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Water quality — Sampling —

Part 3: Guidance on the preservation and handling of water samples

Qualité de l'eau — Échantillonnage —

Partie 3: Lignes directrices pour la conservation et la manipulation des échantillons d'eau

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Foreword

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

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

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

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

ISO 5667-3 was prepared by Technical Committee ISO/TC 147, *Water quality* Subcommittee SC 6, *Sampling (general methods)*.

This third edition cancels and replaces the second edition (ISO 5667-3:1994), which has been technically revised.

ISO 5667 consists of the following parts, under the general title *Water quality — Sampling*:

— *Part 1: Guidance on the design of sampling programmes*

— *Part 2: Guidance on sampling techniques*

— *Part 3: Guidance on the preservation and handling of water samples*

— *Part 4: Guidance on sampling from lakes, natural and man-made*

— *Part 5: Guidance on sampling of drinking water and water used for food and beverage processing*

— *Part 6: Guidance on sampling of rivers and streams*

— *Part 7: Guidance on sampling of water and steam in boiler plants*

— *Part 8: Guidance on the sampling of wet deposition*

— *Part 9: Guidance on sampling from marine waters*

— *Part 10: Guidance on sampling of waste waters*

— *Part 11: Guidance on sampling of groundwaters*

— *Part 12: Guidance on sampling of bottom sediments*

— *Part 13: Guidance on sampling of sludges from sewage and water-treatment works*

— *Part 14: Guidance on quality assurance of environmental water-sampling and handling*

— *Part 15: Guidance on preservation and handling of sludge and sediment samples*

— *Part 16: Guidance on biotesting of samples*

— *Part 17: Guidance on sampling of suspended sediments*

— *Part 18: Guidance on sampling of groundwater at contaminated sites*

— *Part 19: Guidance on sediment sampling in marine areas*

Introduction

This part of ISO 5667 is intended to be used in conjunction with ISO 5667-1 and ISO 5667-2, which deal with the design of sampling programmes and sampling techniques respectively.

Water quality — Sampling —

Part 3: Guidance on the preservation and handling of water samples

1 Scope

This part of ISO 5667 gives general guidelines on the precautions to be taken to preserve and transport all water samples including those for biological analyses but not those intended for microbiological analysis.

These guidelines are particularly appropriate when spot or composite samples cannot be analysed on-site and have to be transported to a laboratory for analysis.

2 Normative references

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

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 5667-14:1998, *Water quality — Sampling — Part 14: Guidance on quality assurance of environmental water sampling and handling*

ISO 5667-16:1998, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO Guide 34:2000, *General requirements for the competence of reference material procedures*

3 Preservation of samples

3.1 General considerations

Waters, particularly fresh waters, waste waters and groundwaters, are susceptible to changes as a result of physical, chemical or biological reactions which may take place between the time of sampling and the commencement of analysis. The nature and rate of these reactions are often such that, if precautions are not taken during sampling, transport and storage (for specific determinands), the concentrations determined may be different to those existing at the time of sampling.

The extent of these changes is dependent on the chemical and biological nature of the sample, its temperature, its exposure to light, the nature of the container in which it is placed, the time between sampling and analysis, and the conditions to which it is subjected, for example agitation during transport. Further specific causes of variation are as follows.

a) The presence of bacteria, algae and other organisms can consume certain constituents of the samples. These organisms can also modify the nature of the constituents to produce new constituents. This biological activity affects, for example, the concentrations of dissolved oxygen, carbon dioxide and compounds, of nitrogen, phosphorus and sometimes silicon.

b) Certain compounds can be oxidized by the dissolved oxygen present in the samples or by atmospheric oxygen (e.g. organic compounds, Fe (II) and sulfides).

c) Certain substances can precipitate out of solution [for example calcium carbonate, metals and metallic compounds such as $Al(OH)_3$] or be lost to the vapour phase (for example oxygen, cyanides and mercury).

d) The pH and conductivity can be modified and the dissolved carbon dioxide changed by the absorption of carbon dioxide from air.

e) Dissolved metals or metals in a colloidal state, as well as certain organic compounds can be irreversibly adsorbed onto the surface of the containers or solid materials in the samples.

f) Polymerized products can depolymerize and conversely, simple compounds can polymerize.

Changes to particular constituents vary both in degree and rate, not only as a function of the type of water, but also, for the same water type, as a function of seasonal conditions.

It should be emphasized that these changes are often sufficiently rapid to modify the sample considerably in a short time. In all cases, it is essential to take precautions to minimize these reactions and, in the case of many determinands, to analyse the sample with a minimum of delay.

Preservation of water samples is necessary for a number of reasons, therefore it is generally necessary to choose, from the various possible methods of preservation, a method that does not introduce contamination.

Fresh waters and groundwaters can be stored more successfully. In the case of potable waters, storage can be solved easily by cooling, because these waters are less susceptible to biological and chemical reactions.

In many cases, if samples are analysed within 24 h, the preservation technique of cooling to between 1 °C to 5 °C is sufficient. Municipal or industrial sewage plant effluents should be preserved immediately after sampling, because of the high biological activities in these samples.

This part of ISO 5667 describes the most commonly used preservation techniques and storage times.

In spite of investigations[4] which have been carried out in order to recommend methods that enable water samples to be stored without changes occurring to their composition, no guidance has been reported that covers all situations. Users of particular test methods and analytical techniques described in International Standards prepared by ISO/TC 147 are encouraged to take into account any relevant guidance offered in this part of ISO 5667 when making decisions in relation to sample preservation and handling for such methods and techniques.

3.2 Precautions to be taken

3.2.1 Container selection

The choice of sample container is of major importance and ISO 5667-2 provides some guidance on this subject. Details of the type of container used for the collection and storage of samples are given in Tables 1 to 4. The same considerations given to this selection of suitable container material should also be given to the selection of cap-liner materials. The guidance given here is to help in the selection of containers for general use.

The containers used to collect and store the samples should be selected after taking into account the following predominant criteria (especially when the analytes are present in trace quantities).

a) Minimizing sample contamination by the container or cap material, for example leaching of inorganic constituents from glass (especially soda glass) and organic compounds and metals from plastics. Some coloured caps may contain significant levels of heavy metals.

b) Ability to clean and treat the walls of the container to reduce surface contamination by trace constituents such as heavy metals or radionuclides.

c) Chemical and biological inertness of the container or cap material in order to prevent or minimize reaction between sample constituents and the container.

d) Containers may also cause changes to constituent concentrations by adsorption or absorption of analytes. Trace metals are particularly susceptible to these effects but other analytes (for example detergents, pesticides, phosphates) may also be affected.

Guidance should be sought from laboratory staff on the selection of sample containers and sampling equipment.

Other factors should also be considered, e.g. resistance to temperature extremes, resistance to breakage, ease of sealing and reopening, size, shape, mass, availability, cost, potential for cleaning and re-use.

Container blanks should always be taken, preserved and analysed as a check on the suitability of the container and preservation procedures (see ISO 5667-14).

