
**Water quality — Determination of selected
organic nitrogen and phosphorus
compounds — Gas chromatographic
methods**

*Qualité de l'eau — Dosage de certains composés organiques azotés et
phosphorés sélectionnés — Méthodes par chromatographie en phase
gazeuse*

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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 10695 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Annexes A, B, C and D of this International Standard are for information only.

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Water quality — Determination of selected organic nitrogen and phosphorus compounds — Gas chromatographic methods

WARNING — This International Standard makes use of flammable and toxic organic solvents and some toxic organic and phosphorus compounds. Observe the safety regulations in effect.

1 Scope

This International Standard specifies two methods for the determination of certain organic nitrogen and phosphorus compounds in waters by gas chromatography (see Table 1).

The methods may be extended to include additional substances, provided the methods are validated for each individual case.

Clause 3 describes the liquid/liquid extraction method, which is applicable to samples of drinking waters, ground waters, surface waters and waste waters containing up to 0,05 g/l of suspended solids. In the presence of organic matter, suspended matter and colloids, interferences are more numerous and consequently the detection limits of this method can be higher.

NOTE Because of the very low concentrations normally present in the waters, the problem of contamination is extremely important. The lower the level measured, the more precautions have to be observed.

Clause 4 describes the liquid/solid extraction method which is applicable to samples of ground water, surface water and drinking water containing mass concentrations of about $\geq 0,05 \mu\text{g/l}$. Interferences occurring with the examination of some types of surface water can prevent the application of this method.

Detection limits are given for information in annex A.

NOTE When applying this International Standard, the guide on analytical quality control for water analysis (see ISO/TR 13530) should be followed.

Table 1 — Organic nitrogen and phosphorus compounds analysed by these methods

Name	Molecular formula	Molar mass	CAS No. ^a
Atrazine	C ₈ H ₁₄ ClN ₅	215,7	001912-24-9
Cyanazine	C ₉ H ₁₃ ClN ₆	240,7	021725-46-2
Metazachlor	C ₁₄ H ₁₆ ClN ₃ O	277,8	067129-08-2
Parathion (ethyl)	C ₁₀ H ₁₄ NO ₅ PS	291,3	00056-38-2
Parathion (methyl)	C ₈ H ₁₀ NO ₅ PS	263,2	298-00-0
Pendimethalin	C ₁₃ H ₁₉ N ₃ O ₄	281,3	040487-42-1
Propazine	C ₉ H ₁₆ ClN ₅	229,7	000139-40-2
Sebuthylazine	C ₉ H ₁₆ ClN ₅	229,7	007286-69-3
Simazine	C ₇ H ₁₂ ClN ₅	201,7	000122-34-9
Terbuthylazine	C ₉ H ₁₆ ClN ₅	229,7	005915-41-3
Trifluralin	C ₁₃ H ₁₆ F ₃ N ₃ O ₄	335,3	001582-09-8
Vinclozolin	C ₁₂ H ₉ Cl ₂ NO ₃	286,1	050471-44-8

^a Chemical Abstracts Registry Number.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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/TR 13530:1997, *Water quality — Guide to analytical quality control for water analysis*.

3 Liquid/liquid extraction

3.1 Principle

The organic nitrogen and phosphorus compounds in the water sample are extracted by liquid-liquid extraction with dichloromethane. After concentration, the sample extracts are analysed by gas chromatography, using a nitrogen-phosphorus detector.

3.2 Reagents

All reagents, including water, shall be of sufficient purity that they do not give rise to significant interfering peaks in the gas chromatograms of the blanks. The purity of reagents used in the procedure shall be verified for each batch of material by running blank determinations (3.5.6).

Reagents shall be stored in glass containers.

NOTE Commercial "pesticide grade" solvents are available. The use of these products is recommended only after verifying their quality. The quality of a solvent is checked by evaporation of about 200 ml down to 1 ml and analysis of the concentrate to determine the compounds subsequently analysed. The solvent should be considered acceptable if it does not give any detectable interfering peaks in the chromatogram for the substance of interest.

