For determination in aerosols

$$A_{sp} = \frac{W}{w\, V_f} A \qquad \text{(III-31)}$$

where:

w is the measured sample ash weight (g);
W is the total ash weight of the incinerated sample (g);
V_f is the volume of filtered air (m^3);
A_{sp} is the specific activity ($Bq \cdot m^{-3}$).

For determination in atmospheric precipitation

$$A_{sp} = \frac{W\ 30}{w\ T_e\ a} A \qquad \text{(III-32)}$$

where:

w is the measured sample ash weight (g);
W is the total ash weight of the incinerated sample (g);
a is the tray area (m^2);
T_e is the exposure time (d);
A_{sp} is the specific activity ($Bq \cdot m^{-2} \cdot month^{-1}$).

III.5. PREPARING SAMPLES FOR GRAIN SIZE ANALYSIS

III.5.1. Objective

To condition samples of dry marine sediment for grain size analysis by laser diffraction.

III.5.2. Scope of application

This procedure can be used to prepare the sediment samples from project RLA/7/012, which have been dried at 45°C, for grain size analysis.

III.5.3. Reference documents

Standard procedure for preparing samples for grain size analysis.

III.5.4. Description

This procedure describes and prescribes the steps to be followed for processing marine sediment samples that have been dried at 45°C for grain size analysis.

1. Weigh out approximately 0.5 g of dried sediment in a clean, dry beaker, which has already been labelled and tared, using a three-digit balance (0.001 g).
2. Dampen the sample with approximately 15 mL of water at room temperature.
3. Place the beaker in an ultrasonic bath and apply ultrasound for 15 min per hour for 5 hours.
4. Allow the sample to stand and remove the excess water with a Pasteur pipette.

III.6. DETERMINING ELEMENTAL COMPOSITION BY X-RAY FLUORESCENCE (XRF) SPECTROMETRY

Major components and heavy metals are analysed using the Spectrolab 2000 X-ray fluorescence system by SPECTRO Analytical Instruments.

- Put 4 g of dried and ground sediment in a low-density polyethylene container in which the bottom is lined with Prolene™ membrane. The sample is to be compressed carefully using a Teflon rod of a diameter somewhat smaller than that of the container.
- Perform a spectral analysis and quantitative calculations using the internal evaluation calibration method in the device's software and perform a set of three measurements with different X-ray targets. This allows total concentrations to be determined for elements with atomic numbers between 13 (Al) and 92 (U).
- Perform routine analyses of replicates (n = 6) of aliquots of IAEA reference materials 158, 405 and 433, which exhibit good consistency between analytical and certified values for most of the elements included in the certificate, with accuracies above 90% and uncertainties below 8% for the majority of the elements analysed.

III.7. DETERMINING TOTAL CARBON, TOTAL NITROGEN, TOTAL INORGANIC CARBON AND TOTAL ORGANIC CARBON

III.7.1. Total carbon (C_{tot}) and total nitrogen (N_{tot})

The analysis procedure using a LECO TruSpec elemental analyser is described below:

- Weigh out 100–150 mg of dried, ground and homogenized sediment in a tin container on an analytical balance (having a precision of 0.0001 g).
- Seal the container tightly and place it in the automatic sampler, from which it is transferred to the combustion furnace and remains there for 2–3 min, in several stages, exposed to different amounts of O_2 in the carrier gas stream (combustion profile) in order to ensure complete oxidation.
- The gases generated during this time are collected in a 4.5 L container and, once mixed, a small aliquot of the gases then flows to the detectors, where the CO_2 is determined by an infrared detector and, after the gases have passed through the Cu reduction tube, N_2 is determined by a thermal conductivity detector.

Establishing the calibration curves requires using certified standards with different C and N contents (covering the range of values in the sample).

It is important to perform determinations on blanks (usually an empty Sn capsule) and the values obtained must then be subtracted from those obtained both for the standards (for the calibration curve) and for the sample.

The calibration curve for N is obtained using a first-degree (linear) fit; the calibration curve for C is obtained using a quadratic fit.

The C and N content of the samples is recalculated using the new calibration curves obtained.

III.7.2. Total inorganic carbon (TIC)

This determination is performed using the Shimadzu SSM-5000 solid sampling module along with pre-acidification with phosphoric acid in a furnace at 200°C. The CO_2 produced is transported via air stream and measured in the high sensitivity non-dispersive infrared analyser (NDIR TOC-V). The analytical procedure described in European standard UNE-EN 13137:2002 is followed. A calibration line for $CaCO_3$ ($R^2 = 0.9991$) was used for quantification.

III.7.3. Total organic carbon (TOC)

This value is calculated as the difference between the results obtained for C_{tot} and for TIC, following the above UNE standard.

III.8. DETERMINING TOTAL MERCURY CONCENTRATION IN SEDIMENTS

III.8.1. Scope and application

The following is a description of the methodology for analysing total mercury in samples of sediment and soil using the DMA-80 total mercury analyser. The typical working range of the method is 0.05–600 ng. Mercury vapour is passed through a long-optical-path absorbance cell and then through a short-optical-path absorbance cell (the two cells having a length ratio of 10:1). The same amount of mercury is measured twice at two different sensitivities.

The instrument detection limit (IDL) is 0.01 ng total mercury for this method, which involves wet acid digestion with a mixture of strong acids followed by addition of a reducing agent to produce elemental mercury vapour (Hg^0). The elemental mercury vapour is generated by direct combustion of the sample to be analysed.

Mercury levels in soil are generally below 0.2 mg/kg dry weight (ppm). When the total mercury level in soil exceeds a few mg/kg (ppm), there is a risk that mercury will migrate from the soil into other parts of the environment. In such cases, mercury pollution in the surrounding water systems must be investigated.

III.8.2. Summary of the method

The method involves using controlled heating in an oxygenated decomposition furnace to release mercury from a solid or aqueous sample. The sample is dried and thermally and chemically decomposed in the decomposition furnace. The decomposition products are carried by a stream of oxygen to the catalytic section of the furnace. Oxidation is completed and halogens and nitrogen and sulphur oxides are trapped in the catalyst tube. The remaining decomposition products are carried to an amalgamator that selectively traps mercury. Once the system has been flushed by an oxygen stream to remove any decomposition products or gases produced, the amalgamator is then rapidly heated to release the mercury vapour. The oxygen stream carries the mercury vapour through absorbance cells positioned in the light beam of a single-wavelength atomic absorption spectrophotometer. Absorbance (peak height or peak area) at 253.7 nm is measured as a function of mercury concentration.

III.8.3. General advice for best results

- Use high-purity, mercury-free reagents for reliable results;
- The quartering method of homogenization, which is used only for soil samples, is described below. Take great care when using the method, as the friction involved could lead to mercury losses;
- Mercury can be lost to evaporation if the sample is not kept at a low temperature as soon as it is collected from the sampling point, especially when sampling in the middle of the day;
- The reagents must be checked before performing the analysis, as their properties can change during storage;
- When producing the calibration curve, it is advisable to identify the quartz boats in order to avoid cross-contamination;

— Memory effects between analyses can occur when analysing samples with a high mercury concentrations (200 ng) before analysing a sample with a low concentration (25 ng);
— To minimize memory effects, the samples should usually be analysed in batches having low and high concentrations, with those having a low concentration being analysed first. If the samples cannot be divided into batches, blanks with a decay time could be analysed.

III.8.4. Collecting and preserving samples

Sediments

— For rivers, choose sampling points that allow easy collection of sediments at intervals of 50-200 m downstream from the discharge point of industrial wastewater or city drains. In addition, it is desirable to pick at least two points upstream to collect control samples of sediment. Collection sites for the sediment samples are usually chosen on both riverbanks and the centre of the river. If the river is wide, increase the number of sampling points;
— For lake, wetland and ocean areas, radially centre the sampling points at the starting point or mouth of the river, using a grid as necessary;
— Sampling in lakes at different depths requires the use of a sampling bottle (Van Dorn, Malchanov, etc.);
— It is preferable to use glass or polyethylene containers and they should be properly washed. Store the samples in a cool, dark place. Samples containing metallic or divalent mercury are to be stored in a freezer.

Soil

— Draw a grid on a map of the polluted site. Depending on the extent of the suspected or verified pollution, choose sampling areas ranging from 100 m^2 (10 × 10 m) at more severely polluted sites to 900 m^2 (30 × 30 m) at less severely polluted sites. Choose five points — the centre point of each grid and four associated points around it — and collect individual samples from each. Although the locations of the four associated points do not need to be plotted precisely, it is advisable to collect the four samples at points north, south, east and west of the centre point;
— At each sampling point, collect soil samples between the soil surface and 50 cm below the surface. Be certain to collect individual samples at two separate depths: one between the soil surface and a point 5 cm below the surface, and the other 5–50 cm below the surface. After collecting the soil samples, remove any debris from each sample and homogenize each one using the quartering method of mixing;
— Once the samples have been homogenized, mix an equal weight of material from each sample to obtain a single final composite sample. Similarly, for the five-site mixing method, mix an equal weight of material from each sample (homogenized using the method mentioned above for preprocessing soil) to obtain a single sample for mercury analysis.

III.8.5. Safety precautions

- A lab coat, gloves and safety glasses must be worn.
- Work very carefully, as the process involves the use of H_2SO_4, HNO_3, HCl and $HClO_4$ and the oxidizing effect of $KMnO_4$ causes staining. If staining occurs, rinse with hydroxylamine solution that has been prepared in advance, followed by plenty of tap water. If the skin is exposed to acid, rinse with plenty of tap water, and if acid is ingested, seek medical attention. Anyone exposed to vapours should remove themselves from the place of exposure and go where there is fresh air.
- Exercise extreme caution when handling samples, as these may come from sites with a high concentration of contaminants, and when handling certified reference materials (CRMs). Should there be exposure to the skin, rinse it with plenty of tap water, and if ingestion occurs seek medical attention.
- Store waste from reagents and samples and other waste in containers labelled for this purpose, taking care not to mix waste. Do not flush down the drain. When handling the waste wear a lab coat, latex gloves, safety glasses and a mask.
- Always keep the work area clean. Be sure to retain all reagents used in the analysis.

III.8.6. Reagents

- Mercury-free HNO_3 for trace analysis;
- Mercury-free kaolin;
- Mercury-free silica;
- Mercury-free HCl for trace analysis.

III.8.7. Instruments and materials

- DMA-80 direct total mercury analyser (Milestone);
- Quartz boats;
- Nickel boats;
- Muffle with a temperature range of 150–1000°C;
- Volumetric flasks in sizes 10, 100 and 1000 mL;
- Serological and volumetric pipettes, class A, in sizes 0.2, 0.5, 1.5 and 10 mL;
- Mettler AE160 analytical balance having a precision of 0.001 mg;
- Vials, 20 mL capacity;
- Latex gloves;
- High-purity oxygen (99%);
- Automatic pipettes in sizes 20–1000 μL;
- Agate mortars;
- Plastic or metal trays for sample quartering;
- Microspatulas;
- Safety bulbs;
- Wash bottles;
- Paper towels.

All glassware must be washed using the appropriate procedure.

III.8.8. Homogenizing the samples

Soil samples must be homogenized using the quartering method, but only after they have been passed through a mesh sieve to prepare them for analysis. Given that the samples are taken using a grid yielding a total of five samples per site (one each for the centre and the four cardinal points), they need to be homogenized and then mixed to obtain a single final representative sample for that site. This is done by quartering.

a. First, homogenize each sample individually by quartering, which consists in placing the sample in a plastic tray measuring around 25 × 40 cm and crushing it to a fine consistency using a mortar or spatula.
b. Once this consistency is obtained, spread the sample evenly over the entire surface of the tray and quarter it through the middle with a spatula to obtain four nearly equal parts. Mix each pair of opposite quarters to obtain two nearly equal halves and then mix the two halves.
c. Repeat the previous step 8–12 times.
d. Once the samples from all five points have been quartered, mix together equal weights of material (e.g., 20–25 g) from each sample and repeat the quartering as described in step (b).
e. If one is working with sediment and the sample has a high water content, centrifuge it to remove the top layer of water and homogenize it well before analysis.

III.8.9. Calibrating the method

The instrument must be put in a state in which the baseline values are as low as possible — Abs (Height) <0.005. This is done by alternating quartz boats containing (freshly prepared) 5% HNO_3 with empty boats until an appropriate baseline is established.

Before starting calibration, the Hg lamp must be in a state of optimal stability. It is therefore advisable to switch on the device first thing in the morning and let the lamp warm up for at least 2 hours.

Prepare the standards on the day of calibration. This should be done by successively diluting a certified standard solution of 1000 mg/L Hg. Use a 5% v/v HNO_3 blank as the solvent unless a different solvent is required for a specific use. The normal order of dilution with the relative volumes is shown in Figure III-1.

It is important that the pipettes used to prepare the standards are properly calibrated. It is recommended to prepare the standards in 25 mL disposable vials made of polyethylene (or other suitable material).

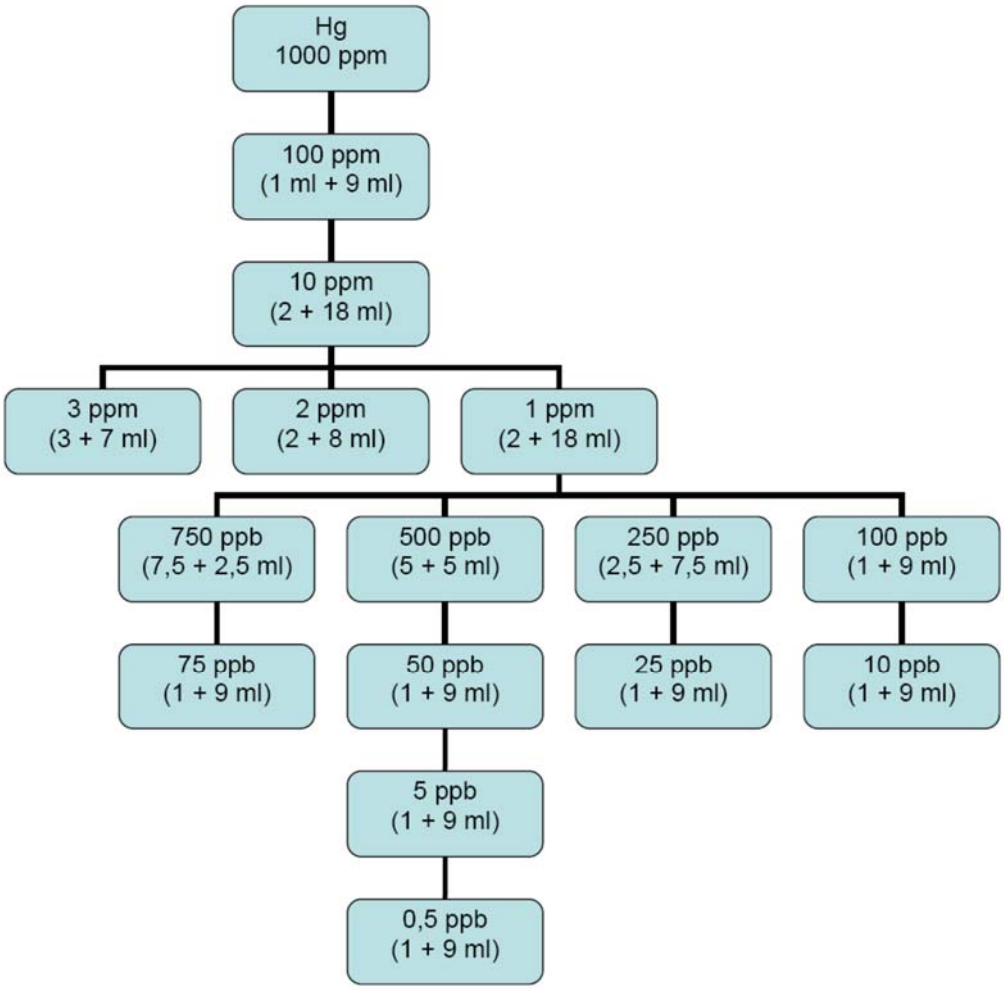


FIG. III-1.

Initial calibration

- Measure successive blanks until appropriate background values are obtained (Abs <0.005);
- Measure at least three replicates for each standard;
- Set the signal measurement to calibration standards (‘Calibrator’ icon) in the software, and then in the ‘Result’ section enter the concentration of the standard. Also enter the weight of the sample in grams, taking:

$$\text{Solution } 1\text{g/mL} \rightarrow \text{volume (mL)} = \text{weight (g)} \qquad \text{(III-33)}$$

- Observe the spread of the results after the replicate measurements. If one or more replicates is far from the others, measure a fourth or fifth replicate in order to estimate the mean absorbance value for the standard being measured;
- Accept or reject measurements based on the spread of the results;
- Adjust the curve-fitting method. It will normally be a quadratic fit;
- Plot each measurement on the assigned curve;
- Once all the replicates for the standards have been completed for one of the curves (low or high concentration), establish the acceptance criteria for the results, assessing whether any standard should be remeasured, depending on the proportionality of the absorbances;
- NEVER ACCEPT A CURVE WITH $R^2 < 0.99$;

— If the calibration lasts more than one day, on the second day clean the boats again and then resume calibration from the point at which it was interrupted, under the same lamp stability conditions. It is also necessary once again to prepare the standards to be processed that day.

III.8.10. Analysis procedure

— To start the analysis, first make sure that the calibration curve is loaded. Open the 'DMA-80 Calibration' tab and select the curve to be used;
— Return to the 'Methods' option;
— Use an existing method or create a new one. Assign a name to the method in the 'Method Name' field. Change the method parameters, if necessary, and save them;
— In the 'Program' section, change the device operating parameters for each sample, such as drying, decomposition and waiting time, if necessary. The drying time varies with the sample volume or with the percentage of water in the sample and has to be calculated using one of the following equations:

$$\text{Drying time (s)} = \text{sample volume (μL)} \times 0.6 \quad \text{(III-34)}$$

$$\text{Drying time (s)} = \text{sample weight (mg)} \times 0.6 \times \%\ H_2O \quad \text{(III-35)}$$

— For dry inorganic samples: Drying time = 10 s;
— For samples with high organic content: Drying time = 30–90 s;
— Drying temperature is 200°C for most samples. (Reduce the drying time if working with flammable samples). See application notes;
— Decomposition time is 3 min for most samples;
— Increase the decomposition time if the analysis result has a high RSD (>5%).
— Add 30 seconds and analyse the samples, recalculating the RSD;
— For example, coal requires 300 seconds (for others, see the application notes).
— Decomposition temperature = 650°C for most samples (also see application notes). Purge time is 60 seconds for most samples;
— Once all samples have been loaded into the tray (if automatic mode is being used) and the data have been entered in the 'DMA-80 Measurement' tab, open the 'Result' section in the 'DMA-80 Measurement/Data' tab and press 'Start';
— As the sample is analysed, the absorbance will start to appear in the 'Height' column of the 'Result' section, the amount of Hg in the sample in the 'Hg [ng]' column, and the Hg concentration in the 'C [μg/kg]' column;
— Once the equipment is ready for operation, weigh the appropriate amount of sample in triplicate. The sample weight is chosen based on the concentration range: the higher the concentration, the greater the memory effect;
— For soils and sediments, start with 10 mg. If the absorbance signal is very low and reproducibility is low, increase to 25 mg; if there is still no change, try 50–100 mg;
— All replicates must be weighed by the same operator, and one should try to make sure that the sample is spread over the entire boat by continuing to use the spatula. If the weight is not exact, do not remove the sample and just note the quantity weighed;
— In the device software, select the method to be applied to the samples or create a new method;
— Create a new samples file to record the sample data to be read. Select single or automatic mode;
— Check that the calibration file is correct, as the device automatically loads the file last used;

— Run a few blanks to make sure that the system is clean;
— Check the calibration curve by measuring a standard or a certified reference sample.
— Measure the samples in triplicate.

III.8.11. Time/temperature ramp

Time for temperature rise at start-up: About 30 seconds

For solid samples (sediments):

TEST NO.	RAMP	PLATEAU
1	30 s	30 s
2	30 s	90 s
3	30 s	120 s

Acceptance criteria:

— If test 1 = test 3 → accept test 1
— If test 1 < test 2 = test 3 → accept test 2

III.8.12. Combustion time and temperature

— The longer the time, the more confident one can be that all the Hg has been atomized. However, various other elements may be present with the Hg, resulting in deterioration of the catalyst and the amalgam;
— Default temperature: 650°C;
— In principle, the procedure is suitable for sediments and for other matrices that do not have a very high percentage of Hg associated with silicates. For such matrices a higher combustion temperature should be used;
— If the Hg cannot be fully atomized because it remains partly occluded in silicates, the sample must be wet digested with HF, after which the HF is removed using H_3BO_3 or $HClO_4$ (not recommended);
— In general, the decomposition time is usually 1 min longer than the drying time (ramp + plateau):

  - Ramp: 1 min
  - Plateau: Total time – 1 min.
  - For solid samples: drying time + 1 min

— When measuring a sample with a very low concentration, one can use the pre-concentration option provided by the software (‘}+’);
— In an analysis file, enter as many replicates as there are to be pre-concentrated (for liquids, it is not recommended to exceed 4 replicates, owing to possible Hg losses). Select ‘}+’, followed by the first and last replica to be pre-concentrated. This allows all the replicates to be pre-concentrated in the amalgam without thermal desorption from the amalgam (at 900°C) being carried out until the last replicate. The result will be an average value for all the replicates entered;

— In practice, it is always advisable to compare the results with a reference material. An appropriate, sufficiently tested and homogenized sample can be used as a reference or control sample. It is recommended that samples be stored cold in order to preserve the Hg content (Hg is quite volatile and can be lost via the container lid);
— Start reading the samples.