3.2.2 Container preparation

3.2.2.1 General

All preparation procedures should be validated to ensure positive or negative interferences do not occur. As a minimum, this should include the analysis of:

a) blanks;

b) samples containing known levels of relevant analytes.

If disposable or single-use containers cannot be used, it is preferable to reserve a set of containers for a particular determinand, thereby minimizing risks of cross-contamination. Care should be taken to prevent a container, formerly holding a sample with a high concentration of a determinand, from contaminating a subsequent sample containing a low concentration of the same determinand.

It may be necessary to wash new containers with water containing a detergent, in order to remove dust and residues of packing materials, followed by thorough rinsing with water of an appropriate quality. The use of cleansing reagents and solvents may cause interferences, e.g. residual contamination by phosphate-containing detergents when undertaking nutrient analyses. If used, all cleaning reagents and solvents should be of an appropriate quality. For the determination of silicon, boron and surfactants, detergents should not be used for cleaning purposes.

3.2.2.2 Detergent-washed plastic or glass containers

The procedure should be as follows.

a) Wash the container and cap with a dilute solution of detergent and water.

b) Rinse thoroughly with tap water.

c) Successively rinse twice with water of an appropriate quality.

d) Drain thoroughly and replace cap.

Automatic dish washing machines may be used for this procedure.

3.2.2.3 Solvent-washed glass containers

WARNING — Organic solvents may be hazardous. Provide suitable handling facilities and handle with care.

The procedure should be as follows.

a) Wash the container and cap with a dilute solution of detergent and tap water.

b) Rinse thoroughly with tap water.

c) Successively rinse twice with water of an appropriate quality and dry.

d) Rinse with acetone of an appropriate quality and drain.

e) Rinse with a suitable solvent of an appropriate quality, dry and immediately replace cap.

The solvent should be compatible with the analytes of interest and the analytical method to be used.

3.2.2.4 Acid-washed containers in plastic or glass

The procedure should be as follows.

a) Wash the container and cap with a dilute solution of detergent and tap water.

b) Rinse thoroughly with tap water.

c) Rinse with an aqueous 10 % nitric acid solution.

d) Drain and completely fill with an aqueous 10 % nitric acid solution.

e) Cap and store for at least 24 h.

f) Empty the container, rinse with water of an appropriate quality and immediately replace cap.

Some manufactures will supply containers with a certificate of cleanliness. Such containers may not need further cleaning or rinsing, provided the manufacturer supplies the containers with caps attached.

Automatic hot acid washers may be used for this procedure.

3.2.3 Filling the container

For samples requiring the determination of physico-chemical determinands, fill the container completely and stopper it in such a way that there is no air space above the sample. This reduces interaction with the gas phase, and minimizes agitation of the sample during transport.

Where samples are frozen as part of their preservation, sample containers should not be completely filled (see 3.2.6).

3.2.4 Handling and preservation of samples for biological examination

The handling of samples for biological examination is different to that for samples requiring chemical analysis. The addition of chemicals to the sample for biological examination can be used for either fixation or preservation of the sample. The term "fixation" is used to describe the protection of morphological structures, while the term "preservation" is used for the protection of organic matter from biochemical or chemical degradation. Preservatives, by definition, are toxic and the addition of preservatives may lead to the death of living organisms. Prior to death, irritation may cause the most delicate organisms, which do not have strong cell walls, to collapse before fixation is complete. To minimize this effect, it is important that the fixation agent

enters the cell quickly. Some preservatives, for instance acid solutions of Lugol, may lead to the loss of some taxonomical groups of organisms, which can be a problem during certain parts of the year in certain areas. This can be addressed by using an additional preservative, such as alkaline solutions of Lugol, during, for example, the summer period when the appearance of silico-flagellates may be frequently observed.

The preservation of samples for biological examination should meet the following criteria:

a) the effect of the preservative on the loss of the organism should be known beforehand;

b) the preservative should effectively prevent the biological degradation of organic matter at least during the storage period of the samples;

c) the preservative should enable the taxonomical groups of organisms to be adequately studied during the storage period of the samples.

3.2.5 Handling and preservation of samples for radiochemical analysis

WARNING — Safety precautions and shielding depend on the activity of the sample.

There is little difference between the handling of samples for radiochemical analysis and the handling of samples for physico-chemical analysis. Safety precautions depend on the nature of the radioactivity of the sample. The preservation techniques for these samples depend on the type of emitter and the half-life of the radionuclide of interest.

3.2.6 Cooling or freezing of samples

The cooling or freezing of samples is only effective if the process is applied immediately after the collection of the samples. This necessitates the use of cool-boxes or refrigerators at the sampling location. Wherever a temperature is given for cooling, the temperature of the sample environment is meant (not the temperature of sample itself).

Simple cooling of the sample (in melting ice or in a refrigerator at a temperature between 1 °C and 5 °C) and storage of the sample in the dark is, in most cases, sufficient to preserve the sample during transport to the laboratory. Cooling cannot be considered as a means of long-term storage, particularly in the case of wastewater samples (see Table 1). The sample should be kept and stored at a temperature lower than that observed during the process of collection or filling of the container.

A small volume of ice does not have much cooling effect upon a large volume of warm water. Where a sample contains determinands that are likely to be affected by biological activity, and where preservation on-site is not possible, the temperature of the sample should be taken immediately on arrival at the laboratory. This is particularly important when samples require transporting for several hours. Samples should be analysed or cooled immediately at receipt in the laboratory. During transport, the temperature of the cooling system should be monitored.

In general, storage of samples at temperatures below – 20 °C allows the samples to be stored for longer periods of time. If samples are to be frozen, the container should be made of plastic and not be filled completely. This reduces the risk to the sample container from being damaged. For some analytes, such as nutrient determinands, freezing of the sample is the preferred method of preservation. In these cases, quick-freezing with dry ice is a satisfactory procedure. The freezing of samples is not an appropriate procedure for samples requiring analysis of volatile substances or if samples contain cells or bacteria or microalgae, which can fracture and lose cell constituents during the freezing process. Nevertheless, it is necessary to control the freezing and thawing technique in order to return the sample to its initial equilibrium after thawing. In this case, the use of plastic containers (for example polyvinyl chloride or polyethene) is strongly recommended. For thawing of samples, see ISO 5667-16.

3.2.7 Filtration or centrifugation of samples

Suspended matter, sediment, algae and other micro-organisms may be removed, either at the time of taking the sample or immediately afterwards, by filtering the sample through membrane filter material (e.g. paper,

polytetrafluoroethylene, glass) or by centrifuging. Filtration is, of course, not applicable if the membrane filter is likely to retain one or more of the constituents to be analysed. It is equally essential that the membrane filter assembly system not be a cause of contamination and be carefully washed before use, but in a manner consistent with the final method of analysis.

Alternatively, the reason for filtering the sample may be to enable the proportion of soluble and insoluble forms of an analyte to be determined (e.g. soluble and insoluble metal fractions).

Decanting the sample is not recommended as an alternative to filtration.

Membrane filters should be used with caution as various heavy metal compounds and organic material may be adsorbed on the membrane filter surface, and soluble compounds (e.g. surfactants) within the membrane filter can be leached out into the sample.