3.2.1 Water, purified using, for example, ion exchange and carbon column adsorption.

3.2.2 Dichloromethane (CH_2Cl_2) extraction solvent.

3.2.3 Solvents for dissolution, e.g. acetone or ethyl acetate.

3.2.4 Sodium sulfate, anhydrous

Heat Na_2SO_4 powder at $500\text{ }^\circ\text{C} \pm 20\text{ }^\circ\text{C}$ for $4\text{ h} \pm 30\text{ min}$, cool to about $200\text{ }^\circ\text{C}$ in a muffle furnace and then to ambient temperature in a desiccator containing magnesium perchlorate or equivalent alternative.

3.2.5 Solutions for neutralization

a) **Sodium hydroxide**, aqueous solution, $c(\text{NaOH}) = 1\text{ mol/l}$.

b) **Hydrochloric acid**, aqueous solution, $c(\text{HCl}) = 1\text{ mol/l}$.

3.2.6 Standard stock solutions

Standard stock solutions shall be prepared by dissolving pure or, if available, certified organic nitrogen and phosphorus compounds in acetone (3.2.3).

Unless manufacturer's information or stability trials indicate otherwise, store the solutions at around $+4\text{ }^\circ\text{C}$ in the dark (they can be stored at around $-18\text{ }^\circ\text{C}$, but some septa closures may harden at such a temperature with possible losses of solvent).

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For compounds listed in Table 1, the stock solutions are stable for around six months. For other compounds, the laboratory shall check the stability of the solutions.

Prior to use, the solutions shall be brought to ambient temperature.

NOTE 1 A convenient concentration of standard stock solution is obtained by weighing accurately approximately 50,0 mg of each determinand and dissolving it in 100 ml of acetone (3.2.3).

NOTE 2 Certified commercial standards may be used.

3.2.7 Intermediate standard solutions

Prepare intermediate standard solutions by a suitable dilution of the stock solution (3.2.6) with the solvent for dissolution (3.2.3). A typical value is 1 mg/100 ml.

Unless manufacturer's information or stability trials indicate otherwise, store these solutions at around $+4\text{ }^\circ\text{C}$ in the dark.

For compounds listed in Table 1, the intermediate standard solutions are stable up to two months in acetone, except for cyanazine and vinclozolin (one week). For other compounds, the laboratory shall check the stability of the solutions.

3.2.8 Working standard solutions

Prepare at least five different concentrations by suitable dilutions of the intermediate standard solutions (3.2.7) with the solvent for dissolution (3.2.3). A suitable concentration range is 1 μg to 100 μg per 100 ml.

Unless manufacturer's information or stability trials indicate otherwise, store these solutions at around + 4 °C in the dark.

The lifetime of these solutions is limited to one week for the compounds listed in Table 1. For other compounds, the laboratory shall check the stability of the solutions.

NOTE At these low concentrations, degradation by light and adsorption onto the glass become more apparent.

3.3 Apparatus

3.3.1 Gas chromatograph, fitted with a capillary column, a nitrogen-phosphorus detector and a data processing system.

At least two glass or fused-silica capillary columns shall be used, with an inside diameter of less than 0,4 mm and a length of 25 m to 60 m, coated with stationary phases of different polarity allowing the separation of the compounds of interest.

NOTE If a mass spectrometer is used for confirmation, one capillary column is normally sufficient.

Annex B provides examples of gas chromatographic conditions and the corresponding chromatograms.

3.3.2 Separating funnels, nominal capacities 1 l to 5 l, with grease-free glass or polytetrafluoroethylene (PTFE) tap.

3.3.3 High-speed stirrer and dichloromethane-washed **magnetic stirring bar**, coated with PTFE.

3.3.4 Any suitable system for evaporation.

3.3.5 Microlitre syringes.

3.3.6 Miscellaneous glassware

Laboratory glassware may be cleaned for example using a cleaning agent (laboratory detergent), followed by either a treatment with chromium(VI)/sulfuric acid mixture, or peroxodisulfate/sulfuric acid mixture and after rinsing with water subsequently washed with dichloromethane.

The efficacy of the treatment shall be randomly verified experimentally, using blank determinations to ensure that no interfering contamination has occurred.

3.4 Sampling and sample preparation

Take samples in accordance with ISO 5667-1 and ISO 5667-2.