III.8.13. Calculating and outputting results

— The calculations are shown automatically in the recorder of the device;
— Be sure always to print out a results sheet for filing and make an electronic backup in the folder 'Total mercury analysis result DMA-80' on the computer located in the office of the Environmental Mercury Laboratory.

III.8.14. Checking accuracy

— To check the accuracy of the analysis, one must analyse a sample of some certified reference material that is suitable for the matrix of the analysed samples, following the procedure described above. Compare the result with the reference value and acceptance limits. If it lies outside the limits, review the procedure and repeat the analysis;
— For each group of samples analysed, measure one or two standards that are within the range of results obtained for the samples analysed.

III.8.15. Checking precision

Samples are always to be tested in batches of at least five replicates and the results are to be accepted if the RSD is less than 10%. If this is not the case, the analysis should be repeated.

III.8.16. Recovery rate

Analyse a sample with a spike *(spiked sample)* according to the concentration obtained from the samples analysed.

III.8.17. Control standard

Verify the calibration curve by running two standards part way through each working session.

III.9 DETERMINING ORGANIC POLLUTANTS IN SEDIMENTS

III.9.1. Introduction

The method described below is based on [1]. To determine organic pollutants such as aliphatic, polycyclic aromatic and total hydrocarbons and chlorinated pesticides in sediment samples, these pollutants must be extracted and isolated from the matrix (see Fig. III-2). For this purpose, a portion of the sediment sample is dried at a low temperature (ideally <25°C) until just dry (alternatively, the sample can be freeze-dried or mixed in its wet state with anhydrous sodium sulphate) and extraction is performed using appropriate solvents in a Soxhlet extractor. The extract is then concentrated and purified using column chromatography before undergoing instrumental analysis. Quality control samples are processed along with each batch of samples in exactly the same manner as the samples under study.

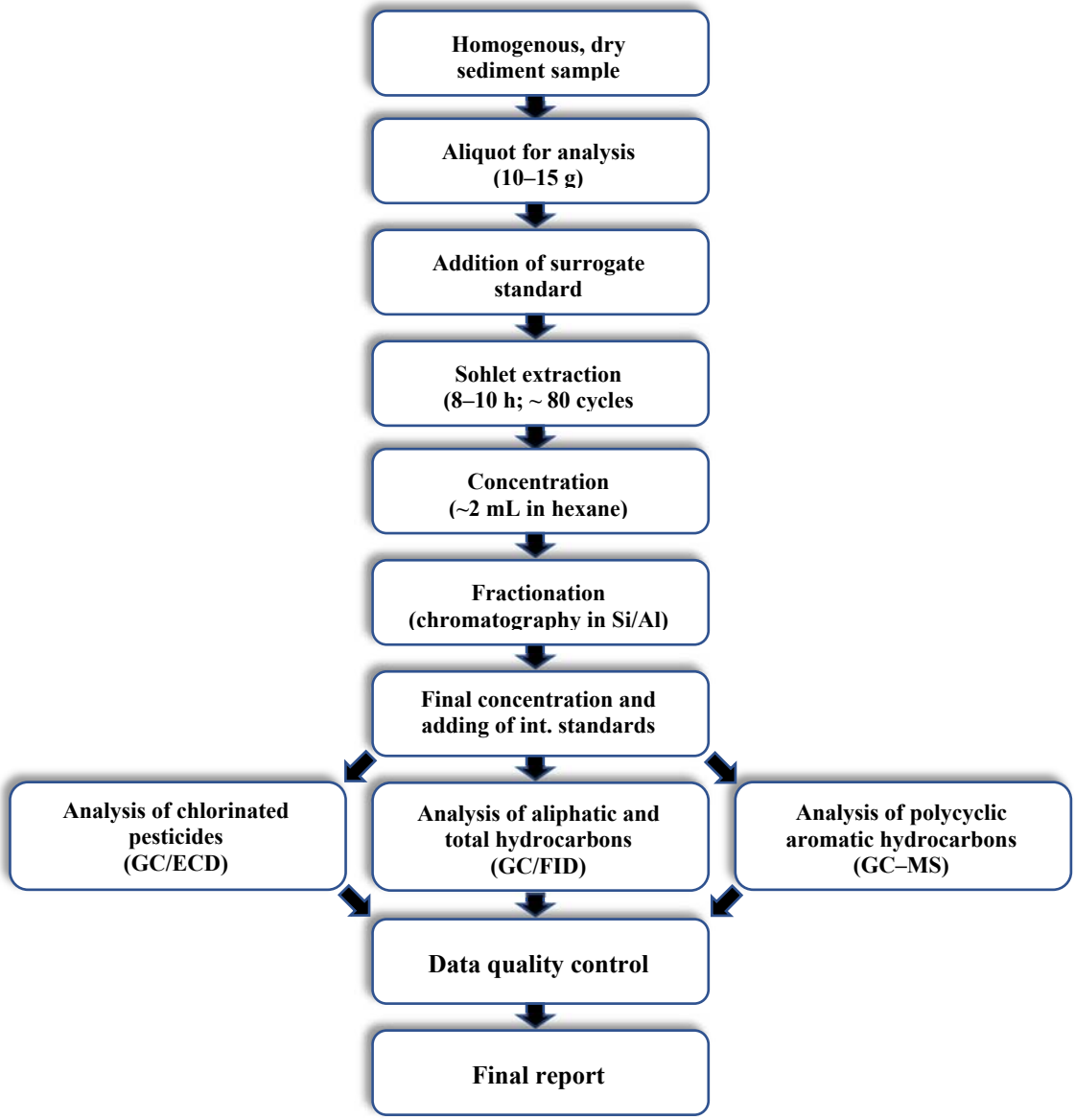


FIG. III-2. Flow diagram illustrating the analysis of chlorinated pesticides, aliphatic and total hydrocarbons and PAHs in sediment samples.

III.9.2. Preprocessing, preserving and storing the sample

The collected sediment sample must be placed in glass containers free of organic contaminants (see section III.9.3.1). Ideally, the sample should be frozen immediately or kept on ice until arrival at the laboratory; once there, it should be stored at -20°C in its original container until it undergoes drying. The extracts from samples must be kept at 4°C prior to instrumental analysis, following which they must be stored in a dark place at -20°C.

III.9.3. Extraction and purification

III.9.3.1. Laboratory equipment

All laboratory glassware must be washed with water and laboratory soap/detergent, dried with acetone and then rinsed in this sequence: once with acetone, then twice with hexane and then twice with methylene chloride. Pesticide-grade solvents should be used for these final rinses. Alternatively, non-volumetric equipment can be left to dry uncovered and, once dry, covered with aluminium foil and heated in a muffle furnace at 440°C for 4 hours. Without removing the aluminium foil, leave them to cool and store them in a protected area until needed.

Laboratory equipment required for the analysis of organic pollutants in sediments:

- Analytical balance with an accuracy of 0.0001 mg;
- Analytical balance with an accuracy of 0.1 g;
- 250 mL and 500 mL flat or round-bottom flasks;
- Temperature controlled water bath set to 60–70°C;
- Cellulose or ceramic thimbles pre-extracted in a Soxhlet extractor;
- Graduated cylinders in sizes 250 mL and 1000 mL;
- 300 mm long chromatography columns with a 13 mm inside diameter, 250 mL upper reservoir and Teflon stopcock;
- Three-ball Snyder distillation columns;
- Desiccator;
- Borosilicate glass funnels of various sizes;
- Stainless steel spatulas;
- Convection oven set to 63–65°C;
- Soxhlet extractors;
- Nitrogen gas for evaporation;
- Glass wool calcined at 440°C for 4 hours or pre-extracted using solvents;
- 100 µL micropipette;
- Glass or Teflon boiling chips, pre-washed with solvents;
- Calcined aluminium foil;
- Forceps;
- 1 mL disposable Pasteur pipettes and rubber bulbs;
- Scissors;
- Graduated 25 mL Kuderna–Danish concentrator tubes with ground glass stoppers;
- 50 cm glass rod;
- 10 mL beakers for dry weight determination;
- 50 mL, 250 mL and 500 mL beakers;
- 1 mL and 7 mL dark glass vials with Teflon-lined caps.

All volumetric equipment used to measure samples or add standards and spike solutions must be calibrated prior to use.

III.9.3.2. Reagents

- Acetone, pesticide grade or equivalent;
- Hydrochloric acid, 12N, reagent grade;
- Water free of organic contaminants;
- Basic chromatography grade alumina, Brockmann grade I, ~150 mesh or equivalent, activated at 440°C for 4 hours and kept in an oven at 120°C (allow to cool to room temperature in a desiccator before use);
- Sand, washed and calcined at 440°C for 4 hours;
- Copper, analytical grade, 20–30 mesh;
- Methylene chloride, pesticide grade or equivalent;
- Hexane, pesticide grade or equivalent;
- Pentane, pesticide grade or equivalent;
- Silica, grade 923, 100–200 mesh or equivalent, activated in an oven at 170°C for 24 hours (allow to cool to room temperature before use);
- Spike solution;
- Internal standard solution;
- Surrogate standard solution;
- Granular anhydrous sodium sulphate (Na_2SO_4), calcined at 440°C for 4 hours and kept in an oven at 120°C (allow to cool to room temperature in a desiccator before use).

III.9.3.3. Determining dry weight (moisture content)

An aliquot of approximately 1 g of perfectly homogenized sediment sample is weighed in a tared 10 mL beaker. After drying in a convection oven at 63–65°C for 24 hours, the aliquot is weighed and returned to the oven for 2 hours before being weighed again. If the difference between the two weight measurements is less than or equal to 0.02 g, the second reading is used to calculate the percentage dry weight of the sample. If the difference is greater than 0.02 g, the sample is returned to the oven for another two hours and weighed again. This process is repeated until the difference between two consecutive weights is less than 0.02 g.

Determining dry weight (moisture content) — Step by step

- Calibrate the balance before use according to the manufacturer's instructions and indications in the laboratory's calibration notebook. Record the date of calibration, weight used and operator's initials.
- Tare the empty balance until it reads '0.000 g'.
- Place a 10 mL vial or beaker on the balance and record its weight ('vial weight').
- Carefully stir the homogenized sediment sample with a stainless steel spatula that has been pre-washed with solvents and, without re-taring the balance, weigh out approximately 1 g of the sample in the vial ('vial + wet sample').
- Prepare the quality control samples (blank, duplicate sample, spiked sample and reference material) in the same manner. For the blank, 'vial weight' and 'vial + wet sample' will be the same.
- Note down any sample characteristics that you notice while working (e.g. odour, colour, bits of vegetation, shells, etc.). The sample to be dried should not contain any foreign material (e.g. bits of vegetation, shells, organisms).
- Once all the samples have been prepared, place the vials containing the wet samples in a laboratory tray and leave the tray in a convection oven at 63–65°C for 24 hours.

— After 24 hours, using suitable protection, carefully remove the tray from the oven and place it in a desiccator. Allow the vials to cool to laboratory temperature for 30 min.
— Calibrate the balance before use according to the manufacturer's instructions and indications in the laboratory's calibration notebook. Record the date of calibration, weight used and operator's initials.
— Tare the empty balance until it reads '0.000 g'.
— Place one of the vials on the balance and record its weight ('vial + dry sample #1').
— Once all the vials have been weighed, return the tray to the oven at 63–65°C for 2 hours.
— After 2 hours, using suitable protection, carefully remove the tray from the oven and place it in a desiccator. Allow the vials to cool to laboratory temperature for 30 min.
— Calibrate the balance before use according to the manufacturer's instructions and indications in the laboratory's calibration notebook. Record the date of calibration, weight used and operator's initials.
— Tare the empty balance until it reads '0.000 g'.
— Place one of the vials on the balance and record its weight ('vial + dry sample #2').
— If the difference between the last two weight readings is less than or equal to 0.02 g, calculate the percentage dry weight using the second reading.
— If the difference is greater than 0.02 g, return the sample to the oven for 2 hours and repeat the weighing process as indicated.
— Determine the relative percent difference (RPD) between the calculated dry weights of the original sample and the duplicate sample. The RPD should be within the acceptable range specified by the laboratory — usually ±25%. If the RPD is greater than ±25%, re-weigh both samples to confirm the weight readings and/or check the calculations. If, after recalculating the dry weights, the RPD is still greater than ±25%, it may be that the sample is not properly homogenized. Notify your supervisor before proceeding.
— For the blank, the absolute difference between the vial weights before and after drying should be less than or equal to 0.02 g.

Calculations

$$\%\ \text{Dry weight} = \left(\frac{(\text{Vial} + \text{Dry sample}) - (\text{Weight of vial})}{(\text{Vial} + \text{Wet sample}) - (\text{Weight of vial})}\right) \times 100 \quad \text{(III-36)}$$

$$\text{Relative percent difference} = \left[\frac{\%\ \text{Dry wt. of orig. sample} - \%\ \text{Dry wt. of dup. sample}}{\left(\frac{\%\ \text{Dry wt. of orig. sample} + \%\ \text{Dry wt. of dup. sample}}{2}\right)}\right] \times 100 \quad \text{(III-37)}$$

III.9.3.4. Obtaining an extract from the sample

A weighed amount of dried and perfectly homogeneous sample is transferred to a cellulose or ceramic thimble, which is placed in a Soxhlet extractor. Note: It is important that there be no foreign material (e.g. bits of vegetation, shells, organisms) in the sample to undergo extraction. Three hundred mL of methylene chloride and 3–5 boiling chips are put in a 500 mL flask. The extractor is then connected to the flask, and the thimble, with the sample inside, is moistened with approximately 50 mL of methylene chloride.

Once the Soxhlet extraction unit is set up, appropriate surrogate standards are added to each sample and the corresponding quality control samples, and a known analyte solution is added to the samples selected to be spiked. The Soxhlet extractor is placed over a heat source with an extraction cycle every 5–6 min (i.e. 10–12 cycles per hour) for a minimum of 8 hours or 80 cycles.

After extraction, a three-ball Snyder column is connected to the 500 mL flask and the extract is concentrated to 10–15 mL over a water bath at 60–65°C.

Alternatively, the extract can be concentrated using a rotary evaporator, taking care that the temperature of the water bath does not exceed 35°C and avoiding any cross-contamination between samples. If the extract contains any sample material or particulate matter, it must be filtered, prior to concentration, through a glass funnel containing sodium sulphate on a calcined glass wool plug. The extract, concentrated to 10–15 mL, is transferred to a 25 mL concentrator tube. The 500 mL flask is rinsed 2–3 times with methylene chloride and the rinse liquids are transferred to the concentrator tube. A boiling chip is added, the extract is concentrated and the solvent is replaced by hexane through the gradual addition of small amounts of hexane, over a water bath at 60–65°C and under a gentle stream of nitrogen. The final volume of the extract prior to column chromatography cleanup must be approximately 2 mL in hexane.

Obtaining an extract from the sample — Step by step

— Weigh, to two decimal places, the amount of sediment to undergo extraction. It is important that foreign material (e.g. bits of vegetation, shells, organisms) not be weighed along with the sediment.
— Pour 300 mL of methylene chloride into a flask clearly labelled with the code for the sample and analytical batch, add 5–6 boiling chips and connect the Soxhlet extractor to the flask.
— Using a spatula that has been washed with methylene chloride, transfer the dry sample to a cellulose or ceramic extraction thimble (pre-extracted using methylene chloride) for 8 hours at 10–12 extraction cycles per hour. Using forceps that have been rinsed with methylene chloride, place the thimble inside the Soxhlet extractor and add 50 mL of methylene chloride in order to moisten the sample, taking care not to push it out of the thimble.
— Add surrogate standards to all the samples, including the quality control samples, and add the target analyte solutions to the samples to be spiked.

Surrogate and internal standard solutions

Surrogate and internal standard solutions are prepared by weighing out the appropriate amounts of pure compounds, quantitatively transferring them to a volumetric flask and diluting them with hexane or a similar non-chlorinated solvent. Such solutions are for individual use and must be prepared and stored as such. The surrogate and internal standard solutions must be prepared in such a way that the addition of 0.100 mL to the sample, prior to extraction and instrumental analysis, respectively, results in final concentrations that are within the maximum and minimum of the calibration curve when the final volume of the extract is adjusted to approximately 1 mL. All target analytes present in the sample are quantified on the basis of a surrogate standard and surrogate standard recovery is established on the basis of the recovery of an internal standard. For this reason, if very high or very low concentrations of the target compounds are expected in the samples, the amounts of surrogate and internal standards added and the final extract volumes must be adjusted accordingly.

Spike solution

The spike solution contains all, or a good selection of, the target analytes that will be used to prepare a spiked blank or spiked sample. This solution is prepared by weighing out the appropriate amounts of pure compounds, quantitatively transferring them to a volumetric flask and diluting them with hexane or a similar non-chlorinated solvent. The spike solution must be prepared in such a way that the addition of 0.100 mL to the blank or the sample, prior to extraction, results in final analyte concentrations that, in the final extract volume (1 mL), are between the maximum and minimum of the calibration curve.

— Connect a condenser to the Soxhlet apparatus, place over a heat source and extract under a hood for at least 8 hours. Adjust the temperature to achieve an extraction cycle every 5–6 min (10–12 cycles per hour). Take special care to check periodically that a new cycle is initiated each time the level of solvent in the Soxhlet extractor reaches the siphon and that the solvent does not simply overflow drop by drop.

— As a rule, a minimum of 80 cycles can be expected. If the cycles are longer, the extraction process can last longer. In this case, take care to ensure that there is enough methylene chloride in the flask to complete the cycle, plus a reserve to avoid losses due to the amount of heating or drying out. Add more solvent to the flask as necessary during extraction.
— Once the extraction process is complete, allow the Soxhlet extractor to cool down until cycling stops, and remove the condenser.
— Add around 4–5 fresh boiling chips to the flask, connect a three-ball Snyder distillation column and place the flask in a water bath at 60–65°C to concentrate the extract to 10–15 mL. Alternatively, the extract can be concentrated using a rotary evaporator, taking care that the temperature of the water bath does not exceed 35°C. Avoid cross-contamination between samples.
— Transfer the extract to a 25 mL concentrator tube and rinse the flask with small amounts of methylene chloride. Collect the rinse liquids in the same concentrator tube. Add a boiling chip to the concentrator tube and place over a water bath at 60–65°C, under a gentle stream of nitrogen, until the extract is concentrated to approximately 1 mL in methylene chloride.
— Without removing the concentrator tube from the bath, add 10 mL of hexane and concentrate the extract again to 1 mL under a gentle stream of nitrogen. Repeat this step as many times as necessary until all the methylene chloride is replaced by hexane, at which point the liquid stops boiling.

III.9.3.5. Cleanup using silica gel/alumina in a chromatography column

The chromatography column is filled with methylene chloride, a plug of calcined glass wool is positioned at the bottom of the column to retain the adsorbent material and a 1 cm layer of anhydrous sodium sulphate or washed and calcined sand is added on top of the glass wool to create an even surface. Ten grams of dry alumina (previously activated and partially deactivated with 1% (w/w) water) are added at the top of the methylene chloride and allowed to settle in the column. Twenty grams of silica gel (previously activated and partially deactivated with 5% (w/w) water) are suspended in methylene chloride before being added to the column. Once the reaction between the silica gel and the methylene chloride is complete (i.e. no gas bubbles are seen to form), the silica gel is added on top of the alumina and allowed to settle. Approximately 2 cm of anhydrous sodium sulphate or washed and calcined sand are added at the top of the column and a 1–2 cm layer of activated copper is added on top of that (see Cleanup using silica gel/alumina in a chromatography column — Step by step). At this point, the methylene chloride-filled column should contain, from the bottom upwards, 1 glass wool plug, 1 cm of washed sand, 10 g of partially deactivated alumina, 20 g of partially deactivated silica, 2 cm of anhydrous sodium sulphate or washed sand and 1–2 cm of activated copper to remove sulphur. The methylene chloride is then allowed to flow through the column until the surface of the activated copper is reached, and 50 mL of pentane are added. The pentane is allowed to flow through the column and replace the methylene chloride until the activated copper is reached. At this point, the column is ready to receive the concentrated sample extract.

The sample extract, concentrated to 2 mL in hexane, is transferred to the column using a disposable Pasteur pipette. The liquid added is allowed to flow through the column until the surface of the activated copper is reached, and the solvent is collected in a 250 mL flask placed under the column. The concentrator tube that contained the sample is rinsed 2–3 times with small amounts (~1 mL) of a methylene chloride:pentane (1:1) mixture and the rinse liquids are added to the column following the procedure described above between additions. Two hundred mL of the methylene chloride:pentane mixture are added to the column and collected in the 250 mL flask drop by drop, at a rate of approximately 2 mL/min. This fraction will contain aliphatic, polycyclic and total aromatic hydrocarbons and chlorinated pesticides.