3.2.8 Addition of preservatives

Certain physical and chemical constituents can be stabilized by the addition of selective chemical compounds, either directly to the sample after it has been taken, or beforehand, to the empty container.

Particular reagents, necessary for the specific preservation of certain constituents (e.g. the determination of oxygen, total cyanides and sulfides) require the sample to be preserved on-site.

It is essential that the preservatives used do not interfere with the analysis; tests intended to check their compatibility are necessary in case of doubt. Any dilution of the sample with added preservative solutions should be taken into account during the analysis and calculation of results. It is preferable that the addition of preservatives to samples be made using concentrated solutions so that only small volumes are used. In most cases, this enables the corresponding dilution to be disregarded. The use of solid preservatives, for example sodium hydroxide, is to be avoided as local heating may occur, adversely affecting the sample.

The fact that the addition of these agents can modify or change the chemical or physical nature of the constituents means that these changes are not incompatible with the purpose of later determinations. For example, acidification can solubilize colloidal constituents or solids, and should therefore be used with caution if the aim of the analysis is the determination of dissolved constituents and then only for that purpose. Filtration of the sample prior to the addition of preservative is essential for dissolved ions. Similarly, caution should be applied if the aim of the analysis is to determine the toxicity of the sample to aquatic animals, as certain components, particularly heavy metal compounds, are more toxic in the ionic form. Samples should therefore be analysed as soon as possible.

It is essential to carry out a blank test, particularly in determinations for trace elements, to take into account the possible introduction of an additional quantity of the determinand (for example acids can introduce a significant amount of arsenic, lead and mercury) by the preservatives. In such cases, samples of the preservatives used for the treatment of the water samples should be retained for use in the preparation of blank tests.

3.3 Reagents

WARNING — Certain preservatives (e.g. acids, alkalis, formaldehyde) need to be used with caution. Sampling personnel should be warned of potential dangers and that appropriate safety procedures should be followed.

The following reagents are used for the preservation of samples and shall only be prepared according to individual sampling requirements. Unless otherwise specified, all reagents used should be of at least analytical reagent grade and water should be of at least ISO 3696:1987 Grade 2 purity. Acids referred to in this part of ISO 5667 are the commercially available "concentrated" acids.

All reagents should be labelled with a "shelf life" which should not be exceeded. The "shelf life" represents the period for which the reagent is suitable for use, if stored correctly. Any reagents that are not completely used by the expiry of the "shelf life" date should be discarded.

Check the reagent dispensers periodically and discard any reagent where dispensers are shown to be unsuitable.

Between field trips, reagents should be stored in clean, secure cabinets in order to prevent contamination.

It is essential that all samples requiring the same determinand determination be preserved together.

Each sample should be labelled accordingly after the addition of the preservative, since usually there may be no visible indication as to which samples have been preserved and which have not.

3.3.1 Solids

3.3.1.1 **Sodium thiosulfate pentahydrate**, $Na_2S_2O_3 \cdot 5H_2O$.

3.3.1.2 **Ascorbic acid**, $C_6H_8O_6$.

3.3.1.3 **Sodium hydroxide**, NaOH.

3.3.1.4 **Potassium dichromate**, $K_2Cr_2O_7$.

3.3.1.5 **Copper sulfate**, $CuSO_4$.

3.3.1.6 **Sodium tetraborate**, $Na_2B_4O_7 \cdot 10H_2O$.

3.3.1.7 **Hexamethylenetetramine** (hexamine, urotropine), $C_6H_{12}N_4$.

3.3.2 Solutions

3.3.2.1 **Zinc acetate solution** (ρ = 0,10 g/ml), $C_4H_6O_4Zn$.

3.3.2.2 **Orthophosphoric acid** (ρ = 1,7 g/ml), H_3PO_4.

3.3.2.3 **Hydrochloric acid** (ρ = 1,16 g/ml), HCl.

3.3.2.4 **Nitric acid** (ρ = 1,42 g/ml), HNO_3.

3.3.2.5 **Sulfuric acid** (8 mol/l), H_2SO_4.

3.3.2.6 **Sodium hydroxide solution** (ρ = 0,40 g/ml), NaOH.

3.3.2.7 **Formaldehyde solution** (volume fraction of 37 %) (Formalin), CH_2O.

WARNING — Beware of formaldehyde vapours. Do not store large numbers of samples in small work areas.

3.3.2.8 **Aqueous solution of disodium salt of ethylenediaminetetraacetic** (EDTA) (ρ = 0,025 g/ml), $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$.

3.3.2.9 **Ethanol** (volume fraction of 96 %).

3.3.2.10 **Alkaline Lugol solution**, with sodium acetate.

3.3.2.11 **Acid Lugol solution**, with acetic acid.

3.4 Prolonged storage of samples

The timing of the maximum recommended preservation time before commencement of analysis begins immediately after the sample has been taken.

For certain regulatory situations, there is a requirement for samples to be retained for a prescribed period of time. Notwithstanding the analytical implications of this, this legal requirement takes precedence over the guidance given in this part of ISO 5667.

Where samples are analysed after the maximum recommended preservation time, it is essential that the results be accompanied with a statement to the effect that the analytical results may not be reflective of the concentration present at the time of sampling.

In addition, where laboratories report data analysed after the maximum recommended preservation time, it is essential that the analytical result be accompanied with a statement to the effect that the maximum recommended preservation time has been exceeded.

Prolonged storage of samples may be appropriate if the laboratory can demonstrate that there is no difference between the test result obtained after extended preservation time and the test result obtained within the preservation time given in this part of ISO 5667. Procedures to be used for establishing homogeneity and stability are outlined in ISO Guide 34.

3.5 General guidance

Staff should not be allowed to smoke near samples; in addition, samples should not be placed near any source of engine exhaust.

Open samples should neither be placed (e.g. while samples are being filtered or preserved) near a fan or air conditioner, nor near food and beverages.

Decontamination and cleaning is appropriate if reusable equipment (such as sample scoops) is used between and during use.

The inner surfaces of bottles or caps should not be touched with fingers or other objects.

It is essential that empty bottles be stored and transported with caps tightly in place.

Extraneous matter should be kept out of sample bottles. If a measurement (such as temperature or pH) needs to be made outside of a bottle, then a specific container should be used for that purpose, and the sample used in the measurement discarded. Under no circumstances should a field measurement be made and the sample returned to the sample container that is then subsequently forwarded to a laboratory for analysis.

Samples should be scrutinized for large particles, such as leaves or detritus and if these are observed, the sample should be discarded and a new sample taken.

Preservation reagents should be scrutinized as contamination can sometimes be indicated by, for example, a change in colour. If contamination is suspected, the reagent should be discarded.

4 Recommendations

For samples requiring analysis for certain organic determinands, an initial on-site extraction may be advantageous. Alternative procedures such as on-site adsorption techniques or on-site headspace collection may also be employed where appropriate.

As stated in 3.1, it is impossible to give guidance for storage times or the nature of the sample containers for all preservation techniques. The efficiency of the preservation process depends not only on the constituents that require analysis and their concentration levels, but also on the nature of the sample. In all cases, it is essential that the method of storage be compatible with the analytical technique used. One objective of Tables 1 to 4 is to describe the most common preservation techniques.

