HELCOM

Guidelines for the determination of persistent organic compounds (POPs) in seawater

1. Introduction

These guidelines concentrate on the sampling and extraction of lipophilic persistent organic pollutants from seawater and also address special aspects of the sampling matrix. Those pollutants comprise the group of polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons (e.g., HCH, HCB, DDT group, chlorinated biphenyls (PCBs)).

Usually, similar analytical methods are used for the determination of lipophilic pollutants in extracts from water samples and from sediments. Therefore, it is meaningful to harmonize analytical procedures and to refer to the respective references. (see 7. CHROMATOGRAPHIC DETERMINATION)

However, it should be taken into consideration (e.g., for calibration) that the relative concentrations of the individual pollutants are different in water and sediment samples which is basically attributed to the compound's polarity and, thus, their octanol/water partition coefficient (log K_{ow}; Kow = Concentration in octanol phase / Concentration in aqueous phase). Thus, in water samples the more hydrophilic compounds with log Kow values of 3 to 4 predominate (e.g., 2- and 3-ring aromatics and HCH isomers), while in sediments and biota pollutants with log Kow values >5 are enriched (4- to 6-ring aromatics, DDT group, PCBs).

These guidelines provide advice for the analysis of lipophilic persistent organic pollutant (POPs) in total seawater which basically includes the following steps:

- sampling and extraction of the water;
- clean-up; and
- analytical determination.

The extraction of the POPs simultaneously enables enrichment of the analytes which is a crucial step in the procedure as the expected concentrations in seawater are often only in the pg l^{-1} range. Extraction and enrichment are usually conducted through solid phase extraction (SPE) or liquid-liquid extraction (LLE).

Determination depends on the chemical structure of the compounds. PAHs can be separated by high performance liquid chromatography (HPLC) with fluorescence detection or gas chromatographic (GC) separation with flame ionization (FID) or mass spectrometric (MS) detection (Fetzer and Vo-Dinh, 1989; Wise et al., 1995). Chlorinated hydrocarbons are generally analysed by gas chromatographic (GC) separation with mass spectrometric (MS) detection.

All steps of the procedure are susceptible to insufficient recovery and contamination. Therefore, regular quality control measures must be applied to monitor method performance. These guidelines are intended to encourage and assist analytical chemists to critically reconsider and improve established methods and associated quality control measures, where necessary.

These guidelines are not intended as complete laboratory manual. If necessary, guidance should be sought from specialized laboratories. Laboratories should demonstrate validity of each methodological step. Moreover, use of an alternative method, carried out concurrently to the routine procedure, is recommended for validation. The participation in analytical proficiency tests is also highly recommended.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part in intercomparison studies or proficiency testing schemes.

2. Sampling and storage

Plastic materials should not be used for sampling and storage due to the risk of adsorption of target compounds onto the container material, in particular for the highly lipophilic compounds such as the 4- to 6-ring containing aromatic hydrocarbons or DDT and higher chlorinated PCB congeners. Therefore, seawater samples should not be stored longer than 2 h and should not be transferred into further containers before extraction. It is highly recommended to extract the water sample as soon as possible after sampling without further manipulation. It is also recommended to extract directly in the sampling device. Extracts in organic solvents are less susceptible to adsorption onto surfaces.

Sampling bottles should be cleaned with detergent, water and subsequently with organic solvents such as acetone, hexane or pentane before use. It is recommended to obtain blank samples during every sampling campaign for the cleaned sampling bottles using the extraction solvent which is then further processed as described below (see 3. BLANKS AND CONTAMINATION).

3. Blanks and contamination

Basically, care should be taken to avoid contaminations during sampling, extraction and clean-up.

Concentrations of the PAHs and chlorinated hydrocarbons in seawater are very low. Therefore, possible blank and contamination problems are more pronounced for seawater samples than for sediment samples.

In order to reduce blank and sample contaminations to a minimum it is strongly recommended to pretreat all used glassware, solvents, chemicals, adsorption materials, etc., as described as follows:

- Glassware should be thoroughly washed with detergents and rinsed with an organic solvent prior to use. Further cleaning of the glassware, other than calibrated instruments, can be carried out by heating at temperatures > 250 °C.
- All solvents should be analyzed for impurities by concentrating to 10 % of the regular final volume. This concentrate is then analysed similarly to a sample by HPLC or GC. The solvent blank should not

contain target analyte or other interfering compounds in higher concentrations than specified through the laboratory.

- All chemicals and adsorption materials should be analyzed for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glassfiber thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used.

Storage of these supercleaned materials for a longer period is not recommended, as laboratory air might contain PAHs which can adsorb onto these materials. Therefore, contaminated blank samples might occur despite precautionary measures due to contamination from the air. The most volatile compounds, in particular naphthalene and phenanthrene, usually are the highest contaminations in blank samples (Gremm and Frimmel, 1990). Therefore, if possible, critical steps should be done in a clean bench.