Cleanup using silica gel/alumina in a chromatography column — Step by Step

Preparing materials for the column

— Remove the sodium sulphate, alumina and silica gel from the oven and place them in a desiccator to cool to room temperature (see Extraction and purification, Laboratory equipment, Reagents).
— Remove the washed and calcined sand from the oven and let it sit on the bench to cool to room temperature.

Note: When removing materials from the oven, always wear protective gloves or use long tongs suitable for handling heavy objects. The containers holding the sand, silica and alumina can weigh up to 1 kg and will be hot. Make sure that all the containers are covered with aluminium foil.

Preparing the deactivated silica and alumina

— Calibrate the balance before use according to the manufacturer's instructions and indications in the laboratory's calibration notebook. Record the date of calibration, weight used and operator's initials.
— Place a 1000 mL flask with a ground glass mouth and a funnel on the balance and tare until the balance reads '0.000 g'
— Weigh the alumina into the flask. The total weight of the alumina should be equal to 10 g × n samples. As a rule, an extra amount of alumina (10–15 g) is weighed out in case of accidents or in case more is needed when building the columns. Record this weight.
— Re-tare the flask containing the alumina until the balance reads '0.000 g' and partially deactivate the adsorbent by adding 1% (w/w) water free of organic contaminants to the flask. Note: The amount of water is calculated by multiplying the total weight of the alumina in the flask by 0.01. For example, if the total weight of alumina in the flask is 171.2 g, the amount of water added should be 171.2 g × 0.01 = 1.712 ≈ 1.7 g. To avoid lumps forming in the mixture, it is best not to add all the water in the same place.
— Place another 1000 mL flask with a ground glass mouth and a funnel on the balance and tare until the balance reads '0.000 g'.
— Weigh the silica into the flask. The total weight of the silica should be equal to 20 g × n samples. As a rule, an extra amount of silica (20–25 g) is weighed out in case of accidents or in case more is needed when building the columns. Record this weight.
— Re-tare the flask containing silica gel until the balance reads '0.000 g' and partially deactivate the adsorbent by adding 5% (w/w) water free of organic contaminants to the flask. Note: The amount of water is calculated by multiplying the total weight of silica in the flask by 0.05. For example, if the total weight of silica in the flask is 363.1 g, the amount of water added should be 363.1 g × 0.05 = 18.15 ≈ 18.2 g. To avoid lumps forming in the mixture, it is best not to add all the water in the same place.
— Cover the two flasks and shake briskly to break up any lumps that may have formed, then shake more gently every 5–10 min for an hour to ensure that the liquid is uniformly distributed.

Preparing the activated copper

Note: Activated copper is used to remove any sulphur that could interfere in the analysis of extracts using gas chromatography coupled with an electron capture detector (chlorinated pesticides).

— Place approximately 50 g (for 24 samples) of metallic copper in a 250 mL beaker.
— Under a hood, very carefully add enough hydrochloric acid to cover the copper.
— Stir the copper with a glass rod for a few seconds and allow to stand for 5 min.
— Position a glass funnel plugged with glass wool over a 500 mL beaker and, under a hood, carefully transfer the copper/acid mixture into the beaker.

— Wash the activated copper with plenty of water free of organic contaminants until all the acid is removed. The acid:wash water mixture should be neutralized using sodium bicarbonate and disposed of according to laboratory procedures.
— Rinse the washed copper 3–4 times with 20 mL portions of methanol, 3–4 times with 20 mL portions of methylene chloride and 3–4 times with 20 mL portions of hexane. Dispose of the solvents according to laboratory procedures.
— Store the activated copper, covered with hexane, in a covered and properly labelled beaker. It is important to activate the copper just before use, as it becomes less effective or completely deactivated over time.

Preparing the methylene chloride:pentane (1:1) mixture

— The methylene chloride:pentane (1:1) mixture is prepared by mixing equal amounts of pentane and methylene chloride.
— Measure out 2 L of pentane using a graduated cylinder and pour into a 4 L bottle.
— Using the same graduated cylinder, measure out 2 L of methylene chloride and add it to the 4 L bottle. Seal the bottle and shake vigorously to ensure thorough mixing. Shake again before each use. This amount is sufficient for approximately 18 samples.

Setting up the column

— Secure the chromatography columns (each measuring 30 cm × 13 mm with a Teflon stopcock and a 250 mL reservoir) to their racks inside the hood. The total number of columns should be the same as the number of extracts to be cleaned up. For each column, follow the below procedure:
— Place a 250 mL container under the column to collect the waste solvents.
— Open the stopcock and, using a rinsing bottle, rinse the column 3 times using approximately 10 mL of methanol each time, and then rinse 3 times using approximately 10 mL of methylene chloride each time.
— Rinse the tips of a pair of forceps and a pair of scissors with methylene chloride and cut an sufficient amount of calcined glass wool to cover the bottom of the column. Using a glass rod that has been rinsed with methylene chloride, push the glass wool to the bottom of the column.
— Close the stopcock and fill part of the chromatography column with methylene chloride.
— Position a funnel over the column and add a 1 cm layer of washed sand to the column using a stainless steel spatula that has been rinsed with methylene chloride.
— Place a 50 mL beaker on a calibrated balance and tare it.
— Weigh out approximately 10 g (±0.1 g) of partially deactivated alumina (per column).
— Pour the alumina into the column using a funnel. Rinse the funnel and the inside of the column reservoir with small amounts of methylene chloride to ensure that all the alumina is transferred to the column. Briefly open the column stopcock to help the alumina to move downward.
— After adding the alumina, place a 50 mL beaker on the calibrated balance and tare its weight. Weigh out approximately 20 g (±0.2 g) of partially deactivated silica (per column).
— Add methylene chloride to the beaker and mix thoroughly with a glass rod until there no gas bubbles are seen to form.
— Pour the silica gel/methylene chloride mixture into the column using a funnel. Rinse the beaker, the funnel and the inside of the column reservoir with small amounts of methylene chloride to ensure that all the silica is transferred to the column. Briefly open the column stopcock to help the silica to move downward.
— Add a 2 cm layer of anhydrous sodium sulphate or washed sand on top of the silica. If necessary, rinse the reservoir with small amounts of methylene chloride to ensure that all the anhydrous sodium sulphate or sand is transferred to the column.
— Taking care to avoid disturbing the surface of the column, add 1–2 cm of activated copper to remove sulphur.
— Open the column stopcock and let the methylene chloride flow through the column until the surface of the activated copper is reached, collecting the waste liquid in a suitable container.

— Add 50 mL of pentane and allow it to flow through the column until the surface of the activated copper is reached.
— Close the stopcock. The sample extract can now be added to the column.

Purifying the extract

— Rinse the lower end of the chromatography column (below the stopcock) with methylene chloride.
— Replace the waste container with a 250 mL flask. The flask should be properly labelled with the code for the sample and analytical batch.
— Transfer the extract from the concentrator tube to the column using a disposable Pasteur pipette. At this point, the sample extracts are concentrated to approximately 2 mL in hexane.
— Open the column stopcock and let the extract flow into the column. Close the stopcock.
— Rinse the concentrator tube with 1 mL of the elution solvent (1:1 methylene chloride:pentane) and add the rinse liquid to the column using the same Pasteur pipette used to transfer the sample.
— Open the column stopcock and let the rinse liquid flow into the column. Close the stopcock.
— Rinse the concentrator tube twice more with the elution mixture, each time repeating the previous two steps and allowing the rinse liquid to flow completely into the column before the next rinse is added.
— After the extracts and their respective rinse liquids have been completely transferred to the columns, add 200 mL of the methylene chloride:pentane mixture using a graduated cylinder, taking care not to disturb the surface of the column so as not to resuspend the sample.
— Open the stopcock and elute the sample at a rate of about 2 mL/min, adjusting the stopcock as necessary.
— Once all the methylene chloride:pentane mixture has passed through the column, close the stopcock. Remove and cover the flask. Remove the chromatography columns and properly dispose of the used silica and alumina.
— The cleaned up sample extracts are now ready to be concentrated for instrumental analysis.

Final concentration of the extract

— Add around 4–5 boiling chips to the flask, connect a three-ball Snyder distillation column and place the flask in a water bath at 60–65°C to concentrate the extract to 10–15 mL. Alternatively, the extract can be concentrated using a rotary evaporator, taking care that the temperature of the water bath does not exceed 35°C. Avoid cross-contamination between samples.
— Transfer the extract to a 25 mL concentrator tube and rinse the flask with small amounts of methylene chloride. Collect the rinse liquids in the same concentrator tube. Add a boiling chip to the concentrator tube and place over a water bath at 60–65°C, under a gentle stream of nitrogen, until the extract is concentrated to approximately 1 mL in methylene chloride.
— Without removing the concentrator tube from the bath, add 10 mL of hexane and concentrate the extract again to 1 mL under a gentle stream of nitrogen. Repeat this step as many times as necessary until all the methylene chloride is replaced by hexane, at which point the liquid stops boiling.
— Quantitatively transfer the liquid to chromatography vials, taking care to rinse the concentrator tube 2–3 times with small amounts of hexane, and add the appropriate internal standards to all the samples, including the quality control samples (see Annexes).
— Add hexane or allow any excess solvent to evaporate under the hood in order to adjust the amount to approximately 1 mL and proceed with instrumental analysis.

III.9.4. Instrumental analysis

The concepts discussed in the following sections are of a general nature and apply to the analysis of aliphatic and total hydrocarbons using gas chromatography coupled with a flame ionization detector, the determination of chlorinated pesticides using gas chromatography coupled with an electron capture detector, and the determination of PAHs using gas chromatography coupled with a mass spectrometer. The only exception concerns checking for degradation of chlorinated pesticides in the injection port.

III.9.4.1. Detection limits

Method detection limits for routine analyses should be determined before starting such analyses, following the procedures set out in the Federal Register (1984), as summarized below, or similar procedures. Otherwise, the concentrations of the lowest-level calibration solution used for the analyses can be used, after adjusting for the weight of sample extracted.

Definition and procedures for the determination of method detection limits

Definition

The method detection limit (MDL) is defined as the lowest measurable concentration of a substance that can be reported with 99% confidence as being non-zero. The MDL should be determined based on the analysis of a specific matrix containing the analyte in question.

Application

This method is designed for use with a variety of samples, ranging from the method blank to various matrices. The MDL for a well-defined analytical method may vary according to the type of sample; it is essential that all steps specified in the analytical method be included when determining the MDL.

Procedure

1. Estimate the MDL as the concentration corresponding to an instrument signal:noise ratio between 3 and 5.
2. Before determining the MDL, analyse the sample to determine the native concentration of the analyte being studied.
   - ✓ If the concentration is in the range of 2–5 times the estimated detection limit, proceed with the study as indicated below.
   - ✓ If the presence of the analyte is not detected, proceed with the study as indicated below.
   - ✓ If the concentration exceeds the maximum value in the recommended range, obtain a sample of the same matrix from a less polluted or unpolluted area and do the analysis again.
3. Once the native concentration of the analyte has been determined, analyse a minimum of seven aliquots (spike appropriately if the analyte being studied was not detected in the sample, or analyse without spiking if the analyte concentration is within the recommended range) and process according to the analytical method. Include a method blank in the analytical batch.
   - ✓ If this analysis confirms that the concentration of the analyte is within the recommended range, continue with the calculations.
   - ✓ If the analysis indicates that the concentration of the analyte is outside the recommended range, obtain a new sample and repeat steps 2–3.
4. Calculate the variance (S) and standard deviation (s) of the replicate measurements as follows:

$$S^2 = \frac{1}{(n-1)}\left[\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2 / n\right] \quad \text{(III-38)}$$

$$s = \sqrt{S^2} \quad \text{(II-39)}$$

where

X_i, i=1 to n are the final analytical results for the aliquots analysed;
Σ is the sum of the X values for i = 1 to n.

5. Calculate the MDL as follows:

$$LDM = t_{(n-1,\ 1-\alpha = 0.99)}\ (s) \quad \text{(III-40)}$$

where

MDL is the method detection limit;
$t_{(n-1,\ 1-\alpha\ =0.99)}$ is the student's *t*-value appropriate for a 99% confidence level and a standard deviation with n-1 degrees of freedom;
s is the standard deviation of the replicate analyses.

6. The 95% confidence interval for the MDL calculated in step 5 above is calculated using the following equations derived from the Chi-squared distribution for the degrees of freedom.

$$\text{Lower confidence limit} = 0.64\ \text{LDM} \quad \text{(III-41)}$$

$$\text{Upper confidence limit} = 2.20\ \text{LDM} \quad \text{(III-42)}$$

Alternatively, the concentrations of the lowest-level calibration solution used in the analyses, adjusted for the weight of sample extracted, can be used to estimate the detection limits. For example, for the measurement of chlorinated pesticides using gas chromatography coupled with an electron capture detector, a range of calibration concentrations between 200 ng/mL and 5 ng/mL is recommended (see information in Annexes). If the weight of the sample extracted is 10 g and it has been concentrated to a final volume of 1 mL, the estimated detection limit is:

$$\text{MDL}_{\text{estimated}} = \frac{C_{\text{min}}}{W_{\text{sam}}} = \frac{5\ \text{ng/mL}}{10\ \text{g/mL}} = 0.5\ \text{ng/g} \quad \text{(III-43)}$$

where

$\text{MDL}_{\text{estimated}}$ is the estimated method detection limit;
C_{min} is the concentration of the lowest-level calibration solution;
W_{sam} is the weight of sample extracted.

III.9.4.2. Analytical quality control

The quality controls required for the quantitative analysis of target compounds are summarized in Table III-1 and discussed in detail below. These criteria can be modified as necessary at the discretion of the laboratory management.

III.9.4.3. Initial calibration of the instrument

All instruments must be calibrated prior to sample analysis. In the case of chlorinated pesticide analysis, it is best to build the calibration curve with a minimum of four calibration levels, using a quadratic or exponential equation to compensate for the limited linear range of the electron capture detector. The calibration is considered acceptable if it has a correlation coefficient (r) $\geq$ 0.9950 (i.e. $R^2 \geq 0.9900$) for all analytes present in the solutions. If this criterion is not met for a particular analyte and the analyte is present in the samples, the calibration must be performed again before the samples are analysed. When analysing aliphatic and total hydrocarbons or PAHs using, respectively, a flame ionization detector or mass spectrometer — which have a much more stable and linear response than the electron capture detector — it is also possible to perform, in addition to the above calibrations, a calibration based on the relative response factors obtained for the calibration solutions in order to demonstrate the linearity of the detector used.

III.9.4.4. Verifying that the initial calibration remains valid

The initial instrument calibration can remain valid for days or weeks, but this validity must be verified before starting work on each analytical batch and then at least once every 12 hours by injecting a solution with concentrations within the calibration range.

TABLE III-1. QUALITY CONTROL REQUIREMENTS FOR THE QUANTITATIVE ANALYSIS OF ORGANIC POLLUTANTS

Parameter	Quality control criterion	Frequency
— Initial calibration of the instrument (using relative response factors)	Minimum of four calibration solutions, where the relative percent difference between response factors does not exceed ±15%	Prior to the analysis of an analytical batch
— Initial calibration of the instrument (using a quadratic or exponential equation)	Minimum of 4 calibration solutions, with a correlation coefficient ≥0.990	Prior to the analysis of an analytical batch
— Verifying that the initial calibration remains valid	Calibration control; error less than ±25% of the nominal concentrations	After the initial calibration and at least once every 12 hours
— Qualitative identification of the target compounds (GC–ECD and GC–FID)	Retention times within ±4 seconds of the reference times	All samples
— Qualitative identification of the target compounds (GC–MS)	Retention times within ±4 seconds of the reference times Difference between retention times for selected ions not exceeding ±2 seconds	All samples
— Instrument blank	Instruments free of contamination	Before calibration (or calibration control) and before starting sample analysis
— Checking for degradation within the system (pesticide analysis)	Degradation of more labile compounds not exceeding 15%	Before calibration (or calibration control) and before starting sample analysis
— Surrogate standard recovery	Recovery between 40% and 120% for all surrogate standards	All samples
— Laboratory blank	No more than two analytes detected at concentrations more than 3 times the MDL	One per analytical batch
— Duplicate sample	Relative percent difference <30% for all target analytes present at concentrations more than 10 times the method detection limit	One per analytical batch
— Spiked laboratory blank — Duplicate spiked laboratory blank	40–120% recovery for 80% of target analytes Relative percent difference <30% for all target analytes	One per analytical batch Can replace spiked sample and duplicate spiked sample if insufficient material is available
— Spiked sample — Duplicate spiked sample	40–120% recovery for 80% of target analytes Relative percent difference <30% for all target analytes	One per analytical batch Can be replaced by spiked blank and duplicate spiked blank if insufficient material is available
— Certified reference material	Recovery of target analytes present at concentrations more than 10 times the MDL is within the certified range	One per analytical batch if available. Can be replaced by a spiked sample

III.9.4.5. Analytical sequence

If the batch of samples put through extraction by the laboratory includes, for example, 16 original samples in addition to the quality control samples (blank, duplicate sample, spiked blank, spiked sample, duplicate spiked sample, reference material), the instrumental analytical sequence could be as follows:

Analytical sequence		
Position in sequence	I.D.	Description
1	W1000	Solvent
2	W1001	Calibration solution 1
3	W1002	Calibration solution 2
4	W1003	Calibration solution 4
5	W1004	Calibration solution 5
6	W1005	Calibration solution 3 (control)
7	Q3645	Blank
8	Q3646	Spiked blank
9	Q3647	Duplicate sample
10	Q3648	Spiked sample
11	Q3649	Duplicate spiked sample
12	Q3650	Certified reference material
13	K1518	Sample 1
14	W1006	Calibration solution 3 (control)
15	K1519	Sample 2
16	K1522	Sample 3
17	K1523	Sample 4
18	K1525	Sample 5
19	K1526	Sample 6
20	K1527	Sample 7
21	K1540	Sample 8
22	K1541	Sample 9
23	W1007	Calibration solution 3 (control)
24	K1542	Sample 10
25	K1543	Sample 11
26	K1544	Sample 12
27	K1545	Sample 13
28	K1546	Sample 14
29	K1547	Sample 15
30	K1548	Sample 16
31	W1008	Calibration solution 3 (control)

Note that in this example, four levels of calibration solutions are used. The intermediate-level solution (i.e. calibration solution 3) is a separate solution used to check the validity of the initial calibration and check that it remains valid during the analytical run.

III.9.4.6. Qualitative identification of the target compounds

Qualitative identification of an analyte in the sample extract using gas chromatography is based on comparing the retention time for the analyte with the average retention time observed for that compound in the calibration solutions. The difference between the two retention times must not exceed ±4 seconds. The experienced analyst must use manual peak selection and baseline correction where appropriate and adjust the size of the retention time window to ensure that all compounds are correctly identified. The intensity of the selected peak must be at least 3 times the noise level.

It is important to note that, for the qualitative identification of an analyte using gas chromatography–mass spectrometry, in addition to the above-mentioned ±4 second requirement for retention times, the difference between the retention times for the quantification and confirmation ions selected must be less than ±2 seconds.

III.9.4.7. Instrument blank

The instrument blank (or solvent blank) is injected and analysed before each analytical sequence to check the functioning of the chromatograph and verify the absence of contamination in the system. The analyses must not be initiated until the instrument blank is completely free from contaminants. Should issues arise with the instrument blank, corrective action must be taken, e.g. replacement of the syringe; repeated injections of solvent; cleaning of the injection port and replacement of the glass liner; removal of part of the chromatography column; or replacement of the column.

III.9.4.8. Checking for degradation within the system (pesticide analysis)

After checking that the instrument is free of contaminants, the system must be checked to be sure it has no active sites that could degrade the more labile compounds during the analysis of chlorinated pesticides. A solution of selected compounds (e.g. DDT and aldrin, which are known to degrade in the injection port under certain conditions) is used to check whether the system is inert. For the system to be considered ready to start the analytical run, degradation of these compounds in the injection port must not exceed 15% of the injected amount. If degradation of any of these compounds is greater than 15%, the problem must be corrected before starting the analyses. This could be done by replacing the glass liner and lower seal of the injection port or by removing part of the column.

III.9.4.9. Surrogate standard recovery

All samples, including those introduced by the laboratory for quality control purpose, are spiked with suitable amounts of the appropriate surrogate standards for the analysis in question. These standards are used to determine the concentrations of the target analytes and to evaluate the effectiveness of the method. This is done using one or more internal standards as a reference. Different types of analysis may require different surrogate and internal standards, and the acceptable recovery limits for surrogate standards are established when the method is developed.