Some organic nitrogen and phosphorus compounds can degrade rapidly in an aqueous environment. Therefore, unless stability trials indicate otherwise, extract the sample within one day of collection for phosphorus compounds and within two days of collection for nitrogen compounds. If extraction is delayed beyond one day, the extent of the delay shall be noted in the test report.

Collect the water samples in glass bottles cleaned as described in 3.3.6 (do not use plastics bottles) with ground-glass stoppers or with screw caps with PTFE liners. Fill the bottles above the shoulders.

Samples shall be protected from daylight using, for example, brown glass bottles, aluminium foil, etc.

On sample collection, take care that no interfering substances enter the water sample, and no losses of the determinands occur. This is especially important with sampling apparatus using plastics tubings.

When losses by adsorption are suspected, it shall be proved by control tests (3.5.6 and 5.2.3) that no losses by adsorption occur.

Glass and stainless steel devices shall preferably be used.

Measure the sample pH and, if necessary, correct the pH immediately after collection in order to be in the range pH 6 to 9, by adding the appropriate neutralization solutions (3.2.5) (the volume of reagent added shall be negligible to the volume of water collected).

If not done at the time of sampling, this fact shall be stated in the test report.

During transport, keep the sample at about + 4 °C, avoiding contamination.

3.5 Procedure

3.5.1 General

Varying recoveries and reproducibilities can be obtained with different water types. The yields of the procedure shall be checked (see 5.2.4) and the number of extraction steps required to obtain satisfactory yields [more than 60 % (see 5.2.4)] shall be determined by the laboratory. A minimum of three extractions is necessary. See annex A for typical recovery rates.

NOTE The limit of detection depends on the volume of water extracted, the final volume of solvent, volume injected and detector sensitivity. Typically a sample of 500 ml and a final extract volume of 1 ml are used.

3.5.2 Sample pretreatment

Sample pretreatment is normally not necessary.

It is recommended to perform the extraction in the sampling bottle.

Where the sample bottle is completely filled, shake and pour off excess sample in order to obtain sufficient free volume for the subsequent addition of the solvent.

Measure the volume of the water to be extracted by weighing the bottle before extraction and after emptying, or with a volumetric cylinder.

3.5.3 Extraction in the sampling bottle and further separation

Use a sample volume of about 0,5 l to 1 l.

NOTE Some circumstances can require different volumes.

Add the dichloromethane (3.2.2) to the sample (3.5.2) (typically 50 ml of dichloromethane per 500 ml of water) and shake for at least 10 min or use a high-speed stirrer (3.3.3).

Transfer into a separating funnel of suitable capacity (3.3.2) and allow the phases to separate.

Run the aqueous phase back into the sample container. Repeat the extraction twice with 20 ml of the solvent (3.2.2) per 500 ml of sample.

If previous tests indicate yields less than 60 % or strong emulsions are encountered, repeat the extraction with a new portion of the solvent.

Collect the solvent extract and dry it, using an appropriate procedure, for example:

- to the flask, add about 5 g of anhydrous sodium sulfate (3.2.4) and swirl. Leave to stand for 5 min. If the sodium sulfate has aggregated after this period and there are no separate grains, add more sodium sulfate, swirl and leave to stand for a further 5 min. Decant the extract into the concentration apparatus. Wash the sodium sulfate with a further 10 ml to 20 ml of solvent (3.2.2) and add the washings to the evaporating vessel,

or

- freeze the extract at $-18\text{ }^{\circ}\text{C}$ for 2 h. Decant the solvent extract from the ice and transfer to the evaporating vessel. Rapidly wash the vial with a few millilitres of cooled solvent (3.2.2) and add the washings to the evaporating vessel.

3.5.4 Concentration of the extract

Concentrate the combined dried extracts from 3.5.3 using the evaporation system (3.3.4).

Evaporate at a temperature less than $40\text{ }^{\circ}\text{C}$, just to dryness, and add exactly 1 ml of the solvent used for the calibration solutions.

3.5.5 Gas chromatography

Set up the gas chromatograph (3.3.1), fitted with a suitable column, according to the instructions of the manufacturer and ensure it is in a stable condition.