Further guidance is given in Table 2 on suitable preservation techniques used in conjunction with several determinands. However, it is not reasonable or logical to combine organic and inorganic determinands, because of the manner these determinations are treated within the laboratory.

The biological determinands are generally numerous and sometimes vary from one biological species to another. For this reason, it is impossible to draw up an exhaustive checklist of all the precautions that should be taken to preserve samples for biological analysis. The information given in Table 3 therefore relates only to certain determinands generally studied for various animal or plant groups.

It should be noted that before carrying out any detailed study, it is essential to choose the determinands of interest.

Table 4 gives techniques generally suitable for the preservation of radioactive samples.

There should be no significant statistical difference between the results of samples analysed immediately following collection and those analysed after preservation. Results should be verified taking into account the method of analysis and the guidance provided in this part of ISO 5667.

The sample volumes listed in Table 1 represent typical volumes required to perform a single determination on the sample. Where more than one method is available for a particular determinant, the sample volumes pertain to the method that requires the maximum sample volume. In some cases, it may be possible to take a smaller volume of sample; however, this should only be undertaken after consultation with laboratory staff.

For a sample that requires the determination of more than one determinand, sometimes it is necessary to take several sub-samples in order to meet the requirements of sample preservation. It is essential that extreme care be taken to avoid cross-contamination which may occur. For example, nitric acid preservation used for a metal sub-sample will contaminate the sub-sample taken for nitrate analysis.

5 Identification of samples

Sample containers should be labelled in a clear and unambiguous manner that is durable.

Additionally, it may be necessary to note, at the time of sampling, details which will enable a correct interpretation of the information provided (for example, date and hour of sampling, name of person sampling, nature and amount of preservatives added). The use of pre-printed labels, forms, etc. can facilitate the practical attainment of these objectives.

Special samples of anomalous material should be clearly marked and accompanied by a description of the observed anomaly. It is essential that samples containing hazardous or potentially hazardous materials, for example acids, be clearly identified as such.

6 Transport of samples

Containers holding samples should be protected and sealed in such a way that samples do not deteriorate and do not lose any of their constituents during transport. Packaging material should protect the containers from possible external contamination and breakage, particularly near the opening of the container, and should not be a source of contamination. During transportation, the samples should be stored according to the guidance given in Tables 1 to 4. In cases where the storage and transportation time exceeds the maximum recommended preservation time before commencement of analysis, whether or not the samples should be analysed should be checked with the client, and if it is decided to proceed with the analysis, the time between sampling and analysis should be reported.

7 Reception of samples

Laboratory staff should establish whether samples underwent cooling during transportation and if possible whether a sample environmental temperature between 1 °C to 5 °C was maintained.

In all cases, and especially when a "chain of custody" process needs to be established, the count of sample containers received in the laboratory should be verified against the number of sample bottles provided for each sample.

Table 1 — Techniques generally suitable for the preservation of samples — Physico-chemical and chemical analysis

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Acidity and alkalinity	P or G	500 Fill container completely to exclude air.	Cool to between 1 °C and 5 °C.	24 h	14 days[c] Samples should preferably be analysed on-site (particularly for samples high in dissolved gases). Reduction and oxidation during storage can change the sample
Acidic herbicides	G with PTFE cap liner or septum	1 000 Do not pre-rinse the empty container with sample; analytes adhere to the wall of the bottle. Do not completely fill sample container.	Acidify to between pH 1 to 2 with HCl and cool to between 1 °C and 5 °C.	2 weeks	Extract sample container as part of the sample extraction procedure. If the sample is chlorinated, for each 1 000 ml of sample, add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to collection.
Adsorbable organic halides (AOX)	P or G	1 000 Fill container completely to exclude air.	Acidify to between pH 1 to 2 with HNO_3, cool to between 1 °C and 5 °C, keep samples stored in the dark.	5 days	
	P	1 000	Freeze to – 20 °C.	1 month	
Aluminium	P acid-washed G or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	
Ammonia, free and ionized	P or G	500	Acidify to between pH 1 to 2 with H_2SO_4, cool to between 1 °C and 5 °C.	21 days	Filter on-site before preservation
	P	500	Freeze to – 20 °C.	1 month	

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Anions (Br, F, Cl, NO_2, NO_3, SO_4 and PO_4)	P or G	500	Cool to between 1 °C and 5 °C.	24 h	Filter on-site before preservation. See also ISO 10304-1.
	P	500	Freeze to – 20 °C	1 month	
Antimony	P acid-washed G acid-washed	100	Acidify to between pH 1 to 2 with HCl or HNO_3.	1 month	HCl should be used if the hydride technique is used for analysis.
Arsenic	P acid-washed G acid-washed	500	Acidify to pH 1 to 2 with HCl or HNO_3.	1 month	HCl should be used if the hydride technique is used for analysis.
Barium	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3.	1 month	Do not use H_2SO_4
Beryllium	P acid-washed or G acid-washed	100	Acidify to between pH 1 to 2 with HNO_3.	1 month	
Biochemical oxygen demand (BOD)	P or G	1 000 Fill container completely to exclude air.	Cool to between 1 °C and 5 °C	24 h	Keep samples stored in the dark. In case of freezing to – 20 °C: 6 months (1 month if < 50 mg/l)[c]
	P	1 000	Freeze to – 20 °C.	1 month	
Boron	P	100 Fill container completely to exclude air.	None required	1 month	6 months[c]
Bromate	P or G	100	Cool to between 1 °C and 5 °C	1 month	
Bromide and bromine compounds	P or G	100	Cool to between 1 °C and 5 °C	1 month	
Bromine residual	P or G	500	Cool to between 1 °C and 5 °C	24 h	Keep samples stored in the dark. The analysis should be carried out on-site, within 5 min of sample collection.
Cadmium	P acid-washed or BG acid-washed.	100	Acidify to between pH 1 to 2 with HNO_3	1 month	6 months[c]

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Calcium	P or G	100	Acidify to between pH 1 to 2 with HNO_3	1 month	
Carbamate pesticides	G solvent-washed	1 000	Cool to between 1 °C and 5 °C.	14 days	If the sample is chlorinated, for each 1 000 ml of sample add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to analysis.
	P	1 000	Freeze to – 20 °C.	1 month	
Carbon dioxide	P or G	500 Fill container completely to exclude air.	Cool to between 1 °C and 5 °C.	24 h	Determination preferably carried out on-site.
Carbon, total organic (TOC)	P or G	100	Acidify to between pH 1 to 2 with H_2SO_4, cool to between 1 °C and 5 °C.	7 days	Acidification to pH 1 to 2 with H_3PO_4 is suitable. If volatile organic compounds are suspected, acidification is not suitable. Analyse within 8 h.
	P	100	Freeze to – 20 °C	1 month	
Chemical oxygen demand (COD)	P or G	100	Acidify to between pH 1 to 2 with H_2SO_4	1 month	6 months[c]
	P	100	Freeze to – 20 °C	1 month	6 months[c]
Chloramine	P or G	500		5 min	Keep samples stored in the dark. The analysis should be carried out on-site, within 5 min of sample collection.
Chlorate	P or G	500	Cool to between 1 °C and 5 °C.	7 days	
Chloride	P or G	100		1 month	