- In general, recovery levels of standards used in the routine analysis of organic pollutants should be between 40% and 120% of the spike amount. If this is not the case, check the calculations and look for possible interference with either of the two types of standards (surrogate and internal), errors in spiking, problems with the spike solutions, etc. In the case of recoveries consistently above 120%, it must be assumed that the solution(s) could have become concentrated owing to evaporation of the solvent.
- If a standard used to determine the concentrations of target analytes in one or more samples has recoveries exceeding 120%, and if the same standard used in the calibration solutions and in the majority of the samples in the analytical batch is ‘in control’, it must be assumed that interferences are involved. If possible, it is useful to have more than one surrogate standard on hand in order to avoid this issue.
- If the cause of recovery outside the limits is not identified, the extract must be re-analysed. If re-analysis yields a recovery within the established limits, the sample data can be reported; otherwise, the sample must be returned to the laboratory for another cleanup or for re-extraction. If the problem persists, the sample is to be reported with the appropriate note.

III.9.4.10. Laboratory blank

A laboratory blank (method blank) is used to demonstrate the absence of contamination issues in the laboratory during the processing of the analytical batch. A method blank is required for each batch of samples that are processed at the same time.

- An acceptable blank should contain no more than two target analytes at concentrations more than 3 times the established method detection limits (MDL). If the blank contains more than two target analytes at concentrations more than 3 times the MDLs, it may be necessary to redo the extraction for all the samples.
- If a target analyte detected in the laboratory blank at a concentration more than 3 times the detection limit but is not detected in the samples at concentrations above the detection limit, the analytical data for the blank is to be reported as ‘3>MDL’ and analysis and reporting may continue.

— If a target analyte is detected in the laboratory blank at a concentration more than 3 times the detection limit and the concentration of the same compound is 10 times higher in the samples than in the blank, the value for the blank is reported as '3>MDL' and the data for the samples are also reported, because the contamination found in the laboratory blank is considered to be relatively insignificant.
— If a target analyte is detected in the laboratory blank at a concentration more than 3 times the detection limit and the concentration of the same compound in the samples is less than 10 times the concentration in the blank, the samples from that analytical batch must undergo re-extraction re-analysis. If there is insufficient sample material to do so, the concentrations observed can be reported but the analyte in the blank is to be reported as '3>MDL' and in the samples as 'B' (contaminated laboratory blank).

III.9.4.11. Duplicate sample

Duplicate samples are analysed to assess the degree of sample homogeneity and, together with the original sample, the analytical precision. There needs to be one duplicate sample in each analytical batch. The relative differences in percentage terms between the analyte concentrations observed in the original sample and those in the duplicate sample are expected to be less than 25%. If the relative percent difference is greater than that for more than 20% of the analytes detected at concentrations more than 10 times the detection limit, corrective action is required. This may include a review of chromatogram peak integrations and identities, a review of the calculations, re-injection and re-analysis of both samples, maintenance and/or re-calibration of the equipment or re-extraction of the analytical batch.

III.9.4.12. Spiked laboratory blank and duplicate spiked laboratory blank

The spiked laboratory blank is used to assess the analytical precision of the method. It can replace the spiked sample when there is insufficient matrix material to produce a spiked sample. The duplicate spiked laboratory blank is used to assess analytical precision and, together with the original spiked laboratory blank, analytical accuracy. There needs to be one spiked laboratory blank in each analytical batch.

— For the analysis of a spiked laboratory blank to be acceptable, 80% of the target analytes must have recoveries of between 40% and 120% of the spike amount.
— If this criterion is not met, corrective action must be taken. This may include recalculation and/or re-analysis, re-extraction for the group of samples or equipment recalibration or maintenance.
— If the analytical batch includes a duplicate spiked laboratory blank, the relative percent difference (RPD) between recoveries of analytes in this and the spiked laboratory blank must not exceed 30%.

III.9.4.13. Spiked sample and duplicate spiked sample

The spiked sample is analysed to assess analytical accuracy in the presence of the matrix. The duplicate spiked sample is used to assess analytical precision and, together with the original spiked sample, analytical accuracy. There needs to be one spiked sample in each analytical batch. When there is not enough matrix material to produce a spiked sample, the spiked laboratory blank may be used instead.

- For the analysis of a spiked sample to be acceptable, 80% of the target analytes must have recoveries of between 40% and 120% of the spike amount. The recovery of one or more analytes could be invalidated by high concentrations naturally present in the sample (native concentrations). In this case, only valid recoveries should be used in the calculation. If most or all of the analytes present this problem, the sample selected to be spiked should be considered invalid and the analysis of the analytical batch continued.
- If native concentrations are not an issue but more than 20% of the target analytes have recoveries outside the established limits, consideration must be given to reviewing the chromatogram peak integrations and identities, reviewing the calculations or re-analysing the extract. If the quality control criterion is still not met, a decision must be taken to redo the extraction for the samples.
- If the analytical batch includes a duplicate spiked sample, the relative percent difference (RPD) between recoveries of analytes added to this and the spiked sample must not exceed 30%.

III.9.4.14. Certified reference material

A certified reference material is analysed to assess the analytical precision and accuracy of the analytical method. If no certified reference material is available, the spiked laboratory blank may be used for this purpose. One certified reference material must be analysed as part of each analytical batch.

- For the analysis of a certified reference material to be acceptable, 80% of the target analytes present in the material at concentrations greater than 10 times the MDL should be within the certified concentration ranges.
- If the above quality control criterion is not met, the chromatogram peak integrations and identities should be reviewed, the calculations checked or the extract re-injected. If it is still not met, a decision must be taken to redo the extraction for the samples.

III.9.4.15. Evaluating analytical quality control parameters

Surrogate standard recovery: Recovery rates for surrogate standards in sample extracts are assessed by comparing the relative response of the surrogate standard in the sample with that observed in the calibration solutions. The laboratory must take appropriate corrective action whenever surrogate standard recovery is outside the target range (see Analytical quality control, Surrogate standard recovery). As a rule, a recovery is considered acceptable if it is between 40% and 120%, calculated as follows:

$$\text{Standards recovery (\%)} = \frac{\mathrm{A}_{\mathrm{sur/sam}}}{\mathrm{A}_{\mathrm{int/sam}}} \times \frac{\overline{\mathrm{A}}_{\mathrm{int/cal}}}{\overline{\mathrm{A}}_{\mathrm{sur/cal}}} \times 100 \qquad \text{(III-44)}$$

where

$A_{sur/sam}$ is the area for the surrogate standard in the sample;
$A_{int/sam}$ is the area for the internal standard in the sample;
$\bar{A}_{sur/cal}$ is the average area for the surrogate standard in the calibration solutions;
$\bar{A}_{int/ccal}$ is the average area for the internal standard in the calibration solutions.

This assumes that the concentrations of surrogate and internal standards in the calibration solutions are equal to those added to the samples. If the concentrations differ:

$$\text{Standards recovery (\%)} = \frac{(A_{sur/sam}/C_{sur/sam})}{(A_{int/sam}/C_{int/sam})} \times \frac{(\bar{A}_{int/cal}/C_{int/cal})}{(\bar{A}_{sur/cal}/C_{sur/cal})} \times 100 \quad \text{(III-45)}$$

where

$C_{sur/sam}$ is the concentration of the surrogate standard in the sample;
$C_{int/sam}$ is the concentration of the internal standard in the sample;
$C_{sur/cal}$ is the concentration of the surrogate standard in the calibration solutions;
$C_{int/cal}$ is the concentration of the internal standard in the calibration solutions.

Original Sample *versus* Duplicate Sample — Relative Percent Difference

The concentrations observed in the original sample and those in the corresponding duplicate sample must be comparable in order to certify sample homogeneity and analytical precision (see Analytical quality control, Duplicate sample). If the quality control requirements are not met, the laboratory must take the necessary corrective action.

$$\text{Relative Percent Difference (RPD)} = \left[\frac{(C_{orig} - C_{dup})}{\left(\frac{C_{orig} + C_{dup}}{2}\right)}\right] \times 100 \quad \text{(III-46)}$$

where

C_{orig} is the concentration in the original sample;
C_{dup} is the concentration in the duplicate sample.

Recovery of target analytes in spiked samples and certified reference materials: good recovery of analytes added to spiked samples and the duplication of certified concentrations in reference material are indicative of a good laboratory technique. The percent recovery of the various analytes added to the spiked samples is calculated using the following equation:

$$\text{Percent recovery} = \left[\frac{(C_{spiked} \times S_{spiked} - C_{orig} \times S_{orig})}{A_{added}}\right] \times 100 \quad \text{(III-47)}$$

where

C_{spiked} is the concentration in the spiked sample;
C_{orig} is the concentration in the original sample;
S_{spiked} is the size of the spiked sample;
S_{orig} is the size of the original sample;
A_{added} is the concentration of the analyte added to the spiked sample.

Calculate and report the percent recovery of all target analytes added to the spiked samples and all compounds with certified concentrations in the reference materials. Note that, by definition, C_{os} in the enriched laboratory blank and its duplicate is zero. If the quality control requirements are not met, the laboratory must take the necessary corrective action (see Analytical quality control, Spiked sample and duplicate spiked sample and Certified reference material).

III.9.5. Quantitative determination of chlorinated pesticides using gas chromatography coupled with an electron capture detector

III.9.5.1. Introduction

Chlorinated pesticides are routinely quantified using gas chromatography coupled with an electron capture detector (GC–ECD). This technique is very sensitive and is capable of determining these compounds at trace levels (e.g. ng/g, or pg/g). The method described can be applied to sediment sample extracts after appropriate cleanup.

The target chlorinated pesticides for this study are limited to the following:

Chlorinated pesticides	Aldrin	Endosulfan I	beta-HCH
	Alpha-chlordane	Endosulfan II	delta-HCH
	Dieldrin	Endosulfan	gamma-HCH (lindane)
	4,4'-DDD	sulphate	Heptachlor
	4,4'-DDE	Endrin aldehyde	Heptachlor epoxide
	4,4'-DDT	Endrin ketone	Methoxychlor
	Endrin	alpha-HCH	
Additional chlorinated pesticides	gamma-Chlordane	trans-Nonachlor	2,4'-DDE
	cis-Nonachlor	2,4'-DDD	2,4'-DDT

III.9.5.2. Equipment and materials

Gas chromatograph coupled with an electron capture detector

The gas chromatograph must have a split/splitless injector and be suitable for use with a capillary column located inside a programmable oven and coupled with a standard or micro electron capture detector.

The column commonly used for pesticide analysis is a capillary column 30 m long with a 0.25-mm inside diameter and a 0.25-µm thick DB-5 liquid phase. Other columns with similar characteristics can be used. It is best to use an autosampler capable of making 1–4 µL injections. A second column with a different polarity (DB-17ht or equivalent) can be used for confirmation of the target compounds. The programme used for the analysis of pesticides using gas chromatography–electron capture is as follows:

- Injector temperature: 275°C
- Detector temperature: 325°C
- Oven temperature programme:
  - Initial temperature: 100°C, held for 1 min
  - 1st heating ramp 5.0°C/min
  - 1st ramp final temperature 140ºC, held for 1 min
  - 2nd heating ramp 1.5°C/min
  - 2nd ramp final temperature 250°C, held for 1 min
  - 3rd heating ramp 10°C/min
  - 3rd ramp final temperature 300°C, held for 5 min
- Carrier gas Helium at ~1.5 mL/min
- Make-up gas Argon:methane (95:5) at ~40 mL/min
- Total time 94.3 min

These conditions can be modified to improve the chromatographic profile, if necessary.

III.9.5.3. Calibrating the chromatograph

The gas chromatograph used for pesticide analysis must be calibrated using one of the methods listed above (see Quality control, Calibrating the instrument). Due to the limited linearity of the electron capture detector, it is recommended to calibrate the instrument using a quadratic or exponential equation.

III.9.5.4. Chromatographic analysis of the samples

As mentioned above, the quality control samples (blank, duplicate sample, spiked sample and reference material) and the samples making up the analytical batch are analysed as a single analytical sequence along with the calibration solutions or calibration control solutions (see Analytical quality control, Initial calibration of the instrument and Verifying that the initial calibration remains valid). Before initiating the analytical sequence, 1 or 2 µL of hexane must be injected (see Quality control, Instrument blank) followed by a similar volume of degradation control solution (see Quality control, Checking for degradation within the system), to check that the system is free of contaminants and that there is no observable degradation of the more labile compounds in the injection port. To analyse the samples, the extracts are injected in amounts of 1–4 µL.

It is important to stress that the quality control samples in an analytical batch must be evaluated as a set, as the failure of one of these samples to meet the established criteria does not necessarily mean that all the samples in the extraction in question must be rejected.

II.9.5.5. Checking for degradation

A solution containing compounds such as DDT and aldrin, which are known to degrade easily in the injection port under certain conditions, is used to check the system for active sites that could degrade the more labile analytes (see Quality control, Checking for degradation within the system). This solution is prepared by weighing out the appropriate amounts of pure compounds, quantitatively transferring them to a volumetric flask and diluting them with hexane or a similar non-chlorinated solvent to achieve concentrations approximately in the middle of the calibration curve. In its final, ready-to-use form, the solution must contain suitable amounts of surrogate and internal standards.

Checking for degradation — Step by step

— DDT and endrin can easily degrade in the chromatograph injection port if the port or the beginning of the column is soiled. This soiling results from the accumulation of high boiling point residues from previously analysed samples.
— Prepare a solution of 4,4'-DDT and endrin of a known concentration that is in the central region of the calibration curve.
— Inject 2 µL of this solution and analyse using the same method that will be used to analyse the sample extracts.
— Record the presence and amounts of the degradation products of 4,4'-DDT (4,4'-DDE and 4,4'-DDD) and endrin (endrin ketone and endrin aldehyde).
— Calculate the degradation percentage using the following formulas:

$$\%\ \text{Degradation 4,4'-DDT} = \left(\frac{(\text{Area 4,4'-DDE} + \text{Area 4,4'-DDD})}{(\text{Area 4,4'-DDT} + \text{Area 4,4'-DDE} + \text{Area 4,4'-DDD})}\right) \times 100 \quad \text{(III-48)}$$

$$\%\ \text{Degradation endrin} = \left(\frac{(\text{Area endrin ketone} + \text{Area endrin aldehyde})}{(\text{Area endrin} + \text{Area endrin ketone} + \text{Area endrin aldehyde})}\right) \times 100 \quad \text{(III-49)}$$

— If the degradation of either analyte is greater than 15%, the problem needs to be corrected before proceeding with calibration and sample extract analysis.

III.9.5.6. Qualitative identification of the target analytes

To identify a peak as corresponding to a target analyte, the criterion discussed above must be met (see Quality control, Qualitative identification of target compounds). Fig. III-3 shows the elution order and relative responses of the chlorinated insecticides, surrogate standards and internal standard; the concentration of each analyte is 100 ng/mL.

III.9.5.7. Initial calibration, verifying the calibration and quantitative determination of the target analytes

Because the electron capture detector is only slightly linear in behaviour, it is best to calibrate the chromatograph using a curve fitting method that compensates for this behaviour. This means that curve fitting methods that use quadratic or exponential equations are generally more robust than fitting by linear regression or the use of relative response factors.

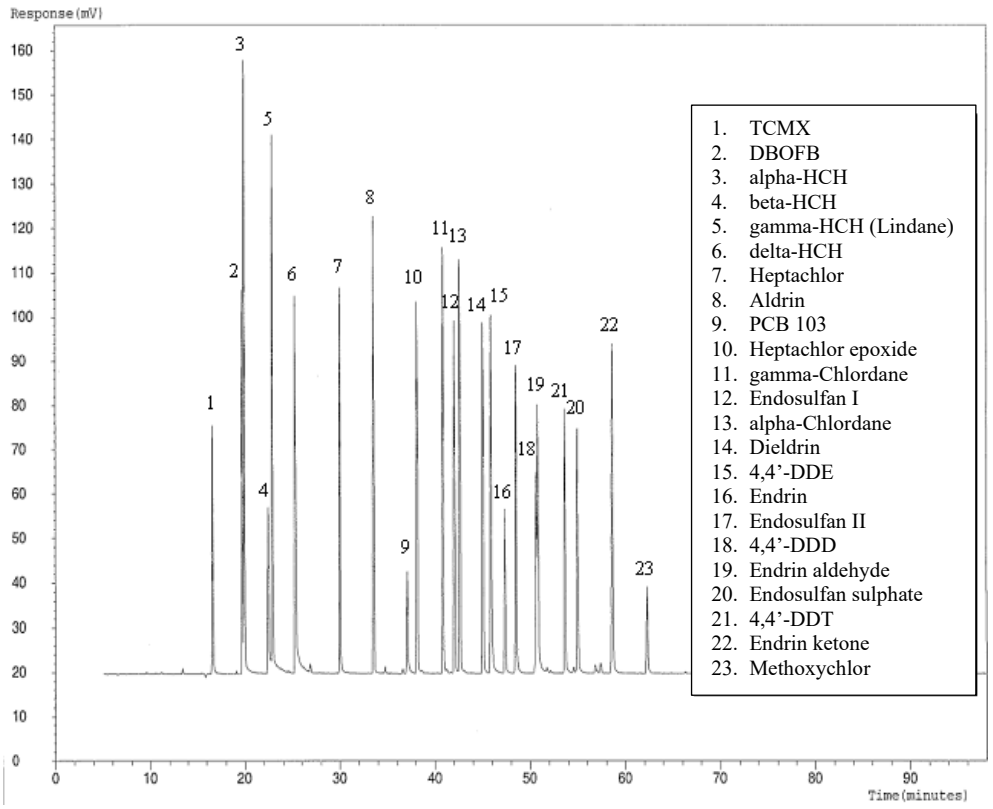


FIG. III-3. Chlorinated insecticides analysed using a gas chromatograph coupled with an electron capture detector.

Quadratic equation

The calibration curve is prepared using the data obtained by injecting the various calibration solutions. Curve fitting can be performed using an equation of the form:

$$Y = b_0 + b_1 x + b_2 x^2 \qquad \text{(III-50)}$$

where:

x is the amount of target analyte in ng;

b_0, b_1, b_2 are the coefficients of the quadratic equation. If the equation is forced through zero, then $b_0 = 0$.

Y is the modified area for the target analyte, defined as:

$$Y = \left(\frac{A_{ana}}{A_{sur}}\right) \times M_{sur} \qquad \text{(III-51)}$$

where:

A_{ana} is the area for the compound;

A_{sur} is the area for the surrogate standard;

M_{sur} is the mass of surrogate standard in ng.

For chromatographic peaks that meet the qualitative identification criterion, see Analytical quality control, Qualitative identification of target compounds.

Exponential equation

As an alternative to the quadratic equation, one can use an exponential equation of the form:

$$Y = A\ X^B \tag{III-52}$$

where

Y is the relative concentration (ratio of C_{ana} to C_{sur});
X is the relative area (ratio of A_{ana} to A_{sur});
A is the slope;
B is the exponent.

III.9.5.8. Initial calibration

The initial calibration is considered acceptable if the RSD (in %) of the response factors for each compound in the calibration solutions is no greater than ±15%.

Verifying the initial calibration

The initial calibration must be checked every 10–12 samples, or at least once every 12 hours, by injecting a specially prepared solution or re-injecting one of the intermediate-level calibration solutions. If the concentrations determined for this solution differ from the nominal value by no more than ± 25 %, the initial calibration is considered to be still acceptable.

III.9.5.9. Quantitative determination of the target analytes

Based on the data obtained by injecting the various calibration solutions, the coefficients satisfying the chosen fitting equation are calculated and are used to calculate the concentrations of the respective target analytes in the samples. The solution to the quadratic equation (Eq. III-5):

$$x = \frac{-b_1 + \sqrt{b_1^2 - 4b_2(b_0 - Y)}}{2b_2} \tag{III-53}$$

will give the amount of each target analyte in the analysed extract. To determine the concentration of the compound in the sample, the value obtained must be divided by the weight of sample extracted in order to express the concentration in terms of ng/g.

When using an exponential equation (Eq. III-52) for calibration, it is possible to write it as:

$$\frac{C_{ana}}{C_{sur}} = A\left(\frac{A_{ana}}{A_{sur}}\right)^B \tag{III-54}$$

where

C_{ana} is the concentration of the analyte (ng/mL);
C_{sur} is the concentration of the surrogate standard used (ng/mL);
A_{ana} is the area for the analyte being measured;
A_{sur} is the area for the surrogate standard used.

Because C_{ana} and C_{sur} refer to the concentrations of analyte and surrogate standard present in the same volume of extract, both can be replaced with their respective quantities. By rearranging the terms of the equation and dividing both sides by the amount of sample extracted, the concentration of the target analyte in ng/g can be expressed as follows:

$$[\text{Analyte}]_{(\text{ng g}^{-1})} = A\left(\frac{A_{ana}}{A_{sur}}\right)^{B} \times \frac{M_{sur}}{M_{sam}} \quad \text{(III-55)}$$

where

A is the slope;
B is the exponent;
A_{ana} is the area for the analyte being measured;
A_{sur} is the area for the surrogate standard used;
M_{sur} is the amount, in ng/g, of surrogate standard added to the sample prior to extraction;
M_{sam} is the sample size in g.