4. Pre-treatment

For the extraction of total water samples, no pre-treatment is necessary.

For the separate analysis of the suspended particulate material (SPM) and the solute phase both must be separated through either filtration or centrifugation. Due to the additional steps, this operation affords a number of additional quality control measures (adsorption losses, contamination problems).

Filtration is done by GF/F glass fibre filters. As flat-bed filters have a very limited capacity, the use of coiled glass fibre filters is recommended for volumes larger than 10 l and water samples with high amounts of suspended matter. A pump is necessary to force the water through the filter.

Centrifugation requires a high volume centrifuge which must be operable onboard. Centrifuges with a throughput of about 1 $m^3\ h^{-1}$ are commercially available and are often used for sampling the SPM. However, separation efficiency should be analyzed in advance.

However, validation of the phase separation procedure is difficult. Therefore, it is recommended to determine the target compounds in the total water sample for monitoring purposes and to determine in addition the amount of SPM in the water for reference or normalization purposes.

The sampled SPM is processed similar to sediment samples (see Annex B-13, Appendix 1, 2) and the solute phase similar to the total water sample.

5. Extraction

The volume of the water sample is the most important parameter influencing the limit of detection of the method. As POP concentrations of even less than 10 pg l^{-1} are observed in seawater, volumes of 10 l to 100l need to be sampled and extracted in order to obtain sufficiently high detector signals and to discriminate from contamination problems.

Basically, there are two different extraction procedures in use: solid phase extraction (SPE) and liquid-liquid extraction (LLE) which, however, do not necessarily yield in comparable results as the extraction principles are different (Sturm et al., 1998, Gomez-Belinchon et al., 1988).

With SPE large volumes of water samples with up to 1000 l can be extracted and, moreover, a phase separation step can be incorporated to obtain separate samples for SPM and the solute phase. However, this method requires longer sampling time and a complex instrumentation.

LLE is a classical extraction technique and a lot of experience is available. The robustness of the principle is proven. With respect to potential limitations in sample volume a number of techniques have been described for the extraction of only 10 l and 100 l sample water on a routine basis with sufficient extraction of the target compounds (Gaul and Ziebarth, 1993; Theobald et al., 1990). Due to the robustness of the

method and less complex instrumentation necessary, LLE might be considered as preferential for routine monitoring purposes of lipophilic organic contaminants.

5.1 Solid phase extraction

The extraction device usually consists of a filter holder, an adsorption column filled with an adsorbing material (e.g., XAD resin, C18 modified silica gel), a pump which forces the water sample through the column, a flow meter, an electronic control unit, and a power supply. Sampling can be done either by deploying the whole extraction device into the water (*in situ* pumping) or by pumping the water with an additional pump onboard which is then forced through the extraction device. A suitable *in situ* system is described in detail in Patrick et al. (1996). After sampling, the columns should be stored at 4 °C and the filters at –20 °C.

For elution of the target compounds organic solvents are used such as acetone or acetonitrile to which internal standards are added prior to elution. The obtained extract should be cleaned-up (see 6 clean-up) and analysed chromatographically (see 7 chromatographic determination).

Analytical procedures for the use of XAD-2 adsorption resins are published by the IOC (1993), Ehrhardt (1987) and Bruhn and McLachlan (2001).

Although the SPE technique has many advantages, one has to be aware of some problems. Especially for large volume sampling, validation of the method is extremely difficult and has not yet been achieved. Some publications have shown that the extraction efficiency is dependent on, e.g., the amount and kind of humic substances which can complex lipophilic compounds (Johnson et al., 1991; Kulovaara, 1993; Sturm et al., 1998).

5.2 Liquid-liquid extraction

The volume of water sampled (10 l, 20 l, or 100 l) usually depends on the expected concentrations of the compounds to be analysed in the samples. For remote sea areas with expected concentration of not more than 10 pg l−1 a volume of 100 l is recommended. The technique and principles are independent of the sample volumes. Details of the sampling and extraction procedures are described by Gaul and Ziebarth (1993) for a 10 l sampler and by Theobald et al. (1990) for a 100 l sampler.

The all-glass bottle sampler is usually fixed in a stainless steel cage and lowered by a hydrographic wire to the sampling depth and then opened. After filling, the sampler is brought on deck of the ship and immediately extracted with a non-polar solvent such as pentane or hexane. Prior to extraction, an appropriate internal standard (e.g., deuterated PAHs, ε-HCH, PCB 185) is added to the water sample. After extraction and phase separation, the organic extract is collected and dried over Na_2SO_4 and concentrated at ambient or mild/gentle vacuum conditions to about 1 ml in a rotary evaporator. Further evaporation is done under a gentle stream of nitrogen.