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Chlorinated solvents	G, head-space vials with PTFE caps	250 Fill container completely to exclude air.	Acidify to between pH 1 to 2 with HCl.	24 h	If the sample is chlorinated, for each 250 ml of sample, add 20 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to analysis. For purge and trap, HCl interferes. See specific standard for preservation.
			Cool to between 1 °C and 5 °C	24 h	
Chlorine dioxide	P or G	500		5 min	Keep samples stored in the dark. The analysis should be carried out in the field, within 5 min of sample collection.
Chlorine, residual	P or G	500		5 min	Keep samples stored in the dark. The analysis should be carried out in the field, within 5 min of sample collection.
Chlorite	P or G	500	Cool to between 1 °C and 5 °C	5 min	Keep samples stored in the dark. The analysis should be carried out on-site, within 5 min of sample collection.
Chlorophyll	P or G	1 000	Cool to between 1 °C and 5 °C	24 h	Transport in amber coloured bottles.
	P	1 000	After filtration and extraction with hot ethanol, freeze to – 20 °C.	1 month	
	P	1 000	After filtration, freeze to – 80 °C	1 month	
Chromium	P acid-washed or G acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	6 months[c]
Chromium (VI)	P acid-washed or G acid-washed	100	Cool to between 1 °C and 5 °C	24 h	Reduction and oxidation during storage can change the sample concentration.

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Cobalt	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	6 months[c]
Colour	P or G	500	Cool to between 1 °C and 5 °C	5 days	Keep samples stored in the dark. In case of groundwater, rich with iron(II), analysis should be carried out on-site, within 5 min of sample collection
Conductivity	P or BG	100 Fill container completely to exclude air.	Cool to between 1 °C and 5 °C	24 h	Analysis preferably be carried out on-site
Copper	P acid-washed or G acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	6 months[c]
Cyanide by diffusion at pH 6	P	500	Add NaOH to pH > 12. Cool to between 1 °C and 5 °C.	24 h	
Cyanide easily liberated	P	500	Add NaOH to pH > 12. Cool to between 1 °C and 5 °C.	7 days 24 h if sulphide is present.	Keep samples stored in the dark.
Cyanide, total	P	500	Add NaOH to pH > 12. Cool to between 1 °C and 5 °C.	7 days 24 h if sulphide is present.	14 days[c] Keep samples stored in the dark.
Cyanochloride	P	500	Cool to between 1 °C and 5 °C.	24 h	
Detergents	See "Surfactants"				
Dissolved solids (dry residue)	See "Total solids (Total residues)"				
Fluorides	P but not PTFE	200		1 month	
Heavy metal compounds (except mercury)	P or BG	500	Acidify to between pH 1 to 2 with HNO_3.	1 month	6 months[c]
Hydrazine	G	500	Acidify with HCl to 1 mol/l	24 h	Keep samples stored in the dark.

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Hydrocarbons	G solvent (e.g. pentane) used for extraction	1 000 Do not pre-rinse container with sample; analytes adhere to the wall of the bottle. Do not completely fill sample container.	Acidify to between pH 1 to 2 with H_2SO_4 or with HCl	1 month	Extract on-site where practical.
Hydrogen-carbonates	See "Acidity and alkalinity"				
Iodide	G	500	Cool to between 1 °C and 5 °C.	1 month	
Iodine	G	500	Cool to between 1 °C and 5 °C.	24 h	Keep samples stored in the dark.
Iron(II)	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HCl and exclusion of atmospheric oxygen.	7 days	
Iron, total	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3.	1 month	
Kjeldahl nitrogen	P or BG	250	Acidify to between pH 1 to 2 with H_2SO_4	1 month	Keep samples stored in the dark. 6 months for both techniques[c]
	P	250	Freeze to – 20 °C.	1 month	
Lead	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3.	1 month	6 months[c]
Lithium	P	100	Acidify to between pH 1 to 2 with HNO_3	1 month	
Magnesium	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	
Manganese	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Mercury	BG acid-washed	500	Acidify to between pH 1 to 2 with HNO_3 and addition of $K_2Cr_2O_7$ [0,05 % by mass final concentration].	1 month	Particular care is needed to ensure that the sample is free from contamination.
Monocyclic aromatic hydrocarbons	G, vials with PTFE-lined septum	500 Fill container completely to exclude air.	Acidify to between pH 1 to 2 with H_2SO_4	7 days	If the sample is chlorinated, for each 1 000 ml of sample add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to container prior to sample collection.
Nickel	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	6 months[c]
Nitrate	P or G	250	Cool to between 1 °C and 5 °C.	24 h	
	P or G	250	Acidify to between pH 1 to 2 with HCl	7 days	
	P	250	Freeze to – 20 °C.	1 month	
Nitrite	P or G	200	Cool to between 1 °C and 5 °C.	24 h	Analysis should preferably be carried out on-site. 2 days[c]
Nitrogen total	P or G	500	Acidify to between pH 1 to 2 with H_2SO_4[d].	1 month	
	P	500	Freeze to – 20 °C.	1 month	
Odour	G	500	Cool to between 1 °C and 5 °C.	6 h	The test can be carried out on site (qualitative analysis).
Oil and grease	G solvent-washed	1 000	Acidify to between pH 1 to 2 with H_2SO_4 or HCl	1 month	
Organic chlorine	See "Adsorbable organic halides (AOX)"				
Organotin compounds	G	500	Cool to between 1 °C and 5 °C.	7 days	Extraction of the sample should be carried out on-site.

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Orthophosphates, dissolved	See "Phosphorus, dissolved"				
Orthophosphates, total	See "Phosphorus, total"				
Oxygen	P or G	300 Container should be filled completely		4 days	Fix the oxygen on-site and keep samples stored in the dark. The electrochemical method may be used as well and can be carried out on-site.
Permanganate index	G or P	500	Acidify to between pH 1 to 2 with H_2SO_4, 8 mol/l	2 days	Analyse as soon as possible.
	G or P	500	Cool to between 1 °C and 5 °C and keep samples stored in the dark.	2 days	
	P	500	Freeze to – 20 °C.	1 month	
Pesticides, organochlorine, organo-phosphorus and organo-nitrogen containing	G solvent washed with PTFE cap liner For glyfosate use P	1 000 to 3 000 Do not pre-rinse container with sample; analytes adhere to the wall of the bottle. Do not completely fill the container	Cool to between 1 °C and 5 °C.	Preservation time of the extract is 5 days	If sample is chlorinated, for each 1 000 ml of sample add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to sample collection. Extraction should be carried out within 24 h after sampling.
Petroleum and derivatives	See "Hydrocarbons"				
pH	P or G Fill container completely to exclude air.	100	Cool to between 1 °C and 5 °C.	6 h	The test should be carried out as soon as possible and preferably immediately on-site after sampling.
Phenol index	G	1 000	Inhibit biochemical oxidation by addition of $CuSO_4$ and acidify to pH < 4 with H_3PO_4	21 days	