III.9.5.10. Quality control for calculations

The concentrations of the target analytes are determined on the basis of the response factors of these compounds in the calibration solutions. In general, modern chromatography systems have software that allows the calculations to be performed almost automatically. However, it is important to understand the concepts involved in these calculations in order to be able to reproduce, manually and as an additional means of quality control, some of the results produced by the software. It is advisable to corroborate the results using independent calculations. Several randomly selected concentrations must be confirmed in a commercial spreadsheet (Excel or similar) using Eq. (53) or Eq. (55), for example, depending on the type of calibration performed, and compared with the reported values. The two concentrations should match exactly, allowing for rounding errors.

Example of a concentration calculation

The following information is compiled from data in the laboratory log book and the printout of the analysis produced using the gas chromatograph software. To provide a general example, the analyte, surrogate standard and internal standard have not been identified:

Sample code	F99999
Weight of the sample:	5.211 g
Mass of surrogate standard added:	90.8 ng
Final volume:	1.0 mL
concentration of surrogate standard (C_{sur})	0.0000908 µg/mL
Calibration curve coefficients:	b0 = 0.0000 b1 = 0.4814 b2 = -205.9000
Area for surrogate standard (A_{sur}):	228.95
Area for analyte 'A' (A_{ana})	24.211
Value calculated by the software	3.863 ng/g

Using the following equation:

$$x = \frac{-b_1 + \sqrt{b_1^2 - 4b_2(b_0 - Y)}}{2b_2} \quad \text{(III-56)}$$

where:

$$Y = \left(\frac{A_{ana}}{A_{sur}}\right) \times C_{sur} \quad \text{(III-57)}$$

Manually calculating the concentration of analyte 'A'

$$Y = \left(\frac{24.211}{228.95}\right) \times 0.0000908\ \mu g\ \mu L^{-1} = 0.000009602\ \mu g\ \mu L^{-1} \quad \text{(III-58)}$$

$$[\text{Analyte A}] = \frac{-0.4814 + \sqrt{0{,}4814^2 - 4\,(-205.9)(0 - 0{,}000009602)}}{2\,(-205.9)} = 0.000020119\ \mu g\ \mu L^{-1} \quad \text{(III-59)}$$

$$[\text{Analyte A}] = \frac{0.000020119\ \mu g\ \mu L^{-1} \times 1000\ ng\ \mu g^{-1} \times 1000\ \mu L\ mL^{-1}}{5.211\ g} = 3.861\ ng\ g^{-1} \quad \text{(III-60)}$$

The value obtained using the gas chromatograph software and the manually obtained value should match exactly, allowing for rounding errors.

$$\text{Relative percent difference} = \left[\frac{(3.863\ ng\ g^{-1} - 3.861\ ng\ g^{-1})}{\left(\frac{3.863\ ng\ g^{-1} + 3.861\ ng\ g^{-1}}{2}\right)}\right] * 100 = 0.05\% \quad \text{(III-61)}$$

III.9.5.11. Diluting an extract

If the instrument response to an analyte exceeds the response observed for the same compound in the most concentrated calibration solution, the extract needs to be diluted accordingly. To obtain a relative response (the ratio of the response for the compound to the response for the corresponding surrogate standard) that is within the range of concentrations used to calibrate the chromatograph, further surrogate standards are added before re-analysing the sample.

Diluting an extract — Step by step

— Before diluting the extract, make sure that its original volume is exactly 1 mL, disregarding the amount injected into the chromatograph (i.e. 1 or 2 µL).
— Depending on the required dilution, transfer an appropriate exact volume of the original extract into a new vial. For example, if the sample needs to be diluted tenfold, take exactly 0.100 mL and dilute to approximately 1 mL (see examples below).

Required dilution	Original volume of extract	Volume of surrogate standard solution	Volume of solvent (2)	Final volume(2)
5x	0.200 mL	0.100 mL	~ 0.700 mL	~ 1 mL
10x	0.100 mL	0.100 mL	~ 0.800 mL	~ 1 mL
20x	0.050 mL(1)	0.100 mL	~ 0.850 mL	~ 1 mL
50x	0.020 mL(1)	0.100 mL	~ 0.880 mL	~ 1 mL
100x	0.010 mL(1)	0.100 mL	~ 0.890 mL	~ 1 mL
500x	0.002 mL(1)	0.100 mL	~ 0.898 mL	~ 1 mL

(1) It is advisable to perform an intermediate dilution.
(2) The use of surrogate standards to determine the concentration of the target analytes results in internal compensation and it is not necessary to bring the final volume to exactly 1 mL.

— Add 0.100 mL of the surrogate standard solution.
— Make up to approximately 1 mL with hexane or a similar non-chlorinated solvent and re-inject into the chromatograph for analysis.
— One must analyse the diluted extract only in order to determine the concentration of those analytes that presented concentrations exceeding the highest calibration level.

III.9.5.12. Maintenance of the gas chromatograph and electron capture detector

There are a number of simple steps that can be taken to help keep the gas chromatograph and associated electron capture detector in good working order:

— The syringe must be rinsed with the appropriate solvent after each injection. This can be done manually or using the autosampler.
— The injection of extracts containing significant residual amounts of chlorinated solvents (e.g. methylene chloride) or high levels of sulphur (e.g. anoxic sediments) should be avoided.
— The injection port seal (septum) is to be replaced after a number of injections, depending on the condition of the syringe needle, to avoid leaks and variations in retention times.
— A new glass liner is to be installed as soon as there are signs of degradation of the more labile compounds. If a significant increase in analyte degradation percentage is observed in the degradation control solution, it is advisable to replace the liner before reaching the 15% action threshold.
— Sometimes it is necessary, in addition to replacing the glass liner, to replace the lower injection port seal (gold seal) in order to avoid degradation or peak tailing.
— If, after replacing the insert and seal, one continues to observe degradation, tailing or a reduced response to the target analytes, the first two coils of the capillary column (the end connected to the injection port) need to be removed.
— The tanks of carrier gases (helium) and auxiliary gases (argon:methane or nitrogen) must be replaced before they are empty to avoid any soiling that could increase the base noise level or contaminate the column and/or detector. As a rule, it is best to replace the tank when the pressure drops below 500 psi.
— All maintenance performed on the gas chromatograph or electron capture detector, including gas tank replacements, is to be recorded in a dedicated logbook for the specific piece of equipment.

III.9.5.13. Documentation

When samples are received for instrumental analysis, the analyst must also receive a properly labelled folder containing copies of the laboratory log sheets for the relevant analytical batch and information documenting the processing of the samples, any problems encountered or accidents that may have occurred and/or any relevant comments (e.g. possible double addition of surrogate standards, sample losses due to spillage, evaporation of the extract to dryness, etc.). The folder must also contain, if available, documentation of the initiation or request for the analysis, identifying the person responsible for the project who can answer any questions (e.g. type of analysis to be performed, amount of sample to undergo extraction, amounts of surrogate and internal standards to be used in spiking and a final report on the concentrations).

Upon performing the instrumental analysis, the analyst must add to the folder a copy of the analytical sequence, information regarding the instrument blank, the degradation control solution and calibration of the instrument, printouts of the chromatograms and analyses thereof, and a copy of the final report. This final report must include, in addition to the results for the samples analysed, information regarding the quality control samples (laboratory blank, duplicate sample, spiked sample and reference material) and their parameters (surrogate standard recovery, relative percent difference between originals and duplicates, recovery of target analytes in blanks and spiked samples, duplication of certified results for the reference material).

III.9.5.14. Reporting concentrations

Concentrations are generally reported as ng/g dry weight to three decimal places.

III.9.6 Quantitative determination of aliphatic and total hydrocarbons using gas chromatography coupled with a flame ionization detector

III.9.6.1. Introduction

The normal alkanes, n-C10 to n-C35, plus pristane and phytane, are quantitatively determined using a gas chromatograph coupled with a flame ionization detector (GC–FID). This technique is very sensitive and is capable of determining these pollutant compounds at trace levels (e.g. ng/g). The method described can be applied to sediment sample extracts after appropriate cleanup.

The target aliphatic hydrocarbons for this study are limited to the following:

n-Decane (n-C_{10})	n-Heptadecane (n-C_{17})	n-Tetracosane (n-C_{24})	n-Hentriacontane (n-C_{31})
n-Undecane (n-C_{11})	n-Octadecane (n-C_{18})	n-Pentacosane (n-C_{25})	n-Dotriacontane (n-C_{32})
n-Dodecane (n-C_{12})	n-Nonadecane (n-C_{19})	n-Hexacosane (n-C_{26})	n-Tritriacontane (n-C_{33})
n-Tridecane (n-C_{13})	n-Eicosane (n-C_{20})	n-Heptacosane (n-C_{27})	n-Tetratriacontane (n-C_{34})
n-Tetradecane (n-C_{14})	n-Heneicosane (n-C_{21})	n-Octacosane (n-C_{28})	n-Pentatriacontane (n-C_{35})
n-Pentadecane (n-C_{15})	n-Docosane (n-C_{22})	n-Nonacosane (n-C_{29})	pristane
n-Hexadecane (n-C_{16})	n-Tricosane (n-C_{23})	n-Triacontane (n-C_{30})	phytane

III.9.6.2. Equipment and materials

Gas chromatograph coupled with a flame ionization detector

The gas chromatograph must have a split/splitless injector and be suitable for use with a capillary column located inside a programmable oven and coupled with a flame ionization detector. The capillary column commonly used for aliphatic and total hydrocarbon analysis is a capillary column 30 m long with a 0.25-mm inside diameter and a 0.25-µm thick DB-5 liquid phase. Other columns with similar characteristics can be used. It is preferable to use an autosampler capable of performing 1–4 µL injections. The programme used for the analysis is as follows:

- Injector temperature: 290°C
- Detector temperature: 320°C
- Oven temperature programme:
  - Initial temperature: 35°C, held for 1 min
  - 1st heating ramp 8.0°C/min
  - 1st ramp final temperature 95ºC, held for 0 min
  - 2nd heating ramp 6.0°C/min
  - 2nd ramp final temperature 300°C, held for 9 min
  - 3rd heating ramp 15°C/min
  - 3rd ramp final temperature 320°C, held for 4 min
- Carrier gas Helium at ~2 mL/min
- Make-up gas Helium at ~33 mL/min
- Combustion gases:
  - Hydrogen ~30 mL/min
  - Air: ~350 mL/min
- Total time: 57 min

These conditions can be modified to improve the chromatographic profile, if necessary.

III.9.6.3. Calibrating the chromatograph

The gas chromatograph used for the analysis of aliphatic and total hydrocarbons must be calibrated using one of the methods indicated above (see Quality control, Calibrating the instrument). Owing to the flame ionization detector's stability and linear response, it is advisable to calibrate the instrument using relative response factors or a linear regression fit.

III.9.6.4. Chromatographic analysis of the samples

As mentioned above, the quality control samples (blank, duplicate sample, spiked sample and reference material) and the samples making up the analytical batch are analysed as a single analytical sequence along with the calibration solutions or calibration control solutions (see Analytical quality control, Initial calibration of the instrument and Verifying that the initial calibration remains valid). Before initiating the analytical sequence, 1 or 2 µL of hexane must be injected (see Quality control, Instrument blank) to check that the system is free of contaminants. To analyse the samples, the extracts are injected in amounts of 1–4 µL.

It is important to stress that the quality control samples in an analytical batch must be evaluated as a set, as the failure of one of these samples to meet the established criteria does not necessarily mean that all the samples in the extraction in question must be rejected.

III.9.6.5. Qualitative identification of the target analytes

To identify a peak as corresponding to a target analyte, the criterion discussed above must be met (see Analytical quality control, Qualitative identification of target compounds). Fig. III-4 shows the elution order and relative responses of the aliphatic hydrocarbons, surrogate standards and internal standard; the concentration of each analyte is 5000 ng/mL.

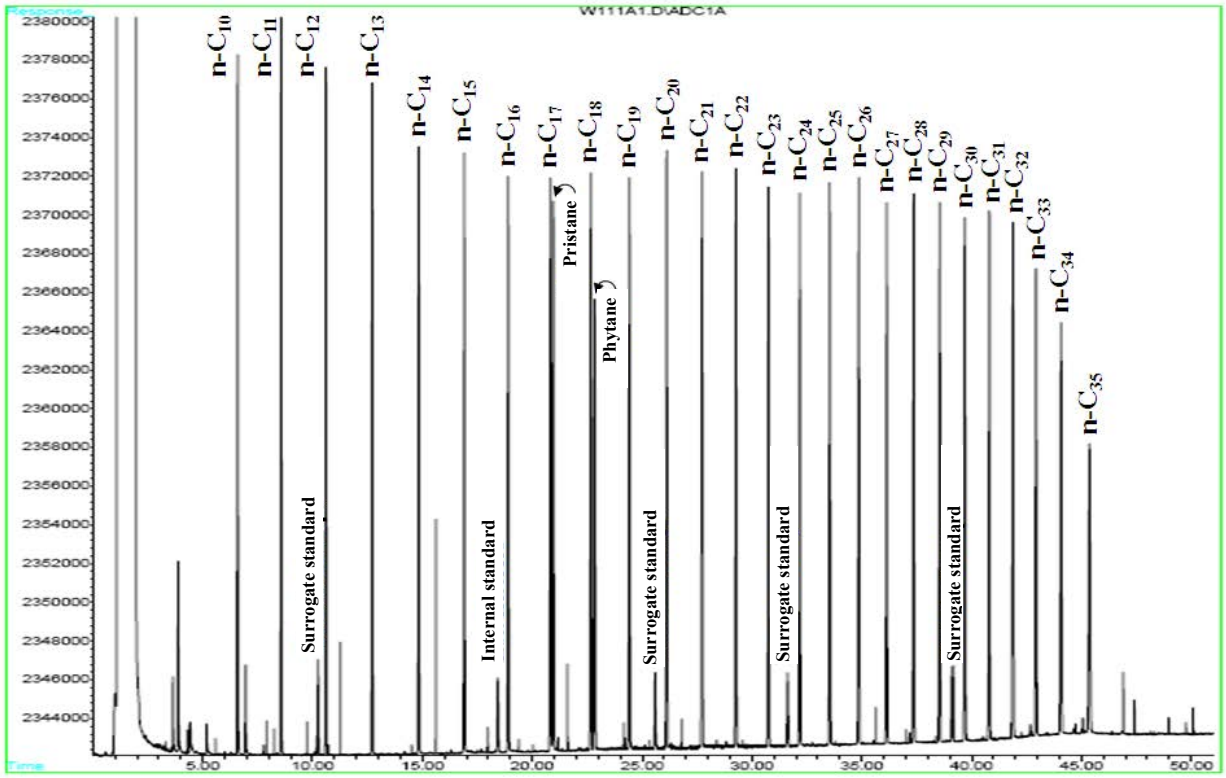


FIG. III-4. Aliphatic hydrocarbons analysed using a gas chromatograph coupled with a flame ionization detector.

III.9.6.6. Initial calibration, verifying the calibration and quantitative determination of the target analytes

As the flame ionization detector is stable and linear in behaviour, the chromatograph can be calibrated using a linear regression fit or relative response factors. The response factor of each compound relative to its surrogate standard is calculated on the basis of the data obtained from the injection of the various calibration solutions using the following equation:

$$RRF = \left(\frac{\frac{A_{ana}}{C_{ana}}}{\frac{A_{sur}}{C_{sur}}}\right) \qquad \text{or} \qquad RRF = \left(\frac{A_{ana}\, C_{sur}}{A_{sur}\, C_{ana}}\right) \tag{III-62}$$

where

RRF is the relative response factor;
A_{ana} is the area for the compound;
A_{sur} is the area for the surrogate standard;
C_{ana} is the concentration of the analyte in ng;
C_{sur} is the concentration of the surrogate standard in ng.

III.9.6.7. Initial calibration

The initial calibration is considered acceptable if the RSD (in %) of the response factors for each compound in the calibration solutions is no greater than ±15%.

Verifying the initial calibration

The initial calibration must be checked every 10–12 samples, or at least once every 12 hours, by injecting a specially prepared solution or re-injecting one of the intermediate-level calibration solutions. If the concentrations determined for this solution differ from the nominal value by no more than ± 25 %, the initial calibration is considered to be still acceptable.

III.9.6.8. Quantitative determination of the target analytes

Using the data obtained by injecting the various calibration solutions, the average relative response factor for each compound in the calibration solutions is calculated. These average relative response factors are used to calculate the concentrations of the respective target analytes and the unresolved complex mixture. The average relative response factors are calculated as follows:

$$\overline{\mathrm{RRF}} = \left(\frac{1}{n}\right) * \sum_{j=1}^{n} \mathrm{RRF}_j \qquad \text{(III-63)}$$

where

$\overline{\mathrm{RRF}}$ is the average relative response factor;
n is the total number of calibration solutions;
j is the solution number.

For the calculation of the concentrations based on these relative response factors:

$$\mathrm{C} = \left(\frac{\mathrm{A_{ana}}\ \mathrm{M_{sur}}}{\mathrm{A_{sur}}\ \overline{\mathrm{RRF}}\ \mathrm{M_{sam}}}\right) \qquad \text{(III-64)}$$

where

C is the concentration of the target analyte in the sample;
A_{ana} is the area for the compound;
M_{sur} is the mass of surrogate standard in ng;
A_{sur} is the area for the surrogate standard;
$\overline{\mathrm{RRF}}$ is the average relative response factor;
M_{sam} is the sample size in g.

Determining the unresolved complex mixture (UCM)

To determine the concentration of UCM in a sample, the average response factor for all alkanes present in the calibration solutions is used:

$$UCM = \left(\frac{A_{corr}\, M_{sur}}{A_{sur}\, \overline{RRF}\, M_{sam}}\right) \quad \text{(III-65)}$$

where

UCM is the concentration of the unresolved complex mixture in the sample;
A_{corr} is the corrected chromatogram area, which is defined as the total area of the chromatogram minus the area corresponding to temperature-related baseline drift and the area of all resolved peaks, including for any surrogate and internal standards added to the sample;
M_{sur} is the amount, in ng, of surrogate standard added to the sample prior to extraction;
A_{sur} is the area for the surrogate standard;
$\overline{RRF}$ is the average relative response factor;
M_{sam} is the sample size in g.

III.9.6.9. Determining total hydrocarbons

To determine the concentration of total hydrocarbons in a sample (Fig. III-5), the average response factor for all alkanes present in the calibration solutions is used:

$$[HcT] = \left(\frac{A_{tcorr}\, M_{sur}}{A_{sur}\, \overline{RRF}\, M_{sam}}\right) \quad \text{(III-65)}$$

where

[HcT] is the concentration of total hydrocarbons in the sample;
A_{tcorr} is the total corrected chromatogram area, which is defined as the total area of the chromatogram minus the area corresponding to the temperature-related baseline drift and the area for the surrogate and internal standards added to the sample;
M_{sur} is the mass, in ng, of surrogate standard added to the sample prior to extraction;
A_{sur} is the area for the surrogate standard;
$\overline{RRF}$ is the average relative response factor;
M_{sam} is the sample size in g.

III.9.6.10. Quality control for calculations

The concentrations of target analytes are calculated based on the response factors of these compounds in the calibration solutions. In general, modern chromatography systems have software that allows the calculations to be performed almost automatically. However, it is important to understand the concepts involved in these calculations in order to be able to reproduce, manually and as an additional means of quality control, some of the results produced by the software.
It is advisable to corroborate the results using independent calculations. Several randomly selected concentrations must be confirmed in a commercial spreadsheet (Excel or similar), using different equations depending on the type of calibration performed, and compared with the reported values. The two concentrations should match exactly, allowing for rounding errors (see Quantitative determination of chlorinated pesticides using gas chromatography coupled with an electron capture detector, Quality control for calculations).

III.9.6.11. Diluting an extract

If the instrument response to an analyte exceeds the response observed for the same compound in the most concentrated calibration solution, the extract needs to be diluted accordingly. To obtain a response within the range of concentrations used to calibrate the chromatograph, further surrogate standards are added before the sample is re-analysed (see Quantitative determination of chlorinated pesticides using gas chromatography coupled with an electron capture detector, Diluting an extract).