Extracts should be stored refrigerator 4°C and in the dark.

6. Clean-up

Interferences arising from matrix compounds in seawater samples are generally smaller than in sediment or biota samples. Nevertheless, the crude extracts require a clean-up before chromatographic separation and determination. The clean-up usually dependents on the compounds to be analysed, the sample, the determination method used and the concentration range expected. For all GC methods, it is essential to remove polar and non-volatile compounds in order to protect the GC column from rapid destruction. A detection system with low selectivity (e.g., GC-FID) needs a better clean-up than a detector with a high selectivity such GC-MS or even GC-MS/MS. HPLC with fluorescence detection (for PAH analyses) is highly selective, but sensitive to petrogenic aromatic compounds (from an oil spill) in the sample.

The clean-up procedure presented here makes use of short silica gel chromatography columns and can be applied with any subsequent analytical method: HPLC, GC or GC-MS. The method is simple and in most cases sufficient for the analysis of PAH and chlorinated hydrocarbon in seawater (ICES, 1996, 1997, 1999).

A 3 ml glass column with glass fibre frit (commercially available for SPE) is filled with 500 mg silica gel which was dried for 2 h at 200 °C and subsequently washed with 30 ml CH_2Cl_2 and 30 ml hexane. The sample extract concentrated to 500 µl is applied onto the column and eluted with 5 ml of CH_2Cl_2/hexane (15/85 v/v) and afterwards with 5 ml of acetone. Fraction 1 contains the lipophilic compounds of interest such as PAHs and chlorinated hydrocarbons (HCB, HCH) and can be used for GC-MS determination after further concentration to about 50 to 300 µl. Newer allow the solvent to evaporate to dryness.

If the water sample is rich in biological material (algae) or if the required detection limits are below 10 pg l^{-1} additional clean-up steps such as LC-GPC might be necessary.

7. Cromatographic determination

Details for the chromatographic determinations are comprehensively described in the 1996 ACME report (ICES, 1996) for chlorobiphenyls in sediments (GC-MS), the 1997 ACME report (ICES, 1997) for PAHs in sediments (HPLC-Fluorescence detection, GC-FID and GC-MS), and the 1998 ACME report (ICES, 1999) for PAHs in biota (HPLC and GC-MS).

More detailed information for the chromatographic separation and detection of PAH and chlorinated hydrocarbons can be obtained from Annex B-13, Appendix 1 and 2.

7.1 Gas chromatography-mass spectrometry

GC-MS is highly selective and, therefore, it is strongly recommended to use it for detection. Due to the required sensitivity, the mass spectrometric detector must be operated in the selected ion mode (SIM) to achieve sensitivities in the range of 1 pg to 10 pg for most compounds. Ion-trap instruments can be operated in full-scan mode and are basically as sensitive as quadrupole detectors. However, the sample matrix may give rise to considerable loss of sensitivity.

With GC-MS, detection limits of 5–30 pg l^{-1} can be reached with water sample volumes of 10 l to 100 l. In most cases, it is not the absolute signal strength of the detector which limits the detection; therefore, the injection of a larger aliquot of the analysis solution would not improve it. For some compounds, blank values are the limiting parameter (especially naphthalene and phenanthrene and, to a lesser extent, other PAHs); for this, only a larger sample volume can improve the detection limits. Many other compounds do not exhibit blank problems, if appropriate care is applied; for these, matrix noise often limits the detection. For such situations, only a better clean-up (e.g., HPLC, GPC) or a more specific detection method (GC-NCI-MS or GC-MS/MS) will improve the detection limit.

For the mass spectrometric detection for highly chlorinated compounds negative chemical ionization (CI-) is useful (e.g., HCB, PCBs with five or more Cl atoms, HCH) and yields in high sensitivity and selectivity for these compounds. Application of tandem mass spectrometry (MS/MS), yields in even higher selectivity than single MS due to further reduction of matrix effects.

Some MS/MS transitions for the detection of selected chlorinated hydrocarbons are listed in Table 1 in Appendix 2 to Annex B-12: Technical note on the determination of polycyclic aromatic hydrocarbons in biota, from the full "Guidelines".

7.2 Quantification

Automatically processed chromatograms should be reviewed if, e.g., the baseline is set correctly. Because the separation of the peaks is often incomplete in HPLC analysis, the use of peak heights is recommended for quantification. In case of GC techniques, peak areas should be used.

For calibration purposes a multilevel calibration with at least five concentration levels is recommended. The calibration curve should be linear and cover the working range. Usually, the response of FID, UV and fluorescence detectors exhibit linearity over a large range.