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Phenols	BG, amber, solvent-washed with PTFE cap liner	1 000 Do not pre-rinse container with sample; analytes adhere to the wall of the bottle. Do not completely fill sample container.	Acidify to between pH < 4 with H_3PO_4 or H_2SO_4	3 weeks	If sample is chlorinated, for each 1 000 ml of sample, add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to sample collection. For chlorophenols the extraction period is 2 days
Phosphorus, dissolved	G or BG or P	250	Cool to between 1 °C and 5 °C.	1 month	The sample should be filtered on-site at the time of sampling. Before analysis, oxidizing agents may be removed by addition of iron(II) sulfate or sodium arsenite.
	P	250	Freeze to – 20 °C.	1 month	
Phosphorus, total	G or BG or P	250	Acidify to between pH 1 to 2 with H_2SO_4 [d]	1 month	See "Phosphorus, dissolved" 6 months for both techniques.[c]
	P	250	Freeze to – 20 °C.	1 month	
Polychlorinated biphenyls (PCBs)	G, solvent-washed with PTFE cap liner	1 000 Do not pre-rinse container with sample; analytes adhere to the wall of the bottle. Do not completely fill sample container.	Cool to between 1 °C and 5 °C.	7 days	Extract on-site where practical. If sample is chlorinated, for each 1 000 ml of sample add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to sample collection
Polycyclic aromatic hydrocarbons (PAHs)	G, solvent-washed with PTFE cap liner	500	Cool to between 1 °C and 5 °C.	7 days	Extract on-site where practicable. If sample is chlorinated, for each 1 000 ml of sample add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to sample collection.
Potassium	P	100	Acidify to between pH 1 to 2 with HNO_3.	1 month	

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Purgeables by purge and trap	G, with PTFE cap liner	100	Acidify to between pH 1 to 2 with H_2SO_4	7 days	14 days[c] If sample is chlorinated, for each 1 000 ml of sample, add 80 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to sample collection.
Selenium	P acid-washed or G acid-washed	500	Acidify to between pH 1 to 2 with HNO_3.	1 month	
Silicates, dissolved	P	200	Cool to between 1 °C and 5 °C.	1 month	The sample should be filtered on-site at the time of sampling.
Silicates, total	P	100	Cool to between 1 °C and 5 °C.	1 month	
Silver	P acid-washed or G acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	
Sodium	P or G	100	Acidify to between pH 1 to 2 with HNO_3	1 month	
Solids, suspended	P or G	500	Cool to between 1 °C and 5 °C.	2 days	
Sulfate	P or G	200	Cool to between 1 °C and 5 °C.	1 month	
Sulfide (easily liberated)	P	500 Fill container completely to exclude air.	Cool to between 1 °C and 5 °C.	1 week	Fix samples immediately on-site by adding 2 ml of 10 % (mass concentration) of zinc acetate solution. If the sample is chlorinated, for each 100 ml of sample add 80 mg of ascorbic acid to the container prior to analysis
Sulfite	P or G	500 Fill container completely to exclude air.		2 days	Fixing on-site by addition of 1 ml of a 2,5 % (by mass) solution of EDTA per 100 ml of sample.

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Surfactants, anionic	G, rinse with methanol.	500	Acidify to between pH 1 to 2 with H_2SO_4 Cool to between 1 °C and 5 °C.	2 days	Glassware should not be detergent-washed. Can be combined with non-ionic.
Surfactants, cationic	G, rinse with methanol.	500	Cool to between 1 °C and 5 °C.	2 days	Glassware should not be detergent-washed.
Surfactants, non-ionic	G	500 Ensure container is filled completely.	Add 37 % (by volume) formaldehyde (see WARNING at end of table) solution to give 1 % (by volume) solution; cool to between 1 °C and 5 °C	1 month	Glassware should not be detergent-washed.
Tin	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HCl	1 month	
Total hardness	See "Calcium"				
Total solids (total residues, dry extract)	P or G	100	Cool to between 1 °C and 5 °C.	24 h	
Trihalomethanes	G, vials with PTFE-faced septum	100 Fill container completely to exclude air.	Cool to between 1 °C and 5 °C	14 days	If sample is chlorinated, for each 100 ml of sample, add 8 mg of $Na_2S_2O_3 \cdot 5H_2O$ to the container prior to sample collection.
Turbidity	P or G	100	Cool to between 1 °C and 5 °C. Keep samples stored in the dark.	24 h	Preferably carried out in the field.
Uranium	P acid-washed or BG acid-washed	200	Acidify to between pH 1 to 2 with HNO_3	1 month	
Vanadium	P acid-washed or BG acid-washed	100	Acidify to pH 1 to 2 with HNO_3	1 month	

Table 1 (*continued*)

Determinand to be studied	Type of container[a]	Typical volume (ml) and filling technique[b]	Preservation technique	Maximum recommended preservation time before analysis after preservation	Comments
Zinc	P acid-washed or BG acid-washed	100	Acidify to between pH 1 to 2 with HNO_3	1 month	6 months[c]

WARNING — Beware of formaldehyde vapours. Do not store large numbers of samples in small work areas.

[a] P = Plastics [e.g. polyethylene, PTFE (polytetrafluoroethylene), PVC (polyvinyl chloride), PET (polyethylene terephthalate)]
G = Glass
BG = Borosilicate glass

[b] The volume is indicative for a single test.

[c] Validated prolonged preservation times.

[d] Not recommended for simultaneous persulfate oxidation/digestion procedures.

Table 2 — Preservation techniques for use with multiple determinands

Preservation technique	Suitable for	Not suitable for
Acidify to between pH 1 to 2 with HNO_3	Alkaline metals (potassium, sodium) Alkaline earth metals (calcium, magnesium) Heavy metals (except mercury) Mercury (with $K_2Cr_2O_7$) Absorbable organic halides (AOX) Aluminium, antimony, arsenic, barium, beryllium, calcium, cadmium, chromium, cobalt, copper, iron (total), lead, lithium, magnesium, manganese, nickel, selenium, silver, uranium, vanadium, zinc Total hardness	Cyanide Sulfide Carbonate, bicarbonate, carbon dioxide Nitrite Soaps and esters Hexamethylenetetramine Thiosulfate
Acidify to between pH 1 to 2 with HCl	Acidic herbicides Antimony Arsenic Chlorinated solvents Hydrocarbons Hydrazine to 1 mol/l Iron(II) Nitrate Oil and grease Petroleum and derivates Tin	Cyanide Silver Thallium Lead Bismuth Mercury(II)

Table 2 (*continued*)

Preservation technique	Suitable for	Not suitable for
Acidify to between pH < 4 with H_3PO_4	Phenols	Cyanide
Acidify to between pH 1 to 2 with H_2SO_4	Adsorbable organic halides (AOX) Ammonia, free and ionized Carbon, total organic (TOC) Chemical oxygen demand (COD) Hydrocarbons Kjeldahl nitrogen Monocyclic aromatic hydrocarbons Nitrogen, total Oil and grease Orthophosphates, total Permanganate index (8 mol/l) Petroleum and derivatives Phenols Phosphorus, total Purgeables by purge and trap Surfactants, anionic	Cyanide Barium Calcium Strontium Radium Lead
Alkali addition to pH > 12 with NaOH	Cyanide total and easily liberated	Most organic compounds Heavy metals, especially in lower valence states Some metals form soluble anions at higher valence states Ammonia/ammonium Amines and amides Hydrazine Hydroxylamine
Freeze (– 20 °C)	Anions Ammonia, free and ionized Nitrate Biological oxygen demand (BOD) Carbamate pesticides Chlorophyll (temperature of – 80 °C required) Chemical oxygen demand (COD) Kjeldahl nitrogen Nitrogen, total Carbon, total organic (TOC) Orthophosphate (total and dissolved) Permanganate index Phosphorus (total and dissolved) Bioassays, toxicity tests	Precipitation (and polymerization) can occur making resolution difficult. Conversely some pesticides depolarize. Suitability should be evaluated before routine use.