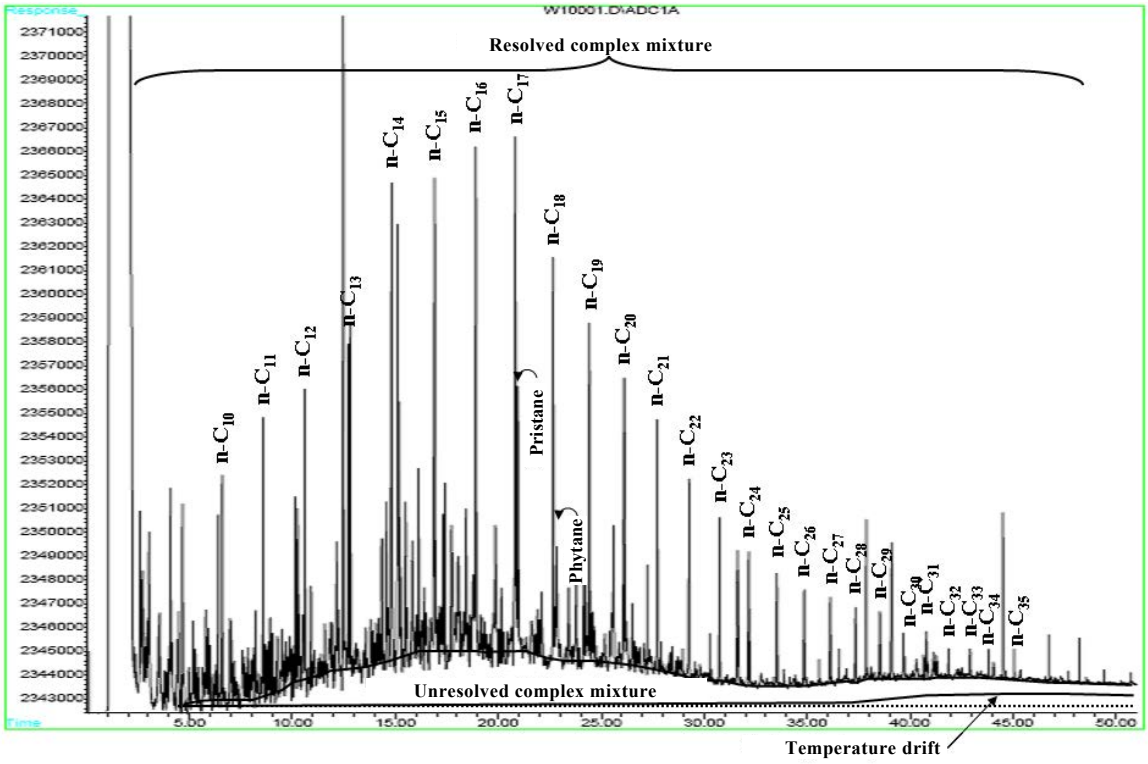


FIG. III-5. Total hydrocarbons analysed using a gas chromatograph coupled with a flame ionization detector.

III.9.6.12. Maintenance of gas chromatograph and flame ionization detector

There are a number of simple steps that can be taken to help keep the gas chromatograph and associated flame ionization detector in good working order:

— The syringe must be rinsed with the appropriate solvent after each injection. This can be done manually or using the autosampler.
— The injection port seal (septum) is to be replaced after a number of injections, depending on the condition of the syringe needle, to avoid leaks and variations in retention times.
— A new glass liner is to be installed as soon as there are signs of degradation or tailing of the target analytes. Sometimes it is necessary, in addition to replacing the glass liner, to replace the lower injection port seal (gold seal).
— If, after replacing the insert and seal, there is still tailing or a reduced response to the target analytes, the first two coils of the capillary column (at the end connected to the injection port) need to be removed.
— The tanks of carrier gases (helium) and auxiliary gases (argon:methane or nitrogen) must be replaced well before they are empty to avoid soiling that could increase the base noise level or contaminate the column and/or detector. As a rule, it is best to replace the tank when the pressure drops below 500 psi.
— All maintenance performed on the gas chromatograph or flame ionization detector, including gas tank replacements, is to be recorded in a dedicated logbook for the specific piece of equipment.

III.9.6.13. Documentation

The documentation that is to be provided to the laboratory together with the sample extracts to be analysed, as well as the documentation that the analyst must add to the relevant folder, is specified in the section on aliphatic hydrocarbon analysis (see Documentation).

Reporting concentrations
Concentrations are generally reported as ng/g dry weight to three decimal places.

III.9.7. Determining PAHs using gas chromatography coupled with a mass spectrometer

III.9.7.1. Introduction

PAHs can be determined using gas chromatography coupled with a mass spectrometer (GC–MS). This technique is capable of determining these pollutants at trace levels (e.g. ng/g). The method described can be applied to sediment extracts after the appropriate processing. The target PAHs for this study are limited to the following:

Acenaphthene	Benzo[k]fluoranthene	Phenanthrene	Pyrene
Acenaphthylene	Benzo[ghi]perylene	Fluoranthene	1-methylnaphthalene
Anthracene	Biphenylene	Fluorene	2-methylnaphthalene
Benzo[a]anthracene	Chrysene	Indeno[1,2,3-cd]pyrene	2,6-dimethylnaphthalene
Benzo[a]pyrene	Dibenz[a,h]anthracene	Naphthalene	2,3,5-trimethylnaphthalene
Benzo[e]pyrene	Dibenzothiophene	Perylene	1-methylphenanthrene
Benzo[b]fluoranthene			

III.9.7.2. Equipment and materials

Gas chromatograph coupled with a mass spectrometer

The gas chromatograph must have a split/splitless injector and be suitable for use with a capillary column located inside a programmable oven and coupled with a mass spectrometer. The capillary column most commonly used for organic contaminant analysis is 30 m long, with a 0.25-mm inside diameter and a 0.25-µm thick DB-5ms liquid phase or similar. It is best to use an autosampler capable of performing 1–4 µL injections. The programme used for the analysis of PAHs using gas chromatography coupled with a mass spectrometer is as follows:

- Injector temperature: 300°C
- Oven temperature programme:
  - Initial temperature: 60°C, held for 0 min
  - 1st heating ramp 15°C/min
  - 1st ramp final temperature 150°C, held for 0 min
  - 2nd heating ramp 5°C/min
  - 2nd ramp final temperature 220°C, held for 0 min
  - 3rd heating ramp 10°C/min
  - 3rd ramp final temperature 300°C, held for 10 min
- Carrier gas Helium at ~1.0 mL/min
- Total time 38 min

These conditions can be modified to improve the chromatographic profile, if necessary.

III.9.7.3. Calibrating the chromatograph

The gas chromatograph for the analysis of PAHs must be calibrated using one of the methods listed above (see Analytical quality control, Calibrating the instrument). Given the good linearity of the mass spectrometer, it is recommended to calibrate this instrument using relative response factors or a linear regression fit.

III.9.7.4. Chromatographic analysis of the samples

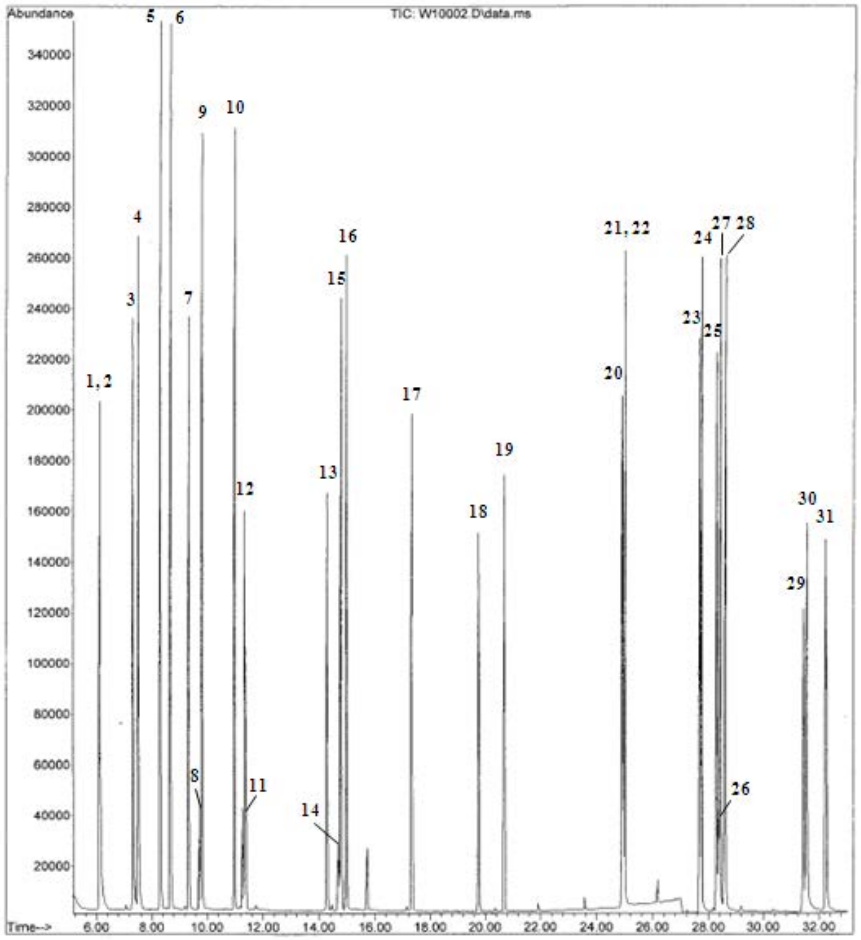
As mentioned above, the quality control samples (blank, duplicate sample, spiked sample and reference material) and the samples making up the analytical batch are analysed as a single analytical sequence along with the calibration solutions or calibration control solutions (see Analytical quality control, Initial calibration of the instrument and Verifying that the initial calibration remains valid). Before starting the analytical sequence, an instrument blank must be run to check that the system is free of contaminants. To analyse the samples, the extracts are injected in amounts of 1–4 µL.

It is important to stress that the quality control samples in an analytical batch must be evaluated as a set, as the failure of one of these samples to meet the established criteria does not necessarily mean that all the samples in the extraction in question must be rejected.

III.9.7.5. Qualitative identification of the target analytes

To identify a peak as corresponding to a target analyte, the criterion discussed above must be met (see Analytical quality control, Qualitative identification of target compounds). Fig. III-6 shows the elution order and relative responses of the PAHs and surrogate and internal standards; the concentration of each analyte is 500 and 100 ng/mL, respectively. In addition, when determining multiple individual ions (e.g. primary ion and 1 or 2 confirmation ions; Table III-2) that exhibit an adequate response and for which there is little chance of interference, the following quality criteria must be met:

- The characteristic masses for each analyte should show the maximum intensities in the same read cycle (spectrum sweep) or separated by at most one cycle;
- The relative intensities of the selected ions for the target analyte must be in line with the values obtained in the same GC–MS, with a maximum tolerance of ±20%;



1. Naphtalene-d8 (surrog. std.)
2. Naphthalene
3. 2-methylnaphthalene
4. 1-methylnaphthalene
5. Biphenyl
6. 2,6-dimethylnaphthalene
7. Acenaphthylene
8. Acenaphthene-d10
9. Acenaphthene
10. 1,6,7-trimethylnaphthalene
11. Fluorene-d10 (internal std.)
12. Fluorene
13. Dibenzothiophene
14. Phenanthrene-d10 (surrog. std.)
15. Phenanthrene
16. Anthracene
17. 1-methylphenanthrene
18. Fluoranthene
19. Pyrene
20. Benzo[a]anthracene
21. Chrysene-d12 (surrog. std.)
22. Chrysene
23. Benzo[b]fluoranthene
24. Benzo[k]fluoranthene
25. Benzo[e]pyrene
26. Benzo[a]pyrene-d12 (int. std.)
27. Benzo[a]pyrene
28. Perylene
29. Indeno[1,2,3-cd]pyrene
30. Dibenzo[a,h]anthracene
31. Benzo[g,h,i]perylene

FIG. III-6. PAHs analysed using mass chromatography.

III.9.7.6. Initial calibration, verifying the calibration and quantitative determination of the target analytes

As the mass spectrometer is stable and linear in behaviour, the chromatograph can be calibrated using a linear regression fit or relative response factors. The response factor of each compound relative to its surrogate standard is calculated on the basis of the data obtained from the injection of the various calibration solutions using the following equation:

$$RRF = \left(\frac{\frac{A_{ana}}{C_{ana}}}{\frac{A_{sur}}{C_{sur}}}\right) \qquad \text{or} \qquad RRF = \left(\frac{A_{ana}\, C_{sur}}{A_{sur}\, C_{ana}}\right) \tag{III-67}$$

where:

RRF is the relative response factor;
A_{ana} is the area for the quantification ion corresponding to the compound;
A_{sur} is the area for the quantification ion corresponding to the surrogate standard;
C_{ana} is the concentration of the analyte in ng;
C_{sur} is the concentration of the surrogate standard in ng.

III.9.7.7. Initial calibration

The initial calibration is considered acceptable if the RSD (in %) of the response factors for each compound in the calibration solutions is no greater than ±15%.

Verifying the initial calibration

The initial calibration must be checked every 10–12 samples, or at least once every 12 hours, by injecting a specially prepared solution or re-injecting one of the intermediate-level calibration solutions. If the concentrations determined for this solution differ from the nominal value by no more than ± 25 %, the initial calibration is considered to be still acceptable.

III.9.7.8. Quantitative determination of the target analytes

Using the data obtained by injecting the various calibration solutions, the average relative response factor for each compound in the calibration solutions is calculated. These average relative response factors are used to calculate the concentrations of the respective target analytes and the unresolved complex mixture. The average relative response factors are calculated as follows:

$$\overline{RRF} = \left(\frac{1}{n}\right) * \sum_{j=1}^{n} RRF_j \tag{III-68}$$

where

RRF is the average relative response factor;
n is the total number of calibration solutions;
j is the solution number.

For the calculation of the concentrations based on these relative response factors:

$$C = \left(\frac{A_{ana}\, C_{sur}}{A_{sur}\, \overline{RRF}\, M_{sam}}\right) \quad \text{(III-69)}$$

where:

C is the concentration of the target analyte in the sample;
A_{ana} is the area for the quantification ion corresponding to the compound;
C_{sur} is the mass of surrogate standard in ng;
A_{sur} is the area for the quantification ion corresponding to the surrogate standard;
$\overline{RRF}$ is the average relative response factor;
M_{sam} is the sample size in g.

TABLE III-2. SELECTED QUANTIFICATION AND CONFIRMATION IONS FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND CORRESPONDING STANDARDS USING MASS SPECTROMETRY

Analyte	Quantification ion	Confirmation ion	Relative abundance (%) of confirmation ion [1]
Naphthalene-d8 (surrogate standard)	136	134	15
Naphthalene	128	127	15
1-methylnaphthalene	142	141	80
2-methylnaphthalene	142	141	80
2,6-dimethylnaphthalene	156	141	75
2,3,5-trimethylnaphthalene	170	155	95
Biphenyl	154	153	35
Acenaphthylene	152	153	15
Acenaphthene-d10 (surrogate standard)	164	162	95
Acenaphthene	154	153	100
Fluorene-d10 (internal standard)	176	174	85
Fluorene	166	165	95
Dibenzothiophene	184	152	15
Phenanthrene-d10 (surrogate standard)	188	184	15
Phenanthrene	178	176	20
1-methylphenanthrene	192	191	55
Anthracene	178	176	20
Fluoranthene	202	101	15
Pyrene	202	101	15
Benzo[a]anthracene	228	226	20
Chrysene-d12 (surrogate standard)	240	236	30
Chrysene	228	226	30
Benzo[b]fluoranthene	252	253	30
Benzo[k]fluoranthene	252	253	30
Benzo[e]pyrene	252	253	30
Benzo[a]pyrene-d12 (internal standard)	264	260	20
Benzo[a]pyrene	252	253	30
Perylene	252	253	20
Indeno[1,2,3-cd]pyrene	276	277	25
Dibenz[a,h]anthracene	278	279	25
Benzo[g,h,i]perylene	276	277	25

[1] Relative abundances are shown for reference only.

III.9.7.9. Quality control for calculations

The concentrations of target analytes are calculated based on the response factors of these compounds in the calibration solutions. In general, modern chromatography systems have software that allows the calculations to be performed almost automatically. However, it is important to understand the concepts involved in these calculations in order to be able to manually reproduce some of the results produced by the software, and as an additional form of quality control. It is advisable to corroborate the results using independent calculations. Several randomly selected concentrations must be confirmed in a commercial spreadsheet (Excel or similar), using different equations depending on the type of calibration performed, and compared with the reported values. The two concentrations should match exactly, allowing for rounding errors (see Quantitative determination of chlorinated pesticides using gas chromatography coupled with an electron capture detector, Quality control for calculations).

III.9.7.10. Diluting an extract

If the instrument response to an analyte exceeds the response observed for the same compound in the most concentrated calibration solution, the extract needs to be diluted accordingly. To obtain a response within the range of concentrations used to calibrate the chromatograph, further surrogate standards are added before the sample is re-analysed (see Quantitative determination of chlorinated pesticides using gas chromatography coupled with an electron capture detector, Diluting an extract).

III.9.7.11. Maintaining the gas chromatograph and the mass spectrometer detector

There are a number of simple steps that can be taken to help keep the gas chromatograph and associated mass spectrometer in good working order: To properly maintain the gas chromatograph:

- Rinse the syringe with the appropriate solvent after each injection. This can be done manually or using the autosampler.
- Replace the injection port seal (septum) after a number of injections, depending on the condition of the syringe needle, to avoid leaks and variations in retention times.
- Install a new glass liner as soon as there are signs of degradation or tailing of the target analytes, in particular the heavier PAHs. It is advisable to use a glass liner with glass wool inside to promote volatilization of the heavier PAHs and their entry into the column.
- Sometimes it is necessary, in addition to replacing the glass liner, to replace the lower injection port seal (gold seal) in order to avoid degradation or peak tailing. If, after replacing the insert and seal, one continues to observe degradation, tailing or a reduced response to the target analytes, the first two coils of the capillary column (at the end connected to the injection port) need to be removed.
- Replace the tank of carrier gas (helium) before it is empty to avoid any contamination that could increase the base noise level or contaminate the column and/or detector. As a rule, it is best to replace the tank when the pressure drops below 500 psi.
- To properly maintain the mass spectrometer:
    - Clean the source and replace the filament whenever necessary.
    - Change the vacuum pump oil once a year, or more frequently if necessary.
    - Check for vacuum leaks after the mass spectrometer is opened and cleaned.

As a rule, all maintenance performed on the gas chromatograph or mass spectrometer, including gas tank replacements, is to be recorded in a dedicated logbook for the specific piece of equipment.

III.9.7.12. Documentation

The documentation that is to be provided to the laboratory together with the sample extracts to be analysed, as well as the documentation that the analyst must add to the relevant folder, is specified in the section on chlorinated pesticide analysis (see Documentation).

Reporting concentrations

Concentrations are generally reported as ng/g dry weight to three decimal places.

III.9.8. Preparing surrogate standards, internal standards and spike solutions

For convenience, the surrogate standards, internal standards and spike solutions are prepared in such a way that their final concentrations in the sample extracts and quality control samples are comparable to those in the calibration solutions. For spiked samples, a 100% recovery of the added analytes will produce an instrumental response similar to that for calibration solution 3.

III.9.8.1. Surrogate standards

Surrogate standards are added to all samples to be analysed and to quality control samples (blank, duplicate sample, spiked blank, spiked sample, duplicate spiked sample, reference material) prior to processing by the laboratory. The use of surrogate standards in the calculations makes it possible to compensate for losses that occur during laboratory processing of the batch of samples and is independent of the final sample extract volume or the volume of extract injected into the chromatograph.

III.9.8.2. Internal standards

Internal standards are added to all extracts to be analysed and to quality control extracts (blank, duplicate sample, spiked blank, spiked sample, duplicate spiked sample, reference material) prior to chromatographic analysis. The use of internal standards provides information regarding the magnitude of the losses that occur during laboratory processing of the batch of samples and is independent of the final extract volume and the volume of extract injected into the chromatograph.

III.9.8.3. Analyte spike solutions

These solutions are used to spike those samples that will be used to assess target compound recovery by the method employed without (e.g. spiked blank) and with (e.g. spiked sample, duplicate spiked sample) the effect of the sediment on which extraction is performed. By comparing the spiked sample with its duplicate, it is possible not only to assess the laboratory's ability to recover and determine concentrations in the presence of the matrix under study, but also its ability to reproduce results.