Inject the extract [usually $1\text{ }\mu\text{l}$ to $10\text{ }\mu\text{l}$, but the same volume as used for calibration (clause 5)] into the gas chromatograph.

The requirements applicable to the extent of the measurements, and the calibration, evaluation and calculation techniques to be used, are described in clauses 5 and 6.

Compare the gas chromatogram obtained to those of the standard solutions (see clause 5).

Evaluate the gas chromatogram qualitatively and quantitatively (see clause 6).

Check the obtained gas chromatogram for the absence of overlapping peaks occurring at the locations of the retention times of the determinands of interest.

Chromatograms of standards should be checked for any retention time and/or peak resolution changes, losses caused by decomposition within the chromatographic system (beware of dirty glass inserts in the injector device). Any change in the solvent composition may affect the detector response.

3.5.6 Blank determination

Carry out the complete procedure (pretreatment, extraction, concentration, gas chromatographic analysis) using a sample of pure water (3.2.1).

If the blank value is too high, namely greater than 10 % of the lowest measured value for any of the compounds of interest, carry out a step-by-step examination of the procedure and eliminate the cause.

If sample concentrations are close to the limit of determination, blank values greater than 10 % of the lowest measured value can be tolerated.

The blank value shall be subtracted only if the between-batch standard deviation of the blank value does not significantly exceed the standard deviation of the calibration function.

4 Liquid/solid extraction

4.1 Principle

The compounds in the water sample are, if necessary after neutralization, enriched on reversed phase (RP)-C18 material or other adsorbent, eluted with a solvent and then determined by gas chromatography, using a nitrogen-phosphorus detector.

4.2 Reagents

All reagents, including water, shall be of sufficient purity that they do not give rise to significant interfering peaks in the gas chromatograms of the blanks. The purity of reagents used in the procedure shall be verified for each batch of material by running blank determinations (3.5.6).

Reagents shall be stored in glass containers.

NOTE Commercial "pesticide grade" solvents are available. The use of these products is recommended only after verifying their quality. The quality of a solvent is checked by evaporation of about 200 ml down to 1 ml and analysis of the concentrate to determine the compounds subsequently analysed. The solvent should be considered acceptable if it does not give any detectable interfering peaks in the chromatogram for the substance of interest.

4.2.1 Water, purified using, for example, ion exchange and carbon column adsorption.

4.2.2 RP-C18 material or other adsorbent.

Adsorbent may be for example in the form of commercially available cartridges or adequately filled glass columns with a minimum packing height of 1 cm.

A higher packing height with the same packing quantity may improve the recovery. The selectivity of the material is dealt with in 4.3.2.

4.2.3 Conditioning solvents, for example, methanol, acetone.

4.2.4 Solvent for the elution, for example, methanol, acetone.

4.2.5 Solutions for neutralization. (standards.iteh.ai)

Identical to those used for liquid/liquid extraction. See 3.2.5.

4.2.6 Standard stock solutions. <https://standards.iteh.ai/catalog/standards/sist/5fac5afe-97a2-4c12-bec1-b8e38356ea42/iso-10695-2000>

Identical to those used for liquid/liquid extraction. See 3.2.6.

4.2.7 Intermediate standard solutions.

Prepare intermediate standard solutions by a suitable dilution of the stock solution (4.2.6) with the solvent used for the elution (4.2.4). A typical value is 1 mg/100 ml.

Unless manufacturer's information or stability trials indicate otherwise, store these solutions at around + 4 °C in the dark.

For compounds listed in Table 1, the intermediate standard solutions are stable up to two months in acetone, except for cyanazine and vinclozolin (one week). For other compounds, the laboratory shall check the stability of the solutions.

4.2.8 Working standards.

Prepare at least five different concentrations by suitable dilutions of the intermediate standard solutions (4.2.7) with the solvent (3.2.3). A suitable concentration range is 1 µg/100 ml to 100 µg/100 ml.

Unless manufacturer's information or stability trials indicate otherwise, store these solutions at around + 4 °C in the dark.

The lifetime of these solutions is limited to one week for compounds listed in Table 1. For other compounds, the laboratory shall check the stability of the solutions.

NOTE At these low concentrations, degradation by light and adsorption onto the glass become more apparent.