Table 3 — Techniques generally suitable for the preservation of samples — Biological analysis

Determinand to be studied	Type of container [a]	Preservation technique	Typical volume ml	Maximum recommended preservation time before analysis [b]	Comments
Counting and identification					
Benthic macro-invertebrates, large samples	P or G	Add ethanol to the sample to give concentration of at least 70 % (volume fraction).	1 000	1 year	Water in samples should first be decanted to maximize the preservative concentration.
	P or G	Add 37 % formaldehyde (see WARNING at end of table) neutralized with sodium tetraborate or hexamethylene-tetramine (100 g/l formalin solution) to give a final solution of 3,7 % formaldehyde (corresponding to a 1 to 10 dilution of formalin solution).	1 000	1 year (3 months minimum preservation time before analysis)	
Benthic macro-invertebrates, small samples (for example reference collections)	G	Transfer to a preservative solution consisting of at least 70 % by volume ethanol, 37 % by volume formaldehyde (see WARNING at end of table) and glycerol (in the proportions 100:2:1 respectively).	100	Indefinitely	Special methods are required for invertebrate groups that are distorted by normal preservative treatment (for example platyhelminthes[6]).

Table 3 (*continued*)

Determinand to be studied	Type of container [a]	Preservation technique	Typical volume ml	Maximum recommended preservation time before analysis [b]	Comments
Algae	G or P with tight fitting lid	Addition of 0,5 part to 1 part by volume of (acid or alkaline) Lugol's solution to 200 parts by volume of sample. Cool to 1 °C to 5 °C	200	6 months	Keep samples stored in the dark. Alkaline Lugol is generally applicable in fresh water and acid Lugol in marine water with delicate flagellates. For specific determination see specific standard. Addition of more Lugol's solution may be necessary if decolourization occurs.
Phytoplankton	G	See "Algae"	200	6 months	Keep samples stored in the dark.
Zooplankton	P or G	Addition of 37 % by volume formaldehyde (see WARNING at end of table) neutralized with sodium borate to give a final solution of 3,7 % formaldehyde or addition of Lugol's solution as for algae	200	1 year	Addition of more Lugol's solution may be necessary if decolourisation occurs.

Table 3 (*continued*)

Determinand to be studied	Type of container [a]	Preservation technique	Typical volume ml	Maximum recommended preservation time before analysis [b]	Comments
Fresh and dry mass					
Benthic macro-invertebrates Macrophytes Algae Phytoplankton Zooplankton Fish	P or G	Cool to between 1 °C and 5 °C.	1 000	24 h	Do not freeze to – 20 °C. The analysis should be carried out as soon as possible and not later than 24 h.
	P or G	Add 37 % formaldehyde (see WARNING at end of table) neutralized with sodium tetraborate or hexamethylene-tetramine (100 g/l formalin solution to give a final solution of 3,7 % formaldehyde (corresponding to a 1 to 10 dilution of formalin solution).	1 000	3 months minimum preservation time before analysis	Note that fresh and dry (bio)mass determinations of periphyton and phytoplankton are usually based on the cell volume measurements made during counting and identification procedure from the preserved sample.
Mass of ash					
Benthic macro-invertebrates Macrophytes Algae Phytoplankton	P or G	Add 37 % formaldehyde (see WARNING at end of table) neutralized with sodium tetraborate or hexamethylene-tetramine (100 g/l formalin solution) to give a final solution of 3,7 % formaldehyde (corresponding to a 1 to 10 dilution of formalin solution).	1 000	3 months minimum preservation time before analysis	Note that fresh and dry (bio)mass determinations of periphyton and phytoplankton are usually based on the cell volume measurements made during counting and identification procedure from the preserved sample.

Table 3 (*continued*)

Determinand to be studied	Type of container [a]	Preservation technique	Typical volume ml	Maximum recommended preservation time before analysis [b]	Comments
Dry mass and mass of ash					
Zooplankton		Freeze to – 20 °C	200	6 months	Sample is filtered through pre-weighed glass-fibre membrane filters and then frozen at – 20 °C.
Toxicity tests					
	P or G	Cool to between 1 °C and 5 °C	1 000	24 h	The preservation period will vary according to the method of analysis to be used. See also ISO 5667-16.
	P	Freeze to – 20 °C	1 000	2 weeks	

WARNING — Beware of formaldehyde vapours. Do not store large numbers of samples in small work areas.

[a] P = Plastics [e.g. polyethylene, PTFE (polytetrafluoroethylene), PVC (polyvinyl chloride), PET (polyethylene terephthalate)]
G = Glass
BG = Borosilicate glass

[b] If a preservation period is not specified, it is generally unimportant. The indication "1 month" represents preservations without particular difficulty.

Table 4 — Techniques generally suitable for the preservation of samples — Radiochemical determinands

Determinand to be studied	Type of container[a]	Preservation technique	Typical volume ml	Maximum recommended preservation time before analysis	Comments
Alpha activity	P	Acidify to between pH 1 to 2 with HNO_3.	2 000	1 month	Do not acidify if the sample is evaporated before analysis. Keep samples stored in the dark.
		Cool to between 1 °C and 5 °C.	2 000	1 month	
Beta activity (except radio-iodine)	P	Acidify to between pH 1 to 2 with HNO_3.	2 000	1 month	Do not acidify if the sample is evaporated before analysis. Keep samples stored in the dark.
		Cool to between 1 °C and 5 °C.	2 000	1 month	
Gamma activity	P	Cool to between 1 °C and 5 °C.	5 000	2 days	
Radio-iodine	P	Cool to between 1 °C and 5 °C.	3 000	2 days	Add 2 ml to 4 ml of sodium hypochlorite solution (10 % by mass) per litre of sample, ensuring an excess of free chlorine.
Radon isotopes Radium by radon in growth	BG	Cool to between 1 °C and 5 °C.	2 000	2 days	Minimum 4 weeks in growth of radium daughters.
Radium by other methods	P	Acidify to a pH < 1 with HNO_3.	2 000	2 months	Minimum 4 weeks in growth of radium daughters.
		Cool to between 1 °C and 5 °C.	2 000	2 months	
Radio-strontium	P	Cool to between 1 °C and 5 °C.	1 000	1 month	Minimum 2 weeks in growth of yttrium-90.
Radio-caesium	P	Cool to between 1 °C and 5 °C.	5 000	2 days	
Tritiated water	P	Cool to between 1 °C and 5 °C.	250	2 months	Sample is distilled before analysis.
Uranium	P	Acidify to a pH < 1 with HNO_3	2 000	1 month	
		Cool to between 1 °C and 5 °C.	2 000	1 month	
Plutonium	P	Acidify to a pH < 1 with HNO_3	2 000	1 month	
		Cool to between 1 °C and 5 °C.	2 000	1 month	

WARNING — Safety precautions and shielding depend on the activity of the sample.