III.9.8.4. Chlorinated pesticides

Surrogate standards (ULTRA Scientific, CUS-9738)

Analyte	CAS No.	Concentration
4,4'-dibromooctafluorobiphenyl	010386-84-2	200.0 ± 1.0 µg/mL
2,2',4,5',6-pentachlorobiphenyl (PCB 103)	060145-21-3	200.0 ± 1.0 µg/mL
2,2',3,3',4,5,5',6-octachlorobiphenyl (PCB 198)	068194-17-2	200.0 ± 1.0 µg/mL

Internal standard (~1 gram of solid drug)

Analyte	CAS No.	Concentration
2,4,5,6-tetrachloro-m-xylene (TCMX)	010386-84-2	N/A

Chlorinated pesticides (ULTRA Scientific, CUS-9736)

Analyte	CAS No.	Concentration
Aldrin	000309-00-2	201.0 ± 1.0 µg/mL
Alpha-chlordane	005103-71-9	201.0 ± 1.0 µg/mL
gamma-Chlordane	005103-74-2	201.0 ± 1.0 µg/mL
Dieldrin	000060-57-1	201.0 ± 1.0 µg/mL
Endosulfan I	000959-98-8	201.0 ± 1.0 µg/mL
Endosulfan II	033213-65-9	201.0 ± 1.0 µg/mL
Endosulfan sulphate	001031-07-8	201.0 ± 1.0 µg/mL
Endrin	00072-20-8	201.0 ± 1.0 µg/mL
Endrin aldehyde	007421-93-4	201.0 ± 1.0 µg/mL
alpha-HCH	000319-84-6	201.0 ± 1.0 µg/mL
beta-HCH	000319-85-7	201.0 ± 1.0 µg/mL
delta-HCH	000319-86-8	201.0 ± 1.0 µg/mL
gamma-HCH	000058-89-9	201.0 ± 1.0 µg/mL
Heptachlor	000076-44-8	201.0 ± 1.0 µg/mL
Heptachlor epoxide	001024-57-3	201.0 ± 1.0 µg/mL
Methoxychlor	00072-43-5	201.0 ± 1.0 µg/mL
cis-Nonachlor	005103-73-1	201.0 ± 1.0 µg/mL
trans-Nonachlor	039765-80-5	201.0 ± 1.0 µg/mL
2,4'-DDD	000053-19-0	201.0 ± 1.0 µg/mL
2,4'-DDE	003424-82-6	201.0 ± 1.0 µg/mL
2,4'-DDT	000789-02-6	201.0 ± 1.0 µg/mL
4,4'-DDD	000072-54-8	201.0 ± 1.0 µg/mL
4,4'-DDE	000072-55-9	201.0 ± 1.0 µg/mL
4,4'-DDT	000050-29-3	201.0 ± 1.0 µg/mL
Endrin ketone	053494-70-5	201.0 ± 1.0 µg/mL

III.9.8.5. Chlorinated pesticides analysed using GC–ECD

Calibration solutions

Calibration solutions	Volume of analyte solution CUS-9736 (200 µg/mL)	Volume of surrogate standard solution CUS-9738 (200 µg/mL)	Volume of internal standard solution (Solid) (2000 µg/mL)	Volume of solvent	Final volume
Level 1	0.050 mL[(a)]	1.000 mL[(b)]	0.500 mL[(c)]	6.450 mL	10.000 mL
Level 2	0.200 mL[(a)]	1.000 mL[(b)]	0.500 mL[(c)]	6.300 mL	10.000 mL
Level 3	4.000 mL[(a)]	0.050 mL	5.000 mL[(c)]	90.700 mL	100.000 mL
Level 4	0.800 mL[(a)]	1.000 mL[(b)]	0.500 mL[(c)]	5.700 mL	10.000 mL
Level 5	2.000 mL[(a)]	1.000 mL[(b)]	0.500 mL[(c)]	4.500 mL	10.000 mL

(a) Requires prior dilution of 1:200 (e.g. 0.050 mL of solution made up to exactly 10 mL).
(b) Requires prior dilution of 1:200 (e.g. 0.050 mL of solution made up to exactly 10 mL).
(c) Requires prior dilution of 1:1000 (e.g. 0.010 mL of solution made up to exactly 10 mL).

Note that the level 3 calibration solution is prepared in a larger volume, so that it can be used each time the calibration of the chromatograph needs to be checked. The resulting concentrations of the analytes, surrogate standards and internal standard in each calibration solution are given below:

Calibration solutions	Concentration of each analyte (ng/mL)	Concentration of each surrogate standard (ng/mL)	Concentration of the internal standard (ng/mL)
Level 1	5	100	100
Level 2	20	100	100
Level 3	40	100	100
Level 4	80	100	100
Level 5	200	100	100

It should be noted that the concentrations of the various calibration solutions, in particular Level 1, depend on the type of analyte, the method used and the sensitivity of the equipment used and can be adjusted as necessary.

III.9.8.6. Fortification (spiking) solutions

These fortification or spiking solutions must be prepared using the solutions received. The recommended preparation, fortification or spiking concentration and use of the various solutions are as follows:

Surrogate standard:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) \;=\; 200\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.250\ \text{mL}}{50\ \text{mL}}\right) \;=\; 1000\ \text{ng/mL} \qquad \text{(III-70)}$$

Solution for use:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) \;=\; 1000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \;=\; 100\ \text{ng/mL} \qquad \text{(III-71)}$$

Internal standard:

$$[\text{Int. std.}]\ (\text{ng/mL}) \;=\; 2000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.025\ \text{mL}}{50\ \text{mL}}\right) \;=\; 1000\ \text{ng/mL} \qquad \text{(III-72)}$$

Solution for use:

$$[\text{Int. std.}]\ (\text{ng/mL}) \ = \ 1000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \ = \ 100\ \text{ng/mL} \qquad \text{(III-73)}$$

Analytes:

$$[\text{Analyte}]\ (\text{ng/mL}) \ = \ 200\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.100\ \text{mL}}{50\ \text{mL}}\right) \ = \ 400\ \text{ng/mL} \qquad \text{(III-74)}$$

Solution for use:

$$[\text{Analyte}]\ (\text{ng/mL}) \ = \ 400\ \text{ng/mL} * \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \ = \ 40\ \text{ng/mL} \qquad \text{(III-75)}$$

III.9.8.7. Aliphatic and total hydrocarbons

Surrogate standards (Absolute Standards Nos 72056, 72069 and 72072, respectively)

Analyte	CAS No.	Concentration
n-Dodecane-d26		1000 µg/mL
n-Eicosane-d42		1000 µg/mL
n-Tetracosane-d50		1000 µg/mL

Internal standard (Absolute Standards No. 72065)

Analyte	CAS No.	Concentration
n-Hexadecane-d34		1000 µg/mL

Aliphatic hydrocarbons (ULTRA Scientific, Custom Standard)

Analyte	CAS No.	Concentration
n-Decane	000124-18-5	200 µg/mL
n-Undecane	001120-21-4	200 µg/mL
n-Dodecane	000112-40-3	200 µg/mL
n-Tridecane	000629-50-5	200 µg/mL
n-Tetradecane	000629-59-4	200 µg/mL
n-Pentadecane	000629-62-9	200 µg/mL
n-Hexadecane	000544-76-3	200 µg/mL
n-Heptadecane	000629-78-7	200 µg/mL
n-Octadecane	000593-45-3	200 µg/mL
n-Nonadecane	000629-92-5	200 µg/mL
n-Eicosane	000112-95-8	200 µg/mL
n-Heneicosane	000629-94-7	200 µg/mL
n-Docosane	000629-97-0	200 µg/mL
n-Tricosane	000638-67-5	200 µg/mL

Analyte	CAS No.	Concentration
n-Tetracosane	000646-31-1	200 µg/mL
n-Pentacosane	000629-99-2	200 µg/mL
n-Hexacosane	000630-01-3	200 µg/mL
n-Heptacosane	000593-49-7	200 µg/mL
n-Octacosane	000630-02-4	200 µg/mL
n-Nonacosane	000630-03-5	200 µg/mL
n-Triacontane	000638-68-6	200 µg/mL
n-Hentriacontane	000630-04-6	200 µg/mL
n-Dotriacontane	000544-85-4	200 µg/mL
n-Tritriacontane	000630-05-7	200 µg/mL
n-Tetratriacontane	014167-59-0	200 µg/mL
n-Pentatriacontane	000630-07-9	200 µg/mL
Pristane	001921-70-6	200 µg/mL
Phytane	000638-36-8	200 µg/mL

III.9.8.8. Aliphatic and total hydrocarbons analysed using GC–FID

Calibration solutions:

Calibration solutions	Volume of analyte solution (200 µg/mL)	Volume of surrogate and internal standard solution (1000 µg/mL)	Volume of solvent	Final volume
Level 1	1.000 mL[(a)]	0.500 mL[(b)]	8.500 mL	10.000 mL
Level 2	5.000 mL[(a)]	0.500 mL[(b)]	4.500 mL	10.000 mL
Level 3	0.625 mL	1.250 mL[(b)]	23.125 mL	25.000 mL
Level 4	0.750 mL	0.500 mL[(b)]	8.500 mL	10.000 mL
Level 5	2.000 mL	0.500 mL[(b)]	7.000 mL	10.000 mL

(a) Requires prior dilution of 1:100 (e.g. 0.100 mL of solution made up to exactly 10 mL).
(b) Each of the solutions in the same flask requires prior dilution of 1:25 (e.g. 0.200 mL of each solution made up to exactly 5 mL).

Note that the level 3 calibration solution is prepared in a larger volume, so that it can be used each time the calibration of the chromatograph needs to be checked. The resulting concentrations of the analytes, surrogate standards and internal standard in each calibration solution are given below:

Calibration solutions	Concentration of each analyte (ng/mL)	Concentration of each surrogate standard (ng/mL)	Concentration of the internal standard (ng/mL)
Level 1	200	2 000	2 000
Level 2	1 000	2 000	2 000
Level 3	5 000	2 000	2 000
Level 4	15 000	2 000	2 000
Level 5	40 000	2 000	2 000

It should be noted that the concentrations of the various calibration solutions, in particular Level 1, depend on the type of analyte, the method used and the sensitivity of the equipment used and can be adjusted as necessary.

Fortification (spiking) solutions

These fortification or spiking solutions must be prepared using the solutions received. The recommended preparation, fortification or spiking concentration and use of the various solutions are as follows:

Surrogate standards:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) = 1000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.500\ \text{mL}}{25\ \text{mL}}\right) = 20\,000\ \text{ng/mL} \qquad \text{(III-76)}$$

Solution for use:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) = 20\,000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) = 2000\ \text{ng/mL} \qquad \text{(III-77)}$$

Internal standard:

$$[\text{Int. std.}]\ (\text{ng/mL}) = 1000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.500\ \text{mL}}{25\ \text{mL}}\right) = 20\,000\ \text{ng/mL} \qquad \text{(III-78)}$$

Solution for use:

$$[\text{Int. std.}]\ (\text{ng/mL}) \ = \ 20\ 000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \ = \ 2000\ \text{ng/mL} \qquad \text{(III-79)}$$

Analytes

$$[\text{Analyte}]\ (\text{ng/mL}) \ = \ 200\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{1.250\ \text{mL}}{5\ \text{mL}}\right) \ = \ 50000\ \text{ng/mL} \qquad \text{(III-80)}$$

Solution for use:

$$[\text{Analito}]\ (\text{ng/mL}) \ = \ 50\ 000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \ = \ 5000\ \text{ng/mL} \qquad \text{(III-81)}$$

III.9.8.9. Aromatic hydrocarbons

Surrogate standards (ULTRA Scientific, CUS-9737)

Analyte	CAS No.	Concentration
Naphthalene-d8	001146-65-2	1002 ± 5 µg/mL
Acenaphthene-d10	015067-26-2	1000 ± 5 µg/mL
Phenanthrene-d10	001517-22-2	1000 ± 5 µg/mL
Chrysene-d12	001719-03-5	1000 ± 5 µg/mL

Internal standards (ULTRA Scientific, CUS-9739)

Analyte	CAS No.	Concentration
Fluorene-d10	081103-79-9	1000 ± 5 µg/mL
Benzo[a]pyrene-d12	063466-71-7	1000 ± 5 µg/mL

Polycyclic aromatic hydrocarbons (ULTRA Scientific, CUS-9735)

Analyte	CAS No.	Concentration
Acenaphthene	000083-32-9	2008 ± 10 µg/mL
Acenaphthylene	000208-96-8	2008 ± 10 µg/mL
Anthracene	000120-12-7	2008 ± 10 µg/mL
Benzo[a]anthracene	000056-55-3	2008 ± 10 µg/mL
Benzo[a]pyrene	000050-32-8	2008 ± 10 µg/mL
Benzo[b]fluoranthene	000205-99-2	2008 ± 10 µg/mL
Benzo[e]pyrene	000192-97-2	2008 ± 10 µg/mL
Benzo[ghi]perylene	000191-24-2	2008 ± 10 µg/mL
Benzo[k]fluoranthene	000207-08-9	2008 ± 10 µg/mL
Biphenyl	000092-52-4	2008 ± 10 µg/mL
Chrysene	000218-01-9	2008 ± 10 µg/mL
Dibenz[a,h]anthracene	000053-70-3	2008 ± 10 µg/mL
Dibenzothiophene	000132-65-0	2008 ± 10 µg/mL
Fluoranthene	000206-44-0	2008 ± 10 µg/mL
Fluorene	000086-73-7	2008 ± 10 µg/mL
Indeno[1,2,3-cd]pyrene	000193-39-5	2008 ± 10 µg/mL
Naphthalene	000091-20-3	2008 ± 10 µg/mL
1-methylnaphthalene	000090-12-0	2008 ± 10 µg/mL
2-methylnaphthalene	000091-57-6	2008 ± 10 µg/mL
2,6-dimethylnaphthalene	000581-42-0	2008 ± 10 µg/mL
1,6,7-trimethylnaphthalene	002245-38-7	2008 ± 10 µg/mL
Perylene	000198-55-0	2008 ± 10 µg/mL
Phenanthrene	000085-01-8	2008 ± 10 µg/mL
1-methylphenanthrene	000832-69-9	2008 ± 10 µg/mL
Pyrene	000129-00-0	2008 ± 10 µg/mL

III.9.8.10. Aromatic hydrocarbons analysed using GC–MS

Calibration solutions

Calibration solutions	Volume of analyte solution CUS-9735 (2000 µg/mL)	Volume of surrogate standard solution CUS-9737 (1000 µg/mL)	Volume of internal standard solution CUS-9739 (1000 µg/mL)	Volume of solvent (mL)	Final volume (mL)
Level 1	0.100 mL[(a)]	1.000 mL[(b)]	1.000 mL[(b)]	96.900 mL	100.000 mL
Level 2	0.500 mL[(a)]	1.000 mL[(b)]	1.000 mL[(b)]	96.500 mL	100.000 mL
Level 3	1.250 mL[(a)]	1.000 mL[(b)]	1.000 mL[(b)]	95.750 mL	100.000 mL
Level 4	2.500 mL[(a)]	1.000 mL[(b)]	1.000 mL[(b)]	94.500 mL	100.000 mL
Level 5	5.000 mL[(a)]	1.000 mL[(b)]	1.000 mL[(b)]	92.000 mL	100.000 mL

(a) Requires prior dilution of 1:100 (e.g. 0.100 mL of solution made up to exactly 10 mL).
(b) Requires prior dilution of 1:100 (e.g. 0.100 mL of solution made up to exactly 10 mL).

The resulting concentrations of the analytes, surrogate standards and internal standards, in each calibration solution, are as follows:

Calibration solutions	Concentration of each analyte (ng/mL)	Concentration of each surrogate standard (ng/mL)	Concentration of each internal standard (ng/mL)
Level 1	20	100	100
Level 2	100	100	100
Level 3	250	100	100
Level 4	500	100	100
Level 5	1 000	100	100

It should be noted that the concentrations of the various calibration solutions, in particular Level 1, depend on the type of analyte, the method used and the sensitivity of the equipment used and can be adjusted as necessary.

Fortification (spiking) solutions

These fortification or spiking solutions must be prepared using the solutions received. The recommended preparation, fortification or spiking concentration and use of the various solutions are as follows:

Surrogate standards:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) \ = \ 1000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.100\ \text{mL}}{100\ \text{mL}}\right) \ = \ 1000\ \text{ng/mL} \qquad \text{(III-82)}$$

Solution for use:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) \ = \ 1000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \ = \ 100\ \text{ng/mL} \qquad \text{(III-83)}$$

Internal standards:

$$[\text{Int. std.}]\ (\text{ng/mL}) \ = \ 1000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.100\ \text{mL}}{100\ \text{mL}}\right) \ = \ 1000\ \text{ng/mL} \qquad \text{(III-84)}$$

Solution for use:

$$[\text{Int. std.}]\ (\text{ng/mL}) \ = \ 1000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \ = \ 100\ \text{ng/mL} \qquad \text{(III-85)}$$

Analytes:

$$[\text{Analyte}]\ (\text{ng/mL}) \ = \ 2000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.125\ \text{mL}}{100\ \text{mL}}\right) \ = \ 2500\ \text{ng/mL} \qquad \text{(III-86)}$$

Solution for use:

$$[\text{Analyte}]\ (\text{ng/mL}) \ = \ 2500\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \ = \ 250\ \text{ng/mL} \qquad \text{(III-87)}$$

III.9.8.11. Aromatic hydrocarbons using GC–FID

Calibration solutions

Calibration solutions	Volume of analyte solution CUS-9735 (2000 µg/mL)	Volume of surrogate standard solution CUS-9737 (1000 µg/mL)	Volume of internal standard solution CUS-9739 (1000 µg/mL)	Volume of solvent (mL)	Final volume (mL)
Level 1	0.100 mL[(a)]	2.000 mL[(b)]	2.000 mL[(b)]	7.900 mL	10.000 mL
Level 2	0.500 mL[(a)]	2.000 mL[(b)]	2.000 mL[(b)]	7.500 mL	10.000 mL
Level 3	0.250 mL	0.200 mL	0.200 mL	99.350 mL	100.000 mL
Level 4	0.100 mL	2.000 mL[(b)]	2.000 mL[(b)]	7.900 mL	10.000 mL
Level 5	0.250 mL	2.000 mL[(b)]	2.000 mL[(b)]	7.750 mL	10.000 mL

(a) Requires prior dilution of 1:100 (e.g. 0.100 mL of solution made up to exactly 10 mL).
(b) Requires prior dilution of 1:100 (e.g. 0.100 mL of solution made up to exactly 10 mL).

Note that the level 3 calibration solution is prepared in a larger volume, so that it can be used each time the calibration of the chromatograph needs to be checked. The resulting concentrations of the analytes, surrogate standards and internal standards, in each calibration solution, are as follows:

Calibration solutions	Concentration of each analyte (ng/mL)	Concentration of each surrogate standard (ng/mL)	Concentration of each internal standard (ng/mL)
Level 1	200	2 000	2 000
Level 2	1 000	2 000	2 000
Level 3	5 000	2 000	2 000
Level 4	20 000	2 000	2 000
Level 5	50 000	2 000	2 000

Fortification (spiking) solutions

These fortification or spiking solutions must be prepared using the solutions received. The recommended preparation, fortification or spiking concentration and use of the various solutions are as follows:

Surrogate standards:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) = 1000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.500\ \text{mL}}{25\ \text{mL}}\right) = 20\,000\ \text{ng/mL} \qquad \text{(III-88)}$$

Solution for use:

$$[\text{Surrog. std.}]\ (\text{ng/mL}) = 20\,000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) = 2000\ \text{ng/mL} \qquad \text{(III-89)}$$

Internal standards:

$$[\text{Int. std.}]\ (\text{ng/mL}) = 1000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.500\ \text{mL}}{25\ \text{mL}}\right) = 20\,000\ \text{ng/mL} \qquad \text{(III-90)}$$

Solution for use:

$$[\text{Int. std.}]\ (\text{ng/mL}) = 20\,000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) = 2000\ \text{ng/mL} \qquad \text{(III-91)}$$

Analytes:

$$[\text{Analyte}]\ (\text{ng/mL}) \;=\; 2000\ \mu\text{g/mL} \times \left(\frac{1000\ \text{ng/mL}}{1\ \mu\text{g/mL}}\right) \times \left(\frac{0.250\ \text{mL}}{10\ \text{mL}}\right) \;=\; 50\ 000\ \text{ng/mL} \qquad \text{(III-92)}$$

Solution for use:

$$[\text{Analyte}]\ (\text{ng/mL}) \;=\; 50\ 000\ \text{ng/mL} \times \left(\frac{0.100\ \text{mL}}{1\ \text{mL}}\right) \;=\; 5000\ \text{ng/mL} \qquad \text{(III-93)}$$

III.9.9. Examples of calibration curves and calculations

This section illustrates how calibration curves are constructed and how equations for calculations are used. The following example illustrates the procedures to be followed in situations involving the addition of surrogate standards and four levels of calibration solutions (in this case, the middle level (level 3) is used as a separate solution for calibration control. Irrespective of the method used to calibrate the gas chromatograph, the information take the form shown in (Fig. III-7) below:

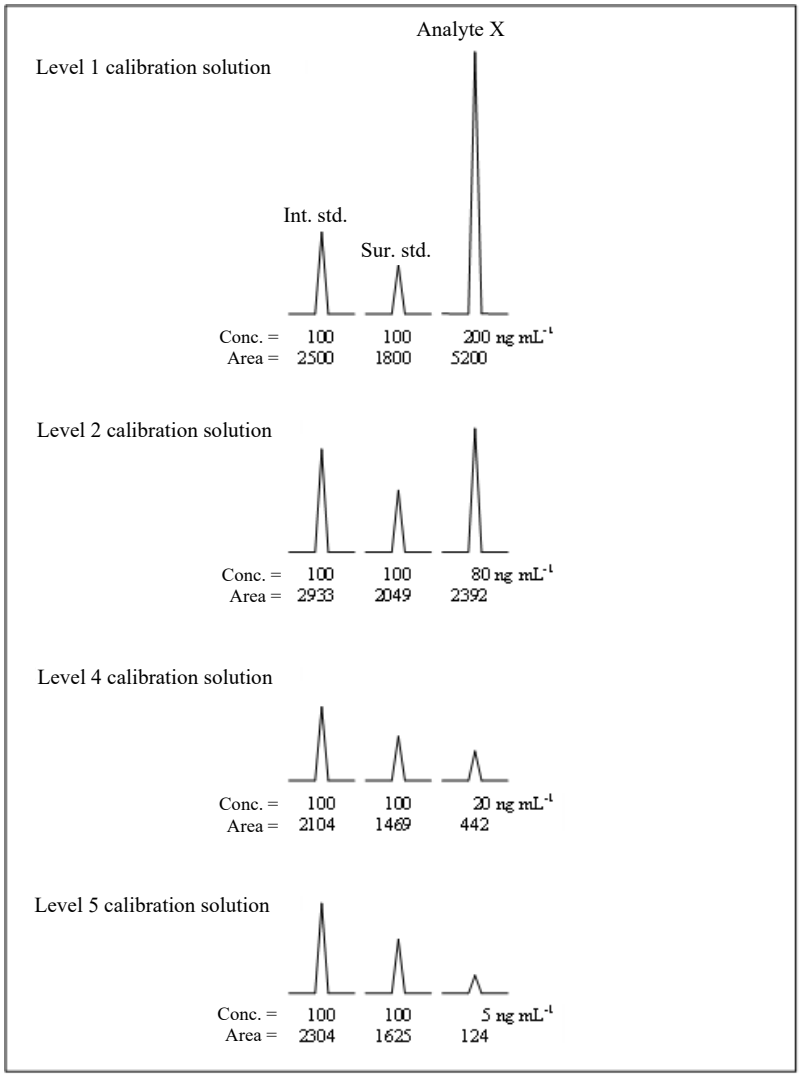


FIG. III-7. Examples of gas chromatograph calibration results.

III.9.9.1. Calibration curve using the surrogate standard

	Relative area $= \left(\frac{\text{Area for analyte X}}{\text{Area for surrog. std.}}\right)$	Relative concentration $= \left(\frac{\text{Analyte X conc.}}{\text{Surr. std. conc.}}\right)$
Level 1 calibration solution	(5200/1800) = 2.8889	(200 ng/mL)/(100 ng/mL) = 2.000
Level 2 calibration solution	(2392/2049) = 1.1674	(80 ng/mL)/(100 ng/mL) = 0.800
Level 3 calibration solution	(442/1469) = 0.3009	(20 ng/mL)/(100 ng/mL) = 0.200
Level 4 calibration solution	(124/1625) = 0.0763	(5 ng/mL)/(100 ng/mL) = 0.050

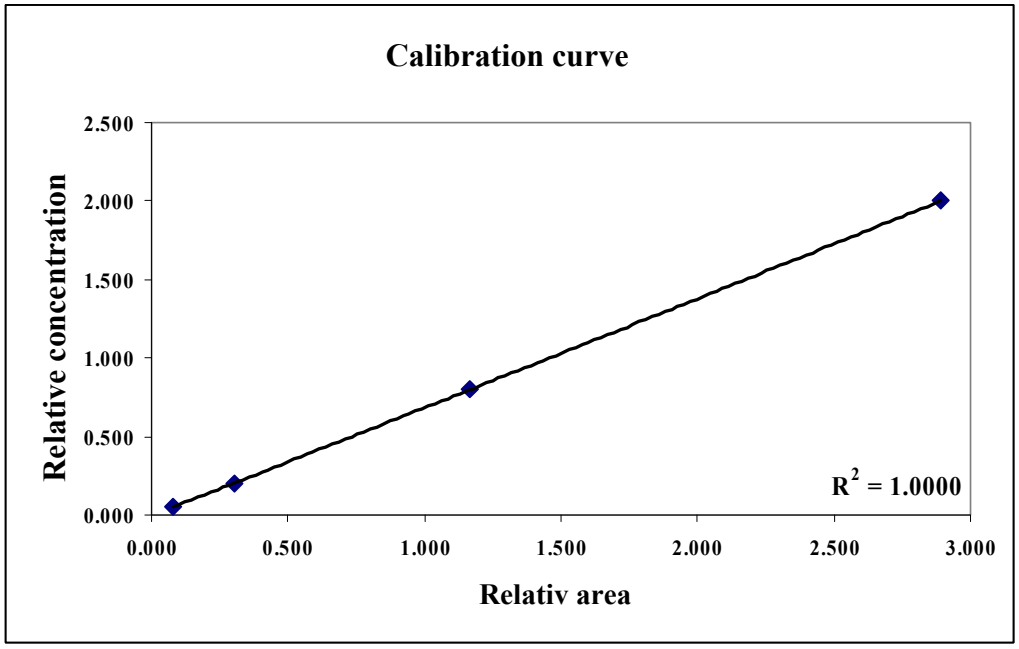


FIG. III-8. Example of a calibration curve.

The calibration curve (see example in Fig. III-8) could correspond to equations of different forms. For example:

$$Y = A\ X^B \qquad \text{(III-94)}$$

where

Y is the relative concentration;
X is the relative area;
A & B are the slope and exponent, respectively.

In this example, the values for A and B that produce a fit are 0.6811 and 1.016, respectively, and R^2 is 1.0000.

Equations for calculations using the surrogate standard

$$Y = A X^{B} \quad \text{(III-95)}$$

$$\text{Relative conc.} = A \times (\text{Relative area})^{B} \quad \text{(III-96)}$$

$$\frac{[\text{Analyte}]\ (\text{ng mL}^{-1})}{[\text{Sur. std.}]\ (\text{ng mL}^{-1})} = A \times \left(\frac{\text{Area for analyte}}{\text{Area for sur. std.}}\right)^{B} \quad \text{(III-97)}$$

$$\frac{\left(\frac{\text{Mass of analyte (ng)}}{\text{Volume (mL)}}\right)}{\left(\frac{\text{Mass of sur. std. (ng)}}{\text{Volume (mL)}}\right)} = A \times \left(\frac{\text{Area for analyte}}{\text{Area for sur. std.}}\right)^{B} \quad \text{(III-98)}$$

$$\frac{\text{Mass of analyte (ng)}}{\text{Volume (mL)}} = A \times \left(\frac{\text{Area for analyte}}{\text{Area for sur. std.}}\right)^{B} \times \left(\frac{\text{Mass of sur. std. (ng)}}{\text{Volume (mL)}}\right) \quad \text{(III-99)}$$

$$\frac{\left(\frac{\text{Mass of analyte (ng)}}{\text{Volume (mL)}}\right)}{\left(\frac{\text{Wt. of sample (g)}}{\text{Volume (mL)}}\right)} = A \times \left(\frac{\text{Area for analyte}}{\text{Area for sur. std.}}\right)^{B} \times \frac{\left(\frac{\text{Mass of sur. std. (ng)}}{\text{Volume (mL)}}\right)}{\left(\frac{\text{Wt. of sample (g)}}{\text{Volume (mL)}}\right)} \quad \text{(III-100)}$$

$$[\text{Analyte}]\ (\text{ng g}^{-1}) = A \times \left(\frac{\text{Area for analyte}}{\text{Area for surr. std.}}\right)^{B} \times \left(\frac{\text{Mass of sur. std. (ng)}}{\text{Wt. of sample (g)}}\right) \quad \text{(III-101)}$$

$$[\text{Analyte}](\text{ng g}^{-1}\ \text{dry wt.}) = A \times \left(\frac{\text{Area for analyte}}{\text{Area for sur. std.}}\right)^{B} \times \left(\frac{\text{Mass of sur. std. (ng)}}{\text{Wt. of sample (g)}}\right) \times \left(\frac{100}{\text{Dry wt. (\%)}}\right) \quad \text{(III-102)}$$

III.9.9.2. Examples of calculations

Consider the following laboratory data:

Example A:

Sample	=	sediment
Sample weight	=	10.82 g wet weight
Dry weight percentage	=	12.9%
Surrogate standard added	=	0.100 mL
[Surrogate standard]	=	1000 ng/mL
Final volume of extract	=	~1 mL
Internal standard added	=	0.100 mL
[Internal standard]	=	1000 ng/mL

Taking the information from ‘Calibration curve using the surrogate standard’ and example A, consider the following chromatogram:

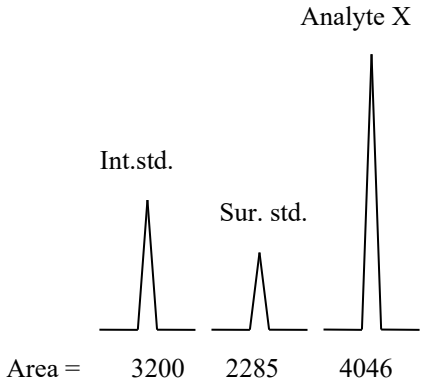


FIG. III-9.

The final equation for calculating concentrations using the surrogate standard, as discussed above (Eq. III-102), is:

$$[\text{Analyte}]\ (\text{ng g}^{-1}\ \text{dry wt.}) = A \times \left(\frac{\text{Area for analyte}}{\text{Area for sur. std.}}\right)^{B} \times \left(\frac{\text{Mass of sur. std. (ng)}}{\text{Wt. of sample (g)}}\right) \times \left(\frac{100}{\text{Dry wt. (\%)}}\right) \quad \text{(III-103)}$$

Plugging the data into this equation:

$$[\text{Analyte}]\ (\text{ng g}^{-1}\ \text{dry wt.}) = 0.6811 \times \left(\frac{4046}{2285}\right)^{1.016} \times \left(\frac{100\ (\text{ng})}{10.82\ (g)}\right) \times \left(\frac{100}{12.9}\right) = 87.20\ \text{ng/g dry wt.} \quad \text{(III-104)}$$

In samples assumed to have a low concentration of the target analytes the extract can be concentrated and its volume reduced (e.g. from 1 mL to approximately 0.5 mL) to strengthen the chromatograph signal. This requires that the added amounts of the recovery and internal standards be adjusted accordingly (i.e. halved) to obtain a response similar to that observed for these standards in the calibration chromatograms and thus facilitate a quick visual comparison of recovery, the quality of the injection etc.:

Example B:

Sample	=	sediment
Sample weight	=	10.82 g wet weight
Dry weight percentage	=	12.9%
Surrogate standard added	=	0.050 mL
[Surrogate standard]	=	1000 ng/mL
Final volume of extract	=	~0.500 mL
Internal standard added	=	0.050 mL
[Internal standard]	=	1000 ng/mL

The following chromatogram is for example B:

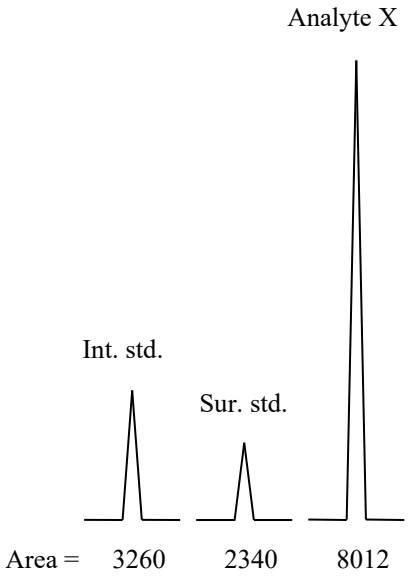


FIG. III-10.

Plugging the data into equation (III-102):

$$[\text{Analyte}]\ (\text{ng g}^{-1}\ \text{dry wt.}) = 0.6811 \times \left(\frac{8012}{2340}\right)^{1.016} \times \left(\frac{50\ (\text{ng})}{10.82\ (g)}\right) \times \left(\frac{100}{12.9}\right) = 85.20\ \text{ng g}^{-1}\ \text{dry wt.} \quad \text{(III-105)}$$

The opposite approach is to be taken if it is suspected that the sample has a high concentration of analytes. The calculations are performed in a similar manner, always using the amount of surrogate standard added to the extract or sample prior to purification or extraction and purification, respectively.

III.9.10. Calculating surrogate standard recovery using the internal standard

The use of internal standards allows one to calculate recovery for surrogate standards and assess the effectiveness of the laboratory method. If surrogate and internal standards are added in the correct amounts, the concentrations of these standards in the final extract and in the calibration solutions will be equal (i.e. 100 ng/mL in each case) and the ratio between them will be maintained regardless of the final volume of the extract. The ratio between the surrogate standard and the internal standard in the calibration solutions represents an ideal recovery of 100%. A simple calculation that uses as a reference any one of these calibration solutions or, better still, uses the average of all the calibration solutions, allows one to calculate the recovery for the surrogate standard in the sample, as illustrated below:

	Area for internal standard (I.S.)	Area for surrogate standard (S.S.)	Ratio (S.S. area/I.S. area)
Calibration solution — Level 1	2500	1800	0.7200
Calibration solution — Level 2	2933	2049	0.6986
Calibration solution — Level 4	2104	1469	0.6981
Calibration solution — Level 5	2304	1625	0.7053
Average	—	—	0.7055
Example A (vol. = 1.0 mL)	3200	2285	0.7141
Example B (vol. = 0.5 mL)	3260	2340	0.7178

$$\text{Sur. std. recovery (\%)} = \frac{0.7141}{0.7055} \times 100 = 101.21\% \quad \text{(Example A)} \qquad \text{(III-106)}$$

$$\text{Sur. std. recovery (\%)} = \frac{0.7178}{0.7055} \times 100 = 101.74\% \quad \text{(Example B)} \qquad \text{(III-107)}$$

III.9.11. Final report on concentrations

As a general rule, the final report should give these concentrations in ng/g dry weight to three decimal places. The qualifiers commonly used to characterize the data are listed in Table III-3.

TABLE III-3. QUALIFIERS COMMONLY USED TO CHARACTERIZE DATA

Qualifier		Description
ND	=	Not detected
J	=	Below the method detection limit
NA	=	Not applicable
Q	=	Result out of control
I	=	Interference
B	=	Contamination present in the blank
D	=	Dilution
EC	=	Exceeds calibration range

The laboratory must have a system for taking corrective action that is activated whenever data quality is compromised and the precision and accuracy targets listed in Table III-4 are not met.

TABLE III-4. PRECISION AND ACCURACY TARGETS

Data quality parameters	Frequency	Targets[(a)]
Surrogate standards	In every sample	Recovery between 40% and 120% of the added amount
Accuracy Spiked blank Spiked sample	5% of the samples in an analytical batch (1:20)[(a, b)]	Recovery between 40% and 120% of the added amount
Precision Duplicate samples Duplicate spiked blank Duplicate spiked sample	5% of the samples in an analytical batch (1:20)[(a, b)]	Relative percent difference between concentrations of the same analyte less than 25%

(a) At least one per analytical batch
(b) Can be disregarded if insufficient sample is available

REFERENCES

[1] UNITED NATIONS ENVIRONMENTAL PROGRAM, Guidelines for the collection, preparation and analysis of organic contaminants in environmental samples (water, soil/sediments, and biota). Coastal Monitoring Manual for the GEF-REPCar Project. UNEP-Caribbean Environment Programme, CEP Technical Report: 57, UNEP, Kingston (2008).

III.10. NORMALIZATION FACTORS FOR METALS AND METALLOIDS

Concentrations of metals and metalloids would normally covary with grain size and organic matter content in sediments. These pollutants show a greater affinity to fine particles than to the coarse size fraction, while organic matter, besides being highly reactive to these compounds, tends to form a film that coats the sedimentary particles and, being concentrated in the finer grain-size particles, becomes a substrate with a very large surface area compared to sand.

Normalization — the process of adjusting metal and metalloid concentrations to a common characteristic in the sediment — aims to compensate for the natural variability of these concentrations. It takes into account differences in the composition of sediment samples, such as grain size distribution and mineralogy, so that anthropogenic contributions can be identified and quantified. A list of the most common normalization factors is presented in Table III-5.

To compensate for variations in metal concentrations due to changes in grain size, or in the mineral fraction of aluminosilicates (abundant in clay minerals), it is recommended to normalize metal concentrations to those of reference elements such as Al, Li or Ti. One of the conditions for deciding which reference element is the most appropriate for normalizing metal concentrations is that there is a significant correlation between the content of this reference element and the abundance of a specific grain size fraction, such as clays, silts, sands.

There are several methods of normalizing by reference elements. The first is to perform a regression analysis between Al, Fe or Li and the metal concentration, using 95% confidence bands [1], where values outside the confidence bands represent anomalous enrichment and, thus, likely contamination (see examples in [2]). Another method involves dividing the metal content by the Al or Li concentration, enabling a comparison of enrichment levels based on the depth in the sediment core (in other words, time). However, this produces a dimensionless value which is not easy to interpret.

One of the most common uses of reference elements when normalizing metal concentrations is in the calculation of the enrichment factor (EF; [3]) using Eq. (III-108):

$$\mathrm{FE} = \left(\frac{\mathrm{M_i^+}}{\mathrm{Al_i}}\right) \Big/ \left(\frac{\mathrm{M_{bckg}^+}}{\mathrm{Al_{bckg}}}\right) \qquad \text{(III-108)}$$

where:

$[M^+_i]$ and $[Al_i]$ are the concentrations of metals and Al (or an alternative reference element) respectively at the i-th depth of the core, and $[M^+_{bckg}]$ and $[Al_{bckg}]$ are the natural or pre-anthropogenic concentrations of the metals and Al (or an alternative reference element) established for the sampling site.

One of these alternative reference elements for normalization is Fe, although it is not always found in cores at sufficient levels due to its potential diagenetic mobility caused by changes in redox conditions in sediments (see [4] for more information). An analysis of the correlation between the concentration levels of Fe (or Mn, another redox-sensitive element) and of metal pollutants may be helpful in determining whether the metal enrichment in the upper layers of a sediment core is the result of anthropogenic pollution or of the formation of Fe and Mn oxy-hydroxides due to diagenetic remobilization.

Organic matter is an important constituent of sediments owing to its high reactivity with organic and inorganic components, including ^{210}Pb and metal pollutants. A variation in organic matter content in the strata of sediment cores can therefore create apparent changes in ^{210}Pb and heavy metal profiles, even if rates of influx have not changed. Determination of the organic matter in the sediments (estimated, for example, using loss on ignition, as discussed in Annex III.2, or by determining total organic carbon, as discussed in Annex III.7) can be used to normalize the concentrations of metallic pollutants with the aid of regression analysis and of confidence bands (as described above for the reference elements) as well as to correct the concentrations of ^{210}Pb and pollutants of interest by calculating the concentrations of ^{210}Pb free of organic matter, as shown in Eq. (III-111):

$$\left[M^{+},\, ^{210}Pb\right]_{\text{normalized}} = \left[M^{+},\, ^{210}Pb\right] \times \left[(100 - OM)/100\right] \qquad \text{(III-109)}$$

where $[M^{+}, {}^{210}Pb]_{\text{normalized}}$ are the normalized values for for the heavy metal concentration or ^{210}Pb activity, [M+, ^{210}Pb] are the measured values for the heavy metal concentration or ^{210}Pb activity, and OM are the percentages of organic matter.

Although some metals can be associated with carbonate minerals, the sedimentary fractions with the highest capacity for concentrating metals are clay minerals, organic matter, and Mn and Fe oxy-hydroxides [5], while quartz, feldspars and carbonates are some of the mineral constituents that are largely chemically inert [6]. The carbonate content is generally considered to be a diluent of metal and metalloid concentrations in sediments, and it is therefore important to assess whether an apparent drop in the trends of these pollutants is the result of a decrease in their deposition in the sediment or an increase in the concentrations of carbonates. One strategy for performing this assessment is to check if there is a significant correlation ($p < 0.05$) between the concentration levels of carbonates (estimated, for example, using loss on ignition, as discussed in connection with PPI_{950} in Annex III.2, or by determining total organic carbon, as discussed in Annex III.7) and the concentration levels of pollutants and then normalize the concentrations of the elements by calculating the dilution factor [5], as shown in equations (III-110) and (III-111):

$$\text{Dilution factor} = 100/(100 - \text{carbonates (\%)}) \qquad \text{(III-110)}$$

$$\text{Normalized concentration} = (\text{dilution factor}) \times (\text{metal concentration, mg/kg}) \qquad \text{(III-111)}$$

TABLE III-5. NORMALIZATION FACTORS FOR METALS AND METALLOIDS *(based on [7])*.

Factor	Indicator	Role
Textural		
Grain size <2000 μm	Grain size variability of metal-bearing minerals and compounds	Determines sorting and depositional patterns of metals
Sand 2000–63 μm	Coarse-grained, metal-poor minerals/compounds	Diluent of metal concentrations
Mud <63 μm	Silt and clay size metal-bearing minerals/compounds	Main concentrators of metal elements
Clay <2 μm	Metal-rich clay minerals	Accumulator of metals, except in sediments derived from glacial erosion of igneous rocks.
Chemical		
Si	Amount and distribution of metal-poor quartz	Diluter of trace-metal concentrations (mainly in coarse-grained form)
Al	Al-silicates, used to compensate for grain size variability of fine Al-silicates (silts and clays) rich in metals	Chemical tracer of Al-silicates, particularly in clay minerals
Fe	Sludges and clays rich in metals and in Fe-bearing minerals (hydrous oxides)	Tracer for Fe-rich clay minerals
Sc	Sc structurally combined in clay minerals	Tracer of clay minerals which are concentrators of trace metals
Cs	Cs structurally combined in clays and feldspars	Tracer of clay minerals which are concentrators of trace metals
Li	Li structurally combined in clay minerals and micas	Tracer of clay minerals, particularly in sediments containing Al-silicates in all size fractions
Ti	Ti present in stable primary minerals (rutile, ilmenite, titanite)	Indicative of terrigenous source, tracer of heavy minerals; present mainly in sands and silts.
Organic carbon	Organic matter associated with fine-grained material	Frequent accumulator of metals, such as Hg and Cd, and organic pollutants

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