Since mass spectrometric detectors often lack sufficient linear response, the use of stable isotopes is a prerequisite. Furthermore, the response of PAHs in standard solutions is often much lower than in sample extracts. A combination of different methods, e.g., use of internal standards and standard addition, might give reliable quantitative results.

Obtained calibrations should be regularly validated in terms of precision and accuracy.

Prior to running a series of samples and standards, the GC or HPLC systems should be equilibrated by injecting at least one sample extract. In addition, standards used for multilevel calibrations should be regularly distributed over the sample series so that matrix- and non-matrix-containing injections alternate. A sample series should include:

- a procedural blank,
- a laboratory reference material,
- at least five standards,
- one standard sample treated similarly to the samples for determination of the recovery

The limit of quantification usually depends on the purpose of the investigation. The limit of quantification that can be achieved depends on the blank sample, the sample matrix, concentrations of interfering compounds, and the volume of sample water. However, a limit of quantification of 2 ng g^{-1} (dry weight) or better should be attained for single compound analysis. A method for calculating the limit of quantification can be obtained from a QUASIMEME advice (Topping et al., 1992).

The typical concentration ranges of PAHs and other POPs in seawater can be found in HELCOM assessments (HELCOM, 2003a, 2003b).

8. Quality assurance

A number of measures should be taken to ensure sufficient quality of the analysis. Six main areas can be identified:

1. extraction efficiency and clean-up;
2. calibrant and calibration;
3. system performance;
4. long-term stability;
5. internal standards; and
6. frequent participation in interlaboratory proficiency testing schemes (e.g. QUASIMEME two times a year, www.quasimeme.org).

8.1 Extraction efficiency and clean-up

Extraction efficiency and clean-up can be controlled by analysing reference materials (Annex B-7). To determine the recovery rates of the clean-up and concentration steps, it is recommended to pass a standard solution through the entire procedure.

If major losses have occurred, the results should not be reported.

CB29 is suggested as a recovery standard because, owing to its high volatility, losses due to evaporation are easily detected. CB29 elutes late from alumina and silica columns. Small peaks that may be present in the

gas chromatogram at the retention time of CB29 do not hinder use of this CB because the recovery standard only indicates major errors in extraction or clean-up.

For GC/MS analysis, labelled CBs might be used as recovery standards. This allows correction for recovery, provided that each chlorination stage is represented.

8.2 Calibrant and calibration

Basically, calibration solutions should be stored in ampoules at a cool, dark place. Weight loss during storage should be recorded for all standards.

For PAH and CB determination preferentially calibration solutions from certified crystalline PAHs should be used. However, the laboratory should have the appropriate equipment and expertise to handle these hazardous crystalline substances. Alternatively, certified PAH solutions, can be used. Preparation of two independent stock solutions allows cross-checks of the standard solutions if necessary.

After clean-up and before GC analysis, both in PAH and CB analysis, an additional internal standard is added for volume correction, such as CB29.

8.3 System performance

The performance of the HPLC or GC system can be monitored through regularly analyzing the resolution of two closely eluting PAHs or CB compounds. A decrease in resolution indicates deteriorating HPLC or GC conditions.

The signal-to-noise ratio of a low concentrated standard gives information on the condition of the detector. For example, a dirty MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio.

8.4 Long-term stability

One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected PAH and CB compounds, e.g., fluoranthene (stable results), pyrene (sensitive to quenching), benzo[a]pyrene (sensitive to light). If warning limits are exceeded, the method should be checked for possible errors and the obtained sample results should not be reported.

If available, a certified reference material (CRM) should be analysed regularly and in particular, if the procedure was changed.

Each laboratory analysing PAHs and CBs in water should participate in interlaboratory analytical performance tests on a regular basis, e.g. QUASIMEME are providing two interlaboratory proficiency tests on most hazardous substances per year (www.quasimeme.org).

8.5 Internal standards

Internal standards should be added to all standards and samples either in a fixed volume or by weight and should not interfere with the target analytes.

A number of deuterated PAH compounds were proven to be suitable for GC-MS as well as for HPLC analysis. For GC-MS analysis it is recommended to add four internal standards representing the different ring-sizes of the PAH compounds.

The following compounds can be used (Wise et al., 1995):

- for HPLC analysis: phenanthrene-d10, fluoranthene-d10, perylene-d12, 6-methyl-chrysene;
- for GC-MS analysis: naphthalene-d8, phenanthrene-d10, chrysene-d12, perylene-d12;
- for GC-FID analysis: 1-butylpropylene, m-tetraphenyl.

Suggested internal standards for CB analysis are the CB 29, 112, 155, 198 or all 2,4,6-substituted CB congeners; alternatively, 1,2,3,4-tetrachloronaphthalene can be used.

9. References

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