Contamination of the sample should be avoided, especially if the sample activity is very low. Some sample sites can have measurable activity in the soil or air, or in waters other than those being sampled. Laboratories, as well as some items of domestic equipment, can contain radioactive material.

When sampling precipitation, any special requirements in this table are additional to those given in ISO 5667-8. As the collection of sufficient sample can require a period of days, both the starting and finishing times and dates should also be recorded. A record of precipitation collection for the sample station for the appropriate period should be appended. Stabilizer or carrier may be added, if appropriate for the determinands being measured.

NOTE Some plastics bottles slowly concentrate samples over a period of many months by being very slightly permeable to water. Also see the comments for radon.

[a] P = Plastics [e.g. polyethylene, PTFE (polytetrafluoroethylene), PVC (polyvinyl chloride), PET (polyethylene terephthalate)]
G = Glass
BG = Borosilicate glass

Annex A
(informative)

Dutch investigation on prolonged preservation times

A.1 Introduction

Studies have been carried out in the Netherlands in 1999 and 2000 on the maximum preservation times given in the second edition of ISO 5667-3 (ISO 5667-3:1994) [1].

This work was funded by the Foundation STOWA (Dutch acronym for the Foundation of Applied Water Management Research for Wastewater Analysis) and by the Netherlands Ministry of Housing, Spatial Planning and the Environment (VROM).

The project concerned the following determinands:

Desk study and laboratory study:
- COD;
- BOD;
- Kjeldahl nitrogen;
- total phosphate;
- heavy metals (arsenic, cadmium, chromium, copper, lead, nickel, zinc and mercury).

Desk study only:
- nitrate;
- ammonium;
- chloride;
- extractable organohalogen compounds;
- volatile organohalogen compounds;
- mineral oil;
- volatile aromatic compounds;
- PAHs;
- organochlorine pesticides and PCBs;
- (chlorinated) phenols;
- nitrogen- and phosphate-pesticides;
- phenyl urea;
- chlorophenoxy-carbonic acids;
- carbamates;
- phthalates;
- oil and fat;
- chlorophyll/phaeophytine;
- organotin compounds.

The desk study included a literature search, an inquiry among laboratories and an inventory of International Standards. In the laboratory study, the preservation techniques as stated in ISO 5667-3:1994 were evaluated, especially with respect to the maximum preservation times.

A.2 Desk study

Within the scope of the project, an inquiry has been held among 25 regional and governmental water quality laboratories and commercial laboratories with respect to the matrices, waste water, surface water, sediment and sewage sludge.

From the 18 returned inquiry forms the following conclusions were made:

- The use of preservation techniques, package materials and storage of water samples by Dutch laboratories are in accordance with ISO 5667-3:1994.

— The maximum preservation times specified in ISO 5667-3:1994 are not always respected because in practice, analysis cannot always be carried out within the maximum time specified in ISO 5667-3:1994.

— The preservation techniques stated in different International Standards are identical, except for mercury (with respect to the application of an oxidizing agent) and, to a lesser extent, total phosphate. Differences exist however in the maximum preservation times.

— Methods of preservation, mentioned in ISO 5667-3:1994 and USA standards can be found in the literature. A basic difference exists in the approach given to methods of preservation. In the USA, preservatives are added to the sample whenever possible, while in ISO 5667-3:1994, cooling of samples is recommended leading to shorter maximum storage times.

— Sufficient scientific foundation is available to verify the preservation techniques stated in ISO 5667-3:1994, except for some of the maximum preservation times.

— In the Netherlands, deep-freezing is allowed as a possible preservation technique and was validated in the laboratory study.

— For sediment and sewage sludge matrices, simple cooling is generally applied as a suitable preservation technique.

— For BOD, cooling for 24 h is generally applied as a suitable preservation technique, although all International and European standards state that analysis should begin as soon as possible.

— Validation results for certain preservation techniques and maximum storage times are available. Based on these results, in some cases it is advisable to use an alternative preservation technique in order to achieve a longer storage time.

A.3 Laboratory study

The study involved 10 water samples, comprising wastewater and surface water samples. The work involved analysis of metals, namely arsenic, cadmium, chromium, copper, lead, nickel, zinc and mercury, and the determinands COD, BOD, Kjeldahl nitrogen and total phosphate. For these determinands, all preservation techniques stated in ISO 5667-3 have been investigated, including:

— addition of sulfuric acid to a pH < 2, cooling to between 2 °C and 4 °C, stored in the dark: determinands COD, Kjeldahl nitrogen and total phosphate;

— deep-freezing at – 18 °C: determinands COD, BOD and Kjeldahl nitrogen;

— addition of nitric acid to a pH < 2: determinands heavy metals and mercury;

— addition of nitric acid to a pH < 2, addition of $K_2Cr_2O_7$ (final concentration 0,05 %): determinand mercury.

At day zero, each determinand in each individual sub-sample was analysed 9 times. The mean and standard deviation of each determinand was calculated. Stored sub-samples were analysed at different pre-determined dates up to 224 days. At several intervals, each sub-sample was analysed in duplicate. The test results at each interval were compared with the mean and standard deviation at day zero. The preservation time was considered to have been exceeded when the mean test results of the stored sub-sample differed from the mean test results of the sub-sample at day zero by more than the standard deviation.

With both deep-freezing and acidification techniques, flocculation of particles occurred, as well as adsorption of these particles onto the wall of the sample container. This effect could give rise to a higher standard deviation in the analytical results. Therefore, extra attention should be paid to the sub-sampling of stored samples. The effect differed in intensity for glass bottles and polypropylene jars.

A.4 Conclusions of the Dutch study

From the results of the Dutch study, it was concluded that:

a) The preservation techniques stated in ISO 5667-3:1994 are adequate and provide longer preservation times than stated in the standard.

b) The techniques investigated in this work are adequate to preserve samples during the whole storage time of 224 days with the exception of determinands BOD and Kjeldahl nitrogen, where the maximum preservation time depends upon the sample matrix for samples having a nitrogen concentration < 8 mg/l or a BOD < 50 mg/l;

c) For samples containing particles, the preservation technique used can give rise to discrepancies in the analytical results. This is probably caused by difficulties encountered in sub-sampling. Sub-sampling therefore requires extra attention to detail.

d) It may be worthwhile to review the guidance given in this part of ISO 5667